

**COLD DIGESTION OF CATTLE RUMEN CONTENT AND WATER
HYACINTH FOR BIOGAS PRODUCTION**

BY

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(CHEMICAL/PETROLEUM TECHNIQUES)

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NOVEMBER, 2025.

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**A PROJECT SUBMITTED TO THE DEPARTMENT OF
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CERTIFICATION

This is to certify that, this project work was carried out by **Adaeze Deborah OSAYANDE** with Matriculation Number **LSC2007350**, under the supervision of **Engr O. Salokun** of the Department of Science Laboratory Technology (Chemical/Petroleum Techniques), University of Benin, Benin City, Edo state, Nigeria.

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DEDICATION

This project is dedicated to ALMIGHTY GOD for his care, mercy, favor, provision, protection and inspiration all through my stay in school as well as the duration of this research

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I give all glory and honor to God Almighty for his unmerited favor, presence, and strength that enabled me to complete this project. I appreciate his guidance and provision throughout this journey.

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ABSTRACT

The need for sustainable energy and waste management solutions around the world has made it essential to look into locally available and affordable organic feedstocks for biogas production. This study looks at the possibility of creating biogas through cold (psychrophilic) anaerobic co-digestion of two common and often problematic wastes in Nigeria: water hyacinth(WH) and cattle rumen content (CRC), As part of the study, the feedstocks were characterized, daily biogas production and pH variation were calculated, and the efficacy of a 2:1 (CRC:WH) mixture at room temperature without external heating was evaluated. The experiment was carried out with a 5kg batch digester over a 30-day retention time. The feedstock analysis revealed a high moisture content (80.25%) and a chemical oxygen demand (COD) of 69 g/L, indicating a substantial organic load suitable for digestion. After an 11-day lag period, biogas production began, coinciding with a pH increase from 6.13 to 6.73, putting it in the optimal range for methanogenic activity. On the 30th day, a peak biogas yield of 2090 ml was recorded at a pH of 8.46, indicating a direct correlation between increasing pH and increased biogas production. The cumulative production profile clearly demonstrated the sequence of hydrolysis, acidogenesis, and methanogenesis. The study comes to the conclusion that it is both technically possible and efficient to cold co-digest water hyacinth and cattle rumen content in a 2:1 ratio. A balanced microbial environment was produced by the carbon-rich water hyacinth and nitrogen-rich rumen content working together to support long-term biogas production.

CHAPTER ONE

INTRODUCTION

1.1 Background of study

The role of biogas technology has expanded significantly in recent years, spurred by heightened awareness of sustainable practices and the global push toward eco-friendly energy sources (Anukam *et al.*, 2019; Bijarchiyan *et al.*, 2020). Biogas transforms organic waste into cleaner energy while solving critical environmental challenges: it captures methane (a greenhouse gas 84 times more potent than CO₂) before it enters into the atmosphere and converts it into reliable renewable power. This circular energy solution not only prevents harmful emissions from landfills and farms but also generates on-demand electricity, complementing intermittent sources like solar and wind. As nations pursue net-zero goals, biogas emerges as an essential technology that turns waste into value while supporting sustainable development (Pramanik *et al.*, 2019; O'Connor *et al.*, 2021). Africa's development depends on switching to clean energy. This change will help reach important energy goals. Most importantly, all Africans must have access to affordable, clean power (IEA. Africa Energy Outlook 2022; 2023). Africa stands at the forefront of renewable energy solutions, with biomass and waste-derived fuels already contributing 45.4% of the continent's total energy mix as of 2021. Current projections suggest the planet will produce approximately 6 million metric tons of waste annually by 2025, positioning Africa's abundant organic resources as a vital component in achieving global carbon neutrality goals (Archana *et al.*, 2024). Generating biogas from organic waste such as animal manure and agricultural residues presents a sustainable energy alternative. This approach not only facilitates clean cooking solutions but also contributes to a

greener energy transition (Archana *et al.*, 2024; Shiv *et al.*, 2017; Ibrahim *et al.*, 2021; Aduroja, 2021).

In 2018, electricity and heat generation accounted for the bulk of global biogas consumption, representing over 90% of total usage. Meanwhile, less than 10% was processed into biomethane for transportation fuel or integration into natural gas infrastructure (International energy agency, 2020). As biomethane production capacity grows globally, industry analysts project its adoption will accelerate across energy markets (Banja *et al.*, 2019). Biogas systems are implemented differently worldwide, reflecting each region's unique energy landscape and development priorities (Capodaglio *et al.*, 2016). Biogas production systems exhibit significant regional variations, with distinct approaches to feedstock selection, pre-processing techniques, facility scale, and end-use applications of the generated biogas across different nations (Sturmer *et al.*, 2021a). Biogas emerges as a sustainable energy alternative when microorganisms digest organic substances—such as crop leftovers, animal dung, food scraps, and waste water—in an environment devoid of oxygen. This anaerobic process converts biomass into usable fuel while minimizing waste (Shiv *et al.*, 2017; Sarvari *et al.*, 2016; Thokchom *et al.*, 2015; Ghosh *et al.*, 2020). Bejor (2020) explains the term “anaerobic” as conditions or processes that occur in the absence of oxygen (Ebaye, 2020). Four specialized microbial communities work in succession to drive this biochemical conversion: initial decomposition by hydrolytic bacteria, followed by acidification through acidogenic microbes, subsequent conversion to acetate by acetogenic organisms, and final methane production by methanogenic archaea (Kwietniewska, 2014).

Anaerobic digestion (AD) is a clean and sustainable biological method used to manage and convert complex organic materials and toxic waste. The process takes place without oxygen, where microorganisms break down the organic content to produce biogas—a fuel-rich mixture primarily

composed of methane (CH₄) and carbon dioxide (CO₂), along with small amounts of hydrogen sulfide (H₂S), ammonia (NH₃), and water vapor. Modern waste valorization strategies prioritize anaerobic digestion (AD) systems due to their unique ability to simultaneously address organic waste accumulation while generating renewable energy – a dual solution that supports both climate change mitigation through methane capture and circular economy objectives by closing nutrient loops (De Baere & Mattheeuws, 2020). The microbial transformations underlying anaerobic digestion share biochemical pathways with natural methane-producing environments, particularly in anoxic wetland soils and herbivore digestive tracts. However, engineered digestion systems significantly enhance these natural processes through precise operational controls, achieving substantially higher organic matter conversion rates than occur in unmanaged environments (Weiland, 2020).

The implementation of anaerobic digestion systems faces three persistent obstacles: biological process sensitivity to operational variables, substantial initial capital outlays, stringent feedstock quality requirements, and seasonal variability in substrate availability (Khalid *et al.*, 2021). Contemporary scientific investigations are overcoming these limitations through three innovative pathways: engineered microbial community augmentation, strategic multi-substrate integration, and targeted government support mechanisms – collectively advancing the practicality and output efficiency of AD technologies (Appels *et al.*, 2021).

Anaerobic digesters have emerged as innovative waste-to-resource technologies that harness microbial metabolism to valorize organic residues into renewable fuel and biofertilizers. These engineered bioreactor systems enhance nature's decomposition mechanisms – similar to those occurring in anoxic lakebeds and enteric fermentation processes – through optimized environmental controls, achieving conversion efficiencies up to three times greater than

uncontrolled anaerobic environments (Weiland, 2020). The technology's global implementation has accelerated dramatically to address three pressing contemporary challenges: environmentally sound waste disposal methods, escalating clean energy demands, and international climate change mitigation targets. Modern anaerobic digestion systems have undergone transformative improvements in feedstock versatility through advanced bioprocessing innovations. These next-generation systems now effectively convert both conventional agricultural residues and unconventional organic sources – including microalgae biomass and food industry side streams – into valuable bioenergy products (Appels *et al.*, 2021). Significant progress in high- solids anaerobic bioreactor technology and optimized high temperature microbial systems has been particularly groundbreaking, enabling stable processing of challenging substrates with dry matter content ranging from 15-40% while achieving superior biogas production (Weiland, 2020)

1.2. AIM AND OBJECTIVES

The aim of this study is to evaluate the potential of cold digestion of cattle rumen content and water hyacinth for efficient biogas production.

The specific objectives are to:

1. to characterize the feedstock so as to determine its suitability for anaerobic digestion.
2. to determine the daily gas production and pH variation during the digestion process using water displacement method.
3. produce biogas from a mixture of cattle rumen content and water hyacinth in a 2:1 ratio.

1.3. Statement of Problem

This project aims to explore a cost-effective method of biogas generation by using cattle rumen content and water hyacinth under natural temperature conditions, without any external heating. The focus is on addressing the energy needs of rural communities where modern biogas systems

are often inaccessible due to high setup and maintenance costs. By converting these commonly discarded organic wastes into usable energy, the study proposes a sustainable solution that supports both waste management and renewable energy production in low-resource environments. This approach can serve as a low-cost, eco-friendly energy solution for underserved communities with limited access to traditional power sources.

1.4. Significance of the study

This study is significant as it provides an innovative approach to addressing energy shortages by exploring biogas production from easily accessible organic wastes—cattle rumen content and water hyacinth—without relying on external heating. The study presents a practical solution for communities with limited energy infrastructure by demonstrating that biogas can be produced under natural temperature conditions. It also promotes efficient waste utilization, helping to reduce environmental pollution. The findings can support adoption of simple, low-maintenance biogas systems, reduce dependence on non-renewable fuels, and contribute to environmental conservation and energy security in underserved communities.

CHAPTER TWO

LITERATURE REVIEW

2.1. OVERVIEW

The world is producing more waste than ever, particularly from farming activities, where organic waste contributes to serious environmental problems. These include inefficient land use, environmental contamination, and worsening global warming. A key concern is that when this organic waste decomposition in landfills, it emits methane-a potent greenhouse gas (Archana *et al.*, 2024). Implementing eco-friendly waste disposal methods has become increasingly necessary. Compounding this issue, the widespread dependence on carbon-based energy resources remains a dominant factor in rising atmospheric pollution levels, directly impacting global warming and ecosystem health (Nimesh *et al.*, 2022). The adoption of renewable energy technologies, particularly biogas systems, can help address these environmental concerns by providing an alternative to emissions-intensive energy production (Alengebawy *et al.*, 2022). Anaerobic digestion technology enables communities to convert organic waste into renewable energy, creating localized power systems that buffer against unpredictable fossil fuel market shocks (Piadeh *et al.*, 2024). In off-grid and underserved rural communities, biogas emerges as a practical renewable energy alternative. Its consistent availability not only addresses energy scarcity but also fosters socioeconomic development by enabling productive activities and improving living conditions (Balcioglu *et al.*, 2022). The process of anaerobic digestion helps create a closed-loop system where discarded materials are converted into usable products, minimizing waste accumulation while boosting sustainable resource management (Alengebawy *et al.*, 2022). Anaerobic digestion emerges as an innovative biological process that converts organic waste into

valuable renewable resources. The technology delivers a dual output system – producing clean biogas energy alongside a potent organic amendment that revitalizes agricultural soils. This natural fertilizer alternative not only reduces chemical inputs but also closes the nutrient loop, transforming waste into sustainable agricultural solutions while mitigating environmental impacts. (Alengebawy *et al.*, 2022). Through anaerobic digestion of organic waste materials, biogas emerges as a sustainable alternative to conventional fossil based gas. This renewable fuel offers dual utilization pathways: it can undergo purification to produce biomethane sustainable for injection into gas grids or serve as direct fuel for combined heat and power units that simultaneously generate usable thermal and electrical outputs with optimized energy efficiency (Alengebawy *et al.*, 2022), emerging as a carbon-neutral energy carrier, biogas can be refined through advanced gas conditioning systems to yield biomethane of exceptional purity (>97% CH₄). This renewable fuel alternative demonstrates combustion characteristics and energy density parameters nearly identical to fossil-derived natural gas, while providing substantial lifecycle emissions reductions (Abd *et al.*, 2022).

2.2. HISTORY OF BIOGAS

Historical evidence suggests that the use of biogas dates as far back as the 10th century B.C., when it was reportedly employed for heating bath water in ancient Assyria, while early indications of anaerobic decomposition of organic waste have been documented in ancient China (He, 2010). Although these early instances were largely empirical, systematic human attempts to control and apply anaerobic digestion processes became more evident during the mid-19th century. At this time, rudimentary digesters were constructed in New Zealand and India, marking some of the earliest engineered efforts to capture and utilize biogas. In the United Kingdom, one of the most notable historical developments was the construction of a sewage sludge digester in Exeter during

the 1890s, which generated biogas for illuminating street lamps (University of Adelaide, 2010). In China, the modern history of biogas technology is closely associated with Guorui Luo, who, in 1921, designed and built an 8 m³ biogas tank that utilized domestic waste as feedstock. His initiative later led to the founding of a company aimed at promoting the widespread use of biogas technology (He, 2010). By the early 20th century, Europe also began to witness significant progress. In 1920, Germany established its first sewage treatment facility capable of supplying biogas to the public energy grid, followed by the development of its first large-scale agricultural biogas plant in 1950.

A major acceleration in the global expansion of biogas technology occurred during the 1970s as a response to escalating petroleum prices, which drove the search for alternative renewable energy sources. Between the 1970s and early 1980s, biogas utilization grew rapidly throughout Asia, Latin America, and Africa, with particularly strong government involvement in China. During this period, the Chinese government initiated extensive rural energy programs that supported the installation of over seven million household digesters (He, 2010). However, as biogas technology later became integrated into urban waste management systems and energy conservation initiatives, the momentum in rural development began to decline. By 1988, the number of operational household digesters in China had decreased to approximately 4.7 million (Lebuhn *et al.*, 2014). From the early 2000s onward, biogas technology entered a new phase of expansion, characterized by widespread governmental and private-sector engagement. By 2007, China reported more than 26.5 million biogas plants, most of which were small-scale household digesters with capacities ranging between 6 and 10 m³ (Chen *et al.*, 2010). India followed a similar trajectory, reporting over three million family-sized biogas systems by 1999, and nearly four million by 2007, largely supported through national subsidies and rural development schemes. The National Project on

Biogas Development (NPBD), established in 1981-1982, played a central role in advancing the technology by providing financial assistance, capacity-building programs, and technical training for users. Throughout the 1980s and 1990s, government incentives covered between 30% and 100% of the installation costs for household bioreactors, underscoring India's commitment to expanding sustainable rural energy access (Yadav *et al.*, 2020).

2.3. BIOGAS AS AN ALTERNATIVE SOURCE OF ENERGY

Energy is the cornerstone of modern development, powering advancements across all sectors while elevating quality of life. However, humanity's insatiable energy appetite has sparked an environmental crisis, manifesting in climate change, biodiversity loss and ecosystem degradation. Confronted with this challenges, the global scientific community is spearheading a transition toward sustainable power generation, championing renewables like photovoltaic systems, wind turbines, hydroelectric plants, bioenergy solutions, atomic energy, and earth-derived thermal power as viable replacements for carbon-intensive fuels (Buraimoh *et al.*, 2020). Energy drives modern civilization, fueling economic growth and technological progress. As populations expand and industries evolve, global demand surges relentlessly-challenging sustainability while powering human advancement (Kang *et al.*, 2020). As global energy demands rise, biogas stands out as a versatile and eco-friendly solution for power generation. This renewable fuel source, created from organic waste, offers a practical alternative to conventional energy while addressing critical environmental challenges (Achinas *et al.*, 2017; Kumar *et al.*, 2018). The world's escalating energy demands coupled with worsening ecological consequences have prompted serious reconsideration of biogas systems as a sustainable power solution that bridges energy access and climate action (Pasternak *et al.*, 2021). Globally, biogas systems currently produce enough electricity to power millions of homes, with annual generation capacity above 7 GW. Beyond

power production, this versatile fuel meets daily energy needs through cooking and heating applications while also offering a sustainable pathway for biofuel manufacturing (Topolewski, 2021).

The bioenergy sector utilizes three interrelated renewable resources differentiated by their transformation state. Biogas generation employs controlled microbial breakdown of cellulosic feedstocks (animal waste, crop byproducts) in oxygen-free environments. Subsequent upgrading yields biomethane meeting specifications for grid injection as renewable natural gas (Abanades, 2022). Biogas made from decomposing plants and waste can power homes and factories, fuel clean vehicles, and make green products (Pasternak *et al.*, 2021). Biogas systems tackle climate change by preventing waste emissions and providing clean alternatives to polluting energy sources (Chen *et al.*, 2007; Kabeyi *et al.*, 2020). As a case in point, 2014 data shows biogas met 0.29% of Switzerland's comprehensive energy needs while supplying nearly one-twelfth of non-hydro renewable generation (Holliger *et al.*, 2017). Modern biogas technology offers a sustainable replacement for conventional wood-burning stoves, providing cleaner cooking solutions for underserved populations. Current projections suggest this renewable energy source could serve approximately 200 million individuals in developing economies, particularly across sub-Saharan Africa and South Asia, within the next two decades (International energy agency, 2020). Biogas is increasingly recognized as a stable and sustainable energy source that facilitates the transition toward renewable-dominated energy systems while reducing carbon emissions (Kabeyi *et al.*, 2020; Tabatabaei *et al.*, 2015). The anaerobic digestion (AD) process generates biogas as a renewable fuel source while simultaneously providing two additional environmental benefits: effective treatment of organic feedstocks through microbial decomposition, and production of nutrient-rich digestate. This liquid byproduct serves as a valuable organic fertilizer, offering a

sustainable alternative to synthetic fertilizers in agricultural systems. (Kabeyi *et al.*, 2020; Lebuhn *et al.*, 2014). As electricity grids worldwide evolve to incorporate more renewable sources, biogas provides critical advantages due to its scalable production from organic matter and multiple energy conversion pathways. This positions it as an essential component in the energy transition, capable of supplying renewable power while also serving as a raw material for sustainable fuel and chemical production. (Machado *et al.*, 2021; Mydeen *et al.*, 2016; Waş *et al.*, 2020; Kabeyi *et al.*, 2022; Liu *et al.*, 2017). Biogas technology represents a proven renewable energy solution with extensive scalability prospects. While its alignment with multiple sustainable development objectives through clean energy production, waste valorization, and energy access expansion is well-established, global energy systems have been slow to capitalize on these advantages. Realizing biogas's full contribution requires targeted capacity-building and market development initiatives. (Gürsan *et al.*, 2021; Kabeyi *et al.*, 2022; Phuangpornpitak *et al.*, 2013; Sahota *et al.*, 2018). Traditional societies maintained an exclusively biomass-based energy regime until the technological breakthroughs of the early industrial era introduced alternative energy options (Moriarty *et al.*, 2019). Industrialization ushered in an era dominated by coal and petroleum-based energy systems. The volatility of oil markets in the 1970s and 1980s, however, forced nations to explore cleaner energy solutions, leading to the recognition of biogas as a viable and eco-friendly substitute (Kabeyi *et al.*, 2022; Ouahabi *et al.*, 2021). According to (Chen *et al.*, 2017), environmental protection necessitates waste treatment methods that avoid ecological damage and emissions. Biogas production offers a practical solution for developing countries' rural farming communities, enabling them to convert agricultural waste into clean energy through locally manageable systems. India's population distribution shows a significant rural predominance, with approximately seven out of every ten residents living in countryside areas rather than urban centers

(Kabeyi *et al.*, 2020), the world continues to overlook biogas's considerable energy potential. Smallholder farmers in developing countries' rural regions could particularly benefit from implementing this technology to fulfill their diverse energy needs in an environmentally sustainable manner (Kariuki, 2009; Tabaa *et al.*, 2020).

2.4. ANAEROBIC DIGESTION

Anaerobic digestion (AD) is a sustainable and efficient biological process used to break down various organic and hazardous waste materials. By harnessing microbial activity in an oxygen-free environment, it converts complex organic matter into biogas, a renewable energy source. Biogas primarily contains methane (CH₄) and carbon dioxide (CO₂), with trace amount of hydrogen sulphide (H₂S), ammonia (NH₃), and water vapor. The conversion of readily available organic wastes to bioenergy offers a sustainable approach to both waste management and renewable fuel production. Dominant agricultural residues include rice straw (47% of total crop waste), corn stover (28%), and wheat straw (25%), which collectively represent significant potential for decentralized energy systems (Qin *et al.*, 2018; Zhao, 2018). The complete mineralization of organic matter under anaerobic conditions occurs through four metabolically linked stages: polymeric substrate breakdown (hydrolysis), monomer conversion to volatile acids (acidogenesis), intermediate transformation to acetic acid (acetogenesis), and terminal methane formation (methanogenesis), each facilitated by distinct microbial groups. The hydrolysis phase, where bacteria initiate the breakdown of complex organic structures into simpler compounds, is paradoxically both the most important and most restrictive step in biogas generation. Research consistently shows that many substrates experience slowed degradation rates during this phase due to the liberation of problematic byproducts, including inhibitory heterocyclic compounds and excessive volatile fatty acids. (Batstone *et al.*, 2018; Buffière *et al.*, 2018; Gonzalez *et al.*, 2018;

Wang *et al.*, 2018). The acidogenesis stage converts hydrolyzed monomers into various organic acids, while acetogenesis further reduces these to acetic acid and hydrogen. The process culminates in methanogenesis, where these intermediates are biologically transformed into methane and other minor gaseous components as the final output of the anaerobic system. Wang, Shen *et al.*, 2018; Wang, Wang *et al.*, 2018; Wang, Hawkins *et al.*, 2018). Anaerobic digestion performance and stability depend on multiple operational and environmental factors, including temperature fluctuations, pH levels, hydraulic retention time, carbon-nitrogen balance, organic loading rates, and feedstock characteristics. These parameters require careful control as minor deviations can substantially impact both biogas quantity and methane purity. Among these influential factors, trace mineral supplementation plays a particularly crucial role in maintaining microbial metabolic functions and promoting volatile fatty acid synthesis. Research has consistently identified iron, nickel and cobalt as the most biologically significant trace elements for optimizing anaerobic digestion processes, with numerous studies confirming their importance in maintaining system stability and enhancing biogas production (Cai *et al.*, 2018; Zhao, Mu *et al.*, 2018; Zhao, Westerholm *et al.*, 2018; Zhao, Ji *et al.*, 2018).

2.5. FACTORS AFFECTING ANAEROBIC DIGESTION

2.5.1 Temperature

Temperature play a crucial role in determining the microbial activity and the decomposition of organic matter (Angelidaki *et al.*, 2003). Temperature variations in anaerobic digestion (AD) systems can significantly influence methane (CH₄) production by modifying key physical characteristics of the organic substrate, including its viscosity, interfacial properties and mass transfer dynamics (Dev *et al.*, 2019). Variations in these properties may alter the substrate's physical

and chemical nature, potentially impacting microbial uptake and processing during anaerobic digestion, thereby influencing the final methane (CH₄) output (Dev *et al.*, 2019; Adekunle *et al.*, 2015). Microorganisms and enzymes are often grouped by their optimal temperature ranges: thermophiles thrive in hot conditions (46-60°C, optimally around 45°C), mesophiles prefer moderate warmth (20-45°C, ideally 35°C), and psychrophiles function best in cooler environments (10-20°C, typically 20°C) (Dev *et al.*, 2019; Wang *et al.*, 2018). Current scientific literature reveals a strong research bias in freshwater anaerobic digestion (FW-AD) studies, with the majority conducted under mesophilic (20-45°C) and thermophilic (46-60°C) conditions, while psychrophilic operation (10-20°C) remains significantly underrepresented in experimental investigation (Dev *et al.*, 2019). Thermophilic anaerobic digestion systems display significantly higher process instability under variable environmental conditions compared to their mesophilic counterparts, necessitating greater energy expenditure to maintain system equilibrium (kim *et al.*, 2002; Rajagopal *et al.*, 2017).

2.5.2 Hydraulic retention time and solid retention time

Hydraulic Retention Time (HRT) and Solid Retention Time (SRT) serve as fundamental control parameters in anaerobic digestion processes. HRT represents the duration organic feedstock remains in the digester, with shorter periods (under 15 days) potentially causing incomplete digestion while optimal ranges (25-35 days) enhance methane conversion efficiency (Gao *et al.*, 2023). SRT governs microbial population dynamics, where extended retention (30-45 days) promotes methanogen activity and system stability compared to shorter SRTs that risk process acidification (Liu *et al.*, 2022). Modern high-rate configurations including membrane bioreactors enable SRT extension independent of HRT, significantly improving volumetric biogas

productivity (Zhao *et al.*, 2023). Strategic manipulation of these temporal factors can elevate methane content to 65-72% while preventing operational failures.

2.5.3 pH and alkalinity

Anaerobic digestion efficiency is highly dependent on maintaining proper pH and alkalinity levels throughout the process. The ideal pH range of 6.8-7.5 creates optimal conditions for microbial communities, particularly methanogens that are sensitive to acidic environments (Gupta *et al.*, 2024). When pH falls below 6.5, the digestion process becomes unstable as acid-producing bacteria outcompete methane-generating archaea, leading to accumulation of volatile fatty acids (VFAs) (Patel *et al.*, 2023). Conversely, pH levels exceeding 8.0 may cause ammonia toxicity, especially when processing nitrogen-rich feedstocks like poultry manure or food waste.

Alkalinity acts as the system's biochemical buffer, with optimal concentrations between 2,000-5,000mg/L as CaCO₃ needed to maintain stable operations (Sharma *et al.*, 2023). The bicarbonate buffer system (HCO₃⁻/CO₂) plays a particularly vital role in neutralizing acids generated during the initial stages of digestion. Inadequate alkalinity results in rapid pH fluctuations that can collapse the entire process, while excessive alkalinity may indicate accumulation of inhibitory compounds (Meegoda *et al.*, 2024). Proper management of pH and alkalinity can increase methane content in biogas to 65-72% while improving process stability and organic loading process stability (Yadav *et al.*, 2024). Regular monitoring through advanced techniques like Fourier-transform infrared spectroscopy (FTIR) allows for real-time process optimization (Kumar *et al.*, 2023).

2.6. BIOGAS DIGESTERS

According to Uddin and Wright (Uddin and Wright, 2022), “Methanogenic decomposition of organic substrates occurs under anaerobic conditions within a closed reactor system, technically referred to as a biogas digester.” A biogas digester constitutes any engineered apparatus designed to facilitate the anaerobic degradation of organic substrates, thereby yielding methane-rich gaseous fuel through controlled microbial decomposition processes. Variations in biodigester size and form arise from differences in user specifications and the logistical constraints of material sourcing (Abubakar, 2022). Biogas technology has been widely studied for its potential in sustainable waste management and renewable energy production. Three primary digester designs have emerged as the most prevalent systems in both research and practical applications: fixed dome, floating drum and balloon digesters. Each configuration presents distinct structural characteristics and operational parameters that influence their suitability for different implementation scenarios. A fixed-dome anaerobic digester is a sealed, dome-shaped bioreactor designed for biogas production, featuring an integrated static gas storage compartment and a connected effluent displacement chamber to accommodate substrate overflow during the digestion process. The anaerobic digestion system employs a sealed, dome-shaped reactor with a permanently attached gas collection space in its upper section, where methane-rich biogas accumulates during organic matter breakdown (Ioannou *et al.*, 2020). The anaerobic digestion process commences with the transfer of organic slurry into either the compensation tank or primary digester chamber, where subsequent biogas production occurs. Fixed-dome digesters are economical and user-friendly due to their stationary, no-moving-parts construction. The fixed-dome biogas digester was intentionally constructed without any iron components that could corrode, enabling it to remain operational for over 20 years. In most applications, the fixed-dome digester is constructed below the ground surface to

optimize functionality. This implementation ensures structural protection for the digester and economical use of land resources (Zaki *et al.*, 2020). The floating drum biogas digester offers a practical and decentralized approach to treating blackwater in a variety of applications. This biogas system is an excellent option due to its wide range of applications, straightforward maintenance and ability to serve diverse needs- whether for individual homes, public facilities, businesses or industrial operations. The biogas system features a feeding tank for organic waste, an airtight digester for anaerobic decomposition, a gas holder to collect methane-rich biogas, and an outlet for digested slurry-efficiently converting waste to renewable energy. The gas generated during the initial phase of anaerobic digestion is predominantly carbon dioxide (CO₂). Since CO₂ is non-combustible, it poses no risk and can be safely discharged into the atmosphere. During anaerobic digestion, the gas mixture gradually shifts from carbon dioxide to methane. The biogas becomes suitable for fuel use only when methane concentrations exceed 45% - the threshold where flammability becomes sufficient for practical energy applications (Anusuyadevi *et al.*, 2023). The floating drum biogas digester offers a sustainable circular solution by transforming organic waste into both renewable energy and agricultural nutrients. This innovative system provides communities with an affordable, decentralized approach to waste management that simultaneously addresses energy poverty and soil fertility challenges. Its simple floating gas holder mechanism ensures reliable operation while making the technology accessible for rural implementation across varying climatic conditions (Sudiartha *et al.*, 2023). A flexible biogas digester is a eco-friendly energy device that transforms organic waste into cooking fuel using durable, airtight polymer membranes. By harnessing natural microbial breakdown in an oxygen-free environment, this portable system provides a sustainable way to convert everyday waste into usable clean energy (Hasan *et al.*, 2021). One of the main advantages of the balloon digester is its inexpensive,

standardized production, enabling affordable biogas solutions. For developing regions with warm climates and high water tables, this system offers a practical solution through its easily transportable components and minimal excavation requirements, which are necessary for maintaining optimal digestion temperatures (Obileke *et al.*, 2021). The balloon digester stands out as an effective biogas solution because of its simple design, ease of construction, and ability to function well in suitable environments where its potential drawbacks can be properly addressed (Tangwe *et al.*, 2022).

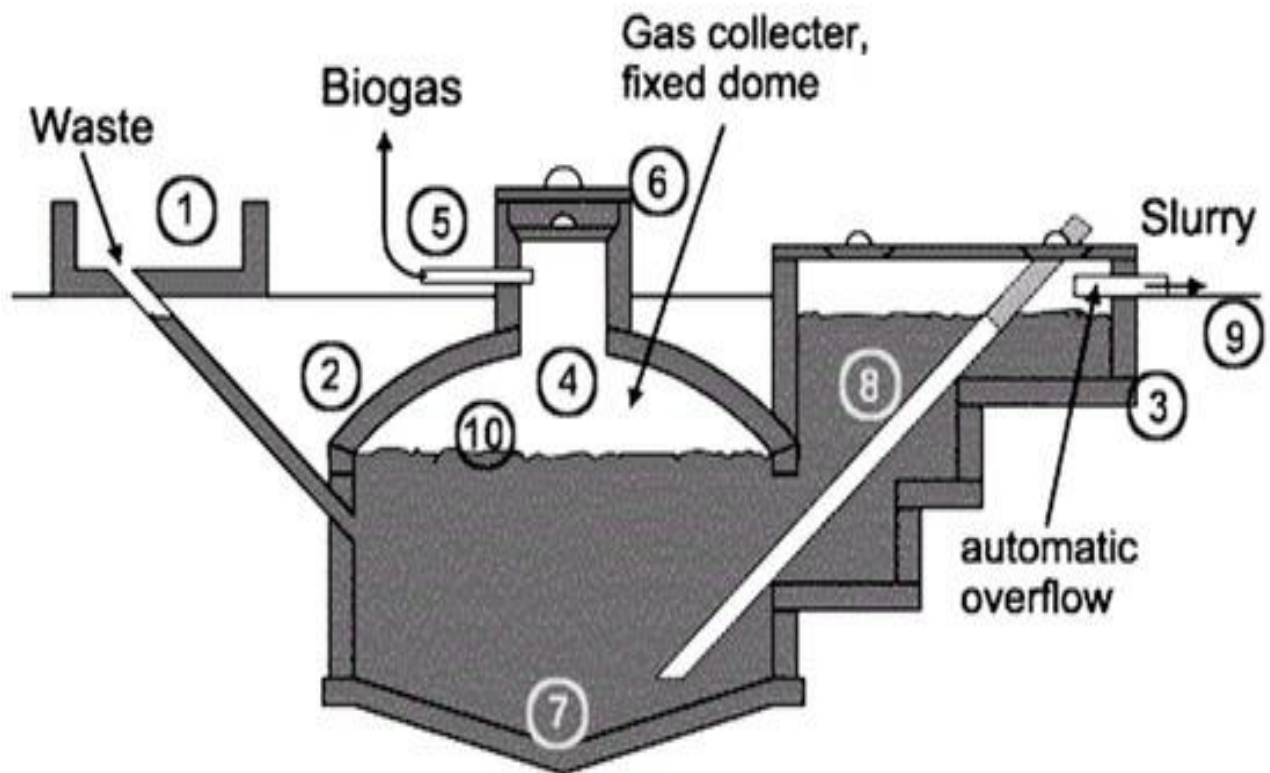


Figure 2.1: Fixed Dome Plant

Source: (Sawyerr *et al.*, 2020)

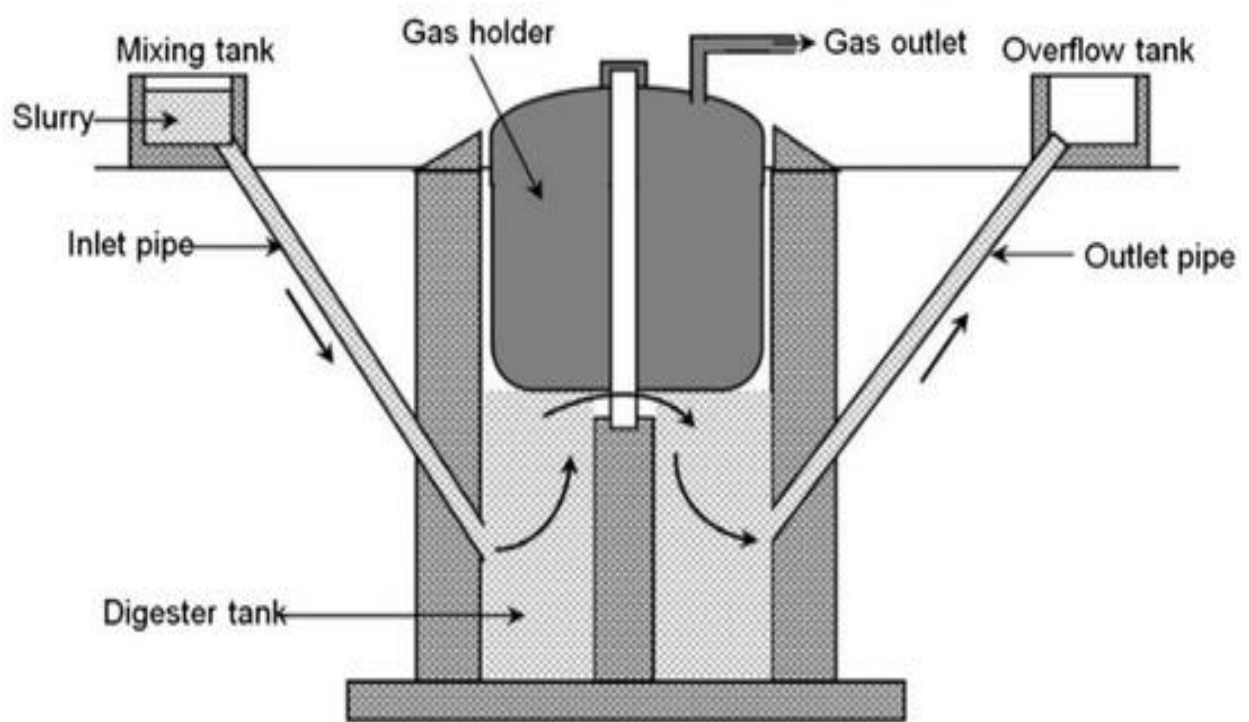


Figure 2.2: Floating Drum Biogas Digester

Source: (Oji *et al.*, 2023)

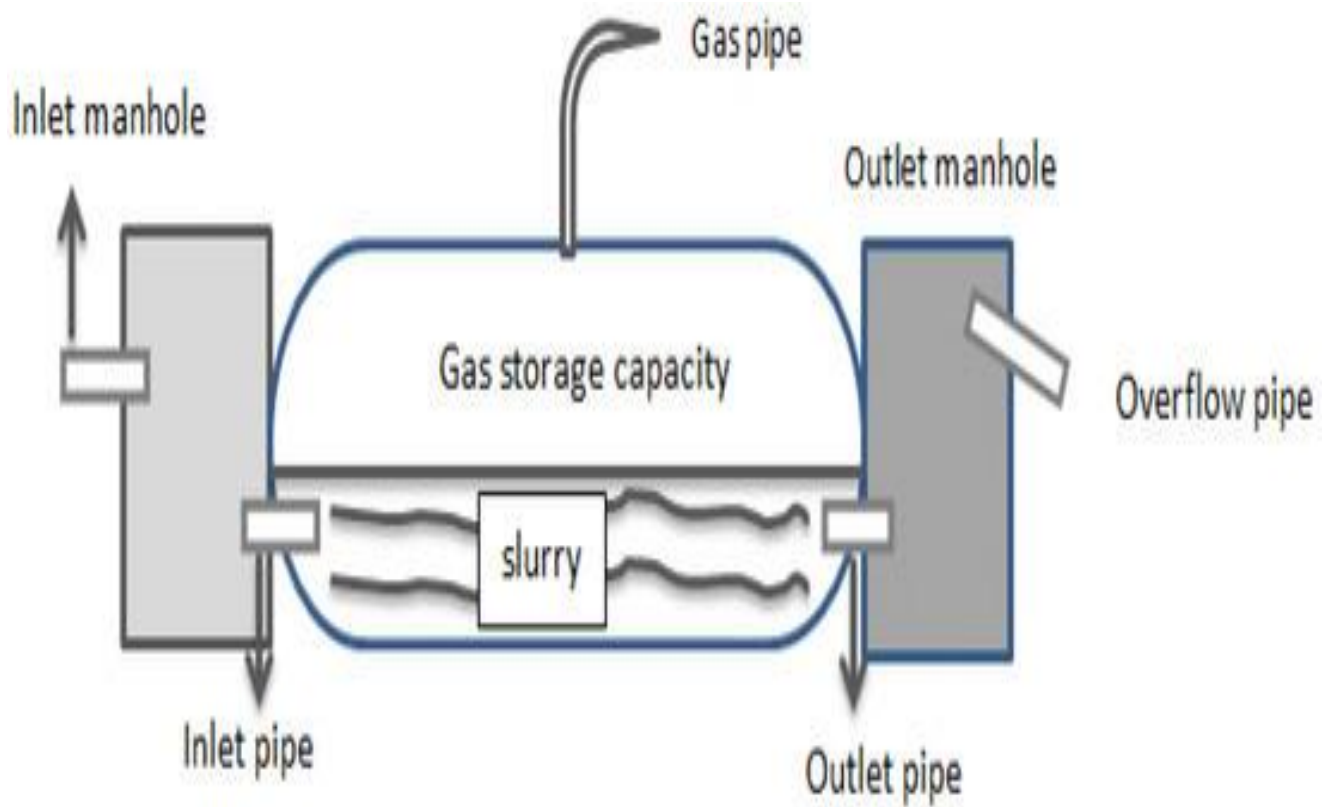


Figure 2.3: Balloon Biogas Digester

Source: (Mutungwazi *et al.*, 2018)

2.7. BIOGAS PROCESSES

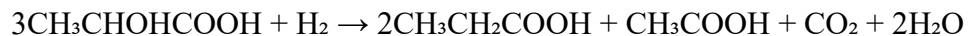
2.7.1 Hydrolysis

The hydrolysis stage marks the first and often rate-determining step in anaerobic digestion. At this stage, fermentative anaerobic bacteria enzymatically decompose high molecular weight organic compounds such as carbohydrates, lipids, proteins, and cellulose into simpler, soluble molecules that can be readily utilized by subsequent microbial communities. Through the secretion of extracellular enzymes, complex macromolecules are broken down: polysaccharides into simple sugars (e.g., glucose), proteins into amino acids, and fats into long-chain fatty acids. These hydrolytic reactions are crucial, as they convert insoluble organic matter into forms accessible to acidogenic and acetogenic microorganisms. The resulting monomers and oligomers are subsequently fermented into volatile fatty acids (VFAs) and other intermediate metabolites, including alcohols, lactic acid, carbon dioxide (CO₂), hydrogen (H₂), and organic acids such as acetic, butyric, and propionic acids. Earlier assumptions posited that methanogenic microorganisms might directly influence the rate of hydrolysis; however, findings by Luo *et al.* (2012) indicated that hydrolysis itself often constitutes the rate-limiting phase of anaerobic digestion. In their study, the degradation of insoluble cellulose was shown to follow first-order kinetics, yielding a rate constant of approximately 1.18 per day under continuous culture conditions with a 1% concentration of soluble reducing sugars. Hydrolysis kinetics vary significantly with substrate composition and environmental conditions. Yu *et al.* (2003) demonstrated that carbohydrates are generally more rapidly hydrolyzed than proteins or fats under anaerobic conditions. In their up-flow reactor system equipped with an agitator and gas-liquid-solid separator acidification efficiencies ranged from 31-65% for carbohydrates, 20-45% for

proteins, and 14-24% for lipids. Corresponding hydrolysis rate constants were 0.02-0.03 day⁻¹ for proteins, 0.08-1.7 day⁻¹ for fats, and 0.04-0.13 day⁻¹ for cellulose.

2.7.2 Acidification

Following hydrolysis, the soluble monomers and intermediate products undergo fermentation during the acidification (or acidogenesis) phase. This stage involves a diverse community of acidogenic bacteria, including *Propionibacterium* species, which convert soluble organic matter into various low molecular weight compounds. Lactic acid-producing bacteria initiate this transformation by breaking down carbohydrates, proteins, and lipids into organic acids, hydrogen sulfide (H₂S), ammonia (NH₃), carbon dioxide (CO₂), and hydrogen gas (H₂). As the process continues, acid-producing bacteria further metabolize these intermediates into volatile fatty acids (VFAs), particularly acetate, butyrate, and propionate, along with gases such as H₂ and CO₂. These compounds serve as essential substrates for acetogenic and methanogenic microorganisms in subsequent stages (Yadav *et al.*, 2020). Propionic acid formation can also occur during the metabolism of long-chain fatty acids especially those containing an odd number of carbon atoms through reactions such as:



These reactions illustrate the fermentative pathways that lead to the generation of propionic and acetic acids, which later serve as precursors for methane production. Acidification plays a pivotal role in regulating the pH and redox balance within the digester, thereby influencing the metabolic activity of subsequent microbial populations.

2.7.3 Acetogenesis

Acetogenesis constitutes the third stage of anaerobic digestion and serves as a vital biochemical link between acidogenesis and methanogenesis. In this stage, *acetogenic* or hydrogen-producing bacteria oxidize intermediate VFAs such as propionic and butyric acids into simpler molecules chiefly acetate, hydrogen, and carbon dioxide. These reactions are thermodynamically unfavorable unless the partial pressure of hydrogen remains low, which is typically maintained through its continuous utilization by hydrogenotrophic methanogens. Pallathadka *et al.* (2022) explain that acetogenic bacteria metabolize higher VFAs into acetate through oxidation reactions that simultaneously release molecular hydrogen as a byproduct. The acetate and hydrogen produced during this phase are critical substrates for methanogenic archaea in the final step of biogas formation. Representative reactions of acetogenesis and their Gibbs free energy changes are summarized in **Table 2.1**, showing that the conversion of butyrate and propionate to acetate is energetically less favorable compared to lactate oxidation.

Table 2.1: Acetogenesis Equation

Equation and Standard Gibbs Free Energy Changes During Acetogenesis of biomass

Reaction	ΔG° (KJ/reaction)
Ethanol + H ₂ O → acetate ⁻ + 2H ₂ + H ⁺	+9.6
Lactate ⁻ + 2H ₂ O → acetate ⁻ + 2H ₂ + H ⁺	+9.6
2H ₂ + HCO ₃ ⁻ + H ⁺	-3.96
Butyrate ⁻ + 2H ₂ O → acetate ⁻ + 2H ₂ + H ⁺	+48.1
Propionate ⁻ + 3H ₂ O → acetate ⁻ + HCO ₃ ⁻ + 3H ₂ + H ⁺	+76.1

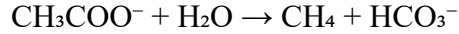
Batstone et al. (2002) identified a special subgroup of acetogenic microorganisms—*H₂*-acetogenic and homoacetogenic bacteria that can synthesize acetate directly from carbon dioxide and hydrogen through the following reaction:



In this biochemical process, hydrogen transfer and consumption play decisive roles in controlling the rate of acetogenesis. Variations in hydrogen concentration affect the metabolic pathways adopted by fermentative bacteria, ultimately determining the proportions of acetate, hydrogen, and other end products generated.

2.7.4. Methanogenesis

Methanogenesis represents the terminal phase of anaerobic digestion and is primarily responsible for methane production. This stage is mediated by methanogenic archaea, which are strictly anaerobic microorganisms capable of utilizing a limited range of substrates mainly acetate, hydrogen, and carbon dioxide. The process converts these substrates into methane (CH₄) and carbon dioxide (CO₂), constituting the principal components of biogas. According to Zhang *et al.* (2021), methanogens exhibit extreme sensitivity to oxygen exposure, reflecting their obligate anaerobic physiology. Methanogenesis typically requires a higher pH than preceding stages and occurs under highly reducing (low redox potential) conditions, which are often difficult to reproduce under laboratory cultivation (Florencio *et al.*, 2016). Two major pathways dominate this phase: acetoclastic methanogenesis, where acetate is split into methane and bicarbonate, and hydrogenotrophic methanogenesis, where carbon dioxide is reduced by hydrogen. These can be represented by the following equations:



Additionally, formate (HCO_2^-) can serve as a substrate for methane production through:

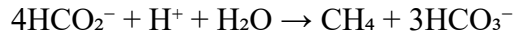


Table 2.2: Methanogenesis Equation

Equation and Standard Gibbs Free Energy Changes During Methanogenesis of biomass	
Reaction	ΔG° (KJ/reaction)
$\text{CH}_3\text{COO}^- + \text{H}_2\text{O} \rightarrow \text{CH}_4 + \text{HCO}_3^-$	-31.0
$4\text{H}_2 + \text{HCO}_3^- + \text{H}^+ \rightarrow \text{CH}_4 + 3\text{H}_2\text{O}$	-135.6
$4\text{HCO}_2^- + \text{H}^+ + \text{H}_2\text{O} \rightarrow \text{CH}_4 + 3\text{HCO}_3^-$	-130.4

The standard Gibbs free energy changes for these reactions, summarized in **Table 2.2**, indicate that methanogenesis is energetically favorable under anaerobic conditions. The efficiency of methanogenesis and overall biogas yield depends on the synergistic interactions among microbial consortia in preceding stages, the maintenance of optimal pH (6.8-7.5), temperature, and the balance between hydrogen-producing and hydrogen-consuming microorganisms.

2.8. BIOGAS PRODUCTION FROM CATTLE RUMEN CONTENT

Greenhouse gases such as carbon dioxide, methane, nitrous oxide, and ozone are key contributors to global warming and climate change due to their capacity to absorb infrared radiation within the atmosphere. Methane is categorized as a trace gas, with an estimated global atmospheric

concentration of 1774 ± 1.8 parts per billion (ppb) and a recorded increase of about 11 ppb since 1998. It is a particularly potent greenhouse gas, exhibiting a global warming potential approximately 25 times greater than that of carbon dioxide and an atmospheric lifetime of about 12 years, making it the second most significant anthropogenic greenhouse gas after carbon dioxide. In addition, methane enhances ozone formation in the troposphere and elevates stratospheric water vapor levels, thereby increasing its overall radiative forcing by nearly 70% (Shinkai *et al.*, 2024). Globally, between 50% and 60% of methane emissions originate from agricultural activities, particularly livestock production systems, with ruminant animals serving as the principal source. Domesticated ruminants including cattle, sheep, and goats collectively emit approximately 86 million metric tons (Tg) of methane annually. Of this total, dairy cattle account for roughly 18.9 Tg, beef cattle 55.9 Tg, and sheep and goats 9.5 Tg. Global methane emissions from buffalo range between 6.2 and 8.1 Tg per year, while camels contribute about 0.9–1.1 Tg. Methane generated in the hindgut of pigs and horses is estimated at approximately 0.9–1.0 Tg and 1.7 Tg, respectively (Afazeli *et al.*, 2014).

Methane production in ruminants occurs primarily in the rumen as a by-product of normal feed fermentation. Although some production also takes place in the lower gastrointestinal tract, approximately 89% of the methane emitted by ruminants originates from the rumen and is expelled through exhalation via the mouth and nostrils. The release of methane represents a direct loss of feed-derived energy, typically between 2% and 12% depending on dietary composition. The proportion of energy lost as methane varies among ruminant species. For example, estimated losses in dairy cattle, range cattle, and feedlot cattle are approximately 5.5–9.0%, 6.0–7.5%, and 3.5–6.5% of gross dietary energy, respectively. In buffalo and camels, the corresponding losses are between 7.5–9.0% and 7.0–9.0%. These variations are influenced by several factors including

geographical location, feed quality, feed intake, nutrient composition, and the degree of feed processing (Hook *et al.*, 2010). Methanogens are members of the domain Archaea and the phylum Euryarchaeota. Unlike bacteria, methanogens lack peptidoglycan in their cell walls; instead, they possess structural substitutes such as pseudomurein in *Methanobrevibacter* and *Methanobacterium*, heteropolysaccharides in *Methanosarcina*, and proteins in *Methanomicrobium*. All methanogens contain coenzyme F₄₂₀, a vital cofactor for enzymes including hydrogenase and formate dehydrogenase. The coenzyme is named for its 420 nm absorbance peak, which causes it to emit blue-green fluorescence at 470 nm. Another defining cofactor of methanogens is coenzyme M, or 2-mercaptoethanesulfonic acid, which can either be synthesized internally by species such as *Methanobacterium* or acquired from external sources, as in *Methanobrevibacter ruminantium*. This coenzyme is methylated during methanogenesis to form methane (Shinkai *et al.*, 2024). The morphological and physiological traits of methanogens differ across species. *Methanobrevibacter ruminantium* is rod-shaped, variably motile, and utilizes hydrogen, carbon dioxide, and formate as substrates for methane synthesis. *Methanobacterium formicicum*, which belongs to the same order (*Methanobacteriales*), can appear rod-like or filamentous, lacks motility, and utilizes similar substrates. *Methanomicrobium mobile* is motile and rod-shaped, producing methane through the use of hydrogen, carbon dioxide, and formate. In contrast, *Methanosarcina barkeri* and *Methanosarcina mazeii* are coccoid in shape and non-motile (Shinkai *et al.*, 2024). In the bovine rumen, the dominant methanogens primarily utilize hydrogen and carbon dioxide to produce methane. However, certain members of the genus *Methanosarcina* occupy a distinct ecological niche by metabolizing methanol and methylamines instead of hydrogen and carbon dioxide, as they grow more slowly on the latter substrates (Hook *et al.*, 2010). Formate, a by-product of acetate formation, can also serve as a substrate for methanogenesis,

though it is often rapidly converted to hydrogen and carbon dioxide. Volatile fatty acids (VFAs) are rarely used as methanogenic substrates because their conversion to carbon dioxide and hydrogen is slow and inhibited by rumen turnover. Instead, methanogenesis primarily relies on hydrogen and carbon dioxide produced during carbohydrate fermentation, which occurs concurrently with VFA formation. By removing hydrogen from the rumen as the terminal step of carbohydrate fermentation, methanogens facilitate optimal microbial fermentation processes, allowing complete oxidation of substrates. The accumulation of hydrogen can suppress microbial metabolism, underscoring the critical role of methanogens in maintaining rumen fermentation balance (Afazeli *et al.*, 2014).

2.9. BIOGAS PRODUCTION FROM WATER HYACINTH

Biogas represents an environmentally friendly and sustainable energy source capable of improving ecological balance by reducing the exploitation of forest resources for fuel. It can serve as an efficient substitute for conventional energy sources such as firewood, offering applications in cooking and electricity generation. The digestate or residue derived from biogas production is rich in nutrients, trace minerals, amino acids, vitamins, and organic matter, making it a valuable fertilizer that provides both immediate and slow-release nutrient benefits (Su *et al.*, 2018). Water hyacinth, with an approximate carbon-to-nitrogen (C/N) ratio of 20:1, contains nutrients that microorganisms can effectively utilize under anaerobic conditions to produce methane. According to Su *et al.* (2018), water hyacinth and similar aquatic macrophytes are readily degradable under such conditions. The anaerobic breakdown of this plant produces considerable amounts of biogas, thus addressing the problem of its overgrowth while simultaneously converting waste into a renewable energy resource. Shredding or comminuting water hyacinth before digestion increases the surface area for microbial activity, enhancing fermentation efficiency. A particle size of about

6.04 mm yielded higher methane and total gas production than finer (1.6 mm) or coarser (12.7 mm) particles. Because water hyacinth has high levels of cellulose and hemicellulose, its digestion by microbes is often limited. Thermochemical pretreatment significantly improves gas yields and supplementing the digestion process with metal ions such as Fe^{3+} and Zn^{2+} enhances methane concentration and overall gas output (Adelodun *et al.*, 2023). Similarly, Su *et al.* (2018) found that adding nickel or cow dung boosts methane yield. Bote *et al.* (2020) compared single-substrate fermentation using water hyacinth to a 7:3 mixture of water hyacinth and cow dung, observing degradation efficiencies of 38% and 43%, respectively. The gases generated during anaerobic fermentation primarily consist of methane, carbon dioxide, and ammonia, with minor quantities of hydrogen sulfide. Methane typically constitutes about 60% of the gas composition, influenced largely by substrate characteristics, temperature, pH, and pressure. Although water hyacinth has high potential for biogas production, its lignin content can impede total gas yield (Su *et al.*, 2018). The fermentation of agricultural residues such as rice straw, corn stover, cotton stalks, and water hyacinth mixed with cow dung, reveals that water hyacinth blends produced higher biogas volumes per unit of volatile solids than the other residues. Dwivedi and Dwivedi (2018) reported an average gas yield of 100 L/kg of fresh water hyacinth during anaerobic digestion, which increased to 134 L/kg when the biomass was pretreated with dilute acid. Combining animal waste with water hyacinth in a two-stage process further enhanced gas production to about 134 L/kg. The residual slurry from anaerobic digestion is nutrient-rich and contains substantial organic matter. According to Su *et al.* (2018), most nitrogen in the residue exists in organic form, with ammonia nitrogen representing 20–50% and smaller amounts appearing as nitrate nitrogen. Hons *et al.* This residue dehydrates easily, and commercial fertilizer forms are typically in dry form. When compared with traditional farmyard manure, biogas residue can improve crop yields by approximately 30%.

However, improper handling of biogas residue such as surface application or inadequate storage can result in significant nitrogen loss due to ammonia volatilization. The extent of loss depends on residue composition, method of application, and soil characteristics. Nitrogen volatilization tends to rise with increasing pH and ammonia nitrogen concentration. In warmer regions, surface application may cause nitrogen losses as high as 70–80%, making deep soil incorporation essential. When the C/N ratio of biogas residue exceeds 20, the risk of nitrogen volatilization becomes considerably higher (Harun *et al.*, 2021).

2.10. ENVIRONMENTAL IMPACTS OF BIOGAS

The contribution of biogas plants to global warming varies significantly depending on the scale, feedstock type, and management practices, and therefore must be evaluated individually. Bachmaier *et al.* (2010) conducted a comparative analysis of ten agricultural biogas facilities and quantified their greenhouse gas (GHG) emissions. Their results showed that GHG emissions from electricity generation in these plants ranged between –85 and 251 gCO₂-eq per kWh of electricity produced, corresponding to a fossil energy saving of approximately 2.31–3.16 kWh of fossil fuel per kWh of biogas-derived electricity. The study also emphasized that reliable estimates of GHG emissions from biogas-based electricity can only be obtained through plant-specific monitoring. Factors such as direct methane leakage reduction, efficient heat recovery from cogeneration systems, feedstock composition, nitrous oxide emissions from energy crop cultivation, and digestate handling all influence the final GHG balance.

In a related study, Battini *et al.* (2014) evaluated an intensive dairy farm in Italy's Po Valley and found that anaerobic digestion reduced overall GHG emissions by 23.7–36.5%, depending largely on digestate management practices. Similarly, Kaparaju and Rintala (2011) reported annual

reductions of 177.0, 87.7, and 125.6 metric tons of CO₂-equivalent emissions for dairy cow, sow, and pig farms, respectively, in a Finnish case study. Process optimization was identified as a major determinant of environmental performance; for instance, improvements in wastewater treatment operations resulted in daily emission abatements of 1,103 kg CO₂-eq for N₂O, 256 kg CO₂-eq for CO₂, and 87 kg CO₂-eq for CH₄ (Mikosz, 2016).

2.10.1. Carbon Dioxide Emissions

During biogas production and utilization, various harmful gases and pollutants are introduced into the environment through both combustion and diffusion processes. When biogas is combusted, methane is oxidized efficiently to carbon dioxide (CO₂), typically yielding about 83.6 kg of CO₂ per gigajoule of energy for a biogas mixture containing 65% CH₄ and 35% CO₂ (Nielsen *et al.*, 2014). Additional CO₂ emissions arise from biomass transportation, storage, and digestate management. Nevertheless, CO₂ generated during these processes is considered biogenic and thus climate-neutral because it originates from recently fixed atmospheric carbon. When fossil fuel displacement is taken into account, biogas utilization contributes substantially to reducing anthropogenic greenhouse gas accumulation in the atmosphere, as demonstrated by Poeschl *et al.* (2012).

2.10.2. Methane Emissions

Although methane emissions from biogas systems are not considered directly harmful to human health, exposure to hydrocarbon mixtures can have certain adverse physiological effects. Methane itself is chemically inert in biological systems and exhibits negligible toxicological interactions (Prasad *et al.*, 2011). However, its environmental relevance stems from its strong greenhouse

potential. Methane is estimated to have 28–36 times greater global warming potential than carbon dioxide over a 100-year period (IPCC, 2013). Consequently, methane control is critical in assessing the overall climate impact of biogas technologies. Methane can escape during incomplete combustion, but the main sources are diffusive losses during biomass storage and digestate management. Improved storage design and post-treatment of digestate are therefore essential for emission reduction. Poeschl *et al.* (2012) also analyzed methane releases across several biogas plants and found that emission rates were consistently below 5 g per kilogram of input material. In the context of cattle manure, digestate stabilization and proper field application can significantly mitigate methane emissions, which would otherwise be substantial if the manure were applied untreated.

2.10.3. Nitrous Oxide Emissions

In addition to CO₂ and CH₄, nitrous oxide (N₂O) is another potent greenhouse gas associated with anaerobic digestion. Due to its high global warming potential, even minor N₂O emissions can contribute substantially to the total GHG footprint of a biogas plant (Carter *et al.*, 2012; Senbayram *et al.*, 2014). The influence of N₂O depends on the climate metric used; for example, when expressed as Global Temperature change Potential over 100 years (GTP-100), N₂O may exhibit a greater relative impact than either CO₂ or CH₄ (Jordan *et al.*, 2016). Total GHG emissions from biogas-based electricity generation typically fall within 0.10–0.40 kg CO₂-eq per kWh, representing a 22–75% reduction compared to Germany's conventional energy mix (Meyer-Aurich *et al.*, 2012). Nonetheless, variations in N₂O release rates contribute to the uncertainty surrounding the overall mitigation potential of biogas systems.

2.11. GASEOUS POLLUTANTS FROM BIOGAS COMBUSTION

While biogas utilization yields clear GHG reduction benefits, combustion processes also generate atmospheric pollutants that must be carefully managed to maintain environmental and public health standards. Carbon monoxide (CO), for example, is a common by-product of incomplete oxidation of carbonaceous materials. Emission rates have been measured at 0.74 g and 8.46 g CO per Nm³ CH₄ for flaring and combined heat and power (CHP) systems, respectively. Depending on plant efficiency, CO emissions associated with energy generation range between 80 and 265 mg CO per megajoule of output energy (Paolini *et al.*, 2018).

Sulfur dioxide (SO₂) emissions from biogas plants primarily depend on the extent of gas desulfurization prior to combustion. The estimated SO₂ release from CHP units ranges between 19.2 and 25 mg MJ⁻¹ (Nielsen *et al.*, 2014), whereas the UK National Society for Clean Air (NSCA, 2002) reports values of 80 g SO₂ per ton of waste for flaring and 100 g SO₂ per ton for CHP applications. Elevated SO₂ concentrations near biogas facilities may result from several sources, including direct combustion, oxidation of hydrogen sulfide emissions, and vehicular exhausts from transport operations (Petracchini *et al.*, 2017). Nitrogen oxides (NO_x) are also critical pollutants associated with biogas combustion and represent a significant environmental concern (Beylot *et al.*, 2015). paolini *et al.* (2018) reported that average NO_x emission factors for biogas engines were approximately 540 g GJ⁻¹, more than three times higher than those from natural gas systems. Expressed relative to methane consumption, the emission factors were 0.63 g NO_x per Nm³ CH₄ for flaring and 11.6 g NO_x per Nm³ CH₄ for CHP units. The importance of NO_x control is supported by several studies; for instance, Battini *et al.* (2014) observed modest increases in acidification potential (5.5–6.1%), particulate emissions (0.7–1.4%), and eutrophication (0.8%) but a marked rise in photochemical ozone formation potential (41.6–42.3%) in their Italian case

study. Similarly, Carreras-Sospedra *et al.* (2016) predicted up to a 10% rise in NO_x emissions in California by 2020 when considering both biogas and biomass combustion. Although reductions in methane storage emissions and electricity substitution credits partially offset these increases, they were insufficient to fully balance the rise in NO_x levels. Biogas typically contains higher concentrations of volatile organic compounds (VOCs) than natural gas. Reported VOC concentrations range between 5 and 500 mg per Nm³, with peak values up to 1,700 mg per Nm³ in certain cases (Salazar Gomez *et al.*, 2016). Studies generally focus on non-methane VOCs (NMVOCs), as these compounds contribute more significantly to air quality concerns. Assuming a 99% reduction efficiency through combustion, VOC emissions from biogas systems are generally lower than those from liquid or solid biofuels. However, formaldehyde remains a notable exception. Research conducted on anaerobic waste treatment facilities in Barcelona identified VOC emission factors between $0.9 \pm 0.3 \text{ g s}^{-1}$, representing 0.3–0.9% of total VOCs in the area, while formaldehyde emissions ranged from 0.2 to 3.0 mg s⁻¹, accounting for about 2% of the total (Gallego *et al.*, 2016).

CHAPTER THREE

MATERIALS AND METHOD

3.1 MATERIAL

Table 3.1: List of materials

Materials	Sources	Uses
Water Hyacinth	Ikpoba hill river , Benin City , Edo state	Used as a raw material in biogas production and for waste water treatment.
Cattle Rumen Content	Afro Abbatoir , Ewa market , Aduwawa , Benin city , Edo state	Used as a feedstock for biogas production and as an organic fertilizer.
Sodium Hydroxide (NaOH)	Department of Science Laboratory Technology, University of Benin	Added in bioreactors or fermentation processes to promote growth and increase microbial activity.
Distilled water	Department of Science Laboratory Technology , University of Benin	Used to prepare or dilute solutions , guaranteeing that pollutants and impurities are eliminated.
Phenolphthalein	Department of Science Laboratory Technology, University of Benin	Serves as a visual indicator , demonstrating a shift in color from colorless to light pink at the saturation point of sodium hydroxide.
Standard Potassium Dichromate Solution	Department of Science Laboratory Technology, University of Benin	It functions as an oxidizing agent, reacting with organic matter to ascertain the oxygen equivalent needed to oxidize it.
Ferrouin Indication Solution	Department of Science Laboratory Technology, University of Benin	It is used to know the end point of titration.

Standard Ferrous Ammonium Sulphate	Department of Science Laboratory Technology, University of Benin	It is used to determine how much dichromate remained unreacted after oxidizing the organic matter in the sample.
Blank Solution (Deionized water)	Department of Science Laboratory Technology , University of Benin	It is used as a reference or control test.
Mercury Sulphate	Department of Science Laboratory Technology , University of Benin	It eliminates interference from chloride ions in the sample.

TABLE 3.2: List of Apparatus

Apparatus	Uses
Batch Digester	Used for anaerobic digestion of organic materials to produce biogas.
Mixing Equipment	Ensures uniform blending of materials in chemical and biological processes.
Retort Stand	Used for setting up the experiment
Pipette	Used for titrimetric purposes
Burettes	Used for titrimetric purposes
pH Meter	Measures the acidity or alkalinity of a solution.
Weighing Balance	Used for measuring the mass of water hyacinth and cattle rumen content.
Hose	For transporting the biogas from one point to another.

Total dissolved Solid Meter	Used to determine the total dissolved solids in the water hyacinth and cattle rumen content.
Muffle Furnace	Used to determine the ash content of water hyacinth and cattle rumen content.
Oven	Used to determine the moisture content of cattle rumen content and water hyacinth.
Personal Protective Equipment (PPE)	Protect individuals from hazards in the workplace or laboratory.

3.2 METHOD

3.2.1 Sample Collection

Cattle rumen content for this study was collected from Afro abattoir located at Ikpoba Hill, while fresh Water hyacinth growing on Ikpoba Hill river was collected. The cattle rumen content and water hyacinth was used freshly for the experiment.

3.2.2 Experimental Setup

The experimental setup followed the batch digestion method, making use of 5kg gas cylinder. The gas collector, reactor, and water-sealed tank were constructed from aluminum to prevent rusting, with all edges and corners sealed with glue to ensure no leakage. A calibrated ruler measured the dimension of the gas collector, facilitating accurate volume calculations for the biogas produced. A rubber pipe connected the gas collector to a cork in each digester, enabling efficient gas collection. The digester was designed to hold 5 kg but was filled with 4 kg to allow space for gas production.



Plate 3.1: Experimental Set up for the Anaerobic Digestion of Cold Digestion of Cattle Rumen Content and Water Hyacinth using Water Displacement Method

3.3 ANALYSIS OF FEEDSTOCK

3.3.1. Determination of ash content

A crucible was weighed and preheated before a measured amount of sample was added. The crucible was placed in a muffle furnace at a temperature of 550°C for a set duration. After cooling, the crucible and residue was weighed.

3.3.2. Determination of moisture content

The moisture content analysis quantifies the water content in water hyacinth and cattle rumen content, which is crucial for optimizing microbial activity during biogas production. The dry weight of the crucible was taken and known weight of the sample was added. The crucible was put into the oven at a temperature of 150°C and left for an hour to dry, the result was recorded. This process was carried out several times until a constant value was gotten.

3.3.3. Measurement of pH

The pH determines the acidity or basicity of a solution. The pH of the slurry was determined using a pH meter. Methanogens typically thrive within a pH range of 6.5-7.5 (Suyog, 2016).

3.3.4. Determination of nitrogen-free extract (carbohydrate)

To determine the nitrogen-free extract (carbohydrate content), the percentages of moisture, fat, protein, fiber and ash were subtracted from 100% (Suyog, 2010).

3.3.5. Biochemical oxygen demand (BOD)

The microbial digestibility for biogas production is reflected in the BOD analysis, which assesses the oxygen demand by microorganisms to break down biodegradable organic matter in the water

hyacinth and cattle rumen content over a period for five days. After preparing a diluted sample of water hyacinth and cattle rumen, the initial dissolved oxygen(DO) was determined using a DO meter or the Winkler titration method. After the sample was incubated for five days at 20°C in the dark, the final DO was noted.

$$\text{BOD}_5 \text{ (mg/L)} = \text{DO}_{\text{initial}} - \text{DO}_{\text{final}} \text{ (Metcalf and Eddy, 2014).}$$

3.3.6. Determination of crude fat

Miah *et al.* (2016), described the Soxhlet extraction method, which was used to determine the sample's fat content. The solvent was used to extract the sample in a thimble, and the residue was weighed to determine the fat content after the solvent was evaporated.

3.3.7. Determination of crude fibre content

In order to determine the crude fibre content, the sample was treated with sulfuric acid and sodium hydroxide before being burned in a muffle furnace (Suyog, 2016). To determine fiber, the resultant ash was weighed and calculated.

3.3.8. Chemical oxygen demand (COD)

The COD analyses provide an estimate of the possible biogas yield by measuring the total amount of oxygen needed to chemically oxidize the organic matter in water hyacinth and cattle rumen content. In the presence of sulfuric acid, silver sulfate, mercuric sulfate and potassium dichromate, a filtered water hyacinth and cattle rumen sample was heated in a reflux apparatus for two hours at 150°C. The amount of oxidized organic matter was measured by titrating the unreacted dichromate with ferrous ammonium sulfate after it has been cooled. The COD can be calculated using this formula:

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

where A and B are the titrant volumes used for the blank and sample, respectively, C is the normality of ferrous ammonium sulphate.

CHAPTER FOUR

RESULTS

The results for the composition analysis of the cold digestion of cattle rumen content and water hyacinth are listed below.

Table 4.1: Results of the composition analysis of the cattle rumen content and water hyacinth sample.

Composition (%) Analysis	
Nitrogen content	1.07
Moisture content	80.25
Fiber content	4.7
Fat content	1.2
COD	69
Carbohydrate content	4
Ash content	18.94
BOD	35

Table 4.2: Daily yield of biogas production (ml)

Day	pH	Biogas produced(ml)
1.	6.13	-
2.	6.17	-
3.	6.20	-
4.	6.25	-
5.	6.29	Bubbling
6.	6.33	Bubbling
7.	6.38	Bubbling
8.	6.47	Bubbling
9.	6.52	Bubbling
10.	6.61	-
11.	6.66	-
12.	6.73	300
13.	6.79	220
14.	6.83	380
15.	6.88	520
16.	6.9	640
17.	7.07	-
18.	7.12	-
19.	7.19	830
20.	7.26	770
21.	7.35	850
22.	7.41	1210

23.	7.56	1270
24.	7.68	650
25.	7.75	-
26.	7.87	500
27.	7.96	520
28.	8.23	1200
29.	8.34	1670
30.	8.46	2090

A graph of pH of Cattle Rumen Content and Water Hyacinth against Retention Time

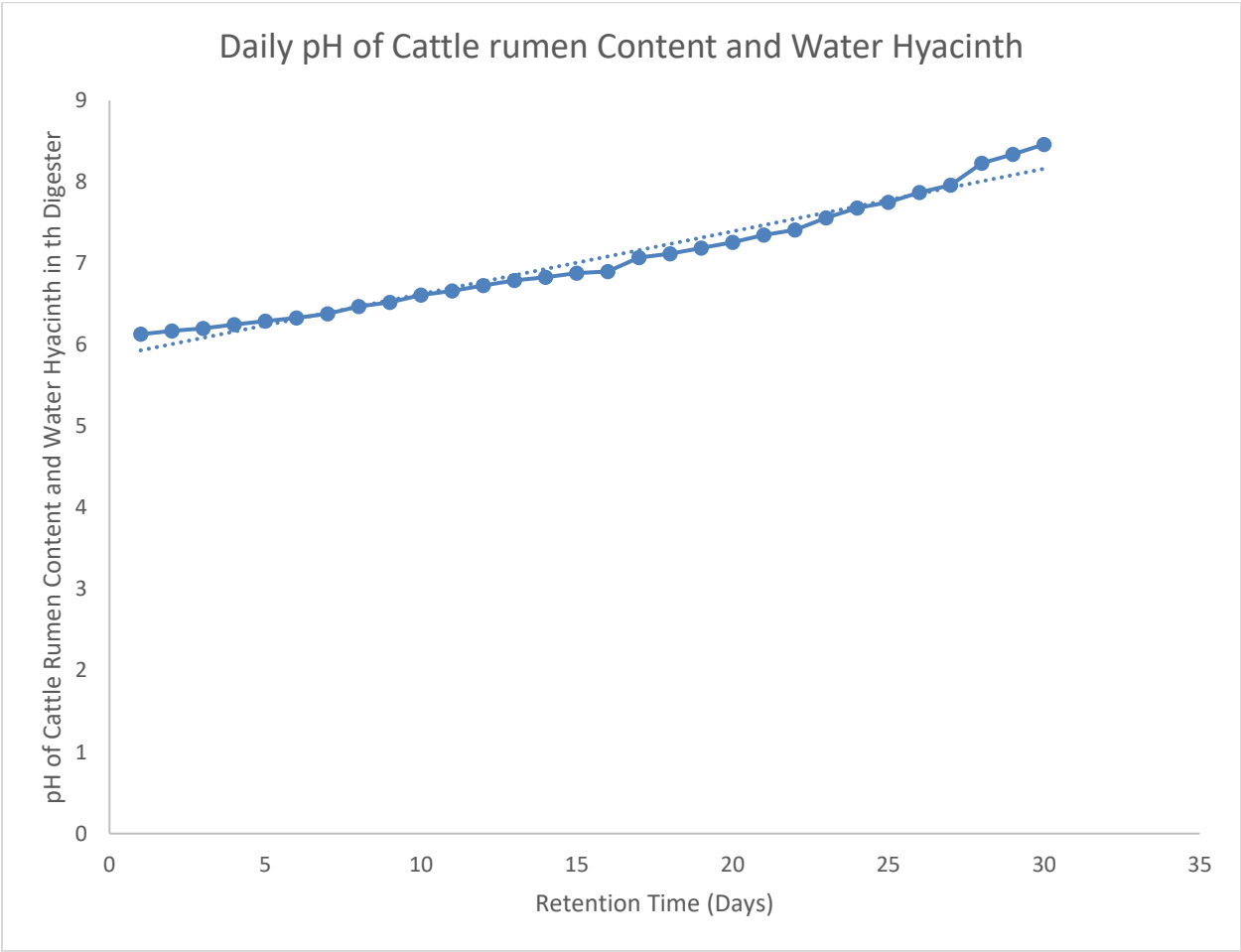


Figure 4.1: Daily pH of Cattle Rumen content and Water Hyacinth in the Digester

A graph of Volume of Biogas produced against Retention Time

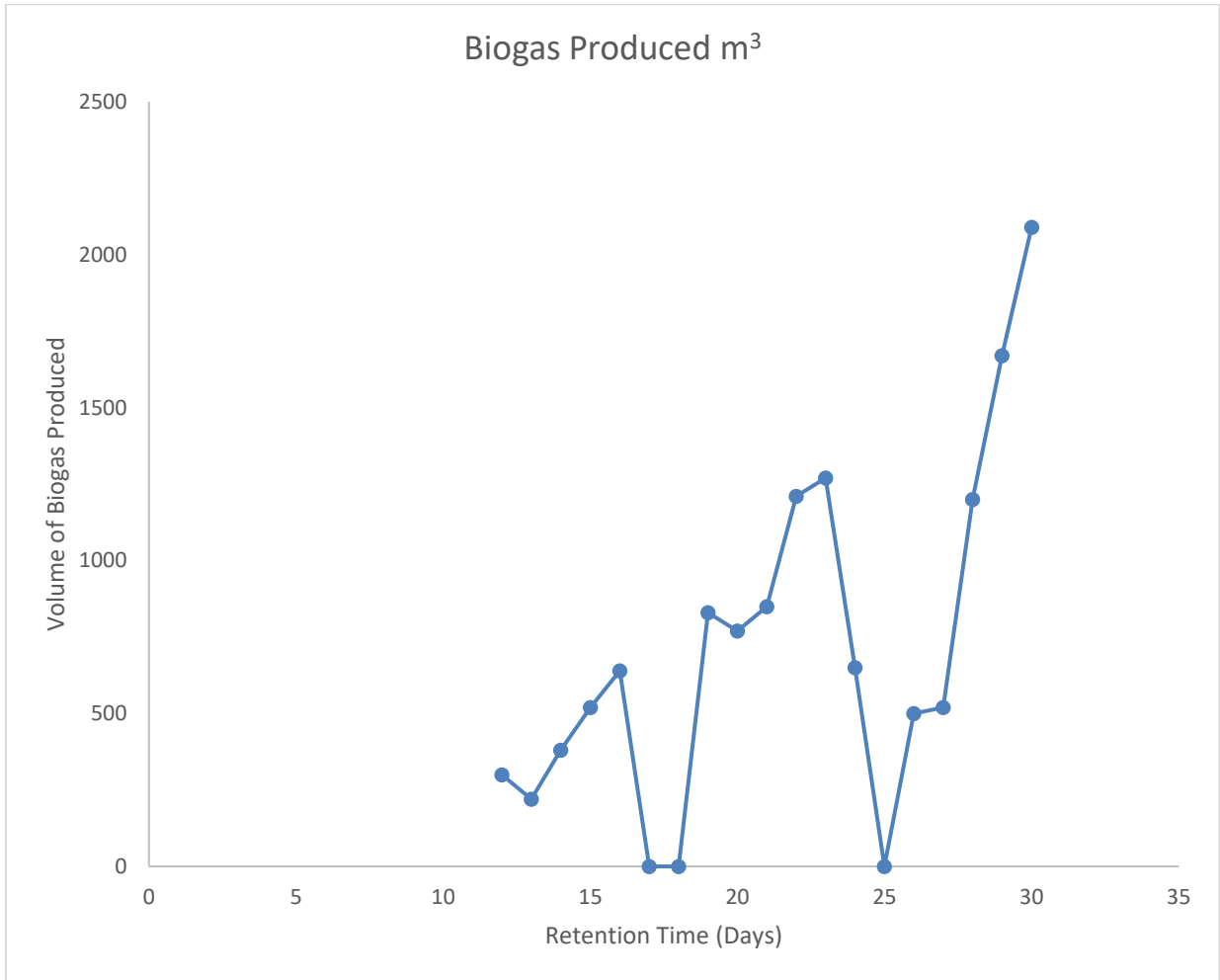


Figure 4.2: Daily volume of biogas produced

CHAPTER FIVE

DISCUSSION OF RESULTS

The Study revealed that it is possible to produce biogas from a mixture of water hyacinth and cattle rumen content. The 30-day retention period's biogas production profile distinctly illustrates the anaerobic digestion process's successive phases. The first 11 days were marked by a lag phase, with no detectable biogas collection, even though bubbling was seen on day five. The complex organic polymers, especially the lignocellulosic structure of water hyacinth, are broken down into simpler sugars and then volatile fatty acids (VFAs) during this time, which is typical of the hydrolysis and acidogenesis stages.

The pH environment was acidic during this phase, ranging from 6.13 to 6.66, and this was directly caused by the production of these VFAs. The observed bubbling was probably not significant methane because the low pH inhibits the methanogenic archaea that produce methane, but rather carbon dioxide (CO₂), a direct product of acidogenic activity (Khalid *et al.*, 2019). The time needed for the microbial community to establish itself and for the required metabolic intermediates to accumulate before methanogenesis can start is highlighted by this first stage.

On day 12, measurable biogas production began at a volume of 300 ml, signaling a significant metabolic shift. When the pH hit 6.73, it entered the lower threshold of the ideal range (6.5–7.5) for methanogens, which is when the gas production started. The pH rises further at this point because acetogenic bacteria break down the accumulated VFAs and produce hydrogen and acetate. The biogas yield then increased steadily, reaching a peak of 1270 ml on day 23. This demonstrated the methanogenic population's established activity and exponential growth. The

information shows a strong relationship between a steady rise in pH and higher biogas production, highlighting how the methanogenic stage depends on the earlier acid-forming stages being successfully completed (Matheri *et al.*, 2018). One of the most significant results of the experiment was the significant increase in biogas production in the final days, with yields reaching 1670 ml on day 29 and 2090 ml on day 30 respectively. This indicates that the methanogenic phase of the digestion process was still active at the 30-day mark

The mixture has a high moisture content (80.25%), according to the compositional analysis, which supports the microbial activity needed for a wet digestion process. Additionally, the high Chemical Oxygen Demand (COD) of 69 g/L indicates the significant organic load. A balanced carbon-to-nitrogen (C/N) ratio avoids ammonia inhibition from the nitrogen-rich rumen content while guaranteeing enough carbon for energy from the water hyacinth. This combination most likely produced a C/N ratio within the ideal range of 20–30, which is crucial for preserving microbial equilibrium (Awe *et al.*, 2017).

CONCLUSION

During the course of this research work, the production of biogas from cold digestion of cattle rumen content and water hyacinth was investigated. The following conclusions can be drawn from the research work.

1. The substrate composition such as, moisture content, ash content, fiber content, carbohydrate, fat content shows the suitability of the cattle rumen and water hyacinth.
2. Optimum pH for maximizing methane production during anaerobic co-digestion of cattle rumen content and water hyacinth was found to be 8.46.
3. Combining both substrates at ratio 2:1 gave a better biogas yield.

RECOMMENDATIONS

- The research work presented above can be greatly improved upon by using buffering strategies (e.g sodium bicarbonate or calcium carbonate) or co-digest.
- More than two or more substrates should be further studied or investigated.
- Additional studies should examine how temperature, pH buffering and nutrient supplementation affect biogas yield in order to improve process stability and efficiency.

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