Enhancing Efficiency of Biofuels from Microalgae Using a Statistical and Mathematical Approach

By

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ABSTRACT

Algae are primary producers in aquatic ecosystems and are thus the most important organisms in maintaining ecosystem functioning and stability. The usage of algae by humans is quite extensive; they act as an ingredient in aquaculture feed, a potential biomedical resource, as a fertiliser and as a nutritional source. Recently, algae have been identified as a third generation biofuel feedstock for fuel generation which essentially means that algae are more efficient, net carbon neutral and have less impacts on the environment. Algae as organisms are extremely sensitive to changes in the immediate environment. The interaction of parameters with each other causes minute changes in the environment which may alter the algae biomass present and the lipids that can be extracted from the biomass. The focus of this study is to model and determine which conditions maximise algal biomass and the subsequent lipids that can be extracted from the biomass. This will allow biofuel producers to understand which conditions are the best for harvesting algae in artificial conditions or harvesting algae from the wild. Furthermore, the model developed has broad application for biofuel specialists, pollution remediation specialists and biologists. This model developed is able to determine the present state of the algal bloom and uses the present state to predict the future state of bloom hence determining the optimal conditions to harvest. The model was developed under optimal ranges described by the Food and Agriculture Organisation (FAO) and designed to replicate the most common combinations of parameters present in the wild. For the purposes of this study, various combinations of parameters within their optimal ranges that is temperature (18 – 24°C), salinity (20 – 24 p.p.t.) and photoperiod (25 – 75% light exposure) were assessed. The model was run for 72 hours with sampling every 6 hours. Every six hours, algal growth was measured by the biomass present (chloro-pigments used as estimators); this was done by fluorescence. Lipids were then extracted from algal biomass using the Bligh and Dyer method (1959). Spline curves were fitted to the data and analysis performed using Mathematica 8.0. It was found that photoperiod was the most important variable in controlling algal growth. Furthermore, lipids extracted from biomass were at their highest when algae were exposed to the conditions 75% light exposure, 21°C and 22 p.p.t. These conditions would allow for the highest amount of biofuel to be produced. Generally, algae biomass trend graphs mimic lipid trend graphs over the 72 hour period that is when lipids are at their maximum, biomass concentrations are at their maximum. It can be concluded from time model that the best time to harvest biomass is 48 hours from the initial start time of algal growth to gain the highest amount of lipids for biofuel production.

PREFACE

The work described in this dissertation was carried out in the Westville campus of the School of Agriculture, Earth and Environmental Sciences, at the University of KwaZulu-Natal, from February 2011 to June 2012. This work was undertaken under the supervision of Dr. Helen Watson (Environmental Sciences) and Prof. Manoj Maharaj (Information Systems and Technology).

This study represents the original work of the author and has not otherwise been submitted in any form, in part or in whole, for any degree or diploma to any other university. Where use has been made of work by others, this has been duly acknowledged in the text.

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DECLARATION 1 - PLAGIARISM

I, ……Kamleshan Pillay.....……., declare that

1. The research reported in this thesis, except where otherwise indicated, is my original research.

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LIST OF ABBREVIATIONS

ATP – Adenosine Triphosphate

CO_2 – Carbon Dioxide

CH_4 – Methane

DIC – Dissolved Inorganic Carbon

DPSIR – Driving Forces-Pressures-State-Impacts-Responses

EU – European Union

FAO – Food and Agriculture Organisation

Fe (III) – Iron (+3 oxidation state)

GHG – Greenhouse Gas

H_2 – Hydrogen

LCA – Life Cycle Assessment

N_2O – Nitrous Oxide

NGO – Non Governmental Organisation

NH_4^+ – Ammonia

NO_2^- – Nitrite

NO_3^- – Nitrate

O_2 – Oxygen

POC – Particulate Organic Carbon

SGR – Specific Growth Rate

U.S.A. – United States of America

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Chapter One: Introduction

1.1. Background

There are many pertinent issues that need to be taken into consideration in attaining a sustainable environment. Two such issues include the degradation of the environment and the energy crisis (Hossain *et al.*, 2008; Brennan and Owende, 2010; Mata *et al.*, 2010; Mohan *et al.*, 2011; Singh and Olsen, 2011). There is general consensus in the literature that elevated concentrations of carbon dioxide (CO_2) and other greenhouse gases (GHGs) are contributing to global climate change hence there is a need to find cleaner energy solutions to replace fossil fuel usage (Amin, 2009). The implementation of renewable energy projects have in several cases yielded positive outcomes such as a reduction in the reliance on fossil fuels (Turner, 1999; World Energy Assessment, 2000; Menegaki, 2008). However, there needs to be more written legalisation and global policies as well as stricter implementation of policy (Singh and Olsen, 2011). There are many alternatives to polluting fossil fuels such as wind, solar, geothermal, marine and biomass sources (World Energy Assessment, 2000). It was also suggested by Elshashed (2010) that the implementation of renewable energies has become a political issue with countries aiming to minimise their dependence on oil rich countries.

Biomass sources may be considered a useful alternative energy if the correct feedstocks are used. The term biomass refers to organic material such as plants, trees and crops (World Energy Assessment, 2000). There are inherent problems with the use of biomass as an energy option. The low conversion rate of sunlight to biomass energy requires large areas where sources (feedstocks) can be grown (World Energy Assessment, 2000; Menegaki, 2008). Tirado *et al.* (2010) note the use of edible agricultural crops for energy production as opposed to feeding poverty-stricken people in developing countries is not a viable option.

In southern Africa, a few projects have been successful in producing appreciable biofuel, namely in Malawi, Zimbabwe and Kenya (Wolde-Georgis and Glantz, 2009; Jumbe *et al.*, 2009; Von Maltitz *et al.*, 2009). There have been very few biofuel projects in South Africa with key projects being the Jatropha Project in the North West Province and ethanol projects in Bothmaville in the Free State (Pillay and Da

Silva, 2009). According to Deenanath *et al.* (2012) there is a need for greater development of biofuel projects since South Africa has plans to become 50% dependent on biofuel by 2013.

A benefit of using algae as a feedstock is that algae have a higher carbon fixation rate than terrestrial plants (Mohan *et al.*, 2011). This means that algae are able to increase their biomass in a shorter time period hence yielding more biofuel products (Puppán, 2002; Brennan and Owende, 2010; Hussian *et al.*, 2010; Mata *et al.*, 2010). The sequestering of carbon by algae rather than terrestrial plants and the potentially high biofuel yield means that algae have the ability to reduce net CO_2 emissions and provide biofuel simultaneously (Chen *et al.*, 2011). Furthermore, most algae have lipid contents of between 20 and 50% hence appreciable amounts of raw materials can be attained for biofuel production (Chen *et al.*, 2011). Other benefits of algae as feedstock are its ability to be cultured in aquatic natural systems hence there is no competition for land being occupied by agriculture (Grobbelar, 1982; Chisti, 2007; Brennan and Owende, 2010; Mata *et al.*, 2010; Pittman *et al.*, 2011). Chen *et al.* (2011) maintain that this will have secondary positive impacts allowing for unsustainable agriculture practices such as intensive monoculture to be minimised. Moreover, the yields per hectare of algal biofuel as compared to terrestrial biofuel sources are tenfold (Groom *et al.*, 2008). Chisti (2007) reports that algae with 70% oil in weight have the potential to yield 136 900 L ha^{-1} of oil as compared to maize and soybean which yield only 172 L ha^{-1} and 446 L ha^{-1} respectively.

Additionally, algae have very high turnover rates with research showing that certain species may double their abundance in 48 hours (Chen *et al.*, 2011). With respect to optimising the amount of biofuel extracted from algae; there are many approaches. Changes in efficiency of biofuel yields from algae may be investigated by evaluating the maximum growth rates under different conditions (Bhola *et al.*, 2011; Chen *et al.*, 2011) or by evaluating the extraction and processing methods of algae to fuel products. Both approaches are equally important in trying to maximise output from algae, however it is important that optimisation tests take place under laboratory conditions first, and then in the field to account for natural variability.

The growth of algae is extremely difficult to assess as there are multiple variables to evaluate which are all intertwined (Converti *et al.*, 2009; Brennan and Owende, 2010; Mata *et al.*, 2010; Pillay and Pillay, 2012). Temperature, depth, pH, salinity, turbidity are some of the physico-chemical parameters that need to be taken into consideration. Furthermore, certain nutrients required for algal growth need to be in specific concentrations to allow for photosynthesis (Salisbury and Ross, 1992; Gruber, 2008; Smit 2008). Also, elements such as iron and silica need to be present for photosynthesis to occur (Sunda and Huntsman, 1995; Das and Chattopadhyay, 2000; Hu *et al.*, 2008). In previous studies, most effects of parameters on algal biomass growth rates have been looked at separately without any variable dependence (Keller, 1989; Boyd, 1991). However this research aims to evaluate three parameters (salinity, temperature and photoperiod) in relation to each other and determine the individual and cumulative effect of these parameters on the algal growth rate and lipid content. These parameters were chosen as they are important to algal growth; this can be easily and accurately manipulated, and allow for a simple yet precise model to be developed.

1.2. Motivation for the Study

With the acknowledgement that microalgae are an efficient source of biofuel (Chisti, 2007; Gouveia and Oliveira, 2009; Mata *et al.*, 2010; Brennan and Owende, 2010); there is need to optimise the raw materials (lipids) from microalgae. The evaluation of parameters affecting microalgal growth affects biofuel yields. This will allow for manufacturers to understand the driving processes behind the biology of algae thereby optimising yields (Converti *et al.*, 2009; Brennan and Owende, 2010; Mata *et al.*, 2010; Pillay and Pillay, 2012). It is imperative that studies be performed in the field and laboratory under controlled conditions. This will allow for a more detailed insight into the processes of algae growth as well as allow for natural variability to be investigated and applied to a laboratory set up. Furthermore, mixed algae cultures as well as individual algae cultures should be assessed. Natural populations consist of multiple species hence the evaluation of mixed algae populations makes this study more realistic than studies using single species (Smit, 2008).

According to Mohan *et al.* (2011) the production of microalgae biomass of one species is more expensive as parameters need to be controlled for *that* particular species. It is thus pertinent to evaluate mixed algae cultures as their may possess potentially higher yields at a lesser cost. With mathematical modelling, time can be taken into consideration and thus act as a determinant to the optimal conditions that produce the highest yields in lipids and algal growth.

The Food and Agriculture Organisation (FAO) stipulates optimal ranges of parameters that are ideal for algal growth. These parameters are seldom analysed simultaneously to evaluate how parameters interact with each other (Coutteau, 1996). It would be more insightful to explore the interaction of parameters as this provides a more realistic representation of nature (Pillay and Pillay, 2012). Aquatic environments that are uniform (Trujillo and Thurman, 2005; Smit, 2008) also aid in acting as a control and are also imperative to sample. Estuaries, lagoons, off shore, fluvial and man-made waterbodies are the aquatic systems that could be sampled, however for the purposes of this research only the estuarine ecosystem will be evaluated as estuaries are the most hydrologically dynamic of all marine environments (Smit, 2008). Several estuaries in South Africa tend to be eutrophic as they are highly polluted by industry and sewage works (Kibirige *et al.*, 2006). Thus the formulation of an algal growth model may aid not only in harvesting biofuel but may inherently allow for pollution remediation and hence ecosystem recovery.

For the purposes of this study, algal growth was evaluated for a 72 hour period. It was suggested by Converti *et al.* (2009) that doubling time of algal growth occurs in 48 hours. Hence a 72 hour study period was used as the time period after the 48 hour cycle also needed to be evaluated to increase accuracy. Additionally, the 72 hour study period was estimated to be long enough to detect algal blooms that occur in the wild. Furthermore, an assumption was made that the model will be cyclic as this allowed for mathematical modelling analysis to be performed on the data.

Models developed in this study will allow for the detection of the present state of algal biomass and lipid productivity and predicts the future state of environment theorising how long one should wait to harvest the maximum amounts of algae and lipid productivity. Consequently, this model will have practical application rather than

just being theoretical in nature. For example, the start time of an algal bloom can be theorised and the waiting time can be calculated to harvest the maximum amount of biomass and lipid productivity. In general, a timing model of this nature, allows for authorities of pollution remediation and biofuel producers to predict algal biomass maximisation which would allow for maximum biofuel products to be extracted. In this way, renewable energy is produced; ecosystem functioning of estuaries improved and pollution is reduced (if the ecosystem is polluted at all).

1.3. Aims and Objectives

This study aims to provide insight into how different combinations of physico-chemical conditions influence the growth of microalgae and to determine which harvesting times yield the maximum amount of lipids for biofuel production.

- To evaluate and model mathematically, physico-chemical conditions that yield the highest amount of algal biomass over a 72 hour period under laboratory conditions.

- To evaluate and model mathematically, physico-chemical conditions that yield the highest amount of products (oil or gas) per unit of microalgae biomass over a 72 hour period under laboratory conditions.

- To evaluate when harvesting of algae would maximise lipids to be attained over a short term study period of 72 hours.

- To extend the results from the laboratory test to real-world situations by modelling the optimal conditions under which naturally occurring algae may be harvested to produce maximal yield.

- To postulate reasons as to why certain trends exist for certain conditions based on a species composition analysis.

1.4. Outline of Thesis

Chapter two outlines a detailed survey of all literature which starts with discussing the general direction that renewable energy has taken globally and in South Africa. Also included in this chapter are details outlining biofuels, their advantages and disadvantages, policies and the potential of algae as a feedstock for biofuels. The biofuel production process is also discussed with facets of harvesting, conversion and methods of optimisation using mathematics. Chapter three evaluates the methodology used for this study which includes a description of the experimental design, lipid and biomass extraction techniques as well as details of the culturing of algae and initial analysis. The mathematical and statistical approach is also examined. Chapter four illustrates the model generated, the statistical tests performed and visual representations of all trends that were evaluated. Within chapter four, all results are explained and reasons put forth to why certain results were attained. The last chapter, chapter 5, concludes the study and incorporates a summation of all major findings. Recommendations for future studies are also outlined in this chapter.

Chapter Two: Literature Review

2.1. Introduction

Global climate change has necessitated a search for cleaner energy technologies to be researched and implemented (Hossain *et al.*, 2008; Brennan and Owende, 2010; Mata *et al.*, 2010; Mohan *et al.*, 2011; Singh and Olsen, 2011). Renewable energies act as alternatives to fossil fuels which have severe repercussions on the greenhouse gas (GHG) emissions: GHGs include nitrous oxides (N_2O), carbon dioxide (CO_2) and methane (CH_4) (Hossain *et al.*, 2008). Furthermore, Brennan and Owende (2010) estimate that fossil fuels account for approximately 88% of all energy consumption (35% oil; 29% coal; 24% natural gas). It is proposed that fossil fuels are responsible for 29 Giga tonnes of CO_2 emissions to the atmosphere with carbon sequestration processes only taking up 12 Giga tonnes of these emissions (Brennan and Owende, 2010). Subsequently, there is critical need to offset the excess carbon emissions *via* the implementation of mitigation strategies (Hossain *et al.*, 2008; Mata *et al.*, 2010; Mohan *et al.*, 2011; Singh and Olsen, 2011). The implementation of renewable energy is one such strategy. Solar, wind, geothermal, marine and biofuels are some of the different types of renewable energies that may be utilized with the common goal of reducing carbon emissions (Turner, 1999; World Energy Assessment, 2000; Menegaki, 2008).

2.2. Renewable Energies

The following subsections detail the three most widely utilized renewable energies in South Africa namely solar, wind and biomass energy (Banks and Schäffler, 2006). Banks and Schäffler (2006) also concluded that geothermal and wave energy were not well utilized in South Africa as the others and hence these energies are not detailed in the following subsections. It must be noted that each renewable energy has advantages and disadvantages and their efficiency is dependent upon on various factors. Some of these factors include where they are implemented, cost effectiveness of their implementation, effects on the natural environment and their conversion efficiency (Groom *et al.*, 2008).

2.2.1. Wind Energy

Wind energy was a major source of energy in the pre-industrial revolution, however the cost-effectiveness of fossil fuels led to their replacement (Ackermann and Söder, 2000; World Energy Assessment, 2000; Pimentel *et al.*, 2004). Recently, however, the installations of the first wind turbines worldwide have allowed wind to become a feasible renewable energy resource and contribute significantly to electricity production (Jebaraj and Iniyan, 2006). In the past, the ratio of the size and weight of wind turbines caused efficiency problems (Pimentel *et al.*, 1994). Theoretically, wind power has the potential to produce 123 petawatt hours per annum, globally (Lu *et al.*, 2009). The potential of wind energy is determined by a multitude of factors ranging from the economics of the wind turbine system to the environmental conditions present (World Energy Assessment, 2000). Parameters such as wind speed, wind distribution, turbulence and terrain roughness play an important role in determining the feasibility of wind power (Gross *et al.*, 2003; Jebaraj and Iniyan, 2006; Lu *et al.*, 2009). Gross *et al.* (2003) suggest that wind speed can be seen to be the most important factor influencing the generation of wind energy however it is actually the time at which the time period over a critical wind speed threshold. In the U.S., only 13% of the land area has wind speeds greater than 22 km/hr, the speed which allows for sufficient amounts of energy to be produced (Gross *et al.*, 2003; Lu *et al.*, 2009). The implementation of wind energy has minimal environmental impacts such as minor losses of insects, birds flying into turbines and blades causing noise pollution (Pimentel *et al.*, 1994).

Studies by Matthies *et al.* (1995) showed that the use of coastal wind energy could contribute significantly (up to 70%) to the European Union's (EU) energy demands (Gross *et al.*, 2003; Archer and Jacobson, 2005). Banks and Schäffler (2006) suggest that wind development projects in Africa and South Africa have been relatively non-existent. This is owing to the lack of funding for pilot projects from international non-governmental organisations (NGOs) with projects having to run on minimal budgets provided by that country (van der Linde, 1996; Ackermann and Söder, 2000). There have been some projects in northern Africa (Egypt and Tunisia) but fossil fuels are still more prominent (Ackermann and Söder, 2000; Elamouri and Ben Amar, 2008)

regardless of the proposal by some researchers (eg. Banks and Schäffler (2006)) that the potential for wind energy is great.

2.2.2. Solar Energy

The basic principle of solar energy involves the conversion of direct sunlight into electricity or the use of sunlight in heating (World Energy Assessment, 2000; Goswami *et al.*, 2004; Alboteanu *et al.*, 2006). This is done by the capturing of sunlight by flat plate and concentrator panels; the solar cell is the most integral component of the system which is responsible for the generation of free electrons using the energy of light (World Energy Assessment, 2000; Goswami *et al.*, 2004; Alboteanu *et al.*, 2006; Crabtree and Lewis, 2007). The capturing of sunlight to the earth's surface is measured as a unit of energy per unit area per a specific amount of time with variations in energy according to location in a horizontal plane (Şen, 2004; Alboteanu *et al.*, 2006; Crabtree and Lewis, 2007). Also seasonality differences and latitude changes influence the amount of sunlight available at the earth's surface. The World Energy Assessment (2000) suggested that the conversion efficiency of solar panels tend to be approximately 10% -15% hence an appreciable amount of solar energy is needed for the generation of electricity. Recently however, more recently Lewis (2007) estimated the solar efficiency limit to be 31%.

Currently, there are many engineering projects that are trying to streamline and make the process more efficient (Şen, 2004; Banks and Schäffler, 2006). Different materials used in the construction of solar panels, altering the shape of the panel and the angle at which sunlight is received and solar tracking are some such methods that are being implemented in order to enhance efficiency and storage of energy (World Energy Assessment, 2000; Şen, 2004; Alboteanu *et al.*, 2006; Crab and Lewis, 2007). Lastly, Borenstein (2008) states that the cost factor of solar panels and their subsequent implementation are the most apparent problems that inhibit solar energy from becoming a widely used renewable energy.

2.2.3. Biomass/Biofuel Energy

Biomass simply refers to all organic material (plants, algae, animals) while biomass sources for renewable energy refer mostly to crops which are cultivated to attain biofuels, heat and electricity (World Energy Assessment, 2000). Biomass contributes

significantly to energy supply in developing countries (20 – 33%) while to a lesser extent in developed countries (~ 3%). Globally, biomass energy only contributes 14% of energy consumption (World Energy Assessment, 2000; Parikka, 2004; Banks and Schäffler, 2006). In developing countries, the use of biomass is primarily for firewood and for basic needs (Watson, 2009). Furthermore, Puppán (2002) and Brennan and Owende (2010) conclude that the use of biomass has environmental consequences on soil and land resources as well as contributing to CO_2 emissions. The low conversion of solar energy captured to biomass energy produced means that there is a need for vast feedstocks to provide appreciable amounts of fuel (Menegaki, 2008).

Scharlemann and Laurance (2008) have suggested that there are many factors that influence how "green" biofuels may actually be. The conventional approach of evaluating how much greenhouse gas emissions are reduced does not allow for an accurate evaluation of how environmentally friendly a feedstock is (Zah *et al.*, 2007). Where a feedstock is grown, what type of ecosystem it has replaced, ecosystem functioning lost (if any), trace gas emissions from fertilisers as well as the "cost and demand" paradigm shift that occurs are all factors that determine how efficient a feedstock is (Scharlemann and Laurance, 2008). A study by Zah *et al.* (2007) showed 80.7% of tested first generation biofuels feedstocks illustrate a reduction in greenhouse gas emissions, however only 46% show a less detrimental compounded effect on the environment than fossil fuels do. Unfortunately, it is rather difficult to quantify compounded environmental effects as can be seen in Table 2.1. Different feedstocks have different overarching effects on world food markets that may change the demand in a particular feedstock. This inherently changes how it is grown in other countries which may lead to further environmental impacts of natural ecosystems (Scharlemann and Laurance, 2008). An example would be the changes to the Amazon rainforest to cultivate more soy to keep up with the demand of soy in the U.S. Hence in the U.S., farmers are changing to corn as a feedstock as they receive a subsidy from the government for growing corn (Scharlemann and Laurance, 2008).

The usage of potential crops for fuel rather than as a food source is a contentious issue especially in poverty stricken countries (Tirado *et al.*, 2010). Tilman *et al.* (2006) demonstrated that non edible resources such as low-input, high-diversity grassland can produce significantly more energy with less GHG emissions and have less

secondary impacts on the environment than agricultural crops. However, it can be concluded that microalgae are the most efficient of all biofuels in terms of its net energy production and secondary environmental impacts (Abbasi and Abbasi, 2010; Elshashed, 2010).

2.3. Types of Biofuel

The common energy products from biomass include alcohols (ethanols, propanols, butanols, propane and butane diols), biodiesel, hydrogen and biogas (Demirbas, 2008; Luque *et al.*, 2008; Elshahed, 2010; Singh and Olsen, 2011). It is generally accepted that there are two streams of biofuel technologies that is, first generation biofuels and second generation biofuels (Luque *et al.*, 2008; Elshahed, 2010; Naik *et al.*, 2010). First generational biofuels refer to those derived from conventional feedstocks and technologies and can be characterised by their ability to be "blended with petroleum based fuels, combusted in existing combustion engines and distributed through existing infrastructure or by their use in existing alternative vehicle technology or natural gas vehicles" (Luque *et al.*, 2008; Naik *et al.*, 2010). Conversely, second generation biofuels refer to those technologies that are relatively new and are most often more efficient than traditional fuels (Luque *et al.*, 2008). Furthermore, second generation biofuels are carbon neutral or carbon negative (Abbasi and Abbasi, 2010; Naik *et al.*, 2010).

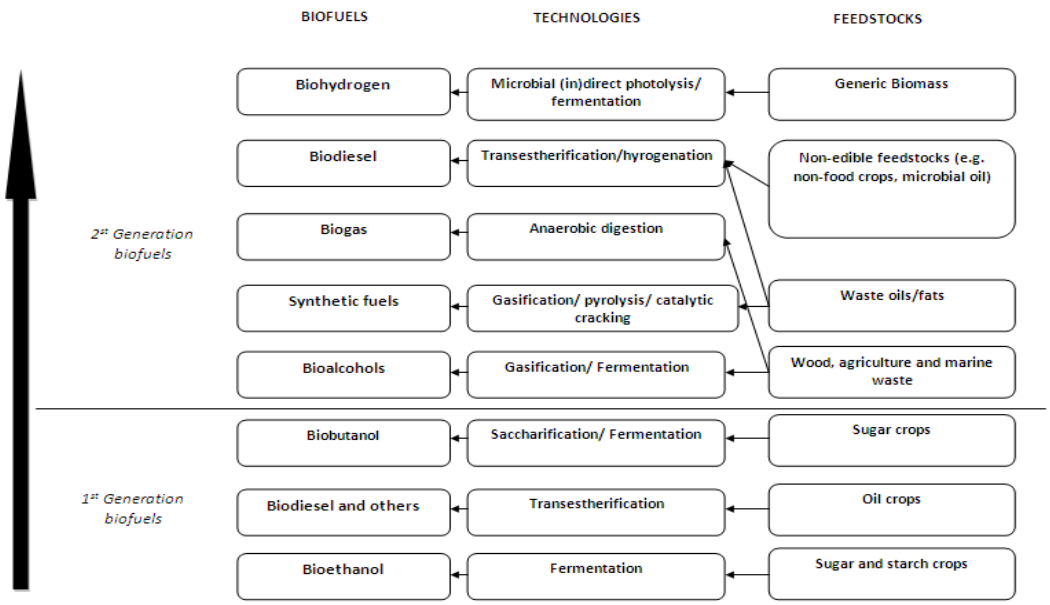

Figure 2.1: An explanation of the biofuels production and technologies with differentiation by generation, technology and feedstocks associated with each fuel (adapted from Luque, 2008).

Figure 2.1. illustrates the biofuels type, technology and feedstock associated with biofuels type. The three main types of first generation biofuels are biodiesel, ethanol and biogas. First and second generation biofuels can be produced from edible oils (rapeseed, canola, soybean, sunflower and palm oil), non-edible oils (J.*curcas*, M. *indica*, F. *elastic*, A. *indica*, silk cotton tree, rubber seed and microalgae) and fats (Demirbas, 2008; Vasudevan and Briggs, 2008; Demirbas, 2009; Naik *et al.*, 2010). After a feedstock is chosen, crops need to be harvested, processed and converted into a usable form and these procedures are outlined in section 2.8 and 2.9. First generation biofuels can be produced by fermentation, transesterfication and saccharification whilst first generation biofuels are produced by transesterfication, gasification, fermentation, pyrolysis, catalytic cracking, anaerobic digestion, hydrogenation and photolysis. Certain second generation biofuels made from crop residues need to be produced by advanced technologies as they are composed of polymers which are more difficult to breakdown than simple sugars (Elshashed, 2010). Lastly, algal-based biofuels are known as third generation fuels and are shown to have distinct efficiency advantages over other feedstocks (Chisti, 2007; Gouveia and Oliveira, 2009; Abbasi and Abbasi, 2010; Elshashed, 2010 Mata *et al.*, 2010; Brennan and Owende, 2010) and these will be discussed in section 2.6.

There are many advantages and disadvantages to biofuel production. Compared to conventional fossil fuels, biofuels exhibit a closed carbon system (Puppán, 2002; Abbasi and Abbasi, 2010; Naik *et al.*, 2010). This means that the carbon emissions from burning are offset by the carbon absorbed by the plant whilst in the growing stages thus making them carbon neutral or carbon negative (Puppán, 2002). Table 2.1 indicates the different types of feedstocks, the environmental impacts on resources as well as whether the implications are either high, medium or low and lastly, the land resources needed to meet transportation needs. Even though there have been mass monoculture projects for corn, they have a poor energy efficiency (1.1 – 1.25) and have high resource usage as compared to other biofuel types (Scharlemann and Laurance, 2008). Energy efficiency refers to the ratio of energy output to fossil fuels input to generate the renewable energy. Also there are secondary implications to consider, that is eutrophication, degradation to land and soil resources, habitat clearing for intensive monoculture (loss of biodiversity), interruption of nutrient

cycles and socio-economic impacts (Groom *et al.*, 2008; Okonko *et al.*, 2009; Abbasi and Abbasi, 2010).

Furthermore, Table 2.1 highlights the other main agricultural crop used as a feedstock, sugar cane. Even though sugar cane has a higher efficiency than corn (8 – 10), it still requires an appreciable amount of land (85 – 105 ha) with a specific climate. In Brazil, the realisation that sugar cane can provide a sizeable amount of fuel contributing of the overall energy needs, has coincided with the issue of habitat clearing to the Amazon rainforest for culturing of sugar cane (Groom *et al.*, 2008). The use of indigenous species that cannot be consumed could be a more viable option (Tilman *et al.*, 2006; Groom *et al.*, 2008; Elshashed, 2010). They usually have less resource needs and hence a higher conversion efficiency (native Prairie, Poplar and Willow spp.) (Groom *et al.*, 2008; Elshashed, 2010). They also increase overall functionality of the ecosystem by retaining indigenous biodiversity as there is no need for habitat clearing (Elshashed, 2010). There is a need to investigate the possibility of polyculture of crops or the use of species that are indigenous to a particular area such that there is no significant strain on the soil, water and land resources (Groom *et al.*, 2008). Additionally, Elshashed (2010) stated that there is a need to incorporate residues that reduce erosion and incorporate tillage practices and crop rotations.

Theoretically, the most efficient types of biofuel are those from microalgae (Chisti, 2007; Gouveia and Oliveira, 2009; Mata *et al.*, 2010; Brennan and Owende, 2010). The most attractive attribute of algae within biofuels is that they are able to produce massive amounts of fuel whilst requiring minimal space (1.5 – 3.2 ha of land area, Table 2.1) without having high resource demands and possess high lipid contents as compared to terrestrial plants (Chisti, 2007; Groom *et al.*, 2008). Some algae species prefer saline or brackish water and this can also be seen as an advantage as it will not compromise freshwater resources (Puppán, 2002; Brennan and Owende, 2010; Mata *et al.*, 2010).

Table 2.1: The various types of biofuels and their relative conversion effeciency, GHG emissions, resource usage and yields (as adapted from Groom *et al.*, 2008).

Biofuel crop	Energy conversion efficiency	GHG emissions (kg CO_2/MJ)	Water use	Fertiliser use	Pesticide use	Energy input	Fuel yield (1/ha)	Land area needed to meet 50% of U.S. transportation fuel demands	
								Million ha	% U.S. cropland
Grasses – ethanol corn	1.1-1.25	81-85	high	high	high	high	1135-1900	290-485	157-262
Sugar cane	8-10.2	4-12	high	high	med	med	5300-6500	85-105	46-57
Switch grass	1.8-4.4	-24	med-low	low	low	low	2750-5000	110-200	60-108
Native prairie grasses	5.44	-88	low	low	low	low	940	585	316
Woody biomass – ethanol/synfuel Poplar and willow spp.	10	-24 to 11	low-med	low-med	low	low	5500-9000	60-100	32-54
Fischer-Tropsch	18-64	-24 to 11	low-med	low-med	low	low	30000-50000	11-18	6-10
Residues biodiesel/ethanol									
Wood residues	20-40	-	med	low	low	low	1150-2000	275-475	150-250
Corn stover	5-11	81	med	high	high	low	0.25-031kg	-	-
Wheat straw	2-5	-	low	med	med	low	0.3-0.51 kg	-	-
Oil crops – biodiesel		-							
Soybeans	1.9-6	49	high	low-med	med	med-low	225-350	330-450	180-240
Rapeseed or canola	1.8-44	37	high	med	med	med-low	2700	55	30
Oil palm	9	51	high	med	low	low	4760	34	18
Microalgae - biodiesel	-	-183	med	low	low	High	49700-108800	1.5-3.2	1.1-1.17

2.4. Biofuel Policy and Economics

Governments have the duty to maintain food security, alleviate poverty, promote and protect social justice whilst at the same time caring for the environment (Pray and Zilberman, 2009). With the resurgence of the use of biofuels in the last decade, there is a need for new environmental governance that prescribes to "ethical consumption" (Davies *et al.*, n.d.). There is a need to demonstrate and implement sustainable production of biofuels. Holistically, economic and environmental concerns need to be taken into consideration in the entire biofuels production system (Watson, 2009). Davies *et al.* (n.d.) suggested the need for a framework to be developed to determine sustainable production, use and distribution within the country and internationally. This can be done by a LCA (life cycle assessment) of the components of a biofuel system (Davies *et al.*, n.d.). Internationally there is a need for biofuels to be produced in a way that subscribes to the policy and laws of countries that are trading in biofuels (Hecht *et al.*, 2009). The DPSIR (driving forces-pressures-state-impacts-responses) system developed by the European Environment Agency is able to evaluate the biofuels system and establish how sustainable it may be (Hecht *et al.*, 2009). Even though Brazil and the U.S. are the biggest producers of biofuel, other countries are also developing their biofuels production systems (Okonko *et al.*, 2009).

"Energy is an essential input driving economic development." (Demirbas, 2008). This statement illustrates the need for energy policies within larger frameworks to allow for competition between renewable and non-renewable energy resources in the economic market (Puppán, 2002). More businesses are introducing the use of renewable energies into businesses to gain a competitive edge by reducing the need for conventional fossil fuels; the use of renewable energy also enables businesses to promote greenness whilst reducing costs (Archbold, 2007).

Walker (2009) declared that one of biofuel policy's main initiatives would be the replacement of transport fossil fuels with biofuels; the use of biofuels in transportation would have a major impact on GHG emission. Biofuels have little or no emissions which would mean a smaller emission footprint for companies (Puppán, 2002; Demirbas, 2008). The main objectives of biofuel policy in general is to promote economic growth in rural areas, conservation for the environment as well as the competitiveness in the job market as discussed earlier (Puppán, 2002;

Archbold, 2007; Demirbas, 2008). The usage of biofuel in rural areas can provide employment as well as electricity whilst promoting other environmental benefits such as soil conservation, run off interception and carbon sequestration when growing (Brennan and Owende, 2010).

Even though there are many benefits to the usage of biofuels, they still cost more to produce than traditional fuels; the use of biofuels within the automotive industry is likely to become more prominent owing to the implementation of tax penalties or commonly referred to carbon taxes for those businesses with higher emissions (Puppán, 2002; Archbold, 2007). There is a strategy in place that by 2050 about half of the energy requirements in the developing world will depend on biomass. Furthermore, there may be a replacement of gasoline and diesel by ethanol and biodiesel, respectively (Demirbas, 2008). Bioenergy from agricultural sources may allow for socio-economic development; unfortunately, natural gas processes are still cheaper than some biofuel processes which is why conventional energies are still prevalent in the market (Archbold, 2007; Demirbas, 2008).

2.5. Biofuel Development in Southern Africa

Owing to the dependence on rainfall for agriculture, Africa as a continent is extremely susceptible to food shortages and irregular crop yields (Wolde-Georgis and Glantz, 2009; Pillay and Da Silva, 2009; Deenanath *et al.*, 2012). With the insecurity of the oil prices and markets, there has been an increasing dependence on imported oil (Wolde-Georgis and Glantz, 2009). On average, African countries spend 30 – 40% of their export earnings on importing oil (Wolde-Georgis and Glantz, 2009). There has been agreement amongst policy makers and environmental analysts that there is a need for renewable energy implementation as opposed to fossil fuels (Brennan and Owende, 2010). Decision makers have seen the need to attract local and international conglomerates to invest in biofuel projects in Africa and attempts have been made to integrate this into policies and frameworks (Wolde-Georgis and Glantz, 2009). There have also been partnerships with African countries and other developing countries that have extensive experience in biofuel implementation (India and Brazil) (Pillay and Da Silva, 2009). Furthermore, there is a move not only for the implementation of biofuel in Africa, but specifically liquid biofuels which are seen to be more environmentally friendly and more efficient in terms of yields (Wolde-Georgis and Glantz, 2009), however this has been shown to

not always be true (Scharlemann and Laurance, 2008). The most important feedstocks that have been suggested in Africa are sugar cane, sweet sorghum and cassava. Hence, the tropical climate is the most important factor in promoting these specific crops (Wolde-Georgis and Glantz, 2009).

There are three development strategies that have been suggested; biofuel production on a large scale by refineries, biofuel crops provided by farmers to refineries; and biofuel feedstocks grown by farmers to provide themselves with energy (Wolde-Georgis and Glantz, 2009). There are inherent problems with these development strategies. Communal and tribal authorities are prominent in Africa and land use decisions are often not easy and may lead to social conflict; furthermore, land usage is normally dependent on environmental impact assessments which are time consuming (Watson, 2009; Wolde-Georgis and Glantz, 2009). The debate of providing crops for energy security rather than alleviating poverty and starvation is quite contentious in Africa (Tirado *et al.*, 2010). Even though there may be job creation for rural areas, it is not certain that these jobs will be permanent (Watson, 2009).

There have been various successful projects such as the Zimbabwean Triangle Ethanol project, the Malawian Dwangwa Estate plant and the Kenyan Muhoroni plant which produces between 15 and 120 million litres of fuel annually (Wolde-Georgis and Glantz, 2009; Jumbe *et al.*, 2009; Von Maltitz *et al.*, 2009). According to Deenanath *et al.* (2012), large scale biofuel projects in southern Africa are quite stagnant. South Africa has specifically proposed to have 20 - 50% of fuel needs provided by biofuel by 2013 (Banks and Schäffler, 2006; Deenanath *et al.*, 2012). In South Africa, there are a few objectives of policy makers which include less dependence on fossil fuels for transportation; use of algae as a feedstock and more awareness (Pillay and Da Silva, 2009). There are few projects in South Africa: The Jatropha Projects in the North West Province and the ethanol projects in Bothmaville, Free State being the major landmark projects (Pillay and Da Silva, 2009).

2.6. The Potential of Microalgae in Biofuels

As stated earlier, microalgae as a biofuel holds distinct advantages over all other biofuels and it is seen to hold the most potential as a renewable energy source in the future (Chisti, 2007;

Gouveia and Oliveira, 2009; Abbasi and Abbasi, 2010; Elshashed, 2010 Mata *et al.*, 2010; Brennan and Owende, 2010). Algae have the potential to produce up to 108 800 L ha^{-1} (Groom *et al.*, 2008, Table 2.1); an appreciable amount more than any other feedstock. Microalgae have the ability to be cultivated on a large scale all year round and have minimal space requirements and hence oil productivity is continuous leading to higher yields as compared to other feedstocks (Grobbelar, 1982; Chisti, 2007; Brennan and Owende, 2010; Mata *et al.*, 2010; Pittman *et al.*, 2011). The needs of algae are less; they are grown in an aqueous medium but they require less water than crops do from irrigation (Brennan and Owende, 2010; Hussian *et al.*, 2010; Pittman *et al.*, 2011).

Furthermore, the use of raceways, ponds and other artificial environments allow for exploitation of non-arable land whilst the cultivation of marine algae allow for the use of brackish and saline aquatic environments placing less strain on freshwater resources (Puppán, 2002; Chisti, 2007; Campbell, 2008; Brennan and Owende, 2010; Mata *et al.*, 2010; Scott *et al.*, 2010; Wahal and Viamajala, 2010; Pittman *et al.*, 2011). The most apparent advantage of microalgae over other biofuels are its rapid growth rate; algal cells may have up to a 50% oil content of their dry weight as well as illustrating exponential growth which can see them double their biomass in less than four hours (Chisti, 2007; Brennan and Owende, 2010; Hussian *et al.*, 2010).

There is a plethora of secondary positive impacts that make algae a more viable and sustainable option. Firstly, algae have the ability to drastically improve air quality by fixating CO_2; furthermore, algae have the ability to grow in polluted waterways which facilitates the uptake of excess nitrogen and phosphorus subsequently allowing for the rehabilitation of effluent contaminated water (Puppán, 2002; Brennan and Owende, 2010; Hussian *et al.*, 2010; Mata *et al.*, 2010). Unlike terrestrially grown crops used for biofuel, there is no need for the use of harmful pesticides and herbicides which is more environmentally friendly and there are range of secondary products that can be obtained from microalgae (feed for aquaculture or fertiliser) (Puppán, 2002; Groom *et al.*, 2008; Brennan and Owende, 2010).

Despite all the benefits and advantages of algae as a biofuel, there are still apparent gaps in our scientific knowledge. Brennan and Owende (2010) concluded that there is a need for more

investigation into the growth rates and lipid productivity of different individual species growth rates (SGRs). Additionally, there is a need for large scale operations to determine whether theoretical maximum yields will equate to actual yields; the use of microalgae within biofuels will surely lead to the adherence of the overall goal to maintain sustainability (Puppán, 2002; Brennan and Owende, 2010; Hussian *et al.*, 2010; Mata *et al.*, 2010). Figure 2.2 delineates the stages of the biofuel process from species and site selection to oil extraction. The inputs of requirements for algal growth are detailed which precedes the algal species and site selection.

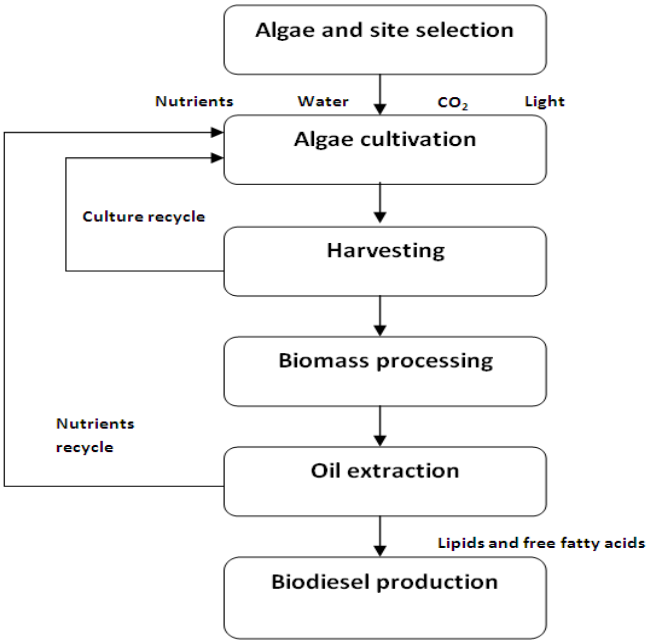

Figure 2.2: An overview of the life cycle of algae through all biofuel processing phases (as adapted from Mata *et al.*, 2010).

2.7. The Biology of Algae

Algae can be defined as aquatic organisms that have the ability to photosynthesise (chlorophyll *a* as the main photosynthetic pigment); they are some of the oldest organisms on earth and may be either eukaryotic or prokaryotic in nature (Grobbelaar, 1982; Campbell, 2008; Converti *et al.*, 2009; Brennan and Owende, 2010; Mata *et al.*, 2010). Alga being unicellular (microalgae) or simply multicellular (macroalgae) in nature allows the adaptation to harsh environments as they can reproduce and subsequently colonise environments rapidly (Mata *et al.*, 2010). The classification of algae into different classes is defined primarily, on their life cycle, basic cellular

structure and pigmentation (Brennan and Owende, 2010). The four most common classes are green algae (Chlorophyta), red algae (Rhodophyta), diatoms (Bacillariophyta) and brown algae (Heterokontophyta) (Smit, 2008). Green and red algae belong to the same group however diatoms and brown algae are within distinct groups (Scott *et al.*, 2010).

Algae may either be autotrophic, heterotrophic or mixotrophic: Autotrophic algae refers to those species that have the ability to photosynthesise by the use of light, CO_2 and salts to form carbohydrates whilst heterotrophic algae are able to obtain nutrients from external sources and mixotrophs refer to those species that are able to grow in either manner (Converti *et al.*, 2009; Brennan and Owende, 2010; Mata *et al.*, 2010). The growth of algae is a very complex process and requires insight to interactions between all growth parameters within the aquatic medium (Pillay and Pillay, 2012). All parameters are intertwined and thus it is crucial to evaluate all parameters simultaneously in order to ascertain the ideal growth conditions of algae (Converti *et al.*, 2009; Brennan and Owende, 2010; Mata *et al.*, 2010; Pillay and Pillay, 2012).

2.8. Physico-Chemical Parameters Affecting Algae Growth

There are a variety of factors that influence algal growth. Even though all factors that contribute to algal growth are detailed below, only the salinity, temperature and light were evaluated in this study. It is important to note that each parameter cannot be evaluated in isolation and that all parameters have effects on the others and there is constant interaction present (Pillay and Pillay, 2012).

2.8.1. Light

Light is the most obvious factor that affects algal growth and photosynthesis (Sorokin and Krauss, 1958; Foy *et al.*, 1976; Salisbury and Ross, 1992; Wahal and Viamajala, 2010; Scott *et al.*, 2010; Sharma *et al.*, 2011). In laboratory settings, light intensity can be controlled to a specific level depending on the need of a particular species in order to maximise algal growth (Wahal and Viamajala, 2010; Sharma *et al.*, 2011). In natural systems, however, light attenuates in the water column according to depth hence the differentiation in zones (euphotic, disphotic and aphotic) (Trujillo and Thurman, 2005). A constant positive relationship is not always present with light and algal growth, as at high light intensities algal growth is inhibited owing to other

environmental parameters not being suitable (CO_2, temperature, salinity, pH, CO_2 partial pressures and nutrient concentrations). (Sorokin and Krauss, 1958; Foy *et al.*, 1976).

Certain algal species that are genetically different are only able to handle a specific range of light intensity (Sorokin and Krauss, 1958; Foy *et al.*, 1976). Species that are not able to handle high light intensities are normally those that are adapted to living at depth or in shaded areas or are incapable of handling certain wavelengths of light at certain depths when light intensities are too high (Sorokin and Krauss, 1958; Foy *et al.*, 1976). Furthermore, light intensities can have a major impact on the formation of polar membranes lipids and storage of neutral lipids (Hu *et al.*, 2008).

2.8.2. Temperature

Temperature within aquatic systems is mostly affected by the penetration of sunlight into the environment (Trujillo and Thurman, 2005). The sunlight allows for the uppermost part of the water column to be warmed up and hence a distinction into three photic zones (euphotic, disphotic and aphotic zones) (Trujillo and Thurman, 2005; Smit, 2008). The depth variation causes warmer water to overlay colder water thus forming a thermocline; the thermocline is important in limnology and marine science as it governs the exchange of nutrients between the depth and surface waters (Trujillo and Thurman, 2005). Colder water lying at depth is denser and subsequently has more capacity to hold nutrients; these nutrients when brought to the surface *via* upwelling processes allow for primary production of algae (Moss, 1973a; Trujillo and Thurman, 2005; Converti *et al.*, 2009). Microalgae will be most productive at the surface as light is in abundance and nutrients will be available *via* upwelling (Moss, 1973 (a, b); Trujillo and Thurman, 2005; Converti *et al.*, 2009).

Temperature is the major contributor to cellular, morphological and physiological changes in microalgal populations (Kumar *et al.*, 2010; Sharma *et al.*, 2011). Algal species generally have an optimal temperature range that they prefer (18 – 24°C) (Stephens *et al.*, 2010). Generally an increase in temperature increases the metabolic and growth rate and a lower temperature means less growth potential (Kumar *et al.*, 2010). However, the interaction of parameters with each other (light and temperature) may cause changes to optimal conditions of algal growth (Kumar *et*

al., 2010; Stephens *et al.*, 2010). Lastly, the temperature may have implications on the fatty acid and lipid content of cells where thermal changes can cause changes to the membrane and cell structures which inherently will affect the amounts of lipids that can be harvested for biofuel production (Hu *et al.*, 2008).

2.8.3. pH, Salinity and Turbidity

Physico-chemical factors such as salinity, pH and turbidity are determinants of the rate of primary production of microalgae (Abril and Borges, 2004; Kumar *et al.*, 2010). According to Garcia-Luque *et al.* (2005), there is a linear relationship between salinity and partial pressures of CO_2. Increases in pH are normally accompanied by increases in salinity (Garcia-Luque *et al.*, 2005). Salinity is subsequently an important mechanism in primary production. It cannot be said that lower salinities equate to higher primary production rates, but rather that lower salinities cause higher partial pressures of CO_2. Hence, there is a possibility that there may be an increase in primary productivity owing the availability of carbon. Turbidity refers to transparency of the water column owing to particles in the water; the evaluation of turbidity is vital as it directly affects the amount of light entering the aquatic body at a particular depth (Trujillo and Thurman, 2005).

pH has consequences for primary productivity: It has consequences for the enzymatic activity within the cell walls of algae and the uptake of nutrients from the environment (Moss, 1973 (a); Kumar *et al.*, 2010). There is a toxic effect when there are increased concentrations of dissolved ions when the pH is too high; also the availability of inorganic carbon ions is affected by higher pH (Moss, 1973 (a); Abril and Borges, 2004; Kumar *et al.*, 2010).

2.8.4. Nutrient Availability

2.8.4.1. Carbon

Carbon fluxes in aquatic systems are controlled by photosynthesis by primary producers and respiration by all biota including microalgae (Duarte *et al.*, 2005; Kumar *et al.*, 2010). Carbon in the form of carbon dioxide enters the ecosystem and forms part of the dissolved inorganic carbon (DIC) component across the air-water interface (Kumar *et al.*, 2010). DIC includes carbonates, bicarbonates and dissolved carbon dioxide (Cai and Wang, 1998; Kumar *et al.*, 2010). DIC

which is assimilated by algae by photosynthesis is converted to particulate organic carbon (POC) (Cai and Wang, 1998). Carbon may be transferred by heterotrophy to other trophic levels. Death of organisms may result in export and burial to sediments in the form of marine snow for assimilation to algae (Duarte *et al.*, 2005). DIC may be transported back across the pynocline *via* diffusion (Cai and Wang, 1998). There are many external sources of carbon such as diffusion of CO_2 across the air-water interface, allochthonous input by rivers, marshlands, mud flats and death of primary producers (Cai and Wang, 1998; Duarte *et al.*, 2005). Internal sources of carbon evolve from the recycling of dead POC as well as cellular exudates, excretion and sloppy feeding (Duarte *et al.*, 2005). The air-water interface however does not allow for uncontrolled entrance of carbon dioxide. For CO_2 entry, there must be a pressure gradient present (Garcia-Luque *et al.*, 2005). It is important to note that the certain species of microalgae can only assimilate carbon in certain forms either carbon dioxide, bi-carbonate or carbonates and the incorrect form of carbon could be potentially toxic to their survival (Moss, 1973a).

2.8.4.2. Nitrogen

Nitrogen is an integral nutrient within all aquatic systems as it essential for protein synthesis in primary producers (Salisbury and Ross, 1992; Gruber, 2008; Smit 2008). They form the building blocks of amino acids and chlorophyll molecules which are vital to photosynthesis and growth (Salisbury and Ross, 1992). However, nitrogen needs to be bioavailable to algae as not all forms are readily assimilated by algae (Smit, 2008). Nitrogen fixation by cyanobacteria (blue-green algae) allow for the production of organic forms of nitrogen (nitrate (NO_3^-), nitrite (NO_2^-) and ammonium (NH_4^+)) which can be used by other photosynthetic organisms (Gruber, 2008). Remineralisation processes of dead organic matter or nitrogen from allochthonous sources may also provide bioavailable nitrogen (Gruber, 2008). The biological pump that exists in natural marine bodies allows for upwelling circulations to constantly bring nitrogen to the euphotic zones where primary production occurs (Trujillo and Thurman, 2005; Gruber, 2008).

2.8.4.3. Phosphorus

Phosphorus is needed as it forms a structural and functional component in all biological organisms and is also responsible for chemical energy transfer and the production of ATP (Salisbury and Ross, 1992). The availability of phosphorus is integral to photosynthesis and can

limit the amount of primary production (and hence the species distribution) (Paytan and McLaughlin, 2007; Hu *et al.*, 2008) if not in sufficient concentrations. Orthophosphate is the specific form of phosphorus that is necessary in the photosynthetic reaction (Smit, 2008). Unlike nitrogen, phosphorus cannot be fixed by any organisms and needs to be recycled within the ecosystem (Paytan and McLaughlin, 2007). Phosphorus is mostly brought into aquatic environments by weathering of rocks with phosphorus content; volcanic activity may also provide allochthonous sources of phosphorus or by upwelling from depth where there may be phosphorus present in the sediment of water bodies (Slomp and Van Cappellen, 2006; Paytan and McLaughlin, 2007).

2.8.4.4. Micronutrients

There are two major micronutrients (that is silica and iron) that are vital to algal growth and primary productivity (Smit, 2008). Iron, like phosphorus, is a limiting micronutrient to growth owing to insolubility (Fe (III)) and hence not being bioavailable to algae (Sunda and Huntsman, 1995). Plant metabolism is dependent of the availability of iron in usable form; iron is also responsible for electron transfer during respiration and photosynthesis and nitrate reduction (annamox) (Salisbury and Ross, 1992; Sunda and Huntsman, 1995). Iron enrichment experiments by Sunda and Huntsman (1997) have shown that algae growth, species abundance and diversity were reduced when iron was not present. Furthermore, the availability of iron also influences competition as species that are more adept at growing in iron deficient waters are able to thrive in conditions where iron concentrations are depleted as compared to other species (Sunda and Huntsman, 1995).

Silica is also a micronutrient that demonstrates accelerated growth in certain species of phytoplankton (Das and Chattopadhyay, 2000; Hu *et al.*, 2008). Soils that have high silica contents such as some sandy soils enter aquatic systems by aeolian forces; iron enters aquatic systems similarly (Garrison *et al.*, 2003). In some scenarios, the use of silica is seen to promote growth (though to a lesser extent) even when carbon is deficient (Das and Chattopadhyay, 2000; Hu *et al.*, 2008).

2.9. Microalgal Biofuel Production

There are various steps during the microalgal biofuel production process and these stages are outlined in the subsections to follow. The subsections aim to summarise the technologies used in the microalgal production and the harvesting, extraction and purification processes.

2.9.1. Technologies in Microalgal Production

All the parameters required for growth can be manipulated to an artificial setting (Brennan and Owende, 2010). Light is seen to be most apparent limiting factor in nature owing to diurnal cycles and seasonality however in an artificial setting the use of fluorescent lamps can be a suitable substitute (Sorokin and Krauss, 1958; Foy *et al.*, 1976; Salisbury and Ross, 1992; Scott *et al.*, 2010; Wahal and Viamajala, 2010). However, this obviously increases the energy demands required. Nitrogen and carbon must be supplied in the correct form that is bioavailable (Moss, 1973 (a,b); Brennan and Owende, 2010). There are three main overarching production types: photoautotrophic, heterotrophic and mixotrophic (Converti *et al.*, 2009; Brennan and Owende, 2010; Mata *et al.*, 2010).

Within photoautotrophic systems, there are two main types, namely open and closed production systems. Open production systems refer to algae cultivated in natural water bodies (rivers, lakes and dams) and in artificial raceways (Puppán, 2002; Chisti, 2007; Campbell, 2008; Brennan and Owende, 2010; Mata *et al.*, 2010; Scott *et al.*, 2010; Wahal and Viamajala, 2010). Open production systems tend to be quite shallow (0.2m – 0.5m) to allow for light penetration whilst the use of paddle wheels aims to reduce turbidity as well as facilitate exchanges in air *via* the air-water interface (Campbell, 2008; Brennan and Owende, 2010). Submerged pumps may also be used to add CO_2 to the system if needed (Brennan and Owende, 2010). There are several disadvantages to open systems since they tend to be more affected by environmental changes (temperature, evaporation loss, CO_2 deficiencies and improper mixing regimes) (Brennan and Owende, 2010). Seasonality and diurnal changes affect light and temperature parameters in open systems whilst improper mixing or aeration can also lead to less gases and nutrients being available (Moss, 1973 (a,b); Trujillo and Thurman, 2005; Smit, 2008; Brennan and Owende, 2010; Wahal and Viamajala, 2010). Open systems do however provide the opportunity to use non-arable land as well as having less energy requirements and maintenance attached to them

(Puppán, 2002; Chisti, 2007; Campbell, 2008; Brennan and Owende, 2010; Mata *et al.*, 2010; Scott *et al.*, 2010; Wahal and Viamajala, 2010).

Closed photobioreactors combat many of the problems that occur in open systems. There is much less cross-contamination and single species can be cultivated much more effectively but they may be more expensive to install and maintain (Puppán, 2002; Chisti, 2007; Campbell, 2008; Brennan and Owende, 2010; Mata *et al.*, 2010; Scott *et al.*, 2010; Wahal and Viamajala, 2010). Closed systems consist of arrays of tubes made of either glass or plastic that aim to capture sunlight while algae are recirculated within to facilitate the exchange of CO_2 and O_2 (Brennan and Owende, 2010).

Mixotrophic and heterotrophic are two lesser used production methods within biofuel production and these methods are outlined as adapted from Mata *et al.* (2010) and Brennan and Owende (2010). In heterotrophic production algae are grown on glucose; there is a high degree of control in the method as well as less energy requirements as compared to the open systems in photautotrophic production. Mixtrophic production involves the use of species that have the ability to photosynthesise or attain organic matter from extraneous sources. The method is not used extensively but it may solve the problem of having light as a limiting factor as growth is not dependent entirely on light. Even though this production method has not been implemented as much as closed and open photoautotrophic methods, they do exhibit high growth rates as growth is still maintained during diurnal times as compared to photoautotophic methods where dark respiration reduces growth. It is possible that in the future mixotrophic production systems will be more prominent.

2.9.2. Harvesting, Extraction and Purification Processes

Microalgae biomass requires the separation of solids and liquids in the medium; this is where a sizeable amount of cost is incurred as some processes tend to be more costly (Brennan and Owende, 2010; Mata *et al.*, 2010). Processes such as flocculation, filtration and centrifugal sedimentation to name a few require a vast amount of energy (Brennan and Owende, 2010). Algal cells that are smaller in size and have low cell densities are harder to harvest thus species

selection is an important consideration when evaluating the energy requirements of microalgal growth and their subsequent conversion efficiency (Smit, 2008; Brennan and Owende, 2010).

There are two main types of harvesting (Brennan and Owende, 2010): bulk harvesting, and thickening as outlined in the following text. Bulk harvesting refers to the extraction of biomass from suspension. This is dependent on the initial cell density and concentration of biomass and is done through flocculation, gravity sedimentation or flotation. Flocculation causes algal cells to aggregate together by reducing the effect of the negative charges which prevent cells from becoming concentrated together. Flotation is different to flocculation as it traps algal cells by the use of air bubbles. Gravity sedimentation is based on Stocks Law of settling attributes of microalgal cells. This method is most suitable when algal cells are large (~ 70 μm and larger). Filtration is the last method of harvesting and is normally performed after flocculation, flotation and gravity sedimentation methods have been implemented. The type of filtration used is dependent on the size of algal cells; conventional methods can be used for larger algal biomass however ultrafiltration or microfiltration techniques need to be employed.

Drying is a key process after the biomass has been harvested. The most common and cost effective technique is sun drying, however this can be a time consuming process and the need for large drying spaces can be problematic. It is important to note that the drying temperature may affect the lipid content of algae and it is imperative that drying temperatures do not exceed 60^{o}C. Lastly, there may be a need to use solvents and homogenisers to extract oils from cells (cell walls and membranes) need to broken down. The use of these breakdown chemicals can be quite costly to the extraction process.

2.10. Biofuel Conversion Technologies

There are two basic types of conversion technologies used in the conversion of microalgae biomass to an energy form (either gas or liquid): they are thermochemical or biochemical (Luque *et al.*, 2008; Brennan and Owende, 2010). The factors influencing the type of conversion technology is type of end product needed, the quality of the feedstock used and economic and conservation considerations (Brennan and Owende, 2010). Outlined in the Figure 2.3 is a summation of the conversion technology process.

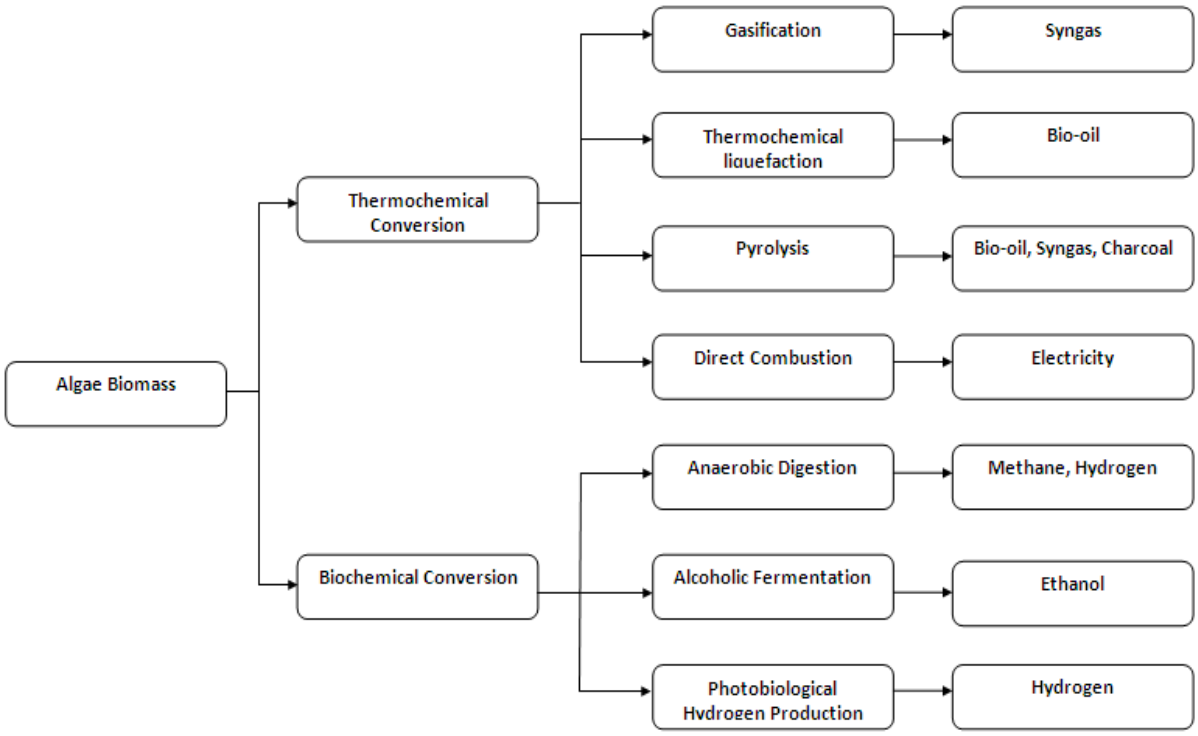

Figure 2.3: The various energy forms derived from thermochemical and biochemical conversion (Luque *et al.*, 2008).

2.10.1. Thermochemical Conversion

Thermochemical conversion is defined as the conversion of organic components within the biomass by thermal means to produce fuel products. The four possible methods of thermochemical conversions are direct combustion, gasification, thermochemical liquefaction and pyrolysis and these will be outlined in the following text as adapted from Luque *et al.* (2008), Amin (2009), Brennan and Owende (2010) and Singh and Olsen (2011).

Gasification is a type of thermochemical conversion which entails the partial oxidation of the microalgae into a gaseous mixture that is combustible. This is done at extremely high temperatures (800 – 1000^{o}C). The reaction with the biomass creates syngas. This method is advantageous as it allows for a variety of feedstocks to be converted and can also be used quite effectively in gas turbines without much secondary conversion. Gasification of microalgae species yielded a 1:1 energy balance (the amount of energy needed to produce a quantity of fuel).

Unfortunately, the gasification process is offset by the needs required to grow the feedstock in terms of emissions.

Thermochemical liquefaction is the process of yielding algal biomass into liquid fuel. Microalgae products are derived by the process of centrifuging algae with a high moisture content. Thermochemical liquefaction involves the use of lower temperatures than in gasification (200 – 500^{o}C), high pressure and catalysts. The reactors used for the thermal liquefaction process tend to be expensive but they do have the ability to convert wet biomass.

Pyrolysis is a complex conversion method in the presence of no air that involves the usage of medium high temperatures (350 – 750^{o}C). The pyrolysis process is able to yield three bio-products namely bio-oil, syngas and charcoal. Variations in temperature and in the use of vapour residence time cause alterations in the amount of syngas, liquid fuel and charcoal. With pyrolysis techniques there is a need for further refinement of oils which maybe acidic in nature, unstable or contain solids. With the adjustment of pyrolysis techniques, slight changes in temperature it was shown that efficiency and quality of bio-oils from algae could be enhanced with as much as 51.8% yield increase in certain species (Demirbas, 2006).

Direct combustion is the last recognised thermochemical conversion method. Biomass is burnt in the presence of air to convert biomass into gases. It is only possible to burn biomass if it has a moisture content of 50% or less and storage of biomass burnt is not an option. Direct combustion processes may be problematic as the energy efficiency tends to decrease if moisture content is too high; furthermore, there may be a reduction in the energy balance as too much energy is required for preprocessing such as drying, chopping and grinding of biomass.

2.10.2. Biochemical Conversion

There are three major biochemical methodologies that are used in the conversion of biomass to fuel products; these methods include anaerobic digestion, alcoholic fermentation and photobiological hydrogen production. Details of these procedures are outlined in the next paragraphs as adapted from Luque *et al.* (2008), Amin (2009), Brennan and Owende (2010) and Singh and Olsen (2011).

Anaerobic digestion involves the breakdown of organic waste to produce biogas in the form of CO_2, methane and hydrogen sulphide. Anaerobic digestion can be more useful than some of the other thermochemical conversions as it able to convert microalgal biomasses that have high moisture content (80 – 90 %). There are three stages of anaerobic digestion: the conversion of biomass to sugars (hydrolysis), the breakdown of sugars to alcohols and acetic acid (fermentation) and the conversion of gases (hydrogen (H_2) and CO_2) into methane (CH_4) by methogens (methanogensis). Anaerobic digestion may be affected by the presence of proteins within microalgae. The presence of proteins affects the C:N ratios which negatively affect the functioning of the digestor. Furthermore, high protein content increases the ammonium present which compromises the digestion process. Sodium ions present similar problems as they are toxic to microorganisms.

Alcoholic fermentation refers to the breakdown of biomass materials that have sugars, starches or cellulose into ethanol. The sugars and starches mix with water and yeast. The yeast breaks down the sugars into ethanol. The ethanol is concentrated by the removal of water (distillation) and the removal of impurities. Microalgae with a high starch or sugar content are ideal in the production of bio-ethanol.

The last type of biochemical conversion is photobiological hydrogen production. H_2 is an efficient energy carrier. They have the ability to photoproduce gas. H_2 is evolved in the light and dark phase as well as during carbon fixation under anaerobic conditions. H_2 may be impeded by the presence of O_2 as aerobic conditions affect the functionality of the enzyme hydrogenase. Subsequently, anaerobic conditions are mostly used for this process. The theoretical maximum of H_2 that can be produced from algae using this method is 198 kg H_2 Ha^{-1}.

2.11. Mathematical Modelling

Defined by Aris (1978), mathematical modelling is the use of mathematics to make predictions about the real world. Mathematics has become the basic language of all other sciences including physics, biology, environmental science and engineering (Barnes and Fulford, 2002). Mathematical modelling is increasingly vital as it provides users with the ability to create

abstractions of scenarios to create complex arguments (Aris, 1978; Fowkes and Mahony, 1994). Whilst, the choice of modelling used is imperative, it must be noted that the use of mathematics within the model does not define the extent to which the model is able to explain phenomena in reality (Fowkes and Mahony, 1994). However, the application of mathematics in the model can define how valid a model is. It is important that as a modelling procedure is undertaken, it is noted that outcome is not to obtain the most comprehensive descriptive model but rather the simplest model that incorporates the major components of the subject (Fowkes and Mahony, 1994; Barnes and Fulford, 2002). The most fundamental requirement of mathematical models is not to lose accuracy when modelling a real life situation.

2.11.1. Types of Modelling

There are several modelling types; mathematical modelling deals primarily with the use of differential equations (Meerschaert, 1999). However, for most modelling exercises, a combination of modelling approaches is needed to gain the level of accuracy to the scenario in reality (Fowkes and Mahony, 1994; Barnes and Fulford, 2002). Empirical models are the least sophisticated of modelling types (Aris, 1978; Barnes and Fulford, 2002). The methodology involves fitting curve to set of data and use of the curve to predict values where none are present. The problem with empirical models is that extrapolation out of the range of the curve can be problematic and hence the accuracy of models can be compromised (Aris, 1978; Barnes and Fulford, 2002).

Stochastic models are based on a probability approach. In this approach, the probability of certain scenarios occurring based on data (Ahmed, 2010). The incorporation of a degree of confidence or uncertainty can also be ascertained from the stochastic model; stochastic models tend to be quite complicated however they have widespread application in remote sensing, biology and economics (Barnes and Fulford, 2002).

Simulation models involve the use of computer programs to apply a set of rules. It can be said that simulation models are more “real” as they have a component to account for random events however simulation models are not necessarily the best types of models to use even though they are the most real (Barnes and Fulford, 2002). Deterministic models are the opposite of simulation

models where no random variation is taken into consideration and only the variables are used to formulate a mathematical equation to explain the problem; this kind of modelling technique is extremely accurate and can provide useful insight into understanding the process (Barnes and Fulford, 2002). The last type of modelling is the statistical. This type of modelling involves the testing of empirical data and what category they fall into according to particular statistical distributions (Aris, 1978; Barnes and Fulford, 2002).

2.11.2. Optimisation Models

Optimisation models are quite extensively used in mathematical modelling. In most real life scenarios, there is always a need to maximise or minimise the process (Giordano *et al.*, 1997). This outcome is no different in environmental scenarios. Managers of renewable energies such as fisheries, feedstocks or forests are always trying to optimise yields; optimisation models have a clear mathematical structure (Meerchaert, 1999). Single or multiple variables are controlled to produce the best result in another variable (dependent variables) subject to changes in the independent variables (Meerchaert, 1999; Mackey, 2008).

2.11.3. Mathematical Modelling of Algae

There has been extensive use of modelling within phycology studies. Studies by Keller (1989) and Boyd (1991) illustrate the use of empirical models to relate algal growth with some environmental parameters (light, temperature and nutrients (Keller (1989)) and dissolved O_2 (Boyd (1991))). Boyd (1991) evaluated a single variable (dissolved O_2) with regards to algal growth similar to a one variable optimisation model. However Keller (1989) took a more statistical approach using regression analysis. Collins (1980), Laws and Chalup (1990), Van Duin *et al.* (2001) and James and Boriah (2010) all use the method of building onto existing models (submodels) with regards to algal growth. However, the model generated from Laws and Chalup (1990) exclude the use of other physico-chemical parameters beside light. There have been attempts to perform other modelling techniques such as non linear methods used by Pisman *et al.* (2005) and equation discovery by Todorovski *et al.* (1998). Also there is a need to overcome the static nature of models by incorporating a non-steady growth function and this was investigated by Davidson *et al.* (1999). Furthermore, the development of dynamic models such

DYPHORA by Pahl-Wostl and Imboden (1990) also aims to correct for the static nature of existing models.

2.12. Summary

With contemporary issues such as climate change taking precedence, there is a need to investigate cleaner technologies. The use of renewable energies such as wind, solar and biomass all provide useful alternatives; however there is a need to investigate the feasibility of the implementation of these renewable energies. The use of biomass is questionable currently, as crop agriculture is used extensively (corn and sugar cane) and this provides the ethical dilemma of food shortage versus fuel shortage. However, the usage of microalgae is an option. Microalgae are not only more efficient in terms of space and resource consumption but also provide more fuel product yield per unit area per unit of biomass. However, there is always a need to streamline the process to optimise results. The investigation into photosynthesis of microalgae that yield the most biomass and most fuel products will be conducted by mathematical modelling and will aim to find these optimum physicochemical conditions in the proceeding chapters.

Chapter Three: Materials and Methods

3.1. Introduction

This chapter discusses the materials and methods that were used in this study. A brief outline of the initial culture system (the conditions that algae were initially grown under before experimentation) was evaluated. The biomass and lipid extraction techniques used are also discussed. The experimental design and the seven combinations of parameters and their effects on lipid content and algal growth are also evaluated. Additionally, the experimental design that assesses the effects of artificial light and natural light on algal growth was outlined. Furthermore, the statistical and mathematical approaches to be taken are assessed with the relative experimental design.

3.2. Description of Culture Systems

Estuarine phytoplankton samples were collected from the Mpenjati estuary which is located on the east coast of South Africa on the KwaZulu-Natal coast (30°55′59″S, 30°16′00″E) as shown in Figure 3.1. In each case, algal samples were taken from the euphotic zone; that is, microphytobenthos sampling was not considered. Approximately 75 litres of estuarine water was sampled per site and brought back to the laboratory. Furthermore, samples were sieved using glass fibre filters of 2 µm and 4 µm. This allowed for zooplankton to be removed whilst retaining phytoplankton. Phytoplankton was then cultured in three different 110 litre tanks with 85 litres of filtered seawater and 25 litres of estuarine sample to represent three replicates. This ratio was chosen as it is allows for sufficient living space for microalgae to grow in. Culture systems were fitted with artificial lighting systems as well as air pumps and given a recommended dosage of nutrients according to the FAO manual on algal production by Coutteau (1996) known as the Walne Medium. These nutrients were added to culture tanks and left for two weeks to allow for an appreciable amount of algal biomass to be produced.

Figure 3.1: Map illustrating the study site where initial samples were taken for culture tanks, Mpenjati estuary in KwaZulu-Natal, South Africa.

3.3. Initial Analysis

There is a need for parameter ranges to be streamlined as it is impossible for physico-chemical parameters to be evaluated across their entire range for the purposes of this study. Following a survey of relevant literature, parameter ranges of mixed algae communities were established as shown in Table 3.1. (Coutteau, 1996).The physico-chemical parameters that were evaluated for the study were temperature (^{o}C), salinity (p.p.t) and photoperiod (% of light exposure). The optimal value for temperature, salinity and photoperiod were hypothesised to be the midpoint of the ranges. According to Pillay and Pillay (2012), physico-chemical parameters in marine systems are extremely complex, intertwined and interlinked. Most models, however, do not account for dependence of parameters upon each other.

Table 3.1: Physico-chemical parameters with their ideal ranges for mixed algal populations.

Parameter	Range	Optimal
Temperature (^{o}C)	18-24	21
Photoperiod (%)	25-75	50
Salinity (p.p.t.)	20-24	22

3.4. Experimental Setup

Seven tanks (in different combinations of hypothesised optimal conditions, Table 3.2) were used for each estuarine sample where three litres of cultured mixed algae were placed in 20 litre tanks with 17 litres of filtered seawater. An initial water sample was taken and recorded as the initial biomass concentration at T_o at the start of each treatment as well as an extracted sample of lipids from the initial biomass concentration.

The physico-chemical parameters to be evaluated needed to be changed in different combinations to decipher the effect of that parameter on biomass whilst keeping the others constant. Temperature was altered by the use of aquatic heaters to 18, 21 and 24^{o}C whilst salinity was altered by the addition of freshwater and filtered seawater to either decrease or increase salinity, respectively. Salinity was altered before the addition of the cultured mixed algae to attain salinities of 20, 22 and 24 p.p.t. Photoperiod was altered by the covering of tanks with black plastic sheets for 25%, 50% and 75% of the six hours between sampling times. It must be

noted that each of the seven combinations of physico-chemical parameters at different settings is referred to as a scheme. These schemes are depicted in the Table 3.2. Turnover of algal biomass is extremely rapid hence the experiment needed to be run for a shorter time period as compared to experimental types using terrestrial plants.

The model was run three times in its entirety, independently to ensure accuracy and precision. In summation, for all three model runs, there are 13 time intervals for all seven schemes. A total of 21 samples were taken for lipid extraction and 21 samples for biomass concentrations at these time intervals (chlorophyll *a* and pheophytin).

For the evaluation of effects of natural and artificial light on algal growth and lipid content: sunlight was not removed as a confounding factor that is during daily sampling. Algae were exposed to sunlight and artificial light. In later experiments, sunlight was excluded as a confounding factor by only exposing schemes to artificial light of a known light intensity.

Table 3.2: Combinations of temperature, photoperiod and salinity altered for each scheme.

Scheme	Temperature (^{o}C)	Photoperiod (%)	Salinity (p.p.t)
1	18	50	22
2	21	50	22
3	24	50	22
4	21	50	20
5	21	50	24
6	21	25	22
7	21	75	22

** - Shaded cells indicate parameters that were held constant whilst the unshaded cells indicate parameters that were changed for that particular scheme.

3.5. Evaluation of the Effects of Parameters on Algal Growth and Lipid Content

60 ml of water was sampled every six hours from each experimental tank for a period of 72 hours. Each tank used was exposed to the same conditions where parameters that were not evaluated in this study (light intensity and carbon availability) were kept constant and

standardised by using the same artificial lighting source and air pumps respectively for each tank. As with culture systems, nutrient concentrations ideal for algal growth were placed in each tank to standardise nutrient concentrations (Walne Medium).

Each 60ml water sample was divided into six equal aliquots of 10 ml each at every defined interval. The latter were centrifuged for fifteen minutes at 4000 rpm. Algae was then extracted from three of these centrifuged sub-samples and placed into polyethylene test tubes with 10 ml of 90% acetone extraction. Acetone acts as a catalyst for the extraction of chloropigments, namely pheophytin and chlorophyll *a*. These pigments were used as estimators for biomass. Samples were left in a freezer for 24 hours to allow for the extraction of chlorophyll pigments in a freezer. Biomass was calculated by the use of a Turner's Fluorometer which measures fluorescence where measurements are calculated automatically and reported in µg per litre.

The lipid fraction of the algae was extracted by using the Bligh and Dyer method (1959) as outlined in Smedes and Thomasen (1996). This was done for the remaining three centrifuged aliquots. 1 ml of water with remaining algal biomass at the bottom of the centrifuge tube was mixed with 3.75 ml of 1:2 $CHCL_3$: MeOH solution, 1.25 ml of pure $CHCL_3$ and 1.25 ml of distilled water. The test tubes containing this mixture were then centrifuged for another five minutes at 1500 rpm until the solution was developed into a two phase system (aqueous top, organic-bottom). 2 ml of bottom phase containing lipids and chloroform was then recovered by inserting a pasteur pipette through gentle bubbling so that the upper phase was not disturbed. The bottom phase contains lipids and chloroform. This 2 ml mixture was weighed before being placed in an oven at 70^oC for 10 minutes. The percentage decrease of known aliquot of chloroform (2 ml) was compared to the remaining chloroform and lipid mixture after being in the oven for 10 minutes at 70^oC and the difference was estimated to be the lipid content.

3.6. Species Composition Analysis

A live subsample of 2.973 ml was decanted into an Utermöhl counting chamber and viewed with an inverted microscope (Nikon Ti-S) at 10x, 40x and 100x magnification using Differential Interference Contrast. Images were taken of the organisms that were seen to be most abundant. It was not possible to photograph organisms that moved too quickly such as Oxyrrhis *marina* and

some Cilliates. On conclusion of the live examination, samples were fixed using Lugol and settled overnight. The samples were counted by analysing five fields of view for the abundant cells and 10 fields of view for those taxa that only occurred sporadically. Cell concentrations (cells/ml) per species were calculated by the equation:

$$\frac{\text{count}}{((F \times A_{fw})/(A_{cc}) \times 2.937} \qquad (1)$$

Where:

Count = The total count of each taxon

F = Number of fields of view analysed

A_{fw} = Area of each field of view

A_{cc} = Total area of the counting camber

Counting camber volume = 2.973 ml

3.7. Mathematical Modelling and Statistical Analysis

There is general consensus amongst mathematicians that modelling of physical phenomena can be extremely difficult (Aris, 1978). It has become necessary to simplify situations of physical events to be able to model characteristics and gain suitable mathematical outcomes. Therefore, it is possible in mathematical modelling for sophisticated models to be developed from simple models. For the purposes of this research, visual trends graphs were generated in Microsoft Excel. From these trend graphs, theoretical extrapolations were assessed. Furthermore, statistical analyses were performed in SPSS v 18.0 (Statistical Package for the Social Sciences). ANOVA tests were used to evaluate whether statistical differences were present for chlorophyll *a* SGRs Specific Growth Rates (SGRs), pheophytin SGRs and lipid productivity between time intervals for each scheme. This was done to determine at which time interval the maxima exists for chlorophyll *a* SGRs, pheophytin SGRs and lipid productivity for all schemes. ANOVA tests were also performed for chlorophyll *a*, pheophytin and lipid productivity between each scheme at every time interval. This was done to evaluate how conditions could be changed in dynamic aquaculture systems at each time interval to maximise chlorophyll *a*, pheophytin SGRs and lipid

productivity. This would enhance the overall efficiency of algal growth and lipid productivity in dynamic aquaculture systems.

A maximum gains and losses analysis was also performed using ANOVA tests. This was done by evaluating the maximum and minimum chlorophyll *a* SGR, pheophytin SGR and lipid productivity and determining whether they were significantly different to all other positive and negative chlorophyll *a* SGRs, pheophytin SGRs and lipid productivities, respectively. From this analysis, a true theoretical maximum could be hypothesised. Pearson's correlation tests were run between chlorophyll *a* SGRs, pheophytin SGRs and lipid productivity for each scheme. This was done to determine whether key parameters covaried and whether this co-variance intimated a casual relationship. Assumption tests were performed for all statistical analysis. The Kolmogrov-Smirnov tests for normality and the Levene's test for equality variance were satisfied if $p > 0.05$. If these assumptions were not met, parameters were log transformed. In all statistical analysis, all assumption tests were met either before or after log transformations and hence there was no need for non-parametric tests. For the Pearson's correlations, the test for linearity was evaluated visually be the use of scatterplots.

The use of statistical analysis and mathematical modelling can be too descriptive and hence for these analyses it was desirable to have some predictive value within the analyses. This research has practical application in the field and it can be used to solve real-world problems. A scenario that this model can be applied to is the harvesting of algae when growth and lipid productivity is at its maximum under normal conditions and under algal bloom conditions (sometimes caused by eutrophication). The use of this model under algal bloom conditions makes this model extremely dynamic as it allows for researchers, biofuel producers and pollution biologists to determine the stage of the algal bloom. Based upon the experimental results in this dissertation, it would then be possible to ascertain the optimal time at which this bloom could be harvested for maximal yield of lipids. As an illustration of this process, two interpolations of scheme 1 were performed as an example of how the model would be derived using Mathematica 8.0. Note that once the observed data are obtained, the specific interpolating polynomial is used to fix the position of the observations on the experimental curves, for the particular combination of measured parameters.

3.8. Conclusion

This chapter illustrated the methodology used to mathematically model the algal growth and lipid products. All statistical tests used are described as well as details of the assumption tests that were either satisfied or not for each statistical test. The experimental set-up, initial preprocessing and the culture system are also detailed in this chapter. The following chapter will discuss the results and discussion attained from the study.

Chapter Four: Results and Discussion

4.1. Introduction

This chapter deals with the results obtained and a subsequently presents a discussion related to the results. It is important to note why the experiment was run over 72 hours. An objective of this research was to obtain a model that would be applicable in the field to harvest during algal blooms as well as under controlled conditions. When algal blooms occur, they are expected to be remediated as soon as possible thus harvesting becomes important such that algal blooms can be controlled and maximum lipids can be harvested for biofuel production. For long term harvesting of algae for lipid production (regardless of algal blooms), it would be imperative to evaluate growth and lipid productivity over days as ecosystems need to be "mature" which means that algae populations need to be present over a long period to produce the highest growth rates which would produce the largest amount of lipids in the long term (Moss, 1973b). Several studies have been done over the long term time period (Baldia *et al.*, 1991; Widjaja *et al.*, 2009; Packer *et al.*, 2011) for assessing the effects of physico-chemical parameters on algal growth but thus far none of them have looked at harvesting during algal blooms.

4.2. Natural and Artificial Light Experiments

Light is seen to be the most important parameter in controlling algal growth (Foy *et al.*, 1976). Prior to running the main experiment where temperature, salinity and photoperiod were controlled stringently against chlorophyll *a*, pheophytin and lipid concentrations; the same experiment was run where the photoperiod included natural light and artificial light. Light was set to mimic the natural daily cycles with artificial lights of 36W fluorescent lamps. It was found that changes in natural and artificial light caused appreciable fluctuations with cyclic responses of algal growth (chlorophyll *a* and pheophytin content) and lipid production to changes in light intensity as the day progressed (as seen in trend graphs 4.1 – 4.3).

This would inherently cause major fluctuations in the results as the light intensity for harvesting in artificial production would be unknown. Usually, it is expected that algal growth curves exhibit a Malthusian growth form (sometimes referred to a logistic growth form) even when physico-chemical parameters are changed (Coutteau, 1996). Alteration of the parameters would

illustrate a change in range of chlorophyll *a*, pheophytin and lipids produced but not the distribution of the data. Consequently, light was corrected by darkening the laboratory such that only artificial light was present (36W fluorescent lamps). This would allow for light to be controlled completely. If this model would be applicable in the field, then the light intensities could be corrected for using daily cyclic light intensities.

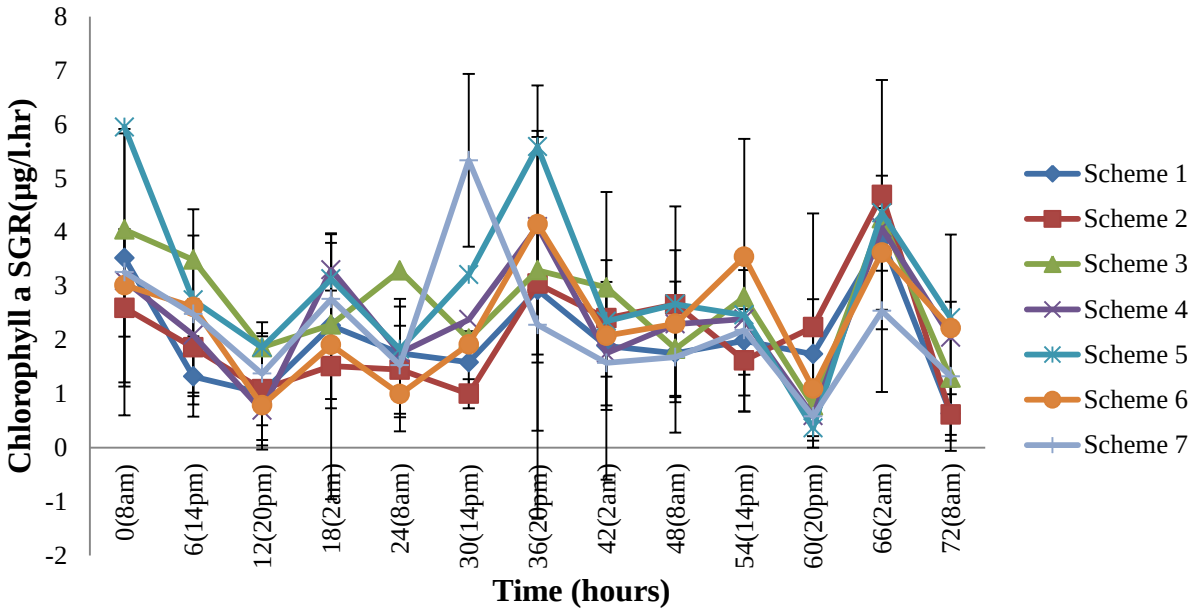

Figure 4.1: Trend graphs for each scheme over a 72 hour period for chlorophyll *a* when artificial light and natural light interact.

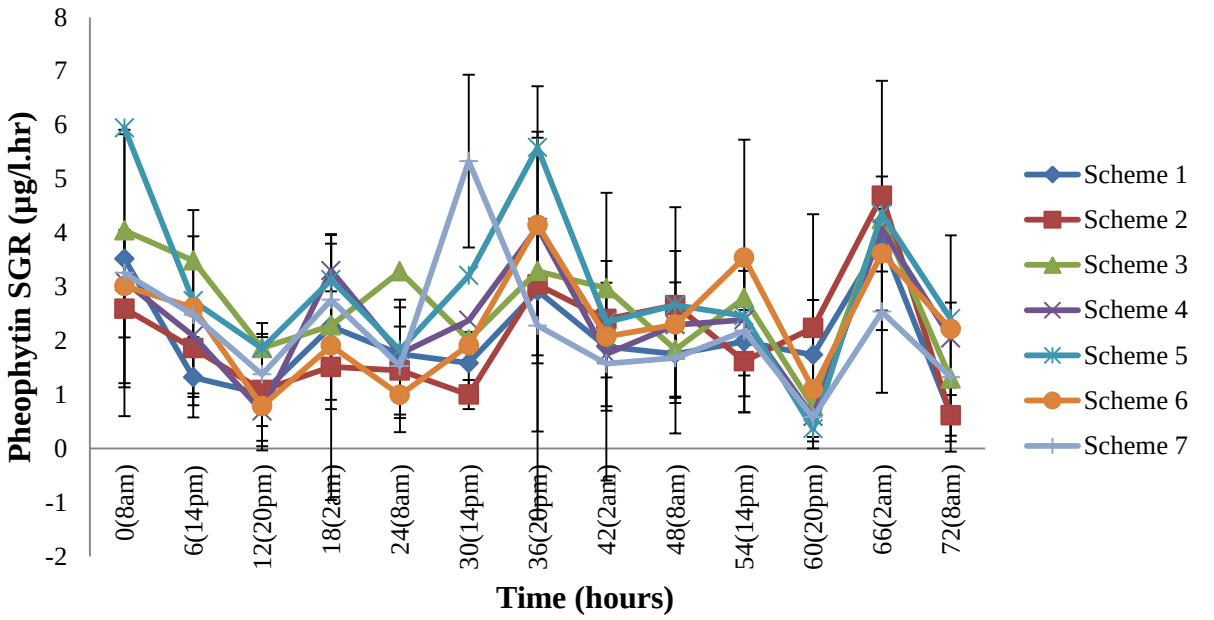

Figure 4.2: Trend graphs for each scheme for the over a 72 hour period for pheophytin when artificial light and natural light interact.

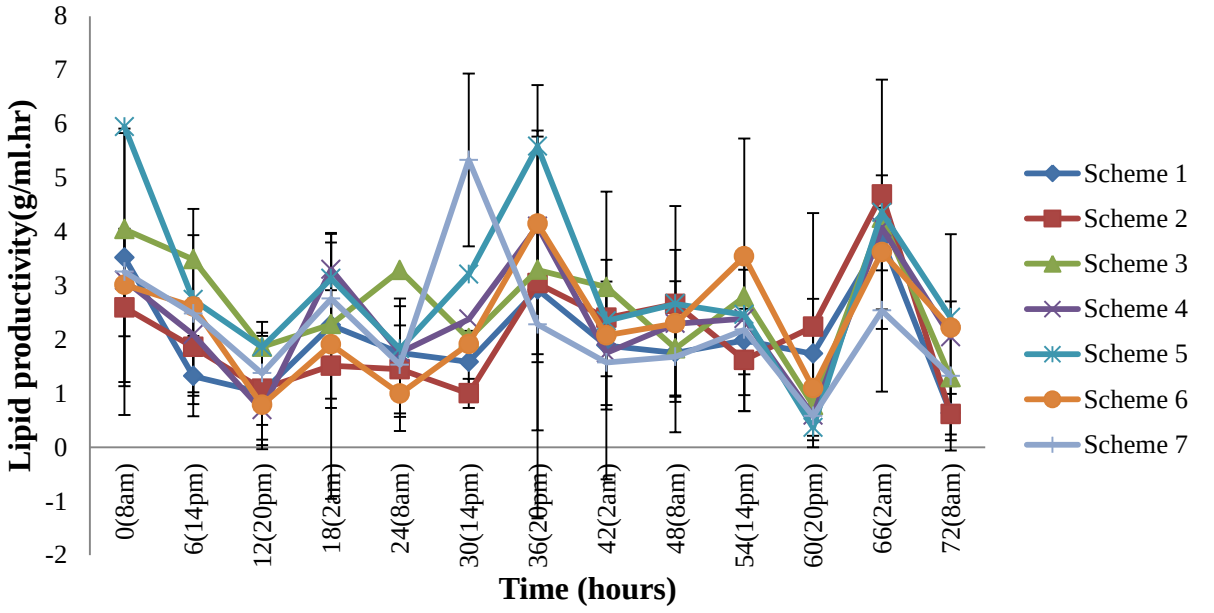

Figure 4.3: Trend graphs for each scheme over a 72 hour period for lipid productivity when artificial light and natural light interact.

4.3. Trend Analysis

Table 4.1: Pheophytin specific growth (μg/l.hr) over a 72 hour period by intervals for each scheme.

Time interval	Scheme						
	1	**2**	**3**	**4**	**5**	**6**	**7**
0-6	-0.0482	0.0125	0.0197	-0.0139	-0.0730	-0.1771	-0.0462
	0.0161	0.2085	0.1496	0.1331	0.0388	0.1101	0.1008
6-12	0.0550	-0.0035	-0.0218	0.0832*	0.0535	0.1755	0.2161
	0.1489	0.1426	0.0238	0.0529	0.1141	0.1589	0.0981
12-18	-0.1525*	-0.0140	-0.0338	-0.1221*	-0.0918	-0.1533	-0.1533
	0.2238	0.0305	0.0689	0.0438	0.1220	0.2506	0.2506
18-24	0.1512	-0.0156	-0.0740	-0.0166	0.0910	0.0517	0.0011
	0.0351	0.0555	0.0459	0.0109	0.1211	0.1106	0.1091
24-30	-0.0759*	0.0915	0.0575	0.1670*	-0.0077	0.0558	0.1491
	0.0141	0.0541	0.1131	0.1670	0.0542	0.0397	0.1644
30-36	0.0900	0.0077	0.1955*	0.0157*	0.0111	0.1075	0.0539
	0.0062	0.1034	0.1219	0.0453	0.0231	0.2002	0.2299
36-42	-0.2345*	-0.3292*	-0.2627*	-0.3017*	-0.2097*	-0.1643	0.0024
	0.1233	0.1768	0.1792	0.0434	0.0652	0.2205	0.1226
42-48	0.3967*^	0.2982*^	0.2242*	0.2083*	0.1851*	0.1669	-0.0451^
	0.0367	0.1903	0.0101	0.1238	0.1269	0.0731	0.1252
48-54	-0.1141*	-0.1892	-0.0862	0.1975*	0.1053	-0.0374	-0.0390
	0.1037	0.1908	0.1250	0.1480	0.1805	0.1210	0.0486
54-60	-0.2775*	0.0008	-0.1633*	-0.1294*	-0.2301*	-0.0084	-0.0262
	0.1906	0.1227	0.1438	0.1171	0.2309	0.0439	0.1351
60-66	0.0713	0.1205	0.0925*	-0.0770	0.0629	-0.1140	0.0769
	0.0587	0.2718	0.1512	0.0438	0.1356	0.0897	0.1665
66-72	0.0886	0.0511	0.0828	-0.0315	0.2955*	0.1513	0.0792
	0.3765	0.2740	0.1568	0.1400	0.2171	0.1612	0.1409

* - Statistically significant (p<0.05) between intervals in the same scheme, ^- Statistically significant between schemes in the same time interval. Shaded cells indicate standard deviations.

ANOVAs were performed between schemes for each time interval (Table 4.1 - Table 4.3). ANOVAs performed showed that pheophytin SGR (specific growth rate) at 42-48 hours for scheme 7 were statistically significantly lower than scheme 1 and 2 (p<0.05, Table 4.1). To allow for optimisation for scheme 7, at time periods 42-48, conditions need to be changed to those in scheme 1 as the SGR is at its highest (0.3967±0.0367 μg/l.hr). The reasoning for the reduction in the SGR at scheme 7 is that a 25% photoperiod as compared to a 50% photoperiod at constant light intensity will cause a reduction in algal growth (Foy *et al.*, 1976). For scheme 6, there were no specific growth rates for chlorophyll *a* and pheophytin that were found to be statistically lower against other schemes for every time interval. This suggests that a photoperiod of 75% at a constant light intensity does not necessarily result in significantly higher specific growth rates for both chlorophyll *a* and pheophytin.

ANOVAs were also performed within each scheme to determine at which time interval the maximum SGR for chlorophyll *a* and pheophytin and lipid productivity is present. These maxima would be the ideal time for harvesting. It was found that for scheme 1, a maximum chlorophyll SGR exists at 42-48 hours as the SGR was significantly different from time intervals 12-18,36-42,48-54,54-60 and 66-72 ($p<0.05$). This can also be seen on the trend graph for scheme 1 (Figure 4.4). For scheme 3, the maxima was determined to be present at 30-36 hours as it was significantly different from several time intervals ($p<0.05$, Table 4.1, Figure 4.6). However lipid production is at its lowest at this time interval. For scheme 7, the maxima are at time interval 12-18 ($p<0.05$, Table 4.1, Figure 4.10). For all other schemes, no maxima could be determined for pheophytin SGRs.

Table 4.2: Chlorophyll *a* specific growth (μg/l.hr) over a 72 hour period by intervals for each scheme.

Time interval	Scheme						
	1	**2**	**3**	**4**	**5**	**6**	**7**
0-6	0.0140	0.1114	0.0578	0.0215	0.0163	-0.0345	0.0107
	0.1081	0.0238	0.1647	0.1129	0.1275	0.0075	0.0555
6-12	0.0062	-0.1022	0.0272	0.0726	-0.0974	0.0143	0.2061*
	0.0975	0.0698	0.0638	0.1364	0.2825	0.1823	0.1093
12-18	-0.1222*	0.0100	-0.1119	-0.1362*	0.0448	-0.1369	-0.2363*
	0.1419	0.1665	0.0905	0.0962	0.2032	0.2165	0.1434
18-24	0.1242	-0.0352	-0.0947	-0.0117*	0.0679	0.1048	0.0427
	0.0217	0.0055	0.0898	0.0902	0.1245	0.1130	0.0313
24-30	-0.0108	0.0485	0.0665	0.1955	-0.0434	-0.0505	0.1181*
	0.1086	0.0663	0.0361	0.2002	0.1290	0.1059	0.0888
30-36	0.0044	0.0844	0.2203	-0.1140*	0.0273	0.1906	0.0260
	0.0266	0.0929	0.2231	0.0457	0.1083	0.1719	0.1804
36-42	-0.0743*	-0.1473	-0.1847	-0.0680*	-0.0693	-0.1035	0.0191
	0.2287	0.1727	0.2180	0.0491	0.0727	0.2305	0.1304
42-48	0.2806*^	0.1163	0.1243	0.0663	0.0177	0.1313	-0.0796^
	0.0789	0.1075	0.0189	0.1375	0.0127	0.0876	0.1566
48-54	-0.1491*	-0.0792	-0.0463	0.1566	0.1655	-0.0458	-0.0587
	0.0918	0.0383	0.0685	0.1727	0.0928	0.1461	0.1301
54-60	-0.2277*^	-0.1118	-0.0265	-0.0956	-0.0856	0.0136^	0.0926*^
	0.1461	0.1126	0.0138	0.0613	0.0160	0.0287	0.0510
60-66	0.1299*^	0.0775^	-0.0463	-0.0708^	-0.0117	-0.1579^	-0.0612^
	0.1024	0.0233	0.0255	0.0526	0.0905	0.0905	0.0405
66-72	-0.0357	0.0821	0.0838	-0.0130	0.1319	0.1578	0.0190
	0.1424	0.1400	0.0232	0.1092	0.0258	0.1479	0.0509

* - Statistically significant ($p<0.05$) between intervals in the same scheme, ^- Statistically significant ($p<0.05$) between schemes in the same time interval. Shaded cells indicate standard deviations.

A similar outcome was found for the ANOVAs done for chlorophyll *a* SGRs, where at 42-48 hours, a significant difference was found between scheme 1 and 7 ($p<0.05$,Table 4.2). As with pheophytin SGR, the growth rate of chlorophyll *a* is significantly higher in scheme 1 than 7, and hence there should also be changes to conditions at this time interval to scheme 1 to maximise yields. Furthermore, for chlorophyll *a* maximisation, at time intervals 54-60, scheme 1 should be changed to conditions in scheme 7 and also at time intervals 60-66. Schemes 4, 6 and 7 should be changed to the conditions of scheme 1 as SGRs were significantly higher ($p<0.05$). For lipid productivity, there was shown to be no statistically significant difference between any of the schemes for all time intervals over the 72 hour study ($p>0.05$).

With chlorophyll *a* SGRs, for scheme 1, the maximum occurs at time interval 42-48 hours. For scheme 3, there are 2 distinct maxima that are statistically significant (p<0.05) that is, time intervals 30-36 and 42-48 hours (Figure 4.6, Table 4.1). From the Figure 4.7, SGRs are higher for chlorophyll *a* at time interval 42-48 and 24-30 and are statistically significant (p<0.05, Table 4.1). For all the other schemes, no apparent maxima are present for chlorophyll *a* SGRs.

Table 4.3.: Lipid productivity (g/ml.hr) over a 72 hour period by intervals for each scheme.

Time interval	**Scheme**						
	1	**2**	**3**	**4**	**5**	**6**	**7**
0-6	0.0264	0.0244	0.0295*	0.0240*	0.0192*	0.0237*	0.0210*
	0.0023	0.0034	0.0091	0.0057	0.0024	0.0033	0.0049
6-12	0.0426	0.0334*	0.0302*	0.0419	0.0370	0.0484	0.0428
	0.0058	0.0024	0.0084	0.0043	0.0114	0.0160	0.0127
12-18	0.0512	0.0543*	0.0603*	0.0484*	0.0484	0.0554*	0.0668*
	0.0025	0.0085	0.0138	0.0072	0.0029	0.0053	0.0098
18-24	0.0461	0.0403*	0.0428*	0.0374*	0.0459	0.0407	0.0375
	0.0027	0.0071	0.0037	0.0098	0.0083	0.0026	0.0097
24-30	0.0451	0.0397*	0.0338*	0.0705*	0.0673	0.0440	0.0682*
	0.0111	0.0269	0.0178	0.0173	0.0026	0.0065	0.0301
30-36	0.0372	0.0403	0.0178*	0.0366	0.0203*	0.0287*	0.0303
	0.0024	0.0183	0.0082	0.0013	0.0031	0.0139	0.0137
36-42	0.0290*	0.0301	0.0205*	0.0273*	0.0291	0.0354	0.0191*
	0.0192	0.0020	0.0018	0.0087	0.0067	0.0086	0.0142
42-48	0.0885*	0.0612*	0.0874*	0.1294*	0.1066*	0.1252*	0.0959*
	0.0605	0.0509	0.0498	0.0968	0.0841	0.0954	0.0644
48-54	0.0248*	0.0122*	0.0341*	0.0180*	0.0217*	0.0358	0.0545*
	0.0013	0.0047	0.0140	0.0015	0.0080	0.0237	0.0348
54-60	0.0828	0.0817*	0.0399*	0.0787*	0.0477	0.0607*	0.0726*
	0.0728	0.0504	0.0231	0.0528	0.0407	0.0325	0.0578
60-66	0.0576	0.0453*	0.0455*	0.0387*	0.0673*	0.0643*	0.0776*
	0.0033	0.0067	0.0035	0.0102	0.0257	0.0177	0.0336
66-72	0.0276	0.0314*	0.0219*	0.0272*	0.0172*	0.0171*	0.0388*
	0.0031	0.0066	0.0067	0.0030	0.0083	0.0083	0.0171

* - Statistically significant (p<0.05) between intervals in the same scheme, ^- Statistically significant(p<0.05) between schemes in the same time interval. Shaded cells indicate standard deviations.

Statistically, only lipid productivity rates differ significantly between time intervals for scheme 2, 3 and 4. This maximum occurs at 42-48 hours. This time interval is significantly different to all the other time intervals (p<0.05). This can be seen in Table 4.3 and Figure 4.5, 4.6 and 4.7.

Owing to high variability in the data, maximum gains and losses were evaluated separately. This would allow for a greater understanding of the cyclic effect and allow for a maximum to be

established if significantly different (p<0.05). The maximum gain was compared to the entire set of the gains (positive growths) over the entire time series for each scheme for chlorophyll *a*, pheophytin SGRs and lipid productivity to determine whether it is significantly different to other gains. There is a very interesting observation present in scheme 7, where there is statistical difference between the pheophytin SGR at 12-18 and 54-60 where growth rate is significantly higher. This is probably owing to photo-acclimation as proposed by Bernard (2010) where over time species adjust to the prevailing conditions with the ecosystem becoming mature (Moss, 1973b).

Table 4.4: Maximum gains and losses for chlorophyll *a* SGR, pheophytin SGR and lipid productivity for each scheme.

Scheme	**Chlorophyll *a* specific growth rate (µg/l.hr)**		**Pheophytin specific growth rate(µg/l.hr)**		**Lipid productivity (g/ml.hr)**	
	Max gain	Max loss	Max gain	Max loss	Max gain	Max loss
1	0.280571*	-0.22766	0.039686*	-0.27745	0.390137	-0.12
2	0.11626*	-0.14732	0.298181*	-0.3292	0.951376*	-0.13349
3	0.220265*	-0.18467	0.224164*	-0.26274	0.545388*	-0.10172
4	0.195473*	-0.13624	0.208347	-0.30169	0.62202*	-0.14342
5	0.165456*	-0.09735	0.2955*	-0.23013	0.444137*	-0.13274
6	0.190559*	-0.01579	0.17549	-0.17705	0.423018*	-0.122225
7	0.0206081*	-0.05866	0.21606*	-0.15329	0.669854*	-0.09276

* - Statistically significant between intervals in the same scheme

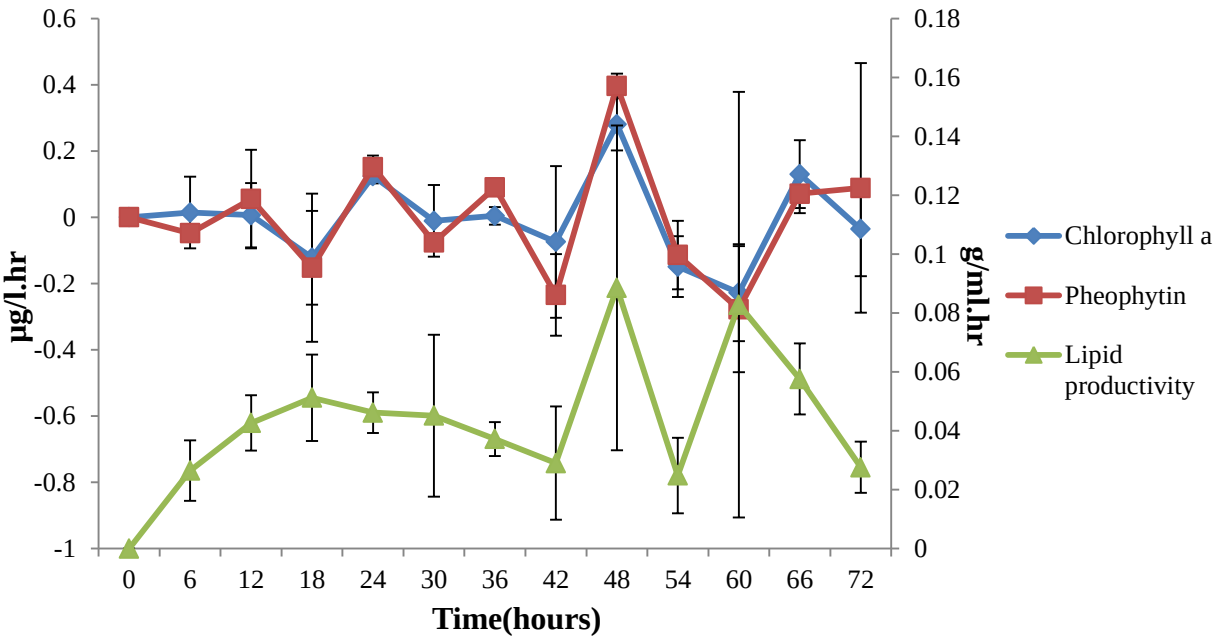

Figure 4.4: Trend graphs with chlorophyll *a* SGR, pheophytin SGR and lipid productivity over 72 hours for scheme 1.

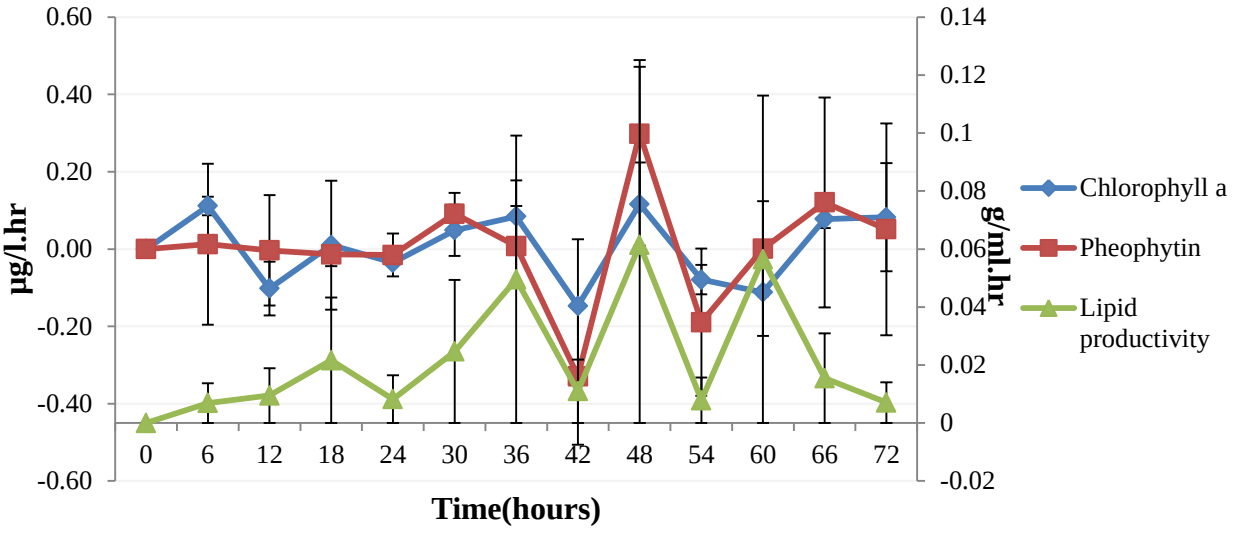

Figure 4.5: Trend graphs with chlorophyll *a* SGR, pheophytin SGR and lipid productivity over 72 hours for scheme 2.

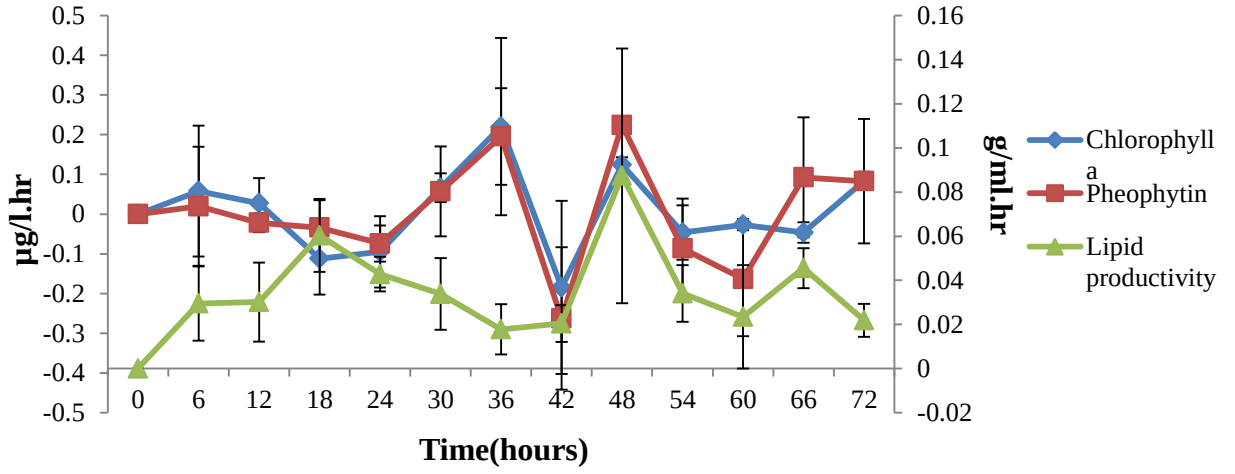

Figure 4.6: Trend graphs with chlorophyll *a* SGR, pheophytin SGR and lipid productivity over 72 hours for scheme 3.

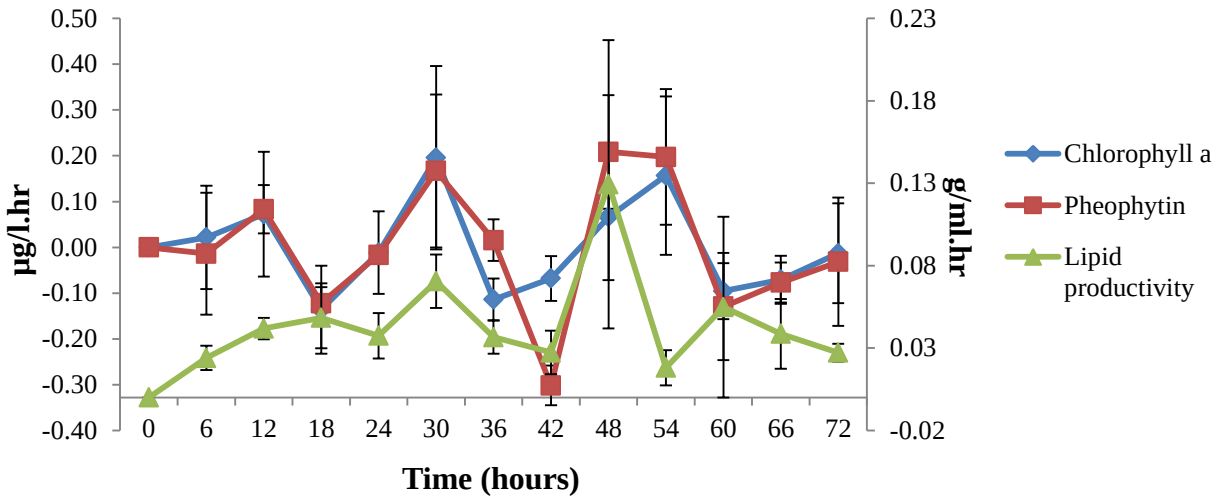

Figure 4.7: Trend graphs with chlorophyll *a* SGR, pheophytin SGR and lipid productivity over 72 hours for scheme 4.

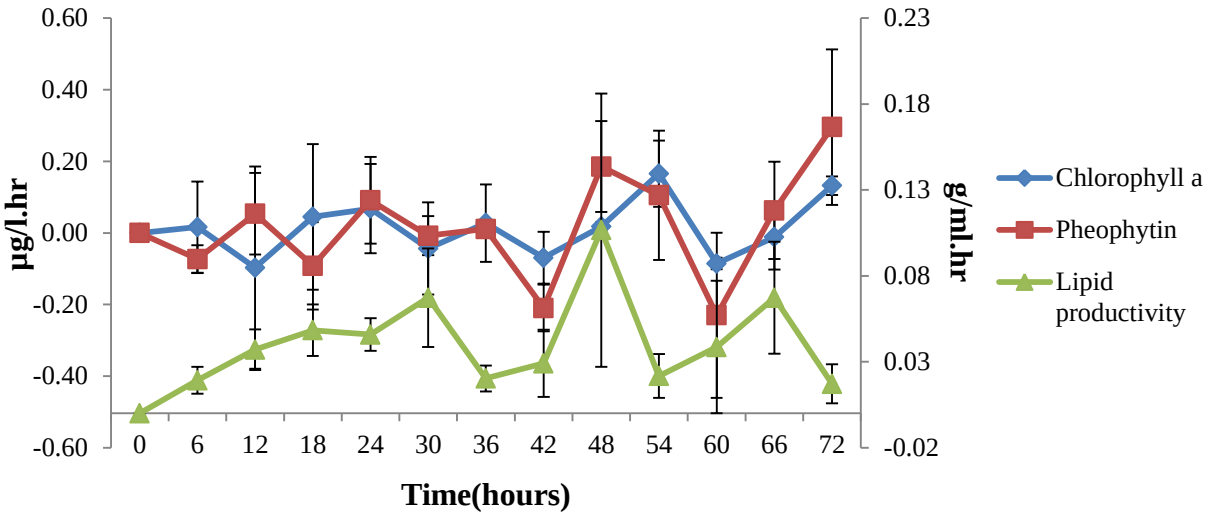

Figure 4.8: Trend graphs with chlorophyll *a* SGR, pheophytin SGR and lipid productivity over 72 hours for scheme 5.

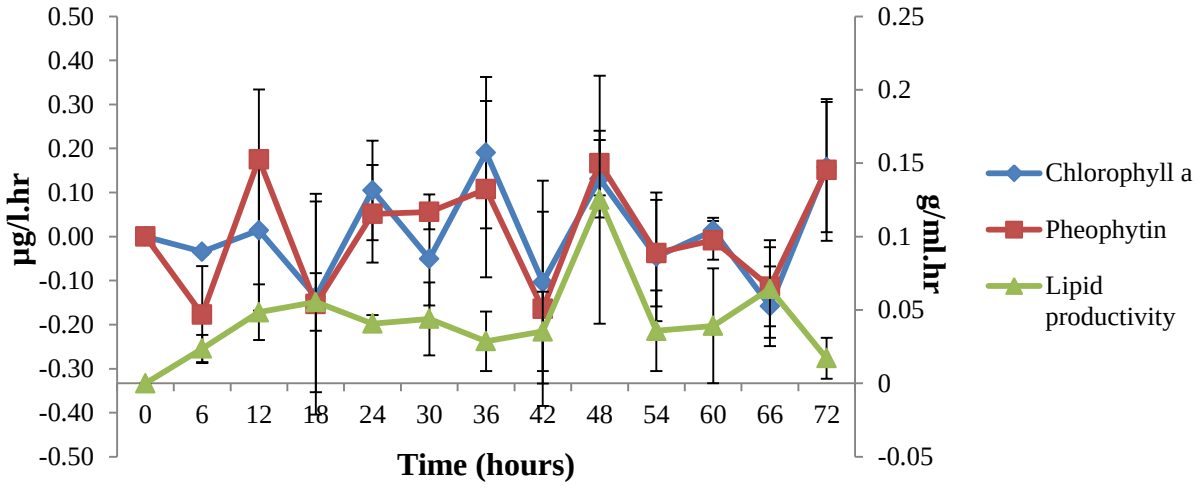

Figure 4.9: Trend graphs with chlorophyll *a* SGR, pheophytin SGR and lipid productivity over 72 hours for scheme 6.

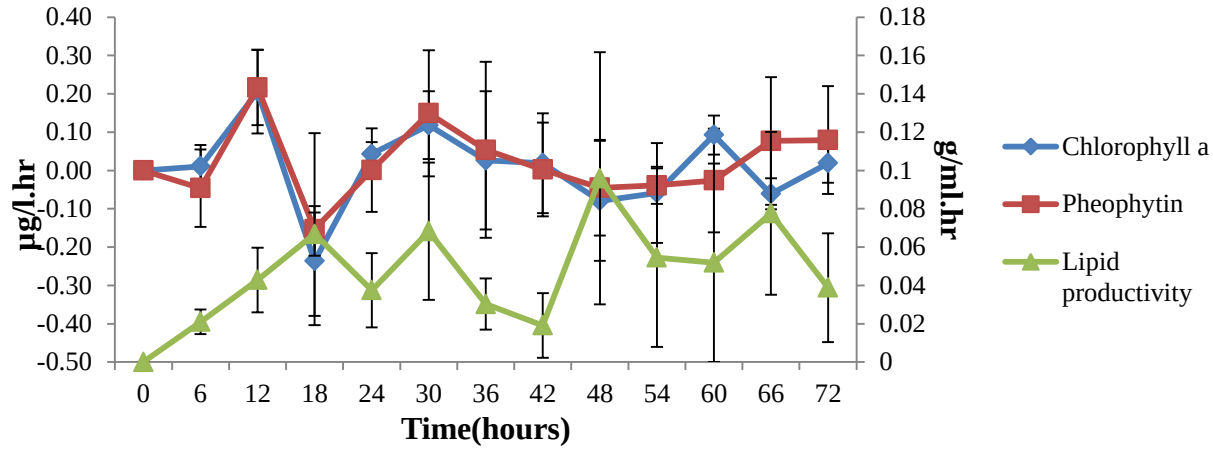

Figure 4.10: Trend graphs with chlorophyll *a* SGR, pheophytin SGR and lipid productivity over 72 hours for scheme 7.

In trend graphs (Figures 4.4 to 4.10) for all seven schemes, there is a notable cyclic trend for pheophytin and chlorophyll *a* SGRs. In schemes 1, 4, 5 and 6, the cyclic trend is very pronounced and present for most of the 72 hour experimental period. According to the FAO, a logistic growth form for algal growth is expected (Coutteau, 1996), however this growth form was not observed in this study. There are several reasons that can be proposed for this cyclic effect. Since nutrients were only added at the beginning of the experiment, nutrient recycling could be responsible for this trend. Nutrient recycling will cause gains and loss cycles in

chlorophyll *a* and pheophytin SGRs. When algal cells die, they release nitrogen and phosphorus back into the water column that is reused, so creating a cycling effect (Carr *et al.*, 1997; Smit, 2008).

Cyclic trends can also be caused by two ecological mechanisms. Firstly, algae productivity is inhibited by predator-prey interactions (Pisman *et al.*, 2005). According to the species composition analysis, zooplankton which feed on microalgae were found in minute concentrations as compared to phytoplankton species (*Oxyrrhis marina*, *Chlorella* and *Amphora* species) and are thus unlikely to be responsible for cyclic effects in the trend graphs. Secondly, interspecific interactions need to be evaluated as algal species react uniquely in different growth media (Smit, 2008).

It is logical to assume that an increased rate of chlorophyll *a* and pheophytin SGR will coincide with higher lipid productivity. However, the converse is true. Nitrogen is one of the essential nutrients that promote algal growth (Smit, 2008). However, nitrogen limitation caused decreased growth rates which led to increased cellular lipid content (Chen *et al.*, 2011). This is done by the limiting protein biosynthesis which increases the ratio of lipid to proteins ratio (Converti *et al.*, 2009). Subsequently, there needs to be a balance of nitrogen present such that enough algal growth is promoted to maximise lipid productivity (Mairet *et al.*, 2010). It would be expected that drops in algae growth (pheophytin and chlorophyll *a* SGRs) would lead to increased lipid productivity. This can be seen especially well in scheme 7, where at time intervals 12-18, 42-48 and 60-66 lipid productivity was at its maximum and growth rates illustrate the maximum loss SGR (-0.05866 µg/l.hr, Table 4.4) after a maximum gain 0.0206081µg/l.hr SGR for pheophytin and chlorophyll *a*. For scheme 1-6, there is a distinct maxima or maximum gain (at 42-48) according to (Figure 4.4 – 4.9, Table 4.4) that is shared by chlorophyll *a* SGR, pheophytin SGR and lipid productivity. Even though scheme 3 and 4 may be bi-modal, at 24-30 and 30-36 hours for scheme 3 and 4, respectively, there is a drop in the lipid production rate. A harvesting time of 42-48 hours to obtain the maximum lipids can be seen as the optimal time. Chen *et al.* (2011) suggests that doubling time may indeed be 38 hours whilst Packer *et al.* (2011) determined that doubling time was actually 48 hours and this research concurs that mixed algae also illustrate the same trend.

A possible optimisation strategy would be the use of indigenous algae instead of alien species of algae (Maharajh and Lalloo, 2008). There are various characteristics that may inhibit finding a maximum growth rate and lipid productivity. Different taxonomic levels, functional groups and phylogenetic diversity are such characteristics that need to be taken into account and can affect the SGRs: This was modelled by Moisan *et al.* (2002). It is likely that indigenous algae would have higher SGRs as compared to alien species as they would be more acclimated to the conditions. If biofuel producers are to harvest in the wild then perhaps it is necessary to understand the local conditions to determine when lipid productivity is at its maximum in a local context. Under laboratory conditions, if indigenous algae are to be cultivated then local conditions would need to be simulated under controlled conditions to yield the most lipids for biofuel production. However, it is possible that algae introduced to unfamiliar conditions can acclimate quickly to a new environment. This was shown by Bernard *et al.* (2010) that photo-acclimation to different light intensities occurs in certain algae species in less than 24 hours.

Table 4.5: Correlations between chlorophyll *a*, pheophytin and lipid productivity for all schemes.

Scheme		**Chlorophyll *a* SGR**	**Pheophytin SGR**	**Lipid productivity**
1	Chlorophyll *a*		0.567*	0.014
	Pheophytin	0.567*		0.004
	Lipid productivity	0.014	0.004	
2	Chlorophyll *a*		0.464*	0.124
	Pheophytin	0.464*		0.201*
	Lipid productivity	0.124	0.201*	
3	Chlorophyll *a*		0.310*	0.065
	Pheophytin	0.310*		0.489
	Lipid productivity	0.065	0.107	
4	Chlorophyll *a*		0.712*	0.083
	Pheophytin	0.712*		0.084
	Lipid productivity	0.083	0.084	
5	Chlorophyll *a*		0.247*	0.075
	Pheophytin	0.247*		0.130
	Lipid productivity	0.075	0.130	
6	Chlorophyll *a*		0.619*	0.092
	Pheophytin	0.619*		0.208*
	Lipid productivity	0.092	0.208*	
7	Chlorophyll *a*		0.536*	0.005
	Pheophytin	0.536*		0.019
	Lipid productivity	0.005	0.019	

* - Statistically significant ($p<0.05$) between intervals in the same scheme. Chlorophyll *a* and pheophytin were measured in μg/l.hr.

On visual inspection of the graphs and according to literature, lower algal SGRs owing to nitrogen limitation may lead to higher lipid productivity. Correlations were run for chlorophyll *a*

SGRs, pheophytin SGRs and lipid productivity for each. Pheophytin and chlorophyll *a* SGRs are significantly correlated ($p<0.05$) in all schemes and this is expected as both are algae photosynthetic pigments. However, in schemes 2 and 6 there are significant correlations present between pheophytin SGR and lipid productivity although these correlations are weak r=0.201 and r=0.208. Since these relationships were weak, it was concluded that three parameters cannot be related and hence neither of them could be used as indicators of each other. This is owing to the complex relationship that nitrogen, lipid productivity and algal growth possess and hence there is no linear trend between the chloropigments and lipid productivity (Converti *et al.,* 2009; Chen *et al.*, 2011).

4.4. Effects of Parameters on the Chlorophyll *a*, Pheophytin SGRs and Lipid Productivity

The effect of temperature on lipid productivity and growth rates can be determined by evaluating the first 3 schemes (Figures 4.4 - 4.6). All temperatures showed an increased chlorophyll *a*, pheophytin SGRs and lipid productivity of algae at the 42-48 hour time interval thus for the first three schemes a common maximum is present. At this point the nitrogen is probably at its optimal to promote maximum growth and lipid production. However, there are other maximum lipid productivity rates; one of which is present at 54-60 hours, where chlorophyll *a* and pheophytin SGR have decreased to their maximum SGR loss. At increased temperatures in scheme 2 (21°C) and 3 (24°C), increased chlorophyll *a* and pheophytin SGRs were present at 60-66 and 24-30 time intervals, respectively, coincided with increased lipid productivity rates. Studies by Converti *et al.* (2009), illustrated that increase in temperature for *Chlorella vulgaris* which is of the same genus that was dominant in the experiment (Plate 4.1., (a)) caused increased growth rates caused a decrease in lipid productivity.

The effects of changing temperature cannot be underestimated as can be seen in schemes 1 to 3 (Figure 4.4. – 4.6). Temperature changes the density of water and the ability of the water to hold CO_2, HCO_3^- and CO_3^-; this will inherently affect the pH of the medium (Moss, 1973a). Algae species have different tolerances of pH and thus effects of temperature on pH will need to evaluate within schemes 1, 2 and 3 as an optimal level of where pH and temperature will need to be evaluated to attain the maximum SGR and lipid productivity (Mayo, 1997). pH and

temperature also influences the toxicity of ammonium and nitrates (Mayo, 1997). Within the mixed algae, certain algae species may be more sensitive to temperature changes as nutrients become toxic at higher temperature as this may only be shown later in the growth curve.

The effect of salinity is not apparent. There are no statistical significant changes amongst schemes 2, 4 and 5 between ANOVAs run between time intervals and between schemes for chlorophyll *a*, pheophytin and lipid productivity. As stated earlier, in scheme 7, where there is statistical difference between the pheophytin SGR at time intervals 12-18 and 54-60, growth rate is significantly higher. This is probably owing to photo-acclimation as proposed by Bernard (2010) where over time species adjust to the prevailing conditions with the ecosystem becoming mature (Moss, 1973b). It can be proposed that at the 12-18 time interval, photo-acclimation is reached.

There is a need to investigate the usage of other lipid extraction techniques. It was found by Lee *et al.* (2010) that different disruption methods of cell biomass mixtures cause changes to the lipid content. This could be done in future studies to enhance the accuracy with regards to lipid production rates. In the field, it is highly unlikely to find one type of algal species yet most literature looks at only at one type of algal species and how they are affected by multiple parameters under laboratory or field conditions. Multiple species will compete with each other for nutrients at different rates (Moisan *et al.*, 2002). Hence it is difficult to deduce what the exact optimal conditions are as the model will be site specific unless a comprehensive species analysis with interaction effects are understood. It was found by Moisan *et al.* (2002) that the phytoplankton species that dominates generally exhibits the most amount of growth in a temperature controlled medium. This argument can be superimposed on this experiment for scheme 1 to 3 however there is no research that evaluates multiple parameter effects on growth rates with specifics in interspecific competition hence this argument cannot be used to reason cyclic trends in schemes 4 to 7.

Another important consideration is elemental stoichiometry: certain ratios of nutrients are needed to promote growth (Packer *et al.*, 2011). The elemental ratios of N:P that is the Redfield ratio is important in determining whether growth will prevail or not (Rhee, 1978; Wynne and Rhee,

1986; Packer *et al.*, 2011). In all the schemes, nutrients were added at T_0, however the relative species composition in each scheme between time intervals will influence how nutrients are depleted in the scheme. From Moisan *et al.* (2002) it can be gathered that interspecific competition may cause changes to the growth rates which will inherently affect the lipid productivity.

4.5. Mathematical Model Development

Each of the following graphs were generated using the Mathematica function BSplineFunction[data], where data represents the observed values of the densities of each of chlorophyll *a* SGR, pheophytin SGR and lipid productivity. Curves were fit to chlorophyll *a* SGR, pheophytin SGR and lipid productivities of schemes one to seven. Figure 4.11 demonstrates how spline curves and 1st order derivatives were fit to data, in this case scheme 1 is used as an example. Each of the graphs range from 0 to 1 which maps continuously to the range [0, 72].

The value of the SGR of chlorophyll *a*, pheophytin and lipid productivity at any time may be obtained by taking the time modulo 72 and dividing by 72 and then using this as an argument for the interpolating spline function. The second member of each pair of graphs above is the first derivative indicating where and how fast growth is increasing or decreasing. The rate of change is an indication of where in the 72 hour period, one should sample algal biomass and extract lipids. It is imperative to note that all interpolation curves for pheophytin, chlorophyll *a* and lipid productivity need to be evaluated simultaneously when deciding which times are the most suitable in terms of attaining the maximum amount of biofuel products. Spline curve interpolation was used because of their simplicity and explaining complex shapes. The use of spline curves is more appropriate as the data do not illustrate a uniform growth curve and consequently a spline curve is able to explain the data more effectively.

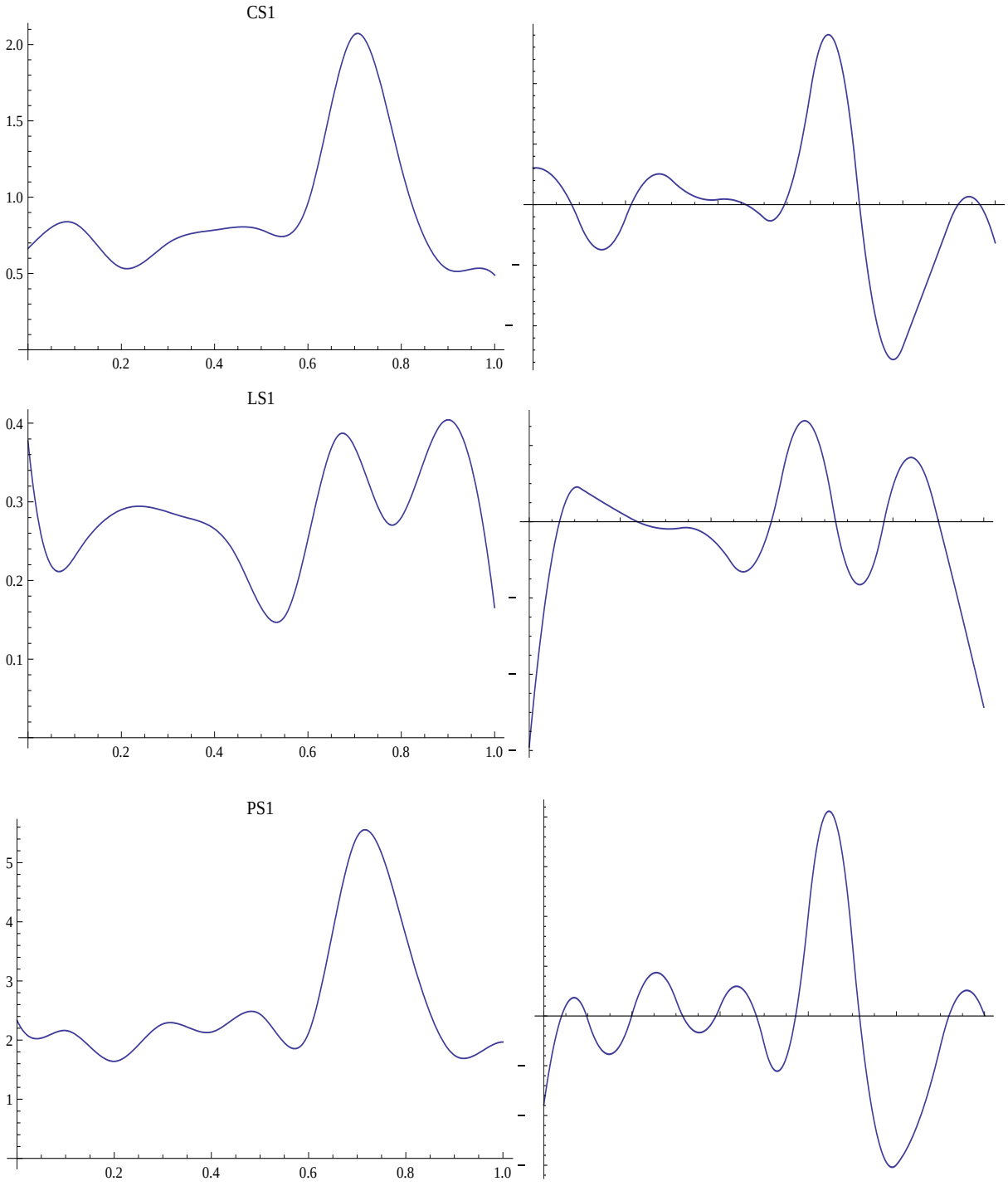

Figure 4.11: Spline interpolation and first derivative graphs for chlorophyll *a*, pheophytin and lipid productivity for scheme 1 where CS refers to chlorophyll *a* SGR, LS refers to lipid productivity and PS refers to pheophytin SGR for scheme 1.

It is envisaged that the model will be used as follows in the field:

- The first step would be to measure the parameters (temperature, salinity, etc) present. This would then place the natural event within one or more of the experimental schemes isolated in this work.
- Thereafter samples of the algae are measured for key components at regular intervals (say two hourly) for up to 6 – 8 hours.
- The characteristics of the component concentrations are then interpolated with the related modelled interpolants from the experiment.
- The stage of the observed event is then determined by matching where on the experimental curve, the observed results lie.
- It is then a simple matter to determine how long to wait before optimal harvest. Note that as it is assumed that all the events are cyclical (with period 72 hours) it is a simple matter to wait for the maximum yield time.

4.6. Species Composition Analysis

The main phytoplankton taxa found during the examination of water samples were *Chlorella* species (Chlorophyta), *Amphora* species (Bacillariophyta), *Oxyrrhis marina* (Dinophyta), *Melosira* species (Bacillariophyta) and *Navicula* species (Bacillariophyta). Furthermore, zooplankton taxa such as Ameoba, Nematodes and Ciliates were found in minuscule concentrations. Even though zooplankton species were initially removed by passing water samples through glass fibre filters with a mesh size of 42µm, species such as Ameoba, Nematodes and Ciliates are of a similar size class compared to phytoplankton species hence they could not be removed. *Chlorella* species were most abundant at an average of 15E+06 ±1.51E+06 cells/ml while *Amphora* and *Oxyrrhis marina* species were also present in high concentrations with average concentrations of 2876.4±2151.58 cells/ml and 750.5±106.54 cells/ml, respectively. Illustrated in Plate 4.1 are images of the major phytoplankton taxa present (Plate 4.1: a-d).

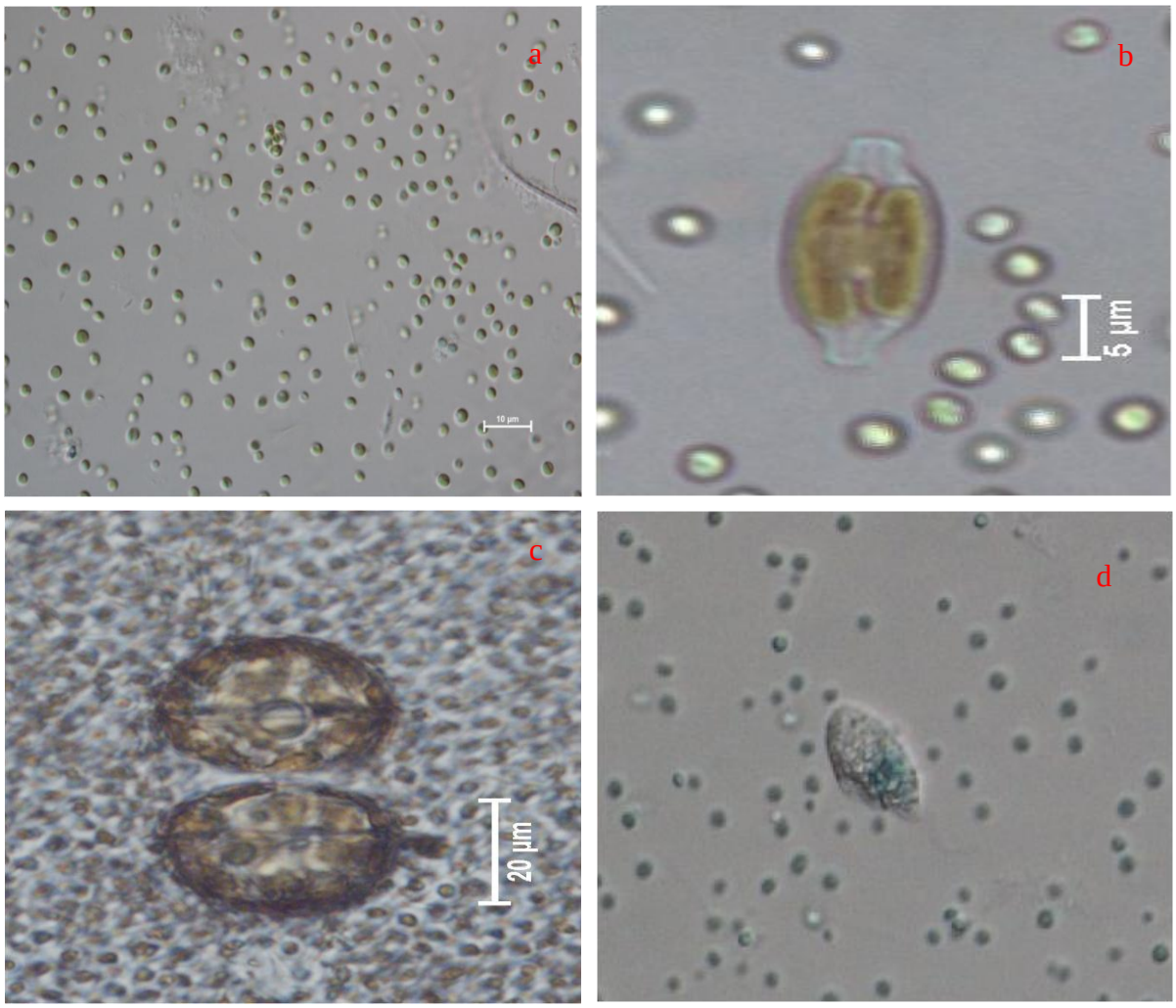

Plate 4.1: Images of the major phytoplankton taxa found, (a.) *Chlorella* species (Chlorophyta), (b.) *Amphora* species (Bacillariophyta), (c.) *Melosira* species (Bacillariophyta) and (d.) *Oxyrrhis marina* (Dinophyta).

The effect of using mixed algae cannot be understated. A single species model would be less complex as there is no interspecific competition within the population for resources. Furthermore, the presence of zooplankton demonstrates that in nature there will be more complexity as there is the presence of predators to consider that will inherently affect the growth of algae biomass. This makes the model more realistic.

4.7. Conclusion

In conclusion, the most favourable time to harvest is at 42-48 hours as this time is seen to produce the maximum SGR and lipid productivity. Harvesting at 42-48 hours can be done at any of the schemes except scheme 7 hence a photoperiod of 25% yields a quicker maxima at time

interval 12-18 hours. The application of harvesting should be done in the field rather than under lab conditions that are controlled in design. Aquaculture facilities for algal production that are dynamic in nature can allow for changes to the conditions at different time intervals which would allow for higher specific growths at time intervals that are statistically significantly lower (p>0.05). Furthermore, the earlier prediction that maximisation of harvesting at high growth rates would lead to lead higher lipid productivity cannot be accepted. According to Chen *et al.* (2011), lipid productivity is highest in nutrient limiting conditions that are in areas where nitrogen is limiting. However, a reduction of nitrogen decreases the growth rate as it is an essential nutrient: thus marine systems that are highly polluted where nitrogen is abundant will not allow for the maximum lipid productivity for harvesting. Subsequently, in the wild, during pollution events, the optimal algal growth and lipid production rates need to be evaluated with nitrogen content under eutrophic conditions to understand when to harvest algae for maximum lipids to be produced. However, in laboratory conditions where biofuel producers may alter conditions in dynamic aquaculture systems: nitrogen fluxes can be controlled such that optimal growth rates are achieved whilst still promoting maximum lipid production.

Chapter Five: Conclusion and Recommendations

5.1. General Conclusion

From this research it is evident that there is a need for interdisciplinary studies that integrate different areas of expertise to solve real-world problems. This study aimed to integrate mathematics, marine biology, renewable energy and environmental science into one research project to assess the objectives for the optimisation of biofuel products from algae. The overall aim of this project was to provide insight into how different combinations of parameters influence the growth of microalgae and determine which harvesting times yield the maximum amount of lipids for biofuel production.

For all schemes that were evaluated during this research it was found that a theoretical maximum does exist at the 42 – 48 hour interval for chlorophyll *a* SGR, pheophytin SGR and lipid productivity. This was shown by the detailed statistical and maximum gains and losses analysis. A doubling time of 38 – 48 hours for algal growth proposed by Converti *et al.* (2009) and Chen *et al.* (2011) illustrates that there is indeed a significant increase in growth in this amount of time. This research demonstrates that an optimal growth time of 42 – 48 hours also exists when mixed algae are grown. Furthermore, a maximum yield was gained in a quicker time (12-18 hours) for scheme 7 (at a photoperiod of 25 %) however an additional maximum also exists at 42 – 48 hours for the same scheme.

The statistical analysis showed that certain schemes were found to have lower algal growth and lipid productivity at certain time intervals. For example, to allow for optimisation for scheme 7, at time periods 42-48 hours, conditions need to be changed to those in scheme 1 as the SGR is significantly higher (0.3967 ± 0.0367 μg/l.hr ($p < 0.05$) as indicated by pheophytin. These conclusions will allow for optimisation in an aquaculture setting that has dynamic systems that would allow for changes in conditions at different time intervals. This would allow for overall increased growth rates and lipid productivity. The finding of a theoretical maximum time interval which allows for maximum algal growth and lipid productivity allowed for the model to be developed for all schemes. Models developed allow for the detection of the present state of algal biomass and lipid productivity and predicts the future state of environment by theorising how long one should wait to harvest the maximum amounts of algae. This model is extremely

dynamic as it can be applied under normal conditions in the field, under algal bloom conditions in the field and under controlled laboratory conditions for aquaculture purposes. Thus this model is extremely useful for biofuel producers, aquaculturists and pollution biologists (ecosystem recovery).

5.2. Recommendations and Limitations

Algal growth is extremely complex to understand and there are a multitude of factors that influence their growth. Additionally, growth parameters cannot be evaluated in isolation and that the interaction of parameters with each other must be considered (Pillay and Pillay, 2012). Within this research only three growth parameters were evaluated. However, for future research more parameters can be evaluated and added to schemes to make the model more realistic in nature. Further, more research is needed to evaluate the cyclic behaviour of mixed algal growth. Consequently, in future models, interspecific competition, predator-prey interactions and nutrient recycling need to be accounted for to determine what causes cyclic trends and whether these factors influence the overall chlorophyll *a* SGR, pheophytin SGR and lipid productivity. Additionally, ecological modelling at each time interval can be used to determine how species composition affects the growth rates and lipid productivity. Additionally, Lotka-Volterra models can be built into these simple models to account for predator-prey interactions amongst algae.

The assessment of nutrient recycling can be done by evaluating nutrient concentrations between time intervals. A key point that needs to be accounted for during the modelling process is that of the interaction of lipids, nitrogen and algal growth. Algal growth is promoted by nitrogen as the vital nutrient that promotes growth however it has been shown to cause lipid concentrations within algae to decrease (Chen *et al.*, 2011). Consequently, an optimisation model would need to be built into this model that evaluates the specific nitrogen concentration that yields the highest of algal growth and that promotes the largest amounts of lipids for biofuel production. Furthermore, in this study, 72 hours was assumed to be a complete growth cycle to allow for modelling. However in future research, it is suggested that studies be longer in duration to allow for more insight into growth cycles of mixed algal populations.

5.3. Summary

In summation, optimisation projects are of extreme importance to enhance the efficiency of renewable energies. The use of mathematical modelling within energy research can aid in providing quantitative solutions to real-world problems. In this research, the inter-disciplinary nature of the project has allowed for the model developed to be dynamic and have wide scale application in marine biology, aquaculture, pollution biology and renewable energy.

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