ASSESSMENT OF BIOGAS PRODUCTION FROM WASTE CASSAVA PROCESSING WATER

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CERTIFICATION

This is to certify that this research tilted assessment of biogas production from waste cassava processing water was carried out by BANJO Oluwaseyi Daniel (AGE/2017/036) in partial fulfillments of the requirement for the award of Bachelor of Science Degree (B.Sc.) in Agricultural and Environmental Engineering, Faculty of Technology, Obafemi Awolowo University, Ile-Ife Nigeria

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ABSTRACT

This study investigates the potential of biogas production from waste cassava processing water using anaerobic digestion. The primary objectives are to evaluate the efficiency of anaerobic digestion in converting organic content into biogas, identify optimal operating conditions for maximizing production, and assess the economic and environmental feasibility of the process. Laboratory-scale experiments were conducted to analyze the biogas production from cassava processing water, its mixture with cow dung, and cow dung alone as a control. The study revealed that the mixture of cassava processing water and cow dung (TV2) consistently produced the highest biogas volumes, with notable peaks on days 8, 9, and 18. In contrast, cassava processing water alone (TV1) yielded the lowest production levels, while cow dung alone (TV3) demonstrated moderate and stable performance. Despite the variability in biogas output, TV2 emerged as the most effective treatment for high production peaks. The temperature range (25.5 to 28.4 °C) did not significantly impact the biogas production rates. The findings suggest that the mixture of cassava processing water and cow dung is the most promising method for efficient biogas production. Future research should focus on scaling up the process, testing different ratios, exploring other agricultural wastes, and developing automated systems for data collection.

CHAPTER ONE

INTRODUCTION

1.1 General Background

The utilization of renewable energy sources has become increasingly imperative in addressing global energy demands while mitigating environmental concerns (Sen and Ganguly, 2017). According to Afonja (2020), fossil fuels have been powering economies for over 150 years, and currently supply about 80 percent of the world's energy. These formed millions of years ago from the carbon-rich remains of animals and plants, as they decomposed and were compressed and heated underground. When fossil fuels are burned, the stored carbon and other greenhouse gases are released into the atmosphere. Mikhaylov, *et. al.* (2020) lamented that an excess buildup of greenhouse gases in the atmosphere has caused dramatic changes to Earth's climate, a trend that will worsen as more fossil fuels are burned. To reduce the emission of greenhouse gases, the world will need to resort to using renewable energy.

Renewable energies represent a pivotal shift towards sustainable and environmentally conscious energy production (Al-Shetwi, 2022). This involves harnessing natural resources such as sunlight, wind, water, and geothermal heat, (these forms of energy offer a viable alternative to fossil fuels). Solar power, derived from photovoltaic cells converting sunlight into electricity, stands as a symbol of this transition, powering everything from homes to large-scale industrial facilities. Similarly, wind energy, captured through turbines, has become increasingly competitive in the energy market, boasting significant growth and technological advancements. Hydroelectricity, geothermal, biomass, and emerging tidal and wave energy further diversify the renewable energy portfolio, each with its unique advantages and challenges. Medina, *et al* (2022) however explained that despite the progress, optimizing renewable energy deployment requires addressing issues like intermittency and grid integration while fostering continued innovation and supportive policies. Meanwhile, by prioritizing renewable energies, we can mitigate climate change, enhance energy security, and pave the way toward a sustainable future.

Among these sources, biogas stands out as a promising avenue due to its dual benefits of waste management and energy production. Singh and Kalamdhad, (2021) established that waste streams from various industries present untapped potential for biogas generation, contributing to sustainable development objectives.

Biogas has been used on a small scale for heating, cooking, and lighting in low-tech environments around the world for centuries. Indeed, the World Biogas Association says biogas was used to heat Assyrian bath waters in 900 BC. However, the discovery of methane is generally attributed to an Italian scientist, Alessando Volta, in the 1770s. (IRENA 2018).

Biogas is a production made from the fermentation of biodegradable materials such as sewage, manure, wastewater from livestock farms and industrial plants. It is produced by the anaerobic digestion of organic substances with anaerobic bacteria in the absence of oxygen (anaerobic condition. Biogas comprises 60% - 70 %methane (CH_4), 28-30% carbon dioxide (CO_2), and around 2% of hydrogen sulphide (H_2S), nitrogen (N_2), and steam. The property of biogas depends largely on the quantity of combustible methane. The methane content can range from 50% to 80% (on a volumetric basis). The high amounts of carbon dioxide in biogas typically reduce the heating value to between 18 and 26 MJ/m^3 (GCV) compared with natural gas typically around 40 MJ/m^3 (GCV). The organic substances that are typically found in waste include protein, carbohydrate, and fat compound, both in the forms of solid and solution. Biogas can be used for electricity production on sewage works, in a combined heat and power

(CHP) power gas engine, where the waste heat from the engine is conveniently used for heating the digester; cooking; space heating; water heating; and process heating. If compressed, it can replace compressed natural gas for use in vehicles, where it can fuel an internal combustion engine or fuel cells and is a much more effective displacer of carbon dioxide than the normal use in on-site CHP plants.

Summarily, Biogas can be produced from waste such as manure, sewage, waste water from livestock farm and industrial plant, as well as agricultural waste. The Organic waste from agricultural processing presents a promising resource for biogas production through anaerobic digestion. Various types of agricultural residues, including crop residues like stalks and leaves, livestock manure from dairy and poultry farms, and food processing waste such as fruit peels and vegetable trimmings, can serve as valuable feedstock for biogas generation. Additionally, dairy waste, wastewater sludge from treatment plants, silage, alcohol industry byproducts, and aquaculture waste contribute to the diverse array of materials suitable for biogas production. By harnessing these organic wastes, agricultural processing facilities can not only mitigate environmental impacts associated with waste disposal but also generate renewable energy in the form of biogas, thus promoting sustainability and reducing dependence on fossil fuels. Efficient management and preprocessing of feedstock are essential for optimizing biogas yields and maintaining the stability of anaerobic digestion processes.

In the sphere of agricultural processing, cassava (figure 1.1) stands out as a vital staple crop across numerous regions globally, yet its processing entails a significant byproduct in the form of organic waste (Anwar *et. al,* 2023). The processing of cassava results in the generation of copious amounts of organic waste, particularly in the form of cassava processing water. This residual liquid, abundant in organic content, presents considerable environmental concerns if left unattended. However, amidst these challenges lies a

Figure 1.1: Cassava Tuber

remarkable opportunity: cassava processing water, with its rich organic composition, serves as a potent resource for the production of biogas through the process of anaerobic digestion.

The intricate process of cassava processing yields a substantial volume of wastewater laden with organic compounds (Amara *et al* 2023). Without proper management, this wastewater can pollute water bodies, degrade soil quality, and emit foul odors, posing significant environmental hazards. Moreover, the disposal of such organic waste often entails logistical challenges and financial burdens for processing facilities and local communities alike.

Nevertheless, within this seemingly daunting scenario lies a beacon of sustainability. Through the application of anaerobic digestion technology, the organic matter present in cassava processing water can be efficiently converted into biogas, a renewable energy source rich in methane. This process not only mitigates the environmental impact of cassava processing but also offers a tangible solution for energy generation and waste management.

Biogas derived from cassava processing water presents a dual benefit: it offers a renewable energy source while concurrently addressing environmental concerns associated with organic waste disposal. This symbiotic relationship between waste management and energy production exemplifies the principles of circular economy and sustainable development.

In summary, while cassava processing may pose challenges in terms of organic waste management, the inherent potential of cassava processing water as a feedstock for biogas production presents a compelling opportunity. By embracing anaerobic digestion technology, stakeholders can not only mitigate environmental impacts but also unlock a sustainable pathway towards energy production and resource efficiency in the agricultural sector.

Therefore, there is need to explore the feasibility and potential of harnessing biogas from waste cassava processing water. It is also necessary to delve into the intricate interplay of factors involved in the anaerobic digestion process, from the characterization of cassava processing water to the optimization of biogas production. By examining the technical, economic, and environmental dimensions, we aim to provide insights into the viability and sustainability of biogas production from this underutilized resource.

Through this exploration, we not only seek to address the pressing issue of waste management in cassava processing but also to unlock a renewable energy source that can contribute to local energy security and resilience. Furthermore, the integration of biogas production into cassava processing operations has the potential to create additional revenue streams and enhance the overall sustainability profile of the agricultural sector.

1.2 Significance of the Study

Cassava, a staple food crop in many parts of the world, is processed into various products, leaving behind significant amounts of wastewater. This waste stream poses environmental challenges due to its high organic content and chemical composition. However, it also presents an opportunity for sustainable energy production through biogas generation. Understanding the feasibility and efficiency of biogas production from waste cassava processing water is crucial for both environmental sustainability and economic development.

1.3 Objectives of the Study

The primary objective of this research is to assess the potential for biogas production from waste cassava processing water. Specifically, the study aims to:

i. Evaluate the performance of anaerobic digestion processes in converting the organic content of cassava processing water into biogas,

ii. Determine the optimal operating conditions and parameters for maximizing biogas production,

1.4 Scope of the Study

This study will focus on the assessment of biogas production from waste cassava processing water, with particular emphasis on the anaerobic digestion process. The research will involve laboratory-scale experiments to analyze the composition of cassava processing water, mixture of the waste water with cow dung and only cow dung as the control experiment. It also extended to optimizing the anaerobic digestion conditions, and measure biogas production rates. Additionally, economic and environmental assessments will be conducted to evaluate the feasibility and sustainability of the proposed biogas production system.

CHAPTER TWO

LITERATURE REVIEW

2.1 Anaerobic Digestion

Anaerobic digestion is widely used for treating various waste streams such as municipal sludge or animal waste (biomass). Anaerobic digestion is a sequence of processes by which microorganisms break down biodegradable material (such as plant or animal waste) in the absence of oxygen.

Anaerobic digestion (AD) is a biological method used to convert organic wastes into biogas and a stable product for land application without adverse environmental effects. The Biogas produced from this process can then be used as a renewable energy source. Anaerobic digestion that utilizes manure for biogas production is one of the most promising uses of biomass waste because it provides a source of energy which simultaneously resolving ecological and agrochemical issue. The rate and efficiency of the Anaerobic Digestion process is determined by type of waste being digested, concentration, temperature, the present of toxic material, the pH and alkalinity, Hydraulic Retention Time (HRT), the Solid Retention Time (SRT), the ratio of food to microorganism (F/M ratio), the rate of digester loading and the rate at which toxic end product of digestion are removed (Burke, 2001).

The anaerobic digestion process takes place through four (4) successive stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. The anaerobic digestion process is dependent on the interactions between the diverse microorganisms that are able to carry out the four aforementioned stages. In single-stage batch reactors, all wastes are loaded simultaneously, and all four processes are allowed to occur in the same reactor sequentially; the compost is then emptied after at the conclusion of a given retention period or the cessation of biogas production. The Digestion process starts with the Bacterial Hydrolysis (also known as the Liquefaction of Complex molecules) of the input materials. Complex molecules have complex structures with a large number of chains. Hydrolysis is the process of breaking the chains with the help of hydrolysing enzymes. High molecular weight polymeric components are broken down into simple sugars and monomers which can be readily accessible to bacteria. Acetate, hydrogen, and some Volatile Fatty Acid (VFA) are produced during these steps. VFAs cannot be directly used by the microorganisms so they are first catabolized into small molecules that can be utilized by the bacteria. Acidogenesis (which is the process of acidic breakdown of oligo polymers and compounds into simpler molecules) is performed by acidogenic bacteria. During this reaction, Ammonia, Carbondioxide, and Hydrogen Sulphide, as well as other byproducts, are formed. The resulting organic acids from Acidogensis is then converted to Acetic acid with the aid of Acetogens. This reaction produces Carbondioxide and Hydrogen as its main byproduct, alongside additional ammonia. Methanogenesis is the final step of the anaerobic digestion. During this step, Methanogens converts the products from Acetogens to methane, and carbondioxide. It is a pH sensitive reaction that occurs between the range of pH 6.5 to pH 8.

The resulting biogas produced from the digestion can then be used directly as fuel, in combined heat and power gas engines or upgraded to natural gas-quality biomethane. The nutrient-rich digestate also produced can be used as fertilizer. Figure 2.1 shows a breakdown of the complete anaerobic digestion process.

Anaerobic digestion helps to reduce production of landfill gas, which when damaged leads to an outburst of methane (major greenhouse gas). It also helps in reducing the

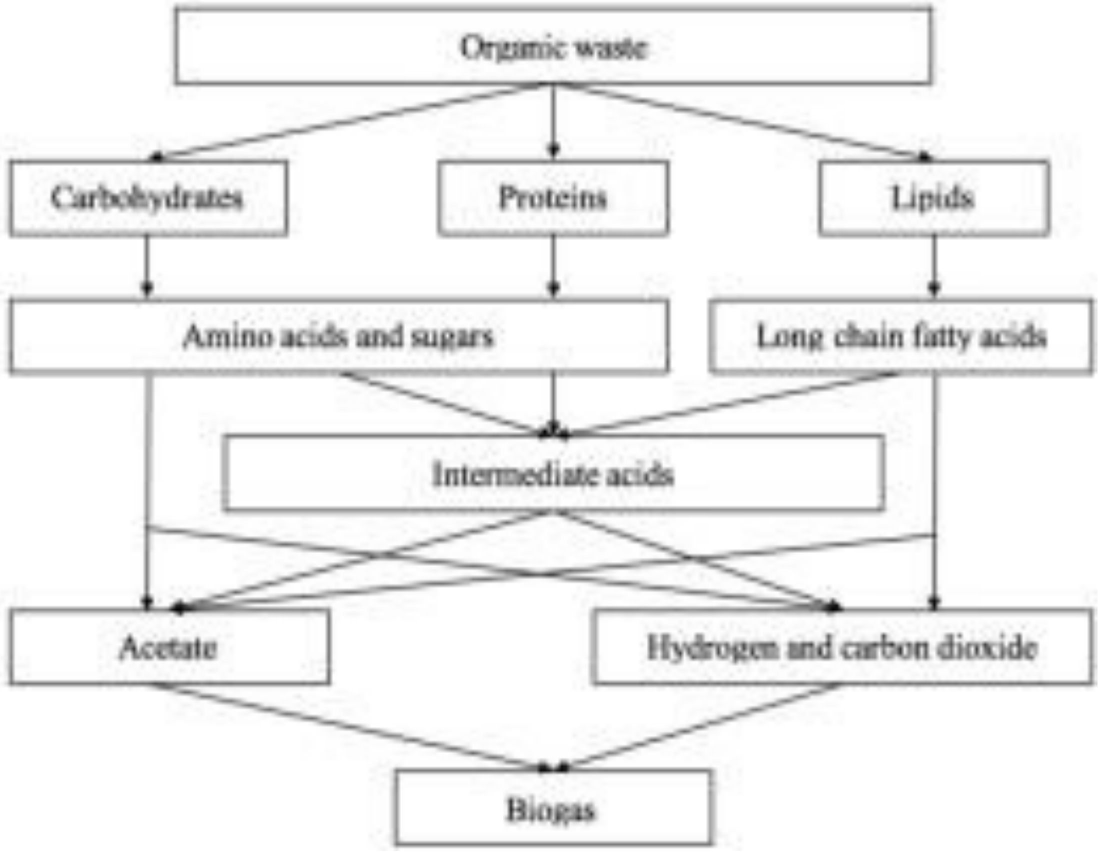

Figure 2.1: A breakdown of Anaerobic Digestion

energy footprint of conventional wastewater treatment technology. It has reduced the use of chemical fertilizer as the digestate (the content of the reactor after completion of digestion can be used as fertilizer).

2.2 Biogas

Biogas is a type of renewable energy that is produced from organic materials through a process known as anaerobic digestion. The organic materials used to produce biogas can include food waste, agricultural waste, animal manure, sewage sludge, and other types of biomass.

During the anaerobic digestion process, bacteria break down the organic materials in the absence of oxygen, releasing methane gas (CH_4, which has a calorific value of 9.97 kWh/m^3) as a by-product. Biogas consists mainly of methane (CH4) and carbon dioxide (CO2), as well as trace amounts of other gases.

2.2.1 Components of biogas

The main components of biogas are:

a) Methane (CH_4): Methane is the primary component of biogas and typically makes up between 50-70% of the gas produced. It is a potent greenhouse gas, but when captured and used as a fuel, it can displace the use of fossil fuels and reduce greenhouse gas emissions.

b) Carbon dioxide (CO_2): Carbon dioxide is the second most abundant component of biogas, typically making up between 30-50% of the gas produced. While it is also a greenhouse gas, it is less potent than methane and can be captured and used for other purposes.

c) Trace gases: Biogas also contains trace amounts of other gases such as hydrogen (H_2), nitrogen (N_2), oxygen (O_2), and hydrogen sulphide (H_2S), which give the gas its characteristic odour.

d) Water vapour**:** Biogas also contains water vapour, which can condense in pipelines and equipment if not managed properly.

Biogas is considered a renewable energy source because the organic materials used to produce it are constantly being generated by various processes such as food production and waste disposal. Additionally, biogas production can help reduce greenhouse gas emissions by diverting organic waste from landfills where it would otherwise decompose and release methane into the atmosphere. The components of biogas is represented in Table 2.1. Overall, biogas has the potential to be a valuable source of clean energy, particularly in rural areas where there is a ready supply of organic materials.

2.2.2 Uses of Biogas

Biogas can be used readily in all applications designed for natural gas such as direct combustion including absorption heating and cooling, cooking, space and water heating, drying, and gas turbines. It may also be used in fueling internal combustion engines and fuel cells for production of mechanical work and/or electricity. If cleaned up to adequate standards, it may be injected into gas pipelines and provide illumination and steam production. Through a catalytic chemical oxidation methane can be used in the production of methanol production.

Biogas, if compressed for use as an alternative transportation fuel in light and heavy-duty vehicles, can use the same existing technique for fueling already being used for compressed natural gas vehicles. In many countries, biogas is viewed as an environmentally attractive alternative to diesel and gasoline for operating buses and other local transit vehicles. The sound level generated by methane-powdered engines is

Table 2.1: Typical composition of biogas

Typical Composition of Biogas		
COMPOUND	MOLECULAR FORMULA	PERCENTAGE
Methane	CH_4	50–75
Carbon Dioxide	CO_2	25–50
Nitrogen	N_2	0–10
Hydrogen	H_2	0–1
Hydrogen Sulphide	H_2S	0–3
Oxygen	O_2	0–0

generally lower than that generated by diesel engines and the exhaust fume emissions are considered lower than the emission from diesel engines, and the emission of nitrogen oxides is very low. Application of biogas in mobile engines requires compression to high pressure gas (>3000 psig) and may be best applied in fleet vehicles. A refuelling station may be required to lower fuelling time and provide adequate fuel storage.

Biogas can be compressed after removal of carbon dioxide, the same way as natural gas is compressed to compressed natural gas (CNG), and used to power motor vehicles. In the United Kingdom, for example, biogas is estimated to have the potential to replace around 17% of vehicle fuel. It qualifies for renewable energy subsidies in some parts of the world. Biogas can be cleaned and upgraded to natural gas standards, when it becomes bio-methane. Biogas is considered to be a renewable resource because its production-and-use cycle is continuous, and it generates no net carbon dioxide. As the organic material grows, it is converted and used. It then regrows in a continually repeating cycle. From a carbon perspective, as much carbon dioxide is absorbed from the atmosphere in the growth of the primary bio-resource as is released, when the material is ultimately converted to energy.

2.3 Processes involved in biogas production

The biological conversion of organic waste into biogas (as shown in Figure 2.2) consists of 4 main stages: hydrolysis, acidogenesis, acetogenesis and methanogenesis

a) Hydrolysis

Hydrolysis is the first step in the anaerobic decomposition of organic matter, and here fats, cellulose and proteins in insoluble forms are decomposed into soluble compounds. Fats are decomposed by fat decomposing organisms, cellulose is decomposed by cellulose decomposing organisms, proteins are decomposed by protein decomposing organisms. All these are decomposed into soluble compounds. These decomposing organisms can be called microbes. It involves the conversion of polymeric organic matter (e.g., polysaccharides, lipids, proteins) to monomers (e.g., sugars, fatty acids, amino acids) by hydrolases secreted to the environment by microorganisms. Bacteria decompose the long chains of the complex carbohydrates, proteins and lipids into shorter parts. For example, polysaccharides are converted into monosaccharides. Proteins are split unto peptides and amino acids. Three key groups of hydrolases are involved in the process of anaerobic digestion: esterases, glycosidases and peptidases, which catalyse the cleavage of ester bonds, glycoside bonds and peptide bonds, respectively.

Examples of hydrolytic bacteria involved in hydrolysis are; *Bacillus, Cellulomonas, Eubacterium* (Figure 2.2). While some of the products of hydrolysis, including hydrogen and acetate, may be used by methanogens later in the anaerobic digestion process, the majority of the molecules, which are still relatively large, must be further broken down in the process of acidogenesis so that they may be used to create methane.

b) Acidogenesis

During acidogenesis, Acid-producing bacteria converts the intermediates of fermenting bacteria – complex organic compositions into more simple compounds. The products of hydrolysis are converted to non-gaseous short-chain fatty acids, alcohols, aldehydes, Hydrogen Sulphide (H_2S), Ammonia (NH_3) and the gases, such as carbon dioxide (CO_2) and hydrogen (H_2). These organic substances act as nutrients for methane-producing bacteria that converts organic acids into Biogas. Examples of Fermenting Bacteria (Figure 2.2) that takes part in Acidogenesis are; *Propionibacterium, Butyrivibrio, Acetivibrio.*

c) Acetogenesis

Acetogenesis is one of the biogas production processes steps that volatile fatty acids formed by acidogenesis are converted to acetic acid, hydrogen molecule (H_2) and carbon

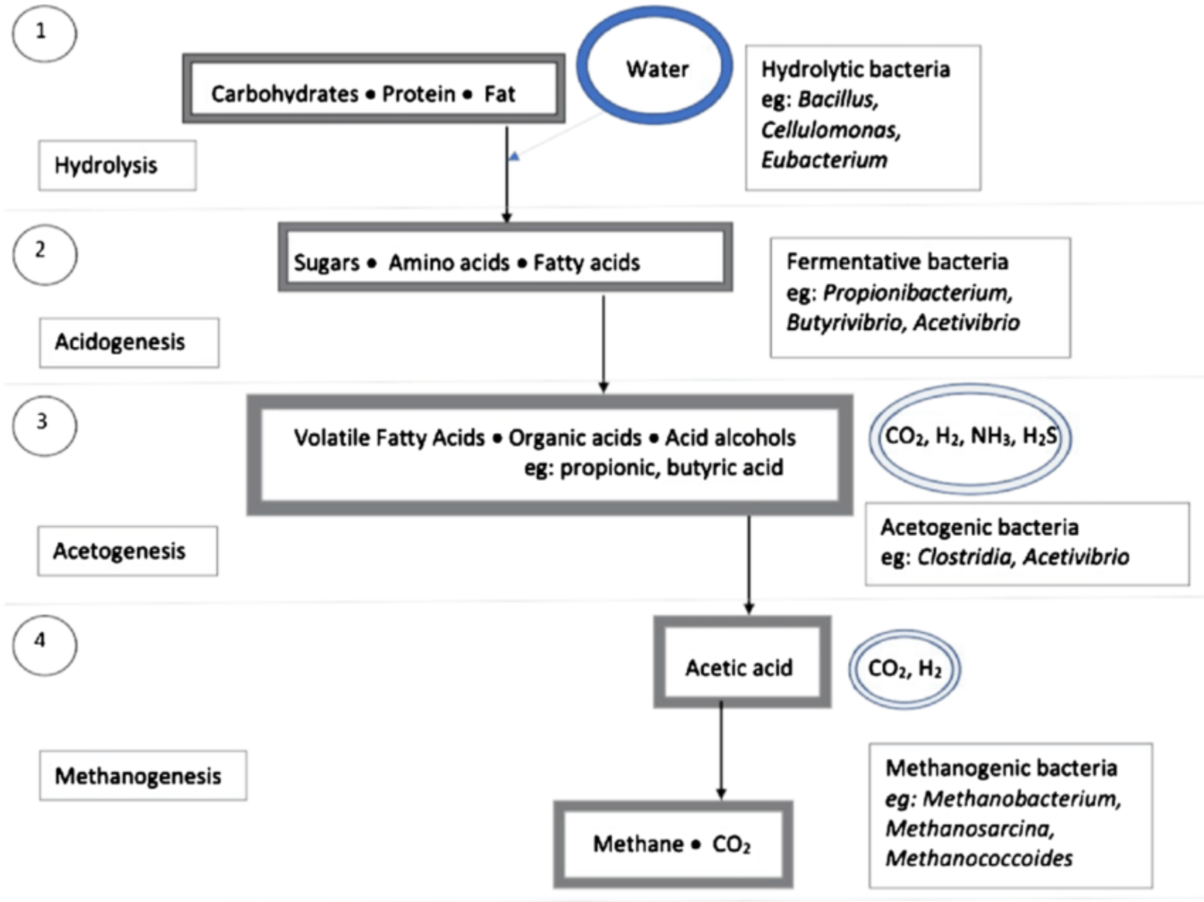

Figure 2.2: A breakdown of the processes involved in biogas production.

dioxide (CO_2) by acetogenic bacteria via anaerobic oxidation. Acetate can also be produced from H_2 and CO_2 by homoacetogens, an H-oxidising acetogenic bacteria. Acetogenesis is essential because during this process, substrates are produced for Methanogens to produce Methane used for biofuel.

d) Methanogenesis

Methanogenesis constitutes the final stage of anaerobic digestion in which methanogens create methane from the final products of acetogenesis as well as from some of the intermediate products from hydrolysis and acidogenesis. There are two general pathways involving the use of acetic acid and carbon dioxide, the two main products of the first three steps of anaerobic digestion, to create methane in methanogenesis:

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$$

$$CH_3COOH \rightarrow CH_4 + CO_2$$

While CO_2 can be converted into methane and water through the reaction, the main mechanism to create methane in methanogenesis is the path involving acetic acid. This step creates methane and CO_2, the two main products of anaerobic digestion.

2.4 Factors Affecting Biogas Production

There are several factors such as biogas potential of feedstock, inoculums, nature of substrate, pH, temperature, loading rate, hydraulic retention time (HRT), C:N ratio, volatile fatty acids (VFA), inhibitory substances, etc. influence the biogas production (Gashaw, 2014; Dioha et al., 2013).

2.4.1 Temperature

Temperature is an important factor for determining the efficiency of anaerobic digestion (AD) process. The process can be operated under three temperature ranges:

- Thermophilic (40 °C – 70 °C)
- Mesophilic (25 °C – 40 °C)
- Psychrophilic (below 25 °C)

Rise in temperature aids increased gas production but results in lesser methane content and increased percentage of CO_2 leading to lower heating value of biogas. Hence, the optimum temperature was found to be 32 °C – 35 °C for efficient and continuous biogas production (Al Mamum etal., 2015). The operating temperature ranges are much debated as some researchers prefer Mesophilic and others Thermophilic for the anaerobic treatment. It has also been reported that the anaerobes are most active in the Mesophilic and Thermophilic ranges (Desai etal., 1994; Zennaki, *et al.,* 1996).

Insulating the digester with insulating material can help retain desired temperature. Digester coated with charcoal has been observed to have improved biogas production by 7-15% in KVIC model (Al Mamun et al., 2015; Anand et al., 1993). Maintaining the temperature to 40 °C can help reduce the retention time in digester by 40 % (Desai et al., 1994).

Thermophilic conditions (> 45 °C) were reported to better than Mesophilic conditions (25 °C – 40 °C) as higher temperature helps reduce pathogens and also eliminate odour problem (Sahlström, 2003; Johansen, et al., 2013; Moset, *et al.,* 2015).

2.4.2 pH value

pH plays a pivotal role in the operation as the pH changes at different stages of the anaerobic digestion. pH and temperature are interdependent. The optimum pH which helps to enhance biogas yield lies between 6.5 - 7.2 (Sunny *et. al.,* 2018). The pH changes when total VFA concentration exceeds 4 g/l and glucose is inhibited for fermentation (Siegert *et al.,* 2005; Nazmi, et al, 2009). The concentration of VFA and acetic acid should be < 200 mg/l for maintaining optimum level of pH (Yadvika *et al.,* 2004; Gashaw, 2016).

The pH within the digester can be maintained within the range of 6.5- 7.2 by determining adequate organic loading rate. If the process leads to a decrease in the pH of the substrate inside the bio-digester then it can be controlled by addition of lime or recycled filtrate (Al Mamun *et al.,* 2015).

Figure 2.3 shows that the biogas productivity is affected by change in pH with respect to the retention time. Thus, we can interpret that the optimum pH for enhanced biogas production lies in the neutral range i.e., 7.0. Researchers comprehended that when pH is in the methanogenic range then it valorizes the methane content in the biogas which is more than 60%. They observed that the pH of the effluent leachate of CSTR digester was in the range of 7.75-8 at a loading rate of 1.4 kg VS/m^3 and COD of 2150 mg/l (Babaee *et al.,* 2011).

2.4.3 Carbon/Nitrogen (C/N) ratio

C/N ratio plays an important role to determine the suitability of organic matter (OM) for anaerobic digestion. High C/N ratio indicates low nitrogen content for microbial growth and as a result methanogens uptake the nitrogen for protein production thereby leading to carbon wastage which ultimately leads low biogas yield. Whereas, low C/N ratio can lead to accumulation of ammonia, nitrogen which may cause inhibition in the anaerobic digestion process. (Aworanti, *et. al* 2017).

Gerardi, (2003) reported that C/N ratio of 25:1 was optimum for good biogas production. The optimum range of C/N for proper functioning of bi-digester was found to be *20–35:1.* Higher temperatures require higher C/N ratio to lessen the possibility of ammonia inhibition. Typical C/N ratios were recorded for few feedstocks: chicken manure 15:1, grass silage 25:1, cattle manure 13:1and rice husks 47:1 for obtaining maximum biogas yield.   The optimal C/N ratio ensures better methane yield

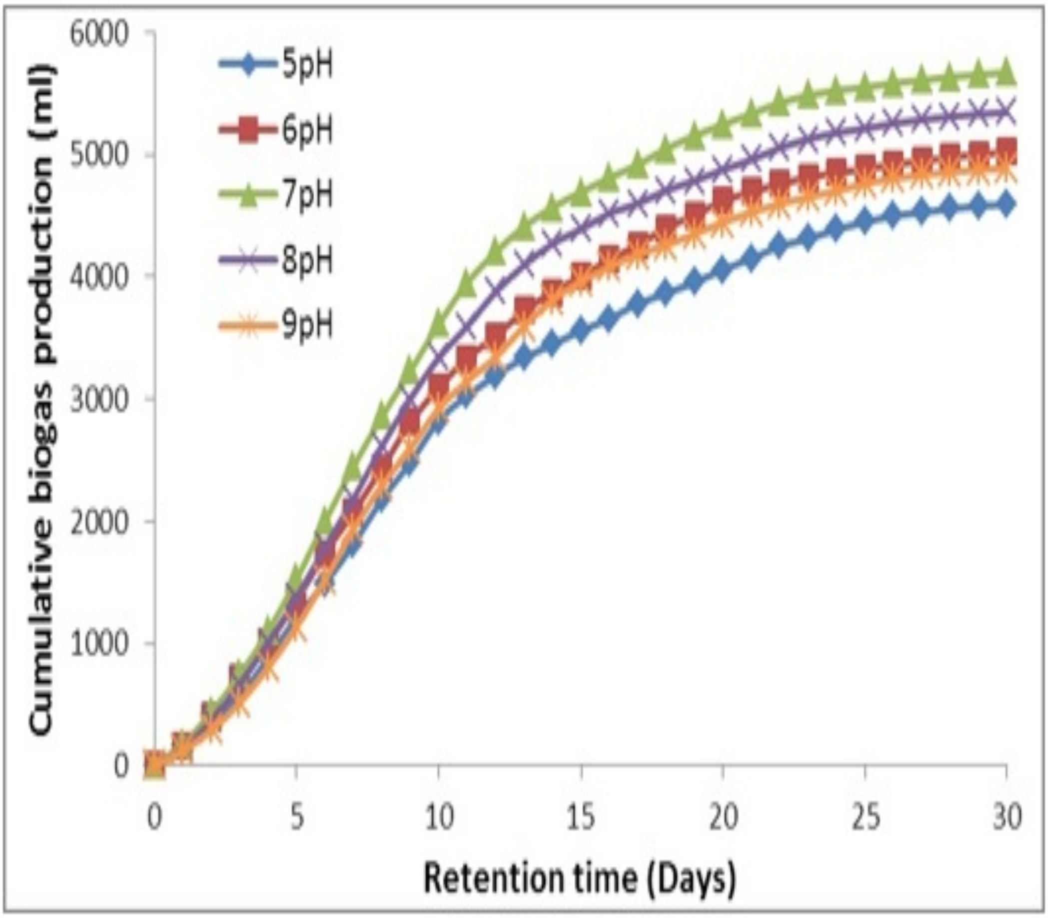

Figure 2.3: pH vs Biogas Production

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2.4.4 Hydraulic retention time (HRT)

Hydraulic retention time (HRT) is the time for which the biodegradable matter remains inside the reactor. HRT is influenced by the temperature inside the digester, the type of the feedstock and the technologies applied. The HRT in case of mesophilic digester is 10- 40 days and thermophilic is of 14 days. Too short retention time might leave the bacteria getting washed out of the digester without they getting multiplied thus leaving the digester coming to standstill state and longer retention time would increase the volume of the reactor. Thus, in order to reduce the retention time and reactor volume, the optimum loading rate is to be maintained for optimizing the methane gas generation. 2-3 weeks of time is considered optimum for lignocellulosic material to degrade and generate biogas.

Studies of treating a co-digested cattle waste, poultry waste and cheese whey (2:1:3) gave highest biogas production of 2.2 l/day having 62% methane content with an HRT of 10 days and OLR of 6 g TS/ l (Al Mamun etal 2015; Desai etal., 1994).

2.4.5 Total and volatile solids and their particle size

Solid contents are the total amount of fermentable substrate present in a unit volume of slurry. Higher level of dry solids, especially lignocellulosic content, affect the hydrolysis process. The optimum level solid content for improving the productivity was found to be 7-9% (Zennak *et al.,* 1996).

Total solids (TS) encompass both organic as well as inorganic matter. The percentage of volatile solids (VS) present in the substrate is directly proportional to the methane yield. (Moody et al., 2009). It is recommended that 8% TS resulted better biogas yield. (Baserja, 1984) reported that the biogas production increased to 0.46 m^3/ (m^3 day) at 37 °C and 0.68 m^3/ (m^3 day) at 55 °C respectively. It was observed that when TS content decreases below 7% system becomes unstable whereas above 10% TS content the digester becomes overloaded hindering its performance.

The size of the particle influences the overall fermentation of the organic matter in the digester. Smaller particle size enhances greater adsorption on the substrate resulting in increase in the microbial activity leading to greater biogas yield. Crushing of the feedstock into smaller and uniform particle size can significantly reduce the volume of the digester without compromising on the quantity of biogas produced.

2.4.6 Moisture content

The moisture content of the substrate affects the process of anaerobic digestion. The highest methane yield has been reported at 60-80% humidity. The experimental comparison between 70% and 80% moisture content showed that maximum biogas was produced in former i.e. 83 ml CH_4/ g dry matter as compared to later i.e. 71 ml CH_4/ g dry matter (Gashaw, 2016; Khalid et al., 2011).

2.4.7 Mixing

Mixing greatly helps to ensure intimate contact between micro-organisms which leads to improved fermentation efficiency. Mixing can be carried out in a number of ways.

For instance, if slurry is fed every day instead of feeding periodically at a certain interval, it causes more frequent contact between micro-organisms thus giving desired mixing effect. It can also be achieved by carrying out certain alterations in inlet and outlet pipes of a plant.

Mixing can also be carried out by installing certain stirring or mixing devices in the plant. Alternatively, mixing can be achieved by installing wooden conical beams which help to break down scum following up and down movement of slurry surface at the time of filling and emptying of digester. Where no mixing device is provided, stratification can be reduced by using a horizontal displacement digester which stimulates a plug flow. In digesters which utilize municipal refuse, part of the gas produced can be re-circulated to mix digester contents.

It is possible to introduce this feature in large sized biogas plants such as community type. It does not seem to be practical to introduce this feature in family sized plants mainly due to unaffordable costs and skilled supervision needed

A number of experiments have been carried out to analyse the effect of mixing on gas yield. One of the early attempts to study the effect of gas recirculation as an aid to mixing, on biogas yield showed that the gas recirculation helps to improve gas yield as reported by (Marsh and Voice 2017).

2.5 Bio-digester

A bio-digester is a closed system that biologically digests organic material, either anaerobic (without oxygen) or aerobically (with oxygen). Microbes and other bacteria break down organic materials in a bio-digester. Most food, including fat, greases, and even animal manure, can be processed in a bio-digester.

2.5.1 Types of Bio-digesters

a) Fixed Dome Digesters

The fixed dome digesters (Figure 2.4) also called “Chinese” or “hydraulic” digesters are the most common model developed (Santerre, et al.,1982). The produced biogas is accumulated at the upper part of the digester called storage part. The difference in the level between slurry inside of the digester and the expansion chamber creates a gas pressure. The collected gas requires space and presses a part of the substrate into an expansion chamber. The slurry flows back into the digester immediately after gas is released (Sasse, et al., 1991). Fixed dome digesters are usually built underground. The size of the digester depends on the location, number of households, and the amount of substrate available every day. For instance, the size of these digesters can typically vary

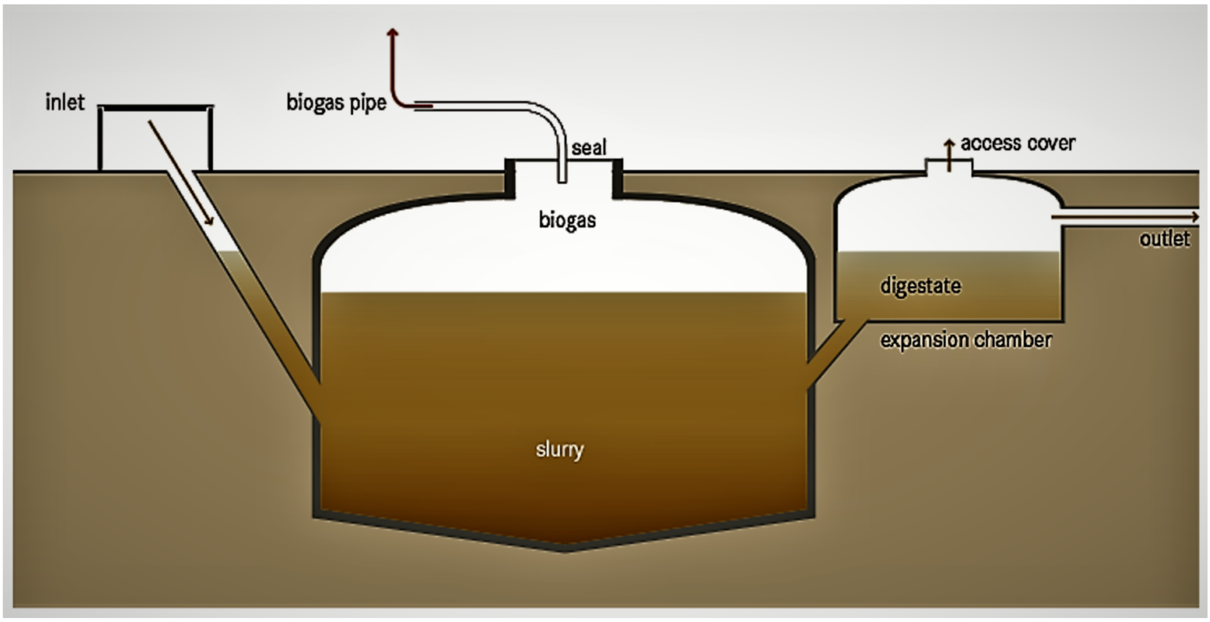


Figure 2.4: Fixed Dome Digester.

between 4 and 20 m^3 in Nepal (Gautam, *et. al* 2009), between 6 and 10 m^3 in China (Daxiong, *et. al* 1990), between 1 and 150 m3 in India (Tomar, 1994) and in Nigeria it is around 6 m3 for a family of 9 (Adeoti, et al, 2000). Instead of having a digester for each individual home, a large volume digester is used to produce biogas for 10–20 homes, and is called community type biogas digesters. In countries where houses are clustered as in Nigeria, these types of biogas digesters are more feasible (Akinbami, *et al,* 2001).

The costs of a fixed-dome biogas plant are relatively low. It is simple as no moving parts exist. There are also no rusting steel parts and hence a long life of the plant (20 years or

more) can be expected. The plant is constructed underground, protecting it from physical damage and saving space. While the underground digester is protected from low temperatures at night and during cold seasons, sunshine and warm seasons take longer to heat up the digester. No day/night fluctuations of temperature in the digester positively influence the bacteriological processes. The construction of fixed dome plants is labor-intensive, thus creating local employment. Fixed-dome plants are not easy to build. They should only be built where construction can be supervised by experienced biogas technicians. Otherwise, plants may not be gas-tight (porosity and cracks).

b. Floating drum digesters

Floating-drum plants (Figure 2.5) consist of an underground digester (cylindrical or dome-shaped) and a moving gas-holder. The gas-holder floats either directly on the fermentation slurry or in a water jacket of its own. The gas is collected in the gas drum, which rises or moves down, according to the amount of gas stored. The gas drum is prevented from tilting by a guiding frame. When biogas is produced, the drum moves up and when it is consumed, the drum goes down. For a small-medium size farms the size varies from around 5–15 m^3.

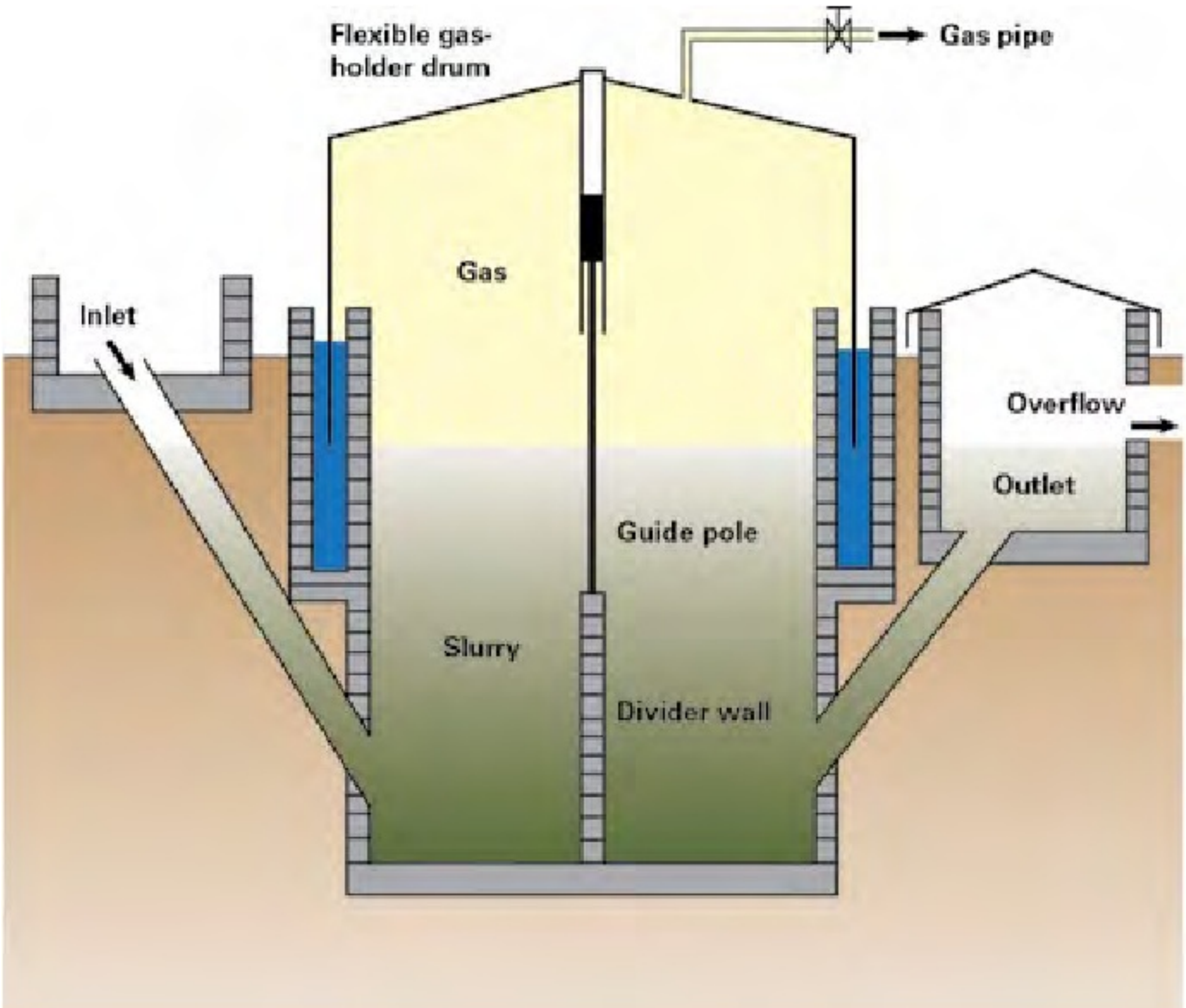


Figure 2.5: A floating drum digester.

Floating drum digesters produce biogas at a constant pressure with variable volume (Green, et al., 2002). From the position of the drum, the amount of biogas accumulated under the drum is easily detectable. However, the floating drum needs to be coated with paint in a constant interval to avoid rust. Additionally, fibrous materials will block the movement of digester. Hence, their accumulation should be avoided if possible (Werner, *et al.,* 1989).

The steel drum is relatively expensive and maintenance-intensive. Removing rust and painting has to be carried out regularly. The life-time of the drum is short (up to 15 years; in tropical coastal regions about five years).

b. Plug flow digesters (Tubular Digester)

The disadvantage with the fixed dome and floating drum models is, once installed they are difficult to move. Hence, portable models built over the ground called tubular or plug flow digesters were developed (Figure 2.6).

Plug flow digesters have a constant volume, but produce biogas at a variable pressure. The size of such digesters varies from 2.4 to 7.5 m^3. Plug-flow digesters consist of a narrow and long tank with, an average length to width ratio of 5:1. The inlet and outlet of the digester are located at opposite ends, kept above ground, while the remaining parts of the digester is buried in the ground in an inclined position. As the fresh substrate is added from the inlet, the digestate flows towards the outlet at the other end of the tank. The inclined position makes it possible to separate acidogenesis and methanogenesis longitudinally, thus producing a two-phase system. In order to avoid temperature fluctuations during the night and maintain the process temperature, a gable or shed roof is placed on top of the digester to cover it, which acts as an insulation both during day and night (Bouallagui, *et al.,* 2003).

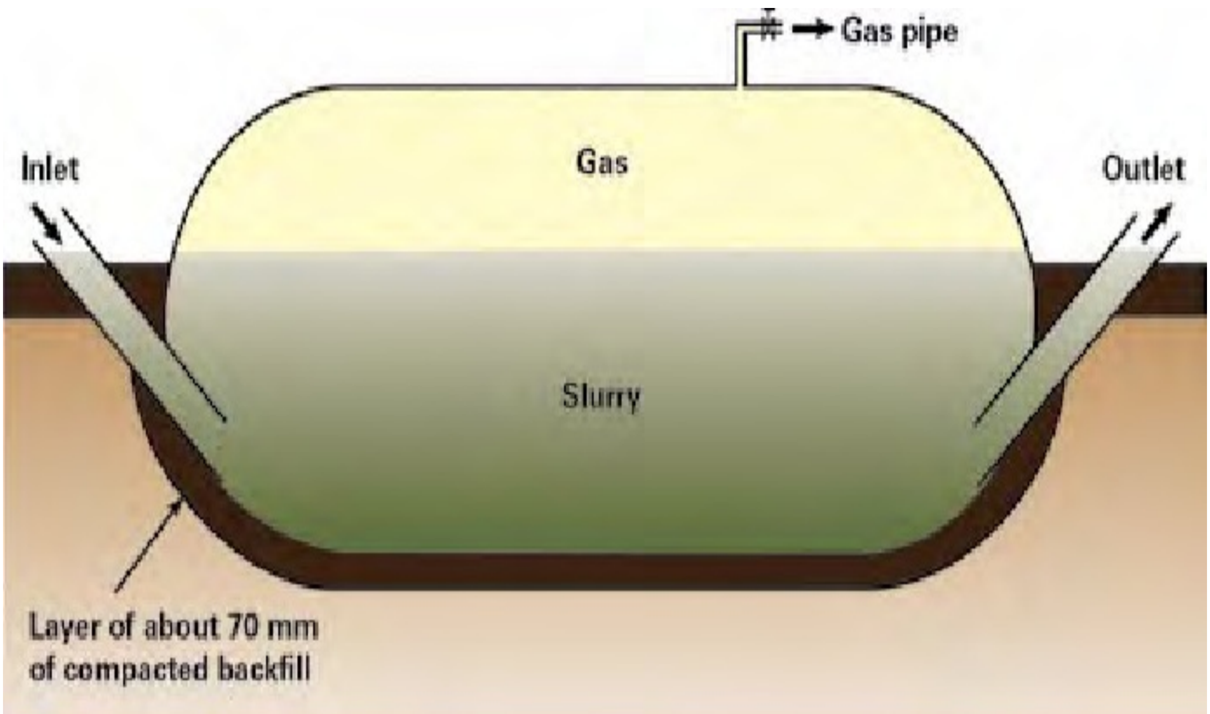


Figure 2.6: A plug flow or tubular digester.

The usefulness of these digesters includes easy installation, easy handling, and adaptation to extreme conditions at high altitudes with low temperatures. The transportation costs for the material to build the digester in hilly areas are high, leading to a high capital cost. On the other hand, plug flow digesters are easy to transport, which ultimately reduces the cost of the digester (Ferrer, et al., 2011).

According to AGSTAR, 51% of all digesters installed by United States Environmental Protection Agency (USEPA) were plug flow digesters. Plug flow designs are suitable for manure, and operating semi-continuously with a HRT between 20 and 30 days, and a solid contents varying between 11 and 14%. These digesters do not have moving parts, reducing risks for failure (Beddoes, *et al.,* 2007)

2.6 Cassava plant

Cassava (*Manihot esculenta* Crantz), synonyms: manioc, yucca, tapioca, is a tubercle 5 to10 cm in diameter and 15 to 35 cm in length. It is produced in almost all mild and tropical countries and grows in degraded soils where almost nothing else can grow. It does not need fertilizers, insecticides, or additional water. Furthermore, cassava can be harvested any time between 8 to 24 months after planting. Native to South America cassava has historically been a human and animal feed

2.6.1 Cassava bioenergy potential

There are three potential biofuels that can be generated at an industrial scale from cassava biomass viz., bioethanol, biodiesel and biogas.

2.6.2 Biogas production from cassava

Biogas is a product of a microbial process known as anaerobic digestion (AD). Anaerobic digestion involves microorganisms decomposing organic matter in the absence of oxygen to produce mainly methane and carbon dioxide. The residues left after AD adds value to the process as they can be used for agricultural purposes as fertilizer. Other important applications of AD include the reduction of sludge volume generated from wastewater treatment processes, sanitation of industrial organic waste, and

2.6.3 Optimization of cassava biogas production

Most of the techniques used to improve biogas production and methane yields at landfills and wastewater treatment plants also apply to cassava biomass because of its organic material content. The rate of biogas production depends on three main factors; the degree of hydrolysis, the rate of production or consumption of intermediates, and the rate of methanogenesis. Therefore, optimization of cassava biogas production is based on the need to improve material digestibility and biological activity

2.7 Benefits of Biogas

i. Biogas is eco-friendly

Biogas is a renewable, as well as a clean, source of energy. Gas generated through biodigestion is Biogas is a renewable, as well as a clean, source of energy. Gas generated through biodigestion is non-polluting; it actually reduces greenhouse emissions (i.e. reduces the greenhouse effect). No combustion takes place in the process, meaning there is zero emission of greenhouse gasses into the atmosphere;

therefore, using gas from waste as a form of energy is a great way to combat global warming.

Unsurprisingly, concern for the environment is a major reason why the use of biogas has become more widespread. Biogas plants significantly curb the greenhouse effect: the plants lower methane emissions by capturing this harmful gas and using it as fuel. Biogas generation helps cut reliance on the use of fossil fuels, such as oil and coal.

Another biogas advantage is that unlike other types of renewable energies, the process to create the gas is natural, not requiring energy for the generation process. In addition, the raw materials used in the production of biogas are renewable, as trees and crops will continue to grow. Manure, food scraps, and crop residue are raw materials that will always be available, which makes it a highly sustainable option.

ii. Biogas generation reduces soil and water pollution

Overflowing landfills don't only spread foul smells- they also allow toxic liquids to drain into underground water sources.

Subsequently, another advantage of biogas is that biogas generation may improve water quality. Moreover, anaerobic digestion deactivates pathogens and parasites; thus, it's also quite effective in reducing the incidence of waterborne diseases. Similarly, waste collection and management significantly improve in areas with biogas plants. This in turn, leads to improvements in the environment, sanitation, and hygiene.

iii. Biogas generation produces organic fertilizer

The by-product of the biogas generation process is enriched organic digestate, which is a perfect supplement to, or substitute for, chemical fertilizers. The fertilizer discharge from the digester can accelerate plant growth and resilience to diseases, whereas commercial fertilizers contain chemicals that have toxic effects and can cause food poisoning, among other things.

iv. It's a simple and low-cost technology that encourages a circular economy

The technology used to produce biogas is quite cheap. It is easy to set up and needs little investment when used on a small scale. Small biodigesters can be used right at home, utilizing kitchen waste and animal manure. A household system pays for itself after a while and the materials used for generation are absolutely free. The gas produced can be used directly for cooking and generation of electricity. This is what allows the cost of biogas production to be relatively low.

Farms can make use of biogas plants and waste products produced by their livestock every day. The waste products of one cow can provide enough energy to power a lightbulb for an entire day. In large plants, biogas can also be compressed to achieve the quality of natural gas and utilized to power automobiles. Building such plants requires relatively low capital investment and creates green jobs. For instance, in India, 10 million jobs were created, mostly in rural areas, in plants and in organic waste collection.

v. Healthy cooking alternative for developing areas

Biogas generators save women and children from the daunting task of firewood collection. As a result, more time is left for cooking and cleaning. More importantly, cooking on a gas stove, instead of over an open fire, prevents the family from being exposed to smoke in the kitchen. This helps prevent deadly respiratory diseases. Sadly, 4.3 million people a year die prematurely from illnesses attributed to the household air pollution caused by the inefficient use of solid fuels for cooking.

CHAPTER THREE

MATERIALS AND METHOD

3.1 Materials

The Fresh sample of cassava waste water was collected from different cassava production in Ile-Ife, Osun State, Nigeria. Plastic keg containers of volume 25, 10 and 5-liters was used during this experiment. While 25 liters plastic kegs served as the bio-digester, the 10 and 5 liters serves the water holder and the displaced water collector respectively. This was be replicated for each unit of the set up. Other accessories used are;

i. Rubber Slippers: This will be used to seal the digesters' openings,
ii. Knives: A kitchen knife would be used to cut the slippers into the required shape and size,
iii. Top Bond and Top Gum: This adhesive would be used to gum the rubber slippers, the hoses and the temperature probe to the plastic biodigester container.

iv. Syringe: the clinical syringe would be used for collecting the samples of substrate to measure the pH.

v. Other accessories: These include Nose Mask, Hand Glove, Bowls, Buckets, temperature probe, the temperature digital display meter. Weighing balance, Hose, Measuring cylinder.

3.2 Cassava Wastewater Substrate Collection

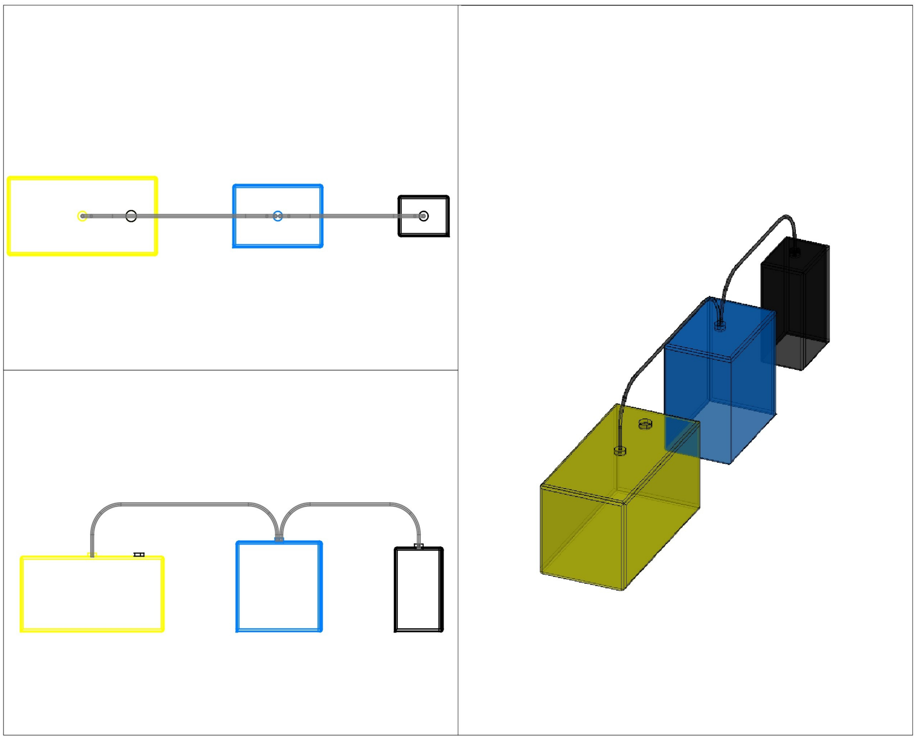
The sample of cassava waste water was obtained from selected local cassava processors in Ife-Ife, Nigeria. The collected sample was stored in airtight containers to prevent contamination and degradation of organic matter.

3.3 Preparation of Inoculum

the wastewater underwent rigorous mixing to ensure a homogeneous substrate, crucial for consistent results. This prepared sample was therefore used in preparing the substrate in three categorized as cassava waste water only at pH value of 8.2 (T1), Mixture of cassava and cow dung at 50 by 50 % proportion (T2) and cow dung only. (T3)

3.4. Experimental Setup

The digestion tests were conducted at the Biogas Laboratory in the Department of Agricultural and Environmental Engineering, Obafemi Awolowo University, Ile-Ife, Nigeria. Prior to commencing the tests, the total solids (TS) and volatile solids (VS) content of the wastewater was determined. Each digestion reactor consists of a 25-liter keg loaded with the substrate for incubation (figure 3.1). Before incubation, the headspace in each vessel will be purged with Nitrogen for three minutes to ensure anaerobic conditions, and immediately capped with a rubber septum. Daily biogas production volume was measured by volumetric displacement through the water display collected attached to the water holder, which linked to the biodigester through



the top rubber septum. Biogas production was therefore quantified volumetrically at normal temperature and pressure conditions using a graduated measuring cylinder. (Figure 3.2) and plate 3.1

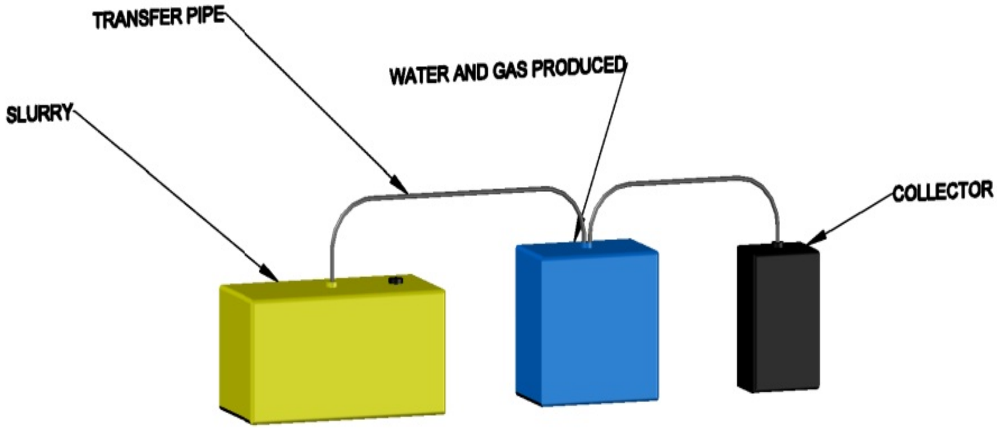


Fig. 3.1: Diagrammatic view of the digestion

Fig. 3.2: Cross section of the digestion setup

Plate 3.1: Pictorial View of the Experimental Set-up

3.5 Monitoring Parameters

The following properties were determined during the course of this experiment.

3.5.1 Total solids (TS)

TS can be measured by weighing a known volume of wastewater before and after drying in an oven at a specific temperature until a constant weight is achieved

3.5.2 Volatile solids (VS)

VS is obtained by combusting the dried solids at high temperature to remove volatile components. The weight loss represents the VS content

3.5.3 Chemical oxygen demand (COD)

Laboratory methods was used which involving digestion with strong oxidizing agents for accurate measurements

3.5.4 pH

Samples of the substrate from the bio-digester was taken with the aid of a syringe and the pH value will be measured with the Digital pH meter. The Initial pH value would be recorded on the first day of loading the substrate into the bio-digester while Subsequent reading would be taken weekly after the day of loading.

3.5.5 Total Kjeldahl nitrogen (TKN)

The samples were dried and analysed for the initial nitrogen concentration using the Kjeldahl Analyser at the Department of Animal Science laboratory, Obafemi Awolowo University, Ile-Ife

3.5.6 Total phosphorus (TP)

A colorimetric method, such as the molybdenum blue method, is employed to measure the orthophosphate concentration spectrophotometrically. Calibration curves using standard solutions are utilized to relate absorbance readings to phosphorus concentrations. Quality control measures, including blank determinations and replicate analyses, ensure accuracy. Finally, the total phosphorus concentration in the original sample is calculated and reported, providing crucial information for wastewater treatment and environmental management

3.5.7 Carbon Content

The gravimetric method was used to determine the carbon content of the sample. The ash would be determined by burning a pre-dried sample in a muffle furnace at 650 ^{o}C for 6 hours. The residue (the ash content), will be calculated using:

$$\text{Ash } (\%) = \frac{weight\ of\ residue\ after\ burning}{weight\ before\ burning}\ X\ 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots.. \quad 3.2$$

The carbon content was estimated using the equation (Mercer and Rose, 1968):

$$\text{Carbon}(\%) = \frac{100 - Ash(\%)}{1.8} \qquad 3.3$$

3.5.8 Carbon to nitrogen ratio (C: N)

The C/N ratio was gotten by dividing the Carbon Content by the Nitrogen Content.

$$\text{C/N ratio} = \frac{Carbon\,Content\ (\%)}{Nitrogen\,Content\ (\%)} \qquad 3.4$$

3.5.9. Biogas production

The biogas production was measure regularly using displacement method.

3.5.10 Composition of biogas

Analyze the composition of biogas (methane, carbon dioxide, etc.) using gas chromatography.

3.5.11. Temperature measurement

Monitor temperature within the reactors to ensure favorable conditions for microbial activity.

The digester temperature and the ambient temperature of the laboratory where the experiment took place was measured with the temperature probe and the digital reader between 12noon to 3pm daily. A digital multimeter temperature within the optimal range for methanogenic bacteria activity (typically 35-40°C) was used.

3.6. Data Collection

The parameters on biogas production (gas composition, pH, and temperature) were taken at regular intervals. Manual agitation of the digesters was repeated daily for uniform temperature distribution and contact between microbes and substrate. The water tank was also filled to the brim. During the period of the study, both the ambient and substrate temperatures were recorded daily, as well as the volume of water displaced as a result of the biogas produced. Samples were collected weekly for pH determination.

3.7 Analysis

The appropriate graph was employed to present the observed differences and similarity among other observations noticed

3.8. Validation

The obtained results will be compared with existing literature or similar studies to validate the experimental findings upon completion of the work

3. 9. Safety Precautions.

Personal Protective Equipment such as gloves, nose cover, and goggles were used during the experiment and when measurements were taken to prevent hazard and contamination

CHAPTER FOUR

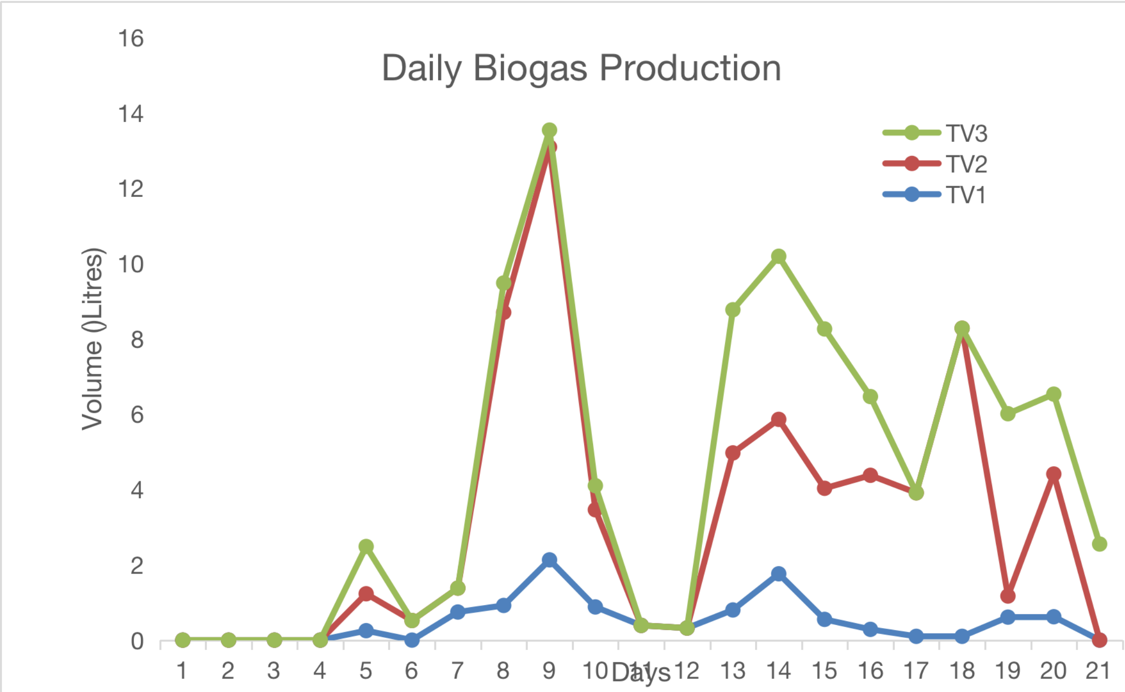
RESULTS AND DISCUSION

This chapter presents a comprehensive summary of the results obtained from the analysis. The trends and patterns identified in the data are illustrated through various visualization techniques, including linear graphs to depict relationships, bar charts for comparative analysis, and detailed tables for precise numerical representation. These visual tools help to effectively communicate the key findings and provide clear insights into the data trends observed.

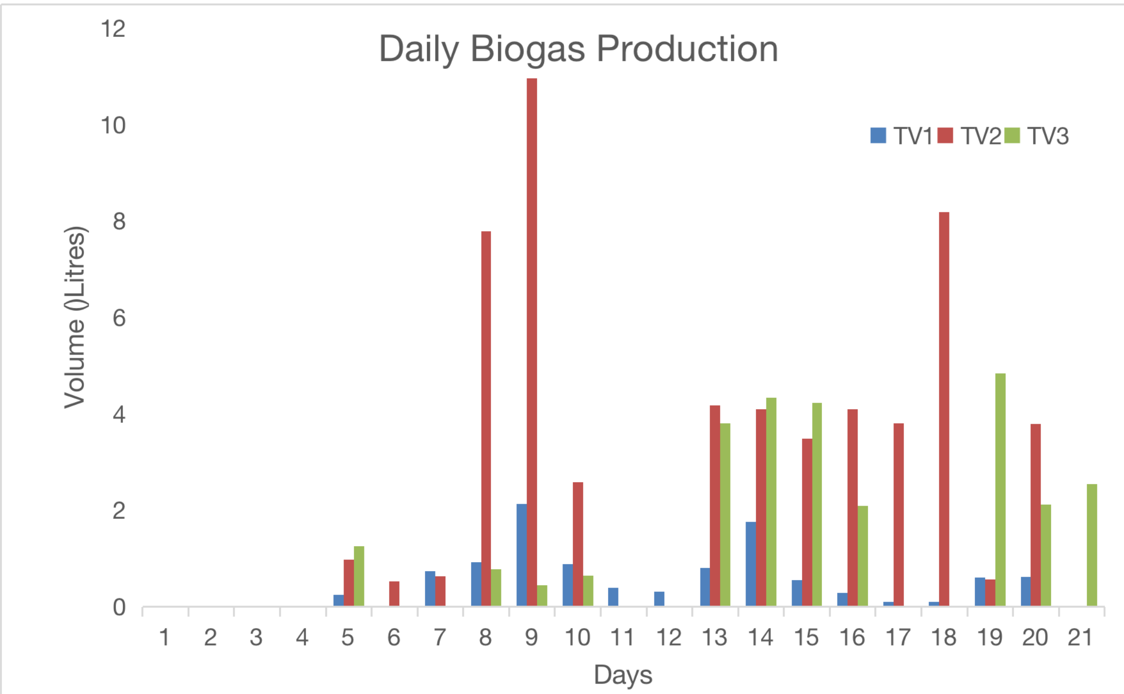
4.1. Results

The daily volume of biogas production for each treatment is presented in Figure 4.1 (a and b). The data was recorded over a period of twenty-one days. The temperature range for all treatments and the ambient conditions during this period was between 25.5 and 28.4 °C.

The vertical axis measures the volume of biogas produced in liters, allowing a direct comparison of daily production levels across the different treatments. TV1 Represents the first treatment variable which consists of only cassava waste water, TV2 Represents the second treatment variable which is the mixture of cassava waste water with cow dung while TV3 Represents the third treatment variable where the digester was filled with cow dung only. Generally, TV2 often shows the highest production peaks while TV3 showing moderate levels of biogas production.



a: Liner representation of the volume of Biogas production



b. Visual representation of the volume of Biogas production

Figure 4.1: Volume of daily Biogas Production

4.2: Discussion

The cursory look at the graphs elucidates that no production was recorded for all the treatment for the first three days. Thereafter, TV1 shows consistently low biogas production across the 21 days. The highest production for TV1 occurs on day 9, reaching

about 3 liters, but it still remains significantly lower compared to TV2 on the same day. Overall, TV1's production is sporadic and generally does not exceed 2 liters on most days. TV2 is the standout performer among the three treatments, showing high production levels, especially on days 8, 9, and 18. Day 9 marks the peak of biogas production for TV2, reaching nearly 11 liters, which is meaningfully higher than any other data point in the graph. There is another notable peak on day 18, where TV2's production spikes again to around 10 liters, demonstrating a recurring high efficiency in biogas output.

TV2's production is more variable, with days of low production interspersed with days of high spikes, suggesting that it may respond strongly to certain conditions. TV3 shows moderate performance overall, with several peaks on days 5, 13, 15, and 19. It has relatively stable production between days 13 to 19, consistently outperforming TV1 and sometimes coming close to TV2's output, especially on day 19. TV3 generally shows a smoother pattern of biogas production compared to the more erratic peaks of TV2, which might suggest a more consistent but less extreme reaction to the experimental conditions.

The TV2 appears to be the most effective treatment for biogas production, showing the highest peaks and a tendency for rapid production increases on certain days. TV3, while not reaching the extreme levels of TV2, demonstrates consistent and moderate production, which may be beneficial for scenarios requiring steady biogas output without large fluctuations.

TV1 is the least effective treatment and consistently produces the lowest volume of biogas, indicating it might not be suitable if high production is desired.

The graph indicates that TV2 is highly responsive under specific conditions, leading to significant spikes in biogas production. Meanwhile, TV3 offers more stable but moderate output, and TV1 shows limited effectiveness. This data can guide decisions on optimizing biogas production by favoring the conditions represented by TV2, while considering TV3 for consistency and TV1 for less demanding scenarios. The pH value (Figure 4.2) follows the same pattern thought the experiment which show no influent on the outcome of the biogas production volume.

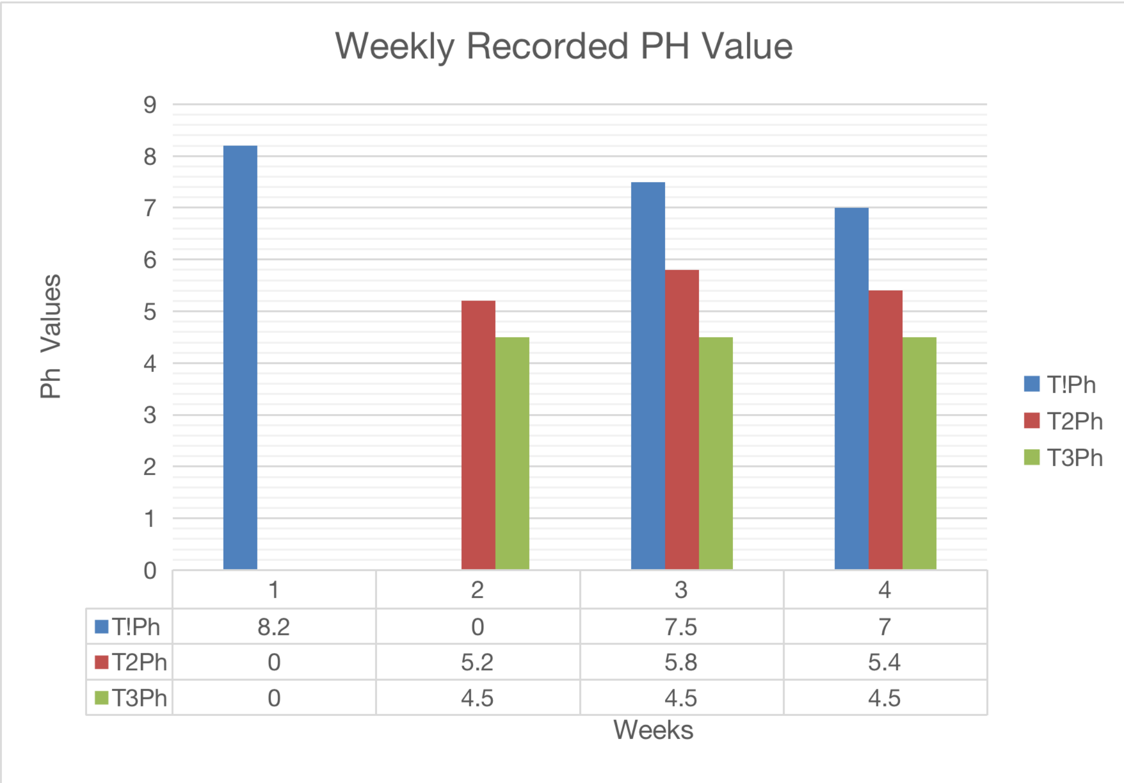


Figure 4.2: PH pattern recorded during the experiment

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATION

5.1. Conclusion

The following findings were documented in the study on assessing biofuel production from cassava wastewater:

i. The mixture of cassava with water and cow dung yielded the highest production, followed by the combination of cassava and cow dung alone, and lastly, cassava with water only.

ii. The temperatures within the ambient environment and the substrate were within the same range during the experiment

iii. The temperature difference has who little influence on the biogas production

5.2. Recommendation

The following suggestions were recommended for the improvement of the system's performance.

i. The experiment should be replicated during high temperature season

ii. The experiment should be scaled up

iii. Different mixture ration should on the volume of the bofas production should be experimented

iv. Other agricultural products should be investigated

v. Smart system should me develop to record the data and reduced frequent interaction with the set-up

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APPENDIX

Appendix 1: Daily Temperature Measurement during the Experiment

Days/ Temp (°C)	Ambient	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5
1	0	0	0	0	0	0
2	27.3	25.8	0	0	27.8	29.5
3	28.4	27.6	0	0		28.4
4	27.5	27	0	0	53.9	29.8
5	26.8	25.8	27.3	28.4	33.3	28.9
6	27.3	26.6	27.3	27.9	27.9	29.5
7	26.9	26.8	26.8	26.8	25.8	30.6
8	26.1	26.4	26.6	27.1	26.3	27.3
9	25.6	26.6	26.6	26.8	30.3	25.6
10	27.9	27.9	0	25.9	30.3	30
11	26.1	26.6	0	26.6	29.5	26.8
12	25.9	25.9	0	26.1	26.6	26.1
13	26.8	26.1	26.3	27.6	25.4	26.6
14	26.1	26.1	26.4	26.3	26.3	26.6
15	26.6	25.6	27.6	27.6	26.6	30.1
16	26.6	26.3	26.9	26.9	33.8	29.5
17	26.6	26.1	26.1	26.1	31.6	29.7
18	26.9	27.1	27.3	27.3	27.9	27.3
19	27.4	26.1	27.6	27.6	26.3	26.9
20	27.3	26.8	22.3	22.3	30.3	26.1
21	27.3	26.8	26.8	26.8	34.6	26.1
22	25.8	26.3	26.3	27.1	30.1	25.8
23	26.1	25.4	25.3	25.6	36.6	26.1
24	26.6	26.9	25.3	25.3	28.6	25.6
25	28.4	29.8	26.8	27.6	24.7	26.6
26	27.9	28.1	27.1	27.9	33.6	25.4
27	26.8	26.4	26.1	25.3	31.7	26.3
28	28.9	27.3	27.1	30.3	28.6	26.8
29	28.8	26.8	27.1	26.8	29	26.8
30	27.1	27.4	26.3	26.3	29.5	27.4

Days/ Temp (°C)	Ambient	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5
31	27.9	27.1	28.4	27.1	25.6	28.9
32	30	28.9	28.1	29.5	28.7	26.8
33	27.6	28.4	28.3	28.4	30.6	29.5
34	27.4	28.1	27.9	27.6	30.3	28.5
35	28.1	28.4	28.6	28.4	30	29.9
36	25.8	27.4	26.9	27.3	31.7	27.9
37	27.9	27.9	27.6	27.6	30	31.1
38	28.3	27.3	26.9	29.7	30.4	29.5
39	28.9	28.4	27.9	30.6	30	28.5
40	27.1	27.8	27.3	27.9	31	25.4
41	31.1	30.4	27.9	27.3	29.1	25.8
42	28.4	28.1	27.4	27.6	30.4	27.1
43	28.4	25.3	28.9	27.9	27.6	26.6
44	27.6	28.1	28.1	28.4	29.7	25.8
45	29.2	27.9	27.9	28.7	30.6	26.3
46	28.9	28.1	27.8	29.5	27.9	28.6
47	30.8	28.8	28.4	31.1	27.3	27.6
48	29.3	27.9	27.8	27.4	27.6	26.1
49	30.4	27.6	28.1	30.3	27.9	27.1
50	29.8	31.1	27.8	27.8	28.4	26.1
51	26.8	27.6	27.3	27.1	28.7	26.4
52	27.9	27.9	27.6	28.7	29.5	27.3
53	30.1	29.2	28.6	30.9	31.1	27.6
54	30.9	27.8	27.3	28.5	27.4	26.8
55	28.3	27.9	25.8	26.3	30.3	26.9
56	52.7	42.8	45.9	49.4	27.8	26.6
57	28.4	29.3	24.5	28.1	27.1	25.6
58	27.5	25.8	26.8	29.1	28.7	25.8
59	29.5	29.4	27.4	29.1	30.9	26.9
60	28.4	27.6	27.3	26.9	28.5	26.1

Days/ Temp (°C)	Ambient	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5
61	29.8	29.7	27.9	29.8	28.4	22.3
62	28.9	28.3	25.1	26.3	27.8	25.8
63	29.5	28.1	20.6	25.3	30.4	28.1
64	30.6	27.6	27.1	27.3	28.1	26.1
65	27.3	26.2	25.3	25.7	25.3	27.1
66	25.6	25.6	25.6	25.8	28.1	26.3
67	30	27.3	26.6	30	27.9	25.8
68	31.4	28.92	27.3	29.3	28.1	25.8
69	29.3	27.9	27.1	30.1	28.8	26.3
70	29.3	27.3	27.1	30.1	27.9	27.9
71	28.1	27.3	27.1	27.8	27.6	26.3
72	31.1	27.6	26.9	29.8	31.1	27.6
73	33.1	29.2	32.6	32.6	27.6	28.4
74	32.8	28.4	26.6	26.1	27.9	28.1
75	28.1	28.3	27.7	28.3	29.2	27.6
76	28.3	26.3	26.4	25.4	28.4	28.1
77	29.7	28.8	26.3	28.8	27.8	29.2
78	30.1	28.6	26.5	28.6	30.4	27.4
79	28.6	27.9	26.7	27.8	28.1	28.1
80	30.3	29.2	28.6	28.7	26.6	26.6
81	30.2	29.1	28.9	29.3	27.9	27.9
82	29.5	29.4	27.4	29.1	26.6	27.9
83	30.1	29.1	28.7	28.4	25.9	26.8
84	29.8	27.8	29.5	28.7	26.1	27.6
85	28.1	27.3	27.1	28.1	26.1	28.1
86	28.5	27.9	27.1	28.5	25.6	29.2
87	30.1	28.6	26.5	28.6	26.3	27.4
88	29.5	29	27.3	28.5	26.1	28.1
89	29.7	30.1	29.6	30.1	27.1	26.6
90	28.5	27.9	27.1	28.5	26.6	22.3

Appendix 2: Daily volume Measurement during the Experiment and ph

Days	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5
1	0	0	0	0	0
2	0	0	0	3.13	1.52
3	0	0	0	0	0
4	0	0	0	3.11	1.3
5	0.25	0.98	1.255	3.1	0
6	0.4	0.52	0	0	0.34
7	0.745	0.635	0	0	5.7
8	0.92	7.78	0.78	2.97	5.07
9	2.13	10.96	0.45	0.1	5.1
10	0.88	2.58	0.64	0.1	5.04
11	0.39	0	0	2.87	4.56
12	0.32	0	0	3.38	5.16
13	0.8	4.17	3.8	2	5.15
14	1.76	4.1	4.33	1.34	5.28
15	0.55	3.48	4.23	3.28	1.97
16	0.285	4.09	2.09	3.28	1.33
17	0.1	3.81	0	3.4	4.85
18	0.1	8.18	0	0.21	5.22
19	0.61	0.56	4.84	0.2	3.53
20	0.62	3.79	2.12	3.45	4.34
21	0	0	2.55	3.35	4.16
22	0.69	6.38	0.24	3.28	5.12
23	0	6.58	0	3.32	3.45
24	0.13	5.15	2.4	4.21	5.24
25	2.78	0.7	2.74	3.32	3.79
26	2.23	5.03	5.5	4.2	4.27
27	n/a	n/a	n/a	3.24	5.39
28	3.1	2.15	n/a	3.3	5.51
29	1.47	4.22	3.43	3.3	2.79
30	2.35	2.22	3.33	2.68	1.1

Days	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5
31	0.7	3.15	1	3.32	0.56
32	0.96	1.17	1.73	2.1	3.73
33	1.86	1.63	4.88	3.12	4.58
34	1.9	2.75	2.83	4.48	3.57
35	3.7	4.77	1.94	0.12	0.24
36	0.91	1.6	0.61	3.06	3.08
37	1.03	2.71	3.98	2.21	1.43
38	1.41	2.64	2.17	7.68	1.5
39	1.14	1.55	3.7	5.26	0
40	0.19	1.62	0.62	4.51	0.09
41	2.8	6.35	1.23	3.52	0.34
42	0.34	2.08	1.7	4.85	0.2
43	5.33	0.79	4.41	5.22	3.78
44	0.8	5.58	1.44	3.53	5.61
45	0.85	3.57	1.63	4.34	5.59
46	1.41	3.24	1.57	4.16	5.79
47	1.34	3.23	2.11	5.12	0.74
48	2.1	1.72	2.27	4.33	5.32
49	0.82	3.14	2.45	5.7	2.61
50	3.83	3.4	2.57	5.07	1.32
51	5.08	3.15	3.16	5.1	5.25
52	2.2	4.36	1.95	5.04	0.6
53	1.03	0.86	1.26	4.56	1.55
54	1.53	2	4.7	5.16	3.57
55	2.3	5.6	2.73	5.15	2.53
56	1.42	1.66	1.3	5.28	2
57	4.1	0.65	4.15	1.97	1.38
58	0.5	0.8	5.6	1.33	7.68
59	3.69	0.65	4.71	5.7	5.26
60	0.42	0.9	2.01	5.4	4.51

Days	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5
61	0.1	3.94	3	4.5	2.95
62	0.2	1.55	3.4	1.68	2.47
63	0.48	1.73	4.75	8.98	4.52
64	0.1	2.89	1.39	5.18	6.39
65	0.1	4.53	5.62	0.62	4.42
66	0.24	5.93	5.04	5.29	2.95
67	0.32	5.61	3.92	3.71	0.25
68	0.2	4.1	4.3	5.31	4.17
69	0.15	3	4.15	5.13	4.01
70	0.1	5.78	2.25	4.81	5.19
71	0.32	5.68	3.6	4.01	5.15
72	0.28	5.88	4.23	5.19	5.28
73	0.11	4.18	4.05	5.15	5.47
74	0.16	6.77	2.72	5.28	0.64
75	1.32	1.46	3.8	5.47	5.18
76	0.12	5.6	3.01	0.64	3.63
77	0.7	2.94	2.42	3.01	5.16
78	0.22	1.34	1.28	2.42	3.22
79	0.21	2.3	2.53	1.28	5.27
80	0.14	1.49	4.5	2.53	5.43
81	2.58	0.86	3.52	0.25	5.09
82	2.95	1.96	3.31	4.17	4.98
83	1.45	5.1	1.9	4.88	4.86
84	0.16	1.43	0.54	2.83	4.82
85	4.85	5.3	0	1.94	5.09
86	2.48	4.78	3.41	0.61	1.1
87	1.77	5.59	4.89	3.98	5.52
89	2.2	4.2	4.5	4.4	5.28
90	3.21	5	3.84	3.01	2.14

Appendix 3: weekly ph Measurement during the Experiment

week	ph 1	ph 2	Tph 3	ph 4	ph 5
1	8.2	nil	nil	4.74	5.1
2	7.5	5.2	4.5	6	5.6
3	7	5.8	4.5	4.5	5.4
4	7.1	5.4	4.5	4.4	5.9
5	7.4	6.5	4.7	4.5	6.5
6	7.6	6.5	4.7	4.6	6.5
7	7.1	6.6	5	4.9	6.5
8	7.3	6.7	5	5	6.5
9	7.8	6.4	5.2	5.2	6.7
10	7.1	6.9	5.6	5.4	6.9
11	7.1	7	5.4	5.5	7.5
12	7.1	7.5	5.5	5.5	7
13	7.1	7.3	5.5	5.5	7.2