THE EFFECTS OF PRETREATMENT ON BIOGAS PRODUCTION FROM MAIZE STRAW AND CATTLE MANURE

MISIR ARTIKLARI VE SIĞIR GÜBRESİNDEN BİYOGAZ ELDESİNDE ÖN ARITIMIN ETKİSİ

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This work titled **"THE EFFECTS OF PRETREATMENT ON BIOGAS PRODUCTION FROM MAIZE STRAW AND CATTLE MANURE"** by **DAUDA KARIMOU** has been approved as a thesis for the Degree of Master of Science in Environmental Engineering by the Examining Committee Members mentioned below.

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ABSTRACT

THE EFFECTS OF PRETREATMENT ON BIOGAS PRODUCTION FROM MAIZE STRAW AND CATTLE MANURE

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The main aim of this study was to determine the effects of physical and chemical pretreatments on biogas production from two agricultural wastes (maize straw and cattle manure). The total solids (TS) were 86.1% and 41.2% for the cattle manure and maize straw respectively while the volatile solids (VS) were 82.0% for cattle manure and 51.6% for maize straw (both interms of total biomass). 5% TS was used for both biomasses in the measurement of reducing sugars and the Biochemical methane potential (BMP) test. Solubility was determined by measuring the reducing sugar concentration using the Miller method (Miller 1959). Biomass samples were seeded with sludge seed on a 50:50 biomass to sludge ratio. The pH of the mixture was regulated to between 7.0 and 7.5 and placed in an incubator at 37°C (mesophilic temperature range). Biogas measurements were done daily and carbon dioxide was measured once in a week.

Two chemical pretreatment methods were applied on both biomasses; acid and alkaline pretreatments assisted with heat. The most effective pretreatment method for maize straw was alkaline pretreatment producing the highest concentrations of daily and cumulative biogas with an average increase of 163%. Acid pretreatment of maize straw lasted between 24 to 27 (the fewest number of days) compared to all other pretreatments applied.

Physical pertreatment methods applied on maize straw include; comminution, microwave and liquid hot water pretreatment. The Microwave pretreated samples produced the highest increase in biogas production of about 3 folds.

Cattle manure was pretreated with H_2SO_4, NaOH, microwave and liquid hot water (LHW). Acid pretreatment was more effective for cattle manure than maize straw with both acid concentrations increasing biogas production. Cattle manure samples treated at LHW 135^{o}C produced the highest cumulative amount of biogas resulting in a 103% increase. Microwave pretreatment was also very effective resulting in 97% increase in methane production which lasted for 32 days.

Keywords: Biogas, pretreatment, lignocellulose, methane, maize straw, cattle manure.

TÜRKÇE ÖZET

Bu çalışmanın amacı, fiziksel ve kimyasal ön arıtımın tarım atıkları (mısır artıkları ve sığır gübre) üzerindeki etkilerini belirlemektir. Sığır gübresi ve mısır samanının toplam katı madde oranları sırasıyla %86.1 ve %41.2 olmuştur. Uçucu katı madde oranları ise sığır gübresinde %82 ve mısır samanında %51.6 olarak ölçülmüştür. Biyokimyasal metan potansiyeli ve indirgen şeker ölçümlerinde atıklar %5 TKM oranında kullanılmıştır. İndirgen şeker Miller (Miller, 1959) metodu kullanılarak ölçülmüştür. Biokütle örnekleri çamurla 50:50 oranında karıştırılmıştır. Karışımın pH değeri 7.0-7.5 değerlerine ayarlanmış ve örnekler 37^{o}C (mezofilik) inkübatörde tutulmuştur. Biyogaz ölçümleri günlük yapılırken karbon dioksit ölçümleri haftada bir yapılmıştır.

Kullanılan atıklara alkali ve asidik olmak üzere iki faklı ön arıtım metodu uygulanmıştır. Mısır samanı için en verimli ön arıtım metodu alkali ön arıtım olmuştur. Alkali ön arıtım sonrası günlük ve kümülatif biyogaz üretiminde %163'lük bir artış olmuştur. Asidik ön arıtım uygulanan mısır samanından biyogaz üretimi prosesi 24-27 (diğer ön arıtım işlemlerine göre daha az) gün sürmüştür.

Mısır samanına ufalama, mikrodalga ve sıcak su gibi üç farklı fiziksel ön arıtım yöntemi uygulanmıştır. Bu yöntemler içerisinde mikrodalga yöntemi diğer yöntemlere göre 3 kat daha fazla biyogaz üretim verimi sağlanmıştır.

Kullanılan sığır gübresine H_2SO_4, NaOH, mikrodalga ve sıcak su ön arıtım yöntemleri uygulanmıştır. Asidik ön arıtım yöntemi mısır atıklarından farklı olarak sığır gübresi çalışmalarında daha verimli olmuştur.

135°C sıcak ön arıtım yöntemi uygulanan sığır gübresinde kümülatif biyogaz üretiminde %103 artış olmuştur. Mikrodalga ön arıtım yöntemi de sığır gübresi çalışmalarında verimli olmuştur. Metan üretiminde % 97 artış yaşanmış ve biyogaz üretimi 32 günde tamamlanmıştır.

Anahtar Kelimeler: Biyogaz, ön arıtma, lignoselüloz, metan, mısır saman, sığır gübresi.

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Above all I thank Allah, the almighty; my creator, the author of my faith, and the provider for his guidance and protection. He has been good to me.

DAUDA KARIMOU

August 2019, Ankara

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ABBREVIATIONS

AD	Anaerobic Digestion
BMP	Biochemical methane Potential
DM	Dry Matter
DNS	Dinitrosalisylic acid
GDP	Gross Domestic Product
HRT	Hydraulic Retention Time
LHW	Liquid Hot Water
MW	Microwave
TS	Total Solids
VS	Volatile Solids
VFAs	Volatile Fatty Acids

1. INTRODUCTION

1.1. Background of Study

Biogas is composed of a combination of gases usually methane (CH_4) and carbon dioxide (CO_2) produced from the degradation of organic materials by anaerobic bacteria. The process leads to the production of bioenergy (biogas) and residues or leftovers which can be used as manure. There are large amounts of organic wastes produced on a day to day basis in both rural and urban environments which makes their use for the production of bioenergy a feasible and cost effective method. Biogas is considered a renewable form of energy because of our ability to grow plants and rear animals within a relatively shorter time frame which can be repeated over and over again. Renewable energy obtained from plants and animals residues is referred to as biomass energy. The part of the biomass that is carbon based and biodegradable is referred to as the organic fraction of the biomass.

In this study, agricultural wastes (maize straw and cattle manure) will be subjected to anaerobic digestion in an attempt to produce biomethane. These substrates have been chosen because of their availability in abundance in my home country and some of the advantages they possess such as; being readily available all year round, their relatively high biogas production rates, the process serving as a waste management technique, the production of highly needed fertilizer and the limited research in this area of renewable energy generation from agricultural residues. Despite being the fifth largest oil producer in Sub-Saharan Africa, agriculture remains the leading contributor to the economy of Cameroon. According to the Ministry of Agriculture and rural development, 60-70% of the country’s workforce is employed in the agricultural sector, which provides 42 percent of its gross domestic product (GDP) and 30 percent of its annual export revenue. This implies that the country has a big potential to use agricultural wastes to produce bioenergy. The problem however with using these substrates is that they contain lignocellulose and therefore are degraded very slowly by microbes. The process becomes too slow sometimes that it’s not economically feasible and hence pretreatment has to be employed. There are three major building blocks of lignocellulosic biomass namely; hemicellulose, lignin and cellulose, but also minute quantities of other components. Cellulose and hemicellulose are larger units of smaller molecules such as glucose, and can therefore be broken-down to their constituent monomers hence serving as a source of fermentable sugars. The cellulose segment is a relatively more repellent polymer and as a consequence requires intense treatment to be dissociated while hemicellulose can dissolve relatively easily after the application of dilute basic and acid solutions. Pretreatment is aimed at making the process faster but even more important to augment the biogas yield of lignocellulosic biomasses. In biogas generation from anaerobic digestion, most if not all of the methane comes from the soluble sugars like glucose with a small percentage coming from other small molecules present in the substrate (Cecilia S., 2013). The origin of these small molecules capable of producing methane within a lignocellulosic substrate is the breakdown of polysaccharides such as starch, cellulose and hemicellulose. The presence of van der Waal forces and H-bonds in cellulose usually results in increased ductility while the possession of molecules with varied arrangements across the structure, leads to high crystallinity and hence difficulty in degradation. Cellulose contains microfibrils held together by hemicellulose with pectin or only pectin in some instances, concealed with lignin which gives rise to the formation of a complicated structure that is very difficult to breakdown by chemical or biological substances (Yi Zheng et al 2014). The third major constituent of lignocellulose, lignin is one of the most ample organic polymers occurring in nature (Yebo Li et al 2014). Lignin is generally non-biodegradable and is sometimes believed to inhibit the degradation of other biopolymers like cellulose but studies carried out by De Angelis have shown that small fractions of lignin can be broken down by microorganisms especially if pretreatment is applied (DeAngelis et al., 2011). It serves as a glue holding together hemicellulose and cellulose to form an adamant three-dimensional formation of the cell wall and hence the more lignin there is in a biomass sample, the greater its resistance to biodegradation.

1.2. Statement of Problem

Humanity’s heavy reliance on fossil materials like coal for its energy supply has led to serious environmental concerns. This is because fossil fuels are finite and due to increasing demand from human population growth, they will get exhausted at some point, so what should be done to replace the excessive use of fossil fuels with cleaner sources of energy?

Some renewables such as agricultural products like seeds, sugars, vegetable oils etc. are food crops and therefore are needed for human consumption which leads to competition while third generation biofuels like algae are very expensive to grow, maintain and harvest. They also contaminate water sources and produce co-products whose environmental impacts are not fully known presently. This has led to the exploitation of what is known as second generation biofuels (Lignocellulosic substrates) as one of the most suitable options. The problem with the utilization of second generation biofuels however is the difficulty in biodegradation due to the composition of their cell walls, so how can we overcome this difficulty to augment the efficiency of the bioenergy production techniques using second generation biofuels?

1.3. Objective of Study

The objective of this study is to use acid, alkaline, comminution, microwave and liquid hot water pretreatments to breakdown lignocellulosic biomass and determine their effects on biogas generation from corn straw/stover and cattle manure.

1.4. Research Hypothesis

The presence of lignocellulose in biomasses such as agricultural residues decreases their degradation by anaerobic microorganisms and hence biomethane production potential. Therefore, the application of acid, alkaline, microwave, comminution and liquid hot water increases their solubility and hence they can be degraded more efficiently by anaerobic bacteria. The faster breakdown of biomasses leads to enhanced rates of anaerobic digestion and a diminished hydraulic retention time. Increased solubility will also result in more complete breakdown and thus a higher biogas production.

1.5. Definition of Concepts

1.5.1. Biofuels

Biofuels generally refer to compact materials (such as wood chips, pellets etc.), fluids like oils, biodiesel and ethanol, or gaseous fuels such as biohydrogen, biogas, and biosyngas that are mainly obtained from biomass sources (Cecilia Sambusiti 2013). They are rich in energy obtained and stored from biomass. Biofuels in their natural solid forms are termed primary biofuels and they are generally utilized in their unprocessed form for example the burning of fuel woods, pellets and wood chips. They include those in which the natural organic matter is used unprocessed (as harvested). They are usually burned directly, mostly to deliver cooking fuel, warming and to yield electricity requirements either in minor or for large scale industrial use.

Secondary biofuels on the other hand are transformed solid materials for example charcoal, fluids like biodiesel, bioethanol and oils or aeriform such as hydrogen and biogas. They are generally obtained from the processing and transformation of biomasses (Cecilia S. 2013) and they can be classified into three different generations (1^{st}, 2^{nd} and 3^{rd} generation biofuels) according to the source of the biomass used in their production (Cecilia S. 2013).

According to the IEA, first generation biofuels are those biofuels originating from agricultural residues which are generally used for food and animal purposes (IEA, 2008). To use them, they are more often than not, further transformed into bioethanol (crops rich in glucose like wheat and maize) or biodiesel (crops such as soy containing fats and oils) and biomethane (from crops equally rich in sugars, oils and fats). First generation biofuels production technologies are well known and the processing and production pathways are equally well understood presently. The US, EU and other developing countries like China, Brazil, Thailand, Colombia and Indonesia, have successfully implemented industries that process and produce 1^{st} generation biofuels like bioethanol and biodiesel. The production of 1^{st} generation biofuels is controversial because of the significant environmental and economic restrictions. A major negative environmental impact for using 1^{st} generation biofuels is the need for large amounts of fertilizers on farmlands and the use of fossil fuels in tractors and other mechanical equipment to grow and maintain them (IEA, 2008). Secondly, 1^{st} generation biofuels must be cultivated in tillable lands leading to competition with food crops and hence the soaring of food prices and scarcity of food in some areas of the world which ends up raising a “food vs. fuel” dilemma (Khanna 2012). The disadvantages mentioned above have led to an erosion of support for first generation biofuels as new research carried out in various parts of the world began to link their production to raising food prices which raised doubts over their ability to replace fossil fuel use. First generation biofuel production has also received criticism for its potential in the contribution to deforestation (Searchinger et al. 2008).

The problems highlighted above have led to the development of 2^{nd} generation biofuels (including biomethane, biodiesel and ethanol) which can be gotten from non-food substrates. Biodiesel for instance can be obtained from uneatable vegetable oils such as citrus oil and Jatropha (Cecilia S. 2013), while others like biogas, biohydrogen and 2^{nd} generation bioethanol are generally produced from biomasses containing lignocellulose like energy plants such as switch grass, miscanthus, poplar etc. cultivated on lands not suitable for growing eatable crops. Second generation biofuels can also be obtained from farm residues or wastes like corn stover, animal manure amongst others. The exploitation of lignocellulosic biomass possesses many advantages such as; they are generally available in excess supply and they are non-food materials. According to the United Nations food and agricultural organization (FAO), the most abundant biomass in the world is cellulosic biomass (FAO 2008), with sum total production of between 10-50 billion dry tons annually (Zacchi, 2002). Cellulose has the potential to contribute about 20% of the current world annual primary energy demand. Also, these biofuels do not clash with food production demands for lands because these energy crops can be grown on areas not used for eatable crops cultivation and crop residues are wastes generated after food extraction (Ohman et al. 2006). The production of 2nd generation biofuels is generally not very fruitful because of the necessity of pretreatment due to the presence of lignocellulose which adds to the operating cost, cost of transportation of feedstock and the acquisition of capital equipment. (Dale and Ong, 2012). The pretreatment stage is estimated to account for between 16-19 % of the entire cost of operating a lignocellulosic energy production plant (Aden 2009). The production of biomethane through anaerobic digestion of biomasses such as maize straw presents a realistic option for increasing energy production and achieving the target fixed by my country.

Third generation biofuels refers to the production of bioenergy from algae. Algae are autotrophic or heterotrophic organisms which require inorganic molecules like CO_2 and sunlight energy to grow and reproduce. Heterotrophic algae cannot produce their own food and they require an external source of carbon and nutrients as energy sources (Owende, 2010) while autotrophic algae can produce their food needs through photosynthesis. There is growing interest and research in algal biomass as a raw material for the generation of biofuels. The merits of using algae is that there is no contest with food crops and animal feed production (Demirbas, 2007). This is because photosynthetic microalgae for instance only requires solar energy, CO_2, phosphorus, potassium and nitrogen for their development. They also produce relatively large amounts of carbohydrates, fats and oils which have a high potential for various biofuels production (Singh et al. 2011). Unsurprisingly, due to the little or no lignin and hemicelluloses in algae biomass, there is a step up in the efficiency of the biomethane production process (Saqib et al., 2013). Both the hydrolysis and fermentation of algal biomass is relatively faster and more efficient. Microalgae are regarded as a useful alternative biomass which is independent from the major problems related to second and first generation biofuels (Dragone et al., 2010). Major merits of using microalgae as a substrates for biofuel production is its enormous oil content (approximately 40% on dry matter basis). Microalgae also has a multi-purpose function and can be used to produce various types of biofuels e.g. biomethane, biohydrogen, biodiesel and bioethanol (Balat, 2011). It equally has a relatively faster growth rate in nature which provides a steady supply of biomass (Lee et al., 2014a). However though, the high demand for water, and sometimes high start-up costs required for their cultivation is a major barrier that limits their large scale production (Cecilia S. 2013). The main advantages of third generation biofuels are their fast growth rate, ability to absorb carbon dioxide and the use of wastewater and non-arable lands. However, growing and harvest of algae is expensive and can also lead to the contamination of water.

1.6. Biogas Production through Anaerobic Digestion (AD)

The production of biogas by AD involves the microbial disintegration of organic matter by bacteria in oxygen free environments. It results in the production of biogas with CH_4 and CO_2 as its principal constituents and traces of other gases in minute quantities generally considered as impurities. It is a well-established technique used to manage wastes and produce fuels both for domestic and industrial purposes. Historically, the anaerobic digestion process has been applied on a diverse range of substrates which include but not limited to municipal solid wastes, industrial and municipal wastewaters and sludge, manures etc., especially from the agro-processing industries because they contain significant amounts of biodegradable organic matter like fats and proteins. Recently, there has been increased interests and research in anaerobic digestion especially in the production of biomethane using lignocellulosic residues such as maize straw (Panagiotopoulos et al., 2009). The biogas production potential from selected agricultural wastes is given in table 1.1.

The anaerobic digestion process is complex and includes a wide range of microbes acting in up to nine stages of transformation of organic matter. The process of methane production can be divided into four major steps of microbial breakdown which are; hydrolysis, acidogenesis, acetogenesis, and methane formation. The various stages are executed by different species of bacteria, which sometimes have a syntrophic relationship (that is, different microorganisms degrade together specific components of substrates which they are not able to decompose on their own) (Angelika S. 2008). The four main stages are shown in figure 1.1.

Table 1. 1 Methane yield from anaerobic digestion of different agricultural wastes (Adapted from Merlin G. and Boileau H. 2013).

Type of agricultural waste	**CH_4 yield (mL/gVS)**
Dairy cattle manure	150
Cow manure	280
Pig manure	360
Straw(maize, rice)	92-330
Vegetable leaves	230-310
Rotten fruits	210-510
Fruit pressings	280-500
Fruit peels	240-520

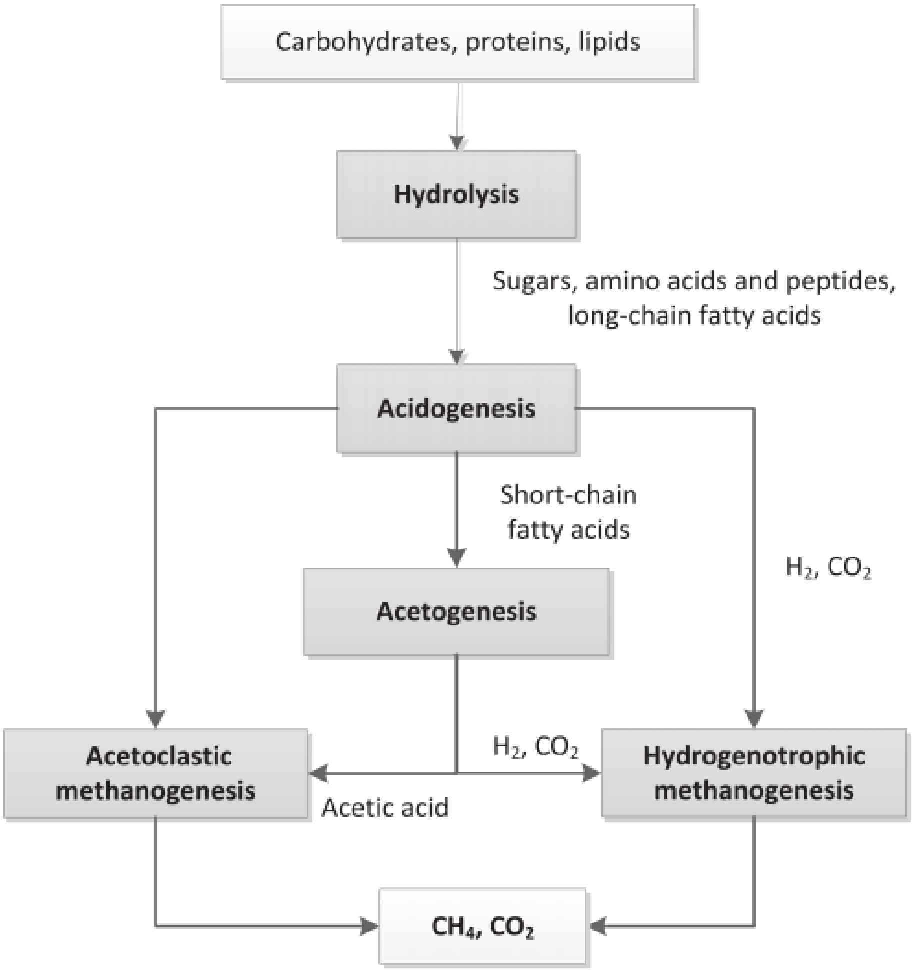


Figure 1. 1 Four stage anaerobic digestion (Zheng et al. 2014)

1.6.1. Hydrolysis

In this phase, water soluble compounds are broken down. The breakdown of simple compounds like carbohydrates is relatively faster while the hydrolysis of oils, fats and proteins can take days to complete. Lignocellulosic biomasses are only broken down partially and slowly hence initial pretreatment is required to increase the dissolution and biogas production. During hydrolysis, long chain carbohydrates present in insoluble structures such as cellulose, hemicellulose, and starch are hydrolysed by *hydrolases*, producing short chain monomers. Proteins are disintegrated to amino acids by *proteases* while fats are dismantled into smaller molecules like glycerine and carboxylic acids by *lipases*. The degree of hydrolysis is also reliant on the availability of enzymes i.e. if the bacteria is able to produce the required enzymes, the dissolution is relatively faster. If the enzymes do not have access to the substrate (the case in lignocellulosic biomass) hydrolysis becomes the rate-limiting step (Karimi 2008).

1.6.2. Acidogenesis

In this phase, monomers resulting from the hydrolysis of complex polymers are taken up by various obligatorily and facultative bacteria and are further broken down to short chained organic acids containing between one and five carbon atoms, molecules (which include but not limited to propionic acid, butyric acid, acetate etc.), NO_2, alcohols, hydrogen sulfide, H_2, and carbon dioxide. The concentration of the hydrogen ions produced during acidogenesis influences the kind of fermentation output that will be formed. If hydrogen’s partial pressure is higher, less reduced compounds such as acetate are produced. In this stage, carbohydrates such as glucose are degraded to pyruvate which is subsequently converted to lactic acid by Lactobacillales and into bioethanol by yeasts. Microorganisms belonging to the group of coli- aerogenes convert pyruvate into formate, ethanol, acetate, acetoin and butanediol. Fatty acids are degraded by, for example, *Acetobacter* by β- oxidation. Therefore, the fatty acid has to be bound on coenzyme A and oxidation then occurs stepwise through the sequential removal of two carbon units, which are set free as acetate while the breakdown of amino acids is catalysed by *Clostridium botulinum* through a reaction called the Stickland reaction in a pairwise manner with one amino acid serving as a hydrogen donor and the other serving as an acceptor leading to the creation of ammonia, acetate, and CO_2. During the breakdown of cysteine, hydrogen sulfide is produced resulting in a foul odour of rotten eggs. Acidogenic bacteria can disintegrate organic material even at very low pH values.

1.6.3. Acetogenesis

The acetogenic phase makes use of the products of the acidogenic phase and this is because methane producing bacteria are unable to utilize the products of acidogenesis directly, thus they have to be processed further during the acetogenic phase, before they can be transformed into biomethane.

In the acetogenic stage, homoacetogenic microorganisms (such as *acetobacterium woodii*) constantly convert hydrogen and carbon dioxide to acetic acid by reduction. Acetogens are obligatory hydrogen generating bacteria. The creation of acetate by the decomposition of propionic acid, butyric acid etc. (long chain fatty acids) is thermodynamically enabled and can run on its own with when the partial pressure of the hydrogen is very low. This means that the condition necessary for acetogens to acquire the energy they need to survive and grow is very low concentrations of hydrogen and as a result, they enter a symbiotic relationship with methane producing microorganisms which survive only in environments with high hydrogen partial pressure The methanogens keep the substrate constantly free of metabolites of the acetogenic bacteria, hence reduced hydrogen partial pressures are maintained within the environment. Acetogens produce mostly carbon dioxide, hydrogen, and acetate in low hydrogen partial pressure environments, and mostly propionic, valeric, butyric, acids and ethanol in environments with high partial pressure of hydrogen. However, the methane producing bacteria only have the ability to process hydrogen, acetate and carbon dioxide. Approximately 30% of the entire methane produced within the anaerobic digester is attributable to the reduction of CO_2 to CH_4 by H_2 and about 5 – 6% of the methane produced is as a result of dissolved hydrogen.

1.6.4. Methanogenic Phase

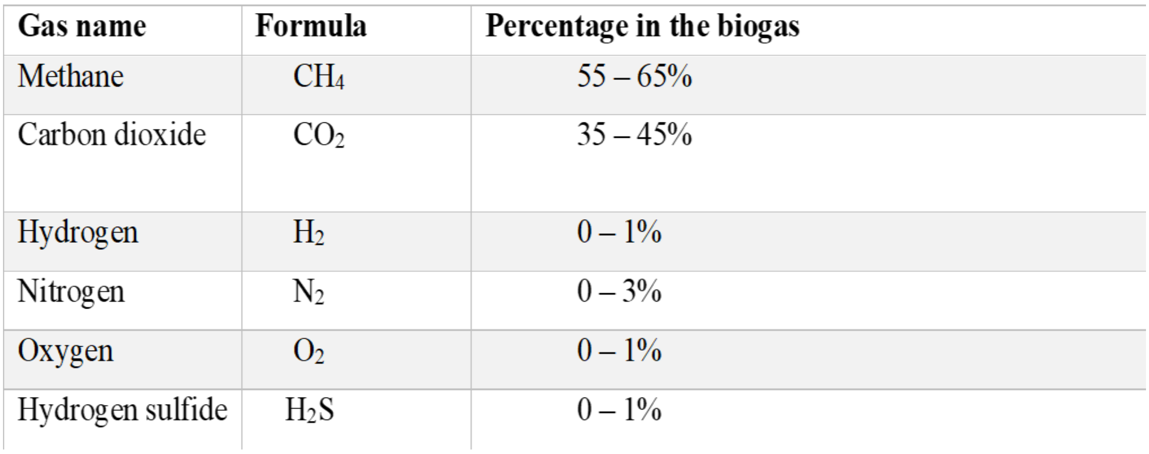
This is the fourth and final step in anaerobic digestion during which biomethane is formed in the absence of oxygen. It generally involves methane producing bacteria that generate methane, either from hydrogen and carbon dioxide or acetate. Here, the carbon present in the substrate is transformed into carbon dioxide in the liquid mixture as a bicarbonate ion (HCO_3^-) plus hydrogen and methane. Methanogenic microbes are selective in the type of substrate they each degrade. The route for the formation of methane from carbon dioxide or acetate in the bacteria involves long chain hydrocarbons such as methanofurans (example given $RC_{24}H_{26}N_4O_8$) plus the co- enzyme F420 as co - factors. Generally, about

65-70% of the methane generated within anaerobic digesters originates from the acetate. Hydrogenotrophic bacteria convert the hydrogen, and the carbon dioxide into methane while maintaining low hydrogen partial pressures to aid the development of acetogenic bacteria.

1.7. Biogas Composition

As mentioned earlier, the main constituents of biogas are methane and carbon dioxide. Methane is the desired component of the mixture which represents the fuel value in it while carbon dioxide is an impurity, so the bigger the fraction of methane it has, the better the quality. The methane content in the biogas produced from anaerobic digestion depends on factors like the substrate type, retention time, particle size, cell composition, dilution etc. Table 1.2 shows a summary of the typical constituents of biogas.

Table 1. 2 Biogas composition



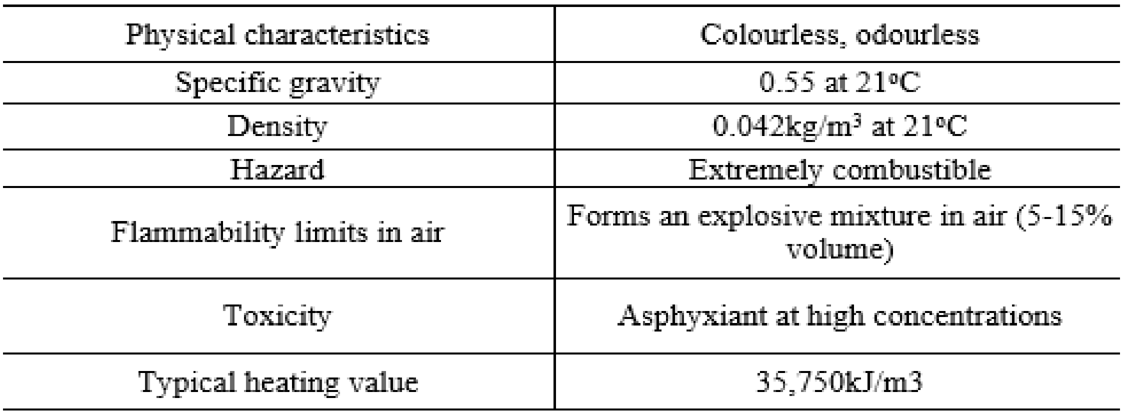
Gas name	Formula	Percentage in the biogas
Methane	CH_4	55 – 65%
Carbon dioxide	CO_2	35 – 45%
Hydrogen	H_2	0 – 1%
Nitrogen	N_2	0 – 3%
Oxygen	O_2	0 – 1%
Hydrogen sulfide	H_2S	0 – 1%

Source: Zishan *et al.* 2013

Methane is a highly combustible gas and therefore should be handled with care. When it mixes with air, it produces an explosive mixture and can also impair respiration if the concentration is too high (Samir K. 2008).

The properties (physical and chemical) of methane are given in table 1.3.

Table 1. 3 Physical and Chemical properties of methane gas



Physical characteristics	Colourless, odourless
Specific gravity	0.55 at 21°C
Density	0.042kg/m^3 at 21°C
Hazard	Extremely combustible
Flammability limits in air	Forms an explosive mixture in air (5-15% volume)
Toxicity	Asphyxiant at high concentrations
Typical heating value	35,750kJ/m3

Source: Samir K. (2008)

Biogas might also possess some impurities like hydrogen sulfide, carbon dioxide, particulates and siloxanes. It also contains some moisture at the normal temperatures that most digesters function. A brief explanation of the impurities is given in the paragraphs that follow.

a) Carbon dioxide

Carbon dioxide is normally found in most digesters so it does not have strong negative effects on the quality of the biogas although it dilutes its energy content and therefore lowers its calorific potential. Power generation equipment are designed such that they are not greatly affected by 30 to 50% carbon dioxide by volume in the biogas hence if it is used for electrical power generation, the carbon dioxide must not be removed. However if the biogas has to be upgraded to higher quality fuels such as natural gas, then the carbon dioxide has to be removed since that will enhance the quality of the gas.

b) Moisture

The methane produced through anaerobic reactors generally contains significant amounts of moisture at the usual operating temperature. Sometimes it condenses as it comes in contact with the pipes because they are cooler and therefore has to be removed. It will also lead to corrosion of the gas pipes if it mixes with hydrogen sulfide found within the biogas because of the production of acids. The removal of moisture from biogas can be achieved by lowering the temperature for it to condense and subsequently removed (D. Deublein and Angelika S. 2008).

c) Hydrogen sulfide

It is an extremely reactive gas present in biogas due to the degradation of proteineous substrates containing the element sulphur. It reacts with moisture (another impurity present in biogas) to form sulphuric acid which has damaging effects like the corroding of gas pipes, utilization equipment and tanks used in storage. Hydrogen sulphide can also be poisonous at high concentrations above 700 ppmv. ((D. Deublein and Angelika S. 2008)) and should therefore be removed from the biogas plants as soon as its starts accumulating. To remove hydrogen sulphide from biogas, certain strategies such as using an iron sponge to suck it, scrubbing with water, and biological oxidation should be used.

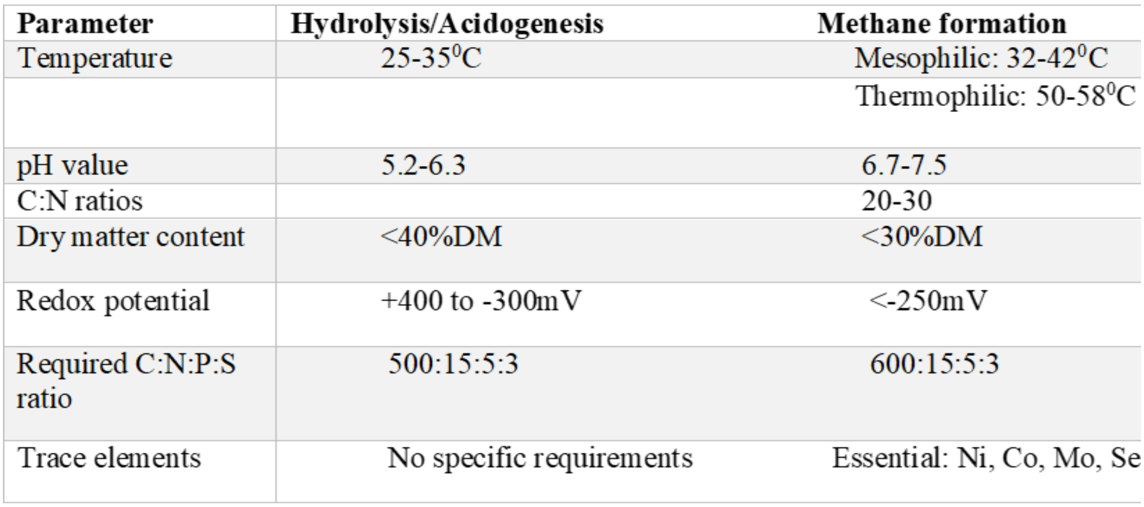
d) Siloxanes

This refers to organic silicon polymers used in diverse industrial, medical, body care/aesthetics, commercial, and food products. They include shampoos, hair conditioners, cosmetics, detergents etc. (Personal care products). Their properties such as high volatility makes them to escape as gases during the anaerobic breakdown of municipal solid waste and municipal sludge. When they are oxidised in the equipment used in the gas, a solid which resembles tiny sand particles is produced and then it accumulates on the mobile parts or on the surfaces of heat exchangers which leads to wear and tear of the material. It equally diminishes the heat exchange efficiency of the equipments. To remove siloxanes from biogas, graphite filters and refrigerant dryers can be used (D. Deublein and Angelika S. 2008).

1.8 Environmental Factors Influencing Anaerobic Digestion

Anaerobic bacteria are very vulnerable to alterations of environmental parameters such as temperature, alkalinity, pH, nutrients etc. The Anaerobic digestion systems are more susceptible to changes than aerobic systems for the same magnitude of change from required environmental conditions. In the AD process, methanogenesis is the rate limiting step because the bacteria present at this stage are highly susceptible to environmental changes and they also grow very slowly. This therefore requires very careful monitoring and maintenance of the environmental conditions. Table 1.4 summarizes the environmental requirements of the anaerobic microbes in anaerobic digestion for biogas production.

Table 1. 4 Environmental requirements of anaerobic microorganisms in anaerobic digestion.



Parameter	**Hydrolysis/Acidogenesis**	**Methane formation**
Temperature	25-35^{0}C	Mesophilic: 32-42^{0}C
		Thermophilic: 50-58^{0}C
pH value	5.2-6.3	6.7-7.5
C:N ratios		20-30
Dry matter content	<40%DM	<30%DM
Redox potential	+400 to -300mV	<-250mV
Required C:N:P:S ratio	500:15:5:3	600:15:5:3
Trace elements	No specific requirements	Essential: Ni, Co, Mo, Se

Adapted from D. Deublein and Angelika S. 2008

A brief description of some of the environmental factors is given in the following paragraphs;

a) Temperature

Anaerobic digestion (AD) like many biological systems is highly reliant on the temperature of its environment. The degradation of organic materials have their maximum efficiency at temperature ranges between 35–40^{o}C for mesophiles and at around 55^{o}C for thermophiles (Lettinga 1994). There are three optimal temperature ranges in anaerobic systems for methane production and the corresponding microbes operating in them are referred to psychrophilic, mesophilic, and thermophilic microorganisms. Generally, anaerobic conversion rates augment with an increase in temperature until around 60^{o}C (Samir K. K 2008). Anaerobic disintegration is most efficient between 5–15^{o}C for psycrophiles, 35–40^{o}C for mesophiles, and approximately 55^{o}C for thermophiles, with undulating rates between these optima as shown in figure 1.2 below (Sami K. K 2008).

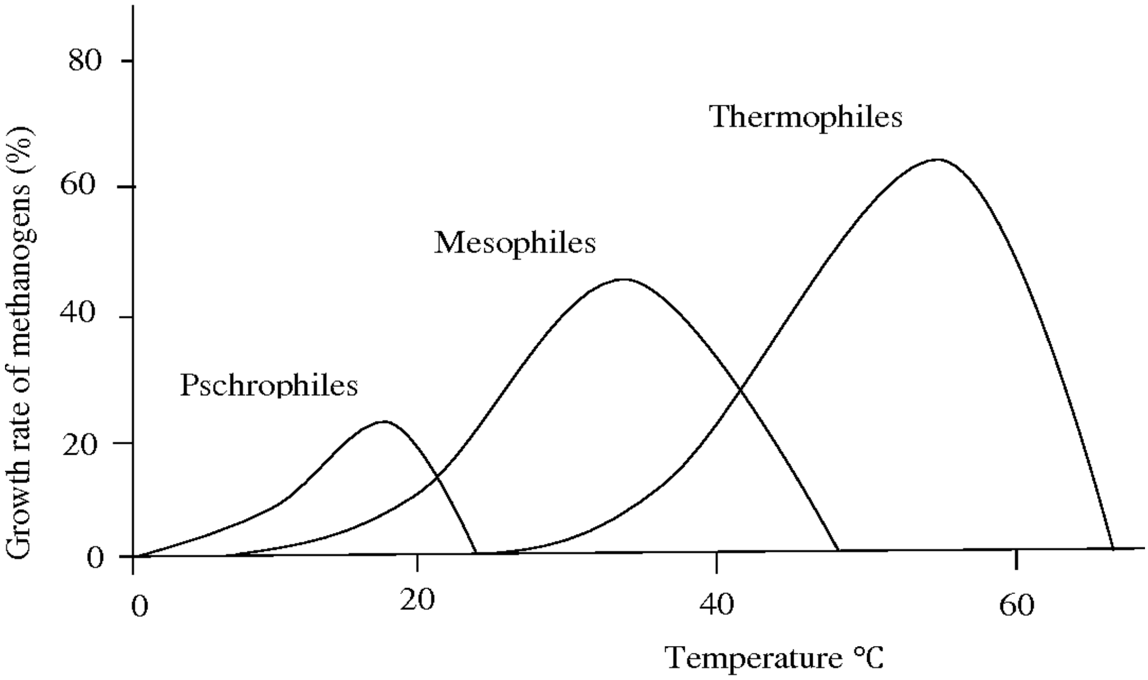


Figure 1. 2 Relative growth rate of bacteria (E. Ogbonna. 2017).

In most laboratory scale and even full scale digesters, the temperature is maintained within the mesophilic range and this study will also be carried out with temperatures within this range. This is because there is between 25–50% more methane production within the mesophilic temperature range, based on the substrate variety (Harremoes 1983). Other reasons why the mesophilic anaerobic digestion is preferred to the thermophilic anaerobic digestion include; susceptibility to loading variations and slow start-ups time for the thermophilic range amongst others. The lysis rate of thermophilic microorganisms is also relatively higher than that of the mesophilic bacteria, and as a result they usually only exist in an exponential growth phase (Sami K. K 2008). However though, there exists some few exceptions, for instance high temperatures destroy pathogens, and also the hydraulic retention time of thermophiles can be reduced in comparison to mesophiles as a result of the rapid growth of thermophilic, acid-consuming microorganisms (Zinder 1988).

b) Operating pH

In terms of pH optima, there exist two bacterial species; acid-producing bacteria and methane producing bacteria. The former operates in pH of 5.5–6.5, while methane producing bacteria grow best between 7.8 and 8.2 (Samir K. 2008). When both cultures coexist, in the same environment, the preferred pH range is adjusted to 6.8–7.4. The pH range is however maintained around 7 which is the optimum for methanogenesis: the rate-limiting step in AD and also given that methanogens are much more likely to be affected by pH variations compared with any other microorganisms (Samir K. K. 2008).

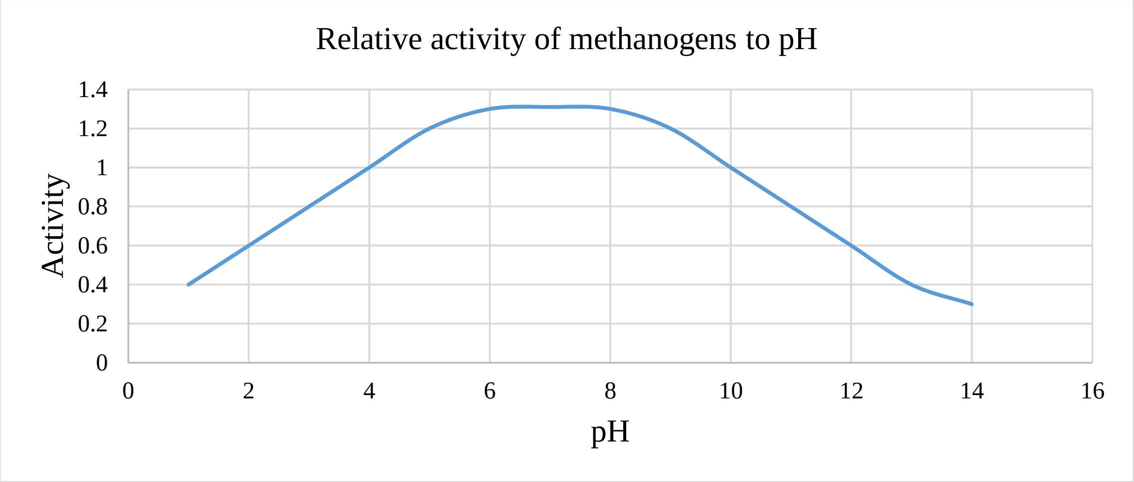


Figure 1. 3 pH dependence of methanogenic activity.

In anaerobic treatment processes, alterations in pH arise due to the accumulation of volatile fatty acids (VFAs) or even the accumulation of carbon dioxide. To solve the problem of VFAs accumulation the volumetric organic loading rate should be reduced such that the accumulated VFAs are consumed faster than their rates of production. The susceptibility of methanogenic bacteria to pH changes means anaerobic reactors need buffering to adjust the fluctuations in pH. The buffer used in this study is sodium bicarbonate due to its advanced dissolution rates, durability, and low toxicity.

b) Oxidation–Reduction potentials

For maximum growth of anaerobic bacteria in many media, the oxidation reduction potential values have to be maintained between -200 to -350 mV at pH 7 (Morris 1975). Methanogenic bacteria requires extremely reducing environments, with redox potential values as low as -400 mV (Samir K. K 2008). In order to maintain a low redox potential in anaerobic digesters, a few oxidising agents such as sulphates, nitrates or nitrites should be supplied.

d) Trace Elements

Methane producing bacteria require certain concentrations of trace elements like iron, cobalt, nickel, magnesium etc. for waste stabilization and sometimes as nutrients. They provide suitable physicochemical conditions for optimum growth of the bacteria. Microbes also require macronutrients (e.g. phosphorus) for the anaerobic digestion process to result in the production of new products. According to a study carried out by Speece, trace elements like cobalt, iron etc. in minute quantities such as the mg/L dose and vitamin B12 in μg/L concentrations lead to an increase in methane creation (Samir K. 2008). The element Nickel is very useful because it is a skeletal component of factor F430, which is unique to methanogens (Samir K. 2008). Another trace element with specific significance is Cobalt because it is the building block of vitamin B12, which catalyses methane production. The most suitable cobalt concentration for methanogenesis is 0.05mg/L (Samir K K.2008).

1.9 Inhibition

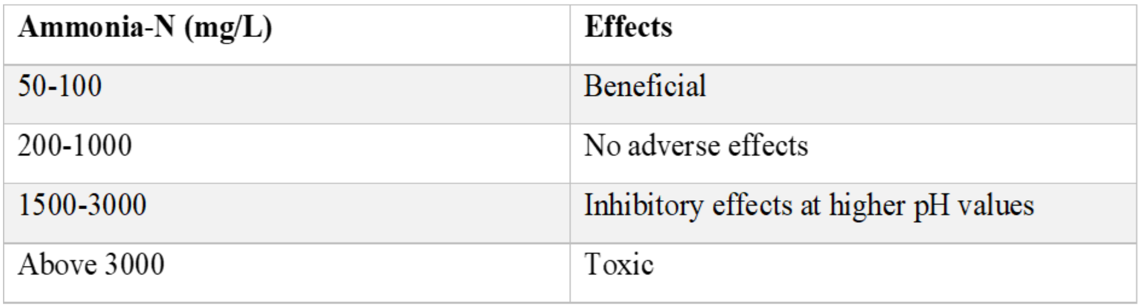
During anaerobic digestion, certain compounds formed from the metabolism of the anaerobic bacteria hinders the biocenosis (that is an association of various types of microorganisms forming a microbial community) and can even be toxic in high concentration. Some examples of inhibitors include sulfide, ammonia and long-chain fatty acids (LCFAs). Some of the toxic materials can also be brought into the digester from the influent waste stream. The degree to which inhibition occurs generally depends on factors like the intensity of the inhibitor, the make-up of the substrate and the habituation of the methanogens to the inhibitor in question. It should however be noted that anaerobic bacteria requires small amounts of these inhibitors as trace elements. Brief descriptions of some of the inhibitors are given below;

a) Ammonia (NH_3)

Ammonia is usually produced in reactors as a results of the abasement of organic wastes containing nitrogen. Complex organic polymers in the waste stream like proteins usually contain about 16% nitrogen and are a major source of ammonia. Also, Agric-based industries, involved in the rearing and refining of animal waste give rise to waste streams with high levels of ammonia. Excessive levels of ammonia in waste streams makes their treatment unsuccessful (Farina et al. 1998). As a general rule, ammonia level exceeding 4,000 mg nitrogen per litre have repressive effects on anaerobic bacteria during cattle manure breakdown (Ahring 1994). Ammonia toxicity to anaerobic microorganisms is reliant on pH and temperature. Reactors operating in the thermophilic temperature range react more to ammonia toxicity than reactors in mesophilic temperatures (Samir K. K. 2008). The concentration of unionized ammonia increases with an increase in temperature which renders the environment more toxic (Gallert 1997). Free ammonia concentrations above 1,100 mg N/L lead to an inhibition and decreased microbial growth in batch reactors at pH value around 8.0 and 55°C temperatures (Hansen et al. 1997).

If the ammonia nitrogen concentrations is above 3,000 mg/L, the NH_4^+ itself becomes toxic independent of the pH (McCarty 1964) as seen in the table 1.5;

Table 1. 5 Ammonia nitrogen concentration and its effects on anaerobic treatment.



Ammonia-N (mg/L)	Effects
50-100	Beneficial
200-1000	No adverse effects
1500-3000	Inhibitory effects at higher pH values
Above 3000	Toxic

Samir K. K. 2008

At concentrations below the threshold, ammonia shields a rise in pH i.e., it produces a hydrogen ion hence making the medium more acidic. Ammonia nitrogen in a bioreactor dwells in two separate forms based on the pH of the reactor. At pH values around 7, free ammonia nitrogen (NH_3-N) makes up about 0.5% of the cumulative ammonia in the system (NH_3-N + NH_4^+-N). Free unionized ammonia is the most problematic form of ammonia nitrogen because even at concentrations as low as 100 mg/L it is still able to enter the cell membrane of methanogens.

b) Heavy Metals

These metals can both be beneficial and problematic to methanogenic bacteria based on their concentration. When the concentration is low, they act as trace elements stimulating the activity of the microorganisms and when the concentration is high, they have inhibitory effects. The ramifications of heavy metals also depend on the solubility of the metals; with soluble heavy metals being more crucial to anaerobic processes than insoluble heavy metals (Dieter D. and Angelika S. 2008). Examples of heavy metals that can lead to disturbances in biogas plants are copper, nickel cadmium, zinc (Dieter D. and Angelika S. 2008). Other elements with potential inhibitory effects are potassium, magnesium, sodium and calcium. Metal toxicity can be reduced by generating insoluble metal sulphides, with only chromium being the exception. The degree of toxicity to methanogens follows the sequence: Ni > Cu > Pb > Cr > Zn (Dieter D. and Angelika S. 2008), while a heavy metal like iron is regarded more as a useful than toxic element because it moderates sulfide toxicity.

c) Sulfide toxicity

Sulfide toxicity occurs when sulphides are produced in the process of anaerobic breakdown of biomasses or wastes having high amounts of sulphur. These wastes are generally degraded by sulphate reducing bacteria. Wastes from industrial sites such as petrochemical industries, coal gasification plants, tanneries etc. have high amounts of sulphides. Sulphur is equally found in agricultural wastes such as animal manure (particularly pig manure) because they contain proteins. According to Samir K. Khanal, hydrogen sulphide (H_2S) i.e. the unionized form has higher toxicity towards methanogenic bacteria compared to the ionized form of hydrogen sulphide.

d) Organic Acids

The organic acids found in anaerobic digesters are short chain fatty acids, long chain fatty acids, and volatile fatty acids. They are toxic to methane producing bacteria at certain concentrations even though they are naturally present in some biomasses. They generally exist in two different forms which include dissociated and undissociated forms (Dieter D. and Angelika S. 2008). The undissociated forms are known to have a stronger inhibitory effect than dissociated forms. This is because they are able to penetrate the lipophilic into cell membranes and then denature proteins in the cells (Dieter D. and Angelika S. 2008). They also reduce the pH value of reactors thus leading the acid aggregation and inhibition of the process of methane formation. In a healthy anaerobic digester, organic polymers such as proteins, fats and carbohydrates are disintegrated into their building blocks (simpler compounds). The breakdown of fats produces short chain fatty acids (containing two to six carbon atoms) which includes propionic acid, acetic acid and butyric acid alongside small quantities of *iso*-butyric, *iso*-valeric, caproic and valeric acids. At pH values less than 7 (acidic environments), acetic acid has an inhibiting threshold of about 1000mg per litre while for iso-butyric and iso-valeric acids, the threshold is around 50mg per litre (Samir K. K. 2008). Propionic acid has the strongest inhibiting effect. It is strongly inhibiting even at concentrations as low as 5mg per litre (Dieter D. et al. 2008). According to Samir Kumar Khanal, healthy anaerobic systems have volatile fatty acids concentration of between 50 to 250 mg/L. The accumulation of volatile fatty acids generally occurs when the symbiosis between methanogens and acidogens breaks down (Samir K. K. 2008). The long chain fatty acids such as octadecanoic acid, palmitic, capric, linoleic, and oleic acids on the other hand are obtained from the disintegration of oils and fats produced from the discard of edible oils, slaughter houses, oil refineries etc.

Theoretically, they have the highest methane production potential in comparisms with proteins and carbohydrates (Pereira et al. 2003).

1.10 Reactor types

There are verities of biogas plants used to produce biomethane from agricultural residues such as maize straw and animal manure. They are classified based on their feeding method as batch plants, semi-batch plants and continuous plants. Biogas plants used for agricultural wastes can equally be categorised based on the way they are constructed as fixed dome reactors and floating drum reactors

In batch type reactors, the digester is completely filled at once with materials (biomass, activated sludge etc.) added into the digester at the same time and closed allowing only the biogas produced to be extracted and then emptied completely after a given amount of time. The substrates are degraded my anaerobic microorganisms without adding or removing anything until the biomass is completely exhausted and gas production ceases. The major disadvantage of batch reactors is the unsteady production of gas; that is there are ups and downs in the quantity and contents of the biogas being produced as time goes on. The biogas produced steadily increases until it reaches its highest point which generally occurs at about half the duration of process but varies with biomass types and pretreatments applied. It then starts decreasing at a steady rate until the biomass is completely exhausted. After completion, the content of the reactor is emptied and the process repeated all over again. In continuous reactors, there is regular feeding of given biomass quantities and regular removal of given quantities of material on the other end of the reactor on a continuous basis. In these types of reactors, materials empty automatically through the overflow whenever new materials are added from the inlet pipe. The major advantage that continuous bioreactors have is that they produce constant amounts of biogas without fluctuations in quantity and composition. They also produce a higher quantity of biogas when compared to batch systems.

Semi batch reactors have the same operating principle as batch reactors but they include an extra component of nonstop addition and/or elimination of one of its components. If two materials having different degradation rates such as manure and straw are to be digested in the same reactor, it can be operated on a semi batch system. The material that digests at a slower rate is added once or twice after a given time while the one that digests faster is added and removed more regularly. Semi batch reactors have a better biogas yield than batch reactors and also, the gradual addition or removal of biomass and digestate assists in controlling temperature. In this study, based on the available materials, batch systems will be used to determine the biogas produced by maize straw and cattle manure.

1.11 Pretreatment of Lignocellulosic Biomass

Lignocellulose simply means the complex conformation of plants cell walls, often regarded as the most bounteous biomass on the surface of the earth. Lignocellulose is built from three major segments which include hemicellulose, cellulose and lignin with some smaller constituents like acetyl groups, phenolic substituents and minerals. It is very resistant to biodegradation due to the properties of the polymers, their orientation within the molecules and the linkages between molecules and functional groups present in them. However though, both cellulose and hemicellulose bio polymers are readily broken down by microorganisms after hydrolysis. Cellulose is the major constituent of lignocellulosic biomass. Cellulose molecules are highly crystalline in nature due to their orientation and this results in high tensile strength. Cellulose consists of two parts; a crystalline and an unstructured part (Zheng et al, 2014). Also, cellulose microfibrils are held together by hemicellulose and sometimes pectin and sealed by lignin molecules producing a very complex and resistant structure (Zhang et al. 2014).

Hemicellulose is composed of pentoses such as arabinose, acids, and hexoses like glucose and galactose. It has a branched, random and amorphous structure. The branches of hemicelluloses form a grid with cellulose microfibrils while also interacting with lignin, resulting in a cellulose-hemicellulose-lignin structure which is very rigid in nature (Zhang et al. 2014). The type of hemicellulose found in different plants cell walls vary in their composition, for example hardwood hemicelluloses consist mainly of xylans while softwood hemicelluloses for instance are made up of glucomannans. The amorphous and branched structure of hemicellulose makes it easily broken down by chemical and biological agents. Hemicelluloses are made up of either a hetero-polymer or a homo-polymer having short branches and held together by glucan bonds. Hemicellulose molecules are generally considered to be easily hydrolysable due to the types of polymers they contain. They can be broken down easily by both dilute basic solutions such as sodium hydroxide and dilute solutions of acids such as sulphuric acid.

Lignin consists of a bulky and complex hydrophobic and aromatic, random diverse polymer build up by phenyl propane units such as, hydroxyl and carbonyl assemblies, coniferyl alcohol with methoxyl etc. (Zheng et al., 2014). Lignin functions as a cellular glue in plant cells cross-linking between cellulose and hemicellulose yielding an inflexible three-dimensional anatomy of the cell wall (Zhao et al., 2014). It does not dissolve even in water and it is also optically inactive though it has been shown to dissociate at very high temperatures of about 180^0C, neutral pH and acidic or alkaline conditions (Zheng et al., 2014). The higher the amount of lignin in a cell, the more resistant it is to attacks from chemical and biological agents.

Broadly speaking, lignocellulosic biomasses are made up of between 10–25% lignin, 35–50% cellulose and 20–35% hemicellulose alongside small fraction of proteins, oils, and ash. Due to the physical and chemical characteristics of lignocellulose highlighted above, pretreatment is required to alter the structure and make cellulose and hemicellulose more accessible to microorganisms for biomethane production. Pretreatments modify the super molecular make-up of the cellulose–hemicellulose–lignin pattern which alters the natural tying features of lignocellulosic materials (Becer et al., 2015). The structural and compositional properties of lignocellulosic cell walls or their constituent polymers decrease its biodegradability. These properties include the reachable surface area, the crystallinity of cellulose, the extent of cellulose polymerization etc. (Zheng et al., 2014). The main objective of pretreatments is to alter the properties of the cell walls to improve the contact between the sugars and the methanogens and enzymes. It is generally reported that diminishing the crystallinity of cellulose and hemicellulose increases biodegradation and biogas production. Reducing the lignin content in biomasses has also been said to augment biogas yield (Liew et al 2012). This study intends to use physical and chemical pretreatments on maize straw and cattle manure. These pretreatment methods are briefly explained below:

I. Physical Pretreatment

Physical pretreatments are used to lessen the crystallinity of cellulose thus increasing the total surface area for enzyme action and also increase the pore size of lignocelluloses. It also plays the role of downgrading the extent of polymerization of the cellulose molecules present in the lignocellulose. The main advantages of adopting mechanical pretreatments is the lack of chemical requirements during its applications, which diminishes the quantity of post processed wastes. Physical pretreatment methods are those that do not use microorganisms or chemicals to alter the structures of lignocellulosic biomasses such as agricultural waste. It involves the physical disintegration of the framework of cellulose by adopting comminution/mechanical methods which includes milling and grinding or the extrusion method (subjecting to heat) which includes microwave, hydrothermolysis, and ultrasound treatment. The physical pretreatment methods that will be used in this study are; comminution (breaking down into small particles of sizes between 0.003mm-30mm), microwave (heating in a microwave oven at 300W for 2 to 4 minutes) and liquid hot water pretreatment (heating in an autoclave under high temperatures and pressure). The Microwave refers to a type of electromagnetic radiations which are nonionizing having frequencies between the infrared and radio waves region of the electromagnetic spectrum. When these radiations are absorbed by the biomass molecules, it produces sufficient energy to excite the vibration of molecules although it cannot break the chemical bonds holding them together. The major advantages of using microwave treatment are: shorter reaction times and lower energy consumption (Jendrzejczyk et al., 2019). Also, prolonged time of microwave treatment has been found to increase the disintegration of polysaccharides (Jendrzejczyk et al., 2019).

Table 1.6 shows selected physical pretreatment methods, the suitable feedstock, pretreatment conditions, results etc.

Table 1. 6 Selected physical pretreatment methods for lignocellulosic feedstock used in biogas production.

Physical pretreatment	**Pretreatment conditions**	**Results**	**Advantages**	**Disadvantages**
Comminution	Particle size: 0.003–30mm	Up to 30% increase in methane yield	-Increases solubility and surface area -Makes substrates easier to handle	-High energy consumption, costly maintenance of equipment
Liquid hot water (LHW)	100–230 °C, few minutes to hours	7 – 220% increase in methane yield	-No need for chemicals or enzymes, increases the activity of microbes	-Highly sensitive to pH changes, high heat demand.
Microwave	300–600W, 2 to 4 minutes	Up to 3 folds increase in methane yield	-Shorter reaction times	Small in size and produces inhibitory phenolic acids

Adapted from Zheng et al. 2014

II. Chemical Pretreatment

It involves the use of chemical substances such as ionic liquids, bases and acids to change the properties and structures of lignocellulosic substrates to increase their rates of biodegradation. It changes the structural and chemical features of the cell walls to make them more accessible to methanogens. The various chemical pretreatment types applied before anaerobic digestion in biogas production include; acid pretreatment, alkaline pretreatment, wet oxidation, catalysed steam explosion, oxidative pretreatment with peroxides and the use of ionic liquids. In this study, acid and alkaline pretreatments will be applied. Acid pretreatments is the use of agents such as sulphuric acid, phosphoric acid, acetic acid etc. assisted with heat while basic pretreatments involves the utilization of basic solutions such as sodium hydroxide, potassium hydroxide, ammonium hydroxide etc. to disintegrate the structural makeup of lignocellulosic biomasses. The application of acid pretreatment on lignocelluloses is one of the most efficient methods used in solubilizing hemicelluloses and increasing the accessibility of cellulose to bacteria. The reactions occurring during acid treatment include dissolution, condensation and precipitation of hemicellulose and solubilization of lignin respectively. The disadvantages of acid pretreatment include but not limited to corrosion of equipment and the possible creation of inhibitory products such as hydroxymethylofurfural (Jendrzejczyk et al., 2019). Acid pretreatment contributes to the output of high amounts of reducing sugars with the application of mild temperatures leading to the hydrolysis of cellulose and hence increased biogas production. Alkaline pretreatment on the other hand is effective in the dissolution and elimination of lignin from the biomass sample. Alkaline pretreatment of biomass is also applicable at room temperature, and its output generally relies on the amount of lignin in the biomass (Kumar et al., 2009). Major advantages of alkaline treatment include; warm reaction conditions, adequate elimination of lignin, the use of relatively cheap chemicals, and the probability of biomass division. The disadvantage of basic pretreatment is that it renders the biomass thick and jelly like and hence very difficult to filter. It also leads to difficulties in neutralization of the post-treatment mixture (Jendrzejczyk et al., 2019). The effects, advantages and disadvantages of acid and alkaline pretreatments are given in table 1.7.

Table 1. 7 Acid and Alkaline pretreatment methods for lignocellulosic feedstock.

Chemical pretreatment	**conditions**	**Results**	**Advantages**	**Disadvantages**
Alkaline	1-10% (w/v), 15-170°C for 1hour to 10 days	3.2% to 2.3 folds increase in CH_4 yield	Increases alkalinity and acts as a buffer, Solubilises lignin	Na^+ produced can inhibit digestion, high cost of chemical
Acid	1-4%(v/v), ambient temperature for a few minutes to hours	20-200% increase in CH_4 yield. Negative results have also been reported in very few cases	Effective in the solubilization of hemicellulose	May reduce pH, Produces inhibitors, corrosion of equipment

Adapted from Zheng et al. 2014

The two main agents that control the enzymatic digestibility of biomasses are the crystallinity of the cellulosic fraction of the substrate and its accessible surface area (McMillan 1992). The application of pretreatments increases the susceptibility of the cellulosic piece of the lignocellulosic biomass to enzymatic attacks and it also decreases the crystallinity of its microfibrils. Enlarging the surface and the accessibility of cellulose to enzyme allows them to bind to cellulose fiber surfaces and decreasing the crystallinity makes cellulose more reactive which increases the rate at which enzymes will be able to hydrolyse glycosidic bonds within the cellulose molecules. A major objective of pretreatment is to maximize the production of reducing sugars or the ability to form sugars by subsequent enzymatic hydrolysis. The production rates of the sugars relative to their rate of destruction must be maximized to achieve a high-yield pretreatment (McMillan 1992).

Figure 1.4 shows the breakdown of lignocellulosic components during pretreatment.

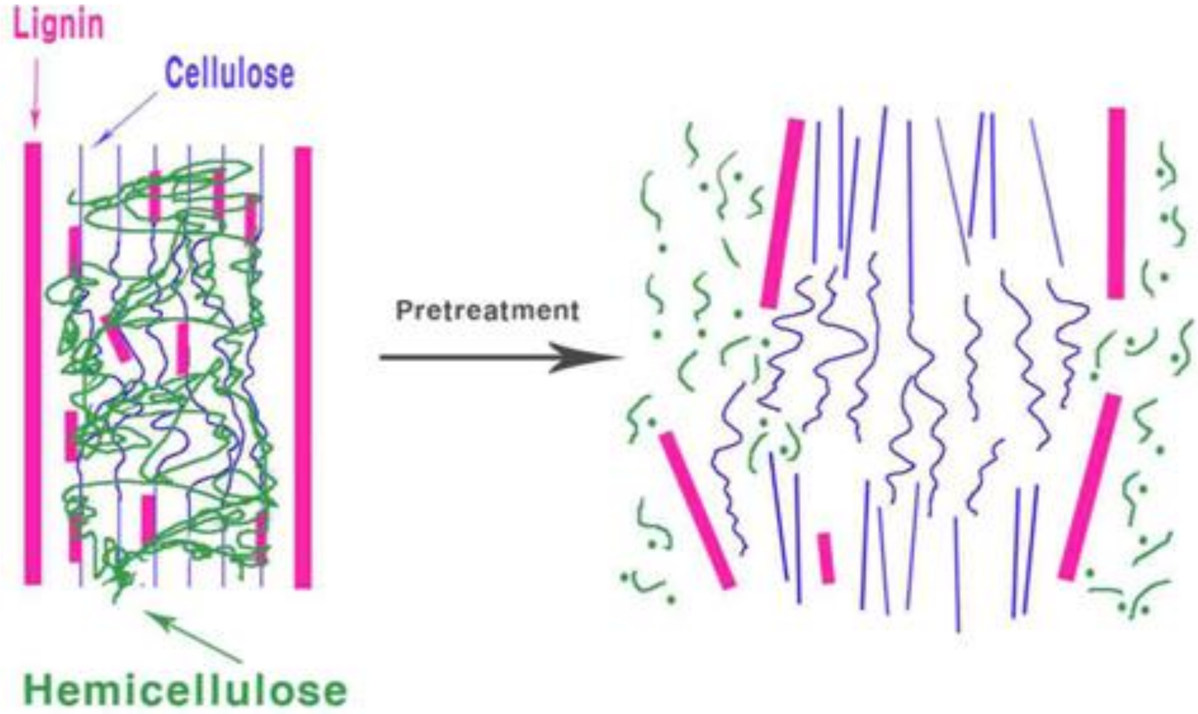


Figure 1. 4 Disruption of recalcitrant structures of lignocellulose upon the application of pretreatment (Puligundla et al. 2016).

2. MATERIALS AND METHODS

In this chapter, there will be a general characterization of the substrates followed by the measurements of physicochemical parameters and the pretreatment studies. It includes the description of the pretreatment types, conditions and the procedure for the preparation of solutions used in pretreatment and how it is applied to each biomass. The final part of the chapter is the description of the Biochemical methane potential test and the formulae for the calculation of biogas and methane produced in the experiments. As stated earlier, three physical pretreatment methods will be used viz. comminution, microwave, and hydrothermal (liquid hot water) pretreatment, and two chemical pretreatment techniques i.e. acid and alkaline pretreatment on two of the main agricultural wastes produced in Cameroon; maize straw and cattle manure.

2.1 Substrates

Two agricultural wastes; corn stover and cattle manure were used in this study.

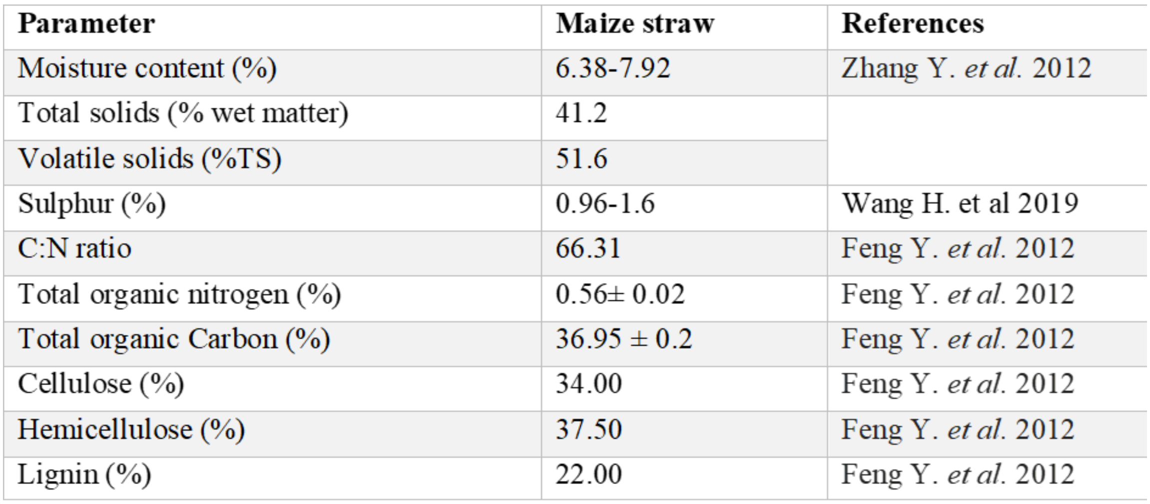
2.1.1 Maize Straw (Corn Stover)

Maize straw or stover consists of the leaves, cobs, and stalks that are left over after harvest and the removal of grains from the cobs. Estimates show that in the production of 1 kilogram of corn grain, approximately one kilogram of maize straw is simultaneously produced (Koundinya, et al. 2017). Maize straw is mostly utilised in the production of bioethanol through fermentation, and as forage for dairy animals in some regions of the world. Maize grains can equally be used as food for human consumption, fuel for bioenergy or as feedstock for bio-products such as starch and sugars. The straw can also be combusted in furnaces to generate heat energy that steam turbines to produce electricity. It equally has the potential for biogas production through anaerobic digestion. However, with the available technology, huge portions of the biofuel potential of the components of the straw such as cellulose is lost due to the strength of glycosidic bonds holding the glucose molecules together and because maize straw consists of approximately 15 to 20 percent lignin and about 70 percent of its total biomass is a combination of hemicellulose and cellulose (Koundinya V. 2009), pretreatment is required. Maize straw used for this study was obtained from a local market in the city of Ankara. It was then taken to the laboratory and preserved in a refrigerator at 4^{o}C for a few days before samples were prepared for pretreatment and biomethane production. First of all, the grains were removed, and then the cob, leaves and stalk cut into small pieces using a knife. A blender was then used to further ground the mixture into small particles which were then placed in a silver dish and kept in an oven for a few hours to reduce the moisture in the blended biomass. After about six hours, the biomass was transferred into a plastic dish and stored in a refrigerator for subsequent use in the measurement of total solids, volatile solids, reducing sugar, pretreatment and anaerobic digestion.

2.1.2 Characteristics of Maize Straw

Table 2.1 gives a summary of typical physicochemical characteristics of maize straw obtained in previous studies.

Table 2. 1 Typical characteristics of maize straw.



Parameter	**Maize straw**	**References**
Moisture content (%)	6.38-7.92	Zhang Y. *et al*. 2012
Total solids (% wet matter)	41.2	
Volatile solids (%TS)	51.6	
Sulphur (%)	0.96-1.6	Wang H. et al 2019
C:N ratio	66.31	Feng Y. *et al*. 2012
Total organic nitrogen (%)	0.56± 0.02	Feng Y. *et al*. 2012
Total organic Carbon (%)	36.95 ± 0.2	Feng Y. *et al*. 2012
Cellulose (%)	34.00	Feng Y. *et al*. 2012
Hemicellulose (%)	37.50	Feng Y. *et al*. 2012
Lignin (%)	22.00	Feng Y. *et al*. 2012

The biogas production from maize straw and other lignocellulosic plants from previous studies is encapsulated in table 2.2.

Table 2. 2 Biogas production from agricultural residues.

Substrate	CH_4 yield (mL/gVS)	Reference
Straw(maize, rice)	92-330	Merlin G. et al. 2013
Maize straw	344-462	Sambusiti Cecilia 2013
Maize stalks	246-267	
Maize silage	370-390	
Vegetable leaves	230-310	Merlin G. et al. 2013
Rotten fruits	210-510	Merlin G. et al. 2013
Fruit pressings	280-500	Merlin G. et al. 2013
Fruit peels	240-520	Merlin G. et al. 2013

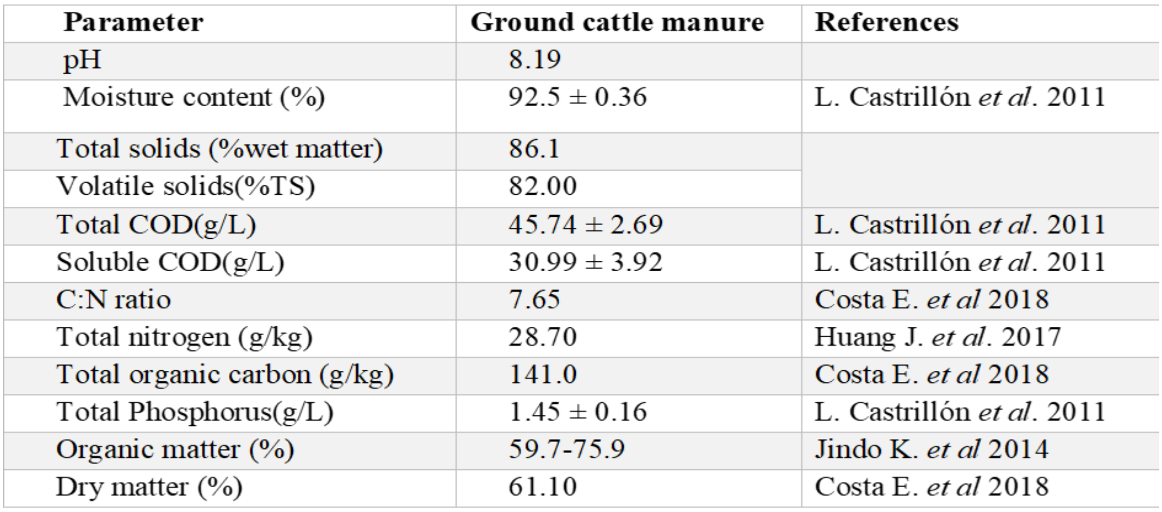
2.1.3 Cattle Manure

Cattle manure refers to the indigestible components of grass, leaves etc. which has passed through the cow's digestive system. Cattle and other livestock manure may contain significant amounts of lignocellulose depending on their diet and the amount of straw it contains. It generally contains between 30% to 80% lignocellulose with biodegradability levels ranging between 0.38% and 0.93% (M. Pilar, et al. 2017). According to the American society of agronomy, one cow, based on the size and age can produce between 20 and 50 kilograms of manure each day (American Society of Agronomy, 2015). It is very plentiful in minerals and can be adopted in farms as a fertilizer and for biogas production by subjecting it to anaerobic digestion. In the absence of appropriate disposal methods, it has the potential to cause negative impacts to public health and the environment because it can contaminate water bodies in its vicinity with pathogens present in the dung. It also produces unpleasant odours and greenhouse gases such as the production of methane and carbon dioxide after its breakdown by microorganisms during storage and the production of air borne ammonia amongst others. This means using cattle manure for biogas production is and attractive waste to energy conversion technology because it has the potential to produce methane that can be adopted as a renewable energy source while producing fertilizers with little adverse impacts on the environment. The cattle manure used in this study was obtained from a biogas plant located at Sincan in the outskirts of Ankara. It was transported to the laboratory and placed in plastic bottles and preserved in a refrigerator at a temperature of 4^{o}C for subsequent use. The total solids, volatile solids were then quantified according to standard methods (APHA 2005) while the reducing sugar was determined before and after pretreatment by the Miller method (Miller 1959). Based on the total solids, the cattle manure was then mixed with seed sludge on a 50:50 ratio in 100mL bottles for the biochemical methane potential (BMP) test.

2.1.4 Characteristics of Cattle Manure

Table 2.3 summarizes typical physicochemical characteristics of cattle manure obtained from previous works.

Table 2. 3 Physicochemical properties of cattle manure.



Parameter	Ground cattle manure	References
pH	8.19	
Moisture content (%)	92.5 ± 0.36	L. Castrillón *et al*. 2011
Total solids (%wet matter)	86.1	
Volatile solids(%TS)	82.00	
Total COD(g/L)	45.74 ± 2.69	L. Castrillón *et al*. 2011
Soluble COD(g/L)	30.99 ± 3.92	L. Castrillón *et al*. 2011
C:N ratio	7.65	Costa E. *et al* 2018
Total nitrogen (g/kg)	28.70	Huang J. *et al*. 2017
Total organic carbon (g/kg)	141.0	Costa E. *et al* 2018
Total Phosphorus(g/L)	1.45 ± 0.16	L. Castrillón *et al*. 2011
Organic matter (%)	59.7-75.9	Jindo K. *et al* 2014
Dry matter (%)	61.10	Costa E. *et al* 2018

Various studies have been done in the area of manure digestion for the generation of biogas. Table 2.4 encapsulates the biomethane production potential of animal manures from recent literature.

Table 2. 4 Summary of methane production values from animal manure in previous studies.

Manure type	Biogas (mLCH4/gVS)	Reference
Cattle manure	333	J. Ramos-Suarez et al. 2019
	240	A.K.P. Meyer et al. 2018
	292	Chen F. et al. 2017
	147-215	Abdullah M. et al. 2018
	150-280	Merlin G. et al. 2013
Goats manure	449	J. Ramos-Suarez et al. 2019
Sheep manure	452	J. Ramos-Suarez et al. 2019
Swine manure	370	A.K.P. Meyer et al. 2018
	461-470	J. Ramos-Suarez et al. 2019
Chicken manure	410-447	J. Ramos-Suarez et al. 2019
	400	A.K.P. Meyer et al. 2018
Cow slurry	200-300	Ertem F.C. 2011
Pig slurry	250-500	Ertem F.C. 2011

2.1.5 Inoculum

Sludge seed used for the biochemical methane potential (BMP) test was also collected from the same biogas plant where cattle manure was obtained. It was transported to the laboratory and kept in the refrigerator at a temperature of 4°C. The total and volatile solids of the sludge seed were measured following the standard methods (APHA 2005). Before using the sludge seed for the BMP test, it was activated by adding a small amount of glucose and placing it in an incubator at a temperature of 37°C for forty eight hours. The glucose is used by the bacteria as food to keep them alive while stored at the mesophilic temperature range which they require for optimal growth. The measured properties of the inoculum are given in table 2.5

Table 2. 5 Measured characteristics of seed sludge.

Total solids(% wet mass)	**Volatile solids(% wet mass)**	**pH**
90.73	96.65	7.43

2.2 Pretreatment studies

This study tests the effects of physical and chemical pretreatments on biogas generation from maize straw and cattle manure. Three physical pretreatment methods were applied which include comminution, microwave pretreatment and hydrothermal pretreatment. These pretreatments were also applied in combination, for instance comminution applied to maize straw before microwave or hydrothermal pretreatment. The pretreatment conditions for physical and chemical pretreatments used in this study are summarized in tables 2.4 and 2.5.

a) Physical Pretreatments

Table 2. 6 Summary of physical pretreatment types and conditions.

Pretreatment type	Conditions		
Comminution	Use of a knife and blender to chop maize straw into tiny pieces of sizes between 0.2-2mm by grinding.		
Liquid hot water	105°C for 30mins	120°C for 30mins	135°C for 30mins
Microwave	300W for 2 to 4 minutes		

b) Chemical Pretreatments

Table 2. 7 Summary of Pretreatment conditions for acid and alkaline pretreatments.

Substrate(g/100mLbottle)	Acid/base dosage(mL)	Temperature(°C)	Time(hours)
4.81g and 6.25g (5%TS)	47.5mL, 0.2M H_2SO_4	70	50
4.81g and 6.25g (5% TS)	47.5mL, 0.2M H_2SO_4	70	50
4.81g and 6.25g (5% TS)	47.5mL , 0.2M H_2SO_4	70	50
4.81g and 6.25g (5%TS)	47.5mL ,0.2M NaOH	120	0.5
4.81g and 6.25g (5% TS)	47.5mL , 0.3M NaOH	120	0.5
4.81g and 6.25g (5% TS)	47.5mL , 0.4M NaOH	120	0.5

5% biomass was used and based on the total solids, it was calculated as 4.81g of Cattle manure and 6.25g of maize straw.

2.2.1 Physical Pretreatment

The physical pretreatment methods used in this study are explained in the sections that follow.

2.2.2 Comminution

This pretreatment method was only applied on maize straw as it is not necessary for cattle manure. First of all, the maize grains were removed from the cob. The leaves, the cob and the stem were then cut into small pieces with the use of a knife. The pieces were then placed in a blender and a small amount of water added to them to facilitate the blending process**.** The combination was then ground into very tiny particles of sizes between 0.2-2mm and placed in a silver tray for drying to allow the moisture resulting from the addition of water to evaporate. It was then placed in a plastic dish, covered and placed in a refrigerator at 4°C for use in other pretreatment methods and for the biochemical methane potential (BMP) test.

2.2.3 Liquid hot water (LHW) treatment

5 % (w/w) total solids i.e. 4.81g cattle manure was weighed on an electronic balance and 95% (v/v) medium of deionized water was measured in a measuring cylinder and mixed with the cattle manure in a 100mL beaker. The mixture was closed using an aluminium foil to prevent the solution from spilling out during bubbling.

5% total solids (6.25g) of the blended maize straw was carefully weighed on an electronic balance. 47.5mL of distilled water was measured in a measuring cylinder and put in the beaker. They were mixed and the solution covered using an aluminium foil. For both biomasses, samples were done in duplicates and placed in an autoclave at three different temperatures i.e. 105°C, 120°C and 135°C for 30 minutes each. The beakers were removed after the autoclave cooled down, the solutions were centrifuged and then sifted using cellulose acetate membrane filters with holes of 0.45µm in size. The filtered liquid was put in 50mL falcon tubes and placed in a refrigerator. Part of it was used to measure the reducing sugar of the biomasses while the remaining part was used for anaerobic digestion.

2.2.4 Microwave pretreatment

Microwave pretreatment was applied to both biomasses: maize straw and cattle manure. Exactly 4.81g of cattle manure and 6.25g of maize straw were weighed on an electronic balance and put in 100mL beakers. Both were performed in duplicates and 47.5mL deionized water was added to each beaker. They were then closed using aluminium foils and placed in the microwave at a power of 300W. One set was performed for 2 minutes and another set for 4 minutes. After 2, and 4 minutes respectively, the beakers were removed from the microwave, allowed to cool down, centrifuged and sifted using cellulose acetate membrane filters of pore size 0.45µm. The filtered liquids were put in 50mL falcon tubes and placed in refrigerator at 4°C for use in the determination of reducing sugar and biogas production.

2.3. Chemical Pretreatment

Two chemical pretreatment methods were used in this study. They include acid and alkaline pretreatments. The procedures for this pretreatments are explained in the sections that follow;

2.3.1. Alkaline Pretreatment

Alkaline pretreatment was performed using dilute solutions of sodium hydroxide (0.2M, 0.3M and 0.4M) representing concentrations of 0.8%w/v, 1.2%w/v and 1.65w/v respectively. Alkaline pretreatment was assisted with heating at temperatures of 120°C for 30 minutes in an autoclave. Heating is recommended because it increases the reaction rate hence increasing the efficiency of the pretreatment process. Sodium hydroxide has been chosen for this study because it is most effective in increasing the digestibility and fractionation of agricultural residues, it increases sugar production, elimination of lignin and the high rate of biomass utilization. The use of dilute NaOH solutions also loosens biomass structures, damages the linkages between lignin and carbohydrates, increases internal surface area and decreases the extent of cellulose crystallinity and polymerization (Zhao et al. 2008). The application of alkaline pretreatment, began with the preparation of dilute NaOH solutions as follows;

Preparation of 0.2M, 0.3M and 0.4M $NaOH_{(aq)}$ solutions: exactly 8g, 12g and 16g (for the preparation of 0.2M (0.8% w/v), 0.3M (1.2% w/v) and 0.4M (1.6%w/v) solutions respectively) of 100% pure sodium hydroxide pellets were weighed on an electronic balance. The pellets were then dissolved in 100mL beakers using distilled water. A stirring rod was used the stir the pellets until they were dissolved completely. The mixture was then moved into 1000mL volumetric flasks. The beakers and stirring rods were cleaned several times and the solution was topped up to the volumetric flasks. A water bottle was then used to make up the solution to the 1L marks in a drop wise manner. The solutions were then mixed and stored at room temperature for subsequent use in biomass pretreatments.

Alkaline protocol

4.81g of cattle manure and 6.25g of maize straw were weighed on an electronic balance. Secondly, 47.5mL of 0.2M, 0.3M and 0.4M solutions of sodium hydroxide were measured using a measuring cylinder. One set of samples were treated in an autoclave at 120^{o}C while there was no heat for the other set. The alkaline solutions were added to the biomasses in 100mL beakers and closed with aluminium foils (for those samples to be treated with heat). They were then kept in an autoclave at a temperature of 120^{o}C for 30 minutes. After half an hour, the specimens were removed kept to cold down and then centrifuged to separate the solid and liquid mixtures. The samples were then sifted using glucose acetate membrane filters with pore sizes of 0.45µm, put into 50mL falcon tubes and stored in a refrigerator. Part of the filtered liquid was used for reducing sugar measurement and the other part used for anaerobic digestion in combination with the unfiltered biomass. For both samples, the measurements were performed in replica and the average of the outcomes were taken in order to minimize random errors.

2.3.2 Acid Pretreatment

Acid pretreatments were performed on both biomasses using dilute solutions of sulphuric acid. Sulphuric acid was chosen because of its high reactivity and its effectiveness in the breakdown of celluloses to their constituent sugars such as glucose. Also, sulphuric acid has been reported to completely dissolve hemicellulose into its component sugars such as galactose (Zheng et al 2014) and hence increases the rate of anaerobic digestion and methane yield from lignocellulosic biomasses. Concentrations of 0.2M (1.1%v/v), 0.3M (1.7%v/v) and 0.4M (2.2% v/v) solutions of sulphuric acid were used with mild heat conditions for 50 hours and high temperatures for 30 minutes in an oven and autoclave respectively.

Preparation of 0.2M, 0.3M and 0.4M $H_2SO_{4(aq)}$ solutions: Exactly 11.01mL, 16.57mL and 22.13mL of the 96.0% sulphuric acid were measured using a measuring cylinder. Distilled water was added to three different 1L volumetric flasks. The sulphuric acid was then transferred from the measuring cylinders to the half-filled volumetric flasks. Distilled water was then added drop wise from a water bottle until it reached the 1000mL marks of the volumetric flasks. The solutions were mixed properly and stored at room temperature for use in the biomass pretreatments.

Acid Protocol

Exactly 4.81g of cattle manure and 6.25g of maize straw were measured on an electronic balance using 100mL beakers. 47.5mL of the 0.2M, 0.3M and 0.4M sulphuric acid solutions were measured using a measuring cylinder. For both concentrations, the samples were performed in duplicates. Maize straw samples were treated in mild heat for 50 hours and also in an autoclave at 120^{o}C for half an hour while cattle manure was treated just once; with the autoclave at 120^{o}C for 30 minutes under pressure.

47.5mL of 0.2M, 0.3M and 0.4M sulphuric acid solutions were added to 100mL beakers containing 6.25g maize straw. The beakers were then closed and kept in an oven at 70^{o}C for 50 hours and the other set placed in an autoclave at 120^{o}C for 0.5hour. After 50 hours, the maize straw in the oven was removed, cleaned with distilled water and dried at room temperature for one day. Meanwhile the maize straw in the autoclave was removed after 30 minutes, it was allowed to get cool, centrifuged and then sifted using cellulose acetate membrane filters and the filtered liquid used for the measurement of reducing sugars.

The cattle manure was also removed from the autoclave after 30 minutes and filtered (for the experiment used in the determination of soluble sugar) while for the experiment used in biogas production, the samples were allowed to cold down and then mixed with seed sludge and used for anaerobic digestion tests.

2.4 Biochemical Methane Potential (BMP) Test

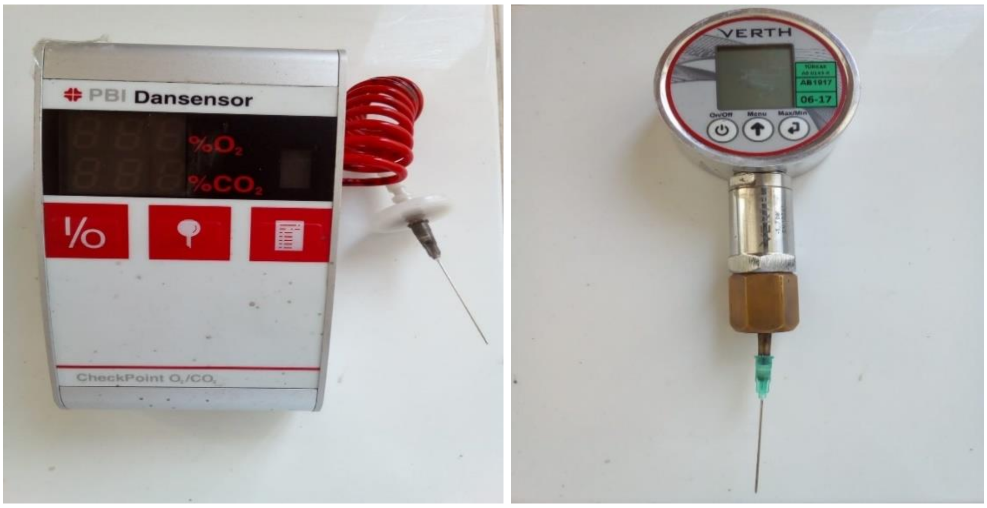
5% biomass and 95% medium were used in the BMP test for both substrates. The various Pretreatments described above were applied on the samples and they were mixed with activated seed sludge in a 50:50 ratio in 100mL bottles. The sludge plus sample made up 70% of the bottle’s volume with 30% empty space left for the biogas produced to be collected. The pH of the mixture was neutralised to between 7.0 and 7.5 using 1M hydrochloric acid and 1M sodium hydroxide solutions. After neutralisation, about 1 gram sodium bicarbonate was added to the mixture to sustain the pH throughout the anaerobic digestion process. The bottles were closed using rubber stoppers and para film plastics to prevent leakage and a syringe was used to extract the air present in the bottle in order to get rid of any oxygen present in it. The bottles were kept in an incubator at 37°C and the biogas produced was measured once every day until the biomass became exhausted and no gas was produced anymore. A summary of the conditions applied in the biochemical methane potential tests is given in table 2.8.

Table 2. 8 summary of conditions applied in BMP tests.

Temperature (°C)	**pH**	**Time (days)**	**Stirring(per minute)**
37	7.0-7.5	30-40	60

The measurement was done by determining the pressure exerted by the gas produced in the bottle using a manometer and the carbon dioxide sensor was used to measure the percentage of methane and carbon dioxide produced by the samples each day. The set up and instruments used in the biogas measurements are given in figure 2.1.

(a) Set of batch experiments in an incubator (b) single bottle



(c) CO_2/O_2 sensor (d) Manometer

Figure 2. 1 BMP tests set up and instruments used in biogas measurement.

The ideal gas equation was used to calculate the quantity of biogas generated in mL of methane per gram volatile solid. In theory, complete biodegradation of 1kg of COD yields 0.35m^3 of methane at standard pressure and temperature. From the ideal gas law, a mole of any gas at STP fills a volume of 22.4L but in practical conditions, this is hardly achieved because some of the biomass is not fully degraded by microorganisms. Some of the biomass may also be used up by the microbes for their metabolic activities. The specific methane production in millilitres per gram of substrate volatile solid was calculated from the following formula;

$$BMP = \frac{V_{CH4s} - V_{CH4b}}{VSs} \qquad equation\ 1$$

Where VCH_4s = the amount of methane gas produced by substrate in mL

$VCH_4\,b$= the quantity of methane generated by the blank/control in mL

VSs= the volatile solids (%) of the substrate

The results of the BMP tests during this study are given in chapter three.

2.5 Analytical Methods

2.5.1 Measurement of pH and Electrical Conductivity

After the pretreatments and filtration, the pH and electrical conductivity were obtained from a pH meter. The meter has two probes: one measures the pH and the other measures the electrical conductivity of the solution. To measure the pH, the electrical conductivity probe is closed or placed in a neutral solution such as distilled water. The pH probe is then immersed in the solution and allowed for a few minutes to stabilise. After stabilization, the pH value on the screen is read and recorded and the probe was removed and rinsed with deionized water. This action was repeated for all samples and their duplicates. The average was then recorded as the pH value of the solution. To measure the electrical conductivity, the pH probe was rinsed and placed in a neutral solution while the conductivity probe was immersed in the solution. After a few seconds, it stabilised and the value on the screen was read and recorded. The probe was then removed, rinsed and the process repeated for all samples and their duplicates with the average for each sample set taken as the electrical conductivity. At the end of these measurements, the solutions were again filled in 50mL falcon tubes and stored in a refrigerator at 4^oC for subsequent use.

2.5.2 Total Solids (TS)

First of all the maize straw was chopped into fine pieces using a blender. It was then kept in a silver can and kept in an oven for 6 hours to reduce the moisture. After that, two porcelain dishes were washed and dried in an oven at 105^oC for one hour. After 60 minutes, the two porcelain dishes were removed and kept in a desiccator to cool down and also to prevent them from trapping some moisture. They were then labelled and placed one after the other on an electronic balance to get the weight of the empty dishes. A known amount of biomass (cattle manure and maize straw) were carefully added to the dishes and the weight of the dish plus the biomass recorded. The two porcelain dishes were kept in an oven at a temperature of 150°C for 12 hours. After 12 hours, the porcelain dishes containing the biomasses were removed and placed in a desiccator for about one hour to get dry free from moisture. They were then weighed on an electronic balance to determine the change in weight from the initial weight of the dish and moist biomass to the dried and moisture free dish and biomass. Calculations were then made to obtain the biomass percentage as an average between the changes in weight of the two porcelain dishes. The calculated total solids in this study in terms of total biomass were; 86.1% for cattle manure and 41.2% for maize straw.

2.5.3 Volatile solids (VS)

To determine the volatile solids of the biomasses, the dried biomass from the total solids measurement above was burned in an oven at 550°C for two hours. The dishes were then removed and kept in a desiccator for one hour to cold down. After cooling, the dishes and the ash left after burning at 550°C for 120 minutes were weighed on an electronic balance and the changes in weight between the dried dish and biomass and the dried dish and ash were recorded. Calculations were made to obtain the volatile solids in percentage as the average between the two dishes for each biomass. The calculated volatile solids in this study were as follows; 82.0% VS (in terms of total solids) for cattle manure and 51.6% VS for maize straw.

2.5.4 Measurement of Soluble Sugar

The method used to determine the reducing sugar loads for both the untreated and treated biomass samples used in the study was the G. Lorenz Miller method (G. L. Miller 1959) using the Dinitrosalicylic acid reagent (DNS). The method is described below.

Media preparation

Requirements: Glucose, DNS, Rochelle salt, spectrophotometer (adjusted to 575µm), test tubes, heater, cells, and 3mL pipettes, heating magnetic stirrer, water etc.

Procedure

The DNS reagent containing 1% of the Dinitrosalicylic acid (DNS), 1% NaOH and 0.05% sodium sulphite was added to a beaker. A stirring rod was used to stir the mixture until it was wholly dissolved. The mixture was then moved into a 1000mL volumetric flask and the beaker and stirring rod cleaned several times into the flak. Deionized water was added drop wise into the flask until it reached the one litre mark. The DNS solution was then mixed and covered with aluminium foil to prevent it from direct light rays. At the same time, 40% of Potassium sodium tartrate (Rochelle salt) was weighed on an electronic balance. The Rochelle salt was placed in a 100mL beaker and dissolved using pure water and a stirring rod until it was wholly dissolved. The mixture was moved into a 250mL volumetric flask. The beaker and stirring rod were cleaned several times into the volumetric flask. A drop bottle was used to add distilled water drop wise until it reached the 250mL mark.

20mg, 40mg, 60mg, 80mg, 100mg, 120mg, 140mg and 160mg of glucose monohydrate powder were measured on an electronic balance. They were each dissolved in 100mL beakers using deionized water and the solution transferred into 100mL volumetric flasks. The beakers and stirring rods were rinsed into the flasks and deionized water added to the solution to make it to the 100mL mark in each of the volumetric flasks. About 500mL of water was heated in a beaker until it reached boiling point.

For the standard curve, 17 test tubes were labelled and placed in a plastic grid because for each concentration, the tests were performed in duplicates. 3mL of the DNS solution was added to 3mL of the various glucose solutions in the test tubes using a pipette while 3mL of deionized water was added to 1 test tube serving as the blank. The test tubes were then kept in the boiling water for 5 minutes. After 5 minutes, they were removed and placed in cool water for a few minutes while 1mL of the Rochelle salt solution was added to each of them.

The addition of Rochelle salt prevents the reagent from absorbing oxygen and also to prevent the Rochelle salt initially found in the DNS reagent from interfering with the defensive action of the sulphide, which is essential to colour stability. The colour intensities were measured using cells placed in a spectrophotometer at 575mµ and width of 0.06 mm. The results obtained for the standard curve are given in table 2.9 and figure 2.2;

Table 2. 9 Absorbance values for the standard curve in the measurement of reducing sugar.

Concentration of glucose(mg)	Absorbance
20	0.09
40	0.152
60	0.227
80	0.324
100	0.404
120	0.530
140	0.500
160	0.655

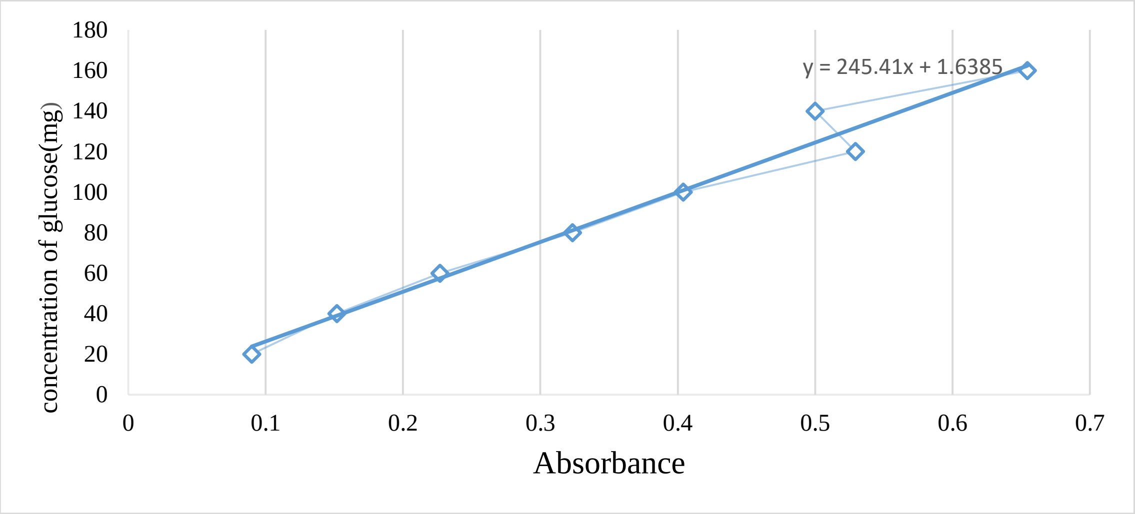


Figure 2. 2 Standard curve for the determination of reducing sugar using the Miller method.

2.6 Data Collection

Data for the physical parameters such as pH and electrical conductivity were determined onsite while the reducing sugar contents for both biomass samples were determined from the spectrophotometer as described in section 3.2 below and calculated based on the standard curve equation. The data for biogas produced (pressure and carbon dioxide were measured daily and weekly respectively until the biomass was exhausted and gas production ceased. The total and volatile solids were measured for both samples as described above using an electronic balance, an oven and porcelain dishes. The biogas produced was calculated per gram VS while the carbon dioxide sensor was used to quantify the percentage of carbon dioxide and hence methane in the biogas produced. It is assumed that the gas produced contains just methane and carbon dioxide and therefore the remainder of whatever the carbon dioxide sensor reads is assumed to be methane.

2.6.1 Biogas Measurement (carbon dioxide concentration)

The carbon dioxide content of the samples was measured once every week using the carbon dioxide sensor. The sensor can measure both the carbon dioxide and oxygen content of a mixture of gases. To measure the carbon dioxide percentage, a syringe is attached to the sensor which is then inserted into the bottle containing the biogas through the rubber stoppers. The sensor is started and carbon dioxide is selected on the top right corner of the sensor. It is allowed for a few seconds to stabilise and then the reading is recorded. This is done in duplicates for each pretreatment method and condition and the average recorded as the percentage of the gas. The main reason for measuring the carbon dioxide is to be able to calculate the quantities of the constituents (methane and carbon dioxide) of the biogas produced.

3. RESULTS AND DISCUSSION

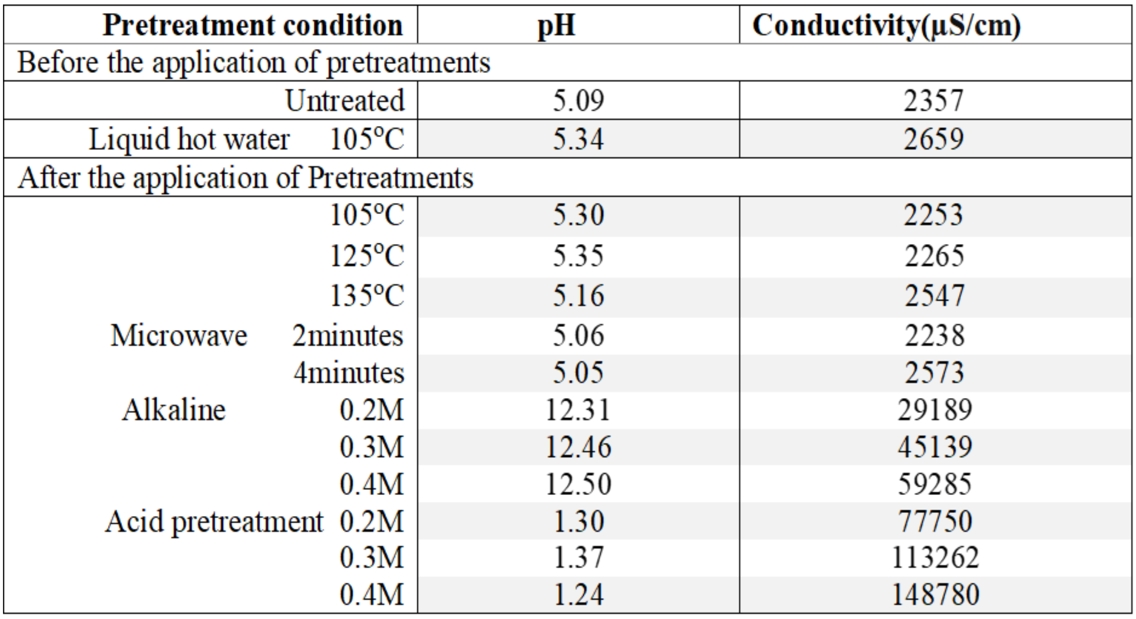
In chapter three, a presentation of the results in tables, charts and graphs will be done. Firstly, physicochemical parameters (pH, electrical conductivity, soluble sugar, and the total and volatile solids) will be presented and discussed followed by the results of the pretreatment studies and BMP tests for both biomasses.

3.1 Electrical Conductivity and pH

The results of the pH and electrical conductivity in this study measured using the pH meter are given in table 3.1. The values for the untreated portions were measured before the application of the pretreatment studies while those for liquid hot water, microwave, alkaline and acid pretreatment were measured after the application of the respective pretreatment studies.

a) Maize straw

Table 3. 1 pH and electrical conductivity for pretreatments of maize straw.



Pretreatment condition		pH	Conductivity(µS/cm)
Before the application of pretreatments			
	Untreated	5.09	2357
Liquid hot water	105°C	5.34	2659
After the application of Pretreatments			
	105°C	5.30	2253
	125°C	5.35	2265
	135°C	5.16	2547
Microwave	2minutes	5.06	2238
	4minutes	5.05	2573
Alkaline	0.2M	12.31	29189
	0.3M	12.46	45139
	0.4M	12.50	59285
Acid pretreatment	0.2M	1.30	77750
	0.3M	1.37	113262
	0.4M	1.24	148780

The pH values measured after the application of different pretreatments to maize straw varied between 1.24 and 12.5 with the samples treated with sulphuric acid showing very low pH values and those treated with sodium hydroxide showing very high pH values. The pH values for the samples pretreated with liquid hot water (LHW) and the microwave were approximately the same (around 5). The pH of both of them was neutralised to between 7.0 and 7.5 which is the range most suitable for methanogenic bacteria to operate.

Also, the samples treated with sulphuric acid showed the highest electrical conductivity followed by those treated with sodium hydroxide solutions. The conductivity values for samples treated with the microwave and liquid hot water were similar. This suggests that sodium hydroxide and sulphuric acid pretreatment breaks down the biomass into finer particles producing more dissolved material in the solution and hence rendering it more biodegradable.

b) Cattle Manure

Table 3. 2 pH and electrical conductivity for pretreatments of cattle manure.

Pretreatment method	**pH**	**Electrical conductivity(µS/cm)**
Before the application of pretreatments		
Untreated	8.19	2552
After the application of Pretreatments		
Liquid hot water 105ºC	8.46	3036
125ºC	8.68	2807
135ºC	8.40	2758
Microwave 2minutes	8.06	3028
4minutes	8.48	3165
Alkaline 0.2M	12.35	30740
0.3M	12.46	44587
0.4M	12.53	55762
Acid 0.2M	1.38	73353
0.3M	1.46	112430
0.4M	1.55	152139

From table 3.2, it can be seen that the pH fluctuates between 1.38 and 12.53 based on the pretreatment conditions that was applied. Raw cattle manure had a pH of 8.19 and electrical conductivity of 2552 µS/cm. The samples treated with acid had very low (acidic) pH values while those treated with sodium hydroxide had very high pH values. The pH of the samples was neutralised to between 7.0 and 7.5 after pretreatments. It can also be seen that the samples treated with sulphuric acid and sodium hydroxide had the highest electrical conductivity while those treated with liquid hot water showed the lowest. This is probably because sodium hydroxide and sulphuric acid pretreatment breaks down the biomass into finer particles producing more dissolved material in the solution and hence rendering it more biodegradable. This might also stem from the dissociation of the base and the acid into their constituent cations and anions i.e. the hydrogen ion (H^+), sulphate ion (SO_4^{++}), sodium ion (Na^+) and the hydroxyl ion (OH^-) which are very mobile ions and hence increased electrical conductivity.

3.2. Effects of Pretreatment on Solubility

The solubility of the organic matter in the biomasses after pretreatment studies were determined by measuring the soluble sugar concentration they produced. To get the reducing sugar of the substrate samples (by Miller method), 3mL of the various biomass samples were added into labelled test tubes containing 3mL of the DNS reagent using a pipette. The test tubes were kept in bubbling hot water for 5 minutes for the colours to come out and then removed. 1mL of the Rochelle salt solution was then added to the entire test tubes which were then allowed to cold down. The colour intensities were measured using cells placed in a spectrophotometer at 575mµ and width of 0.06 mm. The results obtained for each of the pretreatments and the untreated samples are summarised in tables 3.3 and 3.4.

a) Maize Straw

Table 3. 3 Amount of reducing sugar from pretreatment of maize straw.

Pretreatment condition		**Reducing sugar (mg/g biomass)**
Untreated		60
Acid pretreatment	0.2M H_2SO_4	199
	0.3M H_2SO_4	197
	0.4M H_2SO_4	204
Alkaline	0.2M NaOH	290
	0.3M NaOH	251
	0.4M NaOH	240
Liquid hot water	105°C	82
	105°C	92
	120°C	99
	135°C	115
Microwave	2 minutes	216
	4 minutes	237

From table 3.3, it can be seen that pretreatments of maize straw lead to increases in the production of soluble sugar. The pretreatment type/condition that lead to the highest production of soluble sugar was the 0.2M NaOH with a total concentration of 290mg/g biomass representing an increase in solubility of 382% while the pretreatment condition with the relatively lowest soluble sugar production was the liquid hot water 105°C producing an average concentration of 82mg/g biomass representing an increase of about 37%. In general alkaline pretreatment proved to be the most effective pretreatment type producing more reducing sugars which in total were about four times more than the raw sample. Physical pretreatments also proved to be very effective in increasing solubility of maize straw. Microwave pretreatment was the most effective physical pretreatment method producing more than three times the quantity of reducing sugar produced by the raw samples. Liquid hot water pretreatment produced the lowest amount of reducing sugars compared with the microwave, sulphuric acid and alkaline pretreatments. The liquid hot water pretreatment condition that produced the relatively lowest amount of reducing sugar was the 105°C while that which produced the relatively higher amount was the 135°C. This suggests that solubility of maize straw increases when the temperature increase. The comparisms of the effects of pretreatment on the solubility of maize straw is shown on figure 3.1.

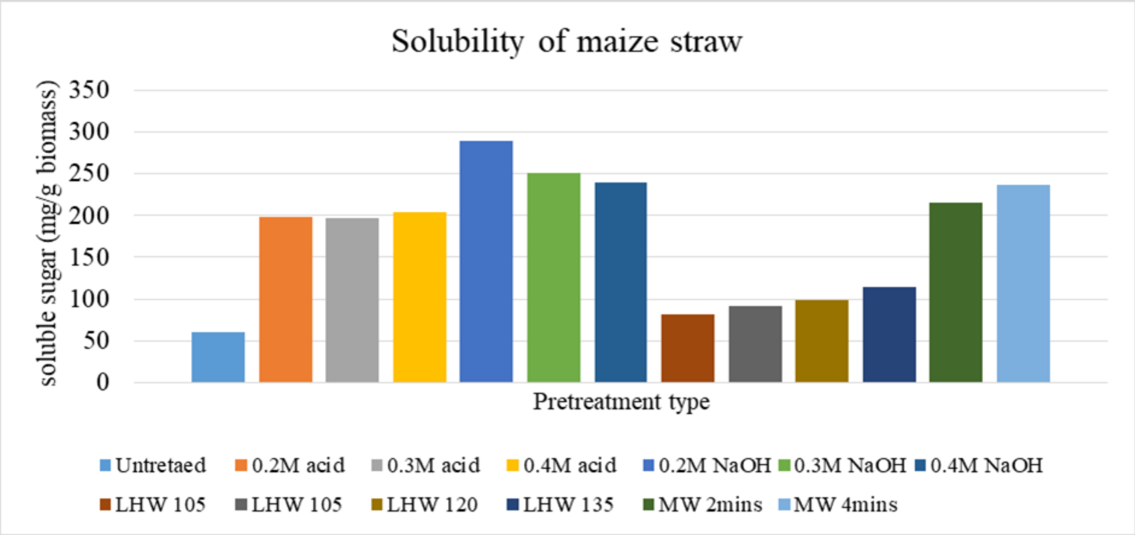


Figure 3. 1 Solubility of maize straw.

b) Cattle manure

Table 3. 4 Amount of reducing sugar from pretreatment of cattle manure.

Pretreatment condition		Reducing sugar (mg/g biomass)
Untreated		56
Acid pretreatment	0.2M H_2SO_4	170
	0.3M H_2SO_4	198
	0.4M H_2SO_4	186
Alkaline	0.2M NaOH	147
	0.3M NaOH	154
	0.4M NaOH	131
Liquid hot water	105°C	77
	120°C	120
	135°C	189
Microwave	2 minutes	190
	4 minutes	171

Table 3.4 shows the effects of pretreatment on the solubility of cattle manure. It can be seen that the pretreatment condition which produced the highest amount of reducing sugars was 0.3M H_2SO_4 with total concentration of 198mg/g biomass representing an increase of about 254% while the pretreatment condition with the relatively lowest soluble sugar production was the liquid hot water 105°C producing an average concentration of 77mg/g biomass which represents an increase in solubility of about 38%. Generally, both chemical pretreatments produced relatively higher amounts of soluble sugar compared to the raw sample. Physical pretreatments also proved to be very effective in increasing solubility of cattle manure. Microwave pretreatment was the most effective physical pretreatment method producing more than three times the amount of soluble sugar produced by the untreated samples. Liquid hot water pretreatment was also very effective; except for the samples treated at a temperature of 105°C, the amount of sugar produced was more than double that of the raw manure samples. This suggests that solubility of cattle manure also increases when the temperature is raised i.e. Higher temperatures, make the manure more soluble and hence it produces more soluble sugars. The comparisms of the effects of pretreatment on the solubility of cattle manure are shown on figure 3.2.

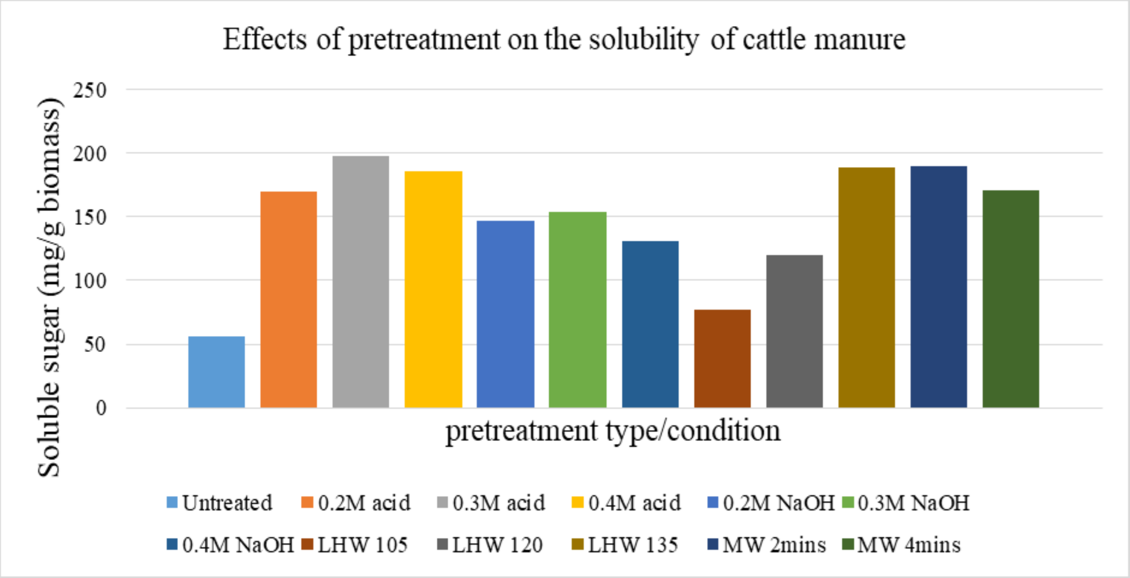


Figure 3. 2 Amount of reducing sugar produced after pretreatment of cattle manure.

3.3. Comparisms of the Solubility of Substrates

Comparatively, maize straw produced higher amounts of soluble sugar than cattle manure. This shows that for reducing sugar production, the applied chemical and physical pretreatments were more effective for maize straw than cattle manure. It also means that for this study, maize straw has a higher biogas production potential than cattle manure. Liquid hot water pretreatments increased the solubility of cattle manure more than maize straw while acid, alkaline and microwave pretreatments increased the solubility of maize straw more than cattle manure.

3.4. The Relationship between Reducing Sugar and Methane production

The relationships between the reducing sugar production and methane production from treated and raw samples of maize straw and cattle manure are given on figures 3.3 and 3.4.

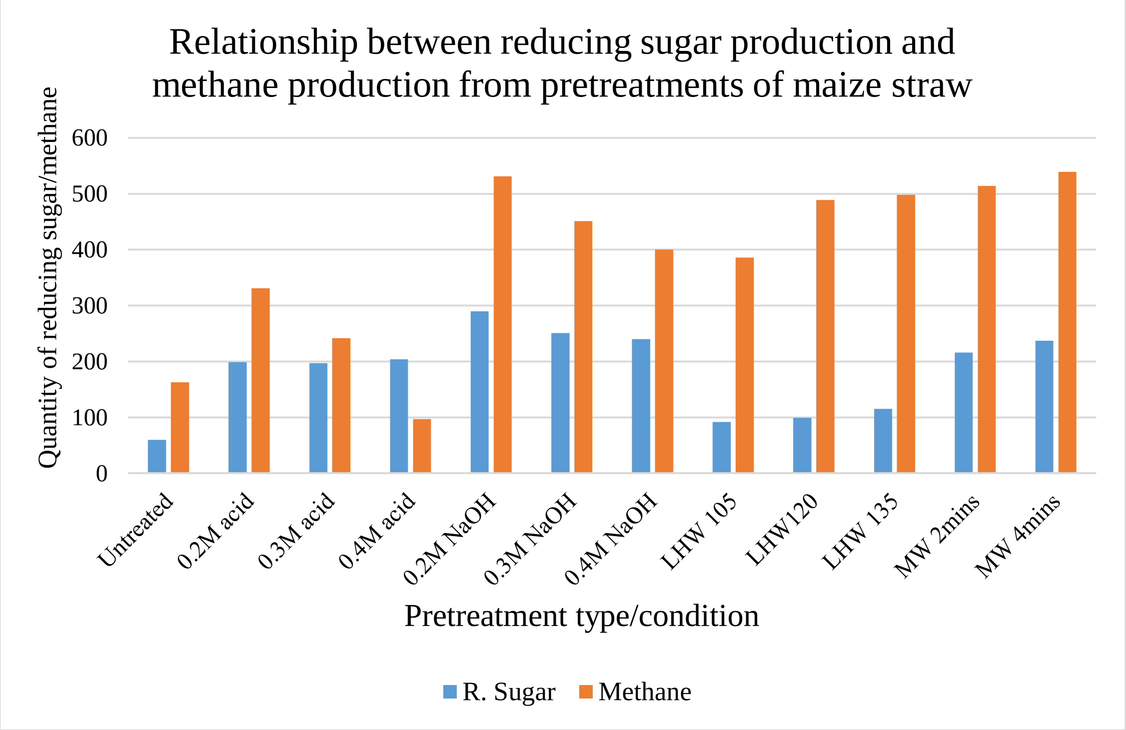


Figure 3. 3 Relationship between measured reducing sugar and methane production from maize straw.

The bar chart on figure 3.3 above shows the relationship between reducing sugar production and cumulative biogas production from raw and pretreated maize straw. It shows that the pretreatment methods which produced the highest quantity of soluble sugar and biogas production are alkaline pretreatment and microwave pretreatments. Acid and liquid hot water pretreatments produced the relatively lowest amounts of reducing sugar and while the methane production for acid pretreatment was relatively lower, the highest temperatures of liquid hot water pretreatments produced higher volumes of methane.

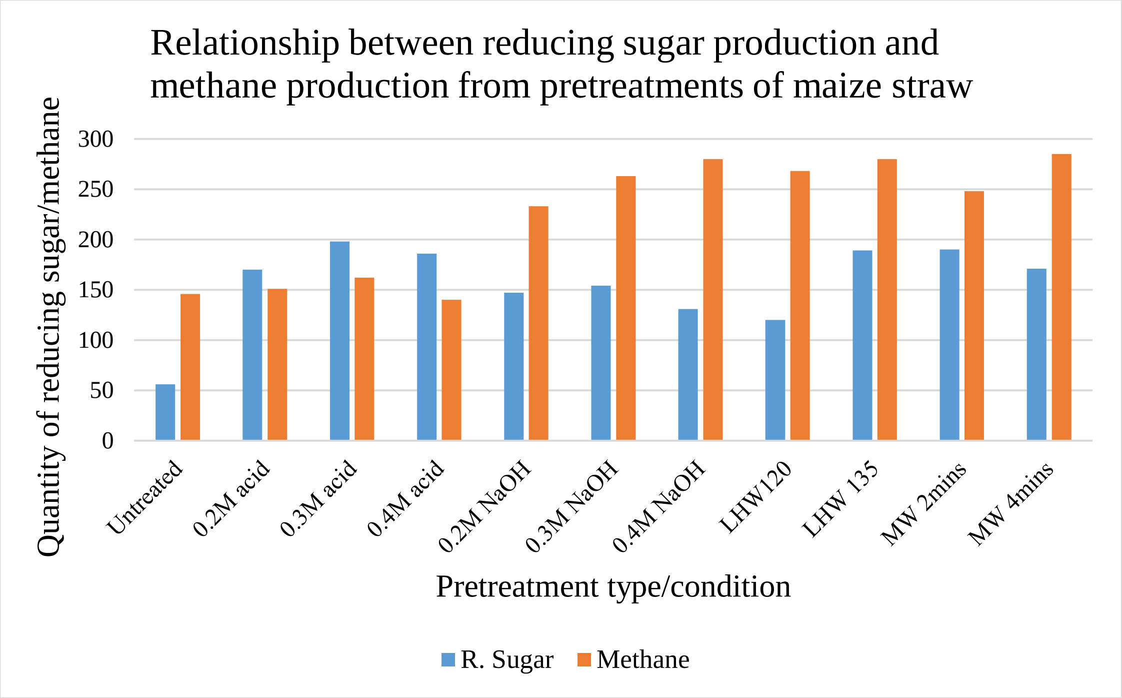


Figure 3. 4 Relationship between measured reducing sugar and biomethane production from cattle manure.

Figure 3.4 shows the relation between the reducing sugar production and cumulative biogas production from untreated and pretreated cattle manure. It can be seen that both physical and chemical pretreatments led to high production of reducing sugars. It can also be seen that with the exception of acid pretreatment, the higher reducing sugar production corresponds to higher methane production.

Comparatively, maize straw produced higher reducing sugar concentrations than cattle manure. It can also be seen that maize straw samples produced higher volumes of methane compared to cattle manure.

3.5. Pretreatment of Maize Straw

The outcome of the biochemical methane potential tests before and after pretreatments of maize straw are given in the following sections.

3.5.1. Acid Pretreatment of Maize Straw

In acid pretreatment, dilute concentrations of sulphuric acid assisted by heat were used. Three different concentrations 0.2M (1.1%v/v), 0.3M (1.6%v/v) and 0.4M (2.2%v/v) were tested on the maize straw. Heat was equally applied to both samples by placing them in an oven and the temperature set at 70°C for 50 hours before the samples were removed, cleaned with deionized water and dried at 60°C before starting the BMP test. The results obtained are presented in table 3.5.

Table 3. 5 Results of the BMP test for Acid pretreatment of maize straw.

Day	Biogas production (mLCH_4/gVS/day)							
	0.2M	Sum	0.3M	Sum	0.4M	Sum	Untreated	Sum
1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	32.20	32.20	26.93	26.93	15.29	15.29	13.10	13.10
6	41.23	73.43	30.21	57.14	18.16	33.45	13.59	26.69
9	43.36	116.79	30.57	87.71	26.56	60.01	14.89	41.58
12	44.60	161.39	29.49	117.20	8.17	68.18	15.70	57.28
15	45.24	206.63	30.97	148.17	10.55	78.73	16.52	73.80
18	45.86	252.49	29.82	177.99	8.53	87.26	21.63	95.43
21	40.77	293.26	29.03	207.02	7.29	94.55	23.22	118.65
24	26.93	320.19	25.42	232.44	3.20	97.75	23.94	142.59
27	11.27	331.46	10.23	242.67	-	-	12.18	154.77
30	-	-	-	-	-	-	8.45	163.22

Biogas measurements were carried out daily or in three day intervals. As seen on the table 3.5, the biogas production increased rapidly from day 3 until it reached the point of maximum production between 15 and 18 days for the samples treated with 0.2M and 0.3M H_2SO_4 while the samples treated with 0.4M H_2SO_4 reached their highest production around the 9th day. The samples treated with 0.2M H_2SO_4 produced their maximum biogas on the 18th day with a total production of 45.86mLCH_4/gVS. It also shows that biogas production started falling on the 21st day and continued to fall until it reached the lowest point around the 27th day. The samples treated with 0.3M H_2SO_4 had the second highest biogas production with its maximum production of 30.97mLCH_4/gVS occurring on the 15th day and then it started falling until it reached the lowest production on the 27th day. The samples treated with 0.4M concentration had the least production and the earliest maximum point. The highest production of 26.56mLCH_4/gVS occurred on the 9th day.

The daily biogas production from acid pretreatment of maize straw is given on figure 3.5

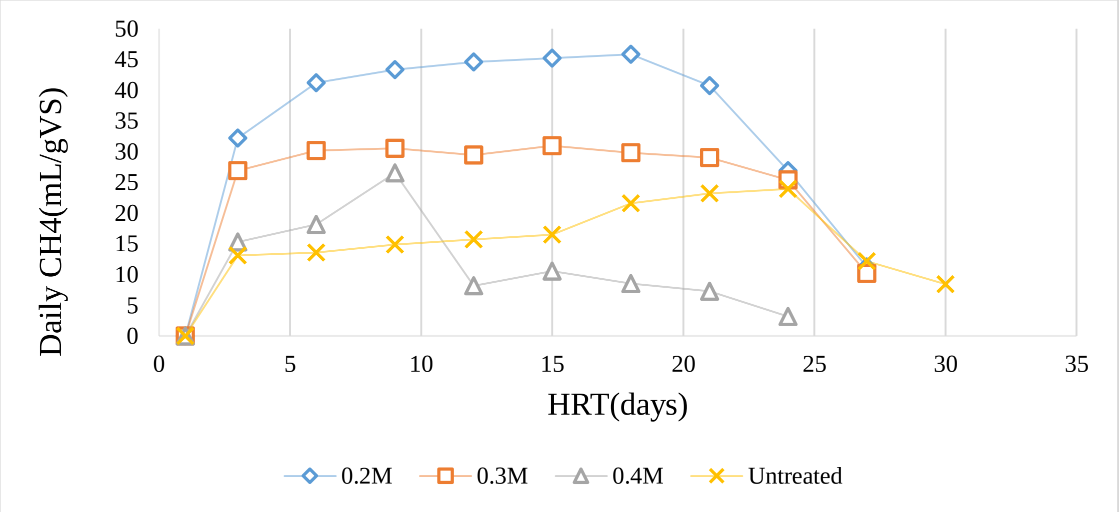


Figure 3. 5 Daily methane production from acid pretreatment of Maize straw.

The cumulative biogas production is given in figure 3.6.

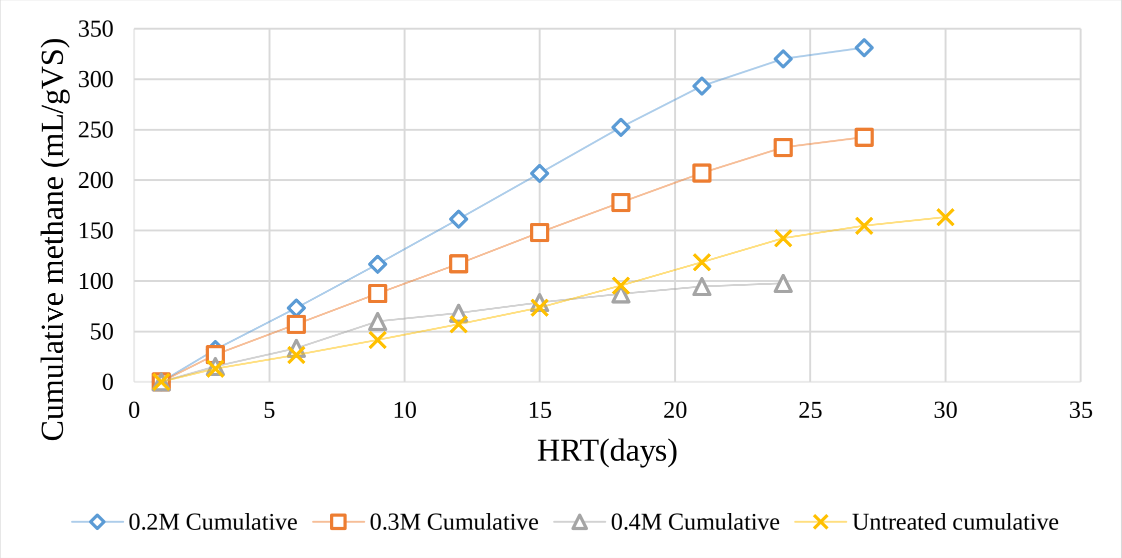


Figure 3. 6 Cumulative methane production from acid treated maize straw.

From the graph on fig. 3.6, there is an increase in biogas production in a logarithmic manner from day 2 untill it reached a maximum production after 15 days, then there was steady production for a few days before production started to fall. Biogas production from acid pretreatment lasted the fewest days (27) compared to other pretreatments. It can also be seen that for all concentrations of acid, the biogas production stopped before that for the untreated maize straw. This is probably because the application of sulphuric acid pretreatment has catalysed the reaction increasing the rate of anaerobic digestion and hence the straw was exhausted faster than the untreated samples. It can equally be seen that the 0.2M concentration had the highest production of all the concentrations with the sum total of 331.46mLCH_4/gVS of biogas produced which represents a 103% raise compared to that of the raw maize straw. The samples treated with 0.3M concentration had the second highest biogas production with a sum total of 242.67mLCH_4/gVS representing 48.5% increase in methane production. The 0.4M concentration reached its point of maximum production first after just 9 days. The biogas production then started falling untill the 12th day and then it stabilised for the following 10 days before it finally started falling again. This also agrees with the hypothesis that pretreatment shortens the hydraulic retention time for biogas production from anaerobic digestion. The biomethane production however fell by 40% on average for the samples treated by 0.4M H_2SO_4 solution which could be as a result of the production of inhibitory by products or a fall in pH to a level that impacted the microorganisms negatively. It might also be as a result of the production of hydrogen sulphide due to the reduction of the sulphate present in the acid.

The bar chart of the cumulative biogas production from suphuric acid pretreatment of maize straw is shown on figure 3.7.

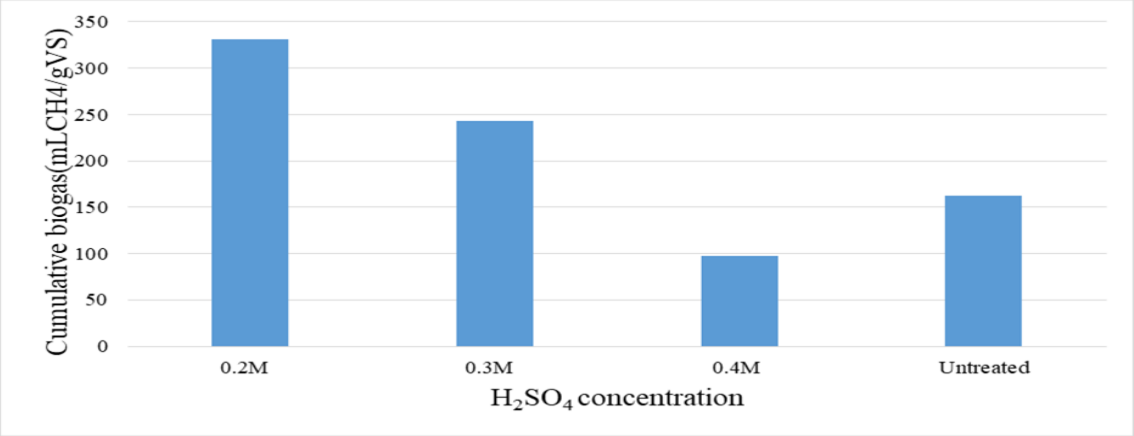


Figure 3. 7 Cumuclative biomethane production from acid pretreatment of maize straw.

3.5.2. Alkaline Pretreatment of Maize Straw

Alkaline/basic pretreatment was applied by using dilute sodium hydroxide solutions assisted by heat and pressure. Three different concentrations of dilute sodium hydroxide solutions while heating in an autoclave at 120°C for thirty minutes were applied on the biomass. Similar to the acid pretreatment, 0.8%w/v, 1.2%w/v and 1.6%w/v sodium hydroxide concentrations were used. The results acquired are given in the table 3.6.

Table 3. 6 Methane production from alkaline pretreatment of maize straw.

Day	Biogas production ($mLCH_4$/gVS/day)							
	0.2M NaOH	Sum	0.3M NaOH	Sum	0.4M NaOH	Sum	Untreated	Sum
1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	16.67	16.67	9.27	9.27	7.79	7.79	13.10	13.10
4	24.23	40.90	16.52	25.79	13.38	21.17	13.59	26.69
6	27.26	68.16	18.18	43.97	15.35	36.52	14.89	41.58
8	29.31	97.47	21.59	65.56	17.83	54.35	15.70	57.28
10	30.94	128.41	22.80	88.36	19.35	73.70	16.52	73.80
12	32.84	161.25	23.57	111.93	23.57	97.27	21.63	95.43
14	34.66	195.91	26.71	138.64	26.87	124.14	23.22	118.65
16	35.44	231.35	30.24	168.88	31.91	156.05	23.94	142.59
18	37.53	268.88	36.64	205.52	37.76	193.81	12.18	154.77
20	40.23	309.11	38.32	243.84	37.45	231.26	12.18	166.95
22	38.20	347.31	37.78	281.62	32.80	264.06	8.45	175.40
24	37.20	384.51	35.46	317.08	28.89	292.95		
26	36.49	421.00	32.53	349.61	31.52	324.47		
28	32.79	453.79	30.84	380.45	24.54	349.01		
30	29.81	483.60	29.62	410.07	21.29	370.30		
32	26.98	510.58	24.93	435.00	17.02	387.32		
34	20.70	531.28	16.13	451.13	13.07	400.39		

Biogas measurements for the alkaline pretreatment were done both daily and in two days intervals. As seen in table 3.6, the biogas production increased rapidly from the second day after the experiment started. The production stabilised between the sixth and the eighteenth day before increasing to its maximum production on the 20th day. The production then started falling steadily until it ceased which is likely as a result of the exhaustion of the biomass by the bacteria.

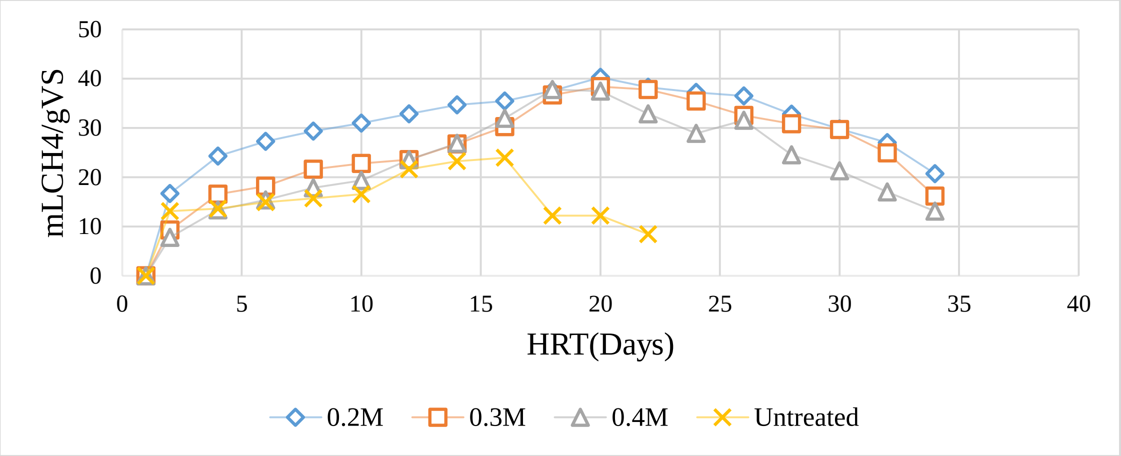


Figure 3. 8 Daily methane production from alkaline pretreatment of maize straw.

The graph in figure 3.8 shows the effects alkaline pretreatment had on biogas production from maize straw. It also distinguishes the consequences of the different pretreatments such as the concentration of the sodium hydroxide solution on biogas production. It can also be seen here that the 0.8%w/v sodium hydroxide solution had the most biogas production with the 0.3M and 0.4M concentrations generally having similar production rates which were also higher than the untreated samples. The maximum methane production for both pretreatment conditions occurred between the 18- 20th day with a total production of 40.23mL, 38.78 mL and 37.76mL for 0.2M, 0.3M and 0.4M sequentially. The cumulative biogas production is shown on figure 3.9.

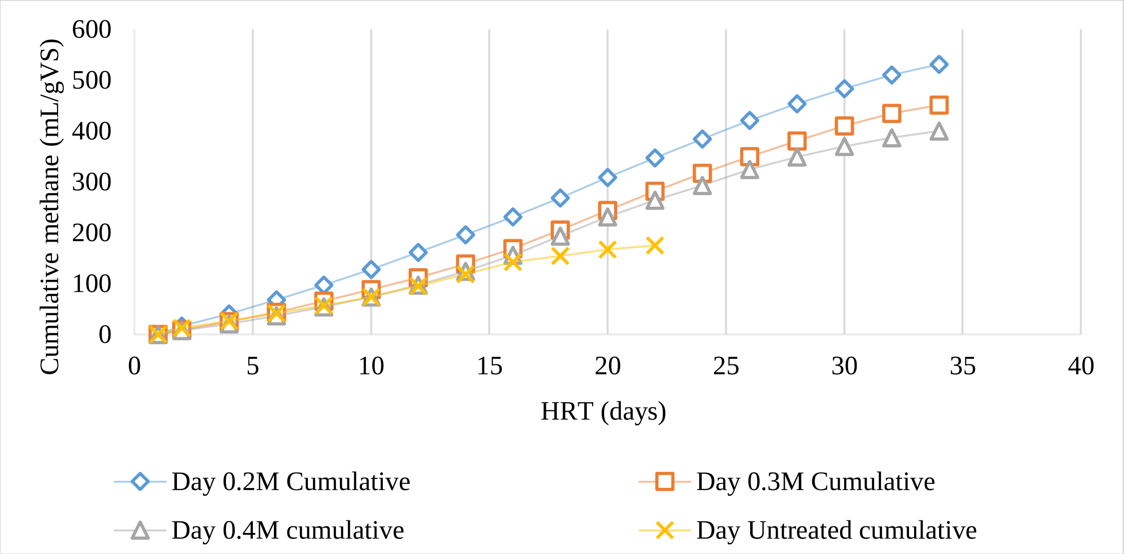


Figure 3. 9 Cumulative methane production from alkaline treated maize straw.

The graph on figure 3.9 ahows the rapid increase in biogas production from the second day untill around the 7th day. After the first week, production was stable untill around the 15th day. After which it increased untill it reached maximum production on the 20th day and then decreased steadily. The overall biogas production lasted about 34 days for the pre-treated samples and about 30 days for the untreated sample. This is likely as a result of the increased hydrolysis or solubilization of the straw which made available more biomass for the microorganisms to feed on. Biogas production from untreated straw lasted for fewer days probably because there were lower amounts of reducing sugars available for the bacteria to feed on. 0.8%w/v NaOH solution led to the highest methane production of all the pretreated samples with the sum total of 531.3mLCH_4/gVS representing an increase of about 203% with while the 1.2%w/v and 1.6%w/v NaOH concentrations had approximately similar biogas production with 158% and 129% increase in biogas production respectively compared to raw straw. Biogas production for the pretreated samples lasted about one week to ten days longer than the untreated and control samples.

The total biogas production from alkaline pretreatment of maize straw is shown in figure 3.11.

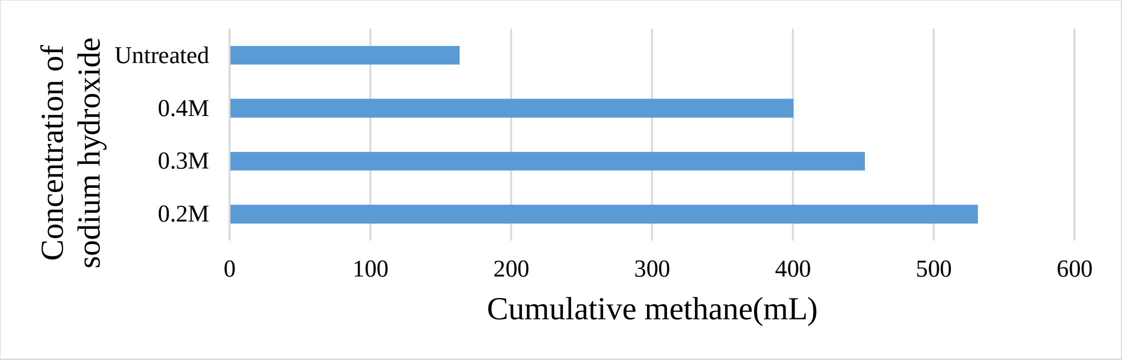


Figure 3. 10 Cumulative CH_4 production from alkaline pretreatment of maize straw.

3.5.3. Microwave pretreatment

The microwave pretreatment applied on maize straw used the same power/intensity of 300W and varying times. Higher intensities were tried but the biomass got burned and hence, 300W was found to be the most appropriate. One set of samples were placed for 2 minutes while another set was placed for 4 minutes. The results obtained are highlighted in table 3.7.

Table 3. 7 Results of the BMP test from microwave pretreatment on maize straw.

Day	Biogas production ($mLCH_4/gVS/day$)					
	300W, 2mins	Sum	300W, 4mins	Sum	Untreated	Sum
1	0.00	0.00	0.00	0.00	0.00	0.00
2	16.05	16.05	16.28	16.28	13.10	13.10
4	17.99	34.04	18.03	34.31	13.59	26.69
6	26.33	60.37	23.69	58.00	14.89	41.58
8	29.97	90.34	26.48	84.48	15.70	57.28
10	32.33	122.67	30.98	115.46	16.52	73.80
12	36.29	158.96	34.93	150.39	21.63	95.43
14	42.96	201.92	43.93	194.32	23.22	118.65
16	51.99	253.91	48.97	243.29	23.94	142.59
18	52.11	306.02	50.87	294.16	12.18	154.77
20	53.04	359.06	51.72	345.88	8.45	163.22
22	55.21	414.27	54.20	400.08		
24	54.70	468.97	46.37	446.45		
26	25.59	494.56	45.32	491.77		
28	12.96	507.52	26.91	518.68		
30	6.55	514.07	13.49	532.17		
32			7.29	539.46		

Table 3.7 shows the biogas production values for lignocellulosic maize straw that has been treated in a microwave oven. Measurements were done daily using the manometer to determine the pressure exerted by the biogas produced each day. The microwave pretreatment was one of the most potent methods for maize straw pretreatment. Biogas production was relatively higher from day one when compared to other pretreatment methods and the untreated maize straw samples. It increased rapidly within the first two weeks until it reached maximum production after around 20 days. The production then started falling steadily until it ceased which is likely as a result of the exhaustion of the straw by bacteria. The higher amount of biogas production by these samples is expected because the microwave pretreated samples produced the highest amounts of reducing sugars. This implies that there were more sugars available for the bacteria and hence they produced higher biogas concentrations. This also, agrees with the hypothesis that pretreatment enhances biogas production by making more sugars available for the microbes to feed on. The daily biogas production is shown in figure 3.11.

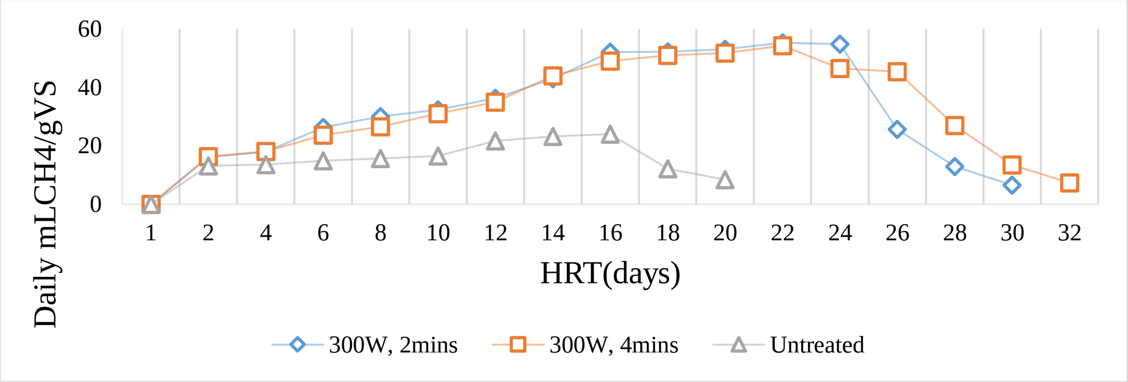


Figure 3. 11 Daily Methane production from microwave pretreatment of maize straw.

Figure 3.11 indicates the effects of microwave pretreatment on daily biomethane production from maize stover. It shows the amount of biogas produced by each pretreatment condition and also highlights the effectiveness of the different pretreatment conditions. It can be seen that the straw treated for 2 and 4 minutes generally had similar production rates which varied only slightly in their peaks as the time went on. The maximum production for both the samples treated for 2 minutes and those treated for 4 minutes occurred on the 22nd day. The overall biogas production lasted for about 32 days for the samples treated for 4 minutes and 30 days for the sample treated for 2 minutes. The cumulative biogas production is shown on figure 3.12.

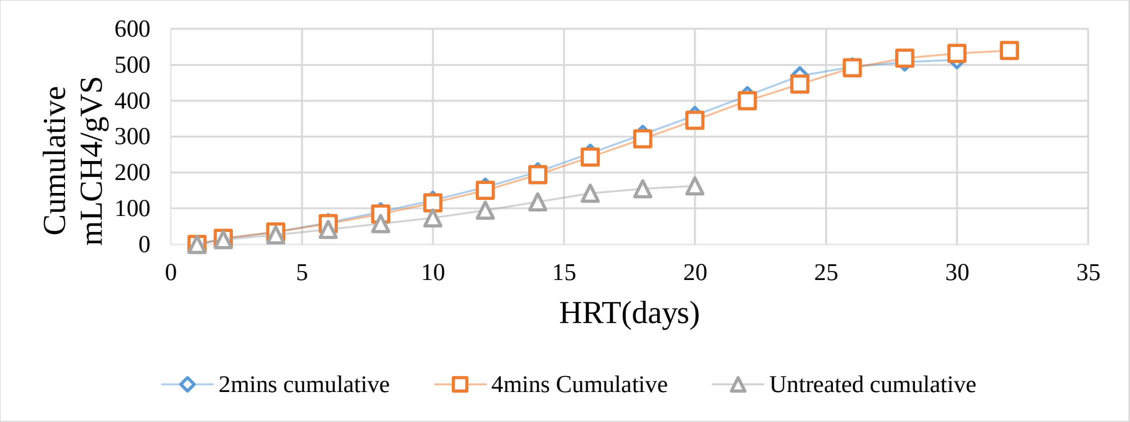


Figure 3. 12 The cumulative methane production from microwave pretreatment of maize straw.

Figures 3.11 and 3.12 show the pattern in the production of biogas from microwave treated maize straw. Biogas production increased from the day of the first measurement untill around the 16th day for both pretreatment conditions. Production then stabilised for the following week before it started falling around the 24th day. The samples that were treated for four minutes yielded the highest amount of biogas with a sum total of 539.46mL which is an increment of 208% compared with that of the raw maize straw. The samples pretreated for two minutes had a biogas production of 514.07mL of methane. This represents a 194% increase in biogas output with respect to the raw samples. Biogas production from pretreated samples lasted about ten days longer the raw samples. The results also hint that the treatment of lignocelluloses for longer times in a microwave increases their breakdown making more sugars avaibale for bacteria to feed. Jendrzejczyk et al. (2019) also found that prolonged time of microwave treatement increased the degradation of polysaccharides like cellulose present in the straw. The cumulative and daily biogas prioduction are represented in figure 3.13.

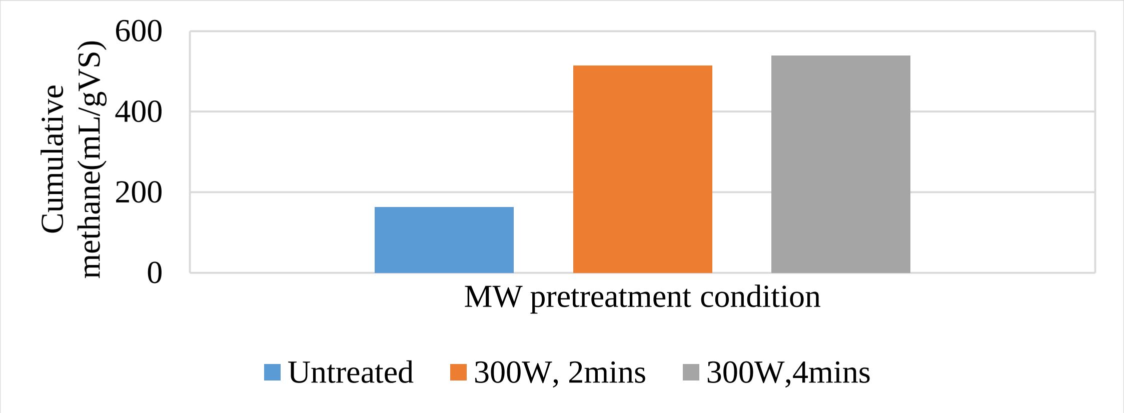


Figure 3. 13 Total biogas production from microwave pretreatment of maize straw.

3.5.4. Liquid Hot Water treatment

The other pretreatment method applied on the organic maize straw was the liquid hot water pretreatment. It involved heating the straw in an autoclave at high temperatures for a thirty minutes period. Maize straw samples were treated at three different temperatures but the same time. The temperatures were increased at 15^{o}C intervals to determine how the changes in temperature affects biogas production. The temperatures used were 105^{o}C, 120^{o}C and 135^{o}C. The results obtained are summarised on the table 3.8

Table 3. 8 The effects of liquid hot water treatment on biogas production from maize straw.

Day	Biogas production (mLCH$_4$/gVS/day)							
	105^{o}C	Sum	120^{o}C	Sum	135^{o}C	Sum	Untreated	Sum
1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	8.45	8.45	15.43	15.43	4.61	4.61	13.10	13.10
4	11.98	20.43	17.10	32.53	14.27	18.27	13.59	26.69
6	16.21	36.64	21.71	54.24	16.24	35.12	14.89	41.58
8	21.71	58.35	23.80	78.04	23.96	59.08	15.70	57.28
10	23.42	81.77	27.60	105.64	27.68	86.76	16.52	73.80
12	26.75	108.52	33.38	139.02	32.29	119.05	21.63	95.43
14	27.64	136.16	33.03	172.05	32.29	151.34	23.22	118.65
16	26.48	162.64	34.27	206.32	37.37	188.71	23.94	142.59
18	27.84	190.48	36.75	243.07	40.55	229.26	12.18	154.77
20	32.22	222.70	37.33	280.40	47.14	276.40	8.45	163.22
22	34.85	257.55	37.22	317.62	49.01	325.41		
24	36.48	294.03	48.27	365.89	46.45	371.86		
26	36.37	330.40	48.42	414.31	42.23	414.09		
28	25.55	355.95	34.74	449.05	36.17	450.26		
30	16.44	372.39	20.90	469.95	23.18	473.44		
32	8.26	380.65	11.55	481.50	12.79	486.23		
34	5.35	386.00	8.37	489.87	11.79	498.02		

Table 3.8 shows the effects of liquid hot water treatment on methane yield from maize straw. This pretreatment method was very effective in eliminating the resistance posed by lignin, cellulose and hemicellulose for maize straw as it produced relatively high amounts of biogas when compared to other pretreatment methods and the untreated maize straw samples. It is also a very economically feasible and environmentally friendly method because it does not involve the purchase, handling and disposal of chemicals that are both costly and potentially harmful to the environment. Liquid hot water pretreatment does not lead to the production of inhibitory by products. Biogas production showed a steady rise within the first two weeks until it reached maximum production after three

weeks before it started to decreased steadily until it ceased after about 34 days. The biogas production patterns are shown on the graph in figure 3.14.

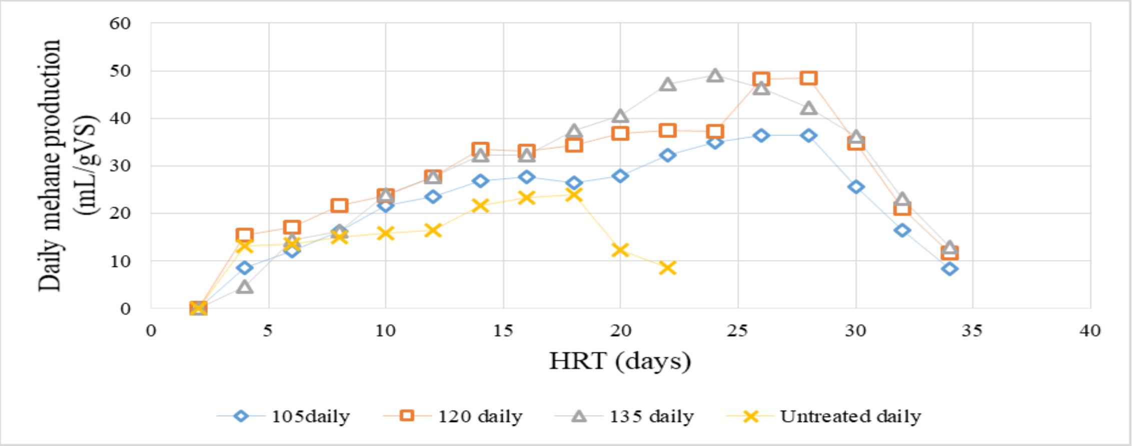


Figure 3. 14 Daily biomethane production from liquid hot water pretreatment of maize straw.

Fig. 3.14 represents the effects of liquid hot water treatment on biogas production from maize straw. It shows daily amounts of biomethane produced by the samples treated at different temperatures. The graph shows that the samples treated at 135°C had the highest daily biogas production with its maximum production of 49mL/d occurring on the 22nd day while the maximum production for the samples treated at 120°C was 48.4mL/d occurring on the 26th day. The samples treated at 105°C had a maximum production of 36.5mL/d on the 24th day. The overall biogas production lasted about 34 days for all pretreatment conditions which is about one week longer than the raw straw. The reason is probably because heating at high temperatures and pressure broke the glycosidic bonds between cellulose molecules and the bridges between lignin and cellulose/hemicellulose. It might also have been as a result of solubility of the biomass which made available more feed and energy for the bacteria. The cumulative biogas production pattern is shown on the graph in fig. 3.15.

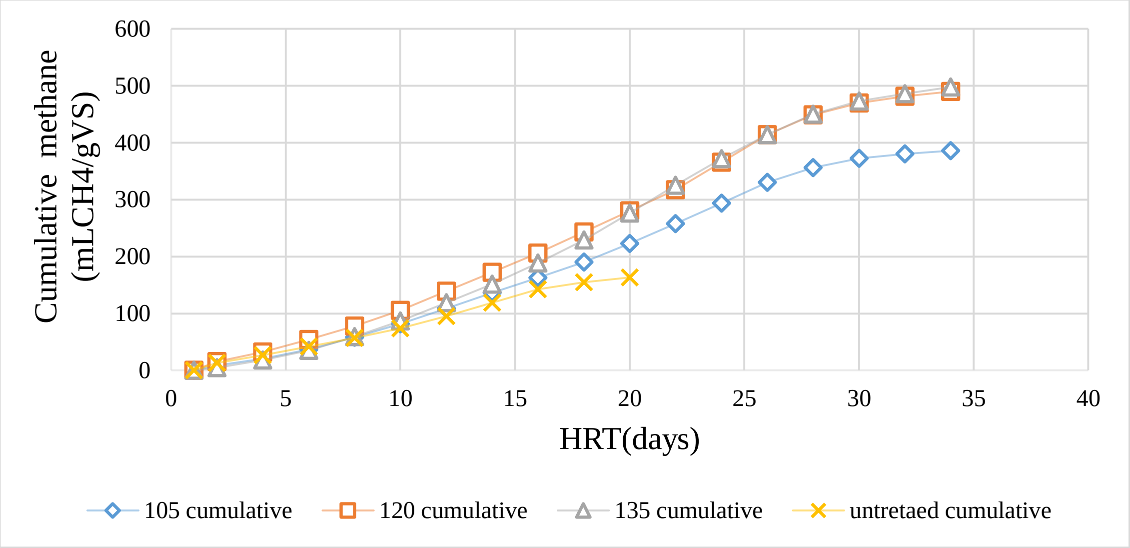


Figure 3. 15 Cumulative biogas production from liquid hot water pretreatment of maize straw.

Figure 3.15 shows the pattern in biogas production from lignocellulosic maize straw treated with liquid hot water. It shows how the biogas production increased steadily within the first fourteen days before stabilising for about a week for the samples treated at a temperature of 120°C while the production for those treated at 135°C kept on increasing until around the 23rd day. It can also be seen that the samples treated at 135°C had the highest biogas production with a sum total of 498.02mL of methane which represents increments of about 185% with respect to the raw maize straw. The samples treated at 120°C and 105°C had sums total of 490mL and 386mL representing increases of 179% and 121% respectively. Figure 3.16 shows the cumulative biogas production from LHW pretreatment of maize straw.

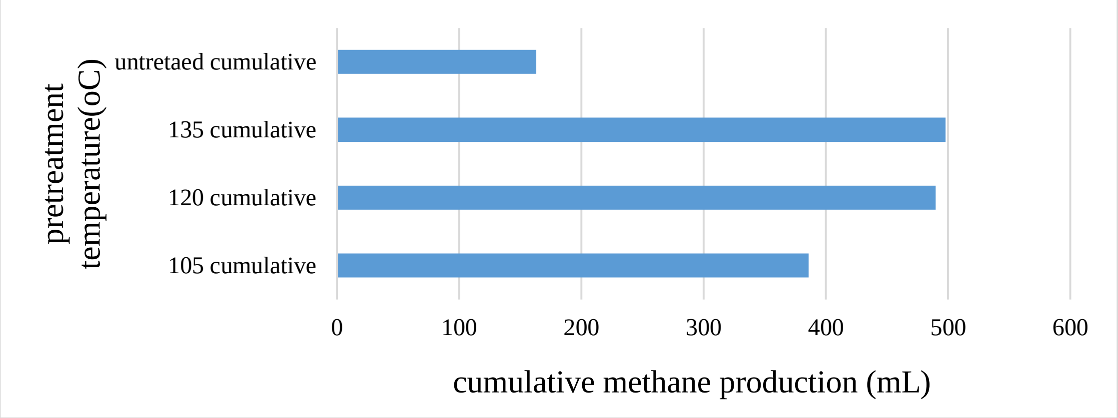


Figure 3. 16 Cumulative methane production from liquid hot water pretreatment of maize straw.

3.6. Comparisms of Total Methane Production from Pretreatments of Maize Straw

Comparatively therefore, the alkaline pretreatment was the most effective pretreatment method applied on maize straw. It yielded the highest cumulative amount of biogas and a relatively higher biomethane to carbon dioxide ratio. The concentrations of sodium hydroxide with the highest biogas production was 0.2M producing 531.3mL of methane. The microwave and liquid hot water pretreatments also produced very high and similar biogas volumes but with a slightly higher carbondioxide production. The pretreatment method with the least biogas production was the acid pretreatment. Also, of the three concentrations of sulphuric acid tested in this pretreatment method, the 0.4M concentration produced the lowest quantity of biogas while the 0.2M concentration produced a relatively higher amount. This is probably as a result of the acid damaging the reducing sugars, which is reduced the available organic material for microorganisms to feed on. Also, it might be as a result of the production of inhibitory compounds or even hydrogen sulphide which resulted in lower methane production.

Table 3.9 and figure 3.17 show the comparisms between the biogas produced by the various pretreatment methods applied on maize straw in this study.

Table 3. 9 Comparative biogas production from pretreatments of maize straw.

Pretreatment condition		Cumulative methane ($mLCH_4/gVS$)
Acid Pretreatment	0.2M H_2SO_4	331.5
	0.3M H_2SO_4	242.7
	0.4M H_2SO_4	97.8
Alkaline pretreatment	0.2M NaOH	531.3
	0.3M NaOH	451.1
	0.4M NaOH	400.4
LHW pretreatment	105°C	386.0
	120°C	489.9
	135°C	498.0
Microwave pretreatment	300W for 2mins	514.1
	300W for 4mins	539.5

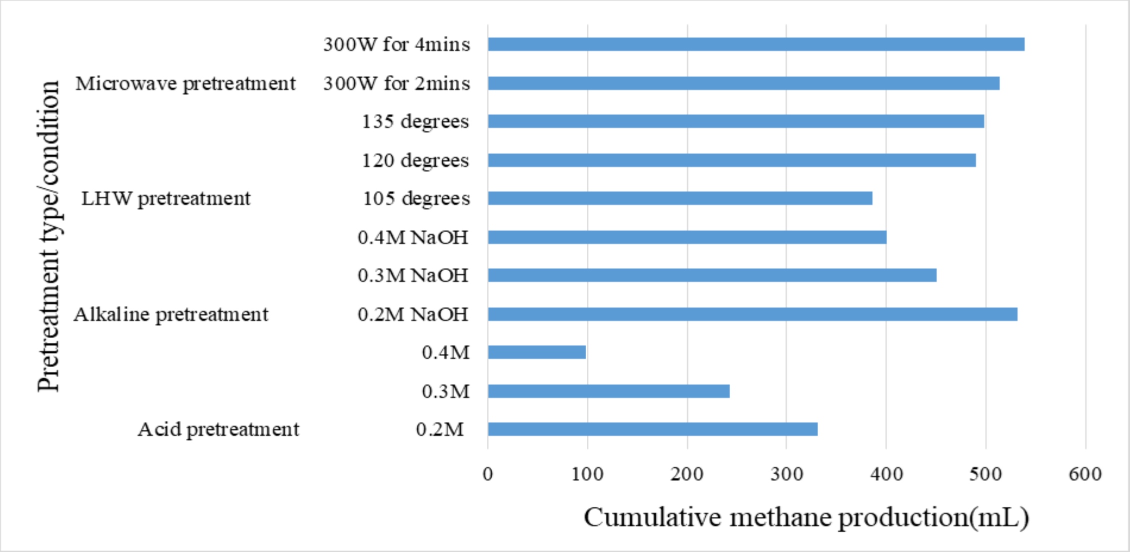


Figure 3. 17 Comparative methane production from pretreatments of maize straw.

3.7. Pretreatment of Cattle Manure

Four types of pretreatments were applied on cattle manure to investigate their effects on biogas production. They include; acid pretreatment, alkaline pretreatment, microwave and liquid hot water pretreatments. Varying concentrations of acids and bases were tested for the chemical pretreatments and varying temperatures for the liquid hot water pretreatment. The microwave pretreatment was performed at the same intensity (300W) but at different exposure times. In both cases, samples were duplicated and the average of the results taken. The results obtained for the various pretreatment types are summarised in the following sections.

3.7.1. Acid pretreatment of Cattle Manure

Three different concentrations were tested for cattle manure. Dilute concentrations of sulphuric acid (1.1%v/v, 1.6%v/v and 2.2%v/v) assisted with heat were applied. The samples were heated in an autoclave for thirty minutes at a temperature of 120^{o}C. The outcomes obtained are given in table 3.10 and figure 3.18, 3.19 and 3.20.

Table 3. 10 The effects of acid pretreatment on biogas production from cattle manure.

Day	Biogas production (mLCH$_4$/gVS)							
	0.2M H_2SO_4	Sum	0.3M H_2SO_4	Sum	0.4M H_2SO_4	Sum	Raw	Sum
1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	5.10	5.10	6.32	6.32	2.40	2.40	13.80	13.80
4	5.88	10.98	6.78	13.10	4.98	7.38	14.30	28.10
6	7.76	18.74	7.61	20.71	6.81	14.17	14.40	42.50
8	11.03	29.77	10.69	31.40	9.78	23.97	18.00	60.50
10	12.54	42.31	13.52	44.92	11.01	34.98	16.40	76.90
12	14.20	56.51	14.39	59.31	14.08	49.06	15.03	91.93
14	16.79	73.30	17.05	76.36	15.02	64.08	8.04	99.97
16	18.52	91.82	18.06	94.42	16.71	80.79	5.34	105.31
18	20.62	112.44	17.79	112.21	16.49	97.28	7.92	113.23
20	15.83	128.27	18.01	130.22	15.85	113.13	5.61	118.84
22	10.00	138.27	14.47	144.69	12.56	125.69	4.22	123.06
24	8.12	146.39	10.78	155.47	8.22	133.91	7.47	130.53
26	4.78	151.17	6.71	162.18	6.49	140.40	5.22	135.75

The biogas produced was measured daily or in two day intervals. As seen in table 3.10, methane production increased gradually within the first week until it reached its point of maximum production after about 15 days and then it started falling. This is probably because the microorganisms reached their optimal production and biomass utilization and as the biomass diminished, due to low amounts of substrate available to the microbes, a fall in biogas production was observed. The overall biogas production lasted about 26 days for the treated samples. The cumulative biomethane production patterns across the 26 days are shown in figure 3.18.

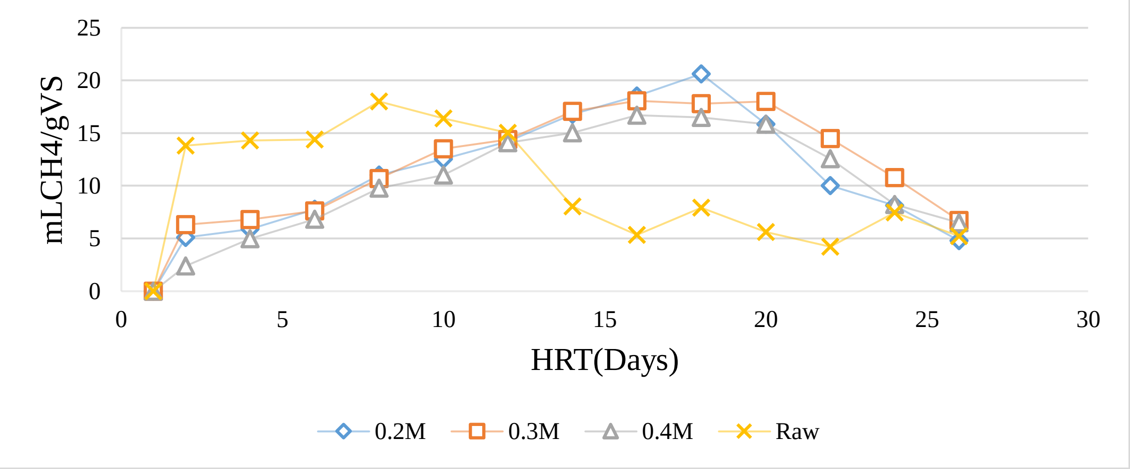


Figure 3. 18 Daily methane production from acid pretreatment of cattle manure.

Figure 3.18 portrays the effects of sulphuric acid pretreatment on methane production from cattle manure. It highlights the effectiveness of the different acid concentrations tested in the study. As seen above, the pretreatment with the maximum biomethane production was the 0.2M acid solution with highest daily biomethane production of 20.62mLCH$_4$/d occurring on day 18. Maximum production for the samples treated with 0.3M and 0.4M sulphuric acid were observed on the 16th day while the maximum biogas production for the raw manure occurred on the 9th day. Figure 3.19 shows the cumulative biogas production.

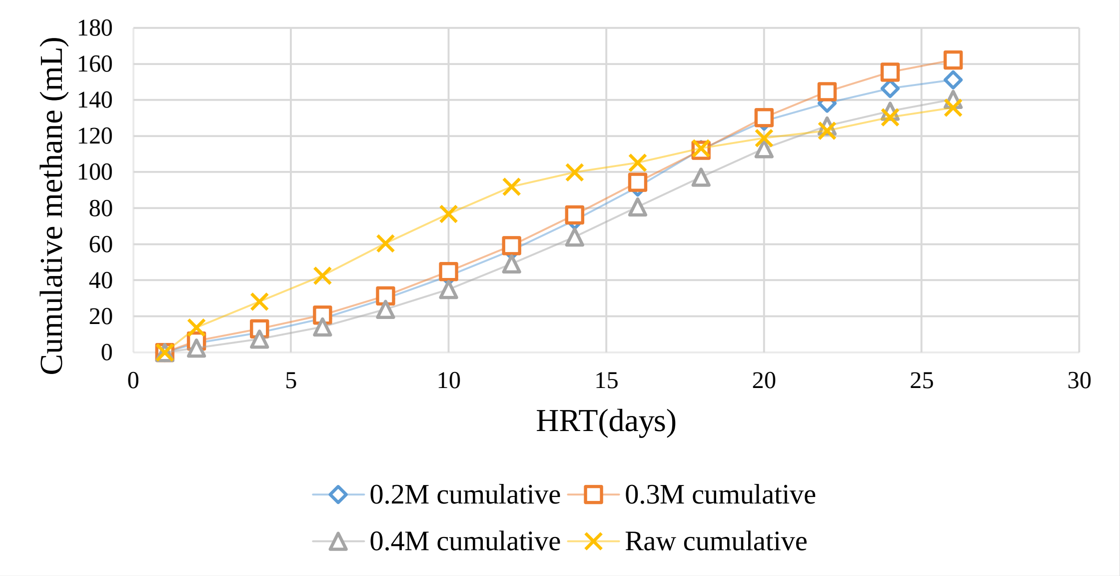


Figure 3. 19 Cumulative methane production from acid pretreatment of cattle manure.

The total methane production from the three pretreatment conditions are represented on the bar chart in figure 3.20.

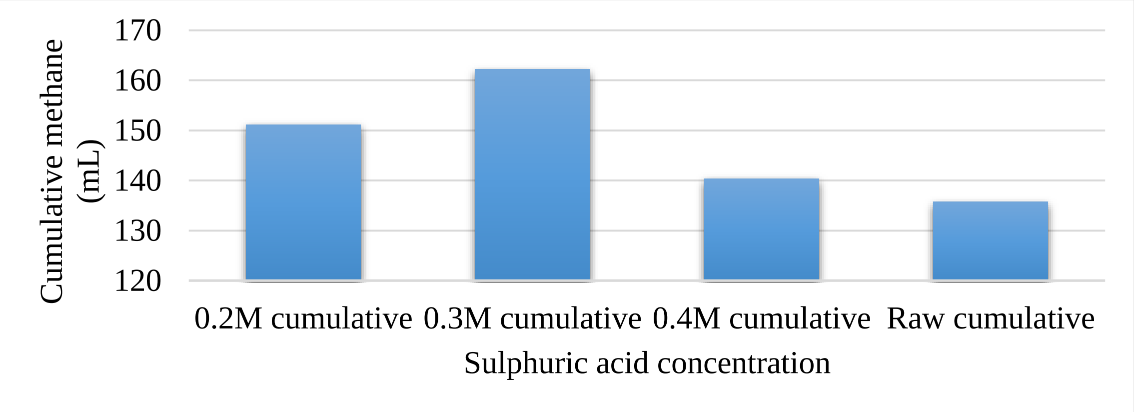


Figure 3. 20 Cumulative methane production from acid pretreatment of cattle manure.

Figure 3.20 shows the action of acid pretreatment on the cumulative methane production from cattle manure. It highlights the efficacy of the different pretreatment conditions in terms of cumulative biomethane yields. As seen above, the pretreatment condition which led to the maximum production of biomethane 0.3M acid solution with a total methane production value of 162.2mLCH_4/gVS representing a 20% more methane production than the raw cattle manure. The second most effective result was the samples treated with 0.2M sulphuric acid solution which lead to an average increase of 12% in biomethane production while the samples treated with 0.4M sulphuric acid resulted in a 4% increase in methane yield. It indicates that the higher the acid concentration, the lower biogas was produced. This could be as a result of the production of H_2S which reduces methane production, lowering the pH or the production of inhibitors.

3.7.2. Alkaline Pretreatment of Cattle Manure

In alkaline pretreatment, three different concentrations of dilute sodium hydroxide solutions assisted with heat were used. Heating was done in an autoclave at 120^{o}C for thirty minutes. Similar to the acid pretreatment, 0.2M (0.8%w/v), 0.3M (1.2%w/v) and 0.4M (1.6%w/v) concentrations of sodium hydroxide were used. The results obtained in the BMP tests are given in table 3.11

Table 3. 11 Results of the BMP test of alkaline treated cattle manure.

Day	Biogas production (mLCH_4/gVS)							
	0.2M NaOH	Sum	0.3M NaOH	Sum	0.4M NaOH	Sum	Raw	Sum
1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	6.90	6.90	6.54	6.54	9.47	9.47	13.80	13.80
4	11.74	18.64	15.08	21.62	18.27	27.74	14.30	28.10
6	13.27	31.91	16.83	38.45	17.18	44.92	14.40	42.50
8	14.71	46.62	17.42	55.87	19.47	64.39	18.00	60.50
10	16.86	63.48	19.59	75.46	19.83	84.22	16.40	76.90
12	19.79	83.27	21.23	96.69	20.35	104.57	15.03	91.93
14	23.96	107.23	23.93	120.62	22.96	127.53	8.04	99.97
16	23.45	130.68	26.93	147.55	24.71	152.24	5.34	105.31
18	26.42	157.10	31.86	179.41	23.49	175.73	7.92	113.23
20	23.96	181.06	27.42	206.83	27.08	202.81	5.61	118.84
22	17.13	198.19	25.20	232.03	25.06	227.87	4.22	123.06
24	13.81	212.00	17.10	249.13	19.42	247.29	7.47	130.53
26	12.49	224.49	14.59	263.72	15.44	262.73	5.22	135.75
28	7.30	231.79	-	-	10.08	272.81	-	-
30	1.54	233.33	-	-	7.27	280.08	-	-

Table 3.11 shows the biogas production values from cattle manure treated with heat assisted alkaline solutions. Measurements were carried out daily and sometimes in two

day intervals and the biogas production lasted for about 30 days. The biogas production generally increased rapidly during the first week and reached its highest values between the 18th and the 20th days. It then started diminishing gradually until it finally stopped after 30 days probably due to the exhaustion of the biomass in the BMP bottles. The daily methane production is shown on figure 3.21.

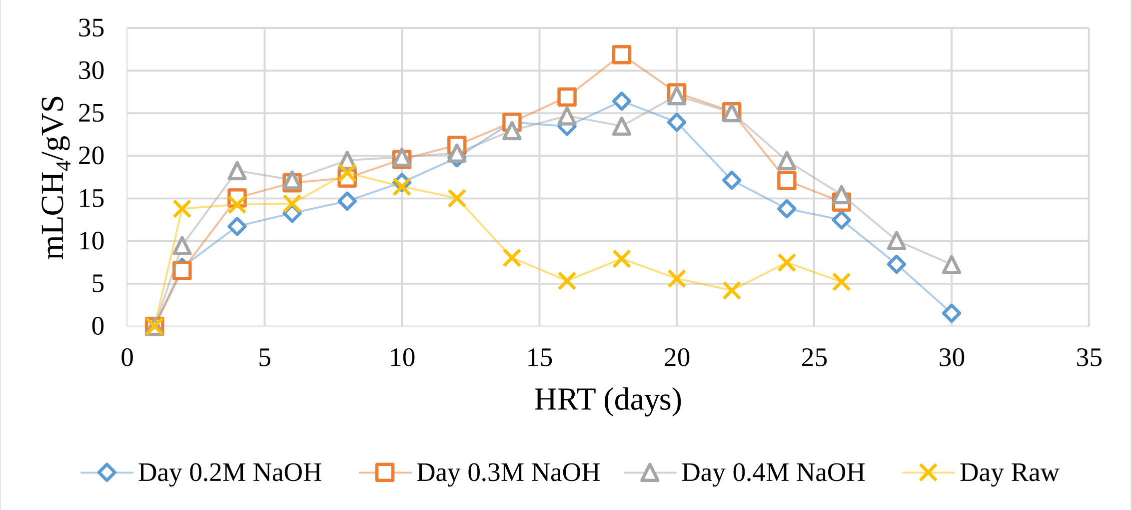


Figure 3. 21 Daily methane production from alkaline pretreatment of cattle manure.

Fig. 3.21 portrays the effects of sodium hydroxide pretreatment on biomethane production from cattle manure taken as the average amount of biogas produced by samples from each pretreatment condition i.e. the concentration of the basic solutions. It also highlights the effectiveness of different pretreatment conditions that were applied on the cattle manure. It can be seen that the pretreatment condition which led to the highest biogas production was the 0.3M NaOH solution with a maximum daily output of 31.9mLCH_4/d occurring on day 18. The cumulative biogas output is shown in figure 3.23.

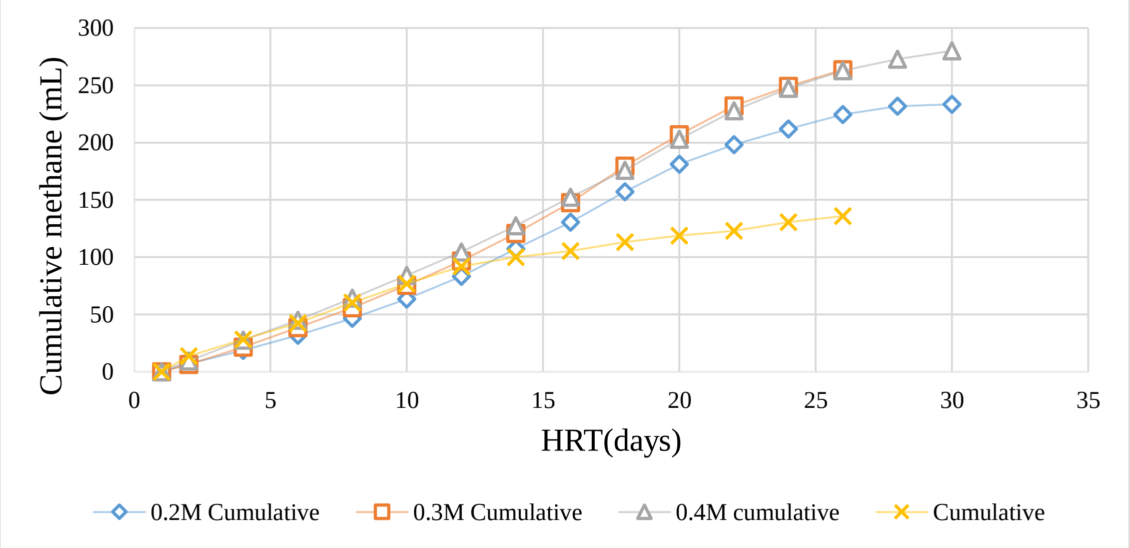


Figure 3. 22 The cumulative methane production from alkaline pretreatment of cattle manure.

Figure 3.21 shows the methane production patterns from alkaline pretreatment of cattle manure i.e. the biomethane production patterns for the three pretreatment conditions and the raw manure as time went on. The production lasted about thirty days for the pretreated samples. It can be seen that biomethane production rose rapidly from the day of the first measurement to the 4th day and then it decreased a little bit before it continued a gradual increase until it reached maximum production between the 16th and the 24th days during which it reached a maximum production of 26.4mLCH_4/d for the samples treated with 0.2M NaOH and 26.9mLCH_4/d and 27.1mLCH_4/d respectively for those treated with 0.3M NaOH and 0.4M NaOH. Overall, the samples treated with 0.4M solution produced the highest cumulative biogas concentration within the 30 days period with a sum total production of 280mL of methane which is an increase of 107% compared to that of the raw cattle manure. The samples treated with 0.2M NaOH and 0.3M NaOH had cumulative biogas productions of 233mLCH_4 and 264mLCH_4 representing an increase of 73% and 96% respectively. The sum total methane productions are represented on figure 3.23.

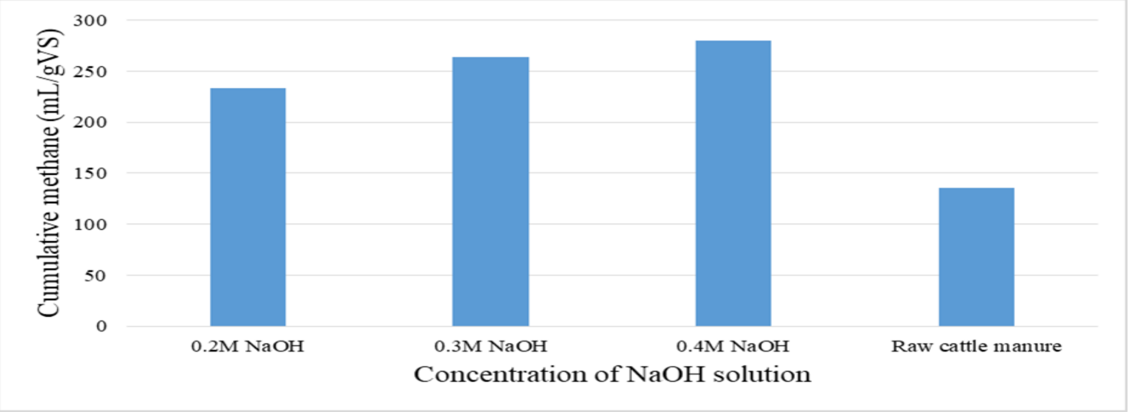


Figure 3. 23 Cumulative and average daily biogas production from alkaline pretreatment of cattle manure.

3.7.3. Microwave Pretreatment of Cattle Manure

Microwave pretreatment was applied on cattle manure samples at a fixed power rate of 300W and treatment time varied between 2 to 4 minutes. This is because higher powers resulted in the burning and loss of the biomass while lower powers proved to have less intensities of the electromagnetic waves to breakdown the lignocellulosic structure of the undigested plants remains found in cattle manure. The outcomes of the BMP tests after the application of microwave pretreatment on cattle manure are given in table 3.12.

Table 3. 12 The effects of microwave pretreatment on biogas production from cattle manure.

Day	Biogas production ($mLCH_4/gVS$)					
	300W, 2mins	Sum	300W, 4mins	Sum	Raw manure	Sum
1	0	0	0	0	0	0
2	5.76	5.76	9.3	9.3	13.8	13.8
4	7.37	13.13	12.49	21.79	14.3	28.1
6	9.37	22.5	14.2	35.99	14.4	42.5
8	12.3	34.8	16.98	52.97	18	60.5
10	13.98	48.78	18.44	71.41	16.4	76.9
12	14.98	63.76	20.49	91.9	15.03	91.93
14	16.93	80.69	20.91	112.81	8.04	99.97
16	17.64	98.33	22.91	135.72	5.34	105.31
18	22.05	120.38	28.67	164.39	7.92	113.23
20	26.81	147.19	27.64	192.03	5.61	118.84
22	25.52	172.71	25.64	217.67	4.22	123.06
24	25.57	198.28	22.57	240.24	7.47	130.53
26	21.05	219.33	16.54	256.78	5.22	135.75
28	10.2	229.53	12.27	269.05		
30	10.3	239.83	9.15	278.2		
32	8.88	248.71	5.32	283.52		

Table 3.12 shows the amount of biogas produced from cattle manure after pretreatment in a microwave. Daily measurements were done using a manometer to determine the pressure exerted by the biogas produced each day. The microwave pretreatment of cattle manure proved to be very effective producing high daily and cumulative biogas concentrations when compared to untreated cattle manure and other pretreatment methods applied in this study. Biogas increased rapidly within the first two weeks until it reached maximum production between the 18th and the 20th day. Production was then stable for about a week before it started falling steadily until it ceased. The production lasted just over thirty two days before it ceased probably because of the exhaustion of the biomass. The daily biogas production from microwave pretreatment of cattle manure is shown on figure 3.24

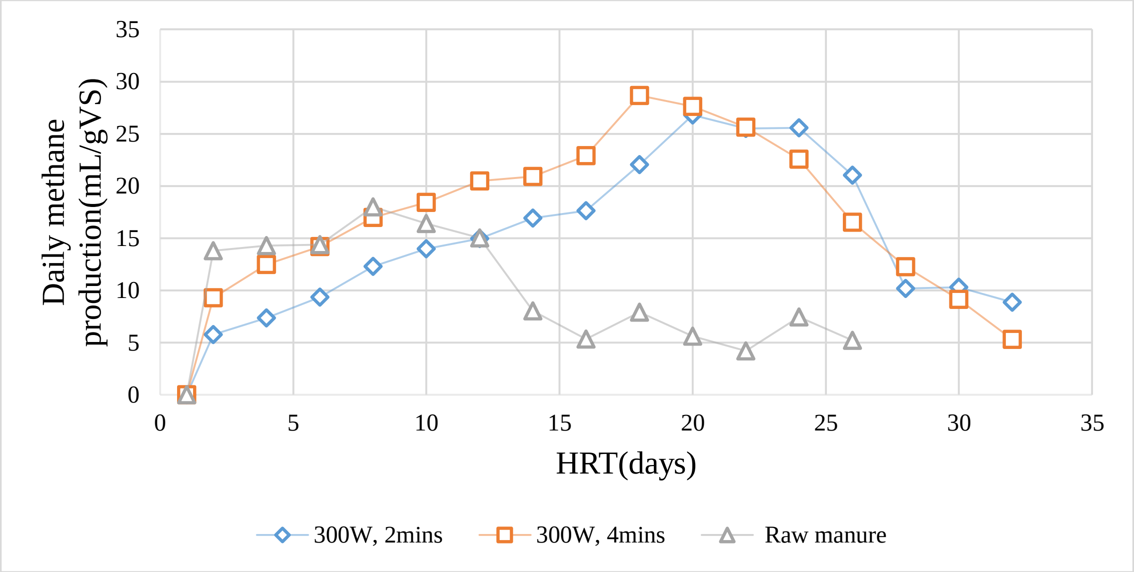


Figure 3. 24 Daily methane production from microwave pretreatment of cattle manure.

Figure 3.24 shows the daily biogas production from two microwave pretreatment conditions applied on cattle manure. Here it can be seen that the pretreatment condition which yielded the highest amount of biomethane was the 300W for 4minutes with maximum production of 26.8mLCH_4/d occurring on day 18 while maximum production for the samples treated for two minutes was 28.7mLCH_4/d recorded on the 20th day. Figure 3.25 shows the cumulative biogas production from microwave pretreatment. The cumulative methane production is shown in figure 3.25.

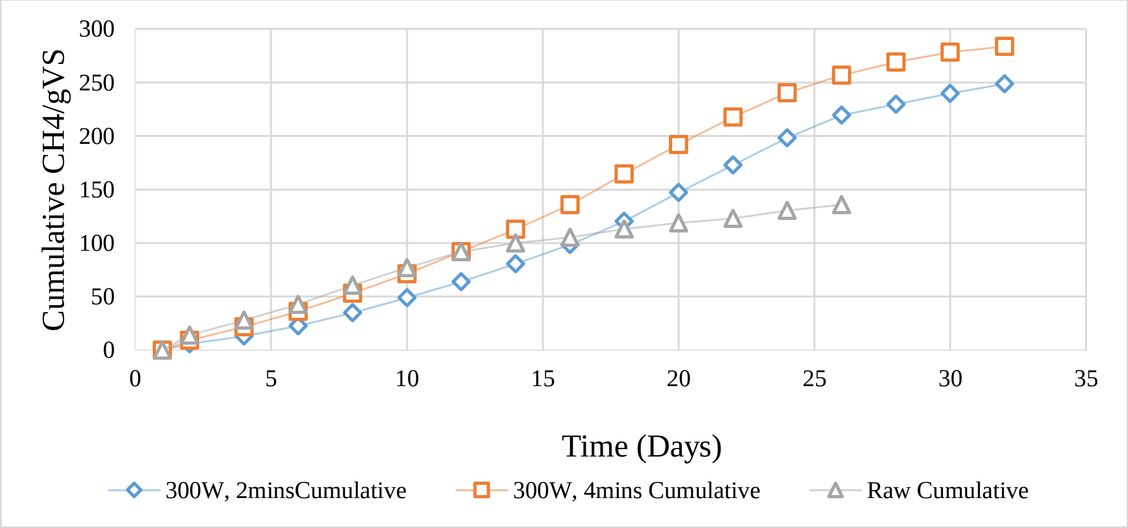


Figure 3. 25 Cumulative methane production from microwave pretreatment of cattle manure.

The graph in figure 3.25 shows the effects of microwave pretreatment on cattle manure. It shows how the biogas production behaved for the two pretreatment conditions and the untreated samples as time went on. Biogas production lasted over 32 days for both pretreatment conditions. From the graph, it can be seen that the production of biogas increased daily from the second day until it reached its highest concentration between the 18th and 20th days. It then started falling gradually until it finally stopped. Overall, both pretreatment conditions yielded similar results with the samples treated for 4 minutes producing a slightly higher amount of biogas over the 32 day period with a sum total methane production of 284mL and the samples treated for two minutes producing a sum total of 249mLCH_4 representing an increase of 110% and 84% respectively compared to the untreated cattle manure.

The cumulative biogas production is shown on the bar diagram in figure 3.26.

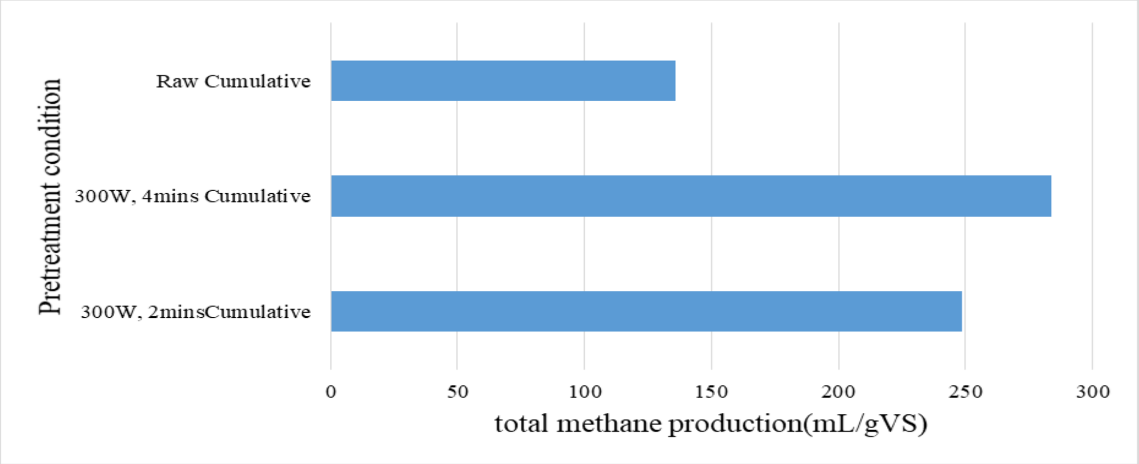


Figure 3. 26 Sum total methane production from microwave pretreatment of cattle manure.

3.7.4. Liquid Hot Water Pretreatment of Cattle Manure

The fourth and last pretreatment method applied on the cattle manure was the liquid hot water pretreatment. This involved heating 5% TS of the raw cattle manure in an autoclave at high temperatures for thirty minutes. Samples were treated at two different temperatures under the same contact time. The temperature was increased in 15°C intervals to find out the effects of heat variation on the production of biogas. The studies were carried out at temperatures 120°C and 135°C. The outcomes obtained from the BMP tests are given on table 3.13

Table 3. 13 The cumulative and daily methane production from LHW pretreatment of cattle manure.

Day	Biogas production (mLCH$_4$/gVS)					
	120°C	Sum	135°C	Sum	Raw	Sum
1	0.00	0.00	0.00	0.00	0.00	0.00
2	5.69	5.69	10.35	10.35	13.8	13.8
4	10.35	16.04	5.32	15.67	14.3	28.1
6	12.64	28.68	15.03	30.70	14.4	42.5
8	18.69	47.37	18.35	49.05	18.0	60.5
10	19.61	66.98	19.81	68.86	16.4	76.9
12	21.98	88.96	21.81	90.67	15.03	91.93
14	23.25	112.21	24.23	114.90	8.04	99.97
16	26.64	138.85	24.47	139.37	5.34	105.31
18	27.15	166.00	27.42	166.79	7.92	113.23
20	28.84	194.84	28.74	195.53	5.61	118.84
22	24.84	219.68	27.42	222.95	4.22	123.06
24	22.15	241.83	23.86	246.81	7.47	130.53
26	14.93	256.76	13.56	260.37	5.22	135.75
28	6.93	263.69	9.61	269.98		
30	2.83	266.52	5.27	275.25		
32	2.20	268.72	5.56	280.81		

Table 3.13 shows the daily and cumulative amounts of biogas produced from cattle manure after pretreatment with liquid hot water. The quantity of biogas yield was quantified daily by using a manometer to determine the pressure exerted by the biogas produced. This pretreatment method proved to be more effective as temperature increased. The cumulative and daily biogas productions for the two pretreatment conditions (120°C and 135°C) were relatively high. Biogas increased rapidly within the first two weeks until it reached maximum production around the 20th day for both pretreatment conditions. Biogas production then started falling in a gradual manner until it ceased after a total time of about 32 days.

The everyday biogas production is shown on the graph in figure 3.27.

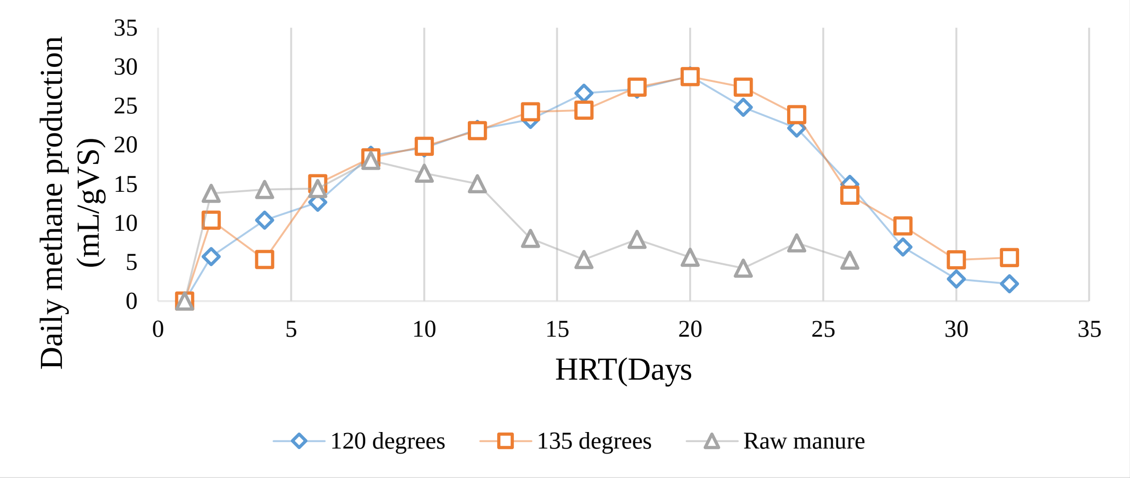


Figure 3. 27 Graph of the daily methane production from LHW pretreatment of cattle manure.

The graph on figure 3.27 shows the daily biogas production from liquid hot water treated cattle manure. The two pretreatment conditions had similar biogas production patterns with a similar maximum production occurring on the 20th day. The total biomethane output is shown on figure 3.28.

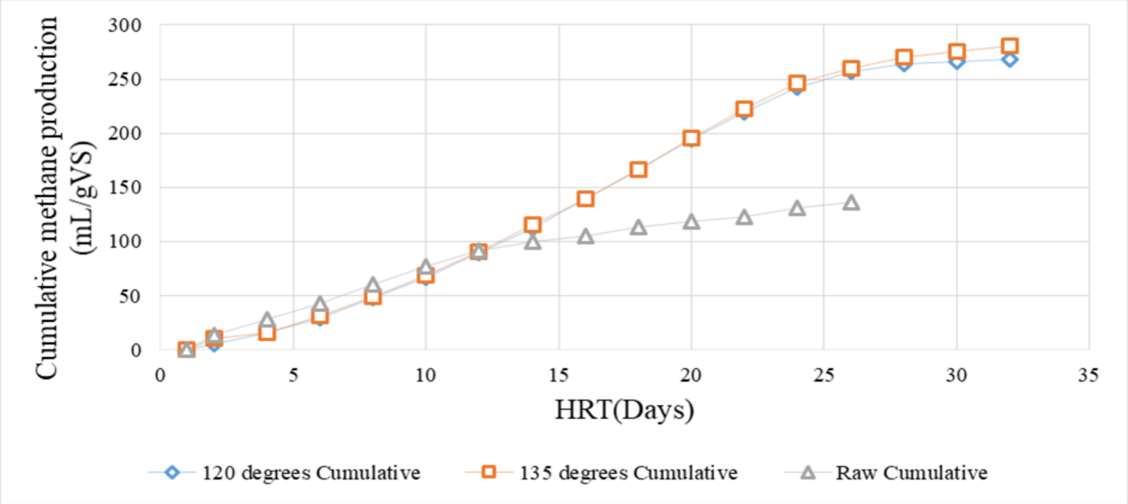


Figure 3. 28 Cumulative methane yield from LHW pretreatment of cattle manure.

Fig. 3.28 demonstrates the effects of liquid hot water pretreatment on cattle manure. It shows how the biogas production increased from the first few days until around the 18th day when it stabilised for the samples treated at 135°C meanwhile the samples treated at 120°C increased steadily until it reached the peak point on the 20th day. The production then started falling lasting for about 32 days for both pretreatment conditions before it finally stopped likely as a result of the exhaustion of the manure in the bottles. The samples treated at a temperature of 120°C produced a sum total of 267mLCH_4 while the samples treated at 135°C produced a sum total of 281mLCH_4 giving about 99% and 108% higher methane respectively when compared to the raw cattle manure. The cumulative methane production is shown in figure 3.29.

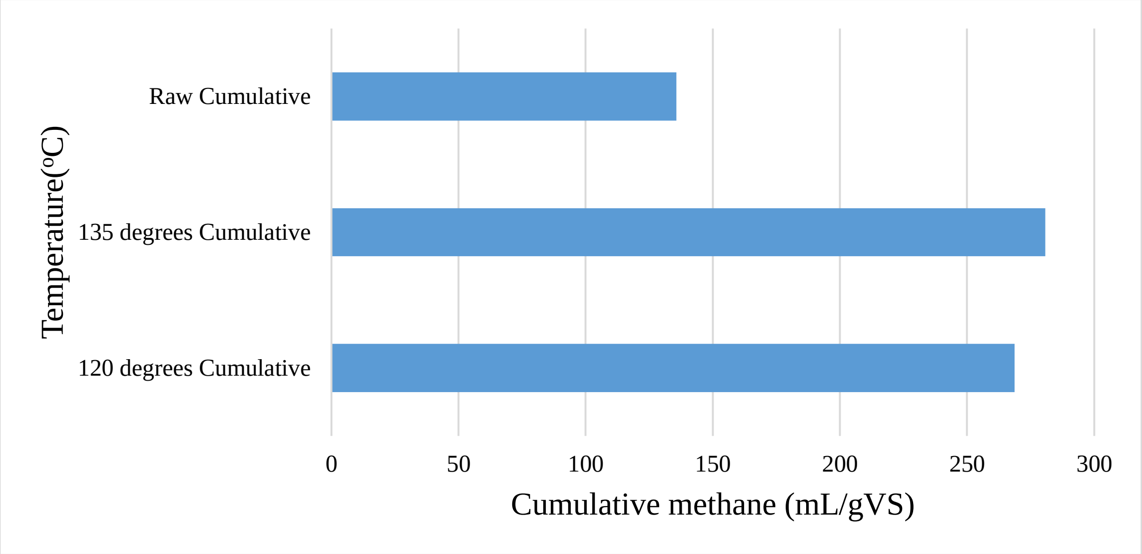


Figure 3. 29 Cumulative biomethane production from LHW pretreatment of cattle manure.

3.8. Comparisms of Total Methane Production from Pretreatments of Cattle Manure

All in all, both chemical and the physical pretreatment methods applied in this study demonstrated high effectiveness in enhancing biomethane yield from cattle manure. In liquid hot water pretreatment, the biomethane yield from cattle manure showed a high dependence on temperature; that is, the higher the temperatures, the more biogas was produced. This is probably because high temperatures are required to breakdown the undigested remains of the grass straw, plants leaves and other substances present in the manure. The pretreatment with the lowest biogas production was the acid pretreatment but it also had the lowest hydraulic retention time. This suggests that acid pretreatment increased the rate of anaerobic digestion by breaking the bonds holding the molecules together in the cell wall of the undigested residue making the biomass to be exhausted at a relatively faster rate. The application of acid pretreatment on cattle manure however produced higher concentration of biogas which also lasted about a week longer than maize straw. Table 3.14 and the bar diagram in figure 3.30 show the alterations in the amounts of biogas produced from each of the pretreatments comparatively applied on cattle manure.

Table 3. 14 Comparative methane production from different pretreatment methods applied on cattle manure.

Pretreatment condition		Cumulative biogas produced(mLCH4/gVS)
Acid Pretreatment	0.2M H_2SO_4	151.2
	0.3M H_2SO_4	162.2
	0.4M H_2SO_4	140.4
Alkaline pretreatment	0.2M NaOH	233.3
	0.3M NaOH	263.7
	0.4M NaOH	280.1
LHW pretreatment	120°C	268.7
	135°C	280.8
Microwave pretreatment	300W for 2mins	248.7
	300W for 4mins	283.5

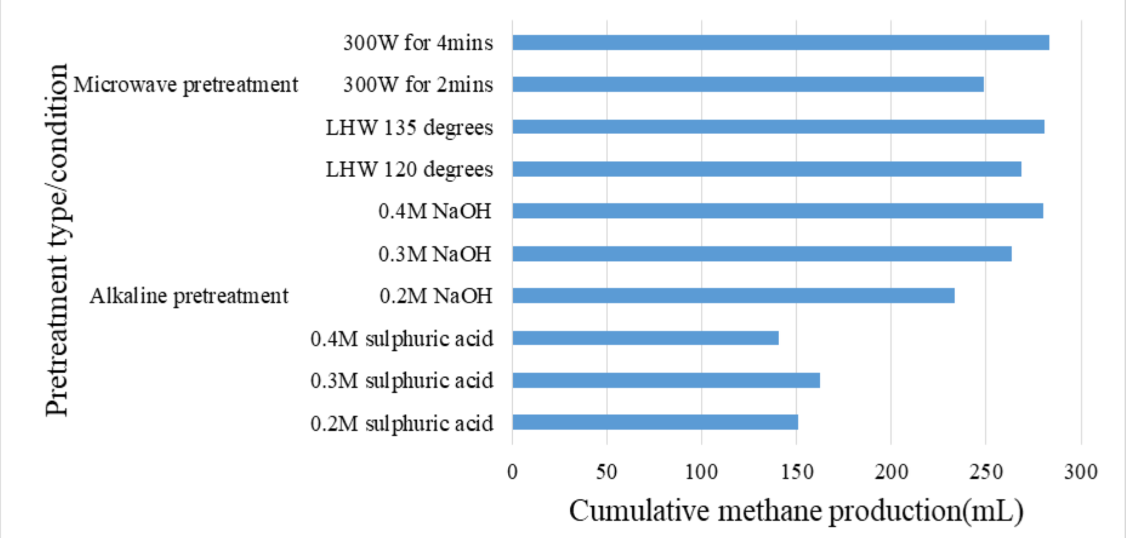


Figure 3. 30 The comparative methane production from pretreatment of cattle manure.

3.9: Comparisms between the total biogas production from current study and literature

Compared to previous studies presented in tables 2.2 and 2.3 in chapter 2, it can be seen that the results in this study are relatively higher for maize straw but generally similar for cattle manure. According to Merlin G. et al. (2013), maize straw produced methane concentration ranging between 92-330mL/gVS. Similar studies cited by Sambusiti Cecilia (2013), report methane yield values for maize stalks in the ranges between 246-267mL/gVS. In the same study, maize silage was reported to produce biomethane concentrations ranging between 379-390mL/gVS. Comparing to this study where methane production was between 224-527mL/gVS, based on the pretreatment type, it could be stated that methane production from maize straw was higher for samples treated with microwave, alkaline and liquid hot water.

The comparative biomethane concentrations are portrayed in tables 3.15 and 3.16.

Table 3. 15 Cumulative methane production from maize straw in current study.

Pretreatment type	Cumulative methane mL/gVS
Acid	224
Alkaline	461
Microwave	527
Liquid hot water	458

Table 3. 16 Cumulative methane production from cattle manure in literature.

Substrate	CH_4 yield (mL/gVS)	Reference
Straw(maize, rice)	92-330	Merlin G. et al. 2013
Maize straw	344-462	Sambusiti Cecilia 2013
Maize stalks	246-267	
Maize silage	370-390	
Vegetable leaves	230-310	Merlin G. et al. 2013
Rotten fruits	210-510	Merlin G. et al. 2013
Fruit pressings	280-500	Merlin G. et al. 2013
Fruit peels	240-520	Merlin G. et al. 2013

In table 2.3 in chapter 2, the results for previous studies and tests using animal manure as substrate for biogas production are presented. It generally varies based and animal type and feeding conditions for example, pig and chicken manure generally produced higher amounts of biogas when compared to grass eating animals like cattle, horses and sheep. According to reports from J. Ramos-Suarez et al. (2019), cattle manure produced about 333 mLCH_4/gVS which is higher than the quantity of 151-275 mLCH_4/gVS obtained in this study. Results from A.K.P. Meyer et al. (2018) stated that cattle manure produced methane concentration of 240 mL/gVS which is higher than the quantity produced by cattle manure samples pretreated with H_2SO_4 but slightly lower than the quantities produced by samples treated with alkaline, microwave and liquid hot water. Abdullah M.

et al. (2018) and Merlin G. et al. (2013) reported methane concentrations between 147-215 mL/gVS and 150-280 mL/gVS both similar to the outcomes in this study.

Table 3. 17 Cumulative biomethane yield from cattle manure in literature.

Manure type	**Biogas (mLCH4/gVS)**	**Reference**
Cattle manure	333	J. Ramos-Suarez et al. 2019
	240	A.K.P. Meyer et al. 2018
	292	Chen F. et al. 2017
	147-215	Abdullah M. et al. 2018
	150-280	Merlin G. et al. 2013
Goats manure	449	J. Ramos-Suarez et al. 2019
Sheep manure	452	J. Ramos-Suarez et al. 2019
Swine manure	370	A.K.P. Meyer et al. 2018
	461-470	J. Ramos-Suarez et al. 2019
Chicken manure	410-447	J. Ramos-Suarez et al. 2019
	400	A.K.P. Meyer et al. 2018
Cow slurry	200-300	Ertem F.C. 2011
Pig slurry	250-500	Ertem F.C. 2011

4. CONCLUSION AND RECOMMENDATIONS

The results obtained from this study lead to the following conclusions;

All in all, both agricultural residues tested in this study (maize straw and cattle manure) produced reasonable amounts of biogas. They both have the potential to be used for bioenergy production and therefore provide a good option for renewable energy production.

- As expected, all pretreatments increased biogas production for both cattle manure and maize straw although the degree of increase varied. Acid pretreatment lasted shorter period compared to all the other pretreatments both for cattle manure pretreatment and maize straw pretreatment. On average, biogas production from acid pretreatment of maize straw lasted 27 days. Also, heat assisted acid pretreatment proved to be more effective especially for maize straw because test samples treated with acid without the application of heat failed to produce biogas probably because of a decrease in pH. The 0.2M H_2SO_4 proved to be the most effective concentration producing about 103% increase when compared to the untreated maize straw. The 0.4M concentration of sulphuric acid produced 40% lower amount of biogas than the untreated samples which is likely because of the higher concentration of H_2SO_4 damaged the reducing sugars in the substrates.
- NaOH treatment of maize straw was best chemical pretreatment method producing the highest daily and cumulative biogas concentration. 0.2M (0.8%w/v) NaOH lead to about 203% more biogas than the raw sample with the methane production lasting 34 days.
- Microwave pretreatment of maize straw was also very effective leading to an increase in biogas production of about 208% and 194%.
- Liquid hot water pretreatment had a similar effect to microwave pretreatment. Three different temperatures (105^{o}C, 120^{o}C and 135^{o}C) were applied by treating the samples in an autoclave and one sample was heated at 105^{o}C in an oven. The results show that the samples treated in the autoclave produced more biogas than the samples treated at the same temperature in an oven. Also, it was noticed that the more temperature was increased, biogas production improved ; that is the

samples treated at 135^{o}C produced the highest concentration of biogas with an increments of about 185% relative to raw maize straw samples.

- All four pretreatments were also applied in cattle manure and the BMP test was also applied on the raw and pretreated cattle manure. Acid pretreatment was more effective for cattle manure than maize straw leading to between 4%, 12% and 20% increase in biogas production which lasted for 26 days.
- Alkaline pretreatment of cattle manure also had a positive effect leading to an average increase of about 92% in biogas production which lasted for 30 days.
- The most effective pretreatment method for cattle manure was however the microwave pretreatment. It lead to a 97% raise in biogas production which also lasted over 32 days in total.
- Liquid hot water pretreatment was also effective on cattle manure producing relatively high amounts of biogas. Two different temperatures were applied on the biomass (120^{o}C and 135^{o}C) with the samples treated at 135^{o}C producing the highest concentration of biogas. The samples treated at 135^{o}C produced a 103% increase in biogas production.

Generally, maize straw proved to have a higher biogas production potential than cattle manure but the handling of maize straw especially the mechanical breakdown (comminution) requires good machinery and high energy input.

From these results, the following recommendation can be made for subsequent research in the field of anaerobic digestion of agricultural residues;

- Acid and alkaline pretreatments should apply lower concentrations such as 0.2M NaOH and H_2SO_4 to maximise methane production from agricultural wastes. It is also advised that acid and alkaline pretreatment should be assisted with heat to make them more efficient. Acid pretreatment of maize straw should also consider washing and drying the biomass after pretreatment to improve biogas production. Heating in an autoclave is recommended as it facilitates the application and adjustments of pressure and temperature which are both crucial for optimal biogas production.
- Microwave pretreatments of maize straw and cattle manure should use intensities of maximum 300W as higher intensities led to the burning of the biomass. For better biogas production, pretreatment should be applied for between two to four

minutes. This is because biomass samples treated at 300W for 4 minutes produced maximum amount of biogas with a better methane to carbon dioxide ratio in this study.

- Liquid hot water pretreatments for both maize straw and cattle manure should use higher temperatures (135^{o}C was the optimum temperature for this study) and pressure. Lower temperatures especially for cattle manure are very ineffective. Pretreatment should preferably be done in an autoclave.
- Future studies and industrial applications should also consider using higher solid loading rates because it can potentially reduce residence time and increase biogas production. Because of time and material constraints, this study could only test the 5% TS.
- Finally, future studies using chemical pretreatments should take into account environmental and health hazards linked with them. They should possess appropriate disposal methods for the used acid, basic etc. solutions because they have adverse impacts on biodiversity. Handling of this chemicals should also be done carefully because concentrated acids can cause severe damage to the human body.

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THESIS/~~DISSERTATION~~ ORIGINALITY REPORT

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