UNIVERSITY OF CAPE COAST

ASSESSMENT OF PERFORMANCE OF MESOPHILIC SINGLE-STAGE BIOGAS DIGESTER FOR TREATING AGRICULTURAL WASTES

BY

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Thesis submitted to the Department of Environmental Science of the School of Biological Sciences, College of Agriculture and Natural Sciences, University of Cape Coast, in partial fulfilment of the requirements for the award of Master of Philosophy degree in Environmental Science

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DECLARATION

Candidate's Declaration

I hereby declare that except for reference to authors who have been acknowledged, this thesis is the result of my own original research under supervision and that no part of it has been presented for another degree in this university or elsewhere.

Candidate's Signature: .... Date: ....

Name: …………………………………………………………..

Supervisor's Declaration

I hereby declare that preparation and presentation of this thesis were supervised in accordance with the guidelines on supervision of thesis laid down by the University of Cape Coast.

Principal Supervisor's Signature: .... Date: ....

Name: ……………………………………………………………….

ABSTRACT

Agricultural activities play a crucial role in global food production and economic growth, but they also generate substantial amounts of organic wastes and by-products. One of the leading causes of environmental pollution and health hazards has been the improper management of agricultural wastes. One promising and environmentally friendly solution for treating agricultural wastes is anaerobic digestion. Although studies have been done in Ghana and Central Region using anaerobic digestion to treat organic wastes, there is little or no knowledge available on the assessment of the performance of a mesophilic single-stage biogas digester for treating agricultural wastes. The primary objective of the research work is to develop such biogas digester to treat agricultural wastes. An 8 m^3 pilot-scale single-stage digester with a manual stirrer operated at a mesophilic condition (30 ^oC) was used to treat agricultural wastes at three different hydraulic retention time (HRT): HRT 20, 23 and 26 days with a hydraulic flow rate of 300 L/d, 260 L/d and 230 L/d respectively. Cow dung was used as inoculum for the digester whiles pig manure, cabbage wastes, carrot leaves, jute leaves, amaranth plant and spinach leaves represented agricultural wastes. Selected physicochemical parameters (BOD, COD, pH, chloride, ammonia, total phosphorus, total solid, volatile solid, total nitrogen, and nitrate), pathogenic microorganisms (*E. coli* and *Salmonella spp*.) and heavy metals (lead, chromium, nickel, zinc and cadmium) were analyzed on the inoculum, influent and effluent. The results from the cow manure made it feasible and preferred inoculum for the anaerobic digestion of agricultural wastes. With regards to physicochemical parameters, the greatest elimination was seen in TS and VS at HRT 26, whereas TN, OC, COD NO_3^-, and TP were at HRT 23. The results of the pathogenic microbial treatment indicated an infinite reduction of *salmonella spp*. and a 2.02 log reduction in *E. Coli* all at HRT 26. Additionally, the data related to heavy metals indicated that all the initial values of these metals were higher in the influent than in the effluent, except for Zn and Pb at an HRT of 23 days, which saw an increment in their effluent concentrations. HRTs 23 and 26 days showed better treatment efficiency as compared to HRT 20. This research is a win-win solution for farmers and policy makers as it addresses waste management, energy, environmental and economic concern, whiles supporting sustainable agricultural and rural development. However, Pb and Zn showed higher effluent values which need additional treatment before using it for cultivation.

KEY WORDS

Anaerobic digester

Single-stage

Heavy metals

Microorganism

Physicochemical parameters

Mesophilic

Hydraulic retention time

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DEDICATION

To my lovely family

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LIST OF ABBREVIATIONS

AD	Anaerobic Digestion
AW	Agricultural wastes
UCC.	University of Cape Coast
OLR	Organic loading rate
BOD	Biological Oxygen Demand
CFU	Coliform Unit
OLR	Organic Loading Rate
COD	Chemical Oxygen Demand
L	Litre
ml.	millilitre
ppm.	Part per million
E. coli	Escherichia coli
EA	Endo Agar
BGA	Brilliant green agar
GHG	Green House Gas
HRT	Hydraulic Retention Time
HRTs	Hydraulic Retention Times
HFR	Hydraulic flow rate
MSSBD	Mesophilic single-stage biogas digester
TN	Total nitrogen
NH_3	Ammonia
OC	Organic Carbon
TP	Phosphorus
TAN	Total Ammonia Nitrogen

TS	Total Solids
VFA	Volatile Fatty Acids
VS	Volatile Solids
Zn	Zinc
Cr	Chromium
Pb	Lead
Ni	Nickel
Cd	Cadmium
KCARP	Kumasi Compost and Recycling Plant
ACARP	Accra Compost and Recycling Plant
SSGL	Sewerage System Ghana Limited
TMP	Theoretical Methane Production
KWh	Kilowatt hour

CHAPTER ONE

INTRODUCTION

Background to the study

Agricultural activities play a crucial role in global food production and economic growth, but they also generate substantial amounts of organic wastes and by-products (Obi et al., 2016; Specht et al., 2014). Improper management of agricultural wastes has led to environmental pollution and health hazards. In order to address these challenges, the implementation of sustainable wastes management strategies has become a critical focus for researchers and policymakers worldwide. One promising and environmentally friendly solution for treating agricultural wastes (AW) is anaerobic digestion (AD) (Khalid *et al.*, 2011).

AD is a biological process in which microorganisms degrade organic matter in the absence of oxygen, leading to the production of biogas, a renewable energy source, which is rich in methane (Ngan et al., 2020; Sibiya & Muzenda, 2014) and a digestate which is nutrient rich organic fertilizer. The final byproduct of anaerobic digestion is biogas, which contains 60–70% methane, 30–40% carbon dioxide, traces of other gases such as hydrogen sulphide, and digestate (Chen & Neibling, 2014). Ofori Boateng (2013), Ulrike et al. (2014), Kemausuor et al. (2014), Miezah et al. (2017), Mohammed et al. (2017), and Bryant (2019) all demonstrated Ghana's enormous potential for energy from wastes production. Due to the ability of the AD process to reduce chemical oxygen demand (COD) and biological oxygen demand (BOD) from waste streams while producing green energy, this technology has been used effectively in the treatment of agricultural wastes, food wastes, and wastewater sludge (Chen et al., 2008). By lowering the levels of physical and chemical indicators like nitrates, phosphorus, chlorides, and ammonia, which are signs indicating the presence of organic contaminants.

AD has the potential to substantially reduce the negative effects of agricultural wastes in addition to producing renewable energy (Dar et al., 2021). This reduction contributes to an overall improvement in water quality, especially when the treated effluent is safely discharged into water bodies or used for irrigation purposes. Another vital aspect of anaerobic digestion is its potential to address the issue of heavy metals in agricultural waste (Alrawashdeh et al., 2020). Heavy metals, such as cadmium, iron, lead, nickel, and chromium, can accumulate in the environment and pose adverse health risks to both humans and wildlife. According to Gupta et al. (2016), through the AD process, certain microorganisms such as *Salmonella sp*. and *Escherichia coli* can assist in immobilizing heavy metals, reducing their mobility and potential for leaching into the soil or groundwater. Furthermore, the microbial community involved in the anaerobic digestion process exhibits pathogen reduction capabilities (Mao et al., 2015). As studied by Zamri et al. (2021), anaerobic conditions hinder the growth of pathogenic microorganisms, hence generating digestate with reduced levels of harmful pathogens, making it safer for agricultural use as an organic fertilizer.

There are various benefits to treating agricultural wastes with anaerobic digestion. The first benefit is that it lessens the environmental impact by transforming organic wastes into useful biogas that can be used for electricity generation, cooking, and other purposes (Bhatt & Tao, 2020). According to Mao et al. (2015), AD also helps to reduce waste volume, which improves waste management systems. Thirdly, it reduces the emissions of dangerous greenhouse gases like methane, which significantly contributes to climate change (Ntinyari & Gweyi-Onyango, 2021). Despite the numerous benefits of anaerobic digestion in treating agricultural wastes, there are challenges with the installation of the biogas digester that need to be addressed for optimal efficiency and implementation. Factors such as feedstock composition, process parameters, and reactor design need careful consideration to achieve maximum biogas yield, effective treatment and minimize potential drawbacks. These include optimizing the feedstock composition to promote a diverse microbial population, carefully adjusting process parameters such as organic loading rate (OLR), hydraulic retention time (HRT), temperature, and pH, and selecting the appropriate reactor design based on the feedstock characteristics (Sarker et al., 2019). Additionally, addressing inhibitory substances and maintaining a well-adapted microbial consortium are crucial for efficient biogas production (Viancelli et al., 2023). Proper mixing and agitation, waste-to-energy integration, and process monitoring, and control are also essential to improve the overall sustainability and performance of the anaerobic digestion system (Zhang et al., 2019). By taking these factors into account and tailoring the process to the specific needs, anaerobic digestion can become a viable and effective solution for organic waste management, providing valuable biogas while effectively treating the waste, thus contributing to environmental and economic benefits.

As we seek sustainable solutions to the pressing global challenges of waste management and energy demand, anaerobic digestion emerges as a viable and efficient technology for transforming agricultural wastes into valuable resources.

Problem statement

Numerous research projects carried out in Ghana and other regions of the world have demonstrated that AD is a potential option for recycling various organic wastes and agricultural wastes (Al-Mamun and Torii 2017; Magomnang et al., 2017; Tsapekos et al., 2017; Bryant, 2019).

A study made by Bryant (2019) in "Terterkessim" slum, Elmina–Ghana, used AD to produce biogas from human faeces co-digested with household food wastes at hyper-thermophilic condition (65 °C), however, no agricultural wastes were used.

Al-Mamun and Torii (2017) studied the use of anaerobic co-digestion using batch system to treat food and vegetable wastes at a mesophilic temperature of 33 ± 3 °C. The findings revealed that the study's average methane content was 61%, which was high for grid injection or vehicle fuel.

At a thermophilic temperature of 54 1 °C, Tsapekos et al. (2017) investigated the co-digestion of manure and lignocellulosic biomass for the formation of biogas. Based on their findings, lignocellulosic silage underwent a mechanical preparation to increase methane output.

Magomnang et al. (2017) conducted research on agricultural biomass wastes for the production of biogas. The study looked at the anaerobic co-digestion of cattle dung with different feedstocks such as rice straw, coconut shell, and sewage sludge to produce biogas at mesophilic condition of 35 °C. The findings indicated that co-digesting cow dung with sewage sludge increased the methane generation by 162 %. However, co-digestion of cow manure with a mixture of coconut shells and rice straw led to ammonia inhibition, The increase in the generation of biogas was due to the addition of sewage sludge which enhanced improvement in the biodegradability.

To date, only limited research has been conducted regarding the treatment efficiency and mitigation of greenhouse gas (GHG) in the treatment of large quantities of agricultural wastes using a single-stage biogas digester. Although studies have been done in Ghana and Central Region on the use of anaerobic digesters to treat organic wastes, there is little or no knowledge and information available on the technical and operational feasibility of treating agricultural wastes using a mesophilic single-stage biogas digester (MSSBD). However, lab-scale single-stage systems are widely used in anaerobic treatment technology applications because they are uncomplicated, simple to design, and less expensive to build and operate (Muşlu, 2011; Meena et al., 2022). Since the efficient conversion of agricultural wastes into renewable energy and utilization is crucial in reducing environmental pollution, it is, therefore, beneficial to carry out this research to produce data that can add more information and knowledge in the sector. Additionally, the digestate produced from the MSSBD which is an organic fertilizer rich in nitrogen will be applied on agricultural lands for the cultivation of some cereals and vegetables. Hence, this research is necessary to solve the issue of Ghana's severe environmental pollution, and at the same time, poor wastes management.

Justification

The results of this project will produce original information about how sustainable AW is, as a potential feedstock for AD on the UCC campus, information that can be reproduced throughout Ghana, Africa, and the rest of the world. The results will further provide data on important optimum parameters to consider when running such a system. Furthermore, treatment of agricultural wastes using a mesophilic single-stage biogas digester will reduce the amount of wastes generated and at the same time reduce GHG released into the atmosphere. This research is a win-win solution for farmers and policy makers as it will address waste management, energy, environmental and economic concerns, whiles supporting sustainable agricultural and rural development.

General objective

The objective of the study is to assess the performance of mesophilic single-stage biogas digester for treating agricultural wastes.

Specific Objectives

1. To assess selected physicochemical parameters (pH, volatile solid, total nitrogen, COD, BOD, organic carbon, chloride, nitrate, ammonia, and total phosphorus) of the influent and the effluent.
2. To determine the optimal parameters (HRT and hydraulic flow rate) that will be more suitable for running the pilot-scale mesophilic digester.
3. To assess the pathogenic microorganisms (*Escherichia. coli* and *Salmonella spp*) present in the influent and effluent samples.
4. To assess the presence of heavy metals (Pb, Ni, Cr, Zn and Cd) in the influent and effluent.
5. To determine treatment efficiency of the reactor

Hypothesis

- Ho: There is no significant difference in the selected parameters (pH, volatile solid, total nitrogen, COD, BOD, organic carbon, chloride, nitrate, ammonia, and total phosphorus) of the influent and the effluent.
- H1: There is a significant difference in the selected parameters (pH, volatile solid, total nitrogen, COD, BOD, organic carbon, chloride, nitrate, ammonia, and total phosphorus) of the influent and the effluent.
- Ho: There is no significant difference in the optimum hydraulic retention time and flow rate for the treatment of agricultural wastes
- H1: There is a significant difference in the optimum hydraulic retention time and flow rate for the treatment of agricultural wastes.
- Ho: There is no significant difference in the heavy metals' concentrations (Pb, Cd, Cr, Zn and Ni) of the influent and effluent.
- H1: There is a significant difference in the heavy metals' concentrations (Pb, Cd, Cr, Zn and Ni) of the influent and effluent.
- Ho: There is no significant difference in the presence of *E. coli* and *Salmonella typhi* of the influent and effluent.
- H1: There is a significant difference in the presence of *E. coli and Salmonella typhi* of the influent and effluent.

Organization of the Study

Thematically, there are six chapters in this research. So far, the study's background, the research problem, justification, the objectives and hypotheses, have been covered in Chapter one. The literature review is discussed in chapter two, while the materials and methods are the subject of chapter three. The findings of the analyses are summarized in chapter four. The fifth chapter discusses the findings of the study. The conclusion and suggestions for authorities and future research are presented in the last chapter, six.

CHAPTER TWO

LITERATURE REVIEW

Overview

This study set out to assess the performance of mesophilic single-stage biogas digester for treating agricultural wastes. The existing literature of related works gathered through this chapter were reviewed journals, research papers and books that have been published. The historical development of anaerobic digester, implementation of anaerobic digesters in Ghana, classification of agricultural wastes, environmental impacts of agricultural wastes, types of anaerobic digesters, as well as anaerobic digestion process, were discussed. Furthermore, important operating parameters in anaerobic digestion and implementation of anaerobic digesters in developing countries were discussed. Again, the economic importance of anaerobic digestion of agricultural wastes is among the topics addressed.

Historical development of anaerobic digester

The historical development of anaerobic digesters can be traced back to ancient civilizations where the potential of harnessing biogas from organic waste was first observed. Ancient Chinese farmers utilized biogas from the fermentation of organic matter to provide heating and lighting for their homes and agricultural activities (Abbasi et al., 2012). Similarly, in ancient Persia (modern-day Iran), evidence suggests that early farmers constructed underground anaerobic digestion pits to convert organic waste into biogas, which was used for cooking and lighting (Casper, 2007). The modern understanding and practical application of anaerobic digesters began to take shape in the latter part of the 19th and the early 20th centuries (Chanakya &

Malayil, 2012). Notable pioneers like Sir Humphry Davy conducted experiments on the fermentation of organic matter to produce combustible gases, making significant contributions to our understanding of the production of biogas (Aggarangsi et al., 2023). During this period, biogas applications for energy generation and waste treatment gained momentum.

The first large-scale application of anaerobic digestion for waste treatment and energy production occurred in Exeter, England, in the late 19th century (Abbasi et al., 2012). In 1895, the Exeter sewage works installed a covered anaerobic digester to treat sewage sludge and generate biogas for street lighting (Rufai, 2010). This marked a significant milestone in the practical utilization of anaerobic digestion technology. In the early 20th century, anaerobic digesters found applications in sewage treatment in Europe and later in the United States. In Germany, Carl von Linde, a pioneer in refrigeration technology, developed large-scale biogas plants for sewage treatment, further advancing the technology's application (Riffat et al., 2017). Biogas applications continued to expand globally, with countries like India and China leading in promoting biogas technology for rural energy supply and waste treatment (Bond & Templeton, 2011).

Anaerobic digestion has been adopted by African countries in the last few decades for a variety of reasons, including energy scarcity, waste management, and agricultural regeneration (Mutezo & Mulopo, 2021). Large-scale digesters are being used by nations including Ghana, Kenya, South Africa, Nigeria, and Kenya to turn municipal and agricultural waste into biogas for heat and power (Kemausuor et al., 2018). By producing biogas from manure and kitchen waste for cooking and lighting, small-scale biogas systems strengthen local communities by lowering their need on fossil fuels. Although there are still obstacles to overcome, these developments demonstrate how anaerobic digestion provides sustainable answers for Africa's energy, waste, agriculture, and community empowerment needs.

Throughout the 20th century, research and development efforts focused on optimizing anaerobic digester designs, enhancing feedstock utilization, and improving biogas utilization systems. These advancements resulted in increased efficiency and scalability of biogas production. In the 21st century, anaerobic digesters have evolved into sophisticated systems used for various applications. The modern era witnesses the utilization of anaerobic digestion for organic waste management, wastewater treatment, and renewable energy production on both small and large scales (Kalyani & Pandey, 2014). Innovative digester designs, enhanced feedstock flexibility, and improved process control have expanded the potential uses of anaerobic digestion in diverse industries and applications (Yu et al., 2013; Nguyen et al., 2019)).

Implementation of anaerobic digesters in Ghana

Ghana has used anaerobic digestion as an environmentally friendly approach for producing renewable energy and managing organic waste (Arthur et al., 2011). In recent years, the Ghanaian government has shown commitment towards promoting renewable energy development, including anaerobic digestion, as a means of achieving energy security, reducing greenhouse gas emissions, and promoting sustainable economic growth (Owusu-Manu et al., 2021). Several anaerobic digestion plants have been put into place in Ghana to manage organic waste such as agricultural waste, faecal sludge, food waste, and municipal solid waste, especially in the cities (Cofie et al., 2016). One of the notable projects is the Kumasi Compost and Recycling Plant (KCARP), Sewerage Systems Ghana Limited (SSGL) and Accra Compost and Recycling Plant (ACARP), which use anaerobic digestion to treat municipal solid waste and produce biogas for electricity generation (Lohri et al., 2013).

The implementation of anaerobic digestion in Ghana, however, faces many challenges such as inadequate financial resources, lack of technical expertise, and poor public awareness (Williams et al., 2023). Despite these challenges, the government has made efforts to address them by providing financial incentives, technical assistance, and creating awareness among the public and stakeholders (Afrane et al., 2021; Williams et al., 2023). The success of anaerobic digestion implementation in Ghana relies on the availability of appropriate feedstocks, the sustainability of the technology, the support of the government and private sector, and the participation of the public (Arthur et al., 2011).

The potential benefits of anaerobic digestion in Ghana include the reduction of waste pollution, the creation of job opportunities, and the production of green energy (Afrane et al., 2021). The implementation of anaerobic digestion in Ghana is still in its early stages, but the government has shown a commitment to promoting sustainable energy development. The implementation of anaerobic digestion in Ghana will be successful if the obstacles are overcome and the technology is sustained with the help of all stakeholders.

Anaerobic digesters in Ghana

Kumasi Compost and Recycling Plant (KCARP)

KCARP was primarily designed to tackle the challenges of waste management in Kumasi, which is a rapidly growing urban center with a significant waste generation issue(Addo-Fordwuor & Seah, 2022). The plant focused on converting organic waste into compost and recycling non-organic materials. KCARP was set up to process organic waste materials, such as kitchen waste, garden waste, and other biodegradable materials, through the composting process (Addo-Fordwuor & Seah, 2022). Compost produced at the facility could be used for agriculture, landscaping, and soil improvement. In addition to composting, KCARP aimed to promote recycling by sorting and processing non-organic waste materials, including plastics, metals, and paper. The recycling component contributed to waste reduction and the recovery of valuable resources. The establishment of KCARP had multiple environmental benefits, including the reduction of landfill waste, decreased environmental pollution, and the promotion of sustainable waste management practices.

Accra Compost and Recycling Plant (ACARP)

The waste management facility known as the Accra Compost and Recycling Plant (ACARP) is situated in Ghana's capital city of Accra (Mudu et al., 2021). The ACARP project is crucial for improving environmental sustainability in the city and handling of waste. The main goals of ACARP are the processing and management of organic waste in addition to the recycling of non-organic materials (Okai, 2020). Redirecting waste from landfills and promoting ethical waste management techniques are its two main objectives. By composting organic waste, including yard and kitchen garbage as well as other biodegradable materials, ACARP manages waste organic material. The compost that is produced is useful as a soil conditioner and can be applied to landscaping, gardening, and farming. Apart from composting, recycling is another area that ACARP prioritizes (Agbefe et al., 2019). Paper, metals, and plastics are among the non-organic waste products that the plant separates and processes. In addition to reducing waste, this helps recover precious resources. In order to overcome its waste management issues and transition to a more environmentally responsible and sustainable method of disposing of waste, Accra needs ACARP's assistance (Agbefe et al., 2019).

Sewerage Systems Ghana Limited (SSGL)

According to Tanoh et al. (2022) SSGL is principally in charge of running and maintaining the sewerage systems in various parts of Ghana. Sewage collection and treatment are included in this, as is making sure wastewater is disposed of properly to avoid contamination and health risks. In order to clean and disinfect sewage before it is safely released into the environment, the corporation runs wastewater treatment plants and facilities (Arthur et al., 2022). Construction and upkeep of sanitation infrastructure, such as sewage pipelines, pumping stations, and related facilities, are tasks performed by SSGL. According to Tanoh et al. (2022) the services offered by SSGL are essential for maintaining public health and protecting the environment. Water sources may be kept clean and waterborne infections can be stopped by practicing proper sanitation and wastewater management.

Classification of Agricultural wastes

The choice of feedstock for anaerobic digestion depends on factors such as availability and suitability for digestion, composition, moisture content, nutrient content, and carbon-to-nitrogen ratio can all affect the rate and extent of digestion (Uddin & Wright, 2022). The most common types of agricultural wastes used for anaerobic digestion include animal manure, crop residues, and food processing wastes. The choice of feedstock for anaerobic digestion can have a significant impact on the efficiency and effectiveness of the process. Therefore, careful consideration must be given to the selection and preparation of feedstocks for anaerobic digestion to ensure optimal performance of the process (Banks & Heaven, 2013).

Animal manure

Animal dung has long been valued as a resource for management of waste and sustainable agriculture. The possible advantages of turning animal dung into biogas and nutrient-rich digestate have been more evident with the development of anaerobic digestion technology (Wang, 2014). A common feedstock for anaerobic digestion is animal manure, especially that from livestock like cows, pigs, and poultry because it is a rich source of organic matter and nutrients (Khoshnevisan et al., 2021). This activity enhances greenhouse gas reduction, nutrient recovery, waste management, odor control, and revenue generation. However, factors including variability in feedstock composition, high solid content, inhibitory substances, process imbalance, and digester foaming from this feedstock can affect digester's efficiency (Naik et al., 2014).

Crop residue

Crop residue are plant materials that are left in the field as wastes after harvesting crops. It includes the parts of the plant that are not harvested, such as leaves, stems, husks, and other vegetative material. Crop residues are a crucial and important component of agricultural ecosystems and have various uses and impacts on soil health, the environment, and sustainable farming practices. Crop residues including straw, corn stover, and sugarcane bagasse are also commonly used for anaerobic digestion (Momayez et al., 2019). Utilizing crop residues for anaerobic digestion shows promise as a sustainable waste management and energy production solution in agriculture. The abundance of crop residues and their potential benefits, such as improved nutrient management, energy generation, and carbon sequestration, make them a desirable substrate for anaerobic digestion (Suhartini et al., 2021). However, challenges related to lignocellulosic structure, nutrient imbalances, pre-treatment requirements, seasonal availability, and mixing complexity must be addressed to fully harness their potential (Gumisiriza et al., 2017).

Food processing wastes

Food processing industries play a critical role in our daily lives by transforming raw agricultural products into a wide range of food products. However, these industries also generate substantial amounts of organic waste, including peels, trimmings, and by-products, vegetable and fruit waste, distillery and brewery waste, and dairy processing waste which can pose significant environmental challenges if not managed properly (Kosseva, 2009). Anaerobic digestion emerges as a promising sustainable waste-to-energy solution, offering numerous benefits and paving the way towards a more circular and eco-friendly economy. However, the variability in waste composition and the presence of high-fat content can lead to process inefficiencies and inhibition (Carucci et al., 2005). Furthermore, the availability of food processing wastes may be seasonal, necessitating appropriate waste management and storage strategies to ensure a continuous supply for the digesters.

Slaughterhouse wastes

Slaughterhouses are integral components of the meat and livestock industry, responsible for processing animal carcasses and generating a substantial amount of organic waste. Slaughterhouse waste, fish waste, and algae can also be used for anaerobic digestion, although they may require special considerations due to their unique characteristics (Samoraj et al., 2020). The anaerobic digestion of slaughterhouse wastes offers a sustainable waste-to-energy solution for the meat and livestock industry. With abundant and consistent feedstock availability, biogas production, waste reduction, and nutrient recycling, this process complies with the concepts of sustainable economy and environmental stewardship. Though challenges exist, such as fat and protein content, pathogen concerns, and inhibitory substances, they can be addressed through pre-treatment, co-digestion, and technological advancements (Moukazis et al., 2018).

Environmental impacts of agricultural wastes

Agricultural waste comprises various residual materials in liquid and solid forms generated from farming activities, including production, processing, and marketing of crops and animals (Maji et al., 2020). The waste materials originate from diverse farming practices such as horticulture, dairy farming, livestock breeding, harvesting, and seed sowing (Koul, Yakoob, & Shah, 2022). This waste category includes both organic and inorganic components, contributing to environmental and health challenges (Pandey et al., 2021). According to the definition provided by the Agriculture Act 1947, agricultural waste encompasses waste materials produced on agricultural premises, which involve activities like livestock breeding, dairy farming, seed growing, horticulture, and woodland use (Vink, 2013). Effective ways to handle waste are required as a result of the intensification of agricultural practices to satisfy the growing worldwide hunger crisis (Vink, 2013). However, agricultural waste negatively impacts environmental sustainability efforts, posing threats to the environment as well as public health (Adomako & Ampadu, 2015). A wide range of waste materials is associated with agriculture, including animal feces, urine, litter, animal carcasses, dairy parlor washings, wasted feed, runoff from feedlots, paunch waste, abattoir wastewater, and crop residues like cereal husks and sugarcane bagasse (Adomako & Ampadu, 2015). The environment and human health are negatively impacted by ineffective disposal methods, such as burning crop leftovers, which produce toxic substances including nitrous oxides, carbon monoxide, and volatile organic compounds (Singh & Singh, 2017). Nevertheless, the proper management and utilization of agricultural waste offer opportunities to convert these waste materials into valuable resources. Technologies like anaerobic digestion can be employed to harness biogas and biofertilizers from animal and crop waste, contributing to sustainable waste management and resource recovery (Audu et al., 2020). In order to guarantee a sustainable agricultural sector and safeguard both human well-being and the natural environment, proper waste management is essential (Amran et al., 2021).

Types of anaerobic digesters

A biogas plant's construction must take into account the importance of selecting a suitable biogas digester. Based on feeding mode (batch or continuous), substrate moisture content (wet or dry), process temperature (mesophilic, psychrophilic or thermophilic) and other variables, there are many various kinds of anaerobic digestion systems. Also, the cost-effectiveness, technical feasibility, and availability of local skills and resources all impact the fundamental Anaerobic Digester (AD) design (Lohri et al., 2013). Anaerobic digesters come in several different general categories, such as Continuous Stirred Tank Reactors (CSTR), Plug Flow Reactors (PFR), Floating Drum Digesters, Fixed Dome Digesters, Balloon Digesters, Horizontal Digesters, and Two-Stage Digesters. The prevailing and established design in the area, which is impacted by climatic, economic, and substrate-specific factors, has a significant impact on design decisions in developing nations like Ghana (Abdel-Shafy & Mansour 2018).

Fixed Dome Digester

According to Khalid et al. (2011), the Fixed Dome Digester is a low-cost, straightforward design that consists of a fixed, dome-shaped structure with an immovable gas container. The digester is continuously supplied feedstock, and the upper portion of the dome is where biogas is collected. According to Orskov et al. (2014), this kind of digester is frequently utilized in small-scale applications in rural locations with limited resources.

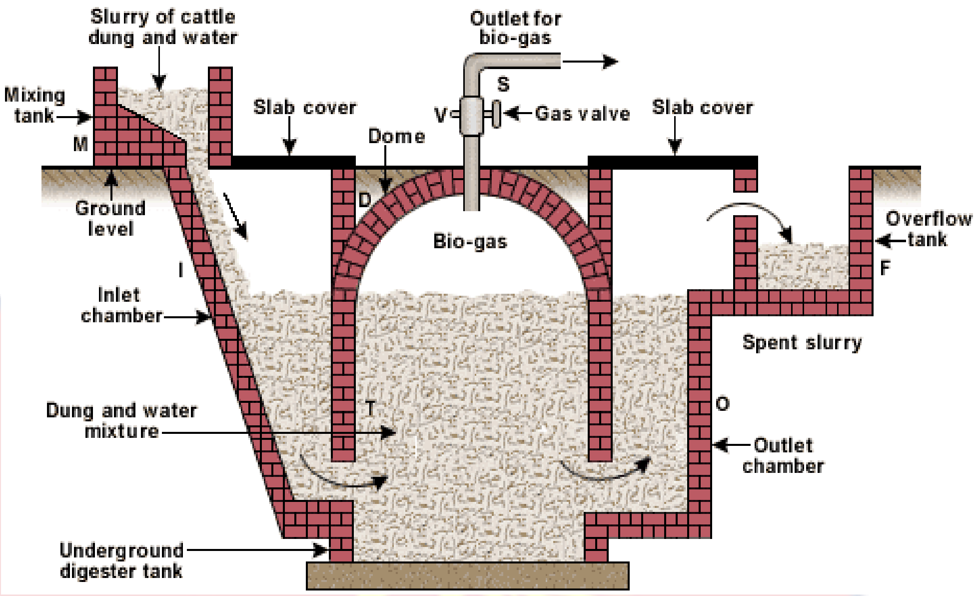


Figure 1: Fixed-dome biogas digester

Floating Drum Digester

In contrast, the Floating Drum Digester also uses a dome-shaped gas holder, but in this design, the gas holder is floating in a water-filled chamber (Deepanraj et al., 2014). As biogas is produced, it displaces water, causing the drum to rise. This type of digester is suitable for varying biogas production rates and fluctuating feedstock volumes, making it adaptable to changing waste loads (Mungwe et al., 2016).

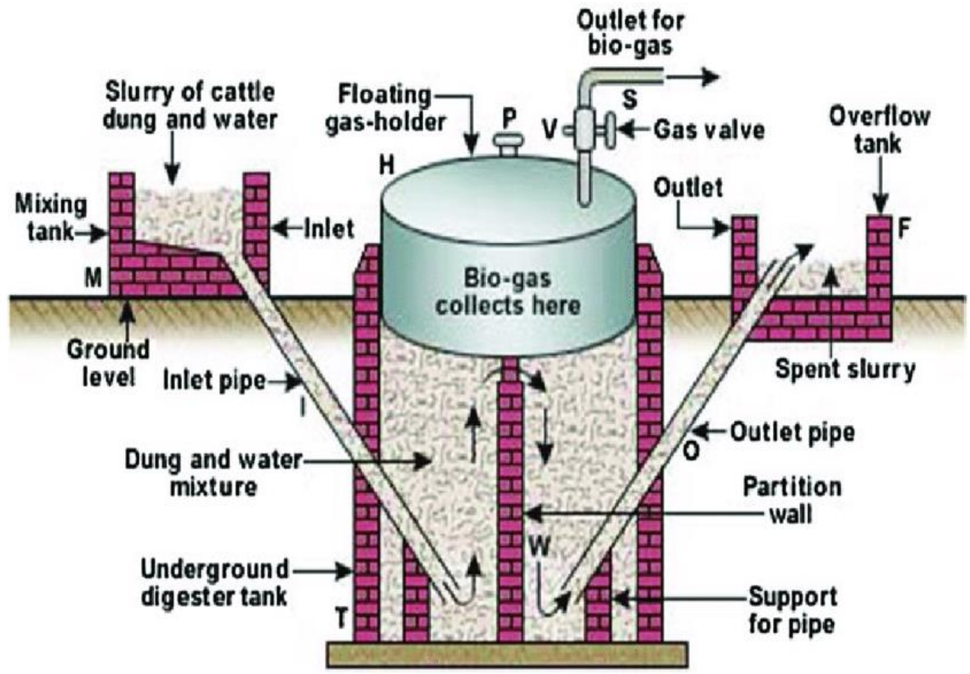


Figure 2: Floating drum bigas digestor

Balloon Digester

The Balloon Digester is a flexible, gas-storage system that expands, and contracts based on biogas production and utilization (Baredar et al., 2020). Often made of high-quality plastic, it is lightweight and easy to install and relocate. Balloon digesters are mostly employed in small-scale applications and in areas with limited resources, making them suitable for household-level waste treatment (Cheng et al., 2014; Orskov et al., 2014).

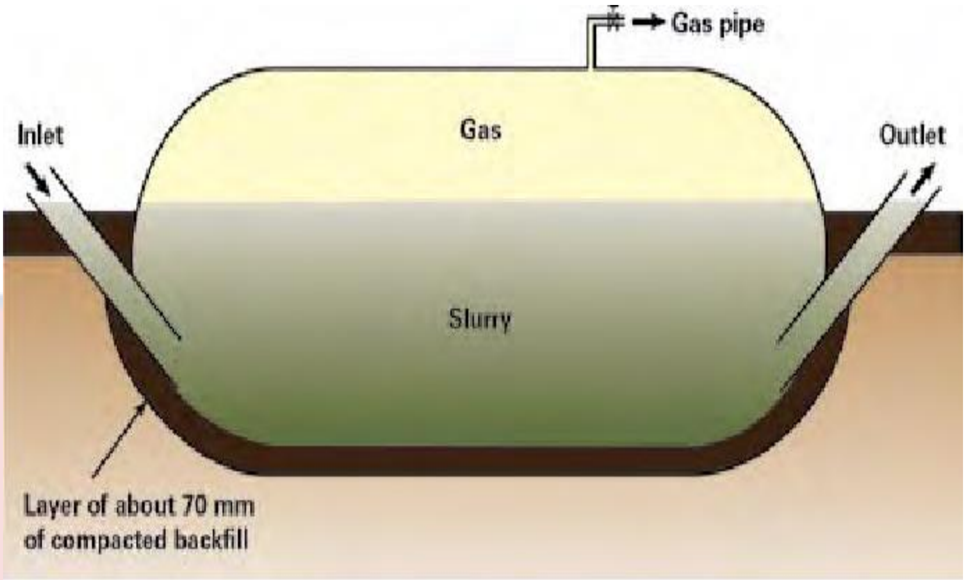


Figure 3: Tubular Digester

Garage-type digester / Horizontal Digester

The Horizontal Digester features a horizontal tank where feedstock is loaded and moved along a slow conveyor system (Samer, 2012). This design provides an extended retention time for the organic matter, enabling thorough digestion and enhanced biogas production. It is well-suited for large-scale agricultural and industrial applications, where a continuous flow of feedstock is available (Mutungwazi et al., 2018).

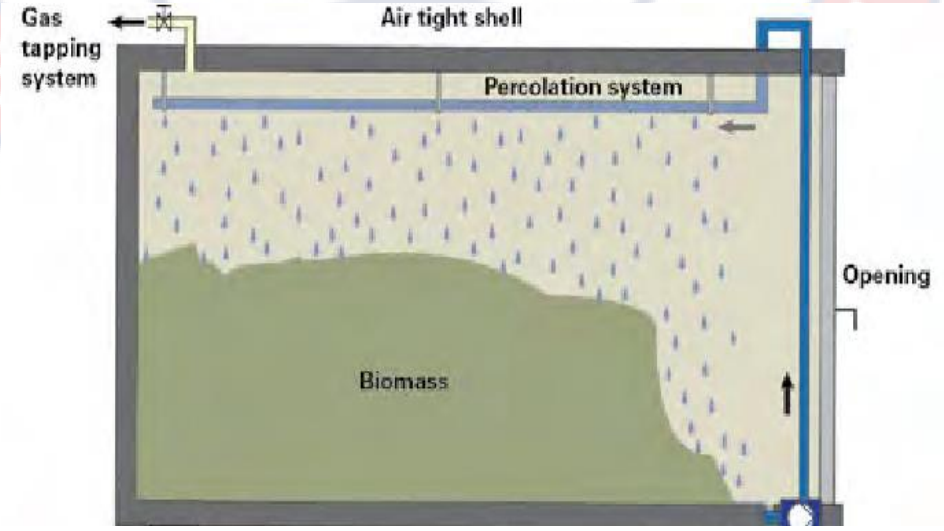


Figure 4: Garage-type biogas Digester

Two-Stage Digester

The Two-Stage Digester is made up of two separate chambers, each optimized for different stages of anaerobic digestion (Ghaly, 1996). The first stage, often referred to as the hydrolysis/acidogenesis stage, breaks down complex organic matter into simpler compounds. The second stage, or methanogenesis stage, converts these compounds into biogas. This design is particularly effective for handling high-solid content and challenging feedstocks, making it suitable for treating a wide range and variety of organic wastes (Srisowmeya et al., 2020).

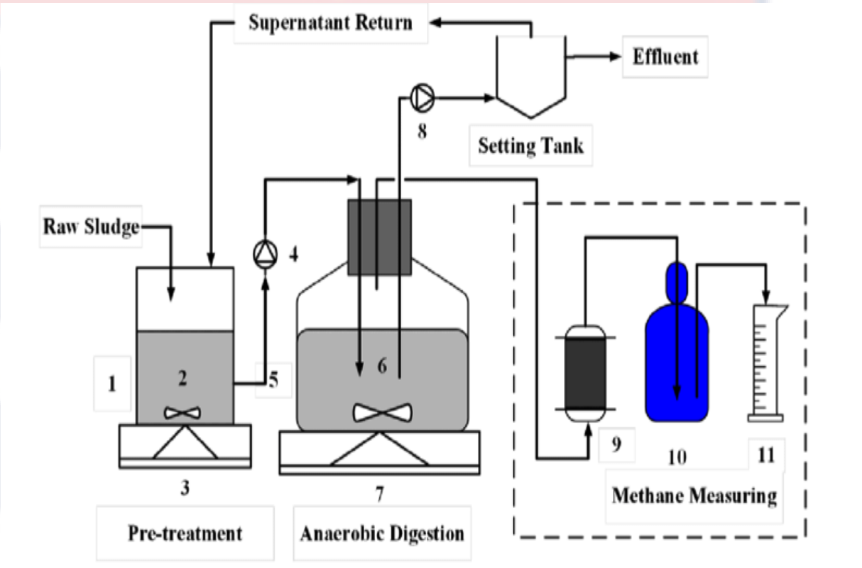


Figure 5: Schematic view of a two-stage anaerobic digester

Plug Flow Reactor (PFR)

The Plug Flow Reactor (PFR) is characterized by the flow of feedstock and microorganisms in a unidirectional manner through the reactor (Nkuna et al., 2022). It promotes a more gradual reduction of organic matter and longer retention time, allowing for better degradation of complex substrates. The PFR is especially suitable for fibrous and lignocellulosic feedstocks, making it an ideal choice for agricultural and forestry waste treatment (Rackemann & Doherty, 2011).

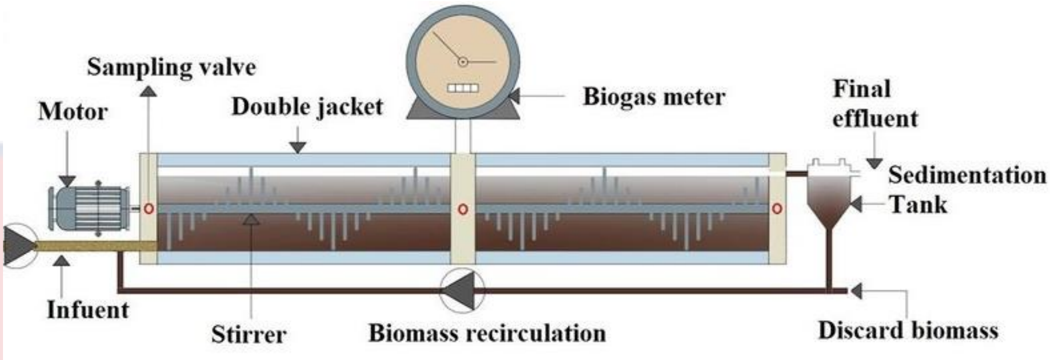


Figure 6: Schematic view of anaerobic Plug-Flow Reactor (PFR)

Continuous stirred tank reactors (CSTRs)

The Continuous Stirred Tank Reactor (CSTR) is one of the most common types of anaerobic digesters (Wendland et al., 2007; Kariyama et al., 2018) It features a well-mixed tank where feedstock is continuously added while biogas and digestate are continuously removed. The continuous mixing ensures a stable and consistent environment for the anaerobic microorganisms, providing steady biogas production (Ward et al., 2008). The CSTR is suitable for a wide range of feedstocks and is often used in medium to large-scale biogas production systems (Mao et al., 2015; Andlar et al., 2021).

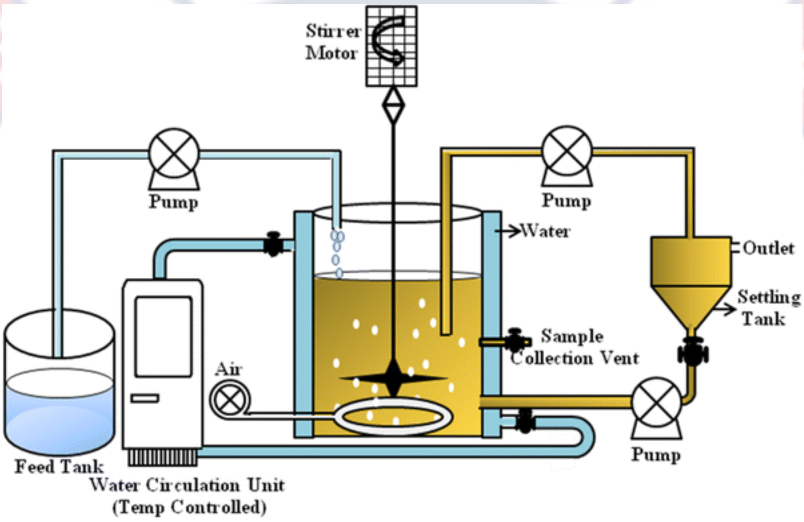


Figure 7: Continuous Stirred Tank Reactor (CSTR) for wastewater treatment

Discontinuous Stirred Tank Reactor (DSTR)

According to Bryant (2019), a continuous stirred tank reactor can be operated on a discontinuous mode making it a Discontinuous Stirred Tank Reactor (DSTR). In his work, a manual stirrer was installed on a fixed-dome digester and was operated discontinuously anytime the substrate and the sludge was mixed. The use of a DSTR has an advantage over the continuous one, in that no electrical energy is required for the stirring and mixing, thus making it less expensive (Bryant and Osei-Marfo, 2021). However, it has a disadvantage of possible scum formation when the individuals who are supposed to stir the digester fail to do so.

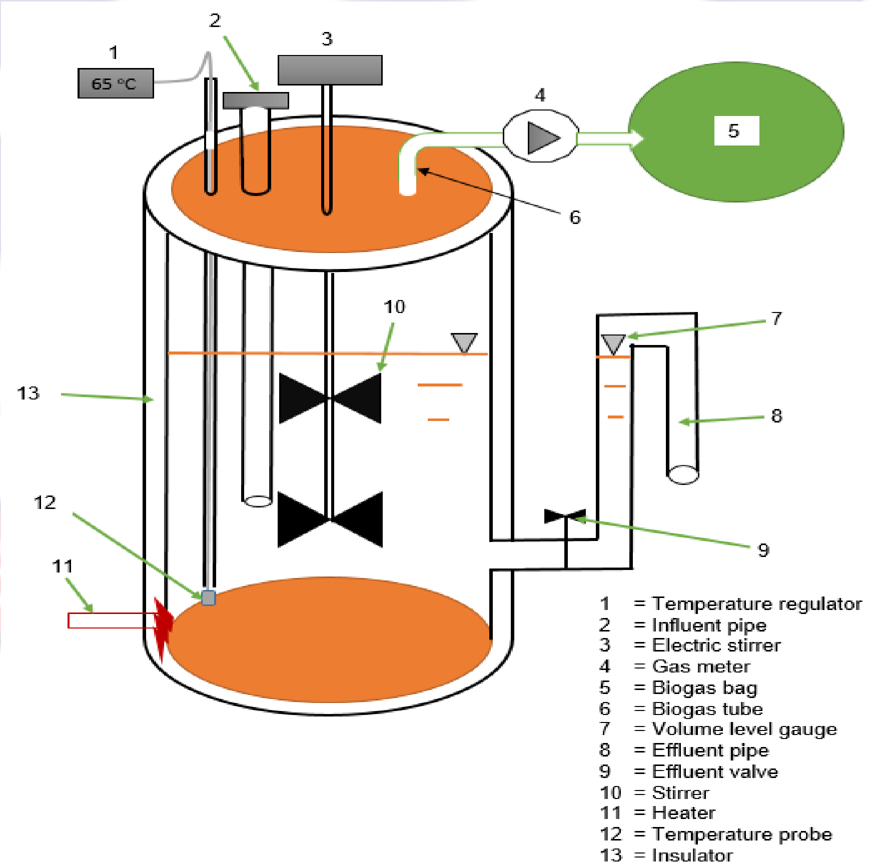


Figure 8: Discontinuous Stirred Tank Reactor (DSTR) for wastewater treatment

Puxin biogas digester

Puxin anaerobic digesters are a type of biogas digester system developed by the Puxin Biogas company in China (Al-Imarah et al., 2022). These digesters are prefabricated, small-scale, and portable units designed to efficiently convert organic waste into biogas for household use (Al-Imarah et al., 2022). The Puxin digester typically consists of a sealed, airtight container where organic waste such as animal manure, kitchen scraps, or agricultural residues is added. Bacteria inside the digester break down this organic matter in the absence of oxygen, producing biogas as a byproduct.

Figure 9: Puxin biogas digester

Single-stage biogas digester

Single-stage biogas digesters, which combine simplicity and efficiency in both design and operation, are a cutting-edge step towards waste management and sustainable energy generation (Bryant 2019). These digesters, distinguished by their single chamber, enable the anaerobic degradation of organic waste without requiring complicated mechanisms or several compartments (Nasr et al., 2012). In addition to lowering building costs, their simplicity makes them useful and accessible, especially for small-scale applications (Azbar & Speece, 2001The adaptability of single-stage digesters is one of their best qualities. They can maximize their potential in a variety of situations by processing a wide range of organic feedstock with efficiency, including agricultural leftovers, animal manure, and kitchen trash (Nasr et al., 2012). Because of their versatility, they can be used as a renewable energy source and a feasible solution for waste management in both urban and rural settings. There are still issues, though, such as the requirement for consistent operating conditions and appropriate feedstock management in order to produce biogas as efficiently as possible. In order to maintain efficiency, it is necessary to pay attention to variations in temperature, pH levels, and feedstock composition that may affect performance (Hagos et al., 2017).

Multi-stage anaerobic Digester

Multi-stage anaerobic digesters are complex machines with divided chambers that are intended to maximize the conversion of organic waste into biogas (Johnson & Mehrvar, 2020). They effectively break down a variety of waste kinds because, in contrast to single-stage digesters, they employ numerous compartments with varying conditions for various stages of microbial activity (Chatterjee & Mazumder, 2016). This clever design increases biogas output and adaptability to manage difficult waste streams. For best results, however, their intricacy necessitates more technical know-how and cautious handling. Although multi-stage digesters show great potential for effective waste management and the production of renewable energy, they necessitate close attention to operational details (Johnson & Mehrvar, 2020).

Anaerobic Digestion Process

Hydrolysis

According to Zhen et al. (2017), the complex polymers found in the organic material used in anaerobic digesters are frequently difficult for microorganisms to reach without extra hydrolysis or pretreatments. The fundamental goal of hydrolysis is to disassemble these complicated organic macromolecules into simpler substances like sugars, long-chain fatty acids (LCFAs), and amino acids so that these substances can be utilized by acidogenic bacteria during anaerobic digestion (Meegoda et al., 2018). Hydrolysis is primarily a biological process in anaerobic digestion, where hydrolytic bacteria secrete extracellular enzymes to facilitate the breakdown of proteins, lipids, and carbohydrates (Menzel et al., 2020). These enzymatic reactions result in the release of soluble products that can diffuse across the cell membranes of acidogenic bacteria for further conversion (Chen et al., 2014). However, certain complex substrates like lignin, cellulose, and hemicellulose may pose challenges in hydrolysis due to their intricate structures, necessitating the addition of enzymes to aid the breakdown of these carbohydrates (Meegoda et al., 2018). While earlier studies suggested that methanogenesis might be the rate-determining step depending on the ratio of hydrolytic to methanogenic bacteria, recent findings emphasize the significance of hydrolysis as a rate-determining process (Atelge et al., 2020). Recognizing the crucial role of hydrolysis in the kinetics of anaerobic digestion, various strategies have been explored to enhance hydrolysis in digesters, particularly for the treatment of highly lignocellulosic wastes (Shrestha et al., 2017). Temperature and pH are crucial factors affecting hydrolysis efficiency, with optimal conditions typically observed at temperatures between 30 °C and 50 °C and pH levels between 5-7, while hydrolytic activity is generally limited below pH 7 (Khan, 2010).

Acidogenesis

Acidogenic bacteria play a crucial role in anaerobic digestion by utilizing the byproducts of hydrolysis, which they absorb through their cell membranes, to synthesize intermediate volatile fatty acids (VFAs) and other compounds. The class of organic acids known as VFAs includes acetates, propionate, and butyrate, typically present in different ratios, ranging from 75:15:10 to 40:40:20 (Meegoda et al., 2018). The specific concentrations of these VFAs produced during the acidogenesis stage can be influenced by the conditions within the digester. Studies have reported significant variations in VFA concentrations in digesters operating at different pH levels, leading to seemingly incongruous results (Meegoda et al., 2018). Acidogenesis progresses rapidly, with acidogenic bacteria having a regeneration time of less than 36 hours, making it one of the fastest stages in anaerobic digestion (Ukaegbu-Obi et al., 2022). Despite the direct production of VFAs as precursors for the final stage of methanogenesis, it is crucial to note that VFAs can also lead to digester failure due to their acidification effects (Meegoda et al., 2018). Moreover, the breakdown of amino acids throughout acidogenesis results in the formation of ammonia through deamination, and at sufficiently high levels, this ammonia can hinder the overall anaerobic digestion process (Xiao et al., 2022). Therefore, carefully managing the acidogenesis stage is crucial for successful anaerobic digestion and optimal biogas production.

Acetogenesis

During the acidogenesis stage of anaerobic digestion, a portion of the initial substrate is transformed into acetate, making it suitable for acetoclastic methanogenesis (Pan et al., 2021). However, other higher-producing volatile fatty acids (VFAs) remain unavailable to methanogenic bacteria at this point. These higher VFAs and other intermediates undergo transformation into acetate during the acetogenesis stage, which is accompanied by the production of hydrogen as a byproduct effects (Meegoda et al., 2018). The generation of hydrogen during acetogenesis unveils an intriguing syntrophic link in the hydrogen interspecies transfer during anaerobic digestion. Despite hydrogen production during acetogenesis, excessive partial pressure of hydrogen negatively impacts acetogenic bacteria (D’Silva et al., 2021). Nevertheless, hydrogen can be rapidly consumed through an exergonic reaction facilitated by the presence of hydrogenotrophic methanogens, thus maintaining hydrogen partial pressures at levels favorable for acetogenesis (Voelklein et al., 2019). Additionally, during acetogenesis, lipids undergo a different process, known as acidogenesis, where glycerol is converted to acetate, and long-chain fatty acids (LCFAs) are transformed into acetate through β-oxidation (Ahmad et al., 2011). It is essential to note that only LCFAs with an even number of carbon atoms can degrade to acetate, while LCFAs with an odd number of carbon atoms initially degrade to propionate before further transformation (Sousa et al., 2007). This knowledge is crucial for understanding the intricate pathways involved in the anaerobic digestion process and the subsequent biogas production.

Methanogenesis

The last stage of anaerobic digestion is known as methanogenesis, during which methanogenic microbes play a critical role in converting available intermediates into methane gas (Li et al., 2019). These methanogenic microorganisms are highly sensitive to oxygen exposure, with studies showing that a significant portion of Methanococcus voltae and Methanococcus vannielli cells can be killed within a short time when exposed to oxygen (Meegoda et al., 2018). As obligate anaerobic archaea, methanogenic bacteria have limited substrate preferences, but they can carry out methanogenesis from methanol, methylamines, and formate (Schnürer, 2016). Acetoclastic methanogenesis, utilizing acetate, typically accounts for about two-thirds of methane production, while hydrogenotrophic methanogenesis makes up the remaining one-third (Leng et al., 2018). Methanogenic bacteria have specific requirements for their activity, including a higher pH compared to earlier stages of anaerobic digestion and a lower redox potential, which can make their laboratory cultivation challenging (Li et al., 2019). These methanogens have a longer regeneration time compared to other microbial groups, ranging from 5 to 16 days, although some hydrogenotrophic organisms like Methanococcus maripaludis have faster doubling times (Li et al., 2019). Methanosarcina spp. have been found to be relatively robust and can withstand ammonia, sodium, acetate concentrations, and pH shocks that would be detrimental to other methanogenic microorganisms (Yenigün & Demirel, 2013; Meegoda et al., 2018). However, overall, methanogenic species are considered to be the most sensitive microbial groups present in anaerobic digestion. In batch reactors, methanogenesis continues until biogas generation ceases, which may take up to 40 days of cultivation (Xin et al., 2014). Monitoring the volatile solids content and dewatering capacity of the sludge can provide valuable insights into the extent of digestion achieved (Chen et al., 2018). The successful functioning of methanogenic microorganisms is crucial for efficient biogas production and sustainable anaerobic digestion processes.

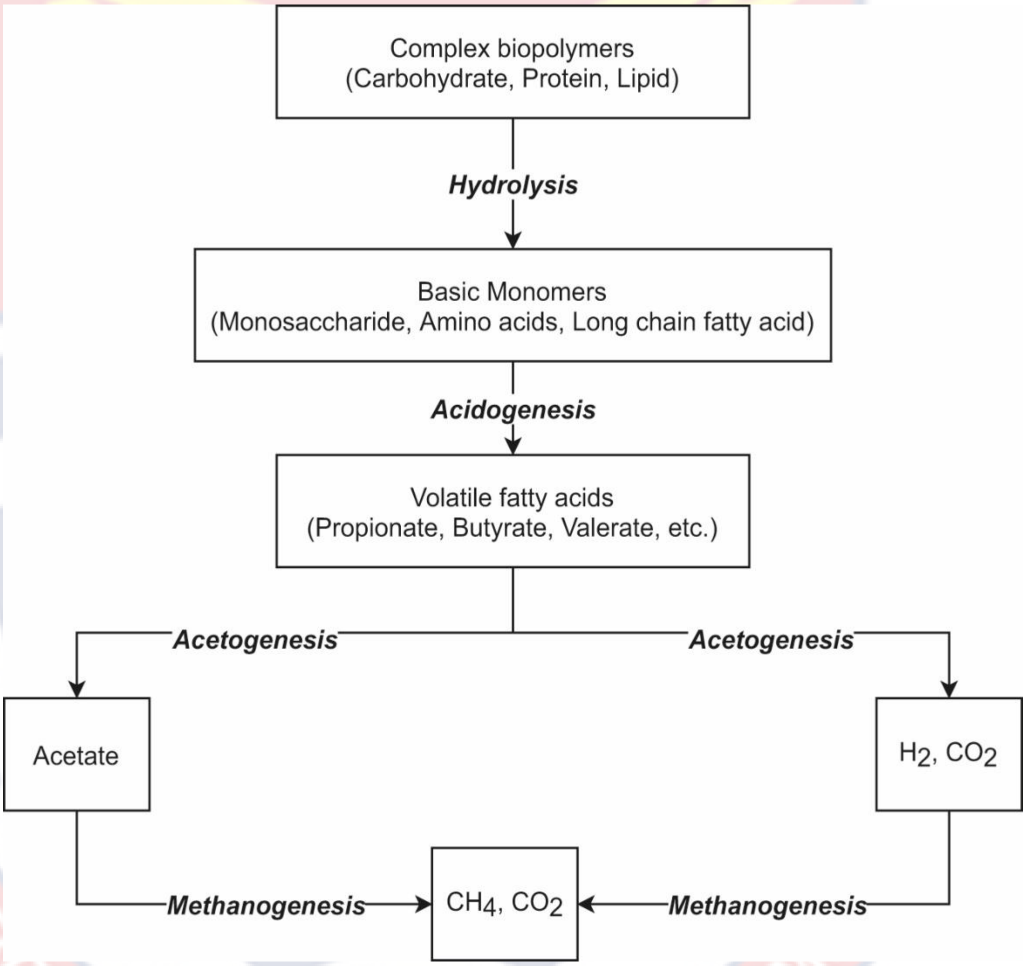


Figure 10: Stages in anaerobic digestion

Important Operating Parameters in Anaerobic digestion of agricultural wastes

Inoculum

Inoculum is among the most important elements, that supplies the bacteria required for the anaerobic digestion process. Its source, sample time, and concentration can all significantly affect the result of AD process (Filer et al., 2019). The inoculum utilized in AD varies greatly across the scientific community and comes from a variety of places, including biogas facilities, agricultural dung, sewage sludge digesters, and biological waste treatment facilities (Koch et al., 2017; Sicchieri et al., 2022). The last few decades have seen a lot of studies on the effects of using different inoculums. The majority of protocol studies contend that variably supplied inoculum can lead to different substrate biodegradability and erroneous results as a result of changes in bacterial population, substrate adaption, and early microorganism activities (Filer et al., 2019; Poursat et al., 2019). When selecting inoculum, there seems to be a widespread understanding that the source that is already adapted to the substrate should be emphasized. Cow dung is the most widely recommended inoculum because of the large diversity of unique and active microorganisms (Gupta et al., 2016; Bryant 2019). The standardization of inoculum requires a quality check to ascertain whether the digester's operational parameters are of high quality. The most common method is to pre-incubate the inoculum at 35 ^{0}C for 1 to 5 days to decrease the impact of methane formation. Li et al. (2013) study on the effect of inoculum pre-incubation found no appreciable difference in produced methane or biodegradability in comparison with inoculum that had not been incubated, with the exception of higher maximum methane production rates when using fresh inoculum at all substrates to inoculum Water ratios. Koch et al. (2017) recommend basing the decision on whether the inoculum has a low or high endogenous methane emission.

Feedstock

The term "feedstock" in the context of anaerobic digestion refers to any substance that anaerobic bacteria may disintegrate into methane. The main constituents of these feedstocks, according to Rabii et al. (2019), are carbon, oxygen, nitrogen, hydrogen, and phosphorus, with microbial cell material having approximately equal amounts of each element at 50%, 20%, 12%, 8%, and 2% respectively. In addition, sulphur is required for the metabolic and anabolic processes that result in vital proteins. Simple high-solid waste and swiftly biodegradable wastes are two examples of different feedstocks. According to Salama et al. (2019), a 25:1 carbon-to-nitrogen (C/N) ratio is best for the effective production of biogas. Low C/N ratios cause excessive nitrogen to build up, which can cause pH levels to rise or prevent methanogenesis (Yenigün & Demirel, 2013). Ammonia (NH_3) accumulation is the result. On the other hand, high C/N ratio, can result in nitrogen depletion, which lowers gas generation (Zhang et al., 2013). The kind, accessibility, and complexity of the substrate have a substantial impact on the rate of anaerobic digestion (Carlsson et al., 2012). Different kinds of carbon sources sustain various microbial communities. Hence, before the digestion process is started, it is essential to characterize the feedstock for its carbohydrate, lipid, protein, and fiber levels (Lesteur et al., 2010). The substrate's ability to generate methane in anaerobic conditions should also be assessed. Carbohydrates are thought to be the most important organic component of municipal solid waste for the production of biogas (Zamri et al., 2021). In comparison to sucrose and glucose, starch has been found to be an efficient low-cost substrate for the production of biogas (Vendruscolo, 2015). Studies have shown that the initial substrate concentration and total solid content of the bioreactor can significantly affect the efficiency of the process and the amount of methane produced (Latifi et al., 2019). The feedstock must be accurately characterized in order to maximize the effectiveness of the anaerobic digestion process and its generation of methane.

Co-digestion

To increase the production of biogas, co-digestion entails the digestion of a mixture of two or more substrates with complementary properties. Animal manures have a high nitrogen concentration, which makes it difficult to achieve the ideal C/N ratio needed for anaerobic digestion (Mao et al., 2015). Before starting the anaerobic digestion, the carbon content of animal faeces must be raised to solve this problem. Agricultural wastes and other lignocellulosic materials could be used as a substitute for animal manure's lack of carbon because of their high carbon content (Neshat et al., 2017). However, they are not likely to be the only substrate for anaerobic digestion. Lignocellulosic materials' slow decomposition and subsequently poor methane output limit anaerobic digestion of them. The rate of digestibility of lignocellulosic feedstock is determined by the slow hydrolysis of cellulose, which is considered to be the rate-controlling phase in the process (Neshat et al., 2017). Although pretreatments like enzymatic hydrolysis or steam explosion can boost the possibility for creating biogas from lignocellulosic materials, the process' economic sustainability may be at risk. Co-digesting lignocellulosic materials and animal manures can balance the C/N ratio of the substrate for anaerobic digestion (Sawatdeenarunat et al., 2015). The procedure enables the conversion of organic waste materials into biogas, and this can be utilized as fertilizer, and leaves behind a nutrient-rich residue. Benefits of lignocellulosic materials and animal manures being digested include an increase in buffering capacity, dilution of potentially hazardous chemicals, utilization of nutrients, bacterial variety, and a decreased danger of ammonia inhibition (Neshat et al., 2017). Additionally, the concentrated organic chemicals found in lignocellulosic wastes are diluted by the high-water content of animal manures, potentially counteracting their inhibitory effects (Tawfik et al., 2023). Co-digestion has been shown to be an economically viable and attractive technology with the potential to increase biogas production rates, provide good synergism in the digestion reactor, and improve process stability (Hagos et al., 2017).

Moisture content

Studies have shown that maintaining an optimal moisture level is crucial for promoting microbial activity within the anaerobic digester (Mir et al., 2016; Mao et al., 2015). Too high or too low moisture content can negatively affect the microbial consortium and lead to reduced biogas production. Optimal moisture levels support the degradation of organic matter and enhance breakdown of complex compounds, contributing to efficient gas production (Khalid et al., 2011). In terms of process control, researchers have explored different methods for adjusting and monitoring moisture content in agricultural waste digesters. Techniques such as adding water or recirculating effluent have been studied to maintain the desired moisture levels and ensure stable digestion (Lorimor et al., 2006). Additionally, researchers have investigated the impact of moisture content on digester performance under different feedstock compositions and operating conditions. The effect of seasonal variations and climatic conditions on moisture content in agricultural waste digestion has also been examined in some studies (Rasi et al., 2011; Gontard et al., 2018). Furthermore, the literature review emphasizes the importance of balancing moisture content with other process parameters, such as temperature, pH, and retention time, to optimize anaerobic digestion performance (Naik et al., 2014).

Ash

The ash content, which comprises inorganic components including minerals and trace elements, holds significant relevance in the context of anaerobic digestion and its subsequent implications (Hagos et al., 2017). It plays a pivotal role in determining the overall efficiency and performance of anaerobic digestion. Unlike the organic components of agricultural waste, the inorganic constituents do not actively participate in the process of biogas production (Hagos et al., 2017). Consequently, the accumulation of these inorganic materials in the digester can result in operational challenges and decreased efficiency. The composition of the agricultural waste feedstock can significantly contribute to the ash content in the effluent. Materials with naturally high mineral content, such as certain types of crop residues or manures, can elevate the overall ash levels when subjected to anaerobic digestion (Antoniou et al., 2019). The presence of high ash content can result in increased maintenance requirements, necessitating more frequent cleaning and potentially causing clogging of pipes and equipment within the system (Van Lier et al., 2010).

Retention (or residence) Time

Solid retention time (SRT) and hydraulic retention time (HRT) are two essential parameters in the anaerobic digestion process, determining the length of time organic material remains in the digester to produce biogas (Chen et al., 2021). SRT refers to the time bacteria and sediments are present inside the digester, while HRT represents the time the input slurry spends within the digester from entry to exit. Different technologies, process temperatures, and waste compositions necessitate varying retention times for the anaerobic digestion reactions to complete (Chen et al., 2021). For mesophilic digesters processing wastes, the typical retention period ranges from 10 to 40 days, while thermophilic-operated digesters require shorter retention durations (Dumitru, 2017). Specifically, the retention period for a high solids' reactor operating in the thermophilic range is usually around 14 days. Overall, the retention duration for anaerobic digestion falls between 14 and 30 days. In order to prevent the washout of methanogens, which have a longer production time, it is crucial to maintain an SRT longer than 12 days (Nges & Liu, 2010). Choosing the appropriate retention time involves striking a balance between maximizing biogas production per volume and achieving sufficient organic matter degradation. While a shorter retention period may lead to greater biogas production, it may compromise the level of organic matter breakdown. Therefore, operational conditions must be carefully considered, and a compromise may be necessary to achieve the desired outcomes, even if it means adjusting the retention time to optimize the digester volume.

Organic Loading Rate (OLR)

The amount of organic matter fed to continuous anaerobic digesters on a daily basis is referred to as the loading rate (Nkuna et al., 2022). However, the anaerobic digestion process might be hampered if a digester is overloaded with waste. Rapid hydrolysis and acidification of wastes results in a buildup of high volatile fatty acids (VFAs), which may restrict methanogenesis and impair the digester's overall functionality (Meegoda et al., 2018). A drop in pH, a reduction in chemical oxygen demand (COD), and a loss in the rates of biogas generation, for instance, were observed in investigations on olive mill waste digesters that were operating at greater loading rates (Meegoda et al., 2018). It is necessary to have a bigger land footprint for high solids batch digesters because the loading rate can only be half that of ordinary single-stage reactors. The digesters' tolerance to higher loading rates allowed methane outputs to eventually revert to normal levels. According to studies on digesters for grease waste that were overloaded, microbial populations may alter when loading rates are suddenly changed. It's interesting to note that after an initial overloading occurrence, it has been suggested that a greater diversity of methanogenic bacteria is the cause of improved digester efficiency and resilience to overloading (Regueiro et al., 2015). It is evident that maintaining an appropriate loading rate is essential for ensuring the stability and efficiency of anaerobic digesters. By carefully managing the loading rate and understanding its impact on microbial communities, digester operators can optimize biogas production and overall system performance while avoiding potential disruptions caused by overloading.

pH

Anaerobic digestion requires careful pH control to ensure its efficiency. There are two distinct groups of bacteria based on their pH preferences: acidogens and methanogens. Acidogens thrive best within a pH range of 5.5–6.5, while methanogens prefer a pH range of 7.8–8.2 (Abouelenien et al., 2014). When these cultures are combined, the optimal operating pH falls within 6.8–7.4, with a neutral pH being the most favorable condition (Bajpai & Bajpai, 2017). Methanogenic bacteria play a critical role in breaking down complex organic substrates during anaerobic digestion, but they are particularly sensitive to low pH levels (Khalid et al., 2011; Christy et al., 2014). Process instabilities and the buildup of volatile fatty acids might result from some changes in the digester conditions of operation or the addition of hazardous substances. The pH can fall below the optimal range if the system's buffer capacity (alkalinity) is insufficient, turning the digester "sour" and resulting in a steady decrease in biogas generation (Ciotola et al., 2014). The pH of the digester's effluent can, however, slightly rise in a well-functioning system because bacteria produce alkalinity when eating organic substances, particularly those high in proteins (Bajpai, 2017).

Temperature

Temperature is a very important and critical environmental factor that significantly impacts the performance of anaerobic digestion (Angelidaki et al., 2003). Different types of methanogens all have distinct ideal temperature ranges, with hyperthermophilic methanogens preferring temperatures above 60 °C, thermophilic methanogens thriving at 45–60 °C, mesophilic methanogens operating best at 20–45 °C, and psychrophilic methanogens functioning below 20 °C (Barasa, 2021). Biogas generation is accelerated at higher temperatures, and anaerobic digestion becomes practically inactive below 10 °C, making mesophilic and thermophilic temperature ranges the most relevant for the process (Deepanraj et al., 2014). Maintaining a steady temperature is crucial as the available bacteria in the digester are sensitive to temperature changes. Thermophilic bacteria exhibit greater efficiency in terms of retention time, loading rate, and gas output compared to mesophilic bacteria, but they need more heat input and are more susceptible to environmental fluctuations

(Deepanraj et al., 2014). Thermophilic digestion, operating at higher temperatures, leads to increased reaction rates, potentially enabling higher loading rates and enhanced biogas production (Meegoda et al., 2018). Moreover, thermophilic digestion is advantageous for pathogen destruction at higher rates, which can be beneficial in regions with strict regulations on effluent pathogen activity. Despite these advantages, mesophilic digesters are still appealing due to their lower heater energy costs, even though they operate at slower rates and produce less biogas compared to thermophilic digesters (Deepanraj et al., 2014). Some digesters rely on ambient temperatures and do not require additional heating, leading to seasonal variations in methane production (Kandhro et al., 2022).

Mixing

To ensure homogeneity and process stability in the digester, mixing is necessary (Singh et al., 2021). By mixing, you may prevent the production of scum and thermal stratification in the digester as well as blend newly incoming material with microorganisms. The process of mixing keeps other environmental parameters, such as substrate concentration and temperature, uniform. Additionally, it avoids solid deposition at the digester's bottom (Bryant, 2019). Mechanical stirrers or centrifugal pumps can be used to recirculate the digester slurry to mix the materials (Kumar & Ramanathan, 2021). The microorganisms can be disrupted by rapid mixing, so slow mixing is recommended. Also, the kind of reactor and the quantity of solids in the digester affect the kind of mixing device utilized and the amount of mixing that is done.

Volatile fatty acids (VFA)

Anaerobic digestion uses volatile fatty acid (VFA) content as a crucial process efficiency indicator, which necessitates vigilant monitoring (Bajpai, 2017). VFAs, with acetic acid/acetate being the most common, play a crucial part in the digesting process, as do propionic acid/propionate, butyric acid/butyrate, valeric acid/valerate, caproic acid/caproate, and enanthic acid/enanthate (Bajpai, 2017). The overall VFA content often stays below 500 mg/L of acetic acid in a digester that is well-designed and operating properly (Labatut & Pronto, 2018). The production of biogas may be inhibited at VFA concentrations greater than 1,500–2,000 mg/L, albeit this concentration may rise if the digester's capacity is insufficient to handle the organic load (Rajagopal et al., 2013; Neshat et al., 2017). Instead of focusing solely on a specific concentration, close attention should be paid to sudden and sustained increases in VFAs in the effluent, as they can indicate potential digestive issues. Regular monitoring of VFAs is essential to detect problems early and implement necessary operational improvements to prevent digester failure.

Molecular hydrogen

Molecular hydrogen, along with VFAs, is one of the most sensitive parameters to process disruptions in anaerobic digestion (Labatut & Gooch, 2014). The degradation of propionate, for instance, requires low energy and demands partial hydrogen pressures below 10-4 atm at 25 °C (Kim et al., 2002). Such low hydrogen partial pressures in AD systems are achievable only through the collaborative interactions of hydrogen-producing bacteria and hydrogen-oxidizing methanogens, a process known as syntrophy (Kim et al., 2002). Maintaining a balance between these two types of microorganisms is crucial to prevent disturbances in the digestion process (Wu et al., 2021). However, accurately measuring molecular hydrogen can be challenging due to its low concentrations in AD systems, requiring specific equipment and techniques (Labatut & Gooch, 2014). Regular monitoring of molecular hydrogen levels is essential to identify potential issues early and implement appropriate adjustments to ensure the stability and efficiency of the anaerobic digestion process (Wu et al., 2019).

Total Solids (TS)

Total solid (TS) is a term utilized to describe the dry matter content in sludge, encompassing both organic and inorganic components (Owusu-Twum & Sharara, 2020). It is commonly expressed either as a percentage or a concentration in various literature. To determine the TS concentration, a sludge sample undergoes a drying process at temperatures of 103-105°C until no further weight change is observed (Drosg, 2013). Besides being an influent parameter, TS plays a crucial role in evaluating digester performance. Recently, there has been significant interest in high-TS anaerobic digestion due to its potential advantages, such as the requirement for fewer digesters and reduced heating demand (Zamri et al., 2021). Studies have shown that continuous high-TS digesters operating under the same retention time can yield higher biogas outputs compared to low-TS digesters (Meegoda et al., 2018). This focus on high-TS digestion highlights its potential to improve the efficiency and sustainability of anaerobic digestion processes.

Volatile solids (VS)

Waste treated by anaerobic digestion (AD) may consist of three main fractions: combustible fraction, biodegradable organic fraction, and inert fraction. The biodegradable organic fraction includes food waste, agricultural waste, and kitchen wastes, which are readily degradable by microorganisms during AD (Ebner et al., 2016). The combustible fraction comprises slowly deteriorating lignocellulosic materials, such as wood, paper, and cardboard, which are better suited for waste-to-energy facilities due to their limited decomposition under anaerobic conditions (Nalo et al., 2014). The inert fraction contains non-biodegradable materials like metal, glass, and sand, which should ideally be eliminated, recycled, or properly disposed of before AD. Failure to remove the inert fraction can increase the volume of the digester and lead to equipment wear (Verma, 2002). The system's structure and the physicochemical properties of the substrate play an important role in determining the percentage of organic matter stabilized. In manure-only digesters, the VS stabilization percentage ranges from 30 to 42% (Labatut & Gooch, 2014). Systems co-digesting manure with other high-strength substrates may exhibit higher percentage stabilization, although the magnitude can vary based on the co-substrates used (Labatut & Gooch, 2014).

Total ammonia-nitrogen (TAN)

Ammonia can be produced during the anaerobic digestion of protein-rich substrates like pig or cow manure. Ammonia and volatile fatty acids (VFAs) have the ability to obstruct and lessen the effectiveness of the digestive process. The digestive process can be inhibited by elevated ammonia-N concentrations exceeding 1,500 mg/L at high pH levels (i.e., > 7.4) (Labatut & Gooch, 2014). The inhibitory effects of ammonia in anaerobic digestion are attributed to its ability to disrupt the microbial activity within the digester. Ammonia can negatively affect methanogenic microorganisms, which are responsible for biogas production, and this will result in a reduction in biogas yields (Gao et al., 2015). Moreover, elevated ammonia concentrations can cause shifts in microbial communities, favoring ammonia-oxidizing bacteria over methane-producing archaea, further hampering the biogas production process (Sitthakarn, 2022). However, some manure systems have shown the ability to adapt to higher ammonia levels (>5,000 mg/L) (Labatut & Gooch, 2014). The adaptability of anaerobic digesters to high ammonia levels could be influenced by several factors, including reactor design, microbial diversity, and process parameters. Proper reactor design, efficient mixing, and adequate retention time can create favorable conditions for ammonia-tolerant microorganisms to thrive (Mao et al., 2015). Additionally, maintaining a diverse and robust microbial community can enhance the resilience of the digester to ammonia inhibition (Carballa et al., 2015). Managing ammonia levels is very important to ensure the successful and optimal performance of anaerobic digestion when dealing with protein-rich substrates.

Production of biogas during anaerobic digestion

The outcome of anaerobic digestion, known as biogas, consists of CH_4 and CO_2, with traces of additional gases such as ammonia nitrogen, and hydrogen sulphide (Demirbas & Ozturk, 2005). When a putrescible material is depleted, most of the biogas is produced in the middle of digestion, when bacterial population has increased (Adegunloye & Oladejo, 2010). The gas is often recovered and kept in a gas container next to the facility or held on top of the digester in an inflatable gas bubble. The amount of methane produced indicates how efficient the digester is operating. The quantity of volatile solids

(VS) as reported by Appels et al. (2011) directly correlates with the amount of methane released during digestion. More crucially, the ability to produce energy (heat and electricity) increases as methane production increases. Over time, biogas production ought to remain constant. It is a strong indication of a digester upset if the biogas production falls below the average daily values (Appels et al., 2011). This may result from altered pH levels, foaming, odors, temperature fluctuations, solids and residue accumulation, floating layers, and pH buffering issues (Zaher et al., 2017).

Digestate

Digestate refers to the solid residue that remains after microorganisms in the digesters have processed the original material, but certain components remain unconsumed (Zupančič & Grilc, 2012). This residue also includes mineralized byproducts of dead bacteria from the digestion process. Anaerobic digestion produces digestate that can be effectively recycled as a sustainable fertilizer for vegetable cultivation in agriculture (Samoraj et al., 2022). During anaerobic digestion, complex organic nitrogen compounds from the feedstocks undergo mineralization, transforming into ammonia. Only minimal amounts of the ammonia nitrogen (less than 1%) are volatilized in the biogas, with the most of it being used for development by the digester bacteria and the creation of struvite and ammonium carbonate. Likely recyclable is the organic phosphorus found in the leafy feedstocks used for anaerobic digestion. According to Möller and Müller, (2012), the degradation mechanisms in anaerobic digestion have improved the availability of phosphorus for plant utilization. Other crucial minerals including potassium, sulphur, organic metals (calcium, magnesium), and micronutrients are also present in anaerobic digestate. Numerous research (Bacenetti et al., 2016, Riya et al., 2020, Tayibi et al., 2021) have investigated the use of digestate from anaerobic digestion as a beneficial fertilizer for different agricultural uses.

Selected physicochemical parameters

The physical and chemical parameters under discussion include COD, BOD nitrate, phosphorus, chloride, ammonia and nitrogen.

Chemical oxygen demand (COD)

Agricultural waste, such as crop residues, animal manure, and food processing by-products, can contain a significant amount of organic compounds, including carbohydrates, fats, and proteins. These compounds contribute to the COD content of the waste. High COD concentrations in agricultural waste can have adverse effects on the environment when released untreated, leading to oxygen depletion in water bodies and subsequent ecosystem impacts (Mushtaq et al., 2020). Anaerobic digestion has shown effectiveness in treating COD in agricultural waste. According to Mathew et al. (2015), anaerobic digestion processes have demonstrated significant reductions in COD concentrations. The microbial consortium in anaerobic digesters plays an important role in the breakdown of organic compounds and the conversion of complex substrates into simpler compounds (Lohani & Havukainen, 2018). The efficiency of COD removal depends on various factors, including feedstock composition, hydraulic retention time (HRT), and temperature. A Study by Mata-Alvarez et al. (2014) has shown anaerobic digestion's efficiency in treating COD in agricultural wastes.

Biochemical Oxygen Demands (BOD)

According to Dogan et al. (2009), BOD is a measure of the amount of oxygen which microorganisms require to biologically degrade organic compounds in wastewater or water. BOD is an important parameter for assessing the organic content and pollution potential of agricultural wastes. According to Koul et al. (2022), agricultural waste, including crop residues, animal manure, and food processing by-products, can contain a significant amount of organic compounds, including carbohydrates, fats, and proteins. These compounds contribute to the BOD content of the waste. High BOD concentrations in agricultural waste can have adverse effects on the environment when released untreated, leading to oxygen depletion in water bodies and subsequent ecosystem impacts (Manasa & Mehta, 2020). Anaerobic digestion has shown effectiveness in treating BOD in agricultural waste as several by Liu and Haynes (2011) and Manasa and Mehta (2020) has demonstrated significant reductions in BOD concentrations during anaerobic digestion processes. The efficiency of BOD removal depends on various factors, including feedstock composition, hydraulic retention time (HRT), and temperature (Selormey et al., 2021).

Nitrogen Compounds

In the anaerobic digestion of agricultural wastes, total nitrogen plays a crucial role as it exists in various organic and inorganic forms. The nitrogen content in agricultural waste can come from proteins, nucleic acids, and other nitrogen-containing compounds present in organic matter. During anaerobic digestion, microorganisms break down the organic matter in the waste in the absence of oxygen, leading to the release of nitrogen compounds (Mata-

Alvarez 2003). The nitrogen content in agricultural waste undergoing anaerobic digestion can undergo complex transformations, with some nitrogen being released as nitrogen gas (N_2) and ammonia (NH_3) or being retained in the digestate as ammonium (NH_4^+) or other nitrogen compounds (Duan et al., 2020). Managing nitrogen in anaerobic digestion is crucial, as excessive nitrogen losses can lead to reduced biogas production, increased greenhouse gas emissions (due to ammonia volatilization), and environmental pollution in the form of nitrogen-rich effluents (Provolo et al., 2017). The presence of specialized microorganisms, such as anammox bacteria and denitrifying bacteria, contributes to the removal of nitrogen compounds by converting them to nitrogen gas (Saha et al., 2022). Barampouti et al. (2020) reported that, technologies such as ammonia stripping and nutrient recovery systems can be employed to recover nitrogen from the digestate, minimizing its release into the environment and providing a valuable resource for fertilizer production.

Ammonia

Organic nitrogen molecules found in agricultural waste can be transformed into ammonium by a variety of microbial activities. Due to its tendency to seep into water bodies and contribute to nutritional imbalances, the presence of ammonium in agricultural waste presents difficulties (Loehr, 2012). Anaerobic digestion procedures have been shown to result in considerable drops in ammonium concentrations. A lab-scale investigation utilising reactors with working volumes of 4.5 L operated at 30 °C was conducted to examine the anaerobic digestion of potato juice at high ammonia concentrations (Koster, & Lettinga 1988). According to the scientists, the microbial community could create methane once it had adapted, which meant it had developed the capacity to do so at ammonia concentrations higher than the threshold level. This proved that toxicity could still be recovered even at extremely high ammonia concentrations. Despite the fact that ammonia can impede a process, Labatut and Gooch (2014) claim that managing and keeping an eye on factors like pH, temperature, and ammonia levels in the substrate and digester can ensure safe and reliable operation, most especially when anaerobic digestion of substrates like animal waste is being conducted (particularly pig waste, cow manure, and poultry manure). It has been demonstrated that the anaerobic digester may function even at extremely high concentrations of ammonia without endangering its safety since the microbial community gradually becomes accustomed to higher volumes of ammonia (Yenigün & Demirel, 2013).

Chloride

A typical anion found in agricultural waste, including animal dung and crop wastes, is chloride (Cl-). According to Ilyas et al. (2019), agricultural waste with high chloride concentrations can degrade the quality of the soil and water. Research has demonstrated significant reductions in chloride concentrations during anaerobic digestion processes. The precipitation of chloride as insoluble salts, such as calcium chloride ($CaCl_2$), helps minimize chloride concentrations in the effluent. The presence of divalent cations and the anaerobic conditions contribute to the reduction of chloride concentrations. Further research is needed to optimize anaerobic digestion processes for chloride removal, considering factors such as waste composition, digester design, and additional treatment technologies. Monitoring and management of chloride levels in the effluent are crucial to ensure environmental compliance and protect the receiving water bodies from any adverse impacts (Ilyas et al., 2019).

Nitrate

The nitrogen ion nitrate (NO3-), which can be found in crop wastes and animal faeces, is typically present in agricultural waste. According to Mateo-Sagasta et al. (2017), inadequate management of high nitrate concentrations in agricultural waste can lead to eutrophication, water contamination, and health risks. The possibility of using anaerobic digestion to handle nitrate in agricultural waste, transform it into a more manageable form, and lessen the environmental impact of the process has been investigated (Szogi et al., 2015). According to studies (Ghyselbrecht et al., 2019; Akunna et al., 1994), anaerobic digestion procedures result in large decreases in nitrate concentrations. According to Kraft et al. (2011), the conversion of nitrate to nitrogen gas depends critically on the presence of particular microbial groups such denitrifying bacteria and anammox bacteria.

Phosphorus Compounds

The nutrient phosphorus, which is crucial for plant growth, is frequently a limiting element in agricultural systems. But too much phosphorus in agricultural waste can contaminate water supplies and cause eutrophication. Various types of phosphorus compounds can be found in agricultural waste, including animal dung, crop wastes, and food processing by-products (Maji et al., 2020). These include both inorganic phosphates like orthophosphate and polyphosphate and organic phosphates like phytate and nucleotides. If not effectively managed, the presence of these chemicals in agricultural waste leads to nutrient imbalances and associated environmental hazards (Silva et al., 2023). By recovering phosphorus from the digestate, struvite, a slow-release phosphorus fertilizer, can offer an extra benefit (Siciliano & Rosa, 2014). Anaerobic digestion is effective in lowering the quantities of phosphorus in agricultural waste, and studies by Xu et al. (2018) and Bryant (2019) have demonstrated this possibility.

The involvement of microorganisms in anaerobic digestion process

A wide variety of microorganisms perform crucial roles in the breakdown of complex chemical molecules into simpler forms during anaerobic digestion (Khalid et al., 2011). Sludge, naturally chosen strains, or purposefully mixed strains of microorganisms can all be used as inoculum sources, which are essential for maximizing the waste-to-inoculum ratio (Ali Shah et al., 2014). Additionally, aggregates of cells with diameters ranging from 0.1 to 100 mm, such as flocs, biofilms, granules, and mats, can be used to facilitate the treatment procedure (De Beer & Stoodley, 2006). Anaerobic digestion is done by diverse communities of microbes, each of which contributes to a different stage of the procedure. For instance, while Clostridium species are frequently dominating among the degraders in anaerobic environments, heterotrophic microbes are principally responsible for degrading organic material (Ali Shah et al., 2014). It is interesting to note, however, that anaerobic digestion rarely depends exclusively on one microbial strain; rather, a wide range of microbial species work together to carry out the process effectively (Mutungwazi et al., 2021).

According to reports, a large number of syntrophic Firmicutes bacteria produce VFAs such acetic and butyric acids by the hydrolysis of different organic substrates found in food waste or agricultural waste (Qin et al., 2021). While butyric acid (C3H7COOH) is used by some of the Firmicutes genera, acetic acid is the main ingredient for biomethane synthesis by acetoclastic methanogenesis. Similar to this, several Porphyromonadaceae species have been shown to be able to use the protein in food waste to produce VFAs like acetic, isobutyric, propionic, and isovaleric acids (Pan et al., 2021). Porphyromonadaceae may contribute to the production of methanogen-related biogas.

According to Kurade et al. (2019), Syntrophomonas is one of the most frequently seen dominant bacteria (Syntrophomonadaceae family) for the quick metabolism of long-chain fatty acids into acetates, which were then converted to methane by acetoclastic methanogens in a syntrophic relationship. If the amount of oils, fats, and greases in the feeding material were raised to 3%, Syntrophomonas was said to grow to make up 15% of the entire bacterial community (Amha et al., 2017). Euryarchaeota, whose genera mostly contain acetoclastic and hydrogenotrophic methanogens, should be the most frequently seen phylum for the archaeal communities in an AD system. Methanosaeta spp. are the most prevalent acetoclastic methanogens and are frequently found to be dominant in stable mesophilic methanogenic systems. A common occurrence in an AD system is the presence of hydrogenotrophic methanogens like Methanolinea, Methanospirillum, Methanobacterium, and Methanoculleus (Gonzalez-Martinez et al., 2016). But it is crucial to remember that large levels of organic acids in the biodigester, such as acetic acid (>5000 mg/L) and butyric acid (>3000 mg/L), can stunt the development of microorganisms and obstruct the creation of molecules rich in energy (Li et al., 2015). Gahlot et al. (2020) also critically examined Direct interspecies electron transfer (DIET), a particular component of AD's microbiology, and subsequently proposed concepts for re-engineering digester design practices. Due to the DIET advancement, they discovered that the use of electrically conductive materials (such carbon fibre and suspended carriers composed of graphite, for instance) may considerably increase the performance of an AD system. However, the economic viability of digesters using these materials which are conductive, must be considered carefully, as they are only doable if the conductive elements are placed permanently inside the digester.

Pathogenic Microorganisms

Microorganisms that have the ability to infect humans, animals, or plants with disease are known as pathogenic microorganisms. They are dangerous and can seriously endanger the health of organisms. The presence of harmful bacteria in the raw waste material is a problem in the context of anaerobic digestion of agricultural wastes. Escherichia coli (E. coli) and Salmonella spp., which are frequently linked to faecal contamination, can be found in agricultural wastes (Jones, 1999). However, the pathogen concentration of the waste can be greatly decreased during the anaerobic digesting process itself.

Total Coliforms

Total coliforms are a group of bacteria that are commonly used as indicators of the microbial quality of water and environmental samples. They are a subset of coliform bacteria, which are gram-negative, rod-shaped bacteria that can ferment lactose to produce gas. Total coliforms include various species, such as E. coli and Enterobacter aerogenes, among others

(Feng et al., 2002). The presence of total coliforms in water or other environmental samples indicates the potential contamination by fecal matter or other sources of bacteria. The presence of total coliforms in water or other environmental samples indicates the potential contamination by fecal matter or other sources of bacteria (Gerba, 2009). In the context of anaerobic digestion of agricultural wastes, total coliforms are often used as a parameter to assess the hygiene and safety of the waste material. Anaerobic digestion can significantly reduce the total coliform content in the treated waste, contributing to the reduction of potential disease transmission and environmental contamination risks (Lin et al., 2022).

Salmonella typhi

Salmonella spp. is a bacterial pathogen commonly found in agricultural waste, including animal manure and crop residues (De Corato, 2020). If not properly treated, Salmonella can contaminate soil and water sources, posing a severe health risk to humans and animals. Anaerobic digestion has been shown to be an effective method for treating agricultural waste and reducing the presence of Salmonella (Costa et al., 2017). The controlled temperature and pH during anaerobic digestion can reduce the survival of pathogenic bacteria such as Salmonella (Sahlström, 2003). Studies have shown that anaerobic digestion can reduce Salmonella levels in animal manure (Manyi-Loh et al., 2014) and crop residues (Guo et al., 2017). In addition to reducing pathogenic bacteria, anaerobic digestion can also help reduce greenhouse gas emissions and the volume of agricultural waste (Wilkie, 2005). However, the effectiveness of anaerobic digestion in reducing Salmonella in agricultural waste may vary depending on the operational conditions and feedstock characteristics (Qi et al., 2018; Lin et al., 2022). Optimization of the anaerobic digestion process, such as selecting the appropriate temperature, pH, and retention time, is crucial for achieving the desired reduction in Salmonella levels. The use of anaerobic digestion in combination with appropriate operational conditions and feedstock characteristics can help ensure safe and sustainable agricultural waste management practices.

Escherichia coli

Agricultural waste, such as crop residues and animal manure, can contain high levels of *E. coli* (Hutchison et al., 2005). When these wastes are improperly treated or disposed of, E. coli can contaminate soil and water sources. E. coli contamination can cause severe health problems in humans, including diarrhea, vomiting, and even kidney failure (Gambushe, et al., 2022). In addition, *E. coli* can also cause infections in livestock, leading to decreased productivity and economic losses for farmers. Pathogenic bacteria like E. coli may not survive as long when the temperature and pH are regulated during anaerobic digestion (Qi et al., 2019). Anaerobic digestion has been shown to be beneficial in lowering E. coli in agricultural waste in a number of investigations. For instance, Matos et al. (2021) discovered that anaerobic digestion decreased the amount of E. coli in pig slurry by 99.9%. Anaerobic digestion was also found to reduce E. coli levels in fuel pellet production wastewater by 89%, according to a study by Cathcart et al. (2022). Additionally, during the course of the entire storage time, Luo et al. (2017) discovered fewer total coliforms and Escherichia coli in both digested slurries. According to them, the higher concentration of ammonium nitrogen, which may inhibit gram-negative bacteria, was to blame for the faster reduction rate that was noticed during the storage of chicken manure-digested slurry. Anaerobic digestion is advantageous for managing agricultural waste since it reduces the amount of E. coli. By generating biogas, which can be utilized to generate electricity, anaerobic digestion can aid in the decrease in greenhouse gas emissions. Agricultural waste can be handled and disposed of more easily by anaerobic digestion since it can be handled in smaller quantities.

Heavy Metals

The longevity, toxicity, and potential for bioaccumulation of heavy metals in agricultural waste raises concerns. Animal waste is rich in organic matter and nutrients like nitrogen and phosphorus (Liu et al., 2017), but it's important to remember that it also contains a significant amount of heavy metals (HMs) like copper, zinc, lead, asernic, chromium, nickel, and cadmium (Li et al., 2020), which are non-degradable and extremely toxic (Awasthi et al., 2021). These HMs originate from plants used as feed for animals that have been tainted by HMs in the soil. Additionally, some HMs derive from additives added to feed for livestock (Chen et al., 2020). In order to prevent sickness, reduce death rates, and improve growth rate, growth promoter compounds are commonly included in animal feed, which contains a high level of HMs (Awasthi et al., 2021). The health of ecosystems, plant development, and soil quality can all be adversely affected by heavy metals. In addition, crops can allow heavy metals to infiltrate the food chain, endangering the well-being of humans. According to studies, anaerobic digestion can lower the amount of heavy metals present in the waste, improving the safety of the resulting digestate for use on land. Heavy metals may be removed from the liquid fraction during anaerobic digestion through precipitation and adsorption onto digester solids. Anaerobic digestion is effective at lowering agricultural waste's levels of heavy metals like Cd, Pb, and Cu, according to research by Inyang et al. (2012). The transformation and immobilization of heavy metals during anaerobic digestion is also greatly aided by microorganisms. Some microbial species have the capacity for complexation, detoxification, and metal reduction, which helps with the removal of heavy metals.

Economic importance of anaerobic digestion of agricultural wastes

Anaerobic digestion (AD) systems have several financial benefits, including a reduction in fossil fuel costs for waste management due to the use of biogas, electricity, and heat; the ability to make money by selling excess energy; and cost savings due to lower fertilizer inputs while simultaneously improving soil fertility and structure (Herbstritt et al., 2023). Nevertheless, a variety of factors, including feedstock types and compositions (Vasco-Correa et al., 2018), digester scale (Mahmudul et al., 2021), operating conditions (Vasco-Correa et al., 2018), incentives from governments, and potential product utilization (Wainaina et al., 2020), have an impact on the economics of AD systems around the world. Furthermore, depending on the region and the time of year, different amounts of energy are required to keep digester temperatures constant (Singhal et al., 2022). The full utilisation of energy products, gate fees for waste acceptance, revenue from co-products like compost/organic fertiliser, and the potential sale of carbon credits earned by offsetting greenhouse gas emissions are all ways that a farm-based AD system can prove to be profitable (Vasco-Correa et al., 2018). Effective management of the AD plant and farm might result in the sharing of resources, such as labour and equipment, which will improve the economics of both systems. Assuring year-round maximum capacity operation for AD systems is a major challenge (Tiwary et al., 2015). Long-distance travel can raise the cost of producing biogas and its related emissions; therefore logistics of feedstock and product transportation are crucial to the economic and environmental viability of AD systems (Gebrezgabher et al., 2010). Additionally, in some areas, such as Europe, most AD systems are built with the aid of government subsidies and various state incentive programmes (Haas et al., 2011). Due to the heterogeneity of these particular parameters, it is very important to assess the economic sustainability of AD systems.

CHAPTER THREE

MATERIALS AND METHODS

The many methods and strategies used for data collection and analyses are covered in this chapter. This chapter covers in detail the research design, study site, construction of the mesophilic single-stage biogas digester, data collection strategy, and laboratory techniques used for parameter analyses.

Study area

The investigation was done at the University of Cape Coast's school of agriculture teaching and research farm, which is in the Cape Coast North District, of Ghana's Central Region. The school farm is a teaching and research center for the university. The location of the research center at sea level is roughly at latitude 5°06'19.3"N and longitude 1°14'47.8"W. The university is situated at five kilometers to the west of Cape Coast and offers views of the Atlantic Ocean. It is divided into two campuses, the Northern Campus (New Site) and the Southern Campus (Old Site). The farm is into rearing animals such as cows, pigs and fowls and growing of crops such all kinds of vegetables and fruits.

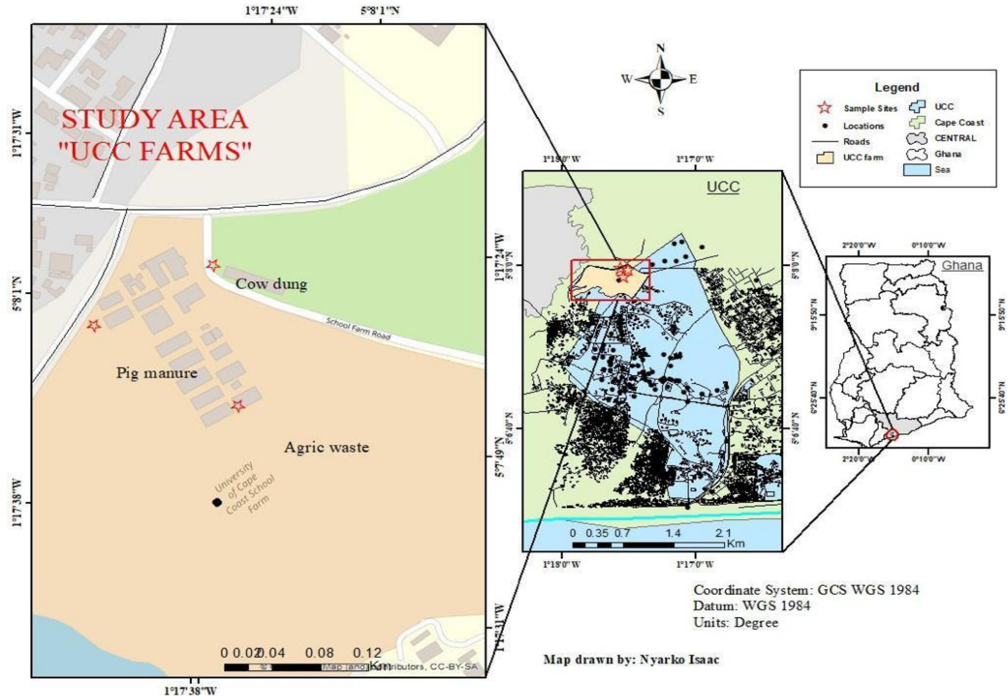


Figure 11: Map of the study area

Design of the pilot-scale single-stage biogas digester

The digester chosen for the study is a fixed dome pilot-scale single-stage biogas digester. This setup consists of a closed dome-shaped structure with a feedstock input, a displacement pit (also known as the compensation tank), and stationary gas holding. The upper section of the digester is a biogas holder used to hold the gas created during anaerobic digestion. A closed outlet gas valve controls the digester's internal gas pressure, which varies according to the amount of gas produced and used. The pressure drops when the gas valve is opened for gas use, and a proportional amount of slurry flows back into the digester from the compensating tank. Depending on the production and use of the gas, this design provides continual adjustment in gas pressure. The fixed-dome plant is often built underground, which insulates from temperature changes and counteracts the internal pressure in the digester with the surrounding earth. The specific single-stage biogas digester used in the study has an active reactor volume of 6 m^3, and its overall capacity is 8 m^3.

The reactor has an inner diameter of 1.1 meters and a height of 2.1 meters, and it is made of concrete and iron rods. It has an effluent outflow which is 0.7 m-in diameter attached to the reactor's side where wastewater flows out of the system. The reactor contains a manually operated stirrer to ensure the influent and active sludge are constantly mixed inside the reactor, as well as a thermometer probe to measure the temperature. A concrete opening with a diameter of 0.5 m is also present for manual feeding of the reactor. The Mesophilic Single-Stage Biogas Digester (MSSBD) used in the experiment is depicted in Figure 11.

Figure 12: Picture of the newly constructed pilot-scale single-stage biogas digester

Collection of Inoculum (cow dung) and preparation

The inoculum was selected based on recommendations made by Bryant (2019) and Gupta et al. (2016). About 5000 L of cow dung collected from the school of agriculture teaching and research cow farm was fed into the system as an inoculum. The quantity of the cow dung collected from the farm was 1667 kg and was mixed 3333 L of tap water. The ratio was 1:2 (cow dung: water). The characteristics of the inoculum were determined using standards provided by (APHA AWWA WEF 2012). The average characteristics of the inoculum were as follows: pH: 7.283±.025, COD: 134000.000±17320.508, BOD: 51166.667± 4041.452, TS: 23.867±0.143, VS: 84.500±0.414.

Collection of Feedstocks and preparation

The AW were collected from the school of agriculture teaching and research piggery and crop farm at the university of Cape Coast. Pig manure (90 %), cabbage leaves (3 %), carrot leaves (3 %), jute leaves (1.5%), amaranth plant (1.5 %), and spinach (1 %) were used as the representative agricultural wastes for the AD. The Cabbage leaves, jute leaves, amaranth plant leaves, spinach and carrot leaves were shredded into smaller sizes using kitchen knife and blended. The pig manure together with the blended cabbage leaves, jute leaves, amaranth plant leaves, spinach and carrot leaves were homogenized before feeding it into the digester. The AW was diluted with tap water in a ratio of 1:1 before feeding it into the digester. The digester was fed with 230 L, 260 L and 300 L of AW per day for all the three different HRTs (26, 23 and 20) respectively. Key parameters selected physicochemical parameters such as, COD, BOD TS VS pH, nitrate chloride, phosphorus, and ammonia, pathogenic microorganisms (*Escherichia. coli and Salmonella* *spp.*), and heavy metals (nickel, chromium, cadmium, lead and zinc) present in these feedstocks were analysed in triplicate before feeding it into the digester.

Data collection

To determine the treatment efficiency of the biogas digester and collection of data, agricultural wastes (AW) samples were collected for laboratory analyses. The analyses were done before and after treatment. The analyses were based on the measurement of some selected physicochemical parameters, (COD, BOD TS VS pH, nitrate chloride, phosphorus, and ammonia), pathogenic microorganisms (*Escherichia. coli and Salmonella spp.*), and heavy metals (nickel, chromium, cadmium, lead and zinc) all before feeding the substrate into and after treatment. Analyses were done in triplicate for each sample at each HRT.

Sampling Procedure

Sampling was performed weekly for each HRT of 20, 23 and 26 days and Flow Rates of 300 L/d, 260 L/d and 230 L/d respectively. All the parameters were analyzed before and after treatment. Collection of agricultural wastes samples was done using pre-treated plastic containers. Samples soon after collection were transported in a disinfected container to the laboratory for analysis. In all, 18 samples were evaluated; including triplicates samples of effluent and influent of AW for each HRT for the parameters to ascertain the efficiency of removal of the treatment system.

Determining of the selected parameters of the influent and the effluent

Moisture content determination

Exactly, 5 g of each sample was weighed using A&D Galaxy Analytical Balance, 252 g/0.1mg (Model: HR-250AZ, 1756 Automation

Parkway, San Jose, CA 95131, USA), and placed into porcelain crucibles, and oven (Memmert Beschickung/ Loading- Modell 100 – 800, Memmert GmbH, 91107 Schwabach, Germany) dried at 105 °C for 24 hours. The crucibles containing the sample was spread over the base of the oven to ensure equal distribution of heat. Thereafter, the heated samples were cooled in a desiccator and reweighed to obtain the mass of the dry sample. After cooling down, the samples were weighed. The moisture content was calculated using the formula below.

$$W = \frac{(a-b)}{a} \times 100 \qquad (1)$$

Where;

W = wet mass moisture content, %

a = initial mass of sample as delivered, kg

b= mass of sample after drying kg

Biochemical Oxygen Demand (BOD_5): Winkler Azide Modification Titrimetric Method

BOD_5 of all samples were analyzed following standard methods (APHA AWWA WEF 2012). About 1 g of the samples were diluted in 99 ml of distilled water. To a 500 ml Erlenmeyer flask, 5 mL of the sample was transferred and filled with deionized water to the brim, 1 mL $MnCl_2$ was added, followed by 3 mL alkali-iodide azide reagent. The bottle was stoppered and inverted severally to ensure proper mixing of the added chemicals with the sample. The precipitate was allowed for some few minutes to settle as flocs to give a clear supernatant of about 50-100 mL. The cork was gently removed, after allowing a brown manganese hydroxide floc to settle (white floc suggests absence of DO), 3.0 ml conc. H_2SO_4 was added and the bottle was restoppered and inverted several times till all the brown flocs were thoroughly dissolved, a clear bright yellow color indicated the end of the process. Titration was performed on 200 mL of the sample using 0.25 N Sodium thiosulfate as a titrant. The sample was titrated to a pale yellow in color. One-two drops of starch was added to the sample in the conical flask, and the color change of blue-black color was observed. The titration continued with the Sodium thiosulphate till a colorless end point was observed. The Average titer value were recorded for all the concordance burette reading. The final readings recorded was reported as the Initial Dissolved Oxygen (DO_1). Similar process was employed for the same sample, after the addition of the 3 mL acid, the obtained sample was incubated for 5 days in a dark bottle closed tightly at 20 ^oC to prevent air. After 5 days the sample was titrated against a 0.25 N Sodium thiosulphate till a colorless end point was achieved. The titer value recorded was the final dissolved Oxygen (DO_5). Samples were performed in triplicates to ensure that DO depletions after 5days was at least 2.0 mg/L DO and at least 1.0 mg/L DO residual. The BOD_5 is therefore calculated as:

BOD_5. 20 $^\circ$C= ($Initial\ concentration\ of\ oxygen\ in\ the\ sample$- $Final\ concentration\ of\ oxygen\ in\ the\ sample$) × $Dilution\ factor$………. (2)

Measurement of Chemical oxygen demand (COD) using Closed Reflux, Titrimetric method

To a 99 mL of distilled water, 1 g of the sample was diluted. Also, 1 ml of the sample was further diluted in 49 mL of distilled water. Approximately 2.5 mL sample was transferred into digestion tubes containing 1.5ml potassium dichromate ($K_2Cr_2O_7$) digestion solution and 3.5mL concentrated sulphuric acid (H_2SO_4). The samples were stoppered and inverted severally, and the ampules were allowed to cool. A sample blank was prepared in the same manner using di-ionized water. The ampules were place in a pre-heated block digester at 150 °C. The samples were refluxed at 150 °C for two hours. After 2 hours, the samples and blank were allowed to cool to room temperature and titrated against 0.10 M ferrous ammonium sulphate (FAS) using ferroin indicator. The endpoint was a sharp color change from blue-green-reddish brown. The COD of Agricultural wastes is calculated as:

COD as mg/L = ((B-A) × M ×8000) / (mL sample) …………………. (3)

Where:

B = mL FAS used for sample

A = mL FAS used for blank

M = molarity of FAS

8000 = milliequivalent weight of oxygen × 1000 mL/L

Determination of total nitrogen (Micro-Kjedahl method) by distillation

For roughly 30 minutes, steam was circulated through a steam distillation apparatus that had been set up. A 100 mL conical flask holding 5 mL of boric acid indicator solution was set underneath a condenser of the distillation equipment after 30 minutes had passed. Through the use of a trap funnel, an aliquot of the sample was introduced into the reaction chamber. 12 mL of the alkali combination were then added, and the distillation process got started right away. A total of 25 mL of the distillate were collected and titrated with M/140 hydrochloric acid (HCl) from green to the indicator's initial red wine colour.

$$N\,(\%) = \frac{(A-B)\times solutiion\ volume\ (mL)}{100\ \times aliquot\ (mL)\times sample\ weight\ (g)} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (4)$$

Where:

A = Sample titre value (mL)

B = Blank titre value (mL)

Determination of organic carbon applying modified Walkley – Black (partial oxidation) method

To determine the organic carbon content in the waste samples, approximately 1g of each sample was carefully weighed using a Mettler Toledo analytical balance (Model: PG203-S, 1900 Polaris Pkwy Columbus, OH 43240-4035, United States) and transferred into a labeled 100 mL conical flask. Next, 10 mL of 5% potassium dichromate solution was added to completely wet or dissolve the sample. Subsequently, 20 mL of sulfuric acid from a fast burette was added to the flask's contents and gently swirled for a minute, allowing it to stand for 30 minutes. After the 30-minute wait, 50 mL of 0.4% barium chloride was added to the mixture and swirled again to ensure thorough mixing. The resulting mixture was then subjected to centrifugation using a Gallenkamp laboratory centrifuge (made in England) for 10 minutes at 3000 rpm. An aliquot of the clear supernatant solution was carefully transferred into a colorimeter cuvette. The absorbance of each standard and sample was measured and recorded. The solution concentrations for each unknown and the blanks were determined using the recorded absorbance values. The organic carbon (OC) content was calculated following the method described by Motsara and Roy (2008).

$$OC\ \% = \frac{value\ \ for\ corrected\ concentration \times 0.1}{weight\ of\ sample \times 0.74} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(5)$$

Determination of total solid (TS), volatile solids (VS) and ash

The crucibles were carefully labeled and weighed using the A&D Galaxy Analytical Balance, Model HR-250AZ (252 g/0.1mg, 1756

Automation Parkway, San Jose, CA 95131, USA), and their weights were recorded as A1. The same analytical balance was used to weigh each waste sample that was collected from the farms, and the weight was recorded as A2. After that, the samples in the crucibles were dried for the entire night in a Memmert Beschickung/Loading-Modell 100-800 oven at 105 °C. After drying, the samples and the crucibles were taken out of the oven and placed in a desiccator to cool. The weight of each cooled sample was then recorded as A3 once it was reweighed. The dried samples were then placed in weighed crucibles and placed in a muffle furnace (Carbolite AAF/3, 1100 °C, S/N. 21-201189, UK) where they were ignited at 550 °C for two hours until they were reduced to ashes. After the two-hour process, the crucibles, along with the ashes, were taken out and cooled in a desiccator. Carefully, the crucibles with the ashes were reweighed, and the weight was recorded as A4. The volatile solids (VS) were then calculated as the difference between the dry weight of the solid waste and the weight of the residue after ignition. The fractions of total solids (TS), volatile solids (VS), and ash were computed following the method described by Baird et al. (2017):

$$TS\ \% = \frac{A3 - A1}{A2} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (6)$$

$$VS\ \% = \frac{mass\ of\ sample - mass\ of\ sample\ after\ ignition}{Mass\ of\ sample} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots (7)$$

$$Ash\ \% = \frac{A4 - A1}{A2} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (8)$$

Where:

A1 = crucible's dry weight (g)

A2 is weight of the wet sample plus the crucible (g)

A3 is weight of (sample + crucible) after 105 °C (g)

A4 is weight of (ash + crucible) after 550 °C (g)

Nitrate by UV Spectrophotometric Method

To quantify nitrate levels, a 1 ml volume of 1N HCl was added to a 50 ml clean and filtered sample and mixed thoroughly. A calibration curve was prepared using standards ranging from 0 to 7 mg NO_3-N/L. The sample's absorbances were measured against distilled water using a UV-6705 UV/VIS Spectrophotometer (Jenway Corporation, Kyoto Japan) at a wavelength of 220 nm to obtain the NO_3^- reading. Interference readings were also taken at 275 nm, representing approximately 10% of the value at 220 nm. The concentration of nitrate in the sample was determined by referring to the calibration curve (APHA AWWA WEF 2012).

Total Phosphorus: Digestion and Ascorbic Acid Spectrophotometric Method

Approximately 50 mL of the sample was transferred into a clean beaker. A precise amount of 0.5 g $K_2S_2O_8$ was added, and the mixture was digested on a heated hot plate for 30 to 40 minutes until the final volume reduced to 10 mL. After allowing it to cool, the solution was diluted to 30 ml using deionized water. To neutralize the solution to a light pink color, exactly 1 drop of phenolphthalein indicator solution was added, followed by further dilution to 100 mL in a 125 mL volumetric flask using deionized water. Then, 50 mL of the digested sample was transferred, and 8 ml of the combined reagent was added and mixed thoroughly. The sample's absorbance was measured at 880 nm using a UV-6705 UV/VIS Spectrophotometer (Jenway Corporation, Kyoto Japan) after waiting for 10 minutes, but no longer than 30 minutes. To create the calibration curve, standards ranging from 0.5 to 1.30 mg P/L (for a 1 cm light path) were subjected to the same persulphate digestion method. A deionized water blank was used in conjunction with the combined reagent. The calibration curve was generated based on the absorbance readings of the standards (APHA AWWA WEF 2012).

Total Phosphorus (P) as mg P/L = mg P from the calibration curve x 1000/sample ml……………………………………………..……………..…. (9)

Ammonia: Phenate Spectrophotometric Method

In a 50 mL conical flask containing 25 mL of the sample, 1 mL of phenol solution and 1 mL of sodium nitroprusside solution were combined with 2.5 mL of oxidizing solution. The samples were then incubated at 37 °C for 30 minutes. Similar preparation of sample blanks and standards was carried out. After 1 hour, the sample absorbance was measured at 640 nm using a UV-6705 UV/VIS Spectrophotometer (Jenway Corporation, Kyoto Japan). A calibration curve was generated by plotting the absorbance readings against the ammonia concentration of the standards. The sample concentration was determined using the standard curve (APHA AWWA WEF 2012).

Chloride determination by thiocyanate colorimetric method

In a platinum crucible, 5 g of sample was mixed with 1.25 g of CaO. Water was added to give a paste and the further evaporated on a water bath. It was then ignited at 500 ^{o}C for 24 hours and later allowed to cool. Hot water was used for extraction through a filter paper. The residue was then ash again in a crucible, dissolved in 20 % HNO_3 and then filtered. Acid was added to it 10 mL of chloride standard pipetted into 50 mL volumetric flask. About 15 mL of the sample solution was measured into the 50 mL volumetric flask. The content was mixed after adding 20 mL buffer solution, 10 mL acid ferric alum solution and 4 mL mercuric thiocyanate regent. The optical density was measured at 460 nm. A calibration curve was prepared from the standard solution, which was used to obtain mg Cl in the sample aliquot.

Cl (mg/L) = $\frac{C\,(mg)*10^3}{aliquot\,(ml)}$………………………………………………... (10)

Determination of optimal parameters

Organic loading rate

The OLR were calculated by dividing the mass of organic matter added to the digester per day by the volume of the active reactor. The daily feedstock input into the digester were measured accurately by weighing the organic waste and measuring the volume of waste added before adding it to the digester. The feedstocks include cow dung, pig dung, cabbage leaves, carrot leaves, jute leave, amaranth plant leave and spinach. The volume of the active reactor was also measured. This refers to the portion of the digester where the anaerobic digestion process occurs. The active volume was taken into consideration, as gas storage space and other components do not actively contribute to the digestion process. The OLR is typically expressed in terms of mass per volume per day, such as kilograms of organic matter per cubic meter of active reactor per day (kg/m^3/day).

The formula for calculating the OLR is as follows:

$OLR = \frac{Mass\ of\ organic\ matter\ applied\ (Kg)}{Volume\ or\ weight\ of\ digester\ (m3)} \times Time\ (Days)$……... (11)

Hydraulic retention time

The hydraulic retention time was calculated by first measuring the total volume of the digester, including the active reactor volume including the gas storage space. It is crucial to consider the total volume, as the feedstock will occupy the entire digester during its retention time. The HRT was calculated by dividing the active volume of the digester by the daily feedstock input:

$\emptyset = \frac{V}{Q}$... (12)

Where Ø is HRT, V is the digester volume, and Q is the flow rate of a digester.

Hydraulic Flow rate

The flow rate was determined by measuring the volume of influent material passing through the digester per unit of time.

$$HFR = \frac{\text{Volume of agricultural wastes}}{HRT} \text{ (L/d)} \quad (13)$$

Where, HFR stands for Hydraulic Flow Rate, and HRT stands for Hydraulic Retention Time.

Temperature and pH

Temperature and pH for influent and effluent were measured using Bench top multi parametric instrument (Eutech, PC 700). Before measurement, the instrument was calibrated using pH buffer 4.01, 7.01 and 10.01. pH probes were rinsed thoroughly with distilled water. Sample to be analyzed were transferred into a beaker, and probes dipped into the sample. Readings were taken after the instrument has stabilized showed by the READY indicator.

Assessment of the microbial content of the influent and effluent

Using Brilliant Green Agar for microbiology from Sigma Aldrich and Endo Agar from VWR BDH Chemicals, Geldenaaksebaan 464B3001 Leuven, Belgium, researchers examined the microorganisms present in the effluent and influent.

Preparation of Endo

The Endo selective media was prepared following the guidelines provided by the manufacturer (Netherlands Institute for Public Health, Utrecht. Approximately 8.3 g of Endo powder was accurately weighed and mixed with 200 mL of distilled water. The mixture was gently swirled, and the pH was adjusted to 7.5 ± 0.2 at 25 °C. The solution was then heated on a water bath at 100 °C for 45 minutes until complete dissolution of the powder was achieved, and autoclaving was not conducted. In a fume chamber that had been air-cleaned for 10 minutes to prevent contamination, the media was cooled to room temperature before being poured into sterile petri dishes and allowed to harden for about 20 minutes. Following a 1:1 initial dilution, further dilutions of the sample were carried out in succession. After that, 1 ml of the diluted samples were evenly spread over the agar's surface and allowed to dry. The cultured samples were stored in a dark place. It's important to note that Salmonella had a pale pink or pinkish-white appearance on Endo agar (EA), but E. coli had a golden green appearance. To ensure the highest level of sterility, strict steps were performed. All samples were then incubated for 16 to 24 hours at 37 °C while being held upside-down to avoid condensation droplets from landing on the agar surface. To calculate the amount of colony-forming units (CFUs) per ml of the samples, bacteria were counted using Stuart scientific colony counter.

Preparation of Brilliant Green agar

The Brilliant Green Agar (BGA) for microbiology obtained from Sigma Aldrich was prepared according to the instructions provided by the manufacturer (Netherlands Institute for Public Health, Utrecht). About 10.54 grams of the Brilliant Green powder were precisely measured and mixed with a measurable volume of distilled water. The mixture was gently swirled, and the pH was adjusted to 6.9 ± 0.2 at 25 °C. The solution was then heated on a water bath at 100 °C for 45 minutes to ensure complete dissolution of the powder, and no autoclaving was carried out. The solution was given 45 minutes to cool to ambient temperature. The prepared media was distributed into sterile Petri plates in a fume chamber that was running under sterile conditions (the fume chamber was air-cleaned with disinfectant for 10 minutes to prevent contamination). The prepared media was then allowed to harden. A serial dilution was performed after a 1:1 dilution of the sample. Then, 1 mL of the diluted samples was distributed evenly across the agar's surface and allowed to dry. A secure location was used to store the solidified agar plates to prevent light exposure. While the development of E. coli was hindered on BGA, Salmonella typhi colonies showed pink coloration. To avoid condensation droplets landing on the agar surface, all samples were then incubated at 37 °C for 16 to 24 hours while being turned upside down. The number of colony-forming units (CFUs) per ml of the samples were calculated through bacterial enumeration using a Stuart scientific colony counter. For further analysis, the averages were computed after the findings were recorded.

$$CFU/mL = \frac{number\ of\ count \times dilution\ factor}{volume\ of\ sample} \qquad (12)$$

Where CFU is coliform forming unit

Test for Heavy Metals: Digestion According to USEPA Method 3010 (Acid digestion of extracts) (1992)

The acid digestion process was used to examine heavy metal residues. This approach was normally achieved by subjecting a sample to a strong acid at a reasonable temperature, that allows the sample to thermally disintegrate and permit analytical techniques to measure the sample due to the solubility of heavy metal ions in solution.

In a 100 mL borosilicate beaker, 40 g of AW sample was placed. In a fume chamber, 5 ml aqua regia was added to the sample. The glassware was placed on a hot plate and digested for 3 hours at 450 °C, capped with a thin cling film. The sample was placed in a 100 mL graduated cylinder after digestion. Deionized water was added to bring the quantity to 30 mL. Digested samples were kept in 15 mL polyethylene tubes in a 40 °C cool environment. The AA-7000 UV-6705 UV/VIS SHIMADZU Atomic Absorption Spectrophotometer and Inductively Coupled-Atomic Emission Spectrophotometer (SHIMADZU Corporation, Kyoto Japan) were used to look for heavy metals in the samples (American Public Health Association 1995).

Determination of the theoretical methane production

The following method was used to compute the theoretical methane potential:

$CH_4 + 2O_2 \rightarrow CO_2 + 2H_2O$……………………………..……… (13)

(16 g) + (64 g) → (44 g) + (36 g) …………………………… (14)

0.25 g CH4 = 1 g COD……………………………………….. (15)

Therefore, ideally, at STP 1 kg COD = 0.350 m^3 CH4. Where STP is standard temperature and pressure.

Since carbon dioxide, the other component of biogas, has zero COD, it is assumed that any COD that is eliminated appears as methane.

Data analysis

Data from the laboratory analysis were presented in tables and graphs using Microsoft Word (2022) and Microsoft Excel (2022). Microsoft Excel 2022 and SPSS version 21 were used for data analysis. The data for the measured parameters were compared to the discharged standards set by the

UK National Environment Regulation (NER 1995), European Wastewater Plant Effluent Standards (EU -98 -15-EC) and the Ghana Environment Protection Agency (EPA 2012). SPSS version 21 software and Microsoft Excel (2022) were used to perform a One-Way ANOVA test to evaluate whether there were any significant differences between the effluent and the influent. In all the statistical tests, the significance threshold was set at $\alpha = 0.05$ and significance level at 95 % i.e., $p < 0.05$. The values obtained were used to assess the efficiency of the reactor. The average means, standard deviations, standard errors of the parameters were calculated, and percentages of treatment were also calculated.

CHAPTER FOUR

RESULT

Introduction

This chapter includes the results obtained in assessing the performance of mesophilic single-stage biogas digester for treating agricultural wastes at UCC farm. The Appendices parts of this work contain the data obtained at the end of the treatment regime.

Average mean values of the selected physicochemical parameters of the influent and the effluent of the AW

The table 1 below provides information on how well the treatment process using the AW performs in reducing various selected parameters from the influent to the effluent. Additionally, guidelines set by Ghana Environmental Protection Agency (EPA), European Union (EU) and National Environment Regulation (NER) are used for comparison to assess whether the effluent meets the required standards for various parameters.

The pH

The mean pH influent value of 6.24 was the lowest when the HRT was 20 days, while the highest mean pH influent value of 7.367 was observed when the HRT was 26 days. Additionally, the mean pH effluent value of 6.49 was the lowest for the HRT of 20 days, but it increased to 7.397 at an HRT of 26 days. Statistical analysis using Analysis of Variance (ANOVA) indicated a significant difference in the mean pH values between the influent and effluent at different HRTs ($p < 0.05$).

Ash

At HRT 26 days, the influent recorded the lowest mean ash value of 24.26%, whereas at HRT 23 days, the highest mean ash value of 31.974 % was observed. On the other hand, for effluent, the least mean ash value of 28.88 % was recorded at HRT 23 days, while the highest mean ash value of 33.52 % was observed at HRT 20 days. There was a significant difference ($p < 0.05$) in the mean ash values between influent and effluent across different HRTs, according to statistical tests.

Moisture

The mean moisture influent value was at its lowest, 88.55 %, when the HRT was 26 days. Conversely, the highest mean moisture influent value of 93.71 % was recorded at an HRT of 20 days. As for the effluent, the lowest mean moisture value of 98.923 % was observed at HRT 23 days, while the highest mean moisture value of 99.033 % was recorded at HRT 26 days. Significant variations in moisture content were found between influent and effluent across the various HRTs, according to the analysis of variance results ($p < 0.05$).

Total solids

When the HRT was 23 days, the average total solids (TS) in the influent were at their lowest point, at 6.29%, and at 26 days, they were at their highest point, at 11.45%. The effluent's average TS value ranged from 0.97% at HRT 26 to 1.21% at HRT 23, with 0.97% being the lowest and 1.21% the highest. Statistical analysis showed significant variations in total solids between influent and effluent across the different HRTs ($p < 0.05$).

Volatile solid

The average influent concentration of volatile solids (VS) was at its lowest, 0.484%, when the HRT was 20 days, while the highest average influent concentration was 2.123% at an HRT of 26 days. As for the effluent, the lowest average concentration of volatile solids was 0.076% at HRT 23 days, and the highest average concentration was 0.198% at HRT 26 days. Statistical analysis revealed significant differences in the mean concentrations of volatile solids between influent and effluent across the various HRTs ($p < 0.05$).

Total nitrogen

At HRT 26 days, the influent recorded the lowest mean total nitrogen (TN) value of 492.433 mg/L, while at HRT 23 days, the highest mean total nitrogen value of 2901.5 mg/L was observed. Similarly, for the effluent, the lowest mean TN value of 151.5 mg/L was recorded at HRT 26 days, while the highest mean TN value of 980.167 mg/L was observed at HRT 23 days. The analysis of variance indicated significant differences in total nitrogen concentrations between influent and effluent across the different HRTs ($p < 0.05$).

Organic carbon

At HRT 23 days, the influent recorded the lowest average concentration of organic carbon, which was 402,105.33 mg/L, while at HRT 26 days, the highest average concentration of 439,325.7 mg/L was observed in the influent. For the effluent, the lowest average concentration of organic carbon was 294,551.33 mg/L at HRT 23 days, while the highest mean concentration was 411,990 mg/L at HRT 26 days. Statistical analysis showed significant differences in the mean concentrations of organic carbon between influent and effluent across the various HRTs ($p < 0.05$).

Biological Oxygen Demand (BOD_5)

The mean values of BOD_5 influent concentration varied from 9333.33 mg/L at HRT 23 days to 25000 mg/L at HRT 26 days. On the other hand, the mean BOD_5 effluent concentration ranged from 3666.67 mg/L at HRT 20 days to 11333.33 mg/L at HRT 26 days EPA and NER effluent discharge standards of 50 mg/L. Statistical analysis showed significant differences in the mean BOD_5 concentrations between influent and effluent across the various HRTs ($p < 0.05$).

Chemical Oxygen Demand (COD)

At HRT 23 days, the influent recorded the lowest mean Chemical Oxygen Demand (COD) value of 1,487,000 mg/L, while at HRT 26 days, the highest mean COD influent value of 3,074,400 mg/L was observed. For the effluent, the least average COD value was 422,000 mg/L at HRT 23 days, and the highest average COD value was 924,000 mg/L at HRT 26 days. The results of the statistical analysis showed that there were significant differences ($p < 0.05$) in the mean COD concentrations between influent and effluent across the different HRTs.

Chloride

The influent chloride concentration ranged from 659.107 mg/L at HRT 23 days to 889.165 mg/L at HRT 26 days, with the highest value observed at the latter. As for the effluent, the chloride concentration ranged from 546.161 mg/L at HRT 23 days to 694.789 mg/L at HRT 26 days, with the highest value recorded at the latter. Statistical analysis revealed significant differences in the mean chloride values between influent and effluent across the various HRTs ($p < 0.05$).

Nitrate

The mean influent concentration of nitrate ranged from 32.706 mg/L at HRT 23 days to 97.630 mg/L at HRT 26 days, with the highest value observed at the latter. Of the effluent, the nitrate concentration ranged from 17.964 mg/L at HRT 23 days to 107 mg/L at HRT 26 days, with the highest value recorded at the latter. The mean nitrate concentrations between influent and effluent throughout the various HRTs varied significantly, according to statistical analysis ($p < 0.05$).

Ammonia

At HRT 23 days, the influent recorded the lowest mean ammonia concentration of 100.71 mg/L, while at HRT 26 days, the highest mean ammonia influent value of 360.656 mg/L was observed. For the effluent, the lowest mean ammonia value of 98.641 mg/L was recorded at HRT 23 days, and the highest mean ammonia value was 175.429 mg/L at HRT 26 days. The ANOVA test indicated that there was significant difference in the mean ammonia concentrations between influent and effluent across the various HRTs ($p > 0.05$).

Phosphorus

At HRT 26 days, the influent recorded the lowest mean phosphorus concentration of 1023.761 mg/L, while at HRT 20 days, the highest mean phosphorus concentration of 1798.656 mg/L was observed. For the effluent, the lowest mean phosphorus value of 286.374 mg/L was recorded at HRT 26 days, and the highest mean phosphorus value was 452.604 mg/L at HRT 20 days. Significant variations in the average phosphorus contents between influent and effluent were observed among the different HRTs, according to statistical analysis ($p < 0.05$).

Table 1: Average means of Physicochemical Parameters for Influent and Effluent at different HRT

Parameter	HRT 20 (days) **Month 1** **Mean± SD**		HRT 23 (days) **Month 2** **Mean± SD**		HRT 26 (days) **Month 3** **Mean± SD**		**Ghana EPA**	**EU**	**NER**
	Influent	Effluent	Influent	Effluent	Influent	Effluent			
pH	6.237±0.015	6.493±0.015	7.220±0.108	7.353±0.025	7.367± 0.015	7.397±0.061	6-9		6-8
Temp (°C)	29	30	30	30	29	30	<30		20-35^{o}C
Ash	30.589±0.475	33.521±0.242	31.974±0.112	28.875±0.745	24.260±0.261	28.974±.262			
Moisture	93.71±0.239	98.923±0.010	92.648±0.3138	98.962±0.824	88.552±0.357	99.033±.003			
Total solid (%)	6.286±0.239	1.077±0.010	7.074±0.062	1.210±0.370	11.447±0.357	0.967±0.003	snf	snf	snf
Volatile solid (%)	.484±0.042	.098±0.010	.578±0.011	.076±0.045	2.123±0.125	0.198±0.005	snf	snf	snf
Total nitrogen (%)	2776.167±31.7223	980.167±15.373	2901.500±43.486	656.833±11.504	492.433±1.030	151.50±0.693	50	15	10
Organic carbon	402617.667±2752.650	385605.000±1401.467	402105.333±6754.433	294551.333±12985.728	439323.33±1516.88	411986.67±1521.72	snf	snf	snf
BOD (mgO_2/L)	9333.333±1154.700	3666.667±577.350	9333.33±1154.700	4333.33±577.350	25000.000±5000.0	11333.333±1527.525	50	25	50
COD (mg/L)	1736000.00±.00	578666.667±64663.230	1487000.000±.00	422000.00±.00	3074400.0±189326.385	924000.000±0.000	250	125	100
Chloride (mg/L)	814.300±7.892	588.986±6.135	659.107±12.565	546.161±11.105	889.165±9.476	694.789±12.407	snf	snf	500
Nitrate (mg/L)	34.439±0.187	22.523±0.375	32.706±0.337	17.964±0.060	97.63033±0.741	107.155±1.152	snf	snf	snf
Ammonia (mg/L)	119.180±0.118	109.513±0.118	100.700±0.600	98.641±1.335	360.656±0.997	175.429±0.189	1		10
Total phosphorus (mg/L)	1798.656±5.499	452.604±0.906	1707.179±33.453	344.432±4.381	1023.761±0.367	286.374±0.367		2	

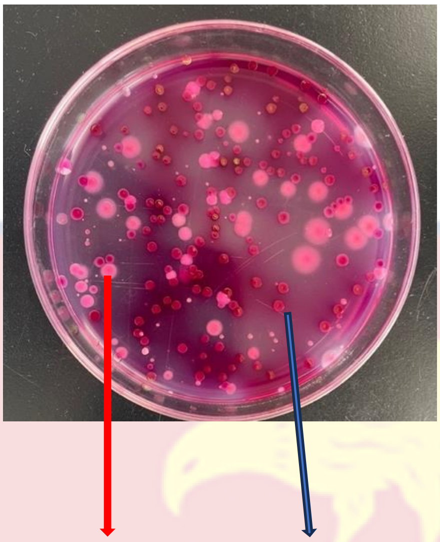
NB: s.n.f = standard not found

Escherichia coli (E. coli)

The mean influent concentration of E. coli in BGA agar ranged from 1.4E+11 CFU/ml at 20 days HRT to 6.04E+11 CFU/ml at 26 days HRT. The mean effluent concentration of E. coli in BGA agar varied from 5.7E+9 CFU/ml at 26 days HRT to 2.3E+10 CFU/ml at 23 days HRT. As for Endo agar, the mean influent concentration of E. coli was lowest at 1.9E+11 CFU/ml at 20 days HRT and highest at 3.2E+11 CFU/ml at 26 days HRT. The mean effluent concentration of E. coli in Endo agar was lowest at 1.3E+10 CFU/ml at 23 days HRT and highest at 3.4E+10 CFU/ml at 26 days HRT.

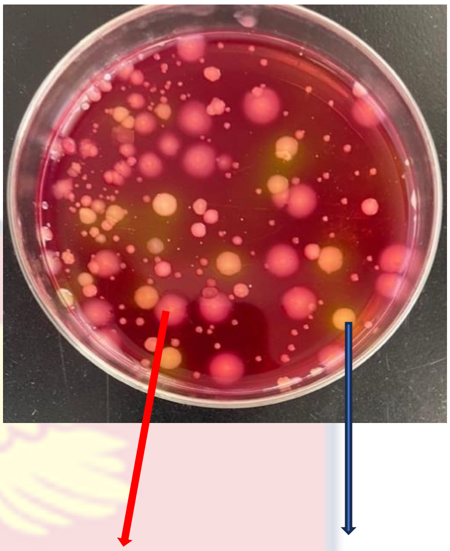
Salmonella spp.

The mean influent concentration of *Salmonella spp.* in BGA agar ranged from 2.1E+11 CFU/ml at 23 days HRT to 2.52E+11 CFU/ml at 26 days HRT. The mean effluent concentration of *Salmonella spp.* in BGA agar was 0 CFU/ml at both 23 days and 26 days HRT, while the highest mean effluent concentration was 6.7E+9 CFU/ml at 20 days HRT. Regarding Endo agar, the lowest mean influent concentration of *Salmonella spp.* was recorded at 2.8E+11 CFU/ml at 26 days HRT, and the highest mean influent concentration was 3.2E+11 CFU/ml at 23 days HRT. For the mean effluent concentration, the lowest value of *Salmonella spp.* was 0 CFU/ml at 23 days HRT, and the highest value was 3.0E+10 CFU/ml at 23 days HRT.



Salmonella spp. *E. coli*

Fig. 13: Growth of *E. coli* and *Salmonella spp*. on BGA before treatment



Salmonella spp. *E. coli*

Fig. 14: Growth *E. coli* and *Salmonella spp.* on BGA before treatment

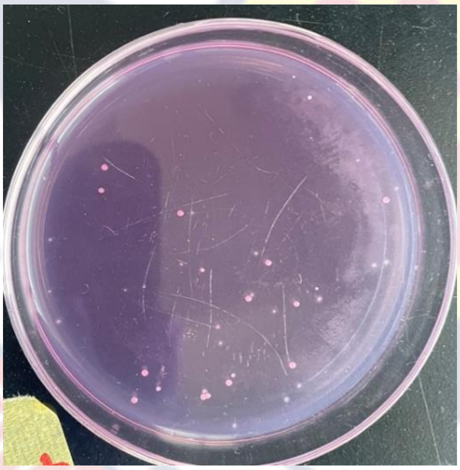


Fig. 15: Growth of *E. coli* and *Salmonella spp*. on BGA after treatment

Fig. 16: Growth of *E. coli* and *Salmonella spp*. on BGA after treatment

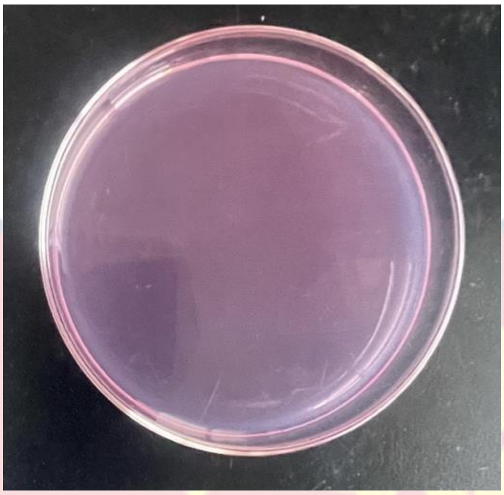


Fig. 17: Negative control EA

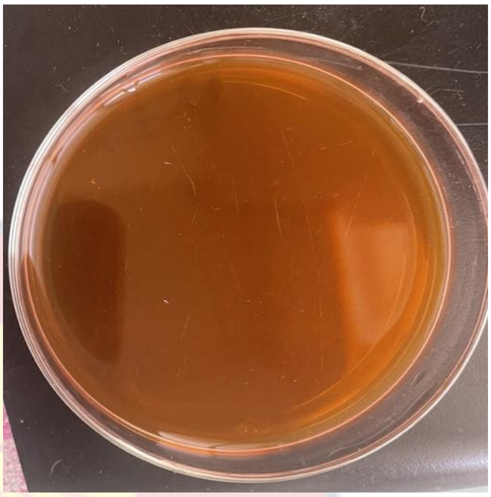


Fig. 18: Negative control BGA

NB: On endo agar, *E. coli* appears golden green whiles *Salmonella spp* appeared as pinkish white. Also, on BGA, *E. coli* appeared as milky yellow whiles *Salmonella spp* appeared as pinkish white.

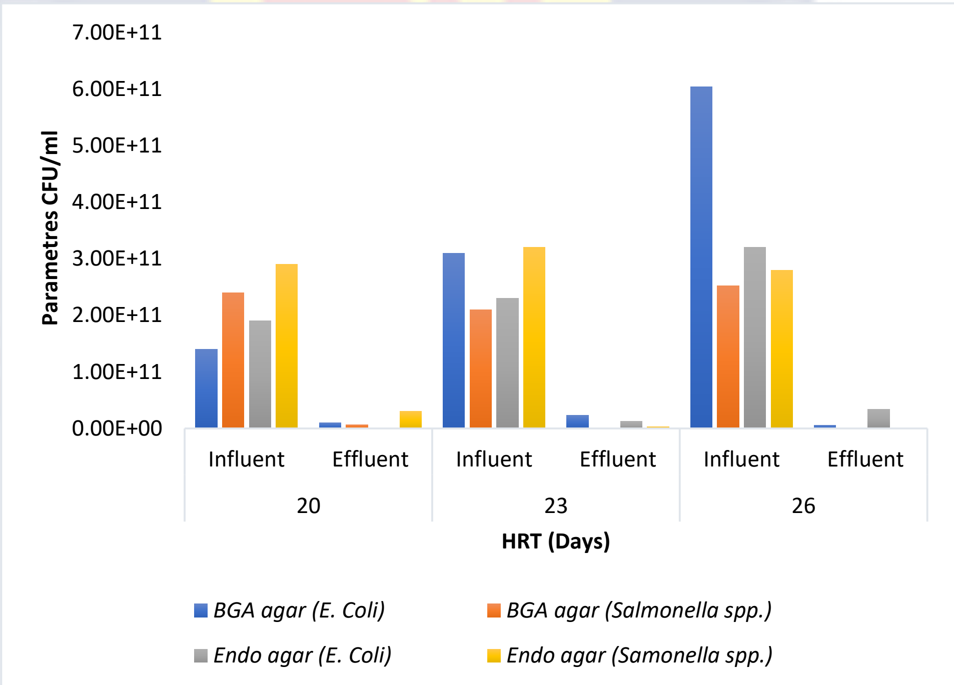


Figure 19: Pathogenic microorganisms' removal at different HRTs

Heavy Metals Concentrations of the influent and the effluent

Table 2 presents the average influent and effluent heavy metal concentrations of agricultural wastes under different Hydraulic Retention Times.

Lead (Pb)

Regarding the concentration of lead in agricultural waste, the lowest average input was 0.1655 ppm, observed at a 20-day HRT. This value increased to 0.3359 ppm when the HRT was extended to 26 days. As for the lowest average lead output, it was found to be below 0.01 ppm at an HRT of 23 days. However, the highest average lead effluent occurred at an HRT of 20 days, with a mean value of 0.1899 ppm. ANOVA test aimed at identifying differences among the means revealed no significant distinction in average input and output across different HRTs ($p > 0.05$).

Cadmium (Cd)

At a Hydraulic Retention Time (HRT) of 26 days, the lowest average input value for cadmium was 0.0105 ppm. This value increased to 0.0336 ppm, observed at both 23-day and 26-day HRTs. The smallest average cadmium output value was <0.01 ppm, recorded at an HRT of 26 days. Conversely, the highest average cadmium output value of 0.0341 ppm was noted at an HRT of 23 days. Comparing the mean input and output values for cadmium, no significant differences were found across various HRTs ($p > 0.05$).

Nickel (Ni)

The average input concentration of nickel, which was below 0.01 ppm, reached its lowest point at both 23-day and 26-day Hydraulic Retention Times

(HRTs). In contrast, the highest mean input concentration of chromium, measuring 0.0605 ppm, was observed at an HRT of 20 days. Throughout the study period, all three HRT durations exhibited a mean output concentration of nickel below 0.01 ppm. Notably, no significant differences were identified among the average input and output concentrations across the various HRTs ($p > 0.05$).

Zinc (Zn)

The average input concentration of zinc, which was 0.1203 ppm, reached its lowest value at an HRT of 20 days. Conversely, the highest average input concentration, measuring 0.4001 ppm, was observed at an HRT of 23 days. Among the different HRTs, the lowest mean zinc output concentration was 0.0523 ppm at an HRT of 23 days, while the highest mean zinc output concentration was 0.2832 ppm at an HRT of 20 days. Despite these variations, no significant differences were noted in the mean zinc values across the various HRTs ($p > 0.05$).

Chromium (Cr)

The average input concentration of chromium, which was below 0.01 ppm, reached its lowest point at both 20-day and 26-day Hydraulic Retention Times (HRTs). Conversely, the highest average input concentration of chromium, measuring 0.0303 ppm, was observed at an HRT of 23 days. Throughout the study period, all three HRT durations exhibited the same mean output concentration of chromium, which was below 0.01 ppm. . Importantly, no significant differences were found among the average input and output concentrations across the various HRTs ($p > 0.05$).

Table 2: Mean values of heavy metal concentrations of influent and effluent at different HRTs

Parameter	Month 1(HRT 20) Mean± SD		Month 2 (HRT 23) Mean± SD		Month 3(HRT 26) Mean± SD		Ghana EPA	EU	NER
	Influent	Effluent	Influent	Effluent	Influent	Effluent			
Pb (mg/L)	0.1655± 0.0002	0.1899± 0.001	0.1899± 0.0011	<0.01±0	0.3359± 0.0023	0.0925± 0.0008	0.1	0.05	0.1
Cd (mg/L)	0.0336± 0.0017	0.0231± 0.0004	0.0366± 0.0023	0.0341± 0.0008	0.0105± 0.0008	0±0	0.1	0.01	0.1
Ni (mg/L)	0.0605± 0.0009	<0.00±0	<0.01±0	<0.01±0	<0.01±0	<0.01±0		0.2	1.0
Zn (mg/L)	0.1203± 0.0066	0.2832± 0.0219	0.4001± 0.0312	0.0523± 0.0036	0.3499± 0.0259	0.083± 0.0072	2.0	5.0	5.0
Cr (mg/L)	<0.01±0	<0.01±0	0.0303± 0.002	<0.01±0	<0.01±0	<0.01±0	0.05	0.0002	0.05

Optimum Hydraulic Retention Time (HRT) and Hydraulic Flow Rate (HFR) for Treatment of agricultural wastes

In this research work, the treatment of agricultural wastes was assessed using different Hydraulic Retention Times (HRTs) and corresponding Hydraulic Flow Rates (HFRs). The HRTs tested were 20 days, 23 days, and 26 days, while the corresponding HFRs were 300 L/d, 260 L/d, and 230 L/d, respectively. The researcher estimated the mean percentage removal of contaminants from agricultural wastes at each HRT with the corresponding HFR. Various parameters such as total solids, organic carbon, nitrogen compounds (ammonia, nitrate), phosphorus, chloride, were monitored to assess the effectiveness of the treatment process. After analyzing the data, the results were compared to determine the optimum HRT and HFR for the treatment process. The optimum HRT and HFR would represent the most efficient and effective operating conditions for treating agricultural wastes and achieving the desired treatment goals.

Total solid

According to Figure 20, the most optimum HRT for total solid treatment was achieved at 26 days with a HFR of 230 L/d. At this HRT and HFR combination, the mean percentage reduction in total solids was 91.6 %. As the HRT was decreased to 23 days with an HFR of 260 L/d, the mean percentage reduction in total solids decreased to 82.9 %, indicating a slightly lower removal of total solids during the treatment process. Reducing the HRT further to 20 days with an HFR of 300 L/d resulted in a similar mean percentage reduction in total solids of 82.87 %.

Volatile solid

According to the results, the most favorable HRT for volatile solids (VS) removal was achieved at 26 days with a HFR of 230 L/d. At this HRT and HFR combination, the percentage removal of VS was 90.7 %, indicating a significant reduction in volatile solids during the treatment process. Following that, at an HRT of 23 days with an HFR of 260 L/d, the percentage removal of VS was slightly lower but still substantial at 86.9 %. Lastly, at an HRT of 20 days, with an HFR of 260 L/d, the percentage removal of VS was 79.8 %, indicating a slightly lower reduction in volatile solids compared to the previous two conditions.

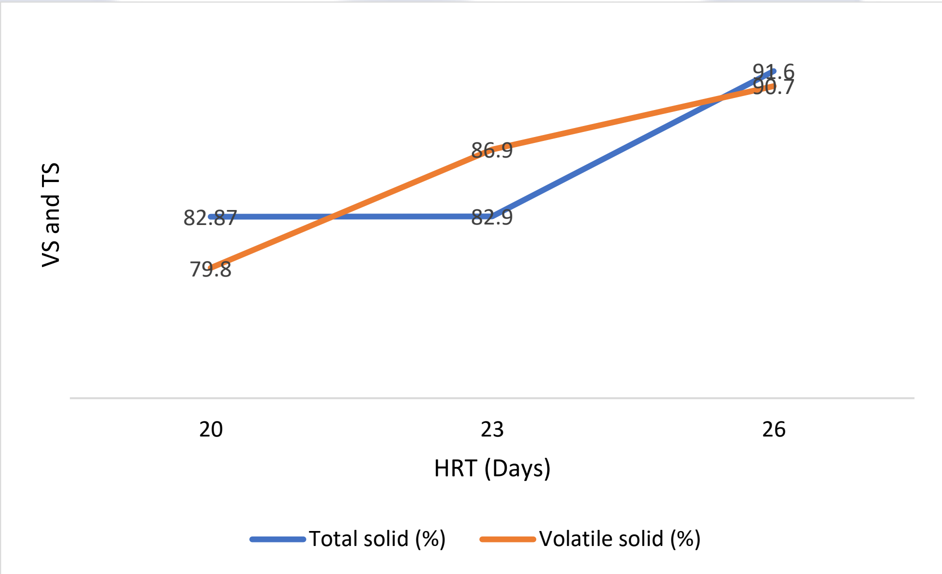


Figure 20: Percentage Removal for total solid and volatile solid content at different HRT

Total nitrogen

The results from Figure 21 indicate that the HRT for total nitrogen removal was observed at 23 days with a HFR of 260 L/d. At this HRT and HFR combination, the mean percentage of total solid removal was 77.4 %. When the HRT was increased to 26 days with an HFR of 230 L/d, the mean percentage of total solid removal decreased to 69.2 %, suggesting a reduction in total solid removal during the treatment process. With a further decrease in HRT to 20 days and an HFR of 300 L/d, the mean percentage of total solid removal decreased further to 64.7 %.

Organic carbon

Based on the data presented in Figure 21, the most favorable HRT for organic carbon removal was achieved at 23 days with a HFR of 260 L/d. At this HRT and HFR combination, the mean percentage of organic carbon removal was 26.7 %. As the HRT was increased to 26 days with an HFR of 230 L/d, the mean percentage of organic carbon removal decreased to 6.2 %, causing a reduction in organic carbon removal during the treatment process. Also, with a further decrease in HRT to 20 days and an HFR of 300 L/d, the mean percentage of organic carbon removal decreased even further to 4.2 %.

Biological Oxygen Demand (BOD_5)

According to Figure 21, the most optimum HRT for total solid removal was recorded at 20 days with a HFR of 300 L/d. At this HRT and HFR combination, the mean percentage of BOD removal was 60.7 %. As the HRT was increased to 26 days with an HFR of 230 L/d, the mean percentage of total solid removal decreased to 54.7 %, indicating a reduction in the efficiency of total solid removal during the treatment process. Furthermore, at an HRT of 23 days, also with an HFR of 230 L/d, the mean percentage of total solid removal further decreased to 53.6 %.

Chemical Oxygen Demand (COD)

According to the data from Figure 21, the most favorable HRT for COD removal was achieved at 23 days with a HFR of 230 L/d. At this HRT and HFR combination, the mean percentage of COD removal was 71.6 %. As the HRT was increased to 26 days, while maintaining an HFR of 230 L/d, the mean percentage of COD removal slightly decreased to 69.9 %. Furthermore, at an HRT of 20 days with an HFR of 300 L/d, the mean percentage of COD removal further decreased to 66.7 %.

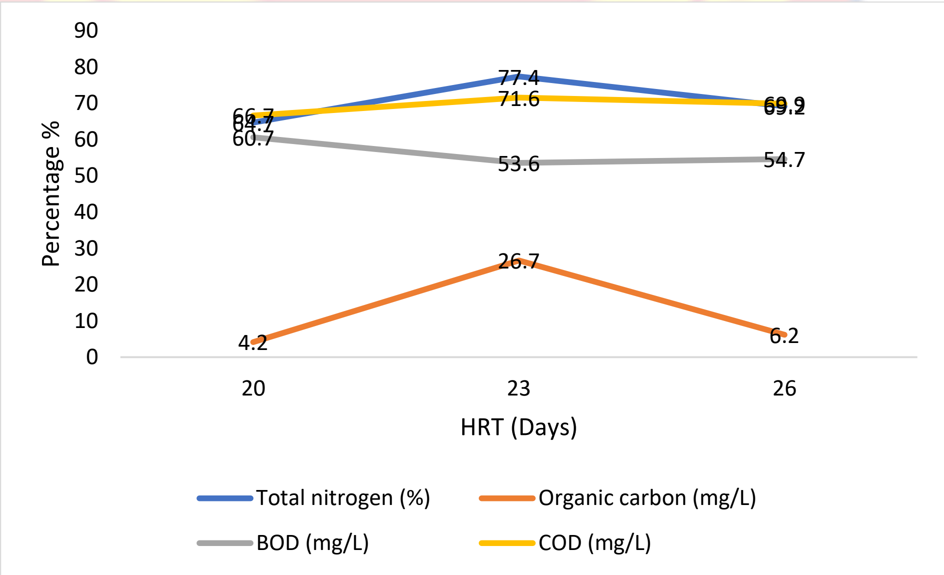


Figure 21: Percentage Removal for TN, OC, BOD and COD at different HRTs

Chloride

According to the data from figure 22, the most favorable HRT for chloride removal was achieved at 20 days with a HFR of 300 L/d. At this HRT and HFR combination, the mean percentage of chloride removal was 27.7 %. As the HRT was increased to 26 days with an HFR of 230 L/d, the mean percentage of chloride removal slightly decreased to 21.9 %. Furthermore, at an HRT of 23 days with an HFR of 260 L/d, the mean percentage of chloride removal further decreased to 17.1 %.

Nitrate

The most favorable HRT for nitrate removal was achieved at 23 days with a HFR of 260 L/d. At this HRT and HFR combination, the mean percentage of nitrate removal was 45.1%. As the HRT was decreased to 20 days with an HFR of 300 L/d, the mean percentage of nitrate removal decreased to 34.6%, indicating a slight reduction in nitrate removal during the treatment process. Also, at an HRT of 26 days with an HFR of 230 L/d, the mean percentage of nitrate removal decreased even further to -9.8%. A negative percentage indicates that there was an increase in nitrate concentration during treatment, which could be attributed to different factors, such as biological processes or changes in influent composition (Figure 22).

Ammonium

Figure 22 illustrates that the most favorable HRT for ammonium removal was observed at 26 days with a HFR of 230 L/d. At this HRT and HFR combination, the mean percentage of ammonium removal was 51.4%. As the HRT was decreased to 20 days with an HFR of 300 L/d, the mean percentage of ammonium removal decreased significantly to 8.1 %. Furthermore, at an HRT of 23 days with an HFR of 260 L/d, the mean percentage of ammonium removal decreased even further to 2.0 %.

Phosphorus

According to figure 22, the most favorable HRT for phosphorus removal was achieved at 23 days with a HFR of 260 L/d. At this HRT and HFR combination, the mean percentage of phosphorus removal was 79.8 %. As the HRT was decreased to 20 days with an HFR of 300 L/d, the mean percentage of phosphorus removal decreased to 74.8 %. Furthermore, at an

HRT of 26 days with an HFR of 230 L/d, the mean percentage of phosphorus removal decreased further to 72.0 %.

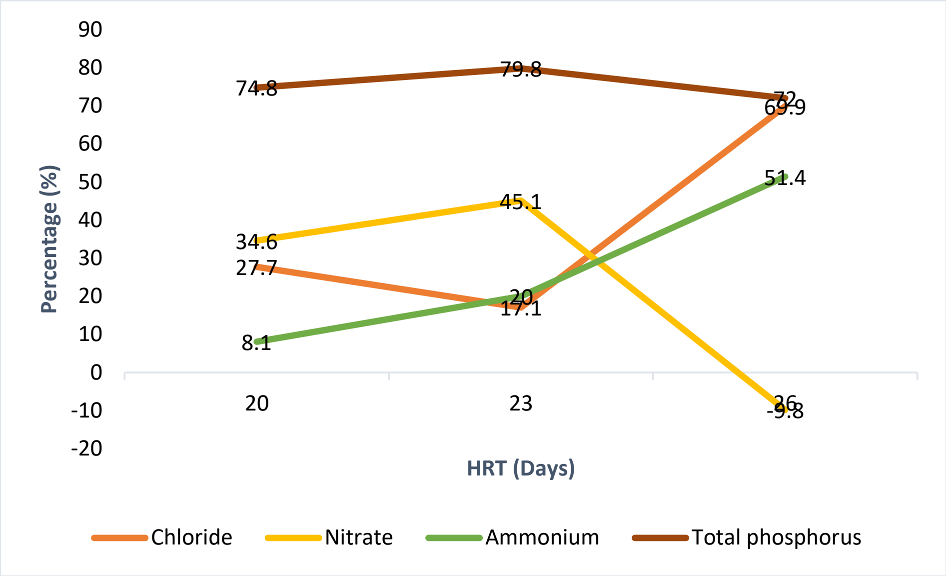


Figure 22: Percentage Removal for Cl, nitrate, ammonium, and TP at different HRTs

Optimum Hydraulic Retention Time (HRT) and Hydraulic Flow Rate (HFR) for Removal of Escherichia coli and Salmonella typhi

Escherichia coli (E. coli)

The results show that the treatment system was highly effective in removing E. coli from the influent with BGA agar. At HRT 23 days with HFR 260 L/d, the percentage removal was 92.5 %. Similarly, at HRT 20 days with HFR 300 L/d, the removal efficiency was 92.9 %. However, the highest removal efficiency of approximately 99 % was achieved at HRT 26 days with HFR 230 L/d.

Using endo agar, both HRT 20 days and HRT 26 days, with HFR 300 L/d and 230 L/d respectively, achieved a treatment removal of 89.3 %, making them equally effective for E. coli removal in the reactor. However, the optimum condition for achieving effective *E. coli* removal was 94.2 % at HRT 23 days with HFR of 260 L/d.

Salmonella spp.

Using BGA, the percentage removal of *Salmonella spp.* was 97.2 % at HRT 20 days with HFR 300 L/d. Remarkably, both HRT 23 days and HRT 26 days, with HFR 260 L/d and 230 L/d respectively, achieved a treatment removal of 100 %, making them equally effective and optimal for E. coli removal in the reactor.

The endo results indicate that at HRT 20 and 23 days with HFR 300 L/d and 260 L/d respectively, the treatment system achieved a significant percentage removal of *Salmonella spp.*, with a removal efficiency of 89.4 % and 99% respectively. Nevertheless, the best condition was achieved at HRT 26 days with HFR of 230 L/d with a 100 %. This shows that the system was effective in reducing the *Salmonella spp.* levels in the influent during that specific operational condition.

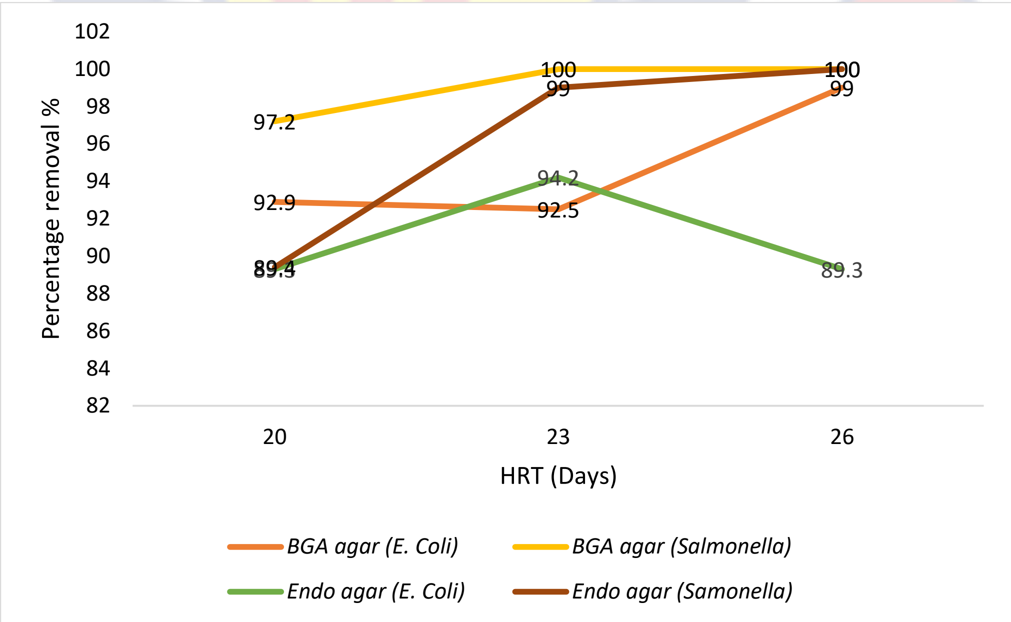


Figure 23: Percentage Removal of *E. coli* and *Salmonella spp*. from the influent and effluent at different HRTs

Optimum Hydraulic Retention Time (HRT) and Hydraulic Flow Rate (HFR) for heavy metal treatment

Lead (Pb)

The lead removal percentages were recorded as -14.7 % at an HRT of 20 days, 72.5 % at an HRT of 26 days, and a complete 100 % at an HRT of 23 days, which was identified as the optimal HRT for lead removal (Figure 24).

Cadmium (Cd)

The cadmium removal percentages were found to be 6.8 % at an HRT of 23 days, 31.3 % at an HRT of 20 days, and a complete 100 % at an HRT of 26 days, which was identified as the optimal HRT for cadmium removal (Figure 24).

Nickel (Ni)

The nickel removal percentages were "Below Detection Limit" (BDL) at both HRT 23 days and HRT 26 days. However, it was 100 % at an HRT of 20 days, which was identified as the optimal HRT for nickel removal (Figure 24).

Zinc (Zn)

The zinc removal percentages were -135.4 % at an HRT of 20 days, 76.3 % at an HRT of 26 days, and 86.9 % at an HRT of 23 days, which was identified as the optimum HRT for zinc removal (Figure 24).

Chromium Cr

The chromium removal percentages were "Below Detection Limit" (BDL) at both HRT 20 days and HRT 26 days, and 100 % at an HRT of 23 days, which was identified as the optimum HRT for chromium removal (Figure 24).

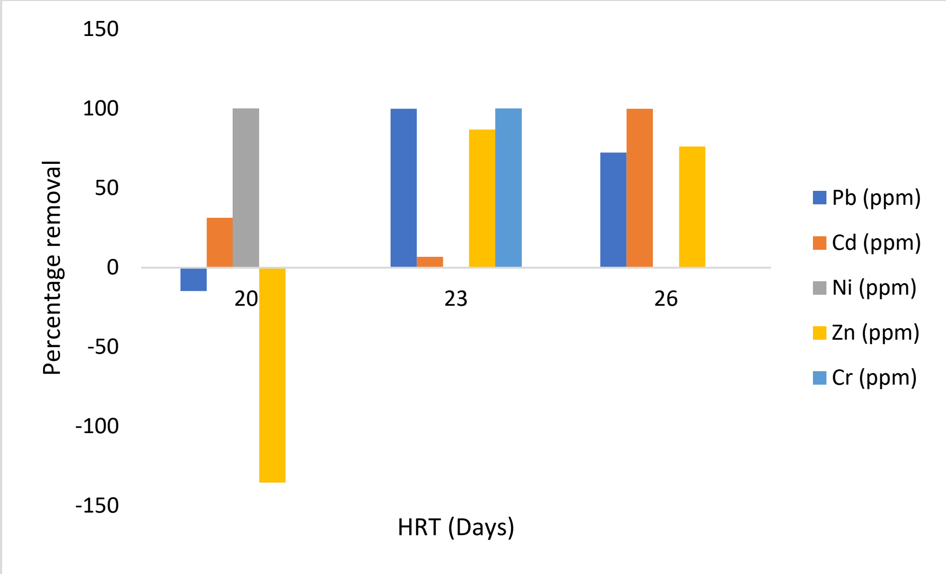


Figure 24: Percentage Removal of heavy metals from the influent and effluent

Removal Efficiency of the pilot-scale single-stage anaerobic digester for treating AW

Removal Efficiency of some selected Parameters

Table 3 presents the efficiency of removal for the treatment system, which was determined by comparing the mean influent and effluent values for each HRT, and then calculating the percentage removal for each parameter. From the results, the highest removal efficiency of the physicochemical parameters occurred at HRT 23 days. The data in Table 4 provides a comprehensive view of how well the treatment system performed in removing various contaminants from the influent. It allows for easy comparison of the removal efficiency across different HRTs and HFRs for each parameter studied.

Table 3: Removal efficiency of some selected parameters of AW from the single-stage biogas digester

	Percentage removal of some selected parameters		
Parameters	HRT 20	HRT 23	HRT 26
Total solid (%)	82.87	82.9	91.6
Volatile solid (%)	79.8	86.9	90.7
Total nitrogen (%)	64.7	77.4	69.2
Organic carbon	4.2	26.7	6.2
BOD (mg/L)	60.7	53.6	54.7
COD (mg/L)	66.7	71.6	69.9
Chloride (mg/L)	27.7	17.1	69.9
Nitrate (mg/L)	34.6	45.1	-9.8
Ammonia (mg/L)	8.1	20	51.4
Total phosphorus (mg/L)	74.8	79.8	72

Removal Efficiency of *Escherichia coli* and *Salmonella spp.* at different HRTs

The concentrations of *Escherichia coli* and *Salmonella spp.* were estimated for both Endo agar (EA) and Brilliant green agar (BGA) and their removal efficiencies were presented in table 4.

E. coli

For BGA agar, the lowest mean influent concentration of *E. coli* was 1.4E+11 CFU/ml at 20 days HRT and the highest mean influent concentration was 6.04E+11 CFU/ml at 26 days HRT. The lowest mean effluent concentration of *E. coli* was 5.7E+9 CFU/ml at 26 days HRT while the highest mean effluent concentration value was 3E+10 CFU/ml at 23 days HRT. The removal efficiency was 99 % at HRT 26 days with HFR 230 L/d with 2log reduction.

For endo agar, the lowest *E. coli* influent concentration were recorded 1.9E+11 CFU/ml at 20 days HRT while the mean influent concentration of 3.2E+11 CFU/ml was the highest at HRT 26 days. The lowest mean effluent concentration *E. coli* was 1.3E+10 CFU/ml at 23 days HRT and the highest mean effluent concentration value was 3.4E+10 CFU/ml at 26 days HRT. The removal efficiency was 94.2 % at HRT 23 days and HFR 260 L/d with 1log reduction.

Salmonella spp.

For BGA, the lowest mean influent concentration of *Salmonella spp.* recorded was 1E+11 CFU/ml at 23 days HRT, with HRT 26 days HRT recording the highest mean influent value of 2.52E+11 CFU/ml. The lowest mean effluent concentration of *Salmonella spp.* was 0 CFU/ml at both 23 days and 26 days HRT while the highest mean effluent concentration of Salmonella typhi was 6.7E+9 CFU/ml at 20 days HRT. The efficiency of removal was100 %, recorded at both HRT 23 days and 26 days with HFR 260 L/d and 230 L/d respectively.

For EA, the lowest mean influent concentration of Salmonella typhi recorded was 2. 2.8E+11 CFU/ml at 26 days HRT and the highest mean *Salmonella spp.* influent was 3.2E+11 CFU/ml at 23 days HRT. With mean effluent, the lowest mean value of Salmonella typhi 0 CFU/ml at 26 days HRT and the highest value was 3.0E+10 CFU/ml at 23 days HR. The removal efficiency recorded was 100 % at HRT 26 days and HFR 230 L/d with 4log reduction.

Table 4: Percentage Removal of Escherichia coli and Salmonella spp. different HRTs

Parameter/ CFU/ml	HRT 20 (Days)	HRT 23(Days)	HRT 26(Days)
BGA agar (*E. Coli*)	92.9	92.5	99
BGA agar (*Salmonella sp*)	97.2	100	100
Endo agar (*E. Coli*)	89.3	94.2	89.3
Endo agar (*Samonella sp*)	89.4	99	100

Removal Efficiency of heavy metals at different HRTs

According to table 5, the highest removal efficiency for Pb, Cd, Ni, Zn and Cr were at HRT 23, 26, 20,23 and 23 days respectively. However, at HRT 20 and 26 days, the concentrations for Nickel were below detectable limit. Likewise, concentrations for Cr were also below detectable limits at HRT 20 and 26 days.

Table 5: Removal Efficiency of heavy metals

Parameters	HRT 20	HRT 23	HRT 26
Pb (ppm)	-14.7	100	72.5
Cd (ppm)	31.3	6.8	100
Ni (ppm)	100	BDL	BDL
Zn (ppm)	-135.4	86.9	76.3
Cr (ppm)	BDL	100	BDL

Theoretical methane production (TMP)

The ability of the theoretical methane production was used to estimate methane yields of the AW across the three HRT. Table 6 represents the lists mass COD converted to methane and TMP results at each HRT using the degraded COD values from the experiment.

Table 6: Theoretical methane production at each HRT

HRT	COD converted to CH_4 (kg)	TMP ($m^3 CH_4$ /d)	KWh
20	1.157	0.405	4.05
23	1.065	0.372	3.72
26	2.150	0.752	7.52

CHAPTER FIVE

DISCUSSION

This chapter presents a comprehensive discussion of the results obtained from the laboratory analysis, as discussed in Chapter four. The findings were analyzed in the context of other related reviewed works to provide a broader understanding of the research outcomes. The laboratory analysis revealed various key parameters, including pH, ash content, total solids, volatile solids, moisture content, total nitrogen, organic carbon, BOD_5, COD, chloride, nitrate, ammonium heavy metal and pathogenic microorganisms. The results showed variations in influent and effluent values across different HRTs and HFRs.

pH

Throughout the treatment period and across different HRTs, the pH values of influent and effluent showed minimal changes. This can be attributed to the fact that the pH values remained within the acidic to neutral range. The effluent pH of agricultural wastes ranged from 6.49 to 7.39, which falls within the recommended discharge standards set by Ghana EPA and NER for effluent. The pH values' stability and adherence to the regulatory standards indicate the effectiveness of the treatment process in maintaining proper pH levels in the effluent, ensuring environmental compliance. The effluent values were in line with Abouelenien et al. (2014) findings, which stated that this range was good for agricultural activities. Similar research conducted by Bajpai and Bajpai, (2017) revealed that, the optimal operating pH falls within 6.8–7.4, which was not a deviation from the pH values obtained in the current study. Overall, the stable pH values within the acceptable range for effluent discharge, as observed in this study, align with the findings and recommendations of other research works, highlighting the importance of pH control in AW treatment processes. This further emphasizes the effectiveness of the treatment system in achieving regulatory compliance and minimizing impacts on the environment.

Moisture content

A thorough assessment of mean moisture values measured showed a slight increment in the mean effluent moisture content of the AW. The research findings showed a statistical significance ($p<0.05$) in the mean moisture content across the HRTs. Nayono et al. (2010) stated that moisture ranging from 60 to 80 % enhances fast degradability and produces high methane. However, the results in this research were slightly above this range which was as a result of seasonal variation. This may have had influence on the removal efficiency of organic matter and contaminant degradation. Alnakeeb et al. (2017) worked with a moisture content ranging from 94 to 99 %. According to the study, adding distilled water to the reaction medium to raise its moisture content improves the generation of biogas. The moisture content in anaerobic digestion of agricultural wastes plays a crucial role in process efficiency, gas production, substrate availability, mixing, temperature control, avoiding inhibition, solids separation, and nutrient recovery. Proper management and control of moisture content are essential for maximizing biogas production and nutrient recovery, ensuring the overall efficiency of the process of digestion.

Ash

A thorough assessment of mean ash values showed an increment in the mean effluent ash content of the AW, except for HRT 23 days which generated a reduction in the effluent ash content. This increment maybe be as a result of recirculation and incomplete digestion of organic materials. Recirculation helps to promote process stability and microbial activity as reported by Chen et al. (2020). However, over time, this can result in the accumulation of minerals and inorganic components, leading to elevated ash levels in the effluent. Also, incomplete digestion as a result of shorter HRT resulted in higher levels of unconverted organic matter, which may contain ash-rich components (Sarker et al., 2019).

Total solids

According to the study's findings, the digested effluent's % TS increased from a mean of 0.967 % to 1.210 %, or from 82.9 % to 91.6 %. The previously mentioned modifications showed that every HRT had decreased a amount of TS or Dry Matter (OM) in their respective effluents. The results demonstrated that the biogas effluent has a lower TS concentration than the undigested wastes in actual use. With respect to the influents, the TS concentration in the effluents fell by more than half throughout all HRT. This supports the conclusions reported by Amenyeku (2021) and Bryant (2019).

Additionally, Masinde et al. (2020) found that the production of biogas grew from 6% TS to a maximum average and then started steadily declining as the total solids increased. This shows that the reduction in TS in the digesters may have been caused by the removal of organic carbon during the digestion process, which led to the generation of CH_4 and CO_2.

Volatile solids

The findings of the study showed that anaerobic digestion can substantially lower the volatile solids in effluent. It was discovered that the influent's volatile solids ranged from 0.484 % to 2.123 %. The concentration of VS in the effluent substantially dropped during the digestion process, going from 0.198% to 0.098%. Throughout the course of the investigation, all HRTs showed a decrease in volatile solids in the effluent, showing that undigested wastes have higher levels of organic matter or VS than anaerobically digested effluent. However, the HRT of 23 days and the hydraulic flow rate of 260 L/d resulted in the elimination of VS at the maximum rate (90.67 %). According to Bryant (2019) and Amenyeku (2021), the reduction may be caused by the anaerobic digestion process, which transforms the organic components of the raw waste into biogas.

Total nitrogen

Results from the influent showed that TN recorded ranged from 492.433 mg/L to 2901.5 mg /L. After the process of anaerobic digestion, TN of the effluent reduced from a mean of 151.5 to 980.167 mg/L. This reduction in the effluent representing 64.7 % to 77.4 %. This agrees with similar finding by John and Kumar (2023) which reveals total nitrogen removal efficacy of anaerobic digester to be 72 % which is slightly lower than the current result. Trouli, et al. (2023) also recorded significant TN (~44 %) reduction values compared with the influent. However, a sequencing batch reactor was used which is a good suggestion for post treatment of the effluent. The reduction in total nitrogen in the current study might be as a result of nitrogen been converted to ammonium during protein degradation as reported by Bareha et al. (2019). Hence, causing the reduction of TN in the digester effluent

Biological oxygen demand (BOD)

The results indicate that the influent BOD_5 concentrations in the agricultural waste were relatively high, ranging from 3666.67 mg/L to 11333.33 mg/L. These high BOD_5 levels are commonly observed in organic-rich waste streams such as agricultural effluents (Koul et al., 2012). This result is in line with a study made by Liu and Haynes, (2011) where effluent from dairy and meat processing factories contain high level of BOD. The optimal mean BOD_5 removal efficiency was 60.7% at the HRT of 20 days and HFR of 300 L/d. This result is below the removal efficiency revealed by McGarvey et al. (2007) where anaerobic digester showed an increased treatment efficacy for the removal of BOD_5 (87%) when bacterial population dynamic was studied for aerobic and anaerobic digesters separately. The lower BOD_5 reduction is as a result of inefficient mixing. Investigations have been carried out in order to monitor the effects of mixing to the performance of anaerobic digesters. According to Poh and Chong (2009), mixing improved the performance of digesters treating waste with higher concentration. The mean BOD_5 effluent concentrations exceeded the Ghana EPA effluent maximum acceptable standard of 50-200 mg/L for discharge into water bodies or use for agricultural activities. The elevated BOD_5 levels in the effluent can potentially pose environmental risks, especially if the discharged effluent enters water bodies or soil, leading to oxygen depletion and water pollution (Manasa & Mehta, 2020). To improve the overall performance and meet the effluent discharge standards, additional treatment or post-treatment processes may be needed to further decrease the BOD_5 concentrations in the effluent. Potential post-treatment options include aerobic treatment, polishing ponds, or further anaerobic digestion stages. It is very crucial to consider the specific characteristics of the agricultural waste and the local environmental regulations when designing the appropriate treatment system.

Chemical oxygen demand (COD)

The mean COD effluent concentrations showed a reduction, ranging from 422000 mg/L to 924000 mg/L, indicating that the anaerobic digestion process was effective in removing organic contaminants from the agricultural wastes. This agrees with Amenyeku, (2021), where there was a reduction in the effluent COD, although the study was on anaerobic co-digestion of faecal sludge with paper or fruit waste for biogas. The significant variation in mean COD values across different HRTs in this research highlights the impact of hydraulic retention time on the treatment efficiency (Santos et al., 2017). The highest removal percentage of COD, 71.3 %, was achieved at an HRT of 23 days and a hydraulic flow rate of 260 L/d. The hydraulic retention time of the reactor and recirculation directly affect how well the reactor removes COD. Increased HRT and recirculation exhibited high-efficiency COD removal of 60.41% and 80.93%, respectively, according to research by Namsree et al. (2012). Dareioti and Kornaros (2014) evaluated the effect of HRT on the anaerobic co-digestion of agro-industrial wastes from a double-stage CSTR that processed liquid cow manure, cheese whey, and olive wastewater, and their findings supported this. Compared to HRT of 20 days, they found that the HRT of 25 days had higher methane generation and, thus, increased COD degradation. Despite the reduction in COD concentrations, the mean effluent values exceeded the Ghana EPA maximum level of permissibility of 250 mg/l – 1000 mg/l for discharge into water bodies or used for irrigation. This indicates that further treatment or post-treatment measures may be essential to achieve effluent quality compliance with environmental regulations. Also, the removal efficiency reported by this result indicates that the anaerobic digestion system performed most efficiently at this specific operating condition in terms of COD removal. However, it also suggests that there is room for further optimization to enhance the overall treatment efficiency.

Chloride

The result analysis showed that the mean chloride influent values ranged from 659.11 mg/L to 814.3 mg/L, while the mean nitrate effluent values ranged from 541.2 mg/L to 694.8 mg/L. The data indicates that there was a significant reduction in the mean chloride concentration in the effluent compared to the influent, indicating the effectiveness of the anaerobic digestion process in removing chloride from the agricultural waste. However, despite the reduction in chloride levels, the mean chloride effluent values still exceeded the effluent discharge guidelines set by the Ghana EPA and NER. This indicates that the treatment system did not fully meet the regulatory requirements for chloride discharge. The overall mean removal efficiency for chloride was 27.7 %, with the highest efficiency recorded at HRT 20 days and HFR of 300 L/d. This suggests that the chosen combination of HRT and HFR resulted in the most effective removal of chloride from the influent. To achieve better compliance with effluent discharge standards, further optimization of the anaerobic digestion process may be necessary. Adjusting process parameters, optimizing reactor design, or implementing additional treatment steps could potentially enhance chloride removal efficiency. Monitoring and management of chloride levels in the effluent are crucial to ensure environmental compliance and protect the receiving water bodies from any adverse impacts (Ilyas et al., 2019).

Nitrate

The result analysis showed that the mean nitrate influent values ranged from 32.71 mg/L to 97.63 mg/L, while the mean nitrate effluent values ranged from 17.96 mg/L to 107.16 mg/L. The data indicates that there was a significant ($p<0.05$) reduction in the mean nitrate concentration in the effluent compared to the influent, indicating the effectiveness of the anaerobic digestion process in removing nitrate from the agricultural waste. However, the mean nitrate effluent values were slightly above the effluent discharge guidelines set by the Ghana EPA and NER. This suggests that further optimization may be required to achieve full compliance with the regulatory standards. The overall mean removal efficiency for nitrate was 45.07 %, with the highest efficiency recorded at HRT 23 days and HFR of 260 L/d. This was a bit higher than the study by Bryant, (2019) where the nitrate removal level 34.4 %. To ensure better compliance with effluent discharge standards, it may be necessary to explore additional treatment measures to further enhance nitrate removal during anaerobic digestion (Chan et al., 2010). Potential strategies may include optimizing the biological processes, considering the use of specific microbial strains that are more effective at nitrate removal, or implementing post-treatment steps to reduce nitrate levels in the effluent.

Ammonia

The findings suggest that anaerobic digestion of agricultural waste effectively decreased ammonia concentrations in the effluent. The average ammonia influent values ranged from 100.7 mg/L to 360.66 mg/L, while the average ammonia effluent values ranged from 98.4 mg/L to 175.43 mg/L, demonstrating a significant ($p < 0.05$) reduction in ammonia levels during treatment. Although ammonia inhibition occurred, the overall average removal efficiency of 51.4% indicates that approximately half of the ammonia present in the influent was successfully removed. Despite this positive result, the effluent ammonia concentrations still exceeded the discharge standards set by Ghana EPA and NER. This indicates the need for further improvements to achieve the required effluent quality regarding ammonia. This outcome is in relation with the study made by Magomnang et al. (2017) where co-digestion of cow manure with a mixture of coconut shells and rice straw led to ammonia inhibition in the reactor.

Phosphorus

Mean phosphorus influent values ranged from 1023.76 mg/L to 1798.66 mg/L which saw a reduction in the mean phosphate effluent values. The mean effluent values ranged from 286.374 mg/L to 452.604 mg/L. Bryant (2019) revealed mean effluent phosphorus value to be 133.7 mg/L and this was below the reported values of the current study. The overall removal efficiency of phosphorus was 79.8 % recorded at HRT 23 days with HFR of 260 L/d. This could be attributed to microbial uptake for their growth and metabolisms and recirculation. This is in line with John and Kumar (2023) result of total phosphorus removal efficacy of anaerobic digester being 61 %, although, slightly lower than the current result. The reduction in phosphorus concentration may be as a result of microorganisms converting the phosphorus to glycogen. This assert that, anaerobic digestion can be used to reduce total phosphorus in agricultural wastes. However, all the mean effluent values were above the Ghana EPA discharge standard where required post treatment such as the application of enhanced biological phosphorus removal before discharged (Lin et al., 2015).

Pathogenic microorganism of the influent and the effluent

After conducting analyses on influent and effluent samples of agricultural wastes, significant reductions in the counts of E. coli and Salmonella typhi were observed in the effluents. The mean values were significant ($P<0.05$) across the various HRTs. The results showed that for *E. coli* on BGA recorded the highest removal efficiency (99 %) at hydraulic retention time of 26 days. Also, the highest removal efficiency of *Salmonella sp.* was 100 % at hydraulic retention time of 26 days. Whiles on endo agar, *E. coli* recorded 94.2 % at HRT 23 days and Salmonella recorded a remarkable result of 100 % at HRT 26 days. Bacterial population dynamic was studied for aerobic and anaerobic digesters separately by McGarvey et al. (2007). The result indicated that anaerobic digester showed an increased treatment efficacy of 99.7% for the removal of coliform which is not a deviation from the current study. According to Saunders et al.'s (2012) research, AD at a mesophilic temperature reduced harmful microorganisms by 90% to 95%. In a similar vein, Harrison et al. (2011) indicated that anaerobic digestion of manure resulted in a 2.5 log drop in E. coli. Additionally, Côte et al. (2006) employed sequencing batch reactors to anaerobically treat manure. They observed a

99.67% to 99 % decrease in the overall population of native E. coli and undetectable levels of native strains of Salmonella and protozoa (Cryptosporidium and Giardia). Salmonella and E. coli levels decreased as a result of the AD process, which has been widely established. Anaerobic digestion and storage of dairy and swine manures are confirmed to be efficient methods to reduce the prevalence of coliforms, according to Costa et al. (2017). Additionally, Saunders et al. (2012) found no evidence that anaerobic digestion increased the longevity of indicator bacteria such E. coli. It has been emphasized, nonetheless, that total pathogen microorganism eradication using AD is improbable (Nag et al., 2019). This contrasts with the present findings, which show that AD had a 100% decrease in efficiency and had the highest effect on Salmonella counts. This might be due to the anaerobic state, which produced an environment unfavorable to harmful bacteria survival (Sahlström, 2003). The digester used in this study operates at mesophilic temperature (30 °C). The results indicate that a reduction in *E. coli* and *Salmonella sp*. numbers can be achieved even without heating the digester artificially. It is crucial to note that not all the pathogenic microbiological quality of the samples analyzed and examined in this study conform with Ghana EPA and NER standard for fertilizers since the goal of reusing digestate as fertilizers in agriculture. In order to use digestate as fertilizer in agriculture, additional treatment is necessary.

Heavy metals

Heavy metals' toxicity and ability to migrate through the environment can be reflected by their bioavailability (Sun et al., 2021). This study revealed that the mean effluent concentrations of lead, cadmium (Cd), nickel (Ni), zinc

(Zn) and chromium (Cr) were within the standard values recommended by the Ghana EPA (2012), and this shows that with regard to heavy metals, the effluent will not pose any toxic effect to agricultural activities and to any water bodies it finds its way into. Also, all the heavy metals had effluent values within the standard recommended by the UK NER (1999). All the mean influent and effluent values were not statistically significant ($P>0.05$) across the various HRTs. The overall removal efficiency of the heavy metals was very encouraging as most the values across the HRTs were above 50 % except lead and zinc, which increased in effluent concentration of -14.7 % and -135.4 % respectively at HRT 23 days. This is because most HMs such Zn and Pb were closely combined with insoluble solids as reported by Jin & Chang (2011). This outcome is consistent with Zheng et al.'s findings from 2021, which showed that anaerobic digestion improved the proportion of bioavailable components in Zn and Cd. Also, Cd, showed a lower effluent removal concentration of 31.3 % and 6.8 % for HRT 20 and 23 days respectively. This could also be attributed to shorter HRT. Among the heavy metals Lead and chromium recorded a remarkable removal efficiency of 100 % at HRT 23 days. On other hand, nickel and cadmium also recorded a remarkable efficiency of 100 % at HRTs 20 and 26 days respectively. Zinc recorded the least removal efficiency and might be due to short HRT.

In practice, the study found that HRTs 23 and 26 days showed a better treatment efficiency as compared to HRT 20. The reason might be due to the shorter HRT and different feeds for pig. According to Awasthi et al. (2021), growth promoter chemicals with high concentrations of HMs are frequently added to feed for animals with the goal of avoiding disease, lowering mortality, and promoting growth rate. The study also demonstrated that the levels of Cd, Cr, Pb, Zn, and Ni in influents and effluents after anaerobic digestion did change. This supports Zheng et al. (2021) assertion that anaerobic digestion makes it possible to reduce heavy metals in digestate or effluent. But the succession will also be influenced by the kind of retention time employed.

Theoretical methane production

The ability of the theoretical methodologies to estimate methane yields of complex AW accurately was evaluated by using the degraded COD obtained from the anaerobic digestion process. Methane production from organic materials, such as agricultural wastes is a valuable process due to its potential as a renewable energy source (Zapalowska & Bashutska, 2019). The comparison between methane production and electricity generation is important because it allows to assess the energy value of methane in relation to other energy sources (Collet et al., 2017). For electricity conversion, 1 m^3 of methane can yield 10 kWh of electricity. This conversion factor is crucial for understanding the energy potential of the methane produced from the feedstock. From the result, the methane yield ranged from 0.372 to 0.752 m^3 CH_4. Comparing it with the electricity conversion factor, it's clear that there is a significant energy potential within your feedstock. Even though the theoretical methane yield from the outcome is slightly lower than the expected value, it's important to note that this discrepancy might arise from factors such as experimental conditions, feedstock composition, and reactor efficiency.

CHAPTER SIX

SUMMARY OF FINDINGS

The research findings indicated that there was a notable decrease in the average effluents for all the assessed parameters. The investigation also demonstrated that adherence to the effluent concentration standards set by the Ghana EPA and NER relied on the specific values of Hydraulic Retention Time (HRT) and Hydraulic Flow Rate (HFR). Some parameters, when considering particular HRTs and HFRs, fell within the allowed limits for effluent discharge, while others surpassed the recommended thresholds. Notable variations were observed in all the physical and chemical parameters. *E. coli* and *Salmonella spp.* similarly displayed significant distinctions across all HRTs. Conversely, all heavy metals did not exhibit statistically significant differences. Although the optimal HRT and HFR varied for each parameter, HRT of 23 days paired with an HFR of 260 L/d demonstrated superior removal efficiency for most factors. Moreover, certain elements like Zn, phosphorus, and lead showcased negative removal efficiencies, indicating elevated average effluent values. Removal efficiencies also demonstrated fluctuations in relation to different HRTs.

Conclusion

The research centered around the assessment of performance of mesophilic single-stage biogas digester for treating agricultural wastes. The study assessed how well the influent and effluent performed in terms of treatment. This assessment focused on the quality of the treated agricultural wastes across three different hydraulic retention times and their corresponding hydraulic flow rates. The MSSAD demonstrated treatment capabilities, surpassing the average performance of some multi-stage digesters.

Regarding the selected physicochemical parameters of the agricultural wastes, the findings indicated decreases in the concentrations of the mean treated wastes (effluents). The optimal HRT and HFR for effectively treating agricultural waste varied depending on the specific parameter being considered. The greatest elimination was seen in TS and VS at HRT 26, whereas TN, OC, COD NO3-, and TP were at HRT 23.

The study also highlighted significant decreases in the mean treated waste concentrations of microorganisms *like E. coli* and *Salmonella spp*. The results of the pathogenic microbial treatment indicated an infinite reduction of *salmonella spp*. and a 2.02 log reduction in *E. Coli* all at HRT 26.

Additionally, the data related to heavy metals indicated that all the initial values of these metals were higher in the influent than in the effluent, except for Zn and Pb at an HRT of 23 days, which saw an increment in their effluent concentrations. HRTs 23 and 26 days showed better treatment efficiency as compared to HRT 20.

Some of the parameters analyzed after treatment displayed mean effluent concentrations that adhered to the discharge standards set by the Ghana EPA and NER, while others exceeded these standards.

While certain parameters demonstrated improved removal efficiencies in the treated waste, others such as Zn and Pb exhibited lower removal efficiencies. For those parameters with subpar removal efficiencies and those that exceeded the discharge standards, additional treatment steps are recommended to prevent environmental contamination.

Based on the available data, the study concluded that utilizing the mesophilic single-stage biogas digester for treating agricultural waste prior to its release into the environment proved effective. This research is a win-win solution for farmers and policy makers as it addresses waste management, energy, environmental and economic concern, whiles supporting sustainable agricultural and rural development.

Recommendations

Based on the data above, the following suggestions are offered to increase the digester's efficiency in order to reach the necessary level for secure utilization or disposal.

1. Future studies should work with thermophilic or hyperthermophilic in order to compare the current study with.
2. Although the mean effluents of some of the parameters were reduced in a sufficient amount, it is still important to perform further treatment to meet required standard before using it for agricultural purposes or discharging it into the environment.
3. Further study should work with batch feeding method in order to compare the current outcome with.

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APPENDICES

APPENDIX A

Definition of Terms

Chemical Oxygen Demand (COD): It calculates how much oxygen is needed overall to chemically oxidise both biodegradable and non-biodegradable organic materials in the wastes.

Biological Oxygen Demand (BOD): shows how much oxygen microbes use when consuming organic matter throughout a five-day incubation period. It provides a clue as to the quantity of biodegradable organic matter in the wastes.

Anaerobic digestion: it is a microbial mechanism that is used by this biological system to break down organic molecules without the presence of oxygen.

Hydraulic Retention Time (HRT): this is the typical period that the feedstock stays in the digester.

Agricultural wastes: these are surplus or unsalable products made entirely in agricultural operations that are connected to raising livestock or cultivating crops primarily for financial gain or survival.

Biogas: is a combination of gas composed of methane, CO2, and trace amounts of other gases because of the anaerobic digestion of organic material in a condition without oxygen.

Hydraulic flow rate: it is the movement of hydraulic fluid within the system. It measures the movement of a particular amount of fluid within a specific time period.

Methane: It is an odorless, colorless, combustible hydrocarbon and among the main ingredients of natural gas.

Heavy metal: are substances with a density that is significantly higher than that of water. Examples are lead, chromium, nickel, cadmium and zinc.

Effluent: liquid that exits a system or limited place (such as treated or untreated wastewater)

Mesophilic: is a range of temperatures between 20 and 45 °C (68 and 113 °F) where an organism can flourish at its finest and is neither too hot nor too cold.

Volatile solid: the percentage of the total solids in the residuals that are lost during combustion at 550 ^{o}C with extra air.

Total solid: consist of all the sample's dissolved, colloidal, and suspended solids.

APPENDIX B

ANOVA for the selected physicochemical parameters

Parameters	*Source of Variation*	*SS*	*df*	*MS*	*F*	*P-value*
	Between Groups	0.005	1	0.0049		
	Within Groups	0.001	2	0.0005	9.8	0.089
pH	Total	0.006	3			
	Between Groups	106.275	1	106.27445		
	Within Groups	0.078	2	0.03897488	2726.74203	0.000
Moisture	Total	106.352	3			
	Between Groups	106.251	1	106.250741		
	Within Groups	0.078	2	0.03896965	2726.49975	0.000
Total solid	Total	106.329	3			
	Between Groups	3.714	1	3.71371441		
	Within Groups	0.031	2	0.01550386	239.534826	0.004
Volatile solid	Total	3.745	3			
	Between Groups	22.482	1	22.4822964		
	Within Groups	0.047	2	0.02333082	963.630682	0.001
Ash	Total	22.529	3			
	Between Groups	116451.563	1	116451.563		
	Within Groups	1.325	2	0.6625	175775.943	5.689E-06
Total nitrogen	Total	116452.888	3			
	Between Groups	543738.85	1	543738.85		
	Within Groups	0.1343225	2	0.06716125	8096020.4	1.2352E-07
Phosphorus	Total	543738.985	3			

	Between Groups	35798.9104	1	35798.9104		
	Within Groups	327.112106	2	163.556053	218.878542	0.005
Chloride	Total	36126.0225	3			
	Between Groups	34413.7746	1	34413.7746		
	Within Groups	1.1531965	2	0.57659825	59684.1468	1.6754E-05
Ammonia	Total	34414.9278	3			
	Between Groups	72.692676	1	72.692676		
	Within Groups	0.690002	2	0.345001	210.702798	0.005
Nitrate	Total	73.382678	3			
	Between Groups	4.41E+12	1	4.41E+12		
	Within Groups	5.6448E+10	2	2.8224E+10	156.25	0.006
COD	Total	4.4664E+12	3			
	Between Groups	144000000	1	144000000		
BOD	Within Groups	13000000	2	6500000	22.1538462	0.042
	Total	157000000	3			
	Between Groups	7.56442512	1	7.56442512		
	Within Groups	0.01569	2	0.007845	964.23489	0.001
Organic carbon	Total	7.58011513	3			

APPENDIX C

ANOVA for heavy microorganisms' analyses at different HRT

HRT	*Source of Variation*	*SS*	*df*	*MS*	*F*	*P-value*
	Between Groups	7.3114E+22	1	7.3114E+22		
	Within Groups	5.2899E+21	4	1.3225E+21	55.2864184	0.001
20	Total	7.8404E+22	5			
	Between Groups	9.2091E+22	1	9.2091E+22		
	Within Groups	6.963E+21	4	1.7407E+21	52.9031915	0.001
23	Total	9.9054E+22	5			
	Between Groups	1.1141E+23	1	1.1141E+23		
	Within Groups	3.1249E+21	4	7.8123E+20	142.611141	0.000
26	Total	1.1454E+23	5			

APPENDIX D

ANOVA for heavy metal analyses at different HRT

HRT	*Source of Variation*	*SS*	*df*	*MS*	*F*	*P-value*
	Between Groups	0.0010557	1	0.0010557		
	Within Groups	0.06505039	6	0.01084173	0.09737386	0.766
20	Total	0.06610609	7			
	Between Groups	0.01810705	1	0.01810705		
	Within Groups	0.10984727	6	0.01830788	0.98903022	0.358
23	Total	0.12795432	7			
	Between Groups	0.00961885	1	0.00961885		
	Within Groups	0.09523497	6	0.0158725	0.60600712	0.466
26	Total	0.10485382	7			

APPENDIX E

Picture 1: Picture of the researcher performing microbiology analyses.

Picture 2: Picture of the researcher performing laboratory analyses.

Picture 6: Picture of the researcher weighing substrate.

Picture 7: Picture of the researcher feeding the digester.

Picture 8: Picture of the researcher feeding the digester

Picture 9: Picture of the researcher mixing substrate

Picture 10: Picture of the researcher performing recirculation.