EFFECTS OF THERMO-CHEMICAL PRETREATMENT ON BIOGAS PRODUCTION FROM SWEET POTATO WASTE

BY

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DECLARATION

Declaration by Candidate

I declare that this thesis is my original work and has not been presented for any degree in any other University. No part of this thesis may be reproduced without a prior written permission from the author and/or Moi University.

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DEDICATION

This thesis is lovingly dedicated to the love of my life, Mr. Langat Leonard and my bundles from heaven Briella, Brian & Barrack for their patience and tolerance in my absence during this study.

I sincerely applaud my lovely parents, Mr. Julius Ng'eno and Mrs. Linner Ng'eno for paying my early education under difficult circumstances, they constantly encouraged me to live to my full potential and to always trust in God.

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ABSTRACT

Sweet potato (SP) is a tuber crop which is grown in most parts of the world; the crop generates lots of waste throughout its supply chain. Currently the waste has little commercial utilization; the unutilized sweet potato root waste (SPW) is highly perishable and release methane as they decompose posing a serious problem of environmental pollution. Biogas potential of sweet potato has been tested as a mono substrate as well as co-substrate. Other studies have also reported that sweet potato has a complex molecular structure which is resistant to enzymatic digestion; however no studies have reported any pretreatment mechanism to alter the complex structure to ease anaerobic degradation. Consequently, the main objective of this research was to evaluate energy recovery through anaerobic digestion of thermochemically pretreated SPW. The following specific objectives were investigated: physicochemical characterization of SPW; thermochemical pretreatment of sweet potato waste; and determination of pretreatment factors combination which produces optimum biogas. The quality of biogas produced in terms of methane content as well as the resultant digestate as a biofertilizer were also analyzed. SPW was milled and then subjected to thermo-chemical pretreatment where sodium hydroxide of concentration (0.6g/L-3.5g/L), temperature (50℃ - 90℃) and pre-treatment time (30-120minutes) were varied. The experimental setup was based on central composite design with all the three factors at five levels and biogas yield as a response. The pretreated SPW was anaerobically digested under mesophilic condition over an incubation period of 22 days. The results from the study revealed that, thermochemical pretreatment on SPW improved both biogas yield and methane. The optimum conditions for biogas production were obtained at: NaOH concentration 2.9g/L, heating temperature 82℃ and treatment time 102 minutes. The pretreated SPW had superior results than the untreated SPW; the untreated SPW cumulatively yielded 28.23 ml/gSPW of biogas, while the thermochemically treated SPW at optimal conditions produced 37.80ml/gSPW, therefore pretreatment improved biogas yield by 33.88%. The untreated SPW produced 42% methane while the thermochemically treated SPW produced 64%, hence pretreatment improved Methane by 22%. SPW in its natural form had a pH value which ranged from 4.8 to 5.0, this was lower than the optimum pH for anaerobic digestion, and hence neutralization step was necessary prior to anaerobic digestion. Carbon nitrogen (C/N) ratio of SPW of 40.86% was obtained in the study was which was higher than the recommended C/N ratio for anaerobic digestion, therefore the use of thermochemically treated SPW as a co-substrate with nitrogen rich substrate could help balance the nutrients. From the analysis of digestate it was observed that, the digestate from the untreated SPW had more macro nutrients (NPK) than the thermochemically treated SPW. It was therefore concluded that, thermochemical pretreatment of SPW improves both biogas yield and methane along with reduction in digester retention time though the quality of digestate as a fertilizer was degraded. Therefore other pretreatment methods on SPW should be tried to obtain a suitable pretreatment method to improve biogas yield without compromising with the quality of fertilizer.

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ACRONYMS

AD	-	Anaerobic Digestion
ANOVA	-	Analysis of Variance
DM	-	Dry Matter
GHG	-	Green House Gases
MC	-	moisture content
SPPP	-	Sweet potato Processing Plant
SPP	-	Sweet potato Processing
SP	-	Sweet Potato
SPWD	-	Sweet potato waste digestate
SPW	-	Sweet potato waste
TS	-	Total Solids
VFA	-	Volatile Fatty Acid
VS	-	Volatile Solids

CHAPTER ONE

GENERAL INTRODUCTION

1.1 Background Information

Energy is one of the most essential factors for growth in all aspects in any nation (Gopinatthan, 2015; Jena et al, 2017). In the recent past, global energy requirement on daily basis has been growing at unexpected rate (Deressa et al., 2016), this is due to population growth, industrialization and transportation. The global energy needs are met by three energy sources: petroleum, natural gas and coal, which together supply approximately 82-88 % of the total energy consumed (Gopinatthan et al, 2015; Schweinberger et al, 2016)**.** In Kenya, the energy sector also relies on three main sources of energy: biomass, petroleum and electricity, at 68%, 21% and 9% respective. Biomass is clearly the country's main source of energy from burning of wood and charcoal (David et al., 2015).

The use of fossil fuel has raised concerns, major issues being: fossil fuel reservoirs depletion which threatens its future supply; emission of greenhouse gases (GHG) which have detrimental effects to both environment and human health; and lastly the high cost of the fossil fuel resource amongst others. To alleviate the adverse effects caused by fossil fuel, active extensive research for more renewable energy sources has become a top priority in many countries (Cesaro & Belgiorno, 2015; Sunarso et al., 2013; Vindis et al., 2009). Renewable energy is a natural resource that is provided by nature, it can be acquired from the sun or natural movements and mechanisms of the environment (Cucchiella & Adamo, 2013). Examples of the renewable energy sources include solar energy, wind energy, thermal and hydrothermal energy sources and fuels from biomass such as biodiesel, biobutanol, bioethanol, biohydrogen and biogas (Okunola et al., 2018).

Among the biofuel, bioethanol, biogas and biodiesel have been produced on large scale for commercial purposes (Comparetti et al., 2017). The use of biomass as a source of renewable energy has attracted lots of interest because it is an economically sustainable technology which meets the energy needs as well as contribute to environmental protection (Ulises et al., 2019). Anaerobic digestion (AD) of organic matter produces biogas through the following four steps: hydrolysis, acidogenesis, acetogenesis and methanogenesis. The AD process occurs in an oxygen free environment and in the presence of highly sensitive microbial groups which are mainly compost of bacteria. Chemical bonding of carbohydrates in a biogas feedstock determines the time required to completely convert the substrate into biogas (Lucy, 2013; Zheng et al., 2014). A bottleneck step among the four AD steps is hydrolysis where complex molecule of organic waste is broken down into monomers, this step takes the longest time (Kasper et al., 2016b). However Zheng et al., (2014) considered methanogenesis as a rate-limiting step in the AD process mainly due to the slowest growth of methanogens which at the same time are sensitive to pH, temperature and inhibitor concentration.

Generally, biogas can be produced from any organic matter, such as wood, crop residue, textile wool, chicken feathers, lignocellulosic waste, industrial food waste and fruit waste (Bochmann & Montgomery, 2013; Deressa et al., 2016; Horváth & Taherzadeh, 2016; Risberg et al, 2017). However, some substrates may not be suitable for biogas production for reasons such as: (a) the substrate could be having complex molecular structure, by either being highly crystalline or is lignin rich hence becoming poorly accessible by microorganisms and their enzymes; (b) substrate may contain chemicals which inhibit growth and biological activity of microorganisms; (c) the feedstock could be light and consequently float in the digester causing physical

problems such as blockages in biogas plants (Drosg & Braun, 2013; Horváth & Taherzadeh, 2016; Montgomery et al, 2015). Sometimes all the three problems exist together. Treatment prior to anaerobic digestion can help solve the digestion barriers (Montgomery, 2013). Several pre-treatment methods for biogas feedstock have been developed and can be broadly classified into four categories- mechanical, chemical, biological and hybrid method (Taherzadeh & Karimi, 2008). All the pre-treatment methods aim at enhancing the digestion process by removing the existing barriers thus making the organic content of the substrate to be easily accessible and degradable by the microbes. Picking out a suitable method of pre-treatment for a biogas feedstock is of great importance since every pre-treatment method produces different effects on the substrate (Gillian, 2011). Ideally according to Horvath & Taherzadeh, (2016) pre-treatment methods should: be cost-effective, expose substrates to microorganisms, not use or produce substances that inhibit biogas production, be energy efficient and finally it should not generate by-products that are toxic to the environment**.**

1.2 Problem Statement

Sweet potato tuber waste is rich in high energy carbohydrates which makes it suitable for biogas production. However, sweet potatoes tuber has starch granules that have double crystalline structure which is complex and resistant to digestive enzymes during hydrolysis. Additionally long and complex amylopectin chains in root and tuber starch make it difficult to hydrolyze into fermentable sugar which might lead to prolonged hydrolysis leading to longer retention time in the digester; therefore SPW is not directly suitable for AD. Consequently there is a need to subject SPW to pretreatment so as to render its complex structure more suitable for bioconversion.

1.3 Justification

In Kenya sweet potato is an important secondary food crop that plays an important role in household food security, it is grown in 43 out of 47 counties. About 763,643 tons of sweet potatoes were produced in 2014 from 61,067 hectares (Abong et al., 2016; W.Kihurani, 2004).

Globally, it is estimated that sweet potato tuber waste generated annually ranges from 5%-7% of the total production which amounts to approximately 5 million tons of waste. The waste currently has little commercial utilisation. The unutilized sweet potato residues are highly perishable and releases methane to the environment as they rot causing serious environmental pollution. Use of SPW as an energy source contributes to environmental protection since methane which would have been released to the environment from self decomposition of SPW is prevented.

Utilisation of SPW for biogas production can help to meet energy needs without competing with food security (Felipe, 2018; Frigon & Guiot, 2010; Montoro et al, 2019)**.**

Furthermore, when biogas is used in place of fossil fuel it contributes to the reduction of emissions of GHG and slows down the climate change.

In addition to biogas production, plant nutrients rich digestate which is a by-product of AD could be used in agricultural fields as a bio-fertilizer.

1.4 Research Objectives

1.4.1 Main objective

- To examine the effects of combined thermal and alkali pre-treatment on biogas production from sweet potato waste

1.4.2 Specific objectives

1. To analyse physicochemical characteristics of sweet potato waste for biogas production
2. To determine the effects of NaOH concentration, temperature and pretreatment time on biogas and methane yield from SPW.
3. To assess fertilizer properties of digestate from anaerobic digestion of thermochemically pretreated SPW.

1.5 The Scope of the Research

This research focuses on the waste generated from orange flesh sweet potato cultivar (Cavington) a variety with red skin and orange flesh. The variety was chosen because it is widely used in sweet potato processing plants and a lot of waste is generated during its processing. It is also narrowed to the waste generated from the root of sweet potato; it does not cover waste from other parts of the crop such as sweet potato leaves and vines.

Milled SPW was subjected to thermochemical pre-treatment with sodium hydroxide of concentration ranging at 0.6 g/L to 3.5 g/L, temperature varied from 50℃ -90℃ and time varied from 30-120 minutes. To achieve the objectives, two steps were involved namely; thermochemical pre-treatment of 20 sweet potato waste samples prior to AD and the AD of all pre-treated samples to determine the conditions for pretreatment of SPW which yields optimum biogas. The anaerobic digestion was carried out under mesophilic conditions 37 ± 1℃. Total solids, volatile solids, carbon and nitrogen ratio were among the parameters examined in the SPW.

1.6 Significance of the Study

Studies have also revealed that, pre-treatment of biogas feedstock enhances both biogas and methane yield and at the same time reducing the retention time in the digester (Zheng et al., 2014).

However, the possibility of enhancing biogas and methane yield from sweet potato root waste by making its complex structure more accessible to AD bacteria by some form of pre-treatment and the analysis of the SPW digestate as a bio-fertilizer has not yet been reported in literature. Besides contributing to the value chain of sweet potato, the present research also seeks to fill the apparent gap in literature.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

The global drive to meet energy requirements in a sustainable way has triggered the search of alternative energy sources which are renewable, affordable and have a minimum impact on the environment (Tamilarasan et al. 2019). Biomass can be described as non-fossil and biodegradable organic material derived from plants, animals and microorganisms that can be used as fuel (Cesaro & Belgiorno, 2015; Karuppiah & Azariah, 2018.; Krus & Lucas, 2014). Among renewable energy sources, biomass has attracted a great extent of interest since its renewable energy can be derived from it by using several techniques (Cesaro et al., 2015).

Three major techniques are involved in biomass transformation into energy source; thermochemical processes such as direct combustion, pyrolysis, gasification and liquefaction; bio-chemical process which include: alcoholic fermentation and anaerobic digestion; and finally physicochemical process such as extraction of biodiesel (Jena et al., 2017; C. Nzil et al, 2010). When selecting a suitable biomass bioconversion method, the nature of the feedstock, the availability of a given technology as well as the demand for a specific energy product are the factors which should be put into consideration (Cesaro et al., 2015). Biomass supplies between 9% and 16% of the world's total energy (Cesaro & Belgiorno, 2015; C. K. . Nzila, 2011; Schweinberger et al., 2016) thus it is an essential substitute for fossil fuel. Biofuel derived from biomass that is currently available in the market still depend on food and oil crops for their production. In this regard they compromise with food security, making them uneconomical and unsustainable (Jung et al. 2015).

According to Frigon et al. (2010) any technological approach that depends on the use of food crops for biofuel production does not meet renewable energy criteria. Its therefore necessary to stop or reduce the use of food and oil crops as energy sources and alternatively focus on the use of organic waste such as municipal waste, industrial waste, agricultural and forest residues in order to meet the renewable energy criteria (Jung et al. 2015). Nzila et al. (2015**)** reported that Kenya produces agricultural waste in massive quantities which are unexploited and when the waste is casted out using conventional methods such as burning, it results in environmental pollution. Therefore, AD of agricultural residues to produce methane is the most suitable method for disposing these organic waste materials at the same time generating energy for domestic use (Gopinatthan et al., 2015; C. Nzila et al., 2015).

2.2 Anaerobic Digestion Process

Anaerobic digestion (AD) is a bio-chemical conversion process in which organic matter is decomposed by various groups of bacteria to produce biogas, it occurs in oxygen (O_2) free environment. The process occurs naturally like in seabed, swamps, volcanic hot springs, flooded rice fields, municipal landfills as well as inside termites and in the digestive tract of rumen animals as shown in Figure 2.1 (Brodeur et al., 2011; Frigon & Guiot, 2010; Krus & Lucas, 2014; Trosgard, 2015; Zheng et al., 2014). However, biogas production process can be controlled in biogas plants to ensure maximum methane production and easy collection of the generated gas (Krus & Lucas, 2014; Lukehurst, 2012).

Figure 2.1: Areas where AD is likely to occur (Bochmann et al. 2012)

Biogas is a mixture of several gases; it is majorly consist of methane and carbon dioxide with small fraction of other gases. The gases and their respective percentages are as shown in the Table 2.1 (Demirel & Scherer, 2008; Uzodinma et al., 2008; Vindis et al., 2009; You et al, 2017).

Table 2.1: Combustible and non-combustible components of biogas

SN	**Combustible components**	Amount (%)
(i)	Methane gas (CH_4)	50- 75
(ii)	Hydrogen (H_2)	1-5
(iii)	Hydrogen Sulphide (H_2S)	0-3
	Non-combustible components	
(iv)	Carbon Dioxide	25-50
(v)	Water vapour	1-5
(vi)	Ammonia (NH_3)	0 – 5
(vii)	Oxygen (O_2)	0.1 - 0.5

Biogas burns with pale blue flame and has a calorific value of between 25.9-30 J/m^3 depending the amount of methane in the gas (Garba & Usman, 2009; Yeboah.,2016).

2.2.1 Stages of AD process

Anaerobic digestion is a complex biochemical process which involves four sequential steps: hydrolysis, acidogenesis, acetogenesis and methanogenesis.

Each stage involves a different group of bacteria, however the bacteria have a correlative co-existence since the compounds released at one stage are used as a

substrate in the next stage, but if the balance is altered, some product accumulation occurs such as volatile fatty acid which causes reduction in pH thus causing bacterial inhibition (Aslanzadeh, 2014; Felipe, 2018; Denisse et al., 2015). Water plays a key role in AD process; it facilitates movement and growth it as well as allowing mass transfer of particulate substrate (Orhorhoro et al., 2017).

2.2.1.1 Hydrolysis

This is the first stage in AD process which takes place outside the microbial cells of facultative bacteria. The bacteria produce hydrolytic enzymes that breaks down complex biopolymers which are insoluble in water and therefore cannot penetrate through cellular membrane, hence they cannot be not directly available to microorganisms (Tamilarasan et al., 2019). In this step, the complex polymers are broken down into their simple forms as follows; carbohydrates into simple sugars, proteins into amino acids and lipids into fatty acids (Gillian, 2011). The resultant compounds are small enough to move across the cell membranes of the acidogenic bacteria (Delatolla, 2012). The hydrolysis step is given in Equation 1 (Aslanzadeh, 2014)

$$\text{Biomass} + H_2O \rightarrow \text{Monomers} + H_2 \quad \ldots\ldots (1)$$

This stage in AD is normally considered as the rate limiting phase in anaerobic digestion of lignocellulosic biomass (Conrad, 1999; C. K.Nzila, 2011).

2.2.1.2 Acidogenesis (acidification/fermentation phase)

At the second stage soluble molecules from hydrolysis stage are further broken down by acidogens to organic acid, alcohols, carbon dioxide and hydrogen.

The organic acids produced are acetic acid (CH_3COOH), propionic acid (CH_3CH_2COOH), butyric acid ($CH_3CH_2CH_2COOH$), shown in Equation 2 and ethanol (C_2H_5OH) in Equation 3. Bacteria that are involved in the conversion of monomers utilise oxygen that was accidentally introduced into the digester (Azariah, 2019; Lyberatos, 2010).

$C_6H_{12}O_6 + 2H_2 \rightarrow 2CH_3CH_2COOH + 2H_2O$ …..... (2)

$C_6H_{12}O_6 \rightarrow 2CH_3CH_2OH + 2CO_2$ …..... (3)

2.2.1.3 Acetogenesis

At this stage, acetate bacteria convert the acid phase products into acetates and hydrogen as shown in Equations 4-7. Products from this phase may be used by methanogenic bacteria.

$CH_3CH_2COO^- + 3H_2O \rightarrow CH_3COO^- + H^+ + HCO_3^- + 3H_2$…… (4)

$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2$……..... (5)

$CH_3CH_2OH + 2H_2O \rightarrow CH_3COO^- + 2H_2 + H^+$……..…..……. (6)

$2HCO_3^- + 4H_2 + H^+ \rightarrow CH_3COO^- + 4H_2O$……..…….…… (7)

This phase determines the efficiency of biogas production because approximately 70% of methane results from reduction process of acetate. In stage phase, approximately 25% of acetates and 11% of hydrogen are produced in the wastes degradation process (Zheng et al., 2014).

2.2.1.4 Methanogenesis

This is the last stage in AD process; at this phase several reactions take place using the intermediate products from all the other phases to form methane. Even though a few bacteria are able to produce methane from acetic acid, most of the methane

produced in anaerobic digestion is from the conversion of acetic acid by heterotrophic methane bacteria (Karakashev et al., 2005). Basing on the methanogenic microorganisms involved, methanogenesis can be classified into three main groups illustrated by Equations 8-10 (Karuppiah & Azariah, 2018).

a) Acetoclastic methanogenesis -Acetate (CH_3COOH) → $CH_4 + CO_2$…… (8)

b) Hydrogenotrophic methanogenesis -$2H_2 + 2CO_2 \rightarrow CH_4 + CO_2$ ……..…..(9)

c) Methylotrophic methanogenesis-$(2C_2H_5OH)+CO_2 \rightarrow CH_4+2CH_3COOH$….(10)

Methanogenesis is a rate limiting step for substrates that are easily biodegradable and those that have low buffering capacity (Rozzi & Remigi, 2004, Horváth & Taherzadeh, 2016)**.** The step is also sensitive to changes in temperature, pH and total solid content in the digester (Jena et al., 2017). The AD process is summarized in the flow chart given Figure 2.2.

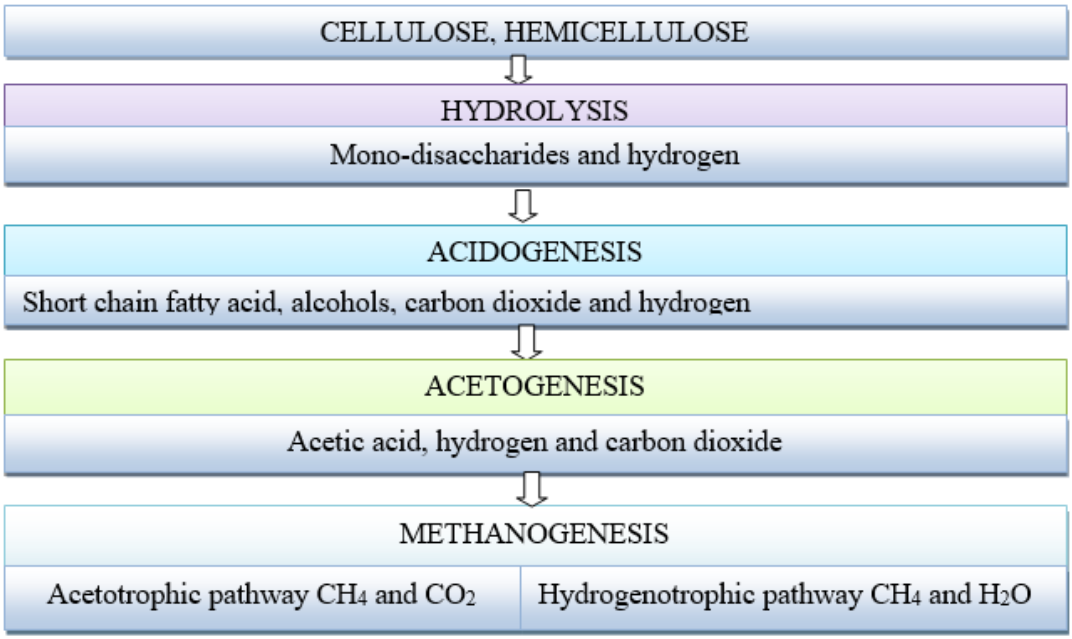

Figure 2.2: Flowchart Sequential steps for AD (Cate et a 2014)

2.3 Major Factors Influencing the AD process

Anaerobic digestion is a biochemical process with a series of steps, the microorganisms that are involved in the process are specific for each degradation step and each may require different environmental conditions; therefore, stability of the living conditions for the microbes is essential for efficient microbial metabolism. Changes in pH, temperature, substrate and organic loading affect anaerobic digestion performance (Gillian, 2011; Khalid et al., 2011):

i Effects of pH

PH is an important parameter in anaerobic digestion; it determines the stability and consistency of AD system. Metabolic activities of AD bacteria are sensitive to changes in pH; its alteration affects performance and growth of the various microorganisms involved in the different stages of the digestion process**.** A range of pH values have been reported by many researchers which have been divided into two groups, 5.5-6.5 for acidogens and 7.8-8.2 for methanogens. For combined cultures the pH ranges from 6.8 to 7.4 is highly favourable (Gillian, 2011; Khalid et al., 2011; Ogunjobi et al., 2018).

ii Effects of Temperature

Anaerobic digestion is strongly influenced by temperature; it has been reported that it affects the microbial community, process kinetics and stability and methane yield (Chen et al.,2008; Khalid et al., 2011). Among the bacterial community volatile acid forming bacteria and the methane forming bacteria are mostly affected by temperature variation (Gerardi, 2003 and Gillian, 2011). Different types of microorganisms exist in different temperature ranges. Basing on the temperature range AD can be categorized into three types namely (Trosgård, 2015):

i. Psychrophilic range (0-15 ℃): This is the slowest among the three temperature ranges in biomethane conversion process. This temperature range is rarely used.

ii. Mesophilic range (30-38 ℃): This is widely used in biogas production as these microorganisms can tolerate temperature variation of 3 ℃ without affecting biogas yield.

iii. Thermophilic range (45-55 ℃) : Thermophilic microbes have rapid growth and are more efficient in methane conversion.

According to Aslanzadeh, 2014, majority of the methane formers are active at two temperature ranges: mesophilic range (30–38 ℃)and the thermophilic range (45–55 ℃). Gavala et al., (2003) reported performance comparison of mesophilic and thermophilic AD of organic matter as shown in the Table 2.2.

Table 2.2: Comparison of mesophilic and thermophilic AD

	Mesophilic digestion	**Thermophilic digestion**
Temperature range	30-38 ℃	45-55 ℃
Gas generation rate	slow	fast
Degradation rate	slow	fast
Hydraulic retention time	20-30 days	10-20 days
Organic loading	low	high
Energy requirement	low	high
Sanitization risk	High	Low

iii Nutrients (C/N ratio)

Carbon to nitrogen for biogas feedstock (C/N) ratio is an important parameter which indicates the nature of biogas feedstock and its ability to biodegrade (Biswabandhu Chatterjee et al, 2016). Macronutrient (carbon, phosphorus, sulfur and nitrogen) and

micronutrients (iron, nickel and molybdenum) are essential for the growth and effective functioning of the bacteria (Trosgård, 2015). The optimum C/N ratio for biogas substrate should be within the range of 16:1-30:1 (Aslanzadeh, 2014). If the C/N ratio is too low, due to the degradation of the proteins and other nitrogenous materials, nitrogen will be released and build up in the form of ammonium ion (NH_4^+) or ammonia (NH_3) in the system (Gillian, 2011). The chemical equilibrium between the ammonium ($\boldsymbol{NH_4^+}$) (weak acid) and the ammonia (weak base) is controlled by temperature and pH as shown in Equation 11. An increase in the temperature or the pH would shift this equilibrium more towards ammonia.

$$\boldsymbol{NH_3} + \boldsymbol{H_2O} \rightleftharpoons NH4^+ + \boldsymbol{OH^-} \quad \text{(11)}$$

Free ammonia causes devastating effects on AD microbes as it capable of diffusing into the cell, causing proton imbalance or leading to a potassium loss. On the other hand, high C/N ratio means that there is low nitrogen in the substrate; consequently, the available nitrogen is quickly consumed by microorganisms in order to meet their protein requirements. Therefore, the carbon content in the substrate which could have been used in biogas production is left out in the substrate thus results in low biogas yield (Aslanzadeh,2014; Trosgard, 2015). The C/N ratio can be improved by co-digestion of organic mixture (Khalid et al., 2011).

iv **Volatile fatty acid**

Volatile fatty acids (VFAs) are important intermediates byproducts of the anaerobic digestion process. VFAs exist in two forms that are, dissociated and undissociated forms. Dissociated form exists when the pH level is high while the undissociated form occurs when the pH level is low.

Accumulation of VFAs leads to the drop in pH hence the VFAs in undissociated form dominates leading inhibition of methanogenesis (Aslanzadeh, 2014).

- v **Light metal ions(**(Na, K, Mg, Ca, and Al)**)**

Salt toxicity has been studied in the biological field for several decades. High salt level causes bacterial cells to dehydrate due to osmotic pressure. The light metal ions including sodium, potassium, calcium, and magnesium are present in the influent of anaerobic digesters. They may be released by the breakdown of organic matter (such as biomass), or added as pH adjustment chemicals. They are required for microbial growth and, consequently, affect specific growth rate like any other nutrient. While moderate concentrations stimulate microbial growth, excessive amounts slow down the growth, and even higher concentrations can cause severe inhibition or toxicity (Gillian, 2011; Ogunjobi et al., 2018; Ye Chen, 2007)

2.4 Digestate from AD process

Biogas residue also known as digestate is the remnant of the original substrates placed into the digester which was not utilized by microbes involved in biogas production process (Logan & Visvanathan, 2019). It is a mixture that is made of degraded organic compounds, inorganic macronutrients and microbial biomass (Alburquerque et al., 2012,). It can either be in liquid or solid form depending on the nature of treated waste and the technology used in AD which could be liquid or semi-dry or dry state anaerobic digestion. Liquid phase digestate is normally spread directly to agricultural field after the necessary cooling (Lukehurst, 2012; Teglia & Tremier, 2010).

The use of digestate as a fertilizer or soil conditioner in crop farms enables the recirculation of plant nutrients which reduces the need to use a chemical fertilizer as well as the need for new landfill (Arthurson, 2009; Risberg et al., 2017). Digestate use

in farm applications results in improved soils' physical properties such as water holding, permeability, water infiltration, aeration and soil structure at the same time suppressing crop disease; these provide good environment for roots for proper plant development. Soil fertility is also improved by introduction of mineralized macro nutrients such as nitrogen, phosphorus, calcium, potassium and other micronutrients (Arthurson, 2009; Evanyloetal., 2008; Teglia et al., 2010).

During AD process, the nutrients in a feedstock are conserved although they are converted to a more organic form that is easily available to plants (Bochmann, 2012). Therefore, the total mass of nutrients such as nitrogen, phosphorus and potassium that is fed into the digester is equal to the mass leaving as digestate. However treatment methods other than AD may lead to loss of nitrogen through volatilization (Logan & Visvanathan, 2019). According to Ascher & Insam, (2015) anaerobically digested animal manure is a better fertilizer than the untreated manure applied directly to the farm; this is because digestate from anaerobic digestion has higher proportion of plant available nutrients due to the mineralization of organic nutrients that are found in feedstock during AD. As an example, the amount of ammonium (NH_4^+) concentration which is more readily available to plants than organic N is generally higher in digestate than feedstock (Arthurson, 2009; Carey, Yang et al., 2016; Orzi et al., 2018). Plants' nutritional content in a digestate depends with the type of the substrate, microbial community, operational conditions and configuration of anaerobic digestion system (Logan & Visvanathan, 2019; Risberg et al., 2017).

For livestock manure, the nutrients, varies with the diet fed on the animal, geographical and climatic conditions as well as gender and the age of the animal (Ascher & Insam, 2015; Frost, 2014). Most developed countries have regulations and

standards for digestate management which help to address issues such as environmental pollution, spread of communicable diseases and offer training services on digestate storage and its applications (Logan & Visvanathan, 2019). A high quality digestate suitable for use as a fertilizer is defined by essential features such as; Total solids, Volatile solids, pH, declared nutrient content, safety to environment and human beings basing on its biological content and chemical pollutants (Bochmann, 2012).

2.5 Substrates for Biogas Production

A great extent of organic matter has been tried out for biogas production and has been classified into five categories;

i. Crop biomass- these include maize, sweet sorghum, barley and wheat
ii. Organic waste- examples are municipal solid and wastewater, industrial waste and animal manure
iii. Energy crops -like sunflower and rape
iv. Crop residues such as banana stem, barley straw, rice straw, softwood spruce and corn stove
v. Non-conventional biomass like glycerol and microalgae (Karuppiah & Azariah,2019)

Even though there are massive organic wastes which are generated by human activities, biogas plants in most families are based on livestock manure (Aslanzadeh, 2014), kitchen wastes such as peels from banana, cassava, sweet potato and potato among others have been neglected (Rajendran et al., 2012).

According to Tumutegyereize et al. (2016) over 90% of the developing countries produce at least two of the three crops (banana, cassava and sweet potatoes) from

which the peels are produced. The peels contribute the highest amount of waste amongst the household waste. Over reliance on livestock manure for biogas production has led to low uptake of biogas technology in developing countries since most households do not have livestock (Tumutegyereize et al., 2016). Agricultural, municipal and industrial wastes are amongst the organic material which are available in large quantities and can be utilized as a substrate for anaerobic digestion (Drosg et al. 2013).

However, not all organic waste products and crops are equally suitable for biogas production and in some cases biogas production might not be profitable at all. Therefore, detailed physical and chemical analysis of substrate is very important to get the exact idea about the quality of waste material before use in biogas production (Okunola et al., 2018).

2.6 Sweet Potato (*Ipomoea Batatas*)

Sweet potato (*Ipomoea batatas L.*) is a tuber crop which belongs to Convolulaceae family (morning glory); it is a herbaceous plant which is believed to have originated from Central and South America (Abong et al., 2016). It is ranked as the world's seventh most important crop after wheat, rice, maize, potato, barley and cassava (Stathers et al., (2005).

Sweet potato roots,vines and leaves are edible (Ma, 2019) and are utilised in a diversity of ways as food, feed and vegetable (Fatunbi, 2018). Starch is the major component in sweet potato root, it accounts upto 80% of dry matter (Zhu & Wang, 2014). The crop is cultivated throughout the world in more than 110 countries; this is attributed to sweet potatoes' distinctive nutritional nature as well as its health promoting effects.

Global annual production of sweet potato is more than 133 million tons from approximately 9 million hectares (Carvalho, 2016; Ivone, 2015; Kathabwalika et al., 2013; Vithu et al., 2019; S. Wang et al., 2016; Waramboi et al., 2011). China is the world's largest producer with 80% of the total global production while Africa is the second leading producer accounting to 10.6 % (Ketnawa et al., 2019; Moorthy, Sajeev et al., 2012; Odisha et al., 2016). In Africa, Tanzania and Nigeria are the leading producers of sweet potato while Uganda is the third and Kenya is the sixth producer (Abong et al., 2016). According Felipe (2018) and Akoetey et al. (2016), sweet potatoes can grow at altitudes ranging from sea level to 2,500 meters.

Preferable characteristics which have made sweet potato to be very attractive to farmers include; high productivity, high calorific content, low input and labour requirements, high tolerance to marginal growing conditions like dry spell and poor soil, it is also resistant to pest and diseases and can be harvested for a prolonged period of time (Dako et al., 2016; Stathers et al.,2005).

International Potato Centre has documented over 8000 sweet potato varieties that have been grown for different purposes**.** The sweet potato genotypes varies in several aspects such as productivity, root structure, root shape, root skin colour, vine colour, flavour, texture and resistance to pests (Felipe, 2018). The edible tuberous root is long and tapered with a smooth skin with colours ranging from red, purple, brown to white. The colour of its flesh ranges from white to yellow, orange and purple (Adegunloye & Oparinde, 2017; Rahman et al., 2013).

When the crop is matured it produces flowers that are either white or purple while its leaves are either green or purple (Christerbel Nicanuru, 2016). Figure 2.3 shows an example of sweet potato root.

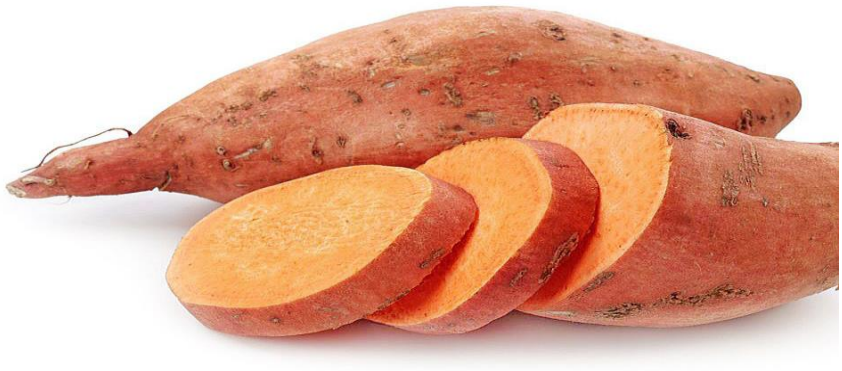

Figure 2.3: Sweet potato tubers

In Kenya sweet potato is an important secondary food crop that plays an important role in household food security, it is grown in 43 out of 47 counties. About 763,643 tons of sweet potatoes were produced in 2014 from 61,067 hectares (Abong et al., 2016; W.Kihurani, 2004). Nationally the annual production of the sweet potato has been expanding over the years due to farmers slowly shifting to the crop for various reasons such as; pest and diseases attacks on major crops such as maize, decreasing soil fertility due to wrong farming practices and a growing understanding by consumers that sweet potato is a healthy crop and not a poor man's crop (Fatunbi, 2018).

For instance, in Bomet County within Rift valley the crop has gained massive acceptance and its production has suddenly grown. Between the years 2012 to 2014 an increase in sweet potato production from 4,650 tons to 30,971 tons was recorded (MoALF. 2018). Even though sweet potato has a lot of appealing characteristics, its root is highly perishable due to high moisture and sugar contents as well as its delicate skin. Under normal conditions sweet potato roots can be kept for five days only and its quality starts to decline with time causing major losses to farmers and users (Ahmed et al., 2010). Makini et al. 2018, reported that in 2011 about 7% of the entire crop was lost globally.

Currently 95% of sweet potato produced in the country are consumed or sold unprocessed it therefore gives low returns to farmers. The challenge of short shelf life of the sweet potato root could be addressed by ensuring that the roots are free of surface wounds and bruises, pest or disease damage (Lu & Gao, 2011). Furthermore the root can be processed to other value added products such as flour or chips that can be stored for a longer duration and be transported with ease (Ngoma et al. 2019). However there are a few potato processers in the country which are already functional while others are in the initial stages of establishment (F. Makini et al., 2018).

Among the few sweet potato processers the County government of Bomet in collaboration with World vision established a sweet potato processing plant (SPPP) which is engaged in value addition activities of the sweet potato root to produce products such as cakes, bread and sweet potato crisp (MoALF. 2017). However, there is no value addition to the waste generated during process. The waste is normally given freely to farmers to feed their animals while more substantial waste is left to decay. Meanwhile anaerobic digestion of the unutilized waste fraction could possibly produce energy that could offset the energy requirements of the SPPP (Akoetey et al., 2016; Montoro et al., 2019). According to Martins et al. (2019) industrial sweet potato and the sweet potatoes that do not meet the market standard of consumption can be considered for energy production.

2.7 Sweet Potato Losses and Waste

Sweet potato losses occur in all sections of sweet potato supply chain; right from the farm to the point of consumption. On average it has been estimated that 15-65% of sweet potatoes are lost in all phases of its supply chain (Ahmed et al., 2010). There are five main aspects in the sweet potato supply chain in which the tuber losses occur;

a) **Losses in the farm**: These are the losses which occur during harvesting of sweet potatoes, they could occur due to mechanical damages caused by harvesting tools and spillages.

   Dewan et al. (2013) claimed that approximately 20% of the total sweet potatoes cultivated were lost in the harvesting field due to damages which occurred while harvesting with ploughs.

b) **Post-harvest handling and storage**: waste in this level include spillages and degradation during handling and transportation and use of improper storage facilities such as sacks (Gustavsson et al., 2011).

c) **Losses in processing**: sweet potato losses may occur when the tubers are sorted out in SPP and any root that is diseased or damaged by insects, partially decayed or any root that is not suitable for human consumption or industrial processing are gotten rid of as culls (Mussoline & Wilkie, 2015). Figure 2.4 shows a sweet potato root that was damaged by sweet potato weevils.

Figure 2.4: Weevil damaged sweet potato roots (Fatunbi, 2018)

Sweet potato processing to produce products such as starch, organic acid, ethanol, chips and sweet potato bread results in generation of massive waste in form of peels,

trimmings, chunks of tuber, and nutrient-rich wastewater. It is estimated that more than a quarter of all the sweet potatoes that goes into potato processing plant as input comes out as waste (Tedesco & Stathers, 2015, Akoetey et al., 2016).

This is because most of the value addition processes of sweet potato start with peeling of the root where the skin and some amount of flesh are discarded as waste; the peel is rarely used in mainstream processing activities for starch and food because of high fibre content and pigment (Vithu et al., 2019). Starch content in sweet potato peel depends on the peeling method, for instance steam peeling results in 28% starch while abrasion peeling such as knife peeling results in higher starch content approximately 58 % as more flesh is removed in abrasion peeling (Adegunloye & Oparinde, 2017; Vithu et al., 2019). The Table 2.3 shows the composition of sweet potato before and after abrasion peeling (Ojewumi et al., 2018).

Table 2.3: Proximate amount of SPW generated

Sweet potato	Weight (Kg)	Percentage %
Sweet potato (before peeling)	5	-
Sweet potato (after peeling)	3.5	70
Loss due to peeling	1.5	30

d) **Losses in distribution**: These are the losses which occur during marketing of the sweet potato root (FAO, 2011). According to literature (Fleming et al., 2009; Martins et al., 2019) up to 40% of a sweet potato crop may be unsuitable for fresh market sales due to poor visual appearance or size.

When sweet potato tuber does not meet the market standards, they are used as animal feed or become waste and is sent to landfills or even left in the field. Furthermore, during rainy season, a lot of sweet potatoes rot, hence generate more

waste. Some retailers reported that during rainy season approximately 20-30% of the fresh sweet potato roots in a sack purchased in Nairobi market may be rotten, while during the other times of the year this percentage is less than 5% (Felipe, 2018).

e) **Consumption:** These are losses and waste during consumption at the household level such as cooked and uncooked sweet potato leftovers (Gustavsson et al., 2011).

2.8 Characteristics of Sweet Potatoes

i. Molecular and structural characteristics of sweet potato

Sweet potato is comprised of two starch molecules that is, amylose and amylopectin (Colonna et al., 2002). Amylose is a minor polymer whose composition in sweet potatoes ranges from 20%-30%. It is made up of linear and slightly branched structure that contains approximately 6000 glucose units and it forms a colloidal suspension in hot water. Amylopectin is the major polymer in sweet potatoes amounting to 70%-80% by weight. Amylopectin is the largest existing natural molecule which has approximately 2,000,000 glucose units per molecule and has side branches. Amylopectin is completely insoluble in water (Duvernay, 2008 ; Chen et al., 2005; Aslanzadeh, 2012; Mu & Zhang, 2019). The structures and the relative amount of both polymers in starch are the two major factors that determine the starch properties (Mu & Zhang, 2019).

According to literature, amylopectin chains have complex structure with 4% to 5% of the total linkages forming branches, this makes the sweet potato structure resistant to digestive enzymes during hydrolysis compared to other cereal starches (Ratnayake,

2001; Srichuwong et al., 2005; Mussoline & Wilkie, 2015; Kim et al., 2013; Abegunde et al., 2013 and Mu & Zhang, 2019).

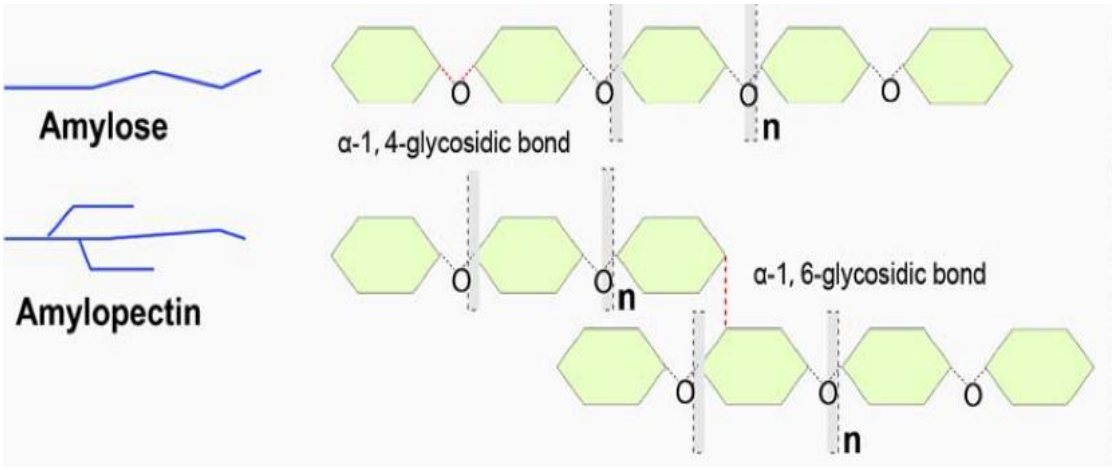

Fig 2.5 molecular structures of amylose and amylopectin (Ruiqing Lyu 1, 2021)

X-ray diffraction studies have revealed that sweet potato starch granules are made up of alternating crystalline and amorphous regions (Zhu & Wang, 2014). Native starch has crystallinity which ranges from 15%-45% (Roberts & Cameron, 2002). The distribution of crystallites in starch granules is an important factor controlling the rate of hydrolysis (Colonna et al., 2002).

ii. Physicochemical characterization of SPW

Characterization of a substrate before subjecting it to AD is important because, knowing properties such as Total solids (TS) and volatile solids (VS) concentrations makes it possible to predict biogas yield as well as the process efficiency (Orhorhoro et al., 2017; Xin Mei, 2010). Table 2.4 shows the physicochemical characteristics of sweet potato peels.

Table 2.4: Chemical composition of sweet potato peels from abrasion peeling method

Sn	Composition	Amount
1	Starch	64%
2	Reducing sugar	1.22%
3	Total carbohydrate	71.1%
4	Dry matter	3-4%
5	Ash content	5.6%
6	Moisture content	61.2%
7	Organic matter	94.4%
8	Cellulose	31.19g/100g of DM
9	Lignin	16.85 g/100g of DM
10	Pectin	15.65 g/100g of DM
11	Hemicellulose	11.38 g/100g of DM

Reducing sugar in various varieties of sweet potatoes ranges from 1.2% to 24.4%. Orange flesh sweet potato (Cavington) in particular has 3.2% reducing sugars (S. Wang et al., 2016). In the Table 2.4 above, the amount of reducing sugar is 1.22% while starch is high 64%. According to Ojewumi et al., (2018) it is not practical to ferment sweet potato peel in its natural form without any pre-treatment since it will produce small amount of ethanol or no ethanol at all, hence the starch needs to be broken down into fermentable sugar.

2.9 Effects of Sweet Potato Waste on Environment

According to Adegunloye & Oparinde, (2017) food processing is a very important industry globally, however disposal of its by-products raises a lot of environmental concerns.

This is because its waste is majorly organic and causes serious environmental pollution if not handled in an appropriate manner. Unutilized sweet potato residues

are highly perishable and release methane as they rot because they are rich in polysaccharides and proteins (F. Wang et al., 2016). Methane gas is the second most common greenhouse gas (GHG) and causes more severe effects to the environment compared to carbon dioxide; 1ton of methane in the air on global warming is equal to the effect of 21 tons CO_2 in a period of 100 year (Haghighat et al., 2019). Therefore, unutilized SPW pose a serious problem of environmental pollution (F. Wang et al., 2016).

2.10 Application of Sweet Potato for Biogas Production

SPW is suitable for AD process because it is rich in high energy carbohydrates (31%)**,** which is the highest among other energy sources for biofuel production and have very good potential for fermentation (Ojewumi et al., 2018**,** Schweinberger et al., 2016). Felipe, (2018**)** compared biogas potential of three sweet potato genotypes and found out that the cumulative amount of biogas produced varied with sweet potato genotypes.

Akoetey et al. (2016) also compared methane production of tropical forestry (albizia) wastes with food wastes including sweet potato, taro and papaya. They observed that the highest biogas yield was from food waste which had sweet potatoes. Montoro et al. (2019) also co-digested sweet potato and dairy cattle manure by varying sweet potato from 0-50 %, they observed that increasing the proportion of sweet potato in co-digestion with dairy cattle manure caused linear increase in biogas production while the methane in biogas decreased in relation to addition of sweet potato. Similarly, Martins et al. (2019) co-digested poultry slaughter wastewater and sweet potato, they discovered that the highest methane yield was obtained when poultry slaughtering waste was at 80% while sweet potato was at 20%. Above the ratio the

biogas production ceased after seven days. According to Akoetey et al. (2016) biogas produced from AD of sweet potato waste can be used to offset energy requirements in a processing plant.

2.11 Pre-treatment of AD feedstock

Lignocellulosic biomass is well suited for renewable energy production because it is abundantly available, low cost and environmentally friendly production (Brodeur et al., 2011). A large percentage of municipal solid waste (MSW), crop residues, animal manures, forest residues or dedicated energy crops are lignocellulosic (Taherzadeh et al., 2008a). However, the inherent characteristic of lignocellulosic materials is the main complication for efficient bioconversion of cellulose and hemicellulose into simple sugars for the next step in biogas production process (Radziah Wahid, 2014).
The main drawback of conventional anaerobic digestion is that, slow hydrolysis during AD process results in high hydraulic retention time in the digester and therefore bigger digester volumes are required (Karuppiah & Azariah, 2019).

To speed up the AD process of lignocellulosic material, pre-treatment step is necessary. The main goals of the pre-treatment are to disorganize the crystalline structure of micro- and macro-fibrils hence improving accessibility of soluble organic materials and modify pores for microbial break down. This leads to increase in the rate of biomass degradation by speeding up hydrolysis phase which take the longest time among AD stages (Brodeur et al., 2011; Sindhu et al., 2015; Zheng et al., 2014).
Biogas yield and reduction of incubation time of substrate in the digester are the two critical factors which should be considered during pre-treatment (Haghighat et al., 2019). Frigon & Guiot (2010**)** reported that pre-treatment of sugar and starch crops

has been neglected yet it produces positive results on methane yield as well as incubation time.

2.11.1 Parameters affected by pre-treatment

a. Effects on crystallinity

The cellulose structure is made up of both crystalline and amorphous regions; approximately 67% of the total cellulose is in the crystalline form. According to literature (Taherzadeh & Karimi, 2008a) cellulase enzyme easily hydrolyses the more accessible amorphous portion of cellulose, but the same enzyme is ineffective in degrading the less accessible crystalline region. The authors therefore concluded that, reducing crystallinity increases digestibility of lignocellulosic biomass, hence yielding more biogas.

b. **Effects on substrate surface area**

Substrate surface area is categorized into two: the internal surface area and the external surface area**.** The external surface area is related to the size and shape of the particles while the internal surface area depends on the porosity of cellulosic fibres.

Removal of lignin and hemicellulose in lignocellulosic material makes the structure to be porous hence become more accessible to enzymes during hydrolysis. Reduction of substrate particle size by physical means increases the external surface area (Taherzadeh & Karimi, 2008a).

c. Effects on lignin

Lignin binds cellulose and hemicellulose together; it is responsible for shape, structural rigidity and prevention of swelling of lignocelluloses. The amount and distribution of lignin in lignocellulosic materials determines the level of resistance to enzymatic degradation (Brodeur et al., 2011). According to Taherzadeh & Karimi,

(2008a) most lignin removal methods also hydrolyses part of hemicellulose hence the delignification does not show the sole effect of lignin. Even though lignin removal is beneficial, the lignin removed dissolves in the substrate and causes inhibitory effects to cellulase, xylanase and glucosidase. Various cellulases are inhibited to different extent by lignin, however the xylanases and glucosidase are less affected by lignin (Zheng et al., 2014).

d. **Effects on hemicellulose**

Hemicellulose is a physical barrier which covers the cellulose fibres; it offers protection to the cellulose from enzymatic attack. Many pre-treatment methods can remove hemicelluloses and hence improve the enzymatic hydrolysis. But most of these processes partly remove the lignin as well, consequently the improvement in hydrolysis is not as a result of removal of hemicellulose alone (Taherzadeh & Karimi, 2008a). Figure 2.5 shows how pretreatment affect the structure of lignocellulosic material.

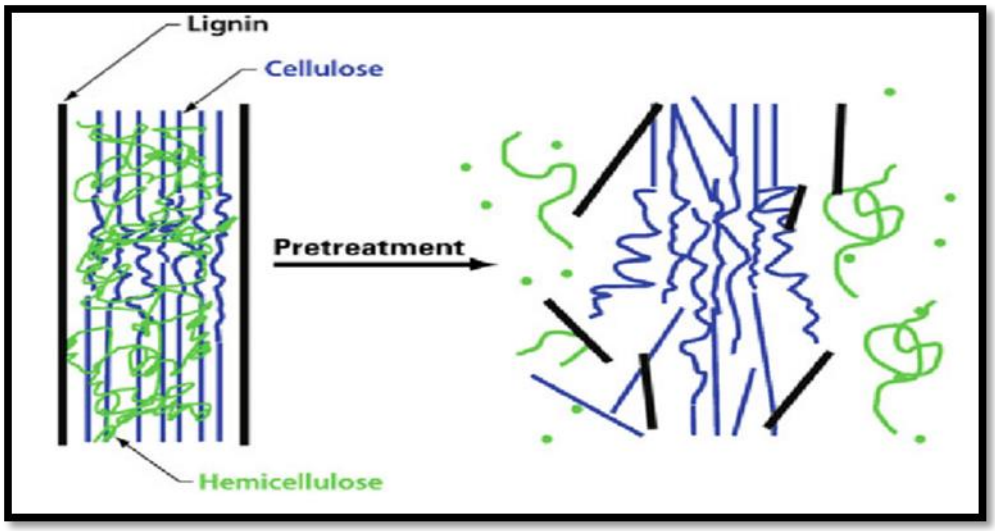

Figure 2.6: Lignocellulosic biomass before and after pre-treatment (Brodeur et al., 2011)

2.11.2 Pre-treatment methods

The effects of pre-treatment on a biogas feedstock depend on the characteristics of the substrate and the pre-treatment method applied. Therefore different pre-treatment methods used on the same substrate could produce different results on biogas yield during AD (Tamilarasan et al., 2019; Chundawat & Balan, 2010). Generally, an ideal pre-treatment method should meet the following requirements: (Brodeur et al., 2011; Gillian, 2011; Taherzadeh & Karimi, 2008b).

- It should produce highly digestible solids that enhances sugar yields during enzyme hydrolysis
- It should not degrade organic matter
- It should limit the formation of inhibitors
- Recovery of lignin for conversion into valuable co-products should be possible
- It should be cost effective in terms of size of the reactors and energy used

Biogas yield should not be the only factor considered when selecting an optimum pre-treatment method for a given biogas feedstock; the effect of the pre-treatment method on the environment and the suitability of a specific pretreatment in large scale applications should also be taken into account (Wahid, 2014).

Pre-treatment methods for biogas feedstock are broadly classified into four methods: mechanical, chemical, biological and hybrid methods (Taherzadeh & Karimi, 2008). The pre-treatment methods have been summarized Figure 2.6.

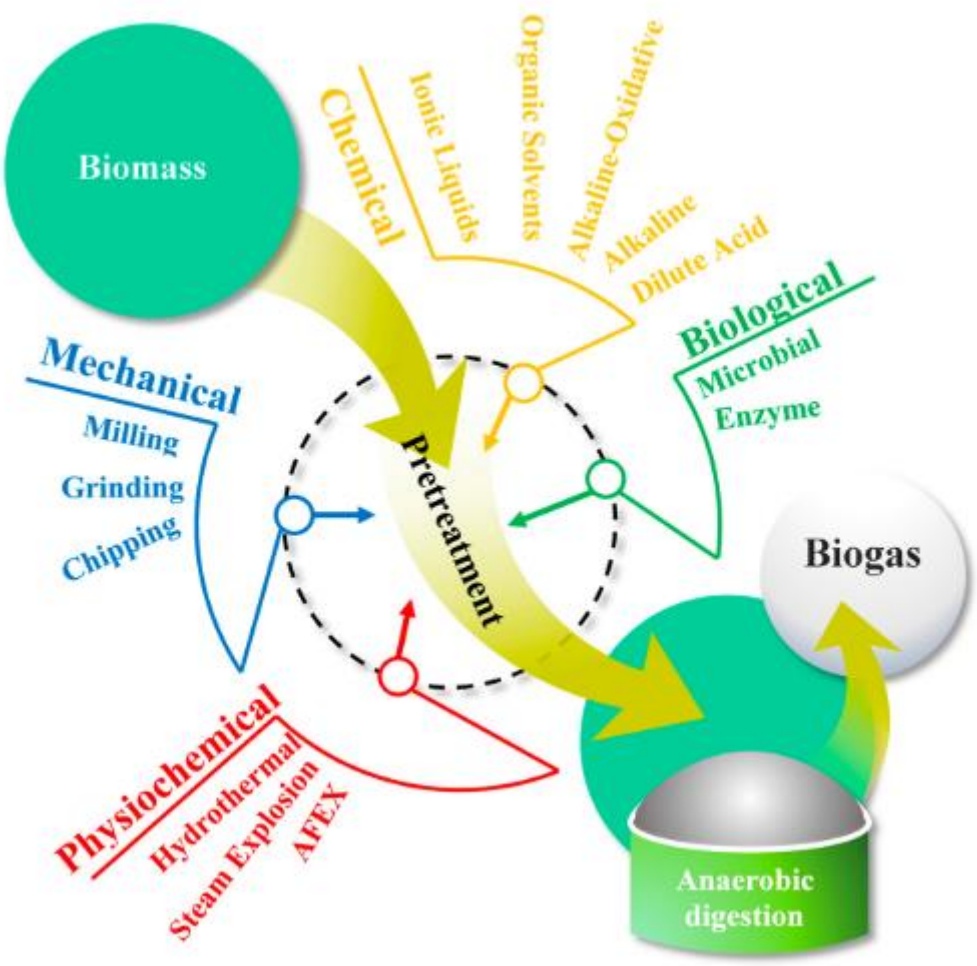

Figure 2.7 : Pre-treatment methods to increase the bioavailability of lignocellulosic biomass (Ulises et al., 2019)

a) Physical/mechanical pre-treatment

This is a treatment method in which the structure and size of particles in biomass are altered by application of physical force; no chemicals or microorganisms are used in the treatment process (Karuppiah & Azariah 2019; Zheng et al., 2014).

It is the simplest form of pre-treatment which involves breakdown of biomass size and crystallinity by milling or grinding in order to increase the specific surface area and bioavailability of biomass (Brodeur et al., 2011).

In addition to increasing biogas yield, particle size reduction also has effects on the viscosity in digesters and reduces the formation of floating layers (Bochmann &

Montgomery, 2013). According to Zheng et al. (2011) and Brodeur et al. (2011) particle size reduction of a substrate leads to an increase in the surface area of the particles thereby making it easily attacked by enzymes resulting to enhanced AD process for methane production. Mechanical pre-treatment is achieved by using methods such as microwave irradiation, sonication, mechanical beating, deflating, dispersing, extruding, refining, milling, grinding and cavitation (Zheng et al., 2014).

a) Thermal pre-treatment

This is a pre-treatment method which involves the application of heat to the lignocellulosic substrate, heating causes solubilization of the lignocelluloses (Gillian, 2011). A wide range of temperature (60 ℃ to 270℃) has been studied, but temperature above 200℃ has been reported to be responsible for the production of inhibitory intermediates during the pre-treatment process which causes sharp reduction in biodegradation (Wilson et al., 2009; Karuppiah & Azariah, 2019). Thermal pre-treatment method can be classified into two categories basing on the amount of heat applied; the temperature below 110 ℃ is considered as low thermal pre-treatment, while temperature above 110 ℃ is considered as high thermal pre-treatment.

Many studies employed at an optimum thermal range of 160–180 ℃ for hydrolysis of wastewater sludge have proved an increase in methane yield during AD (Saragih et al., 2019).

b) **Chemical pre-treatment**

This pre-treatment method involves the use chemicals such as acids, bases and ionic liquids, to alter the physical and chemical characteristics of lignocellulosic biomass.

i. Acid pre-treatment

Acid pre-treatment involves the use of concentrated acid (30-70%) and low temperature or diluted acid (0.1%) and high temperature (230°C) to break the rigid structure of the lignocellulosic material. Both organic and inorganic acid have been used in acid pre-treatment (Zheng et al., 2014). The most commonly used acid is dilute sulphuric acid (H_2SO_4); it has been commercially used to pre-treat a wide variety of biomass types. Other acids that have been applied include: hydrochloric acid (HCl), phosphoric acid (H_3PO_4) and nitric acid (HNO_3). Due to acids' ability to remove hemicellulose, acid pre-treatments have been used as parts of overall processes in fractionating the components of lignocellulosic biomass. Acid pre-treatment to remove hemicellulose followed by alkali pre-treatment which removes lignin, results in a relatively pure cellulose. The optimum pre-treatment conditions for acid are determined by the targeted sugars and the purpose of the pre-treatment. It is very important to ensure that the formation of inhibitory compounds is reduced during acid pre-treatment (Brodeur et al., 2011).

ii. Alkaline pre-treatment

This is a chemical pre-treatment method which involves the use of bases, such as sodium hydroxide, potassium hydroxide, calcium hydroxide and ammonium hydroxide for the pre-treatment of lignocellulosic biomass (Brodeur et al., 2011). Sodium hydroxide is preferred because it is used under mild conditions and it effectively attacks the linkage between lignin and hemicellulose (Ulises et al., 2019). In lignocellulosic biomass, alkaline causes delignification and de-esterification of intermolecular ester bonds causing swelling of lignocelluloses (Bensah & Mensah, 2013; Gumisiriza et al., 2017). When the bonds are broken the porosity and internal surface area of the biomass increases while the degree of polymerization and

crystallinity decreases (Bochmann et al., 2013). Alkaline pre-treatment also reduces the degree of inhibition during methane fermentation and provides a lower production cost compared to other pre-treatment methods (Chandra et al., 2012).

According to Kaparaju & Felby, (2010) and Gillian, (2011) alkaline pre-treatment can convert lignin into substrate suitable for biogas production such as VFA. The most important parameters affecting pre-treatment of lignocellulosic biomass are; the type of alkali, concentration of alkali, biomass loading, pre-treatment temperature and pre-treatment time. The conditions of alkali pre-treatment varies depending on the type of substrate and its composition (Sindhu et al., 2014; Ulises et al., 2019). High concentration of alkali causes degradation and decomposition of polysaccharides and the formation of inhibitory products. Low alkaline concentrations at low temperature and at atmospheric pressure are therefore recommended (Ulises et al., 2019). Compared to acid hydrolysis, NaOH pre-treatment is preferred because enzymatic biodegradability is improved due to its higher delignification ability, sugar degradation and corrosion is less severe in alkali process. In addition to that its environmental impact is low and no special reactors are required (Bensah et al., 2013).

iii. **Oxidative pre-treatment**

Wet air oxidation is a pre-treatment method that enhances contact between molecular oxygen and organic matter for the complete degradation of organic compounds into carbon dioxide and water. To achieve this, high temperature and high pressure conditions are required (Strong et al., 2011).

iv. Ozonation pre-treatment

Ozone is a strong oxidant; it has an ability to degrade lignin in various feedstock; it can reacts with the polysaccharides, proteins, lipids and other recalcitrant compounds and transforms them to monomers which are utilizable by microorganism in anaerobic digestion process, thus the AD process is improved (Zheng et al., 2014).

c) Biological pre-treatment method

This is the biological degradation of substrates by use of microorganism; this pre-treatment method is categorized into three: fungal pre-treatment, pre-treatment by microbial consortium and enzymatic pre-treatment (Zheng et al., 2014).

Complex biopolymers such as carbohydrates and proteins are broken down to simpler end products by enzymes produced by bacteria (Tamilarasan et al., 2019). The advantage of using biological pre-treatment over other methods is that, the biological method is able to solubilizes the organic compounds present in the biomass with minimum energy and has no severe changes in substrate content (Kavitha et al., 2013).

d) Hybrid pre-treatment method

Biodegradability of substrate is determined by several factors which include crystallinity of cellulose, lignin content and the bonds between hemicellulose and lignin. Because of the many factors involved, no single pre-treatment can effectively eliminate all the barriers to biodegradation. Combining two or more pre-treatment methods produce positive results on the substrate and biogas yield (Zheng et al., 2014). Hybrid pre-treatment method therefore consists of different combinations of physical, chemical and/or biological pre-treatment techniques. The commonly used hybrid methods are the hydrothermal and thermochemical while the bio-thermo-

chemical is the least commonly used hybrid method (Azariah, 2019; C. K.Nzila, 2011). This research focuses on thermochemical pre-treatment of sweet potato root waste.

(i) Thermo-chemical pre-treatment

This is a combined pre-treatment method which uses both thermal and chemicals such as acids and/or bases for treatment (Nzila, 2011). Thermochemical pre-treatment of lignocellulosic biomass is known to enhance the yield of fermentable sugars during enzymatic hydrolysis depending on the nature of the substrate and type of pre-treatment (Chundawat et al., 2010). Comparative studies have proved that thermo-alkaline hydrolysis is the most effective in breaking the linkages between lignin, cellulose and hemicellulose (Denisse et al. 2015)**.** Optimum thermo-chemical pre-treatment conditions for selected chemicals are summarized in the Table 2.5.

Table 2.5: Optimum thermo-chemical pre-treatment conditions for alkaline solutions (Brodeur et al., 2011; Charles K . Nzila, 2011)

Chemical	Conc. (%)	Temp. (°C)	Time (hr)	Lignin content	%CH4 enhancement	Advantage /Disadvantage
NaOH	0.08-0.3 (2%)	190	0.33	Low	73%-83%	Provide pH buffering but the resultant digestate cause soil erosion
$Ca(OH)_2$	10%	85	16	High	142%	Cheap and requires low temperature but not suitable for substrate rich in carbohydrates
CaO	6%-8%	15	600	High	59%	Leave no chemical residue
NH_3OH	3%	120	0.33	High /Low	28%	Generate substantial inert COD

Conc.: Concentration and Temp: Temperature

The aim of pre-treating sweet potato waste is to reduce high crystallinity in starch, removal of lignin and hemicellulose that hinders the access of enzymes to cellulose, thus increasing significantly the porosity of substrate and facilitating its subsequent biochemical conversion to fermentable sugars (Vavouraki et al., 2014). Table 2.6 gives a summary of related studies on pre-treatment which resulted in improved biogas and methane yields.

Table 2.6: Related studies for pre-treatment

Substrate	Description	Finding and gaps	Reference
Sweet potato and cow dung	Anaerobic co-digestion of sweet potato and cow-dung	Co-digestion of cow dung and SP up 50% caused linear increase in biogas yield. Co-digestion of cow dung and SP more than 50% should be tried.	Montoro et al. (2019)
potato peels	Potato peels were subjected to thermochemical treatment with NaOH at 50 ℃ for 30 minutes at a pH 10 and 12	Biogas and methane improved by 4.18% and l57.9% respectively in respect to the untreated potato peel	(Krus & Lucas, 2014)
organic food waste	Organic food waste was chemically treated with (equivalent to 6.1 g $Ca(OH)_2$/L) for 1 hour	Calcium hydroxide improved methane from food waste up to 20% compared to untreated	(Kasper & Schiffels, 2016a)
pine wood	Pine wood waste was subjected to chemical treatment with 8.0% w/w NaOH solution at two temperatures (0 and 100℃) and time (10, 30 and 60 min).	NaOH pretreatment caused reduction of cellulose crystallinity and removed lignin. However prolongation of treatment causes decomposition of dissolved polysaccharides and formation of alkali-stable end groups.	(Salehian et al., 2013)
Kitchen waste	Kitchen waste was thermo-chemically pre-treated with 3% and 6% NaOH and Temperature 60, 90 and 120 ℃	Increasing NaOH concentration and temperature improves biogas production	(Haghighat et al., 2019)
Pig manure	Thermal, chemical and thermochemical pre-treatment of dewatered pig manure	The maximum biogas production in thermo-chemically pretreated samples was observed at 70^0C and then decreased with the increase of temperature.	(Rafique et al., 2010)
Sweet Potato peels	Bioconversion of sweet potato peels to ethanol	It is not practical to ferment SP peels due to its low reducing sugar content. Starch conversion to fermentable sugar should be facilitated	(Modupe Elizabeth Ojewumi1*, 2018)

CHAPTER THREE

MATERIALS AND METHODS

3.1 Introduction

The experimental studies in this research were conducted at Moi University Laboratories in Eldoret Kenya and partly in Kenya Agricultural and Livestock Organisation (KALRO) in Kabete, Kenya. The order in which the experiments were carried out and the tasks involved are summarised below in Figure 3.1:

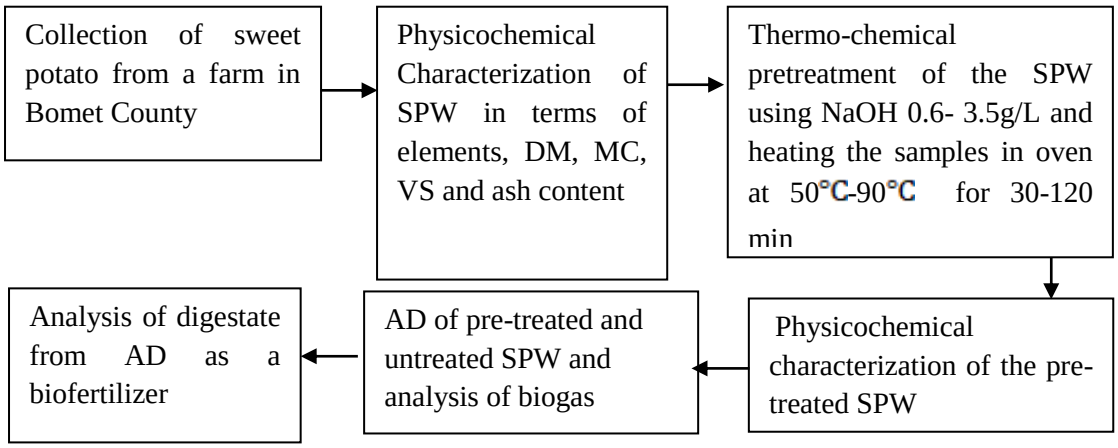

Figure 3.1: General overview of methodology

The following materials, tools and equipment were used:

- Orange fleshed sweet potatoes (Cavington) used in the experiment were obtained from a farm in Ndanai location within Bomet County, Kenya.
- Inoculum based on dairy cattle manure was obtained from a biogas plant in Moi University Main Campus and stored for 10 days in a closed container to reduce its biogas potential.
- 250 ml conical flasks, one holed and two holed rubber coax, rubber tubes, water bath, blender, 250 ml glass beakers, Oven, furnace, gas analyser, measuring cylinders and sodium hydroxide pellets.

3.2 Quantification of Waste Generated from Manual Peeling of Sweet Potato Root

Sweet potatoes were washed thoroughly with clean tap water to remove all the adhering soil, dirt and impurities then left to drain for one hour. The cleaned SP were weighed then subjected to manual peeling using a sharp knife to generate peels in Figure 3.2.

Figure 3.2: sweet potato peels from manual peeling

The edible part of sweet potato and the waste generated from the peeling process were weighed separately and recorded. The amount of waste in percentage was calculated by dividing the weight of waste by the original weight of sweet potato before peeling as shown in the Equation 12.

$$Waste\ \% = (W_2 - W_0)/(W_1 - W_0) \times 100 \quad \text{.....(12)}$$

Where W_0 = mass of empty container, W_1 = mass of unpeeled sweet potatoes+ empty container, W_2 = mass of peels+ empty container

The generated peels were mixed with sweet potato culls and were subjected to size reduction as shown in Figure 3.3 using laboratory blender (NUTRIBULLET 600 series)

Figure 3.3: Milled sweet potato waste

3.3 Physicochemical Characterization of Sweet Potato Waste

3.3.1 Determination of pH

The pH of SPW was determined using method described by (Drosg et al, 2013). 10 g of milled SPW was mixed with 100 ml of distilled water and stirred manually for 30 minutes and left stand still for 1 hour 30 min at room temperature. Hannah pH meter was used to measure the pH value of the mixture.

3.3.2 Determination of dry matter and moisture content

Moisture content (MC) and Dry Matter (DM) content were determined according to the standard methods (Baird et al., 2012) described by Drosg et al. (2013). An empty crucible was weighed and 2g of the milled SPW was added and the sample was placed in an oven (DAIHAN LABTECH) and heated at a temperature of 105 ℃.

Heating, cooling and weighing was done repeated until a constant weight was achieved. MC and DM were calculated by dividing the weight after drying at 105 ℃

with the original weight, as shown in the Equations 13 and 14.

$$\%MC = (W\_2 - W\_3)/(W\_2 - W1) \times 100 \quad (13)$$

$$\%DM = (W\_3 - W\_1)/(W\_2 - W\_1) \times 100 \quad (14)$$

Where;

W_1 = mass of empty crucibles

W_2 = mass of wet SPW sample and crucible

W_3 = dried cooled sample and mass of crucible

3.3.3 Determination of total ash and organic matter

Total ash and organic matter (VS) were determined using standard methods APHA (2005) modified by Ojewumi et al. (2018b). The dried SPW sample from the determination of dry matter was placed in to an already heated furnace (CARBOLITE GERO). The temperature of the furnace was gradually increased from 250℃ to 550℃

after every 20 minutes to avoid incomplete ashing. After one hour of ashing, the crucible was removed from the furnace with a tong and left to cool at room temperature then weighed. The organic matter and ash contents were obtained by using the Equations 15 and 16.

$$\%total\ ash = (W\_4 - W\_1)/(W\_3 - W\_1) \times 100 \quad \ldots\ldots(15)$$

$$\%Organic\ matter = (W\_3 - W\_4)/(W\_3 - W\_1) \times 100 \quad \ldots\ldots(16)$$

W_1= mass of crucible

W_3 = mass of crucible and cooled dried sample

W_4=mass of the crucible and sample after ignition at 550 ℃

3.3.4 Elemental analysis of SPW

(i) **Determination of Kjeldahl Nitrogen content**

Nitrogen content was determined using methodology described by Silva (2002). 5 g of a homogeneous SPW powder was digested with 20 ml of H_2SO_4 98% and selenium

salts was used as a catalyst and K_2PO_4 was added in order to increase the boiling point of H_2S0_4. The aim of the digestion procedure is to break all nitrogen bonds in the sample and convert all of the organically bonded nitrogen into ammonium ions ($NH4^+$) as shown in Equation 17.

Protein (-**N**) + $H_2SO_4 \rightarrow$ ($\mathbf{N}H_4)_2SO_4 + CO_2 + H_2O$……(17)

A blank test was carried out under the same conditions, without the addition of SPW sample. Digestion process was done in four stages 100 ℃ for 30 minutes, 180 ℃ for 30 minutes, 260 ℃ for 30 minutes and 340 ℃ for 90 minutes, in a Velp Scientific digester (Montoro et al., 2019). After digestion the samples were allowed to cool to room temperature, then diluted with 100 ml distilled water and transferred to the Kjeltec System 1002 distillation unit which was highly alkalinized by NaOH at pH more than 8.2, Equation 18 (Montoro et al., 2019)

($\mathbf{N}H_4)_2\ SO_4$+ 2NaOH $\rightleftharpoons$ $\mathbf{N}H_3$ (gas) + Na2SO4 + 2H2O……..(18)

The distilled samples obtained were collected in a boric acid indicator solution, H_3BO_3 (0.32 mol/L), with a purple colour.

The samples which contain organic and ammonium nitrogen change the colour of boric acid solution from purple to green. After the distillation, the solution was titrated with H_2SO_4 (0.020 N) standard solution, until the solution went back to the purple colour. The residual sulfuric acid (the excess not reacted with NH3) is titrated with sodium hydroxide standard solution and by difference the amount of ammonia is calculated based on Equation 19 (Eder et al. 2016).

H_2SO_4 (total) + $2NH_3 \rightarrow SO_4^{2-} + 2NH_4^+$…….. (19)

Determination of Total organic carbon (TOC)

Total organic carbon in the SPW was determined using wet chemistry technique described by (Schumacher, 2002). The method involves the rapid dichromate oxidation of organic matter. In this procedure, potassium dichromate ($K_2Cr_2O_2$) and concentrated H_2SO_4 were added to between 0.5 g and 1 .O g of SPW. The sample and extraction solutions were gently boiled at 150 ℃ for 30 minutes, allowed to cool, and then water was added to halt the reaction. The addition of heat to the system led to a complete digestion of the organic C in the sample; therefore**,** no correction factor was needed. Temperature was strictly controlled because the acid dichromate solution decomposes at temperatures above 150℃. The chemistry of this extraction procedure is presented in Equation 20:

$$2Cr_2O_7^{2-} + 3C^0 + 16H^+ = 4Cr^{3+} + 3CO_2 + 8H_20 \quad \text{.....(20)}$$

Upon completion of the sample extraction phase, the quantity of organic carbon present in the SPW was determined through calorimetric method. Colorimetric quantification of TOC was performed through the measurement of the color change that results from the presence of Cr^{3+} in solution. After sample digestion, the digestate is centrifuged or filtered to remove any suspended particles and then placed in a calorimeter set to measure the light absorbance at a wavelength of 601nm. Quantification was performed by comparison of the results against a standard curve.

(ii) **Determination of phosphorus content**

SPW was dried and milled and then digested in a mixture of sulphuric acid and nitric acid in order to solubilise all existing phosphorous. Ascorbic acid was added as complexing agent, a blue (the colour is proportional to the amount of phosphorus) complex antimony phosphorous molybdate was formed, which was then determined

by a colorimetric method using a HACH DR-2000 spectrophotometer using standards prepared according to the methodology described by (O'Dell, 1993).

(iii) **Determination of potassium content**

2g of milled SPW was transferred into a beaker and 100 ml of demineralised water was added in to the beaker. 3mL of concentrated HNO_3 was then added into the mixture. A blank was prepared by using 3mL concentrated HNO_3 per 100 mL demineralized water.

The sample and the blank were evaporated to dryness. Then cooled and an additional 3 mL concentrated HNO_3 was added to each beaker. Each of the beakers was covered with a watch glass, return to the hotplate, and the samples were gently refluxed. Heating and addition of more acid was done, until the digestion was completed (indicated by a light-colored residue).

After complete digestion the samples were evaporated just to dryness, and 6 mL 6*M* HCl solution was added to the samples and both beakers were warmed to dissolve the residue. The potassium content of the SPW digested extract was then determined using an atomic absorption spectrophotometer (model GBC 932 AA), according to methodology described by (Fishman, 1966; Montoro et al., 2019).

3.4 Effects of Thermochemical Pre-Treatment on SPW

To examine the combined effects of the pre-treatment factors: pre-treatment time, temperature and NaOH concentration, Central Composite Design in Minitab version 17 software was employed to design experiments of three factors at five levels.

RSM was applied in optimization process to reduce the number of runs that can cut extra cost and time needed as compared to conducting one-variable-at-a-time. One-variable at-a-time method requires a change of one parameter while keeping the other

parameters constant, which is time consuming, at the same time interactions between parameters involved cannot be estimated (Junoh, 2015). The three factors at five levels are as tabulated in the Table 3.1

Table 3.1: Independent variables with their level codes

FACTOR	**CODED LEVELS**				
	-2	-1	0	+1	+2
Temperature (℃)	50	58	70	82	90
Time (Minutes)	30	49	75	102	120
NaOH concentration (g/L)	0.6	1.2	2.1	2.9	3.5

For the 3 variables; temperature, time and NaOH concentration a total number of 20 runs were obtained by the expression 2^n (2^3=8 factorial points), 2n (2*3=6 axial points), 6 centre points of replications Table 3.2 in the appendix.

SPW was subjected to size reduction using a laboratory blender for 1 minute. The purpose of size reduction was to increase the surface area for faster degradation. According to Li et al. (2012) ; Chen et al. (2008), NaOH concentrations of 3.5-5 g/L can moderately inhibit the activity of mesophilic methanogens whilst 8 g/L can lead to strong inhibition. Therefore, a maximum NaOH concentration used in the current research was 3.5g/L. Milled SPW amounting to 30 g was placed in a 500 ml beaker and 100 ml NaOH solution was poured into the beaker. The mixture was stirred manually for 10 minutes as shown in Figure 3.4 and then placed in an oven at temperatures ranging from 50 ℃ -90 ℃.

The temperature range was based on other related studies which reported that at a temperature above 100 ℃ Mailard browning reactions takes place between amino acid

and reducing sugar which results in reduction of soluble sugar (Jung et al., 2015; Pedreschi et al., 2007). Temperature of 50 ℃ -90 ℃ was also used by S.Srichuwonga et al. 2005 to analyse swelling properties of starch, among the starches studied was sweet potato. Hence the maximum temperature used in the study was 50- 90℃.

30g of milled SPW was placed in a 500ml beaker and100ml NaOH was added into the beaker. The mixture was stirred manually for 10 minutes Fig.3.4 then placed in an oven set at temperature 50-90℃ for 30-120 minutes Fig 3.5 the mixture was shaken manually for 1 minute every half an hour.

(a) **(b)**

Figure 3.4: (a) Sweet potato waste suspended in NaOH solution and (b) Thermochemical pre-treatment of sweet potato waste

After thermochemical pre-treatment the substrate was analysed for changes in TS and VS (Saragih et al.,2019) ; the same method that was used in characterization of SPW was also applied for the determination of TS and VS of the thermo-chemically treated samples. All pre-treated samples had a pH which ranged at (8-10) hence not suitable for direct use in anaerobic digestion, the pH was adjusted to (7.0 ± 0.2) by adding HCl acid as shown in Figure 3.5. The samples were then stored at 5 ℃ until further use for biogas production.

Figure 3.5: Neutralization with HCl and pH testing of pre-treated SPW

3.4.1 Biomethane potential test (BMP)

Biomethane potential (BMP) tests were carried out in order to further investigate the effect of NaOH and thermal pre-treatment on SPW as a feedstock for biogas production. The experimental BMP test was set according to Braun, (2007). The experiment was based on batch-type digester because of its simplicity. 250 ml conical flasks were used as rectors, 100 ml of the pre-treated neutralized SPW solution was fed in the into the reactor and mixed with active inoculum at feedstock-inoculum (F/I) ratio of 1.2:1 (based on volatile solids 74.9% for inoculum and 96.6 for SPW (Pathak & Srivastava, 2007,Ge et al,2014). Distilled water was added to make a working volume of 150 ml, each reactor was then covered with a coax then tightly sealed with silicon sealant to make it airtight and its outlet tube was connected to a gas collector which was partially filled with water. The reactors were then placed in a water bath set at 37 ± 1℃. During the incubation period, the reactor bottles were shaken for 1 minute every day to prevent scum formation which could inhibit biogas production. The volume of biogas produced was measured daily through downward displacement of water column (Braun, 2007).The data for cumulative biogas yield, was subjected

response optimizer in Minitab 17 to determine optimum pretreatment conditions. 30g of SPW was then thermochemically pretreated at optimum conditions, untreated SPW was used as control, both were then subjected to AD. The biogas production setup Figure 3.6**.**

Figure 3.6: Biogas production set up

3.4.1.1 Biogas composition analysis

Once the gas was produced it was collected and stored in gas bags. In order to prove that the gas produced was biogas and not any other gas, it was analyzed using gas Chromatography method. 2µL of the gas sample was sacked from the gas bag then injected into a gas chromatograph (MRC Scientific instrument: MRC/GC/39621381), equipped with chromatographic column to separate the components of the biogas. When the sample entered through the injection port, it flew through the column which had Nitrogen gas used as carrier gas. The temperature of the injector, column and detector were 200℃, 150℃ and 250℃, respectively. Depending on the chemical and

physical properties of the constituents of the biogas sample and their interaction with the particular column filling, the constituents passed in the carrier gas stream at different rates. The column contains a liquid stationary phase which is adsorbed onto the surface of an inert solid, causing the constituents to exit the column at different times.

A detector then identified and measured methane as it exited the column. Signals from the detector were used to produce chromatogram. The chromatograms are analyzed using Chromeleon software version 6.80. The chromatograph showed series of peaks with the size of peaks directly proportional to the amount of each component. The 1st peak is from inert carrier gas and the subsequent peaks represent compounds in the mixture. Portable gas analyzer was also used to accurately determine methane, oxygen, carbon dioxide and hydrogen sulphide in percentages.

3.5 Assessment of Fertilizer Properties of SPW digestate

After AD, the resultant digestate was analysed for TS, VS, and pH as well as macro nutrients: nitrogen, phosphorus and potassium which are regarded as most important elements in plants' nutrition. The same standards and methods for physicochemical characterization (objective 1) of SPW were also employed in the digestate analysis.

3.6 Statistical Analysis

All the experiments were duplicated in all the above analysis and the average results with ± standard deviations were presented. The Minitab version 17 software was used in analysis of variance (ANOVA) for the data obtained from BMP test. A confidence level of 95% was used to judge their significances. A quadratic model for biogas yield was developed. Moreover, some adequacy measures, such as S, R^2, Adj-R^2 and pred. R^2, were determined to check the adequacy of the developed model.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Introduction

This chapter covers results and detailed discussion of physicochemical characterization SPW, biogas production from BMP test and characterization of digestate as a biofertilizer. The data are presented using tables and graphs.

4.2 Quantification of Waste Generated During Manual Peeling

From manual peeling using a sharp knife, the amount of sweet potato waste generated based on weight were calculated and presented in the table 4.1.

Table 4 1: Quantity of SPW generated from manual peeling

Sweet potato	Weight(g)	Percentage %
Sweet potato (before peeling)	1000	-
Sweet potato (after peeling)	809	80.9
Loss due to peeling	191	19.1

From the table 4.1 above, 19.1% of sweet potato peels was generated from manual peeling, the amount is within the range of 15% to 40% which was reported by Schieber et al. (2002) and Zentek et al. (2014) as losses from potato peeling. The fact that 1Kg of sweet potato generates 19.1% waste means that in large scale sweet potato processing plants, substantial amount of waste in form of peels, chunks and trimmings are produced as by-products which could be used as feedstock for biofuel production.

4.3 Physicochemical Characterization of SPW and Inoculum

Detailed characterization of biogas feedstock is of great importance in order to determine the suitability of a given feedstock for biogas production.

Basic information such as water, volatile solid and fixed solid content can be used to roughly determine the suitability of a given substrate for AD as well as the efficiency

of the AD process (Drosg & Braun, 2013; Krus & Lucas, 2014; Orhorhoro et al., 2017). The physical and elemental analyses of the SPW are presented in Table 4.2.

Table 4 2: Proximate analysis of sweet potato waste

SN	PARAMETER	UNIT	SPW	Inoculum (cow dung)
1	pH	pH unit	4.9 ± 0.1	7.6 ± 0.2
2	Moisture	% (natural matter)	70.7 ± 2.1	89.67 ± 0.3
3	Total solids	% (natural matter)	28.9 ± 1.9	10.33 ± 2
4	Volatile solids	% of the TS	96.6 ± 1	74.9 ± 0.8
5	Fixed solids	% of the TS	3.4 ± 0.2	Nd
6	Total Organic Carbon	% of the TS	38 ± 0.0	Nd
7	Total Kjeldahl Nitrogen	% of the TS	0.93 ± 0.3	Nd
8	C/N ratio	Dimensionless	40.86	Nd
9	Total potassium	g $kgTS^{-1}$	1.09 ± 0.0	Nd
10	Total phosphorus	g $kgTS^{-1}$	0.10 ± 0.0	Nd

Values were presented as means ± standard deviations of duplicates

Nd- not determined

i pH value

The pH in AD plays an important role since the micro-organisms involved in the process are sentive to pH (C. Nzila et al., 2010). The ideal pH for AD ranges from 6.8-7.5 (Drosg et al, 2013), considering the pH value of 4.9 ± 0.1 for untreated SPW obtained in the current study; the value is lower than the generally accepted optimum pH. Accordingly, AD would be less efficient as the performance and growth of anaerobic bacteria is affected by low pH; low pH also leads to the formation of undissociated volatile fatty acid which causes inhibition in methanogenesis step. Nonetheless, other studies have reported a much higher pH for sweet potatoes than the current studies; Martins et al. (2019) and Felipe, (2018) reported pH of sweet potato as 6.20 ± 0.18 and 5.99-6.12 respectively.

ii **Moisture content determination**

Total Solid and MC in a biogas feedstock are crucial in order to assure the balance of all AD stages in the digester (Krus & Lucas, 2014). It has been reported that the highest CH_4 production rates occur at 60–80% of moisture (Khalid et al., 2011). The moisture content (70.7 ± 2%) of SPW obtained in the present work is therefore within the reported ideal range. Moisture is necessary for growth and mobility of microbes (Drosg et al., 2013); thus from SPW, substantial amount of biogas could be produced due to sufficient moisture availability. Likewise Dako et al. (2016) and Hoover (2001) reported similar amount of MC for six sweet potato cultivars which ranged from 68.58%-76.97% and 70%-80% MC for root and tuber crops respectively.

iii. Total solids and Volatile solids

Volatile solid of feedstock is one of the major indicators for biogas production potential while TS is known to affect performance and the behaviour of microbial community (Yi et al., 2014). In this study, Orange flesh SPW has been characterized to contain TS of 28.9 ± 1.9% and VS of 96.6 ± 1%. The high percentage of VS indicates that SPW is rich in biodegradable organic matter, thus keeping other factors constant, SPW could produce lots of biogas.

Other researchers have reported similar amounts: VS 96.99 % and TS 24.76 % for sweet potatoes in Brazil though the cultivar was not specified, Martins et al. (2019); TS 35.5% and VS 97.6% for industrial sweet potato reported by Mussoline & Wilkie, (2015) and TS which ranged from 14.7% to 28% for 25 sweet potato cultivars in Australia reported by Waramboi et al. (2011).

iv. Ash content

The presence of ash in a biomass is an indication that there is inorganic matter in the substrate (Ojewumi et al., 2018). The amount of ash for SPW reported in this study is

3.4 ± 0.2%, presence of large amount of inorganic matter causes inhibition AD. In this case the amount ash content is low, it therefore implies that SPW is ideal for AD (Drosg et al, 2013).

Similar amount of ash content (3.04-4.94%) was reported by Dako et al. (2016) from comparative analysis of three sweet potato varieties for nutritional and anti-nutritional factors. The authors also observed that, sweet potato peels contain more ash than the flesh part of the sweet potato root. Moreover Felipe, (2018), also reported an equal range (2.78%-3.77%) from the study of biogas production potential from four sweet potato genotypes. However, Ivone, (2015) reported a lower ash content, 0.85±0.08% for orange flesh sweet potato.

v. Kjeldahl Nitrogen Content

Sufficient amount of nitrogen in a biogas feedstock is essential for growth of AD microbes (Drosg & Braun, 2013)**.** The nitrogen content obtained in the study 0.93 ± 0.3% and total carbon 38% both based on TS, are equivalent to the results reported by Ivone, (2011); 0.58 ± 0.08% nitrogen and 41.08 ± 0.32 % total carbohydrates from orange flesh sweet potato. The C/N ratio obtained in the present work (40.86) is consistent with the C/N of 46.4:1 of sweet potato reported by Ge, (2014) and 45:1 obtained from culls of industrial sweet potato (Mussoline & Wilkie, 2015). Nevertheless, a much higher C/N ratio (107.80 ± 0.75) was reported by Martin et al. (2019).

These variations could be contributed to factors such as the type of cultivar, harvesting period, soil condition and the storage period. The recommended optimum C/N ratio of the AD substrate should be within the range of 16:1-30:1 (Gillian, 2011). This means that SPW having higher C/N ratio, has low nitrogen which is quickly

consumed by AD bacteria to meet their protein requirements. Consequently, the carbon content in the SPW which was intended to produce biogas is left out unutilized thus resulting to low biogas production, therefore mono-digestion of SPW is inefficient for AD.

vi. Phosphorus and potassium content

Phosphorus and potassium content of sweet potato waste obtained in this research, 0.1 g kg/TS and 1.09 g kg/TS respectively. The amounts are sufficient for microbial growth. The availability of the macro-elements (NPK) in SPW means that, SPW in its natural form could be used as a biofertilizer in farms.

4.4 Effect of Thermochemical Pre-treatment of SPW

4.4.1 Characteristics of Pre-treated SPW

Predominantly, thermochemical pre-treatment of SPW caused the reduction in Total solids and Volatile solids of the pre-treated SPW when compared with the untreated SPW (28.9% TS, 96.6% VS); for the pre-treated SPW, the TS ranged at (21.5%-10%) while their VS ranged at (96%-89%) which is obviously lower when compared to the untreated SPW. The same phenomena was also observed by Jiang et al. (2016), who observed that TS and VS of pre-treated wheat straw reduced with the increase of pre-treatment time and NaOH concentration.

The reduction in both TS and VS of the pre-treated SPW implies that thermochemical pre-treatment is efficient in breaking long chained and complex compounds into monomers for easier digestion. This observation is in agreement with the observation made by F N A Saragih et al. (2019) who reported a reduction of TS and VS of thermal treatment of food waste. Another possible reason for the lower concentration of TS and VS on the pre-treated SPW could be that, some VS content was lost

through volatilisation during thermochemical treatment process, resulting to lower final VS (Gandhi et al., 2018). The resultants TS and VS after pre-treatment of SPW are presented in Figure 4.1.a and Figure 4.1b.

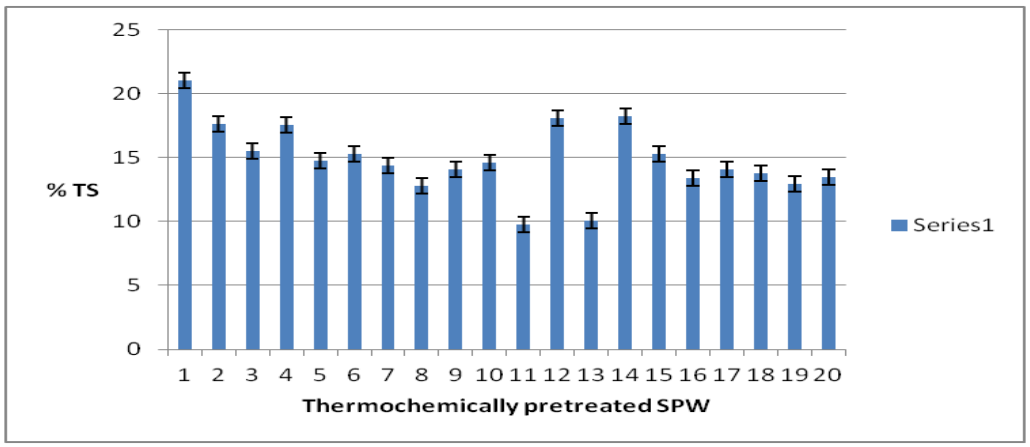

Figure 4.1.a: TS of thermochemically pre-treated SPW

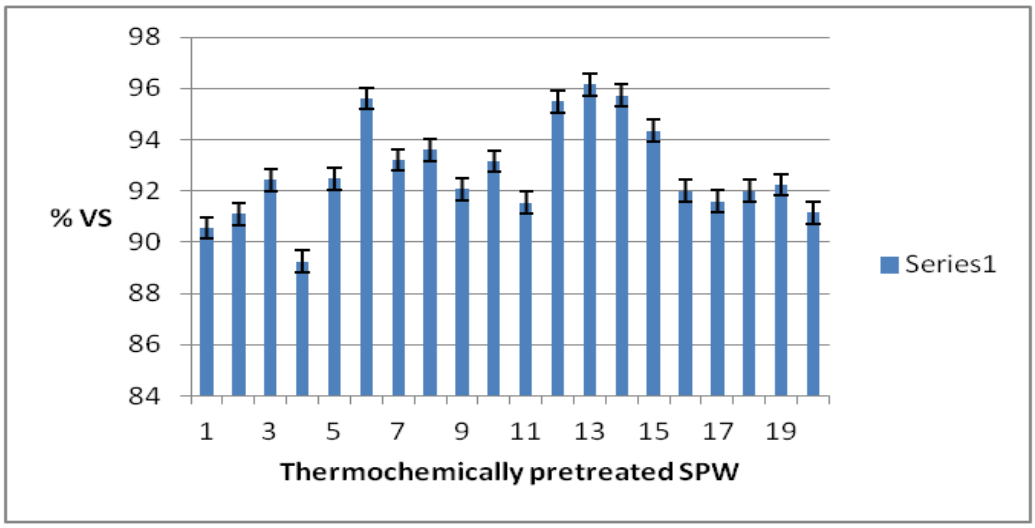

Figure 4.1.b: VS for thermochemically pre-treated SPW

4.4.2 Biomethane Potential test

A Central Composite Design (CCD) under RSM was used to optimize biogas yield based on the interactive effect of thermo-chemical pre-treatment that consisted of three variables- NaOH concentration, temperature and pretreatment time. The cumulative biogas yield produced by 20 thermochemically pre-treated SPW samples is presented in Figure 4.2.

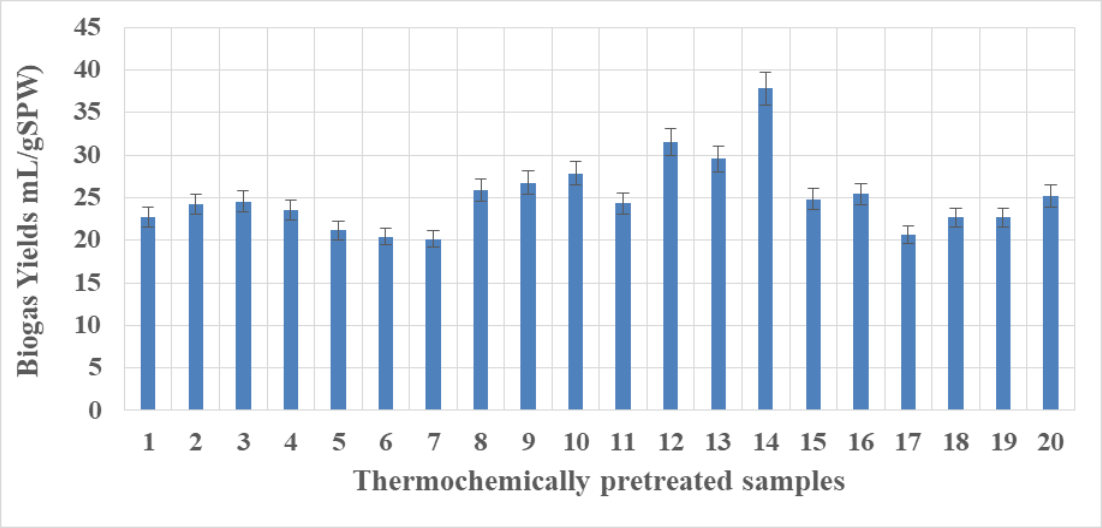

Figure 4.2: Cumulative biogas yields for the pre-treated SPW

The quadratic model obtained from the software (Minitab 17) for biogas yield from the thermochemical pre-treated SPW is given equation 17.

Regression Equation in coded units.....17

$Y = 172.4 - 12.79\ A - 3.935B - 0.441\ C - 0.493\ A^2 + 0.02387\ B^2 + 0.000097\ C^2$

$+ 0.2407\ A \times B + 0.0017\ A \times C + 0.00690\ B \times C$

Where,

Y-Biogas Yield

A-NaOH concentration

B-Pretreatment temperature

C-Pretreatment time

The statistical model was checked by analysis of variance (ANOVA) for the response surface quadratic model is tabulated in the Table 4.3.

Table 4.3: ANOVA for Response Surface quadratic polynomial model

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	9	692.991	76.999	14.17	0.000
Linear	3	428.228	142.743	26.26	0.000
Concentration	1	45.861	45.861	8.44	0.016
Temperature	1	348.087	348.087	64.05	0.000
Time	1	34.280	34.280	6.31	0.031
Square	1	177.991	59.330	10.92	0.002
Concentration*Concentration	1	1.831	1.831	0.34	0.574
Temperature*Temperature	1	170.254	170.254	31.33	0.000
Time*Time	1	0.066	0.066	0.01	0.914
2-Way Interaction	3	86.772	28.924	5.32	0.019
Concentration*Temperature	1	48.216	48.216	8.87	0.014
Concentration*Time	1	0.011	0.011	0.00	0.965
Temperature*Time	1	38.544	38.544	7.09	0.024
Error	10	54.350	5.435		
Lack-Of Fit	5	30.003	6.001	1.23	0.412
Pure Error	5	24.347	4.869		
Total	19	747.341			

Coded Coefficients

Term	Effect	Coef SE	Coef	T-Value	P-Value	VIF
Constant	24.117	0.951	25.36	0.000		
Concentration	3.665	1.833	0.631	2.90	0.016	1.00
Temperature	10.097	5.049	0.631	8.00	0.000	1.00
Time	3.169	1.584	0.631	2.51	0.031	1.00
Concentration*Concentration	-0.713	-0.356	0.614	-0.58	0.574	1.02
Temperature*Temperature	6.874	3.437	0.614	5.60	0.000	1.02
Time*Time	0.136	0.068	0.614	0.11	0.914	1.00
Concentration*Temperature	4.910	2.455	0.824	2.98	0.014	1.00
Concentration*Time	0.075	0.037	0.824	0.05	0.965	1.00
Temperature*Time	4.390	2.195	0.824	2.66	0.024	1.00

Model Summary

S	R-sq	R-sq (adj)	R-sq (pred)
2.33131	92.73%	86.18%	63.96%

The overall p value for the developed model (0.000) is less than the level of significance (0.05). It therefore means that, the full quadratic model of the factors:

NaOH concentration, temperature and time significantly affect the biogas yield. The p-value for the linear terms for all the factors, the concentration (0.016), temperature (0.000) and time (0.031), are also lower than the level of significance. Therefore, the linear terms significantly affect the biogas yield. The p-values for quadratic terms: both NaOH concentration (0.574) and time (0.914) are larger than the level of significance; therefore, the two factors have insignificant effect in respect to biogas yield. On the other hand the p-value for quadratic term for temperature (0.000) is less than the level of significance hence temperature significantly affects the biogas yield. The p value for interaction terms: concentration and temperature (0.014) and temperature and time (0.024) significantly affect the biogas yield. Nevertheless, the interaction between concentration and time (0.965) insignificantly affect the biogas yield.

The model suffers no lack-of-fit since the p-value (0.412) is larger than the level of significance (0.05). Therefore, the quadratic model with the predictor variable concentration, temperature and time significantly predicts the biogas yield. Variance Inflation Factor (VIF) for all factors are observed to be around 1, meaning that there is no multicollinearity between a factor and the other factors.

To further check how well the model fits the data, goodness-of-fit statistics were examined in the model summary. The coefficients S, R^2, adjusted R^2 and predicted R^2 were examined to check the model effectiveness. The coefficient R^2 is the percentage of variation in the response that is explained by the model, it normally ranges between 0% and 100%. The higher the R^2 value, the better the model fits the data, in this case R^2 value is 92.73%, means that the model could explain the variability of the dependent variable (biogas yield).

The coefficient predicted R^2 determine how well a model predicts the response for new observations. Models that have larger predicted R^2 values have better predictive ability, in this case the value of predicted R^2 , 63.96%, was obtained, it implies that the model has 63.96 % ability to predict a correct new observation.

The value of adjusted R^2 (86.18%) means that 86.18% of variance can be predicted from independent variable and only 13.82% of the total variation could not be explained by the model.

Residuals versus fits plot, Residuals versus order plot and Normality plot of the residuals and histogram were used to determine whether the model was adequate and met the assumption of the analysis.

i **Normal probability plot**: This plot was used to verify the assumption that residuals were normally distributed. In the normal probability shown in figure 4.3 all residuals appeared to follow a straight line, indicating normal distribution of residuals.

ii **Residuals versus fits plot:** Versus fits plot is used to verify the assumption that the residuals are randomly distributed and have constant variance. For a model that meets the assumption, the points fall randomly on both sides of 0, with no obvious patterns in the points. The pattern presented in versus plot in Figure 4.3 shows no obvious pattern, indicating no predictability of the residuals; hence the points were randomly distributed.

iii **Histogram of residuals**: The histogram of the residuals shows the distribution of the residuals for all observations. It was used to determine whether data was skewed or had an outlier. Normal distribution plot is

preferred to histogram because the appearance of a histogram depends on the number of intervals used to group the data.

iv **Residuals versus order plot**: this plot was used to verify the assumption that the residuals were independent from one another. Residuals that are independent show no trends or patterns when displayed in time order. Points in the pattern that are close to one another may indicate that they are correlated. The versus order plot shown in Figure 4.3 shows that the model met the assumptions of the analysis since in the residual versus order plot, the residuals fall randomly around the centreline. The plots are shown in Figure 4.3.

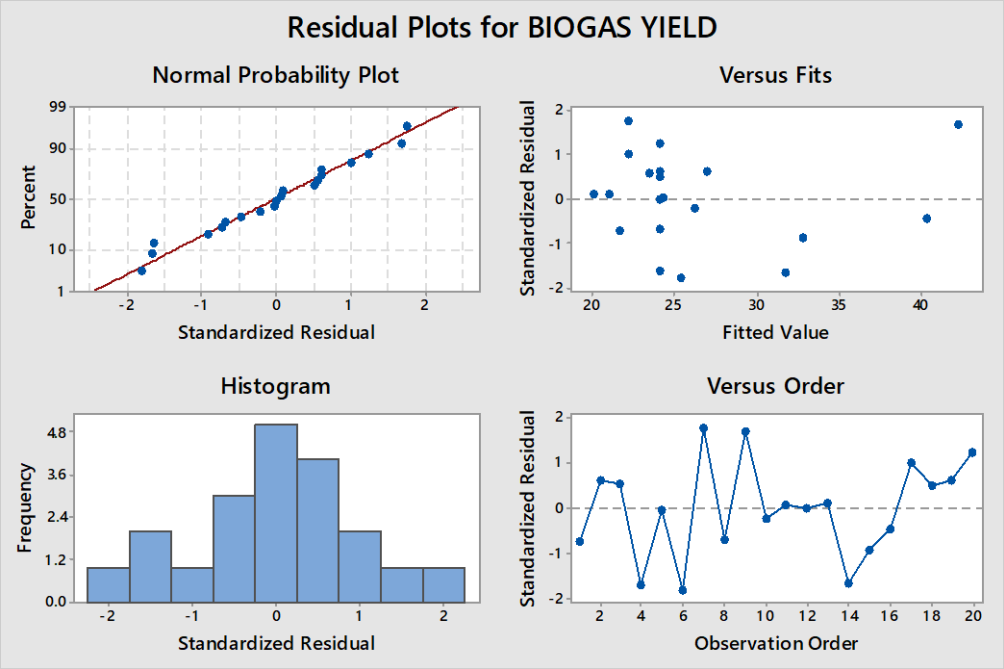

Figure 4.3: Residual plots for biogas yield from thermochemically pretreated SPW

4.4.3 Response Optimization of Biogas Yield

The data obtained from cumulative biogas produced presented in the Table 4.4 in the appendix was subjected to response optimizer in Minitab 17 in order to determine the

potential combination of the input variable settings of the three pre-treatment factors; temperature, time and NaOH concentration for optimum biogas production. The optimization plot in Figure 4.4 was obtained from the software, the software calculated and presented that maximum biogas yield from pre-treated SPW was obtained when NaOH concentration was at 2.9 g/L, temperature 82 ℃ and pretreatment time 102 minutes.

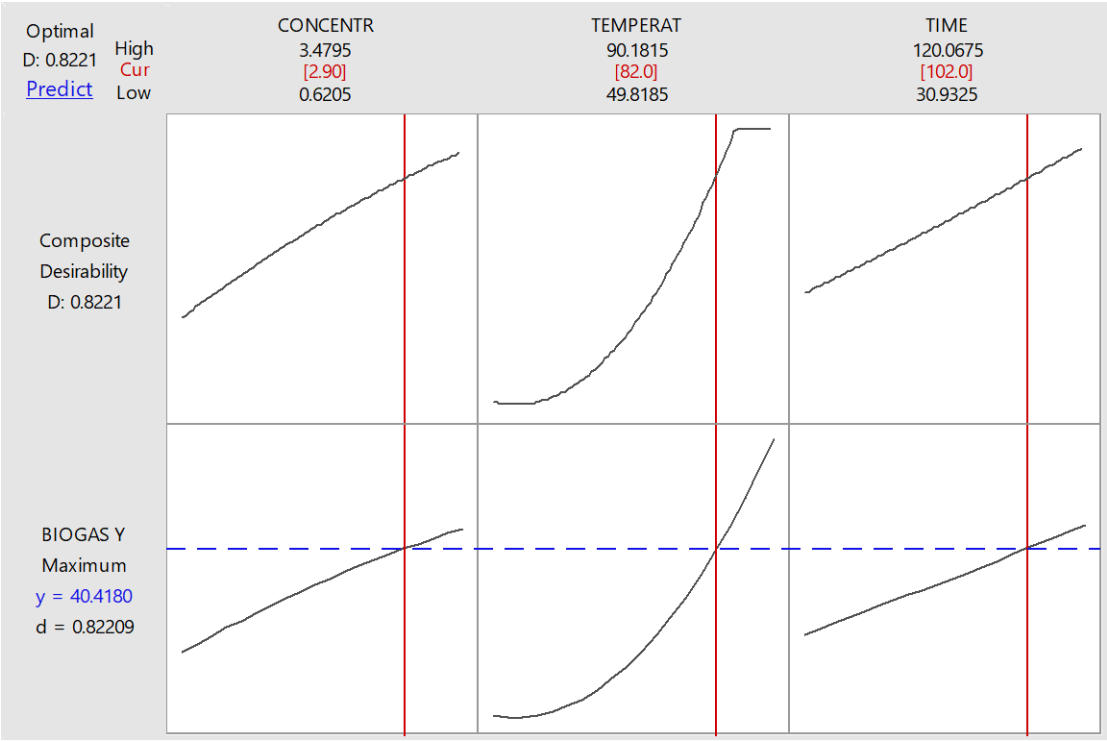

Figure 4 4: Biogas optimization plots

The descriptive data of biogas optimization are presented in Table 4.5 in the appendix. The SPW pre-treated under optimal conditions was then compared with untreated SPW in terms of biogas yield and methane content. It was observed that in both cases, biogas production was high in the first five days and decreased after the period.

For the untreated SPW the peak production was achieved on the 4^{th} day at 7.7 ± 0.03 ml/gSPW while the thermochemically pre-treated sample, the peak was achieved on

the 5th day at 8.3 ± 0.04 ml/gSPW indicated in Figure 4.5. The sharp decrease in biogas production after the 5th day was due to rapid acidification of SPW resulting in low pH in the digester and the production of large volatile fatty acid which together inhibited the metabolic activity of methanogenic bacteria.

The results in the current work are consistent with observation which was made by Martins et al. (2019), they reported that, from the co-digestion of poultry sludge cake and sweet potato at the ratios S40P60, S20P80, and S0P100, lots of biogas was produced in the first seven days of operation and the production ceased after that period. Tumutegyereize et al. (2016) also reported 90% methane yield in less than five days for cassava peels, sweet potato peels and matoke peels.

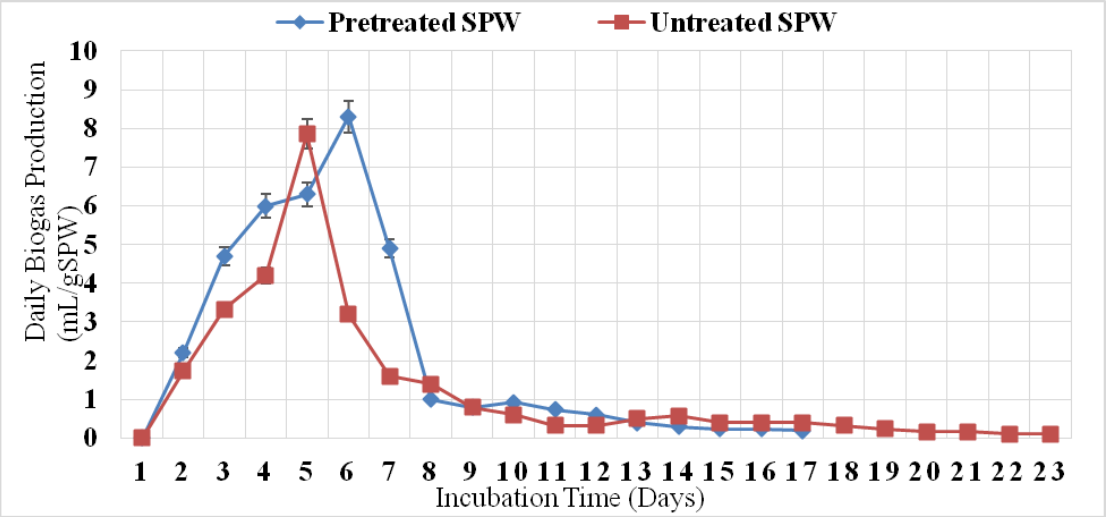

Figure 4 5: Daily biogas productions for the untreated and thermochemically pre-treated SPW for 22 days

Cumulatively the thermochemically pre-treated SPW produced more biogas than the untreated one; untreated SPW produced 28.23 ± 0.07 ml/g SPW of biogas in duration of 22 days while the SPW that was thermochemical pretreated at 2.9 g/L NaOH, 82 °C and 102 minutes produced 37.8 ± 0.21 ml/g SPW in 16 days after which no more biogas was produced. This means that thermochemical pretreatment caused 33.88 % increase on biogas yield in respect to the untreated SPW.

Biogas composition results from Table 4.6 clearly indicates that, the methane content in the biogas also increased from 42% for the untreated SPW to 64% for the thermochemically pre-treated one which is 22% increase. The improvement in biogas and methane production was possibly due to thermochemical pre-treatment which caused delignification of SPW and swelling of sweet potato starch granules which destabilized the amylopectin crystallites facilitating enzymatic conversion of starch into sugars. Alkaline pre-treatment of the SPW could have also reduced the degree of inhibition during AD resulting in more biogas and methane (Chandra et al., 2012). Similar observation on pre-treatment temperature of 82℃ was made by Moorthy et al. (2012) who reported the gelatinization temperature for two orange flesh sweet potato varieties occurred at a temperature range of 79.27 ℃ to 80.15 ℃.

Roberts & Cameron, (2002) also reported that, the addition of NaOH to starch granules causes physicochemical changes in the structure of starch because NaOH causes sudden swelling of the granules and application of heat on the NaOH treated starch caused further swelling leading to rupture of granules making them accessible to anaerobic digestion bacteria. The improvement in biogas yield could also be attributed alkaline nature of NaOH which caused high solubility of sweet potato protein making more nitrogen (nutrient) to be bioavailable for microbial growth (Method,P. 2017). The composition of biogas obtained from portable gas analyser is shown in the Table 4.4.

Table 4.4: Biogas composition

Composition	Treated sample	Untreated sample
Methane %	64 ± 3.5	42 ± 2.8
Carbon Dioxide %	32 ± 5.6	45 ± 6.4
Hydrogen Sulphide ppm	142±9.8	144±10.2
Oxygen %	2.0 ± 0.2	2.0 ± 0.1

The amount of methane from the untreated sweet potato waste 42 ± 2.8 % is comparable to the findings 38.9% methane reported by Martins et al. (2019). Nonetheless, a higher amount of methane ranging from 70-80% for biogas production from industrial sweet potatoes has been reported by Mussoline & Wilkie, (2015).

4.5 Assessment of fertilizer properties of treated and untreated SPW

Physical and elemental characteristics of digestate from the treated and untreated SPW are tabulated in the Table 4.5.

Table 4.5: Digestate characteristics

Digestate type	pH	TS	VS	N %	P %	K %
Treated SPW	3.2 ± 0.14	6.2 ± 1.2	90.74 ± 0.18	0.35	0.08	1.94
Untreated SPW	2.9 ± 0.07	7.36 ± 0.7	91.27 ± 0.5	0.82	0.07	2.37

pH for treated and the untreated sample are 3.2 and 2.9 respectively. A low pH in the SPW digestate is an indication of intensive production of organic acids during anaerobic digestion. Low pH causes higher heavy metal solubility that can cause phyto-toxicity issues on plant growth. National standards, compost should have a pH value within the range 6.0–8.5 to ensure compatibility with most plants (Martel, 2010)

VS in the table 4.7 are high, this indication that both treated and the untreated SPW could be considered as soil amendment on sole criterion of organic matter content. (Martel, 2010). Both the treated and the untreated SPW digestate have low TS hence the digestate could be difficult to store or transport.

NPK are considered as the three most important elements in plants' nutrition. Generally, AD favours the mineralization of these elements hence they become readily available for plants. From digestate analysis, the digestate from the sweet potatoes had considerable amount of NPK table 4.7, hence it could be considered

suitable for farm applications. Among the three macro-elements potassium has the highest percentage, 2.37%, followed by nitrogen 0.82% while phosphorus is the least.

From the comparative analysis of the digestate of untreated SPW and the thermochemically pre-treated SPW, it is clearly indicated that SPW that was not treated had more macro elements than the digestate from thermochemically pre-treated SPW illustrated in table 4.7. This was probably due macronutrients loss which might have occurred during thermochemical pre-treatment of SPW. Ajiboye et al.,(2018) also reported a reduction of N elements from poultry waste which was mixed with NaOH.

The observation confirms the assertion which was made by Logan & Visvanathan, (2019) that; the amount of plant nutrients that enters the biogas plant in the substrate is equals to the amount of nutrients leaving the biogas plant in the digestate, unless other treatments apart AD are carried out on the substrate which might cause the loss of elements such as N through volatilisation. Macro elements are indispensable in plants' nutrition; potassium plays a key role in water balance, enzyme activation, photosynthesis as well as activation of sensitivity to water stress during drought. Phosphorus is also an important element as it ensures plants growth and improve plants yield as well the yield quality (Koszel & Lorencowicz, 2015).

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1 Introduction

Basing on the results and comparison with related information available in literature, for the characterisation, BMP test and the digestate analysis for SPW, conclusions and recommendations were drawn.

5.2 Conclusion

The purpose of this study was to investigate the effects of thermochemical pre-treatment on biogas production from SPW, from the findings it was concluded that; SPW which is available in large quantities in farms, markets and as by-product of sweet potato processing and can be utilized as a renewable energy source through production of biogas.

Physicochemical characterization of the SPW indicates that the pH (4.9 ± 0.1) of SPW was lower than optimum (6.8-7.2) for AD, hence sweet potato tuber in its natural state is not efficient for biogas production. SPW also has reasonable amount of moisture and high organic matter making it suitable for biogas production. In the elemental analysis, it was observed that SPW has C/N ratio of 40.86 which is more than the optimum recommended C/N ratio (16-30). Consequently, mono-digestion of SPW is inefficient for biogas production.

Even though SPW is biodegradable, it is clear from the results that, thermochemical pre-treatment with, NaOH at concentration 2.9g/L, temperature of 82 ℃ and pretreatment time of 102 minutes, enhances biogas and methane yields by 33.88 % and 22% respectively in comparison with untreated SPW. The retention time in the

bio-digester also reduced from 22 days for untreated to 16 days for the thermochemically pre-treated SPW.

Sweet potato waste digestate has considerable amount of macro-nutrients (N 0.82 %, P 0.07% and K 2.37%) and therefore nutrient recovery from the digestate could be considered as renewable fertilizer which could be suitable for farm application after appropriate treatment. However, when SPW was thermochemically pre-treated its nitrogen content reduced. This means that thermochemical pretreatment SPW degrades the quality of digestate as a fertilizer.

5.3 Recommendation

From the study it was observed that sweet potato root waste is acidic in nature pH (4.9 ± 0.1) hence AD of sweet potato in its natural state is inefficient. Neutralization step is therefore recommended to adjust the pH to the ideal level 6.8-7.2 by adding base such as Ca (OH) $_2$, Na_2CO_3, and NaOH. Also, the C/N of SPW 40.86 is higher than the recommended CN ratio of 16:1 to 30:1; hence co-digestion with nitrogen rich waste such as slaughter house waste, meat processing waste and rape seed cake could help to balance the nutrients hence leading to higher biogas yield.

Thermochemical pre-treatment of SPW has proven that pre-treatment of carbohydrate rich substrate improves biogas and methane yields as well as reducing the retention time and therefore should be adopted.

The digestate from the AD of SPW has sufficient nutrients for plants growths; however necessary treatment should be done before applying in the farm to avoid possible transmission of pathogens.

Further research needs to be done to determine the most suitable pre-treatment method for SPW to ensure improved biogas and methane yields without compromising with the quality of biofertilizer.

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APPENDICES

Appendix I: Data Tables

Table 3.1 Design Matrix in actual values

	Factor 1	**Factor 2**	**Factor 3**
Std	A: NaOH. Con(g/l)	B: Temperature(^{0}C)	C: Time (Minutes)
1	2.1	50	75
2	1.2	58	49
3	2.9	58	49
4	1.2	58	102
5	2.9	58	102
6	2.1	70	30
7	0.6	70	75
8	3.5	70	75
9	2.1	70	75
10	2.1	70	120
11	1.2	82	49
12	2.9	82	49
13	1.2	82	102
14	2.9	82	102
15	2.1	90	75
16	2.1	70	75
17	2.1	70	75
18	2.1	70	75
19	2.1	70	75
20	2.1	70	75

Table 4.4 Cumulative biogas yield for thermochemically pre-treated SPW

SN	NaOH. Con	Temperature	Time	Cumulative biogas yield in mL /g SPW
1	2.1	50	75	22.7 ± 0.03
2	1.2	58	49	24.23 ± 0.06
3	2.9	58	49	24.53 ± 0.11
4	1.2	58	102	23.53 ± 0.09
5	2.9	58	102	21.13 ± 0.05
6	2.1	70	30	20.43 ± 0.04
7	0.6	70	75	20.16 ± 0.08
8	3.5	70	75	25.87 ±0.01
9	2.1	70	75	26.77 ± 0.07
10	2.1	70	120	27.87 ± 0.12
11	1.2	82	49	24.3 ± 0.06
12	2.9	82	49	31.57 ± 0.08
13	1.2	82	102	29.53 ± 0.09
14	2.9	82	102	37.8 ± 0.21
15	2.1	90	75	24.8 ± 0.17
16	2.1	70	75	25.43 ± 0.07
17	2.1	70	75	20.6 ± 0.05
18	2.1	70	75	22.67 ± 0.06
19	2.1	70	75	24.06 ± 0.02
20	2.1	70	75	25.2 ± 0.04

Table 4.5: Response optimization: Biogas yield

Parameters					
Response	Goal	Lower Target	Upper	Weight	Importance
Biogas	Maximum	20.17	44.8	1	1
Variable Ranges					
Variable	Values				
Concentration	(1.2, 2.9)				
Temperature	(58, 82)				
Time	(49, 102)				
Starting Values					
Variable	Setting				
Concentration	1.2				
Temperature	58				
Time	49				
Solution					
BIOGAS Composite					
Solution	Concentration	Temperature	Time	Fit	Desirability
1	2.9	82	102	40.4180	0.822085
Multiple Response Prediction					
Variable	Setting				
Concentration	2.9				
Temperature	82				
Time	102				
Response	Fit	SE Fit	95% CI	95% PI	
Biogas	40.42	1.91	(36.17,44.67)	(33.71, 47.13)	

Appendix II: Plagiarism Certificate

SR080

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