OPTIMIZATION OF THERMO-ALKALINE PRETREATMENT OF CATTLE RUMEN CONTENT FOR BIOGAS PRODUCTION USING SODIUM HYDROXIDE

BY

MOSES VICTOR OJO
ENG1403383

DEPARTMENT OF CHEMICAL ENGINEERING
FACULTY OF ENGINEERING
UNIVERSITY OF BENIN
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A RESEARCH PROJECT SUBMITTED TO THE DEPARTMENT OF CHEMICAL ENGINEERING, FACULTY OF ENGINEERING, UNIVERSITY OF BENIN, BENIN CITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF BACHELORS OF ENGINEERING (B.Eng) IN CHEMICAL ENGINEERING

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CERTIFICATION

This is to certify that this research project submitted to the Department of Chemical Engineering was carried out by MOSES VICTOR OJO with matriculation number, ENG1403383 of the Department of Chemical Engineering, Faculty of Engineering, University of Benin, Benin City.

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Mr. Newton A. Ihoeghian **Date**
Project Supervisor

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Dr. Osazuwa **Date**
Project Coordinator

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Engr. Dr. (Mrs.) E. T. Akhihiero **Date**
Head of Department

---------- ----------

EXTERNAL EXAMINER **Date**

DEDICATION

This Project work is dedicated to the Almighty God for his infinite mercy, wisdom, inspiration and provision during the course of this study.

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ABSTRACT

Biogas is a gas mixture consisting mainly of methane and carbon(IV)oxide resulting from the biological process of anaerobic digestion of various organic materials. The percentage of methane in biogas will vary depending on the process conditions and the type of organic matter fermented. This study investigated the effects of thermal and alkaline pretreatment methods on cattle rumen content and increase the biogas yield. In the course of this study, sodium hydroxide (NaOH) was the alkali of choice and temperature ranges of 70ºc 80ºc and 90ºc in a Box Behnken design. Modelling was carried out with using Response Surface Methodology (RSM) which was used for the Analysis of Variance (ANOVA) and multiple regression analysis of the data obtained. The R^2 value of 0.9768 for NaOH, contour plots, ANOVA analysis all shows how suitable the RSM model is for the experiment. The optimum conditions necessary for maximum feedstock degradation for the alkaline was examined and it was found by using NaOH at a temperature of 80.171ºC, timed 13.086 minutes and a molar concentration of 2.05M and the degree of degradation is 56.83%.

CHAPTER ONE

INTRODUCTION

This chapter introduces anaerobic digestion and biogas as well as the reason and importance of this study.

1.1 BACKGROUND OF STUDY

Beef is a major source of animal protein in Nigeria which has a population of over 180 million people, which serves as no surprise why it is documented that Nigeria consumes over 360,000 tons of beef each year, accounting for half of West Africa's beef consumption (Sholatire, 2019). According to reports about 1.3million cattle are slaughtered annually to provide for the country's population, which accounts for about 30% of the country's meat consumption (Gbenga, 2018).

These cattle killings in abattoirs generate wastes from slaughterhouses, which are separated into three categories: solid, liquid, and gaseous. The majority of the solid wastes are made up of bones, feces, and undigested food (rumen content), and hairs and sporadically failed fetishes, but the liquid wastes consist of of dissolved particles, blood, urine, water, and intestinal contents. Odours and emissions make up the gaseous wastes (Adeyomo et al., 2002). These wastes serve as pollutants to the environment and if not properly managed and controlled, have a negative

impact on the nation's economy, public health, animal health, and environment. However, some of these wastes in the form of cattle rumen content can be used to produce energy by acting as a feedstock for the Anaerobic Digestion (AD) technique, which produces biogas.

Anaerobic digestion has been regarded as a waste to energy technology and is an effective method for treating solid wastes and waste water. Introduction of AD into municipal solid waste treatment is one of the most successful and innovative technology developments in the last two decades in waste management field (De Baere, 2000). AD is the proven technology that presents an environment friendly approach to managing bio-waste and in the process generates an energy-value gas like methane which is the main component of natural gas under the influence of microbial activities and in a condition void of oxygen (Kwietniewska and Tys, 2014). AD of organic waste is of increasing interest as it offers an opportunity to deal with some of the problems regarding the reduction of the amount of organic waste, while diminishing environmental impact and facilitating a sustainable development of energy supply (Kholi et al., 2004). Anaerobic digestion of biomass can either be single digestion of substrate or a co-digestion of substrates. Waste made primarily of cellulose is widely available and can be utilized as biomass to produce biogas. However, the recalcitrance natures of these substrates make them very difficult to digest, as their structure opposes microbial hydrolysis

in biogas production (Teghammar, 2013). In order to decrease the biomass recalcitrance and increase the biogas yield, different pretreatment methods can be used (Hendriks et al., 2009). Pretreatments can have effects on the physiochemical properties of a substrate such as particle size, molecular size, cellulose crystallinity, etc (Hendriks et al., 2009). Some pretreatments have an impact on the chemical composition of the substrate, where lignin and/or hemicelluloses, to an extent get soluble, while others have no effect on the chemical composition of the substrate (Johnson and Elander, 2008).

Various ecosystems, including rice fields, wetlands, rumen from ruminants, landfills, and other anaerobic settings, naturally produce biogas. Biogas is an odourless, colourless gas that combust with a clear blue flame as that compared with Liquefied Petroleum Gas (LPG) (Parker, 2007). Biogas comprises majorly of methane and carbon dioxide and other trace gases such as hydrogen sulphide, nitrogen, ammonia, and hydrogen are also formed (Ahring et al., 2003). Since biogas is a renewable energy source, bio-waste occurs naturally, hence it is a free source of energy whose supply does not seem to cease as long as life continues. In contrast to alternative energy sources, biogas is regarded as a non-pollution-causing gas but a bioremediation technique of dealing with the problem of pollution from waste. With the adoption of biogas, deforestation and air pollution from fossil fuel products will be reduced. Waste generated will be minimal,

solving the problem of soil and water pollution (Jingura and Kamusoko, 2017).

1.2 PROBLEM STATEMENT

Nigeria is a country known to not paying so much attention to waste management and still relies majorly on fossil fuels as her energy source; the following problems have been identified;

i. The increase in the rate of cattle killings so as to meet for the beef demand of her population has resulted in environmental pollution as a result of improper disposal methods from abattoirs.

ii. The problem of insufficient energy has been a long-lasting challenge and will continue to be if a a sustainable energy source is not adopted to reduce dependency on fossil fuels and crude oil which are non-renewable.

iii. Cattle rumen content (CRC) can be a pollutant to the environment and if ever considered as feedstock for biogas production can present some difficulty for AD process because of its high lignin content.

1.3 AIMS AND OBJECTIVES

1.3.1 AIM

The aim of this study is to pretreat cattle rumen content for easy biodegradation and increase the biogas yield using thermal and alkaline

pretreatment methods.

1.3.2 OBJECTIVES

The objective of this study includes;

i. Physiochemical characterization of cattle rumen content.

ii. Pretreatment with NaOH.

iii. Determination of maximum soluble chemical oxygen demand ($SCOD_{max}$).

1.4 SCOPE OF STUDY

The scope of this work covers the collection of CRC, preparation of CRC, characterization of CRC and pretreatment of CRC.

1.5 RELEVANCE OF STUDY

This study will help in;

i. Cleaning up the environment.

ii. Enhancing the conversion of cattle rumen content to useful energy.

CHAPTER TWO

LITERATURE REVIEW

2.1 INTRODUCTION

This chapter presents past to present day findings about the concept.

2.2 POLLUTION

Anything that creates an unfavorable or hazardous environment for living things is called pollution. The term also includes the release of toxic substances into the ecosystem. These toxic materials are called pollutants. These pollutants can be any kind of energy, including heat, sound, or radioactivity, as well as solid, liquid, or gas. Air, water, and land contamination are the three main types of pollution. Specific contaminants are also of concern to modern science, giving rise to new forms of pollution like plastic, light, and noise pollution.

2.2.1 Waste and Pollution

Every living creature produces waste. But because of our large population and contemporary, industrialized lives, we have produced more waste than the natural environment can handle and in certain instances, humans have entirely destroyed entire ecosystems. Solid, liquid and gaseous wastes create serious problems for

humans and the environment if not properly treated, transported and managed (Solaanke, 2011). Waste is typically divided into two categories: hazardous waste and non-hazardous waste.

2.2.1.1 Hazardous Wastes

Hazardous wastes are wastes that have the potential to deteriorate the healthy living status of closely living persons or animals if not properly treated, stored or disposed (Gilbert, 1998). Homes, businesses, farms, and the environment all produce poisonous and hazardous materials. Hazardous wastes fall into three main categories: radioactive, chemical, and biological.

2.2.1.1.1 Radioactive Wastes

Radioactive wastes are leftovers from fuel processing plants, nuclear reactors, and establishments like hospitals and research centers. Radioactive pollution can have an impact on the land, water, and air. It takes time for these wastes to decompose and turn harmless. Keeping them properly kept for an extended period of time is a serious worry.

2.2.1.1.2 Chemical Wastes

They aren't organic. These consist of both naturally occurring and man-made metals, such as lead and mercury, which are extremely hazardous and utilized in paints and other substances. Examples of hazardous chemical wastes are batteries,

herbicides, fertilizers, industrial wastes, etc.

2.2.1.1.3 Biological Wastes

Biological wastes are made up of organic molecules; this can include trash from humans and animals as well as kitchen leftovers. These wastes could pose a risk to human and other species' health in different ways, such as being infectious. As such, it needs to be handled carefully and with great attention. However, methane in particular is produced when biological wastes decompose. Examples of biological wastes include faeces, food waste, dead animals, abattoir wastes, etc.

2.2.1.2 Non-hazardous Wastes

Solid waste is categorized as non-hazardous when it doesn't endanger human health or the environment. Paints, plastics, latex, and rubber goods are examples of non-hazardous wastes that are treated with the same care as hazardous wastes by ethical waste processing facilities.

2.2.2 Environmental Implication of the Unhygienic and Improper Discharge of Abattoir Waste

In Nigeria, slaughtering houses are often characterized by unpleasant odour and the inadequate facilities to treat these wastes prior to discharge have been a challenge in ensuring a clean environment.

Abattoir waste is a form of agricultural waste obtained from livestock, slaughtering houses. Abattoir wastes include intestinal contents, rumen, horns, bones, blood (Ezeoha and Ugwuishiwu, 2011)

Olanike (2002) documented that conventional methods of animal waste disposal such as land-filling, combustion and illegal dumping are no longer adequate.

Roberts (2011) also reported that abattoir wastes contain malodorous compounds like amines, mercaptans, sulphides and organic acids. Saturated and unsaturated hydrocarbons, acidic hydrocarbons, organic alcohols, aromatic hydrocarbons, sulphur compounds and inorganic compounds are in trace amount (Jacobus, 2001).

Cattle Rumen Content (CRC, also known as paunch manure) has a typical moisture content of 88% with average chemical oxygen demand (COD) and a 5-day Biochemical Oxygen Demand (BOD5) of 177,300mg/l and 50,200mg/l respectively. The solid mass has the greatest pollution load containing about 73% and 40% of the COD and BOD respectively (Beefland International Inc., 1971).

The microbial load and constituents of CRC exert oxygen demand and suffer aquatic life by depleting oxygen (Ezeoha, 2000), introducing harmful heavy metals and intolerable materials into the aquatic environment. Eutrophication could also occur due to the presence of essential plant nutrients like nitrogen and phosphorus (Ezeoha and Ugwuishiwu, 2011).

2.3 FOSSIL FUEL

Fossil fuels are any class of hydrocarbon containing materials of biological origin occurring within earth's crust that can be used as a source of energy (Otto, 2021). Fossil fuels are hydrocarbons, mostly coal, fuel oil, and natural gas, which are created from the decomposing bodies of plants and animals, according to Wikipedia, the free encyclopedia. Commonly used derivatives of fossil fuels include diesel, kerosene, natural gas and propane.

2.3.1 Diesel

Fossil diesel is a product of crude oil produced from fractional distillation process in a temperature range of 200^0c to 350^0c at atmospheric pressure resulting in liquid mixture at room temperature containing 9 to 25 carbon atoms per molecule (Wikipedia).

2.3.2 Kerosene

Kerosene which is also known as paraffin is a combustible hydrocarbon liquid obtained from the fractional distillation of crude petroleum oil (Anon, 2009). Different varieties of kerosene serve as domestic and transport fuel for jets which are referred to as House Hold Kerosene (HHK) and the Aviation Technical Kerosene (ATK) respectively. (Lawal, 2011).

Kerosene is a yellow or colourless mineral oil with variable density in the range of 0.75-0.85g/cm^3. Kerosene has a flash point in the range of 37^0c to 65^0c, having an auto ignition temperature of 220^0c (Annon, 2009). If not properly combusted with oxygen, it releases fumes which become poisonous at insufficient concentration (Lawal, 2011). Natural gas is used in place of kerosene because it causes less kitchen pollution as it does not produce soot when combusted with oxygen. Also, natural gas is faster compared to kersosene.

2.3.3 Natural Gas

Light hydrocarbons such as methane, ethane, propane, butane, and pentane make up natural gas. Natural gas also contains nitrogen, helium, hydrogen sulfide, and CO_2. Although natural gas's composition varies constantly, methane—which makes up at least 90% of the gas—is usually its main constituent. Methane burns readily and nearly entirely due to its great flammability. It produces less air pollution. When compared to other fuel sources, natural gas is an intrinsically safe fossil fuel since it is neither poisonous nor corrosive, has a high ignition temperature, and a restricted range of flammability. In addition, because of its specific gravity (0.6), this is lower than that of air (1.0), natural gas rises if escaping, and thus dissipates from the site of any leak. (Viswanathan, 2017).

2.4 NON-FOSSIL FUEL

Non fossil fuels are generally known as biofuels because they are produced from or via biological processes. Unlike fossil fuels, the use of renewable sources of energy supports a closed carbon cycle and therefore does not promote carbon (iv) oxide atmospheric concentration. Replacement of fossil fuels reduces greenhouse gas emission and atmospheric pollutant that result in acid rain (Wilkie, 2005). Biofuels cover a range of bioethanol, biodiesel, pure plant oil and biomethane (a constituent of biogas). The following variables affect how biofuels are used: the fuel potential of available feedstock, production of by-products and the process technology involved.

2.4.1 Biodiesel

Biodiesel is produced by alcoholysis also called transesterification. This is a process in which oil molecule is cracked and the resulting glycerine is made to react with methyl or ethyl esters. The products obtained are glycerol and methyl or ethyl of fatty acid (biodesel). The mixture is separated leaving glycerine as the underflow component and biodiesel as the overflow component. The distinguishing characteristics of biodiesel over fossil diesel is nearly sulfur-free and has a low aromatic content, which lowers the amount of harmful hydrocarbons released uncontrollably, Particulate Matter (PM) and nitrous oxide. One of the major biodiesel feedstocks is soy (glycerine max) (Miltelbach and Remschmidt,

2004). Other feedstock includes; palm oil (Elaeis guineensis), coconut (Cocos nucifera), sunflower (Helianthus annuus), micro algae and waste oil.

2.4.2 Bioethanol

Bioethanol can be substituted for petrol. Bioethanol is synthesized from the synthesized from the fermentation of any biological feedstock containing sugar, starch or cellulose that are convertible to sugar. Cotton (Gossypium hirsutum), peanut (Arachis hypogea), mustard (Brassica nigra), castor (Ricinus communis), sugar beets and sugar juice are potential oil crops for biofuel production. Celluse waste (rice straw, bagase, leaves, stalks and cobs) is also an essential bioethanol feedstock (Teodorita et al., 2008).

The decrease in the emission of toxic air pollutant is the resultant effect of a blend of gasoline and ethanol. This is due to the substitutional effect of ethanol on the molecule of gasoline which emits the air pollutants (Mi and Jotanovi, 2015). The energy content of petrol and bioethanol are 32.5 MJ/l and 21.2MJ/l respectively (Teodorita et al., 2008).

2.4.3 Biogas

Biogas is another energy source that is used as car fuel, or for production of heat or electricity in different countries (Sims, 2003). Producing biogas from activated sludge is a well-established and nearly century-old method. Municipal solid waste

(MSW) and certain homogenous wastes, such manures, have also lately been used to create it on an industrial basis.

Anaerobic digestion of a variety of organic materials produces biogas, a gas mixture mostly composed of carbon dioxide and methane. The percentage of methane in biogas will vary depending on the process conditions and the type of organic matter fermented (Vintilla et al., 2012). Very trace amounts of other gases, including hydrogen sulfide, nitrogen, oxygen, water steam, etc., are also present in biogas. The anaerobic digestion is carried naturally in the anaerobic environments such as the bottom of ponds and marshes (Rouse et al., 2008), wetlands, the digestive tract of ruminants and certain species of insects (Rapport et al., 2011).

Biogas is a combustible mixture of gases containing 60%-70% methane, 30% - 40% carbon (iv) oxide, while hydrogen, hydrogen sulphide, nitrogen and ammonia are in minute concentration (Kwietniewska and Tys, 2014). Moreover, the presence of hydrogen sulphide in biogas is lesser than natural gas (Sun et al., 2015). It is possible to convert biogas into fuel for vehicles and use it to generate heat and electricity.

2.5 ANAEROBIC DIGESTION (AD)

Historical evidence from Assyria and Persia indicates the use of biogas for heating bathing water as early as the 10th century B.C. (Lusk, 1998). In the Middle Ages,

Jean Baptiste van Helmont observed the production of combustible gas from the decomposition of organic matter in lakes (Zullo, 2016). Later on, Alessandro Volta conducted a series of experiments on combustible gas that was collected from marsh sediments, observing a direct correlation between degraded organic matter and gas production (Ferry, 1993). In 1808, Humphry Davy discovered that anaerobically digested cattle manure produced methane, which aroused the possibility of producing combustible gas from manure (Lusk, 1993). The industrialization of AD started with the creation of the first digestion plant in Bombay in 1859. With the advance in the knowledge of the advantages of the AD process, improved operating methods, and equipment were adopted, that include the use of closed vessels to optimize anaerobic digestion. However, anaerobic facilities have been found to be a good waste-treating technique alongside an effective energy generating process (Monet, 2003).

Microorganisms break down biodegradable material in a sequence of biological processes known as anaerobic digestion when oxygen is not present. One of the end products is biogas, which is combusted to generate electricity and heat, or can be processed into renewable natural gas (Brewster, 2008).

Every day, a variety of anaerobic digestion methods turn organic waste streams like fats, oils, and grease (FOG), food waste, municipal wastewater, livestock manure, and industrial wastes into biogas. Composting separated digested solids

can be used for dairy breeding. Furthermore, the waste product from the anaerobic digestion of “clean” substrates, such as; manure, municipal solid waste and plant residues can be used as fertilizer on agricultural land (Schnürer and Jarvis, 2009).

2.5.1 Process Stages of Anaerobic Digestion

The process of anaerobic digestion takes place through four successive stages: hydrolysis, acidogenesis, acetogenesis and methanogenesis; the anaerobic digestion process is dependent on the interactions between the diverse micro-organisms that are able to carry out the four aforementioned stages (Verma, 2002). In single-stage batch reactors, all wastes are loaded simultaneously, and all four processes are allowed to occur in the same reactor sequentially; the compost is then emptied after at the conclusion of a given retention period or the cessation of biogas production (Verma, 2002). In each individual stage, different groups of facultative or obligatory anaerobic microorganisms work together (Gerardi, 2003). The microorganisms use their substrate for a source of energy as well as a carbon source for growth (Schnürer and Jarvis, 2009).

2.5.1.1 Hyddrolysis

Anaerobic digesters typically encounter organic biomass that contains complex polymers which are inaccessible to microorganisms without being further broken down through hydrolysis or pretreatments (Gujer and Zehnder, 1983). Therefore,

the objective of hydrolysis is to break down organic macromolecules into smaller pieces so that the acidogenic bacteria may use them. During this phase, polysaccharides, proteins and fats get degraded into their monomers, such as amino acids and fatty acids (Parawira et al., 2008).

Anaerobic digestion involves mostly biological processes; however, hydrolysis can also occur as an electrochemical process. In the process of hydrolysis, hydrolytic bacteria are able to secrete extracellular enzymes that can convert carbohydrates, lipids and proteins into sugars, long chain fatty acids (LCFA) and amino acids respectively (Li et al., 2011). After enzymatic cleavage, the products of hydrolysis are able to diffuse through the cell membranes of acidogenic microorganisms (Zeeman et al., 2008). However, it is important to note that certain substrates, such as, lignin, cellulose and hemicellulose may find it difficult to degrade, and can be inaccessible to microbes due to their complex structures, enzymes are often added to enhance the hydrolysis of these carbohydrates (Lin et al., 2010).

Hydrolysis can be a rate determining step, although prior research has also demonstrated that methagonesis might exist as a rate determining step on the ratio of hydrolytic to methanogenic microorganisms (Wang et al., 2013). A lot of research has been done on how to speed up hydrolysis in anaerobic digesters because it is crucial to the kinetics of anaerobic digestion. A variety of waste pretreatment options are being researched and utilized to optimize hydrolysis,

especially for materials that digest heavily lignocellulosic wastes (Kumar and Sharma, 2017).

Generally speaking, hydrolysis has, on its own, an optimum temperature of 30-50^{0}c band with an optimum pH of 5-7, although there is evidence of improved hydrolytic activity below a pH of 7 (Azman, 2016).

2.5.1.2 Acidogenesis

Acidogenic bacteria can produce intermediate volatile fatty acids (VFA) and other compounds by adsorbing the hydrolysis products via their cell membranes. VFAs constitute a class of organic acids such as acetates, and larger organic acids such as propionate and butyrate, typically in a ratio varying from 75:105:10 to 40:40:20 (Begman, 1990). Even then, smaller amounts of ethanol and lactate may be present (Zeeman et al., 2008). The specific concentration of intermediates produced in the acidogenesis stage may depend on the conditions of the digester; it has been reported that VFA concentrations can fluctuate significantly for digesters operating at different pH, with different studies presenting seemingly contradictory results (Zhu et al., 2010).

As opposed to other stages, acidogenesis is generally believed to proceed at a faster rate than all other stages of anaerobic digestion, with acidogenic bacteria having a regeneration time of fewer than 36hours (Deublein and Steinhauser,

2008). With the rapidity of this stage in mind, it is important to note that while the production of VFAs creates direct precursors for the final stage of methanogenesis, VFA acidification is widely reported to be a cause of digester failure (Akuzawa et al., 2011). A somewhat similar anaerobic process is present in bokashi composting; a composting practice in which food wastes and a microbial innoculant are left to degrade anaerobically, creating a highly acidic final product that can be used as a liquid and dry fertilizer (Yamada et al., 2001).

Lastly, studying the process of generating volatile free acids (VFAs) from amino acids makes sense for wastes high in protein, like sewage wastewaters. Amino acids generally degrades into VFAs in pairs via the Stickland reaction, with single amino acid degradation also possible when hydrogenotrophic bacteria are present, although this latter process is known to be slower than the Stickland reaction (Bagi et al., 2013). One important product of the amino acid breakdown is the production of amino ammonia from deamination, which at sufficiently high concentrations, is known to also be an inhibitor of anaerobic digestion (Park et al., 2014).

2.5.1.3 Acetogenesis

With the production of acetate through acidogenesis, a portion of the original substrate has already been rendered into a substrate suitable for acetoclastic methanogenesis (Fournier and Gogarten, 2008). Methanogenic microbes haven't

yet been able to reach other higher VFA generated. Acetogenesis is the process whereby these higher VFAs and other intermediates are converted into acetate, with hydrogen also being produced (Hansen and Cheong, 2013).

The hydrogen created at this point brings up an intriguing syntrophic link found in AD: the transfer of hydrogen between species. While acetogenesis is a producer of hydrogen, an excessive partial pressure proves to be deleterious to acetogenic microorganisms (Lester et al., 1988). However, due to the presence of hydrogenotrophic methanogens, hydrogen is able to be rapidly consumed while maintaining hydrogen partial pressures at a level favorable to acetogenesis by creating an exergonic reaction (Stams and Plugge, 2009).

At the same time, lipids undergo a separate pathway of acidogenesis via acidogenesis and β-oxidation, where acidogenesis produces acetate from glycerol and β-oxidation produces acetate from LCFAs (Cirne et al., 2007). With this in mind, it would be useful to be mindful that only LCFAs with an even number of carbon atoms can degrade to acetate; LCFAs with an odd number of carbons are first degraded to propionate (Cirne et al., 2007).

2.5.1.4 Methanogenesis

Methanogenesis marks the final stage of anaerobic digestion, where the accessible intermediates are consumed by methanogenic microorganisms to produce methane

(Ferry, 2010). Methanogenic microorganisms represent a group of obligate anaerobic archea; a testament to the acute sensitivity of methanogenic microorganisms to oxygen, it was found that 99% of *Methannococcus voltae* and *Methannococcus vannielli* cells had been killed within ten hours of exposure to oxygen (Kiener and Leisinger, 1983).

Methanogenic microbes can only grow on a limited range of substrates in addition to being oxygen-sensitive. Typically, acetoclastic methanogenesis from acetate accounts for approximately $\frac{2}{3}$ of the methane production, with hydrogenotrophic methanogenesis accounting for the remaining $\frac{1}{3}$ of the methane production; however, methanogenesis from methanol, methylamines and formate has also been observed (Belay et al., 1986).

With regards to the environmental needs of methanogenesis, methanogenic microorganisms tend to require a higher pH than previous stages of anaerobic digestion, in addition to a lower redox potential, the latter requisite having caused significant trouble for laboratory cultivation (Wolfe, 2011). At the same time, methanogens appear to have a significantly slower regeneration time than other microorganisms in anaerobic digestion, upwards of 5 – 16 days (Deublein and Steinhauser, 2008). However, it has been reported that some hydrogenotrophic species have a doubling time of only two hours (Zhang et al., 2016). Since the

methanogens have the longest generation time of all the microorganisms in the reactor, it makes this step the most time-limiting step for easily hydrolyzed materials (Schnüner and Jarvis, 2009).

About 70% of the methane production is from the acetate and about 30% of the methane arises from hydrogen and carbon dioxide (Gerardi, 2003). In batch reactors, the end of methanogenesis is determined when biogas production stops, which can take about 40 days (Verma, 2002).

2.5.2 Quantitative Evaluations of the Anaerobic Digestion Process

The metrics that are frequently employed in quantitative assessments of the anaerobic digestion process are detailed in the following subsections.

2.5.2.1 Biochemical Oxygen Demand

An anaerobic digester's overall efficacy can be measured using biochemical oxygen demand (BOD), which gives an indication of the amount of biodegradable organics contained in a sludge. The values obtained from the microbial metabolism of dissolved oxygen in a specific sludge sample over a period of five days are represented by BOD. Ultimately, BOD is a value that can be used to determine the amount of dissolved oxygen needed to sustain aerobic microorganisms in a sludge sample over a period of 5 days, which in turn can be used to quantify the

concentration of biodegradable organics in the sludge (Delzer and McKenzie, 1999).

In order to stop photosynthesis-induced dissolved oxygen formation, BOD testing is carried out in sealed bottles under dark conditions and at a specific temperature. Therefore, after accounting for dilution, BOD can be determined from the difference in dissolved oxygen at the beginning and end of the incubation period.

2.5.2.2 Chemical Oxygen Demand

The amount of oxygen in a sludge sample that can be consumed in a reaction with oxidizing agents is measured by chemical oxygen demand (COD), just like BOD. COD usually indicates the amount of organics in the sludge during anaerobic digestion. The efficiency of anaerobic digestion can also be evaluated using COD; COD reduction can be reflective of the amount of degradation taking place in an anaerobic digester, as it reflects the consumption of organics (Zeeman et al., 2008).

Sludge is refluxed excessively with a potassium dichromate and sulfuric acid solution during COD testing. Since potassium dichromate cannot convert ammonia into nitrate, nitrification does not need to be taken into consideration while using it. After a reflux is finished, the amount of surplus potassium dichromate can be measured by titrating against ferrous ammonium sulfate; the amount of potassium dichromate used in the original reflux can be used to calculate the final COD value.

2.5.2.3 Carbon/Nitrogen Ratio

One often used method of characterizing nutrients is the carbon/nitrogen ratio (C/N ratio) in a substrate. It makes sense that the breakdown of proteins in an anaerobic digester would be the most abundant source of nitrogen given the makeup of proteins, lipids, and carbohydrates. Just as carbon is necessary at certain concentration to provide a suitable substrate for digestion, nitrogen at a certain concentration is also necessary lest the protein formation for microorganisms can be compromised (Grilc et al., 2012). In a study conducted on dairy manure, it was found that increasing C/N ratios lead to decreasing methane concentrations in biogas, with an optimum at a C/N ratio of 25:1 (Hills, 1979).

With the growing use of co-digestion of several substrates, research on the C/N ratio has been conducted. For example, poultry manures have been known to have a relatively low C/N ratio due to high ammonia content, possibly due to urea, as such, carbon rich substrates such as straw maybe co-digested to obviate the possibility of ammonia inhibition (Wang et al., 2012). In a more recent study conducted on thermophilic and mesophilic digesters for the co-digestion of dairy manure, chicken manure and rice straw, an optimal methane potential and reduced ammonia inhibition were observed at C/N ratio of 25:1 for mesophilic digesters and 35:1 for thermophilic digesters (Wang et al., 2014).

2.5.2.4 Theoretical Methane Yield

The theoretical methane yield for a particular substrate has been attempted to be quantified in summarizing the four stages of anaerobic digestion. Working under the assumption that all substrate is converted to either carbon dioxide or methane, and that the carbon, hydrogen and oxygen composition of the substrate are known, one could use the following equation and the general gas equation to find a theoretical molar and volumetric output of methane (Buswell and Mueller, 1952).

$$C_nH_aO_b + \left(n - \frac{a}{4} - \frac{b}{2}\right)H_2O \rightarrow \left(\frac{n}{2} - \frac{a}{8} + \frac{b}{4}\right)CO_2 + \left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4}\right)CH_4$$

Nevertheless, this model ignores the effects of inhibition and makes the assumption that only carbon dioxide and methane are created. It is clear, therefore, that in real operational situations, neither of these hypotheses is realistic. To account for additional products, a variation of the above equation was proposed, which accounted for the presence of nitrogen and sulphur in wastes and the production of ammonia and hydrogen sulphide (Achinas and Euverick, 2016).

$$C_nH_aO_bN_xS_y + \left(n - \frac{a}{4} - \frac{b}{2} + \frac{3x}{4} + \frac{y}{2}\right)H_2O \rightarrow \left(n - \frac{a}{4} + \frac{b}{2} + \frac{3x}{4} + \frac{y}{2}\right)CO_2 + \left(n + \frac{a}{4} - \frac{b}{2} - \frac{3x}{4} - \frac{y}{2}\right)CH_4 + xNH_3 + yH_2S$$

Comparisons between practical and theoretical methane yield in bottle assays may benefit from these observations, even though they might not be helpful in determining methane yields in a continuous digester. It is expected that there will be deviations from the anticipated methane yield because anaerobic digestion is a dynamic process that can quickly lead to digester upsets and process failures. However, the rate and quantity of biogas generation may depend on amount of organics left, the availability of the organics for digestion, and over –accumulation of inhibitory compounds, and sudden changes in the digester pH (Esposito et al., 2012).

2.5.2.5 Volatile Solids

A more precise definition of volatile solids (VS) would be the quantity of a material in a sludge that is lost upon igniting. Nevertheless, VS can be thought of as a measurement of the organic fraction of the total solids. VS is the amount of biodegradable organic solids that produce methane under anaerobic condition (Moody et al., 2009).

VS is a measure of organics in water that can be compared to COD, albeit COD is a more precise indicator. Still, a digester's organic loading rate can be ascertained using both values as a starting point.

2.5.2.6 Total Solids

Total solids (TS) is a term used in the literature to describe the amount of dry matter in sludge, regardless of whether it is organic or inorganic. It is commonly expressed as a percentage or a concentration. The sludge sample is dried at 103ºC - 105ºC successively until no more weight change is noticed. This process determines the TS content.

TS is a crucial component of digester operation even though it is an assessment of influent. High TS anaerobic digestion needs smaller digester sizes and lower heating needs (Yi et al., 2014). Also improved biogas yields are seen in high TS digesters than in low TS digesters operating on the same retention time (Duan et al., 2012).

2.5.3 Factors Affecting Anaerobic Digestion

Numerous factors, including the type of feedstock (substrate and nutrients) used, as well as operational variables like temperature, pH, alkalinity, organic loading rate, hydraulic retention time, pressure, substrate particle size, and various inhibitors currently in use, can have an impact on the biogas production process. In optimization of an anaerobic system, it is important to examine the effect(s) of the process variables or parameters associated within the system.

2.5.3.1 Substrate and Nutrients

Biogas can be generated from several biological sources. Substrates used today in anaerobic digesters include sewage sludge, waste water, the organic fraction of municipal solid waste (OFMSW), different industrial food streams, slaughter house waste, manure and energy crops (Deublin and Steinhauser, 2008). It takes the right nutrition solution to ensure that the microorganisms grow to a sufficient size and to improve biogas output. In the biogas process, chemical molecules like proteins, lipids, or carbohydrates provide the energy that the bacteria need to grow and function. They furthermore need an electron acceptor, namely carbon dioxide for the anaerobic digester. The energy source is oxidized, while electrons/protons are transferred through different intermediates and in the end of the electron acceptor, where energy is produced (Schnürer and Jarvis, 2009). The nutrients needed for growth are the macronutrients, such as carbon, nitrogen, hydrogen, phosphorus, potassium and sulfur (Rich and Kayhanian, 1995). Biogas yield is strongly influenced by the type of substrate used; for example, digesting lipids produces a larger methane yield than digesting proteins or carbohydrates.

The carbon to nitrogen ratio is a crucial component of the process in addition to the substrate's organic composition. The C/N ratio should be between 10 and 30, and with an optimum ratio of between 25 and 30 (Yadvika et al., 2004) for the digester to work at its full potential. Ammonia inhibition is a possibility with lower C/N,

with methanogens being the most susceptible. As a result, there may be a build-up of volatile fatty acids, which could lower pH and cause a reactor failure. Higher ratios may lead to lower methane yields as there will be a deficiency of nitrogen available for the cell growth (Alvarez and Liden, 2008).

2.5.3.2 Temperature

There are moments during which the growth of various microorganisms is at its best. Three temperature ranges are defined, i.e., the psycrophilic range, where the growth optima is around 10^0c, the mesophilic range with an optima at around 37^0c; and the thermophilic range with an optimum at above 50^0c (Noah and Wiegel, 2008). When compared to mesophilic temperatures, thermophilic temperatures are known to cause the microbes to have 25–50% more activity, which yields methane productivity. However, the microorganisms at thermophilic conditions are more sensitive to disturbances in temperature or to different toxic compounds (Duran and Speece, 1997). On the other hand, mesophilic systems are more robust but with a lower reaction rate (Schnürer and Jarvis, 2009). Generally, there are fewer organisms in the thermophilic temperature compared with the mesophilic (Schnürer et al., 2007), which probably means that the diversity of mesophilic microorganisms can help stabilize the process.

2.5.3.3 System pH and Alkalinity

Because they are sensitive to pH, the microorganisms in the anaerobic digester have various pH optimal values and this appears to be the most determining process variable because any slight change in pH can affect the overall microbial population and also the methane production. The pH of an AD system varies from the initial process stage to the final process stage as the product of each stage contributes to the pH of the system. This occurs when the VS is degraded and methane is formed. While the acetogens function best at a pH of 5.0–8.5, the methanogens have optimal pH ranges of 6.5–8.0. Anaerobic digesters are preferably run at a pH range of 7.0-8.5 (Boe, 2006); outside this neutral range, the process can face imbalances (Schnürer and Jarvis, 2009).

It is crucial to maintain a high and steady alkalinity in the digester in order to maintain a neutral and stable pH. The digester's alkalinity is a gauge of its basic chemical composition. The likelihood of achieving a steady pH increases with alkalinity because it increases buffer capacity. However, substrates rich in proteins liberate ammonia when degraded, which also contributes to the alkalinity (Gerardi, 2003).

2.5.3.4 Organic Loading Rate

The term "organic loading rate" refers to the quantity of biomass or substrate added per reactor or digester volume per unit time (OLR). In terms of VS/m^3/day, it is

typically expressed. A lower OLR is necessary for newly established processes, whereas a greater OLR can be handled by established and reliable systems. Thermophilic processes can handle 4-5kg VS/m^3/day, while mesophilic processes normally work at 2-3kg VS/m^3/day (Schnürer and Jarvis, 2009). An overloading of the system, where easily degradable substrates are added at too high ORL, often results in an inhibition caused by volatile fatty acids (VFA) accumulation (Fang, 2010). OLR also affects other parameters such as Oxidation-Reduction Potential (ORP), pH, VS, TS. However, high OLR can be advantageous in that a lot of biomass is fed into a digester thereby minimizing the cost of getting other digesters (Luste and Luostarinen, 2010; Rai, 2011).

2.5.3.5 Hydraulic Retention Time

It takes time for all of the material in the reactor to change, which is known as the hydraulic retention time (HRT). It depicts how long substrates spend in a digester. HRT has a direct relationship with the OLR, the higher the HRT, the higher the OLR and vice versa (Rao and Singh, 2004; Dareioti and Kornaros, 2014; Liu et al., 2017). The HRT of anaerobic digesters is often 10-25 days or longer (Schnürer and Jarvis, 2009). In comparison to rapidly decomposed compounds like dissolved sugars, slowly degrading materials, such as those containing cellulose, may require a longer HRT. Theoretically, lipids give the highest biogas yield compared with carbohydrates and proteins, and it also has the highest retention time due to its

slow biodegradability compared with carbohydrates and proteins (Esposito et al., 2012). When the ORL is large, a longer HRT is typically required to prevent an excessively low degradation.

2.5.3.6 System Pressure

Reports have shown that the pressure on the surface of the substrates can affect the rate of anaerobic digestion. Lower pressure supports the rate of substrate fermentation, COD removal and methane yield (Cuetos et al., 2010; Vivekanand et al., 2013; Taherzadeh et al., 2008; Li et al., 2017).

2.5.3.7 Substrate Particle Size

Anaerobic digestion is influenced by the size of the substrate particles that are charged into a digester. The hydrolysis stage of the AD process can be improved by reducing the substrate particle size. Esposito et al. (2012) reported a low methane yield using a substrate of large particle size for the process but higher methane production was increased when pretreatment measures were taken to reduce particle size (Zhu et al., 2009; Subramani and Pokumar, 2012.

2.5.3.8 Toxic/Inhibiting Compounds

Inhibitors in the anaerobic digester can be made of a variety of chemicals. Generally speaking, methanogens are thought to be the most delicate type of microbe found in the digester. One of the digester's degradation processes may

produce the hazardous chemicals, or they may come from the substrate itself. The microorganisms can, in some cases, adapt to the system where the microorganisms that are tolerant to the toxic compounds grow, and in later phases of the digester, they can dominate in the reactor (Schnürer and Jarvis, 2009).

Ammonia is the most prevalent inhibitor of the anaerobic process. The urea or protein degradation processes, or the soluble ammonia in the influent, are the sources of the ammonia concentration. The non-ionized form of ammonia is regarded to be the most toxic form, since it can diffuse through the cell wall and cause a proton imbalance as well as potassium deficiency (Chen et al., 2008). Temperature and pH have an impact on the solubility and toxicity of ammonia. The level of toxic concentration further depends on the buffer capacity of the system and on the adaptation of the microorganisms (Alvarez and Liden, 2008; Kayhanian, 1999).

Volatile fatty acids accumulate as a result of ammonia inhibition on the methanogens (VFA). As the methanogens eat the VFAs that the acetogens make, there will be an accumulation of VFAs if the methanogens are inhibited. The accumulation of VFA leads to a pH drop wherein the whole digester could stop working (Kayhanian, 1999). The accumulation of VFA can function can as a process indicator of the performance of the anaerobic digester (Ahring et al., 1995; Murto, 2001).

Other inhibiting compounds are cations at high concentrations such as Na^{2+}, K^{+}, Ca^{2+} and Mg^{2+} (McCarthy and McKinney, 1961), alternative electron acceptors such as SO_4^{2-} and NO^{3-}, phenolic compounds, cyanides, heavy metals, detergents, hydrogen sulphides, antibiotics, etc (Chen et al., 2008).

2.5.4 Suitability of substrate for Anaerobic Digestion

Here, the emphasis is on the biomass's anaerobic digestibility while also taking into account whether or not it contains the elements required for effective digestion.

The carbon to nitrogen ratio (C/N ratio) is commonly mentioned in literature as essential (Carlsson and Uldal, 2009). Of course, it leaves out some crucial facts, but it might still provide useful direction. Typically, it is seen as advantageous to have a C/N ratio between 20 and 30 (Dioha et al., 2013). A high C/N ratio (high C, low N) causes the available nitrogen to be quickly consumed, leaving the remaining carbon unaltered and producing a poor methane production. Conversely, ammonium (NH_4) synthesis and nitrogen release result from a low C/N ratio (low C, high N). This will raise the pH, which if it exceeds 8.5 as noted by Chandra et al. (2012), could have harmful consequences on the methanogens. Toxic effects could also result from elevated ammonia levels. When a substrate has a low C/N ratio, it might be best to digest it in a co-digestion process with other substrates that have higher ratios, or it might be best to digest it with an inoculum that has been

modified to handle high ammonia levels in the process. Another related factor is the C:N:P ratio which is optimal for degradation around 100:5:1 (Steffen et al., 1998).

Apart from the numerical nutrient and carbon viewpoint, various other elements impact the appropriateness for anaerobic digestion and must be taken into account throughout the evaluation process. For instance:

• Its biodegradability (e.g., Batut et al., 2011).

• The content of other nutrients and trace elements (A. Karlsson et al., 2014)

• Other properties having an impact on the digestion. For example, since bacteria can thrive on straw pieces, they may improve digestion.

• Contaminants (substances that hinder digestion and are difficult to eliminate during the pretreatment stage).

In summary, a number of factors affect whether a particular kind of feedstock is appropriate for digestion. For illustration, let's look at woody biomass. The aim of pretreatments is to increase the digestibility of an otherwise hard to digest feedstock, for example by opening up the structure of the lignocellulosic material and remove the lignin to facilitate enzymatic activity (Johnson and Elander, 2008). Yet regardless of the pretreatment technique, some organic and non-organic

compounds, like lignin, are not broken down in the digester and stay undigested. Lignin has very complex (and diverse) three-dimensional molecular structure which is difficult to biodegrade (Taherzadeh and Karimi, 2008). Therefore, the appropriateness for anaerobic digestion is reduced in the presence of such materials. Anaerobic digestion is less suitable when unwanted elements that can act as poisons or inhibitors for AD cannot be readily eliminated by pretreatment.

2.5.5 Bio-Methane Potential and Yield

Bio-methane potential (BMP) is the maximum amount of biomethane produced per unit of volatile solids of a given substrate (Esposito et al., 2012). The volume of gas per unit of weight for each feedstock is used to calculate the biomethane yield. However, there may be discrepancies in the literature because tests may have been done at different pressures and temperatures, and the gas volume may refer to pure methane or include additional content (CO_2, etc.), such as more biogas. However, when looking for information, caution is required. For many types of feedstock categories there are data about the yield, but it can be difficult to value it (Björnsson, 2011), for example to know what type of yield that is stated and if it is trustworthy. Additionally, the weight can be expressed as Organic Dry Matter or Volatile Solids (ODM or VS), Dry Matter or Total Solids (DM or TS, i.e., without water content), or Wet Weight (ww, i.e., all content).

Furthermore, the numbers provided may fall into one of three categories: small-scale laboratory studies, pilot-scale testing, full-scale practical applications, or theoretical values derived from the substrate's chemical composition. Since the feedstock is not entirely digested in reality and part of the carbon will be utilized by the microorganisms to grow, the theoretical values are always significantly overestimated. For example, Schnürer and Carlsson (2011) state that when comparing experimental methane potential to a theoretical, a maximum of 90-95 % can be expected in a batch assay since the rest of the substrate is used for growth of the microorganisms. For a continuous process the methane yield may be only 50-70 % of the theoretical yield (Jarvis and Schnürer, 2009). However, theoretical values could be significant in determining the plausibility of the data found in the literature. Björnsson (2011), states that there are many examples in literature where values are given that are even exceeding the theoretical values. The precise chemical makeup of some biogas feedstock types, such as mixed byproducts, is unknown, which makes it impossible to compute theoretical values.

2.5.6 Pretreatment of Biomass for Anaerobic Digestion

Pretreatment simply entails the process that converts any source of lignocellulosic biomass from its native form, which is recalcitrant to hydrolysis with cellulose enzyme system into a form which enzymatic hydrolysis is effective (Lynd et al., 2002).

The basic reason for pretreatment is to reduce the high molecular chain of stubborn biodegradable substrate thereby improving the solubilization of the complex compounds to increase the hydrolysis rate of the AD process (Eastman and Ferguson, 1981; Noike et al., 1985). Pretreatment helps in the alteration of the lignocellulose structure, hemicellulose partial hydrolysis, and surface area and pore size increase.

Pretreatment methods can be mechanical, thermal, biological or chemical.

2.5.6.1 Mechanical Pretreatment

The main aim of mechanical pretreatment is to reduce the substrate particle size, thereby increasing the surface area for better contact between substrate and microorganisms for better anaerobic digestion (Kumar and Sharma, 2017). Mechanical pretreatment methods include; sonication, lysis-centrifuge, liquid shear, crushing, maceration, high-pressure homogenizer and liquefaction (Ariunbaatar et al., 2014).

Liquid shearing has garnered some degree of research attention, particularly collision plate pretreatment; this method entails jetting a sludge against a smash plate at high pressure, thus causing cell lysis (Nah et al., 2000). This pretreatment was able to reduce the HRT of waste activated sludge from 13 days to six days without any hindrance of process efficiency (Nah et al., 2000). Nonetheless, it

should be noted that research in collision plate pretreatment has been confined to the laboratory scale (Dumas et al., 2010).

Reducing substrate size can also be accomplished mechanically through milling. Although the fine milling of lignocellulosic waste can translate into better conversion than coarse milling, caution must be taken, for excessively fine particles may run the risk of acidification due to high waste solubility (Motte et al., 2014).

High size reduction has been shown to lower the yield and performance of the AD process. This is because of the increase in acidity of the process due to the formation of excess VFA when substrate particle size is reduced less than 0.7mm (Izumi et al., 2010).

The use of mechanical pretreatment allows for easy degradation and better dewatering of the digestate. Mechanical pretreatment is the most commonly used method because energy is moderately consumed and does not generate odour when compared with other pretreatment methods. However, there is a limitation, due to the formation of a slurry substrate, there could be clogging hence there will be need for agitation. Also, no pathogen is removed from mechanical pretreatment methods (Toreci et al., 2009; Perez-Elvira et al.,2006).

2.5.6.2 Thermal Pretreatment

Thermal pretreatment involves exposing wastes to high temperatures to induce hydrolysis while preventing evaporation (Panico et al., 2014). Increased loading rates can be applied to digesters that incorporate thermal pretreatments (Barber, 2016). Furthermore, cell disintegration and hydrolysis are able to create a sludge that is more biodegradable and allows for more stable digestion (Kepp et al., 2000; Tait et al., 2008).

There is a relationship between the temperature of thermal pretreatment and the pretreatment process' effectiveness. Additionally, an excessive temperature could result in the destruction of VS, depleting the available substrate for anaerobic digestion (Panico et al., 2014). Furthermore, the increased solubility of carbohydrates and proteins at high temperatures may cause toxic melanoidins to accumulate from the Maillard reaction (Dwyer et al., 2008). Maillard reaction occurs between carbohydrates and amino acids leading to a substrate-complex formation that is not easily biodegradable. The reaction can occur at very high temperatures exceeding 150^0c or a longer treatment time at lower temperatures ($<100^0$c) (Carrere et al., 2010; Elliot and Mahmood, 2012; Hendriks and Zeeman, 2009).

However, it has been proposed that the mechanism by which low-temperature thermal pretreatment occurs is via enzymatic hydrolysis (Ferrer et al., 2008).

Despite its lower temperature, thermal pretreatment at 70^0C was still able to produce marked pathogen reduction (Lu et al., 2005).

Nevertheless, it can be said that the type of substrates, duration and temperature range are determining factors affecting thermal pretreatment.

2.5.6.3 Biological Pretreatment

Biological pretreatments may include aerobic and anaerobic pretreatments, although these treatments are generally not applied to municipal wastes (Esposito et al., 2014). There have been many researches on the biological pretreatment, experimenting the effects of enzymes and inoculum on the hydrolysis step and on the rate of AD (El-Mashad, 2013). White rot fungi have been investigated as a potential biological agent in the pretreatment of lignocellulosic wastes through enzymatic secretions, although the use of fungi runs the risk of long pretreatment times and there exists a proclivity for certain white rot fungi to destroy cellulose (Wan and Li, 2012). The former disadvantage can be especially troubling, as pretreatment times of several weeks to several months may be necessary for considerable lignin destruction (Wan and Li, 2012).

In addition, temperature-phased anaerobic digestion (TPAD) is another means of biological pretreatment, in which a waste is first digested at thermophilic or hyper-thermophilic conditions to promote hydrolysis (Dumas et al., 2010). TPAD,

particularly thermophilic-mesophilic TPAD, is particularly useful in increasing hydrolysis, which in turn translates to greater VS removal and increased methane production (Ge et al., 2010).

Enzymes produced by industrial fermentation processes can also be used as an accelerant for the pretreatment of lignocellulosic wastes (Bochman et al., 2007). In food waste acidification reactors, it was found that a mixture of carbohydrates, protease, and lipase in a 1:2:1 ratio was most conducive to VS reduction (Kim et al., 2006). However, the efficiency of and long retention times of biological pretreatment has suggested that it is less advantageous than other means of pretreatment (Zheng et al., 2014) Furthermore, the marginal biogas increases may not be able to justify the expensive cost of enzymes (Montgomery and Bochman, 2014).

2.5.6.4 Chemical Pretreatment

Strong acids, alkalis, or oxidants are used in chemical pretreatment to break down complex organic molecules. Positive adjustment in pH scale is achieved by alkali pretreatment method (Li et al., 2012). Acidic pretreatment and oxidative methods (Ozonation) are used to enhance the hydrolysis rate thereby promoting biogas production. Chemical pretreatment does not enhance biodegradability of substrates containing high amounts of carbohydrates, due to their highly degradation

properties and the build-up of VFAs which act as an inhibitor of methane production (Wang et al., 2011).

2.5.6.4.1 Acidic Pretreatment

Acidic pretreatment is one form of chemical pretreatment, in which lignocellulosic substrates are broken down into their respective monosaccharide (Panico et al., 2014). Furthermore, the acidity associated with this kind of pretreatment can be adjusted to by hydrolytic microorganisms (Panico et al., 2014). While acidic pretreatment is able to assist with the degradation of substrates in addition to reducing the time required for digestion, its costs render it less financially effective than utilizing alkaline pretreatments (Kumar and Murthy, 2011). Acid pretreatment has been found to enhance AD that ultimately leads to a higher methane yield due to the hydrolysis of hemicellulose into monosaccharides, while lignin separates out (Hendriks and Zeeman, 2009; Mata-Alvarez et al., 2012). Hence, thermo-chemical method is adopted, the use of dilute acids with temperature below 70^0C. Pretreatments with strong or diluted acids, such as hydrochloric acid, nitric acid, or sulfuric acid, have been carried out at high temperatures. The combination with other methods, such as steam explosion has been performed (Galbe et al., 2013).

2.5.6.4.2 Alkali Pretreatment

Alkaline pretreatment, another form of chemical pretreatment, typically entails ammonia or hydroxide compounds (Wang et al., 2015). These treatments use reagents which are less caustic than acidic pretreatments and can be conducted at ambient temperature (Kim et al., 2016). The principle of alkaline pretreatment is to cause fibers to swell, thus disrupting the structure of lignin and exposing the substrate to enzymatic degradation (Chen et al., 2013). As anaerobic digestion occasionally necessitates the addition of alkaline reagents to balance the pH, alkaline pretreatment is a preferred method to acidic pretreatments (Li et al., 2012).

The biomass itself absorbs some of the alkali, which is a crucial component of the alkali pretreatment process. The residual alkali concentration after the alkali consumption by the biomass is the alkali concentration left over for the reaction (Gossett et al., 1982). In addition to causing lignin to become soluble, redistribute, and condense, alkali extraction can also alter the crystalline state of cellulose. These effects can lower or counteract the positive effects of lignin removal and cellulose swelling (Gregg and Saddler, 1996). Another important aspect of alkaline pretreatment is the change of the cellulose structure to a form that is denser and thermodynamically more stable than the native cellulose (Pettersen, 1984).

2.5.6.4.3 Ozonation

Ozonation is the use of ozone to ease the degradability of biomass taking the advantage of its aftermath effect-free pretreatment characteristics when compared with other chemical pretreatment methods (Carrere et al., 2010; Sri Bala et al., 2011). Ozonation is a highly oxidation process that decomposes into radicals and chemically react with organic matter (Sri Bala et al., 2011). It has been found useful for disinfecting pathogens (Kianmehr, 2010). Hence it has gained application in pretreatment. Ozonation is very effective for the pretreatment of stubborn components of biomass by not only reacting with the structure of the reactant but also the hydroxyl radicals (Carballa et al., 2007).

2.6 LIGNOCELLULOSE

Worldwide, there is an abundance of lignocellulosic materials available. Lignocelluloses have been estimated to account for approximately 50% of the biomass in the world, and to have a yearly production of 200 billion tons per year (Claassen et al., 1999; Zhang, 2008). Anaerobic digestion can be performed with these materials since they are high in carbohydrates.

Lignocelluloses comprise a large fraction of municipal solid waste (MSW), crop residues, animal manures, woodlot arisings, forest residues or dedicated energy crops (Licht, 2015). Lignocelluloses are composed of mainly cellulose, hemi-cellulose and lignin. It also contains pectins, extractives and several inorganic

materials. In lignocellulosic plants, these polymers have distinct roles and chemistry. The table below lists the major lignocellulose groups—grasses, hardwoods, and softwoods—and their compositions.

Table2.1

	Softwood (spruce)	Hardwood (beech)	Grass (switchgrass)
Cellulose	44.7%	45.6%	32.2%
Hemicellulose	22.9%	25.9%	24.4%
Lignin	30.6%	23.8%	23.2%
Others	1.8%	4.7%	20.2%

(Jin et al., 2004; ESteghlalian et al., 1997).

2.6.1 Cellulose

Cellulose is the main component of higher plants, comprising 20-40% of the cell wall (Harris and Stone, 2008). Cellulose is an unbranched polymer chain, constituted by glucose units, which are linked by β-1, 4-glycosidic bonds (Brett, 2000). Up to 14.000 glucose units can be polymerized, with each glucose unit rotating 180 degrees in relation to its neighbor. Three-dimensional microfibrils are formed by the tightly packed cellulose chains. Van der Waals and hydrogen bonds serve to stabilize them. Two intra-chain hydrogen bonds and two to three inter-chain bonds bind each glucose molecule together. Because of this, the configuration is stable and densely packed. Each microfibril consists of 30-36 parallel cellulose chains (Harris and Stone, 2008; O'Sullivan, 1997). Because of its unique and complex structure, cellulose is resistant to chemical and biological

treatments. Cellulose is available in waste streams in the form of lignocelluloses, or partly purified in the form of e.g., papers or pure cellulose such as cotton, or mixed with other materials, in e.g., citrus wastes (Talebnia et al., 2008). The half-life of crystalline cellulose at a neutral pH is 100 million years (Wilson, 2008).

2.6.2 Hemicellulose

Hemicellulose is a complex structure of carbohydrates made up of several polymers such as sugar acids, pentoses (such as xylose and arabinose), and hexoses (such as mannose, glucose, and galactose). The dominant component of hemicellulose from hardwood and agricultural plants, like grasses and straw, is xylan, while this is glucomannan for softwood (Fengel and Wegener, 1984; Saha, 2003). Hemicellulose has a lower molecular weight than cellulose, and branches with short lateral chains that consist of different sugars, which are easy hydrolyzable polymers (Fengel and Wegener, 1984). Hemicellulose serves as a connection between the lignin and the cellulose fibers and gives the whole cellulose–hemicellulose–lignin network more rigidity (Laureano-Perez et al., 2005).

The sequence of solubility for the various hemicellulose compounds is as follows: mannose, xylose, glucose, arabinose, and galactose. As the temperature rises, their solubilities grow. The solubilities of higher molecular polymers could not be predicted, because of unknown melting points (Gray et al., 2003). The

solubilization of hemicellulose compounds into the water starts around 180^0C under neutral conditions according to Bobleter (1994). Garrote et al. (1999) however mentioned that already from 150^0C parts of the hemicellulose solubilize. The solubilization of lignocelluloses components not only depends on temperature, but also on other aspects like moisture content and pH (Fengel and Wegener, 1984). The xylan of hemicellulose can be extracted quite well in an acid or alkaline environment, while glucomannan can hardly be extracted in an acid environment and needs a stronger alkaline environment than xylan to be extracted (Balaban and Ucar, 1999; Fengel and Wegener, 1984; Lawther et al., 1996). It seems that the portion that can be extracted the easiest is xylan.

Of cellulose, hemicellulose and lignin the hemicelluloses are the most thermal-chemically sensitive (Levan et al., 1990; Winandy, 1995). During thermal–chemical pretreatment firstly the side groups of hemicellulose react, followed by the hemicellulose backbone (Sweet and Winandy, 1999).

2.6.3 Lignin

In nature, lignin ranks third in terms of abundance, after cellulose and hemicellulose. Lignins are regarded as complex, amorphous, branched polymers constructed of different phenylpropane units (Saha, 2003; Davin, 2008). The monomeric composition of lignin varies; however, most monolignols are derived

from p-coumaryl alcohol, coniferyl alcohol or sinapyl alcohol (Hendriks and Zeeman, 2009). Lignin can also be designed as a mixture of p-hydroxyphenyl, guaiacyl, and syringyl, based on their aromatic ring substitution pattern (Davin et al., 2008).

The lignocellulosic structure is held together by interlinkages between lignin and hemicellulose and cellulose. Ether, glycosidic bonds, or ester bonds can be used in these interlinkages. Lignin is extremely resistant to biodegradation due to these strong linkages (Davin et al., 2008). Opening up and changing the structure of lignin is one of the main problems with employing lignocelluloses as a feedstock for the production of biogas. High lignin content in the biomass has previously been connected to lower methane yields (Jimenez et al., 1990; Xiao and Clarkson, 1997). Giving a plant structural stability, impermeability, and defense against microbial invasion and oxidative stress is the primary function of lignin. The amorphous heteropolymer is also non-water soluble and optically inactive; all this makes the degradation of lignin very tough (Fengel and Wegener, 1984).

2.6.4 Pectins

Pectins or pectic polysaccharides are another group of polysaccharides that occur in the primary cell wall of, for example, grasses (Harris and Stone, 2008). These polysaccharides come in a wide variety and are hydrophilic. The major

components in pectins are: galacturonic acids, followed by rhamnose, arabinose, galactose, fucose and apiose (Van Dyk and Pletschke, 2012).

2.6.5 Extractives

Non-cell wall components, or extractives, are present in wood, bark, and leaf. These are compounds that contain fewer than 40 carbon atoms in total. They comprise about 1-5% of the wood and the most common extractives are resin acids, fatty acids, and sterols (Kostamo et al., 2004; Leviska et al., 2009). Many of the extractives can be toxic to aquatic organisms and should be removed, for example, from the waste water of pulp and paper plants in order to avoid environmental pollution (Leviska et al., 2009).

2.6.6 Lignocellulosic Materials

Lignocellulosic materials that are suitable for anaerobic digestion can be separated into two categories: cultivated feedstock, which is a plant that is grown specifically for the purpose of producing energy, and lignocelluloses, which are the byproduct of the process. One benefit of using residuals is that they don't compete with the use of land for producing food and feed. The following is a discussion of the two distinct feedstock types:

2.6.6.1 Energy Crops and Forest Production

Energy crops high in lignocellulosic matter are grown specifically with the intention of producing energy from the biomass of the plants. Plants used as energy crops in Europe include, for example reed canary grass, willow, poplar, and miscanthus (Aebiom, 2009). Anaerobic digestion can also be achieved by growing trees in forests. Large amounts of biomass are found in the forests. Approximately, 420 x 109 tons forest biomass is available around the world, of which more than 40% is available in South America (Parikka, 2004). However, there are other uses for wood outside energy generation, therefore using wood for energy production is not a cost-effective option.

2.6.6.2 Forest Residues

The forest residuals include, for example, tops and branches, needles, bark, roots, fuel wood, logging residues, sawdust, as well as stems and trees from clearing and thinning (Thuresson, 2010). Roughly sixty percent of the biomass from the cut tree remains in the forest after logging. A further 8–10% waste is produced during sawing the logs, and a further 30–50% waste is produced when the logs are squared in the forest. Additionally, 45-55% of a log is wasted at the sawmill (Parikka, 2004). Forest residues are abundant, but because they include a large amount of lignin, they are difficult to digest in an anaerobic digestion system.

2.6.6.3 Agricultural Residues

Rice and wheat straw make up the two major waste fractions of the lignocellulosic residuals from agriculture, which are mostly made up of various grains and oilseed straws. The world rice straw production is approximately 730 million tons/year, distributed over Asia, America, Africa, and Europe (Binod et al., 2010). In 2011, 530 million tons of wheat straw were produced globally at the same time. Both straws are often burned on cropland today, causing environmental and health problems (Binod et al., 2010; Zhang et al., 2012), while some are removed for cooking, recycling, or other uses.

Given that they are already gathered at the rice plant, rice husks have a significant potential for anaerobic digestion when combined with straw and manure. Other important agricultural residues are, for example, sugarcane fibers (bagasse), corn stover, coconut husk and shell, groundnut shell, and groundnut straw (Dermibas, 2001). The benefit of agricultural residues over forest residues is that the former can usually be degraded more easily. The reason for this is that the straw's comparatively small dimensions and decreased lignin concentration make the material easier for microbial enzymes to reach.

2.6.7 Lignocellulosic Structure

Primary and secondary cell walls are found in higher plants. The outermost wall, known as the primary wall, is often unlignified. During the cell's growth, the main

cell wall offers both mechanical strength and flexibility. When the cell reaches maturity and full expansion, the secondary wall grows inside the primary cell wall. There is a lot of lignin in the secondary cell wall, which is thicker and stronger. It is frequently separated into three layers, designated as S1, S2, and S3. Each layer's cellulose fibers are arranged differently from the layers above and below it (Figure 2.1). The majority of the carbohydrates in biomass are found in the secondary cell wall. The space between the cells, called the middle lamella, is often also lignified (Harris and Stone, 2008).

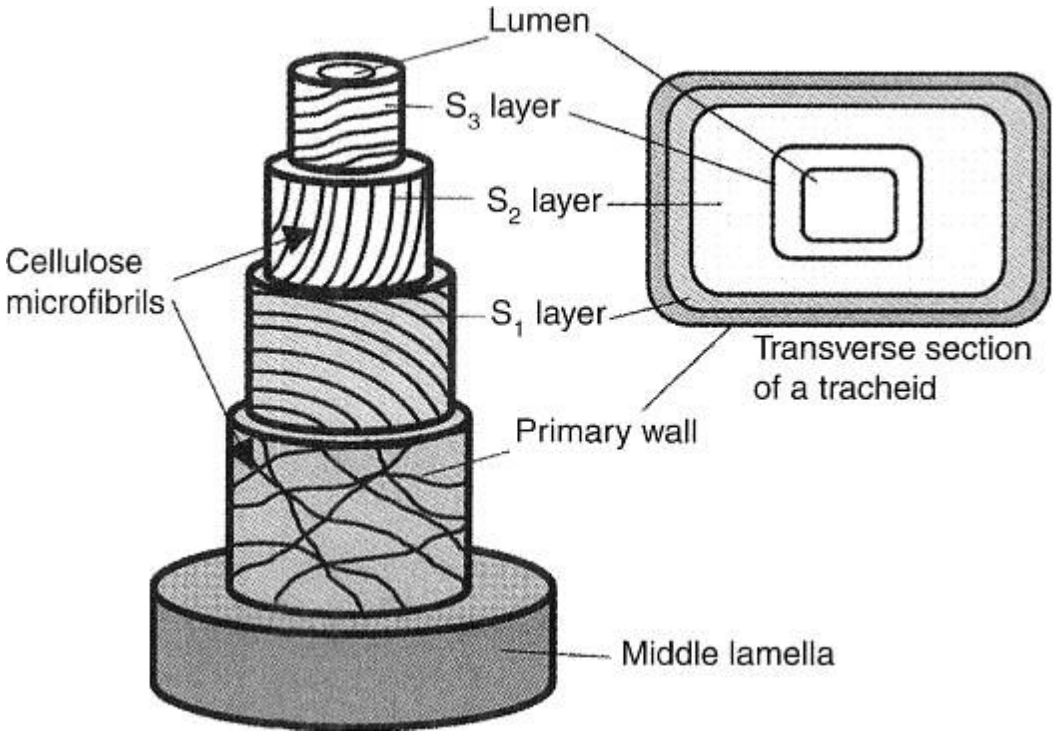

Fig. 2.1 Three-dimensional sketch of a tracheid (xylem cell) (Plomoin et al., 2001)

2.6.8 Biomass Recalcitrance

The complicated lignocellulosic structure within the cell walls is a way for the plants to prevent the microbial and enzymatic degradation. Preventing microbial deterioration of the material is one of the primary goals of the lignocellulosic structure's development. This attribute is defined as the biomass recalcitrance (Himmel et al., 2008). The biomass's level of recalcitrance is dictated by the enzymes' ability to reach the polysaccharides. There are numerous ways in which plants might defend themselves against microbial deterioration. The first protection layer in plants is called epidermis, this is in trees represented by the bark, while in grasses this first protection layer consists of cells with thick cell walls (Himmel et al., 2008). The second protection layer is the structure and organization of the cell walls as well as the vascular tissues (Somerville et al., 2004). The third and probably the main reason for the biomass recalcitrance is the molecular structure within the cell walls (Taherzadeh and Jeihanipour, 2012). The cellulose fibers are arranged crystalline inside the microfibrils. The microfibrils are further surrounded by a matrix of different polymers, such as lignin, hemicelluloses, and pectins (Himmel et al., 2008). Together, the covalent and non-covalent bonds that bind these polysaccharides together create a three-dimensional structure. The various polymers in the gel matrix are related to one another as well as to these cellulose microfibrils and the matrix polymers. This crosslinking between different polymers

in the cell wall has a major contribution to the lignocellulosic biomass recalcitrance (Harris and Stone, 2008). The amount of lignin and the cellulose crystallinity, in addition to the interaction bonds, determine how accessible the substrate is to the enzymes and microorganisms. It is also dependent on the accessible surface area of the material, which has to be high enough in order to make the substrate available for the microorganisms (Cowling and Kirk, 1976; Liu et al., 2001).

2.6.9 Lignocelluloses Pretreatment and Digestion

The high crystallinity and lignin content of lignocelluloses combined with their complicated structure make the material challenging for the microorganisms in the anaerobic digester to naturally break down. The yield of biogas is poor due to this partial or sluggish digestion. In order to decrease the biomass recalcitrance, and thereby increase the biogas yield, different pretreatment methods can be used (Hendriks and Zeeman, 2009). This is critical for a financially successful process, as it allows processes that would otherwise be economically impractical to be transformed into profitable ones. Pretreatments can have effects on the physicochemical properties of a substrate, such as molecular size, cellulose crystallinity, surface accessibility, pore size distribution and particle sizes (Hendriks and Zeeman, 2009). Some pretreatments have an impact on the chemical composition of the substrate, where lignin and/or hemicelluloses, to some extent,

get soluble, while others have no effect on the chemical composition of the substrate (Johnson and Elander, 2008).

2.6.9.1 Pretreatment Methods

Pretreatment methods can be divided into physical, thermal, chemical (with acids, alkalis or organic solvents), and biological methods (enzymatic or microbial) (Cheng and Sun, 2002). Pretreatment of lignocelluloses has been extensively researched, with a particular emphasis on pretreatment before ethanol production. Pretreatments of lignocelluloses before anaerobic digestion serve many of the same purposes as pretreatments before ethanol production, with the possible exception that pretreatments can be less intensive because anaerobic microorganisms can partially degrade hemicelluloses and crystalline cellulose structures on their own. The degradation of the lignin-polysaccharide linkages, together with a general opening of the structure are two of the main goals of the pretreatments prior to anaerobic digestion (Hendriks and Zeeman, 2009).

A pretreatment procedure is required to enhance the creation of biogas or to achieve enzymatic degradation in the enzymatic hydrolysis process used to produce ethanol. An effective and economical pretreatment should meet the following requirements: (a) production of reactive cellulosic fiber for enzymatic attack, (b) avoiding destruction of hemicelluloses and cellulose, (c) avoiding

formation of possible inhibitors for hydrolytic enzymes and fermenting microorganisms, (d) minimizing the energy demand, (e) reducing the cost of size reduction for feedstocks, (f) reducing the cost of material for construction of pretreatment reactors, (g) producing less residues, (h) consumption of little or no chemical and using a cheap chemical (Johnson and Elander, 2008; Cheng and Sun, 2002; Dale et al., 2005).

2.6.9.1.1 Physical Pretreatment

Physical pretreatment can increase the accessible surface area and size of pores, and decrease the crystallinity and degrees of polymerization of cellulose (Muller and Palmowski, 1999; Hendriks and Zeeman, 2009). To increase the enzymatic hydrolysis or biodegradability of lignocellulosic waste materials, a variety of physical processes can be employed, including ultrasound, milling (e.g., ball milling, two-roll milling, hammer milling, colloid milling, and vibro energy milling), and irradiation (e.g., by gamma rays, electron beams, or microwaves).

2.6.9.1.1.1 Ultrasound

A mechanical technique called ultrasound breaks down and destroys biomass, such as waste-water treatment sludge particles. The sludge's properties, energy level, time, and frequency all have an impact on how effective the ultrasound pretreatment is. The method breaks down the structure of the microbial cell and

removes the internal cellular components from the cells. Ultrasonication is used at full-scale sewage sludge plants, where an increase of 50% in biogas production has been found (Peng and Yeow, 2012). A combined pretreatment of alkaline and ultrasound on thickened pulp mill waste activated sludge did not improve the accumulated methane yield; however, the initial digestion rate was substantially improved (Park e al., 2012).

2.6.9.1.1.2 Milling

Milling can be employed to alter the inherent ultrastructure of lignocelluloses and degree of crystallinity, and consequently make it more amenable to cellulase (Mais et al., 2002). Milling and size reduction have been applied prior to enzymatic hydrolysis, or even other pretreatment processes with dilute acid, steam or ammonia, on several lignocellulosic waste materials, MSW and activated sludge (Zhang et al., 1999; Muller et al., 2007; Mais et al., 2002). The extruder, roller mill, cryogenic mill, and hammer mill are typically used for dry materials, whereas the colloid mill, fibrillator, and dissolver are only appropriate for wet materials, such as wet paper from home waste separation or paper pulps. The ball mill can be used for either dry or wet materials. Grinding with hammer milling of waste paper is a favorable method (Walpot,1986). Milling can improve susceptibility to enzymatic hydrolysis by reducing the size of the materials (Zeng et al., 2007), and degree of

crystallinity of lignocelluloses (Fan et al., 1980), which improves enzymatic degradation of these materials toward ethanol or biogas.

2.6.9.1.1.3 Irradiation

The enzymatic hydrolysis of lignocelluloses can be enhanced by irradiation, such as that produced by electron beams, microwaves, and gamma rays. The combination of the radiation and other methods such as acid treatment can further accelerate enzymatic hydrolysis (Kumakura and Kaetsu, 1983). Irradiation has accelerated the cellulose's enzymatic conversion to glucose. However, pre-irradiation is more effective in air than in acid solution (Hadjajdj and Mammar, 1990). The effects of irradiation pretreatment on bagasse before its enzymatic hydrolysis were investigated by Kumakura and Kaetsu. When compared to untreated bagasse, the hydrolysis of the prepared bagasse produced a twofold yield of glucose. The cellulose component of the lignocellulose materials can be degraded by irradiation to fragile fibers and low molecular weight oligosaccharides and even cellobiose (Kumakura and Kaetsu, 1983). It might result from irradiation-induced preferential dissociation of the glucoside linkages in the cellulose molecular chains when lignin is present. A very high irradiation, above 100 MR, can lead to the decomposition of oligosaccharides and the glucose ring structure (Kumakura and Kaetsu, 1983). There was no improvement in the enzymatic hydrolysis of filter paper without lignin after pretreatment with irradiation.

Furthermore, exposure to radiation marginally enhanced the enzymatic hydrolysis of newspapers containing modest levels of lignin. Therefore, the effect of radiation should be correlated with the presence of lignin as well as the structure such as crystallinity and density (Kumakura and Kaetsu, 1978, 1982, 1983). Yet, the industrial implementation of irradiation techniques presents challenges and is costly.

2.6.9.1.2 Chemical Pretreatments

2.6.9.1.2.1 Acidic Pretreatments

One of the lignocellulose pretreatment techniques that has been investigated the most is acidic pretreatment. Elevated temperatures have been used to carry out weak or strong acid pretreatments using substances like nitric acid, hydrochloric acid, or sulfuric acid. The combination with other methods, such as steam explosion has been performed (Galbe et al., 2013). Both lignin and hemicelluloses become soluble at higher acid concentrations; still, acid recovery is necessary. At dilute acid concentrations, lignin is not solubilized but redistributed; on the other hand, neutralization prior to anaerobic digestion is substantial in order to achieve the targeted neutral pH (Hendriks and Zeeman, 2009; Clarkson and Xiao, 1997; Sanchez and Cardona, 2008). Studies have been performed on newspaper, which was treated with nitric and acetic acid, and resulted in 80% lignin removal (Clarkson and Xiao, 1997) and rice straw has been pretreated with acetic and

propionic acid which enhanced the methane production by 36% compared with that of untreated rice straw (Zhao et al., 2010).

2.6.9.1.2.2 Alkaline Pretreatment

The term "alkali pretreatment" describes the process of removing lignin and a portion of the hemicellulose and effectively increasing the accessibility of enzyme to the cellulose by applying alkaline solutions like NaOH, $Ca(OH)_2$ (lime), or ammonia. The alkali pretreatment can result in a sharp increase in saccharification, with manifold yields (Kassim and El-Shahed, 1986). Low temperatures can be used for pretreatment as long as the base concentration is high and the process takes a while.

While retaining a high proportion of cellulose, alkaline pretreatments are excellent in removing lignin. Pretreatment with alkalis can cause swelling of the fibers which results in a bigger accessible surface area: it can decrease the crystallinity and degrade the linkages between lignin and carbohydrates as well as cause a disruption of the lignin structure (Mosier et al., 2005; Duret et al., 2003; Pang et al., 2008). Alkaline pretreatment studies have been performed on wheat straw which resulted in an increase of the methane production by 100% (Pavlostathis and Gossett, 1985). Newspaper has been treated with alkaline subcritical water, resulting in a methane increase of 36-45% (Fox et al., 2003). Rice straw has been

pretreated with sodium hydroxide 4-10%, with a following methane increase of 3-58% (Pang et al., 2009), and with a combination of 4% sodium hydroxide and a hydrothermal pretreatment the methane production increased with up to 112% (Chandra et al., 2012). The methane production from municipal solid waste after pretreatment with calcium hydroxide improved by 172% (Lopez and Espinosa, 2008). The cost of the catalyst is dependent on the amount used in the pretreatment, as well as on the purchase price where, for example, lime is cheaper than sodium hydroxide, together with the cost of recovery and reuse (Johnson and Elander, 2008).

2.6.9.1.2.3 Ozonolysis pretreatment

Pretreatment of lignocellulosic materials can be performed by treatment with ozone, referred to as “ozonolysis” pretreatment. This method can effectively degrade lignin and part of the hemicellulose. The pretreatment is usually carried out at room temperature, and does not lead to inhibitory compounds (Vidal and Molinier, 1988). However, because ozonolysis requires a lot of ozone, it could be costly. Particle size, ozone concentration in the gas flow, and sample moisture content are the three primary ozonolysis pretreatment parameters. The percentage of water in the feed is one of these criteria that is crucial and has the biggest impact on solubilization. It was discovered that the ideal water content was approximately 30%, which is the fibers' saturation point. This is an attractive pretreatment method

since it does not leave acidic, basic, or toxic residues in the treated material (Neely, 1984).

2.6.9.1.2.4 Wet oxidation

The manufacture of biogas and ethanol have both used wet oxidation as a pretreatment. In this process, the materials are treated with water and air or oxygen at temperatures above 120°C (e.g. 148- 200°C) for a period of e.g. 30 min (Palonen et al., 2004; Varga et al., 2004). The temperature, followed by reaction time and oxygen pressure, are the most important parameters in wet oxidation (Schmidt et al., 1998). The process is exothermic, and therefore it becomes self-supporting with respect to heat while the reaction is initiated (Schmidt et al., 1998). A balance between solubilization and degradation is achieved through the wet oxidation of the hemicellulose fraction. This process is an effective method in separating the cellulosic fraction from lignin and hemicellulose (Saha, 2003). Oxygen participates in the degradation reactions and allows operation at comparatively reduced temperatures by enhancing generation of organic acids. However, the control of reactor temperature is critical because of the fast rates of reaction and heat generation (Garrote et al., 1999). In wet oxidation pretreatment, oxidative reactions and the production of acids from hydrolytic processes are the principal reactions. This procedure affects the three lignocellulosic material fractions. There is significant cleavage of the hemicelluloses into monomeric sugars, oxidation and

cleavage of the lignins, and partial degradation of the cellulose. The cellulose becomes highly susceptible to enzymatic hydrolysis (Schultz et al., 1984).

2.6.9.1.3 Thermal pretreatments

Heat is applied to the lignocellulosic biomass during this pretreatment. If the temperature increases above 150–180^{0}C, parts of the lignocellulosic biomass, firstly the hemicelluloses and shortly after that lignin, will start to solubilize (Bobleter, 1994; Garrote et al., 1999). The hemicellulose's stability in terms of heat, acidity, and alkali is determined by the makeup of its branching groups and backbone. Xylan and glucomannan, the two main hemicellulose constituents, differ slightly in terms of thermal stability; still, xylan is the less stable of the two. Above 180^{0}C an exothermal reaction (probably solubilization) of the hemicellulose starts (Beall and Eickner, 1970; Domansky and Rendos, 1962). This temperature of 180^{0}C is probably just an indication of the temperature at which an exothermal reaction of the hemicellulose starts, because the thermal reactivity of lignocellulosic biomass depends largely on its composition (Fengel and Wegener, 1984; Hon and Shiraishi, 1991).

A portion of the hemicellulose is hydrolyzed and turns into acids during thermal operations. These acids are assumed to catalyze the further hydrolysis of the hemicellulose (Gregg and Saddler, 1996). Liu and Wyman (2003) and Zhu et al.

(2004, 2005) conclude that other, so far unknown, factors than the catalyzing effect of in situ formed acids play a role in the solubilization of hemicellulose.

In addition to solubilizing hemicellulose, thermal pretreatment at temperatures of 160°C and above also solubilizes lignin. The produced compounds are almost always phenolic compounds and have in many cases an inhibitory or toxic effect on bacteria, yeast and methanogens/archae (Gossett et al., 1982). These soluble lignin compounds are very reactive and will, if not removed quickly, re-condensate and precipitate on the biomass (Liu and Wyman, 2003). Especially too severe pretreatment conditions promote the condensation and precipitation of soluble lignin compounds, sometimes even with soluble hemicellulosic compounds like furfural and HMF (Bobleter and Concin, 1979; Lora and Wayman, 1978; Negro et al., 2003). Heat pretreatment in which soluble (hemi) cellulose and lignin compounds are formed, has a risk of formation of phenolic and heterocyclic compounds, like vanillin, vanillin alcohol, furfural and HMF, especially in acidic environments (Ramos, 2003). Temperatures of 250^0C and higher should be avoided during pretreatment, as unwanted pyrolysis reactions start to take place at such temperatures (Brownell et al., 1986).

Thermal pretreatment can be further improved by adding chemical pretreatment methods to it to further aid the breakdown of lignocelluloses. These processes include:

2.6.9.1.3.1 Thermal pretreatment in combination with acid pretreatment

By including an external acid, thermal steam or liquid hot water pretreatment can be made more effective. This addition of an external acid catalyzes the solubilization of the hemicellulose, lowers the optimal pretreatment temperature and gives a better enzymatic hydrolysable substrate (Brownell et al., 1986; Gregg and Saddler, 1996). Often, SO_2 or H_2SO_4 is soaked into the lignocellulose. The first 20 seconds of the steam pretreatment process convert SO_2 to H_2SO_4; the hemicellulose is then catalytically hydrolyzed. Another important point is that gradual removal of hemicellulose and lignin can trigger reorientation of cellulose to a more crystalline form (Gregg and Saddler, 1996). The latter is accurate for any pretreatment that progressively eliminates lignin and hemicellulose. Still unclear is the impact of the additional acid. Tengborg et al. (1998) showed a severe inhibition in the ethanol production step at a severity factor of 3 and higher with the addition of an external acid. This is in line with the conclusion of Grohmann et al. (1985) that during steam pretreatment at temperatures of 160^0C and higher with 0.5% sulfuric acid addition an appreciable production of furfural occurs.

2.6.9.1.3.2 Thermal pretreatment in combination with alkaline pretreatment

Adding an external alkali to the procedure in place of an acid is another method to enhance the thermal pretreatment. Lime pretreatment is a highly popular alkaline thermal pretreatment. This pretreatment is usually carried out at temperatures of 100–150^{0}C with lime addition of approximately 0.1 g $Ca(OH)_2$ g substrate 1 (Chang et al., 2001). Chang and Holtzapple (2000) attribute the effectiveness of lime pretreatment to the opening of the 'acetyl valve' and partly opening the 'lignin valve', making the substrate more accessible to hydrolysis. According to Kaar and Holtzapple (2000) lime pretreatment (with heating) is sufficient to increase the digestibility of low-lignin containing biomass, but not for high lignin containing biomass. Chang et al. (2001) mention that lime pretreatment of switchgrass and corn stover did not inhibit the enzymatic saccharification and fermentation steps. However, pretreated softwood was cleaned before the fermentation and enzymatic saccharification stages to avoid any potential inhibition caused by the high solubilized lignin content. A positive effect of lime is that it is relatively cheap and safe (Gandi et al., 1997) and that the calcium can be regained as insoluble calcium carbonate by the reaction with carbon dioxide. This calcium carbonate can be converted to lime again with the lime kiln technology (Chang et al., 1998). Fox et al. (2003) reported an improvement in methane

production with a factor of 3 to 4.5 after pretreating newsprint waste with alkaline heat pretreatment.

2.6.9.1.4 Physico-chemical pretreatment

Physicochemical processes are pretreatments that incorporate both physical and chemical processes. These consist of liquid hot-water pretreatment, ammonia fiber explosion (AFEX), CO_2 explosion, steam explosion (autohydrolysis), and steam explosion with the addition of SO_2.

2.6.9.1.4.1 Steam explosion (autohydrolysis)

Steaming with or without explosion (autohydrolysis), one of the physico-chemical processes, has drawn a lot of interest as a pretreatment for the manufacture of biogas and ethanol. By removing the majority of the hemicellulose, the pretreatment enhances enzymatic digestion. A quick drop in pressure causes the components to decompose explosively in a steam explosion. High pressure and consequently high temperature, typically between 160 and 260°C, for a few seconds (e.g. 30 s) to several minutes (e.g. 20 min), were used in steam explosion (Robinson et al., 1999; Varga et al., 2004; Ruiz et al., 2006). Numerous research groups and businesses have investigated the well-documented steam explosion method in lab and pilot settings. It meets all of the pretreatment process's requirements and has a comparatively low energy cost. It is important to choose

the steam explosion circumstances carefully to prevent the cellulose's chemical and physical qualities from being too severely degraded. Lower lignocellulose enzymatic digestibility following a steam explosion may also be noticed under extremely severe conditions. For instance, generation of condensation substances between the polymers in steam explosion of wheat straw may lead to a more recalcitrant residue (Sun et al., 2005).

2.6.9.1.4.2 Steam explosion with addition of SO_2

Sulfur dioxide (SO2) can be added to steam pretreatment with the intention of better recovering the cellulose and hemicellulose fractions together. The treatment can be carried out by 1-4% SO_2 (w/w substrate) at elevated temperatures, e.g. 160-230°C, for a period of e.g. 10 min (Eklund et al., 1995). In order to extract both cellulose and hemicellulose, Eklund et al. (1995) investigated steam pretreatment of willow with the addition of SO_2 or H_2SO_4. When the willow was treated with 1% SO_2 at 200°C, the maximum glucose yield of 95% was achieved. But compared to pretreatment with diluted sulfuric acid, the yield of xylose recovery by SO_2 was lower.

2.6.9.1.4.3 Ammonia fiber explosion (AFEX)

Among the alkaline physico-chemical pretreatment methods is AFEX. The biomass is subjected to liquid ammonia at a reasonably high temperature (90-

100°C) for a duration of 30 minutes, after which the pressure is immediately reduced. The effective parameters in the AFEX process are ammonia loading, temperature, water loading, blowdown pressure, time, and number of treatments (Jun et al., 1991). The AFEX process produces only a pretreated solid material, while some other pretreatments such as steam explosion produce a slurry that can be separated in solid and liquid fractions (Mosier et al., 2005). The lignin proportion of lignocellulosic materials can be efficiently reduced or modified by the AFEX process, while the hemicellulose and cellulose fractions may stay intact. AFEX has the potential to greatly enhance enzymatic hydrolysis under optimum conditions. The lignocellulosic materials determine the optimum conditions for AFEX. For example, the optimum conditions in pretreatment of switchgrass were reported to be about 100°C, ammonia loading of 1:1 kg of ammonia per kg of dry matter, and 5 min retention time (Gilbert et al., 2005). The absence of some inhibitory by-products formed by conventional pretreatment techniques, such as furans in diluted acid and steam explosion pretreatment, is one of the main benefits of AFEX pretreatment. On the cellulose surface, however, phenolic particles of lignin and other extractives from cell walls might still be present in part. Therefore, washing with water might be necessary to remove part of these inhibitory components, although increasing the amount of wastewater from the process (Dale et al., 2007) However, there are some disadvantages in using the AFEX process

compared to some other processes. When compared to other pretreatment methods like diluted-acid pretreatment, AFEX is more efficient on biomass with lower lignin content and does not considerably solubilize hemicellulose. Furthermore, ammonia must be recycled after the pretreatment to reduce the cost and protect the environment (Sun et al., 2002; Eggeman and Elander., 2005).

2.6.9.1.4.4 CO2 explosion

Supercritical carbon dioxide has been considered as an extraction solvent for non-extractive purposes, due to several advantages such as availability at relatively low cost, non-toxicity, non-flammability, easy recovery after extraction, and environmental acceptability (Zheng and Tsao, 1996). Supercritical carbon dioxide displays gas-like mass transfer properties, besides a liquid-like solvating power (Lin et al, 1995).

Explosion pretreatments of the cellulosic materials by supercritical carbon dioxide were studied by Zheng *et al*. (Lin et al, 1995). The breakdown of the cellulose structure following an abrupt release of the carbon dioxide pressure should increase the substrate's available surface area for enzymatic hydrolysis. An essential component of cellulose hydrolysis is temperature. Either supercritical or subcritical temperature (that is, above or below 31.1°C) can be used for the tests. Experimental results indicated that subcritical carbon dioxide is less effective than

supercritical (Lin et al, 1995). Low diffusion in liquid carbon dioxide is probably one of the causes of this retardation in subcritical carbon dioxide. Subcritical temperatures make it more difficult for carbon dioxide molecules to enter the pores in cellulosic structures than supercritical temperatures do. This makes the pores more susceptible to disruption when the carbon dioxide pressure is abruptly released. The higher pressure of carbon dioxide resulted in the higher glucose yield, which indicates that higher pressure is desirable for faster penetration of the carbon dioxide molecules into the cellulosic pores (Lin et al, 1995). The supercritical CO_2 technique may be too costly for industrial use even with these benefits.

2.6.9.1.4.5 Liquid hot-water pretreatment

For many years, the pulp industries have treated lignocelluloses using liquid hot water as a pretreatment. Thermal pretreatments, both without pressure and under high pressure, have shown to solubilize both hemicellulose and lignin (Taherzadeh and Karimi, 2008; Bobleter, 1994). A major advantage is that in strict thermal pretreatments, there is no need for the addition of chemicals (Taherzadeh and Karimi, 2008). A drawback with pretreatments at high temperatures, however, is the possible production of phenolic compounds as well as furfural and hydroxymethylfurfural (HMF), which are degradation products of lignin as well as sugars, respectively (Hendriks and Zeeman, 2009, Owen et al., 1982). The phenolic compound can be toxic to the microorganisms in the anaerobic digester,

depending on the concentration (Hernandez and Edyvean, 2008), while they can tolerate furfural and HMF to a higher extent. It was reported that hydrothermal pretreatment of rice straw resulted in 222% higher methane yield in a pretreatment study (Chandra et al., 2012).

2.6.9.1.5 Biological pretreatments

Enhancement of digestion in biogas generation is being explored by the use of microbes or enzymes in biological treatments. In addition to removing lignin, the biological pretreatment may also be utilized to remove certain components, such antimicrobial compounds. Biological pretreatments majorly involve the use of enzymes and fungi. Moreover, microorganisms can be employed to improve enzymatic hydrolysis and treat lignocelluloses. Applications of microorganisms often break down lignin and hemicellulose, but very little cellulose, because cellulose is more resistant to biological attack than the other components of lignocelluloses.

2.6.9.1.5.1 Fungi

Fungi that degrade lignin have primarily been used in biological pretreatments. White-rot fungi are a class of basidiomycetes that are known to first break down lignin while preserving the majority of hemicellulose and cellulose. These fungi release laccase, manganese peroxidase, and lignin peroxidase, among other

ligninolytic enzymes. The biomass is pretreated by inoculating it with the fungi at room temperature and letting it sit for a few weeks. These biological pretreatment techniques have the advantages of requiring no chemicals and consuming little energy. However, there are disadvantages as well. For example, during the lengthy incubation period, some cellulose and hemicelluloses are broken down alongside the lignin. White-rot and brown-rot fungi were used as a pretreatment on rice straw, and as a result, methane production rose by 46% and 31%, respectively. In a different investigation, white-rot fungus eliminated phenolic chemicals from olive mill wastewater, which enhanced anaerobic digestion.

2.6.9.1.5.2 Enzymes

Ligninolytic enzymes have been used as a pretreatment technique before anaerobic digestion in very few investigations. Utilized enzymes from white-rot fungus include laccase, manganese peroxidase, and lignin peroxidase. These ligninolytic enzymes are too large to act directly on lignin; instead, they use mediators, which are reactive low molecular weight compounds that attack and break down lignin. The anaerobic digestion of the digested biofibers that followed was positively impacted by the laccase pretreatment and steam explosion. Nevertheless, pretreating the biofibers only with laccase did not result in any improvement.

CHAPTER THREE

MATERIALS AND METHODOLOGY

This chapter of the report presents the materials and methods adopted in the experiment

3.1 MATERIALS USED

The materials and chemicals used in the experiment are shown in the tables Table 3.1 and 3.2 respectively below.

Table 3.1: Table showing materials and equipment used in this study

Materials	Source/Make	Uses
Blender	Obtained from Ecotoxicology laboratory, University of Benin	To blend the cattle rumen contents into smaller sizes
Conical flask	Pyrex	For titrating samples
Round bottom flask	Pyrex	To prepare standard solutions of NaOH and $Ca(OH)_2$
Funnel	Obtained from the laboratory store	To pour the CRC samples into the beakers To pour the acid into the burette
Beaker	Pyrex	For holding the CRC samples
Retort stand	Obtained from the laboratory store	This is used to hold the reflux condenser
10ml measuring cylinder	Pyrex	To measure the volume of NaOH and $Ca(OH)_2$ added to the samples
Gas burner	Obtained from the laboratory, University of Benin	To produce heat for refluxing.
Filter paper	Obtained from commercial shop	For separating the filtrate from the residue
Aluminum foil	Obtained from commercial shop	To ensure the samples are not contaminated from the

		environment
Refrigerator	Haier Thermocool	To store the blended CRC samples
Reflux condenser	Obtained from laboratory store	To ensure homogeneity of the mixture
Thermostatic water cabinet	B-scientific England (Model No. HH-W420)	To heat the samples at specific temperatures over a given time
Burette	Obtained from laboratory store	For titration
Dropper	Obtained from laboratory store	For measuring volumes of reagents
Analytical balance	Metla	To determine experimental weight of samples.
Laboratory oven	Sanfa (Model No.DHG9101.ISA)	For heating at high temperature.
Desiccator	Obtained from laboratory shop	To cool hot samples.
Soxhlet extractor	Quickfit manufacturer	To extract lipid.
Distillation system	Made from glass ware components	To ammonia of crude protein determination.
Fume cupboard	Biobase	To digest samples for crude protein determination without the evolution of poisonous gases

Table 3.2: Table showing chemicals and reagents used in this study

Chemical used	Source/Make	Uses
Potassium dichromate, $K_2Cr_2O_7$	Obtained from laboratory store	Used as oxidizing agent
Perchloric acid, $HClO_4$	Obtained from laboratory store	Used for COD determination of samples.
Mercury sulphate, $HgSO_4$	Obtained from laboratory store	Used for COD determination of samples.
Distilled water	Obtained from laboratory store	Used for COD determination of samples.
Ferroin Indicator	Obtained from laboratory store	Used as indicator for titration
Sodium hydroxide, NaOH	Obtained from laboratory store	Used for alkali pretreatment of the CRC samples
Ferrous ammonium sulphate	Obtained from laboratory store	Used for COD determination of samples.
Sulphuric acid, H_2SO_4	Purchased from Rovet scientific	To determine crude fiber content.
Petroleum ether	Purchased from Rovet scientific	Extraction to determine crude lipid content.
Boric acid	Purchased from Rovet scientific	To determine crude protein content.
Sodium thiosulphate	Purchased from Rovet scientific	To titrate so as to find crude protein content

3.2 METHODS

3.2.1 Sample Collection and Preparation

Fresh cattle rumen content (CRC) was collected from an abattoir at Vegetable Market, Benin City, Edo state, Nigeria. The CRC was then blended with sufficient water so as to mechanically reduce the substrate particle size thereby producing a slurry substrate.

3.2.2 Determination of Physiochemical Properties of CRC

The CRC was characterized according to its content as stated in the following;

3.2.2.1 Moisture Content Determination

Procedure:

a. Weigh a clean dried crucible.
b. Weigh sample into crucible and take weight of both.
c. Put crucible in laboratory oven at a temperature of 105^0C for 2 hours.
d. Remove the crucible from the oven and place in the desiccator.
e. Remove from the desiccator after cooling and take weight, then return to the oven and heat until a constant weight is achieved.

The moisture content is calculated as

$$Percentage\ (\%)\ moisture = \frac{weight\ of\ crucible\ and\ fresh\ sample - weight\ of\ crucible\ and\ dry\ sample}{weight\ of\ fresh\ sample} \times 100\% \quad (3.1)$$

3.2.2.2 Ash Content Determination

Procedure:

a. Weigh a known amount of sample into a tared crucible.

b. Place a crucible in a muffle furnace.

c. Ignite for 4 hours at temperature 550^0C.

d. Turn off muffle furnace and allow cooling to about 250^0C.

e. Transfer crucible carefully with a tong into a desiccator and allow to cool.

f. Take the weight of the crucible and ashed sample.

The ash content is calculated as

$$Percentage(\%)\ ash = \frac{weight\ after\ ashing\ and\ crucible - weight\ of\ tared\ crucible}{weight\ of\ fresh\ sample} \times 100\% \quad (3.2)$$

3.2.2.3 Crude Lipid Content Determination

Procedure:

a. Weigh dried timble and add sample into the timble.
b. Set up the soxhlet extractor and fill the round bottom flask with petroleum ether.
c. Heat the solvent and allow maximum extraction.
d. Detach the setup and dry the flask containing the fat residue in air oven at 100^0C for 5 minutes or on a water bath. Cool in desiccator and weigh.
e. Place timble in a baker and put in the oven and allow drying to a constant weight at temperature of 50^0C.

$$Percentage\ (\%)lipid = \frac{weight\ pof\ beaker\ and\ lipid - weight\ of\ beaker}{weight\ of\ sample\ extracted} \times 100\% \quad (3.3)$$

3.2.2.4 Crude Fibre Content Determination

Procedure:

a. Weigh defatted sample into a conical flask.
b. Add solution of sulphuric acid and heat for about one hour.

c. Remove from heating mantle and filter, thereafter wash residue until no trace of acid.

d. Transfer back to a conical flask and solution of 1.25% sodium hydroxide and heat for about one hour.

e. Filter and wash with distilled water until no trace of the solution.

f. Serve the residue into crucible after drain, dry in oven at 105^0c, cool in desiccator and weigh.

g. Place in muffle furnace at 300^0C for 30 minutes.

h. Remove into desiccator and cool to room temperature, weigh again.

$$Percentage(\%)\ crude\ fibre = \frac{weight\ of\ crucible\ plus\ dried\ sample - (weight\ of\ crucible + weight\ of\ ash)}{weight\ of\ defatted\ sample} \times 100\% \quad (3.4)$$

3.2.2.5 Crude Protein Content Determination

Procedure:

a. Weigh known mass of sample into a conical flask.
b. Add 10ml concentrated sulphuric acid, catalyst and heat until digestion is complete.
c. Cool digest at room temperature, filter and make up volume to 100ml in a volumetric flask.
d. Measure 10ml of the digest into a distillation flask and place the flask on the heater.
e. 5ml of sodium hydroxide was added to the distillation flask and another flask which is the receiving flask has in it 10ml of boric acid and 3 drops of bromocresol indicator.
f. When colour change is observed in the receiving flask, the setup is dismantled.
g. Titrate the distillate with 0.1N sodium thiosulphate solution; the volume of solution used is recorded.

3.2.2.6 Carbohydrate (Nitrogen-Free Extract) Determination

Nitrogen-free extract represents the non-structural carbohydrates. It was obtained by the difference of the total weight of the content (obtained analytically) from the original weight of the sample.

3.2.3 Experimental Design

Thermo-alkaline disintegration of the sludge was done to find out how temperature, NaOH concentration, and retention time affected the sludge's organic contents' swelling and eventual rupture of their cell walls, which improved biodegradation and increased biogas generation. A percentage of the solubilized organic materials was used to gauge the degree of breakdown. In this instance, measurements were taken of the sludge's soluble COD both before and after the disintegration procedure. To prevent unneeded evaporation, the experiment was carried out in a beaker and covered with aluminum foil. The beaker was then placed in a thermostatic water cabinet. A 100ml sample of the sludge was heated to 70, 80, and 90 degrees Celsius, and then combined with 5ml of NaOH at concentrations of 1, 2, and 3M. The various runs were done with retention times of 10, 20, and 30 minutes. To guarantee homogeneity, the substance was extensively mixed.

The following formula provides the degree of disintegration (DD), which is stated as a percentage:

$$DD\% = \frac{SCOD_t - SCOD_r}{SCOD_{max} - SCOD_r} \times 100$$

The soluble COD of the treated (disintegrated) sludge is known as the SCODt. After centrifuging the sample at 5000 rpm for 10 minutes, the quantity of COD that is accessible to the microorganisms is known as the soluble COD, which is the COD that passes through a 0.45µm membrane filter. SCODr is the raw sludge soluble COD, and SCODmax is the maximum soluble COD of the sludge that is obtained by treating the sludge with a 1 M NaOH for 22 h. Total and soluble COD were determined by colorimetry using a HACHDR 890 colorimeter and a COD reactor (Hach, Loveland, CO, USA). (Muhammad et al., 2012).

Response surface methodology (RSM) was used to optimize the thermochemical factors taken into consideration in order to obtain the best biogas yield through the use of sludge disintegration.

3.2.4 Chemical Oxygen Demand (COD) Determination

One quick method to determine how much oxygen is needed to oxidize the organic matter in water and wastewater is to utilize the chemical oxygen demand (COD) measurement. Refluxing most organic molecules in a strong acid solution containing a known amount of a potent oxidizing agent, such potassium dichromate, will destroy them. Measured is the surplus potassium dichromate that remains after the organic substance has been broken down. The quantity of

chemically oxidizable organic matter is directly correlated with the amount of potassium dichromate ingested.

Using orthophenanthroline ferrous complex, or ferroin, as an indicator, the quantity of potassium dichromate remaining after the organic matter has been digested is titrated using a standard ferrous ammonium sulfate (FAS) solution. According to oxygen equivalent measurements, the amount of oxidizable organic matter is directly correlated with the potassium dichromate used in the oxidative reaction.

The CODs were determined via open reflux method (APHA Method 5220 B). The following formula can be used to determine the COD value

$$\frac{mg}{l} of\ COD = \frac{(A - B) \times C\ \times 16000}{ml\ of\ sample}$$

A = mL FAS used for blank

B = mL FAS used for sample

C = Normality of FAS

16000 = milliequivalent weight of oxygen × 1,000 mL/L

3.2.5 Experimental Setup

3.2.5.1 Sample Preparation:

a. The CRC was blended with sufficient water so as to mechanically reduce the substrate particle size thereby producing a slurry substrate.
b. 100ml of the slurry is poured into conical flasks and labeled for each thermo-chemical variable considered.
c. 5ml of NaOH at different concentrations of 1M, 2M and 3M was added to the respective labeled samples and stirred thoroughly to ensure homogeneity.
d. 100ml of the slurry is poured into a conical flask and labeled blank to which no alkali or heat treatment is added.
e. The conical flasks are then covered with aluminum foil so as to avoid evaporation while heating.
f. The labeled samples are put into the thermostatic water cabinet at each desired temperature and retention time respectively.
g. After heating at desired temperatures and retention times in the cabinet, the samples are then taken out of the thermostatic water cabinet and allowed to cool.
h. After cooling, shake the samples thoroughly and with the aid of a 0.45µm membrane filter, filter the sample solution.
i. The blank is also filtered.

j. The filtrates are then taken for titration.

3.2.5.2 Titration and Preparation of Reagent:

- **Reagents**

a. Standard potassium dichromate solution, $K_2Cr_2O_7$

b. Perchloric acid, $HClO_4$

c. Mercuric sulphate: powdered $HgSO_4$

d. Distilled water

e. Standard ferrous ammonium sulphate (FAS), 0.625N:

   i. Dissolve 245g of $Fe(NH_4)_2(SO_4)_2 . 6H_2O$ in distilled water

   ii. Add 20ml of conc. H_2SO_4 of specific gravity 1.84

   iii. Dilute to 1 litre in a standard bottle.

f. Orthophenanthroline ferrous sulphate (ferroin) indicator solution;

   i. Dissolve 1.48g of 1-10 (ortho) phenanthroline monohydrate together with 0.70g of Iron (II) tetraoxosulphate (VI) heptahydrate, $FeSO_4.7H_2O$ in 100ml of water.

- **Titration**

a. With the aid of a dropper, measure 0.5ml of the filtrate into a conical flask.

b. Add 4ml of distilled water and 2ml of $K_2Cr_2O_7$.

c. Add 1ml of perchloric acid, $HClO_4$.

d. Add a pinch of powdered mercury sulphate, $HgSO_4$.

e. Gently swirl the solution thoroughly to ensure proper mixing before heat is applied so as to avoid local heating at the bottom of the flask.
f. Attach the conical flask to the reflux condenser and apply heat to ensure proper mixing and homogeneity.
g. Heat for 3-5 minutes and then allow to cool.
h. Add two drops of ferroin indicator.
i. Titrate with standard ferrous ammonium sulphate. The colour change is sharp, going from blue-green to reddish-brown and should be taken as the end point colour change although the blue-green colour may reappear within minutes.
j. Run a blank for the sample which hasn't undergone thermo-alkaline pretreatment.

CHAPTER FOUR

RESULT AND DISCUSION

This chapter reports the result and discussion on the optimization of thermo-alkaline pretreatment on biogas yield from cattle rumen using NaOH and the physio-chemical characterization of CRC. The experimental data was analyzed using Microsoft Excel.

4.1 PHYSIO-CHEMICAL CHARACTERIZATION OF CATTLE RUMEN CONTENT (CRC)

Quantitative analysis was carried out on samples of CRC and certain parameters were determined as shown in the table 4.1 below:

Table 4.1: Physio-chemical characterization of CRC

Parameter	Percentage (%) (g/100g)
Moisture	64.10
Dry matter/ Total solids	35.90
Crude fat	3.95
Ash content	2.51
Crude fiber	3.24
Carbohydrate/ Nitrogen-free extract (NFE)	14.89
Total nitrogen (TN)	11.31

4.2 CHEMICAL OXYGEN DEMAND (COD) FOR THERMO-ALKALINE PRETREATMENT

For blank, volume of FAS used = 18.7ml

Table 4.2: Showing COD of samples run at different alkali concentration and retention time at 70^{o}C

Sample	Time (mins)	Final reading (ml)	Initial reading (ml)	Volume of FAS used (ml)	mg/l of COD
1M NaOH	20	13.00	12.10	0.90	35,600
2M NaOH	10	13.90	13.00	0.90	35,600
2M NaOH	30	37.00	35.60	1.40	34,600
3M NaOH	20	23.50	19.80	3.70	30,000
Control	10	35.60	23.50	12.10	13,200
Control	20	8.10	0.00	8.10	21,200
control	30	12.10	11.50	0.60	36,200

Table 4.3 showing COD of samples run at different alkali concentration and retention time at 80^{o}c

Sample	Time (mins)	Final reading (ml)	Initial reading (ml)	Volume of FAS used (ml)	mg/l of COD
IM NaOH	10	22.70	22.30	0.40	36,600
1M NaOH	30	24.20	23.50	0.70	36,000
2M NaOH	20	25.30	24.80	0.50	36,400
3M NaOH	10	26.50	26.00	0.50	36,400
3M NaOH	30	27.60	27.10	0.50	36,400
Control	10	28.50	28.00	0.50	36,400
Control	20	30.00	29.10	0.90	35,600
Control	30	29.10	28.50	0.60	36,200

Table 4.4 showing COD of samples run at different alkali concentration and retention time at 90^{o}C

Sample	Time (mins)	Final reading (ml)	Initial reading (ml)	Volume of FAS used (ml)	mg/l of COD
1M NaOH	20	20.30	19.50	0.80	35,800
2M NaOH	10	18.30	17.10	1.20	35,000
2M NaOH	30	21.90	21.00	0.90	35,600
3M NaOH	20	17.10	16.30	0.80	35,800
Control	10	21.00	20.30	0.70	36,000
Control	20	19.50	19.00	0.50	36,400
Control	30	22.30	21.90	0.40	36,600

4.3 EXPERIMENTAL DESIGN VARIABLES FOR OPTIMUM ALKALINE DEGRADATION USING RSM

The data was subjected to multiple regression analysis and analysis of variance (ANOVA) using the RSM program. The overall model significance was ascertained using Fisher's test F and its corresponding probability P, and the fit of the regression model was verified using the coefficient of determination R^2. Using response surface plots, pareto charts, and the p-test, the various independent factors' significance on the dependent variable was examined. Multiple regression analysis was used to get the model coefficients, and the model equation was solved to find the ideal operational variables.

Using Box Behnken Design (BBD) to create a matrix, Design Expert Version 13 was used to optimize the experimental variables. The above-mentioned comprehensive experimental plan and produced Box Behnken design matrix were also taken into consideration when examining the effects of the three independent variables. The design was implemented using six duplicates of the axial and face centers, yielding a standard run of eighteen runs. To find out how different factors affected the ideal pretreatment conditions, the experimental matrix was employed.

Table 4.5 showing the experimental variables for optimum alkaline degradation using Response Surface Methodology (RSM).

Independent variables	Symbol		Coded levels		
	Coded	Uncoded	-1	0	1
Temperature (°C)	X1	Z1	70	80	90
Molar concentration (M)	X2	Z2	1	2	3
Time (min)	X3	Z3	10	20	30

Table 4.6: Experimental Results of RSM using NaOH

Runs	Temperature (◦C)	Time (Min)	Molar Conc. (M)	Treated SCOD (mg/l)	Degree of degradation (%)
1	80	10	3	31000	44.44
2	80	20	2	30000	33.33
3	90	30	2	35600	93.55
4	70	20	3	33000	60.53
5	80	20	2	30000	33.33
6	90	10	2	35000	83.87
7	80	30	1	29500	27.78
8	80	10	1	29800	31.11
9	80	20	2	30000	33.33
10	70	30	2	33300	64.47
11	80	20	2	30000	33.33
12	90	20	1	35800	96.77
13	90	20	3	35500	91.94
14	70	20	1	32300	51.32
15	80	20	2	30000	33.33
16	80	30	3	32000	55.56
17	70	10	2	32200	50
18	80	20	2	30000	33.33

The optimized parameters have been studied using Box Behnken and the calculations involve changing the selected parameter, testing and verifying the design model obtained in the response analysis. Using multiple regression analysis and ANOVA analysis of the experimental data, a second-order or quadratic model was generated to explain and represent the optimal substrate pretreatment conditions. Using multiple experimental regression analysis, polynomial equations were derived to describe the optimal conditions for substrate pretreatment (rumen content of livestock).

The encoded factor equation can be used to predict responses to specific levels of each factor. By default, high factor levels are coded as +1 and low levels as -1. The coded equation is useful for identifying the relative influence of factors by comparing factor coefficients.

The polynomial expression of NaOH is given by:

$$Y=33.33+17.48A+3.99B+5.69C -1.20AB-3.51AC+3.61BC+37.53A^2+2.11B^2+4.28C^2$$

Relationship between Molar Concentration And Time for NaOH

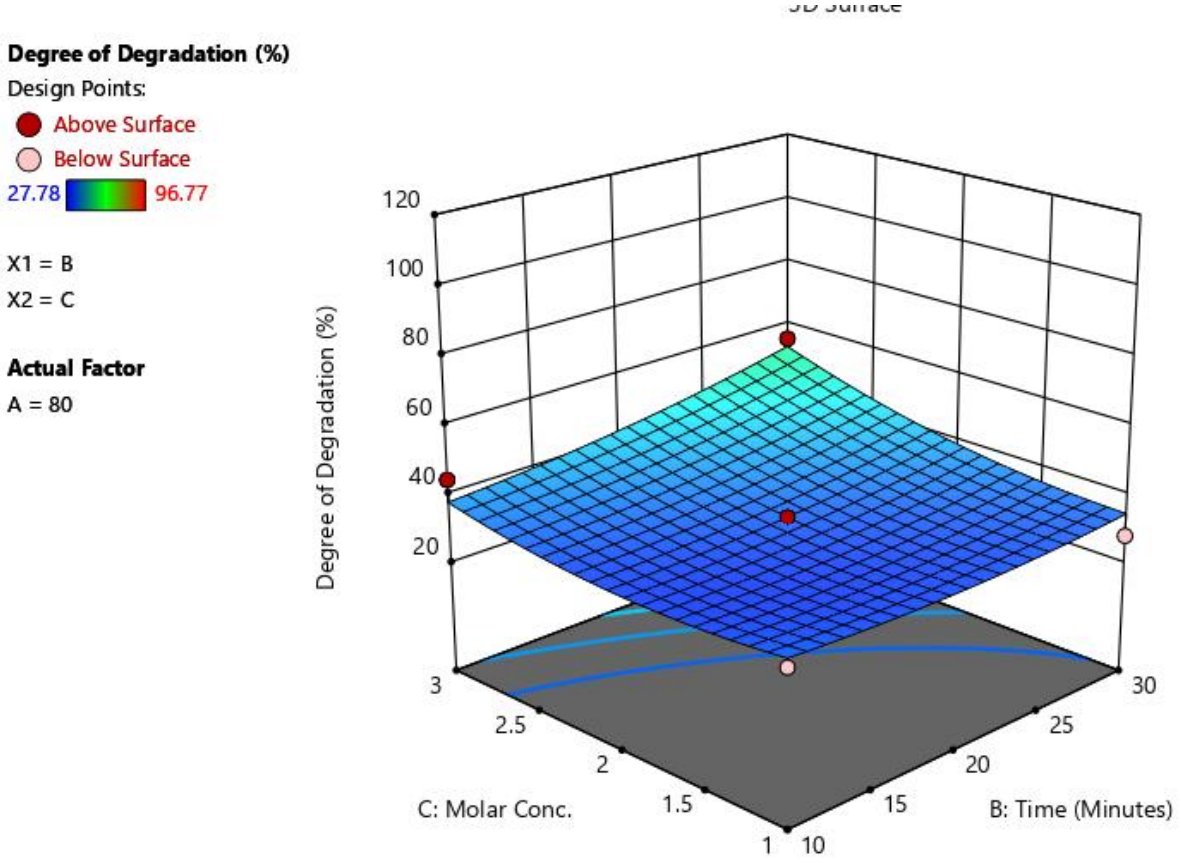

Fig 4.1 Relationship between Molar Concetration and Time For NaOH

Response 2: Degree of Degradation

Table 4.7 Fit Summary

Source	Sequential p-value	Adjusted R^2	Predicted R^2	
Linear	0.1745	0.1383	-0.2481	
2FI	0.9808	-0.0797	-1.6122	
Quadratic	**<0.0001**	**0.9507**	**0.6285**	**Suggested**
Cubic		1.000		Aliased

Table 4.8: Fit Statistics of NaOH

Std. Dev.	5.32
Mean	52.85
C.V.	10.06
R^2	0.9768
Adjusted R^2	0.9507
Predicted R^2	0.6285
Adeq Precision	15.5049

3D Representation for NaOH

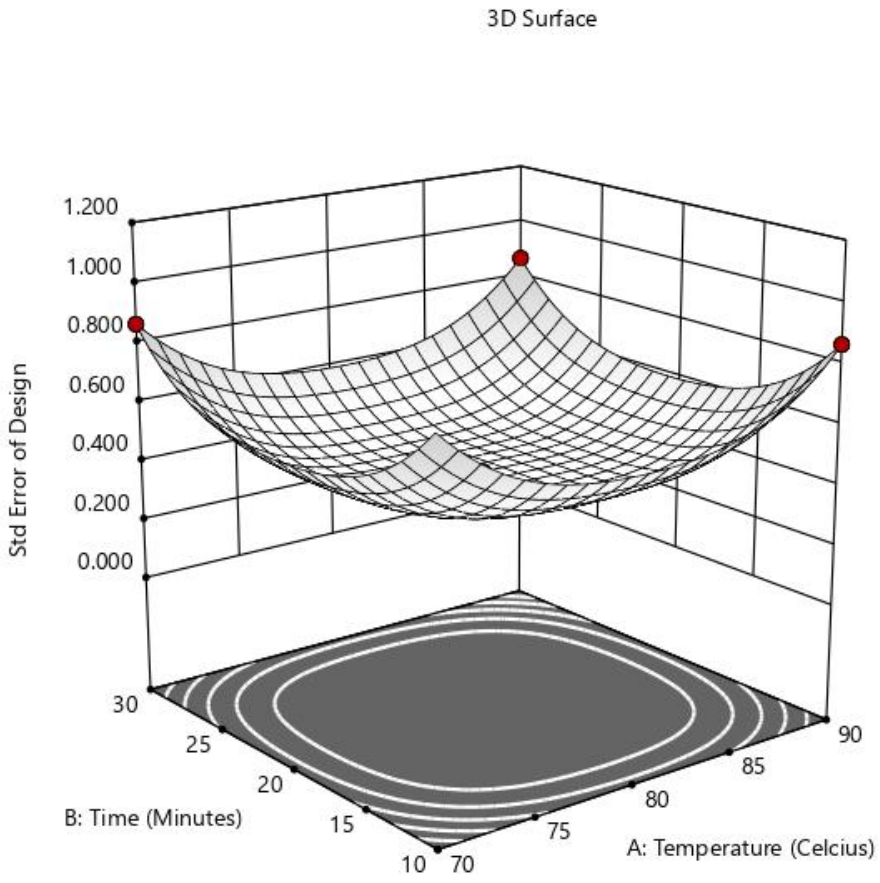

Fig 4.2 3D Representation for NaOH

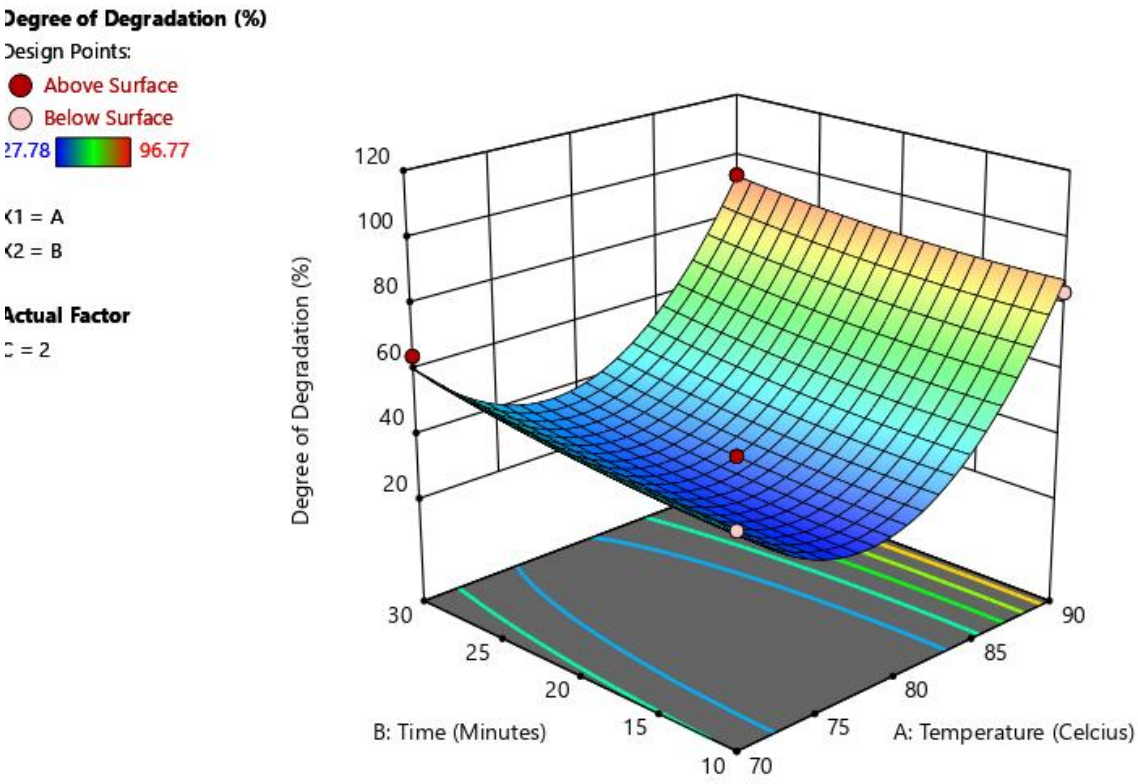

Fig 4.3 Relationship between Temperature and Time for NaOH

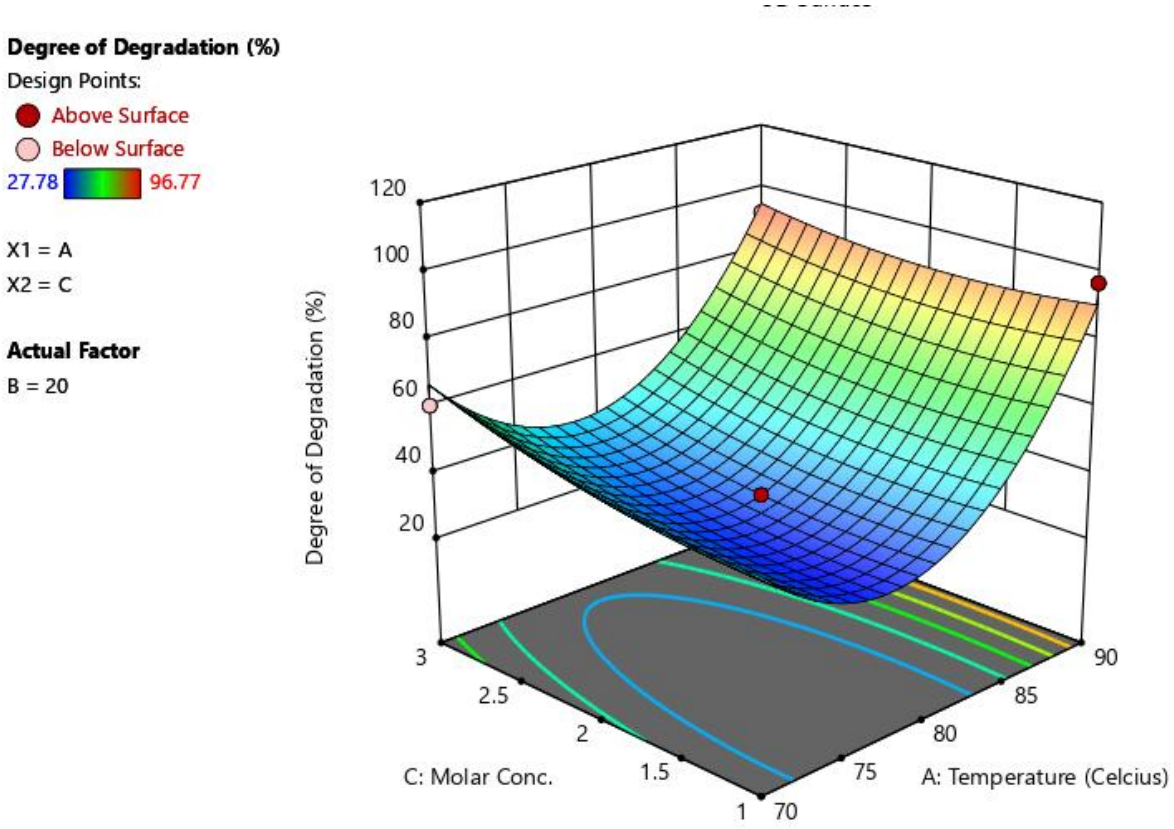

Fig 4.4 Relationship between Molar Concentration and Temperature in NaOH

4.4 ANOVA Analysis of NaOH

Response 2: Degree of degradation

Table 4.9 ANOVA Analysis of NaOH

SOURCE	SUM OF SQUARES	Df	Mean Square	F-value	p-value	
Model	9519.30	9	1057.70	37.39	<0.0001	Significant
A-Temperature	2443.35	1	2443.35	86.38	<0.0001	
B-Time	127.52	1	127.52	4.51	0.0665	
C-Molar Conc.	258.67	1	258.67	9.15	0.0165	
AB	5.74	1	5.74	0.2028	0.6644	
AC	49.28	1	49.28	1.74	0.2234	
BC	52.20	1	52.20	1.85	0.2114	
A^2	6146.19	1	6146.19	217.30	<0.0001	
B^2	19.47	1	19.47	0.6885	0.4307	
C^2	79.93	1	79.93	2.83	0.1313	
Residual	226.28	8	28.28			
Lack of Fit	226.28	3	75.43			
Pure Error	0.0000	5	0.0000			
Cor Total	9745.58	17				

Table 4.10 Optimum condition using NaOH

Number	Temperature	Time	Molar Conc.	Treated SCOD	Degree of Degradation	Desirability	
1	**80.171**	**13.086**	**2.505**	**30250.062**	**56.863**	**1.000**	**Selected**
2	70.000	30.000	2.000	31712.500	48.572	1.000	
3	80.000	10.000	1.000	27475.000	25.426	1.000	
4	90.000	10.000	2.000	32787.500	75.888	1.000	
5	80.000	30.000	1.000	28700.000	38.834	1.000	
6	90.000	30.000	2.000	33662.500	91.000	1.000	
7	90.000	20.00	3.000	32112.50	62.426	1.000	

		0		0			
8	70.000	20.000	3.000	31762.500	49.574	1.000	
9	80.000	10.000	3.000	30000.000	53.726	1.000	
10	70.000	10.000	2.000	32937.500	66.660	1.000	

OPTIMIZATION OF THERMO-ALKALINE PRETREATMENT USING NAOH

During the course of the experiment, the optimum thermos-alkaline pretreatment condition for using sodium hydroxide (NaOH) was found to be at temperature 80.171^{0}C for a duration of 13.086minutes and a molar concentration of 2.505M.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 CONCLUSION

The conclusion from this research work which was aimed at the optimization of thermo-alkaline pretreatment of cattle rumen content using sodium hydroxide (NaOH)

The usage of alkali pretreatment helps to make the constituents of cattle rumen susceptible for hydrolysis. During the course of this research work, the alkaline pretreatment was carried out using sodium hydroxide and the optimum pretreatment condition using NaOH was found to be at Temperature 80.171^{o}C, Time 13.086 minutes and Molar concentration 2.505M and the degree of degradation of 56.863%.

Thus, the use of the optimum pretreatment condition of NaOH made the cattle rumen more susceptible for hydrolysis and of other enzymatic activities which will in turn improve the quantity of biogas yield. The results of the experiment show:

1. The R^2 value of 0.9768 for NaOH, contour plots, ANOVA analysis all shows how suitable the RSM model is for the experiment.
2. The optimum conditions necessary for maximum feedstock degradation for the alkaline was examined and it was found by using NaOH at a

Temperature of 80.171^{0}C, Time 13.086 minutes and Molar concentration 2.505 M and the degree of degradation is 56.863%.

5.2 CONTRIBUTION TO KNOWLEDGE

The result of the experiment shows that thermo-alkaline pretreatment of cattle rumen content with NaOH gives a higher level of degree of degradation which increases the biogas yield. It can serve as reference material for further projects and researches in this area of biogas production.

5.3 FURTHER SCOPE OF STUDY

The study can be furthered by the use of other alkalis such as calcium hydroxide, potassium hydroxide and ammonium hydroxide for the pretreatment of cattle rumen content in order to get best suited alkali for the digestibility of the biomass feedstock.

5.4 LIMITATIONS

Limitation encountered during the course of this study resulting from methodology is inadequate power supply. This project was dependent on electricity supply which was required to power the thermostatic water cabinet. The thermostatic water cabinet is used to raise the cattle rumen contents to required temperatures for a particular period of time. Interruption of power supply would lead to such runs to be restarted and subsequently lead to wastage of materials.

This limitation can be overcome by primarily equipping the laboratory with sufficient backup power supply for example generators and solar panels.

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