PRODUCTION OF BIOGAS FROM TIGER NUT WASTE AND COW DUNG

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CERTIFICATION

This is to certify that this Research project work was carried out by OBOMIGHIE JOAN OMONEGHO with Matriculation number ENG1804652 of the Department of Chemical Engineering at the University of Benin, Benin City, Edo State.

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EXTERNAL EXAMINER	DATE

DEDICATION

I humbly dedicate this research project to God almighty who provided the much needed inspiration, wisdom, knowledge and the strength to carry out this research work successfully. This research project is also dedicated to my family for their unwavering support and contributions. Lastly, I dedicate this research project to the Department of Chemical Engineering, university of Benin.

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I wish to express my heartfelt and profound gratitude to Almighty God for His love and grace in my life. I am also grateful to my Parents and siblings for their immense and unending support and faith in me.

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ABSTRACT

Poor waste management practices have led to significant environmental pollution, with methods like landfilling, burning, and open dumping, contributing to air pollution and public health hazards. Over time, innovative approaches have been developed to tackle these issues. One such approach is anaerobic digestion, which relies on microbial degradation of organic waste material in the absence of oxygen.

The feasibility of generating biogas from combining cow dung and tiger nut waste through anaerobic digestion was investigated. The materials were mixed in a 5:1 ratio with distilled water, and the pH was adjusted from 4.7 to 6.7 using NaOH. Biogas collection was conducted using a water displacement method with a graduated cylinder.

The experiment spanned six days, during which the initial properties of the feed, including volatile solids (14.31%), total solids (14.57%), water content (85.43%), pH (6.7), ash content (0.26%), nitrogen (8.64%), potassium (298 mg/kg), phosphorus (76.28 mg/kg), and ammonium (0.08 mg/kg) were recorded. Biogas volume changes were monitored and recorded daily. The results showed the progressive increase in the volume of biogas produced until the last day of retention. The results demonstrated that anaerobic digestion is an effective method for biogas production, providing a potential solution for improving waste management practices and reducing environmental pollution.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Energy is an essential element that is extremely important to human progress and the global economy. There are several sources of energy and fossil fuels, which is one energy source, have become increasingly important over time, but they also come with disadvantages, including deterioration in environmental quality and human health due to greenhouse gas emissions (Latinwo and Agarry, 2015). According to, (Achinas and Euverink, 2016), there is an increasing interest in converting waste biomass into biogas, an unconventional but ecologically beneficial fuel source, due to the depletion of fossil fuel supplies and the increase in greenhouse gas emissions.

Nigeria is endowed with environmentally friendly but underutilized renewable energy resources such as biomass — Grasses, trees, residues, wastewater, agricultural wastes, municipal wastes and industrial wastes are some types of biomass resources. Nigeria produces roughly 144 million tonnes of biomass annually, according to statistics. Through anaerobic digestion, these resources can be converted into biogas for use as fuel, which is sustainable and replenishable (Oyedepo, 2012).

Anaerobic digestion of organic waste and biomass is becoming increasingly important as a replacement for traditional methods to ensure a steady energy supply due to its ability to reduce greenhouse gas emissions. The Anaerobic digestion or fermentation of organic substances from plant and animal waste produces biogas, a highly efficient and sustainable biofuel (Iweka et al., 2021)

The byproduct known as biogas is created when different types of microorganisms with various environmental requirement break down organic materials through various metabolic processes (Leung and Wang, 2016). Depending on the substrate, the process produces between 55 – 65% methane, 35 – 45% carbon dioxide as well as traces of hydrogen sulfide, nitrogen, and hydrogen (Abate, 2019).

The anaerobic digestion process takes place in the temperature range of 25–60°C (Sandhu and Kaushal, 2019) with the total solids content (TS) being more than 20% for dry solids or less than 10% for wet solids (Karellas et al., 2010). The conversion of large organic molecules in organic wastes into methane and carbon dioxide is the result of the activities of several bacteria that take place in the hydrolytic, acidogenic, acetogenic and methanogenic stages of Anaerobic digestion (Yadav et al., 2022) . The anaerobic digestion process begins with hydrolytic bacteria that hydrolyze or break down carbohydrates into simple sugars, protein into amino acids, and lipid into fatty acids.

Acidogenic bacteria (acetogens) convert sugars, amino acids and fatty acids into intermediate volatile fatty acids, VFAs (propionic acid, butyric acid, acetic acid and ethanol), carbon dioxide and hydrogen. VFAs together with carbon dioxide and hydrogen are then converted into acetic acid/acetate by acetogenic bacteria. The final stage of anaerobic digestion, methanogenesis, is achieved when acetic acid or carbon dioxide and hydrogen from acetogenesis are converted into methane by acetolactic and hydrotropic archaea (Goswami et al., 2016).

In addition to biogas, which is used as an energy source, anaerobic digestion creates another waste stream known as the digestate. This could represent a significant obstacle or limitation for biogas production. Using the digestate as fertilizer is one way to overcome this obstacle. For this to be beneficial, it is important to ensure that the microbial and nutritional composition of the digestate benefits the plants (Mukhuba et al., 2018).

Animal manure is typically characterized by high nitrogen, ammonia, hydrogen sulfide and sulfur content, all of which prevent the production of biogas (Bharathiraja et al., 2018). According to (Odejobi et al., 2022a), animal manure is highly biodegradable due to its diverse microbial forums, moisture content of 75–92% and volatile solids content of 72–93% of total solids. It is an excellent substrate for biogas production due to its strong buffering ability and ability to remove inoculated stages.

According to (Ozor et al., 2014) and (Ben-Iwo et al., 2016), it has been estimated that approximately 0.03 m^3 of biogas is produced from every 1 kg of fresh animal manure in Nigeria. They reported that 6.8 million m^3 of biogas are created daily from 227,500 tons of animal manures. Nigeria as a country generated 197.6 million tonnes cattle manure, 32.6 million tonnes poultry manure, 15.3 million tonnes swine manure and 39.6 million tonnes goat and sheep manure yearly (Oguntoke et al., 2019). The estimated biogas potential per annum from cattle manure, poultry manure, swine manure and goat and sheep manure are respectively 6.25 billion m^3, 2.5 billion m^3, 0.92 billion m^3 and 2.3 billion m^3.

The major limitation of using manures is that animal manures decompose more slowly when used as substrates because high concentrations of ammonia inhibits the action of methanogenic bacteria (Bharathiraja et al., 2018).

Animal manure contains a higher concentration of nitrogen nutrients than other organic wastes, which can hinder the anaerobic digestion process when used as the only substrate in a reactor. Therefore, in order to optimize the C/N ratio and balance the nutrients to boost biogas generation, animal dung can be co-digested with substrate that has a low nitrogen content (but a high carbon content), such as plant biomass or kitchen waste, in this case tiger nut waste (Koryś et al., 2019). According to (Sibiya et al., 2017) insufficient biogas will be produced if anaerobic mono-digestion of manure is practiced.

1.2 PROBLEM STATEMENT

The improper disposal of animal manure, particularly cow dung, and tiger nut waste are growing environmental concerns, contributing to pollution and ecological damage. As livestock production increases and tiger nut processing becomes more widespread, better waste management practices are urgently needed. Additionally, the ongoing reliance on fossil fuels exacerbates environmental degradation, emphasizing the need for sustainable and eco-friendly energy alternatives. This issue is particularly critical in developing countries, where energy demand is rising, yet infrastructure and renewable energy adoption lag behind. Addressing these challenges requires innovative solutions to repurpose waste materials and explore renewable energy options to reduce environmental impact.

1.3 AIM AND OBJECTIVE

The aim of this study is to produce Biogas from the combination of cow dung and Tiger nut waste under anaerobic digestion.

The objective is to investigate the co-digestion of Tiger nut waste with cow dung as a starter in enhancing biogas production.

1.4 SCOPE OF STUDY

1. This project focuses on biogas production through anaerobic digestion using cow dung and Tiger nut waste.
2. The study also examines the variation of biogas production on a daily basis.
3. The experiment includes the measurement of various parameters such as Total solid, volatile solid, volatile fatty acid, pH, ash content, nitrogen, carbon and phosphorous.

1.5 SIGNIFICANCE OF THE PROJECT

1. Waste management: Animal waste is an important source of food and renewable energy. However, if not disposed of properly, they can pose environmental and health risks due to the release of air pollutants. Waste can be further utilized by converting it to biogas.
2. Single feedstock digestion: Single feedstock digestion involves the anaerobic breakdown of a single organic material, limiting the diversity of nutrients and microorganisms. However, co-digestion, combining multiple substrates like Tigernut waste and cow dung, offers significant advantages. Tigernut waste, rich in carbohydrates and lignocellulosic materials, complements the nitrogen-rich and biodegradable properties of cow dung. This synergy enhances microbial diversity, optimizes nutrient balance, and increases biogas yield.
3. Fossil fuels: Research into the production of biogas from cow manure is important due to the rising prices of fossil fuels and the negative environmental impacts of global warming and exhaust emissions. Biogas production from cow manure can help reduce dependence on fossil fuels, reduce greenhouse gas emissions, and thus contribute to sustainable energy solutions and environmental protection.

CHAPTER TWO

LITERATURE REVIEW

2.1 Biogas

The biological degradation of organic matter in the absence of oxygen results in the production of a colorless flammable gas also known as biogas (Umeghalu et al., 2012). Biogas can also be described as the byproduct of the anaerobic fermentation of organic materials, which is a combination of gases mostly carbon dioxide and methane (Abate, 2019). Carbon dioxide (CO_2) and methane (CH_4) are the components that make up the majority of biogas, with trace quantities of nitrogen (N_2), oxygen (O_2), water (H_2O), hydrogen sulfide (H_2S), hydrogen (H_2), and saturated hydrocarbons (ethane, propane) (Sawyerr et al., 2019). Table 2.1 indicates that biogas has a high methane concentration, making it a desirable energy source. CH_4 in its pure form is a colorless, tasteless and odorless gas. However, biogas smells very slightly of rotten eggs due to the presence of a small amount of H_2S. When methane and air mixture undergoes combustion, it results in a blue flame and the generation of significant heat energy (Florian et al., 2013).

Table 2.1: Previously reported studies on biogas composition (Florian et al., 2013)

Component	Formula	Typical analysis (% by volume)
Methane	CH_4	55-65
Carbon dioxide	CO_2	33-45
Hydrogen	H_2	0-1
Nitrogen	N_2	0-3
Hydrogen sulphide	H_2S	0-1
Ammonia	NH_3	0-1

Extensive empirical studies have shown that biogas can be produced from industrial, municipal and agricultural wastes in Nigeria. Manure from animal production, especially from pig and cow farming, is the main source of biomass in the food and feed industry. It provides the microorganisms needed for the biodegradation of biomass and is also one of the largest single sources of biomass (Abate, 2019). As reported by (Ajala and Odejobi, 2023), Nigeria generates a daily quantity of 227,500 tons of animal manure, leading to the production of 6,800,000 cubic meters of biogas per day through conversion processes. An estimated 52 million metric tonnes remain available for biogas production, capable of yielding approximately 21 billion cubic meters of biogas (Odejobi et al., 2022a).

2.1.1 Sources of Biogas

Biogas production is commonly employed for waste treatment, specifically sewage sludge, agricultural waste (such as manure), and organic waste streams from industrial processes (Atelge et al., 2018). The primary source, contributing essential microorganisms for biomass bio-degradation, is manure derived from animal production, mostly sourced from farms specializing in cows and pigs (Ahmed et al., 2015). Furthermore, manure stands out as one of the largest single sources of biomass within the food/feed industry.

2.2 Energy crops and crop residue

2.2.1 Sugarcane

Sugarcane is one of the most significant energy crops used globally, particularly for bio-ethanol production (Mustapha and Havva, 2009). Sugarcane is known for its high yield of biomass and ability to convert sunlight into energy efficiently. It offers both fermentable sugars and fibrous material (bagasse), which can be utilized for energy production. The plant is typically used in biofuel production, especially in countries like Brazil, where sugarcane is fermented to produce bio-ethanol (Boddey et al., 2008). This is a renewable energy source that helps to reduce reliance on fossil fuels.

Sugarcane's example as an energy crop emphasizes the importance of utilizing agricultural waste products in energy production. Other energy crops include, corn, switchgrass, soybeans, and oil palm.

2.2.2 Tiger nut waste

Tiger nut, scientifically known as Cyperus esculentus, has been cultivated since ancient times, predominantly in South Europe and West Africa. Tiger nut is considered a crop residue when discussing the chaff or waste left after extracting milk or oil from the tubers. It is valued for its small tuberous rhizomes, which can be consumed in their raw or roasted form, utilized as livestock feed, or processed to extract juice for beverage production (Edo et al., 2023). One of the byproducts generated during processing is known as Tiger nut chaff (Adejuyitan, 2011). Tiger nut chaff is inherently rich in fiber, presenting a valuable potential for the development of value-added products following the extraction of milk and oil from the tubers (Omodolapo et al., 2020).

Proximate analysis showed that the nut contains the following: Moisture 3.50- 3.78%, Crude Protein 7.15 -9.70%, Lipid 32.13- 35.43%, Crude Fiber 6.26 -5.62%, Carbohydrates 46.99- 41.22%, Ash 3.97- 4.25%, Energy (kJ) 1343- 1511 (Suleiman et al., 2018). The mineral composition of 100g of Tiger nut after analysis: Calcium 43.36mg, Sodium 17.02mg, Potassium 267.18mg, Magnesium 118.14mg, Phosphorus 158.86mg, Iron 2.82mg, Zinc 1.39mg, Copper 0.54mg (Suleiman et al., 2018). Tiger nuts are mildly alkaline, with a pH ranging from around 6.3 to 7.5.

According to (Algieri et al., 2019) The energy potential in these agricultural crop residues has not been fully utilized, despite the global rise in biogas production. Other crop residue include, rice husks, wheat straw, corn stover, and cotton stalks.

2.2.3 Biogas production potential from energy crops and crop residues

Annually, 83 million tonnes of crop residues are produced, with an estimated biogas potential of 4.98 billion m^3 (Oguntoke et al., 2019) and (Sokan-Adeaga and Ana, 2015). Crops can undergo either mono-digestion or co-digestion processes. However, the practice of mono-digestion is uncommon due to the intricate structure of certain crops, causing inhibition in anaerobic digestion and consequently reducing biogas yield. Co-digestion of substrates is reported to significantly improve the cumulative biogas yield compared to the mono-digestion of substrates (Aworanti et al., 2017).

The energy potential of each crop residue is dependent on the production rate of the associated crop, the residue-to-production ratio (RPR), and the energy content. All these residues have high energy potential which greatly contributes to the economy of Nigeria as a nation especially residues from rice, maize and cassava.

The total estimated energy potential derived from agricultural crop residues amounts to 3635.95 (PJ). According to (Ben-Iwo et al., 2016), the utilization of crop residues, including bagasse, straw, and stalk, in biogas production has a positive impact on food supply in the market. This is because only crop residues are utilized as feedstock for the generation of biofuel.

2.3 Animal manure

2.3.1 Cow dung

Livestock production is a key agricultural practice which constitutes the most significant source of animal waste. The volume of waste generated in this process is influenced by factors like the type of animal rearing, feeding methods, animal size, and breed characteristics (Abdeshahian et al., 2016).

To mitigate the environmental risks and odor resulting from the substantial daily disposal of animal waste, a beneficial approach is the transformation of this waste into renewable gas through anaerobic digestion. Various animal wastes, including those from cattle, poultry, pigs, sheep, horses, and goats, can be subjected to this process. According to (Yang et al., 2019) pig and poultry manures are rich in protein, whereas cattle manure is characterized by a high content of lignocellulose.

Nigeria according to (Oguntoke et al., 2019) and (Odejobi et al., 2022a), generates a daily quantity of 227,500 tons of animal manure. Through conversion processes, this leads to the production of 6,800,000 m^3 of biogas per day. (Oguntoke et al., 2019) stated further that, the estimated bioenergy potential of animal manure in Nigeria is reported to be 450.48 petajoules (PJ). Animal waste contributes 61 million tonnes annually to Nigeria's energy reserves (Ben-Iwo et al., 2016).

Improper disposal of this large quantity of manure could pose environmental, human, and animal health risks. However, the conversion of manure from this sizable livestock population through anaerobic digestion has the potential to alleviate the challenges associated with the country's high energy demand.

Cow manure is obtained from abattoirs where cows are processed for human consumption. There are two types of cow manure: intestinal dung and excreted dung. Intestinal dung is extracted from the intestines of slaughtered cows, containing undigested residues of consumed matter. It is characterized by freshness and contains the typical microbial flora found in a cow's rumen.

Excreted dung comes from herbivorous cows and consists of digested residues that have traversed the gastrointestinal system (Mishra et al., 2020). Cow dung is composed of 1.8-2.4% nitrogen (N_2), 1.0-1.2% phosphorous (P_2O_5), 0.6-0.8 potassium (K_2O) and 50-75% organic humus, 39.17% carbon, 53.10% oxygen, 11% fat content (Rath et al., 2016). Predominant fermentative bacteria in cow dung-fed reactors include; Ruminococcus flavefaciens, Eubacterium cellulosolvens, Clostridium cellulosolvens, Clostristium cellulovorans, Clostridium thermocellum, Bacteroides cellulosolvens and Acetivibrio cellulolyticus (Goswami et al., 2016).

2.3.2 Biogas production potential from animal manure

Animal manures are commonly characterized by their elevated nitrogen content and the presence of sulfur, ammonia, and hydrogen sulfide, all of which can impede the process of biogas production. (Chow et al., 2020). (Bharathiraja et al., 2018) reported that animal manures contain diverse microbial fora with a moisture content ranging from 75% to 92% and volatile solids constituting 72% to 93% of total solids. These characteristics render them highly biodegradable. They further emphasized that the excellent buffering capacity and the elimination of the inoculation step make manures an ideal substrate for biogas production. In Nigeria, according to (Ben-Iwo et al., 2016) and (Ozor et al., 2014), it has been estimated that approximately 0.03 m^3 of biogas is generated from every 1 kg of fresh animal manure.

The annual estimated biogas potential from cattle manure, poultry manure, swine manure, and goat and sheep manure are reported as 6.25 billion m^3, 3.5 billion m^3, 0.92 billion m^3, and 2.3 billion m^3, respectively (Odejobi et al., 2022b). These huge energy resources hold significant potential to enhance Nigeria's overall energy supply. However, a major disadvantage in using manures as substrates is the formation of high concentrations of ammonia. This ammonia can impede the activity of methanogenic bacteria and decelerate the digestion process of animal manures according to (Bharathiraja et al., 2018).

Relying solely on raw manure for anaerobic digestion may not be economically viable due to the inhibition caused by elevated nitrogen nutrient levels in animal manure compared to other organic wastes. A more profitable approach involves co-digesting animal manure with substrates that have low nitrogen content (but high carbon content), such as plant biomass or kitchen waste. This approach aims to optimize the C/N ratio and balance nutrient composition, thereby enhancing biogas production (Koryś et al., 2019). Additionally (Sibiya et al., 2017), emphasized that practicing anaerobic mono-digestion of manure may result in insufficient biogas production.

Hence, co-digesting animal manure with other substrates proves beneficial in enhancing biogas production. (Ogunwande et al., 2013) demonstrated that co-digesting swine manure with chicken manure resulted in a remarkable 69.2% increase in biogas production compared to using chicken manure alone. Furthermore, the biogas yield exhibited a substantial 131.6% increase when compared to using swine manure alone. This improvement occurred over a period of 63 days, maintaining a mean substrate temperature and pH of 28.5 ± 2.5°C and 6.80 ± 0.55, respectively.

2.4 Biogas as renewable energy source

Biogas is a sustainable and renewable energy source with the potential to provide energy, environmental improvements, and job creation (Cvetković et al., 2014). Biogas production possesses the flexibility and adaptability to become one of the most versatile energy sources (Dotzauer et al., 2019). The composition and production rate of biogas are generally influenced by the type of digestion process and feedstock utilized. This, in turn, impacts the choice of equipment for biogas utilization (Korres et al., 2013).

In anaerobic digestion, biogas primarily consists of methane (54%-80%), carbon dioxide (20%-45%), and trace amounts of other gases such as hydrogen, carbon monoxide, nitrogen, oxygen, and water vapor. Depending on the feedstock, biogas may also contain hydrogen sulphide (H_2S), ammonia (NH_3), and siloxanes (Sawyerr et al., 2019). While similar to natural gas, biogas lacks the variety of hydrocarbon compounds found in natural gas, resulting in natural gas having 10% higher energy content than biogas (Junne and Kabisch, 2017).

The characteristics of methane make it an excellent fuel for various applications, including heating, cooking, lighting, and engine operation. Additionally, biogas can serve as fuel for small industries.

2.5 Anaerobic digestion

Anaerobic digestion is a series of processes where microorganisms break down biodegradable materials in the absence of oxygen. This complex process demands specific environmental conditions and involves diverse bacterial populations. Within this bacterial population, organic compounds undergo degradation, resulting in the production of a valuable high-energy gas mixture, primarily composed of methane (CH_4) and carbon dioxide (CO_2), known as biogas. The majority of biodegradable organic matter is converted into gases, with only a small proportion (approximately

10%) being transformed into new cell mass through microbial growth. The breakdown of organic compounds occurs during digestion, and it is facilitated by anaerobic microorganisms. Anaerobic digestion of organic matter progresses through stages governed by various categories of microorganisms.

The different species of anaerobic microorganisms has a function to degrade organic matter and complete the carbon cycle. Biogas production is a biochemical process that occurs in the stages, during which different bacteria act upon the organic matter (Mao et al., 2015). The three stages involved are hydrolysis, acidification, and methane formation. Initially, a group of microorganisms converts organic materials into simpler forms, while a second group of organisms utilizes it to form organic acids (Ellacuriaga et al., 2021). The gas production is the end product released during the final stage of anaerobic digestion. In addition to methane, the gas may contain hydrogen, ammonia, and carbon dioxide (Koblenz et al., 2015).

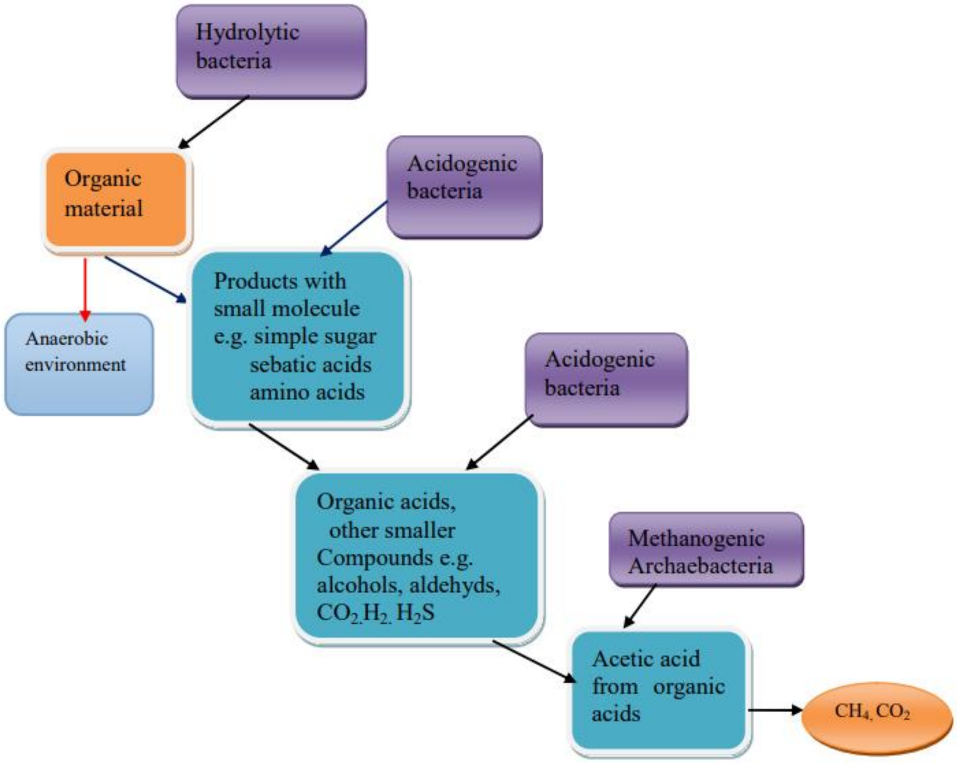
In the first phase (hydrolysis phase), cellulolytic bacteria act upon complex organic matter such as carbohydrates, lipids or fats, proteins, and cellulose (Joseph Igwe, 2014). These organic matters are converted into soluble compounds like sugars, fatty acids, and amino acids. During the second phase (acidogenesis phase), acid-producing bacteria convert the materials produced in the first phase into volatile fatty acids (VFAs), hydrogen (H_2), and carbon dioxide (CO_2). Facultative anaerobic bacteria utilize oxygen and carbon during the acidification process; therefore, anaerobic conditions are crucial for methanogenesis (Ali Shah et al., 2014). In the third phase (methanogenesis stage), methanogenic bacteria produce methane either by fermenting acetic acid to form methane and carbon dioxide or by reducing carbon dioxide to methane using hydrogen gas produced by other bacteria (Ma et al., 2019).

Anaerobic digestion of organic waste is widely employed worldwide as both a pollution mitigation strategy and an energy production method (Gaida et al., 2017). Anaerobic digestion is a waste-to-energy technology and is extensively applied in treating various organic wastes, such as the organic fraction of municipal solid waste, sewage sludge, food waste, and animal manure. The methane generated through anaerobic digestion can be utilized to power a treatment plant, which gives anaerobic digestion an economic edge over aerobic digestion. Additionally, the process yields a nutrient-rich fertilizer suitable for use as compost.

2.6 Stages of Biogas production using Anaerobic digestion

Anaerobic digestion comprises of four fundamental stages essential for biogas production from various organic materials within an anaerobic digester. These stages, namely hydrolysis, acidogenesis, acetogenesis, and methanogenesis are integral to the breakdown of organic matter into methane, carbon dioxide, inorganic nutrients, and compost within an anaerobic environment. The process involves four distinct categories of microorganisms responsible for converting complex organic compounds into simpler molecules like methane and carbon dioxide (Kuan Hwa, 2010).

Figure 1.1: Flow diagram of the process of biogas production (Abate, 2019)



2.6.1 Hydrolysis

Hydrolysis, which is the first phase in the anaerobic digestion process, entails the breakdown and solubilization of biopolymer particulate organic compounds and colloidal wastes into soluble monomeric or oligomeric organic compounds (Teo, 2016). This decomposition mainly targets complex organic polymeric materials like carbohydrates, proteins, and lipids, which are enzymatically hydrolyzed by fermentative bacteria (microorganisms) into smaller, water-soluble compounds such as sugars, amino acids, and long-chain fatty acids (Li et al., 2011).

During hydrolysis, chemical bonds are cleaved through the addition of water molecules. This process involves the reaction of cations and anions with water molecules, leading to changes in pH values and the breaking of H-O bonds. Hydrolysis is typically a slower step in the overall digestion process.

Hydrolytic bacteria exhibit phylogenetic variations, with a significant portion belonging to the Bacteroidetes and Firmicutes phyla (Venkiteshwaran et al., 2015). Hydrolysis is facilitated by extracellular enzymes secreted as byproducts of microorganisms active in this stage (Zhang et al., 2007). While some microorganisms possess the ability to degrade various organic polymers, others specialize in specific types, such as saccharolytic microorganisms targeting sugars and proteolytic microorganisms focusing on proteins. Strict anaerobes like clostridia and facultative bacteria such as streptococci play roles in this phase of anaerobic digestion

At the end of the hydrolysis stage, simple organic compounds are generated. These products subsequently undergo absorption and degradation by various facultative and obligate anaerobic bacteria in the acidogenic phase, yielding short-chain volatile fatty acids (VFAs). These VFAs, along with alcohols, undergo conversion into acetate, hydrogen, and carbon dioxide (Feng et al., 2022). This phase involves the hydrolysis of polysaccharides into monosaccharides, fats into glycerol and fatty acids, and proteins into amino acids (Sawyerr et al., 2019).

Enzymes such as cellulase, cellobilase, xylanase, and amylase are involved in hydrolysis, breaking down carbohydrates into sugars. Proteases degrade proteins into amino acids, while lipases aid in the degradation of lipids into glycerol and long-chain fatty acids (LCFAs).

Enzymatic catalysis accelerates the hydrolysis process by oxidizing organic matter through aerobic biological processes. This hydrolysis and aerobic degradation process occur rapidly, with the biogas produced transforming into carbon dioxide (CO_2) from oxygen. Once the substrate is hydrolyzed, it becomes available for cellular transport, and fermentative bacteria can degrade these substrates during the acidogenesis stage. Optimizing the hydrolysis process is crucial to prevent inefficient degradation of macromolecules, which could negatively impact digestion rates or other biological activities, and subsequently affect biogas yield.

It is important to ensure the active operation of microorganism cultures to facilitate the second process, acidogenesis. Physicochemical treatments can also be employed to enhance the solubilization of organic matter. However, it is necessary to avoid air intake in the system, as the presence of air in the biomass hinders the performance of microorganisms as anaerobic units.

2.6.2 Acidogenesis

The acidogenesis process converts organic acids produced during the second stage into acetic acid, acid derivatives, carbon dioxide, and hydrogen. This stage is also known as the fermentation stage. According to (Fang, 2010), maintaining low levels of H_2 is crucial for favorable thermodynamics in acidogenic reactions. During this phase of the anaerobic digestion process, products from the hydrolysis stage are further broken down by a variety of obligate and facultative fermentative microorganisms to yield weak acids, primarily organic acids such as acetic acid, propionic acid, and butyric acid (VFAs), as well as lactic acid, alcohols, hydrogen, and carbon dioxide (CO_2) (Molaoa, 2017).

The most important acid, CH_3COOH, is formed during this stage, serving as a substrate for microorganisms to produce methane. An elevated pH value (> 5) facilitates the production of volatile fatty acids.

The acidogenesis stage entails the generation of high concentrations of hydrogen by acid-producing bacteria, known as acidogenic microorganisms, and is typically the most rapid step in a balanced anaerobic process. Acidogenesis is characterized by the accumulation of lactate, ethanol, propionate, butyrate, and higher volatile fatty acids (VFAs), termed electron sinks or intermediate products. This phase represents the bacterial response to elevated hydrogen concentrations in the system, leading to the production of acetate by acetogenic microorganisms (Behera et al., 2023).

The degradation of organic matter to produce biogas relies on the complex interaction of various bacterial groups, primarily the acid-producing bacteria (acidogens) and methane-producing bacteria (methanogens). Therefore, maintaining a symbiotic relationship between acidogenic and methanogenic bacteria is crucial for sustaining the successful operation of any anaerobic digester (Pandey et al., 2020). This step is important as it connects the fermentation phase with methane production.

This process is facilitated by various facultative and obligate anaerobic bacteria, including Pseudomonas sp, Bacillus sp, Clostridium, Micrococcus sp, and Flavobacterium sp. The resulting components are products of anaerobic respiration in acidogenic biomass. In essence, simple sugars, fatty acids, and amino acids undergo transformation into organic acids and alcohols. Additionally, the concentration of hydrogen produced in this stage, acting as an intermediate product, determines the type of end product generated in the fermentation stage; an increased partial pressure of hydrogen can influence the ultimate product produced (Angelidaki et al., 2011).

2.6.3 Acetogenesis

Acetogenesis represents the dehydrogenation stage, where hydrogen gas is produced as a byproduct. However, the presence of hydrogen can inhibit the metabolism of acetogenic bacteria. Some of the hydrogen gas may be utilized by methane-producing bacteria, acting as hydrogen-scavenging bacteria, to convert it into methane (CH_4) (Anukam et al., 2019).

During the acetogenesis stage, alcohols (such as ethanol) and volatile fatty acids (VFAs) with more than two carbon atoms are converted by acetate-forming bacteria into acetate, with hydrogen and carbon dioxide being the main byproducts (Khalil 2020). This conversion is crucial, as hydrogen and carbon dioxide are continuously reduced to acetate by homoacetogenic microorganisms (Chandra et al., 2012), thereby mitigating the accumulation of hydrogen that could disrupt the function of acetogenic bacteria.

However, a further increase in hydrogen partial pressure may lead to acetogens losing their ability to produce acetate. To maintain low pressure throughout the acetogenesis stage of the anaerobic digestion process, a mutually symbiotic relationship between acetogens and hydrogenotrophic methanogens must occur, enabling acetogens to produce acetate as a substrate for methanogens (Schnürer, 2016). This step marks the final phase of fermentation before methanogenesis. During this process, acetate bacteria (genera such as Syntrophomonas and Syntrophobacter) convert acidogenesis products (ethanol, propionic acid, butyric acid) into acetates and hydrogen for use by bacteria in the methanogenesis stage.

Methanobacterium suboxydans degrades pentanoic acid to propionic acid, and Methanobacterium propionicum degrades propionic acid to acetic acid. Hydrogen is released in this process, which can be toxic to the microbial community. To mitigate this issue, acetogenic bacteria form a symbiosis with autotrophic methane bacteria, which utilize hydrogen, effectively removing it to prevent toxicity in the microbial community. Importantly, this stage significantly influences the efficiency of biogas production, as the acetate produced serves as the precursor for methane formation through a reduction process (Ali Shah et al., 2014)

2.6.4 Methanogenesis

Methanogenesis is a critical stage in anaerobic digestion, which substantially impacts on the overall process, because approximately 70% of methane utilized in Anaerobic digestion originates from this stage (Mutungwazi et al., 2020). During methanogenesis, carbon dioxide-reducing and hydrogen-oxidizing methanogens convert hydrogen and carbon dioxide into methane, while acetoclastic methanogens utilize acetate to produce methane (Ni et al., 2022).

Methanogens, belonging to the Archaea domain, utilize acetate, hydrogen, CO_2, methanol, methylamines, and formate to produce methane and CO_2 (Enzmann et al., 2018). These end products serve as primary substrates for methanogenic bacteria, resulting in the production of biogas, typically comprising 50 –75% methane (CH_4), 50 –25% CO_2, and trace amounts of nitrogen, hydrogen, and hydrogen sulfide. Methanogenesis serves as an indicator of the biological activity level in an anaerobic system and the digestion state. They are also very sensitive to other environmental factors such as temperature, pH etc.

The stability and performance of the system are enhanced with increased methane production, marking the concluding stage of Anaerobic digestion, during which methanogenic bacteria actively produce methane by converting intermediate products (such as acetic acid, carbon dioxide, and hydrogen) into methane and carbon dioxide (Amin et al., 2021).

Heterotrophic methane bacteria convert acetic acid into methane, while autotrophic methane bacteria reduce carbon dioxide in the presence of hydrogen to produce methane. Bacteria within the Archaea domain primarily contribute to methane production. However, methanogenic bacteria exhibit limited temperature resistance due to their enzymatic structures, making temperature a crucial parameter for their functionality (S. Wang et al., 2019).

Methanogens are categorized into three groups namely: hydrogenotrophic methanogens, acetotrophic methanogens, and methylotrophic methanogens (Demirel and Scherer, 2008). Hydrogenotrophic methanogens utilize hydrogen or formate to reduce carbon dioxide into methane, with genera such as Methanobacterium, Methanobrevibacter, Methanoculleus, Methanospirillum, and Methanothermobacter playing essential roles in anaerobic digesters (Kurt, 2019).

Acetotrophic methanogens, including Methanosaeta and Methanosarcina, convert acetate into methane and CO_2. Methanosaeta, being obligate acetoclastic methanogens, exhibit slow growth but a high affinity for acetate, whereas Methanosarcina, being facultative acetoclastic methanogens, dominate over Methanosaeta in digesters when the acetate concentration is high due to their higher growth rate and lower affinity for acetate (van Haandel et al., 2014).

2.7 Main factors affecting the biogas production

The anaerobic digestion process in biogas plants relies on the complex interaction of various bacterial groups, which are influenced by a range of chemical and physical parameters (Kwietniewska and Tys, 2014). Any alteration in these parameters can disrupt the bacterial environment and the activity of microorganisms within the digester, leading to a failure in the digestion process (Shi et al., 2018).

Therefore, it is crucial to monitor these parameters to optimize biogas production. Several factors impact biogas production, including nutrients, pH of feedstock, temperature, flow rate of feed (loading rate), and retention time. Drastic changes in these parameters can negatively impact biogas production, and neglecting the importance of maintaining them within optimal ranges for efficient operation of the biogas plant can impede or halt biogas production (Yilmaz et al., 2018). This section discusses some of these factors.

2.7.1 Temperature

Biological methanogenesis has been observed within a broad temperature range, from 2°C to over 100°C (M. N. Uddin et al., 2021), with optimal activity typically occurring around 35°C under mesophilic conditions and 55°C under thermophilic conditions (Khoiyangbam et al., 2011). Fermentation can occur within different temperature ranges: psychrophilic (<30°C), mesophilic (30–40°C), and thermophilic (50–60°C), with the most active anaerobes typically operating within the mesophilic and thermophilic ranges. Biogas production is generally faster in the thermophilic range compared to the mesophilic range during anaerobic digestion (Alrowais et al., 2023). Elevating the process temperature from mesophilic (32–42°C) to thermophilic (45–57°C) levels accelerates degradation and enhances substrate health (Da Costa Gomez, 2013). However, thermophilic conditions are more susceptible to disturbances, such as fluctuations between the interior and exterior of the digester, and increased ammonia inhibition for microorganisms inside the digester (Jiang et al., 2019). Conversely, high temperatures can negatively affect microorganisms involved in anaerobic digestion (Nie et al., 2021).

Anaerobic digestion typically operates within two distinct temperature ranges: an optimum of 35°C (mesophilic) and an optimum of 55°C (thermophilic) (Nie et al., 2021). Although thermophilic digestion may offer advantages such as improved reaction rates and pathogen reduction, microorganisms in mesophilic digestion have lower nutrient demands (Labatut et al., 2014), and mesophilic digestion can mimic thermophilic digestion. Temperature governs the rate of biological reactions and requires regular monitoring, especially with changing weather conditions. The choice of temperature (mesophilic or thermophilic) depends on the desired outcome and the type of microorganisms used for waste treatment (Nie et al., 2021).

Methanogenic archaea species, crucial for biogas formation, exhibit sensitivity to temperature changes, with an optimal operational temperature between 33- 40°C. Temperature fluctuations should be minimized to maintain consistent biogas production, often achieved by burying the biogas production structure underground to stabilize temperature (Talia, 2018). However, rapid temperature increases should be avoided to prevent the death of temperature-sensitive archaeal strains, which could decrease methane production. To maintain constant temperature during biomethane production tests, reactors are submerged in a water bath or incubated in a thermostatically controlled environment (Gashaw, 2014).

Higher temperatures accelerate biochemical processes, leading to increased biogas production. However, temperatures below 52°C are more suitable for optimal biogas production, with temperature fluctuations ideally limited to within ±2°C at mesophilic levels (~37°C) and within ±0.5°C at thermophilic levels (~52°C) for optimal performance (Peces et al., 2013).

2.7.2 pH

The pH value denotes the acidity or basicity of an aqueous solution and is expressed as the negative logarithm of the concentration of hydronium ions (Drosg et al., 2013). The buffer capacity, largely influenced by CO_2 concentration, ammonia concentration, and water content, plays a crucial role in maintaining pH stability during anaerobic digestion (Wolff-Boenisch, 2011). If the feedstock's pH deviates significantly from this range, necessitating neutralization, a neutralization step before feeding it into the biogas plant is recommended (Drosg et al., 2013). Acidification during anaerobic digestion can be countered by artificially raising pH through the addition of bases such as lime (Drosg et al., 2013).

The pH value is crucial as methanogenic bacteria are sensitive to acidic conditions, which can impede bacterial growth and methane production (Borja, 2011). Optimal pH varies across different stages of the anaerobic digestion process; for instance, during acetogenesis, pH can drop below 5 due to organic acid production (Alrowais et al., 2023). The optimal pH range for maximizing biogas yield in anaerobic digestion is typically between 6.5 and 7.5, although this range may vary depending on factors such as substrate composition and digestion technique (Conant et al., 2008). Maintaining a constant pH is essential, often achieved by adding equilibrium buffers like calcium carbonate or lime to the system.

The pH value serves as a critical indicator of anaerobic process performance and digestion stability. Lower pH values may signal system failure or insufficient buffering capacity, potentially inhibiting digestion, while higher pH values can limit methanogenesis. Factors influencing pH include volatile fatty acid (VFA) concentration, bicarbonate concentration, system alkalinity, and the fraction of CO_2 in the digester gas (Ali et al., 2019).

Cow dung, commonly used to promote bacterial growth and accelerate biogas generation, plays a role in neutralizing pH, improving buffering capacity, and enhancing digestion performance (Fang, 2010). Despite the need for organic acids as food sources for methanogens, excessively acidic environments are not conducive to their survival. Therefore, maintaining a pH level between 6.5 and 8, ideally around 7.5, ensures optimal biogas plant performance (Aggarangsi et al., 2023).

2.7.3 Particle size

The particle size of the substrate significantly impacts biogas production, as highlighted by (Legonda and Nalinga, 2016). Substrates with excessively large particle sizes pose digestion challenges for microbes and may lead to blockages within the digester. Conversely, smaller particle sizes provide a larger surface area for substrate adsorption, facilitating enhanced microbial activity and subsequently increasing gas production (Zamri et al., 2021).

2.7.4 Nutrients

The unavailability of nutrient concentrations in energy crops has led to issues such as low methane yields, acidification, and process instability in crop mono-digestion, necessitating the application of low organic loading rates (OLRs) and extended hydraulic retention times (HRTs) (Dobre et al., 2014). These factors significantly influence the performance and stability of the anaerobic digestion process. These challenges underscore the importance of adequate levels of both macro- and micronutrients (Hinken et al., 2008) for sustained biogas production.

Nutrients play a crucial role in biological processes, especially in anaerobic digestion. Macronutrients such as nitrogen and phosphorus are required in relatively large quantities by all bacteria, while micronutrients (e.g., K, Mg, Ca, Fe, Na, Cl, Zn, Mn, Mo) are needed in smaller amounts. Inorganic nutrients are particularly critical in the conversion of acetate to methane (Mutyala and Kim, 2022).

2.7.5 Agitation

Agitating the digester contents improves the efficiency of the anaerobic digestion process by enhancing the contact between macronutrients (Kainthola et al., 2019). One of the main issues affecting the performance of popular biogas plant types is the formation of a thick scum layer at the digester surface. This layer hinders the release of biogas, rendering it inaccessible for use. An experiment conducted at IIT Guwahati demonstrated that recirculation markedly enhances gas production at similar pH values compared to setups without circulation (Wu et al., 2018).

2.7.6 Loading rate

The loading rate refers to the quantity of organic materials introduced into the digester per unit volume per day (Gautam et al., 2022). It considers the food-to-bacteria ratio and is typically measured in kilograms of volatile solids fed to the digester per cubic meter of digester volume per day (Liu et al., 2012). Factors influencing the loading rate include the concentration of active bacteria, solid content, retention time, and digester temperature (Khan et al., 2016).

Optimal loading rates vary depending on the digester type and location, with higher rates typically used in warmer climates (Khoiyangbam et al., 2011). It's crucial to express loading rates either by weight of total solids volatile added per unit volume or by weight of total solids volatile added per day per unit weight of total volatile solids in the digester (Both et al., 2021). There exists an optimum loading rate that maximizes biogas production. Overfeeding can lead to acid accumulation and methane inhibition, while underfeeding results in low gas production.

2.7.7 Inhibitors

Some compounds generated as byproducts of anaerobic degradation, albeit in limited quantities, can inhibit biogenesis and become toxic at higher concentrations (Wikandari et al., 2019). Salts contain essential micronutrients like sodium, potassium, and chloride, crucial for bacterial activity. However, elevated concentrations of salts can inhibit bacterial function (Liang et al., 2020).

2.7.8 Retention time

The HRT, or Hydraulic Retention Time, delineates the theoretical duration that substrates remain in the digester (Nathalie Bachmann, 2013). While it represents the average retention time, deviations from this value occur in reality (Bushra, 2018). Selecting an appropriate HRT is crucial to ensure sufficient substrate degradation without excessively increasing digester volume (Nathalie

Bachmann, 2013). It's essential to prevent microbial washout, so the HRT should not fall below 10 days. Additionally, HRT decreases with increasing temperature; for instance, a thermophilic digester has a shorter retention time compared to a mesophilic one (Sudalyandi and Jeyakumar, 2022).

HRT signifies the mean residence time for solid and liquid wastes within a digester to interact with microbial biomass. In flow-through systems without recycling, such as Continuous Stirred Tank Reactors (CSTRs), HRT and Sludge Retention Time (SRT) align. Longer retention times are necessary for influent streams with high solids concentrations to maximize bioenergy production (Morales et al., 2015).

A longer HRT enhances removal efficiency by allowing sufficient contact time between biomass and waste, facilitating the removal of contaminants (Viero and Sant'Anna, 2008). Anaerobic digestion employs two reactor types: batch and continuous processes. In the batch process, substrates are added at the beginning, and the reactor is sealed for digestion duration (Tsapekos et al., 2018). The reaction stages occur consecutively, leading to a bell curve in biogas production.

Retention time typically ranges from 30–60 days, with only a portion of the tank volume utilized for active digestion (Getahun et al., 2014). The retention time is governed by the time taken for complete organic material digestion, as indicated by the Chemical and Biological Oxygen Demand (COD and BOD) of the effluent. Accelerating this process enhances efficiency but also reduces digester size and costs (Ni et al., 2022). Thus, designing systems for complete digestion in shorter times offers cost savings. Practices like continuous mixing and using low solids content aid in reducing retention time.

2.7.9 Carbon to Nitrogen ratio

The carbon-to-nitrogen ratio (C/N ratio) signifies the relationship between carbon and nitrogen in organic materials (Brust, 2019). Anaerobic digestion proceeds most rapidly when the C/N ratio of the raw materials falls within the range of 25 to 35:1 (Khoiyangbam et al., 2011). If the ratio is higher, nitrogen becomes depleted while there's still an abundance of carbon available, leading to bacterial die-off and subsequent nitrogen release, ultimately restoring equilibrium (Devaraj et al., 2022). Achieving optimum C/N ratios in digester materials involves blending materials with high and low C/N ratios, such as mixing organic solid waste with sewage or animal manure.

The C/N ratio plays an important role in organic matter decomposition, with anaerobic processes benefiting from substrates from different sources. The optimal C/N ratio for anaerobic digestion typically falls within the range of 20-30 (Uddin, 2023). A high C/N ratio results in rapid nitrogen consumption by methanogenic bacteria for protein synthesis, diminishing available nitrogen for reacting with the remaining carbon, thus reducing biogas production. Conversely, a low C/N ratio leads to nitrogen liberation, accumulating as ammonia and elevating digester pH.

2.7.10 Seeding

One challenge in anaerobic digestion is the lengthy startup period required for microorganisms to establish activity (Wang et al., 2019). Seeding can speed up and stabilize the digestion process (Mahmudul et al., 2021). Common seeding materials, or inoculums, include cow manure, anaerobic sludge, or biogas slurry, which are rich in microorganisms that enhance the process (Mahmudul et al., 2021). Guidelines suggest using seeding materials twice the amount of fresh manure during startup, gradually decreasing the quantity added over three weeks.

Starting a new anaerobic process requires using microorganism inoculums to initiate fermentation. Common seeding materials comprise digested sludge from operational biogas plants or material from well-rotted manure pits or cow dung slurries (Yadvika et al., 2004). Research indicates that digested sludge serves as the best inoculum source for anaerobic thermophilic digestion, particularly for treating the organic fraction of municipal solid waste under dry conditions (30%TS) (Franca and Bassin, 2020). Rumen fluid inoculums have been shown to double biogas production rates and efficiency compared to manure substrates without rumen fluid inoculums (Budiyono et al., 2014). Additionally, the addition of manure slurry to batch reactors as part of the starter has been found to improve biogas production.

2.7.11 Total and Volatile solids

Biogas production can be insufficient if fermentation materials are either too diluted or too concentrated (Singh et al., 2020). All waste materials fed into a plant consist of solid matter and water. Solid matter comprises volatile and non-volatile components (Xu et al., 2018). During anaerobic fermentation, volatile solids undergo digestion while non-volatiles remain unaffected. Optimal solids concentration for the digestion of sewage sludge is typically in the range of 8% to 10% (Khoiyangbam et al., 2011).

Biogas digesters can be designed to operate with either high solids content, where total suspended solids concentration exceeds 20%, or low solids concentration, below 15% (Saady and Massé, 2015). Using feedstock with very high water content results in a high volume of digestate with low nutrient concentration (Drosg et al., 2013). The moisture content and total suspended solids (TSS) of the feedstock are crucial for increasing material circulation between microorganisms and their food (Pleissner et al., 2014). This facilitates easier access for bacteria to the substances they feed on and increases gas production rates.

Total solids and volatile solids are vital parameters for determining sludge characteristics. For wet anaerobic digestion, total solids should ideally range from 8% to 15% of the total volume, while for dry anaerobic digestion, it should be between 25% and 30% dry solid (Ali et al., 2019). Volatile solids is the weight of organic solids burned off when heated to about 550°C and, it can provide an estimate of the substrate potentially converted into methane (Nielfa et al., 2015). However, this estimate varies due to the different degradability speeds of organic compounds within volatile solids. Nonetheless, volatile solids serve as a measure of organic matter, aiding in determining the suitability of digesters for incoming waste (Mahmoodi et al., 2018). Additionally, the biogas production potential of various organic materials can be calculated based on their volatile solid content.

2.7.12 Water content

Water is a vital element for the life and activity of microorganisms. The movement of bacteria and the activity of extracellular enzymes are highly influenced by the water content in the digester (Kainthola et al., 2019). Optimal moisture content must be maintained in the digester, typically ranging from 60% to 95% (Patel et al., 2024). However, the ideal water content may vary depending on the chemical characteristics of the input materials and the rate of bio-degradation.

The maximum dry content in digester feedstock should not exceed 50%. For biogas digestion, a dry matter content within 8% to 10% is more suitable (Ngan et al., 2019). A low density of feedstock can be easily pumped out as digested slurry due to the increase in pressure caused by the produced biogas.

2.7.13 Substrate for Biogas production

Substrate serves as both a material and energy source for microorganisms. It is consumed by microorganisms and converted into methane, as well as utilized for growth. The type of substrate determines the rate of the digestion process, and the absence of substrate terminates the metabolism of microorganisms, thus determining the digestion time. More complex substrates take longer for degradation by microorganisms (Raczka et al., 2021).

During the digestion process, microorganisms produce intermediate products. These products are usually short-lived and do not accumulate in the reactor. However, the production rate of intermediate products depends on the composition of the substrate and can lead to their accumulation. Changes in operational conditions such as pH or temperature can also induce the accumulation of intermediate products. The accumulation of intermediate products can inhibit the digestion process. For instance, substrates containing high fats can lead to the production of fatty acids, inducing a decrease in pH, which further inhibits microorganism activity (Dahiya et al., 2015).

In general, all types of biomass can be used as substrates as long as they contain carbohydrates, proteins, fats, cellulose, and hemicelluloses as main components.

2.7.14 Mixing condition

In traditional anaerobic digesters, mixing has been observed to generally increase CH_4 yields and render the digester more stable (Singh et al., 2019). Mixing achieves a homogeneous environment and effective use of the entire digester volume by minimizing hydraulic dead zones and preventing the buildup of large pockets of unfavorable environmental conditions such as low pH and high volatile fatty acids (VFA) (Kariyama et al., 2018). Consequently, the concentration of toxic agents throughout the reactor is diluted. Mixing also aids in eliminating excess CO_2, which can inhibit the process when its partial pressures exceed 0.2 atmospheres (Shahi et al., 2022).

Agitation of the digester contents offers the benefit of mixing the materials to even out localized concentrations and prevent the formation of 'dead zones' or crust. Additionally, it enhances bacterial access to waste, facilitates the removal and dispersion of metabolic byproducts, and maintains consistent temperature throughout the digester. While some suggest that efficient mixing boosts methane production, evidence supporting this claim is inconclusive, indicating that noticeable benefits may vary depending on the system or operational conditions (Singh et al., 2020). Furthermore, mixing promotes heat transfer, reduces particle size as digestion progresses, and facilitates the release of produced gas from the digester contents (Kariyama et al., 2018).

2.7.15 Organic loading rate (OLR)

The Organic Loading Rate (OLR), which quantifies the amount of substrate (biomass) introduced into the single-stage reactor system, is typically expressed in terms of chemical oxygen demand (COD) kg/m^3•day, volatile solids (VS) of total solids (TS)/L•day, or VS/m^3•day. Studies have indicated that exceeding the system's capacity by increasing the OLR in the anaerobic digestion of solid wastes can lead to challenges (Wang et al., 2023). The hydrogen and volatile fatty acids (VFAs) generated by acidogenic bacteria may not be consumed at the same rate by methanogens. This is because elevated OLRs and the resultant acidogenic activity, along with VFA intermediates from the acid-forming stages, can foster a proliferation of acidogenic bacteria, thereby inhibiting the growth of methanogenic populations.

The rise in OLR and acidogenic activity, marked by increased VFA, CO_2, and H_2 production, may cause an accumulation of organic acids, resulting in decreased pH and gas production (Paudel et al., 2017). Subsequently, this hampers the biological activity of methane-producing methanogens, as their growth is impeded below a pH of 6.6, thus diminishing methane production—the primary output of biogas (Alejandro and Bejarano, 2021). Therefore, determining the appropriate OLR for a specific substrate is crucial for optimizing reactor performance and maximizing methane production (Adu-Gyamfi et al., 2012).

Methane yield is commonly assessed by quantifying the gas produced per unit volume of VS within the feedstock after subjecting it to Anaerobic digestion for an adequate duration under specified temperature and conditions (Lee et al., 2023). Methane yield serves as an indicator of substrate biodegradability, as materials with low VS/TS, such as lignin, pose challenges for anaerobic degradation (Yasim and Buyong, 2023). Hence, gas production is substantially influenced by the nature of the substrate.

2.7.16 Oxygen

Oxygen poses a toxicity risk to the majority of anaerobic microorganisms, causing a notable reduction in the digestion rate within anaerobic reactors. Nevertheless, there is a possibility that facultative anaerobes may metabolize dissolved oxygen before adverse effects become apparent (Lu, 2021).

2.7.17 VFA

In the initial stages of Anaerobic digestion or when the digester experiences an overload of organic matter, elevated levels of volatile fatty acids (VFAs) are commonly detected. These VFAs are often linked to toxicity and inhibitory effects. While it is commonly believed that VFA inhibition stems from their accumulation and subsequent decrease in pH, certain VFAs possess inherent toxicity to anaerobic microorganisms (Ali et al., 2019).

2.8 Methods of Biogas production through Anaerobic digestion.

Anaerobic digestion is a well-known process that converts organic waste into valuable biogas and fertilizer within an oxygen-deprived environment. There are two primary methods for producing biogas through Anaerobic digestion: wet Anaerobic digestion and dry Anaerobic digestion (Arelli et al., 2022). The main difference between these methods lies in how they handle solid waste. Dry Anaerobic digestion processes organic waste in its solid form, typically employing mechanical sorting, while wet Anaerobic digestion requires the waste to be converted into a homogeneous pulp for pumpable processing (Sawyerr et al., 2019).

2.9 Reactor configuration

2.9.1 Feeding system

The feeding system involves the transportation and mixing of the substrate before it enters the digester (Bacenetti et al., 2013). Substrate is conveyed from storage (aerobic conditions) to the digester (anaerobic conditions), where it is mixed and milled with water to achieve homogenization (Gautam et al., 2022).

Adaptation of the feeding system is necessary to accommodate both the feedstock and the type of reactor (Willeghems and Buysse, 2016). For instance, batch digesters require discontinuous feeding, whereas Plug-flow and CSTR digesters are fed continuously or semi-continuously. Substrates can be introduced separately, through the sidewall or ceiling of the digester, to prevent pump clogging (Liu et al., 2012). Additional mixers may be employed to homogenize the material.

Combining multiple feeding systems may be necessary to handle different feedstock types (Mao et al., 2015). Effective feeding management significantly influences the fermentation process, as an excessive load of organic matter can alter the microbial community and reduce gas production (Wu et al., 2016). When designing a feeding system, consideration must be given to both the volume of the substrate and the storage capacity.

2.9.2 Reactor type

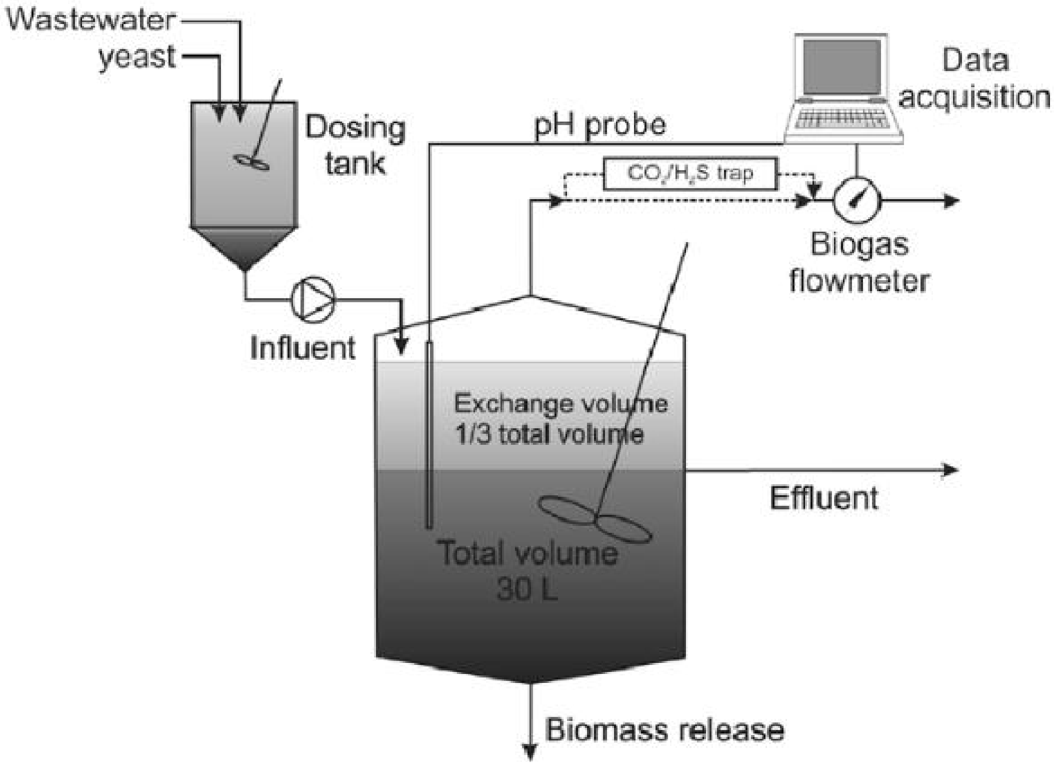
Digestion reactors are distinguished by their feeding mode (batch or continuous) and mixing type (CSTR or plug-flow). The design of the reactor is influenced by the characteristics and type of feedstock (Nathalie, 2013). Plug-flow reactors are employed when the dry matter content of the substrate mix exceeds 20%, whereas CSTRs are utilized when the dry matter content is below 15%. It's noteworthy that approximately 90% of modern biogas plants employ CSTRs (Bensmann et al., 2013).

Reactors may vary in terms of being dry or wet, batch or continuous, one-step or multi-step, and one-phase or multi-phase (Orozco et al., 2013). The various reactor type includes;

1. Batch reactors

Batch reactors are relatively easy to manage as they are loaded with feedstock and remain untouched for extended periods before being cleared. There is no need for mixing, stirring, or pumping, contributing to their low initial investment. Nevertheless, they are prone to issues like channeling and clogging, and they tend to occupy more space while delivering a lower yield of biogas (Figure 2.2). Methane generation usually peaks early in the digestion process and declines as the available substrate is depleted towards the end.

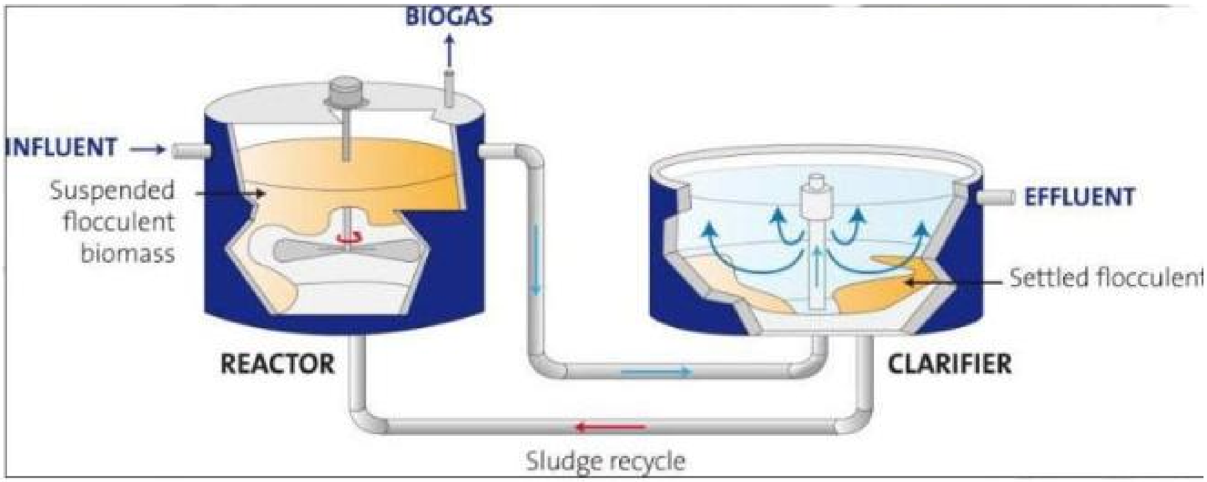
Figure 2.2: Batch reactor



2. Continuous Flow Stirred-tank Reactor (CSTR)

CSTRs (continuous flow stirred-tank reactors) are high-rate digesters commonly utilized for biogas production due to their simpler design compared to other methane digester types. Typically, CSTRs are employed for processing slurries with a total solids content between 5-10% and are particularly effective for treating animal manure and organic industrial wastes. However, they often require longer retention times and may consume more energy than alternative reactor types. The efficiency of CSTRs can be enhanced through the recycling of microbial solids or by boosting the retention of active biomass.

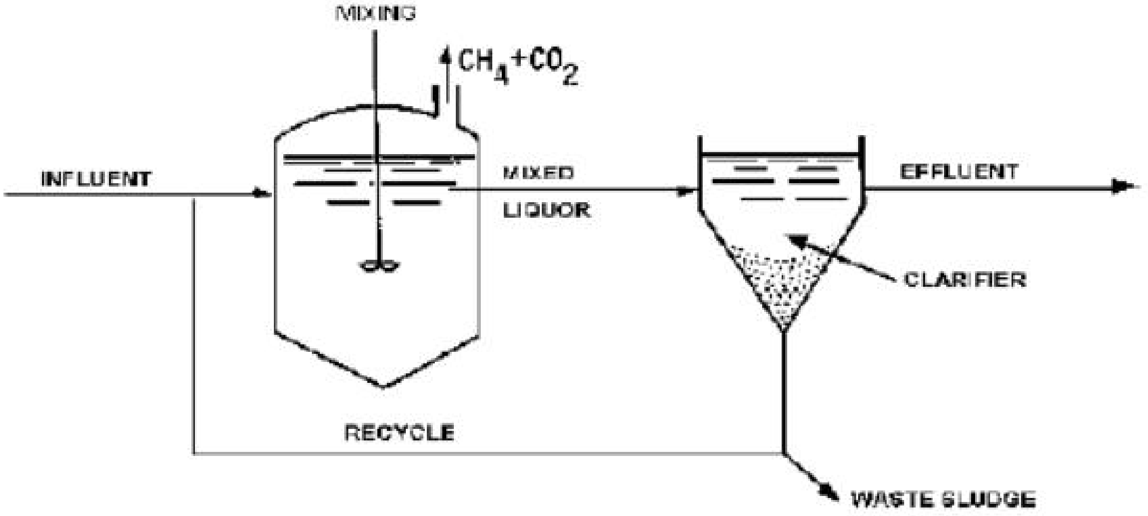
Figure 2.3: Continuous Flow Stirred-tank Reactor (CSTR)



3. Anaerobic Contact Reactor (ACR)

An Anaerobic Contact Reactor (ACR) is a fully mixed tank equipped with mechanical stirring and sludge recycling. The effluent from the tank is directed to a solid-liquid separator—such as a gravity sedimentation tank, sludge flotation device, or lamella clarifier—where solids are separated and returned to the anaerobic digester. ACRs are particularly efficient in treating high-strength waste with a high concentration of digestible solids due to their high concentration of active microbial biomass. They feature a short hydraulic retention time and can adapt easily to changes in organic loading. ACRs are less prone to issues like souring and other inhibitory effects. Additionally, stirred digesters with membrane-based cell retention are highly effective for biogas production.

Figure 2.4: Anaerobic Contact Reactor



4. Anaerobic Plug-Flow Reactors (APFRs)

Anaerobic Plug-Flow Reactors are elongated rectangular channels where flow enters at one end and exits at the other, with infrequent mixing. These reactors are typically situated above ground. They can operate under both thermophilic and mesophilic conditions. APFRs are high-rate digesters used commercially for processing various types of organic wastes, including animal manure slurries, distillery by-products, and the organic fraction of municipal solid waste. Compared to single-stage Continuous Stirred-Tank Reactors (CSTRs), APFRs offer higher efficiency in converting substrates into biogas and exhibit greater operational stability.

2.9.3 Number of phases

Most biogas plants operate with a single phase, wherein all stages of microbial degradation occur within the same tank (Adinurani et al., 2015). These single-phase methods are known for their simplicity and lower cost. In contrast, plants employing a two-phase system separate the hydrolysis stage from the overall process (Hosseini et al., 2022).

This necessitates optimization of pH, temperature, and retention for each phase (Mao et al., 2015), resulting in improved degradation kinetics. The two-phase system is particularly recommended for substrates rich in sugar, starch, or proteins (Nathalie, 2013). An advantage of the two-phase system is the separation of the large amount of acids produced in the hydrolytic phase. These acids can inhibit methane formation in a single-phase system (Yan et al., 2016).

2.9.4 Reactor temperature

Depending on temperature, the digestion process can be categorized into psychrophilic (10-25°C), mesophilic (25-45°C), or thermophilic digestion (50-58°C) (Nie et al., 2021). The temperature within the reactor significantly influences various parameters, thereby impacting the digestion process (Wang et al., 2019).

Higher temperatures in the reactor accelerate the degradation rate, hence thermophilic digestion requires shorter retention times. Moreover, elevated temperatures can eliminate most pathogens present in the digester (Seruga et al., 2020). However, high temperatures also lead to an increase in ammonia levels within the digester. In nitrogen-rich substrates, high temperatures cause most nitrogen to convert into ammonia, inhibiting microbial activity and reducing biogas production (Munk et al., 2017).

Thermophilic temperatures are typically employed for substrates posing hygiene risks, such as food wastes. Plant design may involve a combination of thermophilic and mesophilic conditions to mitigate pathogen risks and reduce energy costs associated with thermophilic reactors (Orozco et al., 2013).

2.9.5 Agitators

Ensuring proper agitation of the digestion material is crucial for effectively distributing substrates, microorganisms, and heat within the digester. Additionally, agitation helps to eliminate gas bubbles and prevent the formation of floating or settling layer (Saini et al., 2021). Agitation methods can vary and may involve mechanical or manual processes, with options for vertical or horizontal placement within the digester (Mahmudul et al., 2021). Furthermore, agitation serves to disrupt any floating scum layers that may form, particularly in feedstock containing large materials such as wood chips, which are commonly found in certain types of manure like chicken manure (Mao et al., 2015). The various types of Agitators include;

1. **Mechanical Agitation**:

Vertical Agitation: This includes equipments such as screw augers or vertical stirrers, which facilitate the movement of material up and down within the digester.

Automated agitation: Automated agitation in horizontal steel vessels primarily relies on mechanical paddle rotors. A horizontal shaft supported by hardwood bearings extends through the entire vessel, with paddles or loop-shaped pipes attached. Rotating the shaft mixes the contents of the vessel, disrupts the floating layer, and pushes sediments toward a drainage outlet.

Hydraulic mixing: Hydraulic mixing involves using a powerful pump to set the entire substrate in motion, as long as the pump's intake and outlet are positioned appropriately to match the shape of the digester. These pumps are frequently installed centrally to perform additional functions as well.

Horizontal Agitation: This involves using devices like horizontal paddles or impellers that stir the material from side to side.

Pneumatic agitation: Pneumatic agitation is achieved by installing a piping system with gas jets at the bottom of the digester and pumping biogas through it. The rising bubbles of biogas create gentle mixing of the substrate. A key issue with these systems is the potential for slurry to enter the piping. This problem can be mitigated by attaching flexible hose sections with stainless steel couplings to the jets.

2. **Manual Agitation**:

This method involves physical stirring or turning of the material, although it is less common in large-scale operations.

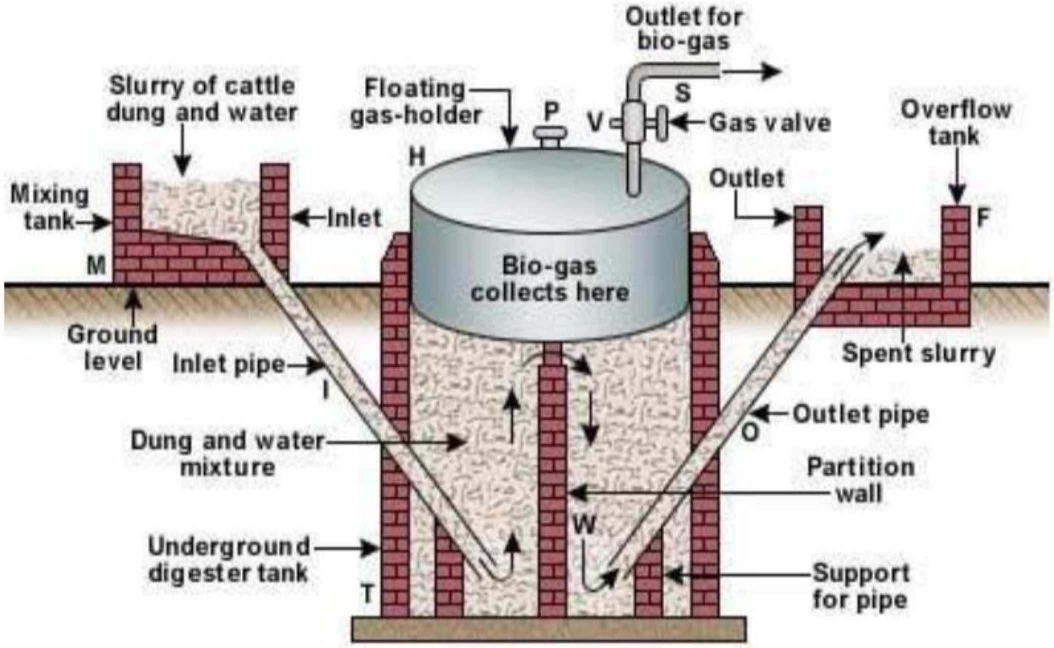
2.10 Types of Biogas digester

Biogas production can occur through various types of digesters utilized for anaerobic digestion. Two common methods are batch mode processing and continuous digestion (Jaysingpure and Khobragade, 2023). The various types of Biogas Digester include;

1. Floating Gas Holder Type Plants

This type of plant features an underground structure constructed from brick and cement, with inlet and outlet connections. A floating steel structure is positioned on top for gas collection. Increased pressure causes the biogas holder to rise along the central axis, releasing the produced gas. The floating gas structure helps maintain a consistent pressure.

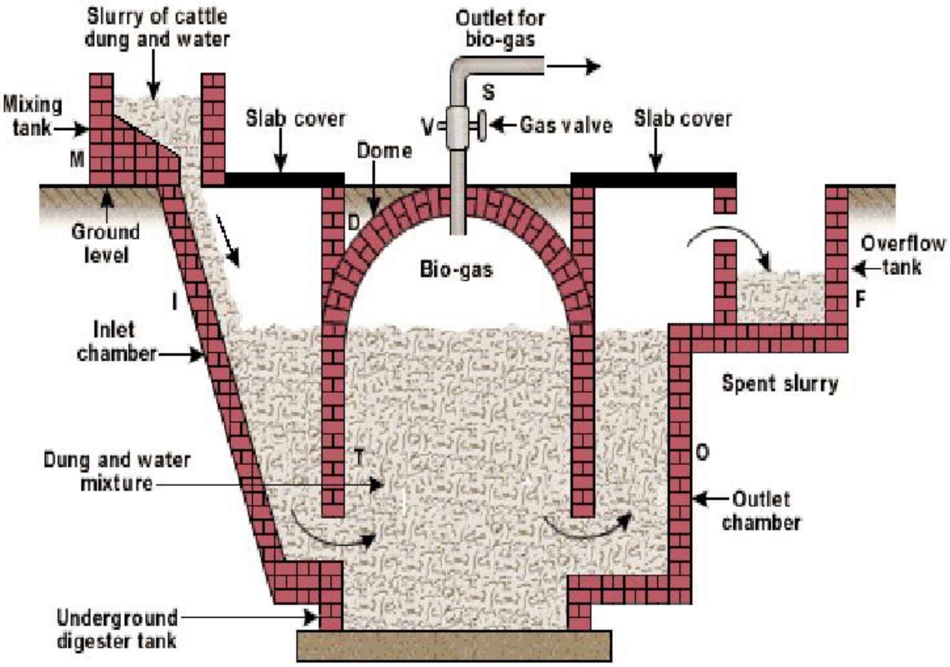
Figure 2.5: A schematic of a floating gas holder type plant.



2. Fixed Dome Type Biogas Plant

In a fixed dome biogas plant, the gas holder and digester are integrated into a single unit, with the upper part of the digester serving as the gas holder. The displacement of slurry generates additional pressure, allowing the release of the produced biogas. The pressure within the biogas digester varies accordingly.

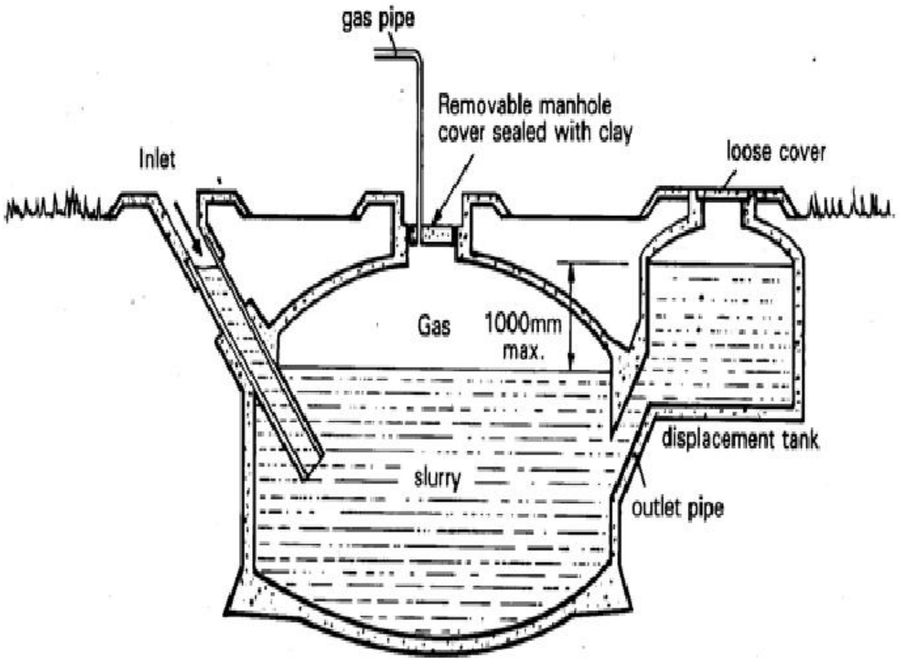
Figure 2.6: Fixed Dome Type Biogas Plant



3. Fixed Dome With Expansion Chamber type Biogas Plant

It features a curved bottom and a hemispherical top that are joined at their bases, eliminating the formation of a cylindrical section. The displaced slurry flows into the outlet tank due to its positioning. An inlet pipe connects the mixing tank to the digester.

Figure 2.7: Fixed Dome With Expansion Chamber type Biogas Plant



2.10.1 Types of Biogas processes

Batch mode process

In the batch-type digester, an airtight reactor tank is filled once with substrate, microbial inoculums, and sometimes a chemical to maintain optimal pH within the reactor. The reactor is then sealed, and fermentation proceeds for a specified period (Zala et al., 2020). In such processes, reactors are filled and emptied completely after a retention time. As a result, daily gas production reaches maximum levels and then declines after several retention days (Rouf et al., 2015). While substrate handling is straightforward with this method, there is significant variation in both the quality and quantity of biogas produced (Dotzauer et al., 2019).

Continuous process

In continuous process, the addition and removal of substrate materials may occur between 1-8 times every day (Demetriades, 2008). In this process, the substrate material is pumped regularly into the digester and an equal volume of digested material is displaced and thus the volume in the digester remains constant. Continuous feeding of substrate is possible with this kind of process which at last gives much more gas production than the batch process. For smaller digester, the feeding of material is often once or twice a day but large digesters are operated more continuously with feeding intervals of less than one hour (Demetriades, 2008)

CHAPTER THREE

MATERIALS AND METHODS

3.1 MATERIALS

Cow dung (inoculum) was obtained from the cattle market in Benin city and Tiger nut was obtained from the market at ring road, Edo state. A gas cylinder was fabricated and designed to be used as the Anaerobic digester.

Plate 3.1: Anaerobic bio-digester and Tiger nut tubers

LIST OF MATERIALS AND THEIR USES

Distilled water: For mixing the Tiger nut waste and Cow dung.

Cow dung: The inoculum that introduces microorganisms for Anaerobic digestion.

Tiger nut: The primary component of the co-digestion feed as a substrate for microbial growth.

REAGENTS AND THEIR USES

Sodium Hydroxide (NaOH): For regulating pH of the mixture.

Hydrochloric Acid (HCL): For regulating pH of the mixture.

LIST OF EQUIPMENT/APPARATUS AND THEIR USES

Gas cylinder: The reactor for the anaerobic digestion process.

Measuring cylinder: For taking measurements of the gas produced daily in the digester using downward displacement of water.

Water bath: For taking measurements of the gas produced daily in the digester using downward displacement of water.

pH meter (electronic device): Used to check the pH of the digester sludge daily.

Weighing balance: For measuring the mass of samples

Hand gloves: For handling samples

Gas hose: For transporting the gas from one point to another.

3.2 METHODS

3.2.1 Construction of Components of Biogas

The digester, purifier, and storage are the three main parts of the biogas system. The engineering workshop at the University of Benin handled the construction, which included material selection, measurement, marking, and cutting of materials into part-sizes whereas I assembled the components and ran the biogas setup test.

Plate 3.2: Experimental set-up for the Anaerobic digestion of Cow dung and Tiger nut waste

3.2.2 Biodigester

A gas cylinder was used as the biodigester. A hole was drilled at the side and an inlet valve was made to fit the hole. An outlet open and close valve which will be used for the discharging gas was already fabricated on the cylinder. Also, the gas hose was fitted to the open and close valve and then passed through the jar filled with NaOH which served as a purifier. Finally, the biodigester was placed on the prepared concrete floor. All Perforations were adequately sealed to prevent gas leakage.

3.2.3 Gas scrubber

The function of the Gas scrubber is to remove unwanted biogas components such as hydrogen sulfide, carbon dioxide and water vapor. This was carried out by passing the raw biogas through a gas hose through NaOH to reduce the percentage of carbon dioxide and hydrogen sulphide. The purified gas is then passed through an inverted measuring cylinder filled with water. The biogas displaced the water in the measuring cylinder and the volume of the biogas produced was measured daily.

3.2.4 Slurry preparation and loading

Fresh cow dung was collected from the cattle market at Uselu, Edo state, Nigeria. Tiger nut was collected from the market in Ring-road. The Tiger nut was prepared by soaking it in water for 24 hours and then grinding it with an industrial grinding mill machine. The grounded tiger nut was then strained with a plastic mesh sieve to collect the chaff. The Tiger nut chaff was weighed to be 5kg and the fresh cow dung was weighed to be 1kg. The Tiger nut chaff and fresh cow dung was mixed with 10 liters of distilled water at ratio 1:2 for maximum biogas production as described by Adeniran et al. (2014).

The pH of the mixture was taken and read to be 4.7. Then, a 40g/l NaOH solution was used as a pretreatment chemical and this was furtherly used to mix the Tiger nut waste and cow dung slurries in the bucket until the pH was 6.7. The chemically pretreated mixed Tiger nut waste and cow dung slurries at pH 6.7 was charged immediately to fill the anaerobic digester. The anaerobic digester was filled so as to create a vacuum afterwards and remove all the oxygen inside the cylinder. 1.75kg of the mixture was removed to create adequate space at the upper part of the fermentation chamber for the storage of the biogas produced before delivery through the hose.

The inlet and outlet openings of the bio digester were properly sealed to avoid oxygen from seeping in. Finally, the experiment was conducted at ambient conditions as there was no any form of control over temperature and pressure.

3.2.5 Gas Collection and Measurement

The graduated cylinder was inverted in a water-filled container. It was ensured that the graduated cylinder was first filled with water and no air bubbles before it was inverted. The gas produced was allowed to flow through the rubber hose, and into the graduated cylinder. As the biogas is produced it displaces the water in the graduated cylinder causing it to move downward. The volume of biogas yield was measured on a daily basis by recording the difference between the initial and final water levels. The pH reading was taken every day by opening the inlet valve to allow small portion of the slurry into a beaker, and measured with a pocket pH meter (Hanna instruments, HI196107, Italy).

3.2.6 Agitators (Manual mixer)

Agitation of the digestion material is important for distributing the substrates, micro-organisms and heat (Bachmann et al., 2013); it increases contact of substrate with the biogas producing microorganisms, resulting in accelerated decomposition and increased production of biogas. The biodigester were subjected to manual agitation every day to ensure thorough mixing of the digester content while maintaining intimate contact between the microorganisms and substrate and to enhance complete digestion of substrate. This would also prevent accumulation of substrates and fatty acids. Agitation of the digestion material breaks the scum on the surface of slurry, it also helps to drive out gas bubbles and avoid the formation of floating or settling layers.

3.3 Analysis of feed stock

3.3.1 Total solid

Production of biogas is inefficient if fermentation materials are too diluted or too concentrated. Therefore, TS is important to determine because you need to know how much water you need to add to the reactor. If the manure has a high TS content, more water needs to be added to the reactor. All waste materials fed into a plant consist of solid matter and water. Solid matter is made of volatile organic matter and non-volatiles. During anaerobic fermentation process, volatile solids undergo digestion and non-volatiles remain unaffected. It has been established that in the digestion of cow dung sludge, the optimum solids concentration is 20% (Makhura et al., 2019).

3.3.2 Nitrogen content

Determination of TKN in a sample is important, primarily to evaluate if there is sufficient nitrogen available for the growth of anaerobic bacteria. In most cases there will be excessive nitrogen in the biogas reactor, so determination of the TKN content in a biogas feedstock helps to estimate nitrogen concentrations in the biogas reactor. This is important to know, because ammonia inhibition can occur if the ammonia concentration in the reactor exceeds certain levels.

CHAPTER FOUR

RESULTS AND DISCUSSION

This chapter will focus on the results from the laboratory analysis, and biogas operation under mesophilic condition using Tiger nut and cow dung as a feed stock. This chapter will also discuss the utilizations of the biogas and bio fertilizers as results from the biogas project.

4.1 Batch Anaerobic Digestion

Cow dung was collected from a cattle farm as inoculum, and the feedstock which is Tiger nut was collected from the market as mentioned in chapter three. The chemical and physical analysis of the samples were carried out and the results are illustrated in (Table 4.1).

Table 4.1: Feed characterization

Parameters	Moisture (%)	TS (%)	VS (%)	Ash content %	pH	Nitrogen %	Potassium mg/kg	Phosphorous mg/kg	Ammonium Mg/kg
Tigernut chaff and cow dung mixture	85.43	14.57	14.31	0.26	6.7	8.64	298	76.28	0.08

Higher water content can facilitate better mixing of the substrate within the biogas reactor, ensuring more uniform digestion and potentially enhancing biogas production efficiency. Insufficient water can inhibit microbial activity and reduce biogas yield. Hence, a water content of 85.43% is enough to promote efficient biogas yield. The rate of digestion may vary depending on the total solid content. Higher solid content can slow down digestion initially as microorganisms work to break down the organic material. However, once digestion is established, it can lead to steady biogas production. The total solid content also affects the availability of nutrients for microorganisms. Proper balance of nutrients such as carbon, nitrogen, and phosphorus are essential for microbial activity and biogas production. Overall, a total solid content of 14.57% provides a moderate amount of organic material for digestion.

Volatile solids are the primary source of methane production in anaerobic digestion. A higher volatile solid content generally correlates with higher biogas yields, as there is more organic material available for microbial conversion into methane. A volatile solids content of 14.31% suggests a moderate amount of organic material available for biogas production. The ash content represents the inorganic materials present in the slurry mixture, such as minerals and metals, that cannot be converted into biogas during anaerobic digestion. Overall, a low ash content of 0.26% suggests that the majority of the slurry mixture consists of organic material that can be converted into biogas. This is favorable for maximizing biogas production efficiency and quality of the digestate.

Nitrogen is an essential nutrient for microbial growth and activity in anaerobic digestion. A nitrogen content of 8.64% suggests a relatively high concentration of nitrogen in the slurry mixture, which can support robust microbial populations and efficient biogas production.

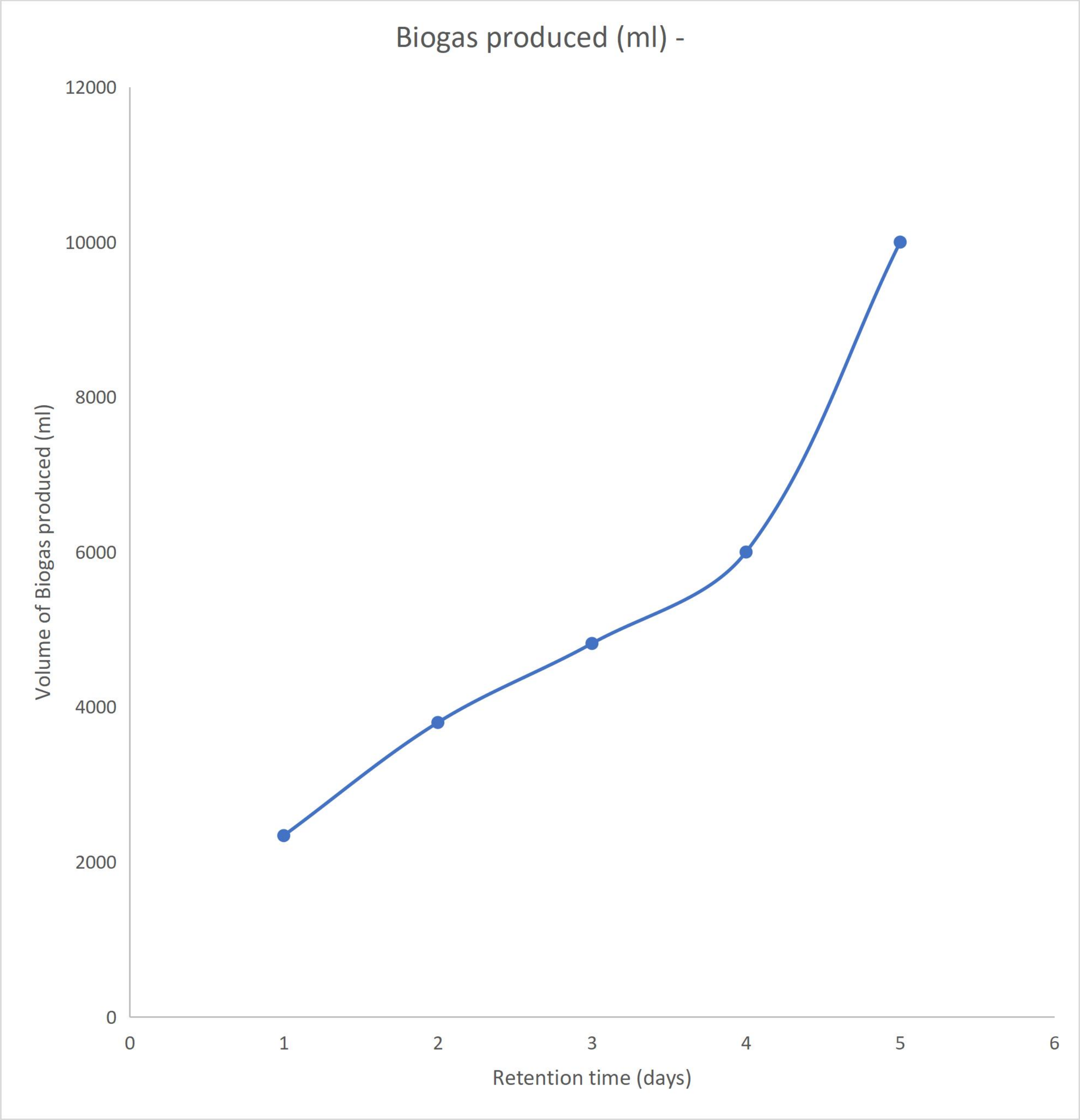
Potassium is an essential nutrient for microbial growth and metabolic activity. It plays a role in enzyme activation, osmoregulation, and cell function. A potassium content of 298 mg/kg suggests a moderate level of potassium availability in the slurry mixture, which can support microbial activity during anaerobic digestion.

Table 4.2: Amount of biogas produced daily and pH taken daily

Day	pH	Biogas produced (ml)
1	6.7	-
2	6.4	2340
3	5.5	3800
4	5.8	4820
5	5.4	6000
6	5.4	10000

Phosphorus plays a crucial role in enzyme activation and function. Many enzymes involved in the breakdown of organic matter and the production of biogas require phosphorus as a cofactor. Optimal phosphorus levels in the slurry mixture can enhance enzymatic activity, facilitating the digestion process. However, the presented parameters could not exactly reflect the degradation and potential of the waste in the anaerobic digestion process. The analysis of the plot of the biogas yield per day showed an instant spike in the production of biogas on the second day of retention with a volume of 2340mL. There was a progressive increase in the volume of biogas produced until the last day of retention.

Figure 4.1: Daily biogas volume readings



This graph shows the volume of biogas produced in milliliters (ml) plotted against the retention time in days. In the X-axis(Retention Time), the days are plotted from 0 to 6, representing the period over which biogas production was measured. In the Y-axis (Volume of Biogas Produced), the volume of biogas is measured in milliliters, ranging from 0 to about 12,000 ml.

From the graph, it's evident that in the initial days (from day 0 to day 3), the production of biogas increases steadily but at a relatively moderate pace. After day 3, there's a sharp increase in biogas production, suggesting a significant acceleration in the biological processes responsible for biogas generation. This could indicate the point at which the microbial activity within the bioreactor becomes highly efficient.

The graph effectively shows the relationship between retention time and the amount of biogas produced, which is crucial for optimizing the operational parameters of biogas production systems.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1 CONCLUSIONS

These are the following conclusion gotten from the Study;

1. The volume of biogas produced during the 6-day period was recorded to be 26960mL.
2. The initial values of the feed properties such as VS (14.31%), TS (14.57%), Water content (85.43%), pH (6.7), Ash content (0.26%), Nitrogen (8.64%), Potassium (298) mg/kg, Phosphorous (76.28mg/kg) and Ammonium (0.08mg/kg).

5.2. RECOMMENDATION

I would recommend that Tiger nut chaff should be co-digested with other feedstock to investigate effect on biogas yield.

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APPENDIX

Feed characterization

Parameters	Tigernut chaff and cow dung mixture
Moisture (%)	85.43
TS (%)	14.57
VS (%)	14.31
Ash content (%)	0.26
pH	6.7
Nitrogen %	8.64
Potassium mg/kg	298
Phosphorous mg/kg	76.28
Ammonium Mg/kg	0.08

Amount of Biogas produced

pH	Biogas produced (ml)
6.7	-
6.4	2340
5.5	3800
5.8	4820
5.4	6000
5.4	10000