PRODUCTION OF JET FUEL FROM MICROALGAE BIOMASS CULTIVATED IN SALINE DOMESTIC WASTEWATER

By

Joseph Kapuku Bwapwa (MSc. Eng.)

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Supervisor: **Professor Cristina Trois**

Co-supervisor: **Professor Akash Anandraj**

SUPERVISOR DECLARATION

As the candidate's Supervisor I agree to the submission of this thesis.

Signed: Date:

Prof. Cristina Trois
Prof. Anandraj Akash

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DECLARATION 2: PUBLICATIONS

All papers listed in this section and presented in this thesis have been first authored by myself under the supervision of Prof. Cristina Trois and Prof Akash Anandraj. My role in the production of these papers included, but not limited to: The conceptualisation and planning of each paper, literature review, the laboratory experiments, data collection, data analysis and the writing of all papers. The role of my co-authors was of an advisory, mentorship, guidance and editorial nature. Some of these papers were designed to be the chapters of this current thesis.

Details of publications:

1. Bwapwa, J.K., Anandraj, A. and Trois, C., 2017. Possibilities for conversion of microalgae oil into aviation fuel: A review. *Renewable and Sustainable Energy Reviews*, *80*, pp.1345-1354. :(see copy in APPENDIX A1)

2. Bwapwa, J.K., Anandraj, A. and Trois, C., 2018. Microalgae processing for jet fuel production. *Biofuels, Bioproducts and Biorefining*, *12*(4), pp.522-535. (see copy in APPENDIX A1)

3. Bwapwa, J.K., Anandraj, A. and Trois, C., 2018. Conceptual process design and simulation of microalgae oil conversion to aviation fuel. *Biofuels, Bioproducts and Biorefining*, *12*(6), pp.935-948 (see copy in APPENDIX A1)

4. Bwapwa, J.K., Anandraj, A. and Trois, C., 2018, jet fuel from blending algal jet fuel and jet a1 in 50/50 volume ratio, International Journal of Low-Carbon Technology (Accepted: to be published)

5. Bwapwa, J.K., Anandraj, A. and Trois, C.,2018 New jet fuel from blending microalgae-based jet fuel and Jet A1 in 20/80 and 80/20 ratios respectively (Under review), international journal of green energy (see copy in APPENDIX A1)

6. Bwapwa, J.K., Anandraj, A. and Trois, C., 2018 jet fuel from domestic wastewater treatment using microalgae, book chapter, editor: SPRINGER NATURE, environmental chemistry for a sustainable world (Under review),(see copy in APPENDIX A1)

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ABSTRACT

Jet fuel from crude petroleum oil is known as the most efficient energy carrier for the aviation sector. Environmental concerns and economic pressure drive major industries to adapt to current global revolution towards alternative, sustainable, and clean fuels. In this study conversion of algae biomass to jet fuel is presented as a novel technology of low carbon footprint and a cost-effective jet fuel. In this current study, it is reported that there is a possibility of substantially converting microalgae oil into aviation fuel by adapting and applying the same processes used for the conversion of fossil crude petroleum oil into conventional jet fuel. The drawback, however, remains the low oil output from used species of microalgae and the general operating costs which are still at developmental stages.

A part from the introduction and the literature review making respectively **chapter 1** and **chapter 2,** this study, therefore, has focused on the magnification of lipid production and the simplification of the conversion processes in **chapter 3**. Microalgae cells were physiologically stressed by totally depriving them of all growth nutrients for three days aiming to modify their genetic physiology which in turn will favour the yield of lipid and bio-oil. An elaborate experiment was established from algae biomass cultivation to jet fuel production. The experiment involved biomass cultivation, harvesting and bio-oil extraction using a solvent mixture made of methanol and chloroform. Thermal cracking without catalyst or pyrolysis of crude bio-oil were undertaken to break down the carbon chains in order to complete the fractionation. ASTM methods and standards related to aviation fuels were used to generate the relevant data.

The conceptual design with a simplified conversion process undertaken **in chapter 4** was established based on the experiment completed **in chapter 3**. It suggested to cultivate the species in domestic wastewater ponds or use the seawater/saline water because the used species was comfortable in saline or marine environment. Parameters such as density, kinematic viscosity, flash point, freezing point, total sulfur, net heat of combustion and distillation were evaluated during the experiment in **chapters 3 and 4**. It was found that the majority of parameters analysed regarding the algae-based jet fuel from the laboratory was complying to ASTM standards. However, it will require additional processes such as upgrading and reforming to enhance the quality of jet fuel and improve the level of some parameters such as density and freezing point which were not rigorously complying with ASTM standards. Also, the improvement involves the use of the same additives used for conventional jet fuels for flow

ability and freezing at higher altitudes. The scaling up of the production process is still a challenge due to operating costs. In this regard, blending algae-based jet fuel and the conventional jet fuel in 50/50, 80/20 and 20/80 ratios was carried out and evaluated in **Chapter 5** and **Chapter 6** as an alternative approach for sustainability.

Keywords: Jet biofuel; algae-based jet fuel; biofuels; microalgae; biomass

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LIST OF ABBREVIATIONS

IEA: International Energy Agency

GHG: Greenhouse Gas

DME: Dimethyl Ether

LCA: Life Cycle Assessment

TAG: Triacylglycerols

ASTM: American Society for Testing and Materials

F-T: Fischer–Tropsch

GFT: Gasification/Fischer-Tropsch

FT-SPK: Fischer-Tropsch synthetic paraffinic Kerosene

FT-SKA: Fischer-Tropsch synthetic Kerosene high in aromatics content

BTL: Biomass to liquids

FTJ: Fermentation to jet

GC/ MS: Gas chromatograph / Mass spectrometry

B20: a mixture composed of 20% of biofuel with 80% of fossil fuel

B50: a mixture composed of 50% of biofuel with 50% of fossil fuel

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CHAPTER 1 PREFACE

The current chapter provides the background information and the methodological approach used in this study. The research problem, research question, hypotheses and expected results including the aims and objectives are outlined. In addition, the knowledge contribution related to algae hyper synthesis of lipids, algae-based jet fuel production and blending options is included. The chapter concludes with the organization of the dissertation and individual chapter content.

CHAPTER 1: INTRODUCTION

1.1 BACKGROUND AND SIGNIFICANCE

The world's demand for energy is increasing yearly and the current production patterns are not sustainable. According to International Energy Agency (IEA), the world uses over 85 million barrels of petroleum per day and global primary energy demand is growing at a rate of 1.6% per year. This implies that global primary energy demand will double over the next 40 years. In addition, the IEA estimates that there is a need to introduce over 30% zero carbon fuels into the global energy pool to stabilize atmospheric CO_2 levels. As the focus on environmental protection is increasingly dominant and the volatility of oil prices is an undeniable reality, many countries are considering the use of renewable energy to prevent any foreseeable and unforeseeable energy crisis in the near future. It is well known that the world is seriously relying on fossil-based fuels for energy production and transportation activities. Unluckily, this type of fuels despite their costs competitiveness compared to biofuels, are known to be non-environmentally friendly with tons of greenhouse emissions being released daily into the atmosphere. Greenhouse emissions are the main cause of global warming and climate change. Hence, the consequences linked to climate change and global warming is immeasurable in today's environmental context. Furthermore, the global increasing demand for energy as mentioned earlier is becoming a challenge today because of the increasing need of products and chemicals used for the production of energy. Also, the limited world reserves in terms of fossil fuels become a cause for concern for future generations. A need for innovative solution to secure a better future in transportation fuels and energy production is very vital. Emerging technologies and continued innovation hold the promise of real solutions through a combination of energy efficiency and new renewable energy. There is a need for solutions that can defy the current definition of energy sources and the way energy is utilised. In recent years,

wind, solar and hydrogen energies have been developed as alternative options to fossil-based fuels, these energy forms are not practical in meeting transportation needs due to size, scale, and costs application. The best alternative could be biofuels, ethanol from corn and crop-based biodiesel. They have positive and energy yields; however, they are inefficient in production. In addition, they increase demand for crops causing inflation of food crisis. Also, they require large acres of agricultural lands. Cost of aviation fuels and its critical handling poses challenges and limits the extent of travel. Altitude and routes set by aviation authorities are constrained by the properties of aviation fuel. The freezing point of a commercial fuel is - 40°C. This temperature is reached sometimes at an altitude of around 10000 m. Beyond this altitude, there is a risk of fuel solidification. Aircraft have heating systems to avoid jet fuel solidification. Similarly, antifreeze additives are added to jet fuel before usage. Algae-based oil was investigated to find out on its eventual freezing properties.

Algae from cold to freezing habitats synthesise oils as energy reserves that have a lower freezing point. This is a highly explored and could be one of the foremost in the renewable energy market more especially for jet fuel production. Though algae-based fuels present some challenges such as low lipid content for many species, high costs for harvesting and drying, algae require fewer inputs, it is the very fastest growing biomass and can be grown anywhere, there is no stress on food or agricultural related products, therefore, solving most problems related to traditional crop-based fuels. The main benefit of using algae compared to other biofuels is that algae do not compete with agricultural or limited resources such as water and lands. Algae have the potential to reduce the generation of greenhouse gas (GHG) and to recycle CO_2 emissions from flue gases generated by power plants and natural gas operations. This is a very important fact because the growth of algae is dependent on the amount of carbon dioxide. Therefore, power plants can reduce reasonably their CO_2 emissions into the atmosphere by supplying CO_2 to algae producers; however, this will require more studies in terms of storage and transportation of CO_2 to the cultivation site. Consequently, algae processing plants will not only produce fuels but will also assist in reducing the effects of climate change in a specific environment. However, it is important to stress the fact that the literature on algae-based transportation fuels is very scarce and also not many studies undertaken in this specific field are accessible to the public domain. Previous studies focusing on processes such as solar conversion into renewable liquid transportation fuel developed by Chisti (2007)[1] were proven to be technically feasible. The economics of producing such biofuels is currently not cost competitive compared to fossil fuels. To alleviate the operation

costs concern, an efficient process that provides high-quality algae-based jet fuel at competitive costs will be a sustainable option. In this regard, there are some documented methods of converting microalgae oil to jet fuel focusing on hydroprocessing. During this process, oil is reacted with hydrogen over a catalyst and then isomerised to produce a targeted mixture of alkanes, water, CO_2. The alkane mixture can be fractionated to produce a synthetic jet fuel. This process was only demonstrated at non-commercial scale and the production of hydrogen was an added cost to the overall process [2]. Also, catalytic cracking and reforming processes were explored on the small scale but not at large scale [2]. Furthermore, a limited number of solvent-based processes was assessed at large scale with some success. However, the economics of the processes have demonstrated that the costs of drying algae biomass added to the overall costs required significant energy. [3] Conversely, another variant of organic solvent-based processes process is extraction by in situ transesterification. [2] In this approach, the bound lipids are released as methyl esters produced by directly adding the catalyst and methanol to the dried algae. While this method has been used at large scales also, the approach works best using dried algae and therefore is not cost effective since energy is also required for drying the biomass [4]. Recently, another ground-breaking study completed in Japan by Kanda (2011) [5] involved the use of Liquefied dimethyl Ether (DME) to skip harvesting and drying processes. During this process, green crude oil was successfully extracted from blue-green microalgae. DME is used as a solvent; its molecules disperse inside cells through cell walls and bind strongly to the oily components. Its boiling point is 25˚C and but liquefied under 20^{o}C.[2] This method was capable of extracting 60 times green oil compared to other methods described previously. DME is recovered as vapour only through depressurisation step because it evaporates at room temperature. There is no commercial plant is operational up to date using this method. [5] The cost aspects and life cycle assessment (LCA) are not part of this study, maturing the conversion technology appears to be a priority before undertaking costs and LCA.

The current study will focus on a laboratory experiment to establish the techniques and generate some operational data essential for scaling up the technology in future studies. A marine algae strain known as *Nannochloropsis sp* contains on average 40 to 60 % of lipids can be a potential candidate for the production of jet fuel. Production of biofuels from oil crops suffers from a handful of drawbacks such as expensive raw material. Marine species are economically ideal alternatives compared to oil crops. Microalgae are known to grow faster in water/wastewater and they contain essential compounds that can be utilized for biofuel production.

Microalgae species such as *Nannochloropsis* are viewed as a promising alternative because they contain extensive fatty acids and lipids.[6,7] *Nannochloropsis* species are challenging to identify because of the cells small size and are characterised by unique features related to the absence of chlorophyll b or c. *Nannochloropsis* is able to attain over 60% lipids increase after nitrogen starvation, this makes their candidacy in a higher spot for biodiesel production. *Nannochloropsis* has the ability to grow briskly and synthesize a large amount of triacylglycerols (TAG) and high-value polyunsaturated fatty acids such as eicosapentaenoic acid that can be converted into jet fuel for the aircraft industry.

Jet-fuel can have 14 to 16 short carbon chains known as middle distillate carbon chains and the TAG is known for a high percentage of short chain fatty acids. This is a key feature of this species to be explored regarding the possibility of producing jet fuel from *Nannochloropsis sp*.

1.2 AIM & OBJECTIVES

The study aims to produce jet fuel from microalgae that complies with stringent regulations and standards currently established in the aviation industry.

To fulfil this aim, there are objectives to be achieved. These objectives are summarized as follows:

1. To increase the lipid content in algae biomass in order to improve the bio-oil output essential for conversion processes. (**Chapter 3**)
2. Develop a simplified process from microalgae cultivation to jet fuel production: a laboratory process from the experiment and its simulation at a larger scale. (**Chapter3 and Chapter 4)**
3. To suggest options to improve the fuel quality to the standards required in the aviation sector (**Chapter 3 and Chapter 4**)
4. Analyse the efficiency and sustainability of blending algae-based jet with Jet A1 using ASTM standards (**Chapter 5 and Chapter 6).**

1.3 HYPOTHESES AND EXPECTED OUTPUTS

1.3. 1 Hypothesis 1

lipid content from *Nannochloropsis sp* will increase by implementing total nutrients starvation of microalgae cells.

1.3.2 Hypothesis 2

The same conversion processes used for conversion of crude petroleum oil to conventional jet fuel can be used and adapted to convert microalgae oil into jet fuel.

1.3.3Expected output

The expected output will include data generated from microalgae cultivation to the final product being jet fuel. The data will consist of physico-chemical parameters such as lipid content, kinematic viscosity, density, net heat of combustion, freezing point, flash point, distillation temperatures, total acidity, total sulfur and Conductivity. All these parameters are analysed using ASTM standards for compliance regarding jet fuel quality.

1.4 RESEARCH PROBLEM AND QUESTION

Globally, energy production is amongst one of the key factors that are essential to economic and industrial development. However, the production of fossil fuels through petrochemical processes is seen as the major cause of environmental pollution and climate change effects.
Furthermore, reserves of world fossil fuels resources are very limited and may lead to scarcity in the near future. In addition, a price fluctuation for petroleum products is another issue that affects the economics of users of petroleum-derived products.
To reduce dependence on fossil fuels and produce cost-effective fuels, microalgae conversion to fuels is one option to be explored in order to meet the costs (capital and production) and environmental requirements. Today, the on-going research for algae-based biofuels seeks to improve technologies in order to enable the production of jet fuel and other biofuels to the commercial level. Several projects on algae-based biofuels have been achieved on laboratory and pilot scales but none of them has been implemented at a larger scale for commercialisation. The feasibility for competitive algae-based fuels remains a challenge because it requires a sustainable conducive technology to lower costs and make algae-based fuels competitive.
In the area of aviation fuels, it is reported that the production of jet fuel can technically be achievable. However, the main challenging concern is to increase algal lipid content and

improve the operation costs for conversion processes. It is important to emphasise that high lipids production is a key aspect of the production of the high amount of oil essential for jet fuel manufacturing. Increasing lipids depends on the type of strains or species type, the cultivation conditions and mainly the starvation technique.

The development of low costs algae-based jet fuel for both small and large scales are greatly needed in the current environmental and economic context. This requires getting operating costs similar or competitive to the ones applied to the manufacturing of conventional jet fuel. It can partly be possible by duplicating and adapting petrochemical processes and operating conditions to produce algae-based jet fuel. However, processes such as cultivation of biomass, biomass harvesting and the physiological modification of microalgae cells require an effective technology to produce cost-competitive algae-based jet fuel. This constitutes one of the main problematics encountered in this field of algae-based fuels particularly for algae-based jet fuel. Technically, it is expected that algae-based jet fuel does not have to freeze at higher altitudes; it has to generate high heat energy and must be lighter or less dense with excellent flow properties. Therefore, the question to be answered in this study is formulated as follows: Given the fact that renewable microalgal oil is similar to crude petroleum oil in terms of quality and it can generate a fuel with a low carbon footprint [8,9]; is it possible to convert microalgae oil from *Nannochloropisis sp* into a jet fuel that complies to relevant ASTM standards?

In case the answer is affirmative some more questions will rise on how and to what extent it is possible. These questions constitute the basis of this study. The appropriate answers are relying on the methodological approach undertaken in this current study.

Costs aspects and life cycle assessment (LCA) are not part of this study, the relevant technology for conversion processes has to reach a level of maturity on a large scale before undertaking costs and LCA studies. The current challenge is focusing more on the development of effective processes or technology to manufacture a competitive and sustainable jet fuel from microalgae.

1.5 METHODOLOGICAL APPROACH

The general approach to achieving the objectives of this study was undertaken on laboratory experiment. The experiment aimed to establish the process required to convert microalgae to a jet fuel that will be in accordance with the aviation fuel standards as mentioned before. The laboratory experiment generated data from various steps. This data provided necessary information to be used for process simulation. The simulation engine assisted on designing the entire process to be used also for large scale production.

The methodological approach was structured as follows:

1.5.1 Cultivation

The growth of cells and biomass productivity were monitored daily. Biomass growth curves were plotted showing all growth phases. Samples were collected in triplicate, using 1 l flasks. The cells of *Nannochloropsis sp* were cultivated under define operating conditions including the temperature, salinity and defined illumination mode. Nutrients were supplied to *Nannochloropsis sp* cells by F/2 media during cultivation for an efficient growth. Microalgae cells were mixed by aeration within the growing biomass to allow the exposure of the cells to the light. The pH of the microalgae was monitored on daily basis to make sure that *Nannochloropsis sp* is growing under acceptable pH values which should neutral. *Nannochloropsis sp* is a marine species, the salinity of the growing environment (domestic saline wastewater) was kept at 30 ppt (gr/l) representing the average salinity for an effective growth relevant to the nature and type of species used. The biomass production was monitored daily with a spectrophotometer. This also assisted in quantifying **Chlorophyll A** needed for the plotting of the growth curve.

1.5.2 Biomass harvesting

This process was undertaken immediately after reaching the stationary growth phase. It was achieved by using a centrifuge at 4000 rpm for 10 minutes.

1.5.3 Physiological modification

Previous studies have used partial starvation of key nutrients to boost the lipid content of species in order to increase their lipid content. In this study, stressing microalgae in an environment free of nutrients was undertaken in order to assist in modifying the species physiology aiming to stimulate the species metabolism aiming to increase its lipid content.

1.5.4 Extraction of crude bio-oil

Solvent-based extraction was used to get the oil stored in the biomass. The mixture of methanol and chloroform was used for crude bio-oil extraction

1.5.5 Conversion of bio-crude oil into jet fuel

A process was simulated using ASPEN HYSIS V8.8 package for the conversion of algae crude bio-oil into jet fuel.

1.5.6 Jet fuel characterisation

This was conducted to verify the compliance of the produced algae-based jet fuel with the required aviation standards.

1.5.7 Blending of algae-based jet fuel with the conventional jet fuel

This was done using various ratios in order to develop a new jet fuel from the mixture of algae-based jet fuel and conventional jet fuel.

Briefly, the methodological approach used for this study is summarized in Figure 1 which includes the main steps from cultivation to jet fuel production**.**

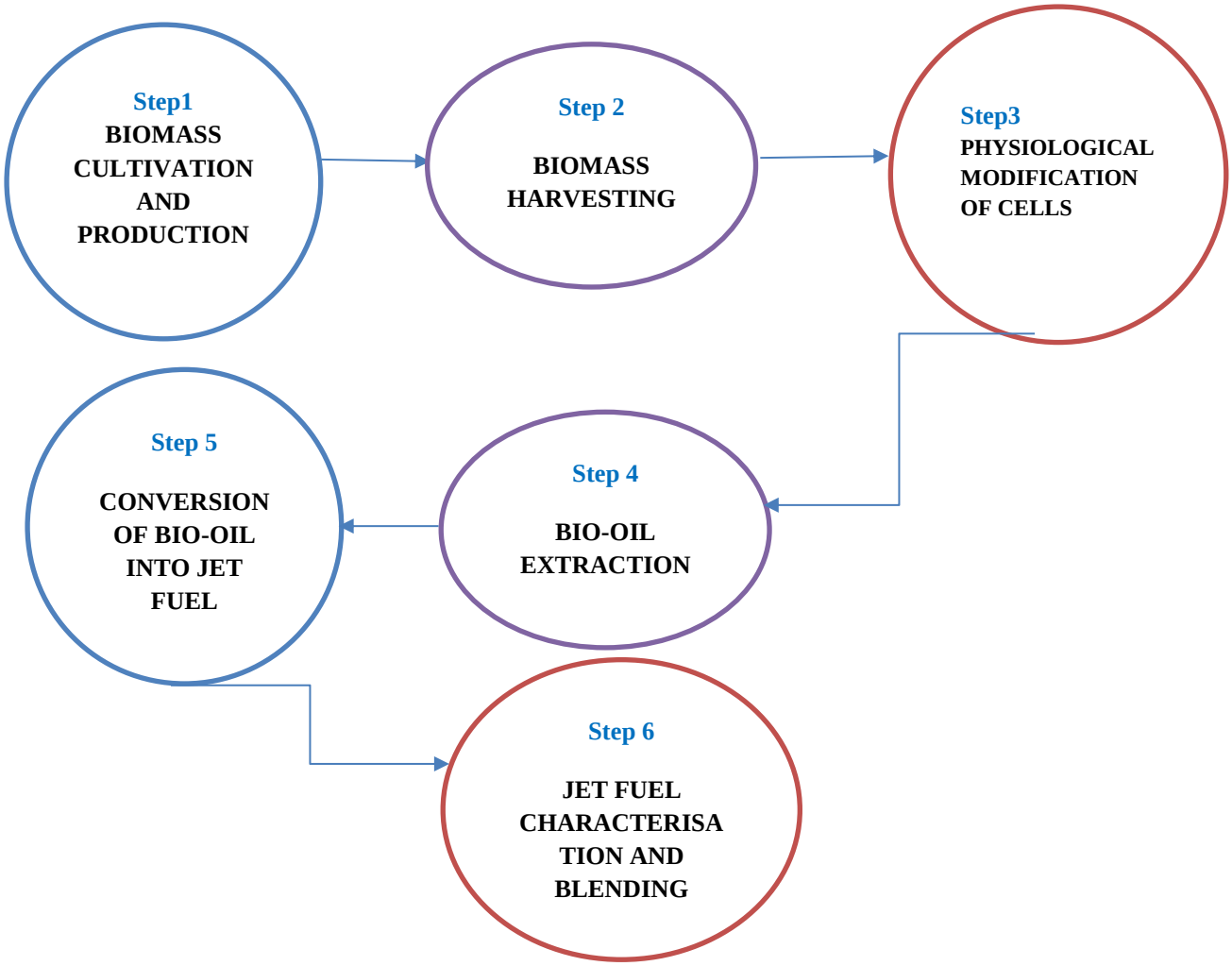

Figure 1: Theoretical framework of the methodological approach for the study

1.6 CONTIBUTION TO KNOWLEDGE

In the past, various studies dealing with algae-based fuel have used physiological manipulation by partial nutrient starvation to increase algae lipid content. In most cases, nitrogen starvation was the most used for many strains by the majority of studies. It was reported that under environmental stress conditions such as nitrogen starvation, microalgal cells accumulate lipids as the main carbon storage compound. Synthesis of lipids and their deposition into cytosolic lipid bodies may be, with exceptions, the default pathway in some species under stress conditions [10]. Producing a high amount of lipids remains a significant challenge and requires novel methods to maximize microalgae lipid content essential for conversion processes. Apart from this challenge, the scaling up of the technology still not yet achieved. Up to date, there is no plant at large scale that produces jet fuel from microalgae, there is a need to design and simulate a process comparable to the one used for conversion of crude oil into conventional jet fuel as mentioned earlier. Therefore, the contribution to knowledge from this study is established on the following facts:

1. In previous studies, the lipid increase was achieved via partial nutrients starvation or enzymatic processes as mentioned earlier. The current study has established another approach for microalgae cells physiological modification to enhance lipid increase based on total nutrients starvation. The increase of lipid content was achieved by genetically manipulating microalgae cells switching back and forth between normal growth and lipid increase in order to maintain high rates for both. Total nutrients starvation was completed in a maximum period of 3 days to avoid cells death.

2. A conceptual design based on the laboratory experiment was developed in order to simulate a complete conversion process to be used on a large scale. This process involved the same processes used to produce conventional jet fuel in a petrochemical plant. The simulation of the conceptual design was successfully completed. Therefore, microalgae oil can be used in a petrochemical refinery plant to produce jet fuel of a similar quality compared to conventional jet fuel

3. Blending in 50/50 ratio algae-based jet fuel and conventional jet fuel. Although this blending option is ASTM certified regarding the blending of biomass led fuels and fossil fuel, this blending option also illustrates the potential of algae-based jet fuel in complying with ASTM standards and the possibility to reduce carbon footprint when blending conventional jet fuel (fossil-based jet fuel) and the algae-based jet fuel.

4. Blending algae-based jet fuel in ratios of 80/20 and 20/80 which are not ASTM certified to provide more insight regarding these blending options. These new blending options have displayed its potential to comply with ASTM standards for aviation fuels. Most parameters have complied with the ASTM standards after blending.

1.7 THESIS OUTLINE

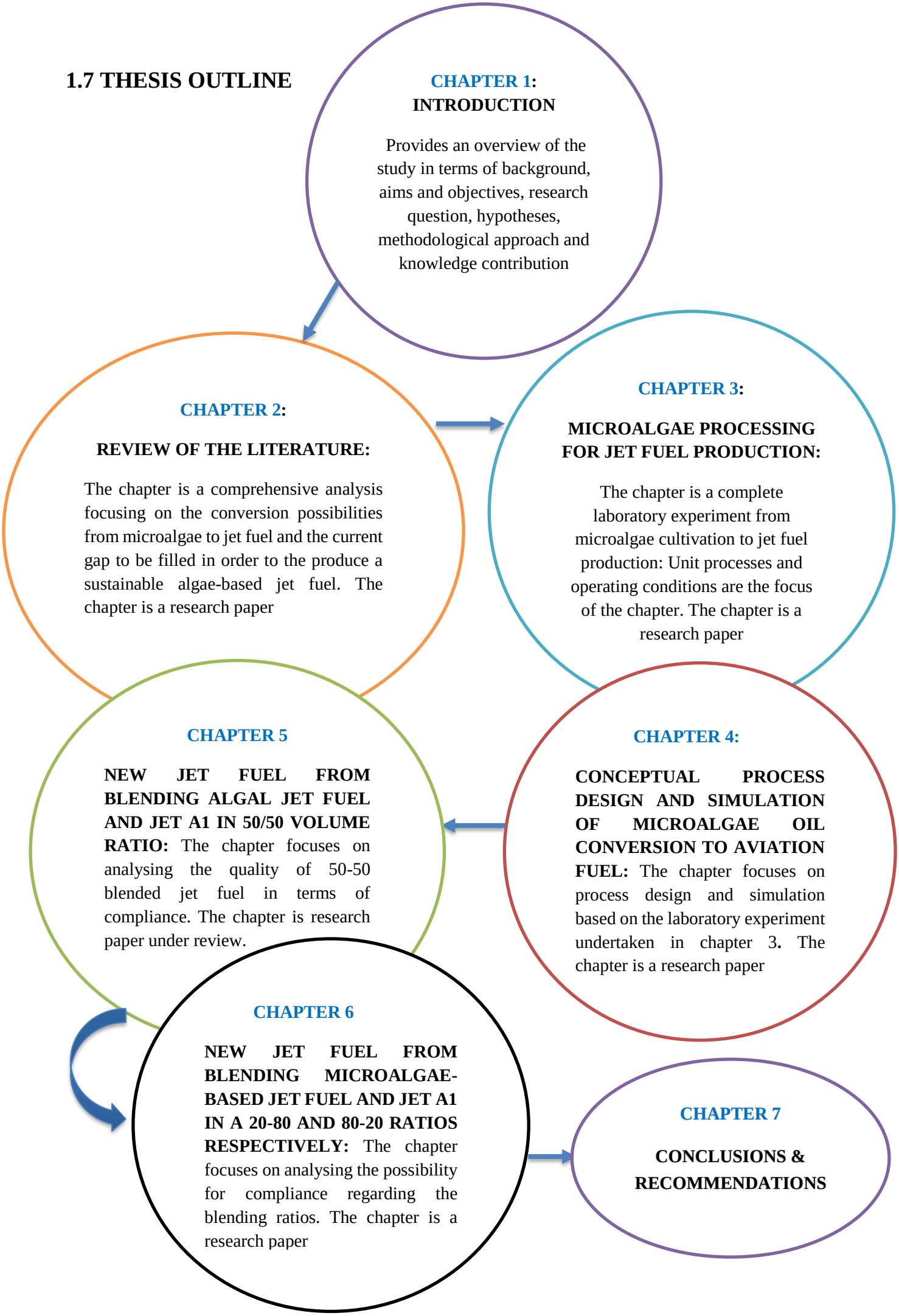

Figure 2: Theoretical framework of the thesis outline

Figure 2 presents the framework of the current thesis made of seven chapters, the content of each chapter is briefly explained.

REFERENCES

1. Chisti Y. Biodiesel from microalgae. Biotechnology advances. 2007 Jun 30;25(3):294-306.

2. Darzins A, Pienkos P, Edye L. Current status and potential for algal biofuels production. A report to IEA Bioenergy Task. 2010 Aug 6;39.

3. Nagle N and Lemke P. Production of methyl ester fuel from microalgae. Applied Biochemistry and Biotechnology. 1990 Mar 1;24(1):355-61.

4. Mendes RL, Nobre BP, Cardoso MT, Pereira AP, Palavra AF. Supercritical carbon dioxide extraction of compounds with pharmaceutical importance from microalgae. Inorganica Chimica Acta. 2003 Dec 3;356: 328-334.

5. Kanda H, Li P. Simple extraction method of green crude from natural blue-green microalgae by dimethyl ether. Fuel. 2011 Mar 1;90(3):1264-1266.

6. Eloka-Eboka AC, Inambao FL. Effects of CO_2 sequestration on lipid and biomass productivity in microalgal biomass production. Applied Energy. 2017 Jun 1;195 :1100-1111.

7. Eloka-Eboka A, Onunka C, Inambao F., Detailed design and optimization of a sustainable micro-algal biofuel process plant. International Journal of Low-Carbon Technologies. 2018 May 26.

8. Bwapwa JK, Anandraj A, Trois C. Possibilities for conversion of microalgae oil into aviation fuel: A review. Renewable and Sustainable Energy Reviews. 2017 Dec 1;80:1345-1354.

9. Liu X, Saydah B, Eranki P, Colosi LM, Mitchell BG, Rhodes J, Clarens AF. Pilot-scale data provide enhanced estimates of the life cycle energy and emissions profile of algae biofuels produced via hydrothermal liquefaction. Bioresource technology. 2013 Nov 1;148:163-171.

10. Hu Q, Sommerfeld M, Jarvis E, Ghirardi M, Posewitz M, Seibert M, Darzins A. Microalgal triacylglycerols as feedstocks for biofuel production: perspectives and advances. The plant Journal, 2008 May 1st; 54(4):621-6

CHAPTER 2 PREFACE

This chapter is written in the form of a research article published in *Renewable and Sustainable Energy reviews* journal (Elsevier), minor changes were made to improve the flow, and clarity of the published review article. The chapter is a gathering of information related to the current state of various possibilities or options to be explored regarding the conversion of algae bio-oil into jet fuel. As a review, it is a comprehensive analysis of various conversion processes including their strengths, weaknesses and the current gap to be filled in this field. It contains some details on various technological developments, fuel readiness, opportunities, challenges for use in aviation and compatibility with existing systems. This review has highlighted and emphasised on the potential that some processes are presenting over the others in order to achieve the conversion of algae bio-oil into jet fuel. From the details gathered in this review algae bio-oil is an excellent candidate to produce aviation with low carbon content. The copy of the original published article is in APPENDIX A1 as indicated before in the declaration section.

CHAPTER 2: LITERATURE REVIEW
POSSIBILITIES FOR CONVERSION OF MICROALGAE OIL INTO AVIATION FUEL: *a Review*

[1,2]Joseph K. Bwapwa, [2]Anandraj Akash, [1]Cristina Trois

[1] University of KwaZulu-Natal, [2] Mangosuthu University of Technology

Corresponding author: joseph@mut.ac.za ; josephkapuku@gmail.com

ABSTRACT

The aviation sector relies on petroleum jet fuel because it is the most efficient energy carrier. Due to environmental and economic concerns, a strong demand for alternative fuels is emerging. There is a need for diversification of energy sources from natural resources. These resources must be environmentally friendly and costs effective. Environmental impacts of fossil fuels on global warming and climate change are being a major concern today. Furthermore, the fluctuations of oil prices and need for sustainable fuel supply are the strong drivers for the economies of fuel users. In the aviation sector, Jet fuel from microalgae is one of the alternatives receiving considerable attention; it offers the potential to diversify energy sources. Microalgae species can produce lipids; they do not require high use of land, do not need freshwater, can grow in marine water or wastewater, grow faster in a very short period of time, the produced oil is not a threat to food security. Similarly, the effect of climate change and global warming due to the generation of greenhouse gases (GHG) from petroleum jet fuel can be considerably reduced due to low carbon footprint generated by algae-based fuels. Therefore, algae-based aviation fuels can be considered as an alternative to producing an efficient fuel compared to conventional fuels. Conversely, the key challenge is: many algae species have lower lipid content. Harvesting and drying processes are costly as well as upstream processes to convert microalgae oil into Jet fuel. Although algae biofuels are still small players in the aviation industry, there is a potential for the future. This review analyses some routes to be explored or already explored, their strengths and weaknesses, the current trends and possible conceptual approaches to get aviation fuel from microalgae oil.

KEYWORDS: microalgae, Bio-jet fuel, harvesting, algal biofuel, microalgae oil, microalgae species

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2.1INTRODUCTION

Global reserves of petroleum oil have decreased significantly during the last decades; a probable energy crisis could severely affect the world in the near future. Environmental and economic concerns are pressurizing the aviation industry to an extent of strongly influencing the industry growth. Despite the implementation of many international treaties aiming for the promotion of environmental sustainability, the aviation industry is requested to work toward the reduction of its environmental impacts. It can only be achieved by developing new technologies or by upgrading the current ones. Therefore, this will successfully promote the concept or idea of green aviation. An average of 705 Mt of CO_2 has been generated in 2013 from airlines operations; it is almost 2 to 3% of global anthropogenic CO_2 emissions.[1] Predictions show that this figure will increase between 1,000 and 3,100 Mt by 2050. The aviation industry has given itself a target to reach a carbon neutral growth status by 2020. [1] It is achievable through the pillars of innovation with the main focus on operations, air traffic management, environmental protection, safety and fuel processing technology and sustainability. Focusing on the last one, renewable energy from biofuels may constitute an acceptable alternative and possibly a reliable option in case the energy crisis deepens in the near future. However, production costs for bio-jet fuels are relatively higher compared to those for conventional jet fuels. Consequently, the selling price may become exorbitant.

Higher production costs are attributed to factors such as relative immaturity of the technology, small number of active producers; producers of biofuels are focusing more on novel end products because of low funding in the biofuel markets. Also, the costs of raw materials may be another cause of higher production cost for biofuels. It is reported that 80% of the operating costs are made up of raw materials costs regarding biofuel/ biodiesel production.[2] Therefore, it is necessary to look for suitable and alternative raw materials or crops capable of producing non-edible oil at low costs to produce biofuels than using edible vegetable oil for biofuel production.

Microalgae species such as *Nannochloropsis sp, Tetraselmis sp, Chlamydomonas sp, Synechococcus sp* and many others can be an acceptable alternative compared to the edible oil used domestically because they do not constitute a threat to food security. They are photosynthetic cells growing at very high rates in an aquatic environment. Also, they are considered as emerging alternatives for biofuel production because their composition reveals a remarkable presence of fatty acids and lipids. Fatty acids and lipids are the main sources of energy for biofuels. Similarly, According to Rodolfi (2009)[3],they have attracted great

attention in the biofuel market because of their high capacity to accumulate abundant amounts of triacylglycerols (TAG). These species are mainly used in the production of biofuels such as biodiesel, bio-hydrogen and bio-jet fuel. Bio-jet fuel from algae is gaining more interest and might become a very important deal with global implications in the near future. Firstly, there is a great focus on aviation biofuels to reduce the impact of climate change and global warming. This will also assist in reducing the operating costs in the aviation industry. Secondly, the petroleum or conventional jet fuel prices and their volatility have a severe impact on the business of airlines: because of fuel price fluctuations, there are rippling repercussions to economies of many airlines as well as many countries. Furthermore, there is a big market for jet fuel which is currently being served partially and the demand has kept its increasing trend due to the need which is growing yearly. The world consumption of jet fuel has almost tripled in 30 years; from 1,837,000 barrels/per day in 1980 to 5,220,000 barrels per day in 2010. About 30% of the world consumption was used in the United States (1,398,130 barrels per day in 2012).[4]These data are used for information regarding the trends but new data should be more informative.

Producing algae-based jet fuel will require a comprehensive identification of the potential technical, economic and environmental challenges. Once these challenges are clearly identified and solved properly, there is a possibility to make bio-jet fuel from algae "a drop in" fuel which will strictly comply with the aviation fuel standards. The objective of this review is to highlight, analyse and suggest some possibilities/ processes or routes that will lead to jet fuel from microalgae oil. This study focuses on downstream processes, the feasibility, their strengths and weaknesses. It is important to stress on the fact that various routes for algae jet fuel production are technically possible but not costs effective and commercially viable to compete with conventional jet fuel. The economic studies and historical developments are not part of this article as they can be part of another detailed and specific study in the future.

2.2 COMMON POINTS BETWEEN PETROLEUM CRUDE OIL AND ALGAE CRUDE BIO-OIL

Algae bio-crude oil extracted from species such as *Nannochloropsis sp, Schizochytrium sp, Botryococcus braunii* and many other species may have many similarities with petroleum crude oil in terms of hydrocarbons content and molecular structure.[5] The majority of petroleum crude oil originates from algae in marine environment or ancient algae deposit.[6] Petroleum crude oil consists largely of liquid hydrocarbons because it is mainly made up of carbon and hydrogen. On average 85% w/w of oil is carbon, 10-14 % of oil is hydrogen, oxygen accounts for 1 to 2 % and sulfur represents up to 4% of the oil total weight.[7] Petroleum crude oil originates from a compound named Kerogen; this compound is created in the marine environment by sedimentary rocks from a series of biochemical and/or chemical reactions called diagenesis and catagenesis.[8] It is transformed into crude oil under specific conditions of pressure and temperature. It is largely composed of algae, biodegraded organic compounds, plankton, bacteria and plant material. Various forms of Kerogen have different amounts of hydrogen relative to carbon and oxygen.[8] Qualitatively, algae crude bio-oil have similar molecules compared to petroleum crude oil, however, there are some differences quantitatively.[5] This is based on the fact that petroleum crude oil is derived from Kerogen and algae is part of Kerogen as mentioned before. However, algae crude bio-oil may differ slightly from petroleum crude oil depending on species features, the type and nature of species, as well as the species lipids content. Therefore, one of the main focuses of algae biotechnology is the development of biofuels making use of microalgae as the main raw material.

2.3 COMPLIANCE AND CURRENT DRIVERS FOR ALTERNATIVE JET FUELS

The biggest challenge for algae biofuel producers is to make jet fuel which is totally compliant with stringent regulations as it is the case with conventional jet fuel. Compliance is mainly focusing on physico-chemical properties or parameters such as cold flow property, energy density, energy content, kinetic viscosity, freezing point, concentration of aromatics, material compatibility, safety properties, flash point and thermal stability. The concept of compliance must also at the time be centred on combustion and kinetic aspects such as ignition and extinction characteristics, chemical kinetics, lubricity flame speed and flammability limits. Table 1 provides an overview of some requirements based on physico-chemical characteristics, the operational purpose as well as the specifications.

Furthermore, compliance implies that jet fuel composition must display specific physico-chemical characteristics that are critical for the fuel to perform efficiently. These features must strongly correlate with the composition of jet fuel knowing that the composition depends mainly on the raw crude oil, the type of refining process used and additives to improve the quality of jet fuel. Generally, the conventional jet fuel composition is roughly made up of 20% paraffin, 40% isoparaffin, 20% naphthenes and 20% aromatics.[9] However, additions are made to jet fuel in order for it to comply with environmental aspects and the expected performance. This data is given for information only and reports only on the main components of jet fuel. These components play a crucial role in the effectiveness of the fuel during operation. For instance, the heat density per unit mass of the fuel is greatly improved when the ratio hydrogen-to-carbon from isoparaffins and paraffins is high; another fact is that naphthenes assist in the reduction of the freezing point, which is very critical at higher altitudes; aromatics contribute largely to material compatibility and prevent leaks in the seals of some aircraft.[9,10,11] Therefore, the composition of jet fuel can be regarded as a key aspect for compliance with the stringent standards used for aviation fuels which are namely ASTM D1655 of the American Society for Testing and Materials (ASTM) and Def Stan 91-91 of the UK Ministry of Defence

Table 1: Summary of important jet fuel properties (derived from [11])

PARAMETERS	**PURPOSE**
High Energy density	Impacts on the aircraft range
Low Freezing point	Influences pumping capacity of the fuel at a lower temperature
High Thermal stability	Clogging or fouling of fuel system and nozzles occur generally due to Coke and gum deposits during combustion. It prevents the chemical decomposition of the fuel.
Viscosity	Influences the ability of the fuel nozzles to spray fuel and the capacity of the engine to restart at higher altitudes.
Combustion features	To minimise the formation of particulates in the combustor and in the exhaust
Adequate Lubricity	Affects the ability of the jet fuel to lubricate fuelling system and engine controls
Material compatibility	The Jet Fuel interacts with metals and many others materials, it necessary to ensure an effective compatibility with materials in contact with jet fuel.
Safety	Avoidance of explosions during handling of the jet fuel and during its storage into containers
High Specific energy	Decreases take-off weight and helps to improve the efficiency of the fuel
High Flash point	Allow the fuel ignition for safe operation
Aromatic compounds	Must be sufficient because it allows acceptable seal swell to prevent leaks in the fuel system.

Alternative jet fuels such as algae-based jet fuels will require to satisfy many expectations which are described as follows:

the reduction of emissions that have an impact on climate change and global warming, the diversification and expansion of energy supplies, jet fuel should be less expensive in terms of capital and operating costs, there should be a possibility of scaling up without major constraints and producing large volumes of fuel without adversative effects on water and land resources, bio- jet fuel must perform as a'' drop-in ''substitution or support for the conventional jet-fuel.[11] These characteristics can be summarized into three main aspects: algae-based jet fuels must contribute to solving problems requiring efficient environmental sustainability, beneficial economic sustainability and energy diversification.[11] To solve this issue related to these characteristics there are challenges that need to be addressed effectively in order to meet compliance requirements.

2.4 ALGAE- BASED AVIATION FUEL: CHALLENGES

Apart from the compliance aspect mentioned earlier, algae aviation fuels are faced with three types of challenges: biological, chemical and mechanical. In terms of biological challenges, strain selection is regarded as one of the main aspects to be considered because each strain is characterised by its lipid content.[5] Lipid content can affect biofuel productivity or yields. Furthermore, Nutrient requirements and circulation, growth rates, lipid productivity and optimization of photosynthetic efficiency are very important. They have to be meticulously monitored because they constitute the basis of an efficient growth. [5,12,13,64] The algal strains to be cultivated should be selected based on lipid production, biomass productivity, harvestability, resistance to contamination, optimal light intensity, tolerance of high oxygen levels and temperature extremes. Individual and collective effects in terms of biomass productivity, lipid contents of four strains of microalgae was recently studied by Eloka-Eboka and Inambao [64], the outcome is that the nature of strain/species has a direct impact on lipid biosynthesis, biomass productivity and the type of lipids produced.

Chemical challenges are faced when dealing with processes such as oil extraction using a solvent, transesterification, chemical harvesting, quality of water and removal of toxic substances in water or wastewater used for algae cultivation.[5,14] Mechanical challenges are mainly based on maintenance issues and costs for cultivation units, harvesting, drying and oil extraction equipment such as oil press or impeller.[5] All these challenges make the production of Jet fuel from microalgae being more challenging but very possible if an efficient process optimization can be undertaken after a viable process design.

2.5 POTENTIAL FOR MICROALGAE OIL TO BE USED FOR JET FUEL PRODUCTION

Carbon chain lengths in microalgae oil are almost similar compared to the ones in the crude petroleum as mentioned earlier. Generally, commercial and military jet fuels have carbon chains length ranging between 8 and 16 (C8 to C16).[15] The conversion of microalgae oil into jet fuel will require reducing the number of carbon chains within the required range as mentioned earlier. It can be achieved through the breaking down of long carbon chains under specific operating conditions defined by temperature, pressure and use of catalysts.

This catalytic process aims to connect carbon molecules in order to get the configuration or chemical structure known for jet fuel. However, carbon chain configuration is not the only requirement to get a compliant fuel; there is also lipids content which is extremely important to acquire high output during downstream processes. It is therefore important to select strains with higher content in lipids in order to generate great output from conversion processes. Lipid characterisation is one of the most important steps to be undertaken before any study involving the production of algae biofuels. It helps determine the suitability of the strain or species for biofuel production. Table 2 presents a summary of some previous studies completed on carbon chain profile for three species. The analysis of the data in table 2 reveals that these species are potentially suitable for aviation fuel or any other biofuel.

The study confirmed that structure or carbon molecule configuration was made up of high energy loaded molecules capable of being converted into biofuels.[16] Therefore, the possibility to produce jet fuel from various microalgae species can be certain to some extent but more work needs to be undertaken regarding the process. The persisting challenges are the costs related to growth, harvesting and drying. Also, the lipid content for many species is relatively low. It is very important to select species with high lipids content for a viable jet fuel production. More studies on species with high lipid content are still progressing especially in the private domain. For low lipid content species, there is a tremendous need for improving lipid content using lipid boosting techniques such as total or partial nutrients starvation or the use of genetically modified organisms. Conventional jet fuel is made up of complex mixtures of hydrocarbons in large numbers. The molecular weights or number of carbon chains is defined by the requirements expected for the final product, for instance, the freezing point, viscosity, density, volatility or smoke point. Biofuel physico-chemical properties improve considerably if both polar and non-polar lipids are involved in the transesterification reaction;

some studies based on making use of total lipids (polar and non-polar lipids) to generate biofuels by transesterification have demonstrated positive outcomes.[17] Furthermore, lipid synthesis can be modified by changing growth conditions, this is particularly valid for non-polar TAGs known as the best substrate for biofuels production.[3]

Table 2: Carbon chains content in algae oil [% w] for some species (adapted from [18]; [19];[20])

Fatty acids	Nannochloropsis salina [18]	Phaeodactylum tricornutum [19]	Botryococcus braunii [20]
C12:0	5.0	-	0.7
C14:0	-	4.5	0.8
C15:0	0.5	-	0.5
C16:0	37.5	25.8	21.0
C16:1	23.3	37.5	2.0
C16:2	-	-	6.5
C16:3	-	-	15.2
C17:0	0.4	-	0.1
C18:0	0.9	1.3	2.9
C18:1	11.9	-	3.2
C18:2	1.5	5.1	13.6
C18:3	-	2.0	33.0
C20:0	0.1	-	0.2
C20:1	-	-	-
C20:4	3.3	1.6	-
C20:5	15.3	13.1	-
C22:0	0.4	-	0.1
C22:1	-	-	-
C24:0	-	-	0.2
C24:1	-	-	-

2.6 OIL YIELDS FOR SPECIES TO BE USED FOR ALGAE -BASED JET FUEL

Table 3 presents outputs for microalgae oil being extracted from dry biomass for twenty different types of species. These species are potentially suitable for biofuels especially for jet fuel production due to their lipid content. This information may be useful for evaluation studies in order to estimate the oil yields to be generated for downstream or conversion processes. It may also be very useful for processes, plant design, mass and energy balance. To increase the species lipid content, a physiological modification can be undertaken as mentioned earlier.

The success of physiological modification or stress will depend on the type, nature and behaviour of each species under stress conditions. Table 3 also indicates that *Botryococcus braunii*, *Nannochloropsis sp* and *Schizochytrium sp* have high lipid content looking at the average between their lower and maximum lipid content. Many other species indicated in table 3 may have also a great potential to produce biofuel or aviation biofuel but not much information is available in the public domain to analyse their potential in producing aviation fuels. *Botryococcus braunii* has a molecular structure which is roughly similar to the gas-oil fraction of the petroleum crude oil; it is potentially fit to be used for blending with other fuels for jet fuel production.[21] However, one of the concerns with *Botryococcus* is that its growth is very slow. It takes nearly a week for one *Botryococcus* cell to double.[22] Generally, many studies are targeting *Nannochloropsis*, *Schizochytrium* and *Botryococcus* to produce biofuels and health-related products. Apart from the challenge of generating high lipid content for many species, there is another challenge: to get sustainable growth of algae biomass with high productivity rate in a short period of time. This can be achieved by implementing effective operating conditions, supplying nutrients adequately and monitoring growth.

Table 3: Output in terms of oil content for some algae species (adapted from [23-26])

Microalgae species	Output: mass of bio-oil per ton of dry biomass [kg/ t]
Botryococcus braunii	250 -750
Nannochloropsis sp.	310-680
Schizochytrium sp.	500-770
Neochloris oleoabundans	350-540
Nitzschia sp	450-470
Ankistrodesmus TR-87	280-400
Chlorella sp	290
Chlorella protothecoides (autotrophic/ heterothrophic)	150-550
Crypthecodinium cohnii	200
Cyclotella DI- 35	420
Dunaliella tertiolecta	360-420

Hantzschia DI-160	660
Nannochloris: 31	60-630
Neochloris oleoabundans	350-540
Nitzschia TR-114	280-500
Phaeodactylum tricornutu	310
Scenedesmus TR-84	450
Stichococcus	90-590
Tetraselmis suecica	150-320
Thalassiosira pseudonana	210-310
Euglena gracilis	140-200
Hormidium sp	38
Phaeodactylum tricornutum	200-300
Pleurochrysis carterae	300–500
Chlamydomonas reinhardtii	210
Prymnesium parvum	220-380
Tetraselmis sueica	150-230
Chlorella emersonii	280-320
Hormidium sp	380
Chlorella pyrenoidosa	470
Chlorella vulgaris	140-220
Crypthecodinium cohnii	200
Dunaliella tertiolecta	360
Dunaliella salina	60
Dunaliella primolecta	230
Cylindrotheca sp.	160-370
Phaeodactylum tricornutum	200-300

Pleurochrysis carterae	300-500
Scenedesmus dimorphus	160-400
Scenedesmus obliquus	120-140

2.7 BIOMASS DRYING AND BIO-OIL EXTRACTION

After cultivation, algae biomass is harvested in order to proceed with downstream processes. Biomass water content increases the biomass volume and can make the dewatering/ harvesting process time and energy consuming. Solar energy for biomass drying can be considered as an alternative; however, the main weakness is the necessity of time and space which are key components for an optimal and efficient process.[27] However, it is possible to optimize the time and create the space using an effective design. Hydrothermal processing is known as an effective substitution approach for biomass drying. In this process, microalgae are exposed to high temperature and pressure to allow the breaking apart of the biomass.[28] Harvesting and drying can be skipped by using the solvent extraction method immediately after cultivation. In this case, a solvent can be added to wet biomass after cultivation. Due to the difference of densities, oil will settle at the top and biomass and water will be at the bottom of the separating funnel or sedimentation tank. Extraction of bio-oil from algae is successfully achieved with a solvent such as dimethyl ether (DME) or with the mixture of chloroform and methanol. The solvent can be recovered by evaporation at lower temperatures; therefore, lower energy consumption is recorded as compared to harvesting and drying options.

Apart from solvent extraction, bio-oil can be extracted mechanically using cell homogenizers or ultrasound.[29] In homogenisers, high pressures are applied to disrupt cells and they are mostly used for proteins extraction.[30] Regarding the ultrasound method cells are disrupted by applying sonic waves at frequencies greater than 20 kHz.[31] Alga cell walls are broken mechanically by ultrasounds due to cavitation shear forces on the walls; therefore, oil molecules are migrating easily from the cells to the solvent. This method is still used at the laboratory level, it is not still yet possible to operate at large scale with ultrasound because of high capital costs and also high acoustic energy is needed for large volumes of microalgae.[30] The effectiveness of ultrasound-assisted extraction was investigated for a long time and it is well known in the field of bio-oil extraction. It is successfully used for extraction of proteins, lipids, phenolic compounds, and enzymes on a laboratory scale as mentioned earlier.

Generally, the extraction of bio-oil from various species has used solvent extraction in most cases because of the simplicity of the process, low costs and low energy consumption.

2.8 CONVERSION OF ALGAE INTO BIO-JET FUEL: EXISTING AND NOVEL CONCEPTUAL APPROACHES FOR CONVERSION FROM ALGAE BIO-OIL TO BIO-JET FUEL

There are several options that may lead to algae-based jet fuel. These include biomass to liquid processes with gasification as the main process, biomass pyrolysis, and biomass fermentation. Some of these processes have reached a commercial level and ASTM certification depending on their maturity, the fuel readiness and the opportunity they may present for the aviation industry. However, new alternatives are arising to close the gap in terms of fuel efficiency and costs, but as mentioned before there is a scarcity of detailed information that can be relevant to a large public.

2.9 BIOMASS TO LIQUIDS PROCESS: GASIFICATION TECHNOLOGY

2. 9.1. The Process

Algae to Jet fuel can take the route known as biomass to liquids (BTL). In this process, the biomass is subjected to the first step of gasification at a temperature between 150°C and 300°C and pressure varying from 10 to 40 bars in which syngas is produced; This syngas is subjected to Fischer-Tropsch synthesis while reacting with hydrogen in a presence of a catalyst (usually iron, cobalt or nickel).[32,33,34] This process is also named gasification/Fischer-Tropsch synthesis (GFT), during this process long chain of alkanes are produced. At this point, a liquid is produced due to the catalytic action. Fractionation can be used to produce a fuel with required fractions for jet fuel. The produced Jet fuel from this pathway based on ASTM certification is known as Fischer-Tropsch synthetic paraffinic Kerosene (FT-SPK). The process is suitable for lignocellulosic, woody, agricultural biomass including algae biomass and municipal solid wastes. However, no fuel has been produced at large scale using this process with algae biomass. New studies are currently being undertaken to develop Fischer-Tropsch synthetic Kerosene high in aromatics content (FT-SKA).

This jet fuel will be an improved version of the previous one. More aromatics can improve the performance of jet fuel more especially the net heat of combustion. Also, they sensibly reduce the possibility of fuel leaks from the aircraft. The biomass is first pre-treated and, in most cases, the drying process is used to reduce the moisture content aiming to facilitate its handling and

transportation. Drying increases reliability and continuity regarding the feeding of biomass through industrial facilities. [35] However, the pre-treatment step must be undertaken remotely to reduce sensibly the costs related to logistics involved in the transportation of biomass. Already biomass drying is costly because it is energy intensive, there is a necessity for optimization of any subsequent process to make the technology cost-effective. Gasification depends mostly on the type of biomass. The moisture and ash content can have an impact on the quality of syngas. High concentrations of volatile substances resulting from high temperatures, low ash content and low moisture can make an ideal BTL process because of fewer impurities in the syngas.[36]

2.9.2 Technological development, commercial activity and fuel readiness

Application of BTL process to algae biomass is relatively new and requires full optimisation. Generally, gasification technologies require substantial improvement, particularly with handling of feedstocks.[32,35] When it comes to Fischer-Tropsch processes, less extensive adaptation is required, due to the similarity in terms of content for syngas produced from biomass and fossil fuels. BTL was expected to be operational in the 2000s, but it has slowly progressed than excepted. Projects using this technology are still at demonstration level or pilot scale, possibly in the next decade a mature process will be established. The readiness of the algae-based jet fuel using this technology is yet to be determined because the technology requires optimisation and important improvements.

2.9.3 Opportunities, challenges for use in aviation and compatibility with existing systems

The FT-SPK process used for biomass conversion into liquid fuels may present some advantages over conventional jet fuel because of its composition. Its specific energy is 2% greater compared to the one for conventional jet fuel because of its paraffinic structure and also the aromatic content is low. Consequently, the weight of jet fuel needed for flying specific distances can be reduced, therefore, the potential payload of the aircraft increases and the consumption of energy per unit of a payload is reduced.[33,37] However, there are many challenges preventing the use of FT-SPK as a neat jet fuel.

For example, its energy density (per unit volume) is 3% lower compared to the one for conventional jet fuel. This is due to lower aromatic content and paraffinic composition. Consequently, the maximum range of the aircraft is reduced, long-distance flights cannot be an option in this case because requiring a full tank of fuel.[34,37] Low aromatic content may end in fuel leaks with elastomers such as nitrile-rubber seals which can expand in the presence of aromatics. The lower lubricity of the fuel also is considered to be another cause that deteriorates

wear on engine components.[32,34] To mitigate these weaknesses, it is recommended blending FT-SPK fuel with conventional jet fuel in a 50-50 % proportion [34]. The costs of this process need optimization since many co-products including diesel and gasoline can be recovered. BTL using this pathway can generate low sulfur content fuels with high cetane number.[34]

2.10 PYROLYSIS

This is a thermal process in which decomposition of organic materials takes place in the absence of oxygen. During pyrolysis, oils, gases, char and water are produced depending on the process conditions. Generally, there is a production of chars at conditions involving slow heating rates with final temperatures lower than 450°C. However, with rapid heating rates and high temperatures greater than 800°C gases are produced. A variant of biomass pyrolysis is known as fast pyrolysis, it is achieved with temperatures between 400 and 600 °C within few seconds as residence time. Thereafter, gas quenching or cooling is rapidly undertaken, the gas is sent to a condenser to produce bio-oil. [35,38,39,40] With fast pyrolysis yields of up to 80% (in weight) can be achieved. To improve the oil quality zeolite is used as a catalyst during the process. The catalyst can influence the increase of aromatic content and cause the decrease of oxygen. The catalytic compared to the non-catalytic pyrolysis produces high-quality oil that requires less upgrading steps. Therefore, it is cost-effective compared to the non-catalytic [41,42]. This process can be effective for algae biomass but the biomass needs to be dried properly before pyrolysis. This is an added cost to be considered, analysed and optimized. Pyrolysis being used on petroleum-derived fuel has been proven successful and its readiness does not need to be proved anymore. However, fast pyrolysis suffers from issues related to slow commercialisation due to the lack of an effective market demand regarding crude bio-oil.[38] Pyrolysis can be an acceptable option for conversion of algae biomass into jet fuel. There is a need for optimization and development of new and low costs upgrading processes. This may be possible through the improvement of the existing hydrodeoxygenation or deoxygenation.

More studies are still being undertaken in this regard. The main areas of these studies may involve the appropriateness of various catalysts as well as their life cycle, reduction of hydrogen intake and the assessment of the required deoxygenation to co-process bio-oil in conventional refineries.[38,43] These new approaches are currently at the laboratory and pilot scale levels.[44,45] In general, it is important to emphasis on the fact that pyrolysis of any biomass to produce crude bio-oil without upgrading is a very mature technology.

2.11 HYDROTHERMAL LIQUEFACTION

This process is similar to pyrolysis and is also used for the conversion of algae biomass into fuels. It is known as a thermochemical process operating under high pressure and medium temperature aiming to produce oil from algae biomass. During this process, algae biomass macromolecules are firstly hydrolysed and broken into smaller molecules. However, unstable and reactive molecules can recombine into larger ones sometimes. Dehydration or decarboxylation is used to remove a substantial amount of oxygen from the biomass to enhance the performance of the produced jet fuel. Bio-oil properties more especially chemical ones are greatly dependent on the biomass composition. Algae biomass is made up of different organic components, each of them generates different ranges of compounds during hydrothermal liquefaction. Generally, a reaction of organic matter takes place in the presence of water and catalysts. The operating conditions for the biomass consist of low final temperatures which are generally between 300 and 400°C under high pressures ranging between 50 and 200 bars with a maximum residence time of 30 minutes or even more.[35,40,46,47] Hydrothermal liquefaction used as a process to generate renewable fuels from algae biomass shows a great potential. However, the technology is still largely used at laboratory and pilot scales, very few cases may have been taken at large scale.

2.11.1 Opportunities and challenges for use in aviation regarding pyrolysis and hydrothermal liquefaction processes

Jet Fuels produced from pyrolysis and hydrothermal processes may have high aromatic content, low oxygen content and few impurities, these are highly required features for jet fuel. [38,48,49] However, upgrading bio-oil to Jet fuel requires extensive deoxygenation which can be a costly process. The high costs may be an obstacle to efficiently produce fractions that are predominantly in the jet fuel range. [35]

2.12 FERMENTATION TO JET (FTJ)

Anaerobic fermentation of algae biomass can generate alkane-based fuels from fermentation. In this method, enzymes are used as catalysts to bring specific biochemical reactions during the fermentation. The method is used to operate directly from sugar to hydrocarbons or fermentation of sugar to jet fuel. The process involves the following steps: pre-treatment, enzymatic hydrolysis, hydrolysate clarification, biological conversion (or bioconversion), product purification and hydroprocessing.[50] During enzymatic hydrolysis, process enzymes facilitate the cleavage of bonds in molecules with the addition of water.

Hydrolysate clarification is used for separation of liquids and solids generated after the hydrolysis process. Bioconversion converts a portion of biomass into sugars which are then fermented into an alcohol. Product purification and hydroprocessing use fractionation and hydrocracking as downstream processes to get the required jet fuel with compliant physico-chemical properties. During the FTJ process, sugar molecules from algae biomass are digested and an alcohol intermediate is produced with the help of enzymes. Direct conversion of alcohols to alkanes is generally challenging. The conversion usually uses a sequence of a two-step process. This sequence involves the conversion of alcohols into leaving groups such as halides and sulfonate esters followed by reduction with metal hydrides.[51] The conversion of alcohol to alkanes can be also achieved through deoxygenation using enzymes. To produce sugars, algae biomass has to be fed with carbon dioxide during growth.

Carbon dioxide can be taken from fossil fuel plants, it is also helpful for growth and lipid boosting. In terms of the fuel readiness, FTJ process is still at the pilot stage but is moving toward demonstration and early commercialisation stages. It involves the conversion of sugars to isoprenoids using genetically modified organisms. These isoprenoids are polymerised to generate hydrocarbons. Thereafter, the hydrocarbons are hydroprocessed to create stocks for blending purposes.[47,52] The fuel produced from this process is ASTM certified only for blending with conventional Jet fuel in proportions lower or equal to 10%.[51] The certified version is named synthesised iso-paraffinic fuel from fermented hydroprocessed sugar.[51] Literature about FTJ fuel produced from algae is very scarce because the process is still new and not yet used in the industry, similarly, the specific operating conditions to get a jet fuel cannot be found in the existing literature. The conversion technologies of alcohol to jet fuels are still at the laboratory and pilot levels of application. The details of operational dedicated pilot plants are not yet identified in the literature. More studies need to be undertaken for the process to be developed at large scale. The studies involve the costs and possibilities to escalate depending on the process simulation and optimisation.

2.12.1 Opportunities and challenges for use in aviation

The process is ASTM certified which confers the confidence for jet fuel to be produced from fermented sugar generated from algae biomass using FTJ. However, the jet fuel produced using this process is still preferred for blending. The quantities to be produced are depending on the biomass growth output. Also, the use of genetically modified organisms to convert sugars and the capture of carbon dioxide for sugar production makes the process expensive compared to the process used for conventional jet fuel production.[51]

2.13 TRANSESTERIFICATION TO BIO-JET FUEL

This route uses the algae biodiesel from the transesterification followed by decarboxylation and deoxygenation to produce high purity jet fuel.[53] Decarboxylation or deoxygenation of methyl esters increases the energy density of the jet fuel and allows the removal of methyl esters which are not needed in jet fuel. Finally, isomerization process allows the breakdown of large molecule chains into small alkanes decreasing the higher freezing point to the normal one for the jet fuel.[53] Generally, biodiesel from algae oil has also high flash point, high freezing point, high density, low kinematic viscosity and high oxygen content compared to the ASTM requirements for jet fuel as indicated in table 4. Transesterification of algae oil followed by deoxygenation or decarboxylation and isomerization to get jet fuel from algae oil is technically feasible but very expensive.[53] This method is still under development, its maturity will depend on the laboratory and pilot scale data.

Table 4: Comparison of selected properties between transesterified algal bio-oil and conventional Jet A and A1 (adapted from [53**])**

Parameter	Jet A	Jet A1	Algal biodiesel
Net heat of combustion[MJ/kg]:	42.8 (minimum)	42.8 (minimum)	35-41
Kinematic viscosity at -20 C[mm^2/s]	8 (Maximum)	8 (Maximum)	7.5
Density at 15 ºC [kg/m^3]	775-840	775-840	864
Flash point º C	38 (minimum)	38 (minimum)	115
Freezing point º C	-40	- 47	Close to 0
Pour point º C	-	-	-12
Sulfur [%]	0.30 (maximum)	0.30 (maximum)	0.05 (maximum)
Oxygen [%w]	1-2	1-2	11.3 (minimum)

2.13.1 Opportunities and challenge in fuel aviation

The process can allow the cracking of long-chain molecules at low temperatures which are the transesterification temperatures mostly around 50-60 ºC. Therefore, transesterification on its own is energy efficient and costs effective. However, the freezing point of the final product might be higher than the one required for jet fuel as indicated in table 5. Also, the purification processes as mentioned before are prohibitively expensive. This is challenging but the process presents an opportunity to develop an innovative way to overcome the challenge.

2.14 SUGGESTED APPROACHES: NEW TRENDS AND CONCEPTUAL APPROACHES

Figure 3 presents various approaches and trends showing the potential for the conversion of algae oil into jet fuel. However, the strengths and weaknesses of most of these approaches are summarized in table 5. These strengths and weaknesses present the critical aspects of the processes that can be explored to develop new alternatives approaches, to improve the current ones and to allow an efficient production of aviation fuel from algae oil. Options such as cracking, hydrocracking, pyrolysis, gasification and Fischer Tropsch are used in the petroleum industry for conventional jet fuel. They are proven to be successful, it is possible to use and adapt them for algae-based jet fuel with defined operating conditions using the same processing plants. Some few processes or technologies under development are also used to get algae-based jet fuel and they may present an opportunity to be explored.

They are described as follows:

2.14.1 Centia^{TM} Process

Developed in the USA at North Carolina State University, patented process that can reuse oil from many feedstocks including agriculture, aquaculture or algae, waste oil, animal fats etc. The process can easily produce bio-jet fuel which is compliant to jet fuel requirements.

High yields and energy density for the produced bio-jet fuel. The process has shown that 85% of energy conversion can be expected.

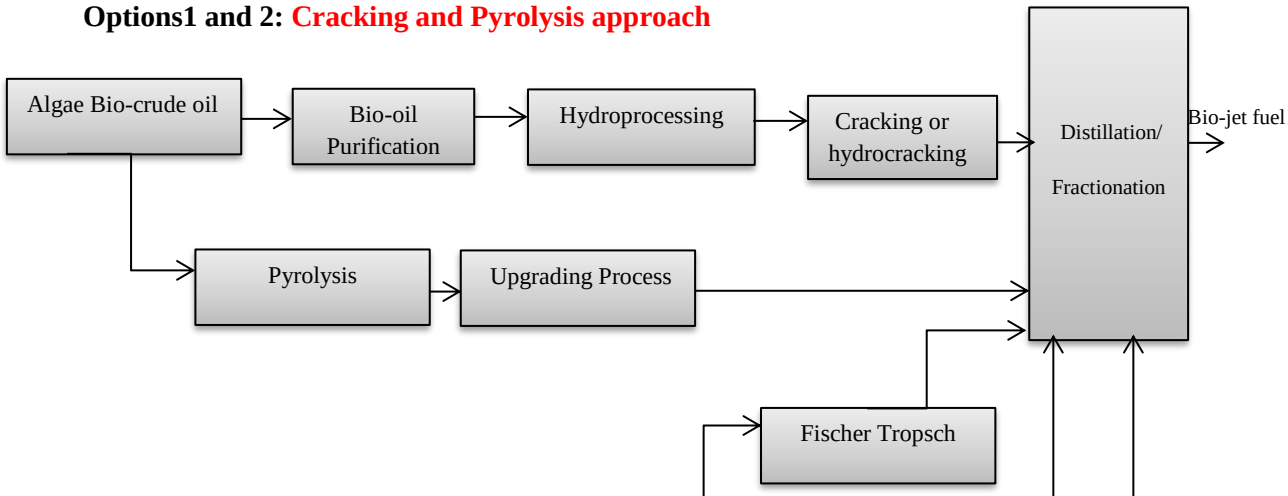
2.14.2 Enzymatic approach (biochemical conversion)

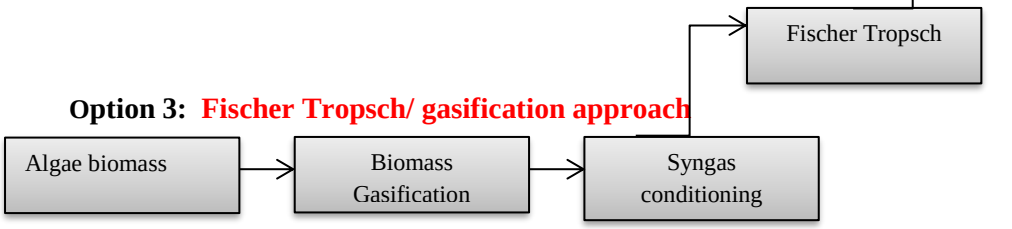
Enzymatic approaches are increasingly becoming attractive, they are not yet at large scale. This is due to the relatively high price of lipase as well as its short operational life. The short operational life is caused by the negative effects of excessive methanol and co-product glycerol. One of the greatest challenges to demonstrating the validity of this approach lies in the conversion of algal oil extracts at a commercial scale and at competitive prices.

2.14.3 Plasma technology

Promising technology that can be used with plasmas to open chemical pathways prohibited at conventional temperatures. This technology is not yet at the level of maturity in order to be used for large scales because still under development.

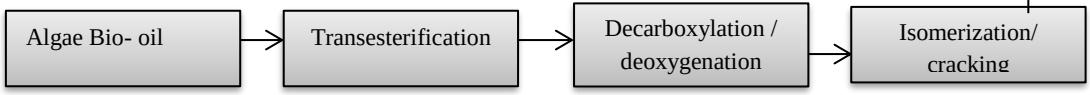
Options1 and 2: Cracking and Pyrolysis approach

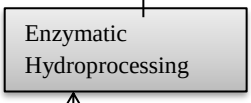




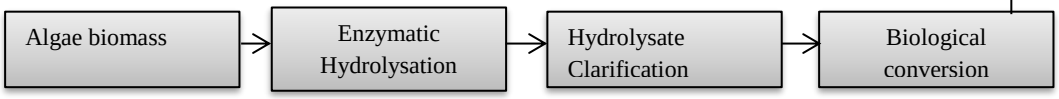
Option 3: Fischer Tropsch/ gasification approach

Option 4: Biodiesel approach





Option 5: Enzymatic approach (1st possibility)



Option 6: Centia ™ Process

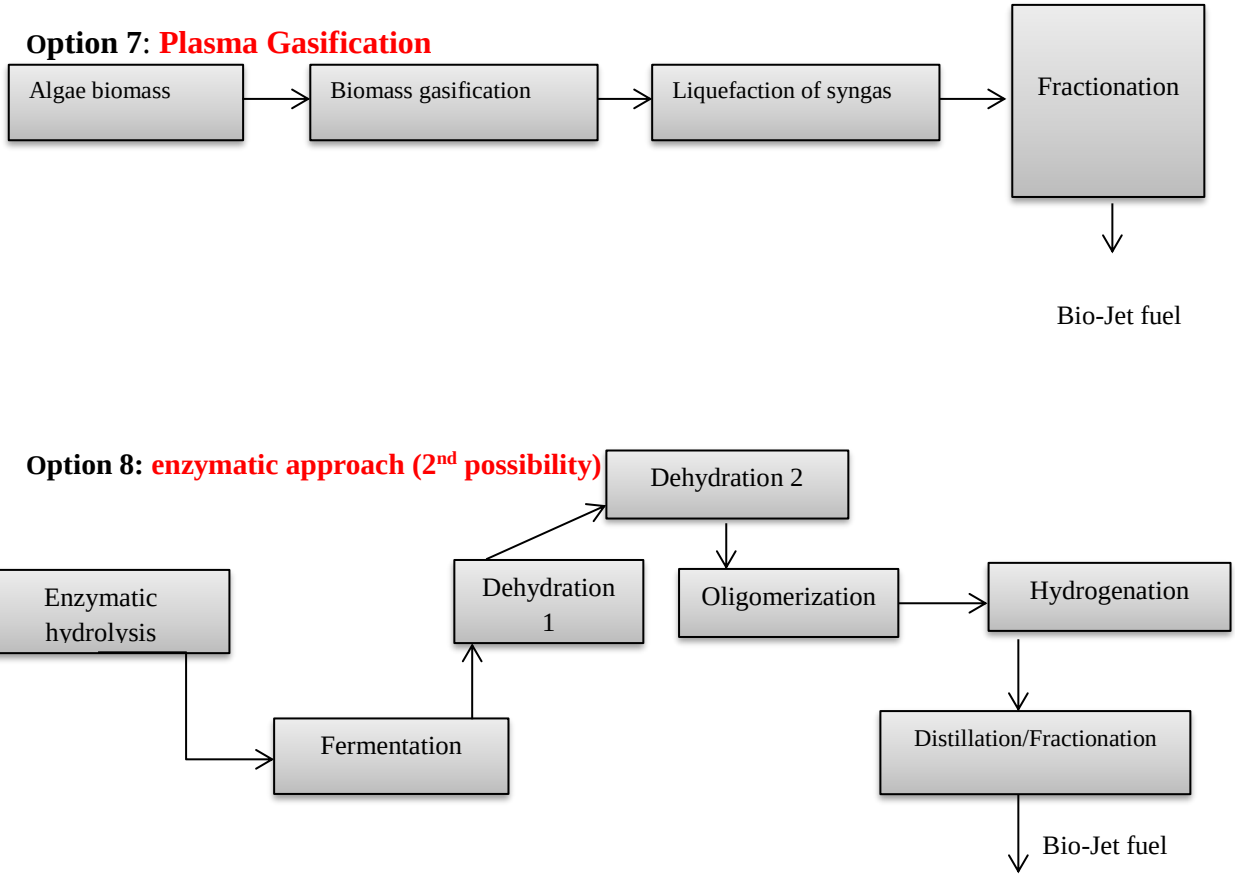


Figure 3: Conversion processes to get algae bio-jet fuel (compiled from [32-36,51-53]).

Table 5: **Advantages and weakness of processes in Figure 3** (compiled from [32-36,42,44,46,51-53]**).**

Process	Advantages / strengths	Weaknesses
Cracking/ Hydrocracking	-Efficient process with high yield. -Can be achieved with achievable temperatures (300 to 450 C) -Achieved on large scale. -Affordable and proven successful	-Impact on costs because of the use of catalysts. -Can be time-consuming.
Pyrolysis	-Very fast process, can be achieved in a fraction of seconds or few minutes -Proven successful and affordable on large scale for conventional petroleum products.	-Challenging to control. -Requires very high temperatures. -Not recommended for small hydrocarbons because of their volatility. -Oil produced from this process has high oxygen and water content, low heating value. -The oil produced may require upgrading using hydroprocessing and hydrocracking. -May generate asphalt or bitumen. -Dehydration must be undertaken upstream to allow an efficient process. Possibility of GHG gas emission
Fischer Tropsch / Gasification	-Used for wide selection of feedstocks (eg biomass and coal) for syngas generation. -Proven successful process for aviation biofuel.	-May be costly and energy-intensive depending on the type of gasification (fluidised bed, hydrothermal, entrained flow etc). -Costs effective when only operated at large scale.

Biodiesel (Transesterification) to bio-jet fuel	-Achievable technically because of the use of lower temperatures compared to previous processes. -Decarboxylation and deoxygenation are undertaken to increase the energy density of the fuel Isomerization is used to decrease a freezing point of the generated jet fuel	-The process can be expensive. -Large quantities of CO_2 might be produced during decarboxylation depending on volumes of biodiesel.
Enzymatic process	-Attractive and interesting process because of low energy input. There is still a tremendous prospect in this area. - Promising technology	-Not a mature process as yet, still at the pilot stage. -Could be very expensive.
Centia ™.	-Proven maturity, scalability and affordability. -The process can generate biodiesel and bio-gasoline while processing bio-jet fuel.	-CO_2 is produced during decarboxylation. -May be costly because of the use of catalysts.
Plasma gasification	-It can generate clean syngas. Promising technology	-Not a mature process as yet. -Very costly.

2.15 UNSOLVED ISSUES AND FUTURE PERSPECTIVES FOR ALGAE BASED AVIATION FUELS

The aviation industry is faced with many challenges that need to be overcome. The demand for aviation fuel is facing an increasing demand which is expected from a range of 1.5 to 3 % annually.[54] In the European Union, an annual growth estimated at 3 % is expected in the aviation transportation sector until 2050. In the meantime, the annual growth for fuel consumption is expected to be at 2%.[55] This represents a great opportunity for algae-based aviation fuel. Algae-based jet fuel will have to fit in the current market characterized by fast growth and high demand. In the case of high quality and efficient algae-based jet fuel is

produced, the fuel will be able to supply the balance needed by the industry and to solve the issue of the increasing demand mentioned earlier. However, some key unsolved issues need to be addressed to advance the agenda of producing sustainable and competitive algae-based jet fuel : the identification of species that can produce very high quantities of lipids with less oxygen content, the development of physiological modification techniques or use of genetically modified organisms to improve the lipid production for various species at higher rates, the development of novel growth processes to reduce sensibly the costs related to growth and updated bio-oil extraction techniques with low energy intake, the implementation of low costs and very efficient harvesting techniques, and the development of novel biomass drying process. Briefly, to reduce the costs related to the production of algae-based jet fuel without compromising the quality needed for compliance. Some of the processes mentioned in Figure 1 can be used to produce algae-based jet fuel. However, the costs remain a challenge, it has been estimated that a barrel of algae fuel can range from 300 to 2600 US dollars [26, 56] (valid from 2005 to 2011), this price was brought down to 84 US dollars by modifying the downstream processes [53] (for the same period). These costs are still high compared to ones related to petroleum fuels. The estimated costs to produce a barrel of petroleum fuels are between 40-80 US Dollars [26,56] (this was valid from 2005 to 2011). Therefore, the costs and the quality issues compulsory for compliance are the main hurdles to overcome in order for algae-based jet fuel to be able to compete with petroleum jet fuel. Technology for species growth, oil extraction, conversion processes need to be completely improved, optimized, and tested. This aims to improve fuel molecules and reduce sensibly the costs and environmental impacts in order to ensure a market-ready algae-based jet fuel.

The implementation of low energy intake processes generating high output should be the basis of new studies to overcome these challenges. Therefore, economic sustainability and environmental sustainability are the driving forces that need to be satisfied in order to have algae-based jet fuel at commercial stage.

2.16 ALGAE CULTIVATION: IMPORTANCE AND TECHNOLOGIES

Cultivation of microalgae highly affects the quantity of produced biomass, the costs of the output and also the increase of lipid quantity. The increase of lipids content for algae biomass is currently one of the major challenges faced by algae biofuels. Once this barrier can be overcome algae-based fuels will be a sustainable alternative to fossil fuels.

Many strategies to improve the lipid production have been used in the past, these include the starvation of nutrients mainly nitrogen, the addition of CO_2 or sugar to the growing culture as mentioned earlier.

Cultivation of algae can be accomplished in open ponds and in photo-bioreactor (PBR) which is a closed culture system.[57] In an open pond system, the culture medium is directly exposed to the natural environment. Open pond systems use solar energy as the light source for microalgae cultivation.[58] The open system has an advantage of regulating the temperature by liquid evaporation. This system incorporates paddle wheels for mixing and circulation of gas or liquid with low investment.[59] Open pond system is less expensive since their fabrication involves lower costs material and they require less energy. Major drawbacks of open pond systems are less effectual temperature control and the usage of light. They require relatively larger area and only handful of microalgae can be cultivation in this system. The system also suffers from higher risk of culture contamination as well as the low density of microalgae.[60,61,27] Photobioreactors are closed systems that are frequently used in commercial scales for the cultivation of microalgae. In photobioreactors, microalgae growth is controlled in order to achieve specific biological modification.[27,59] They are convenient to handle compared to open pond systems for mixing and mass transfer of gas or liquid. Photobioreactors show productivity due to most effective use of the cultivation area and efficient energy consumption.[61,62]

An earlier study reported that cultivation of microalgae in photobioreactors yields higher lipid content [63], this can be beneficial to get a higher yield for jet fuel production. However, a major problem is artificial illumination. Light conversion performance of photobioreactors is restricted because of heat generation due to contact with light sources.[63] The facility installation expenditure is much higher and their functioning costs are higher since they necessitate more power.[58] Although the costs for photobioreactors are higher, many studies are undertaken with the aim to optimise the costs and make it less expensive, therefore, there is still room for research in this area. Once costs are sensibly reduced this option can present many advantages for bio-jet fuel production because of the higher lipid content generated when using photobioreactors.

CONCLUSIONS

Production of biofuels for the aviation industry may be challenged by a handful of shortcomings on the technical feasibility and costs. These limitations hamper the technology readiness, its commercial maturity as well as the technology certification. There is a limited availability of data and information on the public domain regarding the development of alternative jet fuels. The limitation mainly includes economic data which seems insufficient to estimate and question whether it is capital intensive or not together with the technical feasibility. However, in the private domain, many studies are undertaken to get bio-jet fuel from feedstocks with high oil content. Technical feasibility and economics remain a secret for the producer. Therefore, technical and economic considerations including environmental aspects are the backbones for any new development regarding alternative jet-fuels. Algae-based fuels may be one of the most prominent ways to get jet fuel more especially some marine species such as *Nannochloropsis sp*, *Schizochytrium sp*, *Botryococcus braunii*, *Neochloris oleoabundans, Nitzschia sp* and many other. It appears that these species can technically be advantageous for an effective bio-jet fuel production compared jet fuel from other oil crops. Algae biomass has the ability to grow faster, they do not constitute a threat for food crops, they do not need excessive land to grow, they are not a threat for water resources and their crude bio-oil is very similar to petroleum crude oil in terms of carbon chains. However, the challenge is to address effectively this issue of boosting lipid content through the species physiological manipulation. This is an area that needs more studies to improve the ability of many species to increase their oil content during growth and during starvation. The starvation should define the operating conditions and the duration of the process of increasing the lipid content. Another challenge is on the engineering side and deals with the appropriate technology that is cost-effective and sustainable to bring algae aviation to a point of ‘’drop in ‘’fuel. This will involve the development of techniques that may allow the production of a fuel similar to the petroleum jet fuel known as conventional jet fuel. Compliance is the main reference in producing alternative jet fuel, mostly jet fuel from algae biomass. jet-fuel has generally carbon chains ranging from 8 to 16 depending on the jet fuel type. TAGs are recognised for a high percentage of short chain fatty acids that can reach a high number of carbon chain lengths. Many species seem to position themselves as the potential sources of biofuel to be used in aviation. Algae-based jet fuel has achieved many key milestones by identifying various species with high potential to generate oil and developing various routes or downstream processes to get algae-based jet fuel. The technological concept is a milestone which has also been clearly formulated; this means the way to go for successful technologies

must mostly be copied on the petroleum jet fuel processes. These downstream processes used on petroleum fuels have been demonstrated on large scales, the capital and operating costs are no more a big challenge. Furthermore, another milestone is the established valid concept for conversion of algae oil into fuels which already exists within the circles of many stakeholders in the private domain. The public domain needs to step into it and get the relevant information. Consequently, the scaling up to industrial or large scale will only require appropriate process optimization and modelling to reduce the operating costs. The scaling is possible if the technologies used can demonstrate maturity and can show potential for commercialisation. Hence, if these milestones are achieved, certification and commercialisation can follow. Blending algae oil with petroleum oil products is also another option to be considered for algae-based jet fuel to be operational on the market while costs optimization is still being considered in many studies. The B10, B20 and B50 are the most advised ratios for blending. This strategy can be explored because it slows down the speed of depletion of petroleum fuels by using less petroleum products and reduces carbon footprint. In definitive, algae-based aviation could be cost-effective within the next decade providing that high lipid content species are identified and there is development of new processes that are cost competitive and sustainable. For algae-based jet fuel to be a ‘’drop-in’’ fuel, testing the fuel should be simulating all the characteristics of the current conventional jet fuel in terms of engine performance, operability, characteristics such as fuel consumption and engine start. Environmental impact and life cycle assessment studies should be undertaken to add value to the advantages that algae-based jet fuel can present over the conventional jet fuel. Therefore, to meet energy growth new and alternative fuels have to be developed.

To meet this goal and avoid an energy crisis in the near future there should be a criterion including the ‘’drop-in fuel aspect’’ as well as environmental and economic sustainability. The objectives of papers were therefore met by establishing many options and routes that are possible to produce jet fuel from algae oil. Details of the processes, their strengths and weaknesses were discussed. Algae fuels are still small players compared to petroleum-based fuel in the energy sector, there is more to be achieved.

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REFERENCES

[1] Air Transport Action Group [ATAG] Aviation Benefits Beyond Borders. Geneva, Switzerland, 2014: Available online: ⟨http://aviationbenefits.org/media/26786/ ATAG__AviationBenefits2014_FULL_LowRes.pdf⟩ [accessed 1 June 2016].

[2] Demirbas A. Importance of biodiesel as transportation fuel. Energy Policy 2007;35:4661–4670.

[3] Rodolfi L, Chini Zittelli G, Bassi N, Padovani G, Biondi N, Bonini G, Tredici MR. Microalgae for oil: strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor. Biotechnol Bioeng 2009;102:100–112.

[4] Jet fuel consumption on Index Mundi; 2012, available online; ⟨www.indexmundi. com/energy⟩ / [accessed 19 June 2016].

[5] Hannon M, Gimpel J, Tran M, Rasala B, Mayfield S. Biofuels from algae: challenges and potential. Biofuels 2010;1(5):763–84.

[6] Energy Information Administration, International Energy Outlook, 284, 2009, EIA; DC, USA.

[7] Hunt JM. Petroleum Geochemistry and Geology, 2nd edition. W. H. Freeman and Co, New York, 24; 1996.

[8] Gize, A P., and., Manning DA C., Aspects of the organic geochemistry and petrology of metalliferous ores, *In Organic Geochemistry: Principles and Applications*, M. H. Engel and S. A. Macko editors, 1993, 565–580

[9] Blakey S., Rye L., and Wilson, C W., Aviation gas turbine alternative fuels: A review, Proceedings *of the Combustion Institute*, 2011, 33, 2863-2885.

[10] Liu G., Yan B., and Chen G., Technical review on jet fuel production, *Renewable and Sustainable Energy Reviews*, 2013, 25, 59-70.

[11] Bauen A., Howes J., Bertuccioli, L., Chudziak, C., Review of the potential for biofuels in aviation, Report, 2009, London:E4tech. https://www.theccc.org.uk/archive/aws2/Aviation (Accessed on 1st June 2016)

[12] Lehr F., Posten C., Closed photo-bioreactors as tools for biofuel production, *Current Opinion in Biotechnology*; 2009, 20(3):280–285.

[13] Ruiz-Marin A., Mendoza-Espinosa LG., Stephenson T., Growth and nutrient removal in free and immobilized green algae in batch and semi-continuous cultures treating real wastewater, *Bioresource Technology*; 2009, 101(1):58–64

[14] Gerbens-Leenes W., Hoekstra AY., Van Der Meer TH., The water footprint of bioenergy, *Proc Natl Acad Sci* USA; 2009, 106(25):10219–10223

[15] Alternative Jet Fuels, Addendum 1 to Aviation Fuels Technical Review (FTR3/A1), A supplement to Chevron's Aviation Fuels Technical Review, Chevron Corporation, 2006 Available online: www.cgabusinessdesk.com/document/5719_Aviation_Addendum._webpdf.pdf, (Accessed on 16 th May 2016)

[16] Steen, E., Kang, Y., Bokinsky, G., Hu, Z., Schirmer, A., McClure, A., Del Cardayre, S., and Keasling, J., Microbial production of fatty-acid-derived fuels and chemicals from plant biomass, *Nature*, 2010, 463(7280), 559--562.

[17] Wahlen, B D., Willis, R M., and Seefeldt, L C., Biodiesel production by simultaneous extraction and conversion of total lipids from microalgae, cyanobacteria, and wild mixed-cultures. *Bioresource Technology*, 2011, 102, 2724–2730

[18] Hillen LW., Pollard, G., Wake L V., and White N., Hydrocracking of the Oils of Botryococcus braunii to Transport Fuels, *Biotechnology and Bioengineering*, 1982, XXIV, 193-205

[19] Hem R., Thapa, Mandar T., Naik, Okada S., Takada K., Molnár, I., Xu Y., Devarenne T P., "A squalene synthase-like enzyme initiates production of tetraterpenoid hydrocarbons in Botryococcus braunii Race L" *Nature Communications*, 2016 ,7, 11198, doi: 10.1038/ncomms11198

[20] Schenk P., Second generation biofuels: high-efficiency microalgae for biodiesel production, *BioEnergy Research*, 2008, 1, 20–43, 2008.

[21] Becker, E.W., Microalgae in human and animal nutrition, Richmond, A. Edition, Handbook of Microagal Culture. *Blackwell Publishing*, Oxford, 2004, 312-351.

[22] Moheimani, N., Borowitzka, M., Culturing Coccolithophorid Algae for Carbon Dioxide Bioremediation 7th Annual Post-Graduate Symposium, The Royal Society of Western Australia, 2005, Perth.

[23] Chisti, Y., Biodiesel from microalgae, Biotechnology *Advances*, 2007, 25, 294–306.

[24] Georgianna, D., and Mayfield, S., Exploiting diversity and synthetic biology for the production of algal biofuels, *Nature*, 2012, 488 (7411), 329-335.

[25] Brown, T., Duan, P., and Savage, P., Hydrothermal liquefaction and gasification of Nannochloropsis sp, *Energy and Fuels*, 2010, 24(6), 3639-3646.

[26] Brennan, L., Owende, P., Biofuels from microalgae; a review of technologies for production, processing, and extractions of biofuels and co-products. *Renewable and Sustainable Energy Reviews*. 2010, 14, 557–577.

[27] Richmond, A., Handbook of Microalgae Culture: *Biotechnology and Applied Phycology*, Blackwell Publishing, 2004, Oxford, 566

[28] Packer, M., Algal capture of carbon dioxide; biomass generation as a tool for greenhouse gas mitigation with reference to New Zealand, *Energy Policy*, 2009, 37, 9, 3428–3437, New Zealand Energy Strategy, doi : 10.1016/j.enpol.2008.12.025

[29] Maniatis, K., Weitz, M., and Zschocke, A., 2 million tons per year: a performing biofuels supply chain for EU aviation, August 2013 Update, European Commission. Available online: https://ec.europa.eu/energy/sites/ener/files/20130911_a_performing_biofuels_supply_chain.pdf , (Accessed on 10th June 2016)

[30] ALFA-BIRD, Alternative Fuels and Biofuels for Aircraft Development, ICAO Workshop Report, 2011, Available online:

http://www.icao.int/Meetings/EnvironmentalWorkshops/Documents/2011SUSTAF/18_Jeuland.pdf (Accessed 10th June 2016)

[31] Hileman, J. I. O., D.S., Bartis, J. T., Wong, H. M., Donohoo, P. E., Weiss, M. A., Waitz, I. A., Near-Term Feasibility of Alternative Jet Fuels, Technical Report, 2009. Availableonline:http://web.mit.edu/aeroastro/partner/reports/proj17/altfuelfeasrpt.pdf, (Accessed on 10th June 2016).

[32] Güell, B. M., Bugge, M., Kempegowda, R. S., George, A., Paap, S. M., Benchmark of conversion and production technologies for synthetic biofuels for aviation, Norway: SINTEF Energy Research, 2012, Report available online: http://www.flygreenfund.se/wp-content/uploads/2015/05/Benchmark-of-conversion-and-Production-technologies-for-synthetic-biofuels-for-aviation-Sintef-2013.pdf, (Accessed on 11th June 2016)

[33] Arup URS Consortium, Advanced Biofuel Feedstocks; An Assessment of Sustainability, Report submitted by Arup URS consortium, Report available online: http:// www.gov.uk/government/uploads/attachement_data/file/277436/feedstock-sustainability.pdf, 2014, (Accessed 20th May 2016)

[34] Hileman, J. I., Stratton, R. W., and Donohoo, P. E., Energy Content and Alternative Jet Fuel Viability, *Journal of Propulsion and Power*, 2010, 26, 1184-1195

[35] Radlein, D., and Quignard, A., , A short historical review of fast pyrolysis of biomass, *Oil and Gas Science and Technology*, Revue d'IFP Energies nouvelles, Institut Français du Petrole, 2013, 68 (4) ,765-783.

[36] CSIRO, Flight path to sustainable aviation, Towards establishing a sustainable aviation fuels industry in Australia and New Zealand, Sustainable Aviation Fuel Road Map, 2011, Report available online: http://www.licella.com.au/wpcontent/uploads/licella/news/flightpathsustainableaviation , (Accessed on 3nd May 2016)

[37] Pandey, A., Larroche, C., Ricke, S., Dussap, CG., Gnansounou, Biofuels: Alternative Feedstocks and Conversion Processes, 2011, First edition, Elsevier, ISBN: 978-0-12-385099-7

[38] SkyNRG. Technology section, Report Available online: http://skynrg.com/technology-section , 2014 (Accessed on 5 th march 2016)

[39] Brown, TR., Thilakaratne, R., Brown, RC., Hu, G P., Techno-economic analysis of biomass to transportation fuels and electricity via fast pyrolysis and hydroprocessing. *Fuel*, 2013, 106, 463-469.

[40] Arbogast, S., Bellman, D., Paynter, J. D., Wykowski, J., Advanced biofuels from pyrolysis oil, Opportunities for cost reduction. *Fuel Processing Technology*, 2013, 106, 518-525.

[41] VTT Synthetic fuels and bio-refining. Available online: http://www.vtt.fi/research/technology/synthetic_fuels_and_biorefining.jsp?lang=en , ,2014, (Accessed on the 5 th March 2016)

[42] Venderbosch, R. H., and Prins, W., Fast pyrolysis technology development. *Biofuels, Bioproducts and Biorefining*, 2010, 4, 178-208.

[43] Balan, V., Chiaramonti, D., and Kumar, S., Review of US and EU initiatives toward development, demonstration, and commercialization of lignocellulosic biofuels. *Biofuels, Bioproducts and Biorefining*, 2013, 7, 732-759.

[44] National Alternative Biofuels Consortium [NABC]. Making Oils with Low Oxygen Content, Catalytic Fast Pyrolysis. Available online: http://www.nabcprojects.org/pdfs/making_oils_with_low_oxygen_content.pdf,2012, (Accessed on 13th March 2016).

[45] Biomass Technology Group [BTG] online: http://www.btgworld.com/en/rtd/technologies/biofuels , 2014a , (Accessed 13th march 2016).

[46] Zhang, Y., Brown, T R., Hu, G., Brown, R. C., Techno-economic analysis of two bio-oil upgrading pathways, *Chemical Engineering Journal*, 2013, 225, 895-904.

[47] Davis, R., Biddy, M., Tan, E., Tao, L., Jones, S., Biological Conversion of Sugars to Hydrocarbons Technology Pathway, Washington, USA, National Renewable Energy Laboratory, Technical Report, 2013, Available online: http://www.nrel.gov/docs/fy13osti/58054.pdf , (Accessed on the 15th April 2016)

[48] Rebecca M, Adriana R C., Raphael S., Establishing a European renewable jet fuel supply chain: the techno-economic potential of biomass conversion technologies,

Imperial College London, Final Report, available online: http://www.climate-kic.org/projects/renewable-jet-fuel-supply-chain-development-and- flight-operations/ , 2014, (Accessed on 20 June 2016)

[49] William L. R., The 5th International Biofuels Conference, North Carolina State University Raleigh, 2008, NC27695, USA, available online: http://www.competebioafrica.net/events/events2/seminar_india/ppt/4-2-Roberts.pdf (Accessed on 21st June 2016)

[50] International Energy Agency (IEA), Bioenergy Annual report, 2012 available online: http:www.iea.org/publications/freepublications/publication/IEA (Accessed on 15th June 2016)

[51] Zampara M., and Capros P., European Union, Energy, transport and GHG emissions trends to 2050 reference scenario, Updated Report, available online: http://www.e3mlab.eu/e3mlab/presentations/EU%20Energy,%20transport%20and%20GHG%20emissions%20Trends_ECRAN_UPDATE.pdf,2013 (Accessed on 16 th June 2016)

[52] Alabi, AO., Tampier, M., Bibeau E., Microalgae Technologies and Processes for Biofuels/Bioenergy Production in British Columbia, BC Innovation Council; BC, Canada, Report available online: http://www.fao.org/uploads/media/0901_Seed_Science_Microalgae_technologies_and_processes_for_biofuelsbioenergy_production_in_British_Columbia.pdf,2009, (Accessed on 16th 2016)

[53] Huntley ME., Redalje DG., CO_2 mitigation and renewable oil from photosynthetic microbes: a new appraisal. *Mitigation Adaptation Strategies Global Change*, 2006, 12, 573–608.

[54] Huerlimann, R., de Nys, and Heimann K., Growth, lipid content, productivity, and fatty acid composition of tropical microalgae for scale-up production," *Biotechnology and bioengineering*, 2010, 107, 2, 245–257

[55] Jiang H., and Gao, K., Effects of lowering temperature during culture on the production of polyunsaturated fatty acids in the marine diatom Phaeodactylum tricornutum (Bacillariophyceae), *Journal of Phycology*,2004, 40, 4, 651–654

[56] Zhila, NO., Kalacheva, GS., Volova, T.G., Effect of salinity on the biochemical composition of the alga Botryococcus braunii Kütz IPPAS H-252, *Journal of Applied Phycol*ogy,2011, 23, 47–52

[57] Mata, T., Martins, A. and Caetano, N., Microalgae for biodiesel production and other applications: a review. *Renewable and Sustainable Energy Reviews*, 2010, **14(1)**, 217--232.

[58] Widjaja A., C. C. Chien and Y. H. Ju., Study of increasing lipid production from fresh water microalgae Chlorella vulgaris. *J. Taiwan Inst. Chem. Eng*., 2009, **40**, 13.

[59] Cheng-Wu, Z., Zmora, O., Kopel, R., Richmond, A., An industrial-size flat plate glass reactor for mass production of *Nannochloropsis* sp. (Eustigmatophyceae). *Aquaculture*, 2001, **195**, 35–49.

[60] Pulz, O., Photobioreactors: production systems for phototrophic microorganisms. Appl. *Microbiol. Biotechnol.*, 2001, **57**, 287–293.

[61] Carvalho, A.P., Meireles, L.A., Malcata, F.X., Microalgae reactors: a review of enclosed system designs and performances. *Biotechnol. Prog*., 2006, **22**, 1490–1506.

[62] Sierra, E., Acién, F.G., Fernández, J.M., García, J.L., González, C., Molina, E., Characterization of flat plate photobioreactors for the production of microalgae. *Chem.Eng. J*., 2008, **138**, 136–147

[63] Steen, E., Kang, Y., Bokinsky, G., Hu, Z., Schirmer, A., McClure, A., Del Cardayre, S. and Keasling, J., Microbial production of fatty-acid-derived fuels and chemicals from plant biomass, *Nature*, 2010, 463,(7280), pp.559--562.

[64] Eloka-Eboka, A.C. and Inambao, F.L. Effects of CO_2 Sequestration on Lipid and Biomass Productivity in Microalgal Biomass Production. Applied Energy195 (2017): 1100 -1111: http://dx.doi.org/10.1016/j.apenergy.2017.03.071

CHAPTER 3 PREFACE

The following chapter is presented as a research paper accepted and published *in Biofuel, Biorefinery and Bioproducts* journal. The experimental work completed in the laboratory from biomass cultivation to jet fuel production is presented here. It is a detailed experimental work involving all the important steps needed for algae-based jet fuel production. The chapter focuses is on the establishment of effective operating conditions and conversion processes from the laboratory experiment. The aim is, firstly, to increase the lipid content in algae biomass in order to improve the bio-oil output essential for conversion processes. Secondly, to develop a simplified laboratory process from microalgae cultivation to jet fuel production. The chapter is presented with some minor modifications and additions for clarity in order to keep the flow and connect with the previous and next chapters. The copy of the original published article is in APPENDIX A1 as mentioned before (in Declaration 2 section).

CHAPTER 3: MICROALGAE PROCESSING FOR JET FUEL PRODUCTION

[1,2]Joseph K. Bwapwa, [2]Akash Anandraj, [1]Cristina Trois

[1] University of KwaZulu-Natal, [2] Mangosuthu University of Technology

Corresponding author: joseph@mut.ac.za ; josephkapuku@gmail.com

ABSTRACT

This study focuses on the laboratory production of jet fuel from microalgae. In contrast with many studies that use partial nutrient starvation to boost lipid content of the species, physiological modification was undertaken under complete nutrient starvation for 3 days to increase lipid content beyond 80%. This was followed by biomass harvesting, which was necessary for downstream processes. Large amounts of biomass were achieved between day 8 and day 10 during the cultivation period, with temperatures ranging between 15 and 35 °C under constant luminance of 1000 lux and daily supply of CO_2 for 15 days. It was found that *Nannochloropsis sp.* grew effectively between 15 °C and 25 °C with more biomass produced in the same temperature range. Conversion processes involved steps such as oil extraction, thermal cracking without catalyst at 300 °C and fractionation between 70 °C and 250 °C. The pyrolysis of bio-oil was also undertaken as a fast cracking process for the temperature ranging between 350 °C and 450 °C within 12 s. Some parameters such as flash point, net heat of combustion, Sulfur, and viscosity complied with ASTM standards. Jet fuel from microalgae therefore shows potential despite many challenges related to cost-effectiveness and sustainability. In order to obtain a bio-jet fuel that is completely compliant with ASTM standards, upgrading, reforming processes, and the use of additives will be needed more, especially for pilot and large-scale production once fuel sustainability is achieved

KEYWORDS: Bio-jet fuel; biofuel; microalgae; Nannochloropsis sp; biomass

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3.1INTRODUCTION

3.1.1 Background

Industrial interest in the production of alternative fuels and in reducing dependence on fossil fuels is growing. A greater demand for energy has led to fluctuations in the prices of fossil fuels. Adverse environmental effects resulting from the combustion of fossil fuels have increased interest in the search for environmentally friendly renewable resources. The use of biomass as a feedstock for energy production has consequently emerged as an area of importance.[1,2,3] As a result, many feedstocks have been used to produce biofuels.[4,5] However, algae biomass is one of the bioresources that can produce clean and sustainable fuel despite a few challenges.[6,7,8] Today it has technically been proved that there is a real possibility of converting algae or any biomass into bioethanol, bio-jet fuel, and bio-diesel.[3] Nonetheless, algae-based fuels are still expensive when compared with conventional fuels.

Unlike other first- and second-generation biofuels, algae can grow easily and reproduce very quickly under photosynthetic conditions. [9] Microalgae are microscopic and photosynthetic organisms that grow in marine, freshwater, or wastewater environments. The production of algae biomass generally depends on the type of species or strain used during cultivation. [10] The type and nature of the strain or species, the growth conditions, and the nutrients supplied to the culture directly influence biomass productivity and lipid content. [11] Microalgae grow very fast, as mentioned before, and some species are exceptionally rich in oil, but not most of them.[12,13]

Although the growth of the microalgae depends on many characteristics of the species, they can double the amount of biomass within 24 hours.[14] Growing microalgae under well-defined operating conditions will therefore result in high biomass production and eventually, more lipids can be collected.[15,16,17] Microalgae grow like any other microorganisms; however, their growth cycle is made up of four phases: lag, exponential, stationary, and the death phase or lysis. They convert photonic energy, water, and CO_2 to sugars; sugars are therefore converted to macromolecules such as lipids and triacylglycerols (TAG).[18,19] During the cultivation phase, once microalgae culture has reached the stationary phase, biomass harvesting must be undertaken in order to collect the highest amount of biomass needed for conversion processes. In this study, a marine microalga known as *Nannochloropsis sp.* is grown under photosynthetic conditions.

The effectiveness of photosynthesis is one of the crucial aspects required for effective biomass productivity; it depends on the amount of light supplied to the culture, but also on the nature of the species.[20,21]

Photosynthetic efficiency affects the growth rate and potentially biomass production.[14] Similarly, environmental parameters such as the pH of the growing culture, its temperature and salinity (especially for a marine species), the amount of carbon dioxide supplied to the growing culture and the availability of nutrients in the growing media are very important for effective growth. [22]

These parameters directly influence the growth and proliferation of algae cells and consequently the rate of biomass production.[11,20,21,23] The study's main objective is to produce bio-jet fuel from a microalgae species (known as *Nannochloropsis sp*.) under defined operating conditions in order to establish a laboratory process from cultivation to the production of jet fuel.

3.I.2 Knowledge gap and Knowledge contribution from this study

The literature on algae-based transportation fuels is scarce but many studies have been completed in this field; however, most of their outcomes are not available to the public. These studies have focused on conversion processes, biomass productivity, optimising growth conditions, and dewatering processes. [11,20,21,23] Nevertheless, there are still some gaps to be covered regarding the production of algae-based fuels. Effective genetic modification to increase the lipid output is one of the major issues that needs to be addressed. This study contributes to the process of filling the gap by establishing a total (complete) nutrient starvation technique.

This study's contribution to knowledge is its examination of the way in which total nutrient starvation can stimulate an increase in lipid in microalgae cells. In many previous studies, the physiological modification of species is completed under partial nutrient starvation. [11,20,21,23] However, in this study, the physiological modification is achieved under total nutrient starvation of the species after cultivation. Most algae species have a very low lipid content; it is therefore challenging to have a high output for algae-based jet fuel with the low crude bio-oil output.[11] Another way in which the study contributes to knowledge is based on the cracking process of the crude bio-oil, which is completed without a catalyst at an achievable temperature with a high oil recovery rate.

3.1.3 Overview of the species and bio-oil type used for the experiment

Nannochloropsis bio-oil is a reliable source of eicosapentaenoic acid / oil (EPA) and also contains dodecahexanoic acid / oil (DHA). *Nannochloropsis sp.* strains are characterised by various unique biochemical and ultrastructure features such as the absence of chlorophyll b or c, the composition of the cellular xanthophyll pigments [24,25,26] relatively high EPA content [27,28] as well as the presence of specific sterols.[29,30] The unique ultrastructure features of these strains are the presence of lamellate vesicles in the cytoplasm and the connection of the chloroplast envelope with the nuclear envelope. *Nannochloropsis sp.* is widely cultivated because of its higher EPA content (EPA long chain omega-3 fatty acids with 20-carbon chain including 5 cis double bonds) representing between 4% and 5 % of the biomass, and also because of its small cells (2–3 μm diameter) known as feed for rotifers in the 'green water' technique.

The modulation of fatty acid composition by varying culture parameters such as light intensity, light-dark cycles, temperature, salinity, and nutrients has been widely investigated and reviewed in many studies.[17,31,32,33] Generally, the higher the biomass productivity, the higher the EPA productivity.[34,35,36] However, this is not always the case for all species because the lipid content is lower in most cases, as mentioned earlier. Increasing lipid content using a physiological manipulation technique is therefore very important for many species in order to generate the considerable amount of lipids necessary for biofuels production. *Nannochloropsis sp* was also suggested for biofuel production because the physiological manipulation undertaken with nitrogen starvation only showed an increase of approximately 60% in lipid content compared to its initial lipid content before starvation.[37,38,39,40] These lipids are mainly TAGs and they contain saturated and monounsaturated fatty acids.[19,41]

3.2MATERIAL AND METHODS

3.2.1 Sampling and culture maintenance

To obtain pure *Nannochloropsis sp.* cells for cultivation and the rest of the experiment, new cells were cultivated on petri dishes. On these petri dishes, colonies of *Nannochloropsis sp.* cells from a stock solution made up of *Nannochloropsis sp.* cells were spread in parallel lines crossing each other. Parallel lines were drawn in one direction to reduce the risk of contamination. These lines of cells in solution were spread on a viscous and thick mixture made of F/2 media and 15 g of agar added to the petri dishes presented in Figure 4. Thereafter, petri

dishes with colonies of cells were kept in an incubator at 25 °C with the aim of generating pure *Nannochloropsis sp.* cells.

After 5 to 7 days, cells appeared as pellets on the surface of the thick mixture. Some cells were pure and others were not due to probable contamination of growth. To minimise contamination of pure cells on the petri dishes, pure cells were isolated and put on new petri dishes with the thick mixture of F/2 media-agar. Lastly, they were kept at 25 °C in the incubator again. After a period of 7 days, pure cells of *Nannochloropsis sp* appeared on the surface of the thick mixture. These pure cells or pellets were collected and added to F/2 media solution to run a cultivation batch in a photobioreactor, presented in the left part of Figure 5. The photobioreactor produced the biomass under photosynthetic conditions for a cultivation period of 15 days. The stock solution was also collected for future use in case of sample loss or contamination or any other incident occurring during experiments.

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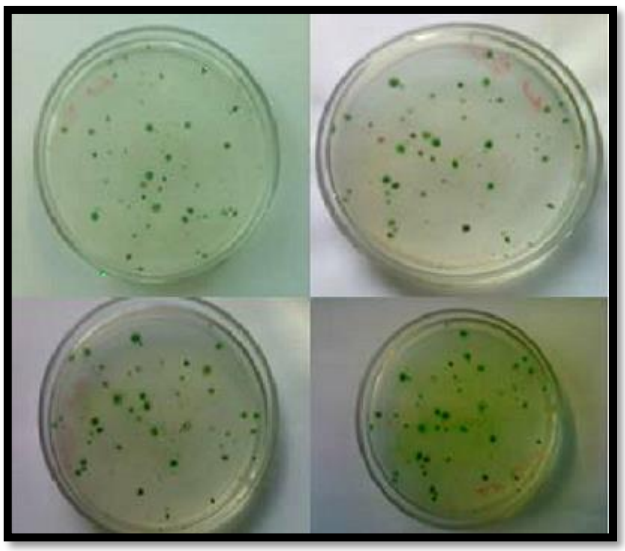

Figure 4: Cells of *Nannochloropsis sp*. on petri dishes for cultivation

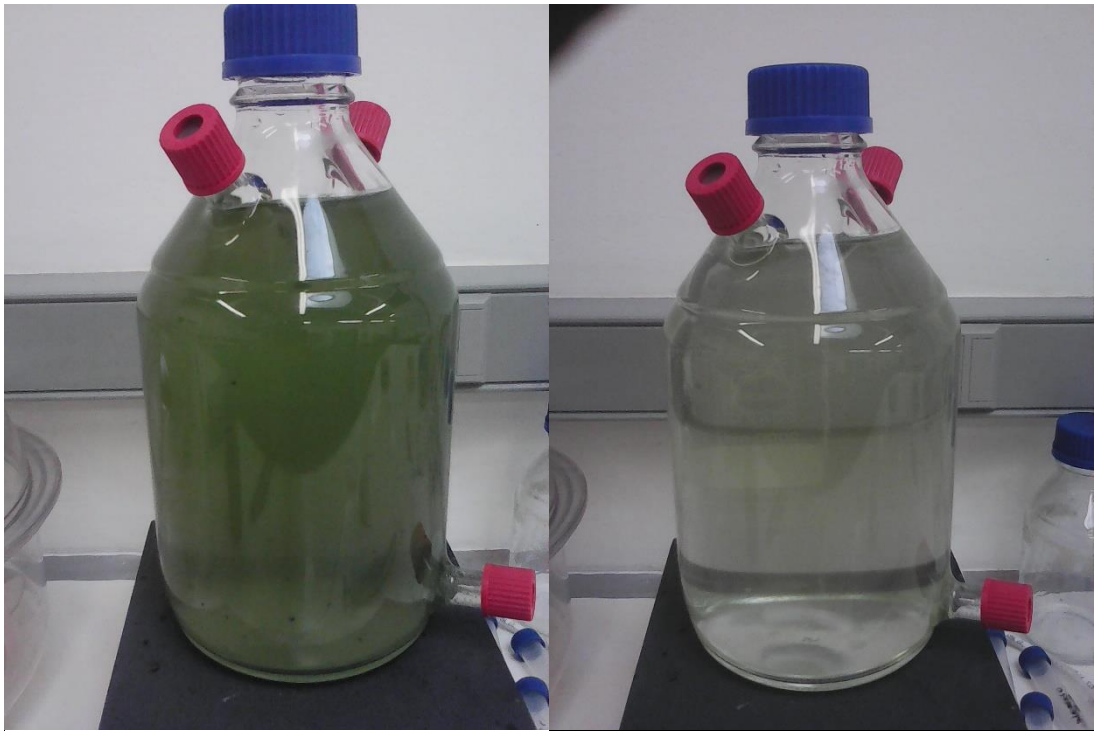

Figure 5: cultivation batch (left) and F/2 media solution (right)

3.2.2 Media preparation for species cultivation

The importance of F/2 media regarding algae biomass production is to provide nutrients effectively to microalgae cells during cultivation. This allows effective growth of cells under operating conditions defined by light, temperature, pH, and salinity. Microalgae need nitrates, phosphates, and trace elements including heavy metals for an effective growth. Media solutions such as F/2 are an ideal environment in which these elements are mixed and supplied to microalgae during cultivation. Nitrates allow the production of nucleic acid and proteins, and contribute greatly to synthesis and lipid production, while phosphates are acting as energy carriers.

The saline wastewater was prepared in the laboratory to suit the cultivation purposes in domestic wastewater which is rich in nutrients such as nitrates and phosphates. The salinity of wastewater was important because *Nannochloropsis sp* is a marine species that grows well in a saline environment.

F/2 media is prepared according to Guillard and Ryther's (1962) method.[42] It is widely used with saline water or seawater for the cultivation of many species including marine species. In this study, the F/2 media preparation begins with 950 ml of saline water. This is prepared from tap water mixed with commercial salt at 30 ppt salinity, which is considered as the average

salinity for seawater in which *Nannochloropsis sp*. can effectively grow. Defined quantities of $NaNO_3$, $NaH_2PO_4.H_2O$, $Na_2SiO_3.9H_2O$, trace elements, and vitamins were added to saline water following Guillard and Ryther's method.[42] The final volume is brought to 1 l by adding saline water or natural filtered seawater to the photobioreactor. Finally, the mixture is sterilised for 1 hour at 105 °C in an autoclave. The uptake of nutrients by microalgae cells takes place during cultivation, so microalgae cells grow as much as they absorb nutrients up to the time the growth process reaches the stationary phase. At this stage, harvesting can be undertaken and a new batch can be started.

3. 2.3 Cultivation of the species and growth under proficient monitoring of the system

Cultivation of *Nannochloropsis sp.* aims to produce as much as biomass possible to be used for downstream processes. These processes need more biomass with high lipid content in order to generate more crude bio-oil. An effective, monitored system is required with defined operating conditions. Effective monitoring is very important because it can assist in the optimisation of the biomass production. In this study, cultivation was undertaken for a maximum of 15 days, as mentioned above, and is presented in Figures 6 to 10. Experiments were carried out in a 1 l photobioreactor (see Figure 4). The salinity of 30 ppt was chosen with the aim to simulate species growth conditions in the marine environment. Generally, the average seawater salinity favourable for species growth varies between 25 and 35 ppt. *Nannochloropsis sp.* being a marine species, it was essential to grow it under conditions of salinity similar to the marine environment. Concerning the pH, the culture was maintained at values equal to or slightly above 7. The biomass was growing at an ambient temperature between 15 °C and 35 °C with an average light intensity of 1000 lux using fluorescent light for 24 hours of illumination. CO_2 was added daily to these batches and its volume was equivalent to 15 % of the photobioreactor.[43] The addition of CO_2 was intended to boost the lipid content of the growing biomass. To expose the cells to maximum illumination, facilitate gas exchange, and provide effective mixing, biomass aeration was undertaken during cultivation. Growth data are summarised in table 6.

Table 6: summary of growth data for *Nannochloropsis sp* biomass

Parameter	Salinity	Temperature range	Average pH	Light intensity (average)	CO_2 addition	Cultivation cycle and mode
Value	30 ppt	Controlled: 15,20,25,30 and 35 ^{O}C	Neutral	1000 lux	5 % of the total volume	15 days in a photobioreactor

3.3RESULTS AND DISCUSSIONS

3.3.1 Unit operations

3.3.1.1 Microalgae cultivation and growth curves

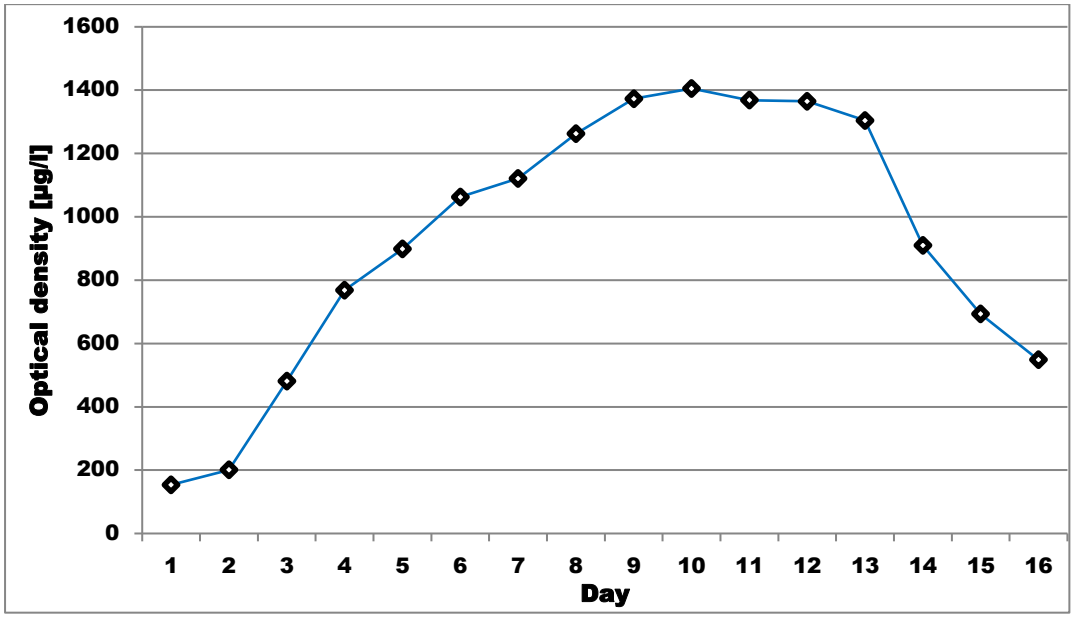

Figure 6: growth curves recorded for *Nannochloropsis sp*.at 15 ^{o} C

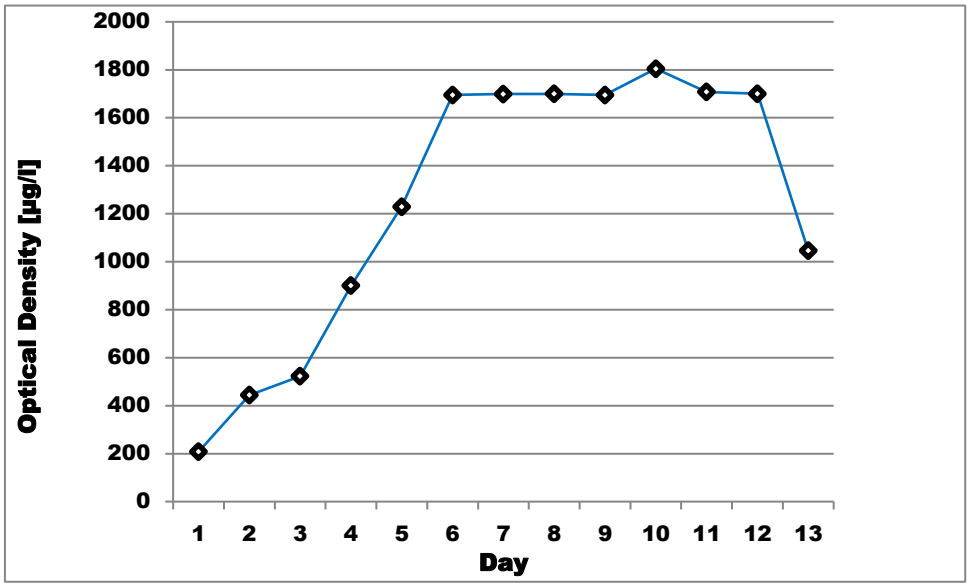

Figure 7: growth curves recorded for *Nannochloropsis sp* at 20 ºC

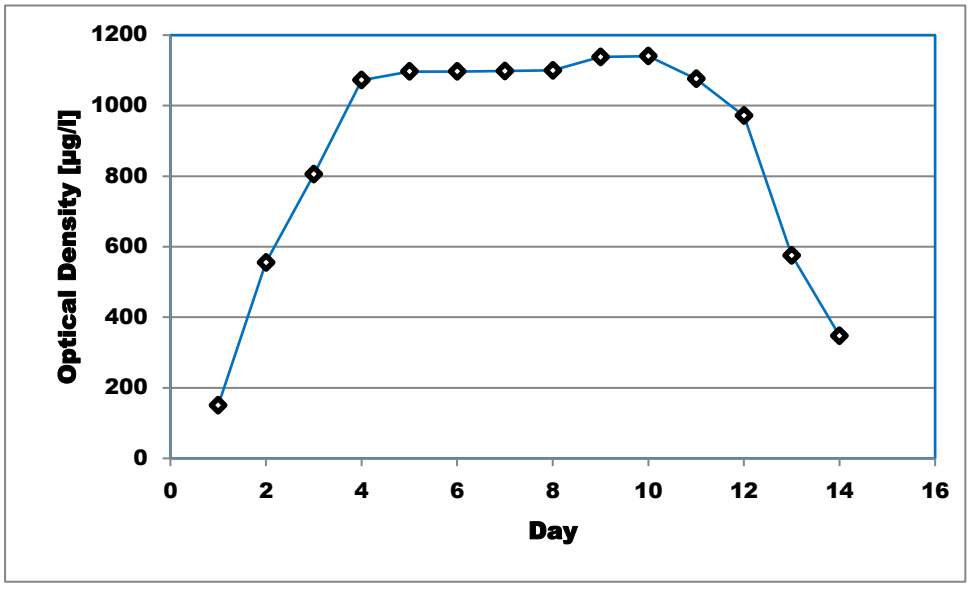

Figure 8: growth curves recorded for *Nannochloropsis sp*.at 25 ºC

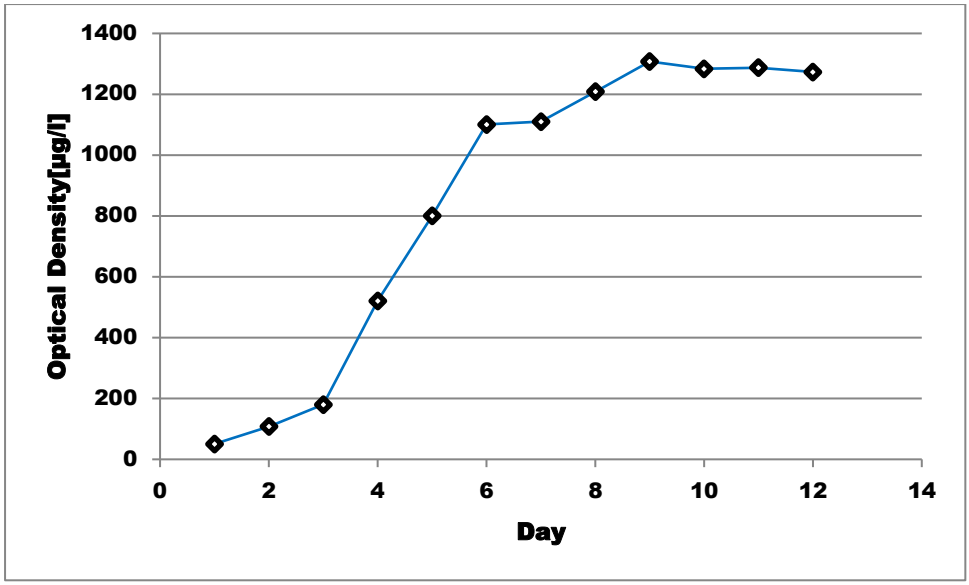

Figure 9: growth curves recorded for *Nannochloropsis sp* at 30 ºC

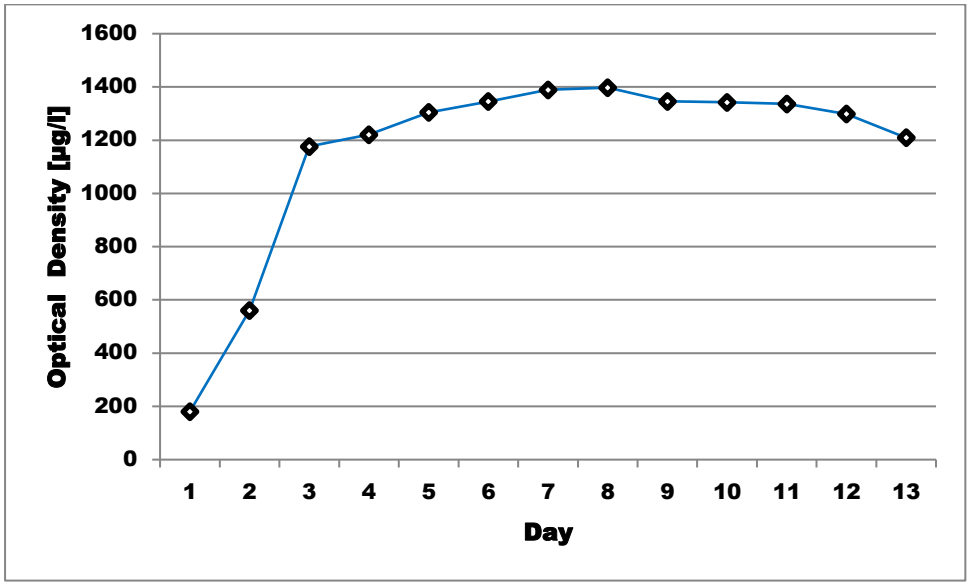

Figure 10: growth curves recorded for *Nannochloropsis sp* at 35ºC

Growth measurements can be monitored using various methods. In this study, optical density was recorded on a daily basis for a period of 15 days. This was done to monitor the species' growth accurately. A wet microalgae biomass was collected from the filtration of 20 ml taken from the growing culture. This was mixed with 10 ml of 85% acetone. The filtration media used for separation of water and wet biomass was a GFF40 filter paper. The mixture of wet biomass and acetone was kept in vials at −4 °C in a refrigerant for 24 hours. A digital spectrophotometer Turner Designs serial number #7200-000 manufactured in San Jose, CA, with Optical Kits P/N 10-040R, was used for the optical density measurements.

In this device the fluorescence was set at 680 nm from a light source excited at 436 nm. The method is based on the direct reading of the fluorescence of optical density expressed in terms of the concentration of the pigment, which in this case was the amount of cells represented by the mass of chlorophyll A non-acid per unit volume. It was expressed in µg/l and represents the degree of absorption of light at a specified wavelength by the solution or suspension containing microalgae cells. The average of the daily measurements recorded in triplicate was used to plot the growth curves represented in Figures 6 to 10.

The analysis of these growth curves shows that *Nannochloropsis sp.* reached the highest amounts of biomass between day 8 and 10 for the temperature ranging from 15 to 35 °C. The highest optical density was obtained within the stationary phases mainly on the tenth day of the culture growing at 15 °C, 20 °C, and 25 °C. The stationary phase during cultivation was very short for these temperatures. Regarding the temperatures of 30 °C and 35 °C, the highest optical density values were recorded on day 9 and day 8 respectively; thereafter, the lysis phase was taking place. It was recorded that *Nannochloropsis sp* was growing efficiently within the temperature range from 15 to 25°C. Similarly, it was recorded that the optical densities recorded during this temperature range were greater than those recorded for 30 °C and 35 °C. More cells were produced within the temperature range between 15 °C and 25 °C than between 30 °C and 35 °C, where the cells number decreased.

3.3.1.2 Biomass harvesting

Algae biomass was harvested during the stationary phase of the cultivation period. It was done on days where high values of optical densities were recorded because large amounts of biomass were produced on these days. It was therefore completed on day 10 for cultivation batches operating between 15 to 25 °C, and on day 9 for cultivation batches operating at 30 °C and on day 8 for cultivation batches operating at 35 °C. The biomass was harvested by centrifugation using a 'Hermle' centrifuge (HERMLE Labortechnik GmbH, Wehingen) running at 4000 rpm for 10 minutes. On average 1800 mg/l of wet biomass was recorded after 10 days, with an average of 600 mg/l of lipid recorded during the same growth period. This almost corresponds to data recorded for *Nannochloropsis sp.* F&M-24 by Rodolfi *et al.* in 2009.[39]

3.3.1.3 Physiological modification: hyper lipid synthesis

To increase microalgae lipid content, it is necessary to modify the physiology of cells. In many studies, increased lipid was obtained by partial nutrient starvation of cells. In most cases this was achieved by depriving species of nitrogen. In this study, total or complete nutrient

starvation is undertaken to modify the physiology of the species in order to stimulate the synthesis of more lipids within cells. Deprivation of nutrients, or cell stressing, was undertaken for 3 days in a pure and nutrient-free environment. After starving microalgae cells for few days, a substantial increase in lipids was recorded. The lipid increase is displayed by the data recorded from the mass balances presented in Figures 12 and 13.

The Nile Red technique was used to observe the changes regarding stressing or physiological modification of microalgae cells. The right side of Figure 11 presents the configuration of microalgae cells before starvation. The left side of Figure 11 shows the changes that took place after genetic stressing or physiological modification caused by complete (or total) nutrient starvation of microalgae cells. It can be observed that the cells are yellow with more lipids after being totally deprived of nutrients for few days. The procedure for Nile Red consists of cells being incubated in a Nile Red solution made of 0.25 mg/l in acetone. The incubating time was 5 minutes and, thereafter, cells were viewed under a fluorescent microscope. A 450–490 nm excitation filter, a 510 nm diachronic mirror, and a 515 nm barrier filter with a 100x lens were also used.

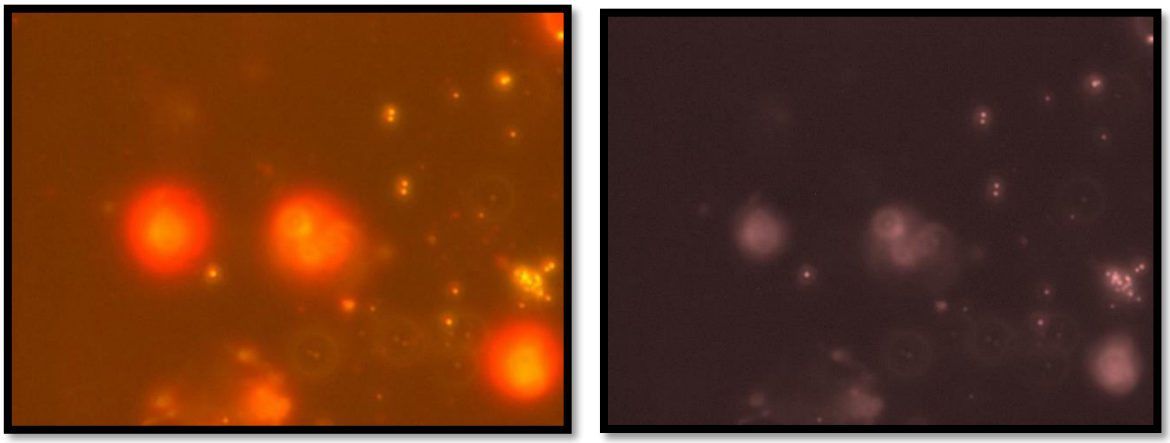

Figure 11: cells of biomass before total nutrients starvation (right) and cells of biomass after total nutrients starvation (left)

3.3.1.4 Bio-oil extraction

Extraction of crude bio-oil from harvested biomass was completed using a modified Bligh and Dyer method [44]. In this modified method a solvent mixture made with chloroform and methanol in a 1:1volume ratio is used. This mixture was added to the wet biomass. The volume ratio between the solvent mixture and wet biomass was also brought to a volume ratio of 1:1. The crude bio-oil extraction was undertaken on wet biomass.

It was aiming to skip the drying process, which is energy and time-consuming. Bio-oil was collected after solvent evaporation in an evaporator from 65 °C.

3.3.1.4.1 Determination of neutral lipids

Lipid content for the control and the stressed samples were determined using a gravimetric method. To determine the total amount of lipids accumulated in microalgae cells during the experiment, the following procedure reported by Guckert *et al.*,45 Wagner *et al.*,46 and Lee *et al.*47 was undertaken: 50 ml of microalgae was harvested by centrifugation for 20 min after cultivation. The extraction of lipids was completed by adding 28 ml of 50 mM phosphate buffer (pH 7.4) into the cell pellets. Thereafter, cells were resuspended and sonicated for 1 min. The samples were transferred to separating funnels; 35 ml of chloroform and 70 ml of methanol were added to each of them, then they were shaken in order to homogenise the mixtures and finally they were kept for 18 hours. After those 18 hours, 35 ml of chloroform and 35 ml of distilled water were added to the samples. The samples were kept for a further 18 hours and the lower chloroform layer in a round-bottom flask was collected. The weight of the empty flask was recorded at the beginning of the experiment. The extract was evaporated in a water bath at 65 °C using a rotary evaporator to remove the solvents; the lipid content, as mass, was then worked out from the difference between the weight of the flask with bio-oil and the weight of the empty flask. This procedure has assisted in establishing the mass balances presented in Figures 12 and 13.

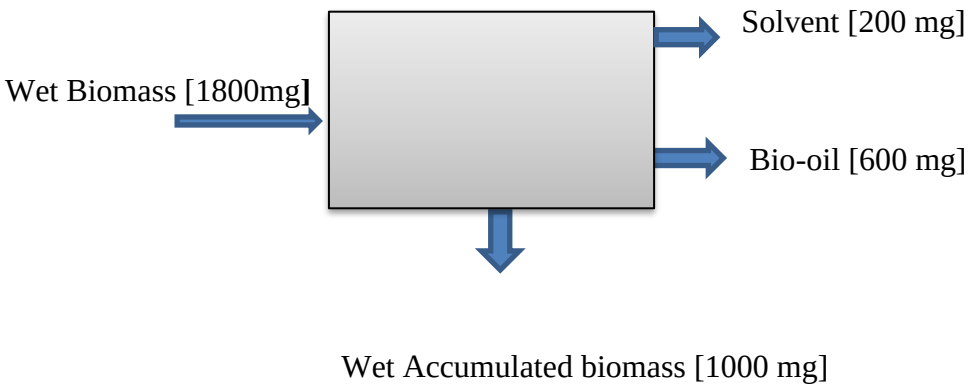

Figure 12: Overall mass balance for bio-oil production/ l of *Nannochloropsis* biomass before total nutrients starvation

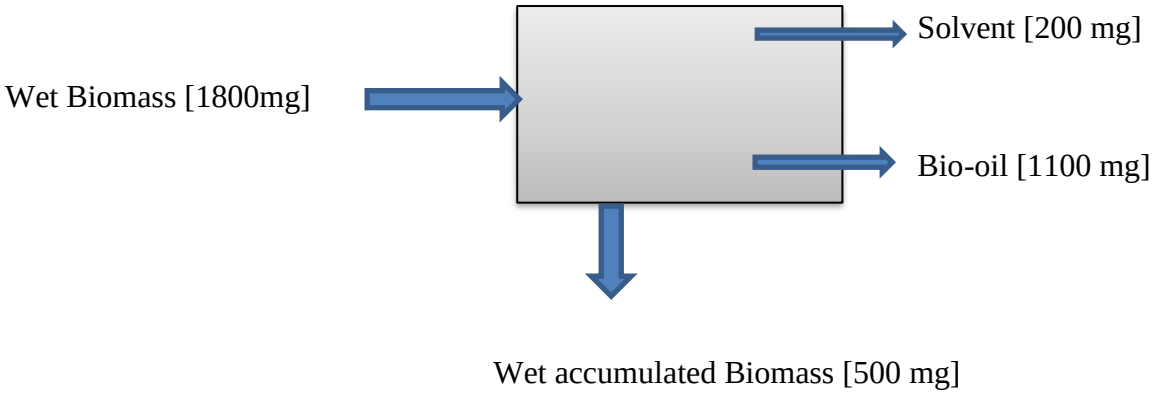

Figure 13: Overall mass balance for bio-oil production/l of *Nannochloropsis* biomass after total nutrients starvation

Analysing these mass balances, it may be observed that, before nutrient starvation, the amount of bio-oil recorded represents only 30% of the total biomass used for oil extraction (Figure 12). After physiological modification undertaken with total starvation of nutrients, there is an increase of more than 80% when comparing the lipid content (bio-oil) before starvation (Figure 12) and after starvation (Figure 13).

3.3.1.4.2 Characterisation of crude bio-oil

The characterisation of microalgae crude bio-oil was completed using a gas chromatograph (GC/ MS). The sample of crude bio-oil was purified by filtration. This was followed by the transesterification of the bio-oil, after which it was injected into the GC/MS for characterisation. After running the standard, vegetable oils such as sunflower and canola oils were also used for trials. These trials were undertaken before running the bio-oil from *Nannochloropsis sp.* The purpose of the trials with vegetable oils was based on the fact that microalgae bio-oil is also a vegetable oil. It has qualitative similarities with many vegetable oils including sunflower and canola oils. These trials have assisted in establishing the method for the bio-oil characterisation with GC/MS. Subsequently, the sample of microalgae bio-oil was run successfully with the GC/ MS. Figure 14 shows the chromatograph and results from a sample of crude bio-oil from microalgae on GC/MS. Table 7 shows the presence of various fatty methyl esters (FAMEs) and other hydrocarbons found in the crude bio-oil from *Nannochloropsis sp*. (see also APPENDIX A5: GC method for bio-oil characterisation)

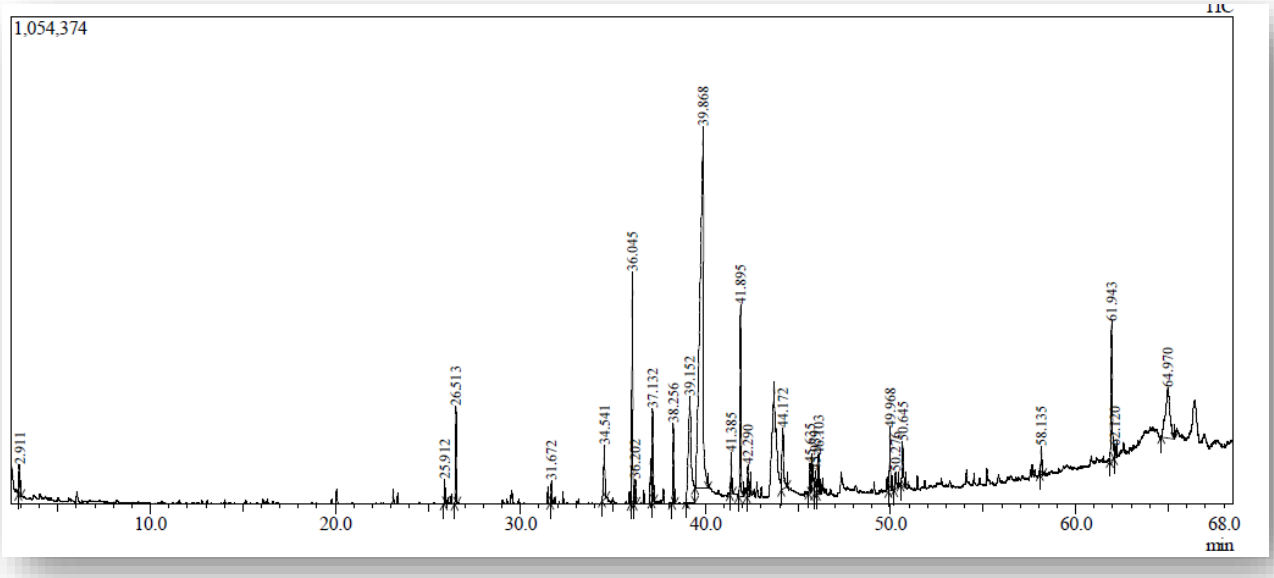

Figure 14: Chromatograph of *Nannochloropsis sp* crude bio- oil

Table 7: Data of crude bio-oil from *Nannochloropsis* completed on GC/MS

Peak #	Retention time [min]	Area %	Height %	A/H	Compound Formula and name
1	2.911	0.94	1.60	3.83	C_6H_8 : 1,4-Cyclohexadiene :
2	25.912	0.55	1.24	2.88	$C_{16}H_{32}$: 3-Hexadecene, (Z)-
3	26.513	2.39	5.14	3.02	$C_{14}H_{30}$: Tetradecane
4	31.672	0.61	1.21	3.30	$C_{16}H_{32}$: 3-Hexadecene, (Z)-
5	34.541	2.27	2.86	5.13	$C_{14}H_{28}O_2$: Tetradecanoic acid
6	36.045	6.27	12.31	3.30	$C_{22}H_{42}O_2$: Phytol, acetate
7	36.202	0.62	1.29	3.10	$C_{20}H_{40}$: 2-Hexadecene, 3,7,11,15-tetramethyl-, [R-[R*;
8	37.132	3.08	4.92	4.06	$C_{20}H_{40}O$ 3,7,11,15-Tetramethyl-2-hexadecen-1-ol
9	38.256	2.04	4.25	3.11	$C_{17}H_{34}O_2$: Hexadecanoic acid, methyl ester
10	39.152	9.77	5.61	11.29	$C_{16}H_{30}O_2$: Palmitoleic acid
11	39.868	39.18	19.14	13.26	$C_{15}H_{30}O_2$: Pentadecanoic acid
12	41.385	1.02,	2.14	3.09	$C_{57}H_{104}O_6$ 9-Octadecenoic acid, 1,2,3-propanetriyl ester,
13	41.895	5.87	9.89	3.85	$C_{21}H_{40}O_2$: Octadecanoic acid, 2-propenyl ester
14	42.290	0.99	1.67	3.85	$C_{18}H_{34}O$: 9,12-Octadecadien-1-ol, (Z,Z)-
15	44.172	2.82	3.18	5.74	$C_{18}H_{36}O_2$: Octadecanoic acid
16	45.635	0.81	1.57	3.35	$C_{21}H_{38}O_2$: Isopropyl linoleate
17	45.939	0.85	1.22	4.47	$C_{26}H_{44}O_6$:Ethyl iso-allocholate
18	46.103	1.35	2.12	4.11	$C_{22}H_{34}O_2$:5,8,11,14,17-Eicosapentaenoic acid, methyl ester
19	49.968	1.85	3.32	3.60	$C_{23}H_{34}O_2$: 4,7,10,13,16,19-Docosahexaenoic acid, methyl
20	50.276	0.68	0.89	4.92	$C_{16}H_{30}O$cis-9-Hexadecenal
21	50.645	1.94	2.34	5.36	$C_{19}H_{38}O_4$ Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl
22	58.135	0.77	1.45	3.43	$C_{27}H_{44}$: Cholesta-3,5-diene
23	61.943	5.75	7.28	5.12	$C_{27}H_{46}O$: Cholesterol

24	62.120	0.55	0.67	5.29	$C_{29}H_{50}O_2$:Vitamin E
25	64.970	7.04	2.67	17.09	$C_{55}H_{106}O_6$: Eicosanoic acid, 2-[(1-oxohexadecyl)oxy]-1-[[(1
		100	100		

From the characterisation indicated in table 7, FAMEs were identified as having carbon chains ranging from C6 to C57. This situation is very favourable for jet fuel production. Cracking is therefore needed in order to break down long carbon chains including C55 and C57 into short and medium ones essential for jet fuel production. Fractionation or distillation will follow, to separate different carbon chains based on the difference in boiling points. Jet fuel is a complex blend of alkanes made mainly from middle-end carbon chains between C14 to C16 and other alkanes from low-middle-end carbon chains in the case of jet B. Table 7 indicates that fractions between C14 and C16 have together recorded high percentages in terms of areas of the peaks compared to the other remaining fractions.

3.3.1.5 Thermal cracking: Thermolysis

Compounds from the characterisation presented in table 7 have to be broken down to produce middle-end fractions or carbon chains needed for jet fuel. These fractions must be mainly alkanes, as mentioned earlier, in order to obtain algae-based jet fuel similar to the conventional jet fuel. Firstly, cracking trials on vegetable oils such as sunflower and canola oils were undertaken at the following temperatures: 300, 350, 400, and 450 °C. It was done with the following times: 30, 45 and 60 min for each temperature. From these trials, it was recorded that when cracking at the temperature of 300 °C all compounds were disintegrated or broken after a maximum time of 30 minutes. The experiment was simulated on GC/MS to determine the cracking temperature and time without a catalyst for a complete disintegration of carbon chains for sunflower and canola oils. Secondly, after using vegetable oils, the same experience was undertaken on microalgae bio-oil. The simulated cracking on GC/MS for microalgae bio-oil generated similar results. The cracking temperature was recorded at 300 °C within a time of 31.06 min. The pyrolysis of purified bio-oil undertaken as fast cracking was achieved at 350 ^{o}C during a time of 12 s while 450 ^{O}C was recorded as the pyrolysis temperature for crude bio-oil achieved within the same amount of time (see APPENDIX A7 for GC methods and various chromatographs related to pyrolysis). The recovered quantity of oil recorded after thermal cracking was between 85% and 90%. Cracking temperature and time can be improved with the assistance of catalysts. Studies can be undertaken to choose the relevant catalyst for an effective cracking temperature and time. Figure 15 presents various peaks of the cracked bio-crude oil recorded on a GC/MS.

This is a simulated cracking achieved at 300 °C. (see APPENDIX A3 including the compounds on the peaks of the chromatograph presented in Figure 15)

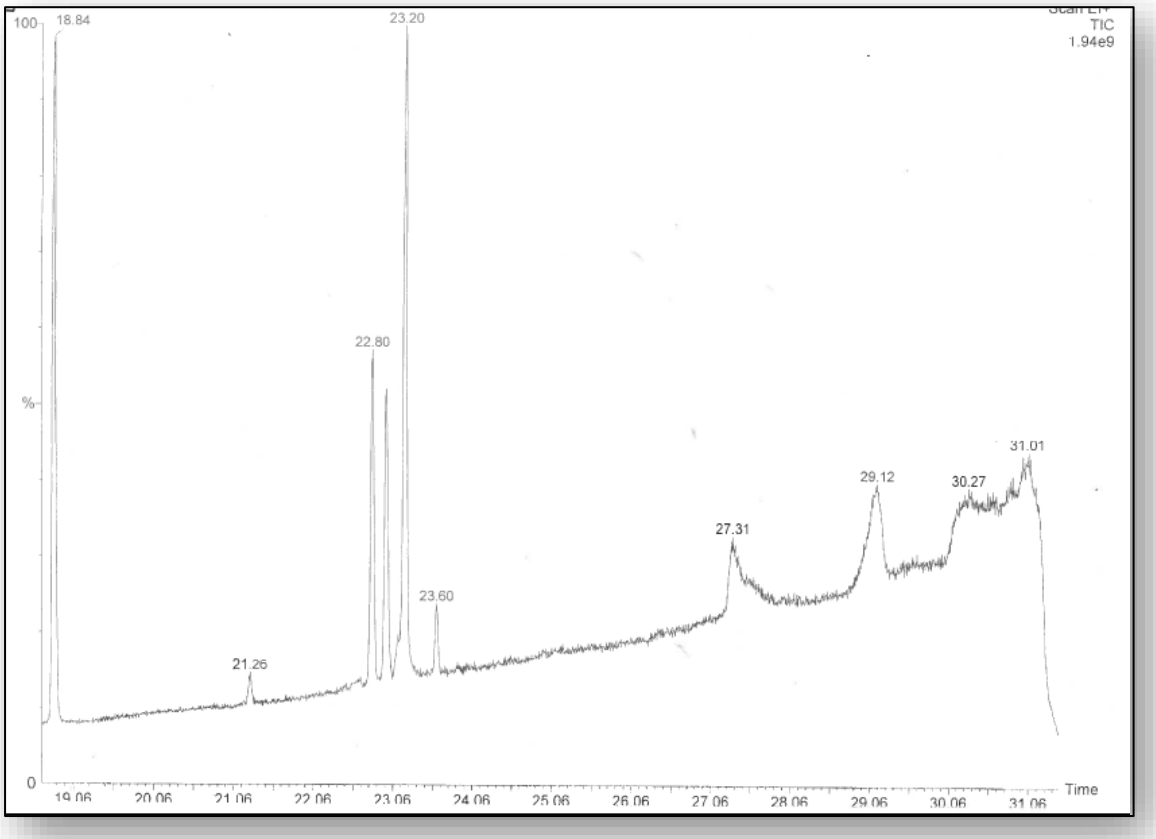

Figure 15: Chromatograph of simulated cracking for crude bio-oil from *Nannochloropsis sp*

3.3.1.6 Fractionation

A distillation unit was used for fractionation. This process was conducted at atmospheric pressure. During fractionation, solvent recovery took place between 60 °C and 65 °C. From 70 °C to an end-point of 250 °C, middle distillate alkanes making a jet fuel from microalgae oil were collected.

3.3.2 Overall Flowchart

Figure 16 presents the entire process undertaken in the laboratory to produce bio-jet fuel from microalgae cultivation. Future research can examine the possibility of adding upgrading and reforming unit processes to obtain a more purified final product.

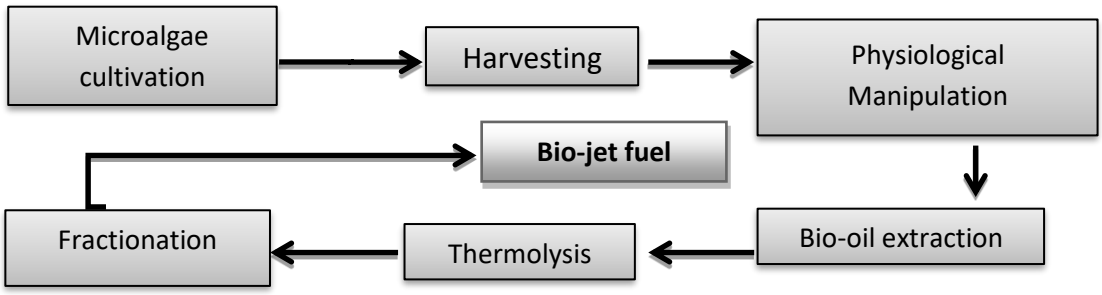

Figure 16: Flowchart of processing operations from algal cultivation to bio-jet fuel

3.3.3 Characterisation of the algae-based jet fuel from *Nannochloropsis*

The jet fuel produced was characterised with GC/MS. This was followed by physico-chemical analyses to determine if the product was similar to the conventional Jet A1 used as a reference. Physico-chemical analyses were also completed, in compliance with ASTM standards for aviation fuels. Figures 17 and 18 present the chromatographs resulting from the characterisation of jet A1 and the bio-jet fuel produced in this study. Table 8 shows details about different compounds found on peaks in the chromatographs generated by the GC/MS.

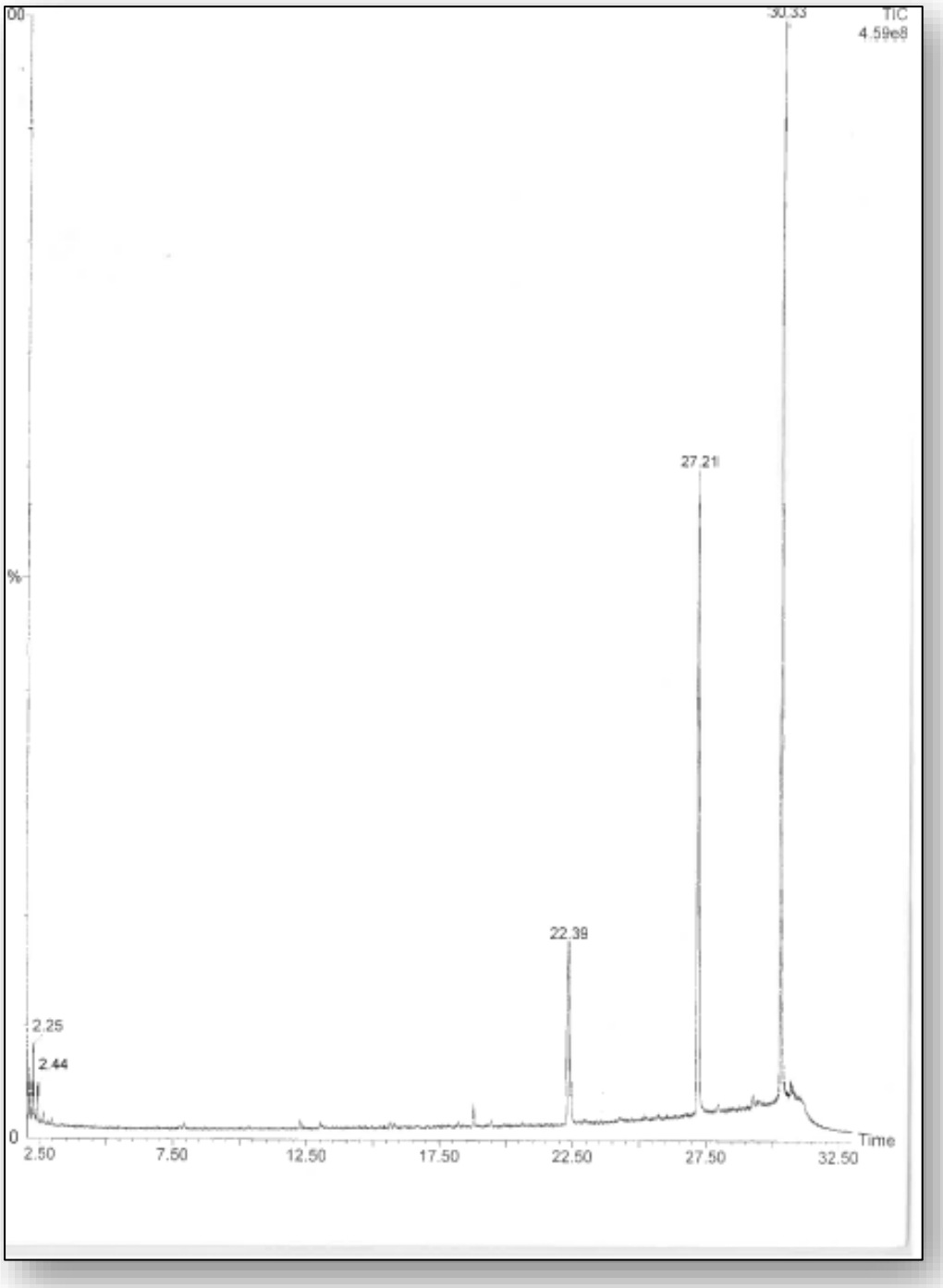

Figure 17: Chromatograph profile of produced bio-jet fuel

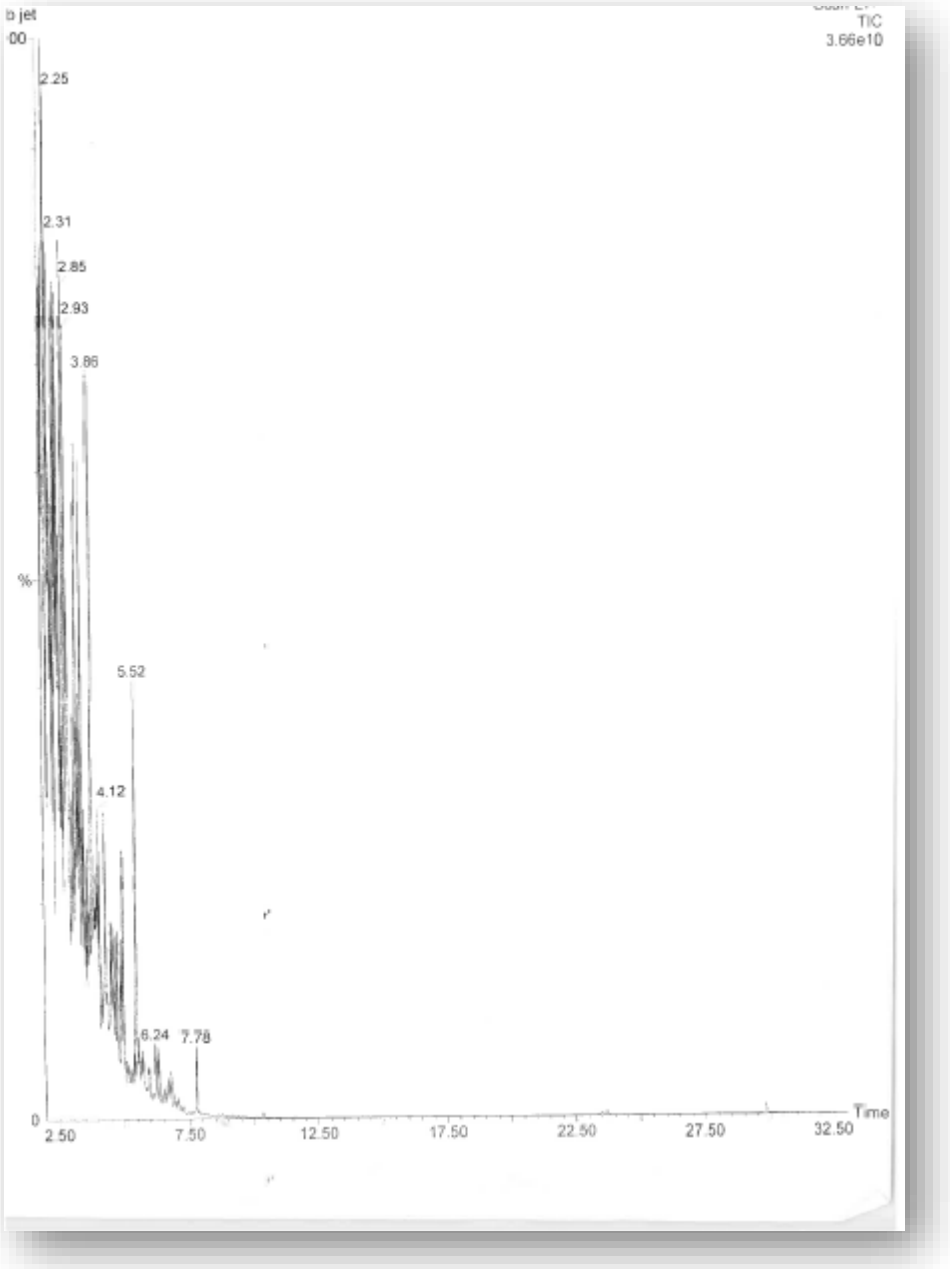

Figure 18: Chromatograph profile of conventional jet fuel (Jet A1)

Table 8: Comparison between peaks from chromatographs of bio-jet fuel (a) and conventional jet fuel (b)

(a)

Hit	Compound
1	$C_{31}H_{64}$
2	$C_{54}H_{110}$
3	$C_{23}H_{48}$
4	$C_{25}H_{52}$
5	$C_{22}H_{46}$
6	$C_{24}H_{50}$
7	$C_{30}H_{62}$
8	$C_{32}H_{66}$
9	$C_{39}H_{80}$
10	$C_{30}H_{62}$
11	$C_{34}H_{70}$
12	$C_{40}H_{82}$
13	$C_{30}H_{62}$
14	$C_{25}H_{52}$
15	$C_{54}H_{110}$
16	$C_{35}H_{72}$
17	$C_{24}H_{50}$
18	$C_{36}H_{74}$
19	$C_{24}H_{50}$
20	$C_{40}H_{82}$

(b)

Hit	Compound
1	$C_{31}H_{64}$
2	$C_{22}H_{46}$
3	$C_{54}H_{110}$
4	$C_{23}H_{48}$
5	$C_{27}H_{56}$
6	$C_{30}H_{62}$
7	$C_{24}H_{50}$
8	$C_{35}H_{72}$
9	$C_{29}H_{60}$
10	$C_{19}H_{40}$
11	$C_{23}H_{48}$
12	$C_{21}H_{44}$
13	$C_{18}H_{38}O_3S$
14	$C_{21}H_{44}$
15	$C_{12}H_{26}$
16	$C_{15}H_{32}O_3S$
17	$C_{22}H_{46}$
18	$C_{16}H_{34}O_3S$
19	$C_{16}H_{34}$
20	$C_{20}H_{42}$

There is a similarity between both jet fuels in terms of content when analysing all the peaks and the compounds from the chromatographs. This is confirmed by the fact that most compounds found in bio-jet fuel, and from jet A1, are alkanes as indicated in both versions of table 8. These alkanes have been recorded on all peaks for both Jet A1 and algae-based jet fuel. A few exceptions were found with the Jet A1: some compounds appear with oxygen and sulfur; these come from the petroleum crude oil and the additives used for improving the physico-chemical proprieties of Jet A1. Table 9 presents the average for measurements done in triplicate during characterisation. It includes the physico-chemical data of both the bio-jet fuel produced in this study and the various conventional jet fuels. The importance of each parameter is explained in terms of the energy needed by the aircraft.

Table 9: Comparison between conventional jet fuels and bio-jet fuel produced in the current study

Parameter (Min or Max for ASTM standards)	Algae bio-jet (fuel from this study)	Jet A1 (ASTM D 1655 standards)	Jet A (ASTM standards D 1655)	Jet B (ASTM standards D 1655) :wide cut Kerosene	Importance of the parameter in terms of Energy production
Heating Value [MJ/kg] (Minimum)	**44**	**42.8**	**42.8**	**42.8**	Represents the total energy released for fuel combustion. It is the energy content of the fuel
Freezing point [^{o}C] (Maximum)	**-30**	**-47**	**-40**	**-50**	Assists in fuel flow at lower temperatures. Consequently, the level of energy produced will not be affected because the mass of the fuel will not be affected
Flash point [^{o}C] (Minimum)	**68**	**38**	**38**	**Not reported**	Related to the fuel flammability and ignition
Kinematics Viscosity at -20^{O}C [Cst] (Maximum)	**2.8**	**8**	**8**	**Not reported**	Influences the capacity of the engine to restart and consume less fuel at higher altitudes.
Density at 15 ^{O}C [g/ml]	**1.38**	**0.775-0.840**	**0.775-0.840**	**0.751-0.802**	Affects the engine performance and fuel consumption because the mass of fuel injected depends upon its density, therefore, the energy to propel the aircraft can be affected
Sulfur (total) [wt %] (Maximum)	**0.27**	**0.30**	**0.30**	**0.30**	Affects the fuel efficient, therefore, the generated energy can be affected
End point for Distillation [^{O}C] (Maximum)	250	**300**	**300**	**Not reported**	Influences the fuel quality and the energy to be generated by the fuel

Analysis of table 9 shows that most parameters recorded for algae-based jet fuel comply with the ASTM standards for aviation fuels. The analysis of data for the algae-based jet fuel shows that only density and freezing point need to be improved. This can be achieved by the use of additives. Bio-jet fuel produced in this study can be blended with Jet A 1, Jet A or Jet B. By blending bio-jet fuel with conventional jet fuel it is possible to reduce the fuel carbon

footprint because the refraction index and combustion ability will increase and improve fuel sustainability.

CONCLUSIONS

- High amounts of biomass were produced between day 8 and day 10 of the cultivation period with temperatures ranging between 15 and 35 °C under a luminance of 1000 lux and supply of CO_2 on a daily basis during a cultivation period of 15 days.
- *Nannochloropsis sp* grew effectively between 15 to 25 °C with more biomass produced in the same temperature range.
- The biomass was subjected to physiological modification for 3 days under total nutrient starvation with more than an 80% increase in lipid.
- The microalgae bio-oil produced was subjected to thermal cracking without catalyst at 300 °C for 31 min.
- The pyrolysis of oil was undertaken as a fast cracking at 350 °C for purified bio-oil achieved for a maximum period of 12 s while 450 °C was recorded as the pyrolysis temperature for microalgae crude bio-oil achieved for the same amount of time
- Fractionation was undertaken between 70 ^{o}C and 250 ^{o}C.
- Most physico-chemical parameters were within the range prescribed by ASTM standards, except freezing point and density. The use of antifreeze can assist in solving the issue of a freezing point while polishing processes such as membrane filtration can be used to remove particulate matter in order to improve the density.

There is currently a need to mature the technology by moving from pilot to commercial scale. Once the technology has reached the level of maturity required for commercialisation, life-cycle assessment studies can be undertaken to predict the extent of carbon emissions and fuel sustainability. The pilot scale can involve more investigations on upgrading processes, the use of catalysts, modelling parameters to optimise the bio-jet fuel production and to improve its quality. This will also assist in defining conditions for efficient process design which are essential for scaling up to the commercial plant. It will also assist in optimising the costs for a sustainable bio- jet fuel to compete with the current conventional jet fuel.

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REFERENCES

[1].Srirangan K, Akawi L, Moo-Young M, Chou CP. Towards sustainable production of clean energy carriers from biomass resources. *Applied Energy*; **100**:172-186, (2012). https://doi.org/10.1016/j.apenergy.2012.05.012

[2] Brandt A, Gräsvik J, Hallett JP, Welton T. Deconstruction of lignocellulosic biomass with ionic liquids. *Green Chemistry* **15**(3):550-83, (2013). https://doi.org/10.1039/c2gc36364j

[3] Lakaniemi AM, Tuovinen OH, Puhakka JA. Anaerobic conversion of microalgal biomass to sustainable energy carriers–a review. *Bioresource technology*; **135**:222-231 (2013).https://doi.org/10.1016/j.biortech.2012.08.096

[4] Knothe G, Krahl J, Van Gerpen J, editors. The biodiesel handbook. *Elsevier*; August (2015).

[5] Knothe, G., Razon, L.F., Biodiesel fuels. *Progress in Energy and Combustion Science.;* **58**, pp.36-59, (2017). https://doi.org/10.1016/j.pecs.2016.08.001

[6]Vassilev SV, Vassileva CG. Composition, properties and challenges of algae biomass for biofuel application: An overview. *Fuel* **1**(181**)**:1-33 (2016) https://doi.org/10.1016/j.fuel.2016.04.106

[7] Shankar S. Renewable and Non-renewable Energy Resources: *Bioenergy and Biofuels*. In Principles and Applications of Environmental Biotechnology for a Sustainable Future pp. 293-314, Springer Singapore, (2017), Ed.by R.L **Singh**

[8] Agwa OK, Ibe SN, Abu GO. Economically effective potential of Chlorella sp. for biomass and lipid production. *Journal of Microbiology and Biotechnology Research*.; **2(1):**35-45, (2017)

[9] Blankenship RE. Molecular mechanisms of photosynthesis. *John Wiley & Sons*; May (2013)

[10] Suganya T, Varman M, Masjuki HH, Renganathan S. Macroalgae and microalgae as a potential source for commercial applications along with biofuels production: A biorefinery approach. *Renewable and Sustainable Energy Reviews*; **55**:909-41. (2016) , https://doi.org/10.1016/j.rser.2015.11.026

[11] Eloka-Eboka AC, Inambao FL. Effects of CO_2 sequestration on lipid and biomass productivity in microalgal biomass production. *Applied Energy*.; **195**:1100-11. (2017), https://doi.org/10.1016/j.apenergy.2017.03.071

[12] Demirbas A. Use of algae as biofuel sources. *Energy conversion and management* **51**(12)**:**2738-2749, (2010).https://doi.org/10.1016/j.enconman.2010.06.010

[13] Demirbas A, Demirbas MF. Importance of algae oil as a source of biodiesel. *Energy conversion and management*.; **52** (1):163-170. (2011),https://doi.org/10.1016/j.enconman.2010.06.055

[14] Milano J, Ong HC, Masjuki HH, Chong WT, Lam MK, Loh PK, Vellayan V. Microalgae biofuels as an alternative to fossil fuel for power generation. *Renewable and Sustainable Energy Reviews.* **58**:180-197 (2016), https://doi.org/10.1016/j.rser.2015.12.150

[15] d'Ippolito G, Sardo A, Paris D, Vella FM, Adelfi MG, Botte P, Gallo C, Fontana A. Potential of lipid metabolism in marine diatoms for biofuel production. *Biotechnology for biofuels*. ;**8**(1):28, (2015).https://doi.org/10.1186/s13068-015-0212-4

[16] Arenas EG, Palacio R, Juantorena AU, Fernando SE, Sebastian PJ. Microalgae as a potential source for biodiesel production: techniques, methods, and other challenges. *International Journal of Energy Research*,**41**(6):761-89 (2016).

[17] Moreno-Garcia L, Adjallé K, Barnabé S, Raghavan GS. Microalgae biomass production for a biorefinery system: Recent advances and the way towards sustainability. *Renewable and Sustainable Energy Reviews*.; **76**:493-506, (2017), https://doi.org/10.1016/j.rser.2017.03.024

[18] Patel A, Gami B, Patel P, Patel B. Microalgae: Antiquity to era of integrated technology. *Renewable and Sustainable Energy Reviews*; **71**:535-547, (2017)

[19] Sahay S, Braganza VJ. Microalgae based biodiesel production–current and future scenario. *Journal of Experimental Sciences*; **7**:31-35, (2016)

[20] Demirbas A. Production economics of high-quality microalgae. Energy Sources, Part B: Economics, Planning, and Policy;**12**(5):395-401, (2017), https://doi.org/10.1080/15567249.2015.1057655

[21] Ramachandran, S., Ganesh, P.S., Sivasubramanian, V., Kumar, B.P., Current Developments in Mass Production of Microalgae for Industrial Applications., *Environmental Sustainability Using Green Technologies*, p.147, (2016), edited by V. Sivasubramanian , CRC Press, Nature publishing group, London

[22] Shukla M, Tabassum R, Singh R, Dhar DW. Influence of light intensity, temperature and CO_2 concentration on growth and lipids in green algae and cyanobacteria, *Indian Journal of Experimental Biology, Vol 54*, pp 482-487, (2016)

[23] Griffiths H, Bringing new products from marine microorganisms to the market, in The Marine Microbiome, ed. by Stal LJ and Cretoiu MS. Springer, Cham, pp. 435–452 (2016).

[24] Whittle SJ, Casselton PJ. The chloroplast pigments of the algal classes Eustigmatophyceae and Xanthophyceae. I. Eustigmatophyceae. *British Phycological Journal.*; **10**(2):179-191, (1975).https://doi.org/10.1080/00071617500650171

[25] Dunstan GA, Volkman JK, Barrett SM, Garland CD. Changes in the lipid composition and maximisation of the polyunsaturated fatty acid content of three microalgae grown in mass culture. *Journal of Applied Phycology*.; **5**(1):71-83,(1993), https://doi.org/10.1007/BF02182424

[26] Imamoglu E, Demirel Z, Conk Dalay M. Process optimization and modeling for the cultivation of Nannochloropsis sp. and Tetraselmis striata via response surface methodology. *Journal of phycology*; **51**(3):442-453, (2015),https://doi.org/10.1111/jpy.12286

[27] Mayaruma,I., Nakamura, T., Matsubayashi, T., Ando, Y.,Maeda,T., Identification of the alga known chlorella as a marine Chlorella as a member of the Eustigmatophyceae, *Journal of Phycology*; **34**, 319-325; (1992)

[28] Adarme-Vega TC, Thomas-Hall SR, Lim DK, Schenk PM. Effects of long chain fatty acid synthesis and associated gene expression in microalga Tetraselmis sp. *Marine drugs*; **12**(6):3381-3398, (2014).https://doi.org/10.3390/md12063381

[29] Patterson GW, Tsitsa-Tzardis E, Wikfors GH, Ghosh P, Smith BC, Gladu PK. Sterols of eustigmatophytes. *Lipids;* **29**(9):661-664, (1994). https://doi.org/10.1007/BF02536102

[30] Martin-Creuzburg D, Merkel P. Sterols of freshwater microalgae: potential implications for zooplankton nutrition. *Journal of Plankton Research*.**38**(4):865-877 (2016).https://doi.org/10.1093/plankt/fbw034

[31] Sukenik, A. Production of eicosapentaenoic acid by the marine eustigmatophyte Nannochloropsis. In Chemicals from Microalgae; Cohen, Z., Ed. by Taylor and Francis: London, UK, 1999; pp. 41–56.

[32] Singh P, Kumari S, Guldhe A, Misra R, Rawat I, Bux F. Trends and novel strategies for enhancing lipid accumulation and quality in microalgae. *Renewable and Sustainable Energy Reviews*; **55**:1-6, (2016)

[33] Minhas AK, Hodgson P, Barrow CJ, Adholeya A. A review on the assessment of stress conditions for simultaneous production of microalgal lipids and carotenoids. Frontiers in microbiology; 7:546; (2016). https://doi.org/10.3389/fmicb.2016.00546

[34] Zittelli GC, Lavista F, Bastianini A, Rodolfi L, Vincenzini M, Tredici MR. Production of eicosapentaenoic acid by Nannochloropsis sp. cultures in outdoor tubular photobioreactors. *Journal of Biotechnology*.;**70**(1):299-312, (1999).https://doi.org/10.1016/S0168-1656(99)00082-6

[35] Safafar H, Hass MZ, Møller P, Holdt SL, Jacobsen C. High-EPA Biomass from Nannochloropsis salina Cultivated in a Flat-Panel Photo-Bioreactor on a Process Water-Enriched Growth Medium. *Marine Drugs*.;14(8):144. (2016). https://doi.org/10.3390/md14080144

[36] Remmers IM, Martens DE, Wijffels RH, Lamers PP. Dynamics of triacylglycerol and EPA production in Phaeodactylum tricornutum under nitrogen starvation at different light intensities. *PloS one*.;12(4):e0175630,(2017).https://doi.org/10.1371/journal.pone.0175630

[37] Shifrin NS, Chisholm SW. Phytoplankton lipids: interspecific differences and effects of nitrate, silicate and light-dark cycles. Journal of phycology. 1981 Dec 1;17(4):374-84.

[38] Chisti Y. Biodiesel from microalgae beats bioethanol. *Trends in biotechnology*; **26**(3):126-131, (2008).https://doi.org/10.1016/j.tibtech.2007.12.002

[39] Rodolfi L, Chini Zittelli G, Bassi N, Padovani G, Biondi N, Bonini G, Tredici MR. Microalgae for oil: Strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor. *Biotechnology and bioengineering*.;**102**(1):100-12. (2009). https://doi.org/10.1002/bit.22033

[40] Ma XN, Chen TP, Yang B, Liu J, Chen F. Lipid production from Nannochloropsis. *Marine drugs* ;**14**(4):61, (2016)https://doi.org/10.3390/md14040061

[41] Bondioli P, Della Bella L, Rivolta G, Zittelli GC, Bassi N, Rodolfi L, Casini D, Prussi M, Chiaramonti D, Tredici MR. Oil production by the marine microalgae Nannochloropsis sp. F&M-M24 and Tetraselmis suecica F&M-M33. *Bioresource Technology* ;**114**: 567-572. (2012). https://doi.org/10.1016/j.biortech.2012.02.123

[42] Guillard RR, Ryther JH. Studies of marine planktonic diatoms: I. Cyclotella Nana Hustedt, and Detonula Confervacea (CLEVE) Gran. *Canadian journal of microbiology*. ;**8**(2):229-39, (1962).https://doi.org/10.1139/m62-029

[43] Goswami RC, Kalita N, Kalita MC. A study on growth and carbon dioxide mitigation by microalgae Selenastrum sp.: its growth behavior under different nutrient environments and lipid production. *Annals of Biological Research*;**3**(1):499-510, (2012)

[44] Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. *Canadian journal of biochemistry and physiology* ;**37**(8):911-917. (1959). https://doi.org/10.1139/o59-099

[45] Guckert J.B., Cooksey K.E., and Jackson L.L., Lipid solvent systems are not equivalent for analysis of lipid classes in the microeukaryotic green alga, Chlorella, *Journal of Microbiological Methods*, **8**: 139-149, (1988) http://dx.doi.org/10.1016/0167-7012(88)90015-2

[46] Wagner H., Liu Z., Langner U., Stehfest K., and Wilhelm C., The use of FTIR

spectroscopy to assess quantitative changes in the biochemical composition of microalgae, *Journal of Biophotonic*, **3**: 557-566, (2010) http://dx.doi.org/10.1002/jbio.201000019 PMid:20503222

[47] Lee S.J., Yoon B.D., and Oh H.M., Rapid method for the determination of lipid from the green alga *Botryococcus braunii*, *Biotechnology Techniques*, **12**(7): 553-556, (1998) http://dx.doi.org/10.1023/A:1008811716448

CHAPTER 4 PREFACE

The chapter is presented in a form of a research paper accepted and published in Biofuel, Biorefinery and Bioproduct journal. The chapter focuses on the design of the entire process from the cultivation of microalgae to jet fuel. The basis of the design was the laboratory work undertaken in the previous chapter. The laboratory data were used to simulate a conceptual design which can be used to escalate the project to pilot or commercial level. The chapter is a continuation of work done in previous chapters. It aims to develop a simplified process from microalgae cultivation to jet fuel production and its simulation at a larger scale. Also, suggested options aiming to improve the fuel quality to the required standards in the aviation sector are part of the chapter. A simplified process similar to the petrochemical process used for the manufacturing of conventional jet fuel was established. The simulation test was run successfully, this is an indication that conventional jet fuel technology can be used to manufacture algae-based jet fuel that complies with aviation fuel standards. The chapter is presented as is from the time it was accepted by the journal, there are minor modifications aiming to keep the flow and connection from previous chapters. The copy of the original published article is found in APPENDIX A1 as indicated in declaration section.

CHAPTER 4: CONCEPTUAL PROCESS DESIGN AND SIMULATION OF MICROALGAE OIL CONVERSION TO AVIATION FUEL

[1,2]Joseph K. Bwapwa, [2] Akash Anandraj , [1]Cristina Trois

[1] University of KwaZulu-Natal, [2] Mangosuthu University of Technology

Corresponding author: joseph@mut.ac.za ; josephkapuku@gmail.com

ABSTRACT

Microalgae oil can be converted into aviation fuel to reduce dependence on fossil fuels and decrease the carbon footprint. This could be a significant step toward sustainable energy resources that have the potential to produce 'drop-in' fuels. Algae-based fuels are potential substitutes for fossil fuels due to the quality of their crude oil. However, this is only possible if appropriate conversion processes are undertaken. Conversely, microalgae species have low lipid content and biomass harvesting is still an energy-demanding process. In this study, a conceptual design is developed for the conversion of microalgae oil to jet fuel. It is based on a process undertaken in the laboratory using a species named *Nannochloropsis* sp. Nutrients and CO_2 were supplied to the growing culture for effective growth. Biomass harvesting was completed on the tenth day of the growth cycle. It was followed by physiological modification to improve lipid content. Finally, crude oil extraction was followed by bio-oil hydrocracking at 350 °C, and fractionation of cracked bio-oil between 70 and 300 °C to separate light-, middle-, and heavy-end hydrocarbons for use in the production of jet fuel. During simulation, reforming and upgrading processes were added to the design to enhance the quality of the jet fuel to be produced on large scale in the future. The study, including the results, suggests that it is technically feasible to convert microalgae oil into jet fuel because of the similarity between algae bio-oil and petroleum crude oil.

KEYWORDS: Nannochloropsis; jet fuel; conversion process; microalgae oil; process design; simulation

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4.1 INTRODUCTION

4.1.1 Background, jet fuel and biodiesel

The current need for sustainable energy is focusing on the development of ground-breaking and alternative types of renewable energy. This trend aims to fill the gap created by an increasing demand from the energy industry.[1] Therefore, options related to the production of biofuels such as ethanol, biodiesel and jet fuel from biomass are currently highly considered. Ethanol derived from starch, sugar or any other type of biomass can be used as an alternative or additive to gasoline.[2,3,4,5] Furthermore, in case the cellulosic biomass is successfully used to produce bioethanol at affordable costs, there will consequently be a competition with petroleum-based fuels. Regarding the biodiesel alternative, some edible and non-edible oils can be a substitute for petroleum diesel provided that the production costs are reduced.[6,7] Similarly, it is important to stress the fact that the use of these oils should not be a threat to food production, lubrication and many other industrial applications. Unfortunately, this kind of biofuel cannot be directly used in aircraft engines as substitutes to jet fuel for which high energy density and low-temperature are highly required and very critical parameters. They are among key parameters that define the performance of a jet fuel.

The energy density per unit volume for ethanol is not sufficient to run an aircraft because it is half of the total energy produced by a conventional jet fuel. However, the energy density per unit volume in biodiesel represents almost 80% of the one contained in jet fuel.[8] The major weakness of biodiesel is that at lower temperatures, with an aircraft flying at higher altitudes, there will be fuel solidification taking place.[8,9] This is due to the fact that biodiesel freezing point is much higher than that of conventional jet fuel. Studies to find an appropriate flow improver will be needed for biodiesel.[9] It is a stringent requirement for any aviation fuel to have a very low freezing point to avoid disastrous consequences due to its solidification during the flying time. Therefore, biodiesel cannot be a suitable fuel for aircraft.

4.1.2 Market expectations, current state of technology and requirements to produce algae bio-oil

It is essential to produce algae-based jet fuel with similar physico-chemical properties to conventional jet fuel; it has to be cost-effective and sustainable and have low carbon emissions. A designing and simulating process including modelling and optimizing some parameters could be a key milestone in the production of sustainable jet fuel from microalgae oil. Many studies are currently underway but not many of their outputs are in the public domain.

The field of alternative aviation fuels, and more especially work on microalgae-based jet fuels, is becoming particularly competitive. Demand and market expectations are very high with regard to compliance and costs. 'Drop-in' fuels are the most needed but they are not sustainable as yet; however, the blending of current fossil fuels with algae-derived fuels is considered to be a sustainable option. Due to strong similarity between petroleum crude oil and algae bio-oil qualitatively and quantitatively (depending on the species/strain), it is possible to use a process that is similar to that used in refineries for conventional jet fuel in order to produce algae-based jet fuel [10]. However, the process has to be improved economically for the algae-based jet to be competitive and finally become commercialized. There is currently no jet fuel on the market produced on a larger scale from algae bio-oil. Many projects are still at trial and pilot scale. Algae-based jet fuel is only blended with conventional jet fuel on 50/50 ratio as allowed by ASTM certification.

For microalgae to produce oil, biomass cultivation must take place under defined conditions of temperature and pH. The addition of nutrients and carbon dioxide to allow effective growth should also be part of the cultivation process. Harvesting takes place after the cultivation period and thereafter bio-oil extraction is undertaken to produce bio-oil for the conversion process. In some situations, microalgae grow best in saline water / seawater or wastewater more generally, and in domestic sewage because of the presence of nutrients such as nitrates and phosphates. However, this is not always possible for all species or strains; some will grow easily in any water or wastewater streams and other not, and it also depends on the species or strain's nature or type. Being photosynthetic organisms, microalgae cells require sufficient light to guarantee an effective growth in order to produce enough biomass. Species can therefore multiply in a very short period of time if all growth conditions are gathered. Sunlight can be used, in this regard, as a cheap option for culture illumination; it is an energy-efficient way to cultivate algae, although there could be daily and seasonal variations in terms of light intensity [11,12] conventional jet fuel on 50/50 ratio as allowed by ASTM certification.

4.1.3 Cultivation, lipid production and lipid content

Cultivation takes place in photobioreactors or open ponds. Various microalgae species store energy in the form of hydrocarbon, which can produce lipids or bio-oil. This is obviously happening when nutrient depletion takes place during the cultivation period. This period is characterized by the species growth, which is regarded as the biomass production time.[13,14] The cells' buoyancy is also regulated by the lipids present in them. However, the lipid content for many species is generally low. By manipulating the microalgae cell genetics and the growth

conditions it is possible to increase bio-oil output. Genetic or physiological modification of microalgae cells will stimulate lipid increase. This is needed for conversion processes. The genetic modification of an algal cell is all about stressing the species under adverse conditions to stimulate the metabolism of the microalgae cell. As a result, stressing microalgae cells under defined conditions will allow the production of more lipids.

4.1.4 the need for effective conversion processes and Overview of conversion processes

Algae-based fuels in general and jet fuel particularly require cost-effective processes or technologies that will: first, allow effective cultivation and harvesting of biomass, second, generate a high output of crude oil and finally, assist in optimising conversion processes or technologies to produce compliant jet fuel.[10,15] Process design and simulation including optimisation of parameters is therefore very important to produce a compliant and cost-competitive jet fuel as mentioned earlier. The design of conversion processes should thus take into consideration operating conditions, energy consumption, the cost of additives and the expectations of the aviation sector and the market.[10]

Many approaches can be explored to convert microalgae oil into liquid fuels. Gasification of algae biomass using Fischer–Tropsch (F–T) synthesis is known to be a successful process.[16–20] However, gasification is energy-intensive because it requires higher temperatures to reach an adequate gasification stage. The F–T synthesis is also characterized by low selectivity for liquid fuels – especially for fractions in the range between C6 to C22; there are lighter fractions, such as methane and ethane, and there are middle and heavy fractions.[21] Transesterification is another approach that can be used to convert microalgae oil into aviation fuel; this approach is known as the biodiesel route, although it is not energy intensive; however, it involves catalytic processes such as decarboxylation, deoxygenation, and isomerization, which can be costly compared to the ones used for the production of conventional jet fuel.[10,22–26] Hydroprocessing oil from microalgae can also generate a jet-fuel type using hydrogen as a catalyst during cracking.[27,28] The hydrogen removes oxygen from algae crude bio-oil during the process. Despite the challenge related to low lipid content for many species, these processes are technically achievable. Another challenge is the costs related to the feasibility or economics of the processes. These costs are higher compared to those related to conventional jet fuel production.

4.1.5 Process design and the aim of the study

When designing an efficient process, it is important to take into consideration the type of species, growth conditions, growth time, the rate of biomass production, and the possibility of producing a large amount of lipids. The current study based the process design on laboratory experiment. Most parameters for design were collected from the experimental laboratory work.

Designing and simulating a process for algae-based aviation fuel should focus on developing a sustainable technology. This technology must be developed over time so that its escalation will need few modifications for the fuel to be ready for use. The maturity of the technology will be a major characteristic that will reflect on its readiness. This comes after successful trials at laboratory and pilot scale. It is followed by implementation on a larger scale.

The aim of this study is to establish a conceptual design and simulate the entire process by involving all the steps from cultivation to production of jet fuel using the Aspen HYSIS V8.8 package, which was the only version available for the study. The design can be used as a tool for a larger scale plant in the future once studies of the costs and economics have been completed. The strain used during experimental work to generate the biomass and the crude oil was *Nannochloropsis sp*., a marine species. The steps and operating conditions used in the laboratory constitute the basis for process design, as mentioned earlier. This approach aims to mimic a process close to that used for conventional jet fuel production. The specific aim in simulating the process is to generate a microalgae-based jet fuel that can comply with stringent aviation regulations. This is to some extent possible because of the similarity between algae crude bio-oil and crude petroleum oil as mentioned before.[10] Cost analysis is not the focus of this study; it can be addressed on its own in another study. It is a very interesting aspect of design but it needs to be undertaken after successful design and simulation. This study focuses on conceptual design and simulation based on data generated from a laboratory experiment. Future studies can be undertaken on a very detailed design involving equipment modelling and sensitivity analysis needed for all variables before undertaking cost studies.

4.2 OVERVIEW ABOUT NANNOCHLOROPSIS OIL

Nannochloropsis sp. contains both eicosapentaenoic (EPA) and docosahexaenoic (DHA) acid oils. Its higher percentage of neutral lipids allows *Nannochloropsis sp*. to be an excellent candidate to produce the alternative jet fuel needed for commercial and military aircraft. Furthermore, the high quantity of EPA (20:5 ω3), reaching up to 5% w/w of the biomass, enables *Nannochloropsis sp*. to be widely cultivated for food supplements and

pharmaceuticals. Generally, if the biomass productivity is higher, there is a probability of generating a high EPA productivity rate [29,30]. *Nannochloropsis sp*. is also recommended as a source of the lipid needed to produce biofuels because more than 60% of lipids can be achieved after nitrogen starvation. These lipids are essentially triacylglycerol (TAG) with saturated and monounsaturated fatty acids [31–33]. The greatest advantage of *Nannochloropsis* sp. for high-quality biofuel production can be attributed to its high concentrations of EPA. The percentage of EPA and other fatty acids present in *Nannochloropsis* cells may influence the species growth conditions, and biomass and lipid productivity. These parameters correlate strongly with the species' EPA content. Some studies have reported that *Nannochloropsis sp*. major fatty acids are C 14:0, C16:0, C20:4ω6 and C 20:5ω5.[34–36] They are short chains of fatty acids with a high percentage of saturation and unsaturation and mono-unsaturation configurations. Based on these facts, algae bio-oil in general and *Nannochloropsis sp*. bio-oil in particular can be a potential source for jet fuel production. Table 1 presents a brief composition of algae crude bio-oil compared to the petroleum crude oil.

Analysing the data in table 10, it is obvious that there is no significant difference between the percentages of Sulfur, Oxygen, Nitrogen and Carbon for both crudes. This confirms that there is a good possibility of generating jet fuel from algae oil as mentioned previously.

Table 10: Comparison between algae crude oil and petroleum crude

W %	Algal crude [37]	Petroleum crude [38,39,40,41]
Sulfur	0.5	1.42
Oxygen	5.5	0.1-1.5
Nitrogen	4.4	0.1-2.0
Carbon	78.7	83-87

4.3 RESULTS AND DISCUSSION

4.3.1 Laboratory process for jet fuel production, methodology, operating conditions and results

The laboratory experiment to obtain jet fuel from microalgae using *Nannochloropsis* sp. was completed in a study prior to the current one by Bwapwa *et al.* (2018).[42] (The study is related to the previous Chapter : Chapter 3). The experiment involves cultivation, harvesting (dewatering), oil extraction by solvent, cracking, and fractionation. Figure 19 summarizes the laboratory process used to obtain jet fuel from microalgae biomass. Jet fuel produced from the laboratory experiment includes cultivation and all the conversion processes mentioned earlier. The use of nutrients and CO_2 is completed during cultivation followed by the physiological modification to increase the lipid content of the species after biomass harvesting.

Conversion processes take place after biomass harvesting in order to produce jet fuel. The characterization of jet fuel aiming to determine the level of compliance regarding the production of jet fuel is also included in the study undertaken by Bwapwa *et al*., 2018.[42] (the study is related to the previous Chapter : Chapter 3). The experiment used to produce jet fuel was reproduced in the current study and the data obtained confirmed that most but not all physico-chemical parameters analysed were in accordance with ASTM standards. This current study is a continuation of the laboratory-based experiment to develop a conceptual design for jet fuel production from microalgae species.

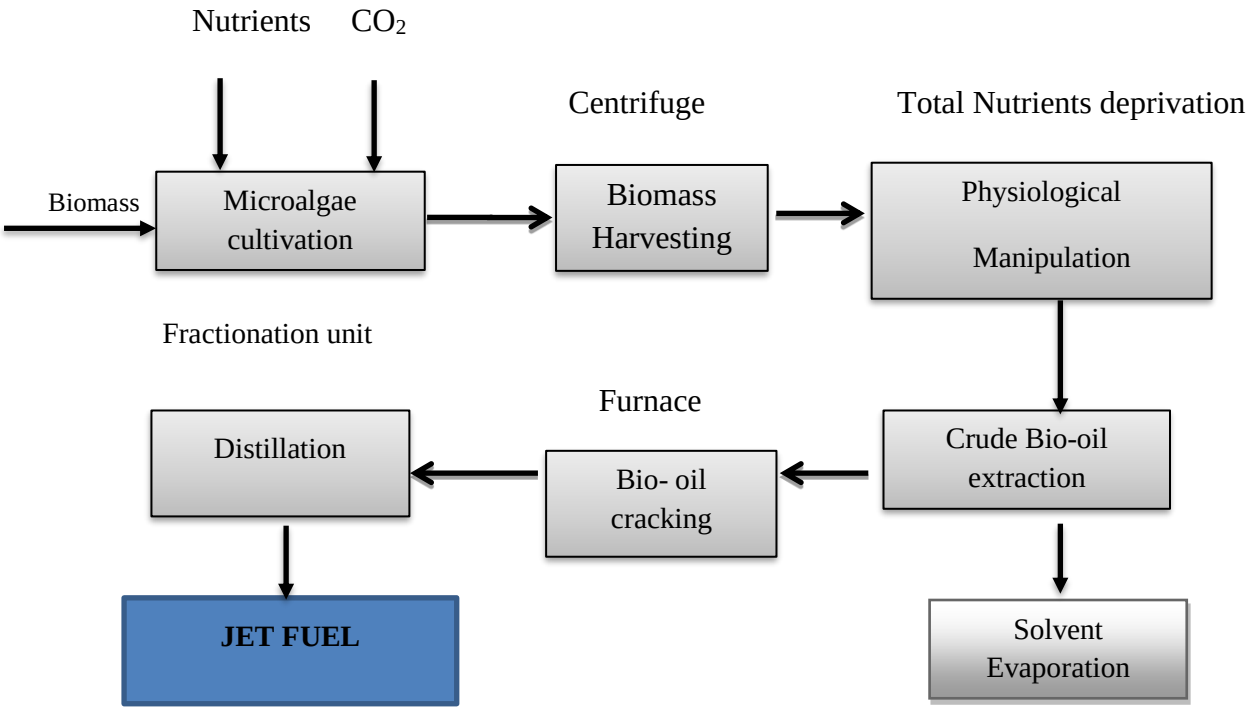

Figure 19: Detailed Approach used to produce jet fuel from nannochloropsis sp in the laboratory (adapted from Bwapwa *et al*., 2018) [42]

Cultivation was undertaken in a 2-l glass bottle photobioreactor. The cultivation environment was made of saline water representing the marine environment in which *Nannochloropsis sp* can grow effectively. Nutrients and CO_2 were supplied to the growing culture to sustain the growth during cultivation as mentioned earlier. CO_2 provides more carbon chains and allows the increase of lipids within the cells. It was added daily to a 2-l photobioreactor for the 15 days cultivation period. Its quantity represented 5 % of the total volume of the photobioreactor.[42] F/2 media was used as a source of nutrients needed for the species growth. This media includes nitrates, phosphates, and trace elements.

The salinity concentration of saline water was 30 ppt representing the average salinity for seawater to facilitate the effective growth of the species. Magnetic stirring was implemented after adding 50 g of dry *Nannochloropsis sp* biomass to the media. The culture was growing at room-controlled temperature of 25 ^{o}C under continuous illumination of 1000 lux for 24 hours. Growth was monitored using a Turner Designs spectrophotometer Model #7200-000, San José, CA (USA) with Optical Kit P/N 10-040R to record the optical density. This was achieved by measuring chlorophyll A non- acid daily as the cells were multiplying very fast. These optical density values were the reflection of culture growth for which the highest value was recorded on day 10. After the tenth day, it was observed that the optical density was decreasing perceptibly. This was the sign that after the 10th day cells were dying; no more cells were produced.

Harvesting was undertaken on the tenth day using a centrifuge for biomass dewatering. The wet biomass in form of heavy slurry was subjected to a physiological modification to increase the lipid content. Physiological modification was achieved by the total deprivation of nutrients, which was undertaken in a separate environment after harvesting biomass. This process aimed to stress microalgae cells by manipulating their physiology in a nutrient-free environment. This influenced the cells metabolism and boosted their lipid content as mentioned earlier. Thereafter, another separation using the centrifuge was undertaken to recover the biomass and use it for bio-oil extraction. From 2 l of culture, 1500 ml of algae on average was collected for several times during cultivation. An average of 900 ml of bio-oil mixed with solvent was recovered and the remaining 600 ml was made of wet biomass. The bio-oil extraction was completed with a mixture of chloroform–methanol in 1/1 volume ratio. This was undertaken in beaker with magnetic stirring for 30 min. Thermal cracking of bio-oil in a close glass container without a catalyst was undertaken in a furnace MSF 12/6, MRC, Ukraine at 300 °C for a duration of about 31 min with the following power characteristics: 220 V / 50 Hz, 2700 W.

After cracking, the bio-oil was fractionated using a distillation unit at a pressure of 1 bar. Mixed solvent made up of chloroform and methanol was recovered from fractionation at between 60 and 65 ^{O}C, chloroform was recovered first and then followed by methanol. The distillate representing the jet fuel from microalgae oil was collected from 70 ^{O}C to an end point of 250 ^{O}C. Table 11 presents the data recorded from the characterisation of the algae-based jet fuel generated from the laboratory experiment. The results were recorded in triplicate, they are presented with the average and standard deviations.

The analysis of this data shows that most parameters are in accordance with the ASTM standards except the freezing point and specific gravity. However, these two parameters can be improved with the use of relevant additives or processes.

Table 11: Characterisation data for Algae-based jet fuel produced from the laboratory process indicated in Figure 19

Parameter	Algal based-jet fuel	ASTM standards for jet fuel (ASTM D1655 standards)
Net Heat of Combustion [MJ/kg]	44±0.5	**42.8 (Minimum)**
Freezing point [ºC]	-32±4	**-40 (Maximum)**
Flash point [ºC]	68± 0.3	**38 (Minimum)**
Final boiling point [ºC]	250±3	**300 (Maximum)**
Kinematics Viscosity @ -20 ºC [cSt]	2.8±0.2	**8 (Maximum)**
Density @ 15 ºC [g/cm^3]	0.96±0.1	**0.775-0.840**
Total acidity mg KOH/g	0.05±0.001	**0.015 (Maximum)**
Total Sulfur %	0.27± 0.02	**0.3 (Maximum)**
Conductivity pS/m	85±0.6	**50-450**

4.3.2. Discussion of the data recorded in table 11 for the algae-based jet fuel

4.3.2.1Net Heat of Combustion

Heating value, also known as Net Heat of Combustion, represents the amount of energy that can be generated by a jet fuel in order to run an aircraft. It is a very important parameter required for all fuels including jet fuel. The data in table 11 has recorded a heating value or Net Heat of Combustion of 44 MJ/kg for algae-derived jet fuel which is above the minimum of 42.8 MJ/kg required by ASTM standard for jet fuel.

4.3.2.2Freezing point

The freezing point is another very important parameter regarding the performance of a jet fuel. It influences jet-fuel fluidity and pumpability at lower temperatures during the flight. At higher altitudes, where temperatures are much lower, the jet fuel will tend to crystallize. It is therefore important to have a jet fuel with the lowest freezing point to avoid the solidification of the fuel at higher altitudes. An ice inhibitor or anti-freezing ingredient can be added to ensure that jet fuel does not solidify. The result, reported in table 11, shows that the freezing point of algae-based jet fuel, which is -32 ^{o}C is higher than the value of -40 ^{o}C required by ASTM. In this case, the use of an ice inhibitor or anti-freezing ingredient is therefore recommended to improve the freezing point of algae- based jet fuel.

4.3.2.3 Flash point

The flash point relates to jet fuel volatility. This can have an impact on combustibility and flammability. It is the main parameter related to fire safety associated with jet-fuel handling at various temperatures. The data reported in table 11 indicate a very high flash point for algae-derived jet fuel. This is an indication that the algae- derived fuel presents lower risks of flammability at lower temperatures and can be handled safely in hot environments.

4.3.2.4 Final Boiling point for distillation

The final boiling point recorded in table 11 is 250 °C. A maximum of 300 °C is prescribed by the ASTM standard for jet fuel. This implies that all hydrocarbon fractions needed for jet fuel can be collected at 250 °C, reducing the energy demands for the fractionation process.

4.3.2.5 Kinematic viscosity

Viscosity affects the fuel's fluidity and also, to a larger extent the fuel's pumpability over an operating temperature range. The ASTM maximum limit for viscosity at -20 ^{o}C is 8 cSt. In this study, algae-based jet fuel recorded a value of 2.8 cSt for viscosity at -20 ^{o}C as indicated in table 11. This parameter complies with the ASTM standard regarding kinematic viscosity.

4.3.2.6 Density

Density relates to volatility and specific energy. The recorded density at 15 °C for the algae-based jet fuel is equal to 0.96 g/cm^3 and it is found beyond the range provided by the ASTM standard for jet fuel, as indicated in table 11. This could be due to the presence of dissolved particulate matter in the algae-based jet fuel. The cleanness of the algae-based jet fuel could be the cause of this situation. It is possible to remedy this situation by using reforming and upgrading processes.

4.3.2.7 Total acidity

The total acidity of algae-based jet fuel was found to be higher than the maximum value required by ASTM standard for jet fuel, as indicated in table 11. This is probably due to the presence of acidic dissolved substances in the algae-based jet fuel. High total acidity will make

the jet fuel very corrosive to metals. To remedy this situation a corrosion inhibitor can be used as an additive.

4.3.2.8 Total Sulfur

Total sulfur was slightly lower than the maximum limit required by ASTM standards for jet fuel as indicated in table 11, so there is compliance in this respect. The origin of Sulfur in the jet fuel is linked to the type of the marine species used to produce the fuel.

4.3.2.9 Conductivity

Conductivity represents the amount of dissolved substances present in the jet fuel. The dissolved substances may come from particulate matter that is not removed from the algae-derived jet fuel. The fuel needs more purification processes, such as rectification or membrane filtration, to keep the level of purity required for an aviation fuel. The data recorded in table 11 show that conductivity complies with the limits required by ASTM standard. However, although it complies with the standard, there is a need to decrease it in order to reduce the acidity and density. An advanced filtration process such as membrane filtration can assist in this regard.

4.3.3 Approaches to design and simulation

A viable design process for algae-based jet fuel must clearly emphasize carbon footprint reduction, costs, and environmentally related issues in order to compete with petroleum-based aviation fuels. In this study, the use of domestic wastewater or seawater for cultivation is suggested. The supply of CO_2 wastes from fossil plants to the growing culture during cultivation period is also suggested. These are inexpensive wastes that can assist the cultivation process and reduce dependence on freshwater resources and manufactured nutrients. This design should be a simple process that can be improved when the quality of the final product needs to be enhanced as mentioned before. This happens when additives are used to improve physico-chemical properties such as freezing point and lubricity of the fuel.

The simulation approach will assist in modeling the data recorded in the future to optimize parameters influencing the effective production of jet fuel from microalgae oil. The simulation work uses an Aspen HYSIS V8.8 package through which physicochemical properties of biomolecules and mixed compounds as well as reaction thermodynamics can be determined. After a successful running of the simulation, a detailed report, including physicochemical and thermodynamic parameters was generated. This report compiled all

aspects related to mass and energy balance, entropy and enthalpy of reactions involved in the combination of many compounds, pressure and temperature at each step or unit process, mole flow, density, reaction kinetics, available equilibria phases, molar concentrations, molecular weight of compounds, and raw materials. To obtain these parameters, there are basic parameters that need to be supplied to the simulation engine.

In this study, the basic parameters were supplied to the simulation engine from the data generated from the laboratory experiment. The report is easily generated by the software package because it includes chemical component libraries, thermodynamic method libraries, unit operation modules, the thermodynamic data manager (TDM), and a programmer. In this case few assumptions were considered. Firstly, not all of the operation units in process design can be found in the Aspen HYSIS V8.8 operation unit libraries. Creative adjustments and simulation process combinations must therefore be used to mimic some of the design processes. Secondly, algal nutrients can be represented by CO_2, HNO_3 and H_3PO_4, which contain the most important elements such as N and P for microalgae cultivation. Thirdly, the feed serving of 1500 kg/h of oil was used as a basis to feed the hydrocracker after cultivation, and a conversion rate of 85% was chosen for the entire process. The design and simulation approaches were also based on the large amount of information collected in studies undertaken by Eloka-Eboka *et al.*[43] and Onunka *et al.*[44]. In both studies the focus was on the process planning and development, criteria / concept selection, and optimization of parameters with required characterizations converted into measurable characteristics for the design of an efficient, operable and sustainable plant using microalgal oil. It was also found that a practical alternative source of energy is conceivable to reduce reliance on fossil-based energy. Some of the elements from these studies were very relevant to the conceptual design of the current study

4.3.3 Summary data for simulation

- **Cultivation temperature**: The simulated temperature was between 20 to 30 ^{o}C for 15 days under photosynthetic conditions, the highest amount of biomass will be collected on the tenth day. Two phases: liquid and solid (wet biomass).
- **Macroalgae harvesting**: Simulated at ambient temperature and atmospheric pressure in sedimentation tank. Coagulants and flocculants can be used in this regard to speed up the process. Two phases: liquid and solid (wet biomass).
- **Physiological modification**: Simulated in highly purified water at ambient temperature for 3 days to help increase the lipid content to 80%. Two phases: liquid and solid.

- **Oil extraction**: Achieved by evaporation, the evaporation temperature should be between 60 to 70 ^{o}C to remove all the solvent. Two phases: solid (wet biomass) and liquid (bio-oil and solvent).
- **Bio-oil storage**: This is done in a collecting tank at ambient temperature and atmospheric pressure. One phase (liquid).
- **Hydrocracking temperature, time and pressure**: The simulated cracking temperature was 300 ^{o}C with a pressure of 1 bar for a duration of 30 minutes. This is almost the same with the data from the GC/MS obtained during the laboratory experiment. One phase (liquid).
- **Distillation or fractionation:** Begins at 70 ^{o}C and ends at 300 ^{o}C under a pressure of 1 bar for 90 % recovery. One phase (liquid).
- **Partial evaporation**: Between 50 and 70 ^{o}C at atmospheric pressure, light vapor or liquid phase will be collected at the outlet of the flash vessel. It will be considered as a waste. Two phases (liquid and vapor)
- **Reforming**: low pressure of 5 bars was chosen to allow partial dehydrogenation, simulated temperature for reforming was 250 ^{o}C to allow the light-end distillate fractions to be amalgamated into high-end distillate fractions. One phase (liquid).
- **Upgrading**: involving hydrocracking, product and by-product separations such as H_2. CO_2 and water. The main variables for this process are hydrocracking temperature, simulated at 350 ^{o}C, low pressure of 1 bar, column operation parameters and the bypass ratio. This ratio is defined as bypassed light-ends hydrocarbons/total hydrotreated crude bio-oil. The simulated amount of hydrogen for the process is 5 to 10 % of the total volume of the reactor. One phase (liquid).

4.4 PROCESS FLOW DESIGN

Figure 20 is the flow chart of the simulated process developed in the current study. Only essential details of the process design and various steps are summarized in table 12 (the 'Process details' section: section 4.5). This process flow chart is developed from the work completed in the laboratory presented in Figure 19. Most simulation results are based on the data generated from the laboratory work presented earlier. The main reason for the use of reported experimental conditions in the laboratory is that these conditions have successfully allowed jet fuel to be produced with the majority of parameters being compliant with ASTM

standards. An attempt to optimize some key parameters was undertaken aiming to enhance the performance and quality of the jet fuel compared with ASTM standards.

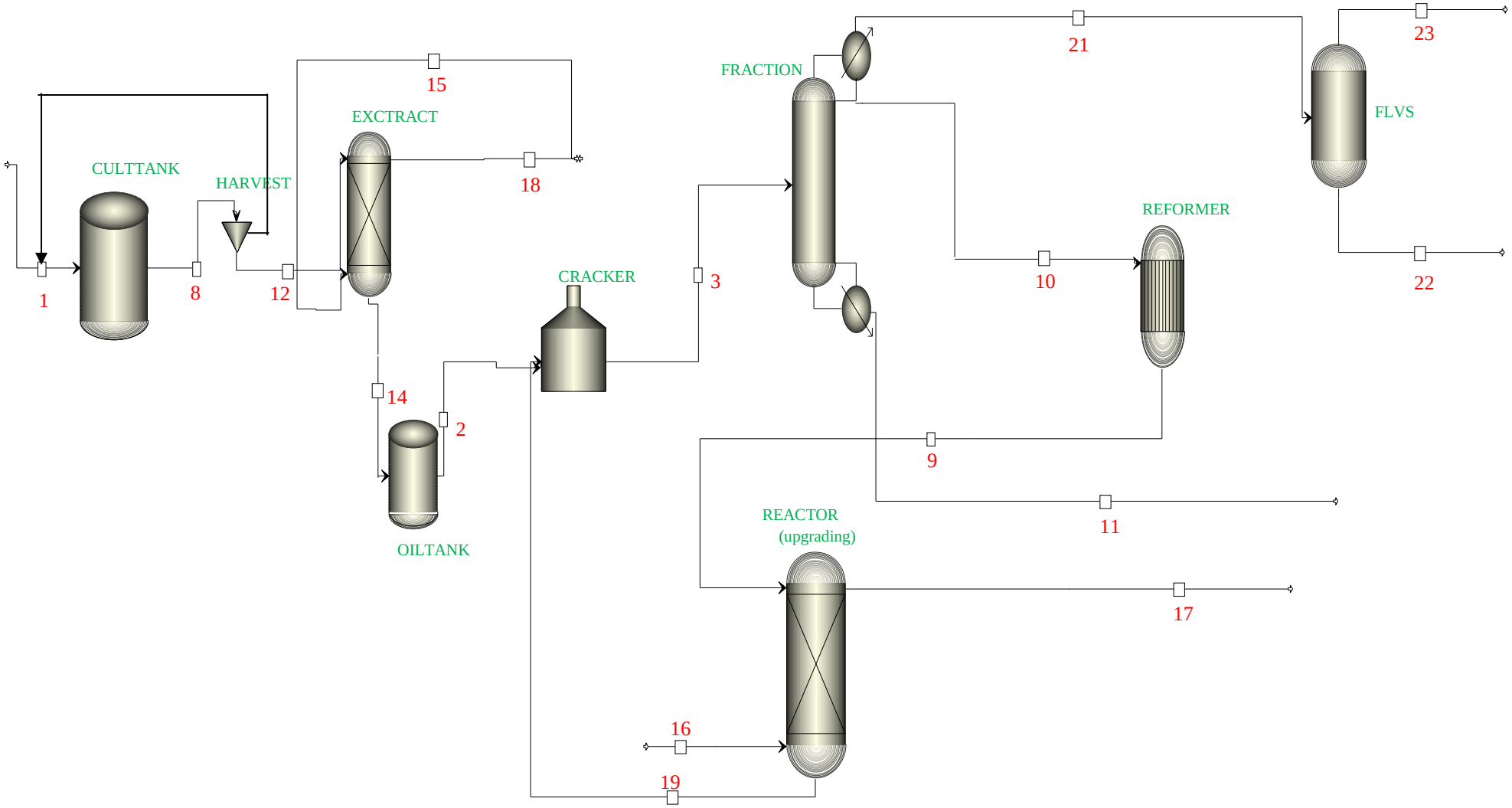

Figure 20: **Conceptual designed process flow diagram for the conversion of microalgae biomass into jet fuel**

4.5 PROCESS DETAILS

The main details regarding the flow chart of the operations are summarized in table 12. The table explains in details how unit processes are structured. These processes are presented in the order in which they take place, from the cultivation of microalgae to the production of jet fuel.

Table 12: details and discussion of the process design

Process (from microalgae cultivation to jet fuel	Unit name /Code On the flow chart	Process aim	Flow of Operations and simulation conditions
Microalgae cultivation	CULTTANK	Cultivation and biomass production	Cultivation of biomass is the first step of the entire process. It is taking place between 20 and 30 ^{o}C. This is the simulated temperature range for a photobioreactor or open pond with culture growing under solar light. The biomass enters the plant via stream 1 in the cultivation tank modelled with one inlet and one outlet. Practically, nutrients such as CO_2, Nitrates and phosphates will be supplied to the growing culture via stream 1. Domestic wastewater or seawater can be used as a growing media. This means that a wastewater treatment plant (also suggested by Roberts et *al*., 2013 [37]; Max et *al*., 2014 [14]) can serve as a cultivation option. Alternatively, a farming algae facility on the sea can be also considered for the same application especially for marine species such as *Nannochloropsis sp.* This can be a prospective option to be studied in detail.

Microalgae Harvesting →		-Collection of biomass needed (Harvesting) for downstream processes	This step can be achieved at ambient temperature and atmospheric pressure. Harvesting will take place in a sedimentation tank modelled with one inlet and two outlets. The biomass generated from the cultivation step should be pumped to the sedimentation tank via stream 8. The use of flocculants or other fast and effective harvesting options can be undertaken to speed up the process. The design is flexible in this regard. Physiological modification can take place in the sedimentation tank after harvesting microalgae. After dewatering the biomass, the volume of the wet biomass can be subjected to the stressing process to manipulate and modify the cells physiology. This is achieved through nutrient starvation as mentioned in the laboratory experiment. The simulation used biomass that is deprived with all nutrients for a period of 3 days as in the laboratory experiment. This will bring a change in cell metabolism, causing an increase in the lipid content. The cells are being stressed by keeping them in harsh conditions. An increase in lipid content of 80% can therefore be expected. This is done in highly purified water, which is nutrient free.
and	HARVEST		
Physiological manipulation →		-To boost lipid content of algae cells (Physiological manipulation)	

Bio-oil extraction	EXTRACT	Extraction of algae bio-oil needed for conversion into jet fuel.	Extraction of bio-oil is completed on wet biomass after cells physiological modification. It uses a mixture of solvent made up of chloroform and methanol in 1/1volume ratio as undertaken during the laboratory experiment. This unit, modelled with one inlet and one outlet, should have a boiling system for solvent evaporation at temperatures depending on the solvent used, in this case the temperature was between 60 and 70 °C. From the harvesting process, the wet biomass is pumped to the extraction unit via stream 12. The solvent is recycled back into the unit via stream 15. Stream 18 is used for the removal of wet biomass sludge. Once the biomass sludge is removed via stream 18, the solvent is taken out via stream 15. A condenser will be needed on stream 15 to liquefy the solvent vapor. Therefore, once the wet biomass and solvent are taken out of the EXTRACT biooil can be collected into the oil tank via stream 14
Bio-Oil storage	OILTANK	Storage of algae bio-oil after extraction. This is done to supply continuously the plant with crude oil needed for conversion processes.	Simulated in such a way that algae bio-oil should be stored in a tank modelled with one outlet and one inlet operating at ambient temperature before it is pumped to conversion processes. The storage time depends on how long the conversion operations can last.
Hydrocracking	CRACKER	Breaking down of larger	

		hydrocarbon chains.	Algae bio-oil is pumped to the cracking unit via stream 2, the simulated cracking temperature has to vary between 200 and 400 ^{o}C. This is consistent with the specifications from the literature for effective cracking and minimizing coke formation [24,45,46]. The upgrading unit supplies hydrogen to the cracking unit via stream 19 to act as a catalyst during cracking. The CO_2 generated from this step can practically be channelled to the cultivation tank to be used as nutrients via stream 1. The cracking unit has one inlet and one outlet.
Distillation (fractionation)	FRACTION	Separation/fractionation of light, middle and heavy fractions of hydrocarbon broken chains	The cracked algae bio-oil is pumped to the fractionation unit via pipe 3, simulated distillation takes place from 70 to 300 ^{o}C at a pressure of 1 bar. Stream 10 will collect the light-end distillates and will send them to the reforming unit. The jet fuel made of middle-end distillates will be collected via stream 11**.** This will happen up to a maximum temperature of 300 ^{o}C considered as end point for middle-end distillates. Heavy-end distillate fractions will be collected at temperatures beyond 300 ^{o}C and sent to the reactor for upgrading. The distillation equipment is modelled using resources found in the simulation package.

Partial evaporation	FLVS	Recovery of light-ends liquid distillates to be sent for reforming.	Light-end distillates and a small fraction of middle-end distillates will get out of the distillation unit in a mixed liquid-vapour phase between 50 and 70 ^{o}C. This mixture is channelled to the flash vessel via stream 21. The pressure within the flash vessel is 1 bar. From this flash vessel a liquid phase will be collected at the bottom via stream 22 and a very light vapour or liquid phase will be released via stream 23. The liquid phase from the flash vessel will also be sent to the reforming unit. In practice this means that stream 22 will be connected to the reforming unit. The flash vessel is modelled with one inlet and two outlets.
Reforming	REFORMER	Partial dehydrogenation and Converting light-ends distillate fractions into high-end distillates fractions which are middle and heavy-end distillates.	From the reforming unit, all liquid phases either from the fractionation unit or flash vessel coming respectively from streams 10 and 22 will be pumped to the reactor or upgrading unit via stream 9, this stream will be made of hydrogen from the dehydrogenation as well as high-end distillate compounds as mentioned earlier. The equipment was modelled according to the resources available in the package with one inlet and one outlet.

Upgrading	REACTOR	Hydrocracking of reformed hydrocarbons. These hydrocarbons are sent to the fractionation unit for separation. This unit is also supplied by steam allowing the production of H_2 that is sent to the cracker for hydrocracking	Upgrading is a catalytic process that will produce another cracked bio-oil to be fractionated again. This process improves the quality of a material by using chemical reactions to remove any compounds present in trace amounts that make the fuel quality undesired. The bio-oil will be sent to the fractionation unit via stream 17. This means this stream will practically be connected to the fractionation unit. The hydrogen from the reformer might be insufficient because depending on the volume of distillates generated by the reforming unit. The upgrading unit will therefore be supplied with more hydrogen via stream 16 to act as catalyst in order to speed up the upgrading process. This hydrogen is needed for the effective reduction of oxygen content from algae oil. Excess of oxygen in the fuel can have a good influence on the fuel combustion but it causes nitrogen oxide (NOx) emissions due to increased combustion temperature and extra oxygen[47] The remaining hydrogen in the unit will be channelled to the cracking unit as catalyst via stream 19 as mentioned earlier after upgrading process is completely done. Two inlets and two outlets were used for this type of equipment.

4.6 OPTIMIZATION ATTEMPT FOR SOME PARAMETERS

Optimization targeted conversion and separation processes such as cracking, distillation, reforming, and upgrading because they have a great impact on the final product quality and performance. The optimizer featured in Aspen HYSIS V8.8 was implemented to maximize the production of the bio-jet fuel, while varying the bypass ratio from 5% to 95%, varying cracking, reforming and upgrading temperatures between 200 and 400 °C, which is consistent with literature specifications for realistic reaction rates and cracking efficiency, while minimizing coke formation. The optimizer predicts an optimal product when using a bypass ratio of 7%, a cracker temperature of 300 °C, which is the same as the one obtained from GC/MS during the laboratory experiment, and bottom to overhead ratio of 20:1.
The temperature range varies between 60 and 70 °C to remove methanol and chloroform. As bio-oil recovery is achieved by solvent extraction and evaporation it was necessary to optimize the solvent extraction temperature at 65 °C to assist in the removal of methanol and chloroform. The maximum distillation temperature was optimized at 300 °C to be able to recover as much as middle carbon fractions needed in the jet fuel.

The data from the laboratory complied with ASTM standards, except for density and freezing point, which can be corrected through upgrading, reforming, and the use of additives. Properties of the jet fuel obtained under the optimized operation conditions are therefore expected to comply with ASTM specifications. This supports the hypothesis that it is technically feasible to begin with microalgae cultivation, produce bio- oil followed by jet fuel production, and finally reform and upgrade it.

4.7 OVERALL MATERIAL BALANCE

The overall mass balance is summarized in table 13. This is generated by Aspen HYSIS V8.8 after running the simulation. It involves the inputs and the outputs from the beginning of the process to the end. It also indicates the phases and flow throughout the entire process. Analysis of table 13 indicates that all parameters have converged. This is an indication that the simulation ran successfully. The convergence is revealed by the presence of zero values recorded at the bottom of the table for each stream.

Table 13: Generated mass balance from the simulation

Aviation Fuel from Algae Oil												
Stream ID		1	2	3	8	9	12	14	15	16	17	19
From			B2	B4	B1	B7	B10	B16			B9	B9
To		B1	B4	B6	B10	B9	B16	B2	B16	B9		B4
Phase		LIQUID	LIQUID	LIQUID	LIQUID	LIQUID	LIQUID	LIQUID	LIQUID	LIQUID	LIQUID	LIQUID
Substream: MIXED												
Mole Flow	kmol/sec											
H2O		.0137057	.0125157	.0255376	.0137057	3.93719E-3	.0137057	.0125157	0.0	.0138771	4.79281E-3	.0130218
CO2		7.01303E-4	6.40409E-4	1.23749E-3	7.01303E-4	1.61168E-3	7.01303E-4	6.40409E-4	0.0	6.31173E-4	1.64579E-3	5.97078E-4
CHLOROFO		0.0	2.32659E-4	2.32659E-4	0.0	0.0	0.0	2.32659E-4	2.32690E-4	0.0	0.0	0.0
METHANOL		0.0	8.66802E-4	8.66802E-4	0.0	0.0	0.0	8.66802E-4	8.66913E-4	0.0	0.0	0.0
Mass Flow	kg/sec											
H2O		.2469136	.2254742	.4600671	.2469136	.0709296	.2469136	.2254742	0.0	.2500000	.0863437	.2345930
CO2		.0308642	.0281842	.0544615	.0308642	.0709296	.0308642	.0281842	0.0	.0277777	.0724310	.0262772
CHLOROFO		0.0	.0277742	.0277742	0.0	0.0	0.0	.0277742	.0277777	0.0	0.0	0.0
METHANOL		0.0	.0277742	.0277742	0.0	0.0	0.0	.0277742	.0277777	0.0	0.0	0.0
Total Flow	kmol/sec	.0144070	.0142555	.0278745	.0144070	5.54887E-3	.0144070	.0142555	1.09960E-3	.0145082	6.43860E-3	.0136189
Total Flow	kg/sec	.2777778	.3092068	.5700771	.2777778	.1418593	.2777778	.3092068	.0555555	.2777778	.1587748	.2608703
Total Flow	cum/sec	2.73365E-4	3.02735E-4	5.59951E-4	2.73365E-4	1.74572E-4	2.73365E-4	3.02735E-4	5.25589E-5	2.75387E-4	1.82387E-4	2.58620E-4
Temperature	K	298.1500	298.1500	298.1500	298.1500	423.1500	298.1500	298.1500	298.1500	303.1500	405.9879	303.2596
Pressure	N/sqm	1.01325E+5	1.01325E+5	1.01325E+5	1.01325E+5	1.01325E+5	1.01325E+5	1.01325E+5	1.01325E+5	1.01325E+5	1.01325E+5	1.01325E+5
Vapor Frac		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Liquid Frac		1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000
Solid Frac		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Enthalpy	J/kmol	-2.9118E+8	-2.8542E+8	-2.8797E+8	-2.9118E+8	-3.0852E+8	-2.9118E+8	-2.8542E+8	-2.1649E+8	-2.9014E+8	-3.0593E+8	-2.9017E+8
Enthalpy	J/kg	-1.5102E+7	-1.3159E+7	-1.4080E+7	-1.5102E+7	-1.2068E+7	-1.5102E+7	-1.3159E+7	-4.2850E+6	-1.5154E+7	-1.2406E+7	-1.5149E+7
Enthalpy	Watt	-4.1951E+6	-4.0688E+6	-8.0269E+6	-4.1951E+6	-1.7120E+6	-4.1951E+6	-4.0688E+6	-2.3806E+5	-4.2095E+6	-1.9698E+6	-3.9518E+6
Entropy	J/kmol-K	-1.5552E+5	-1.5891E+5	-1.5735E+5	-1.5552E+5	-1.0127E+5	-1.5552E+5	-1.5891E+5	-2.2871E+5	-1.5478E+5	-1.0775E+5	-1.5469E+5
Entropy	J/kg-K	-8066.178	-7326.200	-7693.954	-8066.178	-3961.154	-8066.178	-7326.200	-4526.852	-8084.095	-4369.474	-8075.766
Density	kmol/cum	52.70279	47.08939	49.78032	52.70279	31.78558	52.70279	47.08939	20.92136	52.68323	35.30184	52.66025
Density	kg/cum	1016.143	1021.379	1018.083	1016.143	812.6120	1016.143	1021.379	1057.016	1008.681	870.5375	1008.703
Average MW		19.28063	21.69022	20.45152	19.28063	25.56543	19.28063	21.69022	50.52329	19.14615	24.65984	19.15492
Liq Vol 60F	cum/sec	2.84950E-4	3.13900E-4	5.80923E-4	2.84950E-4	1.57384E-4	2.84950E-4	3.13900E-4	5.36992E-5	2.84286E-4	1.74655E-4	2.67023E-4
Substream: $TOTAL												
Total Flow	kg/sec	.2777778	.3092068	.5700771	.2777778	.1418593	.2777778	.3092068	.0555555	.2777778	.1587748	.2608703
Enthalpy	Watt	-4.1951E+6	-4.0688E+6	-8.0269E+6	-4.1951E+6	-1.7120E+6	-4.1951E+6	-4.0688E+6	-2.3806E+5	-4.2095E+6	-1.9698E+6	-3.9518E+6
Substream: CISOLID												
Mole Flow	kmol/sec											
H2O		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CO2		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CHLOROFO		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
METHANOL		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Mass Flow	kg/sec											
H2O		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CO2		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CHLOROFO		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
METHANOL		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Total Flow	kmol/sec	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Total Flow	kg/sec	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Total Flow	cum/sec	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Temperature												
Pressure	N/sqm	1.01325E+5	1.01325E+5	1.01325E+5	1.01325E+5	1.01325E+5	1.01325E+5		1.01325E+5	1.01325E+5		
Vapor Frac												
Liquid Frac												
Solid Frac												
Enthalpy												
Enthalpy												
Enthalpy												
Entropy												
Entropy												
Density												
Density												
Average MW												
Liq Vol 60F												

CONCLUSIONS

This study has focused on the following aspects:

1. Developing the process to produce microalgae-based jet fuel from the laboratory scale.

2.The adaptability of ASPEN HYSIS V8.8 to simulate the unit processes based on the data gathered from the laboratory steps.

The following are the outcomes:

- The unit processes are described as follows: Biomass cultivation, biomass harvesting, physiological modification, bio-oil extraction, hydrocracking, fractionation, reforming, and upgrading. The average cracking temperature in the laboratory experiment is 350 °C; the working pressure for distillation is 1 bar. The same pressure is recorded for the flash vessel collecting some middle-end fractions mixed with the vapor phase. The solvent used is a mixture of chloroform and methanol in a 1/1 ratio. The overall conversion rate is 85%. The data from the laboratory was used to simulate the process including some modifications to run the simulation successfully. The optimization of temperature values for various processes was also completed to enable the simulation and process design.
- Domestic wastewater plants or farming facilities using seawater for marine species can be used to provide a cultivation medium to take advantage of nutrients present in both domestic wastewater and seawater. This will also reduce the use of freshwater resources. Carbon dioxide from fossil sources can also be collected and used as a nutrient for microalgae during cultivation.
- Physiological modification is a very important aspect for algae-based fuels and needs to be incorporated in the process to increase the species' lipid content. The growth medium can be either open ponds or a photobioreactor, depending on the available possibilities and the environmental conditions.

Future studies should work on the detailed design and optimization of a sustainable process plant to be used at the commercial level, including details of modelled equipment. They should also focus on sensitivity analysis, costs, and economic aspects related to the conversion processes, life-cycle assessment and fuel sustainability, blending with other fuels, detailed thermodynamics studies, and modeling of parameters involving effectiveness of some unit processes and costs.

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REFERENCES

[1] Dincer, I., Renewable energy and sustainable development: a crucial review. *Renewable and Sustainable Energy Reviews*, **4**(2):157-175(2000). https://doi.org/10.1016/S1364-0321(99)00011-8

[2] Schmer M.R., Vogel K.P., Mitchell R.B., Perrin R.K., Net energy of cellulosic ethanol from switch grass. *Proceedings of the National Academy of Sciences* ; **105**(2):464-9, (2008). https://doi.org/10.1073/pnas.0704767105

[3] Iodice P, Senatore A, Langella G and Amoresano A, Advantages of ethanol–gasoline blends as fuel substitute for last generation Si engines. *Environ Prog Sustainable Energy* **36**:1173–1179 (2017). https://doi.org/10.1002/ep.12545

[4] Besanko D, Besanko D, Ulan M and Ulan M, Should the ethanol blender's credit be eliminated? in *Kellogg School of Management Cases*, Kellogg School of Management, Northwestern University 2011, USA, pp. 1–36 (2017).

[5] Khatiwada D., Silveira S., Scenarios for bioethanol production in Indonesia: How can we meet mandatory blending targets?. *Energy*;**119**:351-61, (2017) https://doi.org/10.1016/j.energy.2016.12.073

[6] Chisti Y., Biodiesel from microalgae beats bioethanol. *Trends in biotechnology*. **26** (3):126-31, (2008). https://doi.org/10.1016/j.tibtech.2007.12.002

[7] Riayatsyah T.M., Ong H.C., Chong W.T., Aditya L., Hermansyah H., Mahlia TM., Life Cycle Cost and Sensitivity Analysis of Reutealis trisperma as Non-Edible Feedstock for Future Biodiesel Production. *Energies*.; **10**(7):877, June 29 (2017), https://doi.org/10.3390/en10070877

[8] NREL (National Renewable Energy Laboratory), *A Look Back at the US Department of Energy's Aquatic Species Program: Biodiesel from Algae Close-Out Report*[Online]. http://www. eere.energy.gov/biomass/pdfs/biodiesel_from_algae.pdfNREL/FS-840-40352 Report edited in July 2006 [July 1st , 2017].

[9] Lian X., Xue Y., Xu G., Zhao Z., Sheng H., Lin H., Effect of methyl acetoacetate as a potential cold flow improver for biodiesel. Energy Sources, Part A: *Recovery, Utilization, and Environmental Effects*.; **39**(1):97-102, (2017) https://doi.org/10.1080/15567036.2016.1200164

[10] Bwapwa JK, Anandraj A and Trois C, Possibilities for conversion of microalgae oil into aviation fuel: A review, *Renewable Sustainable Energy Rev* **80**:1345–1354 (2017). https://dx.doi.org/10.1016/j.rser.2017.05.224

[11] Chisti Y., Biodiesel from microalgae. *Biotechnology advances*.; **25**(3):294-306, (2007) https://doi.org/10.1016/j.biotechadv.2007.02.001

[12] Daelman M.R., Sorokin D., Kruse O., van Loosdrecht M.C., Strous M., Haloalkaline bioconversions for methane production from microalgae grown on sunlight. *Trends in biotechnology*; **34**(6):450-7, (2016). https://doi.org/10.1016/j.tibtech.2016.02.008

[13] Slade R., Bauen A., Micro-algae cultivation for biofuels: cost, energy balance, environmental impacts and future prospects. *Biomass and bioenergy*.; **53**:29-38 , (2013). https://doi.org/10.1016/j.biombioe.2012.12.019

[14] Ma X., Zhou W., Fu Z., Cheng Y., Min M., Liu Y., Zhang Y., Chen P., Ruan R., Effect of wastewater-borne bacteria on algal growth and nutrients removal in wastewater-based algae cultivation system. *Bioresource technology*; **167**:8-13, (2014). https://doi.org/10.1016/j.biortech.2014.05.087

[15] Wang H.Y., Bluck D., Van Wie B.J., Conversion of microalgae to jet fuel: Process design and simulation. *Bioresource technology*; **167**:349-57, (2014).https://doi.org/10.1016/j.biortech.2014.05.092

[16] Fiori L., Valbusa M., Castello D., Supercritical water gasification of biomass for H2 production: process design. *Bioresource technology*; **121**: 139-47, (2012). https://doi.org/10.1016/j.biortech.2012.06.116

[17] Guan Q., Savage P.E., Wei C., Gasification of alga Nannochloropsis sp. in supercritical water. *The Journal of Supercritical Fluids*; **61**: 139-45, (2012). https://doi.org/10.1016/j.supflu.2011.09.007

[18] Miller A., Hendry D., Wilkinson N., Venkitasamy C., Jacoby W. Exploration of the gasification of Spirulina algae in supercritical water. *Bioresource technology*; **119**: 41-7, (2012). https://doi.org/10.1016/j.biortech.2012.05.005

[19] Bond J.Q., Upadhye AA., Olcay H., Tompsett G.A., Jae J., Xing R., Alonso D.M., Wang D., Zhang T., Kumar R., Foster A., Production of renewable jet fuel range alkanes and commodity chemicals from integrated catalytic processing of biomass. *Energy & Environmental Science*;**7**(4):1500-23, (2014). https://doi.org/10.1039/C3EE43846E

[20] Ail S.S., Dasappa S., Biomass to liquid transportation fuel via Fischer Tropsch synthesis–Technology review and current scenario. *Renewable and Sustainable Energy Reviews*;**58**: 267-86, (2016).https://doi.org/10.1016/j.rser.2015.12.143

[21] Zhang Q., Cheng K., Kang J., Deng W., Wang Y., Fischer–Tropsch catalysts for the production of hydrocarbon fuels with high selectivity. *Chem Sus Chem*; **7**(5):1251-64,(2014). https://doi.org/10.1002/cssc.201300797

[22] Gunawardena DA and Fernando SD, Methods and applications of deoxygenation for the conversion of biomass to petrochemical products in *Biomass Now-Cultivation and Utilization*, ed. by Miodrag DM. IntechOpen, Book chapter, London, United Kingdom. [Online]. Available : https://www.intechopen. com/books/biomass-now-cultivation-and-utilization/methods-and-applications-of-deoxygenation-for-the-conversion-of-biomass-to-petrochemical-products [July,15 th 2017].https://doi.org/10.5772/53983

[23] Biller P., Sharma B.K., Kunwar B., Ross A.B., Hydroprocessing of bio-crude from continuous hydrothermal liquefaction of microalgae. *Fuel*.;**159**:197-205, (2015). https://doi.org/10.1016/j.fuel.2015.06.077

[24] Wang, H.L., Yan, S.L., Salley, S.O., Ng, K.Y.S., Hydrocarbon fuels production from hydrocracking of soybean oil using transition metal carbides and nitrides supported on ZSM-5. *Ind. Eng. Chem. Res*., **51** :10066–10073, (2012). https://doi.org/10.1021/ie3000776

[25] Narula V., Thakur A., Uniyal A., Kalra S., Jain S., Process parameter optimization of low temperature transesterification of algae-Jatropha Curcas oil blend. *Energy*, 2017;**119**: 983-8, (2017).https://doi.org/10.1016/j.energy.2016.11.043

[26] Pattanaik B.P., Misra R.D., Effect of reaction pathway and operating parameters on the deoxygenation of vegetable oils to produce diesel range hydrocarbon fuels: A review. *Renewable and Sustainable Energy Review*; **73**: 545-57. (2017). https://doi.org/10.1016/j.rser.2017.01.018

[27] Yang C., Li R., Cui C., Liu S., Qiu Q., Ding Y., Wu Y., Zhang B., Catalytic hydroprocessing of microalgae-derived biofuels: a review. *Green Chemistry*. **13**:3684-99, (2016).https://doi.org/10.1039/C6GC01239F

[28] Zhou L., Lawal A., Kinetic study of hydrodeoxygenation of palmitic acid as a model compound for microalgae oil over Pt/γ-Al 2 O 3. *Applied Catalysis* A: General. **25**; 532:40-49, (2017).https://doi.org/10.1016/j.apcata.2016.12.014

[29] Leu S., Boussiba S., Advances in the production of high-value products by microalgae. *Industrial Biotechnology*;**10**(3):169-83, (2014).https://doi.org/10.1089/ind.2013.0039

[30] San Pedro A, González-López CV, Acién FG and Molina-Grima E, Outdoor pilot-scale production of *Nannochloropsis gaditana*: influence of culture parameters and lipid production rates in tubular photobioreactors. *Bioresour Technol* **169**:667–676 (2014). https://doi.org/10.1016/j.biortech.2014.07.052

[31] Lakaniemi AM, Tuovinen OH and Puhakka JA, Anaerobic conversion of microalgal biomass to sustainable energy carriers–a review. *Bioresour Technol* **135**:222–231 (2013). https://doi.org/10.1016/j.biortech.2012.08.096

[32] Ramachandran S, Ganesh PS, Sivasubramanian V and Kumar BP, Current developments in mass production of microalgae for industrial applications, in *Environmental Sustainability Using Green Technologies*, ed. by Sivasubramanian V. Taylor & Francis group, Boca Raton, FLorida, USA, p. 147 (2016).

[33] Shukla M, Tabassum R, Singh R and Dhar DW, Influence of light intensity, temperature and CO2 concentration on growth and lipids in green algae and cyanobacteria. *India J Exp Biol* **54**(7):482–487 (2016).

[34] Sukenik, A., Carmeli, Y., Berner, T., Regulation of fatty acid composition by irradiance level in the eustigmatophyte Nannochloropsis sp. *J. Phycol*., **25 :**686–692, (1989). https://doi.org/10.1111/j.0022-3646.1989.00686.x

[35] Scheneider JC and Roessler P, Radiolabelling studies of lipids and fatty acids in *Nannochloropsis* (Eustigmatophyceae), an oleaginous marine alga. *J Phycol* **30**:594–598 , (1994).https://doi.org/10.1111/j.0022-3646.1994.00594.x

[36] Hulatt C.J., Wijffels R.H., Bolla S., Kiron V., Production of Fatty Acids and Protein by Nannochloropsis in Flat-Plate Photobioreactors. *PloS one*, **12**(1):e0170440 , (2017).https://doi.org/10.1371/journal.pone.0170440

[37] Roberts G.W., Fortier M-OP., Sturm B.S.M., Stagg-Williams SM., Promising pathway for algal biofuels through wastewater cultivation and hydrothermal conversion. *Energy Fuels*; **27**: 857–67, (2013).https://doi.org/10.1021/ef3020603

[38] Jena U., Das K.C., Kastner JR., Effect of operating conditions of thermochemical liquefaction on biocrude production from Spirulina platensis. *Bioresource Technology*;102: 6221–9, (2011). https://doi.org/10.1016/j.biortech.2011.02.057

[39] Brown T.M., Duan P., Savage P.E., Hydrothermal liquefaction and gasification of Nannochloropsis sp. *Energy Fuels*;**24**:3639–46, (2010).https://doi.org/10.1021/ef100203u

[40] Skone TJ and Gerdes K, *Development of Baseline Data and Analysis of Life Cycle Greenhouse Gas Emissions of Petroleum-Based Fuels*. National Energy Technology Laboratory, Department of Energy, Washington, DC (2008).

[41] Matar S and Hatch LF, *Chemistry of Petrochemical Processes*. Gulf Professional Publishing, Woburn, MA (2001).

[42] Bwapwa, JK., Akash A., Trois C., Microalgae processing for jet fuel production, *Biofuels, Bioproducts and Biorefining* , (2018) , in press ,

[43] Eloka-Eboka A, Onunka C and Inambao F Detailed design and optimization of a sustainable micro-algal biofuel process plant. *Int J Low-Carbon Technol* 1–9 (2018). https://doi:10.1093/ijlct/cty004

[44] Onunka C, Juddhoo R and Eloka-Eboka AC Conceptual design and optimisation of a sustainable micro-algal biofuel process plant. Proceedings of the 15th Sustainable Energy Technologies (SET 2016) Conference at the National University of Singapore, Queenstown, July 19–22 (2016).

[45] Al-Sabawi M and Chen JW., Hydroprocessing of biomass-derived oils and their blends with petroleum feedstocks: A review. *Energy Fuels* **26**:5373–5399 (2012).

[46] Robota HJ and Alger JC., Preparation of a research jet fuel composition comprised of nearly exclusively methyl-branched tetradecane isomers having a freezing point below −47 °C. *Ind Eng Chem Res* **51**:10313–10319 (2012).

[47] Baskar P and Senthilkumar A., Effects of oxygen enriched combustion on pollution and performance characteristics of a diesel engine. *Eng Sci Technol Int J* **19**(1):438–443 (2016).

CHAPTER 5 PREFACE

The following chapter is a compiled form of a research paper, it is currently under review in the *Energy* Journal (Elsevier). After producing algae-based jet fuel in the laboratory and developing a conceptual design in the previous chapters, it was important to undertake a study on B50 blending. This ASTM certified blending option was undertaken to examine the performance of the blended jet fuel made of algae-based jet fuel and Jet A1. Due to the costs and sustainability issues faced by alternative jet fuels including algae-based jet fuel, blending conventional jet fuel A1 and algae-based jet-fuel on a 50/50 volume ratio may be seen as a viable option. This chapter is presented with some modifications and very few additions to keep the flow and the consistency of the work completed in this dissertation. It aims to analyse the efficiency and sustainability of blending algae-based jet with Jet A1 in a 50/50 volume ratio using ASTM standards.

CHAPTER 5: JET FUEL BLEND FROM ALGAL JET FUEL AND JET A1 IN 50/50 VOLUME RATIO

[1,2]Joseph K. Bwapwa, [2] Anandraj Akash, [1]Cristina Trois

[1] University of KwaZulu-Natal, [2] Mangosuthu University of Technology

Corresponding author's e-mail: josephkapuku@gmail.com ; joseph@mut.ac.za

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ABSTRACT

Alternative sources of energy are greatly needed to ensure the availability of fuels in the long run. Microalgae-derived fuels are amongst the viable options due to their potential to produce sustainable fuels. However, the costs related to the production of microalgal and alternative fuels are still higher compared to conventional fuels. To deal with costs and environmental issues, the blending of microalgal fuels with conventional fuels can be considered as one of the feasible options. Blending has a positive impact on many fuel characteristics including reduction of carbon footprint, costs, freezing point, viscosity, flow, heating and combustion. In this study, jet fuel from *Nannochloropsis sp* crude bio-oil was blended with Jet A1 in 50/50 volume ratio. The data generated from the 50/50 blend jet fuel was analysed according to the ASTM standards. Samples were tested after 30, 60, 90 and 120 days from the production day. It was reported that majority of parameters including Net heat of combustion, flash point, kinematic viscosity, conductivity and freezing point were compliant with ASTM standards. However, parameters such as density and total acidity were found not complying with ASTM standards. This study can have an implication on carbon footprint reduction because of the blending of fossil-based jet fuel and renewable-based jet fuel known as low carbon footprint fuel. The blending ratio can be beneficial for the green energy world in terms of costs and environmental impacts.

KEYWORDS: microalgae; blended Jet fuel; green Jet fuel; bio-energy; biofuels; *Nannochloropsis sp*

5.1 INTRODUCTION

5.1.1 Background

The world petroleum resources are being depleted and they might reach a critical point of scarcity in the near future due to the fact that they are non-renewable. This can cause a serious energy crisis with consequences on many economies. These consequences will particularly hit the transportation sector more especially the aviation industry. In today's highly competitive energy environment, a flexible approach to deal with issues of reliable sources of energy can help in finding alternative and sustainable fuels in order to compete with fossil-based fuels and to slow down their rapid depletion [1-4] .

It is an undeniable fact that fossil fuels although they are very reliable in terms of their performance and compliance, they are however not renewable and environmentally friendly. Furthermore, they are characterised by unsteady price fluctuations [5,6]. Similarly, there is an imperious necessity to identify alternative resources that can be useful for energy production that will benefit the energy sector more particularly the aviation industry [7,8,9]. Currently, the main challenge remains to develop alternative fuels from natural and renewable resources that can be real competitors as well as reliable and better substitutes for fossil fuels. This situation should be addressed within a reasonable timeframe in order to face an eventual scarcity of petroleum oil. Therefore, two major options can be considered in this regard: Firstly, the production of biofuels that are renewable, sustainable and compliant to standards. They have to be cost-effective, not competing with food, with less water use and low carbon footprint. Secondly, blending of biofuels with fossil fuels can be an option to be considered in a sense that blending will balance and combine the weaknesses and strengths of components involved in blending in order to comply with standards and satisfy the market expectations. [10,11]

Blending fossil fuels and biofuels is one of the options undertaken by fuel producers for costs reduction, environmental compliance including quality assurance and performance related aspects [11][31][32][33]. Also, Fossil fuel costs are generally lower than the ones for biofuels. Biofuels have the advantage of being environmentally friendly but costly while fossil fuels are affordable but not environmentally friendly [12].

One of the major economic issues for the fuel producer is to use relevant conversion processes which are costs effective to produce high-quality fuel that can satisfy the need of the fuel user or market. Therefore, the final product has to comply with the criteria defined by the standards [13,14]. In this regard, blending can provide an advantage over other options by generating a jet fuel with low carbon footprint which should also be compliant with standards [15,16]. However, the costs of the blend will depend on the overall production costs for single fuels involved in the blend and the blending costs.

Fuel blending could be one of the options to be considered in the near future as a sustainable possibility for green energy production and carbon footprint reduction[17]. The greatest expectation is such that the blend should be sustainable, compliant and has to be certified by ASTM or any other certification institution. This implies that blended fuel will be subjected to stringent regulations and standards in order for it to be on the market and compete with the existing fuels.

Microalgae-based fuels are currently positioning themselves on the market to compete with fossil fuels for power generation and energy production. Many companies such as Algenol, Algae Fuel System, Solazyme, Sapphire Energy, Euglena, Alpha Biotech, AlgaFuel S.A, AlgalOilDiesel, Algae.Tec and many others are successfully involved in the production of algae-based fuels. However, the main challenge for microalgae-based fuels remains the low productivity of lipids and high production costs owing to conversion processes[18]. Consequently, blending microalgae-based fuels with fossil fuels can be undertaken and analysed to find out if it can be an acceptable option despite the existing certification from the standardisation institutions. This is more related to the performance of the fuel compared the existing ones. The current study is focusing on compliance from blending algae-based jet fuel and Jet A1.

Currently, regarding jet fuel blending options, there is only 50/50 blend that is certified by ASTM[19]. Jet fuels from microalgae have already successfully been tested in both commercial and military aircraft. ASTM has approved the use of biomass-based jet fuel since July 2011 for commercial flights. The ASTM certification is valid only for blending with conventional Jet fuel in a 50/50 volume ratio (B50). The certification is valid for all fuels from biomass which are cleared for take-off. However, the blended jet fuel has not been used consistently by the aviation sector despite the B50 certification. The issue related species oil content, sustainability and production costs for algae-based jet fuel is still a challenge. Certification

does not necessarily mean that the blend will be operational with high performance. The blended is expected to have a high level of purity. It should not solidify at lower temperatures and must generate a high amount of energy required for propulsion of the aircraft.

The major challenge with algae-based jet fuel is to get species with the ability to generate a high amount of bio-oil with hydrocarbons that are molecularly very identical to petroleum oil. This will allow the production of jet fuel from the same downstream processes used to produce conventional jet fuel. *Nannochloropsis sp* is one of the types of species with the potential to produce algae Jet fuel because of its composition and the cultivation possibilities in the marine environment.

The objective of this study is to prepare and produce a new blend by mixing algae-based Jet fuel with Jet A1 in 50/50 volume ratio. The new Jet fuel blend will be tested according to ASTM standards for aviation fuels to identify parameters which are compliant and non-compliant to ASTM standards chosen as a reference in this study. For parameters which are non- compliant, ways to bring them to comply with ASTM standards will be suggested. However, Costs implications or feasibility aspects of the blending, mathematical modelling aspects, are not part of this work. The study will assist in examining the compliance, analysing the behaviour of physico-chemical parameters and the quality of the blend which are probably affected over time. This will assist in considering whether or not blending is a sustainable option for aviation fuels. The contribution will be reflected by the data indicating whether the compliance is possible or not from the 50/50 blended jet fuel. Therefore, blending jet fuels in 50/50 ratio is technically and economically beneficial, it can be used as a sustainable option to reduce dependence on petroleum-derived fuels. Furthermore, the 50/50 blended jet fuel is expected to have a low carbon footprint and low oxygen content including flow properties complying with required standards.

5.2 CHALLENGES ASSOCIATED WITH JET FUEL BLENDING

Blending jet fuels can be much more complicated than a simple mixing of components [20]. The blend is a complex mixture of different carbon chains from C8 to C16[21,22,23]. This situation can impact on compliance and overall costs. Jet fuels or any other fuel can be blended using the in-line blending method through a manifold system, batch blending in tanks and onboard blending into marine vessels. [20,24] Each method has its strengths and weaknesses.

In-line blending of jet fuels is achieved by injecting proportional amounts of each component into the mainstream where turbulence promotes thorough homogenisation [24,25]. Mixing depends on the speed of agitation and residence time. In many cases, to achieve the specifications required for compliance, additives are used during and/or after blending to improve specific properties related to flow, heating energy and anti-freezing capacity. Blending jet fuels is very a complex process because the blend is expected to be renewable with low carbon footprint compared to the fossil-based fuel.

5.3 JET FUEL BLENDING AND ASTM CERTIFICATION

Blending can assist in using a reduced amount of fossil fuels. Also, it is environmentally safe to burn blended jet fuel. Generally, blended fuels are commercially viable and environmentally friendly. Predominantly, the blended jet fuel should be sustainable and always complies with standards. Microalgae-derived fuels can be used in a blend to produce high-quality fuels. They have an advantage of generating fuels with a low carbon footprint evaluated at 60% less compared to conventional fuels. Therefore, they are an acceptable option for jet fuel blending.

It is compulsory that aviation fuels from any source must be certified before they can be used in any aircraft. They must comply with internationally recognized standards for certification. ASTM certification is the most required. The ASTM approval process is known as a multi-year and multi-million-dollar process [26]. Two different conversion technologies are already certified with ASTM standards for alternative aviation fuels. In 2009, ASTM International approved standards for alternative aviation fuels using the Fisher Tropsch process for the conversion of renewable feedstock sources to Jet fuel.[27] In 2011, the production of jet fuel from hydroprocessed esters and fatty acids (HEFA) was added to the standard under D7566-11. It was recommended that these blended jet fuels can only be used in commercial aviation on a 50/50 blended basis certified under ASTM Standard D1655. Expanding on the approved feedstock list, various biomass resources including algae biomass can be used to produce jet fuel substitutes. Technologies to convert biomass to jet fuel are available but the production capacity is currently small and production costs are not well known, especially for less-developed processes[28]. In this case, blending jet fuels could be an option or an intermediate solution to deal with the issue of production capacity and costs.

5.4MATERIAL AND METHODS

A sample of Jet A 1 was blended with algae-based jet fuel on a 50/50 volume ratio (B50). The algae-based jet fuel was produced in the laboratory after *Nannochloropsis sp* biomass was cultivated in a photobioreactor, followed by oil extraction, purification, thermal cracking using pyrolysis of crude bio-oil at 350^{o}C and fractionation between 70 and 300^{o}C to get the required fractions for jet fuel[19]. The cultivation was completed in 15 days with high biomass produced on the 10th day. After harvesting the biomass in a centrifuge, oil extraction using a mixture of solvent made with chloroform and methanol on 1/1 volume ratio was completed. The bio-oil combined with solvent was purified by evaporation to remove the solvent and collect the bio-oil. Thermal cracking was undertaken at 300^{o}C in a furnace to break down the carbon chains. It was followed by fractionation from 70 to 300^{o}C to collect fractions needed for jet fuel. Microalgae-based jet fuel was characterised according to ASTM standards and the results are presented in Table 1. The petroleum jet fuel (Jet A1) was supplied by Sapref –Engen in Durban, South Africa. It was a pure product ready for commercial use. The characterisation of Jet A1 was not disclosed in details by the manufacturer. However, the manufacturing report confirmed the compliance of all parameters according to ASTM standards. The blending procedure used 500ml of Jet A1 in a beaker and mixed it with 500ml of microalgae-derived jet fuel.

The blending was completed using a magnetic stirrer running at 150rpm for a period of 2h at 25^{o}C.The sample was conserved at in a closed glass container. After mixing, the 50/50 blended jet fuel was characterised according to ASTM. Samples were stored and tested for stability using ASTM procedures.

5.5 RESULTS AND DISCUSSION

Samples were kept at a controlled temperature of 25^{o}C in an incubator. The data generated from the characterisation of microalgae-derived jet fuel and the blended jet fuel are summarized in Tables 14, 15 and 16. The results were generated in triplicate and the recorded data in the tables are mean values.

Table 14: Algae-based jet fuel: characterisation after preparation

Parameter	Results ±standard deviation	ASTM Limits for conventional Jet fuel	Method
Density at 15 ^{O}C [g/l]	0.842± 0.015	0.775-0.840	ASTM D7042
Viscosity at- 20 ^{O}C [cSt]	2.4 ± 0.02	8 (maximum)	ASTM D445
Flash point [^{O}C]	68±0.5	38 (Minimum)	ASTM D93
Water content [%]	0.05±0001	n.d	ASTM D6304
Total acidity [mg KOH/g]	0.05±0.001	0.015(Maximum)	ASTM D3242
Total contamination[mg/kg]	7.6±0.2	24 (Maximum)	IP440/SANS 52662
Total Sulfur [%]	0.27±0.01	0.3 (Maximum)	ASTM D4294

Net heat of combustion [MJ/kg]	44±0.5	42.8 (Minimum)	ASTM D4868
Freezing point [^{O}C]	-32 ± 0.01	-40^{o} C (Maximum)	ASTMD D2386
Conductivity [pS/m]	84±0.5	50-600	ASTM D2624

Table 15: 50/50 Blended jet fuel (Algae-based jet fuel and Jet A1): characterisation after blending

Parameter	Results ±standard deviation	ASTM Limits	Method
Density at 15 ^{O}C [g/l]	0.8727±0.02	0.775-0.840	ASTM D7042
Viscosity at -20^{O}C[cSt]	5.3±0.05	8 (maximum)	ASTM D445
Flash point [^{O}C]	67±0.5	38 (Minimum)	ASTM D93
Water content [%]	0.089±0.05	n.d	ASTM D6304
Total acidity [mgKOH/g]	0.03± 0.001	0.015(Maximum)	ASTM D3242
Total contamination[mg/kg]	5.64±0.1	24 (Maximum)	IP440/SANS 52662
Total Sulfur [%]	0.0159±0.001	0.3 (Maximum)	ASTM D4294
Net heat of combustion [MJ/kg]	43.5±0.5	42.8 (Minimum)	ASTM D4868
Freezing point [^{O}C]	-40±0.001	-40^{o} C(Maximum)	ASTMD D2386
Conductivity [pS/m]	85±0.5	50-600	ASTM D2624

All characterisations were undertaken by Wearcheck an ISO 9001:2008 / ISO 14001:2004 / ISO 17025:2005 registered company. Tests of the 50/50 blended jet fuel were also completed after 30, 60, 90 and 120 days from the blending day in order to check the stability of various parameters according to ASTM standards.

Table 16: 50/50 Blended Jet fuel (algae-based jet fuel and Jet A1): storage stability tests

Parameter	30 days	60 days	90days	120 days	ASTM limits	Method
Density at 15 ^{O}C [g/l]	0.86 ± 0.01	0.86± 0.01	0.862±0.01	0.859±0.01	0.775-0.840	ASTM D7042
Viscosity at -20 ^{O}C [cSt]	5 ±0.05	5.1± 0.05	5.1± 0.05	5±0.05	8 (maximum)	ASTM D445
Flash point [^{O}C]	67 ± 0.5	68± 0.5	68±0.5	68±0.5	38 (Minimum)	ASTM D93
Water content [%]	0.06 ± 0.001	0.06±0.001	0.06±0.001	0.06±0.001	n.d	ASTM D6304
Total acidity [mgKOH/g]	0.03 ±0.001	0.029±0.001	0.030±0.001	0.0289±0.001	0.015 (Maximum)	ASTM D3242
Total contamination [mg/kg]	5.6 ±0.1	5.6±0.1	5.6±0.1	5.6±0.1	24 (Maximum)	IP440/SANS 52662
Total Sulfur [%]	0.0159 ±0.001	0.0159± 0.001	0.158±0.001	0.0157±0.001	0.3 (Maximum)	ASTM D4294
Net heat of combustion [MJ/kg]	44 ±0;5	44±0.5	44±0.5	45.3±0.5	42.8 (Minimum)	ASTM D4868
Freezing point [^{O}C]	-40 ±0.001	-40±0.001	-40±0.001	-40±0.001	-40^{o} C (Maximum)	ASTMD D2386
Conductivity [pS/m]	92 ±0.5	125±0.5	130±0.5	128±0.5	50-600	ASTM D2624

5.5.1 Storage stability test

Generally, medium-term storage stability tests of at least 1000 h (~30 days) [29] [30] are required to confirm if some parameters which are time-dependent do not critically affect the physico-chemical properties of the 50/50 blended jet fuel. In this study, the storage stability test for the 50/50 blended jet fuel was undertaken after 30, 60, 90 and 120days from the day of preparation/production. In general, the analysis of data recorded in Tables 14, 15and 16 shows that the majority of parameters are within or slightly beyond the limits described by ASTM. Particularly, it is observed in Table 16 that the variation of measurements for some few parameters recorded after 30, 60, 90 and 120days were not very significant and they remained within the limits required for ASTM compliance. It can be inferred that the 50/50 blending of jet fuels is a suitable option and can be undertaken at a larger scale. This is supported by the fact that no significant fluctuations in data were recorded for most parameters for a period of up to 120 days from the production. However, some parameters such as density and total acidity should be improved according to ASTM standards.

5.5.2 Density

Density at 15^{o}C is beyond ASTM limits as indicated in Tables 15 and 16 dealing with data for the 50/50 blended jet fuel. However, the difference between the ASTM maximum limit and the recorded data in this study for this parameter is not significant. The density of jet fuel is very important and must be within the limits prescribed by the standards or the aircraft weight loading calculations since jet fuel is customarily metered by volume. Also, it relates to specific energy and volatility of jet fuel. To improve the density of the jet fuel, fuel filtration through a membrane system can assist in removing some particulate matter probably from the algae-based jet fuel that might have caused the increase of density. Undesirable substances will be retained by the filter media and a cleaner blended jet fuel with density within ASTM limits can be collected.

5.5.3Kinematic Viscosity

Kinematic Viscosity is directly linked to fuel fluidity or fuel pumpability over the operating temperature range and relates to droplet size in sprays produced by burner nozzles. The ASTM maximum limit for viscosity at -20^{o}C is 8cSt. In this study, the recorded data for viscosity at -20^{o}C presented in Tables 1, 2 and 3 have ranged from 2 to 5.3cSt and it is compliant with the standards. The lowest value is recorded in Table 15 regarding algae-based

jet fuel. However, it has increased in the 50/50 blended jet fuel as indicated in Table 16 for different measurements.

5.5.4 Flash point

Flash point is one of the parameters that relate to jet fuel volatility, it can, therefore, have an impact on combustibility. It is a leading factor in determining fire safety regarding fuel handling. In the present study, the flash point remains almost stable. It has slightly fluctuated between 67 and 68^{o}C as indicated in Tables 14, 15and 16. These values are compliant with ASTM standards requiring a minimum of 38^{o}C. The analysis of the recorded data on flash point for the 50/50 blended jet fuel shows the 50/50 blended jet fuel has lower flammability and it has less hazardous behaviour because of the higher flash point. This is a strong point regarding the 50/50 blended jet fuel produced in this study which shows its potential for safety and hazard related issues. In this regard, the blend can be used in a hot environment where safety issues for fuels are not easy to be handled

5.5.5 Water content

Water content has to be at the very lowest concentration in jet fuel. So far there are no exact limits defined by ASTM standards regarding this parameter in jet fuel. Determining the level of water content in jet fuel will assist in minimising the water reaction controlled by the presence of materials that react with water and affect the stability of the jet fuel-water interface. It is very important that jet fuel be free from water contamination. During the flight, the temperature of jet fuel in the tanks decreases due to the low temperatures in the upper atmosphere. This causes precipitation of the dissolved water from the jet fuel. Therefore, the separated water drops to the bottom of the tank, because it is denser than the jet fuel.

Since water is no longer in solution, it can form droplets that can supercool to below 0°C. Analysing this parameter, water separation index can be also measured to determine the ability of the jet fuel to release entrained or emulsified water when passed through a fibreglass filter coalescer. The data in Tables 14 and 15 shows lower values of water in the algae-based jet fuel compared to the 50/50 blended jet fuel. During storage the quantity of water has decreased in the 50/50 blended jet fuel from 0.089 to 0.06% and this quantity has remained constant up 120 days for the 50/50 blended jet fuel as indicated in Table 16.

5.5.6 Total acidity

Generally, total acidy is a parameter that indicates the corrosive potential of fuel to metals. Trace organic acids can affect water separation properties. In this study total acidity has been found higher than the limit required by ASTM which is equal to 0.015mgKOH/g.

Table 14 has recorded a total acidity of 0.05 mg KOH/g while in the 50/50 blended jet fuel obtained after production for which the data is presented in Table 15 the total acidity is 0.03mg KOH/g. This is still beyond the maximum limit of 0.015mgKOH/g required by ASTM standards. Table 16 shows that the total acidity is still higher and beyond the ASTM limit. It is between 0.0289 to 0.03mgKOH/g during the storage stability test period. Therefore, because of the higher total acidity found in the algae-based jet fuel as well as the 50/50 blended jet fuel and knowing that the acidity does not have a significant impact on the fuel performance but can mainly cause corrosion, it will be indispensable to use corrosion inhibitor to reduce the potential corrosivity of the jet fuel. The corrosion inhibitor is known as a lubricity improver, consequently, inhibiting corrosion will also improve the lubricity of the jet fuel because many aircraft fuel system components, especially pumps, rely on the fuel to lubricate moving parts.

5.5.7 Total contamination

Contaminants in jet fuel are made of existent gum such as non-volatile residue left on jet fuel evaporation, dissolved and undissolved particulate matters which are undesirable including dirt and rust. The analysis of data recorded in Tables 14, 15, and 16 shows that the level of contaminants has remained below the maximum of 24mg/kg required by SANS. It is imperative to have very pure jet fuel after preparation, therefore, reducing the level of contaminants after obtaining the final product is more than a necessity. In this regard, a membrane filtration as mentioned earlier can be an option not only to improve density but mostly to get pure blended jet fuel.

5.5.8 Total Sulfur

Sulfur must be controlled because sulfur oxides formed during combustion can cause corrosion of turbine metal parts. Generally, the origin of sulfur in the conventional jet fuel may come from crude petroleum oil. In the case of the 50/50 blended jet fuel produced during the course of this study, there is a possibility that a fraction of sulfur originates also from the microalgae strain. Marine algae strains may contain sulfur that is found between layers of sedimentary rocks formed by the layering of seabed deposits and free organic material settling. In this study, sulfur has been found lower than the limit prescribed by ASTM standards. The recorded data for algae-based jet fuel in Table 14 as well as the one for the 50/50 blended jet fuel in Tables

15 and 16 are showing that the levels of total sulfur are below the maximum of 0.3% required by ASTM standards in terms of compliance. However, even if the total sulfur is below the value recommended for compliance, it is imperative to consider desulfurization in order to minimize the effects of sulfur in jet fuel and in the environment. Sulfur causes the formation of secondary particulate matter in jet fuel and the emission of sulfur oxide can because of major health issues.

5.5.9 Net Heat of Combustion

This parameter is directly linked to the amount of energy generated by jet fuel and its performance. It is one of the key parameters that are highly needed for most fuels in general and jet fuel in particular. It is a calculated value denoting the amount of heat energy obtainable from fuel to provide the power needed to run an aircraft from take-off to landing. The ASTM required a minimum value for aviation fuels is 42.8MJ/kg. In this study algae-based, jet fuel recorded a Net heat of combustion of 44 MJ/kg as indicated in Table 14.

The 50/50 blended jet fuel presented in Table 15 has recorded a Net Heat of Combustion equivalent to 43.5MJ/kg while the stored 50/50 blended jet fuel presented in Table 3 has kept its Net Heat of Combustion at 44MJ/kg during the storage test period. A slight increase of the Net Heat of Combustion of up to 45.3MJ/kg was recorded on the 120 the day of the storage test period. Overall, the Net Heat of Combustion has remained compliant with the ASTM limit of 42.8MJ/kg. This is an indication that the 50/50 blended jet fuel has the potential to produce enough energy needed for the aircraft thrust.

5.5.10 Freezing point

This parameter is one of the most important regarding jet fuel performance, it limits higher molecular weight hydrocarbons that crystallise at low temperatures; it, therefore, influences low-temperature pumpability during the flight. At higher altitudes where temperatures are very lower, the jet fuel will have a tendency of solidifying. Therefore, the lowest freezing point is the most needed. The analysis of Table 1 shows that algae-based jet fuel has a high freezing point which does not comply with ASTM standards while during the experiment algal crude bio-oil was exposed to the lowest temperature of -80^{o}C without solidifying. However, the 50/50 blended jet fuel indicates a freezing point that complies with ASTM standards. For the 50/50 blended jet fuel, the freezing point has remained constant and it complies with ASTM standards as indicated in Tables 15 and 16. This complying value of the freezing point may be due to the presence and the action of the anti-freezing or ice inhibitor additive present in the Jet A1 which forms part of the blend. Therefore, blending conventional jet fuel and algae-based jet fuel can

assist in improving the freezing point of the blend in case one of the components from the blend has a higher freezing point not complying with ASTM standards

5.5.11 Conductivity

This parameter represents the concentration of dissolved substances in the jet fuel. It has to be sufficiently high in order to dissipate any electrostatic charges generated during fuel handling operations, so as to prevent fire or explosion hazards. Analysing Table 14 and comparing the data in Tables 15 and 16 it is indicated that the conductivity is within the range required by ASTM standards in all cases. Comparing the conductivities of the 50/50 blended jet fuel after preparation and the ones during the storage test period there is an overall increase of conductivity values. This can be due to the increase of the concentration of dissolved substances caused by the decrease in water content during storage test period as mentioned earlier.

CONCLUSIONS

This study has attempted to establish the possibility of using a blended jet fuel made of algae-based- jet fuel with Jet A1 in 50/50 volume ratio. The algae-based jet fuel was obtained from *Nannochloropsis sp*. The main aim of the study was to verify the extent to which compliance to ASTM standards and performance of aviation fuels was going to be met when blending a biomass-based Jet fuel and a petroleum jet fuel.

Therefore, ASTM standards for aviation fuels D1655 were chosen to characterise the 50/50 blended jet fuel. Conversely, it was not also possible to undertake a complete characterisation because of limited resources and time frame allocated to this study. Nevertheless, it was found that most of the selected parameters were complying with ASTM standards for the 50/50 blended jet fuel. However, parameters such as density at 15^{o}C and total acidity were found non-compliant to ASTM standards. The cause of non-compliant could be due to the fact that the algae-based Jet fuel was not at the same level of cleanness compared to Jet A1 used in the 50/50 blend. It needs more refining processes such as reforming and upgrading to reach the same level of purity as it is for Jet A1. However, there is another way to get highly pure algae-derived Jet fuel by using separation processes such as membrane filtration or rectification in order to improve parameters such as density, total acidity and reduce sensibly the contamination level. However, there will be a need for more studies involving costs modelling and innovative technology to improve all parameters required for the 50/50 blended jet fuel. A storage stability tests undertaken on 30, 60,90 and 120thday after the 50/50 blended jet fuel

preparation indicated that most of the parameters have remained stable and compliant to ASTM standards. The 50/50 blended jet fuel can be produced at large scales and commercialized due to the fact that most of the properties comply with ASTM standards. Furthermore, the presence of algae-based jet fuel into Jet A1 has the potential to make the 50/50 blended jet fuel environmentally friendly but still not economically competitive because the production of algae-based jet fuel is still expensive.

Overall the 50/50 blending for jet fuel using algae-based jet fuel and Jet A1 is a sustainable option for jet fuel. It reduces carbon footprint and it is an economically viable compared to algae-based jet fuel. This supports and reinforces the ASTM certification for the 50/50 blending of jet fuels. More characterisation for 50/50 blending involving parameters such as aromatics content, hydrogen content, distillation tests for product recovery, smoke point, naphthalene content and thermal stability have to be explored in future studies.

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REFERENCES

[1] Alarcon-Rodriguez A, Ault G, Galloway S. Multi-objective planning of distributed energy resources: A review of the state-of-the-art. Renewable and Sustainable Energy Reviews. 2010 Jun 30;14(5):1353-66. https://doi.org/10.1016/j.rser.2010.01.006

[2] Akahori, C., Haller, T., Holloway, M., Riel, N., Abid, Z., Han, S.R., Nguyen, H., Augustus, J., Sadzak, S., Knopf, K. and Shin, W., 2017. A New Nuclear Era: The US Role in the Shifting Global Energy Landscape.

[3] Hussain, Akhtar, Syed Muhammad Arif, and Muhammad Aslam. "Emerging renewable and sustainable energy technologies: State of the art." Renewable and Sustainable Energy Reviews 71 (2017): 12-28. https://doi.org/10.1016/j.rser.2016.12.033

[4] Lowry J. Avoiding Carbon Apocalypse Through Alternative Energy: Life After Fossil Fuels. Springer; 2017 Mar 29.

[5] Kurji, H.J., Fuel Flexibility with Low Emissions for Gas Turbine Engines (Doctoral dissertation, Cardiff University); 2017

[6] Heinberg R. Afterburn: society beyond fossil fuels. New Society Publishers; 2015 Mar 16.

[7] Ramadhas AS, editor. Alternative fuels for transportation. CRC Press; 2016 Apr 19.

[8] Owen NA, Inderwildi OR, King DA. The status of conventional world oil reserves—Hype or cause for concern?. Energy policy. 2010 Aug 31;38(8):4743-9. https://doi.org/10.1016/j.enpol.2010.02.026

[9] Covert T, Greenstone M, Knittel CR. Will we ever stop using fossil fuels?. The Journal of Economic Perspectives. 2016 Jan 1;30(1):117-37. https://doi.org/10.1257/jep.30.1.117

[10] Kavalov B. Market perspectives for products from future energy-driven biorefineries by 2020. JRC scientific and technical reports, European Commission, Office for Official Publications of the European Communities, Luxembourg. 2009.

[11] Larsen LE, Jepsen MR, Frederiksen P. Scenarios for biofuel demands, biomass production and land use–The case of Denmark. Biomass and bioenergy. 2013 Aug 31;55:27-40. https://doi.org/10.1016/j.biombioe.2012.08.015

[12] Chisti Y. Biodiesel from microalgae beats bioethanol. Trends in biotechnology. 2008 Mar 31;26(3):126-31. https://doi.org/10.1016/j.tibtech.2007.12.002

[13] Nigam PS, Singh A. Production of liquid biofuels from renewable resources. Progress in energy and combustion science. 2011 Feb 28;37(1):52-68. https://doi.org/10.1016/j.pecs.2010.01.003

[14] McKendry P. Energy production from biomass (part 2): conversion technologies. Bioresource technology. 2002 May 31;83(1):47-54. https://doi.org/10.1016/S0960-8524(01)00119-5

[15]. Yeh S, Lutsey NP, Parker NC. Assessment of technologies to meet a low carbon fuel standard, 2009

[16] Yeh S, Sperling D. Low carbon fuel standards: Implementation scenarios and challenges. Energy Policy. 2010 Nov 30;38(11):6955-65. https://doi.org/10.1016/j.enpol.2010.07.012

[17] Tietenberg TH, Lewis L. Environmental and natural resource economics. Routledge; 2016 Mar 4.

[18] Milano J, Ong HC, Masjuki HH, Chong WT, Lam MK, Loh PK, Vellayan V. Microalgae biofuels as an alternative to fossil fuel for power generation. Renewable and Sustainable Energy Reviews. 2016 May 31;58: 180-97. https://doi.org/10.1016/j.rser.2015.12.150

[19] Bwapwa JK, Anandraj A, Trois C. Possibilities for conversion of microalgae oil into aviation fuel: A review. Renewable and Sustainable Energy Reviews. 2017 Dec 1;80:1345-54. https://doi.org/10.1016/j.rser.2017.05.224

[20] Dooley, S., Won, S.H., Chaos, M., Heyne, J., Ju, Y., Dryer, F.L., Kumar, K., Sung, C.J., Wang, H., Oehlschlaeger, M.A. and Santoro, R.J., 2010. A jet fuel surrogate formulated by real fuel properties. *Combustion and Flame*, *157*(12), pp.2333-2339. https://doi.org/10.1016/j.combustflame.2010.07.001

[21] Savage N. Fuel options: the ideal biofuel. Nature. 2011 Jun 23;474(7352):S9-11. https://doi.org/10.1038/474S09a

[22] Blakey S, Rye L, Wilson CW. Aviation gas turbine alternative fuels: A review. Proceedings of the combustion institute. 2011 Dec 31;33(2):2863-85. https://doi.org/10.1016/j.proci.2010.09.011

[23] Calvert K, Stephen J, Blair MJ, Cabral L, Baxter R, Mabee W. Assessing the Spatial, Economic and Environmental Implications of Biorefining Technologies: Insights From North America. Handbook on Geographies of Technology. 2017 Feb 24:269.

[24] Burger V. *The influence of fuel properties on threshold combustion in aviation gas turbine engines* (Doctoral dissertation, University of Cape Town), 2017

[25] Soloiu V, Gaubert R, Muinos M, Moncada J, Beyerl T, Molina G, Williams J. Performance of an Indirect Injected Engine Operated with ULSD# 2 Blended with Fischer-Tropsch Synthetic Kerosene. SAE Technical Paper; 2017 Mar 28.

[26] Rumizen, M. (2013). "Certification/Qualification." Presented at the Advanced Biofuels Leadership Conference, April 17, Washington, DC. http://www.caafi.org/files/presentations/Cert-Qual_Rumizen_ABLC_Apr17_2013.pdf.

[27]Enright, C. (2011). "Aviation Fuel Standard Takes Flight." *ASTM International - Standards Worldwide*. Accessed July 17, 2013: http://www.astm.org/SNEWS/SO_2011/enright_so11.html.

[28] Milbrandt A, Kinchin C, McCormick R. Feasibility of Producing and Using Biomass-Based Diesel and Jet Fuel in the United States. NREL (National Renewable Energy Laboratory (NREL), Golden, CO (United States)); 2013 Dec 1.

[29] P. Bondioli, P.A. Gasparoli, A. Lanzani, E. Fedeli, S. Veronese,M. Sala, Storage stability of biodiesel, Journal of the American Oil Chemists Society 72 (1995) 699–702. https://doi.org/10.1007/BF02635658

[30]P. Bondioli, A. Gasparoli, L. Della Bella, S. Tagliabue, G. Toso, Biodiesel stability under commercial storage conditions over one year, European Journal of Lipid Science and Technology 105 (2003) 735–741. https://doi.org/10.1002/ejlt.200300783

[31] Eloka-Eboka, A.C. and Inambao, F.L., 2016. Hybridization of feedstocks—A new approach in biodiesel development: A case of Moringa and Jatropha seed oils. *Energy Sources, Part A: Recovery, Utilization, and Environmental Effects*, *38*(11), pp.1495-1502.

[32] Igbum, O.G., Eloka-Eboka, A.C., Ubwa, S.T. and Inambao, F.L., 2014. Evaluation of environmental impact and gaseous emissions of biodiesel fuels and blends of selected feed-stocks. *International Journal of Global Warming*, *6*(1), pp.99-112.

[33] Eloka-Eboka, A.C. and Inambao, F.L., 2014, April. Blending feedstock—Fresh approach in biodiesel development: Moringa and Jatropha seed oils. In *Domestic Use of Energy (DUE), 2014 Proceedings of the Twenty-Second* (pp. 1-8). IEEE.

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CHAPTER 6 PREFACE

The following chapter is a compiled form of a research paper currently under review in the *International Journal of Green Energy* (Taylor and Francis). After analysing B50 blending which is ASTM certified version. The option of B20 blending being is not a certified for aviation fuels. This option was examined in order to assess the performance of the blended jet fuel made of algae-based jet fuel and Jet A1 in 80/20 and 80/20 volume ratios. This study provides some insight to be used for potential certification. B20 blending ratios completed in this study could be also considered as a viable option to reduce the carbon footprint from conventional jet fuel if approved for ASTM certification. The chapter is presented with some minor changes to maintain the connection, the consistency and flow with previous chapters. It aims to analyse the efficiency and sustainability of blending algae-based jet with conventional jet fuel Jet A1 in an 80/20 and 20/80 volume ratio with regard to ASTM standards.

CHAPTER 6: NEW JET FUEL FROM BLENDING MICROALGAE BASED JET FUEL AND JET A1 IN 20/80 AND 80/20 RATIOS RESPECTIVELY

[1,2]Joseph K. Bwapwa, [2] Akash Anandraj, [1]Cristina Trois

[1] University of KwaZulu-Natal, [2] Mangosuthu University of Technology

Corresponding author: joseph@mut.ac.za; josephkapuku@gmail.com

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ABSTRACT

Blending fuels can potentially be a successful option for fuel producers. It has many advantages related to high fuel quality and low carbon footprint. The quality of fuels can be improved for fuel

performance-based parameters such as flash point, freezing point, net heat of combustion and viscosity. To address stringent requirements from the aviation industry in terms of quality and environmental issues, there is an urgent need to develop alternative ways of complying with the economics and environmental requirements. In this paper, jet fuel derived from microalgae oil was blended with Jet A1 in ratios of 20/80 and 80/20 respectively. The microalgae oil was produced from a marine species known as Nannochloropsis sp. Characterisation of both blends was completed in terms of ASTM specifications required for jet fuel. Parameters such as density, kinematic viscosity, freezing point, the Net heat of combustion, flash point, contamination, total acidity, water content and total sulfur were analysed and most of them have complied with ASTM D1655 standards relevant for jet fuel. However, it is highly recommended to undertake upgrading processes to increase the purity and enhance the performance of the blended jet fuel.

KEYWORDS: microalgae; biofuels; aviation fuel; jet fuel; fuel blending

6.1 INTRODUCTION

Bioenergy is currently a greater part of the global energy mix and it is projected to contribute up to 20-30% of the overall primary energy worldwide by 2035[1]. The use of biofuels and their blending with conventional fuels can contribute to the reduction of greenhouse gases (GHG) affecting the ozone layer and bringing down issues related to climatic changes and global warming.[2, 3, 4] Therefore, the sustainability of renewable energy from biomass can add value to the security of energy supply and assist in reducing GHG emissions when blending fossil fuels with biofuels.[5] There are various legislation, policies and regulatory targets towards establishing sustainability of biofuels via various renewable energy options, much have already been achieved in various countries worldwide in this regard.[6,7,8] This has made biofuels as one of the options to be considered in order to prevent an eventual energy crisis in case petroleum reserves could get to a critical level of shortage.[9, 10] Petroleum or fossil fuels are currently the most reliable source of energy. However, price fluctuations, environmental concerns and the depletion of the world reserves of petroleum crude oil are amongst the major challenges that need to be addressed effectively.[11, 12, 13] Consequently, there is a strong need to establish sustainable sources of energy in order to address the issue related to the production of alternative sources of energy including biofuels.[14, 15] Generally, the need is very pressing in the transportation industry more especially in the aviation sector which is among the biggest consumers of fuels worldwide.

As a result, the aviation sector finds itself under enormous pressure and requires desperately a fuel that is sustainable, affordable, with a low carbon footprint. Finding an alternative to jet

fuel is critically important for any economy. This energy transition can pose many risks ranging from engineering technological shifts, food vs fuel competition, policy and other industrial concerns for commercialisation. Furthermore, there is an ongoing concern over environmental issues involving threatening of climatic patterns due to large usage of fossil and non-renewable fuels with unfriendly emissions. Therefore, in this current circumstance, blending fossil-based fuels and biomass led fuels could be one of the options to be undertaken to prevent some of the issues mentioned earlier. Consequently, blending fossil and non-fossil fuels particularly jet fuels from both sources in various proportions to make it more sustainable in order to reduce negative environmental impacts and to reduce the consumption of the world reserves of petroleum fuels can be an option to be explored. In this study, conventional jet fuel is blended with algae-based jet fuel in 80/20 and 20/80 volume ratios respectively, both options are known as B20.

The study focuses on the establishment of a new fuel with characteristics and performance that enable the production of sufficient energy for the propulsion of an aircraft. The new fuel produced by blending algae-based jet fuel from *Nannochloropsis sp* and Jet A1 using 80/20 and 20/80 volume ratio respectively has to be compliant with ASTM standards related to aviation fuels. Producing a new fuel with compliant physico-chemical parameters with regards to ASTM standards can add value to the sustainability. It can also assist to generate low carbon emissions due to the decrease of the fossil fuel fraction in the blend. Therefore, the blending option can be regarded as one of the ways to address the issue of sustainability. The blended fuel does not use additives; however, additives can be needed in the long run if the fuel is presenting some shortfalls regarding the compliance. The study will contribute to increasing knowledge regarding the new fuel performance, the feasibility and compliance aspects related to the blending of algae-based jet fuel with conventional jet fuel (Jet A1) in 20/80 and 80/20 volume ratio respectively. The data generated from this study can also assist standard bodies to implement a certification process for 20/80 and 80/20 blends.

6.2 PERFORMANCE REQUIREMENTS AND INTERNATIONAL STANDARDS AND ASTM CERTIFICATION FOR BLENDING

Conventional jet fuel comes from the refining of crude petroleum oil. It is achieved through a series of unit processes aiming to remove the unnecessary carbon fractions and molecules. The composition of jet fuel mainly depends on the quality of the raw crude oil. In general, conventional jet fuel are made of 20% paraffins, 40% isoparaffins, 20% naphthenes and 20%

aromatics [16]. Each of these components contributes enormously in providing specific fuel characteristics. For instance, a high hydrogen-to-carbon ratio of paraffins and isoparaffins improves the heat density per unit mass of fuel; naphthenes assist in decreasing the freezing point which is a very critical parameter needed at high altitudes, and aromatics contribute to material compatibility and prevent leaks in the seals of some aircraft[16,17,18]

Jet fuel specifications are established by international standard bodies, however, there are two major standard bodies that are the most known in terms of jet fuel standards: ASTM D1655 and Def Stan 91-91. The standards of both bodies are almost equivalent and they focus mainly on performance properties than chemical composition. Chemical composition is very complex and can be subjected to variability or fluctuations because of the operating and storage conditions that can be subjected to changes or variability.[18] Several physico-chemical parameters are identified as key performance aspects for aviation fuels, they include the following: energy content, freezing point, thermal stability, viscosity, combustion characteristics, lubricity, material compatibility and safety properties.[18] There is only 50/50 blending in volume ratio that is currently ASTM certified when it comes to the blending of conventional jet fuel with biomass-based jet fuel. The production costs of most biofuels can be higher while they have a low carbon footprint. The blending in 50/50 volume ratio being ASTM certified is an effective and successful solution, it has been tested and proven in many cases. However, the 80/20 and 20/80 blending in volume ratio are not yet certified.

This study provides the knowledge and data that are enough to support a prospective certification in the future. Also, to analyse the possibility of compliance for various parameters used for jet fuel characterisation regarding the 80/20 and 20/80 blending.

This will assist in establishing a new blending formula for jet fuels which can be a basis for future ASTM certification. The improvement option for parameters that are not complying with ASTM standards from these blending formulas will be suggested.

6.3 MATERIAL AND METHODS

6.3.1 Methodological approach

To achieve the objectives of the current study the following approach was used:

- Preparation and characterisation of algae-based jet fuel
- Characterisation of Jet A1 collected from a local producer (Engen-Sapref)
- Blending of both algae-based jet fuel and Jet A1 in 80/20 and 20/80 volume ratio

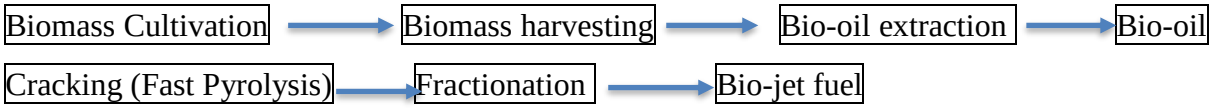
- Characterisation of the blends needed for compliance test according to ASTM standards D1655
- Interpretation and analysis of data generated after blending to find out whether or not parameters are compliant with regards to ASTM specifications or standards.

6.3.2 Experimental procedure

A sample of Jet A1 supplied by a local manufacturer named Sapref-Engen Durban was mixed with algae-based jet fuel in 80/20 and 20/80 volume ratios respectively. The renewable algae-based jet fuel was produced from the biomass from a marine species called *Nannochloropsis sp* in the laboratory after cultivation, oil extraction, purification, thermal cracking and fractionation [21]. The production process started with cultivation to produce the biomass needed for conversion of bio-oil into jet fuel. After the cultivation period of 15 days, the highest amount of biomass was collected between day 8 and day 10. The biomass productivity was approximately 180 mg per day. The strain used has about 30% of the bio-oil content.

An amount of 1800 mg of wet biomass was used for the experiment.[19] The extraction of crude bio-oil was undertaken with the use of a solvent mixture made of methanol and chloroform used as the crude bio-oil extracting agent. The purification of crude bio-oil was achieved through solvent evaporation and filtration. The purified bio-oil was thereafter subjected to fast cracking by pyrolysis at 450^{o}C to break down long hydrocarbon chains found during characterisation of crude bio-oil.[19] Fast pyrolysis was completed during a period of 12 s. Data was gathered from a pyrolyzed gas chromatograph. This equipment is from Frontier Labs but supplied by Shimadzu. Thereafter, fractional distillation was completed within the range between 70 to 300 ^{o}C to collect the middle-end hydrocarbon fractions which are the main fractions needed for jet fuel. However, jet fuel produced in the laboratory will require supplementary processes such as reforming and upgrading to enhance the quality of the final products. The difference between jet fuel and diesel is based on the carbon chain fractions, jet fuel is the range of C14 to C16 while diesel is in the range between C16 and C18. Fractional distillation is appropriate to separate the fractions and collect the needed ones based on the application.

The details for the entire process to get algae-based jet fuel are found in chapter 3. The following scheme summarizes the laboratory process used to produce algae-based jet fuel used in this study as described earlier.



The produced algae-based jet fuel was characterised according to ASTM 1655 standards. The supplier Sapref-Engen certified the Jet A1 by issuing a compliance certificate based on ASTM D1655 standards. The blending of both fuels was completed as follows: 400 ml of Jet A1 in the beaker was mixed with 100 ml of algae-based jet fuel to make the 80/20 blend. Regarding the other sample, 100 ml of Jet A1 was mixed with 400 ml of algae-based jet fuel to make the 20/80 blend. The blends were prepared in the laboratory with the use of a magnetic stirrer running at 150 rpm for a period of 2 hours at ambient temperature. The sample was kept in a closed glass container in an incubator at 25 ^{o}C. The blends were thereafter used for characterisation according to ASTM D1655 standards and all measurements were done in triplicates. The characterisation was completed by the following accredited institutions: Wearcheck laboratories and Chemsciences laboratories situated in Durban. (see some spreadsheet and results in APPENDIX A6)

6.4 RESULTS AND DISCUSSIONS

Tables 17 to 24 are presenting the summary of the data generated during the course of the current study. ASTM limits represent the ASTM D1655

Table 17: Renewable Algae-based Jet fuel data: Physico-chemical data (data collected on preparation day**)**

Parameter	Results	Unit	ASTM Limits	Method
Density @15 ^{O}C	0.842 ±0.02	g/ml	0.775-0.840	ASTM D7042
Viscosity @- 20 ^{O}C	2.4 ± 0.12	cSt	8 (maximum)	ASTM D445
Flash point	68 ± 0.3	^{O}C	38 (Minimum)	ASTM D93
Water content	0.05 ± 0.001	%	n.d	ASTM D6304
Total acidy	0.05 ± 0.001	mg KOH/g	0.015(Maximum)	ASTM D3242
Total Contamination	7.6 ± 0.4	mg/kg	24 (Maximum)	IP440/SANS 52662
Total Sulfur	0.27 ± 0.015	%	0.3 (Maximum)	ASTM D4294
Net heat of combustion	44 ± 0.6	MJ/kg	42.8 (Minimum)	ASTM D4868
Freezing point	-32 ± 4	^{O}C	-40^{o} C (Maximum)	ASTMD D2386
Conductivity	84 ±0.5	pS/m	50-600	ASTM D2624

The analysis of the overall results in table 17 shows that most of the parameters are complying except for the freezing point.

Table 18: Microalgae jet fuel: Organic compounds from GC analyses

Parameter	Results [% weight]	Method	Testing equipment

Aromatics	18±0.1	ASTM D2425	GC-MS
Paraffins	40± 0.2	ASTM D2425	GC-MS
Monocycloparaffins	22± 0.3	ASTM D2425	GC-MS
Dichloroparaffins	4.1± 0.2	ASTM D2425	GC-MS
Trichloroparafins	0.4± 0.02	ASTM D2425	GC-MS
Naphtalenes	1.5± 0.01	ASTM D2425	GC-MS

From the analysis of the data recorded in table 18, it was found that concentration of paraffins are the highest in the algal jet fuel.

Table 19: Blended Jet fuel in 80/20 volume ratio (Jet A1- Algae Jet fuel) (after preparation)

Parameter	Results	Unit	ASTM Limits	Method
Density @15 ^{O}C	0.8294± 0.011	g/ml	0.775-0.840	ASTM D7042
Viscosity @ -20 ^{O}C	4.6± 1.7	cSt	8 (Maximum)	ASTM D7042
Flash point	66± 0.25	^{O}C	38 (Minimum)	ASTM D93
Water content	0.037± 0.0011	%	n.d	ASTM D6304
Total acidity	0.05± 0.001	mg KOH/g	0.015(Maximum)	ASTM D3242
Total Contamination	3.0± 0.022	mg/kg	24 (Maximum)	IP440/SANS 52662
Total Sulfur	0.0052± 0.00015	%	0.3 (Maximum)	ASTM D4294
Net heat of combustion	44± 0.6	MJ/kg	42.8 (Minimum)	ASTM D4868
Freezing point	-40± 0.2	^{O}C	-40º C(Maximum)	ASTMD D2386
Conductivity	78± 0.45	pS/m	50-600	ASTM D2624

Analysing the results in table19, it was found that the 80/20 blended jet fuel is totally complying with the selected ASTM requirements for aviation fuel.

Table 20: Blended Jet fuel in 20/80 volume ratio (JetA1-Algae Jet fuel) (after preparation)

Parameter	Results	Unit	ASTM Limits	Method
Density @15 ^{O}C	0.9095± 0.051	g/ml	0.775-0.840	ASTM D7042
Viscosity @ -20 ^{O}C	5.5± 1.27	cSt	8 (Maximum)	ASTM D7042
Flash point	79± 0.6	^{O}C	38 (Minimum)	ASTM D93
Water content	0.048± 0.001	%	n.d	ASTM D6304
Total acidity	0.08± 0.001	mg KOH/g	0.015(Maximum)	ASTM D3242
Total Contamination	8.1± 0.055	mg/kg	24 (Maximum)	IP440/SANS 52662
Total Sulfur	0.0034± 0.0001	%	0.3 (Maximum)	ASTM D4294
Net heat of combustion	42.9± 0.05	MJ/kg	42.8 (Minimum)	ASTM D4868
Freezing point	-38± 1	^{O}C	-40º C(Maximum)	ASTMD D2386
Conductivity	82± 0.55	pS/m	50-600	ASTM D2624

The data recorded in table 20 indicates that parameters such as density and freezing point have to be adjusted because they are not complying with the aviation requirements defined ASTM standards. This implies that this blend will require an antifreeze as an additive to regulate the freezing point and a polishing step such as membrane filtration to remove particulate matters in order to fine-tune the density to the required value for compliance with ASTM standards.

Table 21: Blended Jet fuel in 80/20 volume ratio (Jet A1- Algae Jet fuel) after 30, 60, 90, 120 days

Parameter	30 days	60 days	90days	120 days	ASTM limits	Method
Density @15 ^{O}C [g/l]	0.83± 0.011	0.83±0.011	0.83± 0.011	0.83± 0.011	0.775-0.840	ASTM D7042
Viscosity @ -20 ^{O}C [cSt]	4.5± 1.75	4.6± 1.7	4.55± 1.725	4.5± 1.8	8 (maximum)	ASTM D445
Flash point [^{O}C]	66± 0.25	66± 0.25	66± 0.25	66± 0.25	38 (Minimum)	ASTM D93
Water content [%]	0.032± 0.001	0.032± 0.001	0.03± 0.001	0.03± 0.001	n.d	ASTM D6304
Total acidity [mgKOH/g]	0.05± 0.001	0.05±0.001	0.05± 0.001	0.05± 0.001	0.015 (Maximum)	ASTM D3242
Total contamination [mg/kgl]	0.30± 0.02	0.31± 0.02	0.3±0.02	0.3±0.02	24(Maximum)	IP440/SANS 52662
Sulfur [%]	0.0052± 0.0001	0.0051±0.001	0.0051±0.001	0.005± 0.001	0.3 (Maximum)	ASTM D4294
Net heat of combustion [MJ/kg]	44.3± 0.75	44±0.6	44±0.6	44.2±0.7	42.8 (Minimum)	ASTM D4868
Freezing point [^{O}C]	-40±0.2	-40±0.2	-40±0.2	-40±0.2	-40^{o} (Maximum)	ASTMD D2386
Conductivity [pS/m]	78±0.45	79±0.5	80±0.52	80±0.5	50-600	ASTM D2624

Overall, table 21 indicates a relative stability and compliance with ASTM standards

Table 22: Blended Jet fuel in 20/80 volume ratio (Jet A1- Algae Jet fuel) after 30,60, 90, 120 days

Parameter	30 days	60 days	90days	120 days	ASTM limits	Method
Density @15 ^{O}C [g/l]	0.90±0.046	0.91±0.051	0.909±0.0507	0.91±0.051	0.775-0.840	ASTM D7042
Viscosity @ -20 ^{O}C [cSt]	5.5±1.25	5.6± 1.2	5.55± 1.23	5.6± 1.2	8 (Maximum)	ASTM D445
Flash point [^{O}C]	79± 0.5	79± 0.5	79± 0.5	79± 0.5	38 (Minimum)	ASTM D93

Water content [%]	0.048±0.0013	0.046± 0.0012	0.045± 0.0011	0.045±0.0011	n.d	ASTM D6304
Total acidity [mgKOH/gl]	0.08±0.0012	0.08± 0.0012	0.08± 0.0012	0.08± 0.0012	0.015 (Maximum)	ASTM D3242
Total contamination [mg/100ml]	8.1±0.55	8.1± 0.55	8.0± 0.52	8.0±0.52	24 (Maximum)	IP440/SANS 52662
Sulfur [%]	0.0034± 0.0001	0.0034±0.0001	0.0034±0.0001	0.0034±0.0001	0.3 (Maximum)	ASTM D4294
Net heat of combustion [MJ/kg]	42.9±0.05	42.9±0.05	43±0.1	43±0.1	42.8 (Minimum)	ASTM D4868
Freezing point [^{O}C]	-38±1	-38±1	-38±1	-38±1	-40^{o} C	ASTMD D2386
Conductivity [pS/m]	82±0.55	83± 0.57	83.5±0.59	84± 0.61	50-600	ASTM D2624

The analysis of the data recorded in table 22 shows that all parameters comply with the standards during the storage period except for the freezing point. The flash is the highest compare for the entire experimental period.

Table 23: Jet A1 characterisation

Parameter	Unit	Results	ASTM D1655 limits	Method
Density @15 ^{O}C	g/ml	0.8±0.004	0.775-0.840	ASTM D7042
Viscosity @- 20 ^{O}C	cSt	7±0.5	8 (Maximum)	ASTM D445
Flash point	^{O}C	38± 0.01	38 (Minimum)	ASTM D93
Water content	%	0	n.d	ASTM D6304
Total acidy	mg KOH/g	0.010± 0.0001	0.015(Maximum)	ASTM D3242
Total Sulfur	%	0.001± 0.0001	0.3 (Maximum)	ASTM D4294
Net heat of combustion	MJ/kg	46±1.6	42.8 (Minimum)	ASTM D4868
Freezing point	^{O}C	-42±1	-40^{o} C (Maximum)	ASTMD D2386
Conductivity	pS/m	180± 2	50-600	ASTM D2624

Table 24: Jet A1 characterisation: organic compounds data

Parameter	Results [% weight]	Method	Testing equipment
Aromatics	19±0.1	ASTM D2425	GC-MS
Paraffins	42± 0.2	ASTM D2425	GC-MS
Monocycloparaffins	26±0.38	ASTM D2425	GC-MS
Dichloroparaffins	5.8±0.4	ASTM D2425	GC-MS
Trichloroparafins	0.6± 0.03	ASTM D2425	GC-MS
Naphtalenes	1.4±0.01	ASTM D2425	GC-MS

Tables 23 and 24 were used as references to examine the differences between conventional jet fuel, algae-based jet fuel and the blended options. Overall, the data generated by the blended jet fuels are almost similar to the one for conventional jet fuel. They are some differences regarding some parameters as mentioned earlier. These are due to the fact that algae-based jet fuel was produced in the laboratory while the conventional jet fuel was produced in the refinery

plant with a high level of purity. This difference has an influence on the quality of blended jet fuels produced in this study.

6.4.1 Storage stability test

Jet fuel instability involves multi-step chemical reactions including some that can be oxidative reactions. A stable jet fuel is regarded as the one with properties that remain unchanged for a long period of time despite the change in environmental conditions.

Factors that lead to damaging changes in fuel properties are time (storage stability) and exposure to high temperatures in the engine (thermal stability). The storage stability test was undertaken in order to establish if physico-chemical properties of the blended jet fuels are time sensitive. Medium-term storage stability tests of at least 1000 h (~30 days) are required to confirm if some parameters that are time-dependent do not critically affect the quality of the blend [20, 21]. The storage stability test was undertaken after 30, 60, 90 and 120 days from the day of preparation. Jet A1 characterisation used in both blends is recorded in table 23 and it was not subjected to storage test because petroleum fuels do not have a problem with biodegradability as it could be the case with biofuels. In general, the analysis of the recorded data has revealed that the majority of parameters has remained compliant with regard to ASTM D1655 standards used for the 80/20 blended jet fuel. However, parameters such as density, total acidity and water content are found in high levels or beyond the limits required by ASTM D 1655 standards regarding the 20/80 blended jet fuel which is made up of a greater volume of algae-based jet fuel. This indicates that the 80/20 blended jet fuel is the most compliant to ASTM D1655 standards, therefore, it is potentially a sustainable option for blending. The 20/80 blending ratio is also an acceptable option to be considered for blending renewable jet fuel and conventional one provided that high level of purity of the algae-based jet fuel is achieved in order to be assured of the compliance level of the blend.

6.4.2 Density

Density at 15 ^{o}C is beyond ASTM limits as indicated in tables 19, 20 and 22. This situation is recorded only on the 20/80 blended jet fuel samples. Overall, it was found in tables 19 and 20 that the density of 80/20 blended jet fuel was lower than the one for the 20/80 blended jet fuel after blending. The same trend was observed during storage test period. This is probably due to the presence of more particulate matters found in the 20/80 blended jet fuel than in the 80/20 blended jet fuel. Particulate matters originate from algae-based jet fuel. Purification of algae-based jet fuel is highly required in this situation. This can be done by membrane filtration

process using microfiltration/ ultrafiltration, or else rectification process can also assist in the removal of particulate matters and water in order to adjust the density of the blended jet fuel.

6.4.3 Kinematic Viscosity

The analysis of data collected for viscosity indicates that kinematic viscosity complies with ASTM standards. It has remained below the maximum of 8cSt required by ASTM D1655 standards for Jet fuel. However, high values of viscosity are recorded for 20/80 blended jet fuel compared to 80/20 blended jet fuel, this is illustrated by the data recorded in tables 19 and 20. The same trend is observed for the data in tables 21 and 22 related to the storage test period where the viscosity has also remained compliant with standards.

6.4.4 Flash point

The analysis of the data presented from tables 17 to 22 (excluding table 18) shows that flash point is above the minimum of 38^{o}C required by ASTM D1655 standards required for jet fuel. It is respectively 66 ^{o}C and 79 ^{o}C for the 80/20 and 20/80 blended jet fuels.

This is one of strongest point for both blends, they have a great potential for safety and hazard related issues. Flash point can assist in terms of thermal stability when the blend is exposed to high temperatures in the engine. In this regard, based on the recorded data, both blends can be used in a hot environment where safety issues for fuels are not easy to be handled. Algae-based jet fuel could be the biggest contributor of the higher flash point. This is observed in tables 17, 20 and 22 where the higher flash points are recorded only in samples with a higher volume of algae-based jet fuel.

6.4.5 Water content

The analysis of data from tables 17 to 22 (excluding table 18) indicated that there is more water in 20/80 blended jet fuel compared to 80/20 blended jet fuel. ASTM standards for jet fuel have no exact range for water content. However, water should totally be removed from jet fuel as mentioned earlier.

6.4.6 Total acidity

The analysis of this parameter from the data in tables 17 to 22 (excluding table 18) revealed that total acidity is beyond the level required by ASTM standards. Total acidity was higher in 20/80 blended jet fuel compared to 80/20 blended jet fuel. Higher acidity is probably due to the presence of organic dissolved substances from the biomass used to produce algae-based jet fuel. Similarly, trace amounts of sodium, potassium, and other alkali metals in the jet fuel can cause corrosion in the turbine section of the engine. Furthermore, some microorganisms can

also generate acidic by-products that can speed up metal corrosion. To remediate to this situation, a corrosion inhibitor must be used to prevent negative effects of acidity on jet fuel distribution and usage network. The corrosion inhibitor is also a lubricity improver, consequently, the lubricity of the jet fuel will be improved. The fuel system components including more especially the pumps rely on the fuel to lubricate moving parts.

6.4.7 Total contamination

It was found that the contamination levels were lower compared to the limit described by IP440/SANS 52662 standards when analysing the data recorded in tables 17 to 22 (excluding table 18). However, despite the fact that total contamination complies with standards, it is higher in 20/80 blended jet fuel than in 80/20 blended jet fuel. The results indicate that algae-based jet fuel requires more purification in order to reduce sensibly the contamination before mixing it with a conventional jet fuel. Therefore, it is possible to conclude that in the current study algae-based jet fuel is the biggest contributor to the contamination for the blended jet fuels.

6.4.8 Total Sulfur

The analysis of data presented in tables 17 to 22(excluding table 18) indicated that sulfur content complies with the maximum required by ASTM standards for jet fuel. It was observed that sulfur content is lower in the 80/20 and 20/80 blended jet fuels after preparation as indicated in tables 19 and 20. The ideal situation will be to have a sulfur-free fuel; therefore, desulfurization can be undertaken if necessary in order to improve the purity of the final product.

6.4.9 Net Heat of Combustion

ASTM standards for jet fuel require a minimum of 42.8 MJ/kg for the net heat of combustion. The analysis of the data recorded in tables 17 to 22 (excluding table 18) shows that the values obtained regarding the net heat of combustion for both blends are complying with the ASTM requirements for jet fuel. The blended jet fuel 80/20 has a net heat of combustion slightly higher compared to the one for 20/80 blended jet fuel. The situation is probably due to the energy content of Jet A1 presented in table 23 which is higher than the one for algae-based jet fuel presented in table 17

6.4.10 Freezing point

The analysis of the freezing point data recorded in tables 17 to 22 (excluding table 18) indicates that values recorded for this parameter vary from -40 to -32 ^{o}C. The 80/20 blended jet fuel has a compliant freezing point. The presence of anti-freezing in high amount in the 80/20 blended

jet fuel coming from Jet A1 is the probable cause of this situation. However, the 20/80 blended jet fuel has a freezing point which does not comply with the ASTM standards, it is slightly higher than the minimum required by ASTM standards for aviation fuels. Algae-based jet fuel has an influence in this blend because of its higher freezing point presented in table 17. On the other hand, it was also recorded during the experiment that the crude algae bio-oil used in this study was not freezing even at the temperature beyond or below -40 ^{o}C. To improve the freezing point for the 20/80 blended jet fuel it is recommended to add more anti-freezing to the blend.

6.4.11 Conductivity

Analysing the data from tables 17 to 22 (excluding table 18), the conductivity remains within the range required by ASTM standards. However, despite the compliance, the 20/80 blended jet fuel has higher conductivities compared to the 80/20 blended jet fuel. This is due to the fact that algae-based jet fuel contains more dissolved substances and it less pure than Jet A1.

6.4.12 Organic compounds

Some few organic compounds were tested in both Jet A1 and algae-based jet fuel. The data is presented in tables 18 and 24. Except for the total aromatics which should be around a maximum value of 25 % in volume regarding ASTM specifications, there are no specified ASTM limits for the other parameters. Analysing the data recorded in both tables 18 and 24, it was found that there was no significant difference between the same organic parameters for both Jet A1 and algae-based jet fuel. The tests were not done on both blends due to limited resources, however, from the data of every single fuel, it is possible to predict that these organic compounds will have the same influence in both blend regarding their impact on physico-chemical parameters such as flash point, freezing point, viscosity etc

CONCLUSION

Blending conventional jet fuel with algae-based jet fuel in 80/20 and 20/80 ratios respectively can assist in reducing the dependence on fossil fuels while keeping the same level of sustainability. The 80/20 blended jet fuel with 80% of conventional jet fuel in volume has totally complied with ASTM D1655 standards for the parameters analysed in this study. The contribution of Jet A1 has had an influence on the outcome of this blend. However, regarding the 20/80 blended jet fuel made with 80% of algae-based jet fuel, the majority of parameters have complied with ASTM D1655 standards except for the freezing point, the density at 15 ^{o}C and the acidity which are slightly beyond the required ASTM D1655 standard values. The storage stability test was also undertaken after 30, 60, 90 and 120 days

from the day of preparation. The data recorded in this regard has revealed that majority of parameters have remained compliant with regard to ASTM D1655 standards for the 80/20 blended jet fuel. However, parameters such as density and total acidity were found to be beyond the limits required by ASTM D 1655 standards for the 20/80 blended jet fuel during the entire storage period. The 80/20 blended jet fuel was fully compliant to ASTM D1655 standards and it can be a sustainable option for certification.

The 20/80 blended jet fuel is also an acceptable option for certification based on the data generated in this study. However, it is imperative to purify or upgrade further the algae-based jet fuel in order to get a highly purified product. This will improve the density, reduce sensibly the contamination, water content, dissolved substances and the particulate matters. From the data collected in the current study, blending in 80/20 and 20/80 can both be explored as one of the options to reduce dependence on fossil fuels and carbon footprint. More studies have to be done in terms of additives to improve the quality of the blends and enhance the possibility for certification for both blends.

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REFERENCES

[1] Jungmeier G, Hingsamer M, van Ree R, Joergenson H, Wellisch M, Stichnothe H, Rauch R, de Jong E, De Bari I. Biofuel-driven Biorefineries A selection of the most promising biorefinery concepts to produce large volumes of road transportation biofuels by 2025. Report of IEA Bioenergy Task. 2013;42.

[2]. Atabani AE, Silitonga AS, Badruddin IA, Mahlia TM, Masjuki HH, Mekhilef S. A comprehensive review on biodiesel as an alternative energy resource and its characteristics. Renewable and sustainable energy reviews. 2012 May 31;16(4):2070-2093.

[3] Moore RH, Thornhill KL, Weinzierl B, Sauer D, D'Ascoli E, Kim J, Lichtenstern M, Scheibe M, Beaton B, Beyersdorf AJ, Barrick J. Biofuel blending reduces particle emissions from aircraft engines at cruise conditions. Nature. 2017 Mar 16;543(7645):411-415.

[4] Agarwal AK, Agarwal RA, Gupta T, Gurjar BR. Introduction to Biofuels. In Biofuels 2017 pp. 3-6, Springer Singapore.

[5] Demirbas MF, Balat M, Balat H. Potential contribution of biomass to the sustainable energy development. Energy Conversion and Management. 2009 Jul 31;50(7):1746-1760

[6] Amigun B, Musango JK, Stafford W. Biofuels and sustainability in Africa. Renewable and sustainable energy reviews. 2011 Feb 28;15(2):1360-1372

[7] Dafnomilis I, Hoefnagels R, Pratama YW, Schott DL, Lodewijks G, Junginger M. Review of solid and liquid biofuel demand and supply in Northwest Europe towards 2030–A comparison of national and regional projections. Renewable and Sustainable Energy Reviews. 2017 Oct 31;78: 31-45

[8] Janda K, Stankus E. Quantification of Biofuels Potential of Post-Soviet Countries in the Context of Global Biofuels Development, 2017

[9] Lesage D, Van de Graaf T. Global energy governance in a multipolar world. Routledge; 2016 Apr 22.

[10] Heinberg R, Fridley D. Our renewable future: laying the path for one hundred percent clean energy. Island Press; 2016 Jun 2

[11] Omer AM. Energy, environment and sustainable development. Renewable and sustainable energy reviews. 2008 Dec 31;12 (9):2265-3000

[12] Panwar NL, Kaushik SC, Kothari S. Role of renewable energy sources in environmental protection: a review. Renewable and Sustainable Energy Reviews. 2011 Apr 30;15(3):1513-24.

[13] Al-Maamary HM, Kazem HA, Chaichan MT. The impact of oil price fluctuations on common renewable energies in GCC countries. Renewable and Sustainable Energy Reviews. 2017 Aug 31;75: 989-1007

[14] Saxena RC, Adhikari DK, Goyal HB. Biomass-based energy fuel through biochemical routes: a review. Renewable and Sustainable Energy Reviews. 2009 Jan 31;13(1):167-178.

[15] Guldhe A, Singh B, Renuka N, Singh P, Misra R, Bux F. Bioenergy: A Sustainable Approach for Cleaner Environment. In Phytoremediation Potential of Bioenergy Plants 2017 pp. 47-62, Springer Singapore

[16] Blakey S, Rye L, Wilson CW. Aviation gas turbine alternative fuels: A review. Proceedings of the combustion institute. 2011 Dec 31;33(2):2863-2885

[17] Liu G, Yan B, Chen G. Technical review on jet fuel production. Renewable and Sustainable Energy Reviews. 2013 Sep 30; 25:59-70

[18] Bauen A, Howes J, Bertuccioli L, Chudziak C. Review of the potential for biofuels in aviation. Prod. E4Tech. 2009 Aug.

[19] Bwapwa, J.K., Anandraj, A. and Trois, C., Microalgae processing for jet fuel production. *Biofuels, Bioproducts and Biorefining*, 2018, in press

[20] Bondioli, P., Gasparoli, A., Lanzani, A., Fedeli, E., Veronese, S. and Sala, M., Storage stability of biodiesel. Journal of the American Oil Chemists' Society,1995, 72(6), pp.699-702

[21]Bondioli, P., Gasparoli, A., Della Bella, L., Tagliabue, S. and Toso, G., Biodiesel stability under commercial storage conditions over one year. European Journal of Lipid Science and Technology, 2003, 105(12), pp.735-741

CHAPTER 7 PREFACE

The current chapter summarizes the main findings and their significance. The overall approach and the findings to produce aviation fuel from microalgae are highlighted. These findings display various aspects of knowledge contribution generated from this study. Recommendations for more studies in the future are presented in the last part of the chapter.

CHAPTER 7: CONCLUSIONS AND RECOMMENDATIONS

7.1 BACKGROUND AND RATIONALE

The total economic value of trade in the algae industry is projected to be worth 1.1 billion $ from 2016 to 2024 according to a market report released by Transparency Market Research in 2016. With an increase in the number of algae start-up companies, many projects are driven by public and private investments. It aims to set up laboratory and pilot facilities to develop cutting-edge technology for sustainable algae-based fuels. Microalgae-based fuels are part of the green solution and of many initiatives from environmental lobby groups to drive alternative fuels to fill this innovation chasm. A commitment to fostering technologies that could yield substantial benefits over time is the current trend that is taking place in the algae biotechnology sector. To date over 50 commercial industries are successfully producing fuel derivatives from microalgae. Among others, Algeniol, Sapphire, Aurora Algae, Eldorado Biofuels, Genifuel, HR Biopetroleum, and Petro Algae are all specialising in algae products. This aims to achieve cost-competitive and sustainable algal biofuels. The current study has focused on the production of jet fuel from algae biomass.

The cultivation, the production of biomass and hyper lipid synthesis, the conversion of algae bio-oil to jet fuel, process design and the blending with commercial Jet A1 showed further

progress towards a biofuel with a superior specification. The product from this study was algae based-jet fuel with most of the higher characteristics complying with ASTM D 1655 standards used to test conventional aviation fuels.

With regard to the processing of microalgae to jet fuel (**Chapter 3**), high amounts of biomass were recorded between day 8 and day 10. Maximising biomass production under defined operating conditions of temperature, light intensity and saline domestic wastewater (nitrate and phosphate loading) contributed to supplement the algae broth, reducing the cost of supplying nutrient for growth. Also, it assists in the removal of nitrates and phosphates from saline domestic wastewater. Microalgae cultivation temperatures ranged between 15 and 35^{o}C under a luminance of 1000 lux and a daily supply of CO_2 for a period of 15 days.

The species *Nannochloropsis sp* grew effectively between 15 to 25^{o}C with more biomass produced within the same temperature range. During hyper lipid synthesis, more than 80% of lipid increase was recorded under total nutrients starvation of microalgae cells in a highly purified water environment after a period of 3 days. Previous studies have so far used a partial starvation with nitrogen or phosphorous deprivation. Once maximum lipids have been synthesised, cells were ruptured releasing oils and through a centrifuge, biomass and lipids were separated. Subsequently, solvents were used for extracting crude oil and then recovered through evaporation. Thermal cracking was initiated without a catalyst at 300 ^{o}C for 31 min. The gas chromatograph showed all long-chain hydrocarbons were broken at this temperature and period. Alternatively, a fast cracking of hydrocarbon chains known as pyrolysis was initiated to compare cracking efficiency. Pyrolysis at 450 ^{o}C was recorded for cracking of crude bio-oil for a time of 12 s. Fractionation of bio-oil between 70 ^{o}C and 250 ^{o}C occurred post cracking, to produce middle-end distillates representing the jet fuel fraction. All carbon fractions collected within the temperature range between 70 and 250 ^{o}C and were identified as alkanes. Most physico-chemical parameters of these alkanes (impure) were within the range prescribed by ASTM standards except freezing point and density. The freezing point was recorded at -30 ^{o}C which was found to extend beyond the standards required by ASTM D1655 (-40 ^{o}C). Impure alkanes made of minute particulates prevented the alkane mixture from reaching -40 ^{o}C. Similarly, the presence of particulates in the alkane mix increased its density to higher than ASTM D1655 standards. The removal of particulate matter can improve the density and the freezing point. Microfiltration, ultrafiltration, reforming and upgrading can remove particulate matter and enhance the quality of algae-based jet with a complying freezing point. The addition of antifreeze is also required to ensure that the jet fuel will not solidify.

A conceptual design using ASPEN HYSIS V8.8 was developed (**Chapter 4**) and the process simulation was successfully completed with data from the laboratory.

The following unit processes were part of the conceptual design: Biomass cultivation, biomass harvesting, physiological modification, bio-oil extraction, hydrocracking, fractionation, reforming and upgrading. The optimization of temperature values for various processes was also completed in order to enable the simulation and process design.

The cracking of crude bio-oil was simulated at 350 ^{o}C. The simulated pressure for the distillation was 1 bar. The same pressure was used for the flash vessel collecting some middle-end fractions mixed with the vapour phase. The solvent used during simulation was a mixture of chloroform and methanol in a 1/1 ratio. The simulated overall conversion rate was 85 %. The simulation used saline domestic wastewater loaded with nutrients as an effective growth media. It will assist in reducing the use of freshwater resources. CO_2 from fossil sources can also be collected and used as an additional nutrient for microalgae during cultivation. The growth medium can be either open ponds or a photobioreactor depending on the available possibilities and the environmental conditions.

Blending algae based-jet fuel and Jet A1 was also part of this study (**Chapters 5 and 6**), blending improves sustainability and carbon footprint. Blending assists also in producing less fossil fuels which are currently being depleted. Two blending options were completed; B50 and B20.

Regarding the 50/50 blending known as B50 (**Chapter 5**), most selected parameters were complying with ASTM standards. However, parameters such as density at 15 ^{o}C and total acidity were found non-compliant to ASTM standards. Non-compliance of properties could be due to the fact that the algae-based jet fuel needed more polishing steps. Therefore, further polishing processes such as reforming and upgrading to reach the same level of purity as Jet A1 will be required for algae-based jet fuel. Membrane filtration or rectification process to improve the density, the total acidity and reduce sensibly the contamination level can be used as a polishing step. Storage stability tests were undertaken on the 30, 60, 90 and 120th days after the 50/50 blended jet fuel preparation. They have indicated that most parameters were stable and complied with ASTM standards. The 50/50 blended jet fuel can be produced at large scales and commercialised, many physico-chemical parameters comply with ASTM standards.

Furthermore, the presence of algae-based jet fuel makes this blend environmentally friendly. However, the costs effectiveness related to the B50 blend should be completed to confirm the affordability aspect.

About the 80/20 and 20/80 blends both known as B20 (**Chapter 6**), it was found that the 80/20 blended jet fuel made with 80% of Jet A1 and 20 % of algae-based jet fuel (in volume), totally complied with the used ASTM D1655 standards. This option is recommendable for ASTM certification. The amount of Jet A1 in the blend had a significant influence on the compliance aspect. However, regarding the 20/80 blended jet fuel made with 20 % Jet A1 and 80% of algae-based jet fuel, all parameters have complied with ASTM standards related to jet fuel except the freezing point, the density at 15^{o}C and the acidity which are slightly beyond the required ASTM D1655 standard. Fuel storage tests for the 80/20 blended jet fuel completed after 30, 60, 90 and 120 days from the production day, have shown that parameters are stable with regards to ASTM D1655 standards. Density and total acidity for the 20/80 blended jet fuel were not complying with ASTM D165. They were beyond ASTM D1655 standards during the storage period. They can be adjusted with advanced filtration and polishing steps mentioned earlier to adjust high density and acidity. Overall, blending in B20 can be explored as one of the options to reduce dependence on fossil fuels and carbon footprint.

Additionally, the connection of all the steps involved from biomass production to jet fuel was the main challenge encountered during the course of this study. It is important to stress the fact that all these steps are interconnected and interdependent. However, lipid hyper synthesis is a very critical step which is more than needed to supply a high amount of bio-crude oil for conversion into jet fuel. Operating conditions during conversion process are vital for each unit process to produce quality jet fuel. The conversion processes used during the experiment were related to the production of conventional jet fuel. This can assist in the future to get algae-based jet fuel similar to conventional jet fuel. The similarity should be in terms of costs and quality to suit the market expectations. It is achievable through innovation only.

The algae industry is currently in expansion and its growth is very fast because of the wide range of products from various species and strains. Products of algae are valuable because traded on major stock markets. It is an indicator of how algae business in general and algae biotechnology is moving toward a great revolution or innovation.

The algae-based fuel industry, especially the one for jet fuel, is among the most exciting, many innovative studies are currently underway. Majority of these studies are generally patented and

hidden in the private domain. A small amount of these innovations is released in the public domain due to high competitivity in the aviation industry.

The price of the barrel fluctuates every so often, producing countries are ruling the market. The current price of the barrel is about 66$ for WTI crude and 73$ for the Brent crude. According to the International Air Transportation Association, the average price for the barrel of jet fuel is about 85 $/bbl for 2018. Also, the impact on 2018 fuel bill of the global airline industry is currently evaluated at 42 billion $. Given the current expansion of the algae market, the size of investments, the competitiveness and innovations in the algae biotechnology industry, algae-based jet fuel can be valuable and competitive in the near future.

7.2 RECOMMENDATION AND FURTHER STUDIES

Based on the findings of this study, the following recommended research have emanated and can be undertaken in future studies:

1. Domestic wastewater is an effective growth media because of the abundant presence of nutrients. Therefore, future studies on the effective growth of species in domestic wastewater to produce more biomass and lipids will be required.

2. The produced algae based-jet fuel is quite similar to conventional jet fuel with regard to the compliance aspects. However, the objective is to make it a ‘’drop in ‘’ jet fuel.

Therefore, polishing processes such as upgrading and membrane filtration can be essential for the removal of particulate matter to improve the density and algae-based jet fuel purity.

3. Freezing point is one of the most important parameters required for jet fuel. Algae-based jet fuel produced had a freezing point superior to the minimum required by ASTM standards.

The addition of an antifreeze to adjust the freezing point at the required by the standards is required.

4. A conceptual design was developed with unit processes related to the production of a conventional jet fuel. The simulation of the process was successfully run as mentioned before. Therefore, the unit process used to produce conventional jet fuel are also relevant for algae-based jet fuel production. A detailed design study including equipment modelling, sensitivity analysis and costs analysis is indispensable using the conceptual design as a basis.

5. Algae-based jet fuel is known as a low carbon footprint because its crude is non-fossil oil, carbon footprint studies should be completed to quantify carbon emission between algae-based jet fuel, blended jet fuel and various conventional jet fuels.

6. Cracking of algae crude bio-oil was successfully completed. Pyrolysis is the fastest and thermal cracking without catalyst was lengthy but both are effective. Other routes such as biomass gasification can be analysed and used for conversion of algae biomass into jet fuel. A study regarding algae biomass gasification can be undertaken in that regard.

7. Life Cycle Assessment (LCA) was not part of the study. However, LCA studies can be completed once the technology has reached a maturity level and it is ready for commercialisation.

8. B20 blended jet fuels are compliant with ASTM aviation standards. Currently, these blending ratios undertaken in the study are not ASTM certified. Further characterisations, blend performance monitoring, sustainability, carbon emissions and cost analyses will provide more insights to persuade the relevant bodies for their certification. Parameters such as aromatics content, hydrogen content, distillation tests for product recovery, smoke point, naphthalene content and thermal stability can also be explored to make these blending ratios more credible for future certification.

APPENDICES

APPENDIX A1

PUBLISHED ARTICLES AND BOOK CHAPTER (under review)

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Possibilities for conversion of microalgae oil into aviation fuel: A review

Joseph K. Bwapwa[a,b,*], Akash Anandraj[b], Cristina Trois[a]

[a] University of Kwazulu-Natal, South Africa
[b] Mangosuthu University of Technology, South Africa

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ABSTRACT

The aviation sector relies on petroleum jet fuel because it is the most efficient energy carrier. Due to environmental and economic concerns a strong demand for alternative fuels is emerging. There is a need for diversification of energy sources from natural resources. These resources must be environmentally friendly and costs effective. Environmental impacts of fossil fuels on global warming and climate change are being a major concern today. Furthermore, the fluctuations of oil prices and need for sustainable fuel supply are the strong drivers for the economies of fuel users. In the aviation sector, Jet fuel from microalgae is one of the alternatives receiving considerable attention; it offers the potential to diversify energy sources. Microalgae species can produce lipids; they do not require high use of land, do not need freshwater, can grow in marine water or wastewater, grow faster in very short period of time, the produced oil is not a threat to food security. Similarly, the effect of climate change and global warming due to the generation of greenhouse gases (GHG) from petroleum jet fuel can be considerably reduced due to low carbon footprint generated by algae based fuels. Therefore, algae based aviation fuels can be considered as an alternative to produce an efficient fuel compared to conventional fuels. Conversely, the key challenge is: many algae species have lower lipid content. Harvesting and drying processes are costly as well as upstream processes to convert microalgae oil into Jet fuel. Although algae biofuels are still small players in the aviation industry, there is a potential for the future. This review analyses some routes to be explored or already explored, their strengths and weaknesses, the current trends and possible conceptual approaches to get aviation fuel from microalgae oil.

1. Introduction

Global reserves of petroleum oil have decreased significantly during the last decades; a probable energy crisis could severely affect the world in the near future. Environmental and economic concerns are pressurizing the aviation industry to an extent of strongly influencing the industry growth. Despite the implementation of many international treaties aiming for the promotion of environmental sustainability, the aviation industry is requested to work toward the reduction of its environmental impacts. It can only be achieved by developing new technologies or by upgrading the current ones. Therefore, this will successfully promote the concept or idea of green aviation. An average of 705 Mt of CO_2 has been generated in 2013 from airlines operations; it is almost 2–3% of global anthropogenic CO_2 emissions [1]. Predictions show that this figure will increase between 1000 and 3100 Mt by 2050. The aviation industry has given itself a target to reach a carbon neutral growth status by 2020 [1]. It is achievable through the pillars of innovation with main focus on operations, air traffic management, environmental protection, safety and fuel processing technology and sustainability. Focusing on the last one, renewable energy from biofuels may constitute an acceptable alternative and possibly a reliable option in case the energy crisis deepens in the near future. However, production costs for bio- Jet fuels are relatively higher compared to those for conventional Jet fuels. Consequently, the selling price may become exorbitant. Higher production costs are attributed to factors such as relative immaturity of the technology, small number of active producers; producers of biofuels are focusing more on novel end products because of low funding in the biofuel markets. Also, the costs of raw materials may be another cause of higher production costs for biofuels. It is reported that 80% of the operating costs is made up of raw materials costs regarding biofuel/ biodiesel production [2]. Therefore, it is necessary to look for suitable and alternative raw materials or crops capable of producing non-edible oil at low costs to produce biofuels than using edible vegetable oil for biofuel production.

Microalgae species such as nannochloropsis sp, tetraselmis sp, Chlamydomonas sp, Synechococcus sp. and many others can be an acceptable alternatives compared to the edible oil used domestically because they do not constitute a threat to food security. They are

* Corresponding author at: University of Kwazulu-Natal, South Africa.
E-mail addresses: joseph@mut.ac.za, josephkapuku@gmail.com (J.K. Bwapwa).

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photosynthetic cells growing at very high rates in an aquatic environment. Also, they are considered as emerging alternatives for biofuel production because their composition reveals a remarkable presence of fatty acids and lipids. Fatty acids and lipids are the main sources of energy for biofuels. Similarly, According to Rodolfi (2009) [3], they have attracted great attention in the biofuel market because of their high capacity to accumulate abundant amounts of triacylglycerols (TAG). These species are mainly used in the production of biofuels such as biodiesel, bio-hydrogen and bio-Jet fuel. Bio-Jet fuel from algae is gaining more interest and might become a very important deal with global implications in the near future. Firstly, there is a great focus on aviation biofuels to reduce the impact of climate change and global warming. This will also assist in reducing the operating costs in the aviation industry. Secondly, the petroleum or conventional Jet fuel prices and their volatility have a severe impact on the business of airlines: because of fuel price fluctuations there are rippling repercussions to economies of many airlines as well as many countries. Furthermore, there is a big market for Jet fuel which is currently being served partially and the demand has kept its increasing trend due to the need which is growing yearly. The world consumption of Jet fuel has almost tripled in 30 years; from 1,837,000 barrels/per day in 1980, to 5,220,000 barrels per day in 2010. About 30% of the world consumption was used in the United States (1,398,130 barrels per day in 2012) [4]. These data are used for information regarding the trends but new data should be more informative.

Producing algae Jet fuel will require a comprehensive identification of the potential technical, economic and environmental challenges. Once these challenges are clearly identified and solved properly, there is a possibility to make bio-Jet fuel from algae “a drop in” fuel which will strictly comply the aviation fuel standards. The objective of this review is to highlight, analyse and suggest some possibilities/ processes or routes that will lead to Jet fuel from microalgae oil. This study focuses on downstream processes, the feasibility, their strengths and weaknesses. It is important to stress on the fact that various routes for algae Jet fuel production are technically possible but not costs effective and commercially viable to compete with conventional Jet fuel. The economic studies and historical developments are not part of this article as they can be part of another detailed and specific study in the future.

2. Common points between petroleum crude oil and algae crude bio-oil

Algae bio-crude oil extracted from species such as Nannochloropsis sp, Schizochytrium sp, Botryococcus braunii and many other species may have many similarities with petroleum crude oil in terms of hydrocarbons content and molecular structure [5]. The majority of petroleum crude oil originates from algae in marine environment or ancient algae deposit [6]. Petroleum crude oil consists largely of liquid hydrocarbons because it is mainly made up of carbon and hydrogen. On average 85% w/w of oil is carbon, 10–14% of oil is hydrogen, oxygen accounts for 1–2% and sulphur represents up to 4% of the oil total weight [7]. Petroleum crude oil originates from a compound named Kerogen; this compound is created in marine environment by sedimentary rocks from a series of biochemical and/or chemical reactions called diagenesis and catagenesis [8]. It is transformed into crude oil under specific conditions of pressure and temperature. It is largely composed with algae, biodegraded organic compounds, plankton, bacteria and plant material. Various forms of Kerogen have different amounts of hydrogen relative to carbon and oxygen [8]. Qualitatively, algae crude bio-oil have a similar molecules compared to petroleum crude oil, however, there are some differences quantitatively [5]. This is based on the fact that petroleum crude oil is derived from Kerogen and algae is part of Kerogen as mentioned before. However, algae crude bio-oil may differ slightly from petroleum crude oil depending on species features: the type and nature of species, as well as the species lipids content. Therefore, one of the main focuses of algae biotechnology is the development of biofuels making use of microalgae as the main raw material.

3. Compliance and current drivers for alternative jet fuels

The biggest challenge for algae biofuel producers is to make a Jet fuel which is totally compliant to stringent regulations as it is the case with conventional Jet fuel. Compliance is mainly focusing on physico-chemical properties or parameters such as cold flow property, energy density, energy content, kinetic viscosity, freezing point, concentration of aromatics, material compatibility, safety properties, flash point and thermal stability. The concept of compliance must also at the time be centred on combustion and kinetic aspects such as ignition and extinction characteristics, chemical kinetics, lubricity flame speed and flammability limits. Table 1 provides an overview of some requirements based on physico-chemical characteristics, the operational purpose as well as the specifications. Furthermore, compliance implies that Jet fuel composition must display specific physico-chemical characteristics that are critical for the fuel to perform efficiently. These features must strongly correlate with the composition of Jet fuel knowing that the composition depends mainly on the raw crude oil, the type of refining process used and additives to improve the quality of Jet fuel. Generally, conventional Jet fuel composition is made up roughly of 20% paraffin, 40% isoparaffin, 20% naphthenes and 20% aromatics [9]. However additions are made to the Jet fuel in order for it to comply with environmental aspects and the expected performance. This data is given for information only and reports only on the main components of jet fuel. These components play a crucial role in the effectiveness of the fuel during operation. For instance, the heat density per unit mass of the fuel is greatly improved when the ratio hydrogen-to-carbon from isoparaffins and paraffins is high; another fact is that naphthenes assist

Table 1
Summary of important jet fuel properties (derived from [11]).

Parameters	Purpose
High energy density	Impacts on the aircraft range
Low freezing point	Influences pumping capacity of the fuel at lower temperature
High thermal stability	Clogging or fouling of fuel system and nozzles occur generally due to Coke and gum deposits during combustion. It prevents the chemical decomposition of the fuel.
Viscosity	Influences the ability of the fuel nozzles to spray fuel and the capacity of the engine to restart at higher altitudes.
Combustion features	To minimise the formation of particulates in combustor and in exhaust
Adequate lubricity	Affects the ability of the jet fuel to lubricate fuelling system and engine controls
Material compatibility	The Jet Fuel interacts with metals and many others materials, it necessary to ensure an effective compatibility with materials in contact with jet fuel.
Safety	Avoidance of explosions during handling of the jet fuel and during its storage into containers
High specific energy	Decreases take-off weight and helps to improve the efficient of the fuel
High flash point	Allow the fuel ignition for safe operation
Aromatic compounds	Must be sufficient because it allows acceptable seal swell to prevent leaks in the fuel system.

Table 2
Carbon chains content in algae oil [% w] for some species (adapted from [18–20]).

Fatty acids	Nannochloropsis salina [18]	Phaeodactylum tricornutum [19]	Botryococcus braunii [20]
C12:0	5.0	–	0.7
C14:0	–	4.5	0.8
C15:0	0.5	–	0.5
C16:0	37.5	25.8	21.0
C16:1	23.3	37.5	2.0
C16:2	–	–	6.5
C16:3	–	–	15.2
C17:0	0.4	–	0.1
C18:0	0.9	1.3	2.9
C18:1	11.9	–	3.2
C18:2	1.5	5.1	13.6
C18:3	–	2.0	33.0
C20:0	0.1	–	0.2
C20:1	–	–	–
C20:4	3.3	1.6	–
C20:5	15.3	13.1	–
C22:0	0.4	–	0.1
C22:1	–	–	–
C24:0	–	–	0.2
C24:1	–	–	–

in the reduction of the freezing point, which is very critical at higher altitudes; aromatics contribute largely to material compatibility and prevent leaks in the seals of some aircraft [9];[10];[11]. Therefore, the composition of Jet fuel can be regarded as a key aspect for compliance with the stringent standards used for aviation fuels which are namely ASTM D1655 of the American Society for Testing and Materials (ASTM) and Def Stan 91-91 of the UK Ministry of Defence.

Alternative Jet fuels such as algae Jet fuels will require to satisfy to many expectations which are described as follows: the reduction of emissions that have an impact on climate change and global warming, the diversification and expansion of energy supplies, Jet fuel should be less expensive in terms of capital and operating costs, there should be a possibility of scaling up without major constraints and producing large volumes of fuel without adversative effects on water and land resources, bio- Jet fuel must perform as a" drop-in '' substitution or support for the conventional Jet-fuel [11]. These characteristics can be summarized into three main aspects: algae based Jet fuels must contribute to solving problems requiring efficient environmental sustainability, beneficial economic sustainability and energy diversification [11]. To solve this issue there are challenges that need to be addressed effectively in order to meet compliance requirements.

4. Algae based aviation fuel: challenges

A part from the compliance aspect mentioned earlier, algae aviation fuels are faced with three types of challenges: biological, chemical and mechanical. In terms of biological challenges, strain selection is regarded as one of the main aspects to be considered because each strain is characterised by its lipid content [5]. Lipid content can affect biofuel productivity or yields. Furthermore, Nutrient requirements and circulation, growth rates, lipid productivity and optimization of photosynthetic efficiency are very important. They have to be meticulously monitored because they constitute the basis of an efficient growth [5,12,13,64]. The algal strains to be cultivated should be selected based on lipid production, biomass productivity, harvest ability, resistance to contamination, optimal light intensity, tolerance of high oxygen levels and temperature extremes. Individual and collective effects in terms of biomass productivity, lipid contents of four strains of microalgae was recently studied by Eloka-Eboka and Inambao[64], the outcome is that the nature of strain/species has direct impact on lipid biosynthesis, biomass productivity and the type of lipids produced. Chemical challenges are faced when dealing with processes such as oil extraction using a solvent, transesterification, chemical harvesting, quality of water and removal of toxic substances in water or wastewater used for algae cultivation [5,14]. Mechanical challenges are mainly based on maintenance issues and costs for cultivation units, harvesting, drying and oil extraction equipment such as oil press or impeller [5]. All these challenges make the production of Jet fuel from microalgae being more challenging but very possible if an efficient process optimization can be undertaken after a viable process design.

5. Potential for microalgae oil to be used for jet fuel production

Carbon chain lengths in microalgae oil are almost similar compared to the ones in the crude petroleum as mentioned earlier. Generally, commercial and military Jet fuels have carbon chains length ranging between 8 and 16 (C8 to C16) [15]. The conversion of microalgae oil into Jet fuel will require reducing the number of carbon chains within the required range as mentioned earlier. It can be achieved through the breaking down of long carbon chains under specific operating conditions defined by temperature, pressure and use of catalysts. This catalytic process aims to connect carbon molecules in order to get the configuration or chemical structure known for Jet fuel. However, carbon chain configuration is not the only requirement to get a compliant fuel; there is also lipids content which is extremely important to acquire high output during downstream processes. It is therefore important to select strains with higher content in lipids in order to generate great output from conversion processes. Lipid characterisation is one of the most important steps to be undertaken before any study involving the production of algae biofuels. It helps determine the suitability of the strain or species for biofuel production. Table 2 presents a summary of some previous studies completed on carbon chain profile for three species. The analysis of the data in Table 2 reveals that these species are potentially suitable for aviation fuel or any other biofuel.

The study confirmed that structure or carbon molecule configuration was made up of high energy loaded molecules capable of being converted into biofuels [16]. Therefore, the possibility to produce Jet fuel from various microalgae species can be certain to some extent but more work needs to be undertake regarding the process. The persisting challenges are the costs related to growth, harvesting and drying. Also, the lipid content for many species is relatively low. It is very important to select species with high lipids content for a viable Jet fuel production. More studies on species with high lipid content are still progressing specially in the private domain. For low lipid content species, there is a tremendous need for improving lipid content using lipid boosting techniques such as total or partial nutrients starvation or the use of genetically modified organisms. Conventional Jet fuel is made up of complex mixtures of hydrocarbons in large numbers. The molecular weights or number of carbon chains is defined by the requirements expected for the final product, for instance the freezing point, viscosity, density, volatility or smoke point. Biofuel physico-chemical properties improve considerably if both polar and non-polar lipids are involved in the transesterification reaction; some studies based on making use of total lipids (polar and non-polar lipids) to generate biofuels by transesterification have demonstrated positive outcomes [17]. Furthermore, lipid synthesis can be modified by changing growth conditions, this is particularly valid for non-polar TAGs known as the best substrate for biofuels production [3].

6. Oil yields for species to be used for algae based jet fuel

Table 3 presents outputs for microalgae oil being extracted from dry biomass for twenty different types of species. These species are potentially suitable for biofuels especially for Jet fuel production due to their lipid content. This information may be useful for evaluation studies in order to estimate the oil yields to be generated for down-

Table 3
Output in terms of oil content for some algae species (adapted from [23–26].

Microalgae species	Output: mass of bio- oil per ton of dry biomass [kg/ t]
Botryococcus braunii	250–750
Nannochloropsis sp.	310–680
Schizochytrium sp.	500–770
Neochloris oleoabundans	350–540
Nitzschia sp	450–470
Ankistrodesmus TR-87	280–400
Chlorella sp	290
Chlorella protothecoides (autotrophic/ heterothrophic)	150–550
Crypthecodinium cohnii	200
Cyclotella DI- 35	420
Dunaliella tertiolecta	360–420
Hantzschia DI-160	660
Nannochloris: 31	60–630
Neochloris oleoabundans	350–540
Nitzschia TR-114	280–500
Phaeodactylum tricornutu	310
Scenedesmus TR–84	450
Stichococcus	90–590
Tetraselmis suecica	150–320
Thalassiosira pseudonana	210–310
Euglena gracilis	140–200
Hormidium sp	38
Phaeodactylum tricornutum	200–300
Pleurochrysis carterae	300–500
Chlamydomonas reinhardtii	210
Prymnesium parvum	220–380
Tetraselmis sueica	150–230
Chlorella emersonii	280–320
Hormidium sp	380
Chlorella pyrenoidosa	470
Chlorella vulgaris	140–220
Crypthecodinium cohnii	200
Dunaliella tertiolecta	360
Dunaliella salina	60
Dunaliella primolecta	230
Cylindrotheca sp.	160–370
Phaeodactylum tricornutum	200–300
Pleurochrysis carterae	300–500
Scenedesmus dimorphus	160–400
Scenedesmus obliquus	120–140

stream or conversion processes. It may also be very useful for processes, plant design, mass and energy balance. To increase the species lipid content, physiological modification can be undertaken as mentioned earlier. The success of physiological modification or stress will depend on the type, nature and behaviour of each species under stress conditions. Table 3 also indicates that Botryococcus braunii, Nannochloropsis sp and schizochytrium sp. have high lipid content looking at the average between their lower and maximum lipid content. Many other species indicated Table 3 may have also a great potential to produce biofuel or aviation biofuel but not much information is available in the public domain to analyse their potential in producing aviation fuels. Botryococcus braunii has a molecule structure which is roughly similar to the gas-oil fraction of the petroleum crude oil; it is potentially fit to be used for blending with other fuels for Jet fuel production [21]. However, one of the concerns with Botryococcus is that its growth is very slow. It takes nearly a week for one Botryococcus cell to double [22]. Generally, many studies are targeting Nannochloropsis, Schizochytrium and Botryococcus to produce biofuels and health related products. Apart from the challenge of generating high lipid content for many species, there is another challenge: to get sustainable growth of algae biomass with high productivity rate in a short period of time. This can be achieved by implementing effective operating conditions, supplying adequately nutrients and monitoring growth.

7. Biomass drying and bio-oil extraction

After cultivation algae biomass is harvested in order to proceed with downstream processes. Biomass water content increases the biomass volume and can make the dewatering/ harvesting process time and energy consuming. Solar energy for biomass drying can be considered as an alternative; however, the main weakness is the necessity of time and space which are key components for an optimal and efficient process [27]. However, it is possible to optimize the time and create the space using an effective design. Hydrothermal processing is known as an effective substitution approach for biomass drying. In this process, microalgae are exposed to high temperature and pressure to allow the breaking apart of the biomass [28]. Harvesting and drying can be skipped by using the solvent extraction method immediately after cultivation. In this case solvent can be added to wet biomass after cultivation. Due to difference of densities, oil will settle at the top and biomass and water will be at the bottom of the separating funnel or sedimentation tank. Extraction of bio-oil from algae is successfully achieved with solvent such as dimethyl ether (DME) or with the mixture of chloroform and methanol. The solvent can be recovered by evaporation at lower temperatures; therefore, lower energy consumption is recorded as compared to harvesting and drying options. A part from solvent extraction, bio- oil can be extracted mechanically using cell homogenizers or ultrasound [29]. In homogenizers high pressures are applied to disrupt cells and they are mostly used for proteins extraction [30]. Regarding the ultrasound method cells are disrupted by applying sonic waves at frequencies greater than 20 kHz [31]. Alga cell walls are broken mechanically by ultrasounds due to cavitation shear forces on the walls; therefore, oil molecules are migrating easily from the cells to the solvent.

This method is still used at laboratory level, it is not still yet possible to operate at large scale with ultrasound because of high capital costs and also high acoustic energy is needed for large volumes of microalgae [30]. The effectiveness of ultrasound-assisted extraction was investigated for a long time and it is well known in the field of bio-oil extraction. It is successfully used for extraction of proteins, lipids, phenolic compounds, and enzymes on laboratory scale as mentioned earlier. Generally, the extraction of bio-oil from various species has used solvent extraction in most cases because of the simplicity of the process, low costs and low energy consumption.

8. Conversion of algae into bio-jet fuel: existing and novel conceptual approaches for conversion form algae bio-oil to bio-jet fuel

There are several options that may lead to algae based Jet fuel. These include biomass to liquid processes with gasification as the main process, biomass pyrolysis, and biomass fermentation. Some of these processes have reached a commercial level and ASTM certification depending on their maturity, the fuel readiness and the opportunity they may present for the aviation industry. However, new alternatives are arising to close the gap in terms of fuel efficiency and costs, but as mentioned before there is scarcity of detailed information that can be relevant to a large public.

8.1. Biomass to liquids process: gasification technology

8.1.1. The process

Algae to Jet fuel can take the route known as biomass to liquids (BTL). In this process, the biomass is subjected to a first step of gasification at a temperature between 150 °C to 300 °C and pressure varying from 10 to 40 bars in which syngas is produced; This syngas is subjected to Fischer-Tropsch synthesis while reacting with hydrogen in a presence of a catalyst (usually iron, cobalt or nickel) [32–34]. This process is also named gasification/Fischer-Tropsch synthesis (GFT), during this process long chain of alkanes are produced. At this point a

liquid is produced due to the catalytic action. Fractionation can be used to produce a fuel with required fractions for Jet fuel. The produced Jet fuel from this pathway based on ASTM certification is known as Fischer-Tropsch synthetic paraffinic Kerosene (FT-SPK). The process is suitable for lignocellulosic, woody, agriculture biomass including algae biomass and municipal solid wastes. However, no fuel has been produced at large scale using this process with algae biomass. New studies are currently being undertaken to develop Fischer-Tropsch synthetic Kerosene high in aromatics content (FT-SKA). This Jet fuel will be an improved version of the previous one. More aromatics can improve the performance of Jet fuel more especially the net heat of combustion. Also, they sensibly reduce the possibility of fuel leaks from the aircrafts. The biomass is first pre-treated and in most cases the drying process is used to reduce the moisture content aiming to facilitate its handling and transportation. Drying increases reliability and continuity regarding the feeding of biomass through industrial facilities [35]. However, the pre-treatment step must be undertaken remotely to reduce sensibly the costs related to logistics involved in transportation of biomass. Already biomass drying is costly because it is energy intensive, there is a necessity for optimization of any subsequent process to make the technology cost effective. Gasification depends mostly on the type of biomass. The moisture and ash content can have an impact on the quality of syngas. High concentrations of volatile substances resulting from high temperatures, low ash content and low moisture can make an ideal BTL process because of fewer impurities in the syngas [36].

8.1.2. Technological development, commercial activity and fuel readiness

Application of BTL process to algae biomass is relatively new and requires full optimization. Generally, gasification technologies require substantial improvement, particularly with handling of feedstocks [32,35]. When it comes to Fischer-Tropsch processes, less extensive adaptation is required, due to the similarity in terms of content for syngas produced from biomass and fossil fuels. BTL was expected to be operational in the 2000s, but it has slowly progressed than excepted. Projects using this technology are still at demonstration level or pilot scale, possibly in the next decade a mature process will be established. The readiness of the algae Jet fuel using this technology is yet to be determined because the technology requires optimization and important improvements.

8.1.3. Opportunities, challenges for use in aviation and compatibility with existing systems

The FT-SPK process used for biomass conversion into liquid fuels may present some advantages over conventional Jet fuel because of its composition. Its specific energy is 2% greater compared to the one for conventional Jet fuel because of its paraffinic structure and also the aromatic content is low. Consequently, the weight of Jet fuel needed for flying specific distances can be reduced, therefore, the potential payload of the aircraft increases and the consumption of energy per unit of payload is reduced [33,37]. However, there are many challenges preventing the use of FT-SPK as a neat Jet fuel. For example its energy density (per unit volume) is 3% lower compared to the one for conventional Jet fuel. This is due to lower aromatic content and paraffinic composition. Consequently, the maximum range of the aircraft is reduced, long distance flights cannot be an option in this case because requiring full tank of fuel [34,37]. Low aromatic content may end in fuel leaks with elastomers such as nitrile-rubber seals which can expand in the presence of aromatics. The lower lubricity of the fuel also is considered to be another cause that deteriorates wear on engine components [32,34]. To mitigate these weaknesses it is recommended blending FT-SPK fuel with conventional Jet fuel in a 50–50% proportion [34]. The costs of this process need optimization since many co-products including diesel and gasoline can be recovered. BTL using this pathway can generate low sulphur content fuels with high cetane number [34].

8.2. Pyrolysis

This is a thermal process in which decomposition of organic materials takes place in the absence of oxygen. During pyrolysis, oils, gases, char and water are produced depending on the process conditions. Generally, there is production of chars at conditions involving slow heating rates with final temperatures lower than 450 °C. However, with rapid heating rates with high temperatures greater than 800 °C gases are produced. A variant of biomass pyrolysis is known as fast pyrolysis, it is achieved with temperatures between 400 and 600 °C within few seconds as residence time. Thereafter, gas quenching or cooling is rapidly undertaken, the gas is sent to a condenser to produce bio-oil [35,38–40]. With fast pyrolysis yields of up to 80% (in weight) can be achieved. To improve the oil quality zeolite is used as catalyst during the process. The catalyst can influence the increase of aromatic content and cause the decrease of oxygen. The catalytic compared to the non-catalytic pyrolysis produces high quality oil that requires less upgrading steps. Therefore, it is cost effective compared to the non-catalytic [41,42]. This process can be effective for algae biomass but the biomass needs to be dried properly before pyrolysis. This is an added cost to be considered, analysed and optimized. Pyrolysis being used on petroleum derived fuel has been proven successful and its readiness does not need to be proved anymore. However, fast pyrolysis suffers with issues related to slow commercialisation due to the lack of an effective market demand regarding crude bio-oil [38]. Pyrolysis can be an acceptable option for conversion of algae biomass into Jet fuel. There is a need for optimization and development of new and low costs upgrading processes. This may be possible through the improvement of the existing hydrodeoxygenation or deoxygenation. More studies are still being undertaken in this regard. The main areas of these studies may involve the appropriateness of various catalysts as well as their life cycle, reduction of hydrogen intake and the assessment of the required deoxygenation to co-process bio-oil in conventional refineries [38,43]. These new approaches are currently at the laboratory and pilot scale levels [44,45]. In general, it is important to emphasis on the fact that pyrolysis of any biomass to produce crude bio-oil without upgrading is a very mature technology.

8.3. Hydrothermal liquefaction

This process is similar to pyrolysis and is also used for the conversion of algae biomass into fuels. It is known as a thermochemical process operating under high pressure and medium temperature aiming to produce oil from algae biomass. During this process, algae biomass macromolecules are firstly hydrolysed and broken into smaller molecules. However, unstable and reactive molecules can recombine into larger ones sometimes. Dehydration or decarboxylation is used to remove substantial amount of oxygen from the biomass to enhance the performance of produced Jet fuel. Bio-oil properties more especially chemical ones are greatly dependent on the biomass composition. Algae biomass is made up of different organic components, each of them generate different ranges of compounds during hydrothermal liquefaction. Generally, a reaction of organic matter takes place in the presence of water and catalysts. The operating conditions for the biomass consist of low final temperatures which are generally between 300 and 400 °C under high pressures ranging between 50 and 200 bars with maximum residence time of 30 min or even more [35,40,46,47]. Hydrothermal liquefaction used as a process to generate renewable fuels from algae biomass shows a great potential. However, the technology is still largely used at laboratory and pilot scales, very few cases may have been taken at large scale.

8.3.1. Opportunities and challenges for use in aviation regarding pyrolysis and hydrothermal liquefaction processes

Jet Fuels produced from pyrolysis and hydrothermal processes may have high aromatic content, low oxygen content and few impurities,

these are highly required features for Jet fuel [38,48,49]. However, upgrading bio-oil to Jet fuel requires extensive deoxygenation which can be a costly process. The high costs may be an obstacle to efficiently produce fractions that are predominantly in the jet fuel range [35].

8.4. Fermentation to jet (FTJ)

Anaerobic fermentation of algae biomass can generate alkane based fuels from fermentation. In this method enzymes are used as catalysts to bring specific biochemical reactions during the fermentation. The method is used to operate directly from sugar to hydrocarbons or fermentation of sugar to Jet fuel. The process involves the following steps: pre-treatment, enzymatic hydrolysis, hydrolysate clarification, biological conversion (or bioconversion), product purification and hydroprocessing [50]. During enzymatic hydrolysis process enzymes facilitate the cleavage of bonds in molecules with the addition of water. Hydrolysate clarification is used for separation of liquids and solids generated after the hydrolysis process. Bioconversion converts a portion of biomass into sugars which is then fermented into an alcohol. Product purification and hydroprocessing use fractionation and hydrocracking as downstream processes to get the required Jet fuel with compliant physico-chemical properties. During the FTJ process sugar molecules from algae biomass are digested and an alcohol intermediate is produced with the help of enzymes. Direct conversion of alcohols to alkanes is generally challenging. The conversion usually uses a sequence of a two-step process. This sequence involves the conversion of alcohols into leaving groups such as halides and sulfonate esters followed by reduction with metal hydrides [51]. The conversion of alcohol to alkanes can be also achieved through deoxygenation using enzymes. To produce sugars, algae biomass have to be fed with carbon dioxide during growth. Carbon dioxide can be taken from fossil fuel plants, it is also helpful for growth and lipid boosting. In terms of the fuel readiness, FTJ process is still at the pilot stage but is moving toward demonstration and early commercialisation stages. It involves the conversion of sugars to isoprenoids using genetically modified organisms. These isoprenoids are polymerised to generate hydrocarbons. Thereafter, the hydrocarbons are hydroprocessed to create stocks for blending purposes [47,52]. The fuel produced from this process is ASTM certified only for blending with conventional Jet fuel in proportions lower or equal to 10% [51]. The certified version is named synthesised iso-paraffinic fuel from fermented hydroprocessed sugar [51]. Literature about FTJ fuel produced from algae is very scarce because the process is still new and not yet used in the industry, similarly, the specific operating conditions to get Jet fuel cannot be found in the existing literature. The conversion technologies of alcohol to jet fuels are still at the laboratory and pilot levels of application. The details of operational dedicated pilot plants are not yet identified in the literature. More studies need to be undertaken for the process to be developed at large scale. The studies involve the costs and possibilities to escalate depending on the process simulation and optimization.

8.4.1. Opportunities and challenges for use in aviation

The process is ASTM certified which confers the confidence for Jet fuel to be produced from fermented sugar generated from algae biomass using FTJ. However, the Jet fuel produced using this process is still preferred for blending. The quantities to be produced are depending on the biomass growth output. Also, the use of genetically modified organisms to convert sugars and the capture of carbon dioxide for sugar production makes the process expensive compared to the process used for conventional Jet fuel production [51].

8.5. Transesterification to bio-jet fuel

This route uses the algae biodiesel from the transesterification followed by decarboxylation and deoxygenation to produce high purity Jet fuel [53]. Decarboxylation or deoxygenation of methyl esters increases energy density of the Jet fuel and allows the removal of methyl esters which are not needed in Jet fuel. Finally, isomerization process allows the breakdown of large molecule chains into small alkanes decreasing the higher freezing point to the normal one for the Jet fuel [53]. Generally, biodiesel from algae oil has also high flash point, high freezing point, high density, low kinematic viscosity and high oxygen content compared to the ASTM requirements for Jet fuel as indicated in Table 4. Transesterification of algae oil followed by deoxygenation or decarboxylation and isomerization to get Jet fuel from algae oil is technically feasible but very expensive [53]. This method is still under development, its maturity will depend on the laboratory and pilot scale data.

8.5.1. Opportunities and challenge in fuel aviation

The process can allow the cracking long chain molecules at low temperatures which are the transesterification temperatures mostly around 50–60 °C. Therefore, transesterification on its own is energy efficient and costs effective. However, the freezing point of the final product might be higher than the one required for Jet fuel as indicated in Table 5. Also, the purification processes as mentioned before are prohibitively expensive. This is challenging but the process presents an opportunity to develop an innovative way to overcome the challenge.

8.6. Suggested approaches: new trends and conceptual approaches

Fig. 1 presents various approaches and trends showing the potential for the conversion of algae oil into Jet fuel. However, the strengths and weaknesses of most of these approaches are summarized in Table 5. These strengths and weaknesses present the critical aspects of the processes that can be explored to develop new alternatives approaches, to improve the current ones and to allow an efficient production of aviation fuel from algae oil. Options such as cracking, hydrocracking, pyrolysis, gasification and Fischer Tropsch are used in the petroleum industry for conventional Jet fuel. They are proven to be successful, it is possible to use and adapt them for algae based Jet fuel with defined operating conditions using the same processing plants. Some few processes or technologies under development are also used to get algae based Jet fuel and they may present an opportunity to be explored. They are described as follows:

- **Centia™ Process**

  Developed in USA at North Carolina State University, patented process that can reuse oil from many feedstocks including agriculture, aquaculture or algae, waste oil, animal fats etc. The process can easily produce bio-Jet fuel which is compliant to Jet fuel requirements. High yields and energy density for the produced bio-Jet fuel.

Table 4
Comparison of selected properties between transesterified algal bio-oil and conventional Jet A and A1 (adapted from [53]).

Parameter	Jet A	Jet A1	Algal biodiesel
Net heat of combustion[MJ/kg]:	42.8 (minimum)	42.8 (minimum)	35–41
Kinematic viscosity at −20C[mm^2/s]	8	8	7.5
Density at 15 °C [kg/m ^{3}]	775–840	775–840	864
Flash point °C	38 (minimum)	38 (minimum)	115
Freezing point °C	−40	− 47	Close to 0
Pour point °C	–	–	−12
Sulphur [%]	0.30 (maximum)	0.30 (maximum)	0.05 (maximum)
Oxygen [%w]	1–2	1–2	11.3 (minimum)

Table 5
Advantages and weakness of processes in Fig. 1 (compiled from [32–36,42,44,46,51–53]).

Process	Advantages/strengths	Weaknesses
Cracking/Hydrocracking	– Efficient process with high yield. – Can be achieved with achievable temperatures (300–450 °C) – Achieved on large scale. – Affordable and proven successful	– Impact on costs because of the use of catalysts. – Can be time consuming.
Pyrolysis	– Very fast process, can be achieved in fraction of seconds or few minutes – Proven successful and affordable on large scale for conventional petroleum products.	– Challenging to control. – Requires very high temperatures. – Not recommended for small hydrocarbons because of their volatility. – Oil produced from this process has high oxygen and water content, low heating value. – The oil produced may require upgrading using hydroprocessing and hydrocracking. – May generate asphalt or bitumen. – Dehydration must be undertaken upstream to allow an efficient process. Possibility of GHG gas emission
Fischer Tropsch/Gasification	– Used for wide selection of feedstocks (eg biomass and coal) for syngas generation. – Proven successful process for aviation biofuel.	– May be costly and energy intensive depending on the type of gasification (fluidised bed, hydrothermal, entrained flow etc). – Costs effective when only operated at large scale.
Biodiesel (Transesterification) to bio-jet fuel	– Achievable technically because of the use of lower temperatures compared to previous processes. – Decarboxylation and deoxygenation are undertaken to increase energy density of the fuel Isomerization is used to decrease a freezing point of the generated jet fuel	– The process can be expensive. – Large quantities of CO2 might be produced during decarboxylation depending on volumes of biodiesel.
Enzymatic process	– Attractive and interesting process because of low energy input. There is still a tremendous prospect in this area. – Promising technology	– Not a mature process as yet, still at pilot stage. – Could be very expensive.
Centia ™.	– Proven maturity, scalability and affordability. – The process can generate biodiesel and bio-gasoline while processing bio-jet fuel.	– CO_2 is produced during decarboxylation. – May be costly because of the use of catalysts.
Plasma gasification	– It can generate clean syngas. Promising technology	– Not a mature process as yet. – Very costly.

The process has shown that 85% of energy conversion can be expected.

- **Enzymatic approach (biochemical conversion)**

  Enzymatic approaches are increasingly becoming attractive, they are not yet at large scale. This is due to the relatively high price of lipase as well as its short operational life. The short operational life is caused by the negative effects of excessive methanol and co-product glycerol. One of the greatest challenges to demonstrating the validity of this approach lies in the conversion of algal oil extracts at a commercial scale and at competitive prices.

- **Plasma technology**

Promising technology that can be used with plasmas to open chemical pathways prohibited at conventional temperatures. This technology is not yet at the level maturity in order to be used for large scales because still under development.

8.7. Unsolved issues and future perspectives for algae based aviation fuels

The aviation industry is faced with many challenges that need to be overcome. The demand for aviation fuel is facing an increasing demand which is expected from a range of 1.5–3% annually [54]. In the European Union, an annual growth estimated at 3% is expected in the aviation transportation sector until 2050. In the meantime the annual growth for fuel consumption is expected to be at 2% [55]. This represents a great opportunity for algae based aviation fuel. Algae Jet fuel will have to fit in the current market characterised by fast growth and high demand. In the case high quality and efficient algae Jet fuel is produced, the fuel will be able to supply the balance needed by the industry and to solve the issue of the increasing demand mentioned earlier. However, some key unsolved issues need to be addressed to advance the agenda of producing sustainable and competitive algae based Jet fuel: the identification of species that can produce very high quantities of lipids with less oxygen content, the development of physiological modification techniques or use of genetically modified organisms to improve the lipid production for various species at higher rates, the development of novel growth processes to reduce sensibly the costs related to growth and updated bio-oil extraction techniques with low energy intake, the implementation of low costs and very efficient harvesting techniques, and the development of novel biomass drying process. Briefly, to reduce the costs related to the production algae Jet fuel without compromising the quality needed for compliance. Some of the processes mentioned in Fig. 1 can be used to produce algae Jet fuel. However, the costs remain a challenge, it has been estimated that a barrel of algae fuel can range between 300 and 2600 US dollars [26,56] (valid from 2005 to 2011), this price was brought down to 84 US dollars by modifying the downstream processes [53] (for the same period). These costs are still high compared to ones related to petroleum fuels. The estimated costs to produce a barrel of petroleum fuels are between 40 and 80 US Dollars [26,56](this was valid from 2005 to 2011). Therefore, the costs and the quality issues compulsory for compliance are the main hurdles to overcome in order for algae Jet fuel to be able to compete with petroleum Jet fuel. Technology for species growth, oil extraction, conversion processes need to be completely improved, optimized, and tested. This aims to improve fuel molecules and reduce sensibly the costs and environmental impacts in order to ensure a market ready algae Jet fuel. The implementation of low energy intake processes generating high output should be the basis of new studies to overcome these challenges. Therefore, economic sustainability and

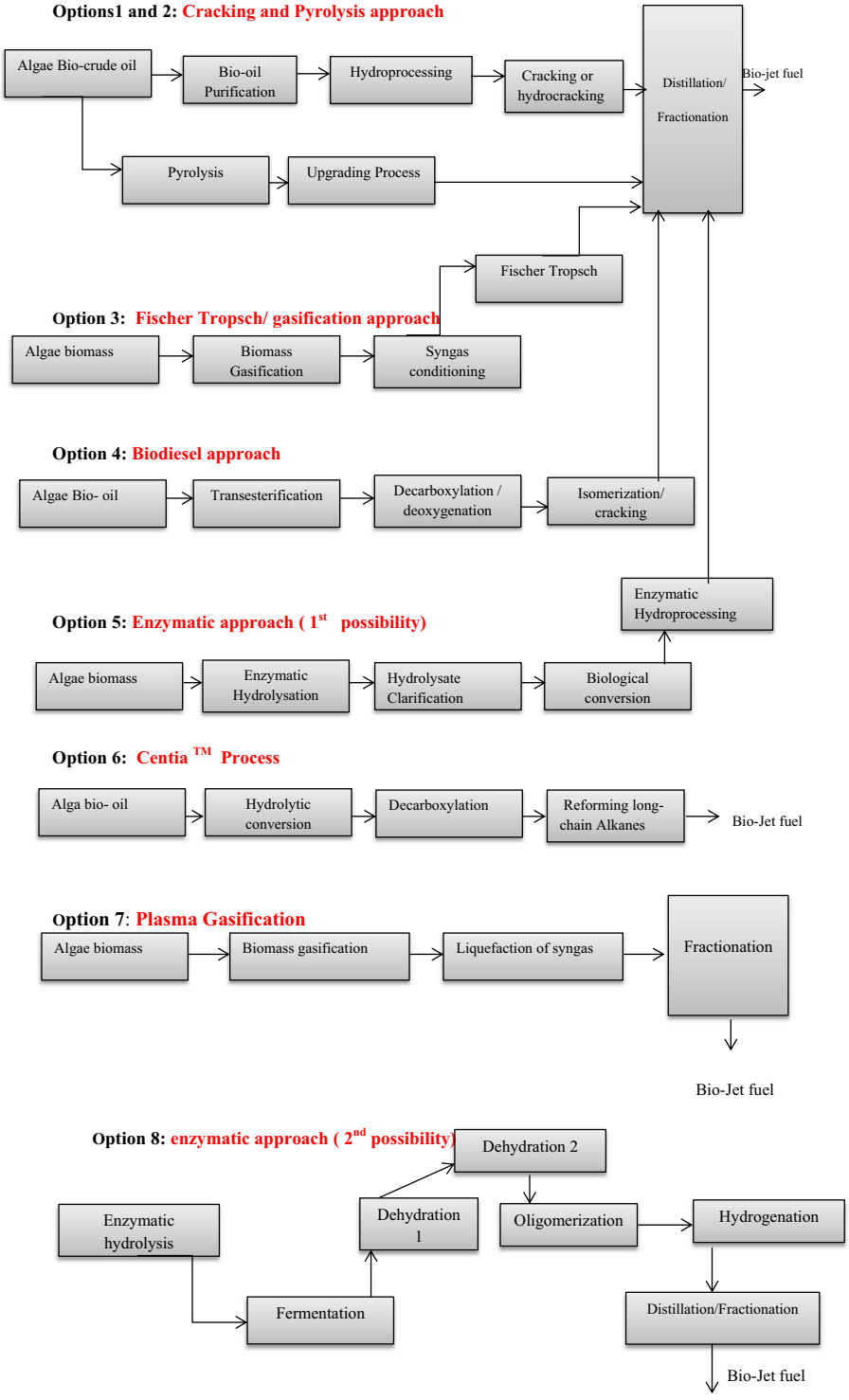

Fig. 1. Conversion processes to get algae bio-jet fuel (compiled from [32–36,51–53]).

environmental sustainability are the driving forces that need to be satisfied in order to have algae Jet fuel at commercial stage.

9. Algae cultivation: importance and technologies

Cultivation of microalgae highly affects the quantity of produced biomass, the costs of the output and also the increase of lipid quantity. The increase of lipids content for algae biomass is currently one of the major challenges faced by algae biofuels. Once this barrier can be overcome algae based fuels will be a sustainable alternative to fossil fuels. Many strategies to improve the lipid production have been used in the past, these include starvation of nutrients mainly nitrogen, addition of CO_2 or sugar to the growing culture as mentioned earlier.

Cultivation of algae can be accomplished in open ponds and in photo-bioreactor (PBR) which is a closed culture systems [57]. In an open pond system the culture medium is directly exposed to the natural environment. Open pond systems use solar energy as the light source for microalgae cultivation [58]. The open system have an advantage of regulating the temperature by liquid evaporation. This system incorporates paddle wheels for mixing and circulation of gas or liquid with

low investment [59]. Open pond system is less expensive since their fabrication involves lower costs material and they require less energy. Major drawbacks of open pond systems are less effectual temperature control and the usage of light. They require relatively larger area and only handful of microalgae can be cultivation in this system. The system also suffers from higher risk of culture contamination as well as low density of microalgae [60,61,27]. Photobioreactors are closed systems that are frequently used in commercial scales for the cultivation of microalgae. In photo-bioreactors microalgae growth is controlled to achieve specific biological modification [27,59]. They are convenient to handle compared to open pond system for mixing and mass transfer of gas or liquid. Photobioreactors show productivity due to most effective use of the cultivation area and efficient energy consumption [61,62].

Earlier study reported that cultivation of microalgae in photobioreactors yield higher lipid content [63], this can be beneficial to get higher yield for bio-Jet fuel production. However, a major problem is artificial illumination. Light conversion performance of photobioreactors is restricted because of heat generation due to contact with light sources [63]. The facility installation expenditure is much higher and their functioning costs are higher since they necessitate more power [58]. Although, the costs for photobioreactors are higher, many studies are undertaken aiming to optimize the costs and make it less expensive, therefore, there is still room for research in this area. Once costs are sensibly reduced this option can present many advantages for bio-Jet fuel production because of the higher lipid content generated when using photobioreactors.

10. Conclusions

Production of biofuels for the aviation industry may be challenged by a handful of shortcomings on the technical feasibility and costs. These limitations hamper the technology readiness, its commercial maturity as well as the technology certification. There is a limited availability of data and information on the public domain regarding the development of alternative Jet fuels. The limitation mainly includes economic data which seems insufficient to estimate and question whether it is capital intensive or not together with the technical feasibility. However, in the private domain many studies are undertaken to get bio-jet fuel from feedstocks with high oil content. Technical feasibility and economics remain a secret for the producer. Therefore, technical and economic considerations including environmental aspects are the back bones for any new development regarding alternative Jet-fuels. Algae based fuels may be one of the most prominent ways to get Jet fuel more especially some marine species such as Nannochloropsis sp, Schizochytrium sp, Botryococcus braunii, Neochloris oleoabundans, Nitzschia sp. and many other. It appears that these species can technically be advantageous for an effective bio-Jet fuel production compared Jet fuel from other oil crops. Algae biomass have the ability to grow faster, they do not constitute a threat for food crops, they do not need excessive land to grow, they are not a threat for water resources and their crude bio-oil is very similar to petroleum crude oil in terms of carbon chains. However, the challenge is to address effectively this issue of boosting lipid content through the species physiological manipulation. This is an area that needs more studies to improve the ability of many species to increase their oil content during growth and during starvation. The starvation should define operating conditions and duration of the process to increasing the lipid content. Another challenge is on the engineering side and deals with the appropriate technology that is costs effective and sustainable to bring algae aviation to a point of ‘‘drop in ‘‘fuel. This will involve the development of techniques that may allow the production of a fuel similar to the petroleum Jet fuel known as conventional jet fuel. Compliance is the main reference in producing alternative Jet fuel, mostly Jet fuel from algae biomass. Jet-fuel has generally carbon chains ranging from 8 to 16 depending on the Jet fuel type. TAGs are recognised for high percentage of short chain fatty acids that can reach high number of carbon chain lengths. Many species seem to position themselves as the potential sources for biofuel to be used in aviation. Algae based Jet fuel has achieved many key milestones by identifying various species with high potential to generate oil and developing various routes or downstream processes to get algae based Jet fuel. Technological concept is a milestone which has also been clearly formulated; this means the way to go for successful technologies must mostly be copied on the petroleum Jet fuel processes. These downstream processes used on petroleum fuels have been demonstrated on large scales, the capital and operating costs are no more a big challenge. Furthermore, another milestone is the established valid concept for conversion of algae oil into fuels which already exists within the circles of many stakeholders in the private domain. The public domain needs to step into it and get the relevant information. Consequently, the scaling up to industrial or large scale will only require appropriate process optimization and modelling to reduce the operating costs. The scaling is possible if the technologies used can demonstrate maturity and can show potential for commercialisation. Hence, if these milestones are achieved, certification and commercialisation can follow. Blending algae oil with petroleum oil products is also another option to be considered for algae based Jet fuel to be operational on the market while costs optimization is still being considered in many studies. The B10, B20 and B50 are the most advised ratios for blending. This strategy can be explored because it slows down the speed of depletion of petroleum fuels by using less petroleum products and reduces carbon footprint. In definitive, algae based aviation could be costs effective within the next decade providing that high lipid content species are identified and there is development of new processes that are costs competitive and sustainable. For algae based Jet fuel to be a ‘‘drop-in’’ fuel, testing the fuel should be simulating all the characteristics of the current conventional Jet fuel in terms of engine performance, operability, characteristics such as fuel consumption and engine start. Environmental impact and life cycle assessment studies should be undertaken to add value to the advantages that algae based jet fuel can present over the conventional Jet fuel. Therefore, to meet energy growth new and alternative fuels have to be developed. To meet this goal and avoid energy crisis in the near future there should be a criteria including the ‘‘drop-in fuel aspect’’ as well as environmental and economic sustainability. The objectives of papers were therefore met by establishing many options and routes that are possible to produce Jet fuel from algae oil. Details of the processes, their strengths and weaknesses were discussed. Algae fuels are still small players compared to petroleum based fuel in the energy sector, there is more to be achieved.

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References

[1] Air Transport Action Group [ATAG] Aviation Benefits Beyond Borders. Geneva, Switzerland, 2014: Available online: ⟨http://aviationbenefits.org/media/26786/ATAG__AviationBenefits2014_FULL_LowRes.pdf⟩ [accessed 1 June 2016].
[2] Demirbas A. Importance of biodiesel as transportation fuel. Energy Policy 2007;35:4661–70.
[3] Rodolfi L, Chini Zittelli G, Bassi N, Padovani G, Biondi N, Bonini G, Tredici MR. Microalgae for oil: strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor. Biotechnol Bioeng 2009;102:100–12.
[4] Jet fuel consumption on Index Mundi; 2012, available online; ⟨www.indexmundi.com/energy⟩ / [accessed 19 June 2016].
[5] Hannon M, Gimpel J, Tran M, Rasala B, Mayfield S. Biofuels from algae: challenges and potential. Biofuels 2010;1(5):763–84.
[6] Energy Information Administration, International Energy Outlook, 284, 2009, EIA; DC, USA.
[7] Hunt JM. Petroleum Geochemistry and Geology, 2nd edition. W. H. Freeman and Co, New York, 24; 1996.

[8] Gize AP, Manning DAC, Aspects of the organic geochemistry and petrology of metalliferous ores. In: Organic Geochemistry: Principles and Applications, Engel MH, Macko SA, (Eds), p. 565–580; 1993.
[9] Blakey S, Rye L, Wilson CW. Aviation gas turbine alternative fuels: a review. Proc Combust Inst 2011;33:2863–85.
[10] Liu G, Yan B, Chen G. Technical review on jet fuel production. Renew Sustain Energy Rev 2013;25:59–70.
[11] Bauen A, Howes J, Bertuccioli L, Chudziak C. Review of the potential for biofuels in aviation, Report, London:E4tech. ⟨https://www.theccc.org.uk/archive/aws2/⟩ Aviation [accessed 1 June 2016]; 2009.
[12] Lehr F, Posten C. Closed photo-bioreactors as tools for biofuel production. Curr Opin Biotechnol 2009;20(3):280–5.
[13] Ruiz-Marin A, Mendoza-Espinosa LG, Stephenson T. Growth and nutrient removal in free and immobilized green algae in batch and semi-continuous cultures treating real wastewater. Bioresour Technol 2009;101(1):58–64.
[14] Gerbens-Leenes W, Hoekstra AY, Van Der , Meer TH. The water footprint of bioenergy. Proc Natl Acad Sci USA 2009;106(25):10219–23.
[15] Alternative Jet Fuels, Addendum 1 to Aviation Fuels Technical Review (FTR3/A1), A supplement to Chevron's Aviation Fuels Technical Review, Chevron Corporation, 2006 Available online: ⟨www.cgabusinessdesk.com/document/5719_Aviation_Addendum._webpdf.pdf⟩, [accessed 16 May 2016].
[16] Steen E, Kang Y, Bokinsky G, Hu Z, Schirmer A, McClure A, Del Cardayre S, Keasling J. Microbial production of fatty-acid-derived fuels and chemicals from plant biomass. Nature 2010;463(7280):559–62.
[17] Wahlen BD, Willis RM, Seefeldt LC. Biodiesel production by simultaneous extraction and conversion of total lipids from microalgae, cyanobacteria, and wild mixed-cultures. Bioresour Technol 2011;102:2724–30.
[18] Hillen LW, Pollard G, Wake LV, White N. Hydrocracking of the oils of *Botryococcus braunii* to transport fuels. Biotechnol Bioeng 1982;XXIV:193–205.
[19] Hem R, Thapa , Mandar T, Naik , Okada S, Takada K, Molnár I, Xu Y, Devarenne TP. A squalene synthase-like enzyme initiates production of tetraterpenoid hydrocarbons in *Botryococcus braunii* Race L. Nat Commun 2016;7:11198. http://dx.doi.org/10.1038/ncomms11198.
[20] Schenk P. Second generation biofuels: high-efficiency microalgae for biodiesel production. BioEnergy Res 2008;1:20–43, [2008].
[21] Becker EW. Microalgae in human and animal nutrition. In: Richmond A, editor. Handbook of microagal culture. Oxford: Blackwell Publishing; 2004. p. 312–51.
[22] Moheimani N, Borowitzka M. Culturing Coccolithophorid Algae for Carbon Dioxide Bioremediation. In: Proceedings of the 7th Annual Post-Graduate Symposium, The Royal Society of Western Australia, Perth; 2005.
[23] Chisti Y. Biodiesel from microalgae. Biotechnol Adv 2007;25:294–306.
[24] Georgianna D, Mayfield S. Exploiting diversity and synthetic biology for the production of algal biofuels. Nature 2012;488(7411):329–35.
[25] Brown T, Duan P, Savage P. Hydrothermal liquefaction and gasification of Nannochloropsis sp. Energy Fuels 2010;24(6):3639–46.
[26] Brennan L, Owende P. Biofuels from microalgae; a review of technologies for production, processing, and extractions of biofuels and co-products. Renew Sustain Energy Rev 2010;14:557–77.
[27] Richmond A. Handbook of microalgae culture: biotechnology and applied phycology, 566. Oxford: Blackwell Publishing; 2004.
[28] Packer M. Algal capture of carbon dioxide; biomass generation as a tool for greenhouse gas mitigation with reference to new Zealand. Energy Policy 2009;37(9):3428–37. http://dx.doi.org/10.1016/j.enpol.2008.12.025.
[29] Maniatis K, Weitz M, Zschocke A, 2 million tons per year: a performing biofuels supply chain for EU aviation, August 2013 Update, European Commission. Available online: ⟨https://ec.europa.eu/energy/sites/ener/files/20130911_a_performing_biofuels_supply_chain.pdf⟩, [accessed 10 June 2016].
[30] ALFA-BIRD, Alternative Fuels and Biofuels for Aircraft Development, ICAO Workshop Report, 2011, Available online: ⟨http://www.icao.int/Meetings/EnvironmentalWorkshops/Documents/2011-⟩ SUSTAF/18_⟨Jeuland.pdf⟩ [accessed 10 June 2016].
[31] Hileman JIO, DS, Bartis JT, Wong HM, Donohoo PE, Weiss MA, Waitz IA, Near-Term Feasibility of Alternative Jet Fuels, Technical Report, 2009. Availableonline: ⟨http://web.mit.edu/aeroastro/partner/reports/proj17/altfuelfeasrpt.pdf⟩, [accessed 10 June 2016].
[32] Güell BM, Bugge M, Kempegowda RS, George A, Paap SM, Benchmark of conversion and production technologies for synthetic biofuels for aviation, Norway: SINTEF Energy Research, 2012, Report available online: ⟨http://www.flygreenfund.se/wp-content/uploads/2015/05/Benchmark-of-conversion-and-Production-technologies-for-synthetic-biofuels-for-aviation-Sintef-2013.pdf⟩, [accessed 11 June 2016].
[33] Arup URS Consortium, Advanced Biofuel Feedstocks; An Assessment of Sustainability, Report submitted by Arup URS consortium, Report available online: ⟨http://www.gov.uk/government/uploads/attachement_data/file/277436/feedstock-sustainability.pdf⟩, 2014, [accessed 20 May 2016].
[34] Hileman JI, Stratton RW, Donohoo PE. Energy content and alternative jet fuel viability. J Propuls Power 2010;26:1184–95.
[35] Radlein D, Quignard A. A short historical review of fast pyrolysis of biomass, Oil and Gas Science and Technology, Revue d'IFP Energies nouvelles. Inst Fr du Pet 2013;68(4):765–83.
[36] CSIRO, Flight path to sustainable aviation, Towards establishing a sustainable aviation fuels industry in Australia and New Zealand, Sustainable Aviation Fuel Road Map, 2011, Report available online: ⟨http://www.licella.com.au/wpcontent/uploads/licella/news/flightpathsustainableaviation⟩, [accessed 3 May 2016].
[37] Pandey A, Larroche C, Ricke S, Dussap CG. Gnansounou, biofuels: alternative feedstocks and conversion processes, First edition. Elsevier; 2011, [ISBN: 978-0-12-385099-7].
[38] SkyNRG. Technology section, Report Available online: ⟨http://skynrg.com/technology-section⟩, 2014 [accessed 5 march 2016].
[39] Brown TR, Thilakaratne R, Brown RC, Hu GP. Techno-economic analysis of biomass to transportation fuels and electricity via fast pyrolysis and hydroprocessing. Fuel 2013;106:463–9.
[40] Arbogast S, Bellman D, Paynter JD, Wykowski J. Advanced biofuels from pyrolysis oil, opportunities for cost reduction. Fuel Process Technol 2013;106:518–25.
[41] VTT Synthetic fuels and bio-refining. Available online: ⟨http://www.vtt.fi/research/technology/synthetic_fuels_and_biorefining.jsp?Lang=en⟩, ,2014, [accessed 5 march 2016].
[42] Venderbosch RH, Prins W. Fast pyrolysis technology development. Biofuels, Bioprod Bioref 2010;4:178–208.
[43] Balan V, Chiaramonti D, Kumar S. Review of US and EU initiatives toward development, demonstration, and commercialization of lignocellulosic biofuels. Biofuels, Bioprod Bioref 2013;7:732–59.
[44] National Alternative Biofuels Consortium [NABC]. Making Oils with Low Oxygen Content, Catalytic Fast Pyrolysis. Available online: ⟨http://www.nabcprojects.org/pdfs/making_oils_with_low_oxygen_content.pdf,2012⟩, [accessed 13 march 2016].
[45] Biomass Technology Group [BTG] online: ⟨http://www.btgworld.com/en/rtd/technologies/biofuels⟩, 2014a, [accessed 13 March 2016].
[46] Zhang Y, Brown TR, Hu G, Brown RC. Techno-economic analysis of two bio-oil upgrading pathways. Chem Eng J 2013;225:895–904.
[47] Davis R, Biddy M, Tan E, Tao L, Jones S, Biological Conversion of Sugars to Hydrocarbons Technology Pathway, Washington, USA, National Renewable Energy Laboratory, Technical Report, 2013, Available online: ⟨http://www.nrel.gov/docs/fy13osti/58054.pdf⟩, [accessed 15 April 2016].
[48] Rebecca M, Adriana RC, Raphael S. Establishing a European renewable jet fuel supply chain: the techno-economic potential of biomass conversion technologies, Imperial College London, Final Report, available online: ⟨http://www.climate-kic.org/projects/renewable-jet-fuel-supply-chain-development-and-flight-operations/⟩, [accessed 20 June 2016]; 2014.
[49] William LR, The5th International Biofuels Conference, North Carolina State University Raleigh, 2008, NC27695, USA, available online: ⟨http://www.competebioafrica.net/events/events2/seminar_india/ppt/4-2-Roberts.pdf⟩ [accessed 21 June 2016].
[50] International Energy Agency (IEA), Bioenergy Annual report, 2012 available online: ⟨http:www.iea.org/publications/freepublications/publication/IEA⟩ [accessed 15 June 2016].
[51] Zampara M, Capros P, European Union, Energy, transport and GHG emissions trends to 2050 reference scenario, Updated Report, available online: ⟨http://www.e3mlab.eu/e3mlab/presentations/EU%20Energy,%20transport%20and%20GHG%20emissions%20Trends_ECRAN_UPDATE.pdf,2013⟩ [accessed 16 June 2016].
[52] Alabi AO, Tampier M, Bibeau E. Microalgae Technologies and Processes for Biofuels/Bioenergy Production in British Columbia, BC Innovation Council; BC, Canada, Report available online: ⟨http://www.fao.org/uploads/media/0901_Seed_Science_Microalgae_technologies_and_processes_for_biofuelsbioenergy_production_in_British_Columbia.pdf⟩, [accessed 16 2016]; 2009.
[53] Huntley ME, Redalje DG. CO_2 mitigation and renewable oil from photosynthetic microbes: a new appraisal. Mitig Adapt Strateg Glob Change 2006;12:573–608.
[54] Huerlimann R, de Nys , Heimann K. Growth, lipid content, productivity, and fatty acid composition of tropical microalgae for scale-up production. Biotechnol Bioeng 2010;107(2):245–57.
[55] Jiang H, Gao K. Effects of lowering temperature during culture on the production of polyunsaturated fatty acids in the marine diatom Phaeodactylum tricornutum (Bacillariophyceae). J Physcol 2004;40(4):651–4.
[56] Zhila NO, Kalacheva GS, Volova TG. Effect of salinity on the biochemical composition of the alga Botryococcus braunii Kütz IPPAS H-252. J Appl Phycol 2011;23:47–52.
[57] Mata T, Martins A, Caetano N. Microalgae for biodiesel production and other applications: a review. Renew Sustain Energy Rev 2010;14(1):217–32.
[58] Widjaja A, Chien CC, Ju YH. Study of increasing lipid production from fresh water microalgae *Chlorella vulgaris*. J Taiwan Inst Chem Eng 2009;40:13.
[59] Cheng-Wu Z, Zmora O, Kopel R, Richmond A. An industrial-size flate plate glass reactor for mass production of *Nannochloropsis* sp. (Eustigmatophyceae). Aquaculture 2001;195:35–49.
[60] Pulz O. Photobioreactors: production systems for phototrophic microorganisms. APPL Microbiol Biotechnol 2001;57:287–93.
[61] Carvalho AP, Meireles LA, Malcata FX. Microalgae reactors: a review of enclosed system designs and performances. Biotechnol Prog 2006;22:1490–506.
[62] Sierra E, Acién FG, Fernández JM, García JL, González C, Molina E. Characterization of flat plate photobioreactors for the production of microalgae. Chem Eng J 2008;138:136–47.
[63] Steen E, Kang Y, Bokinsky G, Hu Z, Schirmer A, McClure A, Del Cardayre S, Keasling J. Microbial production of fatty-acid-derived fuels and chemicals from plant biomass. Nature 2010;463(7280):559–62.
[64] Eloka-Eboka AC, Inambao FL. Effects of CO2 Sequestration on Lipid and Biomass Productivity in Microalgal Biomass Production. Appl Energy 2017;195:1100–1111, http://dx.doi.org/10.1016/j.apenergy.2017.03.071.

In the Field

Biofpr Biofuels Bioproducts & Biorefining

Microalgae processing for jet fuel production

Joseph K. Bwapwa, University of Kwazulu-Natal, South Africa and Mangosuthu University of Technology, Durban, South Africa
Akash Anandraj, Mangosuthu University of Technology, Durban, South Africa
Cristina Trois, University of Kwazulu-Natal, Durban, South Africa

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Abstract: This study focuses on the laboratory production of jet fuel from microalgae. In contrast with many studies that use partial nutrient starvation to boost lipid content of the species, physiological modification was undertaken under complete nutrient starvation for 3 days to increase lipid content beyond 80%. This was followed by biomass harvesting, which was necessary for downstream processes. Large amounts of biomass were achieved between day 8 and day 10 during the cultivation period, with temperatures ranging between 15 and 35 °C under constant luminance of 1000 lux and daily supply of CO_2 for 15 days. It was found that *Nannochloropsis sp.* grew effectively between 15 °C and 25 °C with more biomass produced in the same temperature range. Conversion processes involved steps such as oil extraction, thermal cracking without catalyst at 300 °C and fractionation between 70 °C and 250 °C. The pyrolysis of bio-oil was also undertaken as a fast cracking process for the temperature ranging between 350 °C and 450 °C within 12 s. Some parameters such as flash point, net heat of combustion, sulfur, and viscosity complied with ASTM standards. Jet fuel from microalgae therefore shows potential despite many challenges related to cost effectiveness and sustainability. In order to obtain a bio-jet fuel that is completely compliant with ASTM standards, upgrading, reforming processes, and the use of additives will be needed more, especially for pilot and large-scale production once fuel sustainability is achieved. © 2018 Society of Chemical Industry and John Wiley & Sons, Ltd

Keywords: bio-jet fuel; biofuel; microalgae; *Nannochloropsis sp.*; biomass

Introduction

Background

Industrial interest in the production of alternative fuels and in reducing dependence on fossil fuels is growing. A greater demand for energy has led to fluctuations in the prices of fossil fuels. Adverse environmental effects resulting from the combustion of fossil fuels have increased interest in the search for environmentally friendly renewable resources. The use of biomass as a feedstock for energy production has consequently emerged as an area of importance.[1,2,3] As a result, many feedstocks have been used to produce biofuels.[4,5] However, algae biomass is one of the bioresources that can produce clean and sustainable fuel despite a few challenges.[6,7,8] Today it has technically been proved that there is a real possibility of converting algae or any biomass into bioethanol, bio-jet fuel, and bio-diesel.[3] Nonetheless,

Correspondence to: Joseph K. Bwapwa, 77 Ebor Heights, Bamboo Lane, Pinetown, 3610, Durban, KZN, South Africa.
E-mail: josephkapuku@gmail.com

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algae-based fuels are still expensive when compared with conventional fuels.

Unlike other first- and second-generation biofuels, algae can grow easily and reproduce very quickly under photosynthetic conditions.[9] Microalgae are microscopic and photosynthetic organisms that grow in marine, freshwater, or wastewater environments. The production of algae biomass generally depends on the type of species or strain used during cultivation.[10] The type and nature of the strain or species, the growth conditions, and the nutrients supplied to the culture directly influence biomass productivity and lipid content.[11] Microalgae grow very fast, as mentioned before, and some species are exceptionally rich in oil, but not most of them.[12,13]

Although the growth of the microalgae depends on many characteristics of the species, they can double the amount of biomass within 24 hours.[14] Growing microalgae under well-defined operating conditions will therefore result in high biomass production and eventually more lipids can be collected.[15,16,17] Microalgae grow like any other microorganisms; however, their growth cycle is made of four phases: lag, exponential, stationary, and the death phase or lysis. They convert photonic energy, water, and CO_2 to sugars; sugars are therefore converted to macromolecules such as lipids and triacylglycerols (TAG).[18,19] During the cultivation phase, once microalgae culture has reached the stationary phase, biomass harvesting must be undertaken in order to collect the highest amount of biomass needed for conversion processes. In this study, a marine microalga known as *Nannochloropsis sp.* is grown under photosynthetic conditions. The effectiveness of photosynthesis is one of the crucial aspects required for effective biomass productivity; it depends on the amount of light supplied to the culture, but also on the nature of the species.[20,21] Photosynthetic efficiency affects the growth rate and potentially biomass production.[14] Similarly, environmental parameters such as the pH of the growing culture, its temperature and salinity (especially for a marine species), the amount of carbon dioxide supplied to the growing culture and the availability of nutrients in the growing media are very important for effective growth.[22]

These parameters directly influence the growth and proliferation of algae cells and consequently the rate of biomass production.[11,20,21,23] The study's main objective is to produce bio-jet fuel from a microalgae species (known as *Nannochloropsis sp.*) under defined operating conditions in order to establish a laboratory process from cultivation to the production of jet fuel.

Knowledge gap and the knowledge contribution from this study

The literature on algae-based transportation fuels is scarce but many studies have been completed in this field; however, most of their outcomes are not available to the public. These studies have focused on conversion processes, biomass productivity, optimising growth conditions, and dewatering processes.[11,20,21,23] Nevertheless, there are still some gaps to be covered regarding the production of algae-based fuels. Effective genetic modification to increase the lipid output is one of the major issues that needs to be addressed. This study contributes to the process of filling the gap by establishing a total (complete) nutrient starvation technique.

This study's contribution to knowledge is its examination of the way in which total nutrient starvation can stimulate an increase in lipid in microalgae cells. In many previous studies, the physiological modification of species is completed under partial nutrient starvation.[11,20,21,23] However, in this study, physiological modification is achieved under total nutrient starvation of the species after cultivation. Most algae species have a very low lipid content; it is therefore challenging to have a high output for algae jet fuel with low crude bio-oil output.[11] Another way in which the study contributes to knowledge is based on the cracking process of the crude bio-oil, which is completed without a catalyst at an achievable temperature with a high oil recovery rate.

Overview of the species and bio-oil type used for the experiment

Nannochloropsis bio-oil is a reliable source of eicosapentanoic acid / oil (EPA) and also contains dodecahexanoic acid / oil (DHA). *Nannochloropsis sp.* strains are characterised by various unique biochemical and ultrastructure features such as the absence of chlorophyll b or c, the composition of the cellular xanthophyll pigments,[24,25,26] relatively high EPA content[27,28] as well as the presence of specific sterols.[29,30] The unique ultrastructure features of these strains are the presence of lamellate vesicles in the cytoplasm and the connection of the chloroplast envelope with the nuclear envelope. *Nannochloropsis sp.* is widely cultivated because of its higher EPA content (EPA long chain omega-3 fatty acids with 20-carbon chain including 5 cis double bonds) representing between 4% and 5 % of the biomass, and also because of its small cells (2–3 μm diameter) known as feed for rotifers in the 'green water' technique. The modulation of fatty acid composition by varying culture parameters such as light intensity, light-dark cycles, temperature, salinity, and nutrients has been

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widely investigated and reviewed in many studies.[17,31,32,33] Generally, the higher the biomass productivity, the higher the EPA productivity.[34,35,36] However, this is not always the case for all species because the lipid content is lower in most cases, as mentioned earlier. Increasing lipid content using a physiological manipulation technique is therefore very important for many species in order to generate the considerable amount of lipids necessary for biofuels production. *Nannochloropsis sp.* was suggested for biofuel production because the physiological manipulation undertaken with nitrogen starvation only showed an increase of approximately 60% in lipid content compared to its initial lipid content before starvation.[37,38,39,40] These lipids are mainly TAGs and they contain saturated and monounsaturated fatty acids.[19,41]

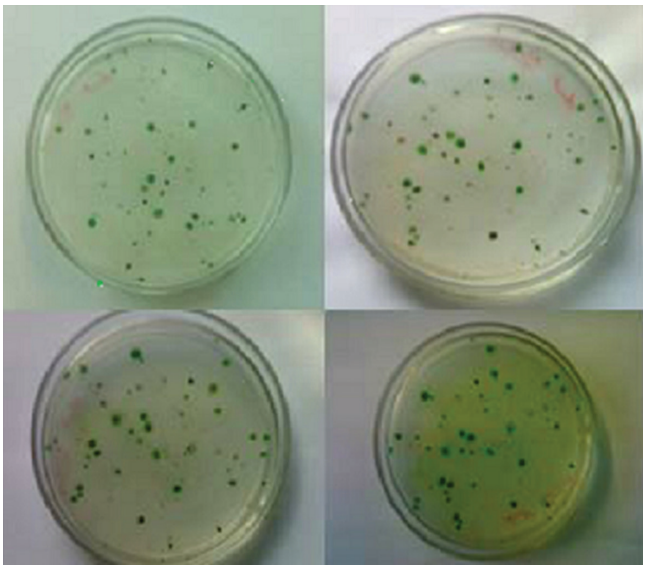

Figure 1. Cells of *Nannochloropsis sp.* on petri dishes for cultivation.

Material and methods

Sampling and culture maintenance

To obtain pure *Nannochloropsis sp.* cells for cultivation and the rest of the experiment, new cells were cultivated on petri dishes. On these petri dishes, colonies of *Nannochloropsis sp.* cells from a stock solution made up of *Nannochloropsis sp.* cells were spread in parallel lines crossing each other. Parallel lines were drawn in one direction to reduce the risk of contamination. These lines of cells in solution were spread on a viscous and thick mixture made of F/2 media and 15 g of agar added to the petri dishes presented in Fig. 1. Thereafter, petri dishes with colonies of cells were kept in an incubator at 25 °C with the aim of generating pure *Nannochloropsis sp.* cells.

After 5 to 7 days, cells appeared as pellets on the surface of the thick mixture. Some cells were pure and others were not due to probable contamination of growth. To minimise contamination of pure cells on the petri dishes, pure cells were isolated and put on new petri dishes with the thick mixture of F/2 media-agar. Lastly, they were kept at 25 °C in the incubator again. After a period of 7 days, pure cells

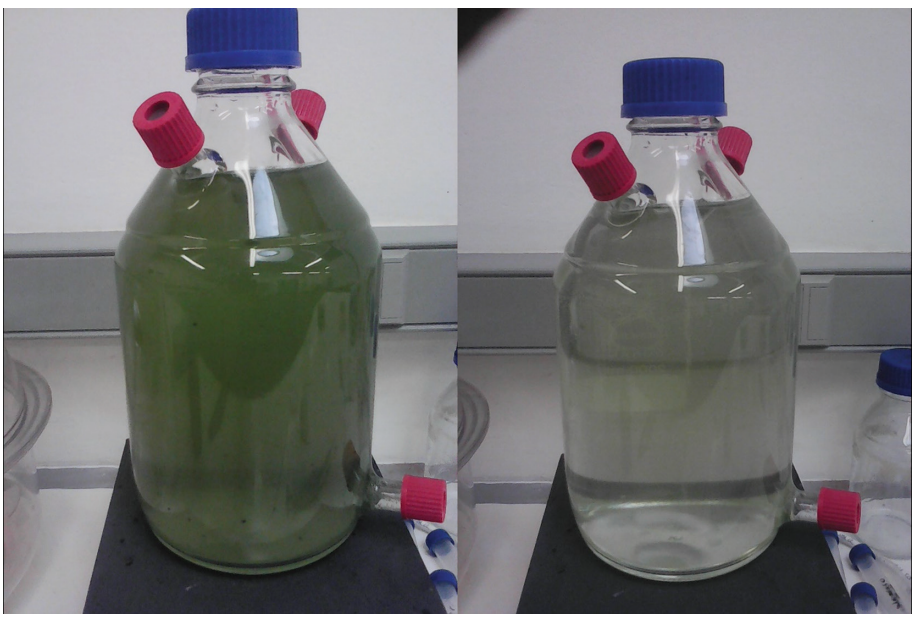

Figure 2. Cultivation batch (left) and F/2 media solution (right).

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of *Nannochloropsis sp.* appeared on the surface of the thick mixture. These pure cells or pellets were collected and added to F/2 media solution to run a cultivation batch in a photobioreactor, presented in the left part of Fig. 2. The photobioreactor will produce biomass under photosynthetic conditions for a cultivation period of 15 days. The stock solution was also collected for future use in case of sample loss or contamination or any other incident occurring during experiments.

Media preparation for species cultivation

The importance of F/2 media regarding algae biomass production is to provide nutrients effectively to microalgae cells during cultivation. This allows effective growth of cells under operating conditions defined by light, temperature, pH, and salinity. Microalgae need nitrates, phosphates, and trace elements including heavy metals for an effective growth. Media solutions such as F/2 are an ideal environment in which these elements are mixed and supplied to microalgae during cultivation. Nitrates allow the production of nucleic acid and proteins, and contribute greatly to synthesis and lipid production, while phosphates are acting as energy carriers.

F/2 media is prepared according to Guillard and Ryther's (1962) method.[42] It is widely used with saline or seawater for cultivation of many species including marine species. In this study the F/2 media preparation begins with 950 ml of saline water. This is prepared from tap water mixed with commercial salt at 30 ppt salinity, which is considered as the average salinity for seawater in which *Nannochloropsis sp.* can effectively grow. Defined quantities of $NaNO_3$, $NaH_2PO_4.H_2O$,

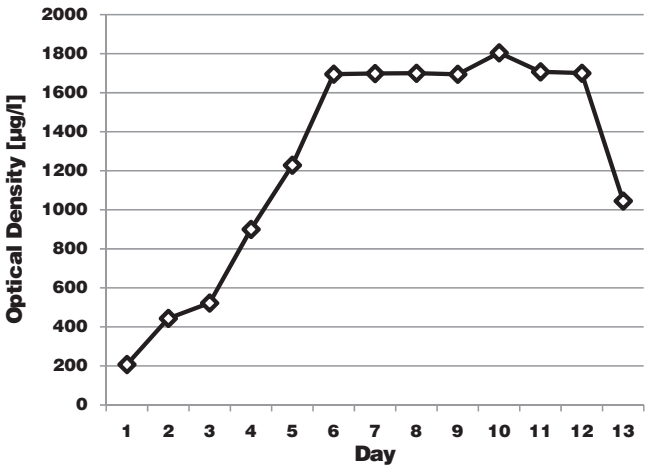

Figure 4. Growth curves recorded for *Nannochloropsis sp.* at 20 °C.

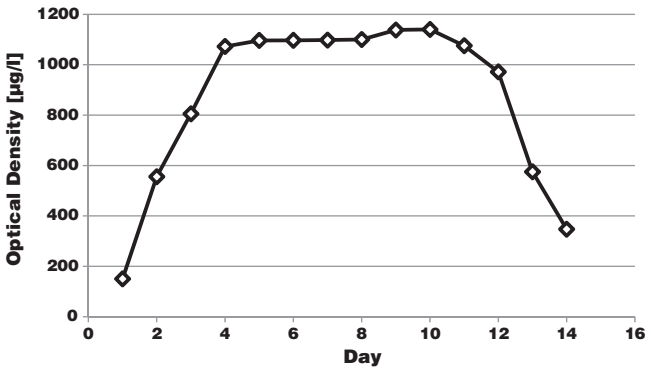

Figure 5. Growth curves recorded for *Nannochloropsis sp.* at 25 °C.

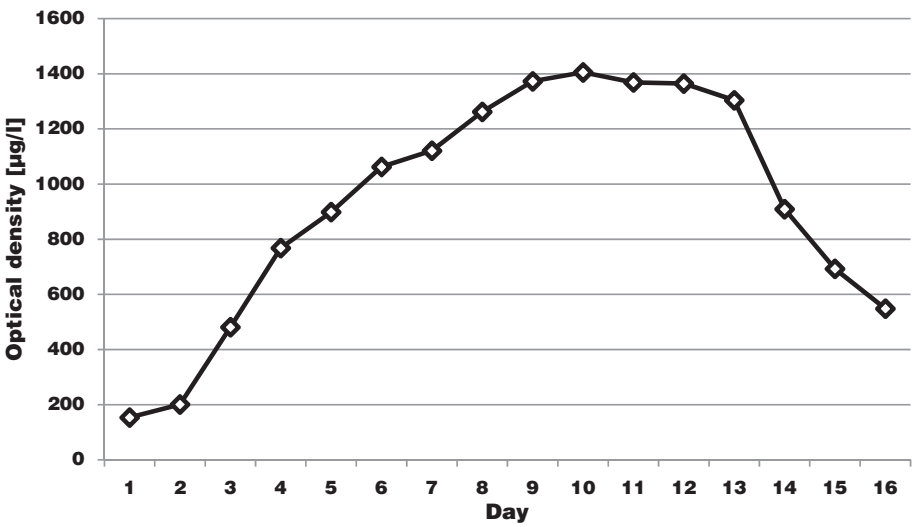

Figure 3. Growth curves recorded for *Nannochloropsis sp.* at 15 °C.

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$Na_2SiO_3.9H_2O$, trace elements, and vitamins were added to saline water following Guillard and Ryther's method.[42] The final volume is brought to 1 L by adding saline water or natural filtered seawater to the photobioreactor. Finally, the mixture is sterilised for 1 hour at 105 °C in an autoclave.

The uptake of nutrients by microalgae cells takes place during cultivation, so microalgae cells grow as much as they absorb nutrients up to the time the growth process reaches the stationary phase. At this stage harvesting can be undertaken and a new batch can be started.

Cultivation of the species and growth under proficient monitoring of the system

Cultivation of *Nannochloropsis sp.* aims to produce as much as biomass possible to be used for downstream processes.

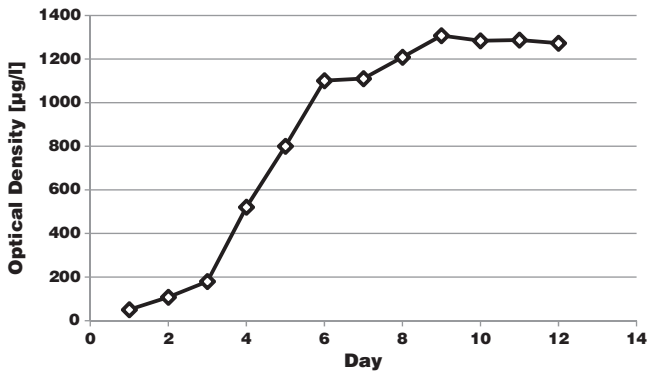

Figure 6. Growth curves recorded for *Nannochloropsis sp.* at 30 °C.

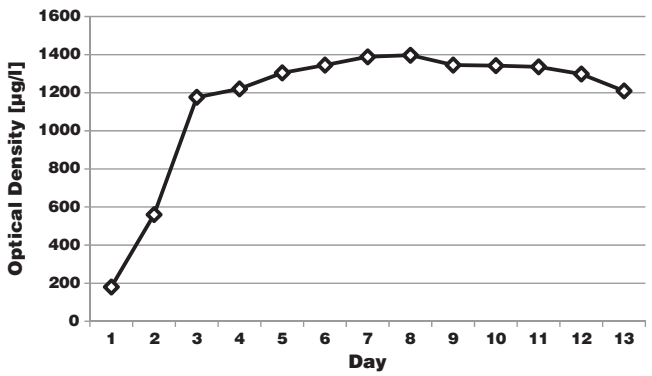

Figure 7. Growth curves recorded for *Nannochloropsis sp.* at 35 °C.

These processes need more biomass with high lipid content in order to generate more crude bio-oil. An effective, monitored system is required with defined operating conditions. Effective monitoring is very important because it can assist in the optimisation of the biomass production. In this study, cultivation was undertaken for a maximum of 15 days, as mentioned above, and is presented in Figs 3 to 7.

Experiments were carried out in a 1 L photobioreactor (see Fig. 2). The salinity of 30 ppt was chosen with the aim to simulate species growth conditions in the marine environment. Generally, the average seawater salinity favourable for species growth varies between 25 and 35 ppt. *Nannochloropsis sp.* being a marine species, it was essential to grow it under conditions of salinity similar to the marine environment. Concerning the pH, the culture was maintained at values equal or slightly above 7. The biomass was growing at an ambient temperature between 15 °C and 35 °C with an average light intensity of 1000 lux using fluorescent light for 24 hours of illumination. CO_2 was added daily to these batches and its volume was equivalent to 15 % of the photobioreactor.[43] The addition of CO_2 was intended to boost the lipid content of the growing biomass. To expose the cells to maximum illumination, facilitate gas exchange, and provide effective mixing, biomass aeration was undertaken during cultivation. Growth data are summarised in Table 1.

Results and discussions

Unit operations

Microalgae cultivation and growth curves

Growth measurements can be monitored using various methods. In this study, optical density was recorded on a daily basis for a period of 15 days. This was done to monitor the species' growth accurately. A wet microalgae biomass was collected from the filtration of 20 ml taken from the growing culture. This was mixed with 10 ml of 85% acetone. The filtration media used for separation of water and wet biomass was a GFF40 filter paper. The mixture of wet biomass and acetone was kept in vials at −4 °C in a refrigerant for 24 hours. A digital spectrophotometer Turner Designs serial number #7200-000 manufactured in San Jose, CA, with Optical Kits P/N 10-040R, was used for the optical

Table 1. Summary of growth data for *Nannochloropsis sp.* biomass.

Parameter	Salinity	Temperature range	Average pH	Light intensity (average)	CO_2 addition	Cultivation cycle and mode
Value	30 ppt	Controlled: 15, 20, 25, 30 and 35 °C	Neutral	1000 lux	5% of the total volume	15 days in a photobioreactor

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density measurements. In this device the fluorescence was set at 680 nm from a light source excited at 436 nm. The method is based on the direct reading of the fluorescence of optical density expressed in terms of the concentration of the pigment, which in this case was the amount of cells represented by the mass of chlorophyll A non-acid per unit volume. It was expressed in µg/L and represents the degree of absorption of light at a specified wavelength by the solution or suspension containing microalgae cells. The average of the daily measurements recorded in triplicate was used to plot the growth curves represented in Figs 3 to 7.

The analysis of these growth curves shows that *Nannochloropsis sp.* reached the highest amounts of biomass between day 8 and 10 for the temperature ranging from 15 to 35 °C. The highest optical density was obtained within the stationary phases mainly on the tenth day of the culture growing at 15 °C, 20 °C, and 25 °C. The stationary phase during cultivation was very short for these temperatures. Regarding the temperatures of 30 °C and 35 °C, the highest optical density values were recorded on day 9 and day 8 respectively; thereafter, the lysis phase was taking place. It was therefore recorded that the *Nannochloropsis sp.* used was growing efficiently within the temperature range from 15 °C to 25 °C. Similarly, it was recorded that the optical densities recorded during this temperature range were greater than those recorded for 30 °C and 35 °C. More cells were produced within the temperature range between 15 °C and 25 °C than between 30 °C and 35 °C, where the cells number decreased.

Biomass harvesting

Algae biomass was harvested during the stationary phase of the cultivation period. It was done on days where high values of optical densities were recorded because large amounts of biomass were produced on these days. It was therefore completed on day 10 for cultivation batches operating between 15 to 25 °C, and on day 9 for cultivation batches operating at 30 °C and on day 8 for cultivation batches operating at 35 °C. The biomass was harvested by centrifugation using a 'Hermle' centrifuge (HERMLE Labortechnik GmbH, Wehingen) running at 4000 rpm for 10 minutes. On average 1800 mg/l of wet biomass was recorded after 10 days, with an average of 600 mg/l of lipid recorded during the same growth period. This almost corresponds to data recorded for *Nannochloropsis sp.* F&M-24 by Rodolfi *et al.* in 2009.[39]

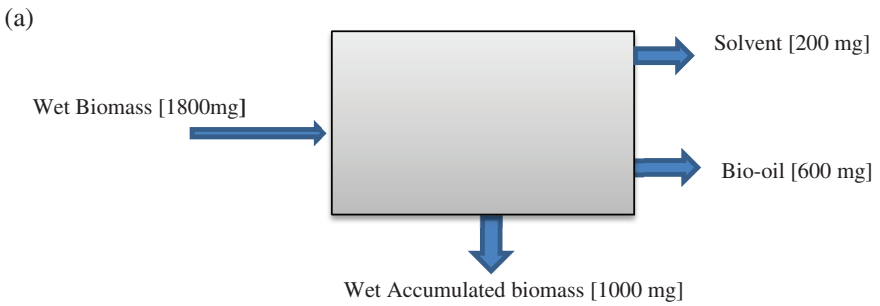

Figure 8a. Overall mass balance for bio-oil production/L of *Nannochloropsis* biomass before total nutrients starvation.

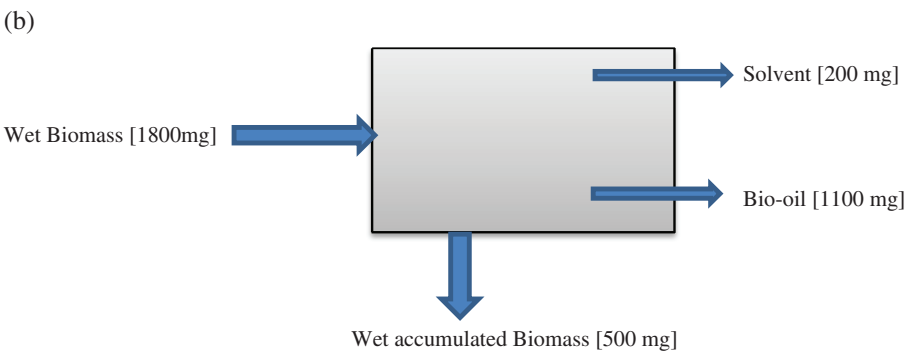

Figure 8b. Overall mass balance for bio-oil production/L of *Nannochloropsis* biomass after total nutrients starvation.

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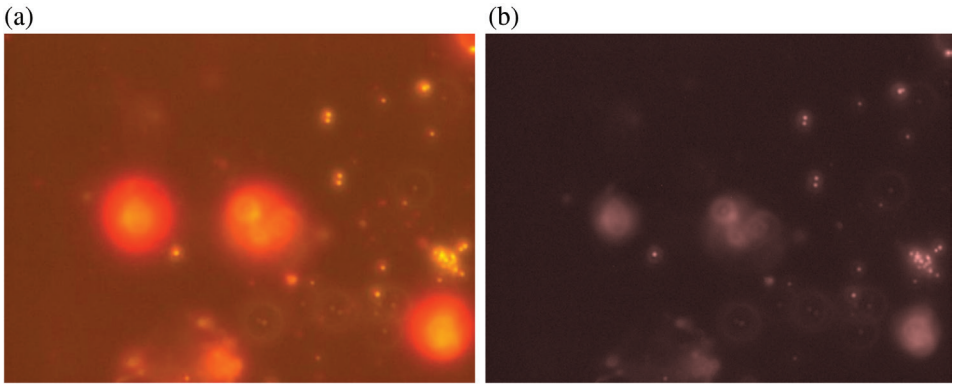

Figure 9. Cells of biomass before total nutrients starvation (right) and cells of biomass after total nutrients starvation (left).

Physiological modification

To increase microalgae lipid content, it is necessary to modify the physiology of cells. In many studies, increased lipid was obtained by partial nutrient starvation of cells. In most cases this was achieved by depriving species of nitrogen. In this study, total or complete nutrient starvation is undertaken to modify the physiology of the species in order to stimulate the synthesis of more lipids within cells. Deprivation of nutrients, or cell stressing, was undertaken for 3 days in a pure and nutrient-free environment. After starving microalgae cells for few days, a substantial increase in lipids was recorded. The lipid increase is displayed by the data recorded from the mass balances presented in Figures 8a and 8b.

The Nile Red technique was used to observe the changes regarding stressing or physiological modification of microalgae cells. The right side of Fig. 9 presents the configuration microalgae cells before starvation. The left side of Fig. 9 shows the changes that took place after genetic stressing or physiological modification caused by complete (or total) nutrient starvation of microalgae cells. It can be observed that the cells are yellow with more lipids after being totally deprived of nutrients for few days. The procedure for Nile Red consists of cells being incubated in a Nile Red solution made of 0.25 mg/l in acetone. The incubating time was 5 minutes and, thereafter, cells were viewed under a fluorescent microscope. A 450–490 nm excitation filter, a 510 nm diachronic mirror, and a 515 nm barrier filter with a 100x lens were also used.

Bio-oil extraction

Extraction of crude bio-oil from harvested biomass was completed using a modified Bligh and Dyer[44] method. In this modified method a solvent mixture made with chloroform and methanol in a 1 : 1 volume ratio is used. This mixture was added to the wet biomass. The volume ratio between the solvent mixture and wet biomass was also brought to a volume ratio of 1 : 1. The crude bio-oil extraction was undertaken on wet biomass. It was aiming to skip the drying process, which is energy and time consuming. Bio-oil was collected after solvent evaporation in an evaporator from 65 °C.

Determination of neutral lipids

Lipid content for the control and the stressed samples were determined using a gravimetric method. To determine the total amount of lipids accumulated in microalgae cells during the experiment the following procedure reported by Guckert *et al.*,[45] Wagner *et al.*,[46] and Lee *et al.*[47] was undertaken: 50 ml of microalgae was harvested by centrifugation for 20 min after cultivation. The extraction of lipids was completed by adding 28 ml of 50 mM phosphate buffer (pH 7.4) into the cell pellets. Thereafter, cells were resuspended and sonicated for 1 min. The samples were transferred to separating funnels; 35 ml chloroform and 70 ml methanol were added to each of them, then they were shaken in order to homogenise the mixtures and finally they were kept for 18 hours. After those 18 hours, 35 ml of chloroform and 35 ml distilled water were added to the samples. The samples were kept for a further 18 hours and the lower chloroform layer in a round-bottom flask was collected. The weight of the empty flask was recorded at the beginning of the experiment. The extract was evaporated in a water bath at 65 °C using a rotary evaporator to remove the solvents; the lipid content, as mass, was then worked out from the difference between the weight of the flask with bio-oil and the weight of the empty flask. This procedure has assisted in establishing the mass balances presented in Figs 8a and 8b.

Analysing these mass balances, it may be observed that, before nutrient starvation, the amount of bio-oil recorded represents only 30% of the total biomass used for oil extraction (Fig. 8a). After physiological modification undertaken

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with total starvation of nutrients, there is an increase of more than 80% when comparing the lipid content (bio-oil) before starvation (Fig. 8a) and after starvation (Fig. 8b).

Characterisation of crude bio-oil

The characterisation of microalgae crude bio-oil was completed using a gas chromatograph (GC/ MS). The sample of crude bio-oil was purified by filtration. This was followed by the transesterification of the bio-oil, after which it was injected into the GC/MS for characterisation. After running the standard, vegetable oils such as sunflower and canola oils were also used for trials. These trials were undertaken before running the bio-oil from *Nannochloropsis*. The purpose of the trials with vegetable oils was based on the fact that microalgae bio-oil is also a vegetable oil. It has qualitative similarities with many vegetable oils including sunflower and canola oils. These trials have assisted in establishing the method for the bio-oil characterisation with GC/MS. Subsequently, the sample of microalgae bio-oil was run successfully with the GC/ MS. Figure 10 shows the chromatograph and results from a sample of crude bio-oil from microalgae on GC/MS. Table 2 shows the presence of various fatty methyl esters (FAMEs) and other hydrocarbons found in the crude bio-oil from *Nannochloropsis sp.*

From the characterisation indicated in Table 2, FAMEs were identified as having carbon chains ranging between C6 to C57. This situation is very favourable for jet fuel production. Cracking is therefore needed in order to break down long carbon chains including C55 and C57 into short and medium ones essential for jet fuel production. Fractionation or distillation will follow, to separate different carbon chains based on the difference in boiling points. Jet fuel is a complex blend of alkanes made mainly from middle-end carbon chains between C14 to C16 and other alkanes from low-middle-end carbon chains in the case of jet B. Table 2 indicates that fractions between C14 and C16 have together recorded high percentages in terms of areas of the peaks compared to the other remaining fractions.

Thermal cracking: thermolysis

Compounds from the characterisation presented in Table 2 have to be broken down to produce middle-end fractions or carbon chains needed for jet fuel. These fractions must be mainly alkanes, as mentioned earlier, in order to obtain a bio-jet fuel similar to the conventional jet fuel. Firstly, cracking trials on vegetable oils such as sunflower and canola oils were undertaken at the following temperatures: 300, 350, 400, and 450 °C. It was done with the following times: 30, 45 and 60 min for each temperature. From these trials it was recorded that when cracking at the temperature of 300 °C all compounds were disintegrated or broken after a maximum time of 30 minutes. The experiment was simulated on GC/MS to determine the cracking temperature and time without catalyst for a complete disintegration of carbon chains for sunflower and canola oils. Secondly, after using vegetable oils, the same experience was undertaken on microalgae bio-oil. The simulated cracking on GC/MS for microalgae bio-oil generated similar results. The cracking temperature was recorded at 300 °C within a time of 31.06 min. The pyrolysis of purified bio-oil undertaken as fast cracking was achieved at

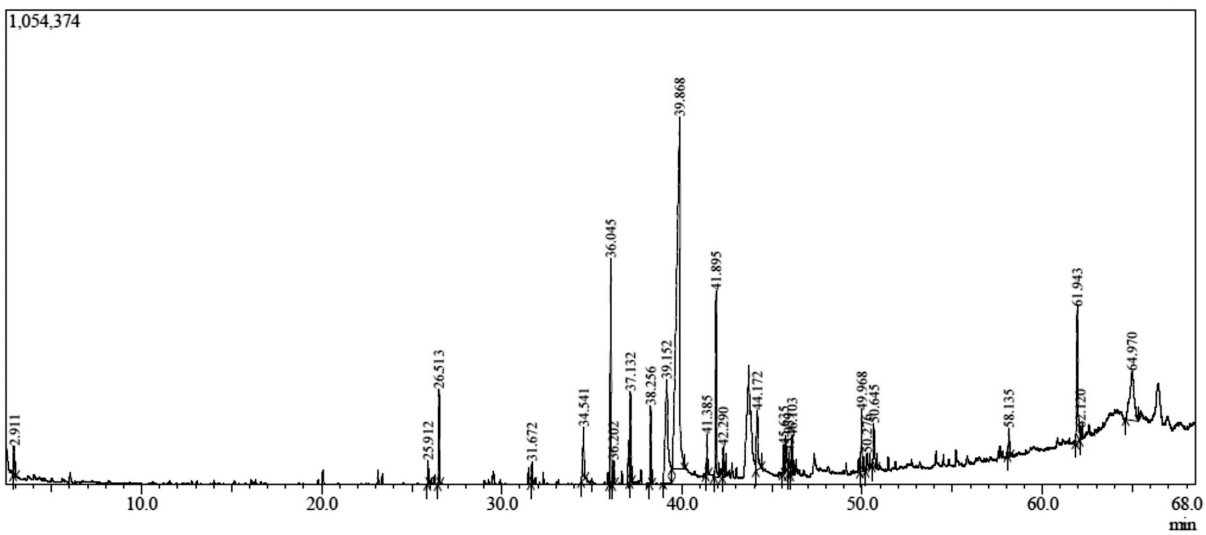

Figure 10. Chromatograph of *Nannochloropsis sp.* crude bio-oil.

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Table 2. Data for crude bio-oil from *Nannochloropsis* completed on GC/MS.

Peak no.	Retention time (min])	Area %	Height %	A/H	Compound formula and name
1	2.911	0.94	1.60	3.83	C_6H_8 : 1,4-cyclohexadiene
2	25.912	0.55	1.24	2.88	$C_{16}H_{32}$: 3-hexadecene, (Z)-
3	26.513	2.39	5.14	3.02	$C_{14}H_{30}$: tetradecane
4	31.672	0.61	1.21	3.30	$C_{16}H_{32}$: 3-hexadecene (Z)-
5	34.541	2.27	2.86	5.13	$C_{14}H_{28}O_2$: tetradecanoic acid
6	36.045	6.27	12.31	3.30	$C_{22}H_{42}O_2$: phytol, acetate
7	36.202	0.62	1.29	3.10	$C_{20}H_{40}$: 2-hexadecene, 3,7,11,15-tetramethyl-, [R-[R*
8	37.132	3.08	4.92	4.06	$C_{20}H_{40}O$ 3,7,11,15-tetramethyl-2-hexadecen-1-ol
9	38.256	2.04	4.25	3.11	$C_{17}H_{34}O_2$: hexadecanoic acid, methyl ester
10	39.152	9.77	5.61	11.29	$C_{16}H_{30}O_2$: palmitoleic acid
11	39.868	39.18	19.14	13.26	$C_{15}H_{30}O_2$: pentadecanoic acid
12	41.385	1.02,	2.14	3.09	$C_{57}H_{104}O_6$ 9-octadecenoic acid, 1,2,3-propanetriyl ester
13	41.895	5.87	9.89	3.85	$C_{21}H_{40}O_2$: octadecanoic acid, 2-propenyl ester
14	42.290	0.99	1.67	3.85	$C_{18}H_{34}O$: 9,12-octadecadien-1-ol, (Z,Z)-
15	44.172	2.82	3.18	5.74	$C_{18}H_{36}O_2$: octadecanoic acid
16	45.635	0.81	1.57	3.35	$C_{21}H_{38}O_2$: isopropyl linoleate
17	45.939	0.85	1.22	4.47	$C_{26}H_{44}O_6$: ethyl iso-allocholate
18	46.103	1.35	2.12	4.11	$C_{22}H_{34}O_2$:5,8,11,14,17-eicosapentaenoic acid, methyl ester
19	49.968	1.85	3.32	3.60	$C_{23}H_{34}O_2$: 4,7,10,13,16,19-docosahexaenoic acid, methyl
20	50.276	0.68	0.89	4.92	$C_{16}H_{30}O$cis-9-hexadecenal
21	50.645	1.94	2.34	5.36	$C_{19}H_{38}O_4$ hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl
22	58.135	0.77	1.45	3.43	$C_{27}H_{44}$: cholesta-3,5-diene
23	61.943	5.75	7.28	5.12	$C_{27}H_{46}O$: cholesterol
24	62.120	0.55	0.67	5.29	$C_{29}H_{50}O_2$: vitamin E
25	64.970	7.04	2.67	17.09	$C_{55}H_{106}O_6$: eicosanoic acid, 2-[(1-oxohexadecyl)oxy]-1-[[(1
		100	100		

350 °C during a time of 12 s while 450 °C was recorded as the pyrolysis temperature for crude bio-oil achieved within the same amount of time. The recovered quantity of oil recorded after thermal cracking was between 85% and 90%. Cracking temperature and time can be improved with the assistance of catalysts. Studies can be undertaken to choose the relevant catalyst for an effective cracking temperature and time. Figure 11 presents various peaks of the cracked bio-crude oil recorded on a GC/MS. This is a simulated cracking achieved at 300 °C.

Fractionation

A distillation unit was used for fractionation. This process was conducted at atmospheric pressure. During fractionation, solvent recovery took place between 60 °C and 65 °C. From 70 °C to an end point of 250 °C, middle distillate alkanes making a jet fuel from microalgae oil were collected.

Overall flowchart

Figure 12 presents the entire process undertaken in the laboratory to produce bio-jet fuel from microalgae cultivation. Future research can examine the possibility of adding upgrading and reforming unit processes to obtain a more purified final product.

Characterisation of the algal jet fuel from *Nannochloropsis*

The jet fuel produced was characterised with GC/MS. This was followed by physico-chemical analyses to determine if the product was similar to the conventional jet A1 used as a reference. Physico-chemical analyses were also completed, in compliance with ASTM standards for aviation fuels.

Figures 13 and 14 present the chromatographs resulting from the characterisation of jet A1 and the bio-jet fuel produced in this study. Table 3 shows details about different

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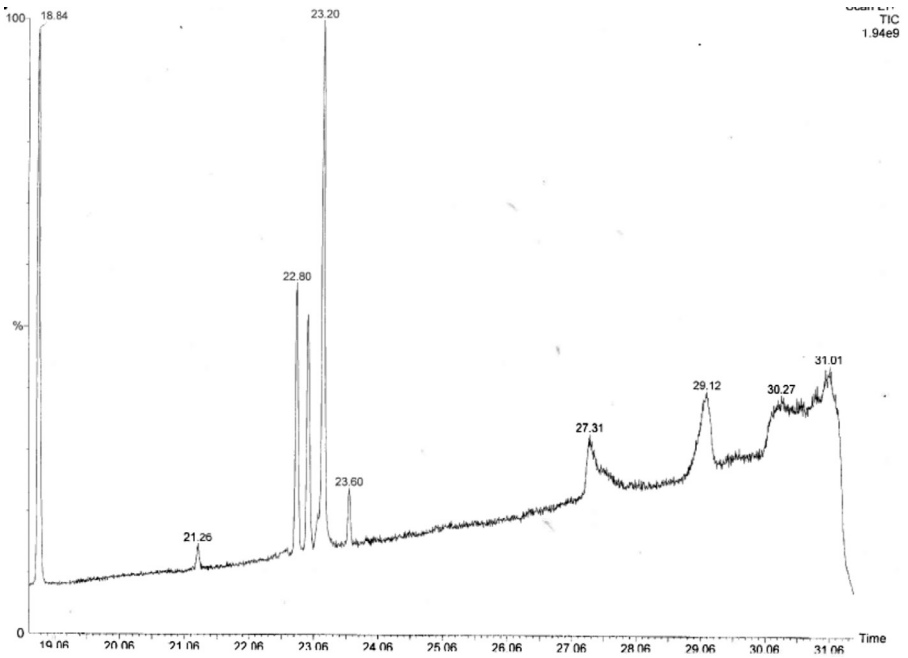

Figure 11. Chromatograph of simulated cracking for crude bio-oil from *Nannochloropsis sp.*

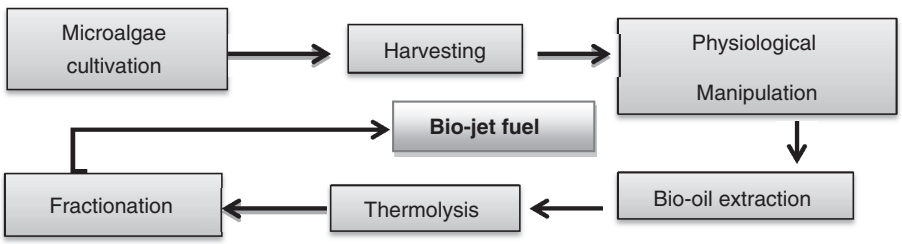

Figure 12. Flowchart of processing operations from algal cultivation to bio-jet fuel.

compounds found on the peaks in the chromatographs generated by the GC/MS.

There is a similarity between both jet fuels in terms of content when analysing all the peaks and the compounds from the chromatographs. This is confirmed by the fact that most compounds found in bio-jet fuel, and from jet A1, are alkanes as indicated in both versions of Table 3. These alkanes have been recorded on all peaks for both jet A1 and bio-jet fuel. A few exceptions were found with the jet A1: some compounds appear with oxygen and sulfur; these come from the petroleum crude oil and the additives used for improving the physico-chemical proprieties of jet A1. Table 4 presents the average for measurements done in triplicate during characterisation. It includes the physico-chemical data of both the bio-jet fuel produced in this study and the various conventional jet fuels. The importance of each parameter is explained in terms of the energy needed by the aircraft.

Analysis of Table 4 shows that most parameters recorded for algae-based jet fuel comply with the ASTM standards for aviation fuels. The analysis of data for the algae jet fuel shows that only density and freezing point need to be improved. This can be achieved by the use of additives. Bio-jet fuel produced in this study can be blended with jet A 1, jet A or jet B. By blending bio-jet fuel with conventional jet fuel it is possible to reduce the fuel carbon footprint, because the refraction index and combustion ability will increase and improve fuel sustainability.

Conclusion

- High amounts of biomass were produced between day 8 and day 10 of the cultivation period with temperatures ranging between 15 and 35 °C under a luminance

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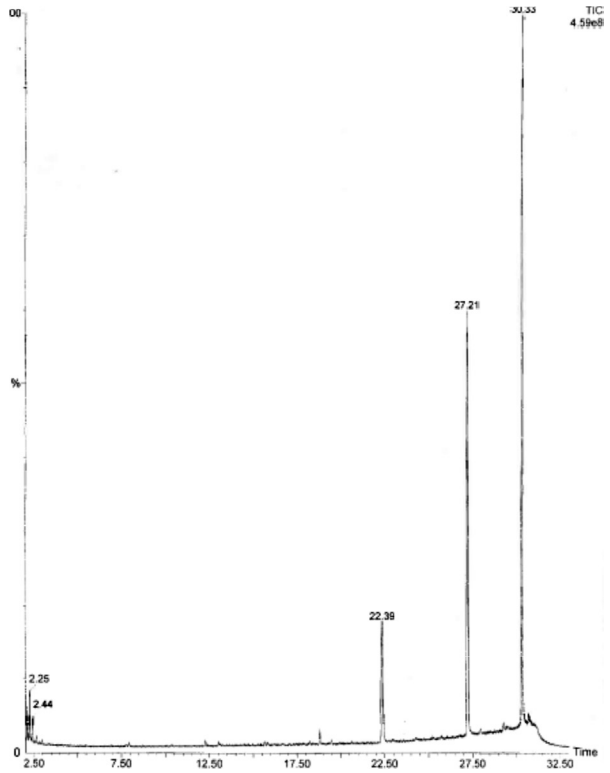

Figure 13. Chromatograph profile of produced bio-jet fuel.

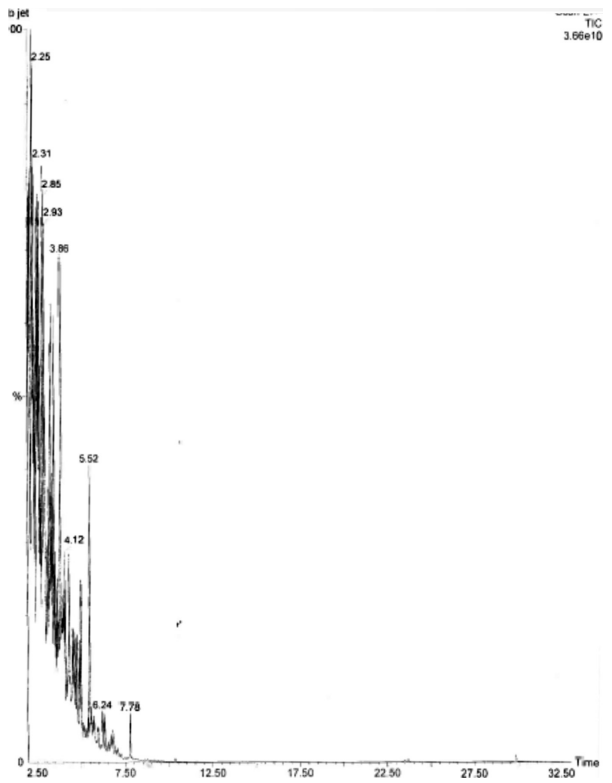

Figure 14. Chromatograph profile of conventional jet fuel (jet A1).

Table 3. Comparison between peaks from chromatographs of bio-jet fuel (a) and conventional jet fuel (b).

(a)		(b)	
Hit	Compound	Hit	Compound
1	$C_{31}H_{64}$	1	$C_{31}H_{64}$
2	$C_{54}H_{110}$	2	$C_{22}H_{46}$
3	$C_{23}H_{48}$	3	$C_{54}H_{110}$
4	$C_{25}H_{52}$	4	$C_{23}H_{48}$
5	$C_{22}H_{46}$	5	$C_{27}H_{56}$
6	$C_{24}H_{50}$	6	$C_{30}H_{62}$
7	$C_{30}H_{62}$	7	$C_{24}H_{50}$
8	$C_{32}H_{66}$	8	$C_{35}H_{72}$
9	$C_{39}H_{80}$	9	$C_{29}H_{60}$
10	$C_{30}H_{62}$	10	$C_{19}H_{40}$
11	$C_{34}H_{70}$	11	$C_{23}H_{48}$
12	$C_{40}H_{82}$	12	$C_{21}H_{44}$
13	$C_{30}H_{62}$	13	$C_{18}H_{38}O_3S$
14	$C_{25}H_{52}$	14	$C_{21}H_{44}$
15	$C_{54}H_{110}$	15	$C_{12}H_{26}$
16	$C_{35}H_{72}$	16	$C_{15}H_{32}O_3S$
17	$C_{24}H_{50}$	17	$C_{22}H_{46}$
18	$C_{36}H_{74}$	18	$C_{16}H_{34}O_3S$
19	$C_{24}H_{50}$	19	$C_{16}H_{34}$
20	$C_{40}H_{82}$	20	$C_{20}H_{42}$

of 1000 lux and supply of CO_2 on a daily basis during a cultivation period of 15 days.

- *Nannochloropsis sp.* grew effectively between 15 to 25 °C with more biomass produced in the same temperature range.
- The biomass was subjected to physiological modification for 3 days under total nutrient starvation with more than an 80% increase in lipid.
- The microalgae bio-oil produced was subjected to thermal cracking without catalyst at 300 °C for 31 min.
- The pyrolysis of oil was undertaken as a fast cracking at 350 °C for purified bio-oil achieved for a maximum period of 12 s while 450 °C was recorded as the pyrolysis temperature for microalgae crude bio-oil achieved for the same amount of time
- Fractionation was undertaken between 70 °C and 250 °C.
- Most physico-chemical parameters were within the range prescribed by ASTM standards, except freezing point and density. The use of antifreezing can assist in solving the issue of freezing point while polishing processes such as membrane filtration can be used to remove particulate matter in order to improve the density.

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Table 4. Comparison between conventional jet fuels and bio-jet fuel produced in the current study.

Parameter (min. or max. for ASTM standards)	Algae bio-jet (fuel from this study)	Jet A1 (ASTM D 1655 standards)	Jet A (ASTM standards D 1655)	Jet B (ASTM standards D 1655): wide cut kerosene	Importance of the parameter in terms of energy production
Heating value (MJ/kg) (minimum)	44	42.8	42.8	42.8	Represents the total energy released for fuel combustion. It is the energy content of the fuel
Freezing point (°C) (maximum)	–30	–47	–40	–50	Assists in fuel flow at lower temperatures. Consequently, the level of energy produced will not be affected because the mass of the fuel will not be affected
Flash point (°C) (minimum)	68	38	38	Not reported	Related to the fuel flammability and ignition
Kinematics viscosity at –20 °C (Cst) (maximum)	2.8	8	8	Not reported	Influences the capacity of the engine to restart and consume less fuel at higher altitudes.
Density at 15 °C (g/mL)	1.38	0.775–0.840	0.775–0.840	0.751–0.802	Affects engine performance and fuel consumption because the mass of fuel injected depends upon its density; the energy to propel the aircraft can therefore be affected
Sulfur total (wt%) (maximum)	0.27	0.30	0.30	0.30	Affects the fuel efficient, therefore, the generated energy can be affected
End point for distillation (°C) (maximum)	250	300	300	Not reported	Influences the fuel quality and the energy to be generated by the fuel

There is currently a need to mature the technology by moving from pilot to commercial scale. Once the technology has reached the level of maturity required for commercialisation, life-cycle assessment studies can be undertaken to predict the extent of carbon emissions and fuel sustainability. The pilot scale can involve more investigations on upgrading processes, the use of catalysts, modelling parameters to optimise the bio- jet fuel production and to improve its quality. This will also assist in defining conditions for efficient process design which are essential for scaling up to commercial plant. It will also assist in optimising the costs for a sustainable bio- jet fuel to compete with the current conventional jet fuel.

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References

1. Srirangan K, Akawi L, Moo-Young M and Chou CP, Towards sustainable production of clean energy carriers from biomass resources. *Appl Energy* **100**:172–186 (2012).
2. Brandt A, Gräsvik J, Hallett JP and Welton T, Deconstruction of lignocellulosic biomass with ionic liquids. *Green Chem* **15**(3):550–583 (2013).
3. Lakaniemi AM, Tuovinen OH and Puhakka JA, Anaerobic conversion of microalgal biomass to sustainable energy carriers – a review. *Bioresour Technol* **135**:222–231 (2013).
4. Knothe G, Krahl J and Van Gerpen J, *The Biodiesel Handbook.* Elsevier, Amsterdam (2015).
5. Knothe G and Razon LF, Biodiesel fuels. *Prog Energy Combust Sci* **58**:36–59 (2017).
6. Vassilev SV and Vassileva CG, Composition, properties and challenges of algae biomass for biofuel application: an overview. *Fuel* **1**(181):1–33 (2016).
7. Shankar S, Renewable and non-renewable energy resources: bioenergy and biofuels, in *Principles and Applications of Environmental Biotechnology for a Sustainable Future,* ed. by R.L Singh. Springer, Singapore, pp. 293–314 (2017).
8. Agwa OK, Ibe SN and Abu GO, Economically effective potential of *Chlorella sp.* for biomass and lipid production. *J Microbiol Biotechnol Res* **2**(1):35–45 (2017).
9. Blankenship RE, *Molecular Mechanisms of Photosynthesis.* Wiley-Blackwell, Chichester (2013).
10. Suganya T, Varman M, Masjuki HH and Renganathan S, Macroalgae and microalgae as a potential source for commercial applications along with biofuels production: a biorefinery approach. *Renewable Sustainable Energy Rev* **55**:909–941 (2016).
11. Eloka-Eboka AC and Inambao FL, Effects of CO_2 sequestration on lipid and biomass productivity in microalgal biomass production. *Appl Energy* **195**:1100–1111 (2017).

 © 2018 Society of Chemical Industry and John Wiley & Sons, Ltd

12. Demirbas A, Use of algae as biofuel sources. *Energy Convers Manage* **51**(12):2738–2749 (2010).
13. Demirbas A and Demirbas MF, Importance of algae oil as a source of biodiesel. *Energy Convers Manage* **52**(1):163–170 (2011).
14. Ong HC, Masjuki HH, Chong WT, Lam MK, Loh PK and Vellayan V, Microalgae biofuels as an alternative to fossil fuel for power generation. *Renewable Sustainable Energy Rev* **58**:180–197 (2016).
15. d'Ippolito G, Sardo A, Paris D, Vella FM, Adelfi MG, Botte P *et al.*, Potential of lipid metabolism in marine diatoms for biofuel production. *Biotechnol Biofuels* **8**(1):28 (2015).
16. Arenas EG, Palacio R, Juantorena AU, Fernando SE and Sebastian PJ, Microalgae as a potential source for biodiesel production: techniques, methods, and other challenges. *Int J Energy Res* **41**(6):761–789 (2016).
17. Moreno-Garcia L, Adjallé K, Barnabé S and Raghavan GS, Microalgae biomass production for a biorefinery system: recent advances and the way towards sustainability. *Renewable Sustainable Energy Rev* **76**:493–506 (2017).
18. Patel A, Gami B, Patel P and Patel B, Microalgae: Antiquity to era of integrated technology. *Renewable Sustainable Energy Rev* **71**:535–547 (2017).
19. Sahay S and Braganza VJ, Microalgae based biodiesel production–current and future scenario. *J Exp Sci* **7**:31–35 (2016).
20. Demirbas A, Production economics of high-quality microalgae. *Energy Sources, Part B: Economics, Planning, and Policy* **12**(5):395–401 (2017).
21. Ramachandran S, Ganesh PS, Sivasubramanian V and Kumar BP, Current developments in mass production of microalgae for industrial applications, in *Environmental Sustainability using Green Technologies*, ed. by V. Sivasubramanian. CRC Press, Nature publishing group, London, p. 147 (2016).
22. Shukla M, Tabassum R, Singh R and Dhar DW, Influence of light intensity, temperature and CO_2 concentration on growth and lipids in green algae and cyanobacteria. *Indian J Exp Biol* **54**:482–487 (2016).
23. Griffiths H, Bringing new products from marine microorganisms to the market, in *The Marine Microbiome,* ed. by Stal LJ and Cretoiu MS. Springer, Cham, pp. 435–452 (2016).
24. Whittle SJ and Casselton PJ, The chloroplast pigments of the algal classes Eustigmatophyceae and Xanthophyceae. I. Eustigmatophyceae. *British Phycological Journal* **10**(2):179–191 (1975).
25. Dunstan GA, Volkman JK, Barrett SM and Garland CD, Changes in the lipid composition and maximisation of the polyunsaturated fatty acid content of three microalgae grown in mass culture. *J Appl Phycol* **5**(1):71–83 (1993).
26. Imamoglu E, Demirel Z and Conk Dalay M, Process optimization and modeling for the cultivation of *Nannochloropsis* sp. and *Tetraselmis striata* via response surface methodology. *J Phycol* **51**(3):442–453 (2015).
27. Mayaruma I, Nakamura T, Matsubayashi T, Ando Y and Maeda T, Identification of the alga known chlorella as a marine Chlorella as a member of the Eustigmatophyceae. *J Phycol* **34**:319–325 (1992).
28. Adarme-Vega TC, Thomas-Hall SR, Lim DK and Schenk PM, Effects of long chain fatty acid synthesis and associated gene expression in microalga *Tetraselmis sp. Mar Drugs* **12**(6):3381–3398 (2014).
29. Patterson GW, Tsitsa-Tzardis E, Wikfors GH, Ghosh P, Smith BC and Gladu PK, Sterols of eustigmatophytes. *Lipids* **29**(9):661–664 (1994).
30. Martin-Creuzburg D and Merkel P, Sterols of freshwater microalgae: potential implications for zooplankton nutrition. *J Plankton Res* **38**(4):865–877 (2016).
31. Sukenik A, Production of eicosapentaenoic acid by the marine eustigmatophyte *Nannochloropsis. Chemicals from Microalgae,* ed. by Taylor and Francis. London, UK, pp. 41–56 (1999).
32. Singh P, Kumari S, Guldhe A, Misra R, Rawat I and Bux F, Trends and novel strategies for enhancing lipid accumulation and quality in microalgae. *Renewable Sustainable Energy Rev* **55**:1–6 (2016).
33. Minhas AK, Hodgson P, Barrow CJ and Adholeya A, A review on the assessment of stress conditions for simultaneous production of microalgal lipids and carotenoids. *Front Microbiol* **7**:546 (2016).
34. Zittelli GC, Lavista F, Bastianini A, Rodolfi L, Vincenzini M and Tredici MR, Production of eicosapentaenoic acid by Nannochloropsis sp. cultures in outdoor tubular photobioreactors. *J Biotechnol* **70**(1):299–312 (1999).
35. Safafar H, Hass MZ, Møller P, Holdt SL and Jacobsen C, High-EPA Biomass from *Nannochloropsis* salina cultivated in a flat-panel photo-bioreactor on a process water-enriched growth medium. *Marine Drugs* **14**(8):144 (2016).
36. Remmers IM, Martens DE, Wijffels RH and Lamers PP, Dynamics of triacylglycerol and EPA production in *Phaeodactylum tricornutum* under nitrogen starvation at different light intensities. *PloS One* **12**(4):e0175630 (2017).
37. Shifrin NS and Chisholm SW, Phytoplankton lipids: interspecific differences and effects of nitrate, silicate and light-dark cycles. *J Phycol* **17**:374–384 (1981).
38. Chisti Y, Biodiesel from microalgae beats bioethanol. *Trends Biotechnol* **26**(3):126–131 (2008).
39. Rodolfi L, Chini Zittelli G, Bassi N, Padovani G, Biondi N, Bonini G *et al*. Microalgae for oil: Strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor. *Biotechnol Bioeng* **102**(1):100–12 (2009).
40. Ma XN, Chen TP, Yang B, Liu J and Chen F, Lipid production from *Nannochloropsis. Marine Drugs* **14**(4):61 (2016).
41. Bondioli P, Della Bella L, Rivolta G, Zittelli GC, Bassi N, Rodolfi L *et al.* Oil production by the marine microalgae *Nannochloropsis sp.* F&M-M24 and *Tetraselmis suecica* F&M-M33. *Bioresour Technol* **114**:567–572 (2012).
42. Guillard RR and Ryther JH. Studies of marine planktonic diatoms: I. Cyclotella Nana Hustedt, and Detonula Confervacea (CLEVE) Gran. *Can J Microbiol* **8**(2):229–239 (1962).
43. Goswami RC, Kalita N and Kalita MC, A study on growth and carbon dioxide mitigation by microalgae *Selenastrum* sp.: its growth behavior under different nutrient environments and lipid production. *Ann Biol Res* **3**(1):499–510 (2012).
44. Bligh EG and Dyer WJ, A rapid method of total lipid extraction and purification. *Can J Biochem Physiol* **37**(8):911–917 (1959).
45. Guckert JB, Cooksey KE and Jackson LL, Lipid solvent systems are not equivalent for analysis of lipid classes in the microeukaryotic green alga, Chlorella. *J Microbiol Meth* **8**:39–149 (1988).
46. Wagner H, Liu Z, Langner U, Stehfest K and Wilhelm C, The use of FTIR spectroscopy to assess quantitative changes in the biochemical composition of microalgae. *J Biophotonics* **3**:557–566 (2010).
47. Lee SJ, Yoon BD and Oh HM, Rapid method for the determination of lipid from the green alga *Botryococcus braunii*. *Biotechnology Tech* **12**(7):553–556 (1998).

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Joseph K. Bwapwa

Joseph K. Bwapwa is a lecturer at the Mangosuthu University of Technology (MUT) and researcher at the University of Kwazulu-Natal (UKZN) in South Africa. His areas of expertise are water and wastewater bioremediation technologies, and bioenergy – especially bioenergy production related to algae biotechnology. He has published papers in these areas and has attended numerous international conferences. He works under the supervision of Prof Anandraj at the Centre of Algal Biotechnology and Prof Cristina Trois at the University of Kwazulu-Natal. His MSc in engineering focused on the treatment of anaerobic digestion of complex particulate wastewater and his PhD research is focusing on the conversion of algae biomass to aviation fuel.

Professor Akash Anandraj

Professor Akash Anandraj has a PhD in biology from the University of Kwazulu-Natal. His research interests include algal biotechnology and biofuels, microalgae physiology, estuarine ecology, phyco-remediation, biogas from algae, value-added products from algae, and algae carbon sequestration rates.

Professor Cristina Trois

Professor Cristina Trois is dean and head of the School of Engineering at the University of Kwazulu-Natal, South Africa. She is an environmental engineer from Italy. Her general area of expertise is in environmental engineering, especially wastewater treatment, solid waste management, landfill emissions control and treatment, acid mine drainage, and geoengineering. She is a founding member of the Centre for Research on Environmental, Coastal and Hydrological Engineering (CRECHE) at the University of KwaZulu-Natal.

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In the Field

Biofpr Biofuels Bioproducts & Biorefining

Conceptual process design and simulation of microalgae oil conversion to aviation fuel

Joseph K. Bwapwa, University of Kwazulu-Natal, School of Engineering, Durban, South Africa and Mangosuthu University of Technology, Department of Civil Engineering, Faculty of Engineering, Jacobs, South Africa
Akash Anandraj, Mangosuthu University of Technology, Department of Nature Conservation, Faculty of Natural Sciences, Umlazi, South Africa
Cristina Trois, University of Kwazulu-Natal, School of Engineering, Durban, South Africa

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Abstract: Microalgae oil can be converted into aviation fuel to reduce dependence on fossil fuels and decrease the carbon footprint. This could be a significant step toward sustainable energy resources that have the potential to produce 'drop-in' fuels. Algae-based fuels are potential substitutes for fossil fuels due to the quality of their crude oil. However, this is only possible if appropriate conversion processes are undertaken. Conversely, microalgae species have low lipid content and biomass harvesting is still an energy-demanding process. In this study, a conceptual design is developed for the conversion of microalgae oil to jet fuel. It is based on a process undertaken in the laboratory using a species named *Nannochloropsis* sp. Nutrients and CO_2 were supplied to the growing culture for effective growth. Biomass harvesting was completed on the tenth day of the growth cycle. It was followed by physiological modification to improve lipid content. Finally, crude oil extraction was followed by bio-oil hydrocracking at 350 °C, and fractionation of cracked bio-oil between 70 and 300 °C to separate light-, middle-, and heavy-end hydrocarbons for use in the production of jet fuel. During simulation, reforming and upgrading processes were added to the design to enhance the quality of the jet fuel to be produced on large scale in the future. The study, including the results, suggests that it is technically feasible to convert microalgae oil into jet fuel because of the similarity between algae bio-oil and petroleum crude oil. © 2018 Society of Chemical Industry and John Wiley & Sons, Ltd

Keywords: *Nannochloropsis*; jet fuel; conversion process; microalgae oil; process design; simulation

Introduction

Background, jet fuel and biodiesel

The current need for sustainable energy has led to a focus on the development of ground-breaking and alternative types of renewable energy. This trend aims to fill the gap created by an increasing demand from the energy industry.[1] Options related to the production of biofuels such as ethanol, biodiesel, and jet fuel from biomass are therefore currently being considered carefully. Ethanol derived from starch, sugar, or any other type of biomass can be used as an alternative or additive to gasoline.[2–5] Furthermore, if cellulosic biomass is used successfully to produce bioethanol at

Correspondence to: Joseph K. Bwapwa, 77 Ebor Heights, Bamboo lane, 3610, Pinetown, South Africa. E-mail: joseph@mut.ac.za; josephkapuku@gmail.com

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affordable costs, there will consequently be competition with petroleum-based fuels. Regarding the biodiesel alternative, some edible and non-edible oils can be a substitute for petroleum diesel if the production costs are reduced.[6,7] Similarly, the use of these oils should not be a threat to food production, lubrication, and many other industrial applications. Unfortunately, this kind of biofuel cannot be used directly in aircraft engines as a substitute for jet fuel, for which high energy density and low-temperature are required and very important parameters apply. They are among key parameters that define the performance of a jet fuel.

The energy density per unit volume for ethanol is not sufficient to run an aircraft because it is half of the total energy produced by a conventional jet fuel. However, the energy density per unit volume in biodiesel represents almost 80% of that contained in jet fuel.[8] The major weakness of biodiesel is that, at lower temperatures, with an aircraft flying at higher altitudes, there will be fuel solidification taking place.[8,9] This is due to the fact that the biodiesel freezing point is much higher than that of conventional jet fuel. Studies to find an appropriate flow improver will be needed for biodiesel.[9] It is a stringent requirement for any aviation fuel to have a very low freezing point to avoid disastrous consequences due to its solidification during flight. Biodiesel therefore cannot be a suitable fuel for aircraft.

Market expectations, current state of technology and requirements to produce algae bio-oil

It is essential to produce algae-based jet fuel with similar physico-chemical properties to conventional jet fuel; it has to be cost effective and sustainable and have low carbon emissions. A designing and simulating process including modeling and optimizing some parameters could be a key milestone in the production of sustainable jet fuel from microalgae oil. Many studies are currently under way but not many of their outputs are in the public domain. The field of alternative aviation fuels, and more especially work on microalgae-derived jet fuels, is becoming particularly competitive. Demand and market expectations are very high with regard to compliance and costs. 'Drop-in' fuels are the most needed but they are not sustainable as yet; however, the blending of current fossil fuels with algae-derived fuels is considered to be a sustainable option. Due to strong similarity between petroleum crude oil and algae bio-oil qualitatively and quantitatively (depending on the species/strain), it is possible to use a process that is similar to that used in refineries for conventional jet fuel in order to produce algae-based jet fuel.[10] However, the process has to be improved economically for algae-derived jet to be competitive and finally become commercialized. There is currently no jet fuel on the market produced on a larger scale from algae bio-oil. Many projects are still at trial and pilot scale. Algae -derived jet fuel is only blended with conventional jet fuel on 50–50 ratio as allowed by ASTM certification.

For microalgae to produce oil, biomass cultivation must take place under defined conditions of temperature and pH. The addition of nutrients and carbon dioxide to allow effective growth should also be part of the cultivation process. Harvesting takes place after the cultivation period and thereafter bio-oil extraction is undertaken to produce bio-oil for the conversion process. In some situations, microalgae grow best in saline water / seawater or wastewater more generally, and in domestic sewage because of the presence of nutrients such as nitrates and phosphates. However, this is not always possible for all species or strains; some will grow easily in any water or wastewater streams and other not, and it also depends on the species or strain's nature or type. Being photosynthetic organisms, microalgae cells require sufficient light to guarantee an effective growth in order to produce enough biomass. Species can therefore multiply in a very short period of time if all growth conditions are gathered. Sunlight can be used, in this regard, as cheap option for culture illumination; it is an energy-efficient way to cultivate algae, although there could be daily and seasonal variations in terms of light intensity.[11,12]

Cultivation, lipid production and lipid content

Cultivation takes place in photobioreactors or open ponds. Various microalgae species store energy in the form of hydrocarbon, which can produce lipids or bio-oil. This is obviously happening when nutrient depletion takes place during the cultivation period. This period is characterized by the species growth, which is regarded as the biomass production time.[13,14] The cells' buoyancy is also regulated by the lipids present in them. However, the lipid content for many species is generally low. By manipulating the microalgae cell genetics and the growth conditions it is possible to increase bio-oil output. Genetic or physiological modification of microalgae cells will stimulate lipid increase. This is needed for conversion processes. The genetic modification of an algal cell is all about stressing the species under adverse conditions to stimulate the metabolism of the microalgae cell. As a result, stressing microalgae cells under defined conditions will allow the production of more lipids.

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The need for effective conversion processes and overview of conversion processes

Algae-based fuels in general, and jet fuel in particular, require cost-effective processes or technologies that will, first, allow effective cultivation and harvesting of biomass, second, generate high output of crude oil, and finally, assist in optimizing conversion processes or technologies to produce compliant jet fuel.[10,15] Process design and simulation, including optimization of parameters, is therefore very important to produce a compliant and cost-competitive jet fuel, as mentioned earlier. The design of conversion processes should thus take into consideration operating conditions, energy consumption, the cost of additives, and the expectations of the aviation sector and the market.[10]

Many approaches can be explored to convert microalgae oil into liquid fuels. Gasification of algae biomass using Fischer–Tropsch (F–T) synthesis is known to be a successful process.[16–20] However, gasification is energy-intensive because it requires higher temperatures to reach an adequate gasification stage. The F–T synthesis is also characterized by low selectivity for liquid fuels – especially for fractions in the range between C6 to C22; there are lighter fractions, such as methane and ethane, and there are middle and heavy fractions.[21] Transesterification is another approach that can be used to convert microalgae oil into aviation fuel; this approach is known as the biodiesel route, although it is not energy intensive; however, it involves catalytic processes such as decarboxylation, deoxygenation, and isomerization, which can be costly compared to the ones used for the production of conventional jet fuel.[10,22–26] Hydroprocessing oil from microalgae can also generate a jet-fuel type using hydrogen as a catalyst during cracking.[27,28] The hydrogen removes oxygen from algae crude bio-oil during the process. Despite the challenge related to low lipid content for many species, these processes are technically achievable. Another challenge is the costs related to the feasibility or economics of the processes. These costs are higher compared to those related to conventional jet-fuel production.

Process design and the aim of the study

When designing an efficient process it is important to take into consideration the type of species, growth conditions, growth time, the rate of biomass production, and the possibility of producing a large amount of lipids. The current study based the process design on laboratory experiment. Most parameters for design were collected from the experimental laboratory work.

Designing and simulating a process for algae-based aviation fuel should focus on developing a sustainable technology. This technology must be developed over time so that its escalation will need few modifications for the fuel to be ready for use. The maturity of the technology will be a major characteristic that will reflect on its readiness. This comes after successful trials at laboratory and pilot scale. It is followed by implementation on a larger scale.

The aim of this study is to establish a conceptual design and simulate the entire process by involving all the steps from cultivation to production of jet fuel using the Aspen HYSIS V8.8 package, which was the only version available for the study. The design can be used as a tool for a larger scale plant in the future once studies of the costs and economics have been completed. The strain used during experimental work to generate the biomass and the crude oil was *Nannochloropsis* sp., a marine species. The steps and operating conditions used in the laboratory constitute the basis for process design, as mentioned earlier. This approach aims to mimic a process close to that used for conventional jet fuel production. The specific aim in simulating the process is to generate a microalgae-based jet fuel that can comply with stringent aviation regulations. This is to some extent possible because of the similarity between algae crude bio-oil and crude petroleum oil as mentioned before.[10] Cost analysis is not the focus of this study; it can be addressed on its own in another study. It is a very interesting aspect of design but it needs to be undertaken after successful design and simulation. This study focuses on conceptual design and simulation based on data generated from a laboratory experiment. Future studies can be undertaken on a very detailed design involving equipment modeling and sensitivity analysis needed for all variables before undertaking cost studies.

Overview of *Nannochloropsis* oil

Nannochloropsis sp. contains both eicosapentaenoic (EPA) and docosahexaenoic (DHA) acid oils. Its higher percentage of neutral lipids allows *Nannochloropsis* sp. to be an excellent candidate to produce alternative jet fuel needed for commercial and military aircrafts. Furthermore, the high quantity of EPA (20:5 ω3), reaching up to 5% w/w of the biomass, enables *Nannochloropsis* sp. to be widely cultivated for food supplements and pharmaceuticals. Generally, if the biomass productivity is higher, there is a probability of generating a high EPA productivity rate.[29,30] *Nannochloropsis* sp. is also recommended as a source of the lipid needed to produce biofuels because more than 60% of lipids can be achieved

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Table 1. Comparison between algae crude oil and petroleum crude.

W%	Algal crude[37]	Petroleum crude[38–41]
Sulfur	0.5	1.42
Oxygen	5.5	0.1–1.5
Nitrogen	4.4	0.1–2.0
Carbon	78.7	83–87

after nitrogen starvation. These lipids are essentially triacylglycerol (TAG) with saturated and monounsaturated fatty acids.[31–33] The greatest advantage of *Nannochloropsis* sp. for high-quality biofuel production can be attributed to its high concentrations of EPA. The percentage of EPA and other fatty acids present in *Nannochloropsis* cells may influence the species growth conditions, and biomass and lipid productivity. These parameters correlate strongly with the species' EPA content. Some studies have reported that *Nannochloropsis* sp. major fatty acids are C 14:0, C16:0, C20:4ω6 and C 20:5ω5.[34–36] They are short chains of fatty acids with a high percentage of saturation and unsaturation and mono-unsaturation configurations. Based on these facts, algae bio-oil in general and *Nannochloropsis* sp. bio-oil in particular can be a potential source for jet fuel production. Table 1 presents a brief composition of algae crude bio-oil compared to the petroleum crude oil. Analyzing the data in Table 1, it is obvious that there is no significant difference between the percentages of sulfur, oxygen, nitrogen, and carbon for both crudes. This confirms that there is a good possibility of generating jet fuel from algae oil as mentioned previously.

Results and discussion

Laboratory process for jet fuel production, methodology, operating conditions and results

The laboratory experiment to obtain jet fuel from microalgae using *Nannochloropsis* sp. was completed in a study prior to the current one by Bwapwa *et al.* (2018).[42] The experiment involves cultivation, harvesting (dewatering), oil extraction by solvent, cracking, and fractionation. Figure 1 summarizes the laboratory process used to obtain jet fuel from microalgae biomass. Jet fuel produced from the laboratory experiment includes cultivation and all the conversion processes mentioned earlier. The use of nutrients and CO_2 is completed during cultivation followed by the physiological modification to increase the lipid content of the species after biomass harvesting. Conversion processes take place after biomass harvesting in order to produce jet fuel. The characterization of jet fuel aiming to determine the level of compliance regarding the production of jet fuel is also included in the study undertaken by Bwapwa *et al.*[42] The experiment used to produce jet fuel was reproduced in the current study and the data obtained confirmed that most, but not all, physico-chemical parameters analyzed were in accordance with ASTM standards. This current study is a continuation of the laboratory-based experiment to develop a conceptual design for jet fuel production from microalgae species.

Cultivation was undertaken in a 2 L glass bottle photobioreactor. The cultivation environment was made of saline water representing the marine environment in which *Nannochloropsis* sp. can grow effectively. Nutrients and CO_2

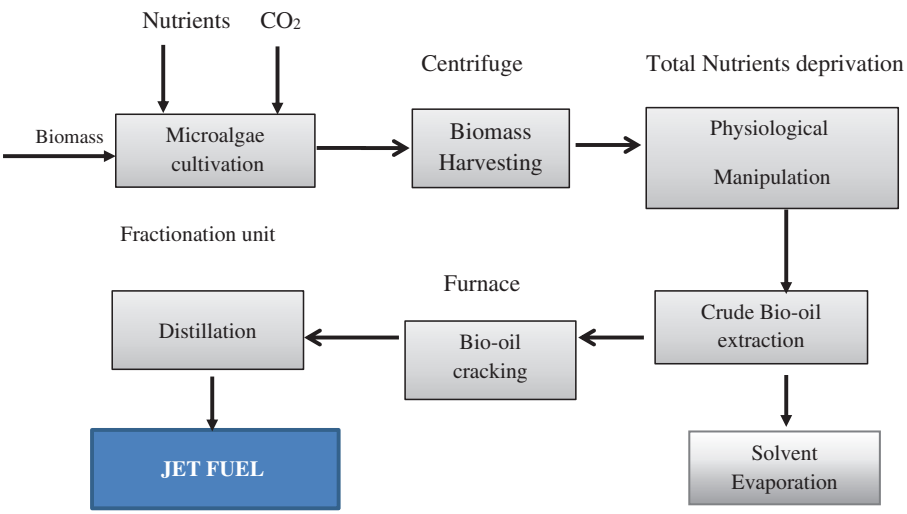

Figure 1. Detailed approach used to produce jet fuel from *Nannochloropsis* sp. in the laboratory (adapted from Bwapwa *et al.*[42]).

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were supplied to the growing culture to sustain the growth during cultivation, as mentioned above. Carbon dioxide provides more carbon chains and allows the increase of lipids within the cells. It was added daily to a 2 L photobioreactor for the 15 days cultivation period. Its quantity represented 5% of the total volume of the photobioreactor.[42] F/2 media were used as a source of nutrients needed for the species growth. These media include nitrates, phosphates, and trace elements. The salinity concentration of saline water was 30 ppt, representing the average salinity for seawater to facilitate the effective growth of the species. Magnetic stirring was implemented after adding 50 g of dry *Nannochloropsis* sp. biomass to the media. The culture was growing at room-controlled temperature of 25 °C under continuous illumination of 1000 lux for 24 h. Growth was monitored using a Turner Designs spectrophotometer, Model #7200-000, San Jose, CA, with Optical Kit P/N 10-040R to record the optical density. This was achieved by measuring chlorophyll A non- acid daily, as the cells were multiplying very fast. These optical density values were the reflection of culture growth for which the highest value was recorded on day 10. After the tenth day it was observed that the optical density was decreasing perceptibly. This was the sign that, after the tenth day, cells were dying; no more cells were produced.

Harvesting was undertaken on the tenth day using a centrifuge for biomass dewatering. The wet biomass in form of heavy slurry was subjected to a physiological modification to increase the lipid content. Physiological modification was achieved by the total deprivation of nutrients, which was undertaken in a separate environment after harvesting biomass. This process aimed to stress microalgae cells by manipulating their physiology in a nutrient-free environment. This influenced the cells metabolism and boosted their lipid content as mentioned earlier.

Thereafter, another separation using the centrifuge was undertaken to recover the biomass and use it for bio-oil extraction. From 2 L of culture, 1500 mL of algae on average was collected for several times during cultivation. An average of 900 mL of bio-oil mixed with solvent was recovered and the remaining 600 mL was made of wet biomass. The bio-oil extraction was completed with a mixture of chloroform–methanol in 1/1 volume ratio. This was undertaken in beaker with magnetic stirring for 30 min.

Thermal cracking of bio-oil in a close glass container without a catalyst was undertaken in a furnace MSF 12/6, MRC , Ukraine at 300 °C for a duration of about 31 min with the following power characteristics: 220 V / 50 Hz, 2700 W. After cracking, the bio-oil was fractionated using a distillation unit at a pressure of 1 bar. Mixed solvent made up of chloroform and methanol was recovered from fractionation at between 60 and 65 °C; chloroform was recovered first and this was followed by methanol. The distillate representing the jet fuel from microalgae oil was collected from 70 °C to an end point of 250 °C. Table 2 presents the data recorded from the characterization of the algae-based jet fuel generated from the laboratory experiment. The results were recorded in triplicate; they are presented with the average and standard deviations.

Analysis of these data shows that most parameters are in accordance with the ASTM standards except the freezing point and specific gravity. However, these two parameters can be improved with the use of relevant additives or processes.

Discussion of the data recorded in Table 2 for the algae-based jet fuel

Net heat of combustion

Heating value, also known as net heat of combustion, represents the amount of energy that can be generated by a jet fuel in order to run an aircraft. It is a very important parameter required for all fuels including jet fuel. The data in Table 2 has recorded a heating value or net heat of combustion of 44 MJ kg^{-1} for algae-derived jet fuel, which is above the minimum of 42.8 MJ kg^{-1} required by ASTM standard for jet fuel.

Freezing point

The freezing point is another very important parameter regarding the performance of a jet fuel. It influences jet-fuel fluidity and pumpability at lower temperatures during the flight. At higher altitudes, where temperatures are much lower, the jet fuel will tend to crystallize. It is therefore important to have a jet fuel with the lowest freezing point to avoid

Table 2. Characterization data for algae-based jet fuel produced from the laboratory process indicated in Fig. 1.

Parameter	Algal-based jet fuel	ASTM D1655 standards for jet fuel
Net heat of combustion (MJ kg^{-1})	44 ± 0.5	**42.8 (minimum)**
Freezing point (°C)	–32 ± 4	**–40** (maximum)
Flash point (°C)	68 ± 0.3	**38** (minimum)
Final boiling point (°C)	250 ± 3	**300 (maximum)**
Kinematics viscosity @ –20 °C (cSt)	2.8 ± 0.2	**8** (maximum)
Density @ 15 °C (g cm^{-3})	0.96 ± 0.1	0.775–0.840
Total acidity mg KOH g^{-1}	0.05 ± 0.001	**0.015 (maximum)**
Total sulfur %	0.27 ± 0.02	**0.3 (maximum)**
Conductivity pS m^{-1}	85 ± 0.6	50–450

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the solidification of the fuel at higher altitudes. An ice inhibitor or anti-freezing ingredient can be added to ensure that jet fuel does not solidify. The result, reported in Table 2, shows that the freezing point of algae- derived jet fuel, which is –32 °C, is higher than the value of –40 °C required by ASTM. In this case, the use of an ice inhibitor or anti-freezing additive is therefore recommended to improve the freezing-point algae-derived jet fuel.

Flash point

The flash point relates to jet fuel volatility. This can have an impact on combustibility and flammability. It is the main parameter related to fire safety associated with jet-fuel handling at various temperatures. The data reported in Table 2 indicate a very high flash point for algae- derived jet fuel. This is an indication that the algae- derived fuel presents lower risks of flammability at lower temperatures and can be handled safely in hot environments.

Final Boiling point for distillation

The final boiling point recorded in Table 2 is 250 °C. A maximum of 300 °C is prescribed by the ASTM standard for jet fuel. This implies that all hydrocarbon fractions needed for jet fuel can be collected at 250 °C, reducing the energy demands for the fractionation process.

Kinematic viscosity

Viscosity affects the fuel's fluidity and also, to a larger extent, the fuel's pumpability over an operating temperature range. The ASTM maximum limit for viscosity at –20 °C is 8 cSt. In this study, algae-derived jet fuel recorded a value of 2.8 cSt for viscosity at –20 °C, as indicated in Table 2. This parameter complies with the ASTM standard regarding kinematic viscosity.

Density

Density relates to volatility and specific energy. The recorded density at 15 °C for the algae-derived jet fuel is equal to 0.96 g cm^{-3} and it is found beyond the range provided by the ASTM standard for jet fuel, as indicated in Table 2. This could be due to the presence of dissolved particulate matter in the algae-derived jet fuel. The cleanness of the algae jet fuel could be the cause of this situation. It is possible to remedy this situation by using reforming and upgrading processes.

Total acidity

The total acidity of algae-derived jet fuel was found to be higher than the maximum value required by ASTM standard for jet fuel, as indicated in Table 2. This is probably due to the presence of acidic dissolved substances in the algae-derived jet fuel. High total acidity will make the jet fuel very corrosive to metals. To remedy this situation a corrosion inhibitor can be used as an additive.

Total sulfur

Total sulfur was slightly lower than the maximum limit required by ASTM standards for jet fuel as indicated in Table 2, so there is compliance in this respect. The origin of sulfur in the jet fuel is linked to the type of the marine species used to produce the fuel.

Conductivity

Conductivity represents the amount of dissolved substances present in the jet fuel. The dissolved substances may come from particulate matter that is not removed from the algae-derived jet fuel. The fuel needs more purification processes, such as rectification or membrane filtration, to keep the level of purity required for an aviation fuel. The data recorded in Table 2 show that conductivity complies with the limits required by ASTM standard. However, although it complies with the standard, there is a need to decrease it in order to reduce the acidity and density. An advanced filtration process such as membrane filtration can assist in this regard.

Approaches to design and simulation

A viable design process for algae-derived jet fuel must clearly emphasize carbon footprint reduction, costs, and environmentally related issues in order to compete with petroleum-based aviation fuels. In this study, the use of domestic wastewater or seawater for cultivation is suggested. The supply of CO_2 wastes from fossil plants to the growing culture during cultivation period is also suggested. These are inexpensive wastes that can assist the cultivation process and reduce dependence on freshwater resources and manufactured nutrients. This design should be a simple process that can be improved when the quality of the final product needs to be enhanced as mentioned before. This happens when additives are used to improve physico-chemical properties such as freezing point and lubricity of the fuel.

The simulation approach will assist in modeling the data recorded in the future to optimize parameters influencing the effective production of jet fuel from microalgae oil. The simulation work uses an Aspen HYSIS V8.8 package through which physicochemical properties of biomolecules and mixed compounds as well as reaction thermodynamics can be determined. After a successful running of the simulation, a detailed report, including physicochemical and

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thermodynamic parameters, was generated. This report compiled all aspects related to mass and energy balance, entropy and enthalpy of reactions involved in the combination of many compounds, pressure and temperature at each step or unit process, mole flow, density, reaction kinetics, available equilibria phases, molar concentrations, molecular weight of compounds, and raw materials. To obtain these parameters, there are basic parameters that need to be supplied to the simulation engine. In this study, the basic parameters were supplied to the simulation engine from the data generated from the laboratory experiment.

The report is easily generated by the software package because it includes chemical component libraries, thermodynamic method libraries, unit operation modules, the thermodynamic data manager (TDM), and a programmer.

In this case few assumptions were considered. Firstly, not all of the operation units in process design can be found in the Aspen HYSIS V8.8 operation unit libraries. Creative adjustments and simulation process combinations must therefore be used to mimic some of the design processes. Secondly, algal nutrients can be represented by CO_2, HNO_3 and H_3PO_4, which contain the most important elements such as N and P for microalgae cultivation. Thirdly, the feed serving of 1500 kg h^{-1} of oil was used as a basis to feed the hydrocracker after cultivation, and a conversion rate of 85% was chosen for the entire process. The design and simulation approaches were also based on the large amount of information collected in studies undertaken by Eloka-Eboka *et al.*[43] and Onunka *et al.*[44] In both studies the focus was on the process planning and development, criteria / concept selection, and optimization of parameters with required characterizations converted into measurable characteristics for the design of an efficient, operable and sustainable plant using microalgal oil. It was also found that a practical alternative source of energy is conceivable to reduce reliance on fossil-based energy. Some of the elements from these studies were very relevant to the conceptual design of the current study.

Summary data for simulation

- **Cultivation temperature**: The simulated temperature was between 20 and 30 °C for 15 days under photosynthetic conditions; the highest amount of biomass is collected on the tenth day; two phases: liquid and solid (wet biomass).
- **Macroalgae harvesting**: Simulated at ambient temperature and atmospheric pressure in sedimentation tank. Coagulants and flocculants can be used in this regard to speed up the process. Two phases: liquid and solid (wet biomass).
- **Physiological modification**: Simulated in highly purified water at ambient temperature for 3 days to help increase the lipid content to 80%. Two phases: liquid and solid.
- **Oil extraction**: Achieved by evaporation, the evaporation temperature should be between 60 and 70 °C to remove all the solvent. Two phases: solid (wet biomass) and liquid (bio-oil and solvent).
- **Bio-oil storage**: This is done in a collecting tank at ambient temperature and atmospheric pressure. One phase (liquid).
- **Hydrocracking temperature, time and pressure**: The simulated cracking temperature was 300 °C with a pressure of 1 bar for a duration of 30 min. This is almost the same with the data from the GC/MS obtained during the laboratory experiment. One phase (liquid).
- **Distillation or fractionation:** Begins at 70 °C and ends at 300 °C under a pressure of 1 bar for 90% recovery. One phase (liquid).
- **Partial evaporation**: Between 50 and 70 °C at atmospheric pressure, light vapor or liquid phase will be collected at the outlet of the flash vessel. It will be considered as a waste. Two phases (liquid and vapor).
- **Reforming**: Low pressure of 5 bars was chosen to allow partial dehydrogenation. The simulated temperature for reforming was 250 °C to allow the light end's distillate fractions to be amalgamated into high-end distillate fractions. One phase (liquid).
- **Upgrading**: involving hydrocracking, product and by-product separations such as H_2. CO_2 and water. The main variables for this process are hydrocracking temperature, simulated at 350 °C, low pressure of 1 bar, column operation parameters, and the bypass ratio. This ratio is defined as bypassed light ends hydrocarbons / total hydrotreated crude bio-oil. The simulated amount of hydrogen for the process is 5–10% of the total volume of the reactor. One phase (liquid).

Process flow design

Figure 2 is the flow chart of the simulated process developed in the current study. Only essential details of the process design and various steps are summarized in Table 3 (the 'Process details' section). This process flow chart is developed from the work completed in the laboratory, presented in Fig. 1. Most simulation results are based on the data generated from the laboratory work presented earlier. The main reason for the use of reported experimental conditions in the laboratory is that these conditions have successfully allowed jet fuel to be produced with the majority of parameters being compliant with ASTM standards. An attempt to optimize some key parameters was

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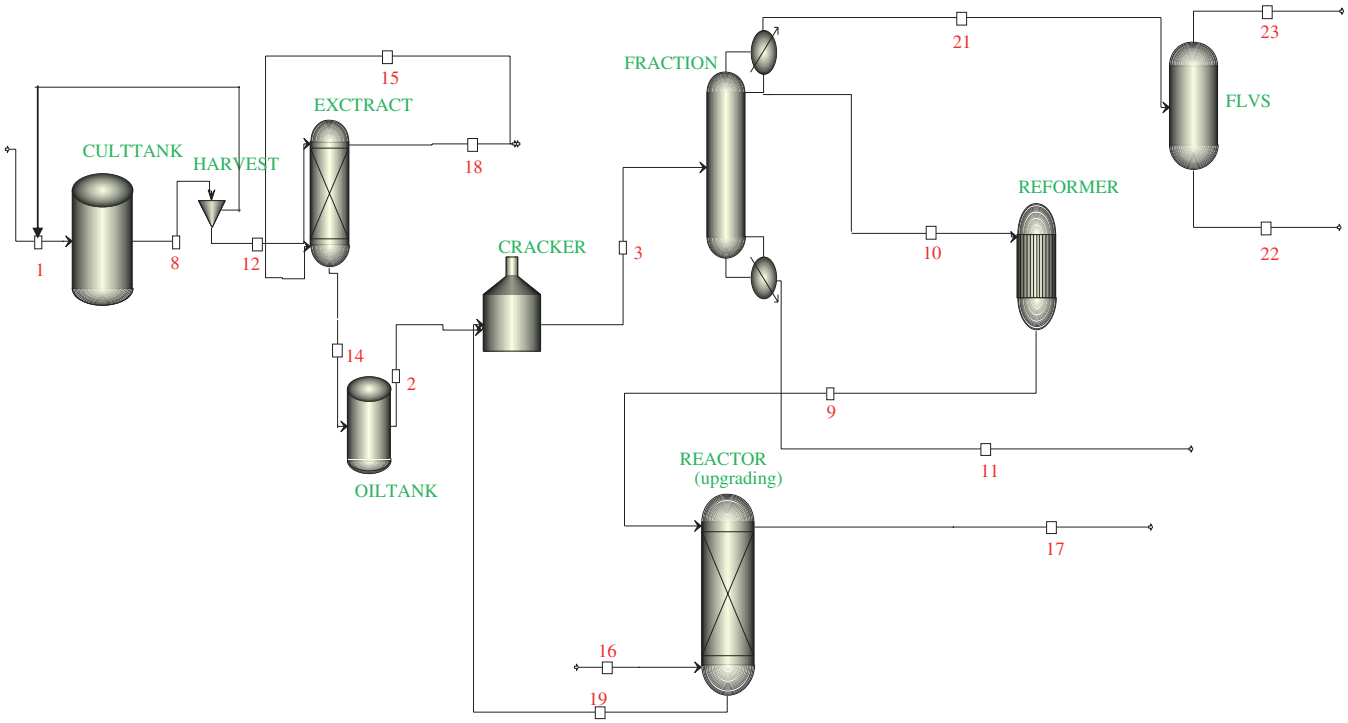

Figure 2. Conceptual design process flow diagram for the conversion of microalgae biomass into jet fuel.

undertaken aiming to enhance the performance and quality of the jet fuel compared with ASTM standards.

Process details

The main details regarding the flow chart of the operations are summarized in Table 3. The table explains in details how unit processes are structured. These processes are presented in the order in which they take place, from the cultivation of microalgae to the production of jet fuel.

Optimization attempt for some parameters

Optimization targeted conversion and separation processes such as cracking, distillation, reforming, and upgrading because they have a great impact on the final product quality and performance. The optimizer featured in Aspen HYSIS V8.8 was implemented to maximize the production of the bio-jet fuel, while varying the bypass ratio from 5% to 95%, varying cracking, reforming and upgrading temperatures between 200 and 400 °C, which is consistent with literature specifications for realistic reaction rates and cracking efficiency, while minimizing coke formation. The optimizer predicts an optimal product when using a bypass ratio of 7%, a cracker temperature of 300 °C, which is the same as the one obtained from GC/MS during the laboratory experiment, and bottom to overhead ratio of 20 : 1. The temperature range varies between 60 and 70 °C to remove methanol and chloroform. As bio-oil recovery is achieved by solvent extraction and evaporation it was necessary to optimize the solvent extraction temperature at 65 °C to assist in the removal of methanol and chloroform.

The maximum distillation temperature was optimized at 300 °C to be able to recover as much as middle carbon fractions needed in the jet fuel.

The data from the laboratory complied with ASTM standards, except for density and freezing point, which can be corrected through upgrading, reforming, and the use of additives. Properties of the jet fuel obtained under the optimized operation conditions are therefore expected to comply with ASTM specifications. This supports the hypothesis that it is technically feasible to begin with microalgae cultivation, produce bio- oil followed by jet fuel production, and finally reform and upgrade it.

Overall material balance

The overall mass balance is summarized in Table 4. This is generated by Aspen HYSIS V8.8 after running the simulation. It involves the inputs and the outputs from the beginning of the process to the end. It also indicates the phases and flow throughout the entire process. Analysis of Table 4 indicates that all parameters have converged. This is an indication that the simulation ran successfully.

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Table 3. Details and discussion of the process design.

Process (from microalgae cultivation to jet fuel	Unit name/ code on the flow chart	Process aim	Flow of operations and simulation conditions
Microalgae cultivation	CULTTANK	Cultivation and biomass production	Cultivation of biomass is the first step of the entire process. It takes place between 20 and 30 °C. This is the simulated temperature range for a photobioreactor or open pond with culture growing under solar light. The biomass enters the plant via stream 1 in the cultivation tank modelled with one inlet and one outlet. Practically, nutrients such as CO_2, Nitrates and phosphates will be supplied to the growing culture via stream 1. Domestic wastewater or seawater can be used as a growing media. This means that a wastewater treatment plant (also suggested by Roberts *et al.*[37] and Max *et al.*[14]) can serve as a cultivation option. Alternatively, a farming algae facility on the sea can be also considered for the same application especially for marine species such as *Nannochloropsis sp*. This can be a prospective option to be studied in detail.
Microalgae harvesting	→	Collection of biomass needed (harvesting) for downstream processes	This step can be achieved at ambient temperature and atmospheric pressure. Harvesting will take place in a sedimentation tank modeled with one inlet and two outlets. The biomass generated from the cultivation step should be pumped to the sedimentation tank via stream 8. The use of flocculants or other fast and effective harvesting options can be undertaken to speed up the process. The design is flexible in this regard. Physiological modification can take place in the sedimentation tank after harvesting microalgae. After dewatering the biomass, the volume of the wet biomass can be subjected to the stressing process to manipulate and modify the cells physiology. This is achieved through nutrient starvation as mentioned in the laboratory experiment. The simulation used biomass that is deprived with all nutrients for a period of 3 days as in the laboratory experiment. This will bring a change in cell metabolism, causing an increase in the lipid content. The cells are being stressed by keeping them in harsh conditions. An increase in lipid content of 80% can therefore be expected. This is done in highly purified water, which is nutrient free.
and	HARVEST		
Physiological manipulation	→	-To boost lipid content of algae cells (Physiological manipulation)	
Bio-oil extraction	EXTRACT	Extraction of algae bio-oil needed for conversion into jet fuel	Extraction of bio-oil is completed on wet biomass after cells physiological modification. It uses a mixture of solvent made up of chloroform and methanol in 1/1 volume ratio as undertaken during the laboratory experiment. This unit, modeled with one inlet and one outlet, should have a boiling system for solvent evaporation at temperatures depending on the solvent used, in this case the temperature was between 60 and 70 °C. From the harvesting process, the wet biomass is pumped to the extraction unit via stream 12. The solvent is recycled back into the unit via stream 15. Stream 18 is used for the removal of wet biomass sludge. Once the biomass sludge is removed via stream 18, the solvent is taken out via stream 15. A condenser will be needed on stream 15 to liquefy the solvent vapor. Therefore, once the wet biomass and solvent are taken out of the EXTRACT bio-oil can be collected into the oil tank via stream 14
Bio-Oil storage	OILTANK	Storage of algae bio-oil after extraction. This is done to supply the plant continuously with the crude oil needed for conversion processes.	Simulated in such a way that algae bio-oil should be stored in a tank modeled with one outlet and one inlet operating at ambient temperature before it is pumped to conversion processes. The storage time depends on how long the conversion operations can last.
Hydrocracking	CRACKER	Breaking down of larger hydrocarbon chains.	Algae bio-oil is pumped to the cracking unit via stream 2, the simulated cracking temperature has to vary between 200 and 400 °C. This is consistent with the specifications from the literature for effective cracking and minimizing coke formation.[24,45,46] The upgrading unit supplies hydrogen to the cracking unit via stream 19 to act as a catalyst during cracking. The CO_2 generated from this step can practically be channeled to the cultivation tank to be used as nutrients via stream 1. The cracking unit has one inlet and one outlet.

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Table 3. (Continued)

Process (from microalgae cultivation to jet fuel	Unit name/ code on the flow chart	Process aim	Flow of operations and simulation conditions
Distillation (fractionation)	FRACTION	Separation / fractionation of light, middle, and heavy fractions of hydrocarbon broken chains	The cracked algae bio-oil is pumped to the fractionation unit via pipe 3. Simulated distillation takes place from 70 to 300 °C at a pressure of 1 bar. Stream 10 will collect the light end distillates and will send them to the reforming unit. The jet fuel made of middle end distillates will be collected via stream 11. This will happen up to a maximum temperature of 300 °C, considered as the end point for middle-end distillates. Heavy-end distillate fractions will be collected at temperatures beyond 300 °C and sent to the reactor for upgrading. The distillation equipment is modeled using resources found in the package.
Partial evaporation	FLVS	Recovery of light-end liquid distillates to be sent for reforming.	Light-end distillates and a small fraction of middle-end distillates will get out of the distillation unit in a mixed liquid-vapor phase between 50 and 70 °C. This mixture is channeled to the flash vessel via stream 21. The pressure within the flash vessel is 1 bar. From this flash vessel a liquid phase will be collected at the bottom via stream 22 and very light vapor or liquid phase will be released via stream 23. The liquid phase from the flash vessel will also be sent to the reforming unit. In practice this means that stream 22 will be connected to the reforming unit. The flash vessel is modeled with one inlet and two outlets.
Reforming	REFORMER	Partial dehydrogenation and Converting light-end distillate fractions into high-end distillate fractions, which are middle- and heavy-end distillates.	From the reforming unit, all liquid phases either from the fractionation unit or flash vessel coming respectively from streams 10 and 22 will be pumped to the reactor or upgrading unit via stream 9, this stream will be made of hydrogen from the dehydrogenation as well as high-end distillate compounds as mentioned earlier. The equipment was modeled according to the resources available in the package with one inlet and one outlet.
Upgrading	REACTOR	Hydrocracking of reformed hydrocarbons. These hydrocarbons are sent to the fractionation unit for separation. This unit is also supplied by steam allowing the production of H_2, which is sent to the cracker for hydrocracking	Upgrading is a catalytic process that will produce another cracked bio-oil to be fractionated again. This process improves the quality of a material by using chemical reactions to remove any compounds present in trace amounts that make the fuel quality undesired. The bio-oil will be sent to the fractionation unit via stream 17. This means this stream will practically be connected to the fractionation unit. The hydrogen from the reformer might be insufficient because depending on the volume of distillates generated by the reforming unit. The upgrading unit will therefore be supplied with more hydrogen via stream 16 to act as catalyst in order to speed up the upgrading process. This hydrogen is needed for the effective reduction of oxygen content from algae oil. Excess oxygen in the fuel can have a good influence on the fuel combustion but it causes nitrogen oxide (NOx) emissions due to increased combustion temperature and extra oxygen[47] The remaining hydrogen in the unit will be channeled to the cracking unit as catalyst via stream 19 as mentioned earlier after upgrading process is completely done. Two inlets and two outlets were used for this type of equipment.

The convergence is revealed by the presence of zero values recorded at the bottom of the table for each stream.

Conclusions

This study has focused on the following aspects:

1. Developing the process to produce microalgae-derived jet fuel at laboratory scale.
2. The adaptability of Aspen HYSIS V8.8 to simulate the unit processes based on the data gathered from the laboratory steps.

The following are the outcomes:

- The unit processes are described as follows: Biomass cultivation, biomass harvesting, physiological modification, bio-oil extraction, hydrocracking, fractionation, reforming, and upgrading. The average cracking temperature in the laboratory experiment is 350 °C; the working pressure for distillation is 1 bar. The same pressure is recorded for the flash vessel collecting some middle-end fractions mixed with the vapor phase. The solvent used is a mixture of chloroform and methanol in a 1/1 ratio. The overall conversion rate is 85%. The data from the laboratory was

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Table 4. Generated mass balance from the simulation.

Aviation fuel from algae oil

Stream ID		1	2	3	8	9	12	14	15	16	17	19
From			B2	B4	B1	B7	B10	B16			B9	B9
To		B1	B4	B6	B10	B9	B16	B2	B16	B9		B4
Phase		LIQUID	LIQUID	LIQUID	LIQUID	LIQUID	LIQUID	LIQUID	LIQUID	LIQUID	LIQUID	LIQUID
Substream: MIXED												
Mole Flow	kmol s^{-1}											
H_2O		0.0137057	0.0125157	0.0255376	0.0137057	3.93719E–3	0.0137057	0.0125157	0.0	0.0138771	4.79281E–3	0.0130218
CO_2		7.01303E–4	6.40409E–4	1.23749E–3	7.01303E–4	1.61168E–3	7.01303E–4	6.40409E–4	0.0	6.31173E–4	1.64579E–3	5.97078E–4
CHLOROFO		0.0	2.32659E–4	2.32659E–4	0.0	0.0	0.0	2.32659E–4	2.32690E–4	0.0	0.0	0.0
METHANOL		0.0	8.66802E–4	8.66802E–4	0.0	0.0	0.0	8.66802E–4	8.66913E–4	0.0	0.0	0.0
Mass Flow	kg s^{-1}											
H_2O		0.2469136	0.2254742	0.4600671	0.2469136	0.0709296	0.2469136	0.2254742	0.0	0.2500000	0.0863437	0.2345930
CO_2		0.0308642	0.0281842	0.0544615	0.0308642	0.0709296	0.0308642	0.0281842	0.0	0.0277777	0.0724310	0.0262772
CHLOROFO		0.0	0.0277742	0.0277742	0.0	0.0	0.0	0.0277742	0.0277777	0.0	0.0	0.0
METHANOL		0.0	0.0277742	0.0277742	0.0	0.0	0.0	0.0277742	0.0277777	0.0	0.0	0.0
Total flow	kmol s^{-1}	0.0144070	0.0142555	0.0278745	0.0144070	5.54887E–3	0.0144070	0.0142555	1.09960E–3	0.0145082	6.43860E–3	0.0136189
Total flow	kg s^{-1}	0.2777778	0.3092068	0.5700771	0.2777778	0.1418593	0.2777778	0.3092068	0.0555555	0.2777778	0.1587748	0.2608703
Total flow	cum s^{-1}	2.73365E–4	3.02735E–4	5.59951E–4	2.73365E–4	1.74572E–4	2.73365E–4	3.02735E–4	5.25589E–5	2.75387E–4	1.82387E–4	2.58620E–4
Temperature	K	298.1500	298.1500	298.1500	298.1500	423.1500	298.1500	298.1500	298.1500	303.1500	405.9879	303.2596
Pressure	N/sqm	1.01325E+5	1.01325E+5	1.01325E+5	1.01325E+5	1.01325E+5	1.01325E+5	1.01325E+5	1.01325E+5	1.01325E+5	1.01325E+5	1.01325E+5
Vapor frac		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Liquid frac		1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000
Solid frac		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Enthalpy	J $kmol^{-1}$	–2.9118E+8	–2.8542E+8	–2.8797E+8	–2.9118E+8	–3.0852E+8	–2.9118E+8	–2.8542E+8	–2.1649E+8	–2.9014E+8	–3.0593E+8	–2.9017E+8
Enthalpy	J kg^{-1}	–1.5102E+7	–1.3159E+7	–1.4080E+7	–1.5102E+7	–1.2068E+7	–1.5102E+7	–1.3159E+7	–4.2850E+6	–1.5154E+7	–1.2406E+7	–1.5149E+7
Enthalpy	Watt	–4.1951E+6	–4.0688E+6	–8.0269E+6	–4.1951E+6	–1.7120E+6	–4.1951E+6	–4.0688E+6	–2.3806E+5	–4.2095E+6	–1.9698E+6	–3.9518E+6
Entropy	J/kmol-K	–1.5552E+5	–1.5891E+5	–1.5735E+5	–1.5552E+5	–1.0127E+5	–1.5552E+5	–1.5891E+5	–2.2871E+5	–1.5478E+5	–1.0775E+5	–1.5469E+5
Entropy	J/kg-K	–8066.178	–7326.200	–7693.954	–8066.178	–3961.154	–8066.178	–7326.200	–4526.852	–8084.095	–4369.474	–8075.766
Density	kmol cum^{-1}	52.70279	47.08939	49.78032	52.70279	31.78558	52.70279	47.08939	20.92136	52.68323	35.30184	52.66025
Density	kg cum^{-1}	1016.143	1021.379	1018.083	1016.143	812.6120	1016.143	1021.379	1057.016	1008.681	870.5375	1008.703
Average MW		19.28063	21.69022	20.45152	19.28063	25.56543	19.28063	21.69022	50.52329	19.14615	24.65984	19.15492

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Table 4. (Continued)

		Aviation fuel from algae oil										
Liq Vol 60F	cum s^{-1}	2.84950E–4	3.13900E–4	5.80923E–4	2.84950E–4	1.57384E–4	2.84950E–4	3.13900E–4	5.36992E–5	2.84286E–4	1.74655E–4	2.67023E–4
Substream: $TOTAL												
Total flow	kg s^{-1}	0.2777778	0.3092068	0.5700771	0.2777778	0.1418593	0.2777778	0.3092068	0.0555555	0.2777778	0.1587748	0.2608703
Enthalpy	Watt	–4.1951E+6	–4.0688E+6	–8.0269E+6	–4.1951E+6	–1.7120E+6	–4.1951E+6	–4.0688E+6	–2.3806E+5	–4.2095E+6	–1.9698E+6	–3.9518E+6
Substream: CISOLID												
Mole flow	kmol s^{-1}											
H_2O		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CO_2		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CHLOROFO		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
METHANOL		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Mass flow	kg s^{-1}											
H_2O		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CO_2		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CHLOROFO		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
METHANOL		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Total flow	kmol s^{-1}	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Total flow	kg s^{-1}	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Total flow	cum s^{-1}	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Temperature												
Pressure	N/sqm	1.01325E+5	1.01325E+5	1.01325E+5	1.01325E+5	1.01325E+5	1.01325E+5		1.01325E+5	1.01325E+5		
Vapor frac												
Liquid frac		s										
Solid frac												
Enthalpy												
Enthalpy												
Enthalpy												
Entropy												
Entropy												
Density												
Density												
Average MW												
Liq Vol 60F												

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used to simulate the process including some modifications to run the simulation successfully. The optimization of temperature values for various processes was also completed to enable the simulation and process design.

- Domestic wastewater plants or farming facilities using seawater for marine species can be used to provide a cultivation medium to take advantage of nutrients present in both domestic wastewater and seawater. This will also reduce the use of freshwater resources. Carbon dioxide from fossil sources can also be collected and used as a nutrient for microalgae during cultivation.
- Physiological modification is a very important aspect for algae-based fuels and needs to be incorporated in the process to increase the species' lipid content. The growth medium can be either open ponds or a photobioreactor, depending on the available possibilities and the environmental conditions.

Future studies should work on the detailed design and optimization of a sustainable process plant to be used at the commercial level, including details of modeled equipment. They should also focus on sensitivity analysis, costs, and economic aspects related to the conversion processes, life-cycle assessment and fuel sustainability, blending with other fuels, detailed thermodynamics studies, and modeling of parameters involving effectiveness of some unit processes and costs.

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References

1. Dincer I, Renewable energy and sustainable development: A crucial review. *Renewable Sustainable Energy Rev* **4**(2):157–175(2000).
2. Schmer MR, Vogel KP, Mitchell RB and Perrin RK, Net energy of cellulosic ethanol from switchgrass. *Proc Natl Acad Sci USA* **105**(2):464–469 (2008).
3. Iodice P, Senatore A, Langella G and Amoresano A, Advantages of ethanol–gasoline blends as fuel substitute for last generation Si engines. *Environ Prog Sustainable Energy* **36**:1173–1179 (2017).
4. Besanko D, Besanko D, Ulan M and Ulan M, Should the ethanol blender's credit be eliminated? in *Kellogg School of Management Cases*, Kellogg School of Management, Northwestern University 2011, USA, pp. 1–36 (2017).
5. Khatiwada D and Silveira S, Scenarios for bioethanol production in Indonesia: How can we meet mandatory blending targets? *Energy* **119**:351–361 (2017).
6. Chisti Y, Biodiesel from microalgae beats bioethanol. *Trends Biotechnol* **26**(3):126–131 (2008).
7. Riayatsyah TM, Ong HC, Chong WT, Aditya L, Hermansyah H and Mahlia TM, Life cycle cost and sensitivity analysis of *Reutealis trisperma* as non-edible feedstock for future biodiesel production. *Energies* **10**(7):877 (2017)
8. NREL (National Renewable Energy Laboratory), *A Look Back at the US Department of Energy's Aquatic Species Program: Biodiesel from Algae Close-Out Report*[Online]. http://www.eere.energy.gov/biomass/pdfs/biodiesel_from_algae.pdfNREL/FS-840-40352 Report edited in July 2006 [1 July 2017].
9. Lian X, Xue Y, Xu G, Zhao Z, Sheng H and Lin H, Effect of methyl acetoacetate as a potential cold flow improver for biodiesel. *Energy Sources, Part A* **39**(1):97–102 (2017).
10. Bwapwa JK, Anandraj A and Trois C, Possibilities for conversion of microalgae oil into aviation fuel: A review, *Renewable Sustainable Energy Rev* **80**:1345–1354 (2017).
11. Chisti Y, Biodiesel from microalgae. *Biotechnol Adv* **25**(3):294–306 (2007).
12. Daelman MRJ, Sorokin D, Kruse O, van Loosdrecht MCM and Strous M, Haloalkaline bioconversions for methane production from microalgae grown on sunlight. *Trends Biotechnol* **34**(6):450–457 (2016).
13. Slade R and Bauen A, Micro-algae cultivation for biofuels: cost, energy balance, environmental impacts and future prospects. *Biomass Bioenergy* **53**:29–38 (2013).
14. Ma X, Zhou W, Fu Z, Cheng Y, Min M, Liu Y *et al*, Effect of wastewater-borne bacteria on algal growth and nutrients removal in wastewater-based algae cultivation system. *Bioresour Technol* **167**:8–13 (2014).
15. Wang HY, Bluck D and Van Wie BJ, Conversion of microalgae to jet fuel: Process design and simulation. *Bioresour Technol* **167**:349–357 (2014).
16. Fiori L, Valbusa M and Castello D, Supercritical water gasification of biomass for H2 production: Process design. *Bioresour Technol* **121**:139–147 (2012).
17. Guan Q, Savage PE and Wei C, Gasification of alga *Nannochloropsis* sp. in supercritical water. *J Supercrit Fluids* **61**:139–145 (2012).
18. Miller A, Hendry D, Wilkinson N, Venkitasamy C and Jacoby W, Exploration of the gasification of *Spirulina* algae in supercritical water. *Bioresour Technol* **119**:41–47 (2012).
19. Bond JQ, Upadhye AA, Olcay H, Tompsett GA, Jae J, Xing R *et al*, Production of renewable jet fuel range alkanes and commodity chemicals from integrated catalytic processing of biomass. *Energy Environ Sci* **7**(4):1500–1523 (2014).
20. Ail SS and Dasappa S, Biomass to liquid transportation fuel via Fischer Tropsch synthesis – technology review and current scenario. *Renewable Sustainable Energy Rev* **58**:267–286 (2016).
21. Zhang Q, Cheng K, Kang J, Deng W and Wang Y, Fischer–Tropsch catalysts for the production of hydrocarbon fuels with high selectivity. *ChemSusChem* **7**(5):1251–1264 (2014).
22. Gunawardena DA and Fernando SD, Methods and applications of deoxygenation for the conversion of biomass to petrochemical products in *Biomass Now-Cultivation and Utilization*, ed. by Miodrag DM. IntechOpen, Book chapter, London, United Kingdom. [Online]. Available : https://www.intechopen.com/books/biomass-now-cultivation-and-utilization/methods-and-applications-of-deoxygenation-for-the-conversion-of-biomass-to-petrochemical-products [15 July 2017].
23. Biller P, Sharma BK, Kunwar B and Ross AB, Hydroprocessing of bio-crude from continuous hydrothermal liquefaction of microalgae. *Fuel* **159**:197–205 (2015).
24. Wang HL, Yan SL, Salley SO and Ng KYS, Hydrocarbon fuels production from hydrocracking of soybean oil using transition metal carbides and nitrides supported on ZSM-5. *Ind Eng Chem Res* **51**:10066–10073 (2012).

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25. Narula V, Thakur A, Uniyal A, Kalra S and Jain S, Process parameter optimization of low temperature transesterification of algae-Jatropha Curcas oil blend. *Energy* **119**:983–938 (2017).
26. Pattanaik BP and Misra RD, Effect of reaction pathway and operating parameters on the deoxygenation of vegetable oils to produce diesel range hydrocarbon fuels: A review. *Renewable Sustainable Energy Rev* **73**:545–557 (2017).
27. Yang C, Li R, Cui C, Liu S, Qiu Q, Ding Y *et al.,* Catalytic hydroprocessing of microalgae-derived biofuels: A review. *Green Chem* **13**:3684–3699 (2016).
28. Zhou L and Lawal A, Kinetic study of hydrodeoxygenation of palmitic acid as a model compound for microalgae oil over Pt/γ-Al_2O_3. *Appl Catal A Gen* **532**:40–49(2017).
29. Leu S and Boussiba S, Advances in the production of high-value products by microalgae. *Ind Biotechnol* **10**(3):169–183 (2014).
30. San Pedro A, González-López CV, Acién FG and Molina-Grima E, Outdoor pilot-scale production of *Nannochloropsis gaditana*: influence of culture parameters and lipid production rates in tubular photobioreactors. *Bioresour Technol* **169**:667–676 (2014).
31. Lakaniemi AM, Tuovinen OH and Puhakka JA, Anaerobic conversion of microalgal biomass to sustainable energy carriers–a review. *Bioresour Technol* **135**:222–231 (2013).
32. Ramachandran S, Ganesh PS, Sivasubramanian V and Kumar BP, Current developments in mass production of microalgae for industrial applications, in *Environmental Sustainability Using Green Technologies*, ed. by Sivasubramanian V. Taylor & Francis group, Boca Raton, FLorida, USA, p. 147 (2016).
33. Shukla M, Tabassum R, Singh R and Dhar DW, Influence of light intensity, temperature and CO_2 concentration on growth and lipids in green algae and cyanobacteria. *India J Exp Biol* **54**(7):482–487 (2016).
34. Sukenik A, Carmeli Y and Berner T, Regulation of fatty acid composition by irradiance level in the eustigmatophyte *Nannochloropsis* sp. *J Phycol* **25**:686–692 (1989).
35. Scheneider JC and Roessler P, Radiolabelling studies of lipids and fatty acids in *Nannochloropsis* (Eustigmatophyceae), an oleaginous marine alga. *J Phycol* **30**:594–598 (1994).
36. Hulatt CJ, Wijffels RH, Bolla S and Kiron V, Production of fatty acids and protein by *Nannochloropsis* in flat-plate photobioreactors. *PLoS One* **12**(1):e0170440 (2017).
37. Roberts GW, Fortier M-OP, Sturm BSM and Stagg-Williams SM, Promising pathway for algal biofuels through wastewater cultivation and hydrothermal conversion. *Energy Fuels* **27**:857–867 (2013).
38. Jena U, Das KC and Kastner JR, Effect of operating conditions of thermochemical liquefaction on biocrude production from *Spirulina platensis. Bioresour Technol* **102:**6221–6229 (2011).
39. Brown TM, Duan P and Savage PE, Hydrothermal liquefaction and gasification of *Nannochloropsis* sp. *Energy Fuels* **24**:3639–3646 (2010).
40. Skone TJ and Gerdes K, *Development of Baseline Data and Analysis of Life Cycle Greenhouse Gas Emissions of Petroleum-Based Fuels*. National Energy Technology Laboratory, Department of Energy, Washington, DC (2008).
41. Matar S and Hatch LF, *Chemistry of Petrochemical Processes*. Gulf Professional Publishing, Woburn, MA (2001).
42. Bwapwa JK, Akash A and Trois C, Microalgae processing for jet fuel production. *Biofuels, Bioprod Biorefin* (2018), in press.
43. Eloka-Eboka A, Onunka C and Inambao F Detailed design and optimization of a sustainable micro-algal biofuel process plant. *Int J Low-Carbon Technol* 1–9 (2018).
44. Onunka C, Juddhoo R and Eloka-Eboka AC Conceptual design and optimisation of a sustainable micro-algal biofuel process plant. Proceedings of the 15th Sustainable Energy Technologies (SET 2016) Conference at the National University of Singapore, Queenstown, July 19–22 (2016).
45. Al-Sabawi M and Chen JW, Hydroprocessing of biomass-derived oils and their blends with petroleum feedstocks: A review. *Energy Fuels* **26**:5373–5399 (2012).
46. Robota HJ and Alger JC, Preparation of a research jet fuel composition comprised of nearly exclusively methyl-branched tetradecane isomers having a freezing point below –47 °C. *Ind Eng Chem Res* **51**:10313–10319 (2012).
47. Baskar P and Senthilkumar A Effects of oxygen enriched combustion on pollution and performance characteristics of a diesel engine. *Eng Sci Technol Int J* **19**(1):438–443 (2016).

Joseph K. Bwapwa

Joseph K. Bwapwa is a lecturer at the Mangosuthu University of Technology (MUT) and researcher at the University of Kwazulu-Natal (UKZN) in South Africa. His areas of expertise are water and wastewater bioremediation technologies, and bioenergy – especially bioenergy production related to algae biotechnology. He has published papers in these areas and has attended numerous international conferences. He works under the supervision of Prof Anandraj at the Centre of Algal Biotechnology and Prof Cristina Trois at the University of Kwazulu-Natal. His MSc in engineering focused on the treatment of anaerobic digestion of complex particulate wastewater and his PhD research is focusing on the conversion of algae biomass to aviation fuel.

Professor Akash Anandraj

Professor Akash Anandraj has a PhD in biology from the University of Kwazulu-Natal. His research interests include algal biotechnology and biofuels, microalgae physiology, estuarine ecology, phyco-remediation, biogas from algae, value-added products from algae, and algae carbon sequestration rates.

Professor Cristina Trois

Professor Cristina Trois is dean and head of the School of Engineering at the University of Kwazulu-Natal, South Africa. She is an environmental engineer from Italy. Her general area of expertise is in environmental engineering, especially wastewater treatment, solid waste management, landfill emissions control and treatment, acid mine drainage, and geoengineering. She is a founding member of the Centre for Research on Environmental, Coastal and Hydrological Engineering (CRECHE) at the University of KwaZulu-Natal.

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CHAPTER: JET FUEL FROM DOMESTIC WASTEWATER TREATMENT USING MICROALGAE: A REVIEW

[1,2]Joseph K. Bwapwa, [2]Anandraj Akash, [1]Cristina Trois

[1] University of Kwazulu-Natal, School of Engineering, Howard College, Durban, South Africa

[2] Mangosuthu University of Technology, Faculty of natural sciences and Faculty of Engineering, Umlazi, Durban, South Africa

Corresponding author: joseph@mut.ac.za; josephkapuku@gmail.com

ABSTRACT

Domestic wastewater can contain numerous toxic substances making it a public health hazard. Recycling wastewater with the possibility of reuse can be considered as a viable option. Various ways of remediation are being used to solve the issue of water pollution. The current review analyses the possibility to use microalgae for wastewater treatment followed by the conversion of biomass into aviation fuel. It is well known that conventional wastewater treatment plants are energy demanding and pollutants removal processes are not always cost effective. Furthermore, sludge management is another issue that requires sustainable solution to reduce its environmental footprint. Algae biotechnology can be an option that can be used to overcome these challenges. Microalgae species such as *chlorella, nannochloropsis, Euglena, Oscillatoria, Chlamydomonas, Scenedesmus, Chlorella, Nitzschia, Navicula and Stigeoclonium* have the ability to accumulate contaminants/pollutants, therefore, reducing the pollutants/contaminants load from wastewater. Removal efficiency can reach up to more than 90% for organic substances, nitrates, phosphates and heavy metals. Microalgae species supply also oxygen to aerobic microorganisms. This can reduce the costs related to oxygen supply in a conventional plant. Once clean effluent is generated, algae biomass is harvested and used for bio-jet fuel production. The biomass is processed to produce crude bio-oil which has similar physical properties compared to the petrochemical crude oil. Algae bio-oil can be processed via transesterification, thermal cracking or pyrolysis and fractionation including reforming and upgrading processes to produce renewable jet fuel. This fuel is expected to have the same physico-chemical properties compared to conventional jet fuel. It is environmentally friendly due to its low carbon footprint. However, the major challenge with algae-based fuels is the low lipids content for many species and the costly biomass harvesting process

Keywords: **wastewater; nutrients removal**, **microalgae; Jet biofuel; conversion processes; algae biofuels, algae biotechnology**

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1. INTRODUCTION

1.1 Energy demand and Biofuels

The world demand for energy is increasing every year and the interest for alternative fuels more especially biofuels including biodiesel, bio-Jet fuel, bioethanol and biohydrogen has grown very fast. However, petroleum derived fuels are still remaining as the leading and sustainable source of energy. According to International Energy Agency (IEA), the world uses over 85 million barrels of petroleum per day, also, the global primary energy demand is growing at a rate of 1.6% per year. Predictions from various models indicate that the primary and global energy demand will double over the next 40 years. In addition, the IEA estimates that there is a strong need to introduce low carbon fuels into the global energy pool in order to stabilize atmospheric CO_2levels. As the focus on environmental protection is increasingly dominant and the volatility of oil prices is an undeniable reality, many countries are considering the use of renewable energy more especially biofuels as backup plan to any eventual energy crisis that could take place in the near future.

It is well known that many countries are relying on fossil fuels in the transportation sector. Unluckily, fossil-based fuels despite their competitive costs compared to biofuels are considered as the most polluting with high volumes of greenhouse emissions released daily in the atmosphere. These emissions are the main cause of global warming and climate change. Hence, the consequences linked to climate change and global warming are immeasurable in today's environmental context. A need for reliable source of energy to generate less carbon is needed in order to remediate to the current environmental challenges. Biofuels generate low

carbon emissions below the environmental standards and they could be considered as an appropriate alternative in the near future despite some of the weaknesses.

They are also expected to be sustainable and have the same level of performance than fossil fuels. In some cases, they are used entirely in engines or are blended with their petroleum counterparts. This last option has presented many advantages economically and technically.

1.2 Necessity of using biofuels in the current context

The global increasing demand for energy is a major challenge today that requires effective solution because various factors such as the level of industrialization, the rapid increase of the population and the lifestyle have an impact on energy consumption. Furthermore, the limited reserves of fossil fuels constitute a cause for concern regarding the availability of energy sources in the near future. A need for innovation to secure a better future for transportation fuels and energy production particularly is very vital. Therefore, emerging technologies and continued innovation hold the promise of real solutions through a combination of energy efficiency and renewable energy such as biofuels (Larkum et al.,2012; Tabatabaei et al., 2015). There is a need for solutions that can defy the current definition of energy sources and the way energy should be utilized or deployed. In recent years wind, solar and hydrogen energy have been developed, these energy forms are not practical in meeting energy and transportation needs due to size, scale, and costs application. However, biofuels such as bioethanol, biohydrogen, and biodiesel, bio-jet fuel from sources that will not cause inflation of food crisis and reduce environmental problems are currently the most regarded alternatives (Mata et al., 2010). There has been a real progress regarding development stages of biofuels in order to improve their effectiveness and sustainability. Biofuels have moved from first to the fourth generation with different improvement and advancement aspects introduced from one generation to another one.

1.3 Different generations of biofuels

The first generation biofuels were generated from food crops by extracting the lipids or oil from seed rape. Thereafter a transesterification reaction was undertaken to produce biodiesel. From biodiesel there is a possibility to produce other biofuels via conversion process such as cracking, decarboxylation, pyrolysis and Isomerisation. For instance, the production of bioethanol was achieved via fermentation using crops such as wheat or sugar as feedstock. These biofuels have encountered some challenges but the most contentious problem is the

threat to the food security. The conflict between food versus fuel was raising. First generation of biofuels has caused the diversion of food crops from the global food market toward fuel market causing price increases for the raw materials used to generate biofuels which are the same used in the food market (Naqvi and Yan,2015; Naik et al., 2010; Mohr and Raman, 2013; Lee and Lavoie, 2013; Dutta et al.,2014)

The second generation of biofuels was initiated with the aim to overcome some limitations encountered from the first-generation biofuels. Non-food crops were chosen to produce biofuels using feedstocks such as wood, food crop wastes or used edible oil and biomass in order to eradicate completely the main the use of food crops. Second generation of biofuels are being cost competitive compared to existing petroleum-based fuels. It has been shown from life cycle assessment that the net energy gains will be increased. This is another aspect that allows to overcome one of the main limitations from the first generation. Second generation of biofuels use mostly biomass from agriculture residues, or non-crop plants such as switch grasss. The use of these biomasses will help to reduce the pressure on the global food supply. Additionally, they require small amount of space, can generate higher biofuel yields compared to the first-generation feedstocks and they can be cultivated on lower quality lands. However, the current data related to the economics of second generation biofuels shows that large-scale commercial production will not be economically viable until significant technological advances are developed. Technological advances will require the investment of time and money in order to use these biofuels. Consequently, second generation biofuels are not likely to play an important role to supply fuels in the short and medium term, unless specific improvement and ground-breaking technologies are developed (Naik et al., 2010; Schenk et al.,2008; Damartzis and Zabaniotou, 2011; Lee and Lavoie, 2013; Dutta et al.,2014).

The third generation of biofuels have aimed to improve the production of biomass. In this category, specially engineered organisms or crops with considerable energy content such as microalgae are highly explored. Algae biomass are cultivated at lower costs and its energy content is very high with the capacity to be renewable in very short periods of time. Therefore, less strain on freshwater resources for algae growth (Dragone et al., 2010; Carere et al., 2008; Lee and Lavoie, 2013; Dutta et al.,2014)

The fourth-generation biofuels focused more on production of highly clean source of energy such as biohydrogen from biomass or feedstocks such as microalgae and organic wastes. Also, the aim is not only to produce sustainable energy but to find ways to capture and store carbon

dioxide. This carbon dioxide will be used to feed the biomass in order to convert it into sustainable fuel. The sequestration of carbon dioxide is part of the process and there are many ways to store carbon dioxide. It can also be stored in gas fields or saline aquifers. The carbon capture can become negative than neutral. In this case more carbon is locked than emitted therefore less carbon emission will be recorded. (Demirbas, 2009; Dutta et al.,2014; Aro ,2016)

1.4 Algae biotechnology and biofuels

Future prospects involve the third and fourth generation of biofuels which are focusing on the identification of feedstocks with very high lipid content and the use of innovative ways to extract oil and produce low costs, low carbon emission and sustainable fuels. Algae biotechnology is a highly explored option and could be one of the foremost players in the renewable energy market for biofuels such as biodiesel, biohydrogen, bioethanol and bio-Jet fuel. Though algae biofuels present few challenges such as high costs for harvesting drying and downstream processes, algae require fewer inputs, it is the very fastest growing biomass and can be grown anywhere, there is no strain on food products, therefore, solving some challenges recorded from the two first fuel generations. The main benefit of using algae-based biofuels compared to other biofuels is that algae do not compete with agricultural or limited resources such as freshwater resources and land. Algae have the potential to reduce greenhouse gas (GHG) effects and recycle CO_2 emissions from power plant and natural gas operations. This is a very important fact because the growth of algae depends not only on nutrients but also on carbon dioxide. Therefore, power plants can reduce sensibly their CO_2 emissions in the atmosphere by supplying CO_2 to algae producers. Consequently, algae processing plants will not only produce fuels but will also reduce the effects of climate change. The main objective for algae biofuel production especially algae-based Jet fuel is to get drop-in-fuels complying with standards and ready to be commercialized. It is evident that the global consumption of bioenergy will continue to increase under the influence of the present renewable energy and climate change policies (Wilhelm and Jakob, 2011; Scott et al., 2010; Huang et al., 2010)

The World Energy Council has indicated that biofuels will meet 30% of the whole world's energy demand by 2050. This shows that there is huge potential for biofuels in general and Jet biofuel particularly to be used as a renewable alternative to fossil fuels. However, more innovative ways are needed to improve the production processes, to identify the most promising feedstocks that can generated more lipids or to develop methods to stimulate the increase of lipids from the various feedstocks. More emphasis can be put on the third and fourth

generation biofuels because they are not competing with the food industry and they can generate low carbon fuels. The economics of these fuels need to be improved by using optimized processes and low costs feedstocks. This chapter is only focusing on biotechnology aspects and conversion processes needed to produce Jet biofuel from microalgae.

Details on the economics of the conversion processes from microalgae to jet fuel can be undertaken in specific studies.

1.5 Rationale of this review

The rationale behind this review is to show if both treatment of domestic sewage assisted by microalgae can generate biomass and lipid essential for jet fuel production. Two streams can be generated: the treated effluent to be reused for other applications and the crude bio-oil from the biomass to be used for jet fuel production. There are challenges to be addressed in order to get a sustainable bio-jet fuel from microalgae.

2. APPROACH TO PRODUCE MICROALGAE JET FUEL AND PROBLEMATIC OF FEASIBILITY

It is important to stress on the fact that the literature on algae-based fuels particularly algae-based jet fuel is very scarce. Although many studies have been undertaken in this specific field but most of the valuable information involving major ground-breaking findings is not in the public domain. Technological improvement is a critical and major step required to produce a competitive algae-based jet fuel being on a market currently dominated by petroleum/conventional jet fuel. The probable approach will be to duplicate and adapt the same technology used to produce conventional jet fuel from crude oil to produce algae-based jet fuel from algae crude bio-oil. This approach will require process modelling and optimisation to produce a drop-in-jet fuel. The demand for jet fuels is very high and will always increase because of the market dynamic reflected by consumption of jet fuel. This consumption is expressed in volume of jet fuel needed on a daily/monthly/ yearly basis by civil and military aviation. The economics of producing algae-based jet fuel is currently still not costs competitive compared to the economics of producing conventional jet fuel. On-going research seeks to improve technologies to enable the production of algae-based jet fuel to commercial level. Many projects on jet biofuel from microalgae are undertaken currently on laboratory and pilot scales but still they are not yet implemented at commercial

scales. Therefore, the challenging concern focuses on developing costs effective conversion processes and increase algae lipids content as mentioned earlier.

Globally, energy production is amongst key factors that are essential to economic and industrial development. However, the production of fossil fuels through traditional petrochemical processes is seen as the major cause of environmental pollution and climate change effects as mentioned earlier. Furthermore, reserves of world fossil fuels resources are very limited and may lead to scarcity in the near future. To reduce dependence on fossil fuels, the production of cost effective biofuels can be considered as one of the options to be explored. The aviation sector is facing challenges involving costs fluctuations and environmental issues related to the use of fossil fuels. These challenges can have an impact on the viability of many businesses. Algal based fuels present many advantages that can be beneficial to the aviation industry. However, to get algal based jet fuel to the level of competing with the current fossil-based jet fuel will require many complex problems and adjustments in terms of cost effectiveness and sustainability.

3. CURRENT CONTEXT FOR JET FUEL PRODUCTION AND CONSUMPTION

Currently, the aviation accounts for 2% of the world global CO_2 emissions. It is anticipated that the global demand for aviation transportation will continue to increase up to mid-term of 2030 (Blakey et al., 2010). Consequently, the environmental impact of aviation regarding the GHG emissions is likely to increase. Furthermore, it was reported that a continued increase in demand will take place in the early 2000's and will range between 3.5 and 5% in passenger km flown (Wickrama,2001; Blakey et al., 2010). Also, other studies since the 2009 economic downturn including the current stagnation of demand, have suggested that the demand will recover to the previous rate once the economic climate has improved from 2010 (Rosenfeld et al., 2009). The air freight will be subjected to the same trend despite the fact that a decline in demand was recorded due the economic downturn (Nygren et al., 2009; Blakey et al., 2010). Up to 2030, it is predicted that the subsequent increase in jet fuel consumption will be at a lower rate than the increase in passenger demand. This is mainly due to two factors: firstly, increases in fuel efficiency of the aircraft fleet which will be estimated at 1.2% reduction per annum (Nygren et al., 2009; Blakey et al., 2010), secondly, through air traffic management optimization. Furthermore, the International Air Transport Association (IATA) has predicted an increase in aviation fuel demand from around 190 million tonnes in 2009 to between 300-350 million tonnes by 2030 (Wickrama,2001; Blakey et al., 2010). As a result, a proportional

increase of CO_2 emissions will be recorded. It is very important to stress on the fact that the environmental sustainability of commercial aviation is not the only concern, the availability of crude oil is another concern, the petroleum oil reserves are decreasing, so far, they are no sustainable and commercialized substitutes to compete with fossil fuels. Several alternative jet fuel products deriving from many sources are emerging and it is felt that their production processes will in time offer routes to produce high quality and sustainable fuels. In this regard, algae-based jet fuel is seen as one of the plausible options to be explored as there is a record of successful attempts regarding its blending with petroleum jet fuel as it is mentioned in the next section.

4. PREVIOUS ATTEMPTS ON THE USE OF ALGAE BASED JET FUEL

Some biofuel producers have attempted to produce jet fuel from microalgae and the they have concluded that there could be a potential in using microalgae species to produce aviation fuel. Some of these producers are: Sapphire Energy; Heliae, Phycal, Cellana, Solazyme and General Atomics , to name a few. The renewable aviation biofuels from microalgae produced by these companies have successfully been used as trials in both commercial and military aircrafts. Their blending with conventional jet fuels on 50-50 basis (known as B50) was approved for use in commercial flights by ASTM international. In January 2009 Continental Airlines made history with the first-ever commercial test flight in the United States. A Continental Boeing 737 departing from Houston International Airport, flew up with a B50 fuel blend made of algae-based jet fuel and conventional jet fuel in one of its two engines. This was part of the biofuel blending plan from Continental Airlines aiming to reduce the impact of fossil jet fuel in the environment. This event was considered as the beginning of a new era regarding the development of alternative jet fuels. In June 2011, the US Navy successfully established a B50 blend made of both microalgae-based Jet fuel and Conventional jet fuel. This blend was produced by Solazyme and it was used in a MH-60S Seahawk helicopter. This was the first time a military aircraft had flown on algae-based jet fuel. In July 2011, ASTM International announced its certification for airlines to fly passenger jets using derivatives of up to 50 % biofuel made from biomass including algal biomass. The certification allows the use of renewable aviation biofuels from biomass to be used by the aviation industry on a B50 basis only. However, this certification is open door for future studies on various blending options and ratios, the conditions of their improvement and optimization that can also lead to new certification. Therefore, there is a gap to be filled with regard to new alternatives for jet fuel blending. In November 2011, United Flight 1403 flew from Houston to Chicago on a 40

% blend of Solazyme's algal jet fuel, became the second U.S. commercial flight powered in part by algae-based biofuel. These trials have sufficiently demonstrated there is potential in using microalgae-based jet fuel to reduce dependence on fossil fuel. However, many challenges mentioned early are still yet to be addressed.

5. REQUIEREMENTS TO PRODUCE MICROALGAL BIOMASS FOR RENEWABLE JET FUEL

5.1 Microalgae selection

It is important to stress on the fact one of the most important steps to a successful production of microalgal based fuel starts with an appropriate selection of microalgal strain (Brennan and Owende, 2010). There are approximately more than 50,000 existing microalgae species but only 30,000 are characterized and some are still under study (Mata *et al.*, 2010). This implies that with the amount of existing species many possibilities could be explored to produce variety of algae-based jet fuel or other algae-based fuels such as biohydrogen, biodiesel and biogasoline. However, it is obvious that several aspects have to be taken into consideration and analysed meticulously in terms of the nature of species, the cultivation mode, the contamination, the lipid content and the possibility to increase it in order to select the most reliable species/strain that has great potential to produce a sustainable jet fuel.

5.2 Lipid content

Microalgal lipid content is one of the most important characteristics required for biofuel production (Schenk *et al.,* 2008). The production of crude bio-oil and eventually the jet fuel will mainly depend on the lipid content of the strain or species. When choosing the strain, one has to decide whether to go for the very high lipid content accompanied with low cell productivity or moderate lipid content accompanied with high cell productivity (Mata *et al.*, 2010), as these two characteristics are mutually exclusive in natural strains (Ratledge and Cohen, 2008). Generally, it has been reported that microalgae accumulate between 10%-50% of their dry weight in oil content (Chisti, 2007). Oil accumulation higher than 50% is usually associated with cell productivity (Schenk *et al.,* 2008). However, there is also a possibility to increase the lipid content by modifying the genetics of the strain. This can be done by nutrients starvation or the use of enzymes or genetically modified organisms to stimulate oil increase in the cell. The process is completed after cultivation, the biomass will be subjected to conditions that involve physiological modification of cells under starvation conditions. This will therefore stimulate the metabolism of cells and induced an increase of biomass quantity and lipid content. The genetic engineering of strains is generally a successful technique used to increase the strain or species lipid content. However, it is depending on the nature and species type. Each species

or strain has its own way of responding to physiological modification involving nutrients starvation or use of enzymes.

5.3 Site selection

Another important step in microalgae cultivation is the choice of an appropriate site. The assessment of a potential site can involve several criteria that have to be inspected and regulated. These criteria are classified into two categories: abiotic and biotic factors (Moheimani, 2005). Generally, abiotic factors are related to characteristics such as the quantity and quality of light and water supply, the water salinity, the amount of dissolved oxygen and carbon dioxide, the pH of water, the surrounding climate including temperature (Schenk *et al.,* 2008), the rate of evaporation and precipitation, and the availability of nutrients and carbon sources (Moheimani, 2005). It is well known that both light and temperature play a major role when it comes to microalgae productivity (Mata *et al.,* 2010). However, the light used for cultivation should be at the optimum value to ensure effective growth of cells. Strong and intense light can lead to photo-inhibition causing harm to effective photosynthesis required for better microalgal growth and biomass production (Moheimani, 2005, Ginzburg, 1993). Therefore, it is vital to match the algal strain with an appropriate environment in which sunlight can sufficiently be provided to help grow the strain effectively (Ginzburg, 1993). Each strain or species has its own optimum growth temperature which allows it to reach its maximum growth rate. However, the optimal growth temperature for microalgae growth ranges between 20 and 30°C (Chisti, 2007). It is assumed that a decline in temperature can be tolerated by most microalgae strains, while an increase by only 2-4 °C from the optimal microalgal temperature can lead to a destruction of the culture (Chisti, 2007, Mata *et al.,* 2010). Creating sufficient space for microalgae cultivation in a conducive environment or site is very important to allow a sustainable biomass production. The topography and geology of the land will also require a careful consideration, the land slope must be less than 10% and soil depth must be minimal to allow the construction of cost effective large cultivation systems (Schenk *et al.,* 2008). The resources required for microalgae growth including carbon and water must be easily accessible, they should be situated within the vicinity of the chosen site in order to reduce the costs related to transport and handling. Various strains/species can grow in different aquatic media such as freshwater, seawater, as well as brackish water (Ratledge and Cohen, 2008), consequently, the stress on freshwater can be reduced. The use of saline water depends on the selected site, this can be situated next to coasts or nearby saline groundwater (Satyanarayana *et al.,* 2010). On the other hand, the biotic factors include the presence of parasites and

predators competing with microalgae growth and preventing nutrients to be assimilated by cells (Moheimani, 2005, Mata *et al.*, 2010).

5.4 Microalgae nutrients

Nutrients are essential to generate significant amounts of biomass from microalgae culture. The nutrients needed are mainly inorganic compounds such as CO_2, phosphorus and nitrogen, (Chisti, 2006). The provision of these elements for microalgae growth is generally inexpensive when using wastewater treatment ponds (Powell *et al.*, 2009). Large portion of hydrocarbons (90%) formed through microalgal photosynthesis are made from carbon, whereas the other 10% is made of hydrogen (Ginzburg, 1993). Moreover, 50% of the dry biomass weight is attributed to carbon content (Patil *et al.*, 2008). The carbon source needed by microalgae through biological uptake can come either from the 0.03% of carbon dioxide available in the atmosphere or from the flue gases generated by heavy industries and fossil fuel power plants. This CO_2 can be fed to the culture to contribute to cells growth and increase biomass production rate. Also, lipid content is increased because of the increase in carbon chains (Brennan and Owende, 2010).

However, in case there is a dependence only on atmospheric CO_2 as carbon source, the productivity of microalgae cells will be very low and not suitable for fuel production because of low biomass generated during cultivation (Posten and Schaub, 2009). Microalgae have a great uptake capacity for CO_2 compared to many terrestrial crops. This is due to their faster growth and high nutrients absorbance capacity. Microalgae can grow on arid land; however, they cannot replace forests which constitute an important carbon sink (Khan *et al.*, 2009). It is reported that 1 kg of microalgae can absorb approximately 1.83 kg of CO_2 (Chisti, 2007, Patil *et al.*, 2008). The efficiency of CO_2 uptake by microalgae ranges between 30 to 99%, depending on the cultivation system used and the rate of culture mixing (Reijnders and Huijbregts, 2009). This mechanism of CO_2 uptake allows the recycling of CO_2 (Mata *et al.*, 2010). The CO_2 absorbed by microalgae during growth is re-emitted during microalgal biofuel combustion (Mata *et al.*, 2010).

Another important nutrient is phosphorus, an energy carrier element and an essential constituent for cellular metabolism and regulation contributing to the production of enzymes and phospholipids in microalgae (Moheimani, 2005). It represents 1% of microalgae dry biomass weight (Powell *et al.*, 2009). It is a major constituent of adenosine triphosphate (ATP) required for short term energy storage and transfer as mentioned before. It is a key factor in the energy metabolism of microalgae and is found in nucleic acids, lipids, proteins, and the intermediates of carbohydrate metabolism. Inorganic phosphates play a significant role in

microalgae cell growth and metabolism. During algae metabolism, phosphorus, preferably is incorporated in to organic compounds through phosphorylation, much of which involves the generation of ATP from adenosine diphosphate (ADP), accompanied by a form of energy input

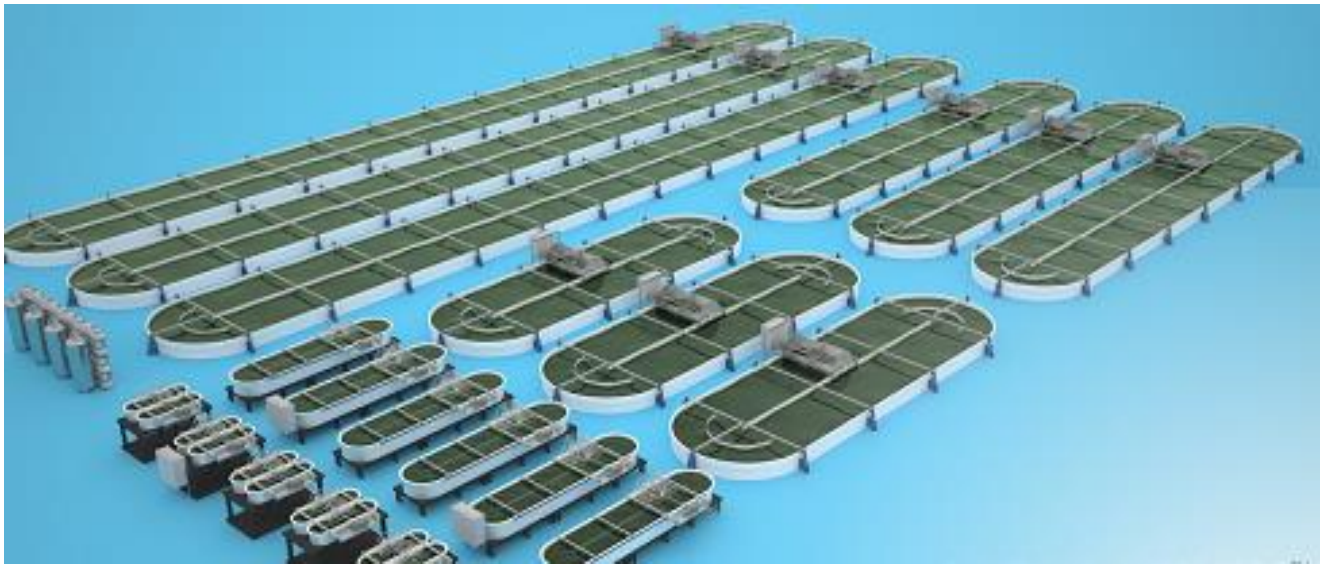
Nitrogen is also one of the most important nutrients for microalgae cells, it is a major component of chlorophyll, the compound by which cells use sunlight energy to produce sugars from water and carbon dioxide during photosynthesis. It is also a major producer of amino acids, the building blocks of proteins contributing to cells lipid content. It is vital for protein and genetic material synthesis. It is an essential macro-nutrient that has significant importance to the growth and metabolism of mixotrophic microalgae. For mixotrophic microalgae, nitrogen can be utilized as $NO3^-$, $NO2^-$, $NH4^+$, or even as N_2 Nitrate, ammonium, and urea are the three most commonly adopted nitrogen sources.

A combination of both phosphorous and Nitrogen in one unit is very advantageous, therefore, a source of nitrogen and phosphorus should be added to the culture, in order to obtain high levels of productivity. Fertilizers seem to an acceptable option, they can be used as a source of nutrients, as they contain nitrogen and phosphorus (Posten and Schaub, 2009). Another option, which can substitute the use of fertilizers, is to grow microalgae in domestic wastewater instead of freshwater (Park *et al.,* 2011). Domestic wastewater can provide the needed nutrients for microalgae growth, since it can contain high concentrations of nitrogen and phosphorus from nitrates and phosphates (Pittman *et al.,* 2011). But prevention mechanism to avoid culture contamination should be put in place to reduce the risk of cells death and low biomass productivity rate.

6. CULTIVATION AND GROWTH MEDIUM

Cultivation of microalgae can take place in open ponds or in photobioreactors (PBR). Generally, the culture medium is directly exposed to the environment when using an open pond. It uses solar energy as the source of light needed for microalgae cultivation (Widjaja et al., 2009). This system can regulate the temperature by liquid evaporation. Paddle wheels are used in open race ponds for mixing and circulation of gas or liquid with low investment. They also allow to expose to light as many cells as possible for an effective photosynthesis (Cheng-Wu et al., 2001). Open pond systems are generally less expensive, their manufacturing requires lower costs material and less energy. However, open ponds are less effective in terms of light and temperature control. They need larger area and only handful of microalgae can be cultivated. There is a possibility of culture contamination including the production of low

density microalgae when using open ponds (Pulz, 2001; Richmond, 2004; Carvalho et al., 2006). Currently, an advanced form of ponds known as raceway ponds are being used because of their effectiveness in terms of biomass production rates. Raceway pond channels are built in concrete, or compacted earth, and possibly lined with white plastic to collect sunlight during daytime. Broth is harvested before the paddle wheel after the circulation loop is completed. The paddle wheel operates all the time to prevent sedimentation. Raceway ponds have been used since 1950s, Figure 1 is an example of raceway ponds used on a site for microalgae cultivation.



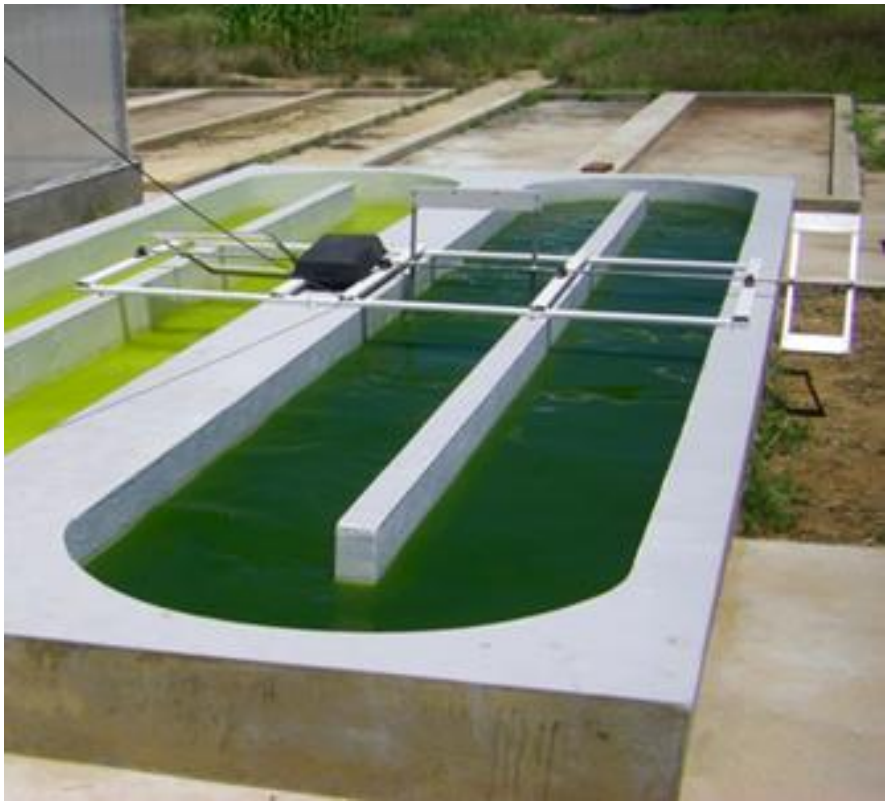


Figure 1: Examples of various raceway ponds for algae cultivation

Photobioreactors are closed systems frequently used in commercial scales for microalgae cultivation. Microalgae growth is well controlled in order to achieve specific biological modification (Richmond, 2004; Cheng-Wu et al., 2001). They are easy to handle compared to open ponds when it comes to mixing and mass transfer of gas or liquid. Photobioreactors are very productive systems due to the most effective use of the cultivation area and efficient energy consumption Carvalho et al., 2006; Sierra et al., 2008). They consist of arrays of straight tubes of 0.1 m or less in diameter to allow an effective penetration of light. Also, the limited diameter is meant to increase the biomass productivity. Generally, the tubes are always oriented North-South in order to maximize the sunlight penetration in the culture. In a typical arrangement, the solar tubes are placed parallel to each other and plane above the ground as presented in Figure 2. Horizontal, parallel straight tubes are sometimes arranged in same way like a fence, in attempts to increase the number of tubes that can be accommodated in a given area.

Figure 2: Photobioreactors used for algae cultivation

To supply nutrients to microalgae, wastewater especially municipal wastewater can be used as source of nutrients as mentioned earlier. This implies that microalgae can be used for wastewater treatment, accumulating contaminants by absorption and adsorption allowing the removal of unwanted substances in wastewater in order to get a clean effluent.

7. POTENTIAL OF MICROALGAE BIOREMEDIATION OF WASTEWATER

Generally, the treatment of domestic sewage aims to remove the pathogen and stabilise the contaminants in order to generate a clean effluent. Therefore, domestic wastewater must go through a series of treatment steps including preliminary treatment, primary treatment, secondary treatment and tertiary treatment. Its composition depends on many factors including the life style in the environment where it is collected. Generally, domestic wastewater contains suspended solids, inorganic and organic substances. Organic components include nitrogenous compounds like proteins and amino acids or non-nitrogenous compounds like carbohydrates and lipids in the form of fatty acids. Nitrogen in domestic wastewater comes from human wastes and from waste food primarily originating from household garbage-disposal units. Human waste is the major source of nitrogen in wastewater. Inorganic components include ammonia, chloride salts, phosphates, potassium and heavy metals in low concentrations. Phosphorus or phosphates compounds in domestic wastewater may originate from human wastes, waste food primarily from household garbage-disposal units, and synthetic detergents. Domestic wastewater also contains a large population of microorganisms, pathogens, emulsions and toxins such as pesticides, poisons, herbicides. The major sources of inorganic components in municipal wastewater are illegal disposal of pesticides. The main source of organic compounds is from food, toilets, bathroom, and kitchen wastes. Generally, many algae species grow well in domestic wastewater. Many of these species have shown potential in bioremediation of wastewater because it is a conducive environment for species. It is offering a possibility of effective cultivation and growth because of to the presence of nutrients in large amounts. The uptake of nutrients and contaminants takes place by biosorption, they accumulate nutrients to allow effective growth (Bwapwa et al., 2017). The following are few examples of species that have proven to be effective in wastewater bioremediation using microalgae:

Chlorella performs well in the removal of lead (II) ions from wastewater. It removes easily nutrients N and P from domestic wastewater. It is also used for the removal of cyanide from wastewater. *Pithophora sp* is very effective in the removal of malachite green dye from wastewater. *Scenedesmus abundans* has the capacity to remove cadmium and copper in wastewater and also in the removal process of cyanide from wastewater. *Sargassum muticum* can remove methylene blue dye from wastewater. *Spirulina sp* is a good candidate for the biosorption of heavy metals like antimony and chromium present in wastewater. *Botryococcus braunii* has shown potential for the removal of nitrogen and phosphorus. *Dunaliella salina* has the ability to remove heavy metals like Cu, Cd, Co and Zn. Also, it is among the best species

for hypersaline wastewater treatment. *Ankistrodesmus sp* has enormous ability to absorb metals, it also removes mercury, arsenic and selenium through methylation. *Microactinium sp* removes zinc and cadmium from wastewater through biosorption by *Microactinium pusillum. Pediastrum sp* is known as an indicator of organic compounds in wastewater, the uptake process of organic contaminants in this case takes place through biosorption. However, these species have been used to production biofuel such as biodiesel. There is possibility to convert biodiesel to bio-jet fuel. Therefore, the production of aviation biofuel from algae species is technically possible.

7.1 Combination of domestic sewage treatment and biofuel production: the advantages, the problematic and the gap

As mentioned earlier domestic wastewater contains large amount of nutrients required for microalgal cultivation and thus can be used for biomass production which will be used to produce jet biofuel or any other biofuel. The nutrients removal efficiency varies depending on the composition of domestic wastewater and environmental conditions, such as the initial nutrient concentration, light intensity, nitrogen/phosphorus ratio and the light/dark cycle or algae species. The protection of the growing culture against the predators should also be included. The use of microalgae for wastewater remediation has attracted many interests due to the effective nutrient removal abilities of microalgae and additional benefits of biofuel production. Microalgae offer the advantages of high growth rates, lipid productivities and ability to bio-sequester carbon dioxide, high possibility to remove contaminants from domestic wastewater and their abilities to produce biofuels as mentioned before. However, large scale, cost-effective and sustainable microalgae biofuel production remains still uncertain. The technology/potential of using microalgae to remove contaminants from domestic wastewater as well as converting the resulting microalgal biomass for biofuel production, are critically reviewed. There is synergy taking place between wastewater and algae cells when they are together in the same environment as shown in Figure 3. The synergy allows the growth of microalgae by the same time removal of nutrients by bioaccumulation takes place to get a clean effluent as mentioned earlier. Figure 3 shows the synergy between microalgae and bacteria in wastewater treatment process. In this synergy biomass is collected by harvesting after wastewater treatment and can therefore be used for biofuel production. The possibility to articulate between wastewater treatment, biomass and biofuel production can be very interesting in terms of cost effective, energy saving and maintenance. This can be a gap for future studies to analyse in details the combination involving biofuel production more

especially jet fuel and domestic wastewater. Therefore, the dual application of microalgae for phycoremediation and the production of biomass in order to generate sustainable biofuels is a feasible option (Rawat et al., 2011). However, there is a need to adapt many parameters for a better articulation between bioremediation and biomass production for biofuel production in general and bio-jet fuel in particular. In this case modelling studies, process improvement and design, choice of species capable of producing high biomass and oil output while removing contaminants can be among the aspects to be studied.

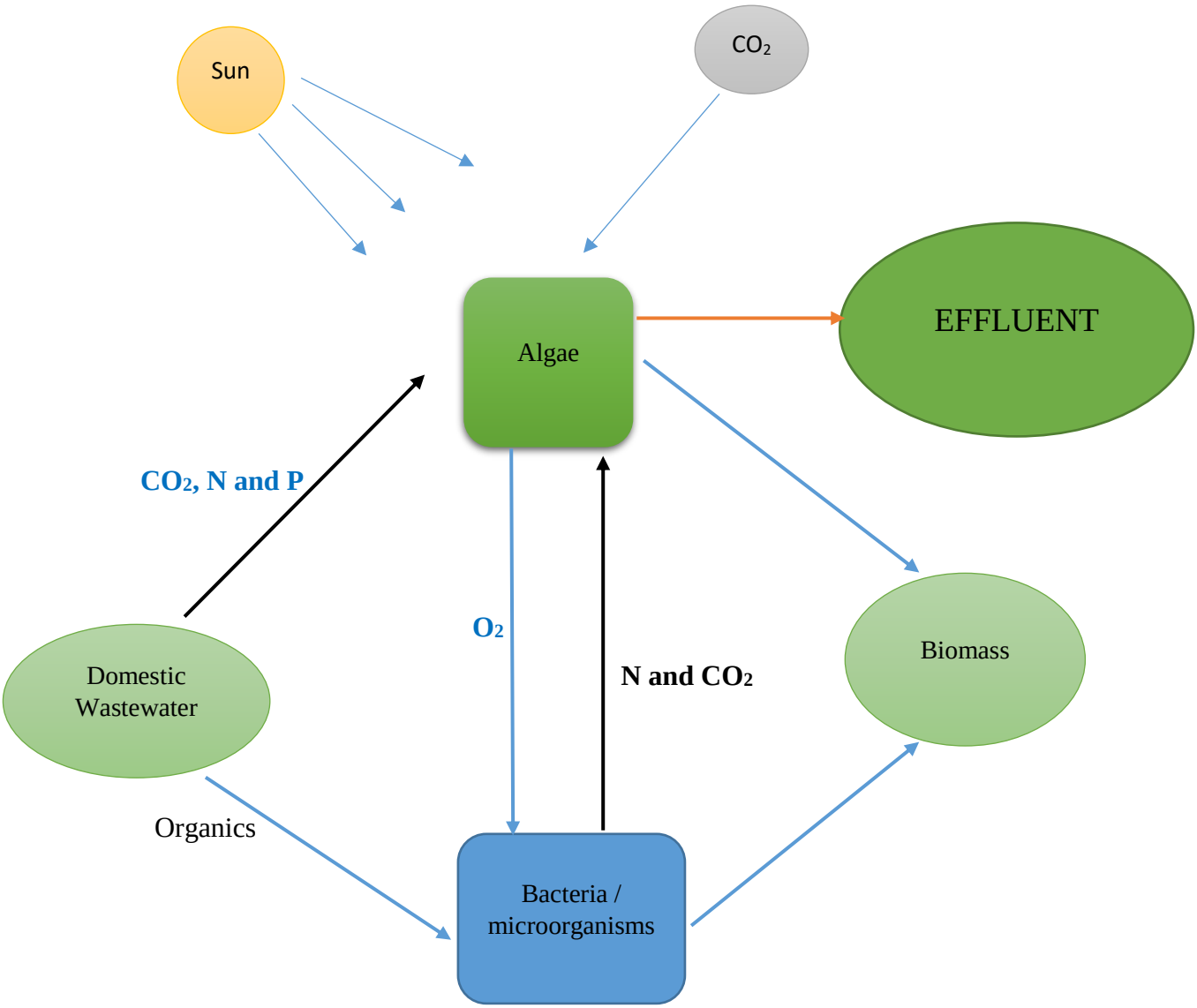


Figure 3: Synergy between algae and bacteria in domestic wastewater for wastewater algal bioremediation and algal biomass production.

7.2 Incorporation of algae-based wastewater treatment in the conventional wastewater treatment plant

Algae-based domestic wastewater treatment can be incorporated in the secondary treatment within a conventional treatment plant, this is suggested as a new conceptual approach to be used for wastewater treatment. The necessary oxygen to feed the microorganisms will be supplied by algae species. In this case algae will provide a way to uptake nutrients and supply required oxygen to aerobic bacteria through photosynthesis. Consequently, this can reduce energy consumption as the source of oxygen is no longer the blowers or pumps but the algae. Aeration process is known as an energy demanding for wastewater treatment plant. Generally, aeration accounts for 45 to 75% of the total energy costs of wastewater treatment plants (Rosso et al., 2008). However, the challenge for algae is its incapacity to produce continuously the needed oxygen for microorganisms to accomplish their work. Therefore, treatment will therefore take longer compared to conventional system as it is a slow process. Therefore, the combination of both conventional and algae-based domestic wastewater treatment as presented in Figure 4 can be an option that need to be deeply examined and explored because it is energy saving and environmentally viable. The excess of oxygen needed to strengthen the treatment performance can be provided by algae when dealing with the combined option, it saves energy as mentioned before and will have a high impact on the removal efficiencies. In this situation algae-based treatment is used as a passive treatment and the conventional system as an active treatment as per normal. However, the costs related to maintenance will be increased. The increase is also due to algae handling and harvesting when there is need to add a new culture. Figure 4 presents the conceptual option suggesting the incorporation algae-based treatment into a conventional treatment plant to achieve the objectives of domestic wastewater treatment and produce biomass for conversion into algae-based fuel such as biodiesel and bio-jet fuel.

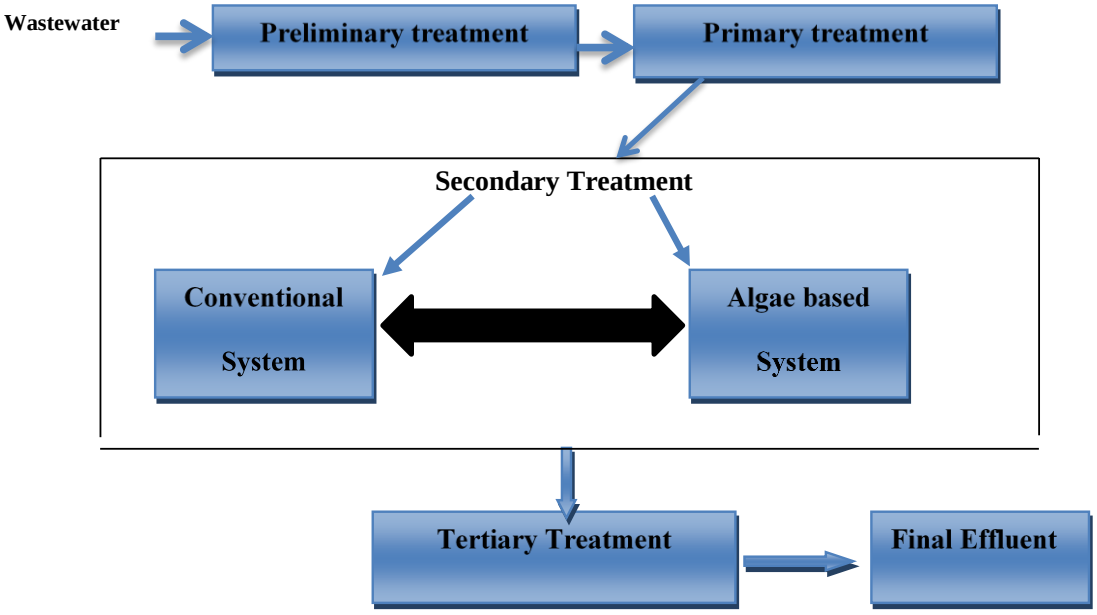


Figure 4: Systematic chart locating the strategic position of algae-based wastewater treatment

7.3 **The effectiveness of algal growth in wastewater to produce biomass**

An efficient growth of microalgae in domestic wastewater depends on many parameters. These parameters include the pH, temperature of the growth medium, the light intensity and salinity. Furthermore, effective algal growth relies mainly on critical parameters such N, P and organic carbon and CO_2. For instance, it was reported that the growth of microalgae in primary settled sewage wastewater increased significantly under long photoperiod conditions and following the addition of CO_2, while increased temperature decreased algal biomass (Pittman et al., 2011). Mostly, the main difference between domestic wastewater used as a growing media and other media is that there is a presence of high concentrations of N and P in domestic wastewater compared to other growing media. Most N is generally found in the form of ammonia. This can at high concentrations cause growth inhibition for algal species (Pittman et al., 2011; Konig et al., 1987; Wrigley and Toerien, 1990). The presence of toxins is another threatening factor for algal growth in domestic wastewater. These toxins are mainly from degraded food, detergents and many other chemicals used domestically. Their presence can

impact negatively on species growth, bacteria and other microorganisms. In addition, microorganisms in wastewater might have a tendency to compete with microalgae for essential nutrients, consequently, the removal efficiency can be affected.

The initial density for microalgae in wastewater is probably one of the critical factors but not the only one that could influence the growth of the entire cells or biomass population (Lau et al., 1995). For instance, *C. vulgaris* removed over 90% of N content and 80% of P content from the primary treated sewage (Lau et al., 1995). This suggests that effective wastewater growth and nutrient removal is not significantly dependent on starting cell density. Wang et al.,2010 also focused on the growth of *Chlorella* in pre- treated wastewater in addition to wastewater from three subsequent treatment phases. It was reported from their study that many of the tested parameters including N and P removal, heavy metals and ion removals including growth rate were equivalent in wastewater prior to and after primary settling.

These variables will differ depending on the wastewater type and from one wastewater treatment site to another. Also, the species type is another factor influencing growth effectiveness. It is related to the ability of the species to accommodate itself to the type of domestic wastewater, its composition and the environmental conditions. In this regard, unicellular chlorophytic microalgae species are particularly tolerant to many wastewater conditions and very efficient at accumulating nutrients from wastewater (Aslan and Kapdan, 2006; Gonzalez et al., 1997; Ruiz-Marin et al., 2010). Consequently, the species ability to survive in wastewater environment plays a vital role in the effectiveness of microalgal growth. For instance, *Chlorella vulgaris* is more effective than *Chlorella kessleri* at accumulating N and P from wastewater (Travieso et al., 1992), while *Scenedesmus obliquus* grows better in municipal wastewater than *C. vulgaris* (Ruiz-Marin et al., 2010). *Chlorella* and *Scenedesmus* are generally known as the predominant species in oxidation ponds (Masseret et al., 2000) and in high-rate algal ponds (Canovas et al., 1996). Overall, the effectiveness of algal growth will mainly depend on growth conditions, the nutrients, the nature of the species and the growth medium. These aspects will assist in producing sufficient biomass with lipid content that can be advantageous for conversion processes to produce a jet fuel complying with aviation standards.

Table1 : Some examples of species used for domestic wastewater treatment

Wastewater type	Species and Parameters	Removal efficiencies	References
Domestic wastewater	Species: *C. vulgaris* Parameters: COD , BOD, NO_3, and PO_4	80.64%, 70.91%, 78.08% and 62.73%, respectively	Ayodhya and Kshirsagar (2013)
Domestic wastewater	Species: *S. quadricauda* Parameters: COD , BOD, NO_3, and PO_4	70.97%, 89.21%, 70.32% and 81.34% respectively	Ayodhya and Kshirsagar (2013)
Domestic wastewater	Species: Chlorococcum humicola Parameters: BOD, TDS,TSS,TS, N, P	59%,66%, 49%,64%,80%, and 100% respectively	Thomas et al., 2016
Domestic wastewater	Species : *Chlorella vulgaris* Parameters : : BOD , TDS,TSS,TS, N, P	67%,67%, 60%,67%,84%, and 100% respectively	Thomas et al., 2016
Domestic wastewater	Species : *Selenastrum sp*. Parameters: BOD , TDS,TSS,TS, N, P	63 %, 49%, 54%, 49%,82%, and 100% respectively	Thomas et al., 2016
Domestic wastewater (settled sewage)	Species : Chlorella pyrenoidosa and Scenedesmus sp Parameters : N and P	More than 80% for both N and P	Tam and Wong (1989)
Domestic wastewater	Species : Botryococcus braunii and Chlorella vulgaris Parameters : N and P	79.63% of the nitrogen and phosphorus	Sydney et al.,2011
Domestic wastewater	Species : Scenedesmus sp. ZTY1 Parameters : N and P	Over 90% for N , 97 % for P.	Zhang et al.,2014

7.4 Algal growth in municipal sewage wastewater

Generally, a conventional municipal sewage treatment involves primary treatment phase for the sedimentation of solids. This is followed by a secondary treatment phase aiming to remove suspended and dissolved organic materials. The tertiary treatment phase in which disinfection of the water is performed prior to discharge into the environment. It is during secondary phase that the removal of many dissolved inorganic compounds including N and P takes place. The potential of microalgae to remove N and P can be explored in this phase. Microalgae are very efficient at removing N and P from sewage-based wastewater because they are the main nutrients needed for microalgae growth.

It is a fact that microalgae cells supply oxygen to microorganisms and at the same time they also absorb contaminants from sewage wastewater, however, the challenge is the adaptation of species in the sewage environment. Some unicellular green microalgae species are particularly tolerant to sewage effluent conditions, heavy metals and other contaminants most notably those

of the *Chlorella* and *Scenedesmus* genus (Chinnasamy et al., 2010; Lau et al., 1995; Ruiz-Marin et al., 2010; Shi et al., 2007; Wang et al., 2010).

Various species of *Chlorella* and *Scenedesmus* can provide high removal efficiency of up to 80% , in many cases complete removal of ammonia, nitrate and total P from secondary treated wastewater can be achieved (Martinez et al., 2000; Ruiz-Marin et al., 2010; Zhang et al., 2008). Generally, many species have a significant removal potential for N and P as well as other substances when used as bioremediation agent for domestic sewage wastewater. Overall, algal growth is strongly related to removal efficiencies of nutrients and other substances. The higher the removal efficiencies the more effective the growth of algal species in domestic wastewater. To have high removal efficiencies optimal growth conditions must be well established and the growing culture must be protected against inhibitor agents such as toxins and pathogenic bacteria.

7.5 Challenges related to the cultivation of algae in domestic wastewater

In conventional wastewater treatment plants, nutrients and treatment process are generally less expensive for small scales and yet represent major technical and economic problems at large scales due to higher levels of contaminants in the effluent, large volume of effluents and energy consumption. Using existing municipal wastewater facilities and streams will reduce nutrient costs on the other hand it presents a high risk of introducing pathogenic bacteria, toxins, complex organic compounds, or heavy metals into the biomass stream (Hoffman et al., 2008; Wilson et al., 2009). Little is known about artificial pond ecology or pathology, more investigations into these areas will be very important to establish large-scale cultivation risk mitigation and remediation strategies. These will also assist in culture stability, nutrients source scaling, monitoring productivity, sustainability and management, as well as water conservation, management and recycling (U.S. DOE, 2010). Treatment of domestic wastewater using algae is more effective in countries dominated by tropical climate where there is presence of abundant sunlight. The climate and sunlight have a significant influence on algae growth and it is challenging to maintain the optimum. Additionally, biological and operational issues causing contamination, auto-inhibition and grazing have to be prevented during cultivation. However, contamination by bacteria and other algal species can be prevented by sterilization and ultra-filtration of the culture medium while grazing can be prevented by chemical treatment. Some of the key challenges are: light requirements, temperature, and rainfall, mixing harvesting, contamination, and oxygen depletion.

8. HARVESTING METHODS

Harvesting aims to collect the biomass needed for downstream processes. It is an important costs component, owing to the challenges involved in the entire process of jet fuel production. It is hence important for those exploring algae-based fuels industry to gain a clear understanding of the various harvesting options available, the challenges related to each option, the current efforts and solutions being attempted in order to overcome these challenges. Also, there are attempts to skip the harvesting process aiming to reduce the costs related to harvesting and drying. However, these attempts are not yet sustainable on large scale. Some major technologies commonly used for microalgae harvesting are centrifugation, filtration, sedimentation and flotation (Danquah *et al.*, 2009; Chen *et al.*, 2011). Table 2 summarizes the strengths and weaknesses of these techniques.

Table 2: Strengths and limitations of harvesting techniques (adapted from Mohn, 1988; Grima et al. 2003; Shen et al. 2009)

Harvesting technique	Strengths	Limitations
Filtration	Less expensive, wide variety of filters and membranes available	Require frequent backwashing, Time consuming. Highly dependent on algal species; best suited to large algal cells. Clogging or fouling an issue
Flotation	Cost efficient and more rapid than sedimentation	Use of chemical, Depend on suspended particles. Less reliable, Algal species specific. High capital and operation costs
Centrifugation	Quick, Highly efficient, Good recovery	Expensive due to high energy consumption and high capital costs
Sedimentation	Low costs, potential for use as a first stage to reduce energy input and costs of subsequent stages	Slow separation, final concentration may be low.
Microfiltration/ ultrafiltration	Capable to handle nannochloropsis cells, very efficient and can reach up 98% dewatering. Can be used as pre-treatment prior to centrifugation.	High operating costs and membrane fouling.

9. ALGAE BIO-OIL EXTRACTION

There are 3 major components that can be extracted from algal biomass: lipids including triglycerides and fatty acids, carbohydrates, and proteins. Lipids and carbohydrates are known as fuel precursors (e.g., gasoline, biodiesel and jet fuel) and proteins can be used for co-products. Most challenges for bio-oil extraction are associated with the industrial scale up of integrated extraction systems. Although many analytical techniques exist and they are being used up to date, it is challenging to optimize extraction systems that will exhibit low energy consumption than the one contained in the algal cells. This is due to the high energy demand associated with both harvesting and drying algae biomass as well as separating out desirable products. Investigations are still underway aiming to develop options that will help to bypass bio-oil extraction, though these are also subjected to a number of unique scale-up challenges. This can be the case of catalytic processes such as biomass gasification and Fisher Tropsch. Lipid extraction from microalgal biomass is largely in the domain of laboratory-scale processes that serve analytical rather than biofuel production goals. However, the extraction process determines the oil output or recovery needed for downstream processes. The more efficient is the extraction, the more oil is collected for conversion processes. The dynamics of extraction in aqueous phase systems serves as a starting place for industrial-scale extraction operations. Lipid extraction includes the following approaches: solvent-based extraction relying on microwaves and or sonication for cell disruption; using solvents to "milk" algal cells without disrupting cellular functions; and extraction bypass schemes that attempt to engineer algal systems that secrete products directly into the growth medium. Conceptually, the cells can be channelled to their original bioreactor for continued growth and production of triglycerides for biofuels production. For example, some have proposed a modified technique to "milk" oils or neutral lipids from algae using biocompatible solvents and applied sonication. Solvent based extraction is the most undertaken technique till date to get bio-oil out of microalgae cells because of its cost effectiveness. The mixture of various solvents is also used for lipid extraction. The method developed by Bligh and Dyer (1959) has been a well-known co-solvent extraction procedure using the mixture chloroform-methanol-water in 1-1 ratio with the algal biomass. Chloroform will assist in extracting more than just the saponifiable lipids, the unsaponifiable lipids such as pigments, lipoproteins, and other lipid and non-lipid contaminants will be also extracted (Fajardo et al., 2007). Other combinations of co-solvents were suggested for the extraction of lipids: hexane/isopropanol for tissue (Hara and Radin, 1978); dimethyl sulfoxide/petroleum ether for yeast (Park et al., 2007); hexane/ethanol for microalgae (Cartens et al., 1996); and hexane/isopropanol for microalgae (Nagle and Lemke,

1990). The hexane system has been endorsed in many cases because hexane and alcohol will readily separate into two separate phases when water is added, thereby improving downstream separations (U.S. DOE, 2010). Also, less volatile and toxic alcohols such as ethanol and isopropanol are suggested instead of methanol. A single solvent can also be used for extraction depending on its effectiveness in extracting oil from the biomass. The latest study using only one solvent was completed by Kanda et al., (2012) using Dimethyl Ether (DME). In this process crude bio-oil was extracted from blue-green microalgae. DME molecules disperse inside cells through cell walls and bind strongly to the oily components. Its boiling point is 25 ^{O}C but it liquefies under 20^{O}C . DME is recovered as vapour only through depressurisation.

10. ORIGIN OF PETROLEUM CRUDE OIL AND COMPARISON WITH ALGAE CRUDE OIL

Algae bio-crude oil extracted from various species is similar in quality to petroleum crude oil (Hannon et al., 2010). Petroleum crude oil originates from algae in marine environment or old algae deposit made of liquid hydrocarbons (EIA, 2009). On average 85% w/w of oil is carbon, 10-14 % of oil is hydrogen, oxygen accounts for 1 to 2 % and sulphur represents up to 4% of the oil total weight (Hunt, 1996). Petroleum crude oil comes from Kerogen a compound present in sedimentary rocks within marine environment. It is formed after a series of biochemical and chemical reactions named diagenesis and catagenesis (Gize and Manning, 1993). Kerogen is transformed into crude oil under specific conditions of pressure and temperature. It is mainly made with algae, organic compounds, bacteria and plant material. Most of these compounds present in crude petroleum oil are also found in crude algae bio-oil (JK Bwapwa et., al 2018). Consequently, there is a possibility to use crude bio-oil in the same processing plant producing conventional jet fuel to produce algae- based jet fuel (Bwapwa et al., 2017).

11. SPECIES WITH POTENTIAL TO BE USED FOR ALGAE -BASED JET FUEL

Table 3 presents the bio-oil outputs from various microalgae species. The quality of bio-oil generated from these species is appropriate for any biofuel as well as bio-jet fuel. They can be successfully cultivated in domestic wastewater to produce the biomass needed for conversion processes.

Table 3: Output in terms of oil content for some algae species (adapted from Chisti, 2007 Georgianna and Mayfield, 2012; Brown et al., 2010; Brennan, and Owende, 2010)

Microalgae species	bio- oil output per mass of dry biomass [%]
Botryococcus braunii	25 -75
Nannochloropsis sp.	31-68
Schizochytrium sp.	50-77
Neochloris oleoabundans	35-54
Nitzschia sp	45-47
Ankistrodesmus TR-87	28-40
Chlorella sp	29
Chlorella protothecoides (autotrophic/ heterothrophic)	15-55
Crypthecodinium cohnii	20
Cyclotella DI- 35	42
Dunaliella tertiolecta	36-42
Hantzschia DI-160	66
Nannochloris: 31	6-63
Neochloris oleoabundans	35-54
Nitzschia TR-114	28-50
Phaeodactylum tricornutu	31
Scenedesmus TR-84	45
Stichococcus	9-59
Tetraselmis suecica	15-32

Thalassiosira pseudonana	210-310
Euglena gracilis	14-20
Hormidium sp	38
Phaeodactylum tricornutum	20-30
Pleurochrysis carterae	30‑50
Chlamydomonas reinhardtii	21
Prymnesium parvum	22-38
Tetraselmis sueica	15-23
Chlorella emersonii	28-32
Hormidium sp	3.8
Chlorella pyrenoidosa	47
Chlorella vulgaris	14-22
Crypthecodinium cohnii	20
Dunaliella tertiolecta	36
Dunaliella salina	6
Dunaliella primolecta	23
Cylindrotheca sp.	16-37
Phaeodactylum tricornutum	20-30
Pleurochrysis carterae	30-50
Scenedesmus dimorphus	16-40
Scenedesmus obliquus	12-14

12. GENERAL REQUIREMENTS AND COMPLIANCE FOR JET FUEL

A complying jet fuel should provide necessary energy for aircraft propulsion and avoid freezing at higher altitudes. Therefore, it must comply with technical and operational specifications. Furthermore, jet engines must be designed accordingly in order to operate under these specific requirements. Both the relatively slow rates for aircraft fleet renewal and the global nature of the aviation sector require that any jet fuel should be produced from high quality crude oil. Also, refinery or conversion processes from crude oil to jet fuel should be costs competitive. They also have to enable the production of a complying jet fuel to established aviation standards which is expected to be highly competitive on the market. In this case, if all these requirements on quality and market are met, the use of an alternative jet fuel would represent

no change or challenge on the ground and supply infrastructure, airframe or engines. This kind of jet fuel is defined as a “drop in” jet fuel. It is the alternative version of jet fuel needed on the market to compete with the conventional jet fuel. On the other side, a “non-drop in” jet fuel implies that new aircrafts and infrastructures would require a substantial investment in a supply chain system which should be kept independent from the one used for conventional jet fuel. Additionally, the engine technology should be specifically designed for the ‘’non-drop in’’ jet fuel. Currently, it is a fact that no manufacturer of aircrafts or engines is ready to restrict the use of their equipment to a particular fuel or way of operating or even developing new equipment to accommodate a new fuel. This will require new studies and new investments from the fuel manufacturer and could be time consuming. Therefore, this suggests that in the near to mid-term, efforts should be made to produce alternative fuels which are “drop in”. to keep using the same equipment or engines without any modification, this is an ideal situation. Nevertheless, ‘’non-drop in" jet fuels should not be automatically rejected, but they have to be carefully evaluated in terms of their quality and performance, and finally improved substantially in order to comply with the standards and meet the market expectations. Consequently, an elaborate improvement approach based on a balance between their potential advantages from efficiency, environmental or economical point of view and their approval and implementation costs should to be undertaken thoroughly.

Producing a microalgae-based jet fuel totally compliant to stringent regulations defined by standard bodies is one of the biggest challenges to be overcome. Compliance focuses mainly on properties or parameters such as cold flow property, energy density, energy content, kinetic viscosity, freezing point, concentration of aromatics, material compatibility, safety properties, flash point, water content and thermal stability (JK Bwapwa et al., 2017). The concept of compliance is also focusing on combustion and kinetic aspects such as ignition and extinction characteristics, chemical kinetics, lubricity flame speed and flammability limits. These features must strongly correlate with the composition of jet fuel knowing that the composition depends mainly on raw crude oil, the type of refining processes used and additives used to improve the quality of jet fuel. Generally, conventional jet fuel is made up roughly of 20% paraffin, 40% isoparaffinic, 20% naphthene’s and 20% aromatics (Blakey et al., 2011). However, additives are made to the jet fuel in order for it to comply with the expected quality and performance. The composition of jet fuel can be regarded as a key aspect for compliance with the stringent standards used for aviation fuels.

Compliance also implies that algae-based jet fuel must perform as a’’ drop-in ‘’ substitution or support for the conventional jet-fuel (Bauen et al., 2009). Table 4 presents ASTM limits and various ASTM

methods used to perform some physico-chemical tests required for jet fuel compliance.**Table 4: Some ASTM standards used for Jet fuel**

Parameter	Unit	ASTM D1655 limits	Method
Density @15 ^{O}C	g/ml	0.775-0.840	ASTM D7042
Viscosity @- 20 ^{O}C	Cost	8 (maximum)	ASTM D445
Flash point	^{O}C	38 (Minimum)	ASTM D93
Water content	%	nd	ASTM D6304
Total acidy	mg KOH/g	0.015(Maximum)	ASTM D3242
Total Sulphur	%	0.3 (Maximum)	ASTM D4294
Net heat of combustion	MJ/kg	42.8 (Minimum)	ASTM D4868
Freezing point	^{O}C	-40^{o} C (Maximum)	ASTMD D2386
Conductivity	pS/m	50-600	ASTM D2624

13. CURRENT AND FUTURE CHALLENGES

A part from the compliance and sustainability issues which are also challenging aspects, the other major challenges faced by algae-based fuels are biological, chemical and mechanical. Biological challenges are related to strain or species ability to grow effectively, lipid production, biomass productivity, resistance to contamination, optimal light intensity, tolerance of high oxygen levels and temperature extremes. Therefore, the selection of the strain is regarded as one of the key aspects because of its link with lipid content and also the nature of strain/species cold have a direct influence on lipid biosynthesis, biomass productivity and the type of lipids produced (Eloka-Eboka and Inambao ,2017). Chemical challenges are related to oil extraction with solvent use, operating conditions of transesterification reaction, the process of chemical harvesting, quality of water and removal of toxic substances in water or wastewater used for algae cultivation (Hannon et al., 2010; Gerbens-Leenes et al., 2009)

Mechanical challenges focus on equipment maintenance and performance related to cultivation equipment, harvesting systems, drying and oil extraction equipment such as oil press or impeller (Hannon et al., 2010). Process optimization and modelling of relevant parameters related to these challenges will also assist to overcome them. Table 5 described further details on the main challenges recorded at each step of the production regarding algae-based fuels. These challenges are part of the main issues faced by algae-based fuels producers. They constitute the basis for research and development in the current context to improve and enhance the sustainability of algae-based fuels including bio-jet fuel from algae.

Table 5: Summary of the main challenges encountered on each process step of microalgae Jet fuel production (adapted from US DOE 2010)

PROCESS STEP	RESEARCH AND DEVELOPMENT CHALLENGES
Algal biology	- Choice of relevant strains with high lipids content - Development of small scale high throughput screening technologies - Development of an open access database and collection of existing strains with detailed characterisation -Investigation of genetics and biochemical pathways for production of fuel precursors - Improvement on strains for desired criteria by gene manipulation techniques and breeding
Algal Cultivation	- Investigation of cultivation approaches depending on the species nature - Achievement of stable and robust cultures at commercial scale - Optimization of algal productivity of fuel precursors - Development of a sustainable and cost-effective models for water and land use and nutrients - Identification and solving environmental impacts and risks
Harvesting and Dewatering	- Investigation of costs effective harvesting process (centrifugation, sedimentation, flocculation, filtration dissolved air floatation. microfiltration etc) - Reduction of harvesting energy intensity

	- Lowering the capital and operating costs - Assessing each harvesting option in terms of compatibility and sustainability - Development of new harvesting technologies to compete with the existing ones
Extraction and Fractionation	- Investigating and optimizing various approaches (sonification, microwave, solvent extraction, etc - Minimizing and optimizing the process energy intensity - Investigating recycling mechanisms to minimize wastes generated after bio-oil extraction - Addressing scaling challenges due to operating parameters such as temperature, pressure, carrying capacity, separations and side reactions
Fuel conversion	- Investigating on various approaches for sustainable processes to get jet fuel from microalgae (thermochemical, catalytic conversion, biochemical conversion etc - Minimizing contamination of the final product and reaction inhibitors - Minimizing process energy consumption and carbon emissions over life cycle - Achieving high conversion rates under scale-up conditions

14.PROPOSED PROCESS FOR CONVERSION OF ALGAE TO JET FUEL FROM WASTEWATER

A set of processes can be explored to convert microalgal crude bio-oil into jet fuel. Prior to conversion processes, sufficient biomass should be produced via cultivation of microalgae under defined operating conditions including temperature, light intensity, salinity and pH depending on the species/strain type and nature as mentioned before. The cultivation process should incorporate the species genetic modification which is vital for lipid content increase. Conversion processes are undertaken after biomass harvesting and/or drying. Currently, there are numbers of conversion processes that can lead to jet fuel production from algal biomass. Some of the ground-breaking processes or techniques are patented and remain under the private domain. Figure 4 is the suggested model including both domestic wastewater treatment and bio-jet fuel production. Conventional wastewater treatment can be combined with algae-based wastewater treatment as suggested before. The generated algae biomass should be collected after treatment and be used for conversion processes. This collection should be done in way that microorganisms are not taken away by the algae biomass. Figure 4 presents a conceptual scheme involving all the processes that are essential to generate jet fuel from domestic wastewater. The feasibility of this combined system should be undertaken in details specially in terms of costs and algae cultivation. From this concept not only jet fuel can be produced but other lighter fractions such as naphtha, biogasoline, diesel distillate depending on carbon chains found in the bio-crude oil from algae.

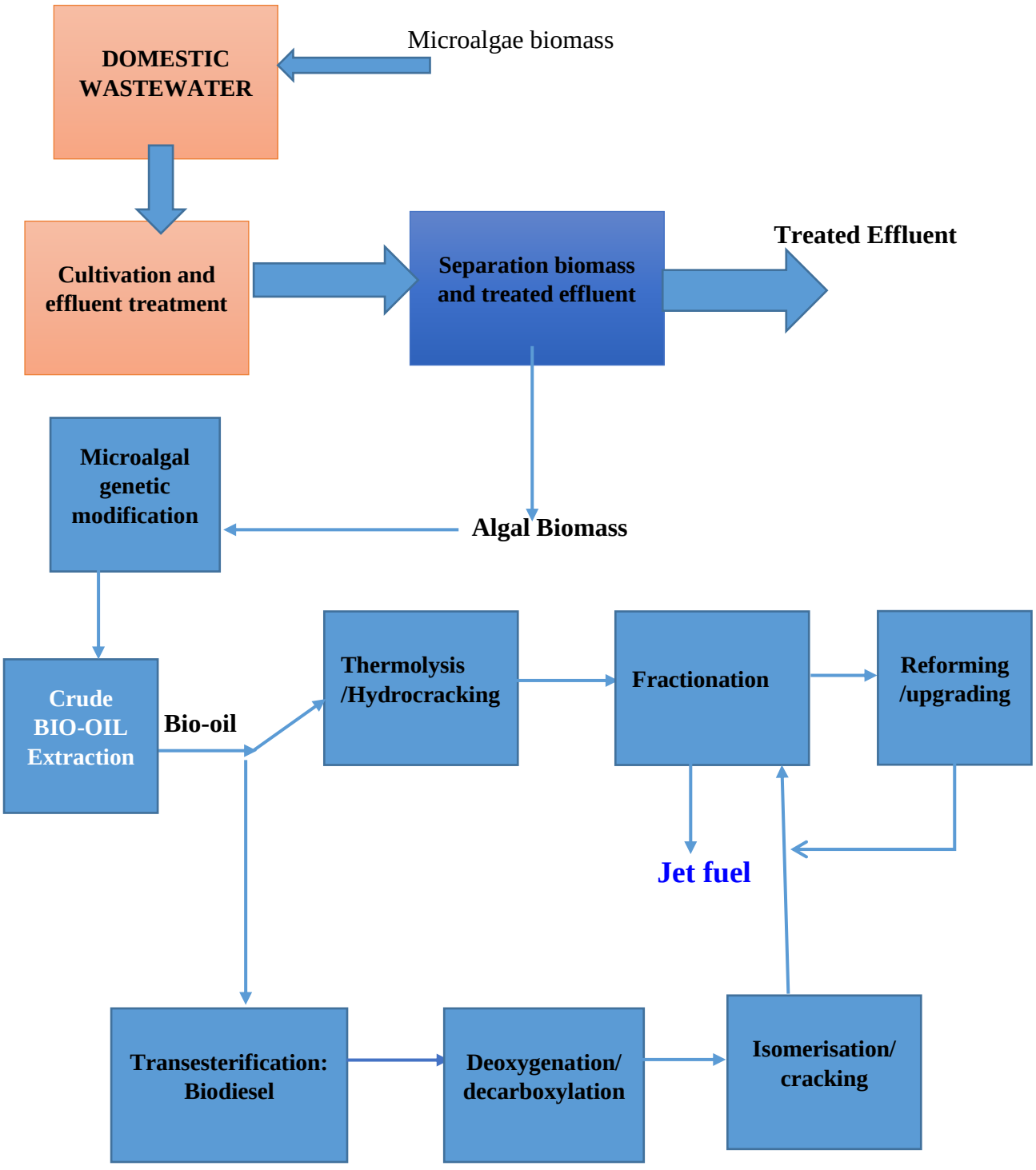


Figure 4: Conceptual scheme to produce jet fuel from microalgae-based wastewater treatment

This conceptual process suggests 2 routes after recycling wastewater: the first is similar to a petrochemical process involving cracking, fractionation, reforming and upgrading. The second route is the biodiesel option which involves deoxygenation and decarboxylation, isomerisation

or cracking and finally the fractionation to get the relevant fractions for jet fuel. This option while it is technically sound but it is still costly and energy demanding.

15. VARIOUS CONVERSION PROCESSES TO BE USED FOR ALGAE BIOMASS INTO JET FUEL

15.1 Hydrothermal liquefaction

This is a thermal depolymerization process used to convert wet biomass into crude bio-oil under moderate temperature and high pressure. The concentration of wet algae should not be less than 20 wt % solids. This slurry is hydrothermally treated in subcritical water, at a pressure range of 2000 to 3000 psia under temperature varying between 300° and 350°C. The reaction temperature dictates the reactor pressure (Biddy et al., 2013). Hydrothermal liquefaction(HTL) is considered as one of the most promising technologies for conversion of biomass including algae biomass into biofuels. This is because of its advantages in rapid reaction using wet feedstocks with no lipid-content restriction. This makes HTL a suitable process for conversion of algae biomass into crude bio-oil. The chemical reactions that take place during the hydrothermal treatment are currently part of many studies; and the effect of main process variables on the liquefaction process is being also studied**.** These process variables include parameters such as temperature, heating rate, holdup time at the maximum temperature, biomass concentration in the water suspension, biochemical and elemental compositions of the microalgae, use of catalysts, etc Bio- oil from HTL process requires additional catalytic processing for oxygen and nitrogen removal. Similarly, Sulphur removal also takes place but then it is found in the HTL bio-oil to a much lower concentration. In this process no energy is spent for drying the algae biomass, the slurry is channelled straight into the reactor and the crude bio-oil is produced without using a solvent after separating it with bio-char. This process can be used in lieu and place of bio-oil extraction. However, HTL process requires an improvement of catalyst performance and the reduction of operating costs to be more efficient.

15.2 Biomass gasification and Fischer Tropsch integrated process (BG-FT)

In this technology there is a combination of carbon monoxide and hydrogen produced from coal, natural gas, or biomass during gasification, and thereafter Fischer–Tropsch(FT) process turns these gases into a synthetic lubrication oil and synthetic fuel. Briefly, in this integrated process, biomass is gasified and the generated bio-syngas is used for FT synthesis to produce long chain hydrocarbons in order to be converted to fractions such as green Diesel and jet fuel

(Boerrigter et al., 2003). Biomass gasification and Fischer Tropsch integrated process (BG-FT) can assist in deriving more varied forms of energy from the thermochemical conversion of biomass than conventional combustion. FT being a well-established process that can be coupled to a biomass gasification system uses cobalt-based or iron-based catalysts. The use of cobalt-based catalysts yields high oil productivity than iron-based catalysts at high conversion level, while the productivity is approximately equal at intermediate levels of conversion, making cobalt the most appropriate catalyst (van Steen and Claeys, 2008). The bio- syngas collected from biomass can be H_2 deficient. Therefore, steam and oxygen are used as gasification units to enhance the H_2 content in the syngas. Although the technology is promising for algae biomass conversion to jet fuel, this integrated process presents some weaknesses such as high oxygen and water content for the produced fuel and it is requiring intensive impurity removal, control and adequate wastewater management approach. There is need for product separation and upgrading when using this technology to produce jet fuel.

15.3 Pyrolysis

This is a thermochemical process used to break down long carbon chains in shorter ones for any organic product at higher temperatures in very short amount of time. In this process, the oil is exposed to high temperature without oxygen. Different new molecules are obtained after a series of chemical and physical changes. A variant of biomass pyrolysis is known as fast pyrolysis, it is achieved with temperatures between 400 and 600 °C within few seconds. Fast pyrolysis can yield up to 80% (in weight). Zeolite is used as catalyst during the process to improve the quality of oil. The catalyst can influence the increase of aromatic content and cause the decrease of oxygen. The catalytic pyrolysis produces high quality oil that requires less upgrading processes than the non-catalytic one. Therefore, it is cost effective compared to the non-catalytic (Venderbosch and Prins, 2010). This process can be effective for algae biomass provided that algae biomass is completely dried before pyrolysis. Consequently, drying is an added cost to be considered. Pyrolysis readiness for biomass does not need to be proved anymore. It has also successfully been used to produce fossil derived fuels. However, there is slow commercialisation of the technology because of the lack of an effective market demand regarding crude bio-oil.

15.4 Cracking, reforming and upgrading

Cracking allows large hydrocarbon molecules to be broken down into smaller alkane molecules which are more useful for fuel production. Fractions containing large hydrocarbon molecules are vaporised and passed over a hot catalyst to allow the breaking of hydrocarbon chains. In

this process temperature and catalyst play a very important role in making the breaking very effective. The effect of pressure during the process is not very significant compared to temperature and catalyst, however, it is still also needed during the process.

There are many types of cracking processes which can be used for algae biomass or any biomass, these processes including isomerisation cracking, hydrocracking, thermal cracking, fluid catalytic cracking and steam cracking. These processes depend on the type of biomass used or oil to be produced. Hydrocracking is one of the most used processes for biomass product, it is a two-stage process combining catalytic cracking and hydrogenation, where heavy feedstock is cracked in the presence of hydrogen to produce more desirable products. Generally cracking process uses moderate pressure, high temperature and a catalyst to achieve the breaking down of hydrocarbon chains. The pressure range is wide and depends on the application and the temperature range is generally between 200 and 500 ^{O}C depending on the type of oil or biomass and the type of the cracking process. However, it is important to stress on the fact that the temperature can go beyond this range depending on the cracking type process. The upgrading process is achieved via fluid catalytic cracking, during the process the heavier or higher-boiling fractions from the crude oil distillation are upgraded by converting them into lighter and lower boiling, more valuable products. Reforming is a catalytic process used to convert low octane distilled from crude oil into high-octane liquid products called reformates. Reforming is also known as aromatisation process, it allows the conversion of open chain hydrocarbons and/or cycloalkanes in the presence of a catalyst, into aromatic hydrocarbons containing the same number of carbon atoms. Aromatisation involves reactions such as dehydrogenation, cyclisation, and isomerisation. In reforming (or aromatisation), cyclic and acyclic alkanes with six to eight carbon atoms are exposed to a temperature of about 670 K in the presence of palladium, platinum or nickel as catalyst. Platinum seems to be the best catalyst and so the process is sometimes called platforming.

15.5 Deoxygenation and decarboxylation

Deoxygenation is a chemical reaction that involves the removal of oxygen from a molecule. The process uses hydrogen and metal catalysts for deoxygenation of C-O bonds which are the most found in algae crude bio-oil. Decarboxylation is a chemical reaction in which a carboxyl group is removed and carbon dioxide is released. Large amount of oxygen and CO_2 are removed from the bio-oil after decarboxylation, this reduces further the carbon footprint of bio-oil. Both are catalytic processes and are conducive with algae biomass however the operating costs are still high. Algae crude bio-oil contains high level of oxygen which can be disturbing

for conversion processes. They are also used for algae bio-oil that has been transesterified (biodiesel) in order to remove methyl esters and allow a suitable conversion of bio-oil into jet fuel.

15.6 Transesterification

Transesterification consists of producing biodiesel from bio-oil. Once biodiesel is produced decarboxylation and deoxygenation can be used to produce high purity jet fuel (Huntley and Redalje, 2006). Decarboxylation or deoxygenation of methyl esters increases energy density of jet fuel and allows the removal of methyl esters considered as impurities in jet fuel. Isomerization cracking is used afterward to breakdown large hydrocarbon chains into small alkanes. This will assist in decreasing the higher freezing point of cracked bio-oil to the one required for the jet fuel (Huntley and Redalje, 2006). Generally, biodiesel from algae oil has high flash point, high freezing point, high density, low kinematic viscosity and high oxygen content compared to the ASTM requirements for aviation fuels. It is technically feasible to combine transesterification, deoxygenation and decarboxylation to produce jet fuel from microalgae but the entire process is very expensive (Huntley and Redalje, 2006). This option has not yet reached the maturity to be commercially viable.

15.7 Fractional distillation

This process aims to separate light ends, middle ends and heavy ends carbon fractions based on their boiling points. Carbon fractions are separated by heating them to a temperature at which one or more fractions of the mixture will vaporize. This will be followed by the condensation of vapor phase to produce the required liquid fuel or substance

Jet fuel is made of middle ends carbon fractions (C14 to C16), for algae bio-oil there is temperature range at which it is possible to collect these fractions. The temperature range begins from 70 ^{O}C and the end point is around 250 ^{O}C at atmospheric pressure. This process is among the most important one during the process of producing any fuel. It plays an important role on the purity of the fuel. There is also a vacuum distillation which is used to further distil the residue bio-oil from the bottom of the crude oil distillation unit. It is performed at a pressure below atmospheric pressure. This operation is undertaken after upgrading process.

CONCLUSIONS

The potential to produce renewable energy from microalgae has reached a considerable interest at the present time, however, there is a need for further optimization of mass culture conditions and operating conditions for conversion processes to make microalgal bio-jet fuel economically viable and sustainable. Sewage wastewater can potentially provide cost-effective and sustainable means of algal growth for algae-based jet fuel in particular and other algae based-fuels in general. There is a potential in combining microalgae-based wastewater treatment and jet fuel production from the generated biomass after sewage treatment. It can happen with nutrient removal and jet fuel production in the same set up provided that operating conditions are well established and the design of the infrastructures is convenient to the type of application. This review has discussed various aspects related to potential benefits and limitations of using domestic wastewater as resource for cost-effective bio-jet fuel production.

Microalgae are known to grow faster in water and they contain essential compounds that can be utilized for bio-fuel production. Domestic wastewater being rich in nutrients needed by microalgae can be used as medium for growth while nutrients and contaminants are being absorbed by microalgae species. There many advantages to use microalgae for wastewater treatment and produce bio-jet fuel out of the biomass produced during treatment. This option can be viable but wastewater should be well protected from contamination created by harmful toxins and toxic substances that can prevent the operation of microalgae cells in reducing the pollution load from wastewater. Using microalgae for domestic wastewater treatment will reduce the costs related to oxygen production and maintenance. Microalgae is effective in removing nitrates, phosphates, heavy metals, COD and BOD. Also, microalgae strains are viewed as promising alternative because they contain extensive fatty acids and lipids. There is possibility to attain over 60% lipids after genetic modification achieved by nutrients starvation. Microalgae has ability to grow briskly and synthesize large amounts of TAGs and high-value polyunsaturated fatty acids such as eicosapentaenoic acid that can be converted in to the bio-jet fuel for the aviation industry. Jet-fuel has 14 to 16 atoms of carbon and triacylglycerol is recognised for high percentage of short chain fatty acids. This key feature of this species allows exploring the possibility of producing bio-jet fuels from microalgae. Production of bio-jet fuel from microalgae requires production system to grow microalgae. The various aspects related to microalgae cultivation in open systems like ponds and closed system like photobioreactors were highlighted. Open pond systems are less expensive and require less energy but the major drawbacks are less efficient temperature control, usage of light, relatively larger area and risk of contamination. Currently high rate algal ponds such as raceway ponds are used to remediate

to many weaknesses observed from open ponds. They have showed great reliability and promise for microalgae cultivation. Photobioreactors are convenient to handle compared to open pond systems for mixing and mass exchange of gas or liquid; however, a major challenge is the use of artificial lighting. In industrial-scale, volumes of culture medium to be prepared and dealt with are highly expensive, particularly when artificial seawater is used. Many environmental factors like light, temperature, pH, salinity and nutrients determine the biochemical content of cultivated microalgae. These factors have an effect on photosynthesis and thus carbon fixation. According to environmental conditions microalgae modify their lipid biosynthesis pathways in nutrient deprivation conditions. The cultivation of microalgae is followed by the separation biomass from the media and subsequent lipids extraction. Harvesting of microalgae involves the extraction of triglycerides by dewatering, drying, and then solvent extraction. There is no fundamental harvesting method in the current situation with perfect advantages of time, energy and cost. Some of the major technologies commonly used are centrifugation, sedimentation filtration, flocculation and flotation. Production of bio-jet fuel from microalgae requires cost effective methods for large scale cultivation and harvesting systems. It is clear that technological development is still needed for better harvesting techniques. Possible biotechnological developments in this procedure might find an appropriate and economical harvesting process. Efficient method for lipid extraction is another key factor for bio-jet fuel production. Transesterification is used in the conventional biodiesel production techniques, but now there are many options available for extraction of neutral lipids. Alternative techniques such as catalytic Megaspores, hydrothermal liquefaction and gasification of the biomass and pyrolysis have been postulated. Although the total bio-fuel production improves significantly when both polar and non-polar lipids are used for fuel production. Some studies regarding this strategy making use of total lipid content for production of bio-fuel by direct transesterification has demonstrated promising results. Bio-jet fuel production from *Nannochloropsis* could be more cost effective in the foreseeable future with aid of modern harvesting technologies and extraction techniques.

REFERENCES

Aslan, S. and Kapdan, I.K., 2006. Batch kinetics of nitrogen and phosphorus removal from synthetic wastewater by algae. Ecological engineering, 28(1), pp.64-70. https://doi.org/10.1016/j.ecoleng.2006.04.003

Aro, E.M., 2016. From first generation biofuels to advanced solar biofuels. Ambio, 45(1), pp.24-31.https://doi.org/10.1007/s13280-015-0730-0

Ayodhya D Kshirsagar, bioremediation of wastewater by using microalgae: an experimental study, International Journal of Environmental Research and Development., ISSN 2249-3131 Volume 2, Number 1 (2012), pp. 35-43 , Research India Publications.

Bauen A., Howes J., Bertuccioli, L., Chudziak, C.,(2009). Review of the potential for biofuels in aviation, Report, London:E4tech. https://www.theccc.org.uk/archive/aws2/Aviation (Accessed on 1st May 2018)

Biddy, M., Davis, R. and Jones, S., 2013. *Whole algae hydrothermal liquefaction technology pathway* (No. PNNL-22314; NREL/TP-5100-58051). National Renewable Energy Laboratory (NREL), Golden, CO.

Blakey, S., Novelli, P., Costes, P., Bringtown, S., Christensen, D., Sakintuna, B., Peineke, C., Jongschaap, R.E.E., Conijn, J.G., Rutgers, B. and Valot, L., 2010. State of the Art on Alternative Fuels in Aviation. SWAFEA. Sustainable Way for Alternative Fuels and Energy in Aviation. Onera.

Blakey S., Rye L., and Wilson, C W., Aviation gas turbine alternative fuels: A review, Proceedings of the Combustion Institute, 2011, 33, 2863-2885. https://doi.org/10.1016/j.proci.2010.09.011

Bligh, E.G. and Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. Canadian journal of biochemistry and physiology, 37(8), pp.911-917. https://doi.org/10.1139/o59-099

Boerrigter, H., Den Uil, H. and Calis, H.P., 2003. Green diesel from biomass via Fischer-Tropsch synthesis: new insights in gas cleaning and process design. *Pyrolysis and gasification of Biomass and waste*, pp.371-383.

Brennan, L. and Owende, P., 2010. Biofuels from microalgae—a review of technologies for production, processing, and extractions of biofuels and co-products. Renewable and sustainable energy reviews, 14(2), pp.557-577.https://doi.org/10.1016/j.rser.2009.10.009

Brown, T.M., Duan, P. and Savage, P.E., 2010. Hydrothermal liquefaction and gasification of Nannochloropsis sp. Energy & Fuels, 24(6), pp.3639-3646.https://doi.org/10.1021/ef100203u

Bwapwa, J.K., Jaiyeola, A.T. and Chetty, R., 2017. Bioremediation of acid mine drainage using algae strains: A review. South African Journal of Chemical Engineering, 24, pp.62-70. https://doi.org/10.1016/j.sajce.2017.06.005

Bwapwa, J.K., Anandraj, A. and Trois, C., 2017. Possibilities for conversion of microalgae oil into aviation fuel: A review. Renewable and Sustainable Energy Reviews, 80, pp.1345-1354. https://doi.org/10.1016/j.rser.2017.05.224

Canovas, S., Picot, B., Casellas, C., Zulkifi, H., Dubois, A. and Bontoux, J., 1996. Seasonal development of phytoplankton and zooplankton in a high-rate algal pond. Water Science and Technology, 33(7), pp.199-206.

Carere, C.R., Sparling, R., Cicek, N. and Levin, D.B., 2008. Third generation biofuels via direct cellulose fermentation. International journal of molecular sciences, 9(7), pp.1342-1360. https://doi.org/10.3390/ijms9071342

Carvalho, A.P., Meireles, L.A. and Malcata, F.X., 2006. Microalgal reactors: a review of enclosed system designs and performances. Biotechnology progress, 22(6), pp.1490-1506. https://doi.org/10.1002/bp060065r

Cartens, M., Moina Grima, E., Robels Medina, A., Gimenez Gimenez, A., Ibanez Gonzalez, J., 1996. Eicosapentaenoic acid (20: 5n–3) from the marine microalgae Phaeodactylum tricornutum. J. Am. Oil Chem. Soc. 73, 1025–1031 https://doi.org/10.1007/BF02523411

Cheah, W.Y., Ling, T.C., Show, P.L., Juan, J.C., Chang, J.S. and Lee, D.J., 2016. Cultivation in wastewaters for energy: A microalgae platform. Applied energy, 179, pp.609-625. https://doi.org/10.1016/j.apenergy.2016.07.015

Chen, C.Y., Yeh, K.L., Aisyah, R., Lee, D.J. and Chang, J.S., 2011. Cultivation, photobioreactor design and harvesting of microalgae for biodiesel production: a critical review. Bioresource technology, 102(1), pp.71-81.https://doi.org/10.1016/j.biortech.2010.06.159

Cheng-Wu, Z., Zmora, O., Kopel, R. and Richmond, A., 2001. An industrial-size flat plate glass reactor for mass production of Nannochloropsis sp.(Eustigmatophyceae). Aquaculture, 195(1-2), pp.35-49. https://doi.org/10.1016/S0044-8486(00)00533-0

Chinnasamy, S., Bhatnagar, A., Hunt, R.W. and Das, K.C., 2010. Microalgae cultivation in a wastewater dominated by carpet mill effluents for biofuel applications. Bioresource technology, 101(9), pp.3097-3105.https://doi.org/10.1016/j.biortech.2009.12.026

Chisti, Y., 2006. MICROALGAE AS SUSTAINABLE CELL FACTORIES. Environmental Engineering & Management Journal (EEMJ), 5(3).

Chisti, Y., 2007. Biodiesel from microalgae. Biotechnology advances, 25(3), pp.294-306. https://doi.org/10.1016/j.biotechadv.2007.02.001

Damartzis, T. and Zabaniotou, A., 2011. Thermochemical conversion of biomass to second generation biofuels through integrated process design—A review. Renewable and Sustainable Energy Reviews, 15(1), pp.366-378. https://doi.org/10.1016/j.rser.2010.08.003

Danquah, M.K., Ang, L., Uduman, N., Moheimani, N. and Forde, G.M., 2009. Dewatering of microalgal culture for biodiesel production: exploring polymer flocculation and tangential flow filtration. Journal of Chemical Technology and Biotechnology, 84(7), pp.1078-1083. https://doi.org/10.1002/jctb.2137

Demirbas MF. Biorefineries for biofuel upgrading: a critical review. Applied Energy. 2009 Nov 1;86:S151-61.https://doi.org/10.1016/j.apenergy.2009.04.043

Dutta, K., Daverey, A. and Lin, J.G., 2014. Evolution retrospective for alternative fuels: First to fourth generation. Renewable energy, 69, pp.114-122. https://doi.org/10.1016/j.renene.2014.02.044

Dragone, G., Fernandes, B.D., Vicente, A.A. and Teixeira, J.A., 2010. Third generation biofuels from microalgae. Current research, technology and education topics in applied microbiology and microbial biotechnology, 2, pp.1355-1366.

Eloka-Eboka, A.C. and Inambao, F.L., 2017. Effects of CO2 sequestration on lipid and biomass productivity in microalgal biomass production. Applied Energy, 195, pp.1100-1111. https://doi.org/10.1016/j.apenergy.2017.03.071

Energy Information Administration (EIA), International Energy Outlook, 284, 2009, EIA; DC, USA Fajardo, A.R., Cerdan, L.E., Medina, A.R., Fernandez, F.G.A., Moreno, P.A.G. and Grima, E.M., 2007. Lipid extraction from the microalga Phaeodactylum tricornutum. European Journal of Lipid Science and Technology, 109(2), pp.120-126.

Georgianna, D.R. and Mayfield, S.P., 2012. Exploiting diversity and synthetic biology for the production of algal biofuels. Nature, 488(7411), p.329. https://doi.org/10.1038/nature11479

Gerbens-Leenes, W., Hoekstra, A.Y. and van der Meer, T.H., 2009. The water footprint of bioenergy. Proceedings of the National Academy of Sciences, 106(25), pp.10219-10223. https://doi.org/10.1073/pnas.0812619106

Ginzburg, B.Z., 1993. Liquid fuel (oil) from halophilic algae: a renewable source of non-polluting energy. Renewable energy, 3(2-3), pp.249-252. https://doi.org/10.1016/0960-1481(93)90031-B

Gize, A.P. and Manning, D.A., 1993. Aspects of the organic geochemistry and petrology of metalliferous ores. In Organic Geochemistry (pp. 565-580). Springer, Boston, MA. https://doi.org/10.1007/978-1-4615-2890-6_26

González, L.E., Ca-izares, R.O. and Baena, S., 1997. Efficiency of ammonia and phosphorus removal from a Colombian agroindustrial wastewater by the microalgae Chlorella vulgaris and Scenedesmus dimorphus. Bioresource Technology, 60(3), pp.259-262. https://doi.org/10.1016/S0960-8524(97)00029-1

Grima, E.M., Belarbi, E.H., Fernández, F.A., Medina, A.R. and Chisti, Y., 2003. Recovery of microalgal biomass and metabolites: process options and economics. Biotechnology advances, 20(7-8), pp.491-515.https://doi.org/10.1016/S0734-9750(02)00050-2

Hannon M., Gimpel J., Tran M., Rasala B., Mayfield S., 2010 . Biofuels from algae: challenges and potential, Biofuels, 1(5): 763–784.https://doi.org/10.4155/bfs.10.44

Hara, A. and Radin, N.S., 1978. Lipid extraction of tissues with a low-toxicity solvent. Analytical biochemistry, 90(1), pp.420-426.https://doi.org/10.1016/0003-2697(78)90046-5

Hoffman, Y., C. Aflalo, A. Zarka, J. Gutman, T. Y. James and S. Boussiba. 2008. Isolation and characterization of a novel chytrid species (phylum Blastocladiomycota), parasitic on the green alga Haermatococcus. Mycological Research. 112, 70-81. https://doi.org/10.1016/j.mycres.2007.09.002

Huang, G., Chen, F., Wei, D., Zhang, X. and Chen, G., 2010. Biodiesel production by microalgal biotechnology. Applied energy, 87(1), pp.38-46. https://doi.org/10.1016/j.apenergy.2009.06.016

Hunt, J.M., 1996. Petroleum geochemistry and geology (Vol. 2, pp. 1-743). New York: WH Freeman.

Huntley ME., Redalje DG., (2006) CO_2 mitigation and renewable oil from photosynthetic microbes: a new appraisal. *Mitigation Adaptation Strategies Global Change*, 12, 573–608.

Kanda, H., Li, P., Ikehara, T. and Yasumoto-Hirose, M., 2012. Lipids extracted from several species of natural blue–green microalgae by dimethyl ether: Extraction yield and properties. Fuel, 95, pp.88-92. https://doi.org/10.1016/j.fuel.2011.11.064

Khan, S.A., Hussain, M.Z., Prasad, S. and Banerjee, U.C., 2009. Prospects of biodiesel production from microalgae in India. Renewable and Sustainable Energy Reviews, 13(9), pp.2361-2372. https://doi.org/10.1016/j.rser.2009.04.005

Konig, A., Pearson, H.W. and Silva, S.A., 1987. Ammonia toxicity to algal growth in waste stabilization ponds. Water Science and Technology, 19(12), pp.115-122.

Larkum, A.W., Ross, I.L., Kruse, O. and Hankamer, B., 2012. Selection, breeding and engineering of microalgae for bioenergy and biofuel production. Trends in biotechnology, 30(4), pp.198-205. https://doi.org/10.1016/j.tibtech.2011.11.003

Lau, P.S., Tam, N.F.Y. and Wong, Y.S., 1995. Effect of algal density on nutrient removal from primary settled wastewater. Environmental Pollution, 89(1), pp.59-66. https://doi.org/10.1016/0269-7491(94)00044-E

Lee, R.A. and Lavoie, J.M., 2013. From first-to third-generation biofuels: Challenges of producing a commodity from a biomass of increasing complexity. Animal Frontiers, 3(2), pp.6-11.https://doi.org/10.2527/af.2013-0010

Mata, T.M., Martins, A.A. and Caetano, N.S., 2010. Microalgae for biodiesel production and other applications: a review. Renewable and sustainable energy reviews, 14(1), pp.217-232. https://doi.org/10.1016/j.rser.2009.07.020

Martınez, M.E., Sánchez, S., Jimenez, J.M., El Yousfi, F. and Munoz, L., 2000. Nitrogen and phosphorus removal from urban wastewater by the microalga Scenedesmus obliquus. Bioresource technology, 73(3), pp.263-272. https://doi.org/10.1016/S0960-8524(99)00121-2

Masseret, E., Amblard, C., Bourdier, G. and Sargos, D., 2000. Effects of a waste stabilization lagoon discharge on bacterial and phytoplanktonic communities of a stream. Water Environment Research, 72(3), pp.285-294. https://doi.org/10.2175/106143000X137509

Moheimani, N.R., 2005. The culture of coccolithophorid algae for carbon dioxide bioremediation (Doctoral dissertation, Murdoch University).

Mohr, A. and Raman, S., 2013. Lessons from first generation biofuels and implications for the sustainability appraisal of second generation biofuels. Energy policy, 63, pp.114-122. https://doi.org/10.1016/j.enpol.2013.08.033

Mohn, F.H., 1988. Harvesting of micro-algal biomass. Micro-algal biotechnology, pp.395-414.

Nagle, N. and Lemke, P., 1990. Production of methyl ester fuel from microalgae. Applied Biochemistry and Biotechnology, 24(1), pp.355-361. https://doi.org/10.1007/BF02920259

Naik, S.N., Goud, V.V., Rout, P.K. and Dalai, A.K., 2010. Production of first and second generation biofuels: a comprehensive review. Renewable and sustainable energy reviews, 14(2), pp.578-597.https://doi.org/10.1016/j.rser.2009.10.003

Naqvi, M. and Yan, J., 2015. First-Generation Biofuels. John Wiley & Sons, Ltd https://doi.org/10.1002/9781118991978.hces207

Nygren, E., Aleklett, K. and Höök, M., 2009. Aviation fuel and future oil production scenarios. Energy Policy, 37(10), pp.4003-4010. https://doi.org/10.1016/j.enpol.2009.04.048

Patil, V., Tran, K.Q. and Giselrød, H.R., 2008. Towards sustainable production of biofuels from microalgae. International journal of molecular sciences, 9(7), pp.1188-1195. https://doi.org/10.3390/ijms9071188

Park, P.K., Kima, E.Y., Chub, K.H., 2007. Chemical disruptions of yeast cells for the isolation of carotenoid pigments. Sep. Purif. Technol. 53, 148–152. https://doi.org/10.1016/j.seppur.2006.06.026

Park, J.B.K., Craggs, R.J. and Shilton, A.N., 2011. Wastewater treatment high rate algal ponds for biofuel production. Bioresource technology, 102(1), pp.35-42. https://doi.org/10.1016/j.biortech.2010.06.158

Posten, C. and Schaub, G., 2009. Microalgae and terrestrial biomass as source for fuels—a process view. Journal of biotechnology, 142(1), pp.64-69. https://doi.org/10.1016/j.jbiotec.2009.03.015

Powell, N., Shilton, A., Chisti, Y. and Pratt, S., 2009. Towards a luxury uptake process via microalgae–defining the polyphosphate dynamics. Water research, 43(17), pp.4207-4213. https://doi.org/10.1016/j.watres.2009.06.011

Pittman, J.K., Dean, A.P. and Osundeko, O., 2011. The potential of sustainable algal biofuel production using wastewater resources. Bioresource technology, 102(1), pp.17-25. https://doi.org/10.1016/j.biortech.2010.06.035

Pulz, O., 2001. Photobioreactors: production systems for phototrophic microorganisms. Applied microbiology and biotechnology, 57(3), pp.287-293. https://doi.org/10.1007/s002530100702

Ratledge, C. and Cohen, Z., 2008. Microbial and algal oils: do they have a future for biodiesel or as commodity oils?. Lipid Technology, 20(7), pp.155-160. https://doi.org/10.1002/lite.200800044

Rawat, I., Kumar, R.R., Mutanda, T. and Bux, F., 2011. Dual role of microalgae: phycoremediation of domestic wastewater and biomass production for sustainable biofuels production. Applied Energy, 88(10), pp.3411-3424. https://doi.org/10.1016/j.apenergy.2010.11.025

Reijnders, L. and Huijbregts, M., 2009. Transport biofuels: their characteristics, production and costs. Biofuels for Road Transport: A Seed to Wheel Perspective, pp.1-48.

Richmond, A., Handbook of Microalgae Culture: Biotechnology and Applied Phycology, Blackwell Publishing, 2004, Oxford, 566

Rosenfeld, J., Remes, J., Mendonca, L., Hu, W., Palani, S., Sethi, U., Nyquist, S., Bozon, I., Roelofsen, O., Haas, P. and Vermeltfoort, K., 2009. Averting the next energy crisis: The demand challenge. McKinsey Group.

Rosso, D., Larson, L.E. and Stenstrom, M.K., 2008. Aeration of large-scale municipal wastewater treatment plants: state of the art. *Water Science and Technology*, *57*(7), pp.973-978. DOI:10.2166/wst.2008.218

Ruiz-Marin, A., Mendoza-Espinosa, L.G. and Stephenson, T., 2010. Growth and nutrient removal in free and immobilized green algae in batch and semi-continuous cultures treating real wastewater. Bioresource Technology, 101(1), pp.58-64. https://doi.org/10.1016/j.biortech.2009.02.076

Ruiz-Martinez, A., Garcia, N.M., Romero, I., Seco, A. and Ferrer, J., 2012. Microalgae cultivation in wastewater: nutrient removal from anaerobic membrane bioreactor effluent. *Bioresource technology*, *126*, pp.247-253.

Schenk, P.M., Thomas-Hall, S.R., Stephens, E., Marx, U.C., Mussgnug, J.H., Posten, C., Kruse, O. and Hankamer, B., 2008. Second generation biofuels: high-efficiency microalgae for biodiesel production. Bioenergy research, 1(1), pp.20-43. https://doi.org/10.1007/s12155-008-9008-8

Scott, S.A., Davey, M.P., Dennis, J.S., Horst, I., Howe, C.J., Lea-Smith, D.J. and Smith, A.G., 2010. Biodiesel from algae: challenges and prospects. Current opinion in biotechnology, 21(3), pp.277-286.https://doi.org/10.1016/j.copbio.2010.03.005

Schenk, P.M., Thomas-Hall, S.R., Stephens, E., Marx, U.C., Mussgnug, J.H., Posten, C., Kruse, O. and Hankamer, B., 2008. Second generation biofuels: high-efficiency microalgae for biodiesel production. Bioenergy research, 1(1), pp.20-43.https://doi.org/10.1007/s12155-008-9008-8

Shen, Y., Yuan, W., Pei, Z.J., Wu, Q. and Mao, E., 2009. Microalgae mass production methods. Transactions of the ASABE, 52(4), pp.1275-1287.https://doi.org/10.13031/2013.27771

Shi, J., Podola, B. and Melkonian, M., 2007. Removal of nitrogen and phosphorus from wastewater using microalgae immobilized on twin layers: an experimental study. Journal of Applied Phycology, 19(5), pp.417-423. https://doi.org/10.1007/s10811-006-9148-1

Sierra, E., Acién, F.G., Fernández, J.M., García, J.L., González, C. and Molina, E., 2008. Characterization of a flat plate photobioreactor for the production of microalgae. Chemical Engineering Journal, 138(1-3), pp.136-147. https://doi.org/10.1016/j.cej.2007.06.004

Sydney, E.D., Da Silva, T.E., Tokarski, A., Novak, A.D., De Carvalho, J.C., Woiciecohwski, A.L., Larroche, C. and Soccol, C.R., 2011. Screening of microalgae with potential for biodiesel production and nutrient removal from treated domestic sewage. *Applied Energy*, *88*(10), pp.3291-32

Tabatabaei, M., Karimi, K., Sárvári Horváth, I. and Kumar, R., 2015. Recent trends in biodiesel production. Biofuel Research Journal, 2(3), pp.258-267. https://doi.org/10.18331/BRJ2015.2.3.4

Tam, N.F.Y. and Wong, Y.S., 1989. Wastewater nutrient removal by Chlorella pyrenoidosa and Scenedesmus sp. *Environmental Pollution*, *58*(1), pp.19-34.

Thomas, D.G., Minj, N., Mohan, N. and Rao, P.H., 2016. Cultivation of microalgae in domestic wastewater for biofuel applications-An upstream approach. J. Algal Biomass Utln, 7(1), pp.62-70.

U.S. DOE 2010. National Algal Biofuels Technology Roadmap. U.S. Department of Energy, Office of Energy Efficiency and Renewable Energy, Biomass Program, Workshop report.

van Steen, E. and Claeys, M., 2008. Fischer-Tropsch Catalysts for the Biomass-to-Liquid (BTL)-Process. *Chemical engineering & technology*, *31*(5), pp.655-666.

Venderbosch, R.H. and Prins, W., 2010. Fast pyrolysis technology development. *Biofuels, bioproducts and biorefining*, *4*(2), pp.178-208.

Wang, L., Min, M., Li, Y., Chen, P., Chen, Y., Liu, Y., Wang, Y. and Ruan, R., 2010. Cultivation of green algae Chlorella sp. in different wastewaters from municipal wastewater treatment plant. Applied biochemistry and biotechnology, 162(4), pp.1174-1186. https://doi.org/10.1007/s12010-009-8866-7

Wickrama, U., Colloquium on Environmental Aspects of Aviation, ICAO FESG, Montreal

Widjaja, A., Chien, C.C. and Ju, Y.H., 2009. Study of increasing lipid production from fresh water microalgae Chlorella vulgaris. Journal of the Taiwan Institute of Chemical Engineers, 40(1), pp.13-20. https://doi.org/10.1016/j.jtice.2008.07.007

Wilhelm, C. and Jakob, T., 2011. From photons to biomass and biofuels: evaluation of different strategies for the improvement of algal biotechnology based on comparative energy balances. Applied microbiology and biotechnology, 92(5), pp.909-919. https://doi.org/10.1007/s00253-011-3627-2

Wilson, W. H., J. L. Van Etten and M. J. Allen. (2009). The Phycodnaviridae: The story of how tiny giants rule the world. Lesser Known Large dsDNA Viruses. Berlin, Springer-Verlag Berlin. 328: 1-42.

Wrigley, T.J. and Toerien, D.F., 1990. Limnological aspects of small sewage ponds. Water Research, 24(1), pp.83-90.https://doi.org/10.1016/0043-1354(90)90068-H

Zhang, E., Wang, B., Wang, Q., Zhang, S. and Zhao, B., 2008. Ammonia–nitrogen and orthophosphate removal by immobilized Scenedesmus sp. isolated from municipal wastewater for potential use in tertiary treatment. Bioresource technology, 99(9), pp.3787-3793.https://doi.org/10.1016/j.biortech.2007.07.011

Zhang, T.Y., Wu, Y.H. and Hu, H.Y., 2014. Domestic wastewater treatment and biofuel production by using microalga Scenedesmus sp. ZTY1. *Water Science and Technology*, *69*(12), pp.2492-2496.

APPENDIX 2

CHROMATOGRAPHS FOR VEGETABLE OILS USED DURING TRIALS FOR THERMAL CRACKING

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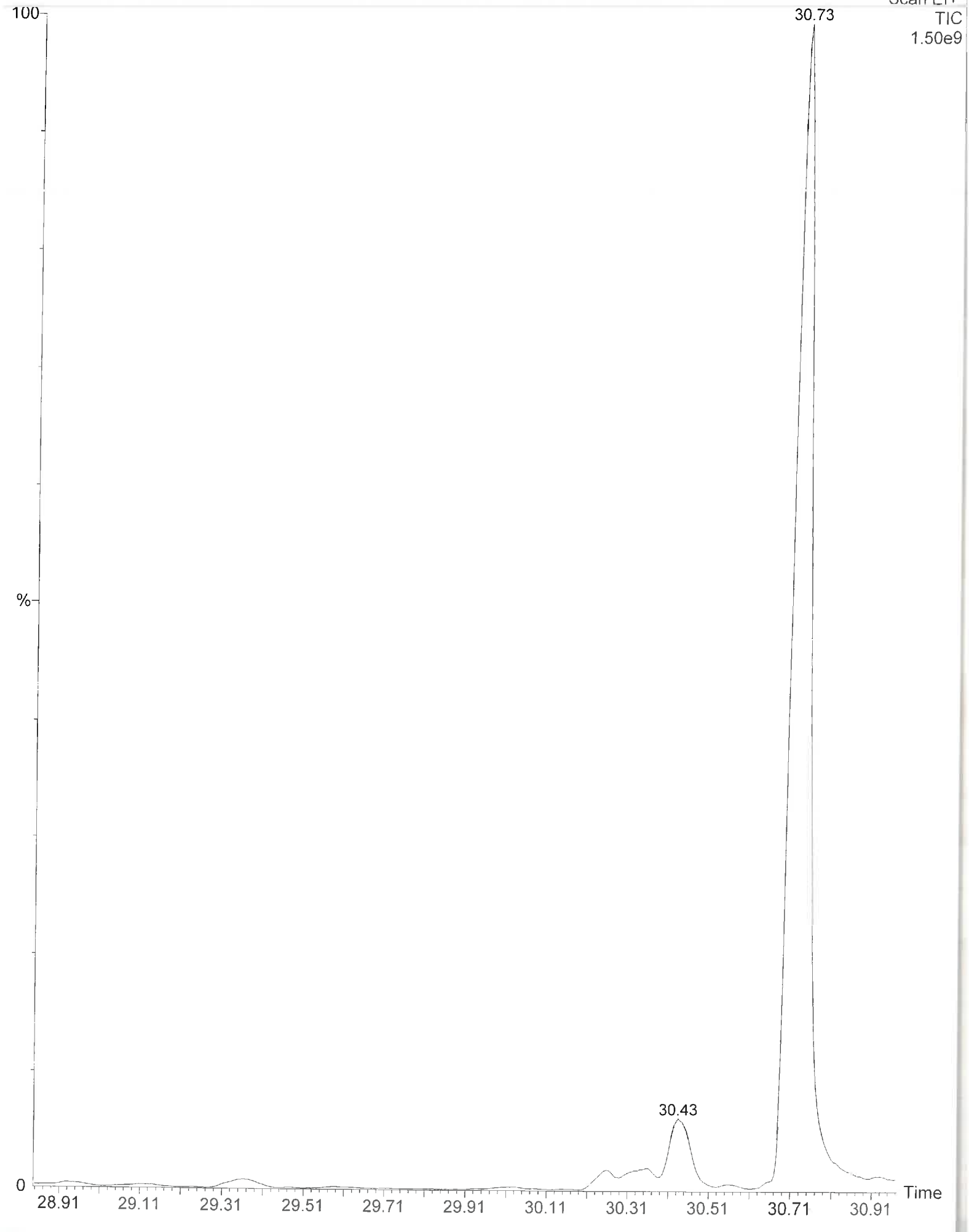
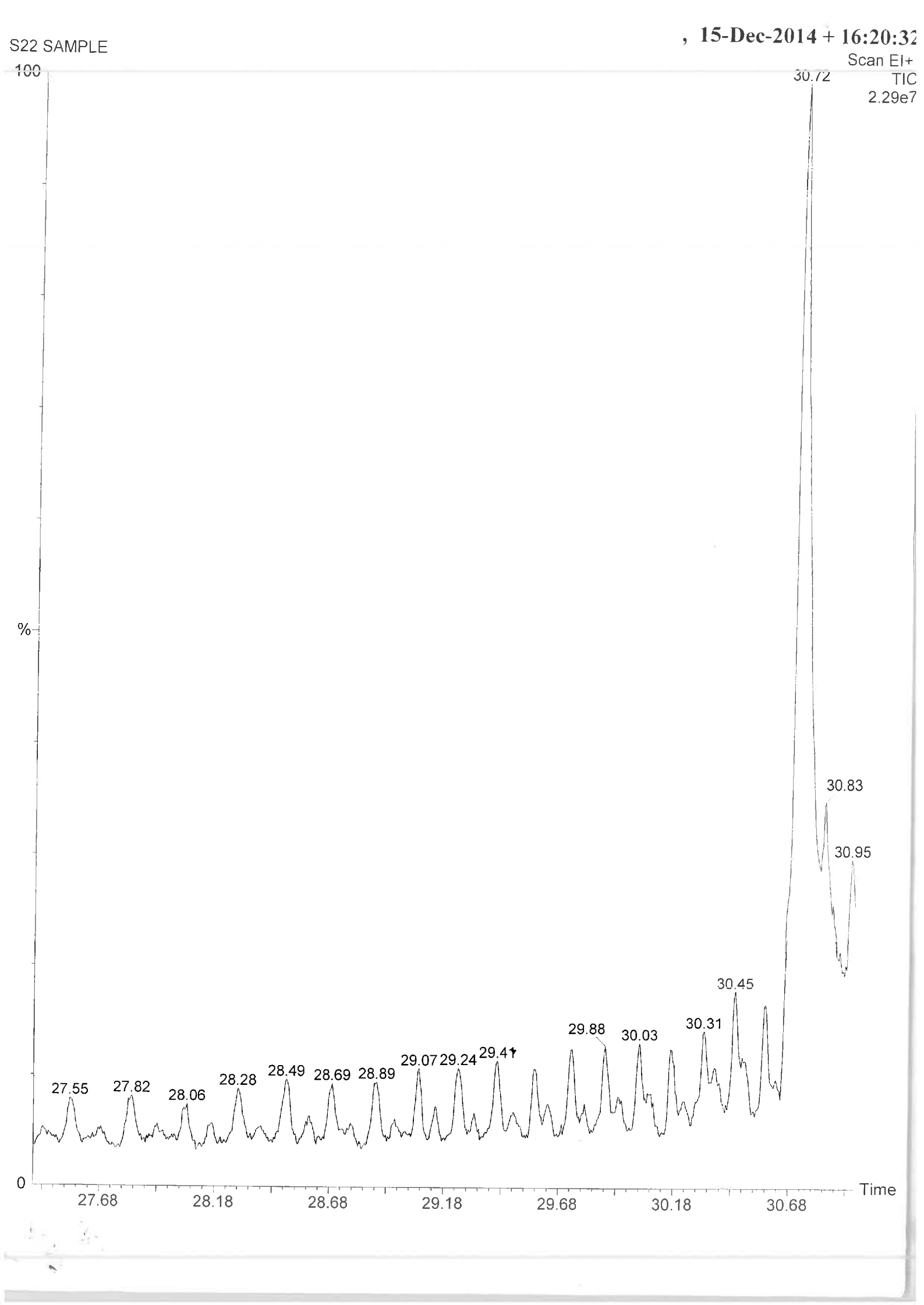


Hit	REV	for	Compound Name	M.W.	Formula	CAS
1	941	894	METHYL 11-HEXADECENOATE	268	C17H32O2	900336-35-
2	922	877	14-METHYLPENTADEC-9-ENOIC ACID METHYL ESTER	268	C17H32O2	900365-89-
3	903	860	9-HEXADECENOIC ACID, METHYL ESTER, (Z)-	268	C17H32O2	1120-25-8
4	899	837	METHYL HEXADEC-9-ENOATE	268	C17H32O2	10030-74-7
5	892	811	METHYL 9-HEPTADECENOATE OR 9-17:1	282	C18H34O2	900336-38-
6	888	865	9-HEXADECENOIC ACID, METHYL ESTER, (Z)-	268	C17H32O2	1120-25-8
7	882	776	13-METHYLTETRADEC-9-ENOIC ACID METHYL ESTER	254	C16H30O2	900365-89-
8	874	784	13-OCTADECENOIC ACID, METHYL ESTER	296	C19H36O2	56554-47-3
9	873	793	METHYL 11-DOCOSENOATE	352	C23H44O2	900336-23-
10	871	783	METHYL 9-EICOSENOATE	324	C21H40O2	900336-50-
11	864	820	CIS-10-NONADECENOIC ACID, METHYL ESTER	310	C20H38O2	900333-64-
12	863	753	METHYL 12-HYDROXY-9-OCTADECENOATE	312	C19H36O3	900336-28-
13	862	764	METHYL 13-EICOSENOATE	324	C21H40O2	900336-48-
14	856	763	11-HEXADECENOIC ACID, METHYL ESTER	268	C17H32O2	55000-42-5
15	853	758	11-EICOSENOIC ACID, METHYL ESTER	324	C21H40O2	3946-08-5
16	853	715	METHYL 18-FLUORO-OCTADEC-9-ENOATE	314	C19H35O2F	900336-48-
17	853	721	METHYL 9-TETRADECENOATE	240	C15H28O2	900336-47-
18	853	750	METHYL 8-HEPTADECENOATE	282	C18H34O2	900336-36-
19	845	769	11-OCTADECENOIC ACID, METHYL ESTER	296	C19H36O2	52380-33-3
20	841	756	CIS-10-HEPTADECENOIC ACID, METHYL ESTER	282	C18H34O2	900333-62-

Hit	REV	for	Compound Name	M.W.	Formula	CAS
1	907	669	TETRADECANOIC ACID, 10,13-DIMETHYL-, METHYL ESTER	270	C17H34O2	267650-23-
2	898	796	TRIDECANOIC ACID, 12-METHYL-, METHYL ESTER	242	C15H30O2	5129-58-8
3	895	788	METHYL 11-METHYL-DODECANOATE	228	C14H28O2	900336-45-
4	884	785	UNDECANOIC ACID, 10-METHYL-, METHYL ESTER	214	C13H26O2	5129-56-6
5	880	741	METHYL 8-METHYL-NONANOATE	186	C11H22O2	900336-43-
6	873	819	METHYL 13-METHYLTETRADECANOATE	256	C16H32O2	900336-31-
7	857	801	HEXADECANOIC ACID, 15-METHYL-, METHYL ESTER	284	C18H36O2	6929-04-0
8	856	725	METHYL 18-FLUORO-OCTADECANOATE	316	C19H37O2F	900336-47-
9	854	836	HEXADECANOIC ACID, METHYL ESTER	270	C17H34O2	112-39-0
10	848	766	TRIDECANOIC ACID, METHYL ESTER	228	C14H28O2	1731-88-0
11	841	746	DODECANOIC ACID, 10-METHYL-, METHYL ESTER	228	C14H28O2	5129-65-7
12	829	741	TRIDECANOIC ACID, METHYL ESTER	228	C14H28O2	1731-88-0
13	824	774	PENTADECANOIC ACID, METHYL ESTER	256	C16H32O2	7132-64-1
14	821	764	HEPTADECANOIC ACID, 16-METHYL-, METHYL ESTER	298	C19H38O2	5129-61-3
15	819	703	METHYL 8-METHYL-DECANOATE	200	C12H24O2	900336-49-
16	815	720	DODECANOIC ACID, METHYL ESTER	214	C13H26O2	111-82-0
17	815	626	HEPTACOSANOIC ACID, 25-METHYL-, METHYL ESTER	438	C29H58O2	900112-14-
18	814	757	PENTADECANOIC ACID, METHYL ESTER	256	C16H32O2	7132-64-1
19	812	792	HEXADECANOIC ACID, METHYL ESTER	270	C17H34O2	112-39-0
20	812	731	METHYL TETRADECANOATE	242	C15H30O2	124-10-7


S22 SAMPLE
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Hit	REV	for	Compound Name	M.W.	Formula	CAS
1	791	600	13-OCTADECENOIC ACID, METHYL ESTER	296	C19H36O2	56554-47-3
2	770	579	13-METHYLTETRADEC-9-ENOIC ACID METHYL ESTER	254	C16H30O2	900365-89-
3	757	561	14-METHYLPENTADEC-9-ENOIC ACID METHYL ESTER	268	C17H32O2	900365-89-
4	746	553	METHYL 16-HYDROXY-HEXADECANOATE	286	C17H34O3	900336-41-
5	736	558	9-OCTADECENOIC ACID, METHYL ESTER, (E)-	296	C19H36O2	1937-62-8
6	736	550	METHYL 13-EICOSENOATE	324	C21H40O2	900336-48-
7	735	558	METHYL 9-TETRADECENOATE	240	C15H28O2	900336-47-
8	735	534	METHYL 11-HEXADECENOATE	268	C17H32O2	900336-35-
9	732	547	11-EICOSENOIC ACID, METHYL ESTER	324	C21H40O2	3946-08-5
10	730	529	CIS-13-OCTADECENOIC ACID, METHYL ESTER	296	C19H36O2	900333-58-
11	729	552	11-OCTADECENOIC ACID, METHYL ESTER	296	C19H36O2	52380-33-3
12	727	551	TRANS-13-OCTADECENOIC ACID, METHYL ESTER	296	C19H36O2	900333-61-
13	724	525	9-OCTADECENOIC ACID (Z)-, METHYL ESTER	296	C19H36O2	112-62-9
14	723	548	9-OCTADECENOIC ACID, METHYL ESTER, (E)-	296	C19H36O2	1937-62-8
15	721	521	METHYL 19-HEXACOSENOATE	408	C27H52O2	900336-27-
16	710	518	METHYL 17-HEXACOSENOATE	408	C27H52O2	900336-27-
17	709	527	METHYL 16-PENTACOSENOATE	394	C26H50O2	900336-24-
18	699	498	9-OCTADECENOIC ACID, METHYL ESTER, (E)-	296	C19H36O2	1937-62-8
19	697	459	DOCOSANEDIOIC ACID, DIMETHYL ESTER	398	C24H46O4	22399-98-0
20	697	521	CIS-11-EICOSENOIC ACID, METHYL ESTER	324	C21H40O2	900333-63-

Hit	REV	for	Compound Name	M.W.	Formula	CAS
1	768	608	13-OCTADECENOIC ACID, METHYL ESTER	296	C19H36O2	56554-47-3
2	749	595	METHYL 9-TETRADECENOATE	240	C15H28O2	900336-47-
3	743	582	METHYL 9-HEPTADECENOATE OR 9-17:1	282	C18H34O2	900336-38-
4	742	586	13-METHYLTETRADEC-9-ENOIC ACID METHYL ESTER	254	C16H30O2	900365-89-
5	738	592	METHYL 12-HYDROXY-9-OCTADECENOATE	312	C19H36O3	900336-28-
6	731	577	METHYL 8-HEPTADECENOATE	282	C18H34O2	900336-36-
7	724	590	METHYL MYRISTOLEATE	240	C15H28O2	56219-06-8
8	724	561	CIS-13-OCTADECENOIC ACID, METHYL ESTER	296	C19H36O2	900333-58-
9	723	556	14-METHYLPENTADEC-9-ENOIC ACID METHYL ESTER	268	C17H32O2	900365-89-
10	722	579	11-OCTADECENOIC ACID, METHYL ESTER	296	C19H36O2	52380-33-3
11	719	538	METHYL 18-FLUORO-OCTADEC-9-ENOATE	314	C19H35O2F	900336-48-
12	718	561	METHYL 13-EICOSENOATE	324	C21H40O2	900336-48-
13	717	536	METHYL 11-HEXADECENOATE	268	C17H32O2	900336-35-
14	716	559	METHYL 9-EICOSENOATE	324	C21H40O2	900336-50-
15	715	574	TRANS-13-OCTADECENOIC ACID, METHYL ESTER	296	C19H36O2	900333-61-
16	714	555	METHYL 11-DOCOSENOATE	352	C23H44O2	900336-23-
17	713	543	9-OCTADECENOIC ACID (Z)-, METHYL ESTER	296	C19H36O2	112-62-9
18	713	559	9-OCTADECENOIC ACID, METHYL ESTER, (E)-	296	C19H36O2	1937-62-8
19	711	571	9-OCTADECENOIC ACID, METHYL ESTER, (E)-	296	C19H36O2	1937-62-8
20	710	564	CIS-10-HEPTADECENOIC ACID, METHYL ESTER	282	C18H34O2	900333-62-

Hit	REV	for	Compound Name	M.W.	Formula	CAS
1	809	680	13-OCTADECENOIC ACID, METHYL ESTER	296	C19H36O2	56554-47-3
2	770	639	9-OCTADECENOIC ACID (Z)-, METHYL ESTER	296	C19H36O2	112-62-9
3	770	632	METHYL 9-HEPTADECENOATE OR 9-17:1	282	C18H34O2	900336-38-
4	770	625	METHYL 8-HEPTADECENOATE	282	C18H34O2	900336-36-
5	765	624	CIS-13-OCTADECENOIC ACID, METHYL ESTER	296	C19H36O2	900333-58-
6	762	591	METHYL 18-FLUORO-OCTADEC-9-ENOATE	314	C19H35O2F	900336-48-
7	761	629	9-OCTADECENOIC ACID, METHYL ESTER, (E)-	296	C19H36O2	1937-62-8
8	750	598	11-EICOSENOIC ACID, METHYL ESTER	324	C21H40O2	3946-08-5
9	746	573	METHYL 11-HEXADECENOATE	268	C17H32O2	900336-35-
10	745	608	11-OCTADECENOIC ACID, METHYL ESTER	296	C19H36O2	52380-33-3
11	739	578	METHYL HEXADEC-9-ENOATE	268	C17H32O2	10030-74-7
12	736	577	METHYL 13-EICOSENOATE	324	C21H40O2	900336-48-
13	736	601	TRANS-13-OCTADECENOIC ACID, METHYL ESTER	296	C19H36O2	900333-61-
14	732	582	14-METHYLPENTADEC-9-ENOIC ACID METHYL ESTER	268	C17H32O2	900365-89-
15	730	582	CIS-10-HEPTADECENOIC ACID, METHYL ESTER	282	C18H34O2	900333-62-
16	729	576	14-METHYLHEXADEC-9-ENOIC ACID, METHYL ESTER	282	C18H34O2	900370-42-
17	727	569	METHYL 9-EICOSENOATE	324	C21H40O2	900336-50-
18	725	566	METHYL 11-DOCOSENOATE	352	C23H44O2	900336-23-
19	724	572	9-OCTADECENOIC ACID, METHYL ESTER, (E)-	296	C19H36O2	1937-62-8
20	723	566	9-HEXADECENOIC ACID, METHYL ESTER, (Z)-	268	C17H32O2	1120-25-8

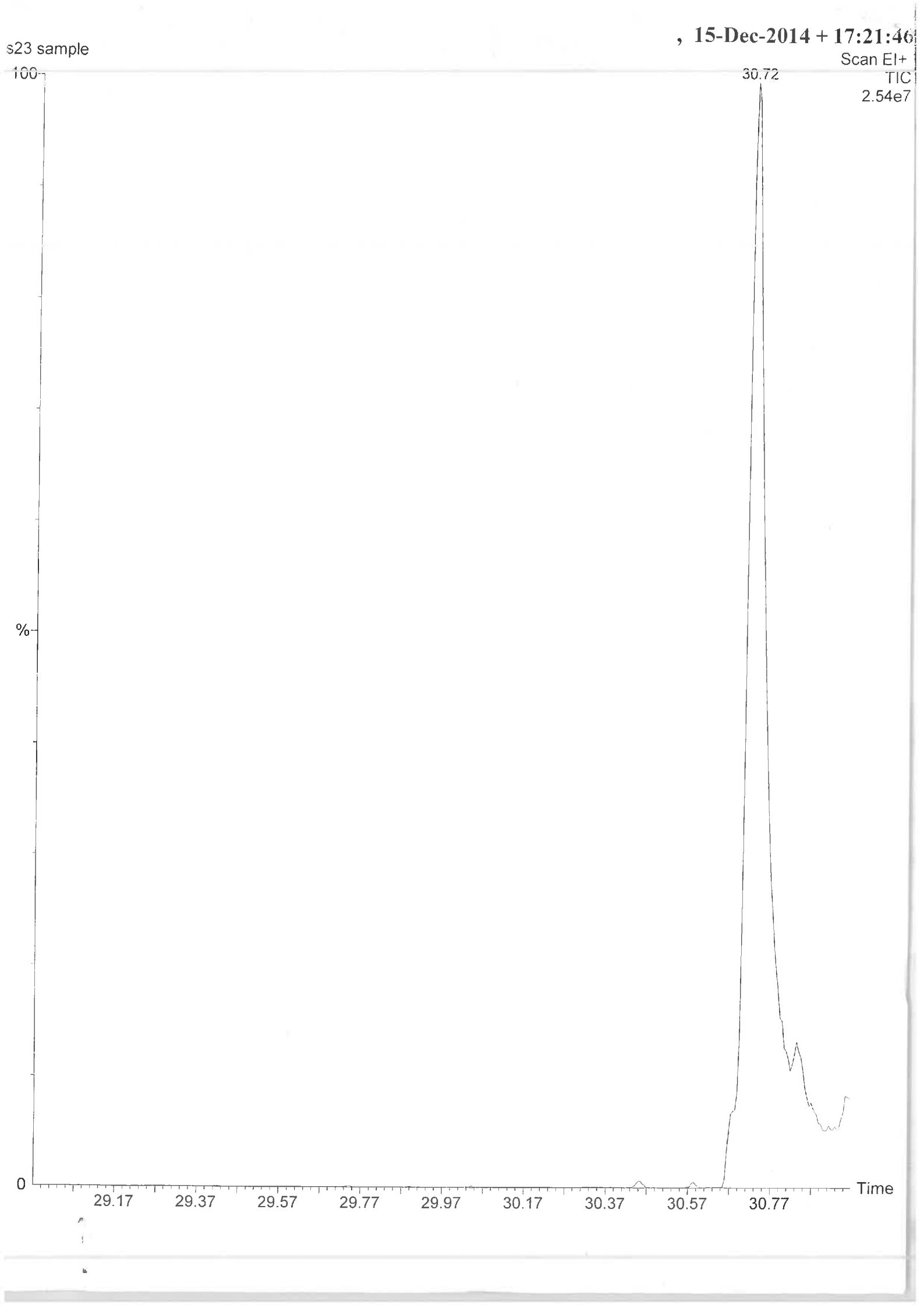
Hit	REV	for	Compound Name	M.W.	Formula	CAS
1	847	721	13-OCTADECENOIC ACID, METHYL ESTER	296	C19H36O2	56554-47-3
2	805	673	METHYL 9-HEPTADECENOATE OR 9-17:1	282	C18H34O2	900336-38-
3	803	653	13-METHYLTETRADEC-9-ENOIC ACID METHYL ESTER	254	C16H30O2	900365-89-
4	786	666	9-OCTADECENOIC ACID, METHYL ESTER, (E)-	296	C19H36O2	1937-62-8
5	779	615	14-METHYLPENTADEC-9-ENOIC ACID METHYL ESTER	268	C17H32O2	900365-89-
6	776	589	METHYL 16-HYDROXY-HEXADECANOATE	286	C17H34O3	900336-41-
7	776	629	CIS-13-OCTADECENOIC ACID, METHYL ESTER	296	C19H36O2	900333-58-
8	775	638	9-OCTADECENOIC ACID (Z)-, METHYL ESTER	296	C19H36O2	112-62-9
9	773	630	11-EICOSENOIC ACID, METHYL ESTER	324	C21H40O2	3946-08-5
10	772	618	METHYL 13-EICOSENOATE	324	C21H40O2	900336-48-
11	771	628	METHYL 9-EICOSENOATE	324	C21H40O2	900336-50-
12	768	621	14-METHYLHEXADEC-9-ENOIC ACID, METHYL ESTER	282	C18H34O2	900370-42-
13	767	650	11-OCTADECENOIC ACID, METHYL ESTER	296	C19H36O2	52380-33-3
14	767	649	TRANS-13-OCTADECENOIC ACID, METHYL ESTER	296	C19H36O2	900333-61-
15	764	598	METHYL 17-HEXACOSENOATE	408	C27H52O2	900336-27-
16	760	603	METHYL 11-DOCOSENOATE	352	C23H44O2	900336-23-
17	759	602	METHYL 8-HEPTADECENOATE	282	C18H34O2	900336-36-
18	758	601	METHYL 11-HEXADECENOATE	268	C17H32O2	900336-35-
19	755	656	16-OCTADECENOIC ACID, METHYL ESTER	296	C19H36O2	56554-49-5
20	751	636	11-OCTADECENOIC ACID, METHYL ESTER	296	C19H36O2	52380-33-3

Hit	REV	for	Compound Name	M.W.	Formula	CAS
1	847	721	13-OCTADECENOIC ACID, METHYL ESTER	296	C19H36O2	56554-47-3
2	840	712	METHYL 9-HEPTADECENOATE OR 9-17:1	282	C18H34O2	900336-38-
3	836	718	METHYL 8-HEPTADECENOATE	282	C18H34O2	900336-36-
4	827	705	11-EICOSENOIC ACID, METHYL ESTER	324	C21H40O2	3946-08-5
5	824	719	9-OCTADECENOIC ACID, METHYL ESTER, (E)-	296	C19H36O2	1937-62-8
6	822	691	14-METHYLPENTADEC-9-ENOIC ACID METHYL ESTER	268	C17H32O2	900365-89-
7	819	676	13-METHYLTETRADEC-9-ENOIC ACID METHYL ESTER	254	C16H30O2	900365-89-
8	809	676	METHYL 13-EICOSENOATE	324	C21H40O2	900336-48-
9	805	668	CIS-13-OCTADECENOIC ACID, METHYL ESTER	296	C19H36O2	900333-58-
10	803	672	METHYL 9-EICOSENOATE	324	C21H40O2	900336-50-
11	801	661	METHYL 11-DOCOSENOATE	352	C23H44O2	900336-23-
12	795	687	11-OCTADECENOIC ACID, METHYL ESTER	296	C19H36O2	52380-33-3
13	793	676	14-METHYLHEXADEC-9-ENOIC ACID, METHYL ESTER	282	C18H34O2	900370-42-
14	792	650	9-OCTADECENOIC ACID (Z)-, METHYL ESTER	296	C19H36O2	112-62-9
15	790	654	METHYL 12-HYDROXY-9-OCTADECENOATE	312	C19H36O3	900336-28-
16	789	682	TRANS-13-OCTADECENOIC ACID, METHYL ESTER	296	C19H36O2	900333-61-
17	788	672	9-OCTADECENOIC ACID, METHYL ESTER, (E)-	296	C19H36O2	1937-62-8
18	788	654	CIS-10-HEPTADECENOIC ACID, METHYL ESTER	282	C18H34O2	900333-62-
19	785	631	METHYL 9-TETRADECENOATE	240	C15H28O2	900336-47-
20	782	620	METHYL 17-HEXACOSENOATE	408	C27H52O2	900336-27-

Hit	REV	for	Compound Name	M.W.	Formula	CAS
1	840	749	13-OCTADECENOIC ACID, METHYL ESTER	296	C19H36O2	56554-47-3
2	835	766	9-OCTADECENOIC ACID, METHYL ESTER, (E)-	296	C19H36O2	1937-62-8
3	806	716	CIS-13-OCTADECENOIC ACID, METHYL ESTER	296	C19H36O2	900333-58-
4	804	688	13-DOCOSENOIC ACID, METHYL ESTER	352	C23H44O2	56630-69-4
5	801	681	METHYL 11-HEXADECENOATE	268	C17H32O2	900336-35-
6	800	702	9-OCTADECENOIC ACID (Z)-, METHYL ESTER	296	C19H36O2	112-62-9
7	799	724	11-OCTADECENOIC ACID, METHYL ESTER	296	C19H36O2	52380-33-3
8	795	692	11-EICOSENOIC ACID, METHYL ESTER	324	C21H40O2	3946-08-5
9	788	692	9-OCTADECENOIC ACID, METHYL ESTER, (E)-	296	C19H36O2	1937-62-8
10	786	652	METHYL 12-HYDROXY-9-OCTADECENOATE	312	C19H36O3	900336-28-
11	785	678	14-METHYLPENTADEC-9-ENOIC ACID METHYL ESTER	268	C17H32O2	900365-89-
12	784	710	TRANS-13-OCTADECENOIC ACID, METHYL ESTER	296	C19H36O2	900333-61-
13	783	660	METHYL 5-EICOSENOATE	324	C21H40O2	900336-48-
14	779	679	13-METHYLTETRADEC-9-ENOIC ACID METHYL ESTER	254	C16H30O2	900365-89-
15	774	664	9-OCTADECENOIC ACID, METHYL ESTER, (E)-	296	C19H36O2	1937-62-8
16	771	677	CIS-10-NONADECENOIC ACID, METHYL ESTER	310	C20H38O2	900333-64-
17	769	569	BUTYL 9-OCTADECENOATE OR 9-18:1	338	C22H42O2	900336-74-
18	768	650	METHYL 13-EICOSENOATE	324	C21H40O2	900336-48-
19	767	629	METHYL HEXADEC-9-ENOATE	268	C17H32O2	10030-74-7
20	765	672	9-HEXADECENOIC ACID, METHYL ESTER, (Z)-	268	C17H32O2	1120-25-8

Hit	REV	for	Compound Name	M.W.	Formula	CAS
1	928	644	TETRADECANOIC ACID, 10,13-DIMETHYL-, METHYL ESTER	270	C17H34O2	267650-23-
2	904	778	TRIDECANOIC ACID, 12-METHYL-, METHYL ESTER	242	C15H30O2	5129-58-8
3	901	766	METHYL 11-METHYL-DODECANOATE	228	C14H28O2	900336-45-
4	890	772	UNDECANOIC ACID, 10-METHYL-, METHYL ESTER	214	C13H26O2	5129-56-6
5	887	739	METHYL 8-METHYL-NONANOATE	186	C11H22O2	900336-43-
6	882	821	METHYL 13-METHYLTETRADECANOATE	256	C16H32O2	900336-31-
7	869	713	METHYL 18-FLUORO-OCTADECANOATE	316	C19H37O2F	900336-47-
8	864	788	HEXADECANOIC ACID, 15-METHYL-, METHYL ESTER	284	C18H36O2	6929-04-0
9	857	740	DODECANOIC ACID, 10-METHYL-, METHYL ESTER	228	C14H28O2	5129-65-7
10	855	729	METHYL 8-METHYL-DECANOATE	200	C12H24O2	900336-49-
11	852	810	HEXADECANOIC ACID, METHYL ESTER	270	C17H34O2	112-39-0
12	850	760	TRIDECANOIC ACID, METHYL ESTER	228	C14H28O2	1731-88-0
13	849	624	HEPTACOSANOIC ACID, 25-METHYL-, METHYL ESTER	438	C29H58O2	900112-14-
14	833	714	CYCLOPENTANEUNDECANOIC ACID, METHYL ESTER	268	C17H32O2	25779-85-5
15	829	747	METHYL 9-METHYLTETRADECANOATE	256	C16H32O2	213617-69-
16	829	735	HEXADECANOIC ACID, 14-METHYL-, METHYL ESTER	284	C18H36O2	2490-49-5
17	828	757	HEPTADECANOIC ACID, 16-METHYL-, METHYL ESTER	298	C19H38O2	5129-61-3
18	825	798	PENTADECANOIC ACID, 14-METHYL-, METHYL ESTER	270	C17H34O2	5129-60-2
19	823	709	TRIDECANOIC ACID, METHYL ESTER	228	C14H28O2	1731-88-0
20	822	759	PENTADECANOIC ACID, METHYL ESTER	256	C16H32O2	7132-64-1

Hit	REV	for	Compound Name	M.W.	Formula	CAS
1	899	509	TETRADECANOIC ACID, 10,13-DIMETHYL-, METHYL ESTER	270	C17H34O2	267650-23-
2	864	597	HEPTACOSANOIC ACID, 25-METHYL-, METHYL ESTER	438	C29H58O2	900112-14-
3	859	701	METHYL 11-METHYL-DODECANOATE	228	C14H28O2	900336-45-
4	853	551	OCTADECANOIC ACID, 11-METHYL-, METHYL ESTER	312	C20H40O2	74484-77-8
5	833	666	METHYL 18-FLUORO-OCTADECANOATE	316	C19H37O2F	900336-47-
6	825	677	TRIDECANOIC ACID, 12-METHYL-, METHYL ESTER	242	C15H30O2	5129-58-8
7	817	689	CYCLOPENTANEUNDECANOIC ACID, METHYL ESTER	268	C17H32O2	25779-85-5
8	813	681	DODECANOIC ACID, 10-METHYL-, METHYL ESTER	228	C14H28O2	5129-65-7
9	796	694	METHYL 14-METHYL-EICOSANOATE	340	C22H44O2	900336-23-
10	793	641	METHYL 8-METHYL-NONANOATE	186	C11H22O2	900336-43-
11	791	494	TETRADECANOIC ACID, 12-METHYL-, METHYL ESTER, (S)-	256	C16H32O2	62691-05-8
12	783	600	TRIACONTANOIC ACID, METHYL ESTER	466	C31H62O2	629-83-4
13	782	653	METHYL 8-METHYL-DECANOATE	200	C12H24O2	900336-49-
14	778	643	HEXADECANOIC ACID, 15-METHYL-, METHYL ESTER	284	C18H36O2	6929-04-0
15	777	666	HEXADECANOIC ACID, METHYL ESTER	270	C17H34O2	112-39-0
16	762	576	METHYL 11-CYCLOHEXYLUNDECANOATE	282	C18H34O2	900336-34-
17	761	640	HEPTADECANOIC ACID, 16-METHYL-, METHYL ESTER	298	C19H38O2	5129-61-3
18	760	651	METHYL 9-METHYLTETRADECANOATE	256	C16H32O2	213617-69-
19	756	674	PENTADECANOIC ACID, 14-METHYL-, METHYL ESTER	270	C17H34O2	5129-60-2
20	744	639	METHYL 8,10-DIMETHYL-HEXADECANOATE OR 8,10-DIMETHYL-16:0	298	C19H38O2	900336-39-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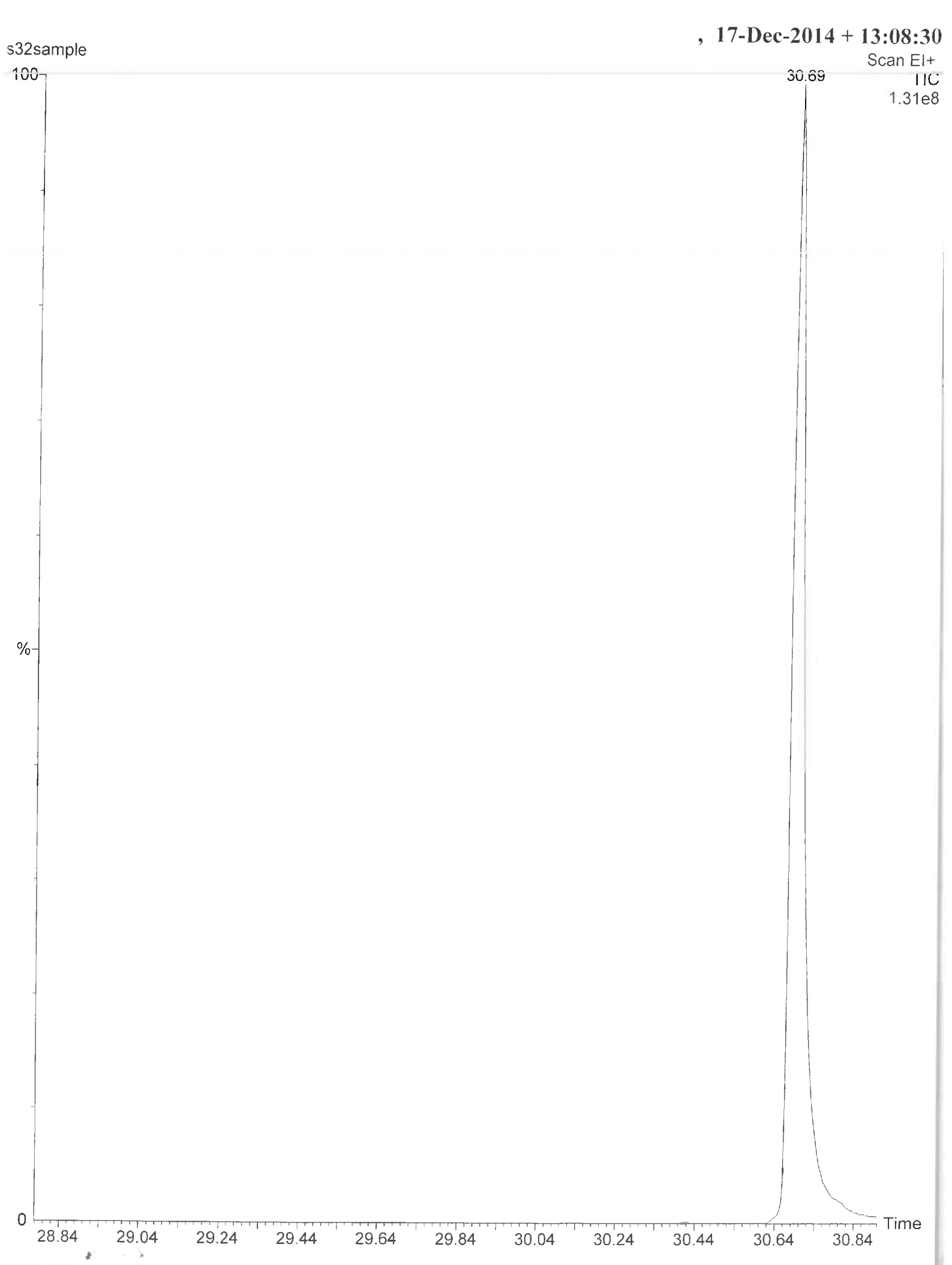
Hit	REV	for	Compound Name	M.W.	Formula	CAS
1	915	711	TETRADECANOIC ACID, 10,13-DIMETHYL-, METHYL ESTER	270	C17H34O2	267650-23-
2	888	831	METHYL 11-METHYL-DODECANOATE	228	C14H28O2	900336-45-
3	883	830	TRIDECANOIC ACID, 12-METHYL-, METHYL ESTER	242	C15H30O2	5129-58-8
4	872	791	METHYL 8-METHYL-NONANOATE	186	C11H22O2	900336-43-
5	870	814	UNDECANOIC ACID, 10-METHYL-, METHYL ESTER	214	C13H26O2	5129-56-6
6	831	824	HEXADECANOIC ACID, METHYL ESTER	270	C17H34O2	112-39-0
7	828	805	METHYL 13-METHYLTETRADECANOATE	256	C16H32O2	900336-31-
8	820	773	TRIDECANOIC ACID, METHYL ESTER	228	C14H28O2	1731-88-0
9	818	814	HEXADECANOIC ACID, METHYL ESTER	270	C17H34O2	112-39-0
10	816	698	HEPTACOSANOIC ACID, 25-METHYL-, METHYL ESTER	438	C29H58O2	900112-14-
11	813	725	METHYL 18-FLUORO-OCTADECANOATE	316	C19H37O2F	900336-47-
12	813	764	TRIDECANOIC ACID, METHYL ESTER	228	C14H28O2	1731-88-0
13	806	739	METHYL 8-METHYL-DECANOATE	200	C12H24O2	900336-49-
14	805	748	METHYL STEARATE	298	C19H38O2	112-61-8
15	803	756	METHYL TETRADECANOATE	242	C15H30O2	124-10-7
16	799	779	PENTADECANOIC ACID, METHYL ESTER	256	C16H32O2	7132-64-1
17	797	722	HEPTADECANOIC ACID, 16-METHYL-, METHYL ESTER	298	C19H38O2	5129-61-3
18	793	762	HEXADECANOIC ACID, 15-METHYL-, METHYL ESTER	284	C18H36O2	6929-04-0
19	792	718	NONANOIC ACID, METHYL ESTER	172	C10H20O2	1731-84-6
20	787	738	METHYL TETRADECANOATE	242	C15H30O2	124-10-7

Hit	REV	for	Compound Name	M.W.	Formula	CAS
1	659	659	METHYL 8-METHYL-NONANOATE	186	C11H22O2	900336-43-
2	659	659	METHYL 11-METHYL-DODECANOATE	228	C14H28O2	900336-45-
3	631	631	TRIDECANOIC ACID, 12-METHYL-, METHYL ESTER	242	C15H30O2	5129-58-8
4	609	609	UNDECANOIC ACID, 10-METHYL-, METHYL ESTER	214	C13H26O2	5129-56-6
5	593	593	METHYL 18-FLUORO-OCTADECANOATE	316	C19H37O2F	900336-47-
6	591	591	HEPTACOSANOIC ACID, 25-METHYL-, METHYL ESTER	438	C29H58O2	900112-14-
7	586	586	PENTADECANOIC ACID, METHYL ESTER	256	C16H32O2	7132-64-1
8	583	583	HEXADECANOIC ACID, METHYL ESTER	270	C17H34O2	112-39-0
9	579	579	METHYL STEARATE	298	C19H38O2	112-61-8
10	575	575	HEXADECANOIC ACID, 15-METHYL-, METHYL ESTER	284	C18H36O2	6929-04-0
11	572	572	NONADECANOIC ACID, METHYL ESTER	312	C20H40O2	1731-94-8
12	568	568	PENTADECANOIC ACID, 14-METHYL-, METHYL ESTER	270	C17H34O2	5129-60-2
13	563	563	HEPTADECANOIC ACID, 16-METHYL-, METHYL ESTER	298	C19H38O2	5129-61-3
14	563	563	METHYL 13-METHYLTETRADECANOATE	256	C16H32O2	900336-31-
15	559	559	NONADECANOIC ACID, METHYL ESTER	312	C20H40O2	1731-94-8
16	554	554	NONADECANOIC ACID, METHYL ESTER	312	C20H40O2	1731-94-8
17	553	553	METHYL 8-METHYL-DECANOATE	200	C12H24O2	900336-49-
18	547	547	HEXADECANOIC ACID, 15-METHYL-, METHYL ESTER	284	C18H36O2	6929-04-0
19	547	547	TETRACOSANOIC ACID, METHYL ESTER	382	C25H50O2	2442-49-1
20	542	542	HEXADECANOIC ACID, 14-METHYL-, METHYL ESTER	284	C18H36O2	2490-49-5

Hit	REV	for	Compound Name	M.W.	Formula	CAS
1	647	647	METHYL 11-METHYL-DODECANOATE	228	C14H28O2	900336-45-
2	643	643	METHYL 8-METHYL-NONANOATE	186	C11H22O2	900336-43-
3	643	641	TETRADECANOIC ACID, 12-METHYL-, METHYL ESTER, (S)-	256	C16H32O2	62691-05-8
4	617	617	TRIDECANOIC ACID, 12-METHYL-, METHYL ESTER	242	C15H30O2	5129-58-8
5	616	616	NONANOIC ACID, METHYL ESTER	172	C10H20O2	1731-84-6
6	610	608	OCTADECANOIC ACID, 11-METHYL-, METHYL ESTER	312	C20H40O2	74484-77-8
7	603	603	UNDECANOIC ACID, METHYL ESTER	200	C12H24O2	1731-86-8
8	599	599	DECANOIC ACID, METHYL ESTER	186	C11H22O2	110-42-9
9	599	599	DODECANOIC ACID, METHYL ESTER	214	C13H26O2	111-82-0
10	593	593	UNDECANOIC ACID, 10-METHYL-, METHYL ESTER	214	C13H26O2	5129-56-6
11	593	593	TRIDECANOIC ACID, METHYL ESTER	228	C14H28O2	1731-88-0
12	589	589	DODECANOIC ACID, METHYL ESTER	214	C13H26O2	111-82-0
13	584	584	METHYL TETRADECANOATE	242	C15H30O2	124-10-7
14	581	581	METHYL TETRADECANOATE	242	C15H30O2	124-10-7
15	580	580	DECANOIC ACID, METHYL ESTER	186	C11H22O2	110-42-9
16	574	574	PENTADECANOIC ACID, METHYL ESTER	256	C16H32O2	7132-64-1
17	573	573	PENTADECANOIC ACID, METHYL ESTER	256	C16H32O2	7132-64-1
18	573	573	HEPTADECANOIC ACID, METHYL ESTER	284	C18H36O2	1731-92-6
19	569	569	HEXADECANOIC ACID, METHYL ESTER	270	C17H34O2	112-39-0
20	569	569	METHYL 18-FLUORO-OCTADECANOATE	316	C19H37O2F	900336-47-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Hit	REV	for	Compound Name	M.W.	Formula	CAS
1	928	837	METHYL 11-METHYL-DODECANOATE	228	C14H28O2	900336-45-
2	927	679	TETRADECANOIC ACID, 10,13-DIMETHYL-, METHYL ESTER	270	C17H34O2	267650-23-
3	922	836	TRIDECANOIC ACID, 12-METHYL-, METHYL ESTER	242	C15H30O2	5129-58-8
4	915	802	METHYL 8-METHYL-NONANOATE	186	C11H22O2	900336-43-
5	911	831	UNDECANOIC ACID, 10-METHYL-, METHYL ESTER	214	C13H26O2	5129-56-6
6	895	851	METHYL 13-METHYLTETRADECANOATE	256	C16H32O2	900336-31-
7	872	810	TRIDECANOIC ACID, METHYL ESTER	228	C14H28O2	1731-88-0
8	866	771	METHYL 8-METHYL-DECANOATE	200	C12H24O2	900336-49-
9	863	836	HEXADECANOIC ACID, METHYL ESTER	270	C17H34O2	112-39-0
10	857	799	HEXADECANOIC ACID, 15-METHYL-, METHYL ESTER	284	C18H36O2	6929-04-0
11	848	721	METHYL 18-FLUORO-OCTADECANOATE	316	C19H37O2F	900336-47-
12	845	770	TRIDECANOIC ACID, METHYL ESTER	228	C14H28O2	1731-88-0
13	840	798	PENTADECANOIC ACID, METHYL ESTER	256	C16H32O2	7132-64-1
14	838	817	HEXADECANOIC ACID, METHYL ESTER	270	C17H34O2	112-39-0
15	837	777	METHYL 9-METHYLTETRADECANOATE	256	C16H32O2	213617-69-
16	837	759	DODECANOIC ACID, METHYL ESTER	214	C13H26O2	111-82-0
17	833	761	METHYL TETRADECANOATE	242	C15H30O2	124-10-7
18	833	650	HEPTACOSANOIC ACID, 25-METHYL-, METHYL ESTER	438	C29H58O2	900112-14-
19	832	767	HEXADECANOIC ACID, 14-METHYL-, METHYL ESTER	284	C18H36O2	2490-49-5
20	829	751	METHYL STEARATE	298	C19H38O2	112-61-8

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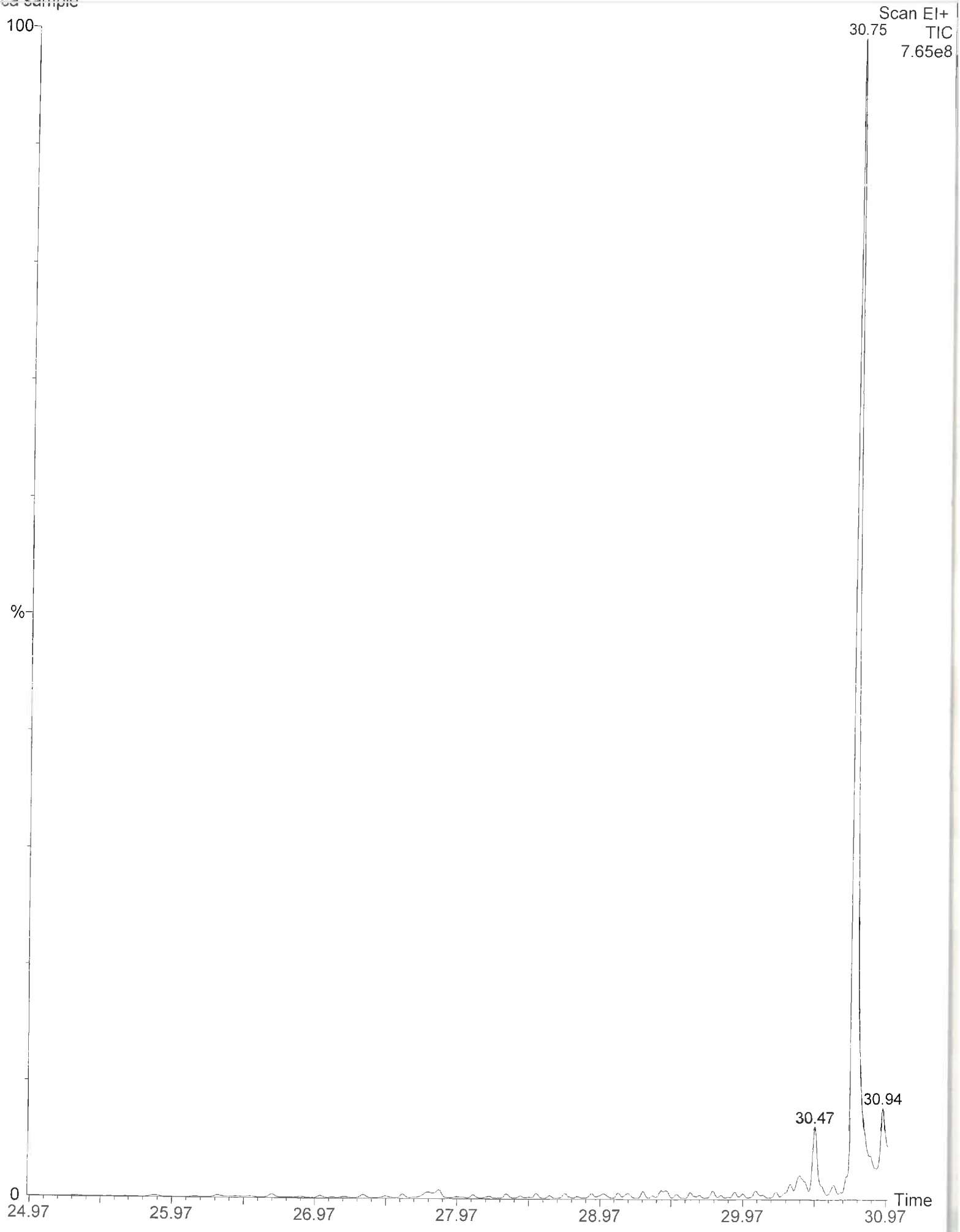

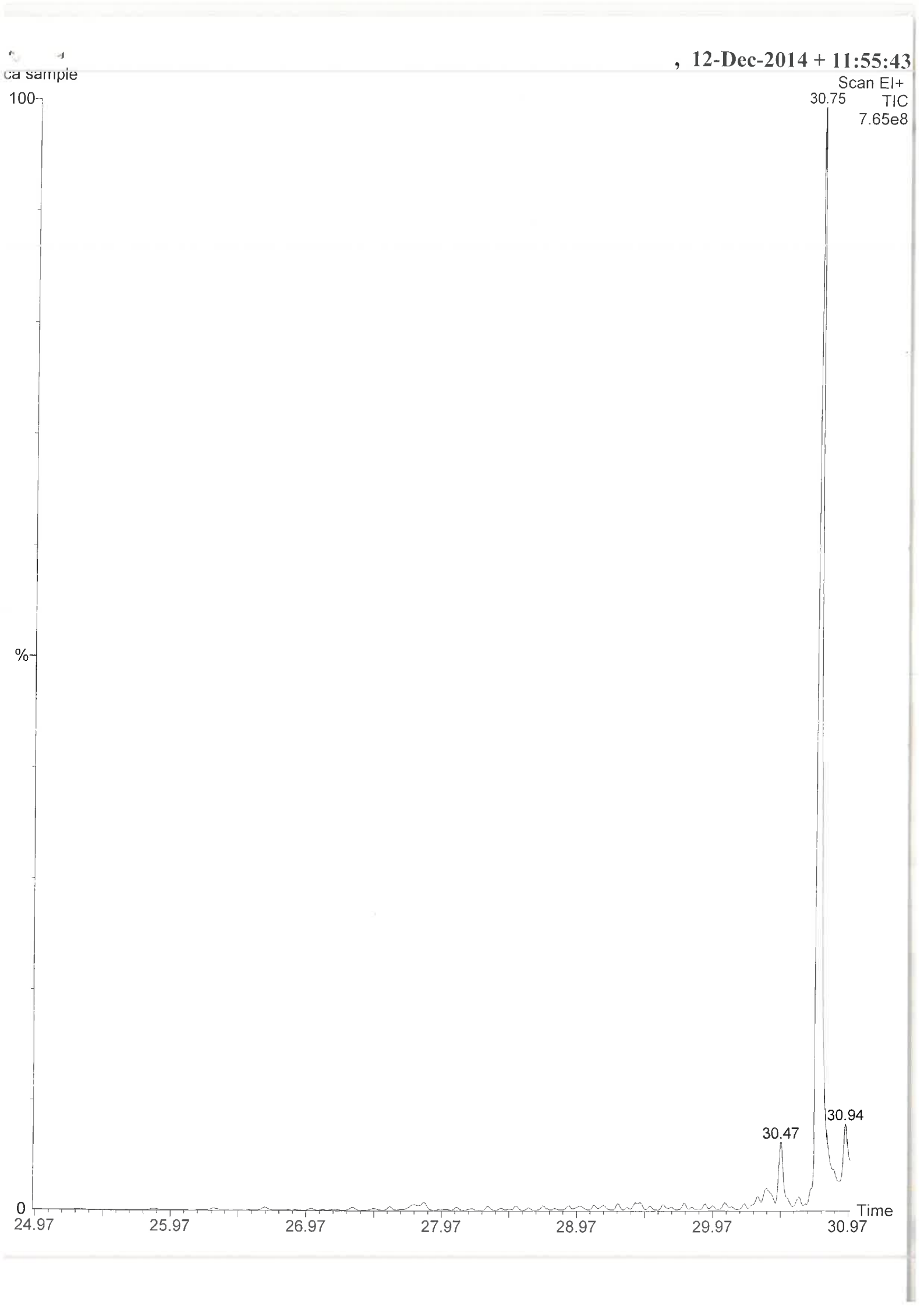
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Hit	REV	for	Compound Name	M.W.	Formula	CAS
1	783	762	METHYL 16-HYDROXY-HEXADECANOATE	286	C17H34O3	900336-41-
2	753	717	CYCLOPENTANEUNDECANOIC ACID, METHYL ESTER	268	C17H32O2	25779-85-5
3	732	648	METHYL 11-CYCLOHEXYLUNDECANOATE	282	C18H34O2	900336-34-
4	728	707	13-OCTADECENOIC ACID, METHYL ESTER	296	C19H36O2	56554-47-3
5	727	707	METHYL 11-HEXADECENOATE	268	C17H32O2	900336-35-
6	722	705	13-METHYLTETRADEC-9-ENOIC ACID METHYL ESTER	254	C16H30O2	900365-89-
7	721	710	14-METHYLPENTADEC-9-ENOIC ACID METHYL ESTER	268	C17H32O2	900365-89-
8	717	699	METHYL 9-HEPTADECENOATE OR 9-17:1	282	C18H34O2	900336-38-
9	716	708	METHYL 11-DOCOSENOATE	352	C23H44O2	900336-23-
10	713	705	METHYL 9-EICOSENOATE	324	C21H40O2	900336-50-
11	703	660	10-UNDECENOIC ACID, METHYL ESTER	198	C12H22O2	111-81-9
12	703	689	METHYL 9-TETRADECENOATE	240	C15H28O2	900336-47-
13	703	682	METHYL TRANS-9-(2-BUTYLCYCLOPENTYL)NONANOATE	296	C19H36O2	108708-61-
14	693	675	METHYL 12-HYDROXY-9-OCTADECENOATE	312	C19H36O3	900336-28-
15	693	673	METHYL 13-EICOSENOATE	324	C21H40O2	900336-48-
16	688	661	OXIRANEOCTANOIC ACID, 3-OCTYL-, METHYL ESTER	312	C19H36O3	2500-59-6
17	680	668	16-OCTADECENOIC ACID, METHYL ESTER	296	C19H36O2	56554-49-5
18	679	662	9-HEXADECENOIC ACID, METHYL ESTER, (Z)-	268	C17H32O2	1120-25-8
19	676	666	14-METHYLHEXADEC-9-ENOIC ACID, METHYL ESTER	282	C18H34O2	900370-42-
20	670	655	9-OCTADECENOIC ACID (Z)-, METHYL ESTER	296	C19H36O2	112-62-9

Hit	REV	for	Compound Name	M.W.	Formula	CAS
1	777	627	TRANS-1-METHYL-2-NONYL-CYCLOHEXANE	224	C16H32	900371-47-
2	690	685	13-OCTADECENOIC ACID, METHYL ESTER	296	C19H36O2	56554-47-3
3	688	682	METHYL 11-HEXADECENOATE	268	C17H32O2	900336-35-
4	682	539	CYCLOHEXANE, 1-METHYL-4-(1-METHYLETHYL)-, CIS-	140	C10H20	6069-98-3
5	671	661	METHYL 17-HEXACOSENOATE	408	C27H52O2	900336-27-
6	671	661	METHYL 16-HYDROXY-HEXADECANOATE	286	C17H34O3	900336-41-
7	666	541	CYCLOHEXANE, 1-METHYL-4-(1-METHYLETHYL)-, TRANS-	140	C10H20	1678-82-6
8	664	659	METHYL 13-EICOSENOATE	324	C21H40O2	900336-48-
9	659	531	OXALIC ACID, DI(CYCLOHEXYLMETHYL) ESTER	282	C16H26O4	900309-68-
10	659	653	METHYL 11-DOCOSENOATE	352	C23H44O2	900336-23-
11	657	652	METHYL 9-HEPTADECENOATE OR 9-17:1	282	C18H34O2	900336-38-
12	655	651	METHYL 9-EICOSENOATE	324	C21H40O2	900336-50-
13	655	650	13-METHYLTETRADEC-9-ENOIC ACID METHYL ESTER	254	C16H30O2	900365-89-
14	654	639	METHYL 19-HEXACOSENOATE	408	C27H52O2	900336-27-
15	652	646	METHYL 9-TETRADECENOATE	240	C15H28O2	900336-47-
16	647	521	OXALIC ACID, BUTYL CYCLOHEXYLMETHYL ESTER	242	C13H22O4	900309-68-
17	646	642	METHYL 12-HYDROXY-9-OCTADECENOATE	312	C19H36O3	900336-28-
18	646	640	14-METHYLPENTADEC-9-ENOIC ACID METHYL ESTER	268	C17H32O2	900365-89-
19	644	534	N-PROPYL 11-OCTADECENOATE	324	C21H40O2	900336-71-
20	642	632	METHYL 16-PENTACOSENOATE	394	C26H50O2	900336-24-

Hit	REV	for	Compound Name	M.W.	Formula	CAS
1	925	657	TETRADECANOIC ACID, 10,13-DIMETHYL-, METHYL ESTER	270	C17H34O2	267650-23-
2	923	812	METHYL 11-METHYL-DODECANOATE	228	C14H28O2	900336-45-
3	917	815	TRIDECANOIC ACID, 12-METHYL-, METHYL ESTER	242	C15H30O2	5129-58-8
4	908	812	UNDECANOIC ACID, 10-METHYL-, METHYL ESTER	214	C13H26O2	5129-56-6
5	906	781	METHYL 8-METHYL-NONANOATE	186	C11H22O2	900336-43-
6	891	834	METHYL 13-METHYLTETRADECANOATE	256	C16H32O2	900336-31-
7	866	794	TRIDECANOIC ACID, METHYL ESTER	228	C14H28O2	1731-88-0
8	866	762	METHYL 8-METHYL-DECANOATE	200	C12H24O2	900336-49-
9	864	724	METHYL 18-FLUORO-OCTADECANOATE	316	C19H37O2F	900336-47-
10	857	814	HEXADECANOIC ACID, METHYL ESTER	270	C17H34O2	112-39-0
11	854	778	HEXADECANOIC ACID, 15-METHYL-, METHYL ESTER	284	C18H36O2	6929-04-0
12	847	774	METHYL 9-METHYLTETRADECANOATE	256	C16H32O2	213617-69-
13	840	634	HEPTACOSANOIC ACID, 25-METHYL-, METHYL ESTER	438	C29H58O2	900112-14-
14	839	755	HEXADECANOIC ACID, 14-METHYL-, METHYL ESTER	284	C18H36O2	2490-49-5
15	837	744	TRIDECANOIC ACID, METHYL ESTER	228	C14H28O2	1731-88-0
16	833	778	PENTADECANOIC ACID, METHYL ESTER	256	C16H32O2	7132-64-1
17	830	740	DODECANOIC ACID, METHYL ESTER	214	C13H26O2	111-82-0
18	830	794	HEXADECANOIC ACID, METHYL ESTER	270	C17H34O2	112-39-0
19	830	719	HEPTADECANOIC ACID, 16-METHYL-, METHYL ESTER	298	C19H38O2	5129-61-3
20	825	739	METHYL TETRADECANOATE	242	C15H30O2	124-10-7

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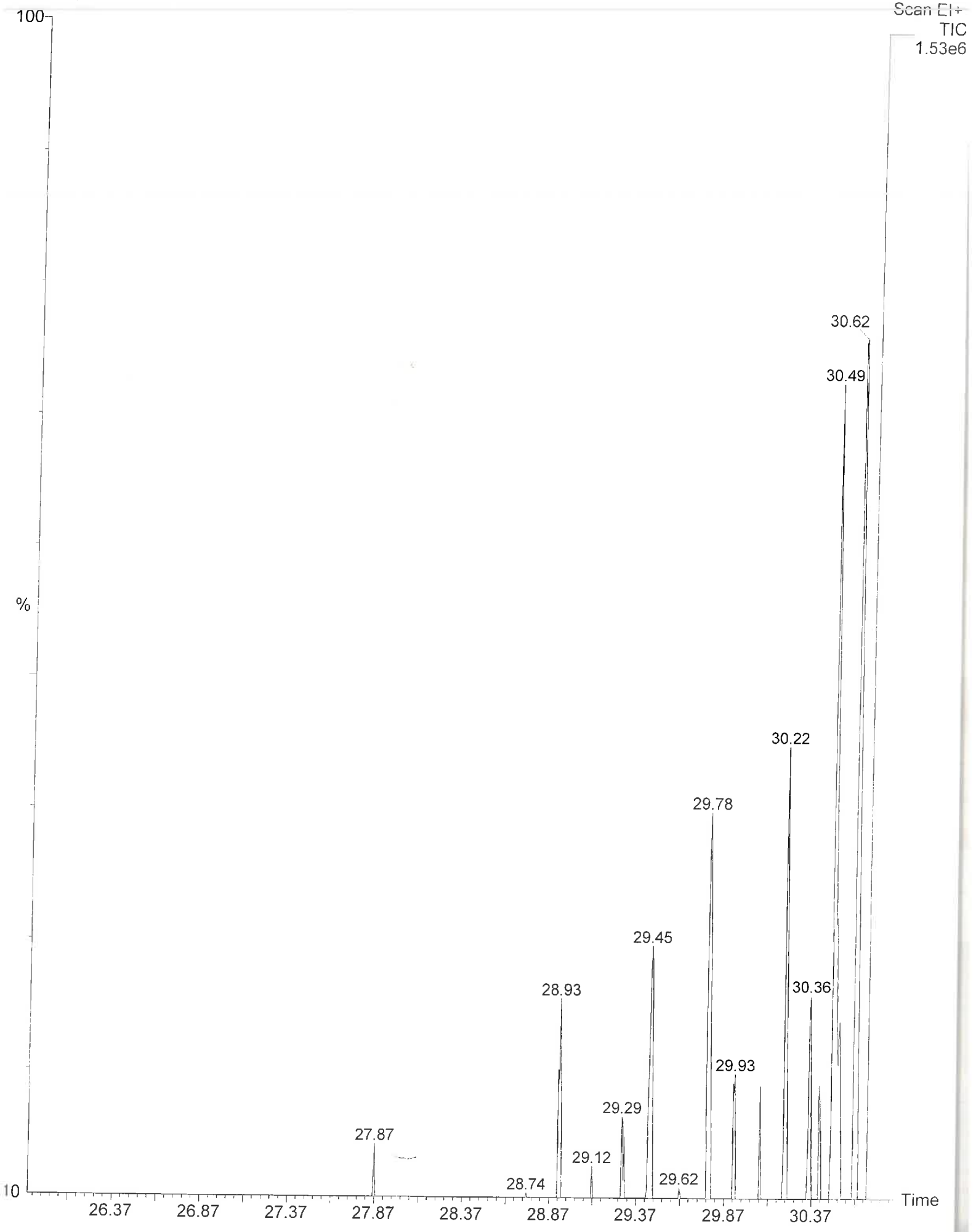

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Hit	REV	for	Compound Name	M.W.	Formula	CAS
1	432	432	METHYL 16-HYDROXY-HEXADECANOATE	286	C17H34O3	900336-41-
2	407	407	10-UNDECENOIC ACID, METHYL ESTER	198	C12H22O2	111-81-9
3	404	404	METHYL 11-HEXADECENOATE	268	C17H32O2	900336-35-
4	401	401	10-UNDECENOIC ACID, METHYL ESTER	198	C12H22O2	111-81-9
5	394	394	10-UNDECENOIC ACID, METHYL ESTER	198	C12H22O2	111-81-9
6	378	378	13-OCTADECENOIC ACID, METHYL ESTER	296	C19H36O2	56554-47-3
7	377	377	10-UNDECENOIC ACID, METHYL ESTER	198	C12H22O2	111-81-9
8	367	367	11-DODECENOIC ACID, METHYL ESTER	212	C13H24O2	29972-79-0
9	364	364	METHYL 19-HEXACOSENOATE	408	C27H52O2	900336-27-
10	360	360	CYCLOPROPANENONANOIC ACID, METHYL ESTER	212	C13H24O2	10152-60-0
11	358	358	METHYL 17-HEXACOSENOATE	408	C27H52O2	900336-27-
12	356	356	16-OCTADECENOIC ACID, METHYL ESTER	296	C19H36O2	56554-49-5
13	353	353	9-HEXADECENOIC ACID, METHYL ESTER, (Z)-	268	C17H32O2	1120-25-8
14	350	350	METHYL 13-EICOSENOATE	324	C21H40O2	900336-48-
15	340	340	METHYL 9-HEPTADECENOATE OR 9-17:1	282	C18H34O2	900336-38-
16	339	339	14-OCTADECENOIC ACID, METHYL ESTER	296	C19H36O2	56554-48-4
17	339	339	13-OCTADECENOIC ACID, METHYL ESTER	296	C19H36O2	56554-47-3
18	338	338	15-OCTADECENOIC ACID, METHYL ESTER	296	C19H36O2	4764-72-1
19	337	337	TRANS-13-OCTADECENOIC ACID, METHYL ESTER	296	C19H36O2	900336-61-
20	335	335	METHYL 12-HYDROXY-9-OCTADECENOATE	312	C19H36O3	900336-28-

Hit	REV	for	Compound Name	M.W.	Formula	CAS
1	600	594	TETRADECANOIC ACID, 12-METHYL-, METHYL ESTER, (S)-	256	C16H32O2	62691-05-8
2	586	583	METHYL 11-METHYL-DODECANOATE	228	C14H28O2	900336-45-
3	578	578	UNDECANOIC ACID, 11-BROMO-, METHYL ESTER	278	C12H23O2Br	6287-90-7
4	576	576	METHYL 8-METHYL-NONANOATE	186	C11H22O2	900336-43-
5	570	564	OCTADECANOIC ACID, 11-METHYL-, METHYL ESTER	312	C20H40O2	74484-77-8
6	561	561	TRIDECANOIC ACID, 12-METHYL-, METHYL ESTER	242	C15H30O2	5129-58-8
7	531	529	UNDECANOIC ACID, 10-METHYL-, METHYL ESTER	214	C13H26O2	5129-56-6
8	530	530	CYCLOPENTANEUNDECANOIC ACID, METHYL ESTER	268	C17H32O2	25779-85-5
9	523	518	HEPTACOSANOIC ACID, 25-METHYL-, METHYL ESTER	438	C29H58O2	900112-14-
10	517	517	PENTADECANOIC ACID, METHYL ESTER	256	C16H32O2	7132-64-1
11	508	506	HEXADECANOIC ACID, METHYL ESTER	270	C17H34O2	112-39-0
12	505	505	HEXADECANOIC ACID, 15-METHYL-, METHYL ESTER	284	C18H36O2	6929-04-0
13	503	503	PENTADECANOIC ACID, 14-METHYL-, METHYL ESTER	270	C17H34O2	5129-60-2
14	501	501	METHYL STEARATE	298	C19H38O2	112-61-8
15	498	496	METHYL 18-FLUORO-OCTADECANOATE	316	C19H37O2F	900336-47-
16	495	495	METHYL 9-METHYLTETRADECANOATE	256	C16H32O2	213617-69-
17	491	491	METHYL 13-METHYLTETRADECANOATE	256	C16H32O2	900336-31-
18	490	477	METHYL 11-CYCLOHEXYLUNDECANOATE	282	C18H34O2	900336-34-
19	485	482	NONADECANOIC ACID, METHYL ESTER	312	C20H40O2	1731-94-8
20	481	481	METHYL 6-METHYL HEPTANOATE	158	C9H18O2	2519-37-1

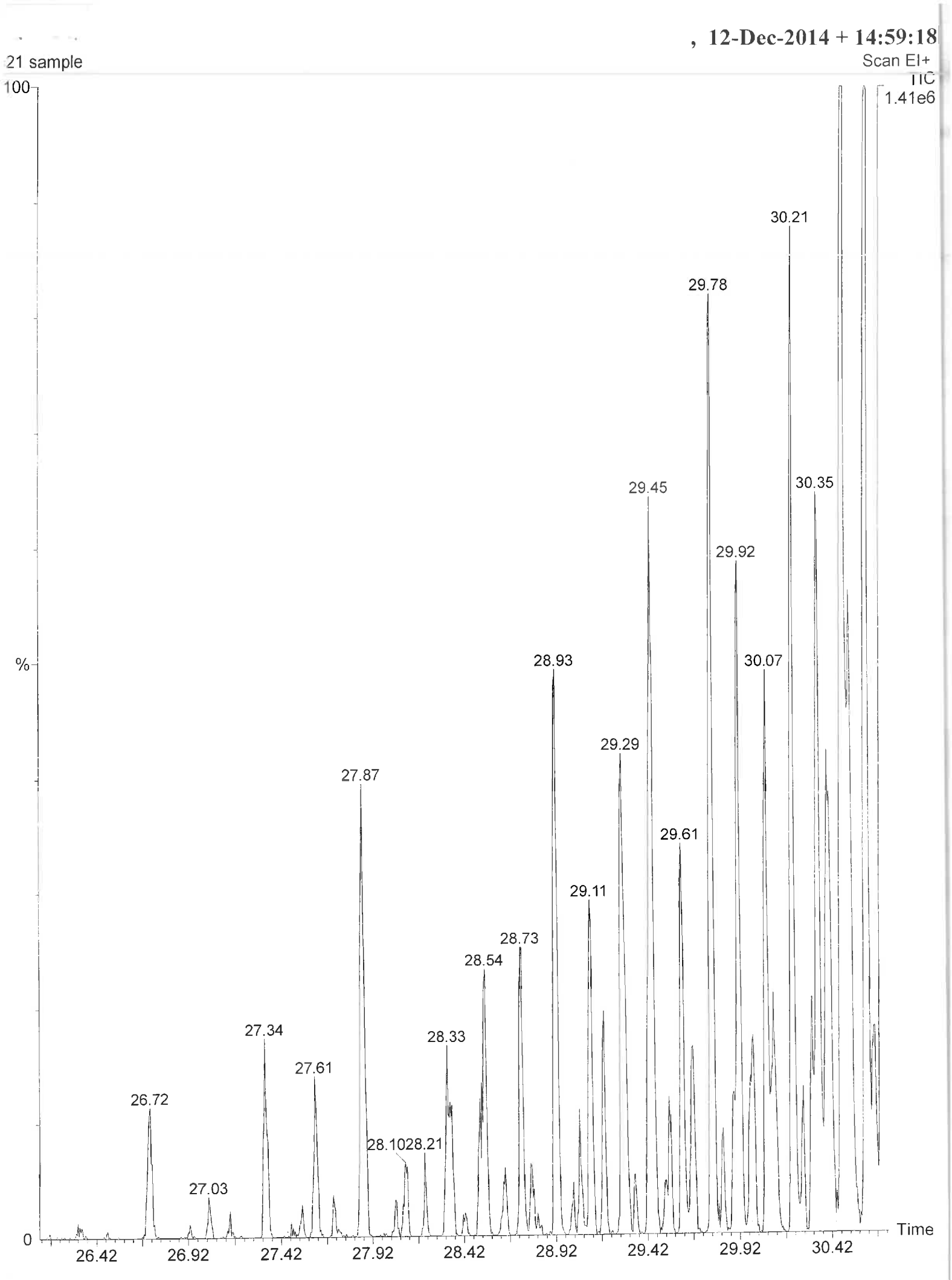
Hit	REV	for	Compound Name	M.W.	Formula	CAS
1	594	594	METHYL 11-HEXADECENOATE	268	C17H32O2	900336-35-
2	577	577	13-OCTADECENOIC ACID, METHYL ESTER	296	C19H36O2	56554-47-3
3	556	556	METHYL 17-HEXACOSENOATE	408	C27H52O2	900336-27-
4	551	551	METHYL 13-EICOSENOATE	324	C21H40O2	900336-48-
5	548	548	METHYL 9-HEPTADECENOATE OR 9-17:1	282	C18H34O2	900336-38-
6	541	541	9-HEXADECENOIC ACID, METHYL ESTER, (Z)-	268	C17H32O2	1120-25-8
7	539	539	METHYL 9-TETRADECENOATE	240	C15H28O2	900336-47-
8	539	539	METHYL 11-DOCOSENOATE	352	C23H44O2	900336-23-
9	535	535	METHYL 9-EICOSENOATE	324	C21H40O2	900336-50-
10	533	533	METHYL 16-PENTACOSENOATE	394	C26H50O2	900336-24-
11	533	533	13-METHYLTETRADEC-9-ENOIC ACID METHYL ESTER	254	C16H30O2	900365-89-
12	531	531	11-DODECENOIC ACID, METHYL ESTER	212	C13H24O2	29972-79-0
13	528	528	14-METHYLPENTADEC-9-ENOIC ACID METHYL ESTER	268	C17H32O2	900365-89-
14	526	526	METHYL 12-HYDROXY-9-OCTADECENOATE	312	C19H36O3	900336-28-
15	522	509	OCTADECANOIC ACID, 9,10-DIHYDROXY-, METHYL ESTER, BIS(TRIFLUOROACETA	522	C23H36O6F6	21987-19-9
16	521	521	TRANS-13-OCTADECENOIC ACID, METHYL ESTER	296	C19H36O2	900333-61-
17	520	515	OXIRANEOCTANOIC ACID, 3-OCTYL-, METHYL ESTER	312	C19H36O3	2500-59-6
18	509	509	9-OCTADECENOIC ACID (Z)-, METHYL ESTER	296	C19H36O2	112-62-9
19	507	507	17-OCTADECYNOIC ACID, METHYL ESTER	294	C19H34O2	900333-54-
20	501	501	11-OCTADECENOIC ACID, METHYL ESTER	296	C19H36O2	52380-33-3

Hit	REV	for	Compound Name	M.W.	Formula	CAS
1	547	547	METHYL 16-HYDROXY-HEXADECANOATE	286	C17H34O3	900336-41-
2	509	506	10-UNDECENOIC ACID, METHYL ESTER	198	C12H22O2	111-81-9
3	508	508	METHYL 11-HEXADECENOATE	268	C17H32O2	900336-35-
4	504	502	10-UNDECENOIC ACID, METHYL ESTER	198	C12H22O2	111-81-9
5	492	492	13-OCTADECENOIC ACID, METHYL ESTER	296	C19H36O2	56554-47-3
6	490	488	10-UNDECENOIC ACID, METHYL ESTER	198	C12H22O2	111-81-9
7	485	482	10-UNDECENOIC ACID, METHYL ESTER	198	C12H22O2	111-81-9
8	477	477	METHYL 19-HEXACOSENOATE	408	C27H52O2	900336-27-
9	472	472	METHYL 17-HEXACOSENOATE	408	C27H52O2	900336-27-
10	469	469	7-NONENOIC ACID, METHYL ESTER	170	C10H18O2	20731-22-0
11	466	466	11-DODECENOIC ACID, METHYL ESTER	212	C13H24O2	29972-79-0
12	462	462	METHYL 9-HEPTADECENOATE OR 9-17:1	282	C18H34O2	900336-38-
13	460	460	16-OCTADECENOIC ACID, METHYL ESTER	296	C19H36O2	56554-49-5
14	459	459	METHYL 13-EICOSENOATE	324	C21H40O2	900336-48-
15	457	457	9-HEXADECENOIC ACID, METHYL ESTER, (Z)-	268	C17H32O2	1120-25-8
16	455	455	14-METHYLPENTADEC-9-ENOIC ACID METHYL ESTER	268	C17H32O2	900365-89-
17	450	450	13-METHYLTETRADEC-9-ENOIC ACID METHYL ESTER	254	C16H30O2	900365-89-
18	448	448	METHYL 11-DOCOSENOATE	352	C23H44O2	900336-23-
19	446	446	15-OCTADECENOIC ACID, METHYL ESTER	296	C19H36O2	4764-72-1
20	444	444	METHYL 12-HYDROXY-9-OCTADECENOATE	312	C19H36O3	900336-28-

Hit	REV	for	Compound Name	M.W.	Formula	CAS
1	824	790	METHYL 8-METHYL-NONANOATE	186	C11H22O2	900336-43-
2	821	789	METHYL 11-METHYL-DODECANOATE	228	C14H28O2	900336-45-
3	812	782	TRIDECANOIC ACID, 12-METHYL-, METHYL ESTER	242	C15H30O2	5129-58-8
4	794	764	UNDECANOIC ACID, 10-METHYL-, METHYL ESTER	214	C13H26O2	5129-56-6
5	769	720	HEPTACOSANOIC ACID, 25-METHYL-, METHYL ESTER	438	C29H58O2	900112-14-
6	758	755	HEXADECANOIC ACID, METHYL ESTER	270	C17H34O2	112-39-0
7	757	726	NONANOIC ACID, METHYL ESTER	172	C10H20O2	1731-84-6
8	757	754	HEXADECANOIC ACID, METHYL ESTER	270	C17H34O2	112-39-0
9	752	711	METHYL 18-FLUORO-OCTADECANOATE	316	C19H37O2F	900336-47-
10	747	719	TRIDECANOIC ACID, METHYL ESTER	228	C14H28O2	1731-88-0
11	746	717	METHYL STEARATE	298	C19H38O2	112-61-8
12	745	715	DECANOIC ACID, METHYL ESTER	186	C11H22O2	110-42-9
13	742	713	METHYL TETRADECANOATE	242	C15H30O2	124-10-7
14	742	737	METHYL 13-METHYLTETRADECANOATE	256	C16H32O2	900336-31-
15	741	711	METHYL 8-METHYL-DECANOATE	200	C12H24O2	900336-49-
16	741	713	METHYL TETRADECANOATE	242	C15H30O2	124-10-7
17	741	736	PENTADECANOIC ACID, METHYL ESTER	256	C16H32O2	7132-64-1
18	738	711	TRIDECANOIC ACID, METHYL ESTER	228	C14H28O2	1731-88-0
19	736	707	DODECANOIC ACID, METHYL ESTER	214	C13H26O2	111-82-0
20	735	734	HEXADECANOIC ACID, METHYL ESTER	270	C17H34O2	112-39-0

Hit	REV	for	Compound Name	M.W.	Formula	CAS
1	916	686	TETRADECANOIC ACID, 10,13-DIMETHYL-, METHYL ESTER	270	C17H34O2	267650-23-
2	911	836	METHYL 11-METHYL-DODECANOATE	228	C14H28O2	900336-45-
3	901	838	TRIDECANOIC ACID, 12-METHYL-, METHYL ESTER	242	C15H30O2	5129-58-8
4	898	809	METHYL 8-METHYL-NONANOATE	186	C11H22O2	900336-43-
5	891	825	UNDECANOIC ACID, 10-METHYL-, METHYL ESTER	214	C13H26O2	5129-56-6
6	850	820	METHYL 13-METHYLTETRADECANOATE	256	C16H32O2	900336-31-
7	842	820	HEXADECANOIC ACID, METHYL ESTER	270	C17H34O2	112-39-0
8	841	791	TRIDECANOIC ACID, METHYL ESTER	228	C14H28O2	1731-88-0
9	834	754	METHYL 8-METHYL-DECANOATE	200	C12H24O2	900336-49-
10	832	733	METHYL 18-FLUORO-OCTADECANOATE	316	C19H37O2F	900336-47-
11	829	765	TRIDECANOIC ACID, METHYL ESTER	228	C14H28O2	1731-88-0
12	828	811	HEXADECANOIC ACID, METHYL ESTER	270	C17H34O2	112-39-0
13	817	772	HEXADECANOIC ACID, 15-METHYL-, METHYL ESTER	284	C18H36O2	6929-04-0
14	817	675	HEPTACOSANOIC ACID, 25-METHYL-, METHYL ESTER	438	C29H58O2	900112-14-
15	816	733	HEPTADECANOIC ACID, 16-METHYL-, METHYL ESTER	298	C19H38O2	5129-61-3
16	814	751	METHYL STEARATE	298	C19H38O2	112-61-8
17	814	759	METHYL TETRADECANOATE	242	C15H30O2	124-10-7
18	814	790	PENTADECANOIC ACID, METHYL ESTER	256	C16H32O2	7132-64-1
19	808	771	METHYL 9-METHYLTETRADECANOATE	256	C16H32O2	213617-69-
20	804	754	HEXADECANOIC ACID, 14-METHYL-, METHYL ESTER	284	C18H36O2	2490-49-5

Hit	REV	for	Compound Name	M.W.	Formula	CAS
1	812	802	METHYL 8-METHYL-NONANOATE	186	C11H22O2	900336-43-
2	801	778	METHYL 11-METHYL-DODECANOATE	228	C14H28O2	900336-45-
3	779	764	TRIDECANOIC ACID, 12-METHYL-, METHYL ESTER	242	C15H30O2	5129-58-8
4	756	731	METHYL 18-FLUORO-OCTADECANOATE	316	C19H37O2F	900336-47-
5	754	712	HEPTACOSANOIC ACID, 25-METHYL-, METHYL ESTER	438	C29H58O2	900112-14-
6	753	733	UNDECANOIC ACID, 10-METHYL-, METHYL ESTER	214	C13H26O2	5129-56-6
7	728	714	METHYL 8-METHYL-DECANOATE	200	C12H24O2	900336-49-
8	719	705	METHYL STEARATE	298	C19H38O2	112-61-8
9	714	702	METHYL 13-METHYLTETRADECANOATE	256	C16H32O2	900336-31-
10	712	687	HEPTADECANOIC ACID, 16-METHYL-, METHYL ESTER	298	C19H38O2	5129-61-3
11	711	702	METHYL 9-METHYLTETRADECANOATE	256	C16H32O2	213617-69-
12	711	698	PENTADECANOIC ACID, METHYL ESTER	256	C16H32O2	7132-64-1
13	710	702	HEXADECANOIC ACID, 15-METHYL-, METHYL ESTER	284	C18H36O2	6929-04-0
14	707	686	HEXADECANOIC ACID, METHYL ESTER	270	C17H34O2	112-39-0
15	700	682	NONADECANOIC ACID, METHYL ESTER	312	C20H40O2	1731-94-8
16	699	689	PENTADECANOIC ACID, 14-METHYL-, METHYL ESTER	270	C17H34O2	5129-60-2
17	695	677	HEXADECANOIC ACID, 14-METHYL-, METHYL ESTER	284	C18H36O2	2490-49-5
18	693	672	NONADECANOIC ACID, METHYL ESTER	312	C20H40O2	1731-94-8
19	691	682	HENEICOSANOIC ACID, METHYL ESTER	340	C22H44O2	6064-90-0
20	691	666	TETRACOSANOIC ACID, METHYL ESTER	382	C25H50O2	2442-49-1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Hit	REV	for	Compound Name	M.W.	Formula	CAS
1	558	556	CYCLOPENTANEUNDECANOIC ACID, METHYL ESTER	268	C17H32O2	25779-85-5
2	532	528	METHYL 11-CYCLOHEXYLUNDECANOATE	282	C18H34O2	900336-34-
3	506	506	METHYL 16-HYDROXY-HEXADECANOATE	286	C17H34O3	900336-41-
4	471	471	METHYL 11-HEXADECENOATE	268	C17H32O2	900336-35-
5	471	471	METHYL TRANS-9-(2-BUTYLCYCLOPENTYL)NONANOATE	296	C19H36O2	108708-61-
6	453	453	13-OCTADECENOIC ACID, METHYL ESTER	296	C19H36O2	56554-47-3
7	443	443	METHYL 19-HEXACOSENOATE	408	C27H52O2	900336-27-
8	439	439	OXIRANEOCTANOIC ACID, 3-OCTYL-, METHYL ESTER	312	C19H36O3	2500-59-6
9	435	433	10-UNDECENOIC ACID, METHYL ESTER	198	C12H22O2	111-81-9
10	435	435	METHYL 17-HEXACOSENOATE	408	C27H52O2	900336-27-
11	434	434	7-NONENOIC ACID, METHYL ESTER	170	C10H18O2	20731-22-0
12	428	428	11-DODECENOIC ACID, METHYL ESTER	212	C13H24O2	29972-79-0
13	425	425	16-OCTADECENOIC ACID, METHYL ESTER	296	C19H36O2	56554-49-5
14	424	424	METHYL 9-TETRADECENOATE	240	C15H28O2	900336-47-
15	422	422	9-HEXADECENOIC ACID, METHYL ESTER, (Z)-	268	C17H32O2	1120-25-8
16	422	422	METHYL 13-EICOSENOATE	324	C21H40O2	900336-48-
17	419	419	METHYL 9-HEPTADECENOATE OR 9-17:1	282	C18H34O2	900336-38-
18	419	419	13-METHYLTETRADEC-9-ENOIC ACID METHYL ESTER	254	C16H30O2	900365-89-
19	419	419	14-METHYLPENTADEC-9-ENOIC ACID METHYL ESTER	268	C17H32O2	900365-89-
20	418	416	METHYL CYCLOHEXANEPROPIONATE	170	C10H18O2	20681-51-0

Hit	REV	for	Compound Name	M.W.	Formula	CAS
1	611	586	TETRADECANOIC ACID, 12-METHYL-, METHYL ESTER, (S)-	256	C16H32O2	62691-05-8
2	597	597	CYCLOPENTANEUNDECANOIC ACID, METHYL ESTER	268	C17H32O2	25779-85-5
3	595	571	OCTADECANOIC ACID, 11-METHYL-, METHYL ESTER	312	C20H40O2	74384-77-8
4	593	577	METHYL 11-METHYL-DODECANOATE	228	C14H28O2	900336-45-
5	592	588	METHYL 8-METHYL-NONANOATE	186	C11H22O2	900336-43-
6	564	554	TRIDECANOIC ACID, 12-METHYL-, METHYL ESTER	242	C15H30O2	5129-58-8
7	558	547	METHYL 11-CYCLOHEXYLUNDECANOATE	282	C18H34O2	900336-34-
8	548	527	HEPTACOSANOIC ACID, 25-METHYL-, METHYL ESTER	438	C29H58O2	900112-14-
9	530	519	METHYL 18-FLUORO-OCTADECANOATE	316	C19H37O2F	900336-47-
10	527	522	METHYL 9-METHYLTETRADECANOATE	256	C16H32O2	213617-69-
11	517	517	CYCLOPENTANETRIDECANOIC ACID, METHYL ESTER	296	C19H36O2	24828-61-3
12	516	505	METHYL 8-METHYL-DECANOATE	200	C12H24O2	900336-49-
13	506	501	HEXADECANOIC ACID, 15-METHYL-, METHYL ESTER	284	C18H36O2	6929-04-0
14	503	498	16-HEXADECANOYL HYDRAZIDE	270	C16H34ON2	2619-88-7
15	503	499	PENTADECANOIC ACID, 14-METHYL-, METHYL ESTER	270	C17H34O2	5129-60-2
16	497	497	METHYL TRANS-9-(2-BUTYLCYCLOPENTYL)NONANOATE	296	C19H36O2	108708-61-
17	493	493	METHYL 11-HEXADECENOATE	268	C17H32O2	900336-35-
18	486	473	HEPTADECANOIC ACID, 16-METHYL-, METHYL ESTER	298	C19H38O2	5129-61-3
19	480	474	HEPTADECANOIC ACID, 16-METHYL-, METHYL ESTER	298	C19H38O2	5129-61-3
20	477	472	HENEICOSANOIC ACID, METHYL ESTER	340	C22H44O2	6064-90-0

Hit	REV	for	Compound Name	M.W.	Formula	CAS
1	704	701	METHYL 11-HEXADECENOATE	268	C17H32O2	900336-35-
2	695	693	13-OCTADECENOIC ACID, METHYL ESTER	296	C19H36O2	56554-47-3
3	684	683	METHYL 11-DOCOSENOATE	352	C23H44O2	900336-23-
4	683	683	METHYL 9-EICOSENOATE	324	C21H40O2	900336-50-
5	682	680	METHYL 13-EICOSENOATE	324	C21H40O2	900336-48-
6	675	672	METHYL 9-HEPTADECENOATE OR 9-17:1	282	C18H34O2	900336-38-
7	667	666	14-METHYLPENTADEC-9-ENOIC ACID METHYL ESTER	268	C17H32O2	900365-89-
8	662	660	13-METHYLTETRADEC-9-ENOIC ACID METHYL ESTER	254	C16H30O2	900365-89-
9	659	658	METHYL 12-HYDROXY-9-OCTADECENOATE	312	C19H36O3	900336-28-
10	655	651	11-METHYLOCTADEC-12-ENOIC ACID, METHYL ESTER	310	C20H38O2	900370-42-
11	653	643	METHYL 9-TETRADECENOATE	240	C15H28O2	900336-47-
12	652	649	9-HEXADECENOIC ACID, METHYL ESTER, (Z)-	268	C17H32O2	1120-25-8
13	648	633	METHYL 17-HEXACOSENOATE	408	C27H52O2	900336-27-
14	647	643	METHYL TRANS-9-(2-BUTYLCYCLOPENTYL)NONANOATE	296	C19H36O2	108708-61-
15	645	643	TRANS-13-OCTADECENOIC ACID, METHYL ESTER	296	C19H36O2	900333-61-
16	635	635	9-OCTADECENOIC ACID (Z)-, METHYL ESTER	296	C19H36O2	112-62-9
17	633	631	11-OCTADECENOIC ACID, METHYL ESTER	296	C19H36O2	52380-33-3
18	630	626	11-EICOSENOIC ACID, METHYL ESTER	324	C21H40O2	3946-08-5
19	627	613	METHYL 16-PENTACOSENOATE	394	C26H50O2	900336-24-
20	624	621	METHYL 8-HEPTADECENOATE	282	C18H34O2	900336-36-

Hit	REV	for	Compound Name	M.W.	Formula	CAS
1	924	669	TETRADECANOIC ACID, 10,13-DIMETHYL-, METHYL ESTER	270	C17H34O2	267650-23-
2	917	827	METHYL 11-METHYL-DODECANOATE	228	C14H28O2	900336-45-
3	912	828	TRIDECANOIC ACID, 12-METHYL-, METHYL ESTER	242	C15H30O2	5129-58-8
4	904	800	METHYL 8-METHYL-NONANOATE	186	C11H22O2	900336-43-
5	902	822	UNDECANOIC ACID, 10-METHYL-, METHYL ESTER	214	C13H26O2	5129-56-6
6	868	818	METHYL 13-METHYLTETRADECANOATE	256	C16H32O2	900336-31-
7	855	765	METHYL 8-METHYL-DECANOATE	200	C12H24O2	900336-49-
8	851	789	TRIDECANOIC ACID, METHYL ESTER	228	C14H28O2	1731-88-0
9	850	813	HEXADECANOIC ACID, METHYL ESTER	270	C17H34O2	112-39-0
10	849	726	METHYL 18-FLUORO-OCTADECANOATE	316	C19H37O2F	900336-47-
11	835	767	HEXADECANOIC ACID, 15-METHYL-, METHYL ESTER	284	C18H36O2	6929-04-0
12	835	806	HEXADECANOIC ACID, METHYL ESTER	270	C17H34O2	112-39-0
13	835	758	TRIDECANOIC ACID, METHYL ESTER	228	C14H28O2	1731-88-0
14	834	779	METHYL 9-METHYLTETRADECANOATE	256	C16H32O2	213617-69-
15	833	736	HEPTADECANOIC ACID, 16-METHYL-, METHYL ESTER	298	C19H38O2	5129-61-3
16	832	655	HEPTACOSANOIC ACID, 25-METHYL-, METHYL ESTER	438	C29H58O2	900112-14-
17	825	780	PENTADECANOIC ACID, METHYL ESTER	256	C16H32O2	7132-64-1
18	822	750	HEXADECANOIC ACID, 14-METHYL-, METHYL ESTER	284	C18H36O2	2490-49-5
19	822	738	METHYL STEARATE	298	C19H38O2	112-61-8
20	822	746	METHYL TETRADECANOATE	242	C15H30O2	124-10-7

Hit	REV	for	Compound Name	M.W.	Formula	CAS
1	853	831	METHYL 8-METHYL-NONANOATE	186	C11H22O2	900336-43-
2	842	788	METHYL 11-METHYL-DODECANOATE	228	C14H28O2	900336-45-
3	823	787	TRIDECANOIC ACID, 12-METHYL-, METHYL ESTER	242	C15H30O2	5129-58-8
4	819	725	HEPTACOSANOIC ACID, 25-METHYL-, METHYL ESTER	438	C29H58O2	900112-14-
5	812	769	UNDECANOIC ACID, 10-METHYL-, METHYL ESTER	214	C13H26O2	5129-56-6
6	803	749	METHYL 18-FLUORO-OCTADECANOATE	316	C19H37O2F	900336-47-
7	799	765	METHYL 8-METHYL-DECANOATE	200	C12H24O2	900336-49-
8	771	748	METHYL 9-METHYLTETRADECANOATE	256	C16H32O2	213617-69-
9	763	714	HEPTADECANOIC ACID, 16-METHYL-, METHYL ESTER	298	C19H38O2	5129-61-3
10	763	738	METHYL 13-METHYLTETRADECANOATE	256	C16H32O2	900336-31-
11	756	692	TRIACONTANOIC ACID, METHYL ESTER	466	C31H62O2	629-33-4
12	756	712	HEXADECANOIC ACID, METHYL ESTER	270	C17H34O2	112-39-0
13	752	719	METHYL STEARATE	298	C19H38O2	112-61-8
14	750	714	HEXADECANOIC ACID, 14-METHYL-, METHYL ESTER	284	C18H36O2	2490-49-5
15	744	718	PENTADECANOIC ACID, METHYL ESTER	256	C16H32O2	7132-64-1
16	740	677	OCTACOSANOIC ACID, METHYL ESTER	438	C29H58O2	55682-92-3
17	739	705	METHYL 14-METHYL-EICOSANOATE	340	C22H44O2	900336-23-
18	739	726	HEXADECANOIC ACID, 15-METHYL-, METHYL ESTER	284	C18H36O2	6929-04-0
19	739	695	NONADECANOIC ACID, METHYL ESTER	312	C20H40O2	1731-94-8
20	738	708	HEPTADECANOIC ACID, 16-METHYL-, METHYL ESTER	298	C19H38O2	5129-61-3

Hit	REV	for	Compound Name	M.W.	Formula	CAS
1	853	831	METHYL 8-METHYL-NONANOATE	186	C11H22O2	900336-43-
2	842	788	METHYL 11-METHYL-DODECANOATE	228	C14H28O2	900336-45-
3	823	787	TRIDECANOIC ACID, 12-METHYL-, METHYL ESTER	242	C15H30O2	5129-58-8
4	819	725	HEPTACOSANOIC ACID, 25-METHYL-, METHYL ESTER	438	C29H58O2	900112-14-
5	812	769	UNDECANOIC ACID, 10-METHYL-, METHYL ESTER	214	C13H26O2	5129-56-6
6	803	749	METHYL 18-FLUORO-OCTADECANOATE	316	C19H37O2F	900336-47-
7	799	765	METHYL 8-METHYL-DECANOATE	200	C12H24O2	900336-49-
8	771	748	METHYL 9-METHYLTETRADECANOATE	256	C16H32O2	213617-69-
9	763	714	HEPTADECANOIC ACID, 16-METHYL-, METHYL ESTER	298	C19H38O2	5129-61-3
10	763	738	METHYL 13-METHYLTETRADECANOATE	256	C16H32O2	900336-31-
11	756	692	TRIACONTANOIC ACID, METHYL ESTER	466	C31H62O2	629-83-4
12	756	712	HEXADECANOIC ACID, METHYL ESTER	270	C17H34O2	112-39-0
13	752	719	METHYL STEARATE	298	C19H38O2	112-61-8
14	750	714	HEXADECANOIC ACID, 14-METHYL-, METHYL ESTER	284	C18H36O2	2490-49-5
15	744	718	PENTADECANOIC ACID, METHYL ESTER	256	C16H32O2	7132-64-1
16	740	677	OCTACOSANOIC ACID, METHYL ESTER	438	C29H58O2	55682-92-3
17	739	705	METHYL 14-METHYL-EICOSANOATE	340	C22H44O2	900336-23-
18	739	726	HEXADECANOIC ACID, 15-METHYL-, METHYL ESTER	284	C18H36O2	6929-04-0
19	739	695	NONADECANOIC ACID, METHYL ESTER	312	C20H40O2	1731-94-8
20	738	708	HEPTADECANOIC ACID, 16-METHYL-, METHYL ESTER	298	C19H38O2	5129-61-3

Hit	REV	for	Compound Name	M.W.	Formula	CAS
1	625	590	TETRADECANOIC ACID, 12-METHYL-, METHYL ESTER, (S)-	256	C16H32O2	62691-05-8
2	605	579	METHYL 11-METHYL-DODECANOATE	228	C14H28O2	90033[illegible]-45-
3	604	570	OCTADECANOIC ACID, 11-METHYL-, METHYL ESTER	312	C20H40O2	74484-77-8
4	596	591	METHYL 8-METHYL-NONANOATE	186	C11H22O2	90033[illegible]-43-
5	590	587	CYCLOPENTANEUNDECANOIC ACID, METHYL ESTER	268	C17H32O2	25779-35-5
6	585	580	UNDECANOIC ACID, 11-BROMO-, METHYL ESTER	278	C12H23O2Br	6287-90-7
7	583	579	TRIDECANOIC ACID, 12-METHYL-, METHYL ESTER	242	C15H30O2	5129-58-8
8	560	529	HEPTACOSANOIC ACID, 25-METHYL-, METHYL ESTER	438	C29H58O2	900112-14-
9	553	532	UNDECANOIC ACID, 10-METHYL-, METHYL ESTER	214	C13H26O2	5129-56-6
10	553	527	METHYL 11-CYCLOHEXYLUNDECANOATE	282	C18H34O2	900336-34-
11	541	537	PENTADECANOIC ACID, METHYL ESTER	256	C16H32O2	7132-64-1
12	534	530	METHYL 16-HYDROXY-HEXADECANOATE	286	C17H34O3	900336-41-
13	532	306	2-BUTENOIC ACID, CYCLOHEXYL ESTER	168	C10H16O2	16491-62-6
14	531	511	METHYL 18-FLUORO-OCTADECANOATE	316	C19H37O2F	900336-47-
15	528	519	METHYL 9-METHYLTETRADECANOATE	256	C16H32O2	213617-69-
16	527	525	CYCLOPENTANEUNDECANOIC ACID, METHYL ESTER	268	C17H32O2	25779-55-5
17	524	504	HEXADECANOIC ACID, METHYL ESTER	270	C17H34O2	112-39-0
18	521	516	METHYL STEARATE	298	C19H38O2	112-61-8
19	521	517	HEXADECANOIC ACID, 15-METHYL-, METHYL ESTER	284	C18H36O2	6929-04-0
20	520	517	METHYL 6-METHYLOCTANOATE	172	C10H20O2	5129-62-4

Hit	REV	for	Compound Name	M.W.	Formula	CAS
1	652	650	METHYL 16-HYDROXY-HEXADECANOATE	286	C17H34O3	900336-41-
2	634	629	CYCLOPENTANEUNDECANOIC ACID, METHYL ESTER	268	C17H32O2	25779-85-5
3	623	623	METHYL 11-HEXADECENOATE	268	C17H32O2	900336-35-
4	613	613	13-OCTADECENOIC ACID, METHYL ESTER	296	C19H36O2	56554-47-3
5	607	607	METHYL TRANS-9-(2-BUTYLCYCLOPENTYL)NONANOATE	296	C19H36O2	108708-61-
6	597	562	METHYL 11-CYCLOHEXYLUNDECANOATE	282	C18H34O2	900336-34-
7	593	590	METHYL 19-HEXACOSENOATE	408	C27H52O2	900336-27-
8	591	588	METHYL 17-HEXACOSENOATE	408	C27H52O2	900336-27-
9	588	588	13-METHYLTETRADEC-9-ENOIC ACID METHYL ESTER	254	C16H30O2	900369-89-
10	586	586	METHYL 13-EICOSENOATE	324	C21H40O2	900336-48-
11	586	586	METHYL 9-HEPTADECENOATE OR 9-17:1	282	C18H34O2	900336-38-
12	586	586	14-METHYLPENTADEC-9-ENOIC ACID METHYL ESTER	268	C17H32O2	900369-89-
13	578	578	METHYL 11-DOCOSENOATE	352	C23H44O2	900336-23-
14	571	571	METHYL 9-EICOSENOATE	324	C21H40O2	900336-50-
15	568	567	METHYL 9-TETRADECENOATE	240	C15H28O2	900336-47-
16	565	562	METHYL 16-PENTACOSENOATE	394	C26H50O2	900336-24-
17	564	562	9-HEXADECENOIC ACID, METHYL ESTER, (Z)-	268	C17H32O2	1120-25-8
18	564	564	METHYL 12-HYDROXY-9-OCTADECENOATE	312	C19H36O3	900336-28-
19	562	562	16-OCTADECENOIC ACID, METHYL ESTER	296	C19H36O2	56554-49-5
20	559	553	DOCOSANEDIOIC ACID, DIMETHYL ESTER	398	C24H46O4	22399-98-0

Hit	REV	for	Compound Name	M.W.	Formula	CAS
1	683	591	2-N-HEXYLTHIOLANE, S,S-DIOXIDE	204	C10H20O2S	71053-04-8
2	672	581	3-N-HEXYLTHIOLANE, S,S-DIOXIDE	204	C10H20O2S	71053-07-1
3	661	661	13-OCTADECENOIC ACID, METHYL ESTER	296	C19H36O2	56554-47-3
4	660	657	METHYL 11-HEXADECENOATE	268	C17H32O2	900336-35-
5	651	645	11-METHYLOCTADEC-12-ENOIC ACID, METHYL ESTER	310	C20H38O2	900370-42-
6	646	644	METHYL 17-HEXACOSENOATE	408	C27H52O2	900336-27-
7	636	636	13-METHYLTETRADEC-9-ENOIC ACID METHYL ESTER	254	C16H30O2	900365-89-
8	634	632	METHYL 13-EICOSENOATE	324	C21H40O2	900336-48-
9	633	547	4-N-HEXYLTHIANE, S,S-DIOXIDE	218	C11H22O2S	70928-62-8
10	626	624	METHYL 11-DOCOSENOATE	352	C23H44O2	900336-23-
11	623	621	METHYL 9-HEPTADECENOATE OR 9-17:1	282	C18H34O2	900336-38-
12	621	620	14-METHYLPENTADEC-9-ENOIC ACID METHYL ESTER	268	C17H32O2	900365-89-
13	619	527	BICYCLO[3.1.1]HEPTAN-3-ONE, 2,6,6-TRIMETHYL-, (1.ALPHA.,2.ALPHA.,5.ALPHA.)-	152	C10H16O	547-60-4
14	617	615	METHYL 9-EICOSENOATE	324	C21H40O2	900336-50-
15	606	604	9-HEXADECENOIC ACID, METHYL ESTER, (Z)-	268	C17H32O2	1120-25-8
16	606	526	N-PROPYL 11-OCTADECENOATE	324	C21H40O2	900336-71-
17	602	602	TRANS-13-OCTADECENOIC ACID, METHYL ESTER	296	C19H36O2	900333-61-
18	591	589	METHYL 12-HYDROXY-9-OCTADECENOATE	312	C19H36O3	900336-28-
19	589	478	(.+/-.)-5-CHLORO-2-(2-PROPENYL)PENTANAL	160	C8H13OCl	79228-14-1
20	583	577	OXIRANEOCTANOIC ACID, 3-OCTYL-, METHYL ESTER	312	C19H36O3	2500-59-6

Hit	REV	for	Compound Name	M.W.	Formula	CAS
1	923	655	TETRADECANOIC ACID, 10,13-DIMETHYL-, METHYL ESTER	270	C17H34O2	267650-23-
2	913	817	METHYL 11-METHYL-DODECANOATE	228	C14H28O2	900336-45-
3	907	816	TRIDECANOIC ACID, 12-METHYL-, METHYL ESTER	242	C15H30O2	5129-58-8
4	898	789	METHYL 8-METHYL-NONANOATE	186	C11H22O2	900336-43-
5	896	809	UNDECANOIC ACID, 10-METHYL-, METHYL ESTER	214	C13H26O2	5129-56-6
6	871	823	METHYL 13-METHYLTETRADECANOATE	256	C16H32O2	900336-31-
7	855	731	METHYL 18-FLUORO-OCTADECANOATE	316	C19H37O2F	900336-47-
8	853	757	METHYL 8-METHYL-DECANOATE	200	C12H24O2	900336-49-
9	850	809	HEXADECANOIC ACID, METHYL ESTER	270	C17H34O2	112-39-0
10	844	778	TRIDECANOIC ACID, METHYL ESTER	228	C14H28O2	1731-88-0
11	840	790	METHYL 9-METHYLTETRADECANOATE	256	C16H32O2	213617-69-
12	837	769	HEXADECANOIC ACID, 15-METHYL-, METHYL ESTER	284	C18H36O2	6929-04-0
13	836	741	HEPTADECANOIC ACID, 16-METHYL-, METHYL ESTER	298	C19H38O2	5129-61-3
14	836	653	HEPTACOSANOIC ACID, 25-METHYL-, METHYL ESTER	438	C29H58O2	900112-14-
15	835	804	HEXADECANOIC ACID, METHYL ESTER	270	C17H34O2	112-39-0
16	832	736	CYCLOPENTANEUNDECANOIC ACID, METHYL ESTER	268	C17H32O2	25779-85-5
17	830	748	TRIDECANOIC ACID, METHYL ESTER	228	C14H28O2	1731-88-0
18	827	752	HEXADECANOIC ACID, 14-METHYL-, METHYL ESTER	284	C18H36O2	2490-49-5
19	825	783	PENTADECANOIC ACID, METHYL ESTER	256	C16H32O2	7132-64-1
20	823	748	METHYL TETRADECANOATE	242	C15H30O2	124-10-7

Hit	REV	for	Compound Name	M.W.	Formula	CAS
1	863	828	METHYL 8-METHYL-NONANOATE	186	C11H22O2	900336-43-
2	852	782	METHYL 11-METHYL-DODECANOATE	228	C14H28O2	900336-45-
3	833	775	TRIDECANOIC ACID, 12-METHYL-, METHYL ESTER	242	C15H30O2	5129-58-8
4	824	693	HEPTACOSANOIC ACID, 25-METHYL-, METHYL ESTER	438	C29H58O2	900112-14-
5	822	761	UNDECANOIC ACID, 10-METHYL-, METHYL ESTER	214	C13H26O2	5129-56-6
6	818	771	METHYL 8-METHYL-DECANOATE	200	C12H24O2	900336-49-
7	811	640	OCTADECANOIC ACID, 11-METHYL-, METHYL ESTER	312	C20H40O2	74484-77-8
8	809	728	METHYL 18-FLUORO-OCTADECANOATE	316	C19H37O2F	900336-47-
9	798	760	CYCLOPENTANEUNDECANOIC ACID, METHYL ESTER	268	C17H32O2	25779-35-5
10	783	753	METHYL 9-METHYLTETRADECANOATE	256	C16H32O2	213617-69-
11	778	754	METHYL 16-HYDROXY-HEXADECANOATE	286	C17H34O3	900336-41-
12	775	707	HEPTADECANOIC ACID, 16-METHYL-, METHYL ESTER	298	C19H38O2	5129-61-3
13	771	731	METHYL 13-METHYLTETRADECANOATE	256	C16H32O2	900336-31-
14	768	708	HEXADECANOIC ACID, METHYL ESTER	270	C17H34O2	112-39-0
15	762	672	TRIACONTANOIC ACID, METHYL ESTER	466	C31H62O2	629-83-4
16	760	597	TETRADECANOIC ACID, 12-METHYL-, METHYL ESTER, (S)-	256	C16H32O2	62691-05-8
17	759	691	DODECANOIC ACID, 10-METHYL-, METHYL ESTER	228	C14H28O2	5129-60-7
18	759	705	METHYL STEARATE	298	C19H38O2	112-61-8
19	755	705	HEXADECANOIC ACID, 14-METHYL-, METHYL ESTER	284	C18H36O2	2490-49-5
20	753	712	PENTADECANOIC ACID, METHYL ESTER	256	C16H32O2	7132-64-1

c33 sample

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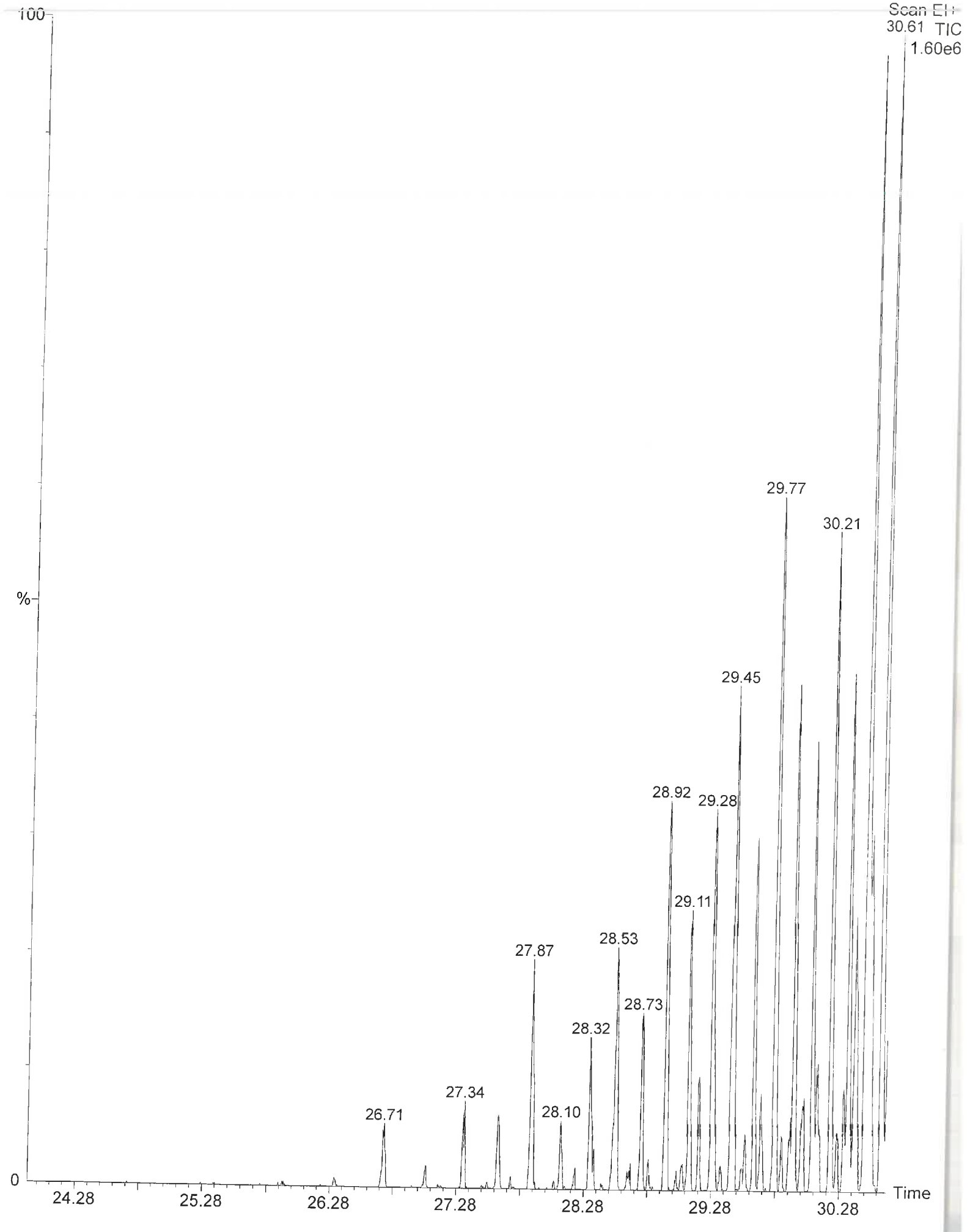


FOR	REV	for	Compound Name	M.W.	Formula	CAS
1	609	607	CYCLOPENTANEUNDECANOIC ACID, METHYL ESTER	268	C17H32O2	25779-85-5
2	587	587	METHYL 16-HYDROXY-HEXADECANOATE	286	C17H34O3	900336-41-
3	583	571	METHYL 11-CYCLOHEXYLUNDECANOATE	282	C18H34O2	900336-34-
4	558	558	METHYL 11-HEXADECENOATE	268	C17H32O2	900336-35-
5	540	540	13-OCTADECENOIC ACID, METHYL ESTER	296	C19H36O2	56554-47-3
6	540	540	METHYL TRANS-9-(2-BUTYLCYCLOPENTYL)NONANOATE	296	C19H36O2	108708-61-
7	516	512	METHYL 19-HEXACOSENOATE	408	C27H52O2	900336-27-
8	513	510	METHYL 17-HEXACOSENOATE	408	C27H52O2	900336-27-
9	511	511	METHYL 13-EICOSENOATE	324	C21H40O2	900336-48-
10	509	509	METHYL 9-HEPTADECENOATE OR 9-17:1	282	C18H34O2	900336-38-
11	502	500	10-UNDECENOIC ACID, METHYL ESTER	198	C12H22O2	111-81-9
12	502	502	9-HEXADECENOIC ACID, METHYL ESTER, (Z)-	268	C17H32O2	1120-25-8
13	500	499	OXIRANEOCTANOIC ACID, 3-OCTYL-, METHYL ESTER	312	C19H36O3	2500-59-6
14	499	499	11-DODECENOIC ACID, METHYL ESTER	212	C13H24O2	29972-79-0
15	499	499	METHYL 11-DOCOSENOATE	352	C23H44O2	900336-23-
16	497	497	16-OCTADECENOIC ACID, METHYL ESTER	296	C19H36O2	56554-49-5
17	495	492	METHYL 9-TETRADECENOATE	240	C15H28O2	900336-47-
18	494	494	METHYL 9-EICOSENOATE	324	C21H40O2	900336-50-
19	493	493	14-METHYLPENTADEC-9-ENOIC ACID METHYL ESTER	268	C17H32O2	900365-89-
20	491	488	DOCOSANEDIOIC ACID, DIMETHYL ESTER	398	C24H46O4	22399-98-0

Hit	REV	for	Compound Name	M.W.	Formula	CAS
1	618	614	CYCLOPENTANEUNDECANOIC ACID, METHYL ESTER	268	C17H32O2	25779-85-5
2	592	572	METHYL 11-CYCLOHEXYLUNDECANOATE	282	C18H34O2	900336-34-
3	587	583	METHYL 16-HYDROXY-HEXADECANOATE	286	C17H34O3	900336-41-
4	545	544	METHYL TRANS-9-(2-BUTYLCYCLOPENTYL)NONANOATE	296	C19H36O2	108708-61-
5	533	528	METHYL 11-HEXADECENOATE	268	C17H32O2	900336-35-
6	530	529	13-OCTADECENOIC ACID, METHYL ESTER	296	C19H36O2	56554-47-3
7	511	508	METHYL 17-HEXACOSENOATE	408	C27H52O2	900336-27-
8	504	500	DOCOSANEDIOIC ACID, DIMETHYL ESTER	398	C24H46O4	22399-98-0
9	504	499	METHYL 19-HEXACOSENOATE	408	C27H52O2	900336-27-
10	498	495	METHYL 13-EICOSENOATE	324	C21H40O2	900336-48-
11	494	492	METHYL 9-HEPTADECENOATE OR 9-17:1	282	C18H34O2	900336-38-
12	493	492	16-OCTADECENOIC ACID, METHYL ESTER	296	C19H36O2	56554-49-5
13	492	486	10-UNDECENOIC ACID, METHYL ESTER	198	C12H22O2	111-81-9
14	489	487	9-HEXADECENOIC ACID, METHYL ESTER, (Z)-	268	C17H32O2	1120-25-8
15	486	482	METHYL 9-TETRADECENOATE	240	C15H28O2	900336-47-
16	485	479	OXIRANEOCTANOIC ACID, 3-OCTYL-, METHYL ESTER	312	C19H36O3	2500-59-6
17	484	482	METHYL 11-DOCOSENOATE	352	C23H44O2	900336-23-
18	484	482	14-METHYLPENTADEC-9-ENOIC ACID METHYL ESTER	268	C17H32O2	900365-89-
19	480	478	METHYL 9-EICOSENOATE	324	C21H40O2	900336-50-
20	480	478	13-METHYLTETRADEC-9-ENOIC ACID METHYL ESTER	254	C16H30O2	900365-89-

Hit	REV	for	Compound Name	M.W.	Formula	CAS
1	636	633	CYCLOPENTANEUNDECANOIC ACID, METHYL ESTER	268	C17H32O2	25779-85-5
2	607	603	METHYL 11-CYCLOHEXYLUNDECANOATE	282	C18H34O2	900336-34-
3	570	569	METHYL 16-HYDROXY-HEXADECANOATE	286	C17H34O3	900336-41-
4	532	532	METHYL TRANS-9-(2-BUTYLCYCLOPENTYL)NONANOATE	296	C19H36O2	108708-61-
5	508	504	METHYL 11-HEXADECENOATE	268	C17H32O2	900336-35-
6	504	500	METHYL CYCLOHEXANEPROPIONATE	170	C10H18O2	20681-51-0
7	502	500	13-OCTADECENOIC ACID, METHYL ESTER	296	C19H36O2	56554-47-3
8	499	497	10-UNDECENOIC ACID, METHYL ESTER	198	C12H22O2	111-81-9
9	490	488	METHYL 17-HEXACOSENOATE	408	C27H52O2	900336-27-
10	485	482	METHYL 19-HEXACOSENOATE	408	C27H52O2	900336-27-
11	484	482	OXIRANEOCTANOIC ACID, 3-OCTYL-, METHYL ESTER	312	C19H36O3	2500-59-6
12	479	479	16-OCTADECENOIC ACID, METHYL ESTER	296	C19H36O2	56554-49-5
13	475	473	METHYL 9-HEPTADECENOATE OR 9-17:1	282	C18H34O2	900336-38-
14	471	470	17-OCTADECYNOIC ACID, METHYL ESTER	294	C19H34O2	900333-54-
15	471	469	METHYL 13-EICOSENOATE	324	C21H40O2	900336-48-
16	469	465	METHYL 9-TETRADECENOATE	240	C15H28O2	900336-47-
17	469	466	11-DODECENOIC ACID, METHYL ESTER	212	C13H24O2	29972-79-0
18	468	466	7-NONENOIC ACID, METHYL ESTER	170	C10H18O2	20731-22-0
19	467	467	15-OCTADECENOIC ACID, METHYL ESTER	296	C19H36O2	4764-72-1
20	465	463	9-HEXADECENOIC ACID, METHYL ESTER, (Z)-	268	C17H32O2	1120-25-8

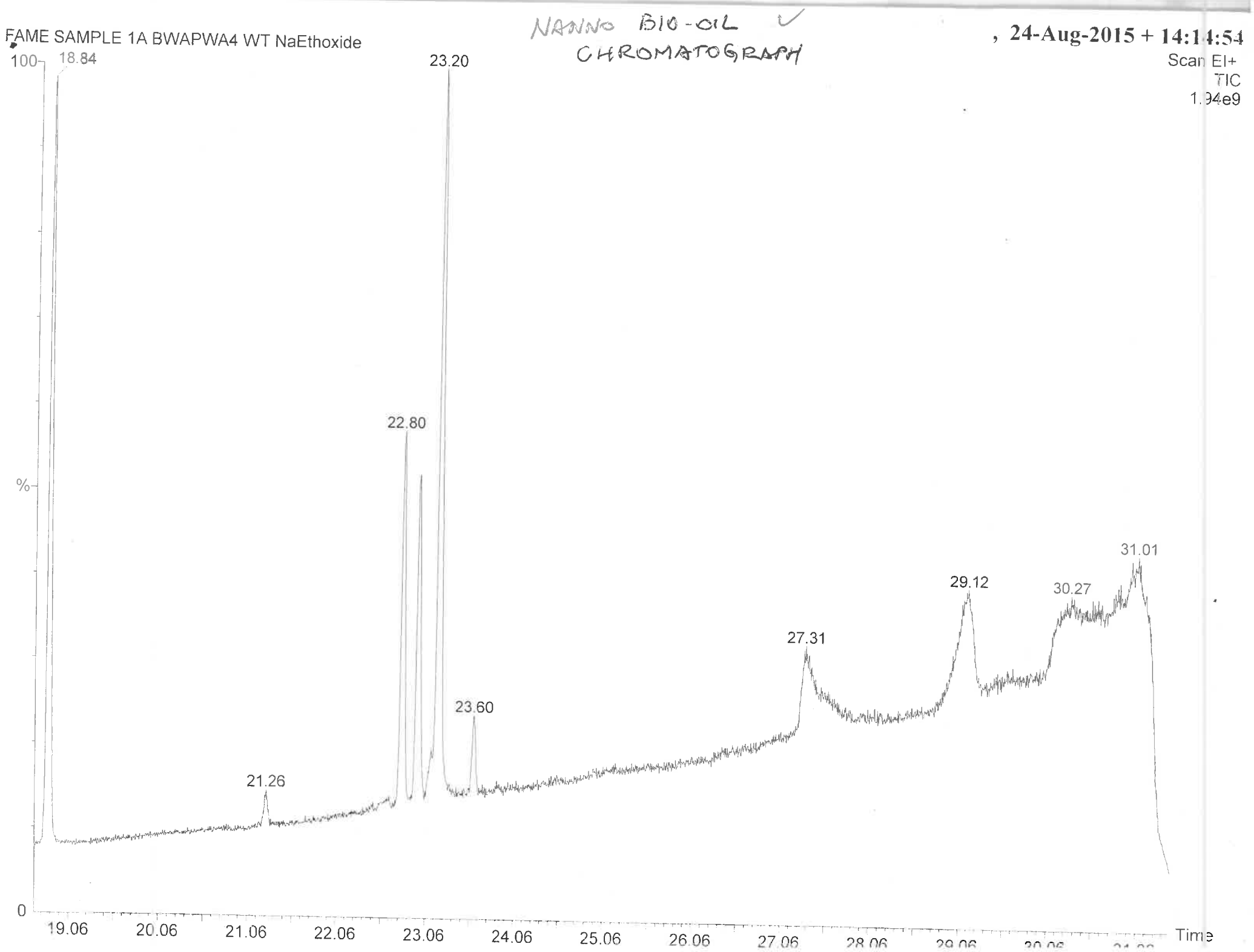
Hit	REV	for	Compound Name	M.W.	Formula	CAS
1	650	644	CYCLOPENTANEUNDECANOIC ACID, METHYL ESTER	268	C17H32O2	25779-85-5
2	647	646	METHYL 16-HYDROXY-HEXADECANOATE	286	C17H34O3	900336-41-
3	623	594	METHYL 11-CYCLOHEXYLUNDECANOATE	282	C18H34O2	900336-34-
4	613	613	13-OCTADECENOIC ACID, METHYL ESTER	296	C19H36O2	56554-47-3
5	608	607	METHYL 11-HEXADECENOATE	268	C17H32O2	900336-35-
6	604	604	METHYL 19-HEXACOSENOATE	408	C27H52O2	900336-27-
7	601	601	METHYL TRANS-9-(2-BUTYLCYCLOPENTYL)NONANOATE	296	C19H36O2	108708-61-
8	600	600	METHYL 17-HEXACOSENOATE	408	C27H52O2	900336-27-
9	581	580	METHYL 9-HEPTADECENOATE OR 9-17:1	282	C18H34O2	900336-38-
10	577	577	METHYL 16-PENTACOSENOATE	394	C26H50O2	900336-24-
11	576	575	METHYL 13-EICOSENOATE	324	C21H40O2	900336-48-
12	573	572	METHYL 9-TETRADECENOATE	240	C15H28O2	900336-47-
13	569	568	METHYL 11-DOCOSENOATE	352	C23H44O2	900336-23-
14	569	568	14-METHYLPENTADEC-9-ENOIC ACID METHYL ESTER	268	C17H32O2	900365-89-
15	566	551	10-UNDECENOIC ACID, METHYL ESTER	198	C12H22O2	111-81-9
16	563	562	METHYL 9-EICOSENOATE	324	C21H40O2	900336-50-
17	563	563	16-OCTADECENOIC ACID, METHYL ESTER	296	C19H36O2	56554-49-5
18	562	561	13-METHYLTETRADEC-9-ENOIC ACID METHYL ESTER	254	C16H30O2	900365-89-
19	556	555	9-HEXADECENOIC ACID, METHYL ESTER, (Z)-	268	C17H32O2	1120-25-8
20	555	555	TRANS-13-OCTADECENOIC ACID, METHYL ESTER	296	C19H36O2	900333-61-

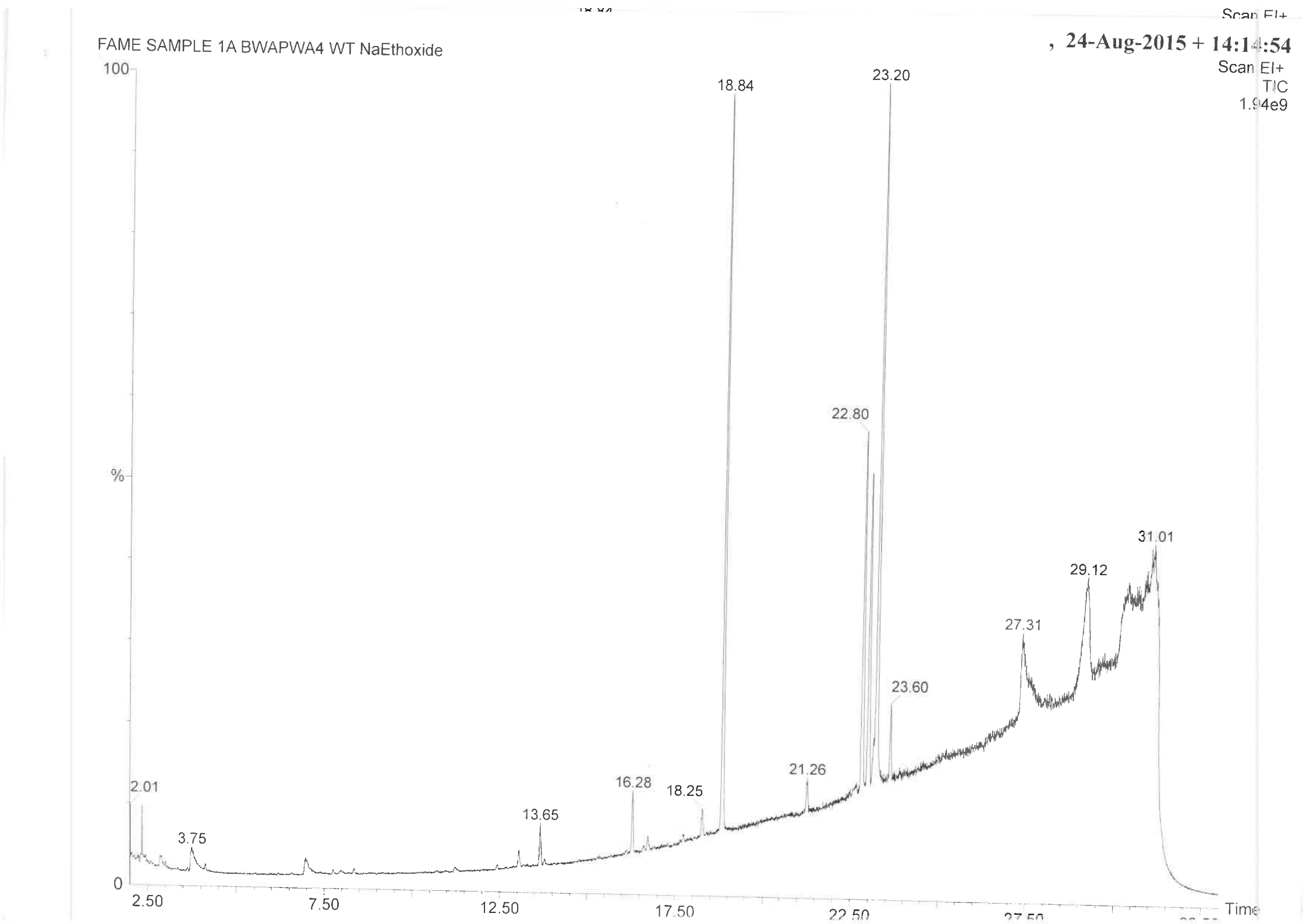
Hit	REV	for	Compound Name	M.W.	Formula	CAS
1	916	666	TETRADECANOIC ACID, 10,13-DIMETHYL-, METHYL ESTER	270	C17H34O2	267650-23-
2	912	821	METHYL 11-METHYL-DODECANOATE	228	C14H28O2	900336-45-
3	903	821	TRIDECANOIC ACID, 12-METHYL-, METHYL ESTER	242	C15H30O2	5129-58-8
4	900	798	METHYL 8-METHYL-NONANOATE	186	C11H22O2	900336-43-
5	899	817	UNDECANOIC ACID, 10-METHYL-, METHYL ESTER	214	C13H26O2	5129-56-6
6	850	758	METHYL 8-METHYL-DECANOATE	200	C12H24O2	900336-49-
7	847	730	METHYL 18-FLUORO-OCTADECANOATE	316	C19H37O2F	900336-47-
8	846	784	TRIDECANOIC ACID, METHYL ESTER	228	C14H28O2	1731-88-0
9	845	797	METHYL 13-METHYLTETRADECANOATE	256	C16H32O2	900336-31-
10	843	808	HEXADECANOIC ACID, METHYL ESTER	270	C17H34O2	112-39-0
11	832	754	TRIDECANOIC ACID, METHYL ESTER	228	C14H28O2	1731-88-0
12	829	801	HEXADECANOIC ACID, METHYL ESTER	270	C17H34O2	112-39-0
13	828	666	HEPTACOSANOIC ACID, 25-METHYL-, METHYL ESTER	438	C29H58O2	900112-14-
14	825	728	HEPTADECANOIC ACID, 16-METHYL-, METHYL ESTER	298	C19H38O2	5129-61-3
15	822	750	DODECANOIC ACID, METHYL ESTER	214	C13H26O2	111-82-0
16	819	755	HEXADECANOIC ACID, 15-METHYL-, METHYL ESTER	284	C18H36O2	6929-04-0
17	817	766	METHYL 9-METHYLTETRADECANOATE	256	C16H32O2	213617-69-
18	817	730	CYCLOPENTANEUNDECANOIC ACID, METHYL ESTER	268	C17H32O2	25779-85-5
19	815	734	METHYL STEARATE	298	C19H38O2	112-61-8
20	813	742	METHYL TETRADECANOATE	242	C15H30O2	124-10-7

Hit	REV	for	Compound Name	M.W.	Formula	CAS
1	842	819	METHYL 8-METHYL-NONANOATE	186	C11H22O2	900336-43-
2	823	769	METHYL 11-METHYL-DODECANOATE	228	C14H28O2	900336-45-
3	806	764	TRIDECANOIC ACID, 12-METHYL-, METHYL ESTER	242	C15H30O2	5129-58-8
4	804	710	HEPTACOSANOIC ACID, 25-METHYL-, METHYL ESTER	438	C29H58O2	900112-14-
5	790	736	METHYL 18-FLUORO-OCTADECANOATE	316	C19H37O2F	900336-47-
6	789	745	UNDECANOIC ACID, 10-METHYL-, METHYL ESTER	214	C13H26O2	5129-56-6
7	787	751	METHYL 8-METHYL-DECANOATE	200	C12H24O2	900336-49-
8	786	763	CYCLOPENTANEUNDECANOIC ACID, METHYL ESTER	268	C17H32O2	25779-85-5
9	784	649	OCTADECANOIC ACID, 11-METHYL-, METHYL ESTER	312	C20H40O2	74484-77-8
10	755	741	METHYL 16-HYDROXY-HEXADECANOATE	286	C17H34O3	900336-41-
11	751	728	METHYL 9-METHYLTETRADECANOATE	256	C16H32O2	213617-69-
12	751	621	TETRADECANOIC ACID, 12-METHYL-, METHYL ESTER, (S)-	256	C16H32O2	62691-05-8
13	747	718	METHYL 13-METHYLTETRADECANOATE	256	C16H32O2	900336-31-
14	746	701	HEPTADECANOIC ACID, 16-METHYL-, METHYL ESTER	298	C19H38O2	5129-61-3
15	743	679	METHYL 11-CYCLOHEXYLUNDECANOATE	282	C18H34O2	900336-34-
16	739	690	HEXADECANOIC ACID, METHYL ESTER	270	C17H34O2	112-39-0
17	738	675	TRIACONTANOIC ACID, METHYL ESTER	466	C31H62O2	629-83-4
18	735	697	METHYL STEARATE	298	C19H38O2	112-61-8
19	732	694	HEXADECANOIC ACID, 14-METHYL-, METHYL ESTER	284	C18H36O2	2490-49-5
20	731	698	PENTADECANOIC ACID, METHYL ESTER	256	C16H32O2	7132-64-1

APPENDIX A3

NANNOCHLOROPSIS BIO-OIL CHROMATOGRAPHS


FAME SAMPLE 1A BWAPWA4 WT NaEthoxide
NANNO BIO-OIL
CHROMATOGRAPH
, 24-Aug-2015 + 14:14:54
Scan EI+
TIC
1.94e9
100
%
0
18.84
23.20
22.80
23.60
21.26
27.31
29.12
30.27
31.01
19.06
20.06
21.06
22.06
23.06
24.06
25.06
26.06
27.06
28.06
Time


FAME SAMPLE 1A BWAPWA4 WT NaEthoxide
, 24-Aug-2015 + 14:14:54
Scan EI+
TIC
1.94e9
100
%
0
18.84
23.20
22.80
31.01
29.12
27.31
23.60
21.26
16.28
18.25
13.65
3.75
2.01
2.50
7.50
12.50
17.50
22.50
Time

FAME SAMPLE 1 BWAPWA WT NaEthoxide

Hit	REV	for	Compound Name	M.W.	Formula	CAS
1	929	661	TETRADECANOIC ACID, 10,13-DIMETHYL-, METHYL ESTER	270	C17H34O2	2676[illegible]50-23-
2	916	820	TRIDECANOIC ACID, 12-METHYL-, METHYL ESTER	242	C15H30O2	5129[illegible]-58-8
3	909	801	METHYL 11-METHYL-DODECANOATE	228	C14H28O2	900[illegible]36-45-
4	893	811	METHYL 13-METHYLTETRADECANOATE	256	C16H32O2	900[illegible]36-31-
5	867	816	HEXADECANOIC ACID, METHYL ESTER	270	C17H34O2	112-[illegible]39-0
6	865	772	HEXADECANOIC ACID, 15-METHYL-, METHYL ESTER	284	C18H36O2	6929[illegible]-04-0
7	862	741	DODECANOIC ACID, 10-METHYL-, METHYL ESTER	228	C14H28O2	5129[illegible]-65-7
8	850	771	TRIDECANOIC ACID, METHYL ESTER	228	C14H28O2	1731[illegible]-88-0
9	835	672	METHYL 18-FLUORO-OCTADECANOATE	316	C19H37O2F	900[illegible]36-47-
10	835	750	TRIDECANOIC ACID, METHYL ESTER	228	C14H28O2	1731[illegible]-88-0
11	832	728	HEXADECANOIC ACID, 14-METHYL-, METHYL ESTER	284	C18H36O2	2490[illegible]-49-5
12	832	787	HEXADECANOIC ACID, METHYL ESTER	270	C17H34O2	112-[illegible]39-0
13	832	732	METHYL TETRADECANOATE	242	C15H30O2	124-[illegible]10-7
14	826	777	HEXADECANOIC ACID, METHYL ESTER	270	C17H34O2	112-[illegible]39-0
15	823	728	HEPTADECANOIC ACID, 16-METHYL-, METHYL ESTER	298	C19H38O2	5129[illegible]-31-3
16	821	770	PENTADECANOIC ACID, 14-METHYL-, METHYL ESTER	270	C17H34O2	5129[illegible]-30-2
17	813	721	HEPTADECANOIC ACID, METHYL ESTER	284	C18H36O2	1731[illegible]-92-6
18	809	701	METHYL 20-METHYL-HENEICOSANOATE	354	C23H46O2	900[illegible]36-47-
19	806	702	METHYL TETRADECANOATE	242	C15H30O2	124-[illegible]10-7
20	799	691	METHYL STEARATE	298	C19H38O2	112-[illegible]61-8

FAME SAMPLE 1 BWAPWA WT NaEthoxide

Hit	REV	for	Compound Name	M.W.	Formula	CAS
1	898	834	13-OCTADECENOIC ACID, METHYL ESTER	296	C19H36O2	56554-47-3
2	893	743	6-OCTADECENOIC ACID	282	C18H34O2	900336-66-
3	886	743	N-PROPYL 11-OCTADECENOATE	324	C21H40O2	900336-71-
4	871	697	N-PROPYL 9-OCTADECENOATE	324	C21H40O2	900336-71-
5	869	708	2-CHLOROETHYL OLEATE	344	C20H37O2Cl	51479-39-1
6	861	770	9-OCTADECENOIC ACID, METHYL ESTER, (E)-	296	C19H36O2	1937-62-8
7	845	701	OLEIC ANHYDRIDE	546	C36H66O3	24909-72-6
8	843	777	11-OCTADECENOIC ACID, METHYL ESTER	296	C19H36O2	52380-33-3
9	837	726	9-OCTADECENOIC ACID (Z)-, METHYL ESTER	296	C19H36O2	112-62-9
10	836	756	9-OCTADECENOIC ACID, METHYL ESTER, (E)-	296	C19H36O2	1937-62-8
11	834	707	9-OCTADECENOIC ACID, (E)-	282	C18H34O2	112-79-8
12	831	677	2,3-DIHYDROXYPROPYL ELAIDATE	356	C21H40O4	2716-53-2
13	830	704	OLEIC ACID	282	C18H34O2	112-80-1
14	829	705	13-DOCOSENOIC ACID, METHYL ESTER	352	C23H44O2	56630-69-4
15	825	708	CIS-13-OCTADECENOIC ACID, METHYL ESTER	296	C19H36O2	900333-58-
16	825	761	TRANS-13-OCTADECENOIC ACID, METHYL ESTER	296	C19H36O2	900333-61-
17	824	634	BUTYL 9-OCTADECENOATE OR 9-18:1	338	C22H42O2	900336-74-
18	819	685	11-EICOSENOIC ACID, METHYL ESTER	324	C21H40O2	3946-08-5
19	819	726	13-METHYLTETRADEC-9-ENOIC ACID METHYL ESTER	254	C16H30O2	900365-89-
20	818	754	11-OCTADECENOIC ACID, METHYL ESTER	296	C19H36O2	52380-33-3

FAME SAMPLE 1 BWAPWA WT NaEthoxide

Hit	REV	for	Compound Name	M.W.	Formula	CAS
8	859	792	TRIDECANOIC ACID, METHYL ESTER	228	C14H28O2	173 -88-0
9	850	770	HEXADECANOIC ACID, 15-METHYL-, METHYL ESTER	284	C18H36O2	6929-04-0
10	843	769	METHYL 9-METHYLTETRADECANOATE	256	C16H32O2	213617-69-
11	843	696	METHYL 18-FLUORO-OCTADECANOATE	316	C19H37O2F	900336-47-
12	841	766	TRIDECANOIC ACID, METHYL ESTER	228	C14H28O2	1731-88-0
13	840	742	METHYL 8-METHYL-DECANOATE	200	C12H24O2	900336-49-
14	840	638	HEPTACOSANOIC ACID, 25-METHYL-, METHYL ESTER	438	C29H58O2	900112-14-
15	836	798	HEXADECANOIC ACID, METHYL ESTER	270	C17H34O2	112-39-0
16	834	751	HEXADECANOIC ACID, 14-METHYL-, METHYL ESTER	284	C18H36O2	2490-49-5
17	833	768	PENTADECANOIC ACID, METHYL ESTER	256	C16H32O2	7132-64-1
18	829	735	DODECANOIC ACID, METHYL ESTER	214	C13H26O2	111-82-0
19	828	744	METHYL TETRADECANOATE	242	C15H30O2	124-10-7
20	824	734	METHYL STEARATE	298	C19H38O2	112-61-8

APPENDIX A 4

GC METHODS FOR VEGETABLE OILS

GC METHOD

Turbochrom Method File C:\TurboMass\THOBEKA.PRO\ACQUDB\BIODIESEL BWAPWA.mth

Printed by : Administrator on: 1/15/2015 11:15:56 AM
Created by : Administrator on: 12/8/2014 2:14:05 PM
Edited by : Administrator on: 12/10/2014 8:24:39 AM
Number of Times Edited : 4
Number of Times Calibrated : 0
Description: BIODIESEL BWAPWA

Instrument Conditions

Instrument Control Method

Instrument Name : inst1
Instrument Type : PE AutoSystem GC

Channel Parameters

Data will be collected from channel B
Delay Time : 0.00 min
Run Time : 34.00 min
Sampling Rate : 1.5625 pts/s

	Channel A	Channel B
Signal Source	DetA	DetB
Analog Output	INT	INT
Attenuation	0	0
Offset	5.0 mV	5.0 mV

Carriers Parameters

Carrier A control : PFlow - He
Column A length : 30.00 m
Diameter : 250 µm
Vacuum Compensation : ON
Split Flow : 20.0 mL/min
Initial Setpoint : 1.00 ML/MIN
Initial Hold : 999.00 min

Valve configuration and settings

Valve 1 : SPLIT On
Valve 2 : NONE
Valve 3 : NONE
Valve 4 : NONE
Valve 5 : NONE
Valve 6 : NONE

Detector Parameters

	Detector A	Detector B
Detector	NONE	NONE
Range	1	1
Time Constant	200	200
Autozero	ON	ON
Polarity		

1/15/2015 11:15:56 AM Method: C:\TurboMass\THOBEKA.PRO\ACQUDB\BIODIESEL BWAPWA.mth

Heated Zones

Injector A: PSSI
Initial Setpoint : 50°C
Initial Hold : 999.00 min

Injector B: NONE
Setpoint : OFF

Detector A : 0°C
Detector B : 0°C
Auxiliary (NONE) : 0°C

Oven Program

Cryogenics : Off
Initial Temp : 60°C
Initial Hold : 10.00 min
Ramp 1 : 10.0 0/min to 150°, hold for 5.00 min
Ramp 2 : 10.0 0/min to 240°, hold for 1.00 min

Total Run Time : 34.00 min
Maximum Temp : 350°C
Equilibration Time : 2.0 min

Timed Events

There are no timed events in the method

MS METHOD

Experiment Report

Experiment File: c:\turbomass\thobeka.pro\acqudb\default.exp

Printed : Thu Jan 15 11:20:55 2015

Name	Default Experiment
Creation Time	Mon 08 Dec 2014 3:19:51 PM
Instrument Identifier	
Version Number	1.0
Duration (min)	31.0
Calibration Filename	C:\TurboMass\ADSORPTION.PRO\ACQUDB\B Tech chemistry autotune file.cal
Calibration Filename	C:\TurboMass\ADSORPTION.PRO\ACQUDB\B Tech chemistry autotune file.cal
From 0.0 to 2.0 minutes	
Number Of Functions	1

Function 1 : MS Scan, Time 2.00 to 31.00, Mass 50.00 to 300.00 EI+

Type	MS Scan
Ion Mode	EI+
Data Format	Centroid
Start Mass	50.00
End Mass	300.00
Scan Time (sec)	0.20
InterScan Time (sec)	0.10
Start Time (min)	2.00
End Time (min)	31.00

APPENDIX A5

GC METHODS FOR ALGAE BIO-OIL

METHOD GC FOR ALGAE BIO-OIL

Method

[Comment]

=== Analytical Line 1 ===

[GC-2010]

Column Oven Temp.	:50.0 °C
Injection Temp.	:250.00 °C
Injection Mode	:Splitless
Sampling Time	:0.00 min
Flow Control Mode	:Linear Velocity
Pressure	:53.5 kPa
Total Flow	:74.0 mL/min
Column Flow	:1.00 mL/min
Linear Velocity	:36.3 cm/sec
Purge Flow	:3.0 mL/min
Split Ratio	:70.0
High Pressure Injection	:OFF
Carrier Gas Saver	:OFF

Oven Temp. Program

Rate	Temperature(°C)	Hold Time(min)
-	50.0	1.00
4.00	300.0	5.00

< Ready Check Heat Unit >

Column Oven	: Yes
SPL2	: Yes
MS	: Yes

< Ready Check Detector(FTD/BID) >
< Ready Check Baseline Drift >
< Ready Check Injection Flow >

SPL2 Carrier	: Yes
SPL2 Purge	: Yes

< Ready Check APC Flow >
< Ready Check Detector APC Flow >

External Wait	:No
Equilibrium Time	:3.0 min

[GC Program]

[GCMS-QP2010 Ultra]

IonSourceTemp	:250.00 °C
Interface Temp.	:280.00 °C
Solvent Cut Time	:2.50 min
Detector Gain Mode	:Absolute
Detector Gain	:0.80 kV
Threshold	:1000

[MS Table]

--Group 1 - Event 1--

Start Time	:2.50min
End Time	:68.50min
ACQ Mode	:Scan
Event Time	:0.30sec
Scan Speed	:1428
Start m/z	:50.00
End m/z	:450.00

Sample Inlet Unit :GC

[MS Program]

Use MS Program :OFF

APPENDIX A6

CHARACTERISATION DATA FROM WEARCHECK AND CHEMSCIENCE LABORATORIES

First incorporated 1990
380 Umbilo Road, Glenwood, Durban, 4001 | P.O.Box 4618, Durban 4000, Republic of South Africa
Telephones: +27(0) 31 2061390, +27(0) 31 2051216 | Fax2email: +27(0) 88 031 2060518 | mobile: 079 300 9829
Email: info@chemsciencelaboratories.com | website: www.chemsciencelaboratories.com

CERTIFICATE OF ANALYSIS

Our certificate number: 161057 *Our quotation number: Q-CL4487*

Your order / reference number: JB19117

Four samples received on 13 February 2017 at 10h30, ***sampled by yourselves.***

Sample Markings: Biodiesel
Sample 1: 3B
Sample 2: 5
Sample 3: 9
Sample 4: 3A

Sample Description: Liquids
Sample Appearance: Sample 1: Yellow turbid liquid
Sample 2: Clear Liquid yellow
Sample 3: yellow turbid liquid
Sample 4: colorless liquid with two layers
Condition of sample as received: Satisfactory,
Date testing commenced: 21 February 2017
Date testing completed: 21 April 2017

The analysis on an "as received" basis showed:

Test	Method (Lab T0161)	Sample 1 Result	Sample 2Result	Sample 3 Result	Sample 4 Result	Units
Freezing Point*	**ASTMD D2386**	**-20°C**	**-32 °C**	**-30 °C**	**-8 °C**	**°C**
Flash Point*	**ASTM D93 (PMcc)**	**68°C**	**65°C**	**68°C**	**68°C**	**°C**
Net heat of combustion*	**ASTM D4868**	**11.01**	**41.34**	**36.18**	**-22.10**	**MJ/kg**

Comment: All Laboratory testing carried out as per client's instruction, letter dated 170203.

Note: Only original reports are considered official. Electronic documents are transmitted "WITHOUT PREJUDICE"

Signed.....VAS.....this 28th day of April 2017
V A Soffiantini, Chartered Chemist;
C.Chem.,M.R.S.C.,Pr.Sci.Nat.
Technical Signatory (Chemistry)

……SG……..……
S. Gaffoor; Cert. Sci. Nat
Nat. Dip. Biotechnology
Technical Manager

To: **Mangosuthu University of Technology**
Department of Nature Conservation
P.O Box 12363
Jacobs
Durban, 4326

Requested by and Reported to: **Joseph Bwapwa**
E**mail**: joseph@mut.ac.za

TESTS MARKED WITH * ARE NOT SANAS ACCREDITED | Tests marked with a # are results from an approved outsourced laboratory.
Reports relate ONLY to the samples tested and are issued in good faith. Opinions & interpretations expressed herein are outside the scope of SANAS accreditation.
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WEAR CHECK Est 1976 40th anniversary
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KWAZULU-NATAL
P O Box 15108
Westmead
3608
Tel: 031 700 5460
Fax: 031 700 5471

GAUTENG
P O Box 284
Isando
1600
Tel: 011 392 6322
Fax: 011 392 6340

BIODIESEL
Fuel Analysis Report

sanas Testing Laboratory TO525

SANS 17025:2005

FAIL

Joseph

Company Name : Wearcheck Africa - Sundry Fuels - Pinetown
Customer Code : FSUNDY | Site: JOSEPH_MANGOSUTHU_UNIV
Equipment Name: JOSEPH_MANGOSUTHU_UNIV_BIO_DIESEL_SAMPLE_1->FUEL
Chassis/Serial:
Registration :
Eq Make/Model :
Cmp Make/Model:
Job No : SAMPLE 1
Brand/Grade : | Sample Condition:
Container : WEARCHECK | Sample Volume: 300

Sampled Date :
Received Date: 01/12/2016
Reported Date: 02/12/2016
Test Date : 02/12/2016

Diagnosis

1.) s/n:BS61849 on SMR:0HRS
Insufficient sample supplied, unable to determine total contamination or capture images of debris. The sample provided does not conform with SANS 1935 specifications with respect to the tests carried out due to a light concentration of free water and water content by Karl Fischer is unacceptably high. Sulphur content exceeds SANS limits. Density too high. Viscosity is too low. TAN too high.

Technical Signatory:Loshini Govender

Tests — Sample Number: BS61849 — SANS 1935 SPECIFICATIONS

	RESULTS	UNITS	LIMITS	COMMENTS
Density @15 ºC (ASTM D7042)*	1.1948	g/mL	0.86-0.9	FAIL
Viscosity@40°C (ASTM D7042)*	1.4	cSt	3.5 - 5	FAIL
Flashpoint (ASTM D93)*		ºC	120 min	CND
Water Content (ASTM D6304)	1.000	%	0.05 max	FAIL
Total Acid Number	1.60	mgKOH/g	0.5 max	FAIL
Total Contamination (IP440/SANS 52662)*		mg/Kg	24 max	CND
Sulphur (ASTM D4294)*	2395	ppm	10 max	FAIL
Sulphur	0.2395	%		

* Not An Accredited SANAS Method

Visual Inspections / Additional Tests

	UNIT	RESULTS	COMMENTS
Free Water		Light Concentration Of Visible Water	FAIL
Colour		Black	
Appearance		Opaque	
Bacteria Content		Not Requested	
Biodiesel	%	3.2	

Particle Count

Iron :
Aluminium:
Magnesium:
Zinc :
Lead :
Silicon :
Manganese:
Sodium :
Vanadium :

Elemental Analysis ppm

Distillation And Graph

Distillation Data

IBP
10
20
30
40
50
60
70
80
90
FBP
Rec %

Issued subject to Standard Conditions(available on request). NOTE: The results relate only to the sample tested - the opinions and interpretation expressed are outside the scope of the accreditation. Uncertainty of the methods are available on request. Where a test method is quoted, it is taken to mean that the test carried out is closely based on, but may not conform exactly to, the quoted method. The test report shall not be reproduced except in full, without written approval of the laboratory.

WEARCHECK IS AN ISO 9001:2008 / ISO 14001:2004 / ISO 17025:2005 REGISTERED COMPANY

WEAR CHECK Est 1976 40th anniversary
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KWAZULU-NATAL
P O Box 15108
Westmead
3608
Tel: 031 700 5460
Fax: 031 700 5471

GAUTENG
P O Box 284
Isando
1600
Tel: 011 392 6322
Fax: 011 392 6340

BIODIESEL
Fuel Analysis Report

sanas Testing Laboratory TO525

SANS 17025:2005

FAIL

Joseph

Company Name : Wearcheck Africa - Sundry Fuels - Pinetown
Customer Code : FSUNDY | Site: JOSEPH_MANGOSUTHU_UNIV
Equipment Name: JOSEPH_MANGOSUTHU_UNIV_BIO_DIESEL_SAMPLE_2->FUEL
Chassis/Serial:
Registration :
Eq Make/Model :
Cmp Make/Model:
Job No :
Brand/Grade : | Sample Condition:
Container : WEARCHECK | Sample Volume: 400

Sampled Date :
Received Date: 01/12/2016
Reported Date: 02/12/2016
Test Date : 02/12/2016

Diagnosis

1.) s/n:BS61841 on SMR:0HRS
The full range of tests could not be carried out due to the nature of the sample. The sample provided does not conform with SANS 1935 specifications with respect to the tests carried out due to a light concentration of free water and water content by Karl Fischer is unacceptably high. Sulphur content exceeds SANS limits. Density too high. Viscosity is too low. TAN too high. Total contamination mass exceeds SANS limits.

Technical Signatory:Loshini Govender

Tests — Sample Number: BS61841 — SANS 1935 SPECIFICATIONS

	RESULTS	UNITS	LIMITS	COMMENTS
Density @15 ºC (ASTM D7042)*	0.9925	g/mL	0.86-0.9	FAIL
Viscosity@40°C (ASTM D7042)*	1.2	cSt	3.5 - 5	FAIL
Flashpoint (ASTM D93)*		ºC	120 min	CND
Water Content (ASTM D6304)	0.750	%	0.05 max	FAIL
Total Acid Number	0.90	mgKOH/g	0.5 max	FAIL
Total Contamination (IP440/SANS 52662)*	365.0	mg/Kg	24 max	FAIL
Sulphur (ASTM D4294)*	2277	ppm	10 max	FAIL
Sulphur	0.2277	%		

* Not An Accredited SANAS Method

Visual Inspections / Additional Tests

	UNIT	RESULTS	COMMENTS
Free Water		Light Concentration Of Visible Water	FAIL
Colour		Black	
Appearance		Opaque	
Bacteria Content		Not Requested	
Biodiesel	%	Not Requested	

Particle Count

Elemental Analysis ppm

Iron	:	Silicon	:
Aluminium	:	Manganese	:
Magnesium	:	Sodium	:
Zinc	:	Vanadium	:
Lead	:		

Distillation And Graph

Distillation Data

IBP
10
20
30
40
50
60
70
80
90
FBP
Rec %

Issued subject to Standard Conditions(available on request). NOTE: The results relate only to the sample tested - the opinions and interpretation expressed are outside the scope of the accreditation. Uncertainty of the methods are available on request. Where a test method is quoted, it is taken to mean that the test carried out is closely based on, but may not conform exactly to, the quoted method. The test report shall not be reproduced except in full, without written approval of the laboratory.

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3608
Tel: 031 700 5460
Fax: 031 700 5471

GAUTENG
P O Box 284
Isando
1600
Tel: 011 392 6322
Fax: 011 392 6340

BIODIESEL
Fuel Analysis Report

sanas Testing Laboratory TO525

SANS 17025:2005

FAIL

Joseph

Company Name : Wearcheck Africa - Sundry Fuels - Pinetown
Customer Code : FSUNDY | Site: JOSEPH_MANGOSUTHU_UNIV
Equipment Name: JOSEPH_MANGOSUTHU_UNIV_BIO_DIESEL_SAMPLE_3->FUEL
Chassis/Serial:
Registration :
Eq Make/Model :
Cmp Make/Model:
Job No :
Brand/Grade : | Sample Condition:
Container : WEARCHECK | Sample Volume: 400

Sampled Date :
Received Date: 01/12/2016
Reported Date: 02/12/2016
Test Date : 02/12/2016

Diagnosis

1.) s/n:BS61843 on SMR:0HRS
The full range of tests could not be carried out due to the nature of the sample. The sample provided does not conform with SANS 1935 specifications with respect to the tests carried out due to the presence of free water and water content by Karl Fischer is unacceptably high. Sulphur content exceeds SANS limits. Density too low. Viscosity is too low.

Technical Signatory:Loshini Govender

Tests — Sample Number: BS61843 — SANS 1935 SPECIFICATIONS

	RESULTS	UNITS	LIMITS	COMMENTS
Density @15 ºC (ASTM D7042)*	0.9557	g/mL	0.86-0.9	FAIL
Viscosity@40°C (ASTM D7042)*	1.2	cSt	3.5 - 5	FAIL
Flashpoint (ASTM D93)*		ºC	120 min	CND
Water Content (ASTM D6304)	99.750	%	0.05 max	FAIL
Total Acid Number	0.05	mgKOH/g	0.5 max	PASS
Total Contamination (IP440/SANS 52662)*	2.0	mg/Kg	24 max	PASS
Sulphur (ASTM D4294)*	623	ppm	10 max	FAIL
Sulphur	0.0623	%		

* Not An Accredited SANAS Method

Visual Inspections / Additional Tests

	UNIT	RESULTS	COMMENTS
Free Water		Free Water	FAIL
Colour		Pale Straw Yellow	
Appearance		Hazy	
Bacteria Content		Not Requested	
Biodiesel	%	Not Requested	

Particle Count

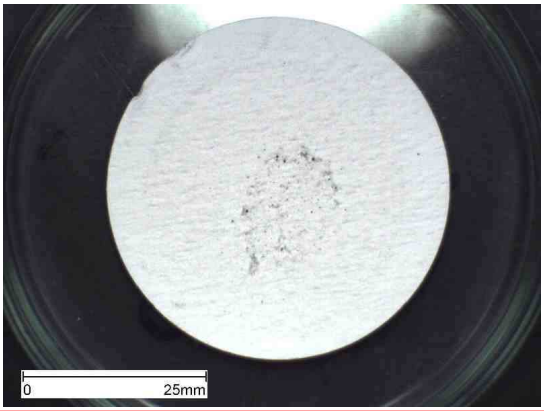
Iron : Silicon :
Aluminium: Manganese:
Magnesium: Sodium :
Zinc : Vanadium :
Lead :

Elemental Analysis ppm

Distillation And Graph

Distillation Data

IBP
10
20
30
40
50
60
70
80
90
FBP
Rec %



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Westmead
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Fax: 031 700 5471

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Isando
1600
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BIODIESEL
Fuel Analysis Report

sanas Testing Laboratory TO525

SANS 17025:2005

FAIL

Joseph

Company Name : Wearcheck Africa - Sundry Fuels - Pinetown
Customer Code : FSUNDY | Site: JOSEPH_MANGOSUTHU_UNIV
Equipment Name: JOSEPH_MANGOSUTHU_UNIV_BIO_DIESEL_SAMPLE_3->FUEL
Chassis/Serial:
Registration :
Eq Make/Model :
Cmp Make/Model:
Job No :
Brand/Grade : | Sample Condition: GOOD
Container : WEARCHECK | Sample Volume: 420

Sampled Date :
Received Date: 09/01/2017
Reported Date: 10/01/2017
Test Date : 10/01/2017

Diagnosis

1.) s/n:BS62114 on SMR:0HRS
The sample provided does not conform with SANS 1935 specifications with respect to the tests carried out. Water content by Karl Fischer is unacceptably high. Sulphur content exceeds SANS limits. Density too high. Viscosity is too low.

Technical Signatory:Loshini Govender

Tests — Sample Number: BS62114 — SANS 1935 SPECIFICATIONS

	RESULTS	UNITS	LIMITS	COMMENTS
Density @15 ºC (ASTM D7042)*	1.3776	g/mL	0.86-0.9	FAIL
Viscosity@40°C (ASTM D7042)*	0.4	cSt	3.5 - 5	FAIL
Flashpoint (ASTM D93)*		ºC	120 min	CND
Water Content (ASTM D6304)	0.077	%	0.05 max	FAIL
Total Acid Number	0.05	mgKOH/g	0.5 max	PASS
Total Contamination (IP440/SANS 52662)*	7.6	mg/Kg	24 max	PASS
Sulphur (ASTM D4294)*	2712	ppm	10 max	FAIL
Sulphur	0.2712	%		

* Not An Accredited SANAS Method

Visual Inspections / Additional Tests

	UNIT	RESULTS	COMMENTS
Free Water		None Observed	PASS
Colour		Pale Straw Yellow	
Appearance		Clear	
Bacteria Content		Not Requested	
Biodiesel	%	0	

Particle Count

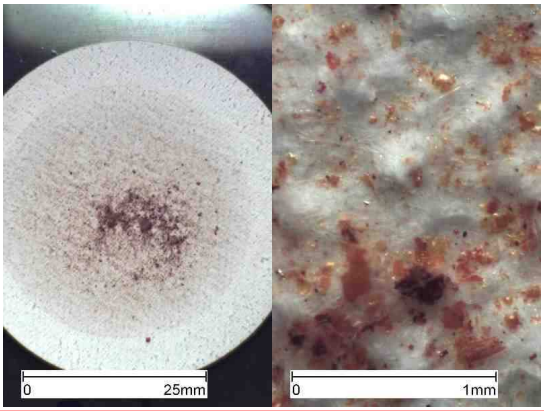
Elemental Analysis ppm

Iron	:	Silicon	:
Aluminium	:	Manganese	:
Magnesium	:	Sodium	:
Zinc	:	Vanadium	:
Lead	:		

Distillation And Graph

Distillation Data

IBP
10
20
30
40
50
60
70
80
90
FBP
Rec %



Issued subject to Standard Conditions(available on request). NOTE: The results relate only to the sample tested - the opinions and interpretation expressed are outside the scope of the accreditation. Uncertainty of the methods are available on request. Where a test method is quoted, it is taken to mean that the test carried out is closely based on, but may not conform exactly to, the quoted method. The test report shall not be reproduced except in full, without written approval of the laboratory.

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Fax: 031 700 5471

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P O Box 284
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1600
Tel: 011 392 6322
Fax: 011 392 6340

BIODIESEL
Fuel Analysis Report

sanas Testing Laboratory TO525

SANS 17025:2005

FAIL

Joseph

Company Name : Wearcheck Africa - Sundry Fuels - Pinetown
Customer Code : FSUNDY | Site: JOSEPH_MANGOSUTHU_UNIV
Equipment Name: JOSEPH_MANGOSUTHU_UNIV_BIO_DIESEL_SAMPLE_4->FUEL
Chassis/Serial:
Registration :
Eq Make/Model :
Cmp Make/Model:
Job No : SAMPLE 4
Brand/Grade : | Sample Condition:
Container : WEARCHECK | Sample Volume: 390

Sampled Date :
Received Date: 01/12/2016
Reported Date: 02/12/2016
Test Date : 02/12/2016

Diagnosis

1.) s/n:BS61842 on SMR:0HRS
The full range of tests could not be carried out due to the nature of the sample. The sample provided does not conform with SANS 1935 specifications with respect to the tests carried out due to a moderate concentration of free water and water content by Karl Fischer is unacceptably high. Sulphur content exceeds SANS limits. Viscosity is too low. Total contamination mass exceeds SANS limits.

Technical Signatory:Loshini Govender

Tests — Sample Number: BS61842 — SANS 1935 SPECIFICATIONS

	RESULTS	UNITS	LIMITS	COMMENTS
Density @15 ºC (ASTM D7042)*	0.8751	g/mL	0.86-0.9	PASS
Viscosity@40°C (ASTM D7042)*	1.2	cSt	3.5 - 5	FAIL
Flashpoint (ASTM D93)*		ºC	120 min	CND
Water Content (ASTM D6304)	1.282	%	0.05 max	FAIL
Total Acid Number	0.45	mgKOH/g	0.5 max	PASS
Total Contamination (IP440/SANS 52662)*	600.7	mg/Kg	24 max	FAIL
Sulphur (ASTM D4294)*	2436	ppm	10 max	FAIL
Sulphur	0.2436	%		

* Not An Accredited SANAS Method

Visual Inspections / Additional Tests

	UNIT	RESULTS	COMMENTS
Free Water		Moderate Concentration Of Visible Water	FAIL
Colour		Black	
Appearance		Very Hazy (Opaque)	
Bacteria Content		Not Requested	
Biodiesel	%	Not Requested	

Particle Count

Elemental Analysis ppm

Iron	:	Silicon	:
Aluminium	:	Manganese	:
Magnesium	:	Sodium	:
Zinc	:	Vanadium	:
Lead	:		

Distillation And Graph

Distillation Data

IBP
10
20
30
40
50
60
70
80
90
FBP
Rec %

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P O Box 284
Isando
1600
Tel: 011 392 6322
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BIODIESEL
Fuel Analysis Report

SANS 17025:2005

FAIL

Joseph

Company Name : Wearcheck Africa - Sundry Fuels - Pinetown
Customer Code : FSUNDY | Site: JOSEPH_MANGOSUTHU_UNIV
Equipment Name: JOSEPH_MANGOSUTHU_UNIV_BIO_DIESEL_SAMPLE_5->FUEL
Chassis/Serial:
Registration :
Eq Make/Model :
Cmp Make/Model:
Job No :
Brand/Grade : | Sample Condition:
Container : WEARCHECK | Sample Volume: 300

Sampled Date :
Received Date: 01/12/2016
Reported Date: 02/12/2016
Test Date : 02/12/2016

Diagnosis

1.) s/n:BS61840 on SMR:0HRS
Insufficient sample supplied to carry out a full range of tests. The sample provided does not conform with SANS 1935 specifications with respect to the tests carried out due to the presence of free water and water content by Karl Fischer is unacceptably high. Sulphur content exceeds SANS limits. Density too high. Viscosity is too low. .

Technical Signatory:Loshini Govender

Tests	Sample Number: BS61840		SANS 1935 SPECIFICATIONS	
	RESULTS	UNITS	LIMITS	COMMENTS
Density @15 ºC (ASTM D7042)*	0.9454	g/mL	0.86-0.9	FAIL
Viscosity@40°C (ASTM D7042)*	2.2	cSt	3.5 - 5	FAIL
Flashpoint (ASTM D93)*		ºC	120 min	CND
Water Content (ASTM D6304)	36.667	%	0.05 max	FAIL
Total Acid Number	0.01	mgKOH/g	0.5 max	PASS
Total Contamination (IP440/SANS 52662)*		mg/Kg	24 max	CND
Sulphur (ASTM D4294)*	1497	ppm	10 max	FAIL
Sulphur	0.1497	%		

* Not An Accredited SANAS Method

Visual Inspections / Additional Tests

	UNIT	RESULTS	COMMENTS
Free Water		Free Water	FAIL
Colour		Dirty Straw Yellow	
Appearance		Hazy	
Bacteria Content		Not Requested	
Biodiesel	%	Not Requested	

Particle Count

Elemental Analysis ppm

Iron	:	Silicon	:
Aluminium	:	Manganese	:
Magnesium	:	Sodium	:
Zinc	:	Vanadium	:
Lead	:		

Distillation And Graph

Distillation Data

IBP
10
20
30
40
50
60
70
80
90
FBP
Rec %

Issued subject to Standard Conditions(available on request). NOTE: The results relate only to the sample tested - the opinions and interpretation expressed are outside the scope of the accreditation. Uncertainty of the methods are available on request. Where a test method is quoted, it is taken to mean that the test carried out is closely based on, but may not conform exactly to, the quoted method. The test report shall not be reproduced except in full, without written approval of the laboratory.

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P O Box 284
Isando
1600
Tel: 011 392 6322
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BIODIESEL
Fuel Analysis Report

sanas Testing Laboratory TO525

SANS 17025:2005

FAIL

Joseph

Company Name : Wearcheck Africa - Sundry Fuels - Pinetown
Customer Code : FSUNDY | Site: JOSEPH_MANGOSUTHU_UNIV
Equipment Name: JOSEPH_MANGOSUTHU_UNIV_BIO_DIESEL_SAMPLE_5->FUEL
Chassis/Serial:
Registration :
Eq Make/Model :
Cmp Make/Model:
Job No :
Brand/Grade : | Sample Condition: GOOD
Container : WEARCHECK | Sample Volume: 470

Sampled Date :
Received Date: 09/01/2017
Reported Date: 09/01/2017
Test Date : 09/01/2017

Diagnosis

1.) s/n:BS62115 on SMR:0HRS
The sample provided does not conform with SANS 1935 specifications with respect to the tests carried out due to a light concentration of free water and water content by Karl Fischer is unacceptably high. Sulphur content exceeds SANS limits too high. Viscosity is too high. Total contamination mass exceeds SANS limits. Flash point too low. REPEAT PROBLEM oil-u/sx2 . REPEAT PROBLEM waterx2 .

Technical Signatory:Loshini Govender

Tests — Sample Number: BS62115 — SANS 1935 SPECIFICATIONS

	RESULTS	UNITS	LIMITS	COMMENTS
Density @15 ºC (ASTM D7042)*	0.9001	g/mL	0.86-0.9	FAIL
Viscosity@40°C (ASTM D7042)*	9.7	cSt	3.5 - 5	FAIL
Flashpoint (ASTM D93)*	89	ºC	120 min	FAIL
Water Content (ASTM D6304)	0.207	%	0.05 max	FAIL
Total Acid Number	0.07	mgKOH/g	0.5 max	PASS
Total Contamination (IP440/SANS 52662)*	99.0	mg/Kg	24 max	FAIL
Sulphur (ASTM D4294)*	219	ppm	10 max	FAIL
Sulphur	0.0219	%		

* Not An Accredited SANAS Method

Visual Inspections / Additional Tests

	UNIT	RESULTS	COMMENTS
Free Water		Light Concentration Of Visible Water	FAIL
Colour		Golden Straw Yellow	
Appearance		Hazy	
Bacteria Content		Not Requested	
Biodiesel	%	65.66	

Particle Count

4 micron	6 micron	14 micron	20 micron	25 micron	50 micron	75 micron	100 micron	Cleanliness
1347	429	30	10	7	22	3	1	18/16/13

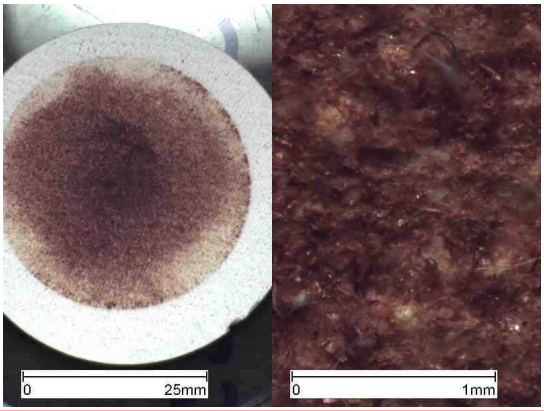
Elemental Analysis ppm

Iron	:	Silicon	:
Aluminium	:	Manganese	:
Magnesium	:	Sodium	:
Zinc	:	Vanadium	:
Lead	:		

Distillation And Graph

Distillation Data

IBP
10
20
30
40
50
60
70
80
90
FBP
Rec %



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1600
Tel: 011 392 6322
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BIODIESEL
Fuel Analysis Report

sanas Testing Laboratory TO525

SANS 17025:2005

FAIL

Joseph

Company Name : Wearcheck Africa - Sundry Fuels - Pinetown
Customer Code : FSUNDY | Site: JOSEPH_MANGOSUTHU_UNIV
Equipment Name: JOSEPH_MANGOSUTHU_UNIV_BIO_DIESEL_SAMPLE_6->FUEL
Chassis/Serial:
Registration :
Eq Make/Model :
Cmp Make/Model:
Job No :
Brand/Grade : | Sample Condition:
Container : WEARCHECK | Sample Volume: 310

Sampled Date :
Received Date: 01/12/2016
Reported Date: 02/12/2016
Test Date : 02/12/2016

Diagnosis

1.) s/n:BS61844 on SMR:0HRS
Insufficient sample supplied, unable to determine total contamination or capture images of debris. The sample provided does not conform with SANS 1935 specifications with respect to the tests carried out due to the presence of free water and water content by Karl Fischer is unacceptably high. Sulphur content exceeds SANS limits. Density too low. Viscosity is.

Technical Signatory:Loshini Govender

Tests — Sample Number: BS61844 — SANS 1935 SPECIFICATIONS

	RESULTS	UNITS	LIMITS	COMMENTS
Density @15 ºC (ASTM D7042)*	0.8164	g/mL	0.86-0.9	FAIL
Viscosity@40°C (ASTM D7042)*	1.3	cSt	3.5 - 5	FAIL
Flashpoint (ASTM D93)*		ºC	120 min	CND
Water Content (ASTM D6304)	99.677	%	0.05 max	FAIL
Total Acid Number	0.02	mgKOH/g	0.5 max	PASS
Total Contamination (IP440/SANS 52662)*		mg/Kg	24 max	CND
Sulphur (ASTM D4294)*	91	ppm	10 max	FAIL
Sulphur	0.0091	%		

* Not An Accredited SANAS Method

Visual Inspections / Additional Tests

	UNIT	RESULTS	COMMENTS
Free Water		Free Water	FAIL
Colour		White Emulsified Diesel	
Appearance		Hazy	
Bacteria Content		Not Requested	
Biodiesel	%	Not Requested	

Particle Count

Elemental Analysis ppm

Iron	:	Silicon	:
Aluminium	:	Manganese	:
Magnesium	:	Sodium	:
Zinc	:	Vanadium	:
Lead	:		

Distillation And Graph

Distillation Data

IBP
10
20
30
40
50
60
70
80
90
FBP
Rec %

Issued subject to Standard Conditions(available on request). NOTE: The results relate only to the sample tested - the opinions and interpretation expressed are outside the scope of the accreditation. Uncertainty of the methods are available on request. Where a test method is quoted, it is taken to mean that the test carried out is closely based on, but may not conform exactly to, the quoted method. The test report shall not be reproduced except in full, without written approval of the laboratory.

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1600
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BIODIESEL
Fuel Analysis Report

sanas Testing Laboratory TO525

SANS 17025:2005

FAIL

Joseph

Company Name : Wearcheck Africa - Sundry Fuels - Pinetown
Customer Code : FSUNDY | Site: JOSEPH_MANGOSUTHU_UNIV
Equipment Name: JOSEPH_MANGOSUTHU_UNIV_BIO_DIESEL_SAMPLE_6->FUEL
Chassis/Serial:
Registration :
Eq Make/Model :
Cmp Make/Model:
Job No :
Brand/Grade : | Sample Condition: GOOD
Container : WEARCHECK | Sample Volume: 480

Sampled Date :
Received Date: 09/01/2017
Reported Date: 10/01/2017
Test Date : 10/01/2017

Diagnosis

1.) s/n:BS62111 on SMR:0HRS
The sample provided does not conform with SANS 1935 specifications with respect to the tests carried out due to a light concentration of free water and water content by Karl Fischer is unacceptably high. Sulphur content exceeds SANS limits . Viscosity is too high. Total contamination mass exceeds SANS limits. Flash point too low. . REPEAT PROBLEM oil-u/sx2 . REPEAT PROBLEM waterx2 .

Technical Signatory:Loshini Govender

Tests — Sample Number: BS62111 — SANS 1935 SPECIFICATIONS

	RESULTS	UNITS	LIMITS	COMMENTS
Density @15 ºC (ASTM D7042)*	0.8727	g/mL	0.86-0.9	PASS
Viscosity@40°C (ASTM D7042)*	5.3	cSt	3.5 - 5	FAIL
Flashpoint (ASTM D93)*	67	ºC	120 min	FAIL
Water Content (ASTM D6304)	0.089	%	0.05 max	FAIL
Total Acid Number	0.03	mgKOH/g	0.5 max	PASS
Total Contamination (IP440/SANS 52662)*	56.4	mg/Kg	24 max	FAIL
Sulphur (ASTM D4294)*	159	ppm	10 max	FAIL
Sulphur	0.0159	%		

* Not An Accredited SANAS Method

Visual Inspections / Additional Tests

	UNIT	RESULTS	COMMENTS
Free Water		Light Concentration Of Visible Water	FAIL
Colour		Straw Yellow	
Appearance		Slight Hazy	
Bacteria Content		Not Requested	
Biodiesel	%	68.67	

Particle Count

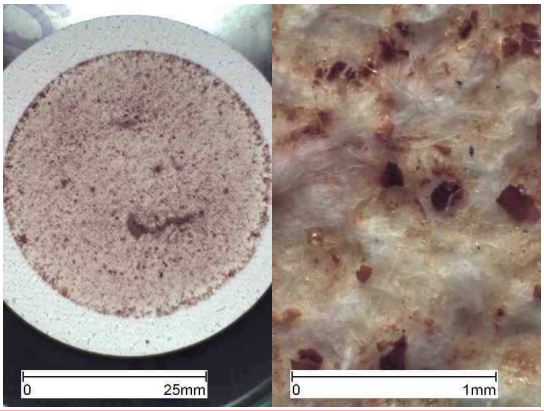
Iron : Silicon :
Aluminium: Manganese:
Magnesium: Sodium :
Zinc : Vanadium :
Lead :

Elemental Analysis ppm

Distillation And Graph

Distillation Data

IBP
10
20
30
40
50
60
70
80
90
FBP
Rec %



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Fax: 031 700 5471

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P O Box 284
Isando
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BIODIESEL
Fuel Analysis Report

sanas Testing Laboratory TO525

SANS 17025:2005

FAIL

Joseph

Company Name : Wearcheck Africa - Sundry Fuels - Pinetown
Customer Code : FSUNDY | Site: JOSEPH_MANGOSUTHU_UNIV
Equipment Name: JOSEPH_MANGOSUTHU_UNIV_BIO_DIESEL_SAMPLE_7->FUEL
Chassis/Serial:
Registration :
Eq Make/Model :
Cmp Make/Model:
Job No :
Brand/Grade : | Sample Condition:
Container : WEARCHECK | Sample Volume: 420

Sampled Date :
Received Date: 01/12/2016
Reported Date: 02/12/2016
Test Date : 02/12/2016

Diagnosis

1.) s/n:BS61845 on SMR:0HRS
The full range of tests could not be carried out due to the nature of the sample. The sample provided does not conform with SANS 1935 specifications with respect to the tests carried out due to the presence of free water and water content by Karl Fischer is unacceptably high. Sulphur content exceeds SANS limits. Density too low. Viscosity is too low.

Technical Signatory:Loshini Govender

Tests — Sample Number: BS61845 — SANS 1935 SPECIFICATIONS

	RESULTS	UNITS	LIMITS	COMMENTS
Density @15 ºC (ASTM D7042)*	0.8015	g/mL	0.86-0.9	FAIL
Viscosity@40°C (ASTM D7042)*	1.3	cSt	3.5 - 5	FAIL
Flashpoint (ASTM D93)*		ºC	120 min	CND
Water Content (ASTM D6304)	23.809	%	0.05 max	FAIL
Total Acid Number	0.01	mgKOH/g	0.5 max	PASS
Total Contamination (IP440/SANS 52662)*	16.2	mg/Kg	24 max	PASS
Sulphur (ASTM D4294)*	201	ppm	10 max	FAIL
Sulphur	0.0201	%		

* Not An Accredited SANAS Method

Visual Inspections / Additional Tests

	UNIT	RESULTS	COMMENTS
Free Water		Free Water	FAIL
Colour		Pale Straw Yellow	
Appearance		Clear	
Bacteria Content		Not Requested	
Biodiesel	%	Not Requested	

Particle Count

4 micron	6 micron	14 micron	20 micron	25 micron	50 micron	75 micron	100 micron	Cleanliness
633	1651	353	209	193	357	7	2	19/19/17

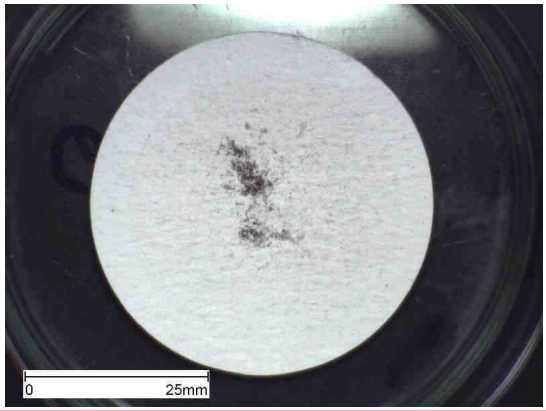
Elemental Analysis ppm

Iron	:	Silicon	:
Aluminium	:	Manganese	:
Magnesium	:	Sodium	:
Zinc	:	Vanadium	:
Lead	:		

Distillation And Graph

Distillation Data

IBP
10
20
30
40
50
60
70
80
90
FBP
Rec %



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KWAZULU-NATAL
P O Box 15108
Westmead
3608
Tel: 031 700 5460
Fax: 031 700 5471

GAUTENG
P O Box 284
Isando
1600
Tel: 011 392 6322
Fax: 011 392 6340

BIODIESEL
Fuel Analysis Report

sanas Testing Laboratory TO525

SANS 17025:2005

FAIL

Joseph

Company Name : Wearcheck Africa - Sundry Fuels - Pinetown
Customer Code : FSUNDY | Site: JOSEPH_MANGOSUTHU_UNIV
Equipment Name: JOSEPH_MANGOSUTHU_UNIV_BIO_DIESEL_SAMPLE_7->FUEL
Chassis/Serial:
Registration :
Eq Make/Model :
Cmp Make/Model:
Job No :
Brand/Grade : | Sample Condition: GOOD
Container : WEARCHECK | Sample Volume: 465

Sampled Date :
Received Date: 09/01/2017
Reported Date: 09/01/2017
Test Date : 09/01/2017

Diagnosis

1.) s/n:BS62112 on SMR:0HRS
The sample provided does not conform with SANS 1935 specifications with respect to the tests carried out due to a light concentration of free water. Sulphur content exceeds SANS limits. Density too low. Viscosity is too low. Total contamination mass exceeds SANS limits. Flash point too low. .

Technical Signatory:Loshini Govender

Tests — Sample Number: BS62112 — SANS 1935 SPECIFICATIONS

	RESULTS	UNITS	LIMITS	COMMENTS
Density @15 ºC (ASTM D7042)*	0.8294	g/mL	0.86-0.9	FAIL
Viscosity@40°C (ASTM D7042)*	2.3	cSt	3.5 - 5	FAIL
Flashpoint (ASTM D93)*	66	ºC	120 min	FAIL
Water Content (ASTM D6304)	0.037	%	0.05 max	PASS
Total Acid Number	0.05	mgKOH/g	0.5 max	PASS
Total Contamination (IP440/SANS 52662)*	30.0	mg/Kg	24 max	FAIL
Sulphur (ASTM D4294)*	52	ppm	10 max	FAIL
Sulphur	0.0052	%		

* Not An Accredited SANAS Method

Visual Inspections / Additional Tests

	UNIT	RESULTS	COMMENTS
Free Water		Light Concentration Of Visible Water	FAIL
Colour		Straw Yellow	
Appearance		Clear	
Bacteria Content		Not Requested	
Biodiesel	%	28.49	

Particle Count

4 micron	6 micron	14 micron	20 micron	25 micron	50 micron	75 micron	100 micron	Cleanliness
822	348	17	5	4	5	0	0	17/16/12

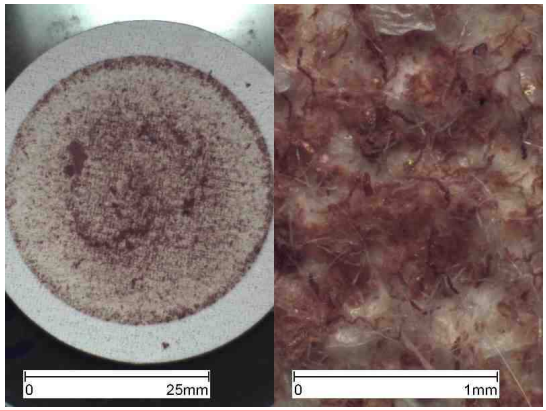
Elemental Analysis ppm

Iron	:	Silicon	:
Aluminium	:	Manganese	:
Magnesium	:	Sodium	:
Zinc	:	Vanadium	:
Lead	:		

Distillation And Graph

Distillation Data

IBP
10
20
30
40
50
60
70
80
90
FBP
Rec %



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KWAZULU-NATAL
P O Box 15108
Westmead
3608
Tel: 031 700 5460
Fax: 031 700 5471

GAUTENG
P O Box 284
Isando
1600
Tel: 011 392 6322
Fax: 011 392 6340

BIODIESEL
Fuel Analysis Report

sanas Testing Laboratory TO525

SANS 17025:2005

FAIL

Joseph

Company Name : Wearcheck Africa - Sundry Fuels - Pinetown
Customer Code : FSUNDY | Site: JOSEPH_MANGOSUTHU_UNIV
Equipment Name: JOSEPH_MANGOSUTHU_UNIV_BIO_DIESEL_SAMPLE_8->FUEL
Chassis/Serial:
Registration :
Eq Make/Model :
Cmp Make/Model:
Job No : SAMPLE 8
Brand/Grade : | Sample Condition:
Container : WEARCHECK | Sample Volume: 300

Sampled Date :
Received Date: 01/12/2016
Reported Date: 02/12/2016
Test Date : 02/12/2016

Diagnosis

1.) s/n:BS61846 on SMR:0HRS
Insufficient sample supplied, unable to determine total contamination or capture images of debris. The sample provided does not conform with SANS 1935 specifications with respect to the tests carried out due to the presence of free water and water content by Karl Fischer is unacceptably high. Sulphur content exceeds SANS limits. Density too high. Viscosity = too low

Technical Signatory:Loshini Govender

Tests — Sample Number: BS61846 — SANS 1935 SPECIFICATIONS

	RESULTS	UNITS	LIMITS	COMMENTS
Density @15 ºC (ASTM D7042)*	0.9576	g/mL	0.86-0.9	FAIL
Viscosity@40°C (ASTM D7042)*	1.1	cSt	3.5 - 5	FAIL
Flashpoint (ASTM D93)*		ºC	120 min	CND
Water Content (ASTM D6304)	96.667	%	0.05 max	FAIL
Total Acid Number	0.03	mgKOH/g	0.5 max	PASS
Total Contamination (IP440/SANS 52662)*		mg/Kg	24 max	CND
Sulphur (ASTM D4294)*	278	ppm	10 max	FAIL
Sulphur	0.0278	%		

* Not An Accredited SANAS Method

Visual Inspections / Additional Tests

	UNIT	RESULTS	COMMENTS
Free Water		Free Water	FAIL
Colour		White Emulsified Diesel	
Appearance		Hazy	
Bacteria Content		Not Requested	
Biodiesel	%	Not Requested	

Particle Count

Elemental Analysis ppm

Iron	:	Silicon	:
Aluminium	:	Manganese	:
Magnesium	:	Sodium	:
Zinc	:	Vanadium	:
Lead	:		

Distillation And Graph

Distillation Data

IBP
10
20
30
40
50
60
70
80
90
FBP
Rec %

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KWAZULU-NATAL
P O Box 15108
Westmead
3608
Tel: 031 700 5460
Fax: 031 700 5471

GAUTENG
P O Box 284
Isando
1600
Tel: 011 392 6322
Fax: 011 392 6340

BIODIESEL
Fuel Analysis Report

SANS 17025:2005

FAIL

Joseph

Company Name : Wearcheck Africa - Sundry Fuels - Pinetown
Customer Code : FSUNDY | Site: JOSEPH_MANGOSUTHU_UNIV
Equipment Name: JOSEPH_MANGOSUTHU_UNIV_BIO_DIESEL_SAMPLE_8->FUEL
Chassis/Serial:
Registration :
Eq Make/Model :
Cmp Make/Model:
Job No :
Brand/Grade : | Sample Condition: GOOD
Container : WEARCHECK | Sample Volume: 465

Sampled Date :
Received Date: 09/01/2017
Reported Date: 09/01/2017
Test Date : 09/01/2017

Diagnosis

1.) s/n:BS62113 on SMR:0HRS
The sample provided does not conform with SANS 1935 specifications with respect to the tests carried out. Sulphur content exceeds SANS limits. Density too high. Viscosity is too high. Flash point too low.

Technical Signatory:Loshini Govender

Tests — Sample Number: BS62113 — SANS 1935 SPECIFICATIONS

	RESULTS	UNITS	LIMITS	COMMENTS
Density @15 ºC (ASTM D7042)*	0.9095	g/mL	0.86-0.9	FAIL
Viscosity@40°C (ASTM D7042)*	11.1	cSt	3.5 - 5	FAIL
Flashpoint (ASTM D93)*	79	ºC	120 min	FAIL
Water Content (ASTM D6304)	0.048	%	0.05 max	PASS
Total Acid Number	0.08	mgKOH/g	0.5 max	PASS
Total Contamination (IP440/SANS 52662)*	8.1	mg/Kg	24 max	PASS
Sulphur (ASTM D4294)*	34	ppm	10 max	FAIL
Sulphur	0.0034	%		

* Not An Accredited SANAS Method

Visual Inspections / Additional Tests

	UNIT	RESULTS	COMMENTS
Free Water		None Observed	PASS
Colour		Straw Yellow	
Appearance		Clear	
Bacteria Content		Not Requested	
Biodiesel	%	82.16	

Particle Count

4 micron	6 micron	14 micron	20 micron	25 micron	50 micron	75 micron	100 micron	Cleanliness
187	70	7	3	3	12	1	0	15/14/12

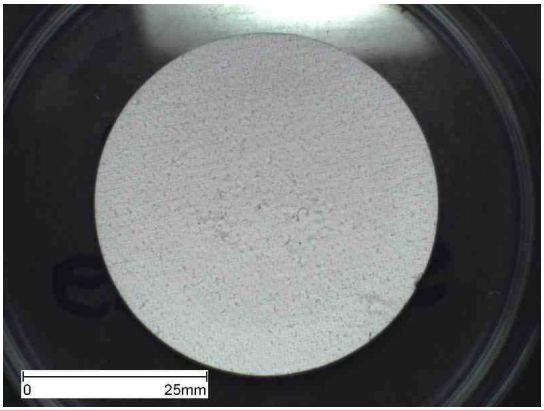
Elemental Analysis ppm

Iron	:	Silicon	:
Aluminium	:	Manganese	:
Magnesium	:	Sodium	:
Zinc	:	Vanadium	:
Lead	:		

Distillation And Graph

Distillation Data

IBP
10
20
30
40
50
60
70
80
90
FBP
Rec %



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Condition Monitoring Specialists
A Part of Torre Industries

KWAZULU-NATAL
P O Box 15108
Westmead
3608
Tel: 031 700 5460
Fax: 031 700 5471

GAUTENG
P O Box 284
Isando
1600
Tel: 011 392 6322
Fax: 011 392 6340

BIODIESEL
Fuel Analysis Report

sanas Testing Laboratory TO525

SANS 17025:2005

FAIL

Joseph

Company Name : Wearcheck Africa - Sundry Fuels - Pinetown
Customer Code : FSUNDY | Site: JOSEPH_MANGOSUTHU_UNIV
Equipment Name: JOSEPH_MANGOSUTHU_UNIV_BIO_DIESEL_SAMPLE_9->FUEL
Chassis/Serial:
Registration :
Eq Make/Model :
Cmp Make/Model:
Job No :
Brand/Grade : | Sample Condition:
Container : WEARCHECK | Sample Volume: 300

Sampled Date :
Received Date: 01/12/2016
Reported Date: 02/12/2016
Test Date : 02/12/2016

Diagnosis

1.) s/n:BS61847 on SMR:0HRS
Insufficient sample supplied, unable to determine total contamination or capture images of debris. The sample provided does not conform with SANS 1935 specifications with respect to the tests carried out due to the presence of free water and water content by Karl Fischer is unacceptably high. Sulphur content exceeds SANS limits. Density too high. Viscosity = light.

Technical Signatory:Loshini Govender

Tests — Sample Number: BS61847 — SANS 1935 SPECIFICATIONS

	RESULTS	UNITS	LIMITS	COMMENTS
Density @15 ºC (ASTM D7042)*	0.9096	g/mL	0.86-0.9	FAIL
Viscosity@40°C (ASTM D7042)*	1.6	cSt	3.5 - 5	FAIL
Flashpoint (ASTM D93)*		ºC	120 min	CND
Water Content (ASTM D6304)	33.333	%	0.05 max	FAIL
Total Acid Number	0.01	mgKOH/g	0.5 max	PASS
Total Contamination (IP440/SANS 52662)*		mg/Kg	24 max	CND
Sulphur (ASTM D4294)*	678	ppm	10 max	FAIL
Sulphur	0.0678	%		

* Not An Accredited SANAS Method

Visual Inspections / Additional Tests

	UNIT	RESULTS	COMMENTS
Free Water		Free Water	FAIL
Colour		Dirty Straw Yellow	
Appearance		Hazy	
Bacteria Content		Not Requested	
Biodiesel	%	Not Requested	

Particle Count

Iron : Silicon :
Aluminium: Manganese:
Magnesium: Sodium :
Zinc : Vanadium :
Lead :

Elemental Analysis ppm

Distillation And Graph

Distillation Data

IBP
10
20
30
40
50
60
70
80
90
FBP
Rec %

Issued subject to Standard Conditions(available on request). NOTE: The results relate only to the sample tested - the opinions and interpretation expressed are outside the scope of the accreditation. Uncertainty of the methods are available on request. Where a test method is quoted, it is taken to mean that the test carried out is closely based on, but may not conform exactly to, the quoted method. The test report shall not be reproduced except in full, without written approval of the laboratory.

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P O Box 15108
Westmead
3608
Tel: 031 700 5460
Fax: 031 700 5471

GAUTENG
P O Box 284
Isando
1600
Tel: 011 392 6322
Fax: 011 392 6340

BIODIESEL
Fuel Analysis Report

sanas Testing Laboratory TO525

SANS 17025:2005

FAIL

Joseph

Company Name : Wearcheck Africa - Sundry Fuels - Pinetown
Customer Code : FSUNDY | Site: <none>
Equipment Name: SAMPLE_9->FUEL
Chassis/Serial:
Registration :
Eq Make/Model :
Cmp Make/Model:
Job No :
Brand/Grade : | Sample Condition: GOOD
Container : WEARCHECK | Sample Volume: 500

Sampled Date :
Received Date: 09/01/2017
Reported Date: 09/01/2017
Test Date : 09/01/2017

Diagnosis

1.) s/n:BS62110 on SMR:0HRS
The sample provided does not conform with SANS 1935 specifications with respect to the tests carried out due to a moderate concentration of free water and water content by Karl Fischer is unacceptably high. Sulphur content exceeds SANS limits. Flash point too low.

Technical Signatory:Loshini Govender

Tests — Sample Number: BS62110 — SANS 1935 SPECIFICATIONS

	RESULTS	UNITS	LIMITS	COMMENTS
Density @15 ºC (ASTM D7042)*	0.8648	g/mL	0.86-0.9	PASS
Viscosity@40°C (ASTM D7042)*	4.5	cSt	3.5 - 5	PASS
Flashpoint (ASTM D93)*	68	ºC	120 min	FAIL
Water Content (ASTM D6304)	0.107	%	0.05 max	FAIL
Total Acid Number	0.04	mgKOH/g	0.5 max	PASS
Total Contamination (IP440/SANS 52662)*	18.7	mg/Kg	24 max	PASS
Sulphur (ASTM D4294)*	144	ppm	10 max	FAIL
Sulphur	0.0144	%		

* Not An Accredited SANAS Method

Visual Inspections / Additional Tests

	UNIT	RESULTS	COMMENTS
Free Water		Moderate Concentration Of Visible Water	FAIL
Colour		Golden Straw Yellow	
Appearance		Clear	
Bacteria Content		Not Requested	
Biodiesel	%	41.33	

Particle Count

4 micron	6 micron	14 micron	20 micron	25 micron	50 micron	75 micron	100 micron	Cleanliness
898	321	32	22	24	145	12	3	18/16/15

Elemental Analysis ppm

Iron	:	Silicon	:
Aluminium	:	Manganese	:
Magnesium	:	Sodium	:
Zinc	:	Vanadium	:
Lead	:		

Distillation And Graph

Distillation Data

IBP
10
20
30
40
50
60
70
80
90
FBP
Rec %

Issued subject to Standard Conditions(available on request). NOTE: The results relate only to the sample tested - the opinions and interpretation expressed are outside the scope of the accreditation. Uncertainty of the methods are available on request. Where a test method is quoted, it is taken to mean that the test carried out is closely based on, but may not conform exactly to, the quoted method. The test report shall not be reproduced except in full, without written approval of the laboratory.

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P O Box 15108
Westmead
3608
Tel: 031 700 5460
Fax: 031 700 5471

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P O Box 284
Isando
1600
Tel: 011 392 6322
Fax: 011 392 6340

BIODIESEL
Fuel Analysis Report

sanas Testing Laboratory TO525

SANS 17025:2005

FAIL

Joseph

Company Name : Wearcheck Africa - Sundry Fuels - Pinetown
Customer Code : FSUNDY | Site: JOSEPH_MANGOSUTHU_UNIV
Equipment Name: JOSEPH_MANGOSUTHU_UNIV_BIO_DIESEL_SAMPLE_10->FUEL
Chassis/Serial:
Registration :
Eq Make/Model :
Cmp Make/Model:
Job No :
Brand/Grade : | Sample Condition: GOOD
Container : WEARCHECK | Sample Volume: 440

Sampled Date :
Received Date: 09/01/2017
Reported Date: 09/01/2017
Test Date : 09/01/2017

Diagnosis

1.) s/n:BS62116 on SMR:0HRS
The sample provided does not conform with SANS 1935 specifications with respect to the tests carried out due to a light concentration of free water and water content by Karl Fischer is unacceptably high. Sulphur content exceeds SANS limits too high. Viscosity is too high. Total contamination mass exceeds SANS limits. Flash point too low.

Technical Signatory:Loshini Govender

Tests — Sample Number: BS62116 — SANS 1935 SPECIFICATIONS

	RESULTS	UNITS	LIMITS	COMMENTS
Density @15 ºC (ASTM D7042)*	0.8836	g/mL	0.86-0.9	PASS
Viscosity@40°C (ASTM D7042)*	6.7	cSt	3.5 - 5	FAIL
Flashpoint (ASTM D93)*	73	ºC	120 min	FAIL
Water Content (ASTM D6304)	0.087	%	0.05 max	FAIL
Total Acid Number	0.06	mgKOH/g	0.5 max	PASS
Total Contamination (IP440/SANS 52662)*	80.4	mg/Kg	24 max	FAIL
Sulphur (ASTM D4294)*	127	ppm	10 max	FAIL
Sulphur	0.0127	%		

* Not An Accredited SANAS Method

Visual Inspections / Additional Tests

	UNIT	RESULTS	COMMENTS
Free Water		Light Concentration Of Visible Water	FAIL
Colour		Golden Straw Yellow	
Appearance		Hazy	
Bacteria Content		Not Requested	
Biodiesel	%	65.12	

Particle Count

4 micron	6 micron	14 micron	20 micron	25 micron	50 micron	75 micron	100 micron	Cleanliness
568	166	9	2	2	5	0	0	17/15/11

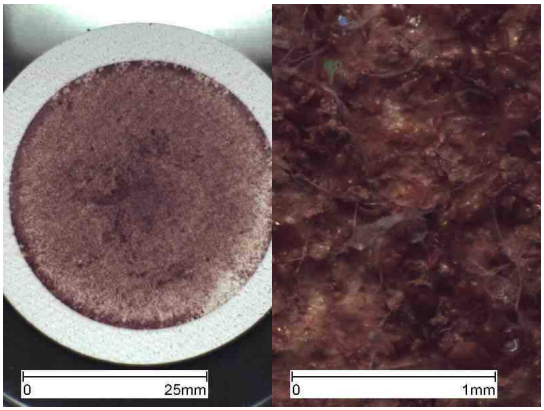
Elemental Analysis ppm

Iron	:	Silicon	:
Aluminium	:	Manganese	:
Magnesium	:	Sodium	:
Zinc	:	Vanadium	:
Lead	:		

Distillation And Graph

Distillation Data

IBP
10
20
30
40
50
60
70
80
90
FBP
Rec %



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		Units	g/ml	cSt	°C	%	mgKOH/g	mg/Kg	ppm	%
		Limits	0.86-0.9	3.5 - 5	120 min	0.05 max	0.5 max	24 max	10 max	
		Name	Density @15 °C (ASTM D7042)*	Viscosity@40°C (ASTM D7042)*	Flashpoint (ASTM D93)*	Water Content (ASTM D6304)	Total Acid Number	Total Contamination	Sulphur (ASTM D4294)*	Sulphur
	Sample 1	Purified algae bio-crude oil	1,1948	1,4		1	1,6		2395	0,2395
	Sample 1a	Non-purified biocrude-oil	1,349	0,8	30	3,551	1,15	16266,7	3236	0,3236
	Sample 2	Crude bio-oil+ Jet A1 : B50	0,9925	1,2	68	0,75	0,9	365	2277	0,2277
	Sample 3	Agae Jet fuel 100%	1.3776	0.4	68	0.077	0.05	7.6	2712	0.2712
	Sample 4	Jet A1 + Crude bio-oil : B20	0,8751	1,2		1,282	0,45	600.7	2436	0,2436
	Sample 5	Algae Jet fuel + Diesel : B50	0.9001	9.7	89	0.207	0.07	99.0	219	0.0219
	Sample 6	Algae Jet fuel + Jet A1 : B50	0.8727	5.3	67	0.089	0.03	56.4 /5.64	159	0.0159
	Sample 7	Jet A1 + Algae Jet fuel : B20	0.8294	2.3 / 4.6	66	0.037	0.05	30.0/ 3.0	52	0.0052
	Sample 8	Jet A1+ Algae Jet fuel :B20 reverse	0.9095	11.1/ 5.5	79	/0.048	/0.08	8.1	34	0.0034
		(20% Jet A1 + 80% Algae jet) volume								
	sample 9	Algae Jet fuel + Diesel +Jet A1	8648	4.5	68	0,107	0,01	18.7	144	0,0144
		(equal volumes)								
	Sample 10	Algae Jet fuel + Diesel + Jet A1	0.8836	6.7	73	0.087	0.06	80.4	127	0.0127
		(50/25/25 respectively in volume)								
	Jet fuel	Jet A1	0.775-0.840	8	38	N.D	0,015	N.D	3000	0,3
				maximum @ -20 C	min		max			max

APPENDIX A7

CHROMATOGRAPHS FROM PYROLYSED BIOMASS AND ALGAE BIO-OIL

Method

[Comment]

===== Analytical Line 1 =====

[GC-2010]

Column Oven Temp.	:50.0 °C
Injection Temp.	:250.00 °C
Injection Mode	:Splitless
Sampling Time	:0.00 min
Flow Control Mode	:Linear Velocity
Pressure	:45.5 kPa
Total Flow	:66.9 mL/min
Column Flow	:0.90 mL/min
Linear Velocity	:34.4 cm/sec
Purge Flow	:3.0 mL/min
Split Ratio	:70.0
High Pressure Injection	:OFF
Carrier Gas Saver	:OFF

Oven Temp. Program

Rate	Temperature(°C)	Hold Time(min)
-	50.0	1.00
4.00	300.0	5.00

< Ready Check Heat Unit >

Column Oven	: Yes
SPL2	: Yes
MS	: Yes

< Ready Check Detector(FTD/BID) >
< Ready Check Baseline Drift >
< Ready Check Injection Flow >

SPL2 Carrier	: Yes
SPL2 Purge	: Yes

< Ready Check APC Flow >
< Ready Check Detector APC Flow >

External Wait	:No
Equilibrium Time	:3.0 min

[GC Program]

[GCMS-QP2010 Ultra]

IonSourceTemp	:250.00 °C
Interface Temp.	:280.00 °C
Solvent Cut Time	:2.50 min
Detector Gain Mode	:Absolute
Detector Gain	:0.80 kV
Threshold	:1000

[MS Table]

--Group 1 - Event 1--

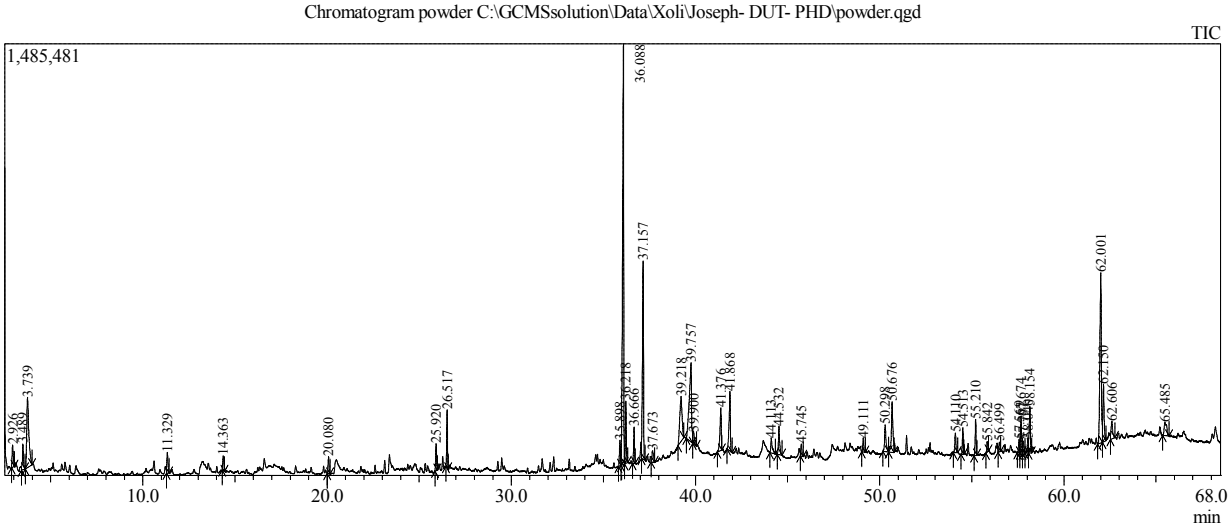
Start Time	:2.50min
End Time	:68.50min
ACQ Mode	:Scan
Event Time	:0.30sec
Scan Speed	:1428
Start m/z	:50.00
End m/z	:450.00

Sample Inlet Unit :GC

[MS Program]

Use MS Program :OFF

Chromatogram powder C:\GCMSsolution\Data\Xoli\Joseph- DUT- PHD\powder.qgd



Peak Report TIC

Peak#	R.Time	Area%	Height%	Name
1	2.926	1.06	1.19	1,4-Cyclohexadiene
2	3.489	1.05	1.38	Ethanamine, 2-chloro-N,N-dimethyl-
3	3.739	6.07	3.74	Toluene
4	11.329	0.91	1.11	1H-Pyrrole, 2-ethyl-4-methyl-
5	14.363	0.65	0.79	1H-Pyrrole, 4-ethyl-2,3-dimethyl-
6	20.080	0.65	0.92	Undecane, 4,7-dimethyl-
7	25.920	0.95	1.45	1-Pentadecene
8	26.517	1.96	3.09	Tetradecane
9	35.898	1.07	1.37	2-Hexadecene, 3,7,11,15-tetramethyl-, [R-[R*
10	36.088	20.17	22.41	Phytol, acetate
11	36.218	2.24	3.32	2-Hexadecene, 3,7,11,15-tetramethyl-, [R-[R*
12	36.666	1.42	1.95	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
13	37.157	9.36	10.58	E-6-Octadecen-1-ol acetate
14	37.673	0.68	0.62	Oxirane, hexadecyl-
15	39.218	4.26	2.40	Palmitoleic acid
16	39.757	7.40	4.22	Pentadecanoic acid
17	39.900	0.94	0.65	Nonanamide
18	41.376	2.23	2.23	i-Propyl 11,12-methylene-octadecanoate
19	41.868	3.09	3.18	Octadecanoic acid, 2-propenyl ester
20	44.113	0.90	0.78	9-Octadecenamide, (Z)-
21	44.532	1.48	1.50	Octadecanamide
22	45.745	0.54	0.63	9-Octadecenoic acid, 1,2,3-propanetriyl ester,
23	49.111	0.55	0.75	Doconexent
24	50.298	1.61	1.41	Oleoyl chloride
25	50.676	3.37	2.68	Hexadecanoic acid, 2-hydroxy-1-(hydroxymet
26	54.110	0.90	1.04	Z-14-Octadecen-1-ol acetate
27	54.513	1.02	1.41	Bacteriochlorophyll-c-stearyl
28	55.210	1.36	1.93	1,4-Eicosadiene
29	55.842	0.41	0.56	Cholest-5-en-3-ol (3.beta.)-, tetradecanoate
30	56.499	0.41	0.52	5-Tetradecen-3-yne, (Z)-
31	57.569	0.57	0.71	Cholesta-2,4-diene
32	57.674	1.51	1.92	(Z)-14-Tricosenyl formate
33	57.797	0.56	0.63	Cholesta-4,6-dien-3-ol, (3.beta.)-
34	58.040	0.70	0.77	Hexadecanedinitrile
35	58.154	2.22	2.45	Cholesta-3,5-diene
36	62.001	10.25	9.04	Cholesterol
37	62.150	2.90	3.06	Vitamin E
38	62.606	1.18	0.92	17-Pentatriacontene
39	65.485	1.38	0.70	Fucosterol
		100.00	100.00	

Method

[Comment]

===== Analytical Line 1 =====

[GC-2010]
Column Oven Temp. :50.0 °C
Injection Temp. :250.00 °C
Injection Mode :Splitless
Sampling Time :0.00 min
Flow Control Mode :Linear Velocity
Pressure :45.5 kPa
Total Flow :66.9 mL/min
Column Flow :0.90 mL/min
Linear Velocity :34.4 cm/sec
Purge Flow :3.0 mL/min
Split Ratio :70.0
High Pressure Injection :OFF
Carrier Gas Saver :OFF
Oven Temp. Program

Rate	Temperature(°C)	Hold Time(min)
-	50.0	1.00
4.00	300.0	5.00

< Ready Check Heat Unit >
Column Oven : Yes
SPL2 : Yes
MS : Yes
< Ready Check Detector(FTD/BID) >
< Ready Check Baseline Drift >
< Ready Check Injection Flow >
SPL2 Carrier : Yes
SPL2 Purge : Yes
< Ready Check APC Flow >
< Ready Check Detector APC Flow >
External Wait :No
Equilibrium Time :3.0 min

[GC Program]

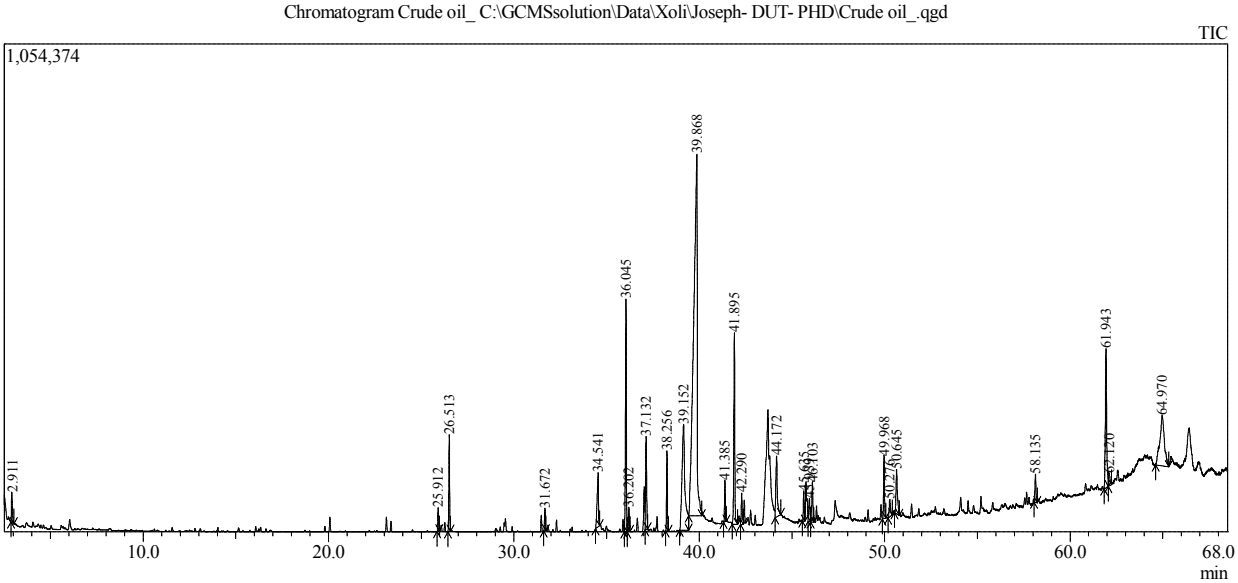
[GCMS-QP2010 Ultra]
IonSourceTemp :250.00 °C
Interface Temp. :280.00 °C
Solvent Cut Time :2.50 min
Detector Gain Mode :Absolute
Detector Gain :0.80 kV
Threshold :1000

[MS Table]
--Group 1 - Event 1--
Start Time :2.50min
End Time :68.50min
ACQ Mode :Scan
Event Time :0.30sec
Scan Speed :1428
Start m/z :50.00
End m/z :450.00

Sample Inlet Unit :GC

[MS Program]
Use MS Program :OFF

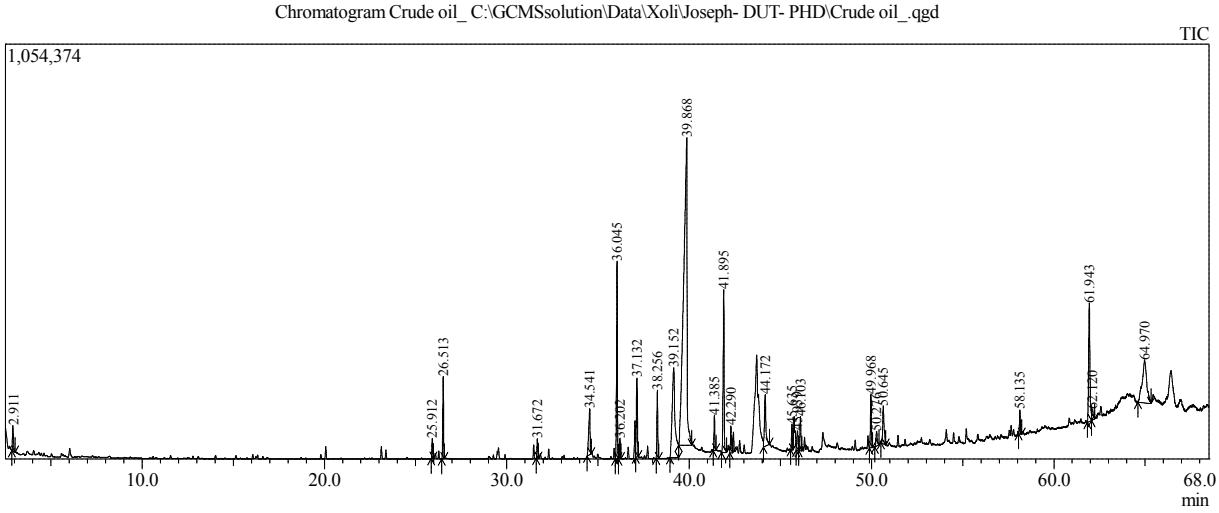
Chromatogram Crude oil_ C:\GCMSsolution\Data\Xoli\Joseph- DUT- PHD\Crude oil_.qgd



Peak Report TIC

Peak#	R.Time	Area%	Height%	Name
1	2.911	0.94	1.60	1,4-Cyclohexadiene
2	25.912	0.55	1.24	3-Hexadecene, (Z)-
3	26.513	2.39	5.14	Tetradecane
4	31.672	0.61	1.21	3-Hexadecene, (Z)-
5	34.541	2.27	2.86	Tetradecanoic acid
6	36.045	6.27	12.31	Phytol, acetate
7	36.202	0.62	1.29	2-Hexadecene, 3,7,11,15-tetramethyl-, [R-[R*
8	37.132	3.08	4.92	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
9	38.256	2.04	4.25	Hexadecanoic acid, methyl ester
10	39.152	9.77	5.61	Palmitoleic acid
11	39.868	39.18	19.14	Pentadecanoic acid
12	41.385	1.02	2.14	9-Octadecenoic acid, 1,2,3-propanetriyl ester, (
13	41.895	5.87	9.89	Octadecanoic acid, 2-propenyl ester
14	42.290	0.99	1.67	9,12-Octadecadien-1-ol, (Z,Z)-
15	44.172	2.82	3.18	Octadecanoic acid
16	45.635	0.81	1.57	Isopropyl linoleate
17	45.939	0.85	1.22	Ethyl iso-allocholate
18	46.103	1.35	2.12	5,8,11,14,17-Eicosapentaenoic acid, methyl es
19	49.968	1.85	3.32	4,7,10,13,16,19-Docosahexaenoic acid, methy
20	50.276	0.68	0.89	cis-9-Hexadecenal
21	50.645	1.94	2.34	Hexadecanoic acid, 2-hydroxy-1-(hydroxymet
22	58.135	0.77	1.45	Cholesta-3,5-diene
23	61.943	5.75	7.28	Cholesterol
24	62.120	0.55	0.67	Vitamin E
25	64.970	7.04	2.67	Eicosanoic acid, 2-[(1-oxohexadecyl)oxy]-1-[[
		100.00	100.00	

Chromatogram Crude oil_ C:\GCMSsolution\Data\Xoli\Joseph- DUT- PHD\Crude oil_.qgd



Peak Report TIC

Peak#	R.Time	Area%	Height%	A/H	Name
1	2.911	0.94	1.60	3.83	1,4-Cyclohexadiene
2	25.912	0.55	1.24	2.88	3-Hexadecene, (Z)-
3	26.513	2.39	5.14	3.02	Tetradecane
4	31.672	0.61	1.21	3.30	3-Hexadecene, (Z)-
5	34.541	2.27	2.86	5.13	Tetradecanoic acid
6	36.045	6.27	12.31	3.30	Phytol, acetate
7	36.202	0.62	1.29	3.10	2-Hexadecene, 3,7,11,15-tetramethyl-, [R-[R*
8	37.132	3.08	4.92	4.06	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
9	38.256	2.04	4.25	3.11	Hexadecanoic acid, methyl ester
10	39.152	9.77	5.61	11.29	Palmitoleic acid
11	39.868	39.18	19.14	13.26	Pentadecanoic acid
12	41.385	1.02	2.14	3.09	9-Octadecenoic acid, 1,2,3-propanetriyl ester, (
13	41.895	5.87	9.89	3.85	Octadecanoic acid, 2-propenyl ester
14	42.290	0.99	1.67	3.85	9,12-Octadecadien-1-ol, (Z,Z)-
15	44.172	2.82	3.18	5.74	Octadecanoic acid
16	45.635	0.81	1.57	3.35	Isopropyl linoleate
17	45.939	0.85	1.22	4.47	Ethyl iso-allocholate
18	46.103	1.35	2.12	4.11	5,8,11,14,17-Eicosapentaenoic acid, methyl es
19	49.968	1.85	3.32	3.60	4,7,10,13,16,19-Docosahexaenoic acid, methy
20	50.276	0.68	0.89	4.92	cis-9-Hexadecenal
21	50.645	1.94	2.34	5.36	Hexadecanoic acid, 2-hydroxy-1-(hydroxymet
22	58.135	0.77	1.45	3.43	Cholesta-3,5-diene
23	61.943	5.75	7.28	5.12	Cholesterol
24	62.120	0.55	0.67	5.29	Vitamin E
25	64.970	7.04	2.67	17.09	Eicosanoic acid, 2-[(1-oxohexadecyl)oxy]-1-[[
		100.00	100.00		

Method

[Comment]

===== Analytical Line 1 =====

[GC-2010]
Column Oven Temp. :50.0 °C
Injection Temp. :250.00 °C
Injection Mode :Splitless
Sampling Time :0.00 min
Flow Control Mode :Linear Velocity
Pressure :53.5 kPa
Total Flow :74.0 mL/min
Column Flow :1.00 mL/min
Linear Velocity :36.3 cm/sec
Purge Flow :3.0 mL/min
Split Ratio :70.0
High Pressure Injection :OFF
Carrier Gas Saver :OFF
Oven Temp. Program

Rate	Temperature(°C)	Hold Time(min)
-	50.0	1.00
4.00	300.0	5.00

< Ready Check Heat Unit >
Column Oven : Yes
SPL2 : Yes
MS : Yes
< Ready Check Detector(FTD/BID) >
< Ready Check Baseline Drift >
< Ready Check Injection Flow >
SPL2 Carrier : Yes
SPL2 Purge : Yes
< Ready Check APC Flow >
< Ready Check Detector APC Flow >
External Wait :No
Equilibrium Time :3.0 min

[GC Program]

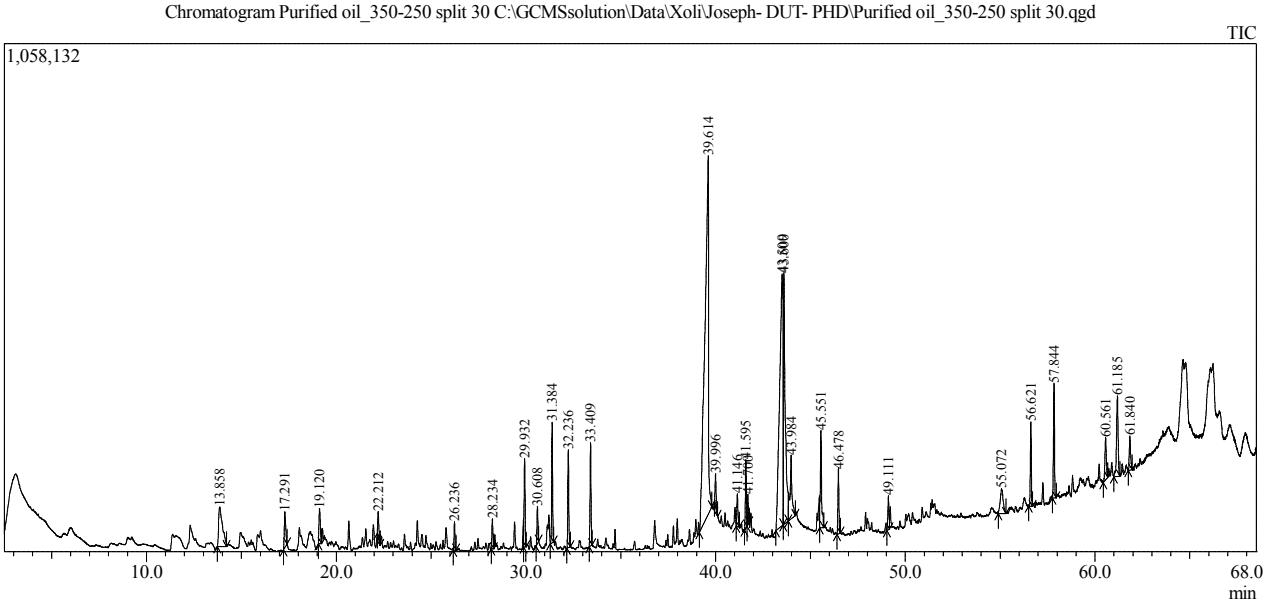
[GCMS-QP2010 Ultra]
IonSourceTemp :250.00 °C
Interface Temp. :280.00 °C
Solvent Cut Time :2.50 min
Detector Gain Mode :Absolute
Detector Gain :0.80 kV
Threshold :1000

[MS Table]
--Group 1 - Event 1--
Start Time :2.50min
End Time :68.50min
ACQ Mode :Scan
Event Time :0.30sec
Scan Speed :1428
Start m/z :50.00
End m/z :450.00

Sample Inlet Unit :GC

[MS Program]
Use MS Program :OFF

Chromatogram Purified oil_350-250 split 30 C:\GCMSsolution\Data\Xoli\Joseph- DUT- PHD\Purified oil_350-250 split 30.qgd



Peak Report TIC

Peak#	R.Time	Area%	Height%	Name
1	13.858	3.47	1.72	1-Phenyl-1-butene
2	17.291	1.17	1.56	trans-1-Phenyl-1-pentene
3	19.120	1.05	1.53	1,3,14,16-Nonadecatetraene
4	22.212	0.62	1.11	7-Phenyl-n-heptanol
5	26.236	0.56	1.24	Undecane, 4,7-dimethyl-
6	28.234	0.68	1.30	Doconexent
7	29.932	1.82	3.85	Benzene, 3-hexenyl-
8	30.608	0.77	1.63	[4.4.2]Propella-3,8,11-triene
9	31.384	2.59	5.24	Benzene, 6-heptynyl-
10	32.236	2.09	4.26	Benzene, 1-octenyl-
11	33.409	2.21	4.48	1,3,5,7-Cyclooctatetraene, 1-butyl-
12	39.614	29.93	15.55	Pentadecanoic acid
13	39.996	0.72	1.45	Tricyclo[7.4.0.0(3,8)]tridec-12-en-2-one, 5,6-e
14	41.146	0.79	1.42	Tricyclo[6.6.0.0(3,6)]tetradeca-1(8),4,11-trien
15	41.595	1.56	2.96	Octadecanoic acid, 2-propenyl ester
16	41.700	0.65	1.32	9-Tricosene, (Z)-
17	43.509	18.81	10.93	9,12-Octadecadienoic acid, methyl ester
18	43.600	11.22	10.87	9-Octadecenoic acid, (E)-
19	43.984	2.98	2.72	Octadecanoic acid
20	45.551	2.79	4.22	Hexadecanoic acid, 2-hydroxy-1-(hydroxymet
21	46.478	1.40	2.84	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-eth
22	49.111	0.79	1.44	9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1
23	55.072	1.67	1.10	8-Hexadecenal, 14-methyl-, (Z)-
24	56.621	1.81	3.65	Squalene
25	57.844	2.66	4.87	Cholesta-3,5-diene
26	60.561	1.31	1.85	.gamma.-Tocopherol
27	61.185	2.87	3.45	Stigmasta-4,7,22-trien-3.alpha.-ol
28	61.840	1.01	1.46	Vitamin E
		100.00	100.00	