THE EFFECT OF MICROWAVE TREATMENT ON THE PHYSICOCHEMICAL CHARACTERISTICS OF FAECAL SLUDGE AND IMPLICATIONS FOR SLUDGE TREATMENT

by

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PREFACE

The research contained in this thesis was completed by the candidate while based in the Discipline of Chemical Engineering, School of Engineering of the College of Agriculture, Engineering and Science, University of KwaZulu-Natal, Howard College, South Africa. The research was financially supported by the Bill and Melinda Gates Foundation.

The contents of this work have not been submitted in any form to another university and, except where the work of others is acknowledged in the text, the results reported are due to investigations by the candidate.

Signed: Jon Pocock

Date: 30th May 2024

DECLARATION 1: PLAGIARISM

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(ii) this dissertation has not been submitted in full or in part for any degree or examination to any other university;

(iii) this dissertation does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons;

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a) their words have been re-written, but the general information attributed to them has been referenced;

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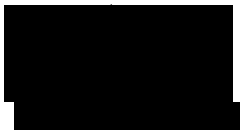

Date: 8th March 2023

DECLARATION 2: PUBLICATIONS

1. Mdolo P*, Velkushanova K, Pocock J (to come). Optimization of the microwave assisted solubilization of faecal sludge. *(Manuscript in preparation)*
2. Mdolo P*, Velkushanova K, Pocock J (to come). The effect of microwave treatment on the physicochemical characteristics of faecal sludge. *(Manuscript in preparation)*
3. Mdolo P*, Velkushanova K, Pocock J (to come). Improvement of the anaerobic digestion of faecal sludge through microwave pretreatment. *(Manuscript in preparation)*
4. Mdolo P*, Velkushanova K, Pocock J 2022. Valorization of faecal sludge through microwave pre-treatment to recover valuable resources and reduce handling costs. Paper presented to the 2022 IHE Delft Institute of Water Education PhD Symposium, Delft, Netherlands, 13th to 14th October 2022. Presented by P Mdolo
5. Mdolo P*, Velkushanova K, Buckley CA 2020. Physicochemical characteristics of ventilated improved pit (VIP) latrine sludge after microwave treatment. Paper presented to the 2020 Postgraduate Research Innovation Symposium (PRIS) UKZN, Durban, South Africa, 10th to 11th December 2020. Presented by P Mdolo

The results reported and presented are based on the data I collected during the study period. I designed the experiments, collected, analyzed the data, and wrote the papers and presentations. Dr Jon Pocock and Dr Konstantina Velkushanova reviewed the experimental design, data analysis and manuscripts and supervised the implementation of the experimental design. Prof Chris Buckley reviewed and supervised the work reported in 5.

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ABSTRACT

Faecal sludge (FS) is generated from non-sewered sanitation technologies such as pit latrines and septic tanks. Over time, FS needs to be emptied from the pit latrine and processed. Resource recovery from FS is growing because it contains organic matter and other materials that can be valorized to plant nutrients and biofuels. Drying, hydrothermal carbonization (HTC) and anaerobic digestion (AD) are some technologies used to treat FS and recover resources from it. Drying is achieved through solar, thermal, infrared or microwave heating. Microwave (MW) treatment is a technology of interest because of its efficient heating mechanism and versatility. It can efficiently meet several treatment objectives, such as volume reduction, nutrient recovery, or pathogen inactivation in a single operation. It can be incorporated into other treatment processes like pyrolysis, hydrothermal carbonization, and biological degradation to improve process efficiency. This study evaluated the effect of MW treatment on FS characteristics and the subsequent anaerobic digestion. A response surface modelling was used to study the MW operating parameters that influenced FS solubilization. The FS was collected from active ventilated improved pit (VIP) latrines and was treated in a domestic MW oven at 630W, 720W and 810W for 1 to 10 minutes. Changes in the properties of the treated FS were analyzed using ultraviolet-visible (UV/VIS) spectrophotometry, carbon, nitrogen and sulfur (CNS) elemental analyzer, Fourier transform infrared (FTIR), scanning electron microscopy (SEM) imaging, flow cytometry (FCM) and Automatic Methane Potential Test System (AMPTS II). The change in chemical oxygen demand (COD) provided a means of evaluating the effect of MW treatment on FS solubilization. The untreated FS had a high soluble COD (sCOD) to total COD (TCOD) ratio (10%), which indicated that the FS underwent stabilization in the pit latrine and during storage. A high sCOD/TCOD ratio was expected because the average age of VIP latrine FS in eThekwini is approximately five years. Although the FS showed properties of stabilization, MW treatment increased the sCOD, showing an initial sCOD release phase (phase 1), degradation of sCOD (phase 2) and a second slight sCOD release (phase 3). The sCOD release profiles were similar to the temperature profiles. In all the treatments (630W, 720W and 810W), the maximum sCOD release was recorded when the FS reached the boiling temperature (~96°C). The highest sCOD/TCOD achieved was 27% when a microwave power (MP) of 630W was applied for 4 minutes. The highest sCOD release was achieved in all treatments during the first phase. Although there was a second sCOD release (phase 3), it did not result in additional sCOD. Therefore, the microwave operation could be stopped after the first phase if the treatment aims to solubilize organic matter. Soluble proteins (sProt) and soluble carbohydrates (sCarbs) also increased after MW treatment and followed a similar trend to the sCOD release. A correlation heat map revealed

that the increase in sCOD resulted from the release of sProt and sCarbs from cell walls and the FS matrix. The untreated samples contained 300g/kg.TS C, 72g/kg.TS N, 1.8g/kg.TS NO_3^- and 10.2g/kg.TS NH_4^+. At low MP applied (630W), the concentration of C, NO_3^- and NH_4^+ slightly increased while N decreased. The C:N ratio ranged between 4.2:1-10:1 which was lower than the recommended C:N ratio for optimum biological treatment. The cumulative methane production in microwave treated FS was 783NmL (~17% more), while it was 672NmL in the reactors treating untreated FS. MW treatment increased the methane potential of FS from 110NmL/g.VS to 128NmL/g.VS (~16%). After microwave treatment, the organic loading and methane production rate also increased by 18% and 33%. This research has shown that MW treatment increased the sCOD and solubilization of organic matter. Organic matter solubilization is frequently used as a metric for improved hydrolysis during AD. Therefore, MW treatment could be incorporated as a pretreatment step to improve the hydrolysis of FS and increase biogas production and process efficiency during AD. The digestate cake and supernatant were analyzed and showed that nutrients were retained in the digestate cake. Thus, making it a suitable product for soil conditioning. On the other hand, the supernatant could be co-treated with wastewater without causing organic and nutrient-loading shocks since these are retained in the cake. MW treatment offers opportunities to valorize FS through reduced processing costs and the production of valuable products such as nutrients and bioenergy.

Keywords: Anaerobic digestion, faecal sludge, microwave treatment, nutrients, organic matter solubilization, soluble COD

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LIST OF ACRONYMS

AD	Anaerobic digestion
AMPTS	Automatic methane potential test system
BMP	Biochemical methane potential
CNS	Carbon, nitrogen, sulfur
COD	Chemical oxygen demand
CST	Capillary suction time
DEWATS	Decentralized wastewater treatment system
DOC	Dissolved organic carbon
DOE	Design of experiments
DS	Dewatered sludge
EPS	Extracellular polymeric substances
ES	Excess sludge
FCM	Flow cytometry
FS	Faecal sludge
FSM	Faecal sludge management
FTIR	Fourier transform infrared
HF	Fresh human faeces without urine
HFS	Fresh human faeces with urine and toilet paper
HRT	Hydraulic retention time
HTC	Hydrothermal carbonization
HTHP	High temperature thermal hydrolysis
ISR	Inoculum to substrate ratio
JMP	Joint monitoring program
LaDePa	Latrine dehydration pasteurisation
LTHP	Low temperature thermal hydrolysis
MDGs	Millennium development goals
MFS	Microwave treated faecal sludge
MFW	Microwave treated faecal sludge with wood chips
MGDS	Malawi growth and development strategy
M-HTC	Microwave hydrothermal carbonization
MIR	Mid infrared

MP	Microwave power
MW	Microwave
NSO	National statistical office
O&M	Operations and maintenance
OH	Hydroxyl
OLR	Organic loading rate
OSS	Onsite sanitation system
PI	Propidium iodide
R&D	Research and development
RFS	Untreated faecal sludge
RFW	Untreated faecal sludge with wood chips
r_G	Rate of methane production
RSM	Response surface modelling
sCarbs	Soluble carbohydrates
sCOD	Soluble chemical oxygen demand
SDG	Sustainable development goal
SE	Specific energy
SEM	Scanning electron microscope
SG	SYBR-Green
SOP	Standard operating procedure
sProt	Soluble protein
sTKN	Soluble total Kjeldahl nitrogen
TCOD	Total chemical oxygen demand
TH	Thermal hydrolysis
TKN	Total Kjeldahl nitrogen
TS	Total solids
TVFA	Total volatile fatty acids
TWAS	Thickened waste activated sludge
UDDT	Urine diversion dry toilet
UDFT	Urine diversion flush toilet
UN	United nations
UNICEF	United nations international children's emergency fund
UV/VIS	Ultraviolet-visible

VFA	Volatile fatty acids
VFCW	Vertical flow constructed wetland
VIP	Ventilated improved pit
VS	Volatile solids
VSS	Volatile suspended solids
WAS	Waste activated sludge
WASH	Water, sanitation and hygiene
WHO	World health organization

CHAPTER 1 : INTRODUCTION

1.1 The rationale for the research

The resources and efforts committed to meet the sanitation goal of the Millennium Development Goals (MDGs) did not yield the desired results. At its expiry, 15 years later, almost 2.3 billion people lacked access to adequate sanitation (WHO/UNICEF, 2021). Hence, the United Nations (UN) committed to ambitious targets in sustainable development goals (SDGs) to transform the world by 2030. Goal number 6 targets to achieve availability and sustainable management of water and sanitation for all. Several objectives highlight the commitment to achieve this purpose, of which SDG 6.2 aims to end open defecation, ensure universal access to a basic toilet, and put in place systems to manage excreta and promote hygiene by 2030 safely. Even with the current, most ambitious, global goals (SDGs), onsite sanitation is considered secondary to the conventional sewerage systems. The Joint Monitoring Programme (JMP) and the United Nations International Children's Emergency Fund (UNICEF) developed a tool to track the progress in achieving SDG 6.2 (Table 1.1).

Table 1.1 JMP global monitoring ladder for sanitation (United Nations 2018)

Service level	Definition
Safely managed	Use of improved facilities that are not shared with other households and where excreta are safely deposited in situ or transported and treated offsite
Basic	Use of improved facilities that are not shared with other households. Excreta is neither safely disposed of in situ nor transported for offsite treatment
Limited	Use of improved facilities that are shared with other households
Unimproved	Use of pit latrines without a slab or platform, hanging latrines or bucket latrines
Open defecation	Disposal of human faeces in fields, forests, bushes, open bodies of water, beaches, or other open spaces or with solid waste

The current rate of progress to achieving SDG 6.2 is slow. It is estimated that about 2.8 billion people will lack access to safely managed sanitation services by 2030 (WHO/UNICEF, 2021). For example, in 2020, almost 67% of the world's rural population did not have access to safely managed sanitation services, of which the majority (>50%) were in sub-Saharan Africa (Fig 1.1).

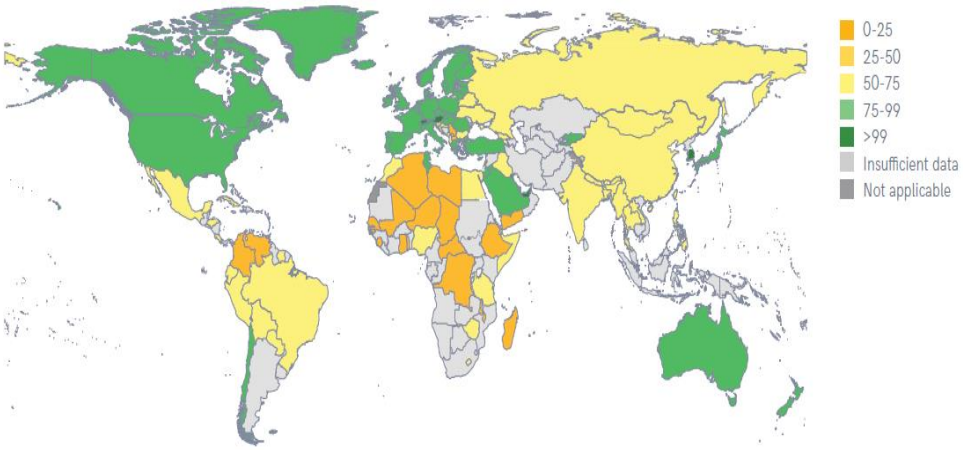


Figure 1.1 Proportion of population using safely managed sanitation service in 2020 (WHO/UNICEF 2021)

The progress to achieving SDG 6.2 varies across the globe, with richer countries progressing fast while poorer countries are the slowest (Fig 1.1). The slow pace observed in low to middle-income countries is worrisome because most of the population lack essential sanitation services. Therefore, the burden of water-related diseases and degraded environments will prevail with severe consequences on public health and global food systems.

Safe treatment of faecal sludge (FS) has received particular attention in the SDGs, and it is emphasized that containment alone is not enough if the world is to achieve SDG 6.2 (UN, 2018). Achieving this target requires investments in sanitation infrastructure that collect FS and safely transport and treat it. Safely managed sanitation involves different sanitation technologies interlinked together and form a complete system referred to as the sanitation service (Fig 1.2).

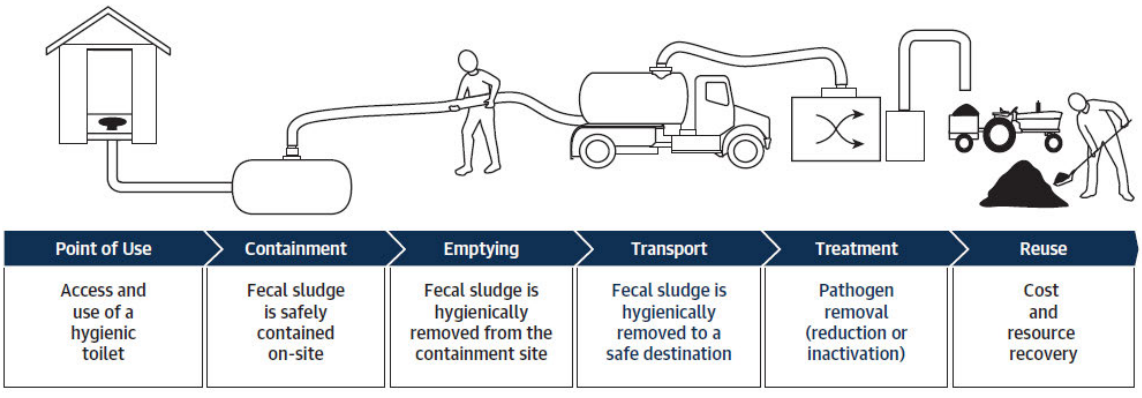


Figure 1.2 The sanitation service chain showing different technologies linked together to achieve safely managed sanitation (Verhagen & Scott, 2019)

A break in any stage of the service chain will potentially introduce FS into the environment and lead to possible exposure to the public. Therefore, the management of the entire sanitation service chain is crucial in protecting the environment and public health.

Wastewater treatment plants have been designed and constructed in many countries worldwide to contain and treat human excreta. The systems consist of a network of sewers that convey the wastewater to a centralized treatment facility. High energy input, operating costs and requirement for skilled personnel for operation and maintenance (O&M) characterize sewer-based technologies (Strande et al., 2014). Consequently, they have not been successfully implemented in many developing countries. In many cases, they are not functioning efficiently or are often non-existent (Munamati et al., 2017). The rise in informal settlements in the developing world makes it even more challenging to expand the sewerage network in such areas. Thus, households use onsite sanitation technologies (e.g., septic tanks, cesspools, and pit latrines) to meet their sanitation needs. The global distribution of onsite sanitation technologies is varied. About 21% of onsite sanitation technologies in sub-Saharan Africa are pit latrines (Munamati et al., 2017). For example, in Malawi, over 66% of households in urban areas either use a ventilated improved pit (VIP) latrine or a traditional pit latrine with a roof, while 14% use a flush toilet (Malawi National Statistical Office (NSO), 2020), which, most often, is connected to a septic tank.

Onsite sanitation technologies produce FS, which, if not adequately managed, poses serious public health and environmental risks (Naido et al., 2020; Farling et al., 2019; Hashemi & Han, 2019). The characteristics of FS are influenced, among others, by the characteristics of the sanitation technology (i.e., whether it is lined or unlined, wet, or dry) and the origin of the FS, e.g., whether it is from a public or private toilet. For example, an unlined pit latrine leads to ingress and egress of water that can affect the solid content of the FS (Gold et al., 2018). FS collected from shared or public toilets is relatively fresh and hence contains higher amounts of readily biodegradable components (Septien et al., 2018a) than that collected from household toilets which are not shared. In the absence of standardized pit sizing, the volume of the pit per number of users and user habits (such as the addition of trash and different anal cleansing materials to the pit) may also impact the freshness and hence biodegradability of the FS. The biodegradability of the FS would greatly depend on the amount of organic matter present in it. Within South African pit latrines, which tend to operate without water, it has been reported that biodegradability decreases from top to bottom (Bakare et al., 2012).

1.2 Context and scope of the research

Biological treatment of waste is a vital process in pollution control and resource recovery. Both aerobic and anaerobic microorganisms are involved in the biodegradation of waste. Aerobic degradation produces carbon dioxide and water as end products, while anaerobic degradation results in biogas (Metcalf & Eddy, 2014). Pit latrines are widely used sanitation technologies in

sub-Saharan Africa (Munamati et al., 2017). The major processes in a pit latrine involve filling with faeces, anal cleansing material and other materials added by the user; transfer of water into and out of the pit; biodegradation of the added organic materials; pathogen die-off and accumulation of the inert material at the bottom of the pit
(Back et al., 2018; Bakare et al., 2012; Graham & Polizzotto 2013; Nyenje et al., 2013). The reported biodegradation in South African pit latrines indicates that they do not only collect and store the excreta, but also treat it to some extent. The biodegradation occurring in pit latrines is attributed to aerobic and anaerobic processes. The first zone (the top-most layer) is predominantly aerobic. This layer is very thin, and the FS undergoes rapid aerobic degradation (Bakare et al., 2012). The layer is covered as more material is added, becomes void of oxygen and evolves to an anaerobic layer. In Tanzania it was postulated that anaerobic degradation is the primary pathway of FS degradation in pit latrines (van Eekert et al., 2019). Yet Bourgault et al., 2019 argued that biodegradation pathways in pit latrines could not be conclusively classified as predominantly anaerobic with the current state of knowledge.

Regardless of which degradation pathway is predominant, the biodegradability of the FS would greatly depend on the amount of organic matter present in it. Compared to wastewater sludge, FS has high organic matter content (Tayler, 2018). Anaerobic digestion could be a useful treatment technology for such types of waste. However, slow hydrolysis of complex organic wastes like FS limits anaerobic digestion and results in long retention times and large reactor volumes.

Pretreatment of the substrate before anaerobic digestion can improve the solubilization of complex organic matter and enhance hydrolysis. Technologies for pretreatment include mechanical, thermal, chemical, biological, or combination of two or more technologies (Zhen et al., 2017). Although the focus of sludge pretreatment has been to enhance hydrolysis and methane production, it also brings several beneficial effects such as sludge sanitization, volume reduction (Mawioo et al., 2017) and increased organic loading rates (OLR) during biological treatment (Serrano et al., 2016).

Microwave (MW) irradiation is a promising technology to treat complex organic materials such as FS. Among other benefits, MW treatment sanitizes sludge, reduces its volume, and improves the solubilization of organic matter (Mawioo et al., 2017; Yu et al., 2010). Hence, the influence of MW treatment on FS was evaluated as a novel technology for improving its physical and biochemical characteristics, which could further aid in downstream process selection and design.

1.3 Justification

This research studied the transformation of FS during microwave treatment and its influence on critical physicochemical characteristics. This dissertation focused on understanding the effect of MP and treatment time on FS characteristics with an emphasis on microwave treatment as a stand-alone technology or integrated with other FS treatment technologies. An extensive review of literature and laboratory investigations were undertaken to provide answers to the following questions:

- What microwave operating parameters are critical in the treatment of FS?
- What is the influence of microwave treatment on the solubilization and biodegradability of organic matter in FS?
- What is the relationship between soluble organic matter and biochemical molecules in FS?
- How does microwave treatment affect the nutrient content and C/N ratio of FS?
- What are the major functional groups in FS before and after microwave treatment?
- What are the effects of microwave treatment on FS surface morphology?
- How does microwave treatment impact the biodegradability of FS and the rate of methane production?
- What is the impact of microwave treatment on the digestate and effluent quality of anaerobic digestion by-products?

The hypotheses tested are summarized below (Table 1.2), together with the measure of success for each hypothesis.

Table 1.2 Summary of hypotheses and their success criteria

Hypothesis	Experiments to test the hypothesis	Success criteria
MW treatment can solubilize organic matter in FS	VIP FS was treated by MW irradiation at different operating conditions.	sCOD, sCarbs, sProt ammonium, and nitrate in microwaved samples higher compared to untreated samples
MW treatment can improve physicochemical parameters for better downstream treatment	Physicochemical characterization of microwaved samples (sCOD, TS, VS, CNS, FTIR, scanning electron microscopy)	Indicator metrics e.g., VS/TS, sCOD/TCOD, C:N, functional groups, surface morphology
MW treatment improves handling of FS	Volume reduction and flow cytometry (FCM) analysis of bacteria viability	Significant reduction in FS volume and decrease in the number of viable bacteria cells
MW treated FS produce more biogas compared to the untreated FS	Biochemical methane potential (BMP) test of both the MW treated and untreated FS	Cumulative and specific methane production higher in MW treated FS than in the untreated FS
MW treatment improves methane production kinetics of FS	Evaluation of the organic loading and methane production rates	Time taken to produce 80% of the cumulative methane lower in MW treated FS than in the untreated FS. Methane production rate higher in MW treated FS than in the untreated FS

1.4 Aims

This research aimed to understand the influence of MW treatment on the physicochemical characteristics of FS and how these changes affect the design and choice of reliable and cost-effective FS treatment technologies.

1.5 Objectives

- To identify important microwave operating parameters that influence the physicochemical characteristics of FS
- To evaluate the effect of MW treatment on physicochemical properties of FS
- To understand the relationship between soluble organic matter and biochemical molecules in FS
- To understand the impact of MW treatment on FS surface morphology and how it impacts the concentration of soluble organic matter
- To understand the effect of MW treatment on subsequent digestion of FS

1.6 The vision and impact of the research

The complexity of FSM drove the research, the location where the FS is generated (mostly informal settlements with no access to sewered sanitation), the volumes generated, and the widely varying characteristics of the generated FS. The difficulty to connect such areas to conventional sewerage networks or deploying traditional sludge treatment technologies put the people living there at risk of contracting water-related diseases. Therefore, deploying novel and flexible FS treatment technologies will bring economic, environmental, and social benefits to the would-be neglected areas. MW treatment is a flexible technology that can be deployed as a stand-alone technology or incorporated with existing sludge technologies to improve their performance efficiency. For example, depending on the objective of the treatment, MW treatment can be used for volume reduction or pathogen inactivation as a stand-alone treatment technology. It can also be incorporated as a pretreatment stage in pyrolysis, hydrothermal carbonization, or anaerobic digestion. MW treatment can be a decentralized technology that removes the need for expensive sewerage networks. MW treatment offers opportunities to recover valuable products from FS such as nutrients, proteins, bioenergy, metals etc. Recovering such value products is an incentive to invest in novel FS treatment technologies. Recovery of methane in anaerobic digestion (AD) and application of the stabilized sludge in agriculture/land, restoration projects contribute to mitigating adverse environmental impacts from using non-renewable resources.

1.7 Outline of the thesis

This thesis is organized into five chapters (Fig 1.3).

Chapter 1: Provides the introduction to the research. It describes the rationale, context, scope, and justification of the research. The hypotheses and their measure of success, aims and objectives are presented. The chapter highlights the vision which drove the research and the envisioned impact it will have on public health, society and the environment.

Chapter 2: Reviews relevant literature on global sanitation, available sanitation solutions (sewered and non-sewered) and the characteristics of the sludge they produce. The characteristics of VIP latrines are discussed in detail because VIP latrines are the most basic form of sanitation in the study area. The degradation process of sludge and the technologies to enhance it are evaluated in detail. Chapter 2 also discusses the general principles of microwave technology and its application in waste management. The chapter further reviews the specific application of microwave treatment to sewage sludge and FS. The chapter concludes by drawing key conclusions from microwave treatment of human biowaste and linking them to the current work.

Chapter 3: This chapter outlines the materials and experimental methods used to test the presented hypotheses.

Chapter 4: Presents the results and discussions from the current research.

Chapter 5: This chapter presents the overall conclusions, recommendations, and areas of further study.

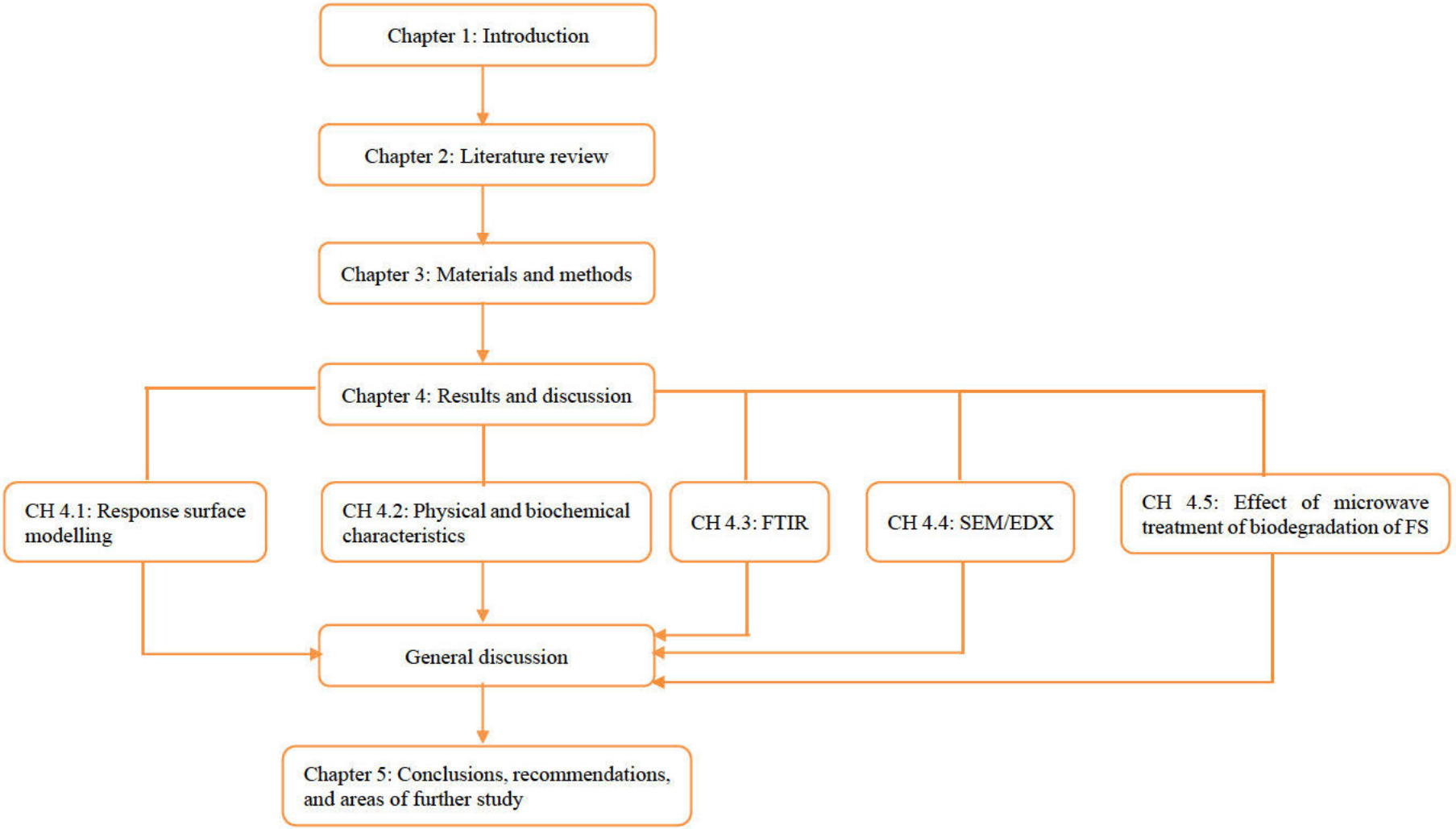


Figure 1.3 Schematic layout of the thesis

In the next chapter (Chapter 2), the literature review used to support this study is discussed.

CHAPTER 2 : LITERATURE REVIEW

2.1 Introduction

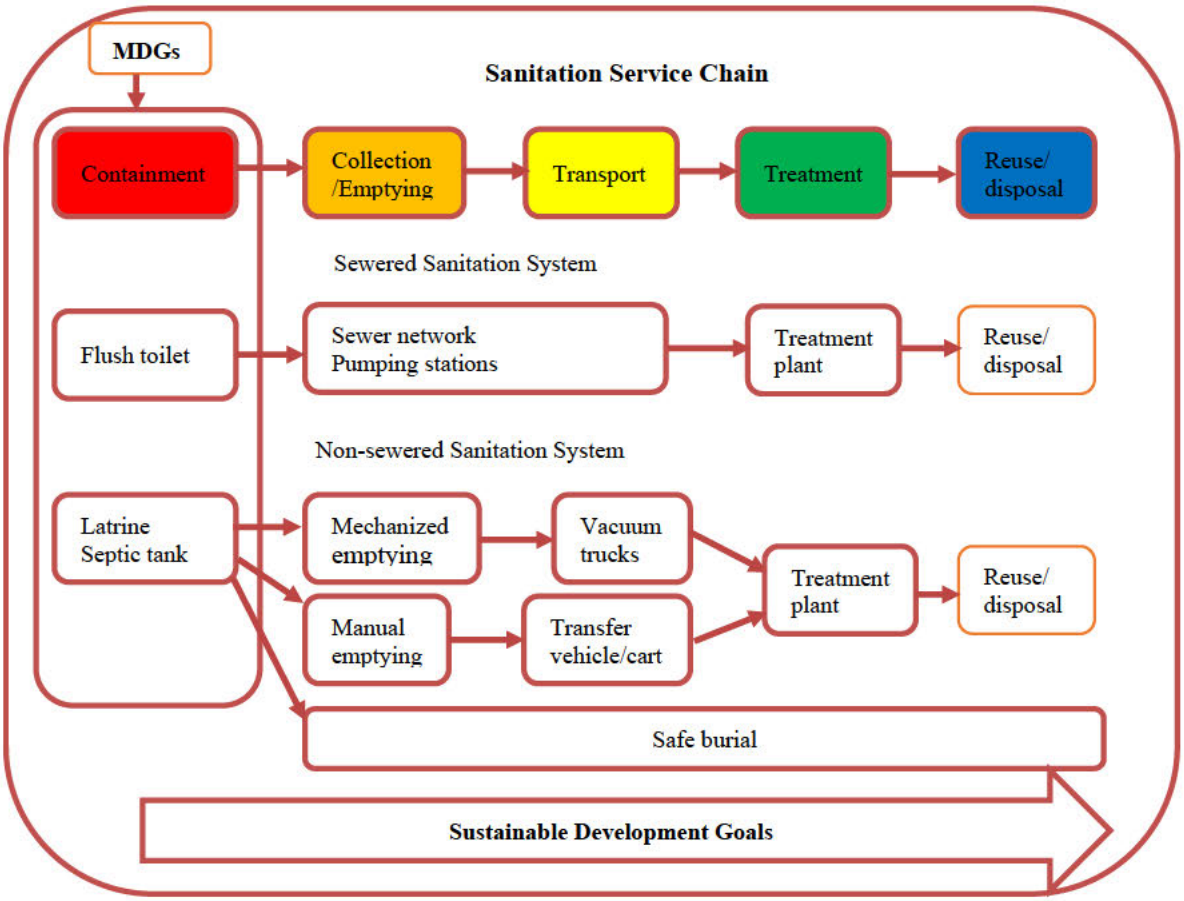
FS is defined as the semi-solid material that is removed from onsite sanitation technologies which are not connected to reticulation sewerage systems (Strande et al., 2014). When this material is deposited in the pit, it rapidly undergoes transformation and is covered as more material is added to the pit. With time, the material becomes partially or completely digested making it variable in consistency, quantity, and concentration throughout the profile of the pit. The primary material added to the pit is excreta which comprise urine and faeces, most often without flush water. The volume of excreta is usually small but highly concentrated in both nutrients and pathogens (Rose et al., 2015). Excreta can have a soft or runny consistency (Strande et al., 2014). The different streams of excreta include faeces; urine; urine mixed with faeces; additives (household garbage); anal cleansing materials (e.g., wet, or dry material); flush water; greywater; cover material; detritus and chemical additives; all, or a combination of which form FS in onsite sanitation systems (OSS) (Tilley et al., 2014).

FSM is a fast-growing concept in the management of excreta and comprises management of FS from its containment, emptying, transport, safe treatment, disposal and/or end-use.

2.2 Type and distribution of sanitation technologies

Sanitation systems comprise networked i.e., sewered (conventional, or non-conventional sewerage network) and loosely networked i.e., non-sewered systems. The networked sanitation system collects wastewater from a large catchment area and conveys it in a network of sewers to a central or decentralized treatment system (Chen et al., 2020).

In decentralized systems wastewater is collected from a group of households and transported in shallow sewers to a treatment plant. The systems comprising shallow sewers are referred to as decentralized wastewater treatment systems (DEWATS) and are an alternative to the non-sewered systems. The loosely networked systems are most common in low-income areas, and they meet the sanitation needs of local community. Whether sewered or non-sewered, a sanitation system comprises several sanitation technologies linked together to form the sanitation service chain. A functional sanitation system comprises five components of the sanitation technologies, i.e., the user interface; collection and storage; conveyance/transport; treatment; and reuse/disposal (Tilley et al., 2014) (Fig 2.1).



MDGs= Millennium Development Goals

Figure 2.1 Sewered and non-sewered sanitation systems and their relationship to MDGs and SDGs (Koottatep et al., 2019)

Both the sewered and non-sewered sanitation systems must comprise the five components of the sanitation service chain to qualify to have provided a safely managed sanitation service. Management of the excreta is quite different in both the sewered and non-sewered systems. For example, in sewered systems excreta is flushed and transported in a network of sewers to a treatment plant. While in a non-sewered system, excreta are contained onsite, in the latrine or septic tank (the collection technology) and requires some form emptying, transport in vehicles/cart etc. to a treatment or reuse/disposal site. Thus, sludge production in a sewered system happens offsite at the wastewater treatment plant while it is produced onsite at household level in a non-sewered sanitation system. Breakages in the sanitation service chain are common in low-and middle-income countries. Hence, excreta are introduced to the environment with serious consequences to environmental and public health.

For example, a study conducted in 39 cities across Africa, East Asia, Latin America, and South Asia indicated that 58% of the population (~42 million people) living there used sanitation systems (both sewered and non-sewered) that were not safely managed (Peal et al., 2020). Meaning that the sanitation systems they used lacked one or more of the five components of the

sanitation service chain. The causes of the breakages in the sanitation service chain are summarized in Table 2.1.

Table 2.1 Causes of failure to attain safely managed sanitation (Peal et al., 2020)

Failure Mode	Cause of breakage (failure)	Percent (%)
1	Sludge not contained and not emptied	14
2	Sludge emptied but not delivered for treatment	18
3	Sludge delivered to treatment but not treated	3
4	Wastewater not delivered to treatment plant	14
5	Non-functional wastewater treatment plants	6
6	Open defecation	3
7	**Total excreta unsafely managed**	**58**

Fig 2.2 a-c shows field examples of the state of some of the sewered and non-sewered sanitation technologies in low- and middle-income countries and how they relate to failures reported in Table 2.1.

Fig 2.2a shows the state of the pit latrine (containment technology in the sanitation service chain) in eThekwini Municipality, South Africa. Here the sludge could be collected and emptied but not delivered to a treatment plant, leading to failure mode number 2 in Table 2.1. Figure 2.2b shows a blocked manhole in the conveyance system (collection and transport technology in the sanitation service chain) in eThekwini Municipality, South Africa. In this scenario, the blockage could lead to backflow and hence the wastewater will not be delivered to the treatment plant leading to failure mode number 4 in Table 2.1.

a

Figure 2.2 Field examples of the causes of failures in the sanitation value chain (Photos taken by the author)

Fig 2.2c shows the state of an anaerobic pond in a waste stabilization pond system (treatment technology in the sanitation service chain) in Lilongwe, Malawi. In this situation, the wastewater is delivered to the treatment plant. However, it is not treated because the treatment technology is not functional, thus leading to failure mode number 5 in Table 2.1.

The failures that can occur at several stages of the sanitation service chain highlight the need to adequately manage it. Although connection to a sewerage network is classified as having access to safely managed sanitation (UN, 2018), failures along the service chain do not guarantee safe management of the excreta. Hence, a holistic approach is required to manage the sanitation service chain for both the sewered and non-sewered sanitation systems. Management of the non-sewered sanitation service chain is what is known as FSM (Strande et al., 2014).

When using a toilet, the user encounters components such as the type of toilet, pedestal, pan, or urinal, known as the frontend technologies which are connected to the backend technologies (the collection and storage technologies). The frontend technologies are either dry or waterborne (e.g., urinal, urine diverting dry toilets (UDDT), urine diverting flush toilets (UDFT), pour flush toilets and cistern flush toilets, traditional pit latrines). The materials added into the frontend technology are collected in the backend technology (e.g., urine storage tank/container, single & double VIP, dehydration vaults, composting toilets, or septic tank (Tilley et al., 2014).

The collection and storage technologies can be categorized as dry or wet, depending on whether excreta and water are collected together or separately. The addition or exclusion of water to and from the system determines how wet or dry the FS will be, which could influence emptying and biodegradation processes taking place within it (Bakare et al., 2012). Other technologies using urine, faeces and greywater diversion create a more targeted treatment and end use option (Tilley et al., 2014) for each individually collected stream.

The distribution of sanitation technologies is varied and depends on several factors. For example, a pit latrine with a slab is the most prevalent technology in sub-Saharan Africa. Its coverage was 21% in 2017. Its prevalence can be attributed to its low cost, allowing more beneficiaries to be reached (Munamati et al., 2017). The flush/pour flush toilet connected to the septic tank is the second preferred technology, especially in households with more income (Munamati et al., 2017; Nakagiri et al., 2016). Despite the potential of using compost from composting toilets for soil conditioning and crop production, the technology is the least adopted in sub-Saharan Africa. The low adoption rate of composting toilets can be attributed to the faecophobic nature of people in this region (Dellström Rosenquist, 2005). In Malawi, over 50% of households still use a traditional pit latrine with a roof. Even within the urban areas of the country, the traditional pit latrine with a roof is still the technology of choice for 59% of the households. High prevalence of pit latrines either with a slab or a VIP and pour flush to a pit latrine is reported in many other developing countries. For example, the pit latrine with a slab is the most prevalent form of sanitation in Burkina Faso, Rwanda, and the Gambia at 83%, 61%, and 56%, respectively, while South Africa, at ~22%, has a pit latrine with slab prevalence of slightly above the sub-Saharan average (Munamati et al., 2017). VIP latrines are most prevalent in Botswana (32%), Nigeria (31%) and Zimbabwe (30%) (Munamati et al., 2017). Low adoption of sustainable sanitation technologies could be linked to lack of commitment by the governments in most of the low-income countries. For example, in Malawi, the country's third mid-term development agenda, the Malawi Growth & Development Strategy (MGDS III) was silent on sanitation development (Government of Malawi, 2017).

Due to the high prevalence of pit latrines and pit-based sanitation technologies in sub-Saharan Africa, different aspects of pit latrines are discussed in detail in the following sections.

2.3 Characteristics of pit latrines

A pit latrine is the most basic and common form of sanitation technology serving the needs of over 1.5 billion people (Graham & Polizzotto, 2013). Numerous advantages (such as being low-cost compared to sewered sanitation, they can be shared by several households, they take long to fill up, they are easy to maintain and can be manually emptied) offered by pit latrines lead to their wide-spread adoption. Also, pit latrines offer flexible technological options to meet the specific

needs of different user groups. Whichever technical option is preferred, the essential components of a pit latrine are a hole, slab with a drop hole and a superstructure built over the pit (Simiyu, 2017). Excreta and other anal cleansing materials drop through the hole dug in the ground or raised above the ground and covered by a slab. The slab with a drop hole offers safety for different user groups while the superstructure ensures privacy while using the toilet.

Simple pit latrines are associated with malodors and flies. Hence, several modifications have been employed to improve them. For example, a VIP latrine was explicitly designed to deal with odor and flies through the action of air currents and light. Air circulates within the superstructure and down the squatting hole flushing out odors through a vent pipe. The flow of air drives flies down the pit, and once there the only light visible is through the vent pipe. As flies are attracted to light, they fly towards it but are prevented from escaping by a fly screen installed on the vent pipe (Fig 2.3).

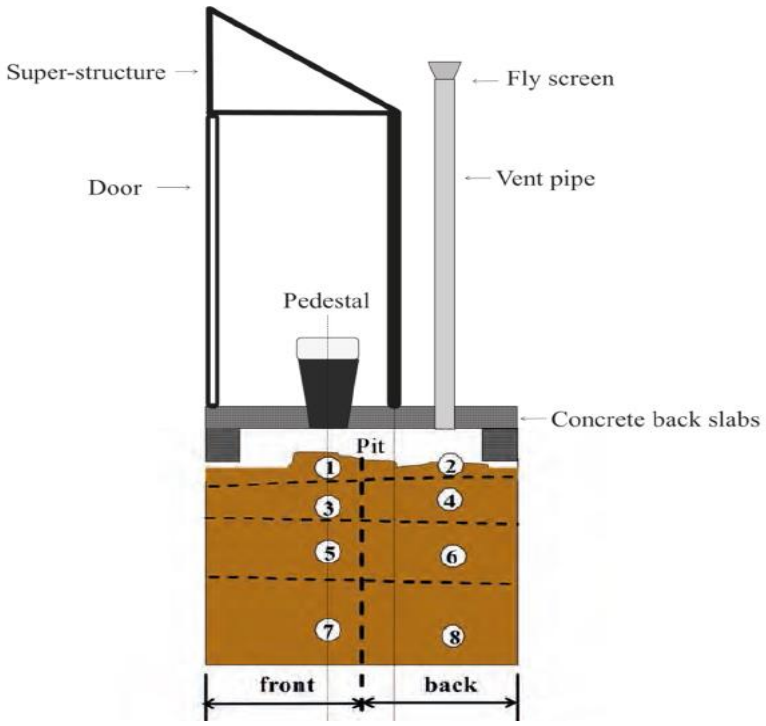


Figure 2.3 Diagram of a VIP latrine showing the mechanisms of how it works (Zuma et al., 2015)

The flow of odor from the pit is by suction, facilitated by the vent pipe. Hence, the dimensions of a vent pipe in respect to ambient air velocity is a critical VIP latrine design parameter (Obeng et al., 2019). In practice, design and construction of pit latrines are not consistent with technical guidelines and has led to their poor performance. Deviations from technical guidelines are expected due to different user preferences; budget constraints; and lack of local masons or recommendations of the local authority. For example, VIP latrines in eThekwini municipality are

single-lined pits with superstructure built slightly offset from the hole (Brouckaert et al., 2013), possibly to facilitate pit emptying. In other instances, the superstructure is built directly above the pit (Nakagiri et al., 2015), in which case FS is emptied through the drop hole.

2.4 Pit latrine microbial ecology and pathogens

The microbiology of pit latrines is complex with a diverse species composition and structure. The content of the pit latrine is heterogeneous, influenced by several factors such as user habits and their diet (Rose et al., 2015), which contribute to a complex microbial structure. Microbial communities play essential roles in biological processes occurring in pit latrines. The processes occurring in the pit are responsible for the intrinsic characteristics of the FS. The characteristics of the FS ultimately influence the choice of the emptying and treatment/disposal technology. Therefore, management of pit latrine sludge requires an understanding of the biology within it.

The human gut and surrounding environment contribute to microbial communities of the pit (Torondel et al., 2016). Faecal excretion introduces microorganisms from the human gut to pit latrines. While household waste, groundwater and soil also introduce microbes to pit latrines. Microbes from the human gut will only survive in pit latrines if they can adapt to their new environment. Diversity of microbial communities differ between regions; between pit latrines in the same area and to a lesser extent at different depths within the same pit latrine (Torondel et al., 2016). Both obligate anaerobes and aerobic species have been isolated in pit latrines which supports the conclusion that both anaerobic and aerobic degradation takes place within them (Bakare et al., 2012).

Other microbial species present in pit latrines are pathogenic and drug resistant (Beukes et al., 2017; Beukes & Schmidt, 2018). High concentrations of helminth eggs and several infectious bacteria, with some species prevalent at specific depths have been identified in several pit latrines (Nabateesa et al., 2017; Nzouebet et al., 2016). Again, microbial communities and diversity are different between regions, and between pit latrines (Kumwenda et al., 2017). The presence of resistant species to clinically proven treatment is a potential public health risk. Therefore, microbiological surveillance of pit latrines is necessary to protect users and sanitation workers, especially manual pit emptiers.

2.5 Sludge treatment

Regardless of the sanitation system deployed in the region, large volumes of sludge are generated. The generated sludge must be properly managed to recover valuable resources and minimize its negative impact on the environment and public health. Sludge treatment is an engineered process that aims at recovering valuable resources and rendering it safe for disposal in the environment. Sludge treatment aims to improve public health, conserve the environment, and generate useful

resources. Income from sludge derived products can improve the livelihoods of the community and create demand for sanitation technologies (Dodane et al., 2012).

Sludge produced in sewered sanitation technologies is known as sewage sludge while that produced in non-sewered technologies is called FS (Metcalf & Eddy, 2014; Strande et al., 2014). Sewage sludge is produced in the treatment stage of the sewered sanitation service chain. Most of the coarse materials are not present in the sewage sludge because they are already removed during the preliminary treatment stages of screening and grit removal. On the other hand, FS is produced in the containment technology (pit or vault) of the non-sewered sanitation service chain. Quite often, FS contains many other non-fecal matters as added by the user (Chipeta et al., 2017; Portiolli et al., 2021; Sisco et al., 2017) (Fig 2.4).

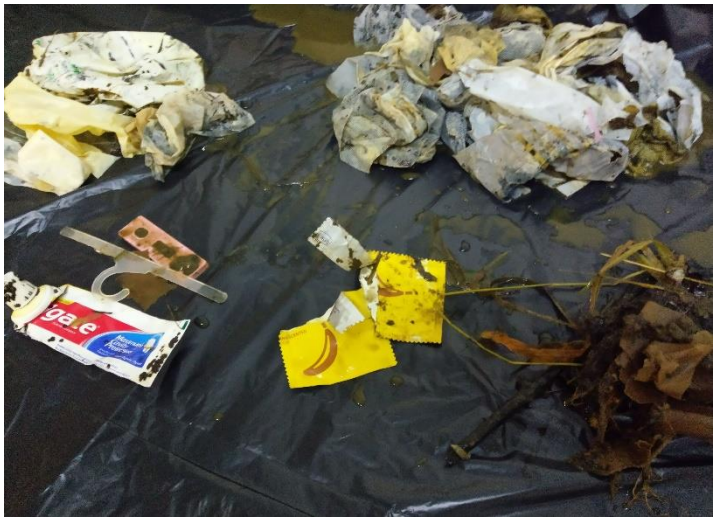


Figure 2.4 Non-faecal matter removed from a VIP latrine in eThekwini municipality, South Africa (Photo taken by the author)

Sewage sludge is described by the wastewater treatment process that led to its generation i.e., primary sludge, secondary sludge or waste activated sludge (WAS). FS is a generic name for all the sludge produced in non-sewered sanitation technologies. However, sludge produced in septic tanks is also called septage. FS is categorized by its total solid content (%TS) such as liquid (< 5 %TS), slurry (5-15 %TS), semi-solid (15-25 %TS or solid (> 25 %TS) (Velkushanova et al., 2021). Sewage sludge is the largest constituent by volume removed during wastewater treatment and presents the most complex challenge for wastewater management.

Characterization of sludge is crucial when considering the selection and design of an appropriate treatment technology. The intended use or disposal of the treated sludge should be considered too when selecting a treatment method. The disposal/intended use methods inform the sludge characteristics that must be evaluated to provide data for designing a treatment method. For example, nutrient content will be of importance if the sludge is to be used in agriculture. The

calorific value becomes crucial where the sludge is to be used for energy production (Afolabi et al., 2017a).

Treatment of FS should not only render it safe for handling or disposal but also recover useful resources from it. This realization is crucial because FS treatment should be viewed as an end-to-end process, functioning as a closed system rather than an open system in which the treated product is disposed of in the environment without resource recovery. This approach enables the identification of synergies between FS treatment with other sectors such as municipal waste management, wastewater management, sewage sludge management, food (agriculture), energy and water (Desmidt et al., 2015; Mdolo & Jóhannsson, 2016).

2.6 Faecal sludge treatment technologies

Treatment technologies for FS are in their infancy. Most of them are restricted to observation either in the laboratory or pilot processes to optimize the design and operating parameters. However, it is clear that finding a suitable treatment technology for FS, especially that from pit latrines, is still a challenge. Whether it is sewage sludge or FS, regardless of the treatment objective, the general flow of sludge treatment process is as in Fig 2.5 (Metcalf & Eddy, 2014)

Sludge treatment involves several mechanisms such as cell lysis, endogenous metabolism, microbial predation, and hydrothermal oxidation. The techniques in Fig 2.5 are employed to achieve the desired sludge treatment objective (Metcalf & Eddy, 2014; Tayler, 2018).

Deciding on a treatment technology is challenging because FS characteristics are varied (Strande et al., 2018). For example, an unlined pit latrine leads to the exchange of water, which could affect the concentration of pollutants in the pit latrine (Gold et al., 2018). Public toilet sludge is relatively fresh and contains higher amounts of biodegradable matter (Septien et al., 2018a).

The absence of standardized pit size, pit volume and user habits all affect the characteristics of pit latrine sludge. Therefore, the design of faecal sludge treatment processes must be based on its characteristics and treatment objectives. Based on its characteristics, pit latrine sludge may be restricted to certain treatment options. For instance, sludge from dry pit latrines (most common in sub-Saharan Africa) may not be suitable for treatment options that depend on sludge flow. In such cases, processes that operate in batch modes are preferred, or some mechanical processes to aid flow (Septien et al., 2018a).

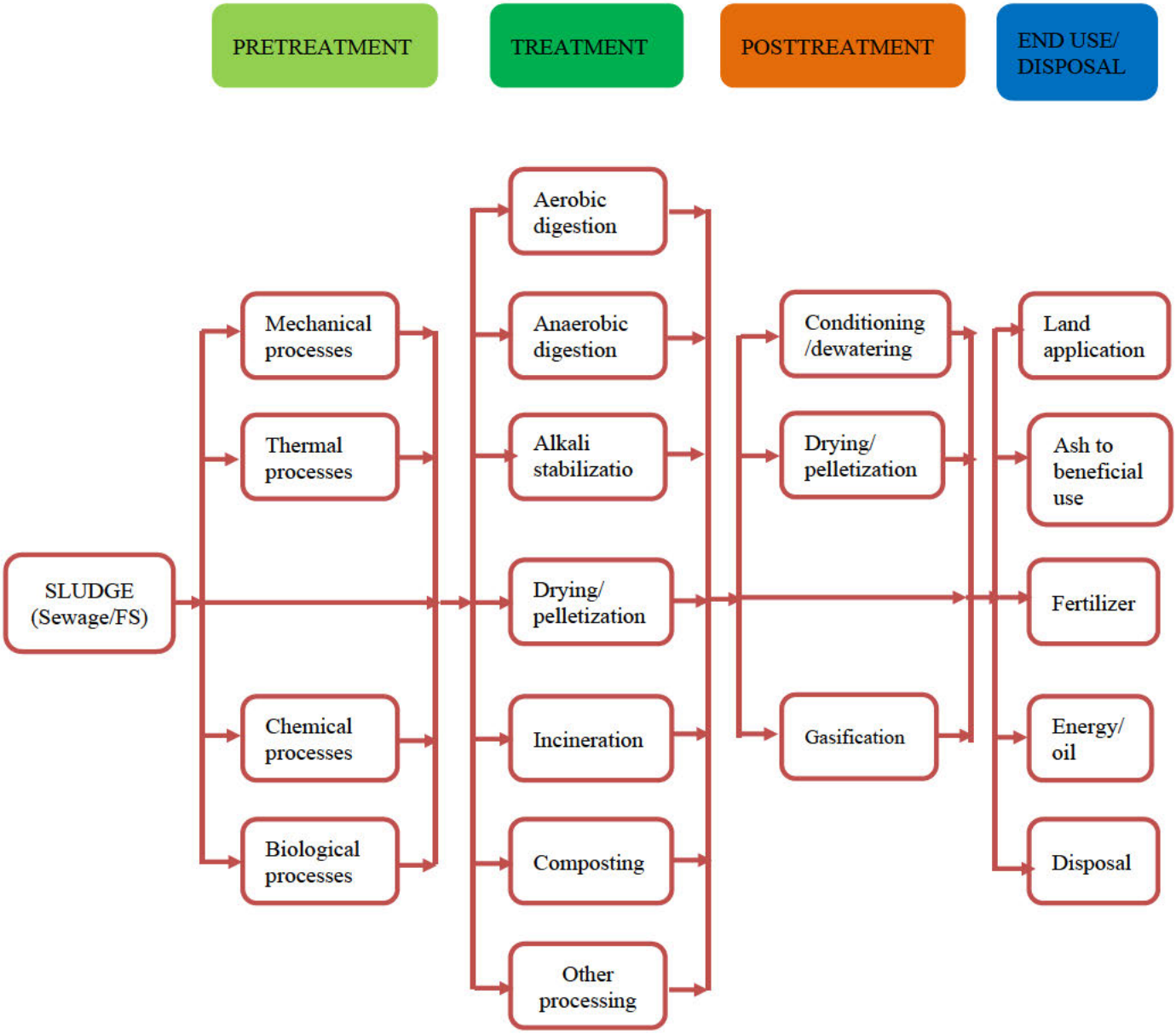


Figure 2.5 General sludge processing flow diagram (Modified from Metcalf & Eddy 2014)

Previous studies on full-scale treatment plants treating pit latrine FS are scarce, there are some laboratory experiments, laboratory-scale and pilot technologies which have shown promising results (Table 2.2).

Table 2.2 Summary of pit latrine sludge treatment technologies

Technology	Key conclusions	Ref
Anaerobic digestion	About 99.9% pathogen elimination in a laboratory-scale study (BMP). Over 90% COD reduction. The cumulative CH_4 production was 99mL/g.VS added. Full-scale AD system did not achieve total pathogen elimination.	(Lansing et al., 2016)
Dewatering	Dewaterability extent was 32% and 19% for the FS from unlined and lined pit latrines, respectively. The dewatering rate was 1122seconds and 2485seconds for the for FS from lined and unlined pit latrines, respectively. Dewatered FS offers opportunities for several downstream treatments. Pit latrine FS does not require thickening before dewatering.	(Semiyaga et al., 2016)
	Sawdust and charcoal dust conditioned FS had better dewaterability rate of 25% and 46%, respectively. The solid fuel characteristics measured by the calorific value of the conditioned FS were improved by 42-49%. The conditioned FS offers several opportunities for further treatment.	(Semiyaga et al., 2018)
	Centrifuge speed was observed to be a key design parameter for centrifugation of pit latrine FS. Centrifuging samples over 20 minutes caused resuspension of solids.	(Semiyaga et al., 2017)
Drying beds	The COD and BOD in the effluent was 1300mg/L and 300mg/L, respectively. While the NO_3^- and NH_3 was 300-800mg/L and 300mg/L, respectively. Both the organic and nutrient loading in the effluent of all drying beds were higher than the WHO guidelines. Drying beds attained the destruction of helminth eggs.	(Manga et al., 2016)
Constructed wetlands	VFCWs achieved a pathogen reduction of over 80 %. The remaining organic and nutrient load was still higher than the WHO guidelines.	(Ngandjui Tchangoue et al., 2019)
Chemical treatment	Lime treatment showed the highest potential to reduce E coli to below detection limit within a short period of exposure (1 hour) at pH>11.	(Anderson et al., 2015)
	Lime stabilization reduced pathogenic bacteria to below detection limits within 1 hour at pH 12. Lime stabilization increased COD of treated sludge.	(Greya et al., 2016)

Thermal treatment	Dried FS from drying beds had a calorific value of 11-13MJ/kg.TS. Kilns reached temperatures of 437℃ and 800℃ which were sufficient for waste oil regeneration and curing clay bricks, respectively. However, the resulting fuel characteristics need further improvements.	(Gold et al., 2017)
Pyrolysis	Biochar yield from pyrolysis of FS was high (84%) compared to sewage sludge (74%). Increasing pyrolysis temperature resulted in low biochar yield.	(Koetlisi & Muchaonyerwa, 2017)
	Char yield decreased with time. The optimal holding time was observed to be 10 minutes. Pyrolysis temperature could be optimized for the intended resource recovery option, i.e., solid fuel (low pyrolysis temperatures); soil enhancement (pyrolysis temperature is not a determining parameter); carbon sequestration (high pyrolysis temperatures, 400-600 ℃)	(Gold et al., 2018)
MIR heating	Dried pellets contained high nutrients (85g/kg.TS P, 10g/kg.TS K and 35g/kg.TS N) and rich in carbon (380g/kg.TS). The calorific value of pellets was similar to wood. Pasteurization can be achieved at 80 ℃ and ≥4 minutes.	(Septien et al., 2018b)

AD = Anaerobic digestion; BMP = Biochemical methane potential; COD = Chemical oxygen demand; CST = Capillary suction time; HRT = Hydraulic retention time; LaDePa = Latrine dehydration pasteurization; MIR = Mid infrared; rpm = Revolution per minute; VFCW = Vertical flow constructed wetland; VIP = Ventilated improve pit; WHO = World Health Organization

Despite their success in achieving high organic and nutrient removal efficiencies, the remaining load in the effluent from drying beds and wetlands required further polishing before it could be discharged into the environment (Manga et al., 2016; Ngandjui Tchangoue et al., 2019). Also, drying beds and constructed wetlands have a high land footprint which makes them unsuitable for informal settlements.

Dewatering is an important treatment stage in sludge management because it reduces sludge volume by concentrating the solids. Laboratory-scale studies showed improved dewatering extent and rate after conditioning the FS from unlined and lined pit latrines with sawdust and charcoal dust. Also, conditioning improved the fuel characteristics and digestibility of the FS (Semiyaga et al., 2018, 2017, 2016). However, after dewatering, the solid and liquid streams may still require further treatment.

In a laboratory-scale experiment, chemical treatment of FS using lactic acid fermentation, urea and lime achieved pathogen destruction to below detection levels at reaction times ranging from 2 to 168 hours. Pilot-scale results using lime only agreed with the observations in laboratory-scale studies (Anderson et al., 2015; Greya et al., 2016). However, high COD was observed in the treated sludge. Hence the treated sludge required further stabilization before disposal into the environment.

The treated FS in thermal and MIR drying studies was rich in nutrients (N, P, K & C) and had fuel potential comparable to solid industrial fuels (Gold et al., 2018; Gold et al., 2017; Koetlisi & Muchaonyerwa, 2017; Septien et al., 2018b).

Most FS treatment technologies reported at pilot- or full-scale were adapted from those applicable for sewage sludge or wastewater. These technologies are suitable for dilute sludge (TS < 5%) and can easily flow. FS from dry toilets like the VIP latrines is highly concentrated (TS>15%) (Zuma et al., 2015). Hence, may require alternative treatment methods that do not rely on sludge flow. Also, pit latrine FS is already highly concentrated, thus may not require further dewatering in its untreated form. Removal of water from this type of sludge would consume large amounts of energy.

2.7 Anaerobic digestion

Despite several advantages associated with AD, there is little information on its application to treat pit latrine sludge. The few studies available reported the high potential for methane production from pit latrine sludge (Changara et al., 2019; Forbis-Stokes et al., 2016; Lansing et al., 2016). One study reported higher methane production from pit latrine sludge compared to septage (Lansing et al., 2016). AD is a multi-stage biochemical process in which long-chain polymeric molecules (e.g., carbohydrates, proteins, and lipids) are converted to short-chain

monomers by a diverse microbial community. Among others, AD offers benefits such as energy recovery***;*** fertilizer recovery; reduced greenhouse gas emission; stabilization of organic matter; reduced amount of waste to landfill; reduce future environmental impacts and removing odor (Afolabi et al., 2015; Beevi et al., 2015).

2.8 Microbiology of anaerobic digestion

During AD, organic matter undergoes cascades of biochemical degradation involving hydrolysis, acidogenesis, acetogenesis and methanogenesis to form carbon dioxide and methane (Metcalf and Eddy, 2014). Enzymes hydrolyze complex polymeric organic compounds (carbohydrates, proteins, and lipids) to soluble monomers (e.g., fatty acids, alcohols, carbon dioxide and ammonia) (Fig 2.6).

The first step in AD is hydrolysis in which complex organic matter is converted to soluble compounds which can be utilized by the microorganisms. Hydrolysis is carried out by extracellular enzymes (exoenzymes) produced by facultative and obligate anaerobes. Exoenzymes are secreted by the cell and functions outside the cell. During hydrolysis, fermentative bacteria convert carbohydrates to soluble sugars, lipids to long chain fatty acids and proteins to amino acids. The acidogenesis step (acid formation) converts the products of hydrolysis to acetic acid, long chain volatile fatty acids (VFAs), alcohols, carbon dioxide and hydrogen. A diverse group of acidogens are involved in this stage including Clostridium, Peptococcus anaerobius, Lactobacillus and Actimomyces. In acetogenesis, propionate and butyrate are converted to acetate, CO_2 and H_2 (Metcalf & Eddy, 2014).

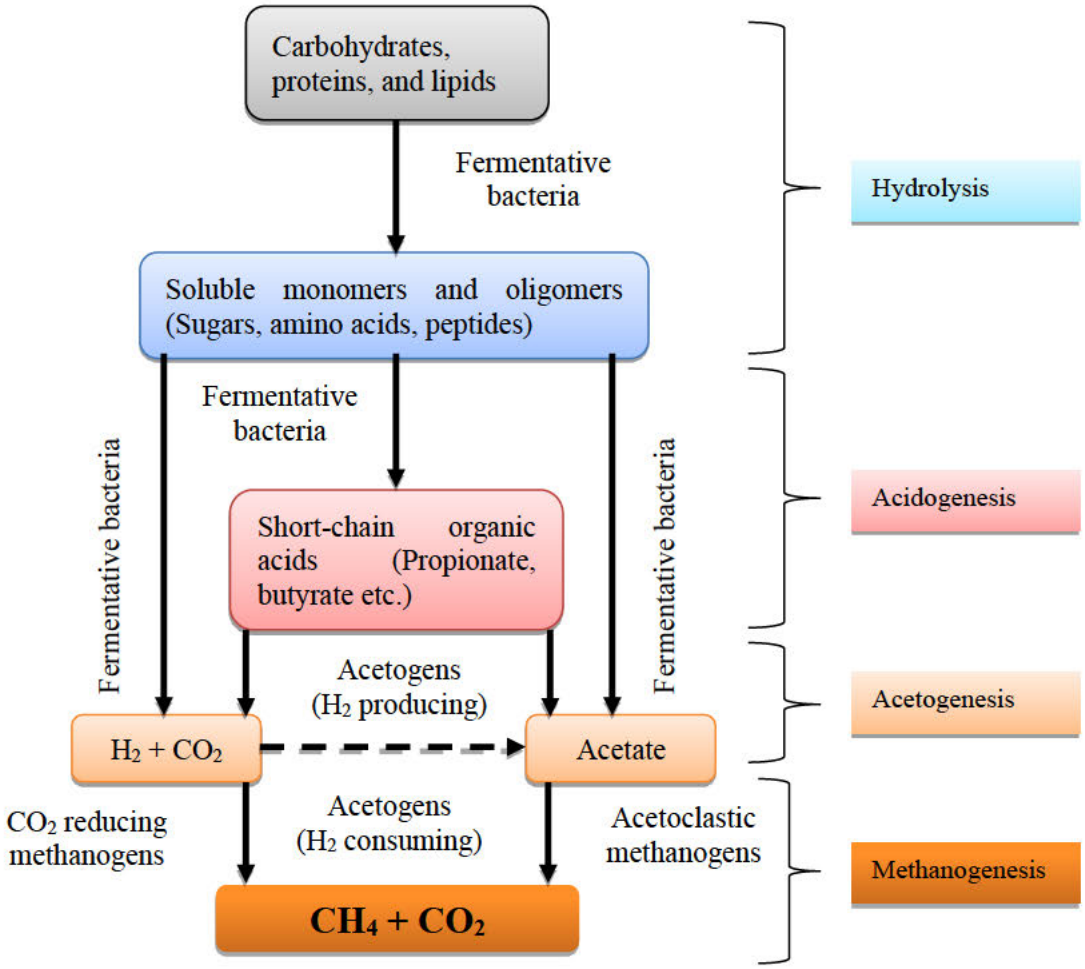


Figure 2.6 Biochemical processes in anaerobic digestion (Karuppiah & Azariah, 2019)

The acetate, CO_2 and H_2 are precursors for CH_4 formation. Most of the H_2 formed comes from long chain fatty acids, while intermediate VFAs form the acetic acid (anaerobic oxidation). Acetogens oxidize long chain fatty acids and alcohols to acetic acid and hydrogen.

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2 \quad (2.1)$$

$$C_2H_5OH + H_2O \rightarrow CH_3COO^- + H^+ + 2H_2 \quad (2.2)$$

Intermediates of anaerobic fermentation are converted to methanogenic substances. For example, propionate and butyrate are converted to acetate and hydrogen:

$$C_2H_5COO^- + 3H_2O \rightarrow CH_3COO^- + HCO_3^- + H^+ + 3H_2 \quad (2.3)$$

$$C_3H_7COO^- + 2H_2O \rightarrow CH_3COO^- + H^+ + 2H_2 \quad (2.4)$$

Methanogenic archaea complete the process by producing methane from CO_2 and H_2; and converting acetate to methane and bicarbonates in three main pathways, e.g., aceticlastic methanogenesis (splitting of acetate); hydrogenotrophic methanogenesis (which utilizes H_2 as an electron donor and CO_2 as electron acceptor to produce CH_4) and methylotrophic methanogenesis.

$$\mathbf{CH_3COO^- + H^+ \rightarrow CH_4 + CO_2} \quad \textbf{: Aceticlastic methanogenesis} \quad \textbf{(2.5)}$$

$$\mathbf{4H_2 + CO_2 \rightarrow CH_4 + 2H_2O} \quad \textbf{: Hydrogenotrophic methanogenesis} \quad \textbf{(2.6)}$$

$$\mathbf{4CH_3OH \rightarrow 3CH_4 + CO_2 + 2H_2O} \quad \textbf{: Methylotrophic methanogenesis} \quad \textbf{(2.7)}$$

Acetate is the most important source of methane in anaerobic process, accounting for almost 70% of its production (Metcalf and Eddy, 2014). A stable AD process produces about 65% CH_4 and 35% CO_2.

The three groups of microorganisms involved in AD are mutually dependent and coexist in anaerobic environments. The syntrophic relationship between hydrogen producers and hydrogen scavengers is crucial in the process of methane production. For example, methanogenic archaea convert the products of fermentation to methane and carbon dioxide, in the process maintaining a low pressure of hydrogen. Methanogenic archaea serve as a hydrogen sink, failing which volatile fatty acids will accumulate (Beevi et al., 2015; Yang et al., 2019) and lower the pH in the digester.

Sulfate reducing bacteria are problematic if the organic material contains high concentration of sulfate. Sulfate reducing bacteria reduce sulfate to sulfide which is toxic to methanogens at high concentration. Iron can be added to precipitate the sulfide to iron sulfide (Metcalf & Eddy, 2014).

A chemical oxygen demand (COD) balance is used to account for COD changes in AD. In AD, COD loss is accounted for by CH_4 production. The COD of CH_4 is determined by stoichiometry and is defined as the amount of O_2 required to oxidize CH_4 to CO_2 and H_2O (Metcalf & Eddy, 2014).

AD is carried out under mesophilic (20-40 ℃) or thermophilic conditions (50-65 ℃). Thermophilic AD has the advantages of being more efficient; allows higher loading rates; higher conversion efficiencies and pathogen destruction. On the other hand, mesophilic AD is more stable; reduces the risk of NH_3-N toxicity and requires less process heat (Metcalf & Eddy, 2014).

Energy recovery (through biogas production) during AD makes it an attractive waste treatment technology. Consequently, it has been investigated using different organic feed materials (Chatterjee & Surampalli, 2017; Kumar et al., 2020; Mirmohamadsadeghi et al., 2019; Montañés & Solera, 2015). The recovered energy would be a viable alternative to non-renewable fuels. Energy recovery from conventional AD of sludge is limited by several factors, and strategies to improve the performance of the process have been recommended by previous literature in the area (Panigrahi & Dubey, 2019). Challenges during AD of sludge can arise from its nature while others are operational. For example, sludge is composed of a complex matrix of flocs and extracellular polymeric substances (EPS) whose hydrolysis and biodegradability are slow while poor operating conditions (e.g., low pH because of build-up of organic acids) result in poor methanogenesis. Sludge pretreatment or co-digestion with other organic substrates has been reported to address the challenges faced in conventional AD (Panigrahi & Dubey, 2019, Zhen et al., 2017). Co-digestion involves the mixing more substrates and has shown to improve the performance of the digestion process, result in high methane yield, decrease sludge water content, increase the calorific value (42%) and improves the C:N ratio (Ciggin, 2016; Semiyaga et al., 2018).

Complex substrates are not readily degradable by microorganisms making the hydrolysis step slow. Thus, making the whole AD process slow and requiring large digester volumes. Also, the slow hydrolysis affects the process reaction kinetics such as methane production and organic loading rates (Serrano et al., 2016). A sludge pre-treatment step disintegrates the sludge floc and disrupts cell walls which release the soluble organics and making them easy to degrade by microorganisms. The following section summarizes the major pretreatment technologies and their effect on sludge characteristics and subsequent AD.

2.9 Sludge pre-treatment technologies

Hydrolysis is the primary limiting step in AD of complex substrates (Serano et al., 2016) and affects energy recovery from it by prolonging the digestion process. Pretreatment is a significant step to speed up hydrolysis and improve biogas production and quality (Bozkurt & Apul, 2020; Zhen et al., 2017). The main objective of pretreatment is to convert soluble organics in the solid phase to a soluble phase, usually measured by an increase in soluble COD (sCOD) and other macromolecules. In addition to organic matter solubilization (measured by sCOD, sCOD/TCOD, soluble proteins (sProt), soluble carbohydrates (sCarbs)) and sludge stabilization (measured by TS/VS ratio), thermal and MW pretreatment also inactivates pathogens, and reduces sludge volume by raising the temperature of the sludge and evaporating moisture (Eskicioglu et al., 2007; Mawioo et al., 2016a; Serrano et al., 2016). By reducing particle size, sludge pretreatment increases the surface area of the substrate and enhances contact with microorganisms. In AD, the performance of the pretreatment technology is evaluated based on biogas yield; process rate; solubility; biodegradability and pathogen reduction (Atelge et al.,

2020). Pretreatment technologies include thermal hydrolysis (TH), chemical treatment (acid or alkaline treatment), ultrasonication treatment, MW treatment or a combination of these methods (Atelge et al., 2020; Nguyen et al., 2021; Zhen et al., 2017). The following sections will discuss these methods in detail. Details of the type of sludge and pretreatment conditions are provided in Table A.1 (In Appendix A).

2.9.1 Thermal hydrolysis

TH is a well-established sludge pretreatment technology which was initially intended to improve the dewaterability of sludge. Conventional heat sources supply the energy to a reactor containing the sludge, which then transfers the energy to the material treated. The transfer of heat from the heat source to the sludge results in temperature and pressure rise which cause the substrate to swell. Swelling breaks hydrogen bonds that keep the sludge matrix together and release the soluble organics. Temperature, pressure, and treatment time are critical operating parameters in TH (Zhen et al., 2017).

TH is categorized based on the operating temperature as either a low temperature TH process (LTHP, ≤100°C) or high temperature TH process (HTHP, >100°C) (Xue et al., 2015). Both LTHP and HTHP have been studied and showed varying results on sludge properties as discussed below.

Xue et al., (2015) compared the effect of LTHP (60-90°C, 1-17h) and HTHP (120-180°C, 15-180 min) on WAS solubilization and noted that sCOD increased with treatment temperature in both process (Fig 2.7a). However, the rate of sCOD increase was initially rapid and then slowed down.

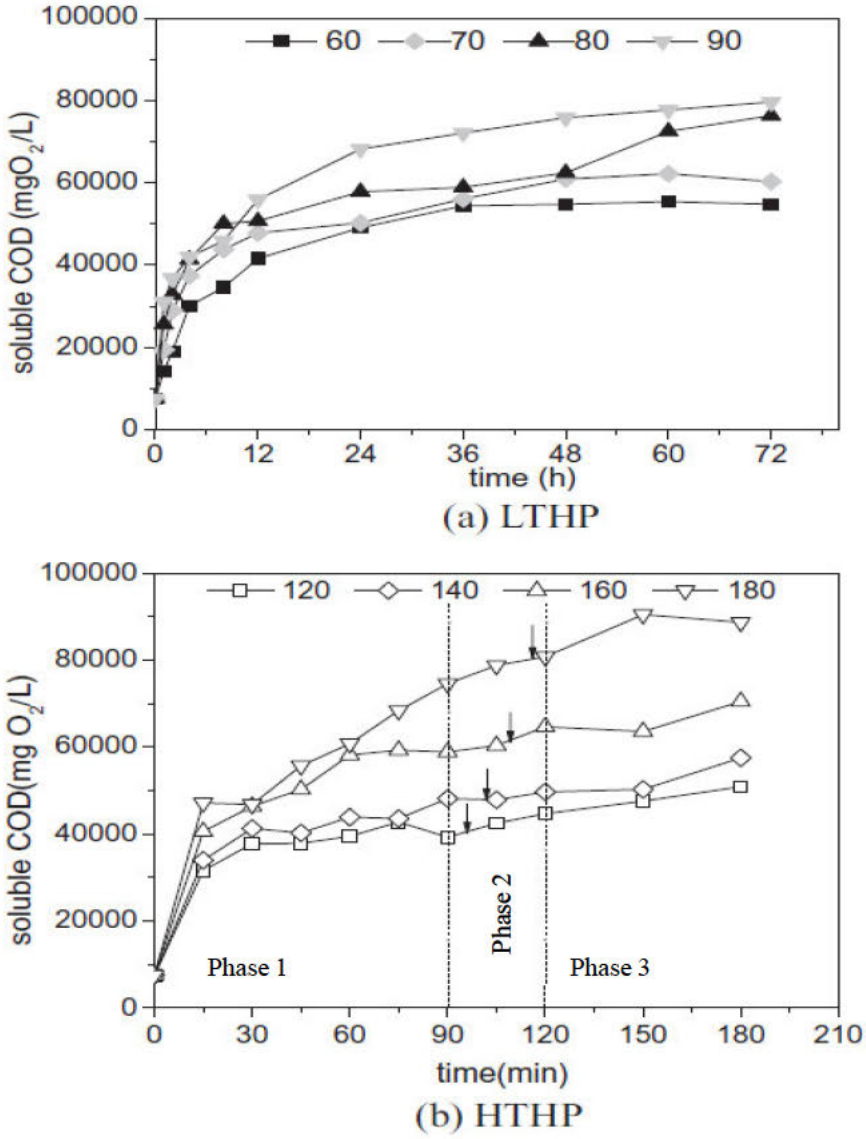


Figure 2.7 The sCOD release profile during thermal hydrolysis (Xue et al., 2015)

A rapid increase in sCOD occurred in the first 24 hours (LTHP) and contributed over 80% of the sCOD (Xue et al., 2015). On the other hand, the sCOD release in HTHP showed three phases (Fig 2.7b), e.g., a rapid sCOD release (phase 1) in the first 90 minutes, a slow sCOD release phase (phase 2) and a second gradual sCOD release (phase 3) (Fig 2.7b). The maximum sCOD/TCOD (53%) was obtained when the sludge was treatment at 180°C during the first phase of sCOD release (Xue et al., 2015).

Pig slurry was treated in a LTHP at 60°C and 80°C for 3 hours to evaluate its influence on solubilization and methane yield. The treatment carried out at 80°C showed superior results, increasing sCOD, soluble total Kjeldahl nitrogen (STKN) and NH_4^+ by 58%, 25% and 12%, respectively while the total volatile fatty acids (TVFA) increment (13% at 80°C and 12% at 60°C) was not significantly different (Bonmatí et al., 2001). The specific methane yield at 80°C was 60% over controls (Bonmatí et al., 2001). Different sludge concentrations (6%TS, 8%TS, 9%TS,

and 12%TS) treated at 90℃ for 30 minutes showed an increase in specific methane and production rate with increase in %TS. However, TS values higher than 8% yielded lower methane, hence 8%TS was concluded to be the optimal and yielded a methane production rate of 0.0074mL/g.VSS.h (Yao et al., 2016).

In another work, LTHP was used to solubilize thickened WAS (TWAS) over a range of temperatures (50℃, 60℃ and 90℃) and time intervals (30, 60 and 90 minutes). Organic matter solubilization and methane yield increased with temperature, reaching a maximum of 20% and 56%, respectively (Azizi et al., 2021). Both organic matter solubilization and methane yield were more sensitive to changes in temperature than treatment time (Azizi et al., 2021).

Using a similar approach, Li & Chen (2022) evaluated the effect of sewage sludge concentration on LTHP. The dewatered sludge (DS) was produced by screw filter centrifugation. In their work, sludge with different concentrations (2%TS, 4%TS, 6%TS, 8%TS, 10%TS and 12%TS) was treated at 90℃ for 60 minutes. The solubilization profile showed an increase (sCOD release) and decrease (sCOD degradation) as TS content increased. The optimum operating condition was 6%TS where the solubilization and methane production was 23% and 115%, respectively (Li & Chen, 2022).

HTHP of WAS increased the sCOD/TCOD from 7% (untreated sludge) to 44% and 60% when the sludge was treated at 135℃ and 190℃ for 35 minutes and 50 minutes, respectively (Bougrier et al., 2007). Keeping the untreated and treated sludge in a cold room at 4℃ for over 60 days deteriorated it by increasing the sCOD/TCOD in the untreated sample to 32% and decreasing it to 34% and 46% for the treated samples at 135℃ and 190℃, respectively. The untreated WAS underwent stabilization during storage while treated sludge reflocculated, which could have underestimated the methane yield (24% at 190℃) (Bougrier et al., 2007).

Kim et al., (2015) reported an increase in organic matter solubilization of WAS with increase in treatment temperature. However, the highest methane production did not correspond to the highest sCOD/TCOD. For example, the methane production at 180℃ and 210℃ was similar (~343mL/mg.VS) although the sCOD/TCOD were widely different (31% at 180℃ and 42% at 210℃). The authors argued that their temperature range and TH enhanced the solubilization and biodegradability of WAS. Also, the treatments altered the chemical composition of the organic solids, which led to an increase in their carbon content through the hydration reaction occurring during the pretreatment phase (Kim et al., 2015).

Working with a wider temperature (130-180℃) and time (5-50 minutes) range, it was reported that the highest sCOD/TCOD of WAS sample was 41% at 180℃ and treatment time of 30 minutes (Sapkaite et al., 2017). However, the highest methane production (72% over control) was recorded

when the WAS sample was treated at 150℃ for 30 minutes (solubilization was 37%) indicating that high solubilization does not always result in high methane yield (Sapkaite et al., 2017).

Lu et al., (2018) treated WAS at 70℃, 121℃ and 172℃ for 30 minutes and noted that the dissolved organic carbon (DOC) and methane production increased with increasing temperature. Specifically, the DOC and methane production at 172℃ was 267mg/g.VS and 212mL/g.TCOD, respectively (Lu et al., 2018). No difference in methane yield was recorded at 70℃ and 121℃ (179mL/g.TCOD).

Choi et al., (2018) also worked with a wide temperature (75℃-225℃) and time (15-105 minutes) range to treat sewage sludge and recorded a highest solubilization (30%) at 180℃ and treatment time of 76 minutes. Again, the maximum solubilization did not correspond to maximum methane yield. The maximum methane yield (273mL/g.COD) was recorded at treatment temperature of 150℃ for 60 minutes (Choi et al., 2018).

Regardless of the thermal hydrolysis process employed (LTHP or HTHP), pretreatment resulted in improved sludge properties such as reduced volume, odor control, pathogen inactivation, increased solubilization, improved biodegradability and improved dewaterability (Atelge et al., 2020; Zhen et al., 2017). The solubilization of organic matter and subsequent increase in methane production result from thermal processes rather than enzyme activity (Bonmatí et al., 2001). It can be further noted from the previous studies that solubilization increased with the treatment temperature and time and that it was more sensitive to temperature than treatment time during TH. The reported studies consistently demonstrated that higher organic matter solubilization did not always result in the highest methane yield. Thus, organic matter solubilization during pretreatment should be used with caution if the main aim of pretreatment is to improve methane production. The major drawback with TH is that it uses energy to get the treated material to temperature and the time taken to heat it.

2.9.2 Chemical treatment

Chemical treatment involves using reagents such acids, alkalis, and oxidants to disrupt cell walls and release intracellular organics. The reagent's affinity for certain compounds makes them suitable for some substrates but not others. Thus, knowledge of the chemistry of the substrate is critical.

Substrates with a high concentration of lignocellulose are best treated by acid treatment during which the hydrolysis of hemicellulose releases soluble sugars but does not affect the hydrolysis of lignin. However, lignin is hydrolyzed by alkaline treatment during solvation and saponification reactions. Also, alkaline treatment can solubilize xylan hemicellulose through saponification of intermolecular ester bonds. Acid and alkaline treatment destroy and liquefy cells with hydroxyl radicals and thus improve sludge characteristics through increased solubilization, inactivation of

pathogens, increased biogas production, improved dewaterability and elevating hydrogen producing bacteria (Zhen et al., 2017).

Chemical dosage, pH and treatment time are the critical operating parameters in chemical treatment as highlighted below.

Tulun & Bilgin, (2019) compared acid treatment (pH-2) and alkaline treatment (pH-10) of WAS and concluded that alkaline treatment was superior to acid treatment in terms of organic matter solubilization and methane yield. Specifically, the sCOD/TCOD and methane yield at pH-10 increased by 34% and 44%, respectively while it was 13% and 12% at pH-2 (Tulun & Bilgin, 2019). Treatment time and the temperature at which the process is operated are critical in acid and alkaline treatment. In chemical treatment, organic matter solubilization is more sensitive to treatment time, with shorter retention times resulting in higher organic matter solubilization (Tulun & Bilgin, 2019).

Working at pH-5 (acid) and pH-10 (alkaline) to treat WAS increased the sCOD/TCOD from 1.7% (untreated sludge) to 8.2% and 23.3% at pH-5 and pH-10, respectively (Chen et al., 2021). The actual sCOD in the untreated sludge was 374mg/L which increased to 1948mg/L (421% increase) and 5563mg/L (1387%) at pH-5 and pH-10, respectively (Chen et al., 2021).

Chemical treatment of WAS at pH-9.5 resulted in a sCOD/TCOD increase of 20% (initial sCOD/TCOD was 13%) with a subsequent increase in methane yield of 65% (He et al., 2021). The concentration of soluble proteins also increased after chemical treatment at pH-9.5 (He et al., 2021).

Alkaline (pH-12) treatment of sequencing batch reactor sludge showed superior results to acid (pH-2) treatment, resulting in 1422% in DOC compared to 33% increase at pH-2 (de Sousa et al., 2021). The concentrations of total Kjeldahl nitrogen (TKN), proteins and carbohydrates were also higher at pH-12 than at pH-2 (Table A.1 in appendix) (de Sousa et al., 2021).

Chemical treatment is an interesting sludge pretreatment method because of its low cost, requires simple device, low energy requirement, ease of operation, can be operated at ambient temperatures and alkaline treatment improves the alkalinity that enhances the buffer capacity of the anaerobic digestion process (Zhen et al., 2017). Despite the advantages, chemical treatments increase the pH of the substrate, leads to build up of salts, destroys lignin instead of separation and corrodes the instrument. Overall, it was noted from literature that alkaline pretreatment leads to more organic matter solublization and subsequent methane yield. However, the high residual alkalinity needs to be neutralized to make the downstream AD more efficient.

2.9.3 Ultrasonication

Ultrasound frequencies range between 20kHz and 10MHz (Zhen et al., 2017). The ultrasound waves cause the periodic compression and rarefaction when propagating through a media and generates liquid-free microbubbles which collapse violently within microseconds after reaching critical size. Thus, causing cavitation which leads to extreme conditions of temperature (over 4700°C) and pressure (over 500 bars) (Chu et al., 2002). The cavitation process initiates hydro-mechanical shear forces and formation of highly reactive hydrogen and hydroxyl radicals (H• and •OH). The hydro-mechanical shear forces and the oxidizing effect of the reactive radicals disintegrates sludge flocs and releases intracellular biopolymers. Although both hydro-mechanical shear forces and oxidation disintegrates sludge flocs, much of it is a result of the former. Cavitation is influenced by the ultrasound frequency, treatment temperature and power density. Previous studies on ultrasonication of sludge are highlighted in the following paragraphs.

Apul & Sanin, (2010) monitored the evolution of sCOD during sonication of WAS at 0.51W/mL for 0-30 minutes. The evolution of the sCOD showed 3 profiles (Fig 2.9) which were related to different processes occurring during sonication.

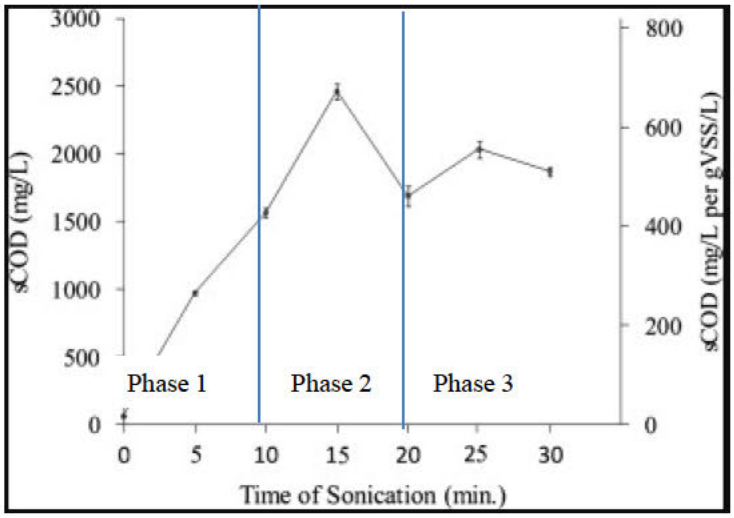


Figure 2.8 The evolution of sCOD during sonication of WAS (from Apul et al., 2010)

The first phase (phase 1) was characterized by a rapid increase in sCOD up to 15 minutes of sonication (Fig 2.9). The sCOD was 4900% of the control after the first 15 minutes (Apul & Sanin, 2010). The second and third phase showed a fluctuating and slightly decreasing concentration of sCOD after 15 minutes (Fig 2.9). The decrease in sCOD after 15 minutes could be a result of complex reaction or the thermal effect of sonication and the entrapment of solubilized organics (Apul & Sanin, 2010).

Şahinkaya & Sevimli, (2013) reported the effect of power density (0.5-1.5W/mL) and treatment time (0.5-10 minutes) on the disintegration degree ($\%DD_{COD}$) and methane production of WAS. A further combined sonication + TH (60, 80 and 100°C, 60 minutes) was evaluated. The results indicated that the degree of disintegration of COD ($\%DD_{COD}$) increased with power density at each sonication time used (Şahinkaya & Sevimli, 2013). The combined sonication + TH had a synergistic effect on the degree of sludge disintegration and the profile was shown to exhibit two profiles i.e., a rapid disintegration step (phase 1) due to the hydro-mechanical shear forces during sonication and a slow disintegration step (phase 2) due to TH (Şahinkaya & Sevimli, 2013). With this analysis, the authors reported optimal sonication conditions of 1.0W/mL, 1 minute and TH of 80°C for 1h (Şahinkaya & Sevimli, 2013). At these operating conditions, the methane production increased by 14%, dewaterability of the digestate improved by 70% and achieved a volatile solid (VS) and COD removal of 38% and 48%, respectively (Şahinkaya & Sevimli, 2013).

In the study of Aylin et al., (2015), ultrasonic treatment of sewage sludge not only improved organic matter solubilization, but also inactivated pathogenic bacteria. Treating a combined wastewater sludge and olive pomace at 15 and 30 minutes resulted in a $\%DD_{COD}$ of 55% and 94%, respectively. The subsequent methane yield increased by 63% at both treatment conditions (Aylin et al., 2015).

Yuan et al., (2019) treated excess sludge (ES) and DS using ultrasound for 30 minutes followed by mesophilic AD of the treated sludge. Ultrasound treatment alone increased the sCOD/TCOD ratio of ES from 5.2% (untreated sludge) to 7.1% (Yuan et al., 2019). The combined CaO + Ultrasound treatment further improved the sCOD/TCOD ratio to 14% with a subsequent methane increase of 41% over the control (Yuan et al., 2019).

In the work of Çelebi et al., (2020), it was reported that ultrasonication of WAS at a power density of 0.73W/mL with different sonication times (0-25 minutes) and specific energies (SE) (19.1-95.5kJ/kg.TS) increased the sCOD. However, the sCOD release profile was distinguished by three regions (Fig 2.8).

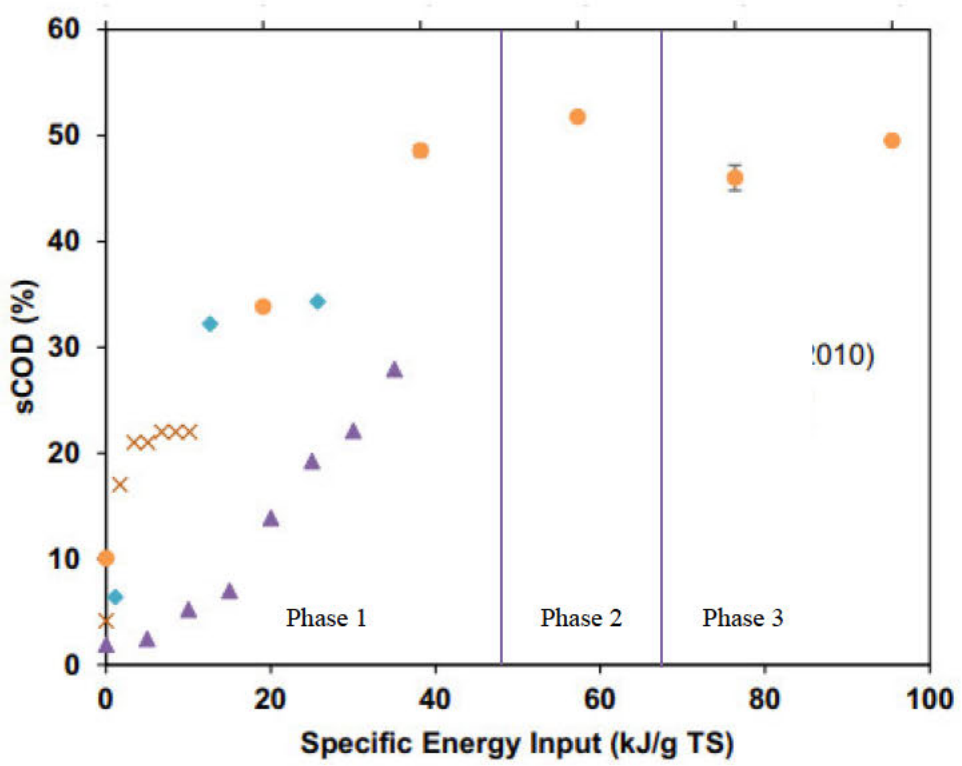


Figure 2.9 The sCOD release profile during ultrasonic treatment (From Celebi et al., 2020)

The first region showed a rapid sCOD release (phase 1) when the sludge was treated for 15 minutes (SE = 57.3kJ/kg.TS) followed by a drop in sCOD (phase 2) after 15 minutes and a second slight sCOD increase (phase 3) at 25 minutes (95.5kJ/kg.TS) (Çelebi et al., 2020). Recapturing of soluble organics, cell damage, local thermal reactions and reflocculation were suspected to cause a decrease in sCOD observed in phase 2 (Çelebi et al., 2020). Although there was a second sCOD increase (phase 3), it did not lead to additional sCOD such that the sCOD after 15 minutes was lower than at 10 minutes (Çelebi et al., 2020). The subsequent anaerobic digestion of the pretreated sludge at 15 minutes resulted in 28% more methane over the control (Çelebi et al., 2020). Longer sonication times than 15 minutes did not result in additional methane. The digestate from reactors fed with pretreated sludge contained lower amounts of carbon compared to the feed sludge, indicating that it was used during methanogenesis. Overall, the optimal sonication conditions were showed to be a power density of 0.73W/mL and sonication time of 5, 10 and 15 minutes. At these operating conditions, the highest sCOD increase, methane yield, VS and COD removal were recorded (Çelebi et al., 2020).

A higher carbon utilization (77%) was reported in the anaerobic digester treating sonicated WAS at a power density of 0.73W/mL for 10 minutes compared to the untreated sludge (65%) (Çelebi et al., 2021). The high carbon utilization corresponded to a high methane yield (32%) and VS reduction (50%) over the control (Çelebi et al., 2021). The high carbon utilization was attributed to the effect of sonication which released intracellular organics and made them readily available for anaerobic microbes during anaerobic digestion.

Xiao et al., 2022 achieved a methane yield of 57% higher than the control after treating the sewage sludge at a power density of 1.5W/mL.

The reviewed literature reports an improved floc disintegration which results in high organic matter solubilization, reduced sludge particles, reduced sludge volume, stimulation of biological activity, release of enzymes, enhanced AD, improved dewaterability and settleability. Despite these benefits, ultrasonication comes with a high energy and maintenance cost.

2.9.4 Combined pretreatment methods

Combined pretreatment can provide synergistic effects, with one pretreatment method benefiting from the other, resulting in higher organic matter solution and improved methane yield compared to mono treatment.

Dogan et al., (2009) compared MW, alkaline treatment and MW + alkaline treatments of WAS. The combined treatment showed superior results to both mono treatments. For example, the sCOD/TCOD ratio in the combined treatment at MW + pH-12.5 was 37%, while it was 18% and 29% for MW alone and pH-12.5 alone, respectively (Doğan & Sanin, 2009). Also, alkaline treatment at pH-12 released more sProt and sCarbs. The treatment at pH-12 was the optimal operating condition, which resulted in 18% more methane produced during digestion over the control (Doğan & Sanin, 2009).

Yeneneh et al., (2015) mixed primary sludge (PS) and WAS at a ratio of 3:1 and treated the mixed sludge using a MW alone and MW + ultrasonic. The sCOD/TCOD ratio in MW + ultrasonic sludge was 12% compared to 8% in the sludge treated by MW alone, while the cumulative biogas production increased by 43% (Yeneneh et al., 2015). However, the digestate from the reactor fed with MW treated sludge alone showed better dewaterability compared to the MW + ultrasonic digestate because combined treatment resulted in the prevalence of smaller particles which could have trapped water, hence reducing the dewaterability (Yeneneh et al., 2015).

In another study, a combined MW + hydrogen peroxide (H_2O_2) treatment of WAS resulted in a sCOD/TCOD ratio of 47% at pH-7 and a H_2O_2 to sludge dose ratio of 1:1 on w/w basis (Wang et al., 2015). The sludge was first treated in a microwave at 600W and heated to 60°C before H_2O_2 treatment.

Another combined MW + H_2O_2 treatment study of dairy WAS reported two soluble organics release profiles after both MW and combined treatments with varying SE during the microwave treatment (Fig 2.10).

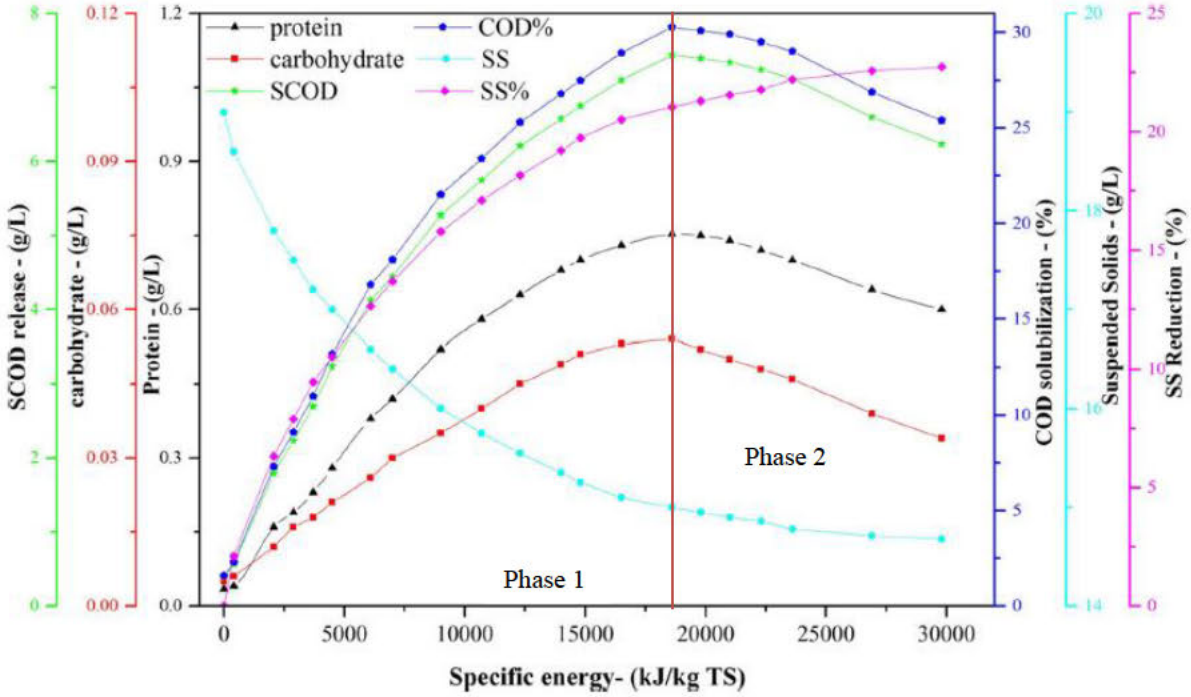


Figure 2.10 The effect of SE on sludge solubilization showing the two distinct profiles (From Eswari et al., 2016)

The observed profiles were similar to those reported above i.e., a rapid sCOD release phase (0-18600kJ/kg.TS) and a slow release (degradation) phase (18600-30000kJ/kg.TS). It was reported that in the first phase, there was a rapid increase of sCOD, sProt and sCarbs due to the disintegration of sludge flocs and cell walls while the application of high SE >18600kJ/kg.TS caused the degradation of sCOD (Eswari et al., 2016; Park et al., 2010; Toreci et al., 2009). H_2O_2 treatment alone had a similar effect on soluble organics i.e., increasing the dosage to 0.3mg/g.SS increased the soluble organics while further increasing the dosage above 0.3mg/g.SS decreased the soluble organics (Eswari et al., 2016). The reason for this observation was that high concentration of H_2O_2 resulted in the scavenging of •OH radicals and released HO_2• which were less reactive, and hence reduced the rate of reaction (Herney-Ramirez et al., 2010). It was further reported that the combined CaO + ultrasonic treatment reduced the time taken to produced 90% of the cumulative methane to 8 days, thus enhancing the rate of methane production (Yuan et al., 2019).

2.10 Microwave technology

MW irradiation is a promising technology for treating complex organic materials such as faecal sludge. The development of microwave technology is characterized by the study and application of electromagnetic wave propagation in different media with a wide range of boundary conditions; interaction of propagating waves with solids, gases, fluids and charged particles; and interaction of microwaves with matter and its conversion to other forms of energy within matter (Gude, 2017).

Early application of MW technology focused on communication using electromagnetic propagation in a vacuum and development of radio, radar, television, long-distance telephony, satellite communication links and wireless access systems. The accidental discovery of MW heating opened the door for its application in this field (Gude, 2017).

MW radiation is electromagnetic radiation in the frequency range of 300 MHz-300 GHz. Domestic and industrial MW ovens are authorized to operate at 915 MHz and 2.45 GHz (Bradshaw et al., 1998) to prevent interference with radar and telecommunication frequencies. Most domestic and industrial MW ovens operate at a frequency of 2.45 GHz (corresponding to a wavelength of 12.24 cm and an energy of 1.02×10^{-5} eV). At this frequency, most of the MW energy is absorbed by liquid water. The energy of MW is lower than the energy of Brownian motion (2.7×10^{-3}eV at 37°C). At the lowest and highest frequencies of 0.3 GHz and 300 GHz, the energies are 1.24×10^{-6} and 1.24×10^{-3} eV, respectively. Therefore, it is not strong enough to break chemical bonds and induce reactions (Gude, 2017).

2.10.1 Microwave heating mechanisms

The heating mechanism in MW treatment is complicated. Heating results from the interaction of dielectric materials with microwaves at a molecular level, without causing molecular structure alteration. The interaction of dielectric materials and microwaves result in the net polarization of dipolar molecules in the sample. The electric field of the MW exerts a force on the charged particles in a compound. Polar molecules are partially charged and restricted in their movement. If they are irradiated with microwaves, dipolar molecules try to align with the applied electromagnetic field (Fig 2.8). However, the electromagnetic field is oscillating, and the dipoles always work to realign themselves with it to follow the oscillation. At the applied MW frequency of 2.45 GHz, they have time to align with the electromagnetic field, but not to follow the oscillating field exactly (Afolabi & Sohail, 2017).

The angle δ (Fig 2.11) represents the phase lag between polarization and the applied electric field. The angle δ is an essential quantity in determining the efficiency of MW heating.

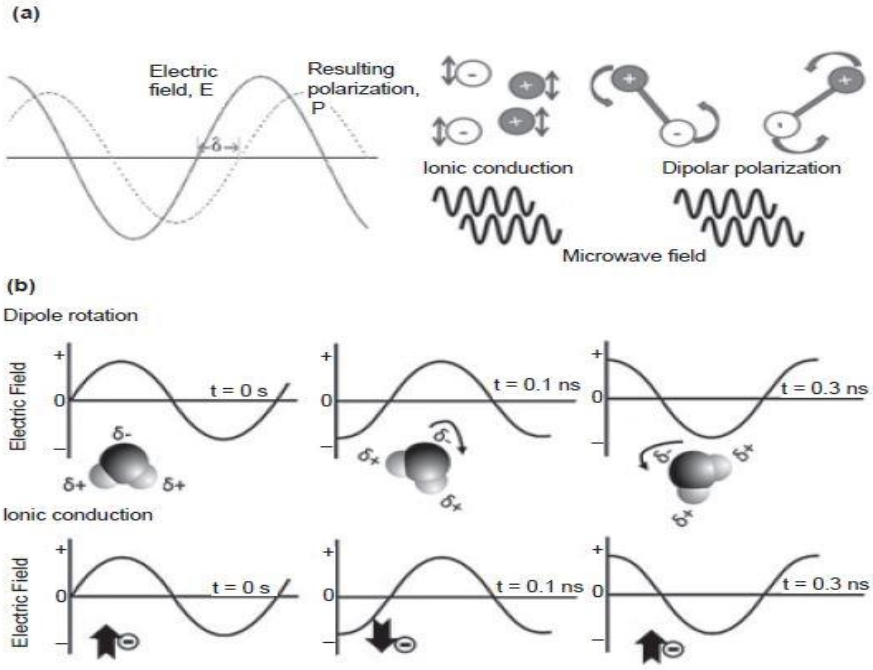


Figure 2.11 Microwave heating mechanisms (a) ionic conduction and dipolar polarization in the microwave field and (b) interaction of a dipolar molecule (water) with electromagnetic field involving dipole rotation and ionic conduction (Gude, 2017)

The continued reorientation of the dipolar molecules causes friction which results in heat generation. If the molecule is charged, the applied electromagnetic field causes the ions to move back and forth (Fig 2.11), creating collisions with each other and thus generating heat.

The orientation is in phase with the applied electric field and results in dielectric polarization. The process causes dissipation of heat within the irradiated material (sample) due to dielectric loss and molecular friction. The process occurs throughout the sample resulting in a uniform and rapid volumetric heating (Bozkurt & Apul, 2020).

MW heating relies on the ability of the sample to absorb microwaves, store the absorbed energy, and convert it to heat. The ability to absorb microwaves is determined by the loss tangent (tan δ) of the substrate. A substance with high tan δ at 2.45 GHz absorbs microwaves better and leads to efficient heating. Dipolar polarization occurs on a time scale in the order of those associated with microwaves. When a dielectric material is subjected to an external electric field, the polarization is related to intrinsic properties of the material (Gude, 2017).

Materials can be categorized as microwave absorbing; transparent or reflect microwaves. Loss tangent (tan δ) is used to predict the behavior of materials when they encounter microwaves. The MW absorption ability of a material is directly proportional to its dissipation factor (tan δ). The MP absorbed per unit volume of material depends on its interaction with the microwave field, which is expressed as dielectric loss factor (Gude, 2017).

Therefore, MW heating is mostly influenced by the dielectric properties of the heated material. However, dielectric properties are not specific to a substance. They change with MW field frequency and temperature. For example, water has different dielectric loss factors at different temperature and frequencies. Even though the frequency is fixed, tan δ is vital because temperature affects the dielectric properties of materials (Gude, 2017).

MW energy is introduced remotely without direct contact between the heat source and the sample. The energy is transferred into the material by dipolar polarization, ionic conduction, and interfacial polarization, leading to localized heating and superheating of the material. The heating results in rapid temperature increases and fast cooling of the sample when the heat source is withdrawn. When a sample is irradiated with microwaves, the temperature of the whole volume rises evenly and simultaneously (Fig 2.12).

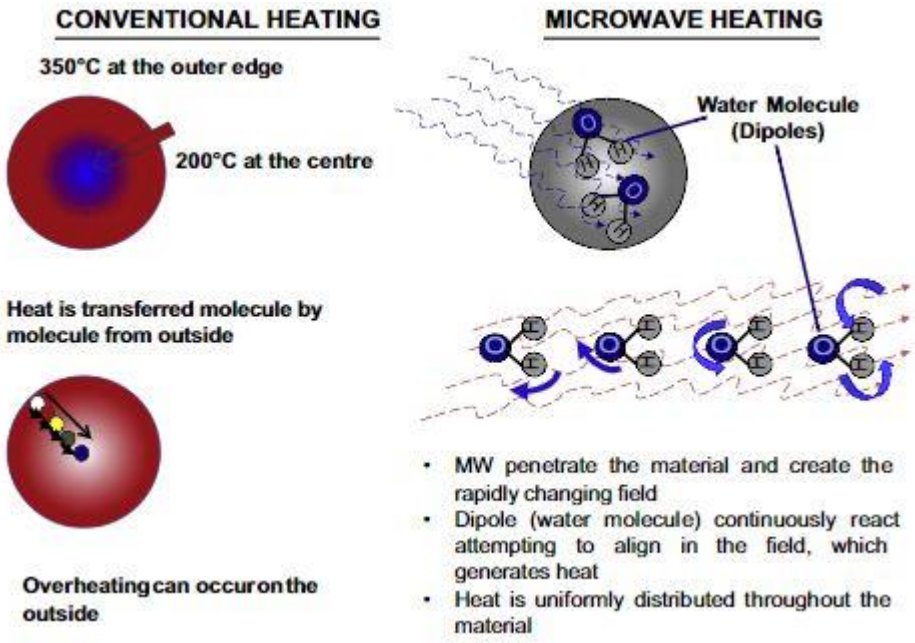


Figure 2.12 Conventional heating versus MW heating (Source: Tyagi & Lo (2013))

Temperature and moisture profiles for MW heating are a direct opposite of conventional heating. In MW heating, temperature builds from the centre of the material, which results in heat transfer from the centre towards the surface. Further, the moisture content is low at the centre because it is transported towards the surface (Tyagi & Lo, 2013)

In conventional heating, heat supplied is first used to increase the temperature of the containment vessel then the material in the vessel. The flow of heat depends on thermal conductivity, specific heat, and density of the material. Hence, there is a high temperature on the surface of the heating vessel, which causes heat transfer to the interior, leading to non-uniform sample temperatures and higher thermal gradients (Guenin, 2016).

MW heating is almost instantaneous, and there is no time spent waiting for the heat source to heat up. Heat is transferred from the outside to the inside of the material by conduction during conventional heating; overheating may occur on the outside while the interior is still cold while MW heating penetrates the material and heats it volumetrically leading to the uniform distribution of energy (Kostas et al., 2017; Tyagi & Lo, 2013).

Microwaves interact directly with the sample, which reduces wastage of energy through heating surfaces. However, the sample must be able to convert microwaves into heat for heating to occur. The temperature profile of heated MW samples is inverse of the conventionally heated sample, i.e., the surface is cooler than the interior (Fig 2.12). The direct heating provides volumetric heating. If the sample is homogenous, volumetric heating leads to uniform heating throughout the sample. However, in most real samples, thermal homogeneity is not guaranteed. Volumetric heating reduces the requirement for heat transfer through thermal conduction. Therefore, large samples can be heated more efficiently with much more thermal history throughout the sample (Gude, 2017).

Direct and volumetric heating leads to fast transfer of MW energy to heat and results in rapid temperature rise and quick reaction times. When the application of MP (MP) is terminated, the heating stops immediately. Direct heating allows sample components that interact strongly with a microwave field to be heated selectively. Hence, MW heating can generate high temperatures in specific regions of the sample while maintaining low temperatures in others (Tyagi & Lo, 2013).

Different materials interact differently with the electromagnetic field depending on the frequency of the electric field and the dielectric properties of the material. Dielectric properties of a material are the dielectric constant (ε'), dielectric loss factor (ε") and tangent loss (tan δ).

The dielectric constant is the ability of the material to store the absorbed MW energy. In contrast, the dielectric loss factor is the ability of the material to convert the electric energy into heat. These characteristics are essential to understanding the heating characteristics, material interactions, and behavior of materials in an electromagnetic field. Among others, dielectric properties depend on microwave frequency, operating temperature and type of material (Brodie et al., 2014).

Since its development, MW heating is proving to be a useful tool in several fields of application. The main uses of MW heating are food processing; drying; polymer synthesis; plastic and rubber treatment; curing and pre-heating of ceramics, and hydrolysis of particulate organic matter in complex substrates (Gude, 2017). MW heating is applied to improve both product quality and quantity; reduce processing time; and improve overall yield and resource efficiency (Bozkurt & Apul, 2020).

2.10.2 Application of microwave technology in sludge treatment

MW heating increases the kinetic energy of the substrate and polarizes molecules, causing rapid generation of heat and pressure which force extracellular organic components out from the sludge matrix. Also, MW treatment disrupts cell walls, releasing the intracellular organic matter into the soluble phase. The intrinsic characteristics of sludge (high water content) efficiently couple with microwaves and produce dielectric heating effects. Dipolar rotation or polarization occur throughout the sludge matrix, generating heat which results in a uniform and rapid volumetric heating. Temperature and pressure build up during MW heating disintegrate the EPS and cell walls (Bozkurt & Apul, 2020). The decay of EPS and cell walls liberates bound organic matter into solution, making them accessible to anaerobic microorganisms.

Several operating parameters (MP, SE, temperature, treatment time etc.,) and sludge properties influence the efficiency of MW heating. Eskicioglu et al., 2007 observed that organic matter solubilization depended on both the temperature reached during MW heating and the duration of exposure to microwaves. sProt and reducing sugars increased until the temperature reached 80°C, beyond which they decreased due to caramelization and Milliard reactions or polymerization (Eskicioglu et al., 2007). The subsequent AD of the sludge treated at 96°C at a heating rate of 1.2°C/min produced 16% more methane than the untreated sludge (Eskicioglu et al., 2007).

At higher temperature (175°C), Toreci et al., (2009) observed that decreasing temperature increase rate from 3.75°C/min to 1.25°C/min almost doubled the sCOD of TWAS because the operation allowed the sludge more time to interact with microwaves. The subsequent AD of the sludge showed a 31% increase in methane production compared to the control (Toreci et al., 2009).

In contrast to this observation, Zheng et al., (2009) argued that the final temperature reached influenced the sCOD and not the rate of temperature increase. They concluded that MP and sludge concentration (%TS) were the critical parameters in sludge solubilization (Zheng et al., 2009). After MW treatment of the PS at 90°C, they observed a methane production increase of 37% and a reduction in digestion time of 30%.

However, the work of Park et al., (2010) concurred with Toreci et al., (2009) and reported that sCOD/TCOD of WAS increased with a decrease in MP because heat exposure time increased at low power.

Yu et al., (2010) varied MP and treatment time during MW processing of WAS and concluded that sCOD/TCOD increased with MP and treatment time. The sCOD/TCOD increased from 6.2% (untreated sludge) to a maximum of 16.1% while proteins and polysaccharides increased by 297% and 654%, respectively when the sludge was treated at 900W and 140 seconds (Yu et al., 2010).

MW treatment of TWAS at different TS, temperature and temperature increase rate showed that the sCOD/TCOD increased when the TS and temperature increased while operating the process at a lower temperature increase rate (Toreci et al., 2010). A sCOD/TCOD of 57% was recorded when the MW treatment was operated at 175°C, 3.75°C/min and a sludge concentration of 11.85%TS (Toreci et al., 2010).

Ebenezer et al., (2015) reported that the sCOD of WAS increased with the SE until the SE reached 14000kJ/kg.TS, after which no significant increase in sCOD happened due to intense boiling that could have evaporated part of the solubilized organics. Also, sProt and sCarbs decreased after 14000kJ/kg.TS due to conversion of proteins to amino acids and caramelization of sCarbs (Ebenezer et al., 2015).

Serrano et al., (2016) evaluated the effect of MW pretreatment on sewage sludge solubilization and subsequent AD performance. For each applied MP, sCOD increased with SE used up to ~20000J/g.TS, after which increasing energy input did not result in more organic matter solublization (Serrano et al., 2016). Methane yield from the MW treated sewage sludge increased by 17% over the control (Serrano et al., 2016). The resulting methane yield after MW pretreatment was regarded as low. However, the rate of methane production (r_G)and OLR was significantly enhanced after MW treatment. Specifically, the methane production rate and OLR were 43% and 38% over the control (Serrano et al., 2016). Therefore, MW pretreatment could significantly reduce the reactor volumes and reduce the digestion time (Serrano et al., 2016).

Treating a relatively fresh WAS (sCOD/TCOD = 1.1%), Liu et al., (2019) reported a maximum sCOD/TCOD of 20% after MW treatment. sProt and sCarbs similarly increased with MW treatment time and were reported to be the most dominant biodegradable components (Liu et al., 2019). The AD of the MW treated sludge resulted in higher methane yield (139%) at 12 minutes, after that the methane yield did not significantly increase (Liu et al., 2019).

MW treatment of wastewater sludge has been widely studied. However, only a few studies have evaluated its potential in FS treatment. A MW hydrothermal carbonization (M-HTC) of fresh faeces showed that elemental composition increased with temperature and treatment time. The carbon content increased from 37% to 49% (32.4% increase) when the M-HTC process was operated at 160°C for 2h or 200°C for 1h, while the C:N ratio increased from 8:1 to between 24:1-62:1 (Afolabi et al., 2015).

In a follow up study, Afolabi et al., 2017 compared the M-HTC process of fresh faeces without urine (HF) and fresh faeces with urine and toilet paper (HFS). Both samples were treated at 180°C and 200°C for 30 minutes. The C content increased with treatment temperature in both samples. In HF, the C content increased from 48% (untreated) to 56% (17% increase) while it increased from 41% to 50% (22% increase) (Afolabi et al., 2017a). The initial C:N ratio in HF and HFS

was 8:1 which had increased to 28:1 and 63:1 after treatment, respectively (Afolabi et al., 2017b). Although HFS contained urine, the C:N ratio was not significantly reduced due to addition of nitrogen, possibly because the addition of toilet paper which could have offset its effect. Also, the addition of toilet paper could have enhanced the efficiency of the microwave process and released more carbon.

Working with yet again relatively fresh faeces (of approximately 24hours) collected from intensively used toilets, MW treatment was used to study the temperature evolution, pathogen inactivation and volume reduction. Different FS masses were treated at different MP levels and time intervals. The temperature evolution showed three distinct regions at each treatment applied. The first phase showed a rapid temperature rise until the FS reached the boiling point, the second phase showed a constant and minimal rise while the last phase showed another rapid temperature rise (Mawioo et al., 2016a; Mawioo et al., 2017).

It was noted that the rate of temperature rise was higher when small amounts were treated at high MP. Pathogenic microorganisms were not detected when the sample reached above 77°C (Mawioo et al., 2016a). The volume reduction was low during the first temperature rise phase because the applied energy increased the temperature of the FS instead of evaporating water (Mawioo et al., 2016a). However, the whole MW treatment process managed to reduce the sludge volume by over 60% (Mawioo et al., 2016a; Mawioo et al., 2017). The flexibility of MW technology to be a stand-alone or integrated with other FS treatment technologies makes it an attractive option for FS treatment.

2.11 Conclusion

It was noted from previous studies that organic matter solubilization and anaerobic digestion are two complex and independent processes. Previous studies demonstrated that the highest organic matter solubilization during pretreatment did not always result in the highest methane yield. The pretreatment method chosen should strive to strike a balance between solubilization, methane yield and energy efficiency. With each pretreatment chosen, working at wider process conditions would improve organic matter solubilization while minimizing energy requirement, its major drawback.

Regardless of its potential benefits, the MW treatment of FS has not been widely studied. A few pilot studies have been reported where the technology was evaluated as a stand-alone treatment unit or incorporated with HTC. Also, the FS used in those studies could best be described as fresh faeces because it was either collected directly from individuals or from intensively used toilets with an average age of approximately 24hours. Further, MW treatment was employed with the aim of reducing sludge volume, inactivating pathogenic microorganisms, or improving the efficiency of the HTC process (Mawioo et at., 2016a, 2017; Afolabi et al., 2015, 2017b).

The effect of MW treatment on the physicochemical characteristics of FS from VIP latrines and the subsequent anaerobic digestion has not been reported yet. The flexibility of MW technology to be independent or integrated with other treatment technologies makes it potential pretreatment option for FS from pit latrines which currently predominate in the non-sewered sanitation solutions used globally.

In the next chapter (Chapter 3), the materials and methods used in this study are presented.

CHAPTER 3 : MATERIALS AND METHODS

3.1 Faecal sludge collection and processing

FS in laboratory experiments to evaluate the influence of microwave treatment on various sludge parameters. The FS was collected from VIP latrines in informal settlements of eThekwini Municipality. Sample 1 (S1) was FS collected from the drop hole of a VIP latrine, sample 2 (S2) was a composite of a single VIP latrine representing all the theoretical layers while sample 3 (S3) was a composite of five VIP latrines as was delivered to a treatment facility. A VIP latrine is the most basic form of sanitation prescribed in South Africa and is widely used in the study area. Most VIP latrines in the study area have reached their full-service life and required emptying.

The samples were collected and processed using standard operating procedures (SOPs) developed by the Water, Sanitation and Hygiene Research & Development (WASH R&D) Centre at University of KwaZulu-Natal. About 5kg of S1 and S2 were collected in 5 L plastic buckets. Similarly, about 3kg of FS was collected from each of the five VIP latrines and mixed to make a composite sample (S3) of about 15kg. Samples were transported to the WASH R&D Centre specialized sanitation laboratory where they were screened to remove trash and other detritus materials (Fig 3.1).

Figure 3.1 Trash removed from VIP sludge (Photo taken by the author)

The screened FS was homogenized and stored at 4°C until required for use.

3.2 Experimental design and microwave treatment

The experiments were conducted in three project phases. The first phase involved optimizing the MW treatment process using sample S1 and S2. The second phase involved a thorough physicochemical characterization of FS after MW treatment of sample S3, while the third phase involved anaerobic digestion of the MW treated FS.

Using sample S1 and S2, design of experiment (DOE) and response surface methodology (RSM) were used to evaluate the relationship between the solubilization (response variable) of FS and the operating parameters (MP and contact time). The results were used to optimize the MW treatment process. Details of the experimental design are presented in Appendix B. The results of experiments using sample S1 and S2 informed the choice of the microwave operating parameters for the treatment of sample S3 in a more detailed experimentation.

MW treatment of sample S1 and S2 involved placing about 50g of a well homogenized sample into a 100mL beaker and covering it with a microwave-safe wrap paper. While using sample S3, about 200g of a well-mixed sample was placed in a 250mL beaker and covered with a microwave-safe wrap paper. An MP of 540W, 630W and 720W was applied to treat S1 and S2 samples for 1, 3 and 5minutes. Sample S3 was treated at an MP of 630W, 720W and 810W for 1, 2, 3, 4, 5, 6, 8 and 10 minutes. All the samples were treated in triplicates and the treatment was randomized. The mass and temperature of the sample were recorded using a benchtop analytical balance and an infrared thermometer, respectively. Using an infrared thermometer, the temperature of the samples was measured immediately after microwave treatment (within 5-10 seconds). The samples were then cooled to room temperature and weighed before further physicochemical analyses. Volume reduction was determined from the difference between the initial and final weight of the sample. The weight loss was mainly attributed to the water evaporating from the FS during MW treatment. Therefore, considering the density of water, the weight loss was assumed to be equivalent to the FS volume reduction (Mawioo et al., 2016b).
MW treatments were carried out in a Hisense domestic MW oven operating at a frequency of 2.45GHz and power ranging from 0 to 900W with 11 power increment levels at 10% intervals. All MW treatments were carried out in a fume hood.

3.3 Analytical methods

To evaluate the effect of MW treatment on FS characteristics, both untreated (untreated) and MW treated samples were analyzed for various parameters as described below. The analytical methods used were adapted from standard methods for the examination of water and wastewater and domesticated as WASH R&D Centre laboratory SOPs. Detailed SOPs on FS collection, sample preparation procedure and the associated conversion of the concentrations to dry weight basis are provided in appendix C and D.

3.3.1 Solids

To determine the solids content of S1, S2 and S3 samples before and after MW treatment, clean crucibles were prepared by igniting them in a furnace at 550℃ for 1hour to remove all the solids that could have remained from previous experiments. The weight of the cooled crucibles was recorded using an analytical balance calibrated to four decimal places. About 20g of the sample

was placed in the weighed crucible and oven heated at 105°C for 24hours. The mass loss after 24hour oven heating at 105°C represented the TS.

The residue of TS was ignited in a furnace at 550°C for 2hours. The mass loss after ignition at 550°C for 2hours represented the VS. All the samples were analyzed in duplicates. The VS/TS ratio was calculated and used to determine the stability of the FS before and after MW treatment.

3.3.2 Chemical oxygen demand

The effect of MW treatment on organic matter solubilization was evaluated by measuring the total COD (TCOD) and sCOD before and after MW treatment of S2 and S3 samples. The COD of FS was determined by the dichromate method using a Hach commercial COD test kit. The TCOD was determined on the stock solution while sCOD was determined on the centrifuged and filtered sample (details in appendix D). Exactly 2mL of a sample was pipetted into a COD cuvette and digested at 150°C for 2hours. All samples were analyzed in duplicates. After cooling, each cuvette was scanned thrice using a portable Hach photometer, DR 900 (Hach®). The solubilization of organic matter was determined as the ratio of sCOD to TCOD (Equation 3.1):

$$\textbf{Solubilization}\ (\%) = \frac{\mathbf{sCOD_t}}{\mathbf{TCOD}}\mathbf{x\ 100}\% \qquad \textbf{(3.1)}$$

3.3.3 Carbohydrates and Nitrogen

The change in soluble macromolecules (sCarbs, sProt, ammonium and nitrates) indicate the success of MW treatment (S3 samples) in disintegrating sludge flocs and disrupting cell walls and are directly related to the change in sCOD. A phenol-sulfuric acid procedure using glucose as a standard (Dubois et al., 1956) was modified and used to determine carbohydrates. Details of the preparation of the standard and calibration curve are provided in appendix D. sCarbs were measured on the filtrate after centrifugation and filtration through a 0.45µm syringe filter.

Total nitrogen, ammonium nitrogen (NH_4^+-N), and nitrate nitrogen (NO_3^--N) were determined using cell tests in a Merck Spectroquant® Prove 300 UV/VIS spectrophotometer. The samples for TN analysis were digested for 1hour at 120°C. Protein content was estimated from TN by using a nitrogen-to-protein ratio of 6.25 (Girault et al., 2012). All the samples were analyzed in duplicates. Details of the analytical procedure are provided in appendix D.

A correlation heat map was generated and used to understand the contribution of the macromolecules to the sCOD release. The nitrogen content values were used together the elemental analysis values to determine the fertilizer value of the microwave treated FS.

3.3.4 Elemental composition

About 0.2g of the FS (S3 samples) was used to determine the carbon and nitrogen using a *Leco TruMac* CNS determinator. The *Leco TruMac* CNS instrument simultaneously determines the C and N in the sample. All samples were analyzed in duplicates. The potential of biological treatment of the FS was determined by evaluating the C:N ratio of the untreated and MW treated samples.

3.3.5 Fourier transform infrared spectroscopy and scanning electron microscopy analysis

The effect of MW treatment on the structural and morphological changes of the FS (S3 samples) was evaluated by comparing the Fourier transform infrared (FTIR) spectra and scanning electron microscope (SEM) images, respectively. The untreated and MW treated FS were frozen in a household-scale freezer for 24hours and then freeze-dried for a further 24hours using a VirTis Bench Top Pro freeze drier. The freeze-dried samples were ground to a fine powder used for the FTIR and SEM analysis at the Analytical Chemistry laboratory and the Microscopy and Microanalysis Unit laboratory, respectively.

An IRSpirit equipped with a QATR 10 single reflection ATR accessory (diamond crystal) collected the spectra at 4000cm^{-1} to 400cm^{-1}. Each sample was scanned 16 times and recorded the average spectra which were processed using the LabSolutions software.

A Zeiss Ultra Plus FEGSEM (Germany) coated with Quorum Q150R ES Sputter Coater captured the images of the untreated and MW treated samples. The dried sample was mounted onto SEM stubs with double-sided adhesive carbon tape and imaged on the Zeiss Ultra Plus FEGSEM (Germany) operating at 20keV.

3.3.6 Flow cytometry

Fluorescent staining coupled with flow cytometry (FCM) was used to identify the viability of bacteria in S3 samples using an Attune^{TM} NXT-Accoustic Focusing Cytometer. A double fluorescent staining using propidium iodide (PI) and SYBR-Green (SG) dyes was used to distinguish dead and live bacteria. The PI dye enters dead cells only while SG enters both dead and live cells. Thus, simultaneous staining with SG and PI activated energy transfer between the fluorochromes which caused live cells to emit green fluorescence while dead cells emitted red fluorescence. Details of the sample preparation and fluorescence staining are provided in appendix E.

3.3.7 Anaerobic digestion

The effect of MW treatment on methane production and the biochemical methane potential (BMP) of FS was evaluated using untreated and MW treated sample S3. Sample S3 only was used because it represented the type of FS to be received at a treatment facility. The MW treated S3

sample for AD was treated at 630W for 4 minutes. The untreated and MW treated S3 samples were stored in cold room at the WASH R&D Centre for 2 months before they were transported to the IHE Delft Institute for Water Education for analysis.

The untreated and MW treated S3 samples were also mixed with wood chips at a ratio of 1:1 to create another stream of substrates in order to evaluate the effect of co-digestion on anaerobic digestion process. The AD batch assays were carried out using the automatic methane potential test system (AMPTS II) system in 500mL bottles with an effective volume of 400mL. The experimental set up was constructed in RStudio software using the "biogas" R package. When the "biogas" package was loaded, a "PlanBMP" function calculated the required inoculum and substrate masses based on an inoculum to substrate ratio (ISR) of 2:1 and VS concentration. The inoculum and substrates (untreated FS, MW treated FS, untreated FS + wood chips and MW treated FS + wood chips) were loaded to the serum bottles of 500mL. A blank containing the inoculum only was carried out to subtract the contribution of the inoculum to methane production in substrate bottles. The inoculum was collected from an active mesophilic AD treating WAS in Delft, Netherlands. All bottles were prepared in triplicate and flushed with N_2 for 2 minutes to create an anaerobic condition and then incubated in a water bath operated at 35±2°C. The biogas produced passed through a 100mL bottle filled with a 3M NaOH solution mixed with a 0.4% Thymolpthalein pH indicator solution to absorb CO_2, leaving methane which was recorded automatically and normalized to standard conditions. The details of the experimental design and calculation of biochemical methane potential (BMP) and yield kinetics is given in appendix F.

3.4 Statistical analysis

Descriptive statistics, linear regression and correlation heat maps were carried out using MS Excel 2019 while RSM was performed in RStudio software. A second-order polynomial was used to evaluate the influence of the control variables (MP and time) on organic matter solubilization (Y_i) (Equation 3.2).

$$\mathbf{Y_i = \beta_0 + \sum \beta_i X_i + \sum \beta_{ii} X_i^2 + \sum \beta_{ij} X_i X_j} \qquad \mathbf{(3.2)}$$

In the equation Y_i is the response variable (sCOD/tCOD), β_0 is a constant, β_i is the linear effect, β_{ii} is the quadratic effect, β_{ij} is the interactive effect, X_i is the control variable. The coefficient of determination (R^2) and statistical significance determined the quality of the model by analysis of variance (ANOVA) at 95%.

In the next chapter (Chapter 4), the results and discussions from this study are presented.

CHAPTER 4 : RESULTS AND DISCUSSION

4.1 Introduction

This chapter presents the results of varying MP (MP) and treatment time on the physicochemical characteristics of faecal sludge (FS). In the initial phase of the experimental study, the effect of MP and treatment time were investigated to provide data to indicate the optimum range for MW pretreatment of the ventilated improved pit (VIP) latrine sludge. Response surface and contour plots were used to determine an optimized range of treatment times and MP levels for further investigation and preparation for anaerobic digestion (AD).

4.2 Effect of MP and treatment time on the physicochemical characteristics of faecal sludge

Characteristics of the untreated FS used in this research are presented in Table 4.1. Sample S1 was collected from the drop hole of the VIP latrine while sample S2 was collected from the entire pit of the VIP latrine. Sample S2 represented the type of material to be received at a treatment plant. Hence it was used for the optimization experiments. The values are averages of two replicates for each parameter.

Table 4.1 Characteristics of the untreated FS

Parameter	Unit	S1 (VIP drop hole)	S2 (entire VIP latrine)
TS	%	21.00±00	31.00±0.00
VS	%	13.00±0.00	13.00±0.00
VS/TS	%	61.90±0.00	41.94±0.00
TCOD	g/kg.TS	1840.14±327.34	711.02±19.14
sCOD	g/kg.TS	405.06±0.00	214.18±5.63
sCOD/TCOD	%	22.37±3.98	30.12±0.02

The TS, VS, TCOD and sCOD were within the range of values reported in the literature for FS collected from VIP latrine in the same study location (Bakare et al., 2012; Zuma et al., 2015). However, the TCOD of 1840.14±327.34gCOD/kg.TS for sample S1 was higher than the values reported by Zuma et al., (2015), possibly due to its freshness as it was mostly less than 24 hours old. Hence it is postulated that it contained higher amounts of readily degradable organic matter that was reported by Zuma et al., 2015.

The high sCOD/TCOD ratio (30.12±0.02%) of sample S2 indicated that it underwent some degradation in the pit latrine during storage. Hence most of the observed COD was associated with the solid phase. Comparing the changes in the physicochemical characteristics before and after microwave pretreatment of the FS provided the means for evaluating the influence of the treatment process. The results are discussed in the following sections.

4.3 Effects of microwave treatment on FS solids and volume reduction

MW treatment increased the TS and VS content of the FS. For each MP increment applied (450-810 W), TS and VS increased linearly with treatment time. The highest TS and VS were 46.00±1.41% and 26.50±0.71%, and 73.50±2.12% and 31.50±0.71% for the S1 and S2 FS, respectively. MW heating led to the agitation of water molecules whose friction caused heat generation and subsequent temperature rise.

MW treatment reduced the volume of the FS by 50% and 58% for the S1 and S2 FS samples, respectively. Microwave's volumetric heating caused a rapid transfer of moisture from the core of the FS to the surface, where it was removed by evaporation, decreasing the remaining FS volume. At high specific energies, the remaining sample mass was low. As the VS/TS ratio remained relatively constant, the observed weight loss resulted from the evaporation of moisture from the sample (Mawioo 2016a).

4.4 Influence of microwave treatment on FS solubilization

To optimize solubilization of sCOD, a representative sample was used. Hence, the effect of MP and treatment time was investigated using FS sample S2 which was collected from the entire pit, representing the type of material that would be received at a treatment plant.

The sCOD in untreated FS was 214.18±5.63gCOD/kg.TS and increased to a maximum of 236.60±4.37gCOD/kg.TS, 274.01±3.31gCOD/kg.TS and 238.77±3.32gCOD/kg.TS after MW treatment at 540W, 630W and 720W, which represented 11%, 39% and 11% increase, respectively.

The operating parameters were normalized to SE (kJ/kg.TS) and plotted against the sCOD/TCOD (Fig 4.1). The sCOD/TCOD ratio in the untreated FS was 30.12±0.02% which increased after MW treatment following two distinct phases i.e., an initial sCOD/TCOD increase up to ~7300 kJ/kg TS ($R^2 = 0.76$) and a decrease in sCOD/TCOD (Fig 4.1). The phase line in Fig 4.1 was duntreatedn to guide the reader.

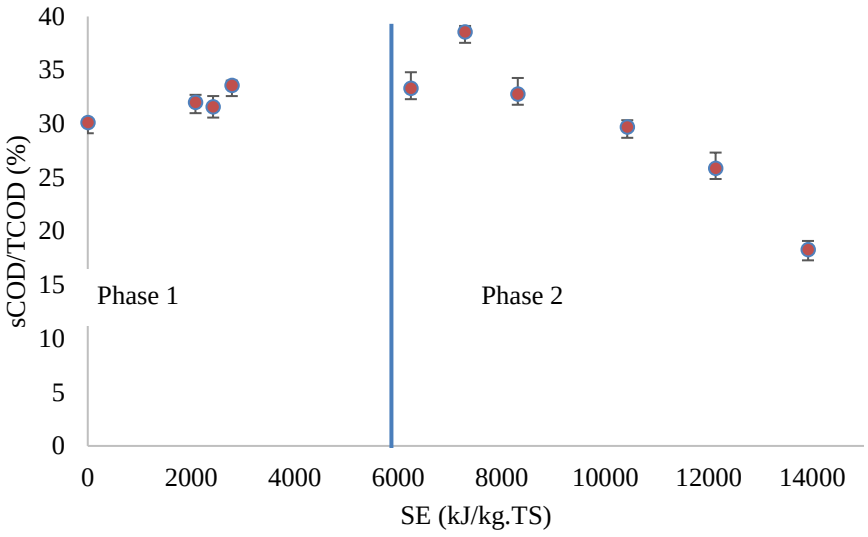


Figure 4.1 COD solubilization showing initial sCOD release (phase 1) and degradation of sCOD (phase 2) as a function of SE. Error bars represent standard deviation

The sCOD/TCOD value in the untreated FS reported here was relatively high compared to wastewater sludge (Doğan & Sanin, 2009; Kor-Bicakci et al., 2019). The high value of initial sCOD/TCOD suggested that the FS underwent considerable solubilization in the pit latrine. Indeed, the age of VIP latrine FS in eThekwini Municipality can be as high as five years before emptying (Brouckaert et al., 2013). Also, solubilization could have occurred during storage in the laboratory (Bougrier et al., 2007). Regardless of the high initial sCOD/TCOD ratio, MW treatment increased the solubilization of organic matter in FS, which initially increased with SE and then decreased (Fig 4.1).

4.5 Characterization of the response surface and contour plot

A statistical analysis was done to understand the factors (MP and time) and interactions (MP: time) that influenced the observed response values (i.e., the effect on the sCOD/TCOD ratio). Details of the experimental design and results of the experiment (response) using sample S2 are outlined in Table B.1 (Appendix B). The results of the statistical analysis in R software are displayed in Tables 4.2 and 4.3. In these Tables, x1 and x2 represents MP and treatment time, respectively

Table 4.2 Model parameters and their estimated values

	Estimate	Std error	t value	pr (>|t|)
Intercept	-1.143×10^{2}	7.1953×10^{1}	-1.5886	0.18735
x1	4.185×10^{-1}	2.2931×10^{-1}	1.8250	0.14204
x2	1.8786×10^{1}	4.5223	4.1540	0.01421*
x1:x2	-1.7886×10^{-2}	6.2280×10^{-3}	-2.8719	0.04538*
$x^2 1$	-3.0382×10^{-4}	1.8121×10^{-4}	-1.6766	0.16892
$x^2 2$	-1.5951	3.6694×10^{-1}	-4.3469	0.01219*

Results of the analysis showed that one linear term (x2), the interaction terms (x1:x2) and one quadratic term ($x^2 2$) significantly influenced the response values ($p < 0.05$) (Table 4.2) with model multiple R^2 and adjusted R^2 of 0.93 and 0.85, respectively. Using the intercept and significant terms only (Table 4.2) yielded the model equation 4.1 (where Y represents the percentage solubilization of COD):

$$\boldsymbol{Y = -1.143\, x\, 10^{2} + 1.8786\, x\, 10^{1} X2 - 1.7886 X1X2 - 1.5951 X^{2}2} \quad (\boldsymbol{4.1})$$

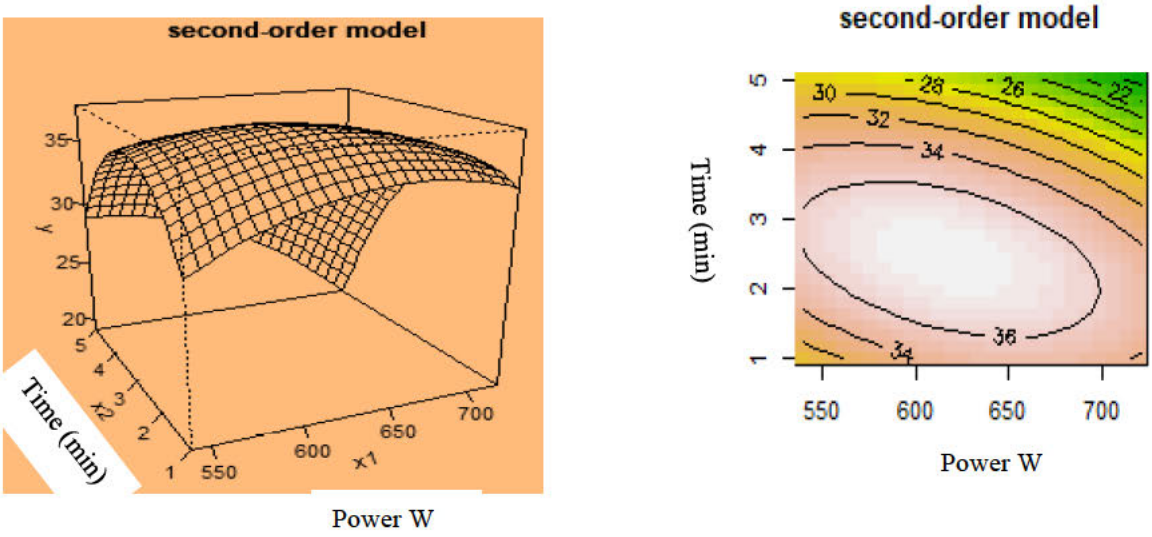
Results of the analysis of variance indicated that the model's lack of fit was not significant ($p > 0.05$) (Table 4.3). Hence, it could explain the percentage solubilization of sCOD (denoted as Y) during MW treatment.

Table 4.3 Analysis of variance (ANOVA) table

	DF	Sum sq.	Mean sq.	F value	pr(>F)
FO (x1, x2)	2	116.854	58.427	11.6230	0.022155
TWI (x1, x2)	1	41.460	41.460	8.2478	0.04538
PQ (x1, x2)	2	124.796	62.398	12.4130	0.01926
Residuals	4	20.107	5.027		
Lack of fit	3	15.649	5.216	1.1699	0.57660*
Pure error	1	4.459	4.459		

FO=First order terms; TWI= Two-way interaction terms; PQ=Pure quadratic terms

Summary results of a canonical analysis yielded all negative eigenvalues (-0.001132643 and -7.122247721). Thus, the second-order polynomial response surface was concave (Fig 4.2a), indicating that the stationary point was a point of maximum response (i.e., maximum sCOD release). The fitted response surface's stationary points were (617.3W, 2.4 minutes), which were within the region of the experimental design. Therefore, within this experimental design, MW treatment at 617.3W for 2.4 minutes would yield the maximum sCOD/TCOD ratio. The contour plot (Fig 4.2b) confirms the above analysis as the response increases towards the centre.



(a) Response surface (b) Contour plot

Figure 4.2 A response surface (a) and contour plot (b) for the solubilization of VIP sludge (Sample S2)

The plot of SE versus percentage solubilization indicated a maximum sCOD percentage that could be achieved, however, as this is due to a combination of MW and treatment time, the response surface rather pinpoints the optimal treatment settings for both as individual factors. So, whilst different combinations of MP and treatment time can result in the same SE, the model fit to the two sets of data can optimize both MP and time.

Both plots (Fig 4.2) indicate that there are maximum MP and time for optimal sCOD solubilization, with increases of sCOD solubilization up to an optimum, after which increasing power will reduce solubilization for a given MW time and increasing time would decrease solubilization at a given power setting. It also indicates that the optimum power for solubilization is as given i.e., around 630W, a higher range of MP settings still result in higher solubilizations but over shorted times. To that end an experimental design based upon a range of MP settings (630W, 720W and 810W with periods of time focused on low time intervals for digestion (from the contour plots, it was expected that solubilization of COD would be optimum in a range of times up to 5 minutes at the lower power, up to slightly lower at the higher power) were selected.

This observation agreed with the findings of Park et al., (2010), who concluded that solubilization of WAS consistently increased with a decrease in MP at all temperatures and sludge concentration (TS %) used. Yu (2010) also observed that MP and treatment time were crucial in the solubilization of WAS. In that research, Yu (2010) observed higher solubilization when the sludge was treated at low MP for a longer time.

4.6 Effect of microwave treatment on the physicochemical characteristics of faecal sludge

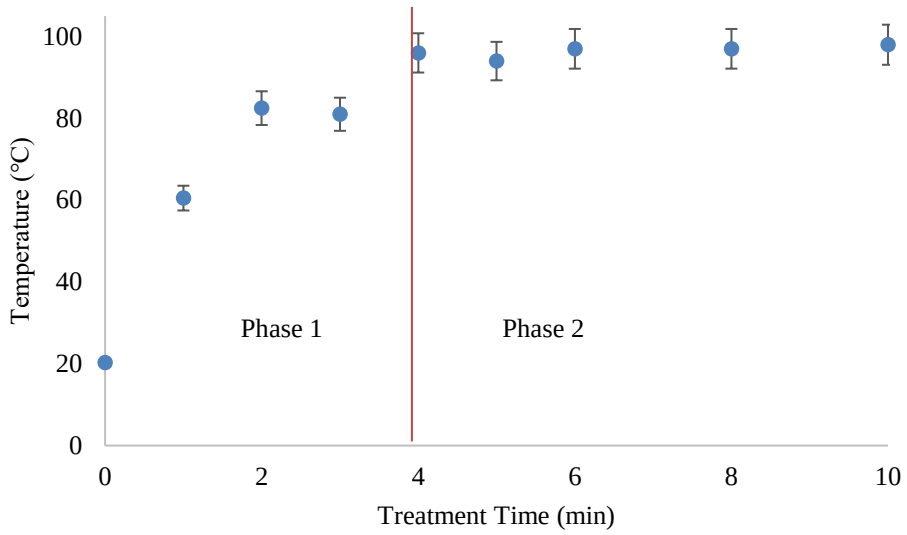
The samples (S3) used for this phase were composite of 5 VIP latrine, which was more representative of what would be received at a treatment facility. The characteristics of the untreated FS samples are presented in Table 4.4 as averages of two replicates together with their standard deviations. The values reported here were in line with the characteristics of untreated sludge collected from the same study area (Zuma et al., 2015).

Table 4.4 Characteristics of the untreated FS (S3)

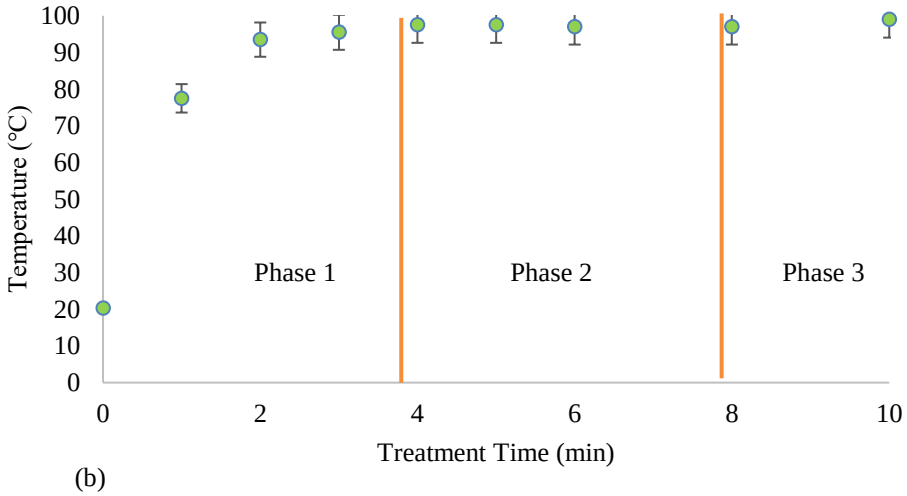
Parameter	Unit	Value (Average)
TS	%	24.21±0.38
VS	%	14.47±0.20
VS/TS	%	59.74±0.54
TCOD	g COD/kg TS	999.29±38.27
sCOD	g COD/kg TS	113.44±4.42
sCOD/TCOD	%	11.35±0.88
NO_3^--N	g NO_3^-/kg TS	1.76±0.74
NH_4^+-N	g NH_4^+/kg TS	10.20±2.06
C	g C/kg TS	300.06±2.29
N	g N/kg TS	71.57±42.49
C:N	-	5.08:1±2.98
Soluble protein	g/kg TS	1105.98±92.01
Soluble carbohydrates	g/kg TS	6.1

4.7 Effect of microwave heating on FS temperature

The temperatures were measured soon after removing the sample (S3) from the MW oven. Fig 4.3 shows the temperature profiles for the FS samples treated at 630W, 720W and 810W for 1, 2, 3, 4, 5, 6, 8 and 10 minutes. In general, MW treatment caused a rise in sludge temperature from ~20.27±2.97℃ in the untreated FS samples to between 60.50±0.71℃ to 99±0.00℃ after MW treatment. Fig 4.3a-c shows three temperature profiles i.e., a rapid linear temperature rise, a constant temperature and a second temperature increase. The rapid temperature rise was observed at all MP levels until the sludge attained the boiling point (~96℃), after which the temperature remained fairly constant before increasing again (Fig 4.3). In Fig 4.3, phase lines were drawn to guide the reader.


100
80
60
40
20
0
Temperature (°C)
Phase 1
Phase 2
0
2
4
6
8
10
Treatment Time (min)

(a)


100
90
80
70
60
50
40
30
20
10
0
Temperature (°C)
Phase 1
Phase 2
Phase 3
0
2
4
6
8
10
Treatment Time (min)

(b)

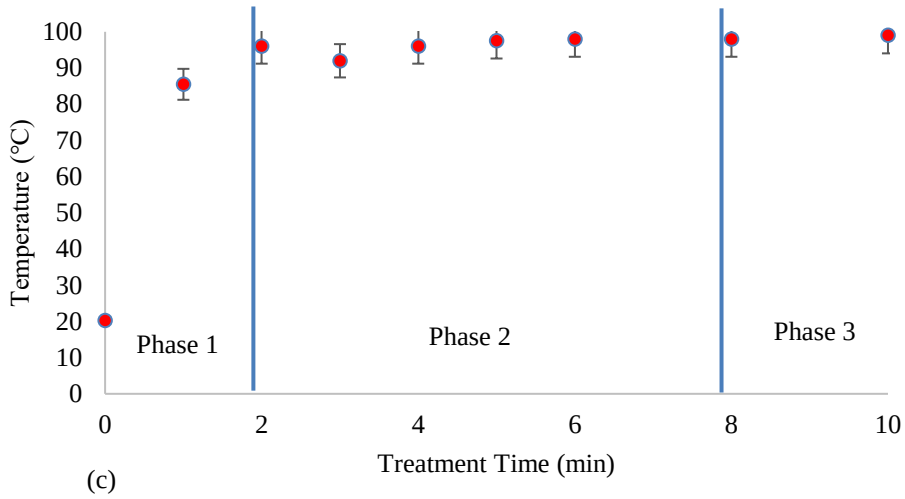


Figure 4.3 Temperature profiles showing initial temperature increase (phase 1), fairly constant temperature phase (phase 2) and second temperature increase (phase 3) for FS sample S3 during MW treatment at 630W (a), 720W (b) and 810W (c). Error bars represent standard deviation

FS samples treated at 630W reached the boiling point after a 4-minute MW treatment, while those treated at 720 W and 810 W reached the boiling point after 2 minutes. Curve fitting the data in a linear regression model showed a temperature increase rate of 13.8°C/min (R^2= 0.81), 16.8°C/min (R^2 = 0.70) and 37.5°C/min (R^2 = 0.86) after MW treatment at 630W, 720W and 810W, respectively.

The temperature profiles reported in this study were similar to those reported in previous studies using FS from intensively used toilets, blackwater, septage, and WAS (Mawioo et al., 2017; Mawioo et al., 2016a; Mawioo et al., 2016b)

4.8 Volume reduction

Fig 4.4 shows the observed volume reduction for FS sample S3 at different MP levels and treatment times. Volume reduction varied with input MP and treatment time. The initial volume reduction between 1-4 minutes was low at all power levels (630W, 720W and 810W), but increased significantly in line with evaporation of moisture once the temperature of the FS stabilized.

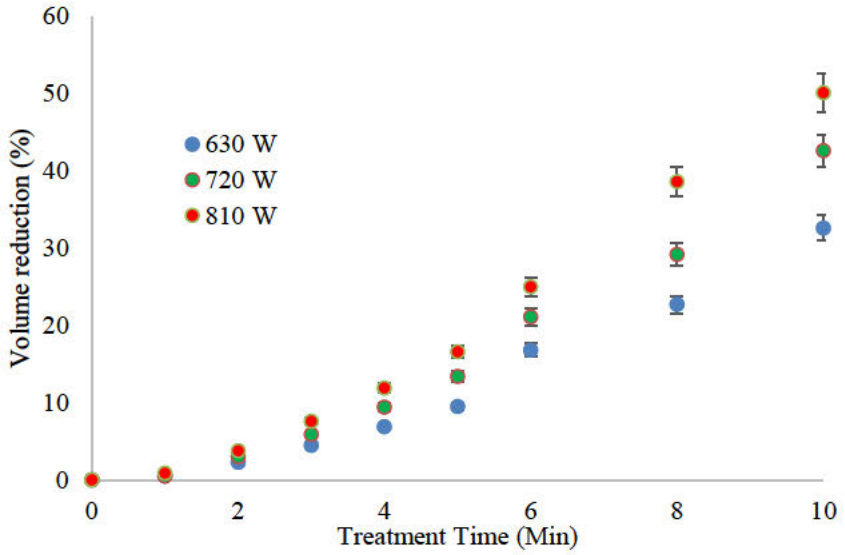


Figure 4.4 FS (S3) volume reduction with contact time with standard deviation error bars

The highest and lowest reduction in FS volume was observed when it was treated at the highest and lowest MP of 810 W and 630 W, respectively. For each MP used, there was a linear increase in volume reduction with increasing contact time. The low volume reductions observed between 1-4 minutes resulted from the heating of the FS water. At this stage, the applied energy raised the temperature of water to the boiling point. After which, the energy applied evaporated the water from the FS causing its volume reduction (Chen et al., 2014). The highest volume reduction was 50% at 810 W and 10 minutes. The SE (which is influenced by MP, treatment time and the amount of sample) was critical in volume reduction. Accordingly, the operating conditions (MP and time) were normalized to SE and plotted against the observed volume reduction (Fig 4.5).

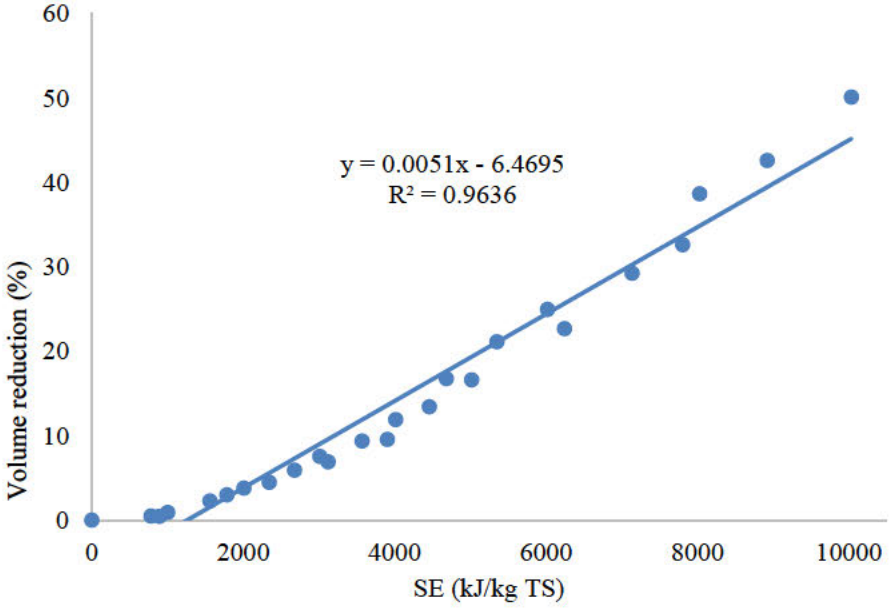


Figure 4.5 FS (S3) volume reduction with SE

A linear relationship was observed between the observed volume reduction and SE ($R^2 = 0.96$). This relationship is important in FS treatment where the main aim is to reduce the volume because the process can be operated at high SE to maximize volume reduction.

4.9 VS/TS ratio

The VS/TS ratio remained constant before and after MW treatment. However, the VS percentage increased with increase in treatment time at each MP level as the moisture was removed from the FS sample. The lowest increments were observed at 630W and 720W while treatment at 810W showed the highest VS increase after 6 minutes (Fig 4.6). At this point, the sludge had reached the boiling point. Therefore, the applied energy disintegrated sludge flocs and released VS components.

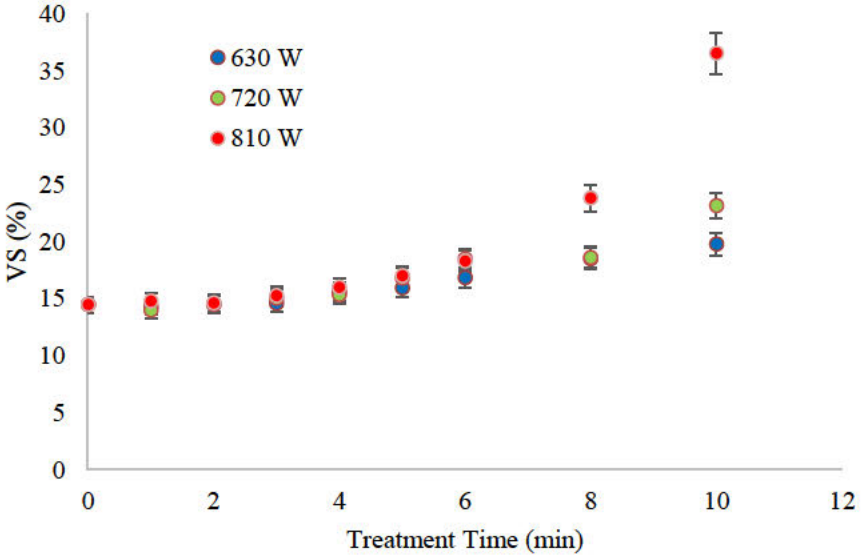


Figure 4.6 Percentage VS evolution during MW treatment of FS (S3). Error bars represent standard deviation

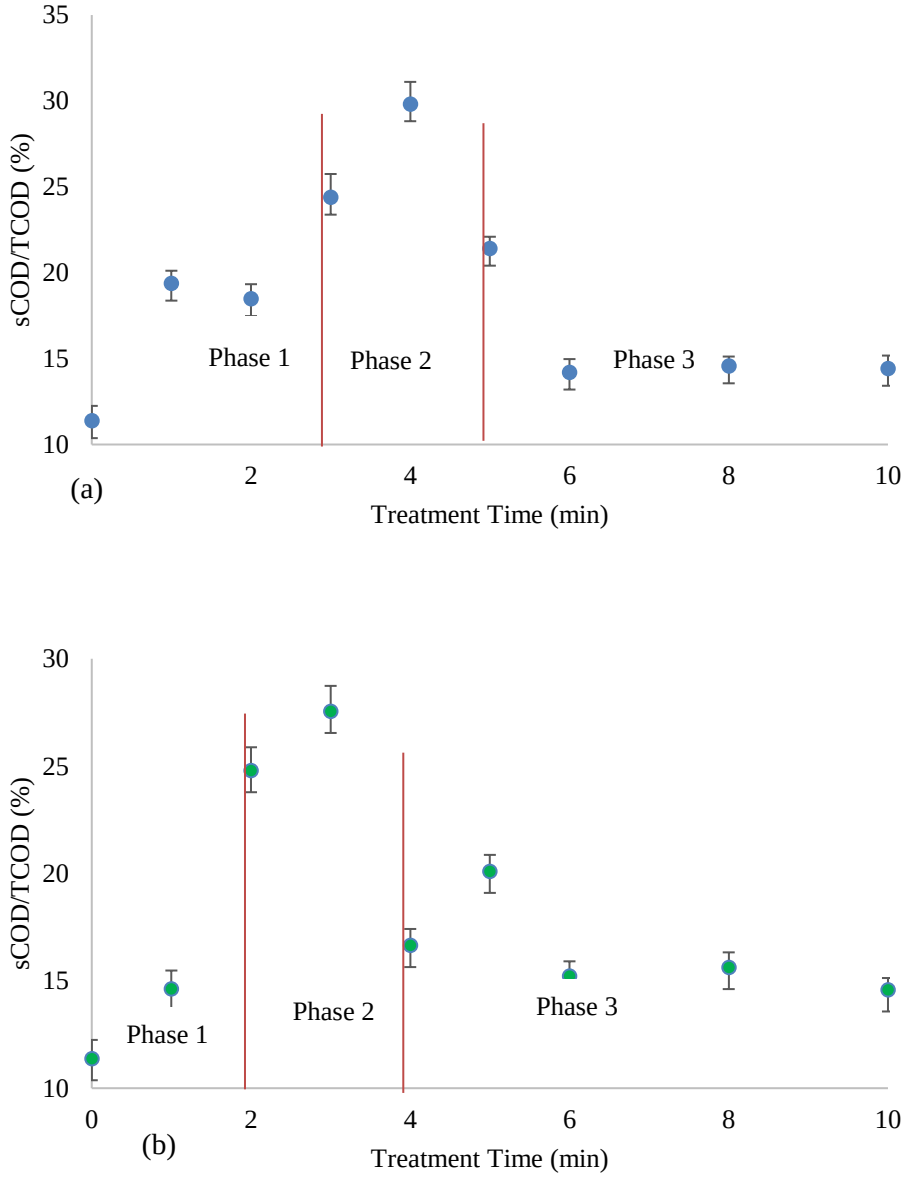
There was no observed loss of VS during treatment and the VS/TS ratio ranged from 55.97% to 67.75%, which was within the range of stable sludge recommended by the European Environmental Agency (Bresters et al., 1997).

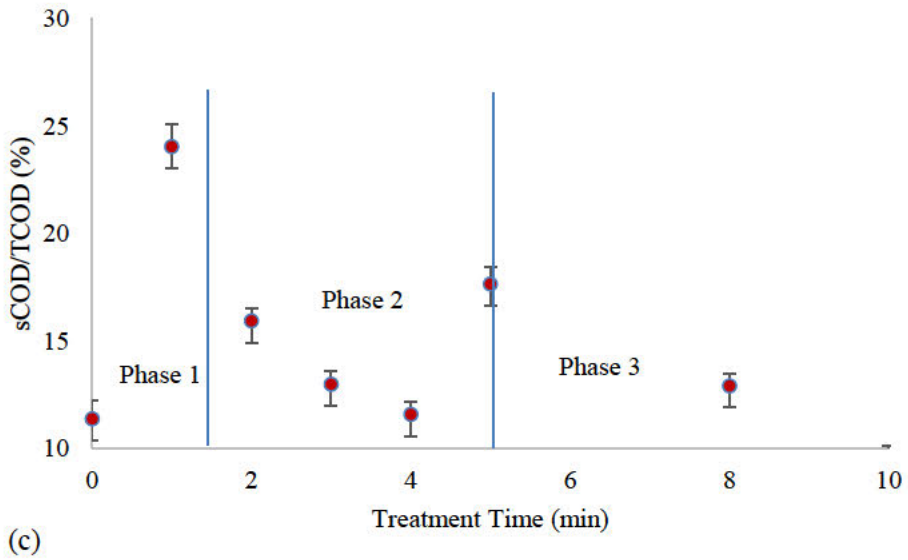
4.10 The effect of microwave treatment on chemical oxygen demand

The change in sCOD during the experiment was used to determine the MW effect on FS (sample S3) solubilization. The COD data were normalized to dry weight (TS) basis to eliminate the influence of dilution on the reported values. The untreated FS had a sCOD of 113.44±4.42gO_2/kg.TS. In general, the sCOD increased after MW treatment depending on the different MP used. The sCOD initially increased with time at all the MP levels applied i.e., 630W, 720W and 810W and the treated samples achieved maximum sCOD after 4, 3 and 1 minutes,

respectively. Compared to the concentration in the untreated FS, sCOD increased by 162%, 142% and 112% at these operating conditions. Comparing the sCOD from all the three MP increments, it was indicated that the highest sCOD was achieved for FS samples irradiated at 630 W for 4 minutes which was in line with the optimization tests.

The sCOD/TCOD profiles for the FS treated at different power levels (630, 720 and 810 W) and varying contact times (1, 2, 3, 4, 5, 6, 8 and 10 minutes) are shown in Fig 4.7a-c. There were three sCOD phases identified for all the samples treated at 630W, 720W and 810W i.e., initial sCOD release phase, degradation of sCOD and a slight sCOD release (Fig 4.7). Phase lines in Fig 4.7 were drawn to guide the reader.





(c)

Figure 4.7 sCOD profile showing initial sCOD release (phase 1), degradation of sCOD (phase 2) and slight sCOD release (phase 3) after MW treatment of FS (S3) at 630W (a), 720W (b) and 810W (c). Error bars represent standard deviation

The sCOD release (phase 1) and degradation (phase 2) varied across the three MPs applied. The initial sCOD release was rapid for the FS treated at 810W and slowest for the 630W MP. Also, the duration phase 1 varied according to the MP (Fig 4.7a-c). It is shown in Fig 4.7 that the sCOD/TCOD reached the peak after 4, 3 and 1 minute of MW treatment at 630W, 720W and 810W, respectively. At these operating conditions, 630W/4 minutes, 720W/3 minutes and 810W/1 minute, the maximum sCOD/TCOD were 29.82±1.29%, 27.54±1.19% and 24.05±1.06%, respectively. The rate of sCOD release was computed from regression analysis and was determined to be 3.9gO_2/kg.TS.minute (R^2 = 0.92) and 5.6gO_2/kg.TS.minute (R^2 = 0.95) at 630W and 720W, respectively. At 630W, the sCOD/TCOD decrease phase (phase 2) continued until 6 minutes and remained similar afterwards. The decrease phase was followed by a slight sCOD release phase (phase 3). The maximum FS solubilization for all treatments coincided with the FS boiling temperature (Fig 4.3). The highest sCOD/TCOD (29.82±1.29%) at 630W and 4 minutes of MW treatment corresponded to a temperature increase rate of 13.8°C/minute (the lowest temperature increase rate). A low temperature increase rate allowed enough time for the microwaves to interact with the FS.

4.11 The influence of microwave treatment on macromolecules and component elements

The concentration of macromolecules in the supernatant of FS after centrifugation and filtration is usually attributed to the MW effect. The effect of MW treatment (using FS sample S3) on sProt, sCarbs, ammonium (NH_4^+), nitrates (NO_3^-), carbon (C), nitrogen (N), and sulfur (S) are shown in Table 4.5.

Table 4.5 Effect microwave treatment on macromolecules and component elements (S3)

Power (W)	Time (min)	sProt (g/kg.TS)	NO_3^- (g/kg.TS)	NH_4^+ (g/kg.TS)	sCarbs (g/kg.TS)	C (g/kg.TS)	N (g/kg.TS)	C/N Ratio
630	1	1248.31±92.91	1.79±0.15	12.82±1.78	7	289.39±9.12	37.07±1.04	7.81±0.03
630	2	1497.80±9.10	2.08±0.00	11.25±0.29	7	-	-	-
630	3	1781.65±0.00	1.73±0.43	12.42±0.00	11	303.97±5.43	36.15±1.37	8.42±0.47
630	4	1958.41±0.00	4.80±3.55	10.76±0.15	18			
630	5	1269.66±85.80	6.38±5.75	10.06±0.27	19	336.91±8.51	44.34±1.51	7.59±0.07
630	6	-	-	10.67±0.35	9	-	-	-
630	8	-	-	9.91±0.00	8	-	-	-
630	10	-	-	6.03±0.10	6	445.70±72.43	49.68±7.58	8.97±0.09
720	1	1824.41±0.00	1.04±0.00	12.41±0.74	13	307.65±9.41	37.57±0.01	8.19±0.25
720	2	1909.06±0.00	1.53±0.14	12.32±3.02	13	-	-	-
720	3	1335.95±0.00	1.85±0.14	10.88±0.82	13	318.95±0.85	37.30±3.39	8.59±0.80
720	4	2064.29±0.00	3.50±1.10	10.49±0.55	12			
720	5	1242.68±159.77	3.62±2.04	9.76±0.26	16	336.20±0.52	40.60±1.93	8.29±0.41
720	6	-	-	8.52±0.00	8	-	-	-
720	8	-	-	7.75±0.00	6	-	-	-
720	10	-	-	6.48±0.00	7	588.10±6.63	61.71±3.16	9.55±0.60
810	1	1618.91±0.00	2.69±0.14	10.06±0.42	12	280.86±4.70	33.80±1.09	8.31±0.13
810	2	1739.87±0.00	4.28±0.14	15.01±6.33	9	-	-	-
810	3	1560.39±0.00	4.51±1.49	10.75±1.09	10	303.98±17.42	35.78±2.06	8.50±0.00
810	4	1791.86±0.00	2.10±0.27	9.65±0.14	9	-	-	-
810	5	1040.62±77.46	1.75±0.00	9.46±0.00	11	365.62±	45.14±2.00	8.10±0.07
810	6	-	-	9.56±0.12	6	-	-	-
810	8	-	-	5.62±0.00	8	-	-	-
810	10	-	-	3.65±0.07	5	593.29±0.00	112.34±9.29	5.30±0.44

The combined composition of proteins and carbohydrates in FS is between 12 and 55% (Rose et al., 2015). The concentration of sProt and sCarbs initially increased with MW treatment then decreased. The untreated FS contained 1105.98±92.01g/kg.TS sProt and 6g/kg.TS sCarbs while their highest concentrations at 630W, 720W and 810W were 1958.41±0.00g/kg.TS, 2064.29±0.00g/kg.TS and 1791.86±0.00g/kg.TS, respectively, all achieved after MW treatment for 4 minutes. Compared to the initial concentration, sProt increased by 77%, 87% and 62% while sCarbs increased by 217%, 167% and 100% after MW treatment at 630W, 720W and 810W, respectively. More carbohydrates were released into soluble form than proteins because carbohydrates are mostly in the sludge flocs while proteins are mostly intracellular compounds (Houtmeyers et al., 2014). Therefore, the temperatures achieved during MW treatment were likely high enough to disrupt sludge flocs but unlikely to achieve a significant disintegration of cell walls.

Nitrate and ammonium concentration in the untreated FS was 1.76±0.74g/kg.TS and 10.20±2.06

g/kg.TS, respectively which initially increased during MW treatment then decreased. The highest concentration of NO_3^- and NH_4^+ recorded were 6.38±5.75g/kg.TS and 15.01±6.33g/kg.TS, respectively.

Analysis of untreated and MW treated sludge was performed to determine the effect of MW treatment on C and N content. The concentration of these elements did not significantly change during MW treatment and ranged between 280.86±4.70-593.29±0.00gC/kg.TS and 33.80±1.09-112.34±9.29gN/kg.TS, respectively which were similar to those reported in other literature (Septien et al., 2020; Septien et al., 2018b) for VIP latrine FS. The nutrient content of the MW treated sludge in terms of C and N were in the same range as those reported for compost and manure (Ghaly & MacDonald, 2012). Therefore, the treated sludge could possibly be used as a soil conditioner.

To determine the biological treatability of the FS, the C:N ratio was computed for both the untreated and MW treated FS. The C:N ratio is an important index used to determine the suitability of organic material for biological treatment. Microorganisms use carbon for energy and nitrogen for growth during the digestion of organic material. Thus, the optimal balance of these elements makes biological treatment efficient. Although a precise C:N ratio is not necessary, the ideal is between 25:1-30:1 (Panigrahi & Dubey, 2019). The C:N ratio calculated in this research ranged between 5.08:1±2.98 in the untreated FS sample and 5.30:1±0.44 to 9.55:1±0.60 in the MW treated FS samples. The C:N ratios were within the range of human faeces and dried FS (Septien et al., 2020, Septien et al.,2018b).

4.12 Correlation between sCOD and macromolecules

A correlation heat map shows a correlation matrix between two discrete dimensions using colored cells to represent data. Correlation values range from +1 to -1 with positive values indicating a linear increase in the two compared variables while negative correlation values show an inverse relationship between the two compared variables. A correlation value of zero mean that there is no relationship between the two variables. The closer is the correlation value to 1 (+ or -), the stronger is the relationship. The intensity of the color in the correlation heat map indicates the strength of the relationship between the two variables.

Pearson's r correlation, presented as a heat map between sCOD and its predictors (sProt, sCarbs, ammonium, and nitrate), is shown in Fig 4.8 (a-c). The concentration of sCOD increased with an increase in sProt *(r = 0.9)* and sCarbs *(r = 0.8)* (Fig 4.8a). The other components (NH_4^+-N and NO_3^--N) also contributed to the increase in sCOD though to a much lesser extent (*i.e., r = 0.5 and 0.3 for* NO_3^- *and* NH_4^+*, respectively*). The increase in sProt, sCarbs, NH_4^+ and NO_3^- confirm the MW effect on disrupting sludge flocs. Their increase is a result of the transfer of biopolymers in solution which increased the sCOD leading to a positive correlation.

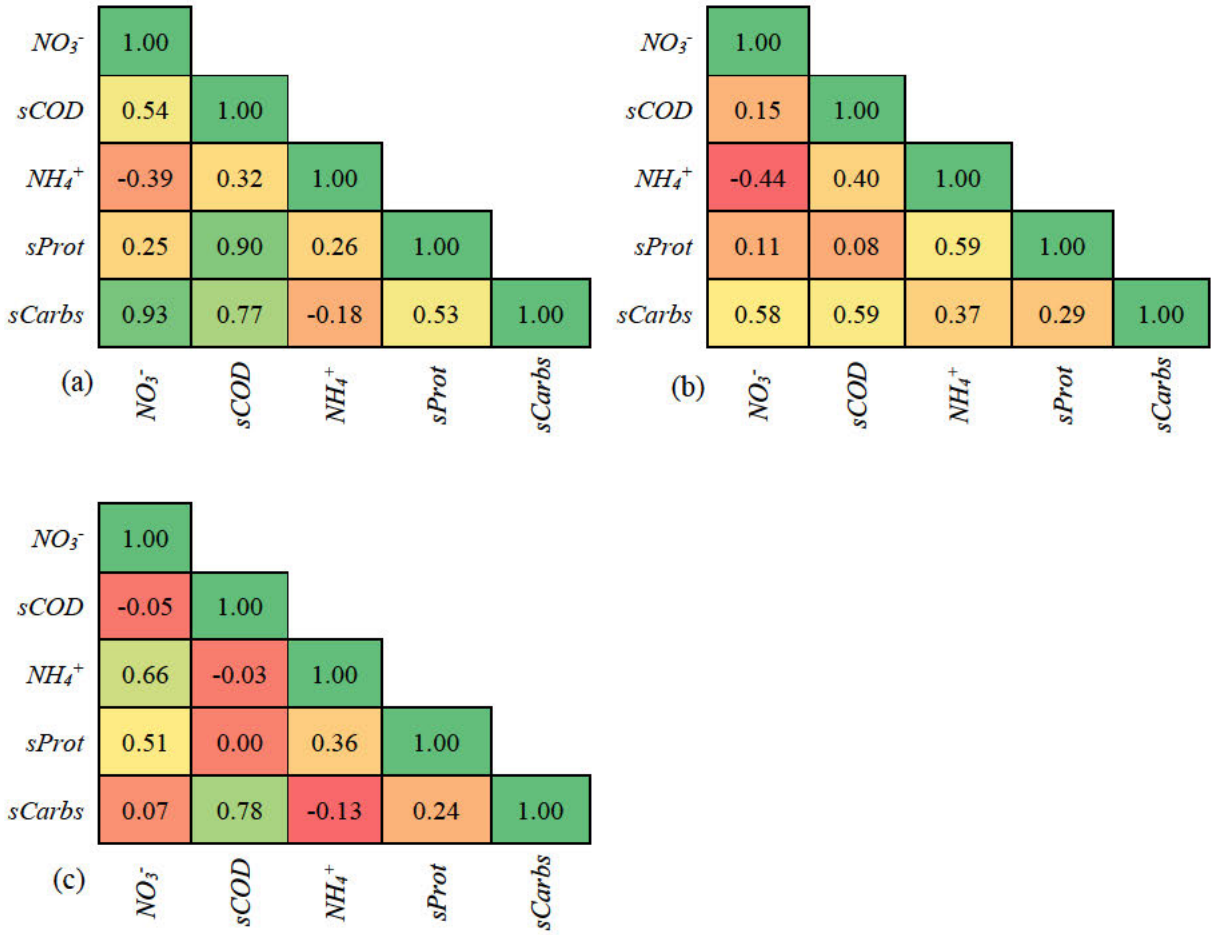


Figure 4.8 Pearson's r correlation heat map between nitrates, sCOD, ammonium, sProt, and sCarbs. The FS sample (S3) was treated by MW at 630W (a) 720W (b) and 810W (c) for 1 to 5 minutes. The level of correlation is represented

The contribution of sProt diminished at MPs of 720W and 810W. There was virtually no sProt contribution to sCOD at 810W (r =0.0). The observation was supported by the negative correlation between sCOD with NH_4^+-N and NO_3^--N at 810W. Also collaborating this observation are the sCOD profiles in section 4.10 (Fig 4.7b-c). Therefore, the observed increase in sCOD is mainly due to the increase of sProt and sCarbs.

4.13 FTIR spectra analysis of untreated and microwave treated VIP latrine sludge

FTIR analysis was used to understand the major functional groups in the untreated FS samples (S3) and their transformation during MW treatment of FS samples. The interpretation of the observed spectra and their spectral band allocation was based on literature on FTIR of human biowastes and reference materials (Afolabi et al., 2017b; Krueger et al., 2021). The observed functional groups and their band allocation is presented in Table 4.6.

Table 4.6 IR absorption band regions for the untreated and microwave treated FS (S3)

cm^{-1} Range	Functional Group
3500-3200	O-H stretch, N-H stretch, alcohol, phenols, primary, secondary amines, amides
3300-2500	O-H stretch, C-H stretch, carboxylic acids, alkanes
2260-2100	−C≡C− stretch, alkynes
1650-1580	N-H bend, primary amines
1550-1475	N-O asymmetric stretch, nitro compounds
1500-1400	C-C stretch (in ring), aromatics
1390-1300	—O—N=O symmetric stretch, nitro compounds
1335-1250	C-N stretch, aromatic amines
1320–1000	C–O stretch, C-H wag (-CH_2X), C-N stretch, alcohols, carboxylic acids, esters, ethers, alkyl halides, aliphatic amines

Several peaks were observed in different regions of the IR spectrum. Absorption band in the region of 3500-3200cm^{-1} were attributed to the presence of O-H and N-H stretch functional groups present in alcohols, amines, and amides. The O-H functional group was also present in the 3300-2500cm^{-1}. Also present in this band was the C-H stretch functional group. These functional groups were attributed to carboxylic acids and alkanes. Other peaks were observed between 2260-2100cm^{-1} and were attributed to the C=C stretch in alkenes. The N-H bend functional group was present in the region of 1650-1580cm^{-1} which indicated the presence of primary amines. Absorption bands in the range of 1550-1475cm^{-1} and 1390-1300cm^{-1} indicated the presence of N-O asymmetric and symmetric stretches, respectively. The N-O functional groups in these regions indicated the presence of nitro compounds in the FS. A C-C stretch associated with aromatic hydrocarbons was observed between 1500-1400cm^{-1}. Also, functional groups associated with aromatic amines were observed at 1335-1250cm^{-1}. Finally, the C-O stretch was observed at 1320-1000cm^{-1}.

The presence of O-H functional groups could be attributed to OH groups in cellulose, hemicellulose and starch which are principal components of carbohydrates. The presence of the C-C and C-O functional groups in the region of 13200-1000cm^{-1} (which is the fingerprint region for carbohydrates) supports the above conclusion (Guo et al., 2018).

Overall, MW treatment did not change the structure of the compounds in the FS as most functional groups were present in both the untreated and MW treated samples. This observation was expected because the temperatures achieved during MW treatment were not high enough to induce structural changes. However, spectral intensities diminished after MW treatment, which implied that the FS stabilized.

4.14 Surface morphology

SEM micrographs of the FS (S3) floc structure provided visual evidence of the series of changes induced by MW treatment (Fig 4.9b-j). Images from SEM showed that the untreated sample had an undisturbed structure with fewer observable pores (Fig 4.9), while the treated samples were more porous, fibrous, rough, and agglomerated (Fig 4.9)

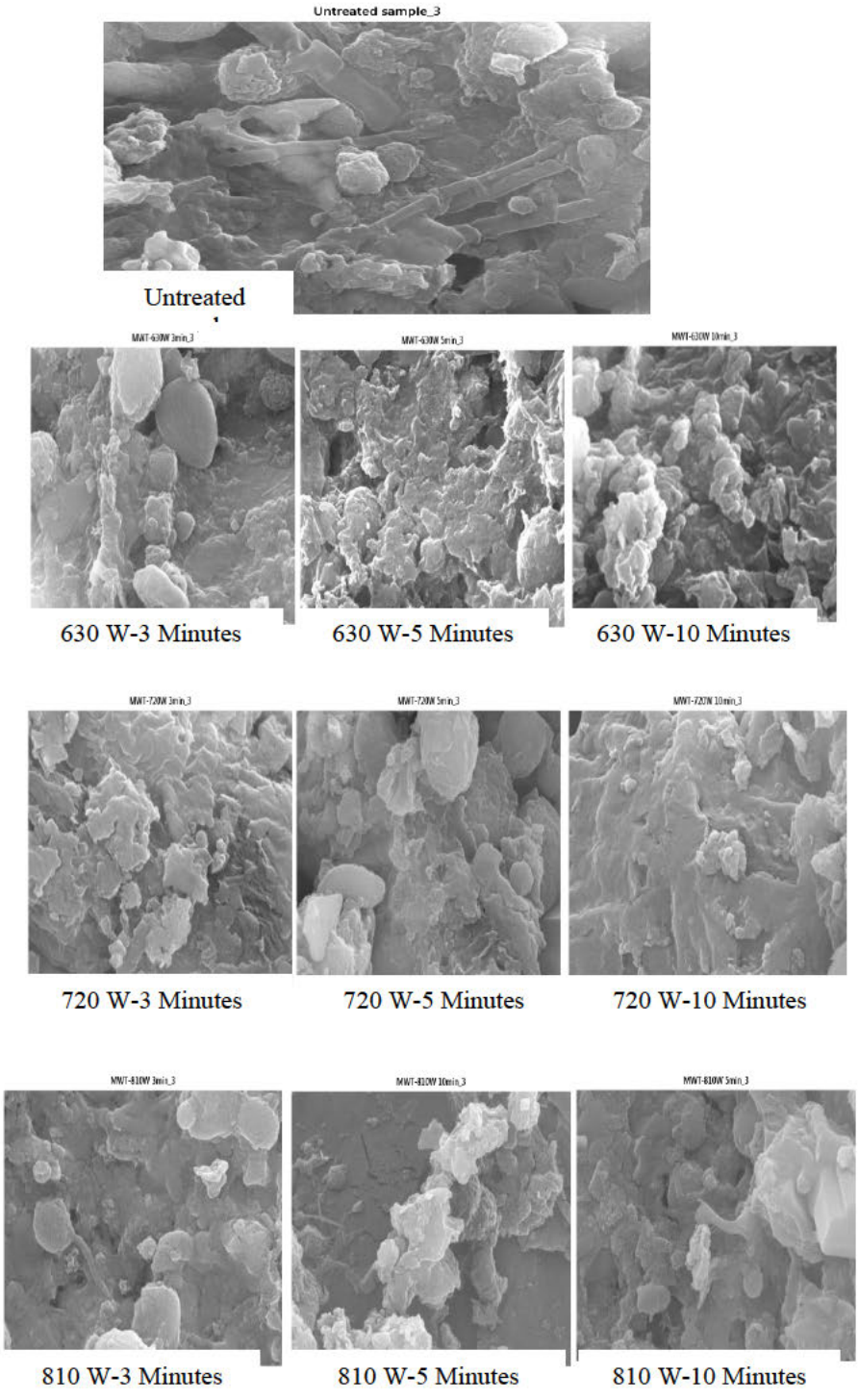


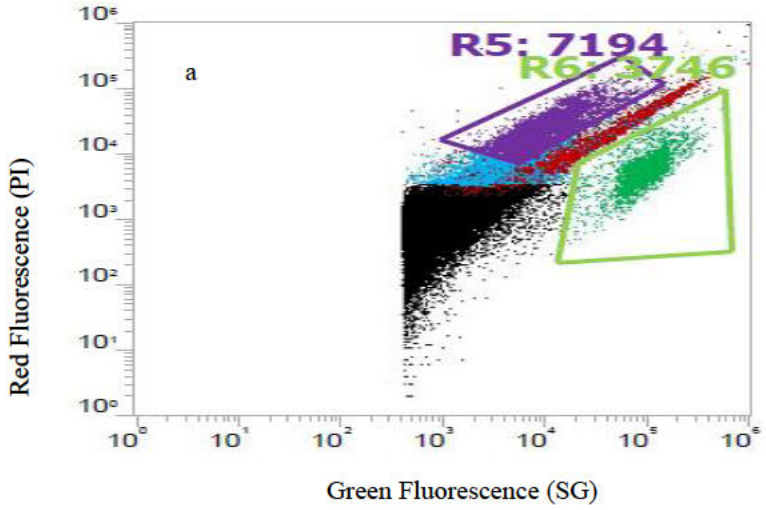
Figure 4.9 SEM images of the untreated and microwave treated FS (S3) samples

The porous microstructures may have resulted from microwave selective heating of polar molecules, which resulted in the dissociation of bound water. Microwave volumetric heating causing a rapid transfer of free water from the core to the surface, before removal by evaporation would leave a porous structure. Roughness and agglomeration can also be caused by fracturing bonds and forming disulfide or isopeptide bonds (Xiang et al., 2020).

The rough and agglomerated structures could explain the sCOD degradation phase (Phase 2 in Fig 4.7) as they could have entrapped the solubilized organics. Porous structures could be advantageous during FS dewatering and drying because the pores would make water flow more easily and result in quicker drying and dewaterability of the digestate after digestion of the MW treated FS (Afolabi et al., 2015). Improved porosity is also advantageous because it enhances air distribution during combustion of MW-derived FS solid chars and results in their fast reactivity during thermal decomposition in the air (Afolabi et al., 2015).

4.15 Effect of microwave pretreatment on microbial viability

Flow cytometry was used to distinguish viable and dead bacteria in FS (S3) samples by simultaneous staining with SYBR-Green (SG) and Propidium Iodide (PI). SG is a dye which enters both viable and dead cells while PI enters dead cells only. Simultaneous staining of the sample with SG and PI activates energy transfer between the fluorochromes (dyes) making live bacteria emit green fluorescence while dead bacteria emit red fluorescence (Foladori et al., 2010; Li et al., 2019). Fig 4.10 shows all events (number of cells and particles that passed through the laser) and gated events (number of cells of interest) in both the untreated and MW pretreated samples.



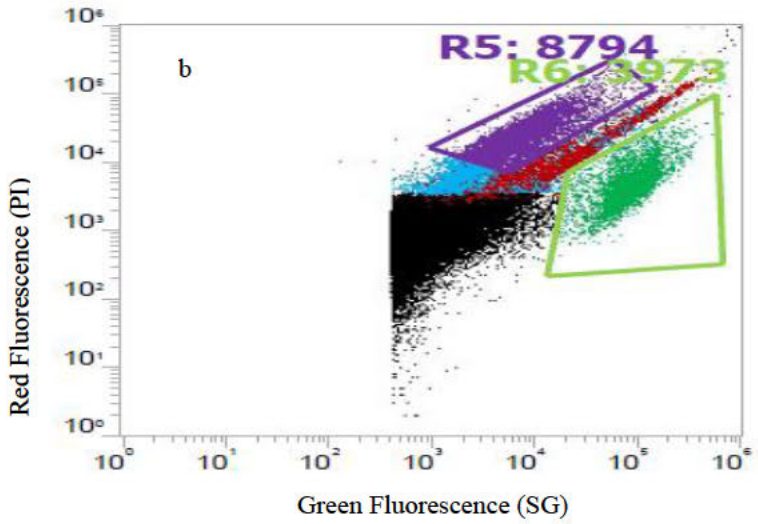


Figure 4.10 FCM cytograms showing dead (red) and live (green) bacteria for untreated (a) and MW treated (b) FS (S3) samples

There were 101095 and 98442 total events in the untreated and MW pretreated FS, respectively. Out of these events, only 10940 (10.82%) and 12767 (12.97%) were found to be associated with live and dead bacteria cells in the untreated and microwave pretreated FS, respectively. Thus, over 85% of the total events were associated with debris in the sample. Further analysis by gating events of interest yielded the statistics that showed the total and percentage of dead and live bacteria cells in the sample. Both samples had more dead bacteria cells than live cells. MW pretreated FS contained a slightly higher number of dead bacteria cells (69%) than the untreated FS (66%).

4.16 Effect of microwave pretreatment on methane production and yield kinetics

BMP tests were used to determine the digestibility of the untreated and MW treated FS (S3) samples. The untreated and MW pretreated FS were also mixed with wood chips at a ratio of 1:1 to evaluate the effect of co-digestion on methane production. FS pretreatment was carried out at 630W for 4 minutes as this had been determined from the sCOD increases to be the optimum pretreatment conditions. Table 4.7 presents the characteristics of the untreated and MW pretreated FS fed into the reactors.

Table 4.7 Characteristics of the feedstock

Parameter	Unit	RFS	RFW	MFS	MFW	Inoculum
pH	-	8.05	8.00	8.09	7.96	7.92
TS	%	29.67±0.52	61.61±0.01	29.65±0.54	60.12±0.81	4.50±0.10
VS	%	17.24±0.36	51.41±0.15	17.74±0.21	49.80±1.86	3.35±0.07
VS/TS	%	58.11±2.25	83.44±0.26	59.84±0.37	82.82±1.97	74.40±0.14
TCOD	g/kg.TS	983.54±3.56		911.54±2.34		

RFS=Untreated FS; RFW=Untreated FS with wood chips; MFS=Microwave pretreated FS; MFW=Microwave pretreated FS with wood chips

The TS and VS in untreated and MW treated FS were comparable. The FS mixed with wood chips had the highest TS and VS due to the addition of dry carbon rich material. The VS/TS of the untreated and MW pretreated FS were within the range of stabilized sludge (~55-60%) and typical of VIP latrine FS in the study area (Zuma et al., 2015). Since the VS/TS in the untreated and treated FS were comparable and the organic loading rate into the reactors were similar, the observed difference in methane production can be attributed to the effect of microwave pretreatment.

4.16.1 Cumulative methane and specific methane production

Cumulative and specific methane production of the untreated and microwave pretreated FS (S3) and their co-substrates were determined from batch tests. The profile of cumulative methane production is presented in Fig 4.11. The batch tests were run for 26 days due to logistical issues. However, the methane production in the untreated and MW pretreated FS had already levelled off during the previous three consecutive days which supported the decision to end the test.

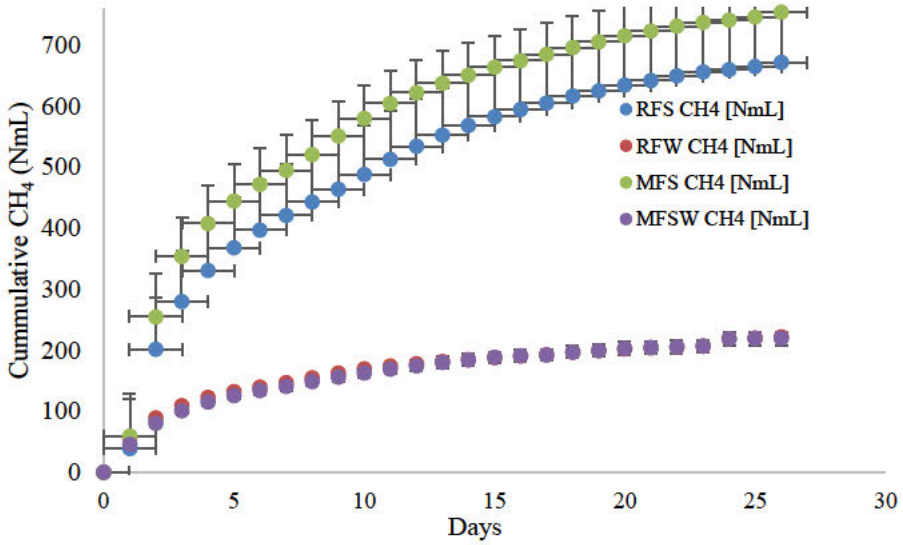


Figure 4.11 Cumulative CH_4 production from the untreated and microwave pretreated FS. In the graph RFS=Untreated FS; RFW=Untreated FS with wood chips; MFS=Microwave pretreated FS; MFW=Microwave pretreated FS with wood chips while error bars represent standard deviation

The cumulative methane production was higher in microwave pretreated FS than in the untreated FS. Cumulative methane production from MW treated FS was 783NmL while the untreated FS produced 672NmL of methane, which was significantly lower ($p<0.05$). Further, the specific methane production of untreated FS was lower than the MW treated FS. While MW treatment resulted in a specific methane production of 128NmL CH_4/g.VS, it was only 110NmL CH_4/g.VS

in the untreated FS. The MW enhancements in cumulative and specific methane production were approximately 17% and 16% over the untreated FS, respectively.

Co-digestion of the untreated and MW treated FS with wood chips was performed in batch assays alongside the other experiments. Wood chips were screened through a 1.6mm sieve. Particles <1.6mm were mixed with the untreated and MW treated FS at a ratio of 1:1. The cumulative methane production from co-digestion during the study period was lower than that in mono-digestion (Fig 4.11). The low methane production from co-digestion could possibly be due to the inoculum used which was collected from an active anaerobic digester treating WAS. Therefore, the microbial community present in it were already acclimatized to that substrate. Thus, it is possible that a longer period was required to adapt to their new substrate (FS with wood chips) than the 26 days used in the current study which was not long enough to produce higher levels of biogas.

4.16.2 Organic loading rate and methane production rate

The OLR and r_G relate to the amount (VS) of substrate added to the reactor with reactor volume (L) and time (days). Although low values of methane production may indicate that pretreatment is not a viable step, OLR and methane production rate should also be considered when evaluating the viability of pretreatment. The OLR was calculated from VS added to the reactor and the time required to reach 80% of the cumulative methane for each feed substrate (Serrano et al., 2016; Yuan et al., 2019). Table 4.8 presents the values of OLR and methane production rate for the various feed materials used in this study.

Table 4.8 Organic loading rate and methane production rate

Substrate	OLR (gVS/L.day)	r_G (NmL/L.day)
RFS	1.18	103.26
RFW	1.35	36.98
MFS	1.39	137.04
MFW	1.25	33.71

RFS=Untreated FS; RFW=Untreated FS with wood chips; MFS=Microwave pretreated FS; MFW=Microwave pretreated FS with wood chips

MW treatment increased the OLR and methane production rate by 18% and 33%, respectively. These results show that the MW treatment of FS can increase the amount of material treated per unit volume of the reactor and increase methane production.

Consequently, smaller reactor volumes could be used and reduce land footprint from utilizing large anaerobic digesters. The enhanced rate of methane production shows that the digestion process is faster. Hence, more material can be digested within a given period.

4.16.3 Digestate analysis

The digestate from all the reactors was collected after the AD tests. Aliquots of 40mL from each reactor were centrifuged for 30 minutes at 4000 rpm. The centrifuged samples were allowed to stand overnight before decanting the supernatant liquid. To understand the distribution of nutrients, carbon and nitrogen content were analyzed on the digestate cake (the solid remaining after decanting the supernatant) and the supernatant. Table 4.9 presents the results of the carbon and nitrogen in the cake and supernatant.

Table 4.9 Carbon and nitrogen content in the digestate

	Digestate cake		Supernatant	
Substrate	C (g/kg.TS)	N (g/kg.TS)	C (g/kg.TS)	N (g/kg.TS)
Inoculum	2092.70±168.61	383.83±39.98	148.85±41.82	17.40±13.20
RFS	1794.62±25.65	213.06±22.81	97.75±34.83	6.31±2.40
RFW	1446.07±82.40	116.73±8.10	308.93±360.92	
MFS	1677.81±22.82	192.61±9.97	183.26±36.36	0.41±0.15
MFW	1758.64±102.17	123.27±14.04	78.74±0.00	

RFS=Untreated FS; RFW=Untreated FS with wood chips; MFS=Microwave pretreated FS; MFW=Microwave pretreated FS with wood chips

It is seen in Table 4.9 that the carbon and nitrogen were more concentrated in the cake and that the values were higher for the samples from reactors treating untreated FS than those treating microwaved FS. This observation confirms that MW treatment made carbon and nitrogen easily accessible to the microorganisms leading to a higher utilization (Çelebi et al., 2021). Almost 95% and 97% of the carbon and nitrogen in the digestate from reactors treating untreated FS were in the solid phase (cake) while the cake from reactors treating microwaved FS contained 90% carbon and almost all the nitrogen (99%). The distribution of carbon and nitrogen after dewatering of the digestate could be interesting to faecal sludge treatment plant operators because it suggests that dewatering of the digestate would be a viable option if the two streams (solid and liquid) would be treated differently. Firstly, dewatering (by centrifugation) concentrated the nutrients in the solid phase. Hence, the cake could be used as a soil conditioner or processed into solid fuel. Secondly, by concentrating the carbon and nitrogen in the solid phase, dewatering reduced the organic and nutrient loading in the liquid phase. Therefore, the supernatant would be co-treated with wastewater without causing a significant upset in the wastewater treatment system.

4.17 Overall discussion of the results in relation to research questions and hypotheses

This study evaluated the effect of MW treatment on FS characteristics and the subsequent biomethanation. Conventional and advanced analytical techniques were used to study the changes in FS characteristics after microwave treatment and compared them to the characteristics in the

untreated samples. The study was designed to provide answers to questions raised in section 1.3, achieve the objectives presented in section 1.5 and confirm or reject the hypotheses in Table 1.2.

The results of this study showed that temperature increased during MW treatment and showed two distinct evolution phases. Understanding temperature profiles during MW treatment is important as they directly relate to the specific FS drying and COD solubilization phases (Mawioo et al., 2016a; Tang et al., 2010a). Also, temperature is a critical operating parameter to optimize the MW process for pathogen inactivation to ensure safe handling of FS (Mawioo et al., 2016a). Heat was generated when microwaves interacted with the unbound moisture in the FS during treatment, allowing for a rapid increase in temperature (Mawioo et al., 2016; Yu et a., 2010). In the initial stage, the supplied energy was used to increase the temperature of the unbound water in the FS resulting in a rapid initial temperature increase (phase 1). When the water reached its boiling point, it was constantly evaporated from the FS surface and replaced by the water from the inside of the FS sample. Hence, the supplied SE evaporated the water than increase the temperature of the FS (phase 2) (Mawioo et al., 2016a).

The rate of temperature evolution increased linearly with input MP. An appropriate MP could therefore be selected depending on the objective of the MW treatment. High MP input result in high temperature increase rates which induce rapid drying and volume reduction of FS (Mawioo et al., 2016a; Mawioo et al., 2017). On the other hand, low input MW power induce low temperature increase rates which result in slow but efficient solubilization (measured as sCOD/TCOD) of organic matter in sludge (Toreci et al., 2010).

The rate of temperature increase is more rapid during MW heating than conventional heating (Eskicioglu et al., 2007). Hence, smaller reactor volumes, high loading rates and shorter contact times could be used. These attributes could make MW treatment a compact process technology to tackle the FS treatment challenge during emergencies and in informal settlements where land is scarce (Mawioo et al., 2016a).

In this study, the temperature was measured using an infrared thermometer, which possibly measured the surface temperature or air close to the FS. Therefore, the reported temperatures could be lower than those attained by the sludge. However, the temperature profiles would be similar, regardless of the temperature measurement technique.

The VS/TS is an essential parameter in AD. It indicates the amount of organic matter potentially available for microorganisms during AD. Although the TS and VS content increased after MW treatment, the VS/TS ratio remained almost similar and ranged between 58-62%, 41-43% and 56-68% for the S1, S2 and S3 FS samples, respectively. The percentage VS increase in the treated FS samples signaled that there was no loss of VS during treatment. This trend indicated that MW treatment neither led to the loss of VS nor accumulated unwanted materials in the FS, but rather

led to moisture evaporation. The highest temperatures recorded (97℃ for S1, 92℃ for S2 and 99℃ for S3) were below the temperature at which moisture analysis is carried out (105℃) to remove moisture, but not either evaporate or ignite volatile solids (APHA/AWWA/WEF, 2017).

Sludge collection and transportation is a significant cost borne by the household in a FSM-based sanitation service (Dodane et al., 2012). Thus, volume reduction could reduce FS collection and transportation frequency, translating to reduced expenses by the household. This study and previous studies (Mawioo et al., 2017; Mawioo et al., 2016a) showed that moisture contributes over 70% to FS weight/volume. Therefore, volume reduction during MW treatment could be attributed to the loss of moisture (Mawioo et al., 2017; Mawioo et al., 2016a). Sludge volume reduction is an important treatment objective as it relates to the volume of solids that can be transported and eventually disposed of (Scholz, 2006). MW treatment at the operating conditions reported here did not lead to the loss of volatile matter and the observed volume reduction resulted from the removal of water from the FS. As already explained, the maximum temperature recorded in this study was below the standard temperature for moisture analysis which aims not to volatize solids (APHA/AWWA/WEF, 2017; Velkushanova et al., 2021).

RSM and DOE were used to optimize solubilization of sCOD by investigating the effect of MP and treatment time was. The collected experimental data fitted first and second-order models. The first-order model was used for exploratory experimentation, i.e., determining important factors to be considered in the experiment while a second-order model determined the significance of model parameters; estimated mean response; and arrived at optimum operating conditions.

The analysis of the response surface and contour plot (Fig 4.2) showed that FS solubilization increased at low MP while increasing the treatment time. The plot of SE versus percentage solubilization (sCOD/TCOD) indicated a maximum sCOD percentage that could be achieved, however, as this is due to a combination of MP and treatment time, the surface response rather pinpoints the optimal treatment settings for both as individual factors. So, whilst different combinations of MP and time can result in the same SE, the model fit to the two sets of data can optimize both input MP and treatment time. Both plots indicate that there are maximum MP and treatment time for optimal sCOD solubilization, with increases of sCOD solubilization up to an optimum, after which increasing input MP will reduce solubilization for a given treatment time and increasing treatment time would decrease solubilization at a given input power setting. It also indicates that the optimum input power for COD solubilization is as given i.e., Around 630W, a higher range of MP settings still result in higher solubilizations but over shorted times. To that end an experimental design based upon a range of input power settings (630W, 720W and 810W) with periods of time focused on low treatment time intervals for digestion (from the contour plots, it was expected that solubilization of COD would be optimum in a range of times up to 5 minutes at the lower power, up to slightly lower at the higher MP) were selected.

The percentage sCOD increase following the optimization tests were lower than those reported in literature for sewage sludge, whose increment could be as high as 215% (Appels et al., 2013; Serrano et al., 2016). The difference is attributed to the nature of the sludge used. Whilst the sludge used by Appels et al., (2013) and Serrano et al., (2016) was relatively fresh (sCOD/TCOD ~ 3%), the FS used in this research had a higher sCOD/TCOD ratio (~ 11%). In addition, the generally low concentration of waste activated sludge (~2.3% TS) meant that the sludge took longer to reach the boiling point because of its high thermal absorption capacity i.e., much of the heat applied is absorbed by the water fraction of the sludge (Mawioo et al., 2016a). Although low sludge concentration makes the MW process inefficient in terms of energy use (Tang et al., 2010), it is advantageous for sCOD release as it increases the MW-sample interaction (Toreci et al., 2010) before it attains the boiling point. The percentage sCOD release in this research, however, was higher than those reported by Kor-Bicakci et al., (2019) who used TWAS and achieved a maximum sCOD increase of 91.2 %. The TWAS used by Kor-Bicakci et al., (2019) was relatively stabilized (sCOD/TCOD ~ 13.5 %) compared to the one used in this study (~11 %). These observations suggests that the initial sludge sCOD/TCOD is an important determinant of the efficiency of organic matter solubilization during MW treatment. Overall, the observed sCOD increase was a clear indication of the microwave effect on FS organic matter solubilization.

The sCOD/TCOD ratio is used to evaluate the effect of MW treatment on the release of soluble organic matter. The sCOD/TCOD ratio is an important index in biological treatment of sludge as it relates to the extent of hydrolysis (Yu et al., 2010), a limiting step in anaerobic digestion. The sCOD/TCOD in the untreated FS used in the optimized testing regime was 11%, which indicated that it underwent stabilization during storage in the pit latrine. Indeed, this was expected for FS collected from VIP latrines in the study area because the average age of the FS in VIP latrines is estimated at five years which coincides with the scheduled pit emptying cycle (Brouckaert et al., 2013). Although the stabilization of the FS in pit latrines is stratified (Bakare et al., 2012), the FS used in this study was a composite. Therefore, the observed sCOD/TCOD represented the nature of the FS that would be delivered to a treatment plant and could provide insights into the design of the FS treatment processes. At the time of reporting these results, only the work of a former WASH R&D Centre MSc student reported sCOD of FS in VIP latrine (Nwaneri, 2009). In that work, FS samples were collected from 16 VIP latrines in same study area and characterized for different COD fractions. Using that data, a sCOD/TCOD ratio of the untreated FS was computed to be 16.6% which was higher than the value for S3 (11%) sample but lower than that of S1 (22%) and S2 (30%) samples. These variations were attributed to the intrinsic variations in FS characteristics, both within and among the pit latrines

(Zuma et al., 2015). Due to limited data on the solubilization of FS, the data in this study were compared to data reported for other types of sludge.

Although the initial sCOD/TCOD ratios for wastewater sludge are generally lower than those for FS, MW treated FS yielded similar sCOD/TCOD profiles to those of wastewater sludge (Apul & Sanin, 2010; Çelebi et al., 2020; Eswari et al., 2016; Xue et al., 2015) (Fig 2.7-2.10). The sCOD release phase (phase 1 in Fig 4.1 and 4.7) was a result of the MW effect that disintegrated sludge flocs and disrupted cell walls and released organic biopolymers (Yu et al., 2010) while the sCOD degradation phase (phase 2 in Fig 4.1 and 4.7) was due to the evaporation of organics (Sólyom et al., 2011). Although there was a second phase of sCOD release (phase 3 in Fig 4.7) during MW treatment, its increment was not higher than the first one. Therefore, the operation can stop at the first peak as continued operation might waste energy and do not result in additional sCOD. The observed sCOD/TCOD profile corresponded to the temperature profiles (Fig 4.3). At each of the MP applied, the first phase resulted in the highest sCOD release and coincided with the FS boiling point. Temperature is quick to measure and can be automated, thus, temperature profiles could be used to provide a quick insight into the operating conditions that would result in high sCOD release.

A correlation heat map confirmed the release of soluble macromolecules and their relationship to sCOD. The relationship was strong and linearly positive at low MP applied and the sCOD release was a result of the release of sProt ($r = 0.90$) and sCarbs ($r = 0.77$) from the FS matrix and cell walls (Charnier et al., 2018). At high MP applied (810W), sProt did not contribute to the observed sCOD release ($r = 0.00$) which suggests that the protein was denatured (Xiang et al., 2020).

sProt, sCarbs, soluble carbohydrates, NH_4^+, NO_3^-, C and N also increased after MW treatment, which confirmed the MW effect on organic matter solubilization. Applying high energy decreased nutrient concentration, possibly due to binding of the nutrients to the solid fraction during drying (Septien et al., 2020; Septien et al., 2018b). The bound nutrients are released slowly to the soil giving crops more time to uptake the nutrients. In addition, the slow release of nutrients minimizes the potential of nutrient loss through leaching or evaporation, and it allows the nutrients to fertilize the soil for long periods. Thus, leading to efficient nutrient uptake by crops, requiring fewer applications, and do not present the risk of root burns (Guertal, 2009; Gutser et al., 2005).

Carbon provides the energy required by microorganisms to digest organic matter while nitrogen is required for microbial growth. Microorganisms require a suitable C:N ratio to function and grow. Hence, biological treatment is sensitive to C:N ratio because it could indicate the release and accumulation of ammonia and volatile fatty acids in the digester. MW treatment increased the C:N ratio (thus improving digestibility of the treated FS samples) but did not raise it to a range (25-30:1) suitable for biological treatment (Panigrahi & Dubey, 2019). The low C:N ratio implied

that the FS contained more nitrogen per the required carbon for microorganisms, hence nitrogen could be lost as ammonia (and possibly causing ammonia inhibition during biological treatment). Therefore, co-composting and/or co-digestion with carbon rich-material would be necessary to improve the C:N ratio and the efficiency of the subsequent digestion process (Borowski, 2015; Panigrahi & Dubey, 2019).

FCM results showed the presence of viable bacterial cells in both untreated and MW treated FS samples. At similar temperatures to the present study, previous studies on MW and conventional heating reported significant reduction in E coli (Mawioo et al., 2016a) and Ascaris eggs (Naidoo et al., 2020). Therefore, the presence of large numbers of bacteria cells (31%) in MW treated FS samples could be attributed to contamination and/or regeneration. The microbial integrity of the MW treated FS samples would likely have been compromised during storage and transportation. As the MW treatment was carried out in the WASH R&D Centre laboratory in South Africa while FCM analysis was done at the IHE Delft Institute for Water Education laboratory in the Netherlands, the long storage period in the WASH R&D Centre laboratory and shipment of the FS to IHE Delft Institute for Water Education may have resulted in the contamination of the FS samples. The results in this case are therefore inconclusive, however, this may be a future technique to consider when determining microbial viability analysis if carried out immediately after treatment of a FS sample. Despite the challenges, FCM showed potential for quick evaluation of the efficiency of FS treatment processes on pathogen inactivation.

BMP tests showed that MW treated FS samples produced more methane than the untreated FS samples. Also, the OLR and r_G of FS increased after MW treatment. Therefore, AD of MW treated FS could be carried out in smaller reactors and for shorter periods (Serrano et al., 2016). Because the untreated and MW treated FS samples had comparable VS/TS ratios, the observed differences in methane production were attributed to the MW effect which liberated extracellular and intracellular biopolymers and made them more easily accessible to the microorganisms during anaerobic digestion (Kor-Bicakci et al., 2019). Although the methane production results in this work were higher than those reported by Couderc et al., (2008) (using FS from the same area) and Rose et al., (2015), they were within the range reported for various types of sludges using different pretreatment methods (Table A.1 in appendix).

The enhancement of methane production after MW treatment was achieved when the FS was treated with an energy of 0.106kWh/kg.TS. Therefore, an energy balance should be undertaken to determine the economic benefits of the pretreatment stage. However, the cost benefit analysis should include the other benefits associated with MW treatment such as volume reduction, pathogen inactivation, reduced digestion time and increased organic loading rates.

In the next chapter (Chapter 5), the conclusions and recommendations for the study are presented.

CHAPTER 5 : CONCLUSION AND RECOMMENDATIONS

The present study evaluated the effect of MW treatment on FS characteristics and the subsequent enhancement of biomethanation. Conventional and advanced analytical techniques were used to study the changes in the FS after MW treatment and compared them to the characteristics in the untreated FS samples.

The rationale behind the choice of MW technology to treat FS was based on the understanding that:

- Faecal sludge can contain up 80% water whose dipolarity would effectively interact with microwaves and result in dielectric heating.
- Faecal sludge is highly concentrated (>15% TS), hence it could lead to efficient MW heating
- Faecal sludge is an organic matter rich resource which could be valorized to nutrients and bioenergy
- MW treatment would achieve several FS treatment objectives in one operation

5.1 Conclusion

The following conclusions can be drawn from the results of this study:

- MW treatment increased the temperature of FS from ~20°C in the untreated samples to a maximum of 99°C. The increase was initially rapid and then remained fairly constant after the FS attained the boiling point temperature.
- Evaporation of water during MW treatment caused a volume reduction of up to a maximum of 58%.
- The percentage VS increased by a maximum of 164% after MW treatment of FS samples while the VS/TS ratios remained fairly constant and ranged between 60% and 69%.
- Although there were signs of bacteria regeneration, MW treated FS samples had lower numbers of viable bacteria cells.
- MW treatment of FS samples increased the sCOD/TCOD from 11% in the untreated FS samples to 30%. Most of the sCOD release occurred in the initial stages (phase 1) of MW treatment. A sCOD degradation phase (phase 2) followed once a certain treatment time was reached at each MW power applied.
- sCOD increase in MW treated samples was positively correlated with the solubilization of sProt and sCarbs. The sCOD release at high MW power used was correlated more to sCarbs than sProt, possibly due to the denaturation of proteins at that MP.
- By applying MW pretreatment, the methane potential of FS was increased by 16% while the kinetic parameters (OLR and r_G) were enhanced by 18% and 33%, respectively.

- Dewatering of the digestate concentrated over 90% of nutrients (C & N) in the cake than in the supernatant.

5.2 Recommendations

- Thorough screening of the FS samples is recommended to remove detritus material. Wet screening using sieves of different sizes could exclude grit from the FS sample thus improving the homogenization of the FS and minimize errors during the analysis of the FS samples.
- The cut off time for FS sample storage in the laboratory should be investigated. Although it is argued that if the FS was not collected it will remain in the pit latrine, analysis of the FS samples after long storage durations does not reflect its characteristics at the time of sampling (mainly done during pit emptying) and hence the quality of the FS delivered for treatment. This is mainly important if the aim of characterization is to design or improve the biological treatment processes like in the present study.
- MW power, temperature and treatment time play an important role in MW treatment of sludge. Temperature could not be controlled in the microwave unit used in the present study. Hence, only MP and treatment time were optimized. It is therefore recommended that a microwave unit which can control not only the applied power and treatment time, but also treatment temperature be used.
- A kitchen type microwave oven was used in the present study and the sCOD profiles that were observed resembled those of wastewater sludge. It is recommended that a pilot-scale microwave oven be used to investigate the feasibility of scale up.
- The long storage duration could have stabilized the FS and the resulting sCOD release after MW treatment could have underestimated the MW effect. Therefore, it is recommended that MW treatment and subsequent physicochemical analyses be done soon after the FS samples are collected and treated, respectively.
- The long storage of FS samples could also have affected the results of the BMP tests. Thus, it is recommended that BMP tests be done soon after the MW treatment of the FS samples.
- The BMP tests were terminated after 26 days of incubation because the time of internship came to an end. It is recommended that the BMP tests be done over a longer period.

5.3 Areas of further research

- Sample preparation (screening and homogenization) is an important step in characterization of FS for designing of biological treatment processes which was the aim of the present study. The current FS sample preparation SOP developed by the WASH R&D Centre laboratory does not specify how screening should be carried out before

homogenization of the FS samples. There is little knowledge on the effect of different screening techniques on the characteristics of FS. Therefore, future research should investigate the effect of manual screening, wet screening and using a sludge trommel (developed by Khanyisa) on FS characteristics. The results of this investigation could form part of the materials included in the revised editions of the Methods for Faecal Sludge Analysis book.

- Long storage duration of the FS could have compromised its biological and physicochemical quality. Therefore, different methods (freeze drying, sterilization and cold storage) and their cut off storage durations should be investigated using different types of FS. The results of this investigation could form part of the materials included in the revised editions of the Methods for Faecal Sludge Analysis book.
- Several advanced analytical techniques such as SEM, FTIR and FCM were used to characterize FS samples in the present study and showed potential application in FS characterization. However, the protocols used followed those of the wastewater and sewage sludge. Therefore, FS specific methods should be developed and included in the future editions of the Methods for Faecal Sludge Analysis book.
- The current study optimized MP and treatment time only. In addition to these, future research should also optimize the specific energy and treatment temperature to investigate their synergistic effect on FS solubilization.
- Although the co-digestion attempted in the present study did not result in significant improvement in methane production, it should be investigated further using different organic waste fractions and over longer digestion periods.
- The present and previous studies have reported that MW treatment of FS produces a material rich in nutrients comparable to composts and manure. Future research should evaluate the effect of application of MW treated FS on soil quality and plant growth.
- A temperature of 99°C was reached in this study, and based on literature, it is postulated that some sort of FS sanitization was achieved. However, this aspect was not evaluated as part of this study and is recommended for future studies.

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APPENDIX A : EFFICIENCY INDICATORS IN SLUDGE PRETREATMENT

Below are the commonly used success criteria to assess the performance of different sludge pre-treatment methods reviewed:

1. COD Solubilization (S_{COD})
   a. $S_{COD}(\%) = \frac{sCOD_t - sCOD_0}{TCOD_0 - sCOD_0}$ x 100
   b. $S_{COD}(\%) = \frac{sCOD_t - sCOD_0}{TCOD_0}$ x 100
   c. $S_{COD}(\%) = \frac{sCOD_t}{TCOD_0}$ x 100

   S_{COD} = COD solubilization

   $TCOD_0$ = Total COD in the untreated sample

   sCOD = Soluble COD at time (t)

   $sCOD_0$ = Soluble COD in the untreated sample
2. Degree of disintegration (DD)
   a. Based on COD solubilization (DD_{COD})

   $DD_{COD}(\%) = \frac{sCOD_t - sCOD_0}{sCOD_{NaOH} - sCOD_0}$ x 100

   DDCOD = COD solubilization

   sCODNaOH = Maximum COD solubilized after alkaline hydrolysis
3. Volume reduction based on the loss of water during treatment
4. Pathogen inactivation
5. Changes in macromolecule concentration
   a. NH4+
   b. NO3-
   c. Proteins
   d. Carbohydrates
6. Enhanced anaerobic digestion performance
   a. Increased cumulative methane production
   b. Increased specific methane production (BMP)
   c. Increased organic loading rate (OLR)
   d. Increased rate of methane production
   e. Increased carbon utilization
   f. Decreased digestion time
   g. Increased VS removal

Table A.1 Effect of different pretreatment methods on the physicochemical characteristics of sludge

Pretreatment	Sludge used	Conditions	Indicator	Unit	Untreated	Treated	Ref
TH	WAS	135 and 190ºC, 35 min and 50 min, held for 30 & 15 min, MAD	sCOD/TCOD	%	7 (32 after 75 days at 4ºC)	44 at 135ºC (34 after 60 days at 4ºC) and 60 at 190°C (46 after 60 days at 4ºC)	Bougrier et al., 2007
			CH_4	mg/g.VS	254	314 (24% at 190ºC)	
TH	WAS	160, 170, 180 & 190°C, MAD	CH4	%		50 (170ºC, 30 min)	(Fdz-Polanco et al., 2008)
TH	WAS	60-180ºC; 0.5-72h	sCOD/TCOD	%		53 (180ºC)	Xue et al., 2015
TH	TWAS	140, 160 & 180ºC; 15, 30, 60 min, MAD	sCOD/TCOD	%	4--13	39-40	(Zhou et al., 2021)
			CH4	mL/gCOD		140--182	
TH	TWAS	50-90ºC; 30-90 min, MAD	sCOD/TCOD	%		20.4	Azizi et al., 2021
			CH_4	%		43 (90ºC, 60 min)	
	TWAS + FPS		CH_4	%		56 (90ºC, 90 min)	
TH	Pig slurry	60 & 80ºC for 3h, MAD	sCOD/TCOD	%		58 (80ºC), 30 (60ºC)	Bonmati et al., 2001
			STKN	%		25 (80ºC), 18 (60ºC)	
			NH_4^+	%		12 (80ºC), 4 (60ºC)	
			TVA	%		13 (80ºC), 12 (60ºC)	
			CH_4	%		60 (80ºC)	
TH	TWAS	50, 80, 100 & 120ºC for 2h	sCOD	%		2600 (120ºC)	(Kang et al., 2016)
			sCOD/TCOD	%	1.04	28 (120ºC)	
TH	DS	6, 8, 9 & 10%TS; 90ºC, 3h, TAD	CH_4	mL/g.VS		210 (8% TS)	Yao et al., 2016
			CH_4	mL/g.VS.d		0.1776 (8%TS)	
			VSS removal	%		23 (8%TS)	
TH	WAS	70, 121, 172ºC, 3h, MAD (35ºC)	DOC	mg/g.VS		267 (172ºC)	Lu et al., 2018
			CH4	mg/g.COD		212 (172ºC)	
TH	DS	2, 4, 6, 8, 10, 12%TS, 90ºC, 1h	sCOD	%		516 (12%TS)	Li et al., 2022
			sCOD/TCOD	%		23 (12%TS)	
			CH_4	mg/g.VS		149 (2%TS)	
			CH_4	mg/g.VS		320 (6%TS)	
			CH_4	mg/g.VS		285 (12%TS)	
			CH_4	%		115 (6%TS)	

			NH_4^+	%		78 (12%TS)	
TH	WAS	150-270ºC, 30 min	sCOD	%		635 (180ºC)	Kim et al., 2015
			sCOD	%		952 (210ºC)	
			sCOD/TCOD	%		31 (180ºC)	
			sCOD/TCOD	%		37 (210ºC)	
			sCOD/TCOD	%		40 (180ºC)	
			sCOD/TCOD	%		42 (210ºC)	
			CH_4	mL/mg.VS	143	343 (180ºC)	
			CH_4	mL/mg.VS	143	344 (210ºC)	
			CH_4	%		60 (180ºC)	
TH	WAS	130, 150, 180ºC, 5, 30, 50 min	sCOD/TCOD	%		37 (150ºC)	Sapkaite et al., 2017
			sCOD/TCOD	%		41 (180ºC)	
			CH_4	%		72 (150ºC)	
			CH_4	%		42 (180ºC)	
TH	Sewage sludge	75, 100, 150, 200, 225ºC, 15, 30, 60, 90, 105 min	sCOD/TCOD	%		30 (180ºC, 76 min)	Choi et al., 2018
			CH_4	mL/g.COD		273 (150ºC, 1h)	
TH	WAS	120-210ºC, 1h	sCOD/TCOD	%		34 (180ºC)	(Lee et al., 2017)
			CH_4	mL/g.VS		322 (180ºC)	
			CH_4	%		39 (180ºC)	
TH	TWAS	140, 160ºC, 40 min	CH_4	mL/g.VS	182.2	250.2 (140ºC)	(Liu et al., 2020)
			CH_4	mL/g.VS	182.2	268.3 (160ºC)	
			CH_4	%		37.3 (140ºC)	
			CH_4	%		47.3 (160ºC)	
TH	WAS	1h, 5h, 80°C, pH-10	sCOD/TCOD	%	1.9	20	(Nazari et al., 2017)
			sProt	mg/L	680		
			sCarbs	mg/L	4090		
			Specific CH_4	%		2	
TH	WAS	100-220°C, 1% (9.9gTS/L), 3% (29.91gTS/L), 5%	sCOD/TCOD	%	2.2-4.6	44-51.2	(Jeong et al., 2019)
			NH_4^+	mg/L	12-228	213-1804	
			Specific CH_4	mL/g.VS	178-187	230-348	

		(49.9gTS/L), 7% (70.06gTS/L)					
Chemical	WAS	pH-5, pH-10 (2M HCl or NaOH to adjust pH)	sCOD	mg/L	374	1948 (pH-5)	Chen et al., 2021
			sCOD	mg/L	374	5563 (pH-10)	
			sCOD	%		421 (pH-5)	
			sCOD	%		1387 (pH-10)	
			sCOD/TCOD	%	1.7	8.2 (pH-5)	
			sCOD/TCOD	%	1.7	23.3 (pH-10)	
Chemical	WAS	pH-9.5, 24h, 0-25% digestion liquid reflux	sCOD	mg/L	1654	2454 (25% reflux)	He et al., 2021
			sCOD	%		48.4 (25% reflux)	
			sCOD/TCOD	%	13	20 (25% reflux)	
			sProt	mg/L	472	1076 (25% reflux)	
			CH_4	mL/g.VSS	172.1	283 (20% reflux)	
			CH_4	%		65 (20% reflux)	
Chemical	SBR	pH-2, pH-10, pH-11, pH-12 (adjusted by NaOH & HCl), 48h	DOC	mg/g.TS	0.9	1.2 (pH-2)	de Sousa et al., 2021
			DOC		0.9	13.7 (pH-12)	
			DOC	%		33 (pH-2)	
			DOC	%		1422 (pH-12)	
			TKN	mg/g.TS	5.9	8.3 (pH-2)	
			TKN	mg/g.TS	5.9	14.2 (pH-12)	
			TKN	%		41 (pH-2)	
			TKN	%		141 (pH-12)	
			sProt	mg/g.TS	2.8	8.9 (pH-2)	
			sProt	mg/g.TS	2.8	131.1 (pH-12)	
			sProt	%		218 (pH-2)	
			sProt	%		4582 (pH-12)	
			sCarbs	mg/g.TS	1.2	4.4 (pH-2)	
			sCarbs	mg/g.TS	1.2	23.7 (pH-12)	
			sCarbs	%		266 (pH-2)	
			sCarbs	%		1875 (pH-12)	
Chemical	WAS	pH-2, pH-5 (H_2SO_4) & pH-10 (NaOH), 25, 40,	sCOD	mg/L		5812 (pH-2, 60ºC, 45 min)	Tulun et al., 2019
			sCOD	mg/L		12702 (pH-5, 60ºC, 45 min)	
			sCOD	mg/L		14680 (pH-10, 60ºC, 15 min)	

		50, 60ºC, 5, 15, 30, 45 min, MAD	sCOD/TCOD	%		13.2 (pH-2, 60ºC, 45 min)	
			sCOD/TCOD	%		29 (pH-5, 60ºC, 45 min)	
			sCOD/TCOD	%		33.5 (pH-10, 60ºC, 15 min)	
			CH_4	mL/g.VS	109.4	122.9 (pH-5, 40ºC, 15 min)	
			CH_4	mL/g.VS	109.4	157.1 (pH-10, 60ºC, 15 min)	
			CH_4	%		12.3 (pH-5, 40ºC, 15 min)	
			CH_4	%		43.6 (pH-10, 60ºC, 15 min)	
Chemical	Secondary SS	pH-10, NH_3 (85mg/L, 250mg/L, 420mg/L, 680mg/L)	sCOD	%		1233	(Wei et al., 2017)
Chemical	WAS	Alkaline ferrate (25-700mg/L), 1h	sCOD	%		244	(He et al., 2018)
			Specific CH_4	%		46	
Chemical	WAS	FNA (2.49mg/L, 3.55mg/L, 4.62mg/L), pH-5.5, 2, 5, 8h	sCOD	%		171	(Zahedi et al., 2017)
			sProt	%		300	
Chemical	Primary SS	FNA (0.77mg/L, 1.54mg/L, 2.31mg/L, 3.08mg/L, 3.85mg/L), pH-5.5, 24h	sCOD	%		233	(Zhang et al., 2016)
US	WAS & DS	150W, 0.04g CaO/g.TS, 30 min, MAD (35ºC)	sCOD/TCOD	%	5.2	7.1 (US WAS alone)	Yuan et al., 2019
			sCOD/TCOD	%	5.2	7.5 (CaO WAS alone)	
			sCOD/TCOD	%	5.2	13.8 (US + CaO WAS)	
			sCOD/TCOD	%	2.1	2.3 (US DS alone)	
			sCOD/TCOD	%	2.1	2.5 (CaO DS alone)	
			sCOD/TCOD	%	2.1	3.3 (US + CaO DS)	
			CH_4	%		40.45 (US + CaO WAS)	
			CH_4	%		36.94 (US + CaO DS)	
			T_{90}	day	15	8 (US + CaO WAS)	
			T_{90}	day	19	4 (US + CaO DS)	
US	WAS	255W, 0.73W/mL, 5, 10, 15, 20, 25 min,	CH_4	mL		278	Celebi et al., 2020
			CH_4	%		28	

		19.1, 38.2, 57.3, 76.4, 95.5kJ/kg.TS					
US	WAS	200W; 0.5, 1.0, 1.5W/mL; 0.5, 1, 2.5, 5, 7.5, 10 min	DD	%		13.4 (0.5W/mL, 10 min), 20.3 (1.0W/mL, 10 min), 21.1 (1.5W/mL, 10 min)	Sahinkaya et al., 2013
			CH_4	%		5.6	
			VS reduction	%	25.8	34.4	
			TCOD reduction	%	37.5	42.9	
			CST	Sec	69.4	52.8	
TH	WAS	60, 80, 100°C; 1h	DD	%		8 (100°C, 1h)	
			CH_4	%		4.2	
			VS reduction	%	25.8	31.1	
			TCOD reduction	%	37.5	40	
			CST	Sec	69.4	50.5	
US + TH	WAS	200W; 0.5, 1.0, 1.5W/mL; 0.5, 1, 2.5, 5, 7.5, 10 min; 60, 80, 100°C, 1h	DD	%		11.8 (0.5W/mL, 1 min, 80°C, 1h), 14.2 (1.0W/mL, 1min, 80°C, 1h), 14.9 (1.5W/mL, 1min, 80°C, 1h), 23.5 (0.5W/mL, 10 min, 80°C, 1h), 31.1 (1.0W/mL, 10 min, 80°C, 1h), 32.1 (1.5W/mL, 10 min, 80°C, 1h)	Sahinkaya et al., 2013
			CH_4	%		13.6 (1.0W/mL, 1min, 80°C, 1h)	
			VS reduction	%	25.8	37.8	
			TCOD reduction	%	37.5	48	
			CST	Sec	69.4	47.5	
US	WAS	0.73W/mL, 10 min, 12.93kJ/g.TS	sCOD	mg/L	3000	13219	Celebi et al., 2021
			sCOD	%		340	
			CH_4	%		32	
			VS reduction	%	38.5	49.5	
			C reduction	%	19	27	
US	Sewage sludge	20, 40, 80, 200, 300W, 2 min	CH_4	%		57 (1.5W/mL)	Xiao et al., 2022

TH	Sewage sludge	30, 60, 90, 120°C, 20 min	CH_4	%		93 (120°C)	
Chemical	Sewage sludge	pH-2, pH-4, pH-7, pH-10, 20 min	CH_4	%		69 (pH-10)	
US	WAS	100W, 200W/kg, 96kJ/kg, 8 min	sCOD	mg/L	104	1923	Houtmeyers et al., 2014
			sCOD	%		1749	
			sCOD/TCOD	%	0.3	4.7	
			sCarbs	mg/L	41	235	
			sProt	mg/L	21.27	21.07	
			CH_4	%		27	
MW	WAS	800W, 96kJ/kg, 1 min	sCOD	mg/L	104	223	
			sCOD	%		117	
			sCOD/TCOD	%	0.3	0.6	
			sCarbs	mg/L	41	56	
			sProt	mg/L	21.27	20.77	
			CH_4	%		20	
MW	Mixed sludge	3.2W/mL, 10 min	sCOD	mg/L		2200	Yeneneh et al., 2015
			sCOD/TCOD	%		8.78	
MW+US	Mixed sludge	3.2W/mL, 10 min + 0.4W/mL, 8min	sCOD	mg/L		3300	
			sCOD/TCOD	%		11.89	
			CH_4	%		43	
MW	WAS	600W, 16 min	sCOD/TCOD	%	0.5	18	Dogan et al., 2009
MW + Alkaline	WAS	600W, 16 min + pH-12	sCOD/TCOD	%	0.5	34	
			CH_4	%		18	
MW + H_2O_2	WAS	600W, 60°C + pH-7, 3g/L TSS, H_2O_2 dose of 1, 100°C	sCOD/TCOD	%		47	Wang et al., 2015
MW + H_2O_2	Dairy WAS	900W, 30-110°C, 1-20 min, 0.1-1mg/g.SS	sCOD	g/L		7.4 (18600 kJ/kg.TS)	Eswari et al., 2016
			sCOD	%		3600 (18600 kJ/kg.TS)	
			sCOD/TCOD	%	0.81	30.14 (18600 kJ/kg.TS)	
			sProt	g/L	0.397	0.7 (18600 kJ/kg.TS)	

			sCarbs	g/L	0.005	0.05 (18600 kJ/kg.TS)	
			sProt	%		1663 (18600 kJ/kg.TS)	
			sCarbs	%		900 (18600 kJ/kg.TS)	
US	WAS	0.51W/mL, 0-30 min	sCOD	mg/L	50	2500 (15 min)	Apul et al., 2010
			sCOD	%		4900	
MW	TWAS	584W & 1168W, 1%TS, 2%TS, 3%TS, 4%TS, 35°C, 65°C & 90°C	sCOD	mg/L	1390	3304 (4%TS, 1168W, 90°C)	Zheng et al., 2009
			sCOD/TCOD	%	6	21.6 (4%TS, 1168W, 90°C)	
	PS		sCOD/TCOD	%	2.4	6.3 (4%TS, 1168W, 90°C)	
			CH_4 Yield	%		37 (4%TS, 1168W, 90°C)	
			Rate of CH_4 Yield	%		38 (4%TS, 1168W, 90°C)	
			Digestionn Time	%		30 (4%TS, 1168W, 90°C)	
MW	TWAS	1200W, 110°C, 150°C, 175°C, 3.75°C/min, 7.5°C/min, 6%TS, 11.85%TS	sCOD/TCOD	%	7	57	Toreci et al., 2010
MW	WAS	400W, 1000W, 1600W, 60°C, 90°C, 120°C, 1%TS, 2%TS, 3%TS	sCOD/TCOD	%	6.9	17.5 (400W, 120°C, 2%TS)	Park et al., 2010
MW	SS	800W, 210s	sCOD	mg/L	1353	4247	Appels et al., 2013
			sCOD/TCOD	%	3	9.2	
			sCarbs	mg/L	77	285	
			sProt	mg/L	390	1317	
			sCarbs/Carbs	%	1.4	5.2	
			sProt/Prot	%	1	3.4	
			Specific CH_4	%		50	
MW	TWAS	700W, 900W, 30-100°C, 8 min	sCOD/TCOD	%	1.1	33	Kuglarz et al., 2013
			sProt	mg/L	122-128	2745-2795	
			NH_4^+	mg/L	15.8	27	
			CH_4	%		52	
MW	WAS		sCOD/TCOD	%	6.2	15.7-16.1	Yu et al., 2010

		500W, 750W, 900W,20-140s	Protein	mg/L	520	2100	
			NO_3^-	mg/L	22	33	
			NH_4^+	mg/L	19	32	
MW	SS	400W, 700W, 0-30kJ/kg.TS	sCOD	%		30	Serrano et al., 2016
			Specific CH_4	mL/g.VS	111	130	
			Specific CH_4	%		17	
			OLR	%		38	
			Rate of CH_4 Yield	%		42	
MW	WAS	800W, 4, 8, 12, 16 min	sCOD/TCOD	%	0.8	5-16.6	Liu et al., 2019
MW	TWAS	1200W, 80°C, 160°C, 2.25°C/min	sCOD/TCOD	%	13.5	17-26	Kor-Bicakci et al., 2019
			CH_4 Yield	%		19	
			Specific CH_4	mL/g.VS		325.65-383.5	
MW	WAS	0.5-5 min, 40-96°C	sCOD/TCOD	%	0.97	21-28	Ebenezer et al., 2015
			sProt	mg/L	12.9	218-535	
			sCarbs	mg/L	4.6	27-64	
			Specific CH_4	mL/g.VS	280	540-970	
			CH_4 Yield	mL/gVS.day	4.5	12--20	
Ultrasonic	WAS	1250-5000kJ/kg.TS, 2, 4, 8 min, 2.1-5%TS	DD	%		9 (2.1%TS, 8 min)	Braguglia et al., 2015
			sCOD	mg/L	48	1598 (2.1%TS, 8 min)	
			sCOD	%		3229 (2.1%TS, 8 min)	
			Protein	mg/L	40	1015 (2.1%TS, 8 min)	
			Protein	%		2438 (2.1%TS, 8 min)	
			Carbohydrates	mg/L	4.4	111 (2.1%TS, 8 min)	
			Carbohydrates	%		2423 (2.1%TS, 8 min)	
			CH_4	mL/kg.VS	170	240 (2.1%TS, 8 min)	
			CH_4	%		41 (2.1%TS, min)	
			VS reduction	%	37	40 (2.1%TS, 8 min)	
TH	WAS	135°C, 20 min, 2.1-5%TS	DD	%		22 (2.1%TS, 20 min, 135°C)	
			sCOD	mg/L	45	4340 (2.1%TS, 20 min, 135°C)	
			sCOD	%		9544 (2.1%TS, 20 min, 135°C)	
			Protein	mg/L	30	1822 (2.1%TS, 20 min, 135°C)	

			Protein	%		5973 (2.1%TS, 20 min, 135°C)	
			Carbohydrates	mg/L	2.7	418 (2.1%TS, 20 min, 135°C)	
			Carbohydrates	%		15381 (2.1%TS, 20 min, 135°C)	
			CH_4	mL/kg.VS	220	240 (2.1%TS, 20 min, 135°C, TAD)	
			CH_4	%		9.1 (2.1%TS, 20 min, 135°C, TAD)	
			VS reduction	%	42	46 (2.1%TS, 20 min, 135°C, TAD)	
MW-Che	WAS	600W, pH-4, pH-5.22, pH-7, pH-8.78, pH-10, H_2O_2 (0.4w/w, 1.0w/w, 1.59w/w, 2.0w/w)	sCOD/TCOD	%		11.6-46.8	Wang et al., 2015
			NH_4^+	mg/L		15.5-65.7	
MW-Che	WAS	Sodium citrate (0.05-0.14g/g.TS, 2h; 850W (30mL), 3-30s, 10-40MJ/kg.TS	DDCOD	%	0	2.8-15	Peng et al., 2018
			Specific CH_4	mL/g.VS	88.4	135.226	
MW-Che	TWAS	TWAS:FOG (20%, 40%, 60%), 5N NaOH, pH-10; 95°C, 135°, 175°C, 3.5°C/min, 1min	sCOD/TCOD	%	21.5	27.5-36.3	Alqaralleh et al., 2019
			Specific CH_4	%		17-137	
MW-HTC	HF	180°C, 200C°, 30 min	C	%	47.8	56 (200°C)	Afolabi et al., 2017b
			N	%	5.9	2 (200°C)	
			C/N	Ratio	8	28 (200°C)	
	HFS		C	%	41	50 (200°C)	
			N	%	5.3	0.8 (200°C)	
			C/N	Ratio	8	63 (200°C)	
MW-HTC	HFS	160°C, 200C°, 0.5, 1, 2h	C	%	36.93	49 (200°C, 2h)	Afolabi et al., 2015
			N	%	4.93	0.9 (200°C, 2h)	
			C/N	Ratio	8	49 (200°C, 2h)	
MW	HFS	465W, 1085W, 1550W, 0.5-14 min	Temp	°C		134 (100g, 1550W, 14 min)	Mawioo et al., 2016a
			Vol reduction	%		>70 (100g, 1550W, 14 min)	
			Pathogen	CFU/g TS		<DL (100g, 1085W, 1 min, 78°C)	

			VS/TS	%	92	82-92	
MW	FS	3.4kW, 0.5, 1, 1.5, 2, 4h	Temp	°C		102	Mawioo et al., 2017
			Vol reduction	%		63	
			Pathogen	CFU/g TS		<DL	
			VS/TS	%	88	88	
			TN	mg/g.TS		42	
	Septage		Temp	°C		96	
			Vol reduction	%		93	
			Pathogen	CFU/g TS		<DL	
			VS/TS	%	55	54	
			TN	mg/g.TS		28	

APPENDIX B : DESIGN OF EXPERIMENT AND RESPONSE SURFACE METHODOLOGY

Response surface methodology (RSM) is a collection of mathematical and statistical techniques concerned with designing appropriate experiments that provide information about a response factor, selection of a suitable model to fit experimental data, and process optimization. The basic characteristics of RSM are sequential experimentation and that information acquired in one stage is used to plan subsequent experiments. The collected experimental data fit first and second-order models. The first-order model is used for exploratory experimentation, i.e., determining important factors to be considered in the experiment. A second-order model is used to determine the significance of model parameters; estimate mean response; and arrive at optimum operating conditions.

A central composite design (CCD) generated experimental data to fit a second-order response surface model. A central composite design consisted of a complete 2^k factorial points; axial portion (star points) of 2k points; and centre points (C) (Eq. A3.1).

$$\boldsymbol{N = 2^k + 2k + C} \qquad \textbf{(A3.1)}$$

Factorial points (2^k) estimated linear terms and factor interactions in the model; centre points (C) provided information about the existence of curvature in the system; and star points (2k) allowed for efficient estimation of pure quadratic terms if curvature existed (Wagner et al., 2014). The axial points were chosen to be within the faces of the cube because it would have been impossible to operate the process outside the faces. For example, the microwave oven used had predefined power increment levels of 10%. Any point outside the predefined power level could not be operated on the microwave oven. Hence, the experimental design was a face centred central composite design (FCCCD). The factorial points (2^k) for MP were set at 60% (540 W) and 80% (720 W) and 1 and 5 minutes for contact time. Hence the axial points were also set at the same operating conditions as the factorial points. The centre points were at 70% (630 W) and 3 minutes for MP and contact time, respectively. The operating parameters were selected to be within the range of values used to study the solubilization of sludges (Park et al., 2010; Yu et al., 2010). Also, preliminary results from the same study guided the choice of operating conditions.

The experimental design and statistical analyses were done in R software, version 4.0.2, (https://cran.r-project.org/) using an rsm package (Lenth, 2009). The total number of experimental treatments (N) required for the two control variables (MP and contact time) was calculated using equation A3.1.

An FCCCD was generated by specifying alpha (α) to "faces" in the ccd function of rsm package. Hence, the coded values had α of ±1. In an FCCCD, one centre run, replicated several times, is

usually adequate to study the second-order effect. All treatments were run in triplicate, giving a total of 27 experimental points.

Design of experiment (DOE) and response surface methodology (RSM) was used to find the optimum sCOD/TCOD profile. A face centred central composite design (FCCCD) generated data to fit a second-degree response surface model. The model describes the most important variables (Xs) and their possible interactions. The model takes the form in equation A3.2:

$$\boldsymbol{Y_i = \beta_0 + \sum \beta_i X_i + \sum \beta_{ii} X_i^2 + \sum \beta_{ij} X_i X_j} \qquad \textbf{(A3.2)}$$

In the equation, Y_i is the response variable (sCOD/TCOD), β_0 is a constant, β_i is the linear effect, β_{ii} is the quadratic effect, β_{ij} is the interactive effect, X_i is the independent variable (factors varied, i.e., MP and contact time). The coefficient of determination (R^2) and statistical significance determined the model's quality by analyzing variance (ANOVA) at 95%. Statistical analyses were done in RStudio using the "rsm" package. For statistical analyses, factors were coded as x1 (for MP) and x2 (for time).

Codes used in the experimental design and data analysis

The R codes used to generate the experimental design and analyze the data are detailed below:

A. Generating and evaluating a face-centred central composite design (FCCCD)

- An “rsm” package from the comprehensive R archive network (CRAN-R project) (https://cran.r-project.org/) generated experimental designs and fitted the collected data in a second-order response surface model
- A “ccd.pick” function generated top 10 designs based on 2 factor interaction
- The chosen design was generated using the procedure outlined below, with (DES2) and without (DES1) blocking
- The design with blocking was implemented, though the effect of blocking on the response surface model was not evaluated
- A “varfcn” function evaluated the suitability of the generated experimental design to fit a second-order response surface model. The function confirmed that the generated experimental design can collect data that can fit the intended model (See the contour plots attached). This is an important rsm function in R that saves time and resources.

```
>library(rsm)
>DES1 = ccd(2, n0 = c(1, 2), oneblock = TRUE, alpha = "faces", wbreps = c(3, 3), randomize = TRUE, coding = list(x1 ~ (MP - 630)/90, x2 ~ (CT - 3)/2))
        >as.data.frame(DES1)
>DES2 = ccd(2, n0 = c(1, 2), oneblock = FALSE, alpha = "faces", wbreps = c(3, 3), coding = list(x1 ~ (MP - 630)/90, x2 ~ (CT - 3)/2))
        >as.data.frame(DES2)
```

>DES1Va = varfcn(DES1, ~ SO(x1, x2))

>DES1Vb = varfcn(DES1, ~ SO(x1, x2), contour = TRUE)

>DES2Va = varfcn(DES2, ~ Block + SO(x1, x2), dist = seq(0, 3, by = .1))

>DES2Vb = varfcn(DES2, ~ Block + SO(x1, x2), dist = seq(0, 3, by = .1), contour = TRUE)

B. Exporting the experimental design to MS Excel

A "writexl" package exported the data from RStudio to an MS Excel format

>library(writexl)

>write_xlsx(DES1, "C:\\Users\\Principal Mdolo\\desktop\\DOE\\DES1.xlsx")

>write_xlsx(DES2, "C:\\Users\\Principal Mdolo\\desktop\\DOE\\DES2.xlsx")

C. Fitting a second-order response surface model

>library(rsm)

>TRYM2 <- rsm(Y2 ~ SO(x1, x2), data = COD_II_Working)

>summary(TRYM2)

D. Generating contour, surface plots and model diagnostics

- Residual plots

>par(mfrow = c(2, 4))

>plot(COD_II_Working$x1, TRYSTUDRES, main = "Residuals vs x1")

>par(mfrow = c(1, 4))

>plot(COD_II_Working$x2, TRYSTUDRES, main = "Residuals vs x2")

- Cook's stat

>par(mfrow = c(1, 4))

>plot(TRYM2, which = c(1,3,5))

- Contour and response surface models

>par(mfrow = c(2, 4))

>contour(TRYM2, ~ x1 + x2, image = TRUE, main="second-order model")

>par(mfrow = c(1, 4))

>persp(TRYM2, x2 ~ x1, zlab = "y", main="second-order model")

The results of the experimental design and the observed responses are shown in Table A3.1.

Table B.1 Design matrix of a 22 full factorial experimental design (FCCCD) with a response factor

		MP (MP)	Contact Time	Coded values			Response (Y)
run.order	**std.order**	**Watts**	**Minutes**	**x1**	**x2**	**Block**	**sCOD/TCOD (%)**
1	2	540	1	-1	-1	1	32.0
2	13	630	3	0	0	1	36.2
3	7	540	5	-1	1	1	29.5
4	4	720	1	1	-1	1	34.5
5	10	720	5	1	1	1	18.1
6	6	720	1	1	-1	1	33.8

7	12	720	5	1	1	1	17.9
8	1	540	1	-1	-1	1	32.7
9	8	540	5	-1	1	1	29.1
10	5	720	1	1	-1	1	34.2
11	9	540	5	-1	1	1	29.3
12	3	540	1	-1	-1	1	32.9
13	11	720	5	1	1	1	18.6
1	12	630	5	0	1	2	26.2
2	6	720	3	1	0	2	32.4
3	4	630	5	0	1	2	27.3
4	1	540	3	-1	0	2	32.9
5	8	630	5	0	1	2	26.4
6	2	720	3	1	0	2	34.2
7	9	540	3	-1	0	2	34.7
8	14	630	3	0	0	2	39.6
9	11	630	1	0	-1	2	33.6
10	5	540	3	-1	0	2	33.8
11	13	630	3	0	0	2	38.9
12	7	630	1	0	-1	2	32.4
13	3	630	1	0	-1	2	32.7
14	10	720	3	1	0	2	33.3

APPENDIX C : STANDARD OPERATION PROCEDURE (SOP) FOR SOLIDS DETERMINATION IN FAECAL SLUDGE

Introduction

Solids refer to matter suspended or dissolved in water or wastewater. Solids may affect water or effluent quality adversely in several ways. Solids analyses are important in the control of biological and physical wastewater treatment processes and for assessing compliance with regulatory agency wastewater effluent limitations.

Total Solids (TS) is the term applied to material residue left in a crucible after evaporation of a sample and its subsequent drying in an oven at 105℃ for 24 hours. Total solids include total suspended solids (TSS), the potion of solids retained by a filter, and total dissolved solids (TDS), the portion that passes through the filter of 2.0µm or smaller. Volatile solids (VS), refer to material lost after igniting the sample at 550℃ for 24 hours. If VS is analyzed following TS analysis, the ignition time can be set at 2 hours.

Total Solids

Scope and field of application

Total Solids are determined in a wide variety of environmental media including portable water, surface water, wastewater, wastewater sludge, septage and faecal sludge. TS are important for the design and efficient operation of a treatment plants.

Principle

For solids analysis in faecal sludge, approximately 20g of a well-mixed sample is evaporated to dryness in a porcelain crucible in a hot air oven at 105ºC for 24 hours. The residues remaining are cooled and weighed. The residual material remaining in the crucible is classified as TS, and may consist of organic, inorganic, dissolved, suspended or volatile matter.

Interferences

- Highly mineralized water with a significant concentration of calcium, magnesium, chloride and sulphate may be hygroscopic and require prolonged drying, proper desiccation and rapid weighing.
- Exclude large, floating particles from the sample if it is determined that their inclusion is not desired in the result.
- Disperse visible floating oil and grease with a blender before withduntreateding sample portion for analysis. Because excessive residue in the dish may form a water-trapping crust.

Sampling

- Mix the sample well to suspend solids uniformly.
- Remove the test portion rapidly before any settling of solid matter occurs.

- Use a measuring cylinder and not a pipette for sludge and wastewater samples.
- Use a crucible for feces.
- Use a volume or mass of sample to ensure a measurable residue- limit sample to no more than 20g residue
- Suitable aliquots: Liquid samples – 100ml, Sludges -30ml, feces and faecal sludge from dry toilets 10-20g.

Safety precautions

- Always use safety goggles, gloves and laboratory coat while working in laboratory
- Wear gloves suitable for withstanding high temperatures when removing crucibles from the oven.
- After the analysis clean bottles and beakers with clear water keep it for drying
- Dispose the used gloves after completion of analysis
- Clean the hands using antiseptic soap
- Disinfect hands after washing with soap
- Avoid spillage and contact with skin. In the latter case use copious washings with cold water and call for medical attention.
- Perform a hazard identification and risk assessment (HIRA) for each activity to be undertaken

Apparatus

- 50ml capacity evaporating porcelain crucibles or aluminum foil cups
- Spatula
- Desiccator
- Drying oven
- Four – place Analytical Balance (Check and make sure its levelled)
- Tray
- Crucible tongs
- Heat resistant gloves

Reagents

- None

Calibration

- Check the temperature throughout the oven area by placing a calibrated thermometer on each shelf, after 30mins, check temperature at each level against oven setting.
- Adjust oven setting if necessary.
- If temperatures are uneven on the shelves, check insulation.

Procedure

Crucible preparation

Switch on the oven and set the temperature to 105℃ and wait until the set temperature is reached. If volatile solids are to be measured ignite clean crucible at 550℃ for 1 hour in the furnace. If only total solids are to be measured, heat clean crucible to 103-105℃ for 1 hour. Store and cool the crucibles in a desiccator until needed.

Sample Analysis

- Weigh and record the mass of crucible (W1g) immediately before use using an analytical balance (Record the mass to 4 decimal places). Tare the mass without removing the crucible from the balance.
- Transfer approximately 10-20g (W2g) of a well-mixed faecal sludge sample into the crucible on the analytical balance.
- Place the weighed sample in a hot oven and dry at 103-105℃ for 24 hours
- Dry sample for at least 1hr in an oven 103-105℃, to dish in desiccator to balance temperature and weigh. Repeat cycle of drying, cooling, desiccating and weighing until a constant weight is obtained, or until weight change is less than 4% of previous weight or 0.5mg, whichever is less.
- After 24 hours, remove the sample from the oven and cool for 15 minutes in a desiccator.
- Weigh and record the mass (W3g) of the cooled sample.
- Place the oven dried sample in a furnace and ignite at 550℃ for 2 hours to determine its VS content
- Remove the sample from the furnace after 2 hours, cool and weigh and record the mass *($W_4 g$)*

Calculations

$$Total\ Solids\ (TS)\ in\ Wet\ Sample\ (g/g) = \frac{(W_3 - W_1)g}{W_2\ (g)}$$

$$Moisture\ Content\ (g) = W_2(g) - [(W_3 - W_1)]g$$

$$Volatile\ Solids\ (VS)\ in\ Wet\ Sample\ (g/g) = \frac{(W_3 - W_4)g}{W_2\ (g)}$$

W_1 = Mass of empty crucible

W_2 = Weighed mass of sample into the crucible

W_3 = Mass of residue after oven drying at 105℃ for 24 hours

W_4 = Mass of residue after furnace drying at 550℃

APPENDIX D : SOP FOR FS SAMPLE COLLECTION, SAMPLE PREPARATION AND WORK INSTRUCTIONS FOR CHEMICAL ANALYSES

SOP: SAMPLING OF FAECAL SLUDGE AND COMMUNITY/SITE VISITS

Co-ordination of Sampling Process

- Student/Researcher sends out and email to lab technician and copy to Tina, Santiago, Edie to request sampling. All specifications are be included.
- Ethical approval in place
- Gatekeeper letter
- Acceptance from EWS
- Specify type of sludge? VIP/UD/pour flush?
- Specify quantity of sludge. E.g 3x 25L/5x 1L • Composite sample?
- Once off or do you require sampling in stages? If so specify month.
- Specify date samples are required. We will arrange to the closest possible date if your specified date is not possible.
- Check with Lungi if EWS has a sampling campaign running and make arrangements accordingly.
- Edie books the EWS vehicle.
- Sampler contacts Dumisani (0735115376) and makes arrangements.
- PRG sampler could either join Dumisani's sampling or schedule a session specific for PRG.
- Dumisani confirms sampling area and date. All done through telephonic conversation.
- PRG sampler to meet Dumisani at Bridge City Mall (offramp after Ethekwini heart hospital).
- Store on vehicle GPS.
- Follow Dumisani to the sampling site.
- At PRG-keys and Sampling form are handed over to Edie.
- For specific sampling areas requested by PRG-Dumasani is contacted and given the details
- Arrangements are made and once sampling is completed, he submits an invoice and payment is made by Kerry/Edie.
- Cost per pit is R1800.00

Preparation for Field Sampling

Prior to the collection of samples, the collection team should prepare the equipment for use:

- Long length elbow gloves

- Respirator
- Mask (safety goggles)
- Overall
- 70% ethanol
- Paper towels
- Black refuse bags
- Latex powder free gloves
- Sample storage box
- Lab coats
- Rubber boots

Travel to field location

- Roadworthy, appropriately insured vehicles with seatbelts for all occupants to be used.
- Driver to have appropriate license.
- Inform person at office of intended destination and estimated return time.(see form attached)

Personnel safety

- Liaise with relevant officials at municipality
- Arrange introductions to caretaker and/or householder in charge of facility before starting sampling, ensure they are kept informed about activities taking place
- Use local facilitators where advised to do so by municipality

Sampling Procedure

- PRG members meet the pit emptying contractors on site with the sample containers.
- **Pit Emptying Contractor: Dumisani Majozi: 0735115376**
- The base concrete cover is removed by the contractors exposing the pit contents.

School Pit toilets

- The pit contents are scooped out with a long handled spade and put into the sample containers.
- The containers are sealed tightly and packed into the vehicle.

Ventilated Latrine School pits Urine Diversion toilets

Ventilated Latrine School pits Urine Diversion toilets

The base concrete cover is removed by the contractors exposing the pit contents.

Samplers to wear full PPE Prepare sampling containers.

Ventilated Latrine School pits

Urine Diversion toilets

Contractors empty the pits/toilets.

Pit contents are transferred to 1L containers.

Pit contents are transferred to bigger containers.

Remaining waste is taken by the contractors.

Sample containers are sealed and contained Disinfection step when sampling is completed. properly.

Transport of sample from field location to lab

- Tight-fitting lids to be fitted to sample containers before being removed from the facility
- Full sample containers to be placed inside bags or another tub inside vehicle. **Storage of sample in lab**
- Samples to be labeled appropriately whilst in the field.
- Full sample containers to be taken from vehicle, through basement access door to lab and placed immediately into cold-room
- Fill in the cold room inventory list.
- Samples must be place in allocated project /student areas.
- All samples must be labeled with: Name of project, name of student, sample type, date and any other useful information.

Sample Handling Safety Precautions

- Cover any small open wounds with waterproof dressings – if large open wounds then do not carry out laboratory work.
- Always use gloves, laboratory coat and closed shoes while working in the laboratory.
- Wear a face-shield when disposing of samples down the sluice (risk of splashback).
- Dispose of samples as specified by the Faeces Sample Disposal SOP.
- Clean all soiled equipment thoroughly after use.
- Any equipment that will be taken out of the laboratory into a ‘clean’ environment should be handled only with clean gloves and disinfected using 70% ethanol spray after use.
- Dispose of the used gloves in the appropriate waste bin after sample handling and disposal and cleaning of equipment is complete.

- Clean hands using antiseptic soap.
- Disinfect hands after washing with soap.

Where mixed samples are being handled (i.e. those from field location sources such as community ablution blocks), additional care must be taken as sharps may be present in the faecal matter. Samples should not be handled directly with gloved hands, but rather with a spoon or spatula.

Maintain 'clean' and 'dirty' work areas

The basement laboratory where excreta samples are processed should be considered in its entirety a 'dirty' area, however within this 'clean zones' should be designated for any items that will later be taken out of the laboratory:

- Sample boxes and equipment used to handle samples should only be placed on wipe-clean surfaces - plastic or metal top workbenches or trays.
- Any 'clean' items that will be taken out of the laboratory – e.g. camera and paper forms used to record results – should be kept on a clean tray or segregated clean area of the workbench.
- 'Clean' items should only be handled whilst wearing clean gloves.

Disposal of samples

- All faeces must be disposed into the sluice. The procedure for the disposal follows:
- Pour unwanted faeces samples into the sluice, and scrape all excess sample from the sample container if necessary
- Flush the sluice once all sample has been disposed of into the sluice
- Clean the sluice of any unflushed faeces
- Clean all containers and equipment used with water and dishwashing detergent, and disinfect with 70% ethanol

1. Inform EWS site contact of intended site visit including:
    - Time, date and expected duration of visit
    - Reason for visit
    - Number of people going to site
2. Get permission from EWS site contact for visit. N.B.: No site visits can take place without permission and EWS site contact has final say on whether a site visit is allowed. This is for the safety of the researchers.
3. Arrange transport to site. Record driver name and vehicle registration on form.
4. Check that at least one person visiting site has a charged mobile phone, phone number of Project Lead and Lab Manager saved and a minimum of R25 phone credit. Record contact name and telephone number on form.
5. Check that you have sufficient water and weather appropriate gear for all people going to site e.g.

hats, sunscreen, waterproof clothing

6. Leave form with Lab Manager.

The following is a general faecal sludge sample preparation procedure for the analysis of chemical oxygen demand (COD), total nitrogen (TN), nitrate (NO_3^-), ammonium (NH_4^+) and carbohydrates.

- Weigh out between 1.8g and 2g of well mixed faecal sludge sample.
- Place the weighed-out sample into a blender with 250ml of distilled water.
- Blend for 30 seconds.
- Transfer the blended mixture into a volumetric flask and top up to 1L with distilled water.
- Transfer the 1L solution (stock solution) to a sample storage bottle and store in the cold room at 4℃ until required.
- To analyze soluble macromolecules:
- Centrifuge about 40mL of the stock solution at 500rpm for 15 minutes
- Decant the supernatant and filter it through a 0.45µm syringe filter
- Analyze soluble macromolecules on the filtrate

Work instruction for chemical oxygen demand (COD) analysis using the cell test

- Switch on the instrument and check that it is working properly
- Switch on and pre-heat the digester (thermos-reactor) at 150℃ for 2 hours.
- Remove prepared samples from the cold room and bring to room temperature
- Assemble the required materials
- Samples
- Beakers (label them)
- 5mL pipette with pipette tips
- Racks
- Test kits (check the range, manufacturer, and expiry date)
- Syringe filters (0.45µm) for soluble COD
- Proceed to carry out the analysis as outlined below
- Label the cuvettes according to the type of sample
- Place the cuvettes in a test tube rack and tilt them
- Open and leave loose the cover on the cuvettes
- Pour about 20 mL of sample in a labelled beaker
- Mix the sample well before pipetting to analyse TCOD
- Pipette 2mL of sample in a tilted cuvette
- In the same way, prepare a blank by pipetting 2mL of distilled water instead of the sample
- Tighten the screw cap on all cuvettes

- Mix the contents
- Place the prepared samples (including a blank) in a pre-heated digester and digest at 150℃ for 2 hours
- Switch off the digester after 2 hours and leave the samples there for about 20 minutes
- Remove the samples and let them cool on a test tube rack for 30 minutes
- Choose the method from the list of methods in the instrument
- Check the test range on the instrument before running samples (make sure it corresponds to the range for which the samples were prepared)
- Analyse the samples and record the results
- Wipe each cell before reading
- Run a blank to get a zero
- Run the samples
- A blank can be run again after analysing a certain number of samples (e.g., every 10 samples)
- Dispose all the cuvettes in a designated COD waste container!!
- Clean all other glassware, pipette tips and dry on a drying rack
- Disinfect all workstations and instruments with 70% ethanol

Work instruction for total nitrogen (TN) analysing using the cell test

- Switch on the instrument and check that it is working properly
- Switch on and pre-heat the digester at 120 ℃ for 30 minutes
- Remove prepared samples from the cold room and bring to room temperature
- Assemble the required materials
- Samples
- Beakers (label them)
- Pipettes (0.1-1mL and 5mL) with pipette tips
- Test tube racks
- Test kits (check the range and manufacturer)
- Proceed as follows to prepare the sample for analysis and analyzing it
- Label the empty cuvettes according to the type of sample
- Place the cuvettes in a test tube rack and tilt them
- Open and leave loose the cover on cuvettes
- Pour about 20mL of sample in a labelled beaker
- Shake (mix) the sample before pipetting
- Pipette 1mL of pretreated sample into an empty cuvette

- Add 9mL of distilled water into cell and mix
- Add 1-level blue microspoon of reagent N-1K and mix
- Add 6 drops of reagent N-2K, close the cuvette and mix.
- Digest the sample at 120℃ in the preheated digester for 1 hour.
- After 1 hour, leave the cuvettes in the digester for about 10 minutes
- Shake the cuvettes briefly after 10 minutes.
- Pipette 1mL of the digested solution into a reaction cuvette. Do not mix.
- Pipette 1mL of reagent N-3K into the reaction cell, close the cuvette and mix. Wear eye protection and hold the cell only at the top.
- Leave the hot reaction cuvettes to stand for 10 min (reaction time). Do not cool with water.
- Measure the samples in the photometer and record the results
- Clean all other glassware and pipette tips
- Disinfect all workstations and instruments with 70% ethanol

Work instruction for the analysing of ammonium (NH_4^+) using the cell test

- Pipette 5mL of the filtered sample into a test tube
- Add 0.6mL of reagent NH4-1 with a pipette and mix
- Add 1-level blue microspoon (in the cap of the NH4-2 reagent bottle)
- Shake vigorously until the reagent is completely dissolved
- Leave the mixture to stand for 5 minutes
- Add 4 drops of Reagent NH4-3 and mix
- Leave the mixture to stand for 5 minutes
- Fill the sample into the cuvette, measure in the photometer and record the results
- Clean all glassware and pipette tips
- Disinfect all workstations and instruments with 70% ethanol

Work instruction for the analysing of nitrates (NO_3^-) using the cell test

- Wear eye protection (the mixture becomes hot) while performing this test
- Pipette 4.0mL of reagent NO3-1 into a dry test tube
- Add 0.50mL of the filtered sample with a pipette to the test tube, do not mix
- Add 0.5ml of reagent NO3-2 with pipette to the test tube and mix, holding only the upper part of the tube
- Leave the hot reaction to stand for 10min
- Do not cool with water

- Fill the sample into the rectangular cuvette, measure in the photometer and record the results
- Clean all glassware and pipette tips
- Disinfect all workstations and instruments with 70% ethanol

Work instruction for the analysing of carbohydrates using the phenol-sulfuric acid method

The carbohydrate test relies on the hydrolysis of complex polysaccharides molecules that could be detected at a visible spectrum range. This method has been developed with sulfuric acid as the hydrolytic agent and phenol as a coloring agent. The hydrolyzed derivatives that form a colored complex with phenol can be detected at 490 nm. Precaution must be taken when dealing with samples containing complex carbohydrates which might carry a charge which causes interferences.

Reagents

- Concentrated Sulfuric acid (H2SO4)
- 5% Phenol (Prepared by dissolving 5.50mL of 99% Phenol in 94.50mL of distilled water)

Preparation of a 1g/L standard glucose solution and 5% phenol

- Preparation of a 1g/L glucose stock solution:
- Weigh 0.1g of glucose into a beaker
- Add 50mL of distilled water and stir till the glucose dissolves
- Transfer the solution into a 100mL volumetric flask
- Add distilled water up to the mark and mix well
- Transfer the mixture into a sample bottle and label it
- Preparation of a 5% phenol solution
- Pipette 5.5mL of 98% phenol into a 100mL volumetric flask
- Add distilled water up to the mark and mix well
- Transfer the mixture into a sample bottle and label it

Constructing a standard curve

- Prepare serial dilutions of glucose from the standard glucose solution as follows:

Table D.1 Preparation of serial dilutions from standard glucose solution

S/N	Volume of standard glucose (mL)	Final volume (mL)	Concentration (g/L)
1	1.25	100	0.0125
2	2.5	100	0.025
3	5.0	100	0.050
4	7.5	100	0.075
5	10	100	0.1

- Pipette 2mL of the prepared glucose solution into a clean dried test tube
- Add 1mL of 5% phenol and mix
- Add 5mL concentrated H2SO4 acid and let the mixture stand for 10 minutes at room temperature.
- Mix thoroughly for 20 seconds and cool to room temperature
- Read light adsorption at 490nm and record the absorbance
- Plot a graph of absorbance vs the known concentration prepared above
- Generate the regression equation from the graph

Analyzing glucose concentration in the sample

- Proceed as in point 2-5 above, pipetting the sample instead of glucose
- Use the generated equation above to determine the concentration of glucose in the sample

Normalizing the measured concentrations to dried solids

All the measured concentrations were normalized to dried solids to minimize the effect of dilutions as follows:

$$\text{Wet Sample Concentration (g/g)} = \frac{\text{A}}{1000} \times \frac{\text{V}}{\text{M}}$$

$$\text{Dry Sample Concentration (g/g)} = \frac{\text{Wet Sample Conc. (g/g)}}{\text{Total Solids (g/g)}}$$

Where:

$\text{A} - \text{Spectroquant Reading Concentration (mg/L)}$

$\text{V} - \text{Volume of Dilution (L)}$ i.e., the final volume to which the sludge was dissolved to

$\text{M} - \text{Mass of Sludge used in sample preperation (g)}$

APPENDIX E : WORK INSTRUCTION FOR FLOW CYTOMETRY ANALYSING OF FAECAL SLUDGE

a) Preparation of the faecal sludge sample
   - Weigh 4g of faecal sludge in a 250mL beaker
   - Add distilled water to dissolve the faecal sludge
   - Blend the mixture for 30 seconds and transfer to a 1L volumetric flask
   - Add distilled water to the mark and mix well
   - Transfer to a storage bottle and label it
   - Store the prepared faecal sludge solution at 4°C

b) Reagents to prepare PBS solution with pH-7.2
   - K2HPO4
   - KH2PO4
   - NaCl

c) Procedure to prepare the PBS solution

- Weigh 3g K2HPO4, 1g KH2PO4 and 8.5g NaCl
- Transfer the weighed reagents into a 250mL beaker
- Add distilled water and stir to dissolve the reagents
- Transfer the solution to a 1L volumetric flask and mark up to the level
- Shake well to mix the solution
- Transfer the prepared solution to a storage bottle and label it
- Store the prepared solution till required

d) Preparation of the sample for analysis

- Prepare a 1:10 dilution of FS sample with PBS solution
- Treat the dilute sample by ultrasonication at 80kJ/L
- Prepare a 1:40 dilution of the treated sample with PBS solution
- Filter the diluted sample through a 20μm filter

e) Analyze the sample using a flow cytometer according to the manufacturer's instructions

APPENDIX F : WORK INSTRUCTION FOR DESIGNING A BIOCHEMICAL METHANE POTENTIAL (BMP) TEST IN RSTUDIO

The experimental design was done in R version 4.0.3 and RStudio version 2022.07.1-554 using the "biogas" R package (8 January 2020). R, RStudio software and a "biogas" package installed were installed in the computer. The "PlanBMP" function in the "biogas" package designs BMP experiments if the inoculum to substrate ratio (ISR), the volatile solids (VS) mass of the substrate and inoculum, and the final volume required are known. PlanBMP determines substrate and inoculum masses to add to the serum bottle based on their VS masses and ISR. It can also determine the ISR based on the masses of the substrate and inoculum. In this research, PlanBMP calculated the masses of substrate and inoculum based on their determined VS concentrations, effective volume and ISR of 2:1.

1. Requirements to generate the experimental set up

- R and RStudio software
- R package "biogas"
- PlanBMP function
- R code to design the experiment
- PlanBMP(vs.inoc, vs.sub, isr=NA, m.inoc=NA, m.sub=NA, m.tot=NA, m.vs.sub=vs.subxm.sub, digits=3, warn=TRUE, nice=TRUE)
- The VS concentration should be in g/g

2. Procedure to generate the experimental set up

- Proceed as follows in RStudio console:
  - >Library(biogas)
  - >PlanBMP(vs.inoc=0.0335, vs.sub=0.1723, isr=2, m.tot=400)
  - The command was repeated for all the substrates by replacing the VS concentration of the substrates in the code
  - The effective volume was 400mL
- The following experimental set up was generated by the above procedure

Table F.1 BMP Experimental set up

Run No.	Substrate	Inoculum mass (g)	Substrate mass (g)	VS mass (g) of the mixture	ISR
1	RFS	365	35.4	0.0458	2
2	RFSW	387	12.6	0.0487	2
3	MFS	365	34.5	0.0459	2
4	MFSW	387	13	0.0486	2

- Each experiment was run in triplicate and the data were presented as average values ± standard deviation.