OPTIMIZATION OF BIOGAS PRODUCTION FROM ANAEROBIC CO-DIGESTION OF FISH WASTE AND WATER HYACINTH

BY

HORTENCE INGABIRE

A Thesis Submitted to the School of Engineering, Department of Mechanical, Production and Energy Engineering in Partial Fulfilment of the Requirement for the Awards of the Degree of Master of Science in Energy studies

,

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DECLARATION

Declaration by Candidate

I hereby declare that this thesis entitled **“Optimization of biogas production from anaerobic co-digestion of fish waste and water hyacinth”** is my own work and has not been presented for a degree in any other university. No part of this thesis may be reproduced without the prior written permission of the author and/or Moi University.

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DEDICATION

This thesis is dedicated to my husband and kids for their care, love, encouragement, support, patience, and sacrifice throughout this level of education. Above all to the Almighty God for providing me health, protection, strength, and favor to see this output.

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MAY GOD BLESS YOU!

ABSTRACT

Fish waste (FW) and water hyacinth (WH) are biodegradable wastes that remain underutilized and unexploited and cause a problem to the environment since the existing disposal techniques result in environmental pollution and health risks. Anaerobic Co-digestion of FW and WH can be used to improve biogas generation as a source of energy to replace fossil fuel consumption. The conversion of wastes to energy can provide an answer to environmental pollution, waste treatment and management, and rising energy costs. The main objective of this study was the optimization of biogas production from anaerobic co-digestion of FW and WH. The specific objectives were to characterize the substrate, evaluate operating conditions to maximize the biogas production, and determine the biogas yield model equation. The WH was collected from Lake Victoria and FW from fish point Eldoret, Kenya, and the inoculum was collected from the Moi University biogas plant. Laboratory scale experiments were carried out in Moi University laboratories (Chemical and Process Engineering Laboratory and Chemistry Laboratory) under mesophilic temperature (37^{o}C). The physiochemical characteristics (Total solids, Moisture content, Volatile solids, and Carbon to nitrogen ratio) of the substrate were tested using standard methods. Design-Expert 13 was used for optimization and results analysis. RSM (Response surface methodology) was used to examine the effects of operating parameters and identify optimum values for biogas yield. Experimental variable levels for biogas were substrate ratio (WH: FW, 25-75g), inoculum concentration (IC, 5-15g), and dilution (85-95mL). The total weight of the substrate was 100 g. The total volume of the biodigesters was made between 190-210 mL. The quantity of biogas produced was measured by the water displacement method on daily basis (20 days). The initial analysis of FW was 61.78, 99.48, and 38.21% for MC, VS, and TS respectively while for WH was 94.4, 83.3, and 5.6 % respectively. The C/N ratio of FW (5.89) was out of range for the accepted C/N ratio (20-30:1). However, the C/N ratio of WH (21.35) and inoculum (23.47) was in a suitable range. Optimum values for maximum biogas yield of 690mL with the highest methane yield of 68.15% were found to be WH: FW ratio, 25:75g, 15g of IC, and 95 mL for dilution. The yield was 16.1% and 32.4% greater than FW and WH mono-digestion, respectively. The biogas yield was expressed as function of operating variables.The model was significant ($P<0.05$). All factors had significant linear and quadratic effects on biogas while only the interaction effects of the two factors were significant. The coefficient of determination(R^2) of 99.9% confirms the good fit of the model with experimental variables. In conclusion, FW and WH were potential feedstock for biogas production. AnCo-digestion of FW and WH feedstock has been shown to enhance biomethane yield. Optimum values for RSM were within the range of experimental results. Biogas yield decreased as substrate ratio increased. FW had a lower C/N ratio, further study needs to consider co-digestion with other higher C/N ratio substrates.

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ABBREVIATIONS

AD : Anaerobic Digestion

AnCo-digestion: Anaerobic co-digestion

ANOVA : Analysis of variance

C : Carbon

℃ : Degree Celsius

C/N : Carbon-to-nitrogen

CCD : Central Composite Design

CH_4 : Methane

CO_2 : Carbon dioxide

DOE : Design of Experiment

Eq : Equation

FW : Fish waste

g : gram

H_2 : Hydrogen

H_2S : Hydrogen sulfur

HCl : Hydrogen chloride

IC : Inoculum Concentration

ISR : Inoculum to Substrate Ratio

Kg : Kilogram

KOH : Potassium hydroxide

L : litre

mL : milliliter

MW : Mega Watt

N : Nitrogen

NaOH : Sodium hydroxide

NH_3 : Ammonia

O_2 : Oxygen

OLR : Organic Loading Rate

pH : potential Hydrogen

ppm : parts per millions

RRR : Reuse Reduce Recycle

RSM : Response Surface Methodology

RT : Retention Time

TAN : Total Ammonia Nitrogen

TS : Total Solid

VFA : Volatile Fatty Acid

VS : Volatile Solid

WH : Water hyacinth

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

The current rapid population growth has increased waste production, which has resulted in the development of toxic wastelands, which are resources that have been left unutilized or wasted (Marchaim, 2007). Because of how wastes are disposed of, there are environmental challenges (Nadu & Nadu, 2017). The global economy is currently transitioning from petroleum-based to bio-based energy.

In light of the environmental issues caused by conventional sources of energy (eg. fossil fuels and oil) as well as their rapid depletion, biogas as a sustainable and clean energy source could be used (Kumar et al., 2013; Tsavkelova & Netrusov, 2012). Biogas from organic waste is widely produced and used worldwide. It aids in attaining sustainability by providing access to modern, clean energy that is inexpensive and dependable and fights climate change and its effects by limiting emissions (Adebayo & Odedele, 2020; Marchaim, 2007; Tsavkelova & Netrusov, 2012).

Anaerobic digestion (AD) is a vital method of using wastes, generating highly efficient energy through biogas technology while the effluent is used as fertilizer, improving the ecology, protecting the environment, eradicating pathogenic microorganisms, and safeguarding human and animal health (Adebayo & Odedele, 2020; Marchaim, 2007; Tsavkelova & Netrusov, 2012). An answer to environmental degradation that results in greenhouse gas emissions, a decrease in fertilizer production, and the neutralization of organic waste can be found in the conversion of wastes into safe and valuable goods (Marchaim, 2007; Tsavkelova & Netrusov, 2012).

In the modern world, wastes that are typically viewed as low-valued are increasingly seen as renewable, ecologically friendly, and sustainable energy sources and are used to revolutionize our resources, safeguard our environment, and preserve the earth from the effects of global warming (Kumar et al., 2013; Olatunde, 2016; State et al., 2016). Technology for producing biogas has developed and proven to be environmentally favorable in these circumstances (Tsavkelova & Netrusov, 2012). Organic wastes like animal waste, agricultural, food, fish, human, industrial, residential, water hyacinth, duckweed, and other plant material are digested by bacteria in an anaerobic environment (absence of oxygen) for biogas production (Makhura et al., 2020; Marchaim, 2007; Nadu & Nadu, 2017; Oke, 2016).

AD will therefore be a greener option for treating these wastes. The procedure typically occurs under predetermined settings in specially constructed plants known as bio-digesters (Nadu & Nadu, 2017). Biogas, a result of the AD processes used to treat organic waste, is mostly composed of the gases methane (CH_4) and carbon dioxide (CO_2) (Araoye et al., 2018; Tsavkelova & Netrusov, 2012). An essential component of the waste management system is the creation of biogas from organic waste (Araoye et al., 2018).

A report from the FAO (Food and Agriculture Organization) estimates that 9.1 million tons of fish waste are thrown away each year. Consequently, fish by-products are now a global issue and pose an issue to the long-term viability of fish aquaculture (Kandyliari et al., 2020). Before being sold, over 70 % of fish is processed. Waste from fish makes up between 20% to 80% of this total (Maghaydah, 2003; Oke, 2016; Owamah, 2010; Pina et al., 2018). Large amounts of waste including heads, intestines, bones, viscera, and scales are created during the processing of fish, and the majority of this waste is made up of lipid and protein which are underutilized and ignored

(Kafle & Kim, 2012; Kandyliari et al., 2020; Maghaydah, 2003; Oke, 2016; Pina et al., 2018). The fish processing business is facing a problem to adhere to federal pollution control requirements (Maghaydah, 2003). Managing fish waste (FW) is a problem that affects the entire world (Pina et al., 2018). If an appropriate method for using and disposing of these wastes is not available, health risks and environmental problems may follow (Kandyliari et al., 2020; Pina et al., 2018). However, there is tremendous potential for FW to be converted into biogas using AD (Pina et al., 2018). FW has a significant potential for producing biomethane because they contain easily biodegradable organic matter that can increase the amount of biogas production (Marchaim, 2007; Pina et al., 2018).

This resource recovery technique also solves the problem of fish waste disposal. A method like that might be advantageous for fish processing everywhere in the nation and the world (Maghaydah, 2003). FW is employed as a feedstock in AD trials in both pure forms and anaerobic co-digestion with waste from strawberry processing, cow manure, water hyacinth, etc. (Pina et al., 2018). Kafle and Kim, (2012) researched the potential of FW to produce biogas. They discovered that fish waste made a promising substrate for biogas production. For increased production of biogas, the FW could be co-digested with additional substances.

Pina et al., (2018) made a study on AD and co-digestion of FW. According to their findings, the generation of biomethane increased from 0.2-0.9 CH_4 m^3/kg VS. Waste generated during fish processing, however, presents technological issues. FW digestion releases a lot of ammonia, which prevents or inhibits substrates from being digested (Pina et al., 2018).

Also, organic wastes like water hyacinth (WH), duckweed, and other plant material are digested by bacteria for biogas production (Makhura et al., 2020; Marchaim, 2007; Nadu & Nadu, 2017; Oke, 2016). WH is one of the most invasive water weed in the world that thrive in freshwater bodies of water and has spread to most nations, has detrimental impacts on the environment, the ecology, and society (Almoustapha & Kenfack, 2019; Armah et al., 2017; Bote et al., 2020; Katima, 2001; Rozy, 2016). It creates mats that obstruct waterways, make fishing impossible, limit water flow, degrades water quality by obstructing sunlight from penetrating the water and sharply lowering oxygen levels in the water, wipes out aquatic life like fish, and significantly reduces biodiversity.

WH used to be the subject of annual expenditures of millions of dollars to manage its growth (Bote et al., 2020; Chanathaworn, 2017; Njogu et al., 2015a; Tham, 2012). However, there are potential advantages of WH, including the creation of biogas, fertilizer, animal food, purified water, fiberboard, and paper (Bote et al., 2020; Dar & Phutela, 2017b; Tham, 2012). In these situations, the weeds' digestion can provide energy while resolving the issue of excessive weed growth in canals and ensuring appropriate management of the plant (Bote et al., 2020; Chanathaworn, 2017; Marchaim, 2007; Rozy, 2016).

WH contains a high cellulose content, low lignin, high carbon to nitrogen (C/N) ratio, and is readily and abundantly available (Bote et al., 2020; Marchaim, 2007). Consequently, it will be easier to digest during digestion (Bote et al., 2020; Chanathaworn, 2017; Marchaim, 2007; Rozy, 2016). With this method of generating biogas from water weed, waste will be reduced in addition to energy production (Pina et al., 2018).

Analytically, the biogas is composed of 40–75% CH_4, 0–3 % N, 25–55 % CO_2, 0–1 % H_2S, and 0–1 % H_2, and other gases (Oke, 2016; Pina et al., 2018). There is no restriction on using a single type of feedstock at a time while producing biogas from biodegradable material. Co-digestion is the process of using various feedstocks for anaerobic digestion. It increases the amount of food accessible for digestion while also stabilizing nutrients in the digester (Makhura et al., 2020). It has been discovered that using the co-digestion principle to produce biogas from a variety of wastes is an efficient way to maximize the amount of biogas produced (Nadu & Nadu, 2017; Owamah, 2010). The co-digestion of different organic materials has been used to increase the C/N ratio, improve gas and methane generation, and enhance good synergy for encouraging bacteria activity, (Owamah, 2010).

Even though co-digestion seeks to improve the quality and quantity of biogas production, the amount varies depending on the co-substrate used. This is possible because the physiochemical composition and mass of the feedstock all affect how quickly an object degrades. However, to ensure the longevity and sustainability of industrial anaerobic digestion facilities, the best possible mix of process variables and substrates must be used most economically (Owamah, 2010). Therefore, it is important to utilize selective and adequate amounts of co-digestion to maximize the biological and nutritional conditions for bacteria in the reactor and increase the production of biogas (Girmaye Kenasa & Ebsa Kena, 2019).

Katima, (2001) reported on the advantages of WH to generate biogas by investigating the impact of substrate concentration (5 to 30 g/l), particle size (1-3mm), and incubation period (1-6 days). Results showed that high biomethane content was 72.53% at a substrate ratio of 25 g/l, particle size below 1 mm, and produced after 5 days of incubation.

Bote et al., (2020) studied the construction of a machine for making briquette and the biogas production from WH. The study has shown that when compared to a 75-25 mixture, a 1:1 ratio of WH to cow dung produced the best yield (71.52 %). Armah et al., (2017) looked at the effects of using varying IC ratios of WH (Eichhornia crassipes) as a substrate for biogas production. At a feedstock to inoculum ratio of 1:4, the maximum biogas output was attained. This suggests that the AD process is favoured by an organic loading that contains more inoculum than the feedstock. Nalinga and Legonda, (2016) reported on the advantages of anaerobic co-digestion of fish waste (FW) and water hyacinth (WH) to produce biogas.

Results showed that there was a high potential for biogas and methane production from the anaerobic co-digestion (AnCo-digestion) of FW and WH than mono-digestion of each feedstock. Not only co-digestion, but also the biogas yield can be improved by optimizing variables such as organic loading rate (OLR), inoculum concentration (IC), pH, dilution, carbon to nitrogen (C/N) ratio, substrate ratio, retention time, dilution, and temperature (Nadu & Nadu, 2017). Additionally, the duration of acclimation of microorganisms in AD will be influenced by these variables as well as the origin of the inoculum, the amount of water present, and the anaerobic digesting conditions (Fathya et al., 2014; Madondo, 2017).

To increase biogas yield, sufficient amounts of active inoculum must be added since it will increase the yield of biogas, the amount of methane it contains, speed up the process, and improve stability (Jnr, 2011; Madondo, 2017; Owamah, 2010; Yadvika et al., 2004). Use active anaerobic inoculum whenever possible to shorten anaerobic digestion time and digester volume (C.Akunna, 2019; Kameswari et al., 2011; Owamah, 2010).

In addition to the type of inoculum used, a digester's inoculum requirement is also crucial. This requirement is sometimes described as the inoculum to substrate (I/S) ratio, inoculum to feed (I/F) ratio, feed to inoculum (F/I) ratio, inoculum concentration (IC), etc. (Abbasi et al., 2017). The choice of inoculum source and IC are crucial operational criteria for determining how quickly organic waste decomposes in anaerobic conditions (Fathya et al., 2014). The content of methane in biogas was observed to rise for IC between 1 and 4 (Owamah, 2010). With various inoculum to feed ratios, Armah et al., (2017) evaluated the effect of WH (Eichhornia crassipes) on the production of biogas. The ratio of feed to inoculum that produced the highest biogas generation was 1:4.

The most biogas was observed with 40 % IC, a WH of 1cm size (Dar & Phutela, 2017a). Having said all of this, there is currently no information in the literature about the right IC for maximizing biogas yield from AnCo-digestion of FW and WH. The rate of organic waste degradation normally increases with increasing dilution, and biogas generation increases with increasing percentage degradation (Jnr, 2011). Simple dilution can be used to enhance the biodegradability of organic waste. Anaerobic digestion is inhibited by large quantities of end products produced by high solids digestion.

Consequently, dilution would be beneficial (Jnr, 2011). Water will reduce the quantity of some elements, such as sulfur, and nitrogen, which result in a byproduct that hinder anaerobic digestion, such as ammonia and hydrogen sulfide. Low biogas is produced by anaerobic digestion without water addition. Anaerobic digesters are divided into three groups based on the amount of solids they contain: high solid digesters, with waste to water ratio of 1:2.5-4.5, medium solid digesters, with a ratio of 1:5-7, and low solid digesters, with a ratio of 1:10 (Hhaygwawu, 2016). Dry AD results in a more compact solution in the reactor and high OLR.

The high organic loading and compactness of the solution improve the level of stabilization and enable the dry AD process to produce high biogas than a wet AD process (Madondo, 2017; Sun, 2015). However, overly dry AD (< 40% relative humidity) will inhibit microorganisms' activity, while preventing bacteria from using organic substances that are not dissolved in water. Having said all of this, there is no literature available regarding the ideal water content (dilution) for FW and WH co-digestion to produce the most amount of biogas. According to Pina et al., (2018), Kafle and Kim, (2012), fish waste made a promising substrate for biogas production.

However, FW produced a lot of amounts of ammonia and volatile fatty acids (VFAs) accumulation which inhibit substrate digestion, when digested alone. For increased production of biogas, the FW could be co-digested with additional substances (Pina et al., 2018). Moreover, AnCo-digestion of FW and WH was a potential technological approach that helped to mitigate that issue (Nalinga & Legonda, 2016). Studies have shown that adding co-substrates to "pure" waste makes it far more valuable from an economic and environmental viewpoint (Tsavkelova & Netrusov, 2012).

Co-digestion of a low C/N ratio substrate (FW) with a high C/N ratio substrate (WH) has been shown to enhance biomethane production (Tsavkelova & Netrusov, 2012).In Kenya and many other African nations, large amounts of FW and WH are produced. Because they are readily available, affordable, and sustainable alternatives, the successful utilization of FW and WH to make biomethane in AD may have significant advantages. However, there is currently a lack of knowledge on the potential and optimal method for co-digesting FW and WH to obtain high biogas output (Kafle & Kim, 2012).

Evaluation of the impact of various operating parameters on biogas generation from AnCo-digestion of FW and WH still needs to be done. Moreover, the process of producing biogas from the co-digestion of FW and WH needs to be optimized. The long-term economic benefit of knowing the right parameters to get the best biogas and methane yields will also benefit fish processors. The best method for optimizing biogas from the AnCo-digestion of FW and WH was assessed. The main goal of this research study was to determine the optimal conditions for optimizing the production of biogas in AD by evaluating the impacts of inoculum concentration (IC), substrate (WH: FW) ratio, and dilution (water content) on biogas production by DOE using RSM approach.

1.2 Problem Statement

Environmental pollution, toxic wastelands, increased energy demand and prices, and fossil fuel depletion has become problems for the entire world. A report from the FAO (Food and Agriculture Organization of the United Nations) estimates that 9.1 million tons of FW are thrown away each year. Consequently, fish by-products are now a global issue and pose an issue to the long-term viability of fish aquaculture (Kandyliari et al., 2020). Before being sold, over 70 % of fish is processed. Waste from fish makes up between 20% to 80% of this total (Maghaydah, 2003; Oke, 2016; Owamah, 2010; Pina et al., 2018).

Large amounts of waste including heads, intestines, bones, viscera, and scales are created during the processing of fish, and the majority of this waste is made up of lipid and protein which are underutilized and ignored (Kafle & Kim, 2012; Kandyliari et al., 2020; Maghaydah, 2003; Oke, 2016; Pina et al., 2018). The fish processing business is facing a problem to adhere to federal pollution control requirements (Maghaydah, 2003). Managing fish waste is a problem that affects the entire world (Pina et al., 2018). If an appropriate method for using and disposing of these wastes is not available, health risks and environmental problems may follow (Kandyliari et al., 2020; Pina et al., 2018).

On the other hand, WH is the most invasive water weed in the world that thrive in freshwater bodies of water and have spread to most nations. It has detrimental impacts on the environment, the ecology, and society (Almoustapha & Kenfack, 2019; Armah et al., 2017; Bote et al., 2020; Katima, 2001; Rozy, 2016). WH slows water flow and forms mats that block streams, making fishing impossible, worsen water quality by obstructing sunlight from penetrating the water and sharply lowers oxygen levels, wipes out aquatic life like fish, and significantly lowers biodiversity. Water hyacinth used to be subject to annual expenditures of millions of dollars to manage its growth (Bote et al., 2020; Chanathaworn, 2017; Njogu et al., 2015a; Tham, 2012).

The current methods of disposing of this highly polluting organic matter such as dumping/discarding onto the open ground and disposing of in sanitary landfills result in health risks/hazards, environmental issues, and a loss of important nutrients (Kafle & Kim, 2012; Kandyliari et al., 2020; Maghaydah, 2003; Oke, 2016; Pina et al., 2018).In Kenya and many other African nations, large amounts of FW and WH are produced. Because they are readily available, affordable, and sustainable alternatives, the successful utilization of FW and WH to make biogas in AD may have significant advantages.

Anaerobic digestion (AD) is a vital method of using wastes, generating highly efficient energy through biogas technology while the effluent is used as fertilizer, improving the ecology, protecting the environment, eradicating pathogenic microorganisms, and safeguarding human and animal health (Adebayo & Odedele, 2020; Marchaim, 2007; Tsavkelova & Netrusov, 2012). An answer to environmental degradation that results in greenhouse gas emissions, a decrease in fertilizer production, and the neutralization of organic waste can be found in the conversion of wastes into safe and valuable goods (Marchaim, 2007; Tsavkelova & Netrusov, 2012).

The research work aimed to optimize the operating conditions for converting these wastes into biogas for maximum energy generation.

1.3 Justification of the Study

The conversion of waste to energy provides an answer to environmental pollution, waste treatment, and management, rising energy costs, and reduction of organic waste and greenhouse gas emissions. More than 9.1 million tons of FW are thrown away each year. 70 % of fish is processed before being sold and 20% to 80% of this total is waste (Kandyliari et al., 2020) (Maghaydah, 2003; Oke, 2016; Owamah, 2010; Pina et al., 2018). However, FW is rich in lipids, and proteins and contains easily biodegradable organic matter that increase the amount of biogas production which is clean and renewable energy (Kafle & Kim, 2012; Kandyliari et al., 2020; Maghaydah, 2003; Oke, 2016; Pina et al., 2018).

Moreover, Millions of dollars are used to be spent to manage the excessive growth of WH because of its effects on the environment, ecology, and irrigation. However, WH contains a high cellulose content, low lignin, and high C/N ratio. It will be easier to digest during AD to produce biogas which can replace fossil fuel (Almoustapha & Kenfack, 2019; Armah et al., 2017; Bote et al., 2020; Katima, 2001; Rozy, 2016). The current methods of disposing of this waste such as dumping/discarding onto the open ground and disposing of in sanitary landfills result in health hazards and environmental issues (Kafle & Kim, 2012; Kandyliari et al., 2020; Maghaydah, 2003; Oke, 2016; Pina et al., 2018).

Biogas can be used as fuel for machines and automobiles as well for cooking, lighting, heating, and other purposes. For effective use of biogas, certain adjustments are required. Biogas is compressed similar to natural gas. The presence of CO_2 is not good and is hazardous to humans and corrodes motors and pipes, its removal is crucial.

The CO_2 can be eliminated by passing the biogas into a solution of calcium hydroxide or washing it with water under pressure (Okonkwo et al., 2016; Rozy, 2016). The byproducts of anaerobic digestion (AD) can be used on farms as compost or bio-fertilizer.

AD is a vital method of using wastes, generating highly efficient energy through biog as technology, improving the ecology, protecting the environment, eradicating pathog enic microorganisms, and safeguarding human and animal health(Adebayo& Odedele, 2020; Marchaim, 2007; Tsavkelova & Netrusov, 2012).

Co-digestion of a low C/N ratio substrate such as FW with a high C/N ratio substrate such as WH has been shown to enhance biomethane production (Tsavkelova & Netrusov, 2012).In Kenya and many other African nations, large amounts of FW and WH are produced. Because they are readily available, affordable, and sustainable alternatives, the successful utilization of FW and WH to make biomethane in AD may have significant advantages.

1.4 Objectives of the study

1.4.1 Main objective

- To optimize biogas production from anaerobic co-digestion of fish waste and water hyacinth.

1.4.2 Specific objectives

1. To characterize the substrate for biogas production.
2. To evaluate operating conditions to maximize the biogas production yield.
3. To determine the biogas yield model equation.

1.5 Significance and Expected Output of the Study

Results showed that there was a high potential for biogas and methane production from the anaerobic co-digestion of FW and WH. Not only co-digestion but also the biogas yield can be improved by optimizing variables such as substrate, inoculum concentration, and dilution. Additionally, the duration of acclimation of microorganisms in AD will be influenced by these variables as well as the origin of the inoculum, the physicochemical characteristics of the substrate, and the anaerobic digesting conditions. Particle size reduction of the substrate such as cutting and blending will help to increase the biodegradability and digestibility of organic matter which will result in a high biogas production rate. The biogas composition analysis will help us to know if the biogas is suitable to be used as fuel which will reduce fossil fuel consumption and environmental pollution. The long-term economic benefit of knowing the right parameters to get the best biogas and methane yields will also benefit fish processors.

1.6. Scope of the Study

This study will characterize the substrates to find out their physicochemical composition. The study will evaluate the effects of operating conditions such as Substrate ratio, IC, and Dilution on biogas production and find out the optimal ratio for maximum biogas yield. Finally, biogas composition and biogas yield model equation will be determined. The substrate will be collected from Lake Victoria, Kenya, and fish point, Eldoret, Kenya.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

Rapid economic and technological development is taking place in many countries throughout the world. Sustainable and efficient energy needs can’t be achieved without using biogas as a fuel (Araoye et al., 2018; Marchaim, 2007; Owamah, 2010). Because AD is one of the few biotechnological processes that provide solutions and exciting opportunities to climate change as an alternative energy source, reducing and handling the amount of organic waste safely, reducing environmental issues, and increasing agricultural productivity by using its digested as fertilizer for soil, biogas production using AD of various organic waste appears to be the most effective and popular (Alfarjani, 2012; Aslanzadeh, 2014; Marchaim, 2007; Owamah, 2010; Tsavkelova & Netrusov, 2012). As the global population grows and the demand for natural resources rises, it is our responsibility to adopt the "RRR" (Reuse, Reduce, and Recycle) attitude (Deressa et al. 2015).

2.2. Biogas Technology

Converting organic waste to energy is provided by biogas technology. This technology is crucial for creating energy from clean, renewable sources. (Araoye et al., 2018; State et al., 2016; Stojkovi et al., 2018; Yadvika et al., 2004). Biogas is a result of microbial anaerobic digestion of an organic substrate (Alfarjani, 2012; Nadu & Nadu, 2017; Stojkovi et al., 2018; Yadvika et al., 2004). It was recognized as a substitute for fossil fuels and may be utilized to address issues with waste treatment and management, rising energy costs, and fostering sustainable growth (Alfarjani, 2012; Araoye et al., 2018; Asam et al., 2018; Madondo, 2017; Ofoefule, Ibeto, & Onukwuli, 2012; Prasad, Rathore, & Singh, 2017; Roslina et al., 2014; Stojkovi et al., 2018).

Biogas mostly consists of CH_4 (methane) and CO_2 (carbon dioxide), while it also contains trace amounts of other gases like ammonia, nitrogen, and hydrogen sulfide (Adebayo & Odedele, 2020; Araoye et al., 2018; Gooch, 2011; Kumar et al., 2013; Madondo, 2017; Ofoefule et al., 2012; Stojkovi et al., 2018; Tsavkelova & Netrusov, 2012).

Biogas can be used as fuel for machines and automobiles as well for cooking, lighting, heating, and other purposes. For effective use of biogas, certain adjustments are required. Biogas is compressed similar to natural gas. The presence of CO_2 is not good and is hazardous to humans and corrodes motors and pipes, its removal is crucial. The CO_2 can be eliminated by passing the biogas through a solution of calcium hydroxide or washing it with water under pressure (Okonkwo et al., 2016; Rozy, 2016).

2.3. Anaerobic Digestion

In the AD system, bacteria break down organic matter in the absence of oxygen to produce biogas (Alfarjani, 2012; Ariunbaatar, 2015; Baredar, Suresh, Kumar, & Krishnakumar, 2016; Carlsson, Lagerkvist, & Morgan-sagastume, 2012; Cioablă, Dumitrel, & Ionel, 2013; Jnr, 2011; Lawal, Dzivama, & Wasinda, 2016; Sinaga, Nasution, & Mel, 2018; Stojkovi et al., 2018). For a variety of biodegradable wastes, such as agricultural (animal manures, vegetable residues, energy crops), weeds (water hyacinth, etc.), industrial (food industry, industries, sludge from industrial processes, waste from fish processing, etc.), municipal residues, aquatic biomass, and other types of organic waste, anaerobic digestion is a desirable and effective alternative. (Cioablă et al., 2013; Madondo, 2017; Mousa & H, 2015; Stojkovi et al., 2018; Toma, Ferdes, Voicu, & Paraschiv, 2016).The benefits of anaerobic digestion make it the most efficient, cost-effective, and environmentally friendly sources of renewable energy (Alfarjani, 2012; Mousa & H, 2015; Stojkovi et al., 2018).

2.3.1 Principal Products of Anaerobic Digestion

Biogas energy, water, and digested slurry are the main three byproducts of the AD system (Jnr, 2011; Stojkovi et al., 2018; Usman, 2018).

The biogas created by the AD process typically contains methane (40-75 %), oxygen (0–2 %), carbon dioxide (25-55 %), hydrogen sulfide (< 1%), ammonia (0–0.05 %), nitrogen (0–2 %), hydrogen (0–1%), and water vapour (2-7%) (Adebayo et al., 2020; Chanathaworn, 2017; Orhorhoro et al., 2017; Pina et al., 2018; Teodorita et al., 2010). The types of feedstock, digestion processes, temperature, dilution, retention period, inoculum source and inoculum concentration, substrate ratio, and other various parameters all affect the quality and composition of biogas production. Digestate is the solid that is left over from the initial input that the microorganisms are unable to utilize. It also consists of the dead microbes from the reactor. The digestate or effluent can be used as an organic fertilizer (Astrid, 2007; Cioablă et al., 2013; Jnr, 2011; Kubaská, M. et al., 2010; Prasad et al., 2017; State et al., 2016; Stojkovi et al., 2018).

The water in the reactor is a result of the organic material's moisture content, and more water is created by microbial processes in the digestive system (Jnr, 2011). By dewatering the digested, this water might be liberated. Njogu et al., (2015a) conducted a study on the Production of biogas using WH (Eichhornia crassipes) to generate biogas. According to the study, WH was a potential feedstock for biogas generation. Armah et al., (2017) made a study on the Impact of using WH (Eichhornia crassipes) for Biogas. The study revealed water hyacinth can make biomethane, which can help reduce reliance on fossil fuels. In this study, AD at mesophilic temperature was proven to be a practical method for producing biogas. According to a study conducted by Kafle and Kim, (2012) on the potential of biogas from fish waste, biomethane produced was 757 and 554ml/g VS respectively.

VS was removed by 77 percent and there was 73 percent methane from the biogas. Fish waste was discovered to provide a highly viable AD substrate. Research on the production of biogas from food waste was conducted by Ojikutuabimbola & O, (2016). According to the study's findings, each form of food waste generated a similar amount of biogas, while the mixed treatment produced the most (8016.67 mL/day). Fish waste had the lowest generation, averaging 1090 mL per day.

Cioablă et al., (2013) studied biogas production from agricultural biomass. Results of a factorial study on the impacts of biomass net calorific value and C/N ratio revealed that these factors had a substantial impact on the overall amount of biogas produced. Almoustapha & Kenfack, (2009) researched on the production of biogas using water hyacinth for meeting energy needs in a Sahelian. Their study has shown that biogas can be generated from Anco-digestion of WH and fresh rumen residue. The facility's yield was 0.52 and 0.29 during warm and cold seasons respectively.

Bote et al., (2020) researched the construction of a machine for making and the biogas generation from WH. The study showed that when compared to a 75-25 mixture, a 1:1 ratio of water hyacinth to cow dung produced the best yield (71.52 %).

The summary of biogas content is shown in table 2.1(Adebayo & Odedele, 2020; Chanathaworn, 2017; Orhorhoro et al., 2017; Pina et al., 2018; Teodorita et al., 2010).

Table 2. 1: Biogas composition

Constituent	Symbol	Composition (Vol %)
Methane	CH_4	40-75
Oxygen	O_2	0-2
Carbone dioxide	CO_2	25-55
Hydrogen sulfide	H_2S	<1
Ammonia	NH_3	<1
Nitrogen	N_2	0-2
Hydrogen	H_2	0-1

2.3.2 Benefits and Limitations of Anaerobic digestion

Methane from anaerobic digestion's biogas when burned can generate power and heat, as well as being frequently used in cooking and lighting and as a fuel for vehicles (Alfarjani, 2012; Jnr, 2011). Methane energy generated during AD can be used as fuel which can help to reduce fossil fuel consumption, consequently greenhouse gas emissions. This is because organic material contains carbon, which is a component of the carbon cycle (Jnr, 2011). Table 2.2 explains the general benefits and limitations of AD (Alfarjani, 2012; Araoye et al., 2018; Ariunbaatar, 2015; Cioablă et al., 2013; Kubaská, M. et al., 2010; Meegoda et al., 2018; Muhammad Rashed, 2015; Nadu & Nadu, 2017; Prasad et al., 2017; Rabii et al., 2019; Stojkovi et al., 2018).

Table 2. 2: Benefits and Limitations of Anaerobic digestion

Benefits

- Organic waste management and treatment system is carried out
- Neutralization of waste is adopted
- Reuse, reduce, and recycling are improved
- Modern, clean, and renewable fuel is produced
- Dependence on fossil fuels is reduced
- Greenhouse gas emissions are reduced
- Less land is used
- Reduction in the production of odorous gases
- Reduction in the need for inorganic fertilizers
- The prevention of the spread of pathogens and
- The Source of electricity and heat is generated from biogas

Limitations

- High capital expense
- Start-up times are lengthy
- Production rate may be long according to the substrate characteristics
- The additive may be necessary
- Environmental changes may cause the process to fail
- Changes in operational parameters may cause the production of biogas to fail
- High explosion risk
- Needs additional treatments
- Corrosive and odors gases may be available.

To lessen or avoid these drawbacks, optimization of the AD process should be used to maintain a steady process and maximize the potential for the production of biogas (Nadu & Nadu, 2017).

2.3.3 Anaerobic Digestion Process

The anaerobic digestion process is classified into four stages which are hydrolysis, acidogenesis, acetogenesis, and methanogenesis. Every stage depends on the metabolic state of the different bacteria (Adebayo & Odedele, 2020; Alfarjani, 2012; Ariunbaatar, 2015; Armah et al., 2017). Figure 2.1 shows the schematic representation of AD (Alfarjani, 2012; Cioablă et al., 2013; Gooch, 2011; Maile, Muzenda, & Mbohwa, 2016; Rabii et al., 2019; Stojkovi et al., 2018)

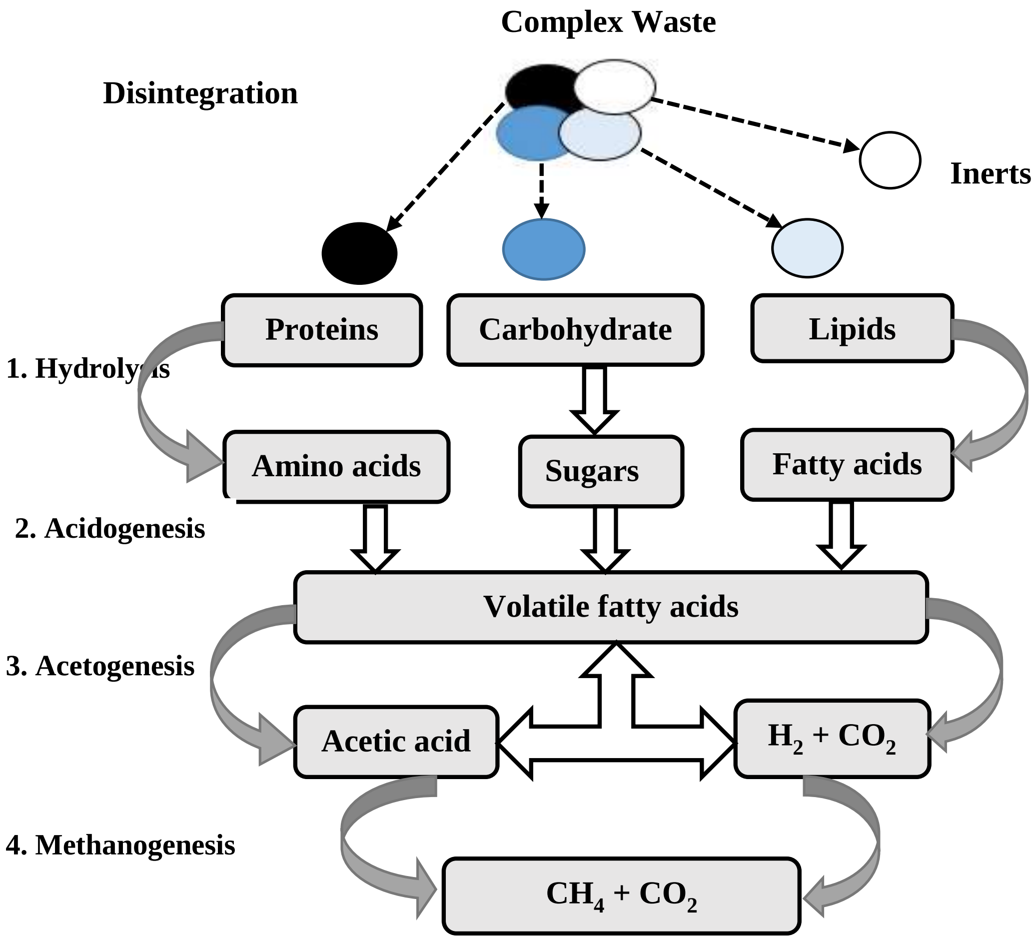


Figure 2. 1: Schematic representation of Anaerobic Biodegradation

2.3.3.1 Hydrolysis

Anaerobic digestion begins with hydrolysis, which includes breaking down and converting complex organic materials into soluble molecules (Astrid du Petit Thouars, 2007; Baredar et al., 2016; C.Akunna, 2019; Jnr, 2011; Nadu & Nadu, 2017; Stojkovi et al., 2018; Xu et al., 2019). Complex organic materials are broken down into smaller components and hydrolyzed to soluble compounds during the hydrolysis stage, making them available for biological degradation. These smaller components and hydrolyzed compounds include carbohydrates, proteins, and lipids (Alfarjani, 2012; Almansa, 2015; Meegoda et al., 2018; Muhammad Rashed, 2015). Cellulases, protoases, and lipases, which are extracellular enzymes, break down lipids, proteins, and carbohydrates into fatty acids, sugars, and amino acids (Almansa, 2015; Madondo, 2017; Muhammad Rashed, 2015). Hydrolytic bacteria are responsible for hydrolysis. Particle size, OLR, pH, retention time, temperature, dilution, substrate ratio, inoculum concentration, and inherent properties of the substrate can all affect the hydrolysis stage (Almansa, 2015). consequently, if the hydrolysis stage can be improved, the anaerobic digestion process may be boosted (Alfarjani, 2012).

2.3.3.2 Acidogenesis

The fatty acids, sugars, and amino acids of the hydrolysis stage are used in the acidogenesis process or acidification to form alcohols, NH_3, CO_2, volatile fatty acids, and H_2 gases (Astrid, 2007; C.Akunna, 2019; Jnr, 2011; Meegoda et al., 2018; Muhammad Rashed, 2015; Nadu & Nadu, 2017). It is carried out by acidogens and is the quickest response in the AD process of compound organic matter. Acidification can appear brought on by out-of-control of acidogenesis (Madondo, 2017; Maile et al., 2016).

2.3.3.3 Acetogenesis

Volatile fatty acids (VFAs) from acidogenesis stage are converted into carbon dioxide, hydrogen, and acetic acid during this stage (Jnr, 2011; Meegoda et al., 2018; Rabii et al., 2019). Acetogen microbes act at this phase (Astrid du Petit Thouars, 2007).

2.3.3.4 Methanogenesis

Methanogens performed the final stage of the AD by converting H_2, CO_2, and acetate to CH_4(Astrid du Petit Thouars, 2007; C.Akunna, 2019; Jnr, 2011; Kumar et al., 2013; Meegoda et al., 2018; Muhammad Rashed, 2015; Nadu & Nadu, 2017; Rabii et al., 2019; Stojkovi et al., 2018; Toma et al., 2016). Microorganisms development and methanogenesis are primarily influenced by different variables including temperature, dilution, pH, RT, C/N ratio, OLR, substrate concentration, digester configuration, IC, etc. (Sathish, S., 2011).

2.3.4 Important Operating Parameters in Anaerobic Digestion Process

To maximize microbial activity and the effectiveness of anaerobic degradation, the operational factors of the reactor can be controlled or adjusted. This is because the microorganisms' growth rate is crucial to the anaerobic digestion process (Jnr, 2011; Stojkovi et al., 2018). The substrate characteristics, temperature, C/N ratio, substrate ratio, pH, dilution, OLR rate, stirring/mixing, retention time, inoculum concentration, and the source of inoculums are only a few examples of the variables that might affect the rate and quantity of biogas output (Alfarjani, 2012; Battista et al., 2016; Girmaye Kenasa et al., 2019; Jnr, 2011; Muhammad Rashed, 2015; Sathish, S., 2011; Stojkovi et al., 2018). These elements are recognized to have an impact on the biogas yield, the digestion system effectiveness, and the cost-effectiveness of running the biogas plant (Alfarjani, 2012; Girmaye Kenasa et al., 2019; Jnr, 2011; Muhammad Rashed, 2015; Stojkovi et al., 2018).

2.3.4.1 pH

A key factor in anaerobic digestion is the pH level (Alfarjani, 2012; Jnr, 2011; Patil & Deshmukh, 2015). Microorganisms that produce methane are affected by it because each group survives at different ranges (Jnr, 2011). It indicates how basic or acidic a solution is (Drosg & Braun, 2013). The pH of more acidic solutions is lower, whereas the pH of more alkaline solutions is greater. The optimum pH range for biogas generation is between 6.5 to 7.2 (Deressa et al., 2015; Rabii et al., 2019; Sathish, S., 2011). Acidic environments hinder the growth of methanogenic bacteria and their ability to produce methane because they are extremely sensitive to acidic conditions (Patil & Deshmukh, 2015).

When the pH was greater than 7.8 or less than 6.3, the rate of methane generation reduced, and if the pH fell below 6.3, the process of methanogenesis may be inhibited (Roslina et al., 2014). The rate of methane production typically decreases for values outside the specified pH range. As a result, at low pH, biogas production is decreased. Methanogen activity is similarly decreased at high pH levels (Dar & Phutela, 2017b). There are situations when the pH level needs to be raised or lowered. Basic solutions such as NaOH or KOH can raise pH, and the addition of lime or the usage of acids like HCl can regulate reduction (Jnr, 2011; Tsavkelova & Netrusov, 2012)

2.3.4.2 Temperature

Given that it affects the rate of reaction, the temperature has a critical impact on the AD process (Alfarjani, 2012; Maile et al., 2016). Various temperatures, such as mesophilic (25 to 45 °C), thermophilic (45 to 65°C), and psychrophilic (20°C) can be used for anaerobic digestion (Twizerimana et al., 2021). In anaerobic digestion, mesophilic and thermophilic temperatures are most frequently utilized and accepted.

Mesophilic operations (25–40°C) benefit from adequate operating performance, stability, and reduced sensitivity to inhibitors. According to some researchers, methane production has a high potential in both mesophilic and thermophilic temperature regimes (Jnr, 2011; Twizerimana et al., 2021; Yadvika et al., 2004). Also at extremely high temperatures, such as those above 90°C, the activity of bacteria is restricted, and microorganisms are destroyed (Dar & Phutela, 2017b). Although thermophilic AD has several benefits, including better pathogen kill, quick digestion, and shorter retention times, it is typically more expensive to run due to the higher operating temperatures, decreased process stability, and more susceptibility to failure or inhibition from environmental changes (Ariunbaatar, 2015; Chanathaworn et al., 2018; Hhaygwawu, 2016; Jnr, 2011).

Different benefits of mesophilic digestion include improved process stability, a higher bacterial richness, and microorganisms that are more resilient and adaptable to changing environmental circumstances (Jnr, 2011; Prasad et al., 2017). Due to higher system stability and less expensive operation management, the mesophilic temperature of the anaerobic digestion process is used more frequently than the thermophilic condition, according to performance statistics (Avs, 2016). In general, mesophilic temperatures maintained in the range of 35 and 37°C were used for anaerobic experiments (Avs, 2016; Patil & Deshmukh, 2015). Several researchers have discovered that mesophilic settings are best for biomethanation investigations, however, the ideal temperature for mesophilic AD is 37°C (Gooch, 2011; Patil & Deshmukh, 2015). The ranges of temperatures for AD are shown in table 2.3 (C.Akunna, 2019; Climent et al., 2007; Twizerimana et al., 2021; Mir et al., 2016; Rutz, 2007).

Table 2. 3: Ranges of temperature and corresponding retention times

Temperature Stages	Operating Temperature (°C)	Retention Time (days)
Mesophilic	<20	11-28
Psychrophilic	25 – 45	6-30
Thermophilic	45 – 65	4-14

2.3.4.3 Particle size

Particle size is among biogas operational factors that affect the production of biogas (Yadvika et al., 2004). Substrates shouldn't be too big, otherwise, will be difficult to carry by bacteria during the digestion process (Kumar et al., 2013; Maile et al., 2016).

Smaller particles enhance the digestibility of the substrate, increasing microbial activity, speeding up the digestion process, and increasing the production of biogas (Kumar et al., 2013; Yadvika et al., 2004).

2.3.4.4. Retention Time (RT)

Retention time is the length of time biodegradable solids fed to the AD system remain or spend in the system (C.Akunna, 2019; Jnr, 2011; Meegoda et al., 2018; Patil & Deshmukh, 2015; Stojkovi et al., 2018; Sun, 2015). The substrate type and its biodegradability determine the proper reaction time (C.Akunna, 2019; Girmaye Kenasa & Ebsa Kena, 2019). The retention period varies depending on several process variables, including process temperature, total solids concentration, mixing intensity, inoculum source and concentration, and waste composition (Hanghome, 2014; Jnr, 2011). Wastes handled in a mesophilic digester have a retention period of 10 to 40 days (Jnr, 2011). Table 2.4 displays the recommended retention time at each temperature (C.Akunna, 2019).

Table 2. 4: Recommended design retention times for AD

Operating Temperature (ºC)	Minimum Retention Time (Day)	Maximum Retention Time (Day)
18	11	28
24	8	20
30	6	14
35	4	10
40	4	10

The ideal retention time (RT) for biogas production is discovered to be between 20 and 30 days (Patil & Deshmukh, 2015). The RT for organic material treated in mesophilic temperatures ranges between 15-30 days and for those treated in thermophilic temperatures ranges between 12-14 days (Hhaygwawu, 2016; Sun, 2015). Since the RT regulates how much digestion occurs in the digester, it must be high enough for biodegradation to occur (Patil & Deshmukh, 2015). Both short and long retention times have an impact on the AD; when too short can generate low gas yield because the organic matter was not fully decomposed, while the longer retention time necessitates a large digester capacity and raises capital costs (C.Akunna, 2019; Jnr, 2011; Patil & Deshmukh, 2015; Stojkovi et al., 2018). The right period will vary depending on the surrounding environment, the feedstock, and the use of the digested material (Patil & Deshmukh, 2015). For instance, RT in tropical regions ranges from 30 to 50 days, whereas in colder areas, RT might reach 100 days (Maile et al., 2016). While digesting vegetable waste, the shortest RT employed was of 15 days, and the longest was of 100 days (Maile et al., 2016).

2.3.4.5 Organic loading rate (OLR)

The ORL, measured as the quantity of organic waste over digester volume over time, is an important operational parameter that helps determine the amount of feedstock being fed in a reactor per day depending on the digester capacity to generate high biogas yield (Maile et al., 2016; Mel et al., 2015; Patil & Deshmukh, 2015; Stojkovi et al., 2018; Teodorita et al., 2010).

Controlling the organic loading to the system is very important as it helps to manage the microbiological process of anaerobic digestion (Marchaim, 2007). Controlling OLR is crucial for maximizing biogas output because of an optimal feedstock rate for a specific plant, beyond where additional increases in the amount of substrate would not create more gas (Kumar et al., 2013; Patil & Deshmukh, 2015). An insufficient loading rate could lead to a decrease in the digester's performance because of the absence of nutrients for microorganisms to grow while system overloading may result in imbalanced methanogenesis activities and the creation of acid that lead to high VFA accumulation that decreases the digester's pH and less biogas production (Jnr, 2011; Rabii et al., 2019; Stojkovi et al., 2018; Sun, 2015).

2.3.4.6 Stirring/Mixing

It is necessary to mix the contents of the digester to guarantee close contact between the substrate and the microorganisms, which can significantly increase the effectiveness of the digestion process (Hanghome, 2014; Yadvika et al., 2004). Additionally, mixing reduces temperature gradients inside the reactor and stops the creation of scum or foam (Hanghome, 2014; Jnr, 2011). Slow mixing is ideal since vigorous mixing might damage the microorganisms and result in the creation of a hard scum on the surface, therefore inhibiting the biogas production (Hanghome, 2014; Jnr, 2011). Mixing digester contents can be done in a variety of methods, such as feeding slurry daily rather than on a regular schedule, adding specific mixing equipment like pistons, etc. in the plant (Kumar et al., 2013; Yadvika et al., 2004).

2.3.4.7 Inoculum

Inoculum is a substance supplied to a digester to provide a live source of microorganisms for the start-up and operation of biogas processes (Madondo, 2017; Yadvika et al., 2004). It aids in the anaerobic digestion process by giving the required bacteria for biodegradation (Almansa, 2015; Jnr, 2011). A common name for this procedure is seeding (Jnr, 2011; Maile et al., 2016; Yadvika et al., 2004). An inoculum must be added since it will increase the yield of biogas, and the amount of methane it contains, speed up the process, and improve stability (Jnr, 2011; Madondo, 2017; Owamah, 2010; Yadvika et al., 2004). To decrease the anaerobic digestion period and digester volume, utilization of active inoculum from animal manure is always preferable (C.Akunna, 2019; Kameswari et al., 2011; Owamah, 2010). Cow manure that has been digested from a functioning biogas plant, well-rotted manure pit material, cow dung slurry, and sewage sludge can be used as inoculum (Kumar et al., 2013; Madondo, 2017; Yadvika et al., 2004). Because it contains an activated microbial consortium, the bio-digested slurry was employed as an inoculum (Dar & Phutela, 2017b).

2.3.4.8 Inoculum concentration (IC)

In addition to the type of inoculum used, another important factor is how much active inoculum is introduced to a reactor. It is usually referring to the inoculum to substrate ratio (ISR), inoculum to feed ratio, or inoculum concentration (IC) (Abbasi et al., 2012; Madondo, 2017). Due to its ability to either improve or hinder the AD of the substrate and result in either high or low biogas and methane outputs, this ratio has a considerable impact on biogas production rates (Lawal et al., 2016). Because methanogenesis is inhibited at higher inoculum concentrations, the amount of methane in the biogas is significantly reduced even at lower inoculum concentrations there are insufficient bacteria to start the AD process (Abbasi et al., 2012; C.Akunna, 2019).

To maximize the benefits of inoculum, an ideal inoculum concentration must be known (Dar & Phutela, 2017b; Madondo, 2017; Maile et al., 2016). Finding the right quantity of inoculum contains the essential microbes for biodegradation to start is crucial since inoculum concentration greatly affects the period of microorganisms' development (Jnr, 2011). The choice of inoculum source and inoculum concentration are important operational criteria for determining how quickly organic waste decomposes in anaerobic conditions (Fathya et al., 2014). Anaerobic digestion is ineffective for inoculum to substrate ratios greater than 4 (Avs, 2016). The ideal inoculum concentration is between 10 and 40%.

It is advised to use inoculum-to-feed ratios larger than ten when first starting (C.Akunna, 2019). It has been proposed that the inoculum to substrate ratio used in laboratories should be optimized (Avs, 2016). Armah et al., (2017) investigated different feed to inoculum ratios to examine the effect of WH (Eichhornia crassipes) on biogas production. The ratio of feed to inoculum that produced the highest biogas generation was 1:4. This suggests that an organic loading with a high ratio of inoculum to feedstock (1:4) favors the anaerobic digestion process. The effect of inoculum concentration on biogas generation of sheep paunch manure was studied by Lawal et al., (2016).

According to the research, the higher inoculum to substrate ratio, the higher the biogas production. The yield increased from 0.57195 -1.46784 Nm^3/kg VS. Quality and quantity of inoculums are essential to the performance, length of time needed, and stability of methanogenesis for the operation of the AD system. The type of substrate, the impact of the I/S ratio, and other factors determine the required quantity of inoculum (Girmaye et al., 2019; Madondo, 2017; Owamah, 2010).

Methane production has increased from 19 to 23% with the use of 10% inoculation as reinforcement of anaerobic digestion. The influence of inoculum concentration on the mesophilic AD of slaughterhouse waste was studied by Fathya et al., (2014). They studied the production of biogas at three different ratios inoculum to feed ratio, 0.3, 0.5, and 1.

2.3.4.9 Carbon to nitrogen (C/ N) Ratio

The C/N ratio of the substrate has a significant impact on how much biogas can be produced from any feedstock. The proportion of nitrogen and carbon in organic waste is known as the carbon to nitrogen ratio (Jnr, 2011). The presence of nitrogen in the waste material has two advantages: (a) it provides nutrients that are necessary for the digestibility of proteins, nucleic and amino acids; and (b) it acts as a strong base to neutralize the volatile acids produced within the digester and maintain the pH levels that are important for microorganisms’ growth. A higher amount of nitrogen in the substrate might cause harmful effects due to high ammonia production.

To prevent either too little nitrogen or too much nitrogen (ammonia toxicity), the feedstock must contain the right quantity of nitrogen (Marchaim, 2007).To carry out their metabolic functions, bacteria require a specific carbon to nitrogen ratio. The C/N ratio higher than 23:1 were shown to be unfavorable for optimum digestion, whereas lower than 10:1 ratios were found to be inhibiting (Marchaim, 2007). However, in anaerobic digesters, the ideal carbon to nitrogen ratio ranges from 20 to 30 (C.Akunna, 2019; Hhaygwawu, 2016; Jnr, 2011; Ojikutuabimbola & O, 2016; Rabii et al., 2019; Stojkovi et al., 2018; Toma et al., 2016; Yadvika et al., 2004). For effective digester operation, the proper C: N ratio must be maintained (Maile et al., 2016).

A high ratio implies that methanogens are fast consuming nitrogen, which slows bacterial development and lowers gas production in the AD system, whereas a low C/N ratio can lead to ammonia toxicity and pH levels above 8.5, which are poisonous for methanogenic bacteria (Avs, 2016; C.Akunna, 2019; Hhaygwawu, 2016; Jnr, 2011; Katima, 2001; Ojikutuabimbola & O, 2016; Rabii et al., 2019; Stojkovi et al., 2018). The organic matter with low C/N ratios can be mixed with an organic matter with high C/N ratios to achieve the digester's ideal C/N ratios. (Abbasi et al., 2012; Hhaygwawu, 2016; Jnr, 2011; Ojikutuabimbola & O, 2016; Owamah, 2010; Rabii et al., 2019).

2.3.4.10 Total Solids or solid concentration

Dry organic and inorganic matter in sludge is referred to as TS (Meegoda et al., 2018)

It measures the overall volume of material that remains after all the moisture has evaporated (Madondo, 2017). Less than 10% of the total solids (TS) in AD systems are low or wet solids, 15-20% are medium or semi-dry solids, and 22–40% are high or dry solids processes (Jnr, 2011; Madondo, 2017; Muhammad Rashed, 2015). Typically, solids concentrations of 7-9 percent are ideal (Muhammad Rashed, 2015; Yadvika et al., 2004). Reactor volume decreases in proportion to an increase in TS in the reactor (Jnr, 2011). The amount of organic matter is dried at a temperature of 105°C till its water content became nil and no further change in mass or weight is noticed to determine the TS of organic matter(Astrid, 2007; Meegoda et al., 2018). It will be necessary to introduce freshwater or other liquid feedstocks to the biogas plant if the feedstocks have a very high TS concentration (Drosg & Braun, 2013).

$$\mathrm{TS}\ (\%) = \frac{\text{weight dried at } 105℃}{\text{wet weight}} \times 100 \qquad \text{eq2.1}$$

2.3.4.11 Volatile Solids (VS)

Volatile solid (VS) is a metric used to determine how much organic matter is in waste (C.Akunna, 2019; Gooch, 2011; Orhorhoro et al., 2017). Organic matter removal is related to methane production (Chanathaworn, 2017). Greater methane productivity will result from organic materials with higher VS ratios (Chanathaworn, 2017; Goswami, 2005; Jnr, 2011). Keep in mind that bacterial action occurs on organic, not inorganic, materials. VS is generally related to digestible biomass in anaerobic digestion (Gooch, 2011; Jnr, 2011; Orhorhoro et al., 2017). The dried substrate is burned in a muffle furnace at a temperature of 550°C to separate the organic to an inorganic fraction of TS; what is left over after burning is the inorganic fraction (Astrid, 2007). By removing the weight of the inorganic fraction from the weight of the dried materiel and dividing it by the weight of the dried material, the VS concentration can be calculated. (Orhorhoro et al., 2017).

$$\mathrm{VS}\ (\%) = \frac{\text{dried weight at } 105℃ - \text{dried weight at } 550℃}{\text{dried weight at } 105℃} \times 100 \qquad \text{eq 2.2}$$

The AD procedure is best suited for waste with low non-biodegradable organic matter and higher VS (Jnr, 2011). The waste composition has an impact on both the quality and yield of biogas as well as the compost. Reduction in VS is the most practical metric for assessing the effectiveness of biogas production in AD (C.Akunna, 2019).

2.3.4.12 Dilution of waste or water content

Water reduces or lessen the effect of some elements, such as sulfur and nitrogen, which result in a byproduct that hinder anaerobic digestion, such as ammonia and hydrogen sulfide. It was determined that anaerobic digestion without the addition of water results in little biogas (Hhaygwawu, 2016).

It’s necessary for the survival and movement of microorganisms. Additionally, it facilitates the digestibility of the substrate (Aslanzadeh, 2014). High concentrations of end products produced by high solids digestion prevent anaerobic breakdown. Consequently, slight dilution may have advantageous benefits. The percentage of waste degradation normally increases with increasing dilution, and biogas generation increases with increasing percentage degradation (Jnr, 2011). The fermentation process could be affected by excessive acid formation caused by the lower water content (Twizerimana et al., 2021). The biodigester's total solid content and substrate concentration have a significant impact on how well the AD works and the amount of biomethane production (Jansson, Patinvoh, & Iiona, 2019; Madondo, 2017).

According to the number of solids they contain, anaerobic digesters are split into three categories: high solid digesters, which have waste to water ratio of 1:2.5–4.5, medium solid digesters, which have a ratio of 1:5-7, and low solid digesters, which have a ratio of 1:10 (Hhaygwawu, 2016). A dry anaerobic system makes the digester's solution more compact, which also offers high loading rates. The dry method can produce more biogas than a wet process because of the high loading rate and compactness of by-products, which increases the level of material digestion (Madondo, 2017; Sun, 2015). As bacteria can use only organic matter that is dissolved in water, overly dry digestion (above 40%) will restrict bacterial activity (Madondo, 2017). Dry AD must be a superior technology to wet AD, requiring less water, and having less water in the residue (digestate) results in a smaller reactor capacity and produces a larger volumetric methane yield for the same solid loading rate (Jansson et al., 2019; Sun, 2015). Hhaygwawu, (2016) studied the co-digestion of cow dung and grass for biogas production. It was determined that little biogas is produced when cow dung and grass are combined anaerobically without the addition of water.

High biogas generation was achieved with the 1:2 ratios. Jnr, (2011) researched the creation of biogas from kitchen trash produced on the Knust campus. The reactor was operated using various water dilutions of 8, 10, 12, 15, and 20 liters. The 20 L dilution produced the maximum amount of biogas (8.91–3.15 L/day), whereas the 8 L dilution produced the least amount (0.65–1.36 L/day). The 8 L dilution showed the least amount of degradation, whereas the 20 L dilution showed the greatest amount. The experiment has shown that the dilution increased as the degradation of material increased which resulted in improved biogas yield.

2.3.5 Key Process Indicators to Prevent Digester Upset

To maintain and improve process efficiency, increase process stability, and prevent system imbalance and failure, control of the system's operational parameters is essential (Astrid, 2007; Gooch, 2011; Madondo, 2017; Rabii et al., 2019). Volatile fatty acids (VFA), pH, alkalinity, ammonia, biogas output, methane content, and a decrease in organic matter are the main process indicators to watch in AD systems (Astrid, 2007; C.Akunna, 2019; Gooch, 2011; Jnr, 2011; Marcham, 2007; Rabii et al., 2019).

2.3.5.1 Volatile fatty acids (VFA)

VFA concentration is the most delicate operational parameter to watch as a process performance indicator (Hhaygwawu, 2016). It is widely known that a process imbalance causes the concentration of VFA to rise during the biogas production process (C.Akunna, 2019; Hhaygwawu, 2016; Kumar et al., 2013). Methane is produced during anaerobic digestions by two types of bacteria: (i) acidogenic bacteria, which turn simple hydrolyzed organic material into volatile fatty acids (VFAs), (ii) and methanogenic bacteria, which break down VFAs into methane and carbon dioxide (CO_2) (Hhaygwawu, 2016).

Butyric, propionic, isobutyric, acetic and isovaleric acids are examples of the volatile fatty acids which are generated during the anaerobic digestion process at acidogenesis stage (Hhaygwawu, 2016; Shin et al., 2019; Teodorita et al., 2010). Acetic acid is a byproduct of the anaerobic process that is eventually digested to produce methane and carbon dioxide (Hhaygwawu, 2016). Most often, AD process instability will result in VFA building up inside the digester, which can also cause the pH level to decrease. High VFA concentrations prevent methanogenesis, which prevents the anaerobic digestion process and can induce systemic discomfort or system upset (Alfarjani, 2012;

Aslanzadeh, 2014; Gooch, 2011; Jnr, 2011). Alkalinity and pH were also proven to be effective monitoring variables, but the VFA was found to be a better predictor of a microbial system overload. Under overloading conditions and in the presence of inhibitors, hydrogen and volatile organic acids cannot be removed in methanogenic activity as quickly as they are produced, causing acids to build up and the pH to drop to the point that impedes the hydrolysis or acidogenesis stage (Gooch, 2011; Hhaygwawu, 2016; Jnr, 2011; Rabii et al., 2019).

2.3.5.2 pH level

To maintain a digester in a stable, equilibrium, and healthy state for a biological process for effective anaerobic digestion, keeping the pH of the system within the right range is crucial (Shen, 2008). Variations depend on VFA and ammonium concentrations. The pH can be increased by ammonia generated during protein breakdown or by ammonia in the feedstock stream, whereas the pH can be decreased by VFA accumulation and a decrease of alkalinity. High CO_2 biogas content is associated with low pH (C.Akunna, 2019; Teodorita et al., 2010). Methanogenic bacteria are very sensitive to pH, which function best at a pH range between 6.9 to 7.2 (Deressa et al., 2015; Rabii et al., 2019; Sathish, S., 2011).

A value of pH below 6.9 can increase the formation of VFAs, and a pH fall below 6.5 can block or inhibits the bacteria by increasing VFAs and leading to process failure (Hhaygwawu, 2016). When there is overloading and the presence of inhibitors, the production of organic acids increases quickly, which inhibits the activity that produces methane. Because of acid accumulation, depletion of the buffer, and subsequent pH decrease brought on by this, the production of biogas will decline and possibly stop altogether (Gooch, 2011; Jnr, 2011; Rabii et al., 2019; Shen, 2008). The series of biological events involved in digestion can stop due to low pH (Marchaim, 2007). There are two basic operational ways to fix a feedstock's low pH condition.

The first strategy involves stopping the feed loading so that to give the methanogenic bacteria enough time to reduce the level of volatile fatty acids and, as a result, elevate the pH to a desirable range of at least 6.8. To prevent further pH drops, feeding can be resumed at decreased levels and then progressively raised after the pH has returned to normal. A second approach entails adding chemicals to increase pH and offer more buffer capacity. Chemical addition has the advantage of stabilizing pH right away and allowing unbalanced populations to adjust more quickly. In anaerobic reactors, lime (calcium hydroxide) and bicarbonate are frequently employed to regulate pH levels (Gooch, 2011; Jnr, 2011; Marchaim, 2007; Rabii et al., 2019; Shen, 2008; Teodorita et al., 2010).

2.3.5.3 Alkalinity (Alk)

The level of alkalinity in the system affects the anaerobic digester process's ability to buffer (C.Akunna, 2019; Sun, 2015). It also symbolizes the digester's capacity to neutralize acids created throughout the digestive process (Madondo, 2017). Even in cases of acid accumulations, high and consistent alkalinity can keep the pH in the neutral or slightly above the neutral range (Sun, 2015). The fatty acid generation will significantly reduce total alkalinity, which lowers the pH (Astrid, 2007; Madondo, 2017).

Alkalinity is present in the gas phase in the form of carbon dioxide. When organic materials deteriorate, carbon dioxide is released, and when proteins and amino acids break down, both carbon dioxide and ammonia are created (Madondo, 2017).

When high-strength and quickly biodegradable wastes are employed as co-substrates in co-digestion systems, cow dung can play a significant role by raising the pH level and the buffer capacity of the mixture (Gooch, 2011).

2.3.5.4 Ammonia

Ammonia (NH_3) is an essential substance that is critical in the AD process (Teodorita et al., 2010). When nitrogen-containing substances and protein-rich substrates are digested, ammonia is generated (Alfarjani, 2012; Hhaygwawu, 2016; Madondo, 2017; Marchaim, 2007; Sun, 2015; Teodorita et al., 2010). Ammonia can be produced through the breakdown of proteinaceous from the processing of fish or meat, as well as from wastewater, animal manure, and agricultural wastewater (Shin et al., 2019).

Ammonia can impede the digestion process and reduce its overall effectiveness, like VFAs (Gooch, 2011). Ammonia is a necessary nutrient for methanogenic bacteria to grow at low concentrations, but at higher concentrations, ammonia may interfere with the processes of anaerobic digestion by increasing intermediate products like VFAs, which will cause the pH to drop and cause the cessation of bacterial activities, which will fail anaerobic (Alfarjani, 2012; Gooch, 2011; Hhaygwawu, 2016; Shin et al., 2019; Teodorita et al., 2010).

When they enter microbial cells, propionic acid (VFA) and ammonia can have an opposing impacts on pH; propionic acid would lower the pH while ammonia could raise it. The two inhibitors operate antagonistically when they coexist (Shin et al., 2019).

2.3.5.5 Temperature

In AD systems, the temperature has a significant effect on the rate of reaction (Alfarjani, 2012; Maile et al., 2016). Mesophilic (37°C) temperature is ideal for the anaerobic digestion (Gooch, 2011). Digester temperature should be performed in a mesophilic environment, maintained in the range between 35 and 37°C (Avs, 2016). The production of gas and the stabilization of organic matter will be reduced when operating at temperatures beyond the specified range. Within a clearly defined range of temperatures, microorganisms grow and function at their best (Marchaim, 2007). Temperature variations can potentially contribute to excessive VFA accumulation and high biogas CO_2 concentration (C.Akunna, 2019). In general, greater temperatures will have a bigger impact on the process than lower ones (Gooch, 2011).

The metabolism will slow down at an excessive temperature because vital enzymes for cellular survival will degrade (Marchaim, 2007). Other species found in digesters are less susceptible to temperature fluctuations than methanogenic bacteria (Marchaim, 2007).

This is due to the ability of other species, like the acetogenic bacteria, to significantly catabolize at low temperatures, and grow more quickly than they do. When temperatures are restored after a brief temperature change of up to two hours, all bacterial populations in digesters quickly resume normal gas production rates. However, frequent or prolonged temperature drops may cause populations to become out of balance and result in the low pH issues mentioned above (Marchaim, 2007).

2.3.5.6 Biogas production

The anaerobic digestion process' efficiency directly relates to biogas production (Jnr, 2011). Methane (CH_4) and carbon dioxide (CO_2) are the main gases that make up biogas, although it also has trace amounts of other gases like ammonia, nitrogen, and hydrogen sulfide (Gooch, 2011).

The biogas yield should remain largely steady over time. When the biogas production yield falls below the daily average values, it means that the other indications mentioned above have also changed, and it is a reliable sign that the digestion process is upset (Gooch, 2011). The most practical metrics for measuring the efficacy of biogas production are variations in the organic constituents' capacity to biodegrade (i.e., VS) (C.Akunna, 2019). Process instability is shown by variations in the biogas CH_4/CO_2 ratios. Increased CO_2 levels may be a sign of methanogenesis inhibition caused by organic overloading and temperature change which results in high levels of VFA accumulation, sulfide, ammonia, etc. Biogas typically contains little hydrogen (H_2), and when it does, it is a symptom of process instability, which is frequently linked to significant VFA accumulation (C.Akunna, 2019).

2.3.5.7 Methane content

A reliable measure of the digestion system stability is shown by the content of methane in the biogas. The amount of VS that has been destroyed (stabilized) directly correlates to the amount of methane gas released throughout the digestion process (Gooch, 2011). Since methanogenic activity is the primary cause of anaerobic digestion imbalance, a decrease in methane content is a crucial parameter to assess the AD system performance (Jnr, 2011). It is also crucial to keep in mind that a low gas/methane content is simply owing to the substrate's poor biodegradability and does not necessarily signal performance deficiencies (Jnr, 2011).

2.3.5.8 Volatile solids (VS)

VS refers to the measurement of the amount of organic matter in waste that can be broken down by a biological process (Battista et al., 2016). The amount of waste that has been destroyed by the digesting process is indicated by the difference between the volatile solid content in the input and that of the output (Gooch, 2011).

The higher biogas and methane generation, the lesser particulates (solids) in the effluent, the higher VS stabilized (destroyed), and the greater odor reduction, those are all positive trends. The physicochemical properties of the substrate and the system configuration are the main determinants of the amount (percent) of organic matter stabilization (Gooch, 2011; Marchaim, 2007). The anaerobic digester's effluent is the ideal sample size to assess the effectiveness of treatment (such as organic matter stabilization) (Gooch, 2011).

2.3.5.9 Hydrogen Sulfide

The breakdown of sulfur-containing compounds is the source of hydrogen sulfide (H_2S) in waste. Inhibition occurs when reducing sulfate bacteria compete with methanogenic and acidogenic bacteria (Hhaygwawu, 2016). They turn sulfate into sulfur by reducing it, and sulfur combines with the hydrogen generated during anaerobic digestion to produce H_2S.

Methanogenic bacteria are at risk from the sulfide that is formed following the reduction of sulfate because it passes their cell membrane and denatures their proteins (Hhaygwawu, 2016). Some hydrogen sulfide escapes with biogas, while other hydrogen sulfides, in dissociated or undissociated forms, remain in the digester. At lower pH the equilibrium changes from the dissociated form to the undissociated form, causing the production of additional $H+$ and raising the digester's acidity (Hhaygwawu, 2016).

2.3.5.10 Toxic compounds

The presence of hazardous substances is a further element that affects the activity of anaerobic microbes. They may be created during the process or added to the AD system along with the feedstock (Teodorita et al., 2010). Heavy metals like Lead, nickel, zinc, copper, chromium, and others, and light metals like sodium, potassium, calcium, magnesium, and aluminum make up the two groups of inhibitory elements.

When present in a digester at a moderate concentration, light metal ions stimulate microbial activity and growth, but when present at larger concentrations, they can stifle or impede growth because they become poisonous. (Hhaygwawu, 2016). When toxic substances are present in low concentrations, they slow down the metabolism rate; when present in large amounts, they poison or kill the organisms. Although all groups engaged in digestion can be impacted, methanogenic bacteria are typically the most vulnerable. In entirely mixed systems, inhibition of the methanogens might result in process failure due to the "washout" of the bacterial mass because of their slow development (i.e drainage of microbes at a faster rate than their production in the digester through the outlet).

Identification of inhibition in the early phases is crucial for controlling and adjusting operations and minimizing hazardous or toxic effects (Marchaim, 2007). The two main signs of inhibition are: (i) a reduction in methane yield, which is demonstrated by some consecutive decreases of more than 10% in daily production at a constant feeding rate, and (ii) an increase in the concentration of VFAs, which typically occurs when acetic acid (volatile acids) are higher than the typical range of roughly 250 to 500 ppm (mg/L).

The term inhibition refers to a reduction or stoppage of microbial growth while toxicity causes microbial mortality (Drosg & Braun, 2013), and buffering capacity, also known as acid neutralizing capacity or alkalinity, describes the feedstock's capacity to withstand rapid pH fluctuations.

Causes of AD process perturbation are shown in figure 2.2 (Alfarjani, 2012; C.Akunna, 2019; Gooch, 2011; Hhaygwawu, 2016; Marchaim, 2007; Shin et al., 2019).

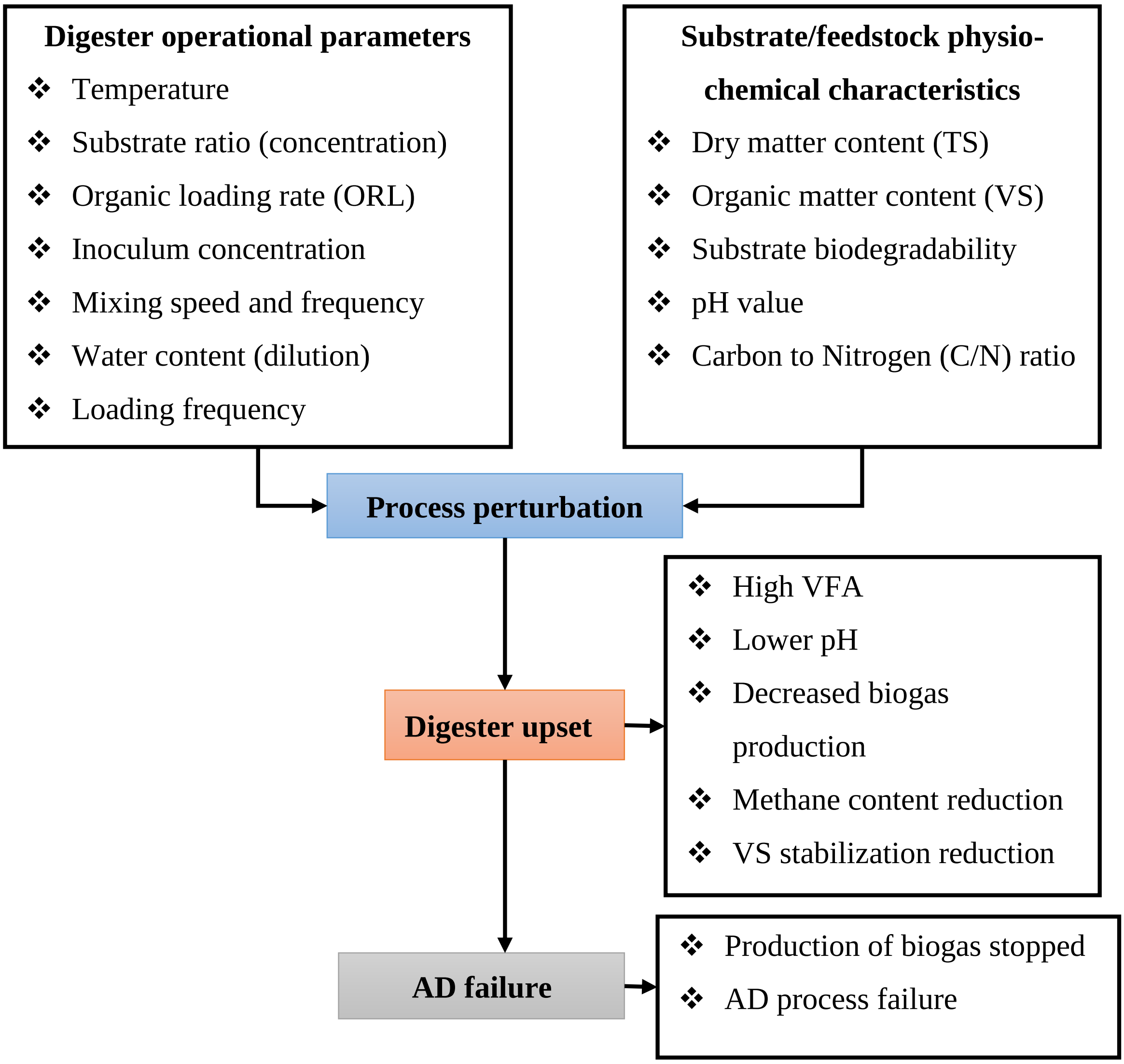


Figure 2. 2: Causes of AD Process Perturbation

2.4 Factors that can Enhance the Anaerobic Digestion Process

Substrate (feed material type and content), Co-digestion, pre-treatment techniques, digester configuration, etc. all have a direct impact on the composition (methane content) and biogas yield (Aslanzadeh, 2014; Kubaská et al., 2010; Maile et al., 2016; Pina et al., 2018).

2.4.1 Substrate

The term "substrate" refers to any organic material that is readily available and renewable that can biodegrade, including food waste, fish waste, animal waste, agricultural waste, water hyacinth, and other waste (Girisuta, 2014). Substrates can be classified into two types: (i)Vegetation, such as floating plant waste, crop leftovers, forest, wood, and agricultural residues, etc., and (ii) Organic waste, such as organic industrial waste, fish waste, kitchen waste, food waste, municipal waste, and animal waste, etc. Figure 2.3 shows the classification of the substrate (Akula, 2013).

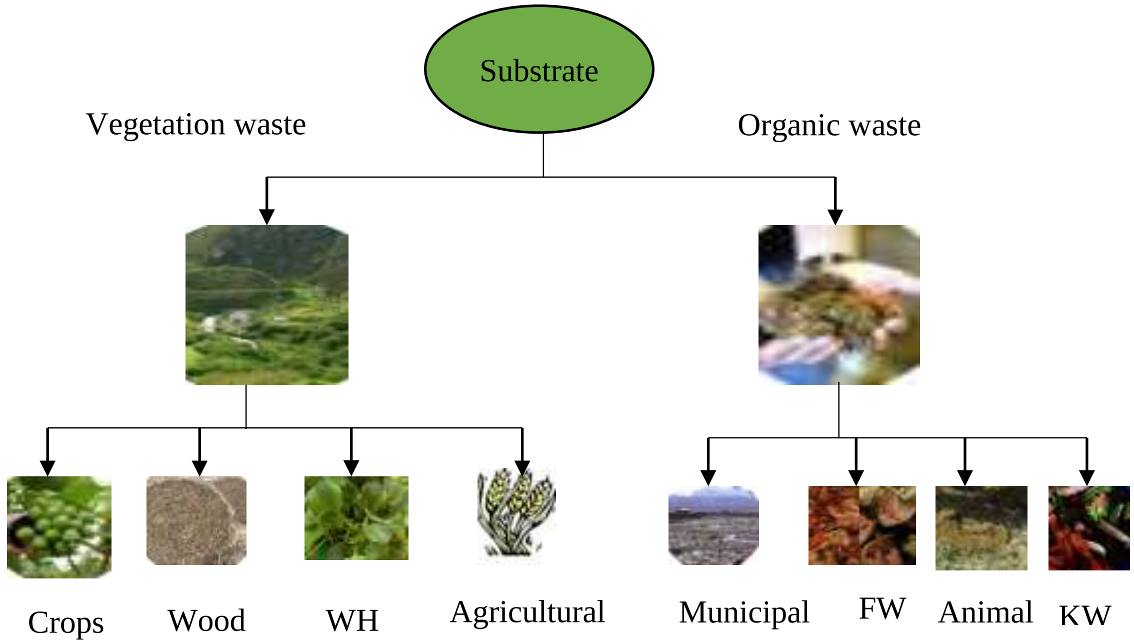


Figure 2. 3: Classification of substrate

The compositions and characteristics of the substrates used affect how well a biogas digester functions (Araoye et al., 2018). The feedstock compositions in terms of their carbohydrate, fat, and protein concentrations affect the biomethane yield of the AD (Aslanzadeh, 2014; Pina et al., 2018; Teodorita et al., 2010).

The quantity of nutrients (lipids, proteins, and carbohydrates) impacts how easily the material degrades, and consequently how much methane may be produced by the AD process (Pina et al., 2018). Additionally, the physicochemical characteristics of the feedstock used, such as total solids (TS) or dry matter, pH, moisture content (MC) and volatile solids (VS), biodegradability, and particle size, can have a significant impact on the anaerobic digestion system (Aslanzadeh, 2014). The substrates for AD can be categorized according to several factors, including dry matter (DM) or total solids content, origin, methane yield, etc., Wet digestion (wet fermentation) uses substrates with DM contents under 20%; dry digestion (dry fermentation) uses substrates with DM contents greater than 35%. DM concentration, the amount of sugars, proteins, and lipids in the feedstock affect the types and quantities of feedstock used in the AD substrate combination (Pina et al., 2018; Teodorita et al., 2010).

Compared to lignocellulosic materials, substrates with high percentages of easily degradable organic matter have more potential for producing biomethane (Pina et al., 2018). Agriculture waste, food waste, fish waste, human waste, industrial trash, residential garbage, organic waste, water hyacinth, and other elements are all digested (Marchaim, 2007; Oke, 2016). Lignin is the main exception to the rule that most naturally occurring organic wastes can be digested (Makhura et al., 2020; Marchaim, 2007; Nadu & Nadu, 2017).

2.4.1.1 Fish waste as a feedstock in Biogas production

A report from the FAO (Food and Agriculture Organization of the United Nations) estimates that 9.1 million tons of FW are thrown away each year. Consequently, fish by-products are now a global issue and pose a danger to the long-term viability of fish aquaculture (Kandyliari et al., 2020).

Before being sold, over 70 % of fish is processed. 20% to 80% of this total are by-products or waste that is not used for direct human consumption (Maghaydah, 2003; Pina et al., 2018). Figure 2.4 illustrates by-products that are rich in lipids and proteins including heads, viscera, intestines, bones, and scales. These by-products are dumped or discarded as waste and stay unexploited (Kafle & Kim, 2012; Kandyliari et al., 2020; Maghaydah, 2003; Oke, 2016; Pina et al., 2018).

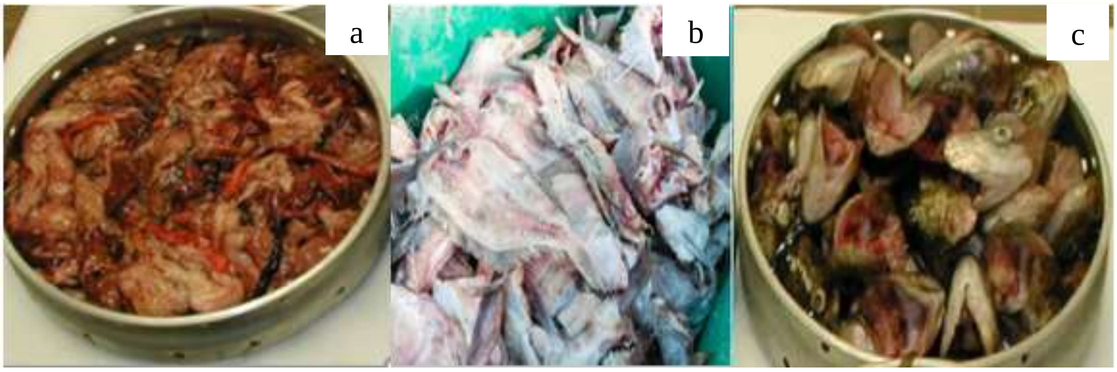


Figure 2. 4: Fish processing by-products, (a) intestines, (b) bones and scales, and (c) heads

The current methods of disposing of this highly polluting organic matter such as dumping/discarding onto the open ground and disposing of in sanitary landfills result in health risks/hazards, environmental issues, and a loss of important nutrients (Kafle & Kim, 2012; Kandyliari et al., 2020; Maghaydah, 2003; Oke, 2016; Pina et al., 2018). Accordingly, any effective development of a by-product utilization technique will lead to the energy recovery of these wasted important nutrients and eradication of the environmental pollution and health hazards or risks brought on by the incorrect disposal of the processing by-products (Maghaydah, 2003; Oke, 2016; Owamah, 2010). When used as substrate in the AD system, fish waste which is abundant in lipids and proteins, has the benefit of producing large methane outputs (Kafle & Kim, 2012).

Consequently, biogas technology may be a useful method for FW utilization and energy production (Kafle & Kim, 2012; Oke, 2016; Pina et al., 2018). The reduction of both fossil fuels and environmental pollution can be accomplished through the AD digestion of this biodegradable waste (Adebayo & Odedele, 2020; Kafle & Kim, 2012; Marchaim, 2007; Nadu & Nadu, 2017; Tsavkelova & Netrusov, 2012). When anaerobic digestion is completed, nutrients like phosphorus and nitrogen are retained in the digestate (effluent). If it meets the appropriate requirements, it can be utilized as a bio-fertilizer or compost in farming production (Adebayo & Odedele, 2020; Kafle & Kim, 2012; Marchaim, 2007; Tsavkelova & Netrusov, 2012).

This resource recovery method also solves the byproduct disposal issues. A technology like that may be advantageous to fish producers everywhere in the nation and the world (Maghaydah, 2003). A Summary of the physicochemical composition of fish waste is given in table 2.5 (Kafle & Kim, 2012; Kandyliari et al., 2020; Maghaydah, 2003; Marchaim, 2007; Nalinga & Legonda, 2016; Ojikutuabimbola & O, 2016; Oke, 2016; Pina et al., 2018).

Table 2.5: By Product Nutrition Composition

Characteristics	**Amount (%)**
Moisture Content (MC)	67.1-81.43
Total Solids (TS)	31.30-32.2
Volatile Solids (VS)	27.50-55.5
Protein	37.23-60
Fat	14-48.6
Nitrogen	5.44-10.85
Carbon	53-54.37
Carbohydrates	16.01-≤ 20
Vitamins	2
Minerals	≤ 12
Ash	2.14-5.7
C/N ratio	3-10.1

2.4.1.2 Water hyacinth as a substrate in the production of biogas

The water hyacinth is the invasive floating water weed in the world that thrive in freshwater water bodies and have spread to most nations. It has detrimental impacts on the environment, the ecology, and society (Almoustapha & Kenfack, 2019; Armah et al., 2017; Bote et al., 2020; Katima, 2001; Rozy, 2016). It slows water flow and forms mats that block streams, making fishing impossible, limiting water flow, degrading water quality by obstructing sunlight from penetrating the water, and sharply lowering oxygen levels in the water, wiping out aquatic life like fish, and significantly reduces biodiversity as shown in figure 2.5. Water hyacinth used to be the subject of annual expenditures of millions of dollars to manage its growth (Bote et al., 2020; Chanathaworn, 2017; Njogu et al., 2015a; Tham, 2012). Figure 2.5 shows different types of WH.

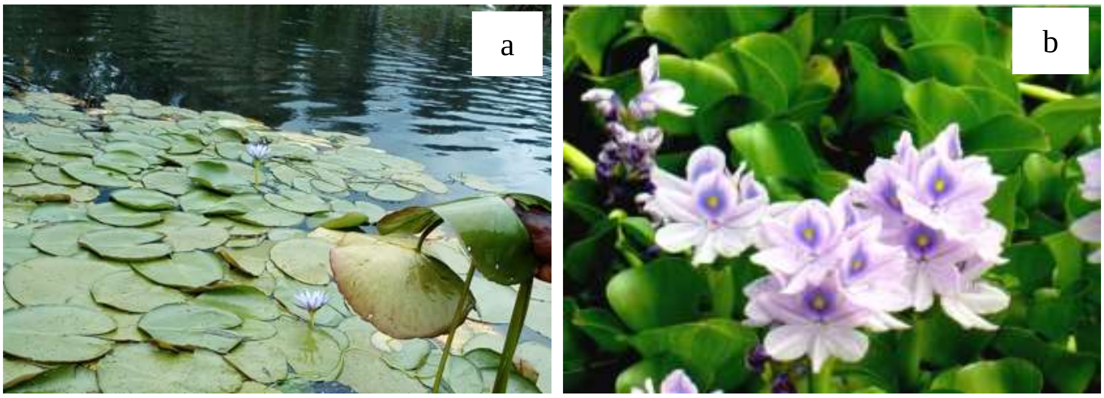


Figure 2. 5:(a) Water hyacinth from different habitats, (b) Water hyacinth from Lake Victoria (Eichhornia crassipes)

Water hyacinth has all the parameters that seem to be necessary for the generation of bioenergy; it has a high carbon to nitrogen (C/N) ratio, is easily and widely accessible, is not a crop plant, is biodegradable, and contains a lot of cellulose and little lignin, however, its disadvantage is that it contains more than 90% water, which makes harvesting and processing more difficult. Water hyacinth can be used to produce biogas, which can be used to power homes (Bote et al., 2020; Marchaim, 2007; Rozy, 2016). Consequently, it will be easier to digest during digestion (Bote et al., 2020; Chanathaworn, 2017; Marchaim, 2007; Rozy, 2016). With this method, waste will be reduced in addition to energy production (Pina et al., 2018). In these situations, the weeds' digestion can provide energy while resolving the issue of excessive weed growth in canals and ensuring appropriate management of the plant (Bote et al., 2020; Chanathaworn, 2017; Marchaim, 2007; Rozy, 2016). However, there are other potential advantages to WH, including fertilizer, animal food, purified water, fiberboard, and paper (Bote et al., 2020; Dar & Phutela, 2017b; Tham, 2012).

A Summary of the typical physicochemical composition of WH is given in table 2.6 (Almoustapha & Kenfack, 2019; Armah et al., 2017; Bote et al., 2020; Chanathaworn, 2017; Girisuta, 2014; Katima, 2001; Nalinga & Legonda, 2016; Njogu et al., 2015a; Rozy, 2016; Tham, 2012).

Table 2. 6: Summary of the typical chemical composition of water hyacinth

Characterization	**Water hyacinth from Lake Victoria (Eichhornia crassipes)**	**Water hyacinth from different habitats**
Moisture Content (MC)	85-95	-
Total Solids (TS)	11.4-27.76	87
Volatile Solids (VS)	80.9-93.2	-
Ash	15-26	131
C/N ratio	10.1-27.4	-
Nitrogen	1.5-4.3	-
Carbon	41.1-43.7	-
Hydrogen	5.3-6.4	-
Fibre	68.1	-
Hemicellulose	18-43	1.1
Lignin	7-26	76
Cellulose	18.33-97	1.1
Protein	15.4	128
Silica	8.2	4
Phosphorus	2.86	-
Potassium	2.89	-

2.4.2 Co-Digestion

Anaerobic co-digestion of organic material leads to the stability of waste as well as the creation of biogas (Esposito, Frunzo, Panico, & Pirozzi, 2011; Roslina et al., 2014).

When two or more organic substrates are digested simultaneously, it's called co-digestion. (Mel et al., 2015; Rabii et al., 2019). Some organic wastes have inappropriate C/N ratios, making them problematic for mono-digestion. Co-digestion's objectives are to balance out any imbalances or downsides of mono-digestion, enhance methane yields, improve biogas quality and yield as co-substrates supply missing nutrients, reduce toxic effects, increase organic matter, improve the system stability due to synergic effect, C/N ratio and biodegradability (Hanghome, 2014; Madondo, 2017; Maile et al., 2016; Makhura et al., 2020; Mel et al., 2015; Owamah, 2010; Rabii et al., 2019; Roslina et al., 2014).

Co-digestion is primarily related to the microbial community's need for balanced macro- and micronutrient availability, adequate moisture content, higher buffering capacity, and a reduction in the impact of toxic substances or dilution of inhibitory compounds (Hhaygwawu, 2016; Kameswari et al., 2011; Kubaská et al., 2010; Makhura et al., 2020; Toma et al., 2016; Zamanzadeh et al., 2017). When multiple substrates are employed in a single digesting process, methane output will be increased; however, if only one substrate is used, methane yield will be low due to the substrate's poor biodegradability and the presence of inhibiting substances (Almansa, 2015; Jnr, 2011; Olatunde, 2016).

More organic wastes being digested in the same digester has beneficial consequences, such as additional nutrients which encourage microbial activities (Roslina et al., 2014;

Zamanzadeh et al., 2017). The co-digestion process's effectiveness determines how much biogas can be generated from organic material, hence improving this process is strategically important (Esposito et al., 2011; Nadu & Nadu, 2017).

The ideal ratio of maize husk (MH) and food waste (FW) for biogas production was found to be 25% MH and 75% FW (w/w) (Owamah, 2010). Makhura et al., (2020) research were done on the impact of co-digestion of food waste and cow dung on biogas generation at mesophilic temperature (37^oC), and results showed that biogas production has increased due to co-digestion. A study on the Anco-digestion of sugarcane press mud with food waste was conducted by Rabii et al., (2019). At a mixing ratio of 80:20, the maximum methane yield (82.36 mL CH4/g VS) was achieved. The production was 22 and 54 % greater than that of mono-digestion alone (sugarcane press mud and food waste), respectively.

A study on the anaerobic mono-digestion and co-digestion of fish waste was conducted by Pina et al., (2018). The generation of biomethane increased, from 0.2-0.9 CH4 m3/kg VS compared mono-digestion to co-digestion. However, because it digests quickly and produces significant amounts of ammonia, waste from the processing of fish presents unique technological challenges. Later, this may slow or prevent the digestion of substrates.

Tasnim et al., (2017) conducted a study on anaerobic co-digestion of kitchen waste, cow manure, and WH at 37^oC. Their research revealed that sewage sludge combined with WH and cow manure was a substantial source of biogas energy for both residential and commercial energy needs. Anaerobic research was done on biogas generation from cow manure and WH by Hassan et al., (2009). At a temperature of 30°C on the fourteenth day, 0.11 m^3 of biogas was optimally produced. Almoustapha and Kenfack,

(2009) conducted research on biogas generation from co-digestion of fresh rumen waste and WH to address societal demands for cooking energy. The facility's daily biogas yield was 0.29 m^3 in the winter and 0.52 m^3 in the summer.

Hhaygwawu, (2016) did a study on the anaerobic co-digestion grass with cow manure for biogas production in semi-arid locations. It was determined that little biogas was produced when cow dung and grass are combined anaerobically without and with lower addition of water. High biogas generation was achieved with the 1:2 waste to water ratios.

To create a ratio that can be easily degraded by microorganisms, the organic waste with a lower carbon to nitrogen (C/N) ratio should be combined with the organic waste materials that are high in carbon to nitrogen ratio, or vice versa. Examples of this would be mixing pig slurry with maize silage, poultry or cow slurry with vegetable waste and fruit, fish waste with water hyacinth, and fruits and vegetables with meat wastes (Hhaygwawu, 2016; Nalinga & Legonda, 2016). According to Gooch, (2011); Hhaygwawu, (2016); Jnr, (2011), and Rabii et al., (2019), feedstocks with low C/N ratios cause the digester pH to rise by producing high concentrations of TAN, which are toxic to microbial growth.

Conversely, substrates with high C/N ratios can lower pH by producing and accumulating VFAs and decreasing TAN, which leads to a gradual inhibition of microorganism growth and ultimately digester failure. When compared to substrates with high ratios, those with low C/N ratios produce a higher yield of methane (Hhaygwawu, 2016). The head, intestines, fin, and scales of fish are excellent biogas plant inoculants. They can be digested alone or used as a fasting inoculant to speed up the digestive process. Fish typically create methane gas; however, it is best employed in bio digestion as an inoculant or in co-digestion with another substrate. This promotes the digestion of other organic compounds that are difficult to break down or non-easily degradable (Oke, 2016).

In anaerobic digestion tests, fish waste can be used as a substrate either alone or in combination with other materials, such as cow manure, WH, waste from strawberry processing, etc. (Pina et al., 2018). There were only about 20 research papers on the AD of FW available at the beginning of 2018 (Pina et al., 2018). Water hyacinth and fish waste harm the environment and the populations' health. However, co-digestion of FW and WH produced a high biogas yield (Nalinga & Legonda, 2016). The digestion and co-digestion of FW produced biomethane of 0.2-0.9 m3/kg VS, respectively (Pina et al., 2018).

Kafle and Kim, (2012); Pina et al., (2018) conducted some research that demonstrates that biomethane can be produced from fish waste. For these reasons, using this waste can produce a significant renewable energy source. However, fish waste presents a unique technological issue. When digested, it produces significant amounts of ammonia and VFAs accumulation which inhibit substrate digestion (Pina et al., 2018). Consequently, the Anco-digestion of FW and WH is a potential technological approach that can help to mitigate this issue.

Studies have shown that adding co-substrates to "pure" waste makes it far more valuable from an economic and environmental viewpoint (Tsavkelova & Netrusov, 2012). In comparison to individual substrate biogas generation, co-digestion of substrates from several sources results in higher biogas output than anticipated (Aslanzadeh, 2014; Tsavkelova & Netrusov, 2012). Nalinga and Legonda, (2016) demonstrated that Anco-digestion of FW and WH feedstock increased the materials' digestibility and biogas yield. Therefore, by co-digesting fish waste with water hyacinth, the production and quality of anaerobic digestion of fish waste can be increased.

Using substrates from various sources and in the proper quantities can improve the performance of the AD process (Aslanzadeh, 2014; Tsavkelova & Netrusov, 2012).

2.4.3 Pre-Treatment

Pre-treatment techniques are necessary for any anaerobic digestion process to produce significant yields of biogas (Alfarjani, 2012; Kratky & Jirout, 2011; Taherzadeh & Karimi, 2008; Xu et al., 2019). Pre-treatment, which is a method used before another process, aids in the breakdown of complex components, boosts substance production, facilitates the digestive process, and speeds up the hydrolysis of the substrate, hence increasing the biogas yield in the anaerobic digestion system (Ariunbaatar, 2015; Kratky & Jirout, 2011; Maile et al., 2016; Yadvika et al., 2004).

According to Ariunbaatar, (2015); Carlsson et al., (2012), and Elbeshbishy et al., (2010), the following are the main effects that pre-treatments have on various substrates: (i)reduction of particle size, (ii) removal of structural barriers to the hydrolysis stage, (iii) solubilization, (iv) enhancement of biodegradability, (v) enhancement of the enzyme hydrolysis rate and (vi) an increase in biogas and methane yields. Four categories of pre-treatment techniques exist: physical, chemical, physicochemical, and biological (Akula, 2013; Aslanzadeh, 2014; Kratky & Jirout, 2011; Marins, 2014; Taherzadeh & Karimi, 2008; Wagner & Illmer, 2018; Xu et al., 2019).

2.4.3.1 Physical pre-treatment

Milling and grinding (particle size reduction), steam explosion, irradiation (such as microwave, gamma ray, etc.), liquid hot water pre-treatment, etc. are examples of physical pre-treatment (Aslanzadeh, 2014; Taherzadeh & Karimi, 2008). The physical pre-treatment method aims to reduce the substrate's particle size to increase its surface area biodegradability and digestibility for enzymatic degradation (Akula, 2013; Ariunbaatar, 2015; Marins, 2014; Xu et al., 2019).

Particle size reduction is typically the pre-treatment technique used most frequently, and it is always the first stage in the entire biomethane manufacturing process. By reducing the particle size, more biogas would be produced. However, too much particle size reduction could lead to inhibitors and reduced biogas generation (Xu et al., 2019)

2.4.3.2 Chemical Pre-treatments

The usage of chemicals like bases (alkali pre-treatment, for instance, using NaOH, ammonia, or ammonium sulfate) or acids (acid pre-treatments), respectively (H_2SO_4, HCl) is referred to as "chemical pre-treatment." Additionally, it makes use of ionic liquid extraction, ozonization, ozonized steam explosion, and oxidation (Akula, 2013; Ariunbaatar, 2015; Aslanzadeh, 2014; Wagner & Illmer, 2018; Xu et al., 2019).

2.4.3.3 Physicochemical pre-treatment

A treatment approach that combines both physical and chemical approaches is called physicochemical pretreatment. Examples include steam explosions, explosions caused by steam containing SO_2 and ammonia fibers, liquid hot water treatments, etc. (Akula, 2013; Aslanzadeh, 2014; Taherzadeh & Karimi, 2008).

2.4.3.4. Biological pretreatment

Fungal, microbial consortium, and enzymatic pretreatment to degrade lignin are three forms of biological pretreatment (Akula, 2013; Aslanzadeh, 2014; Marins, 2014; Xu et al., 2019). The cellulose crystal structure and lignin seal are broken and removed by biological pretreatment, which increases the accessibility of the material to bacteria (Marins, 2014; Taherzadeh & Karimi, 2008; Wagner & Illmer, 2018).

2.4.4 Biogas digesters and process

Anaerobic digestion can use a variety of digesters and processes, which are frequently categorized as follows (Jnr, 2011; Nanda, 2008; Sun, 2015) : (1) Liquid and solid-state processes; (2) Batch and continuous processes; (3) Single and two-stage processes.

2.4.4.1 Batch and Continuous AD processes

A batch digester is a one-stage digestion process in which all stages occur in the same digester (Maile et al., 2016).In a batch process, all substrates are put into the digester at the beginning of the process, sealed during the reaction, and removed when the AD process ends. No substrate is added to or removed from the process (Jnr, 2011; Sun, 2015; Teodorita et al., 2010). The batch digester is the mostly used which provides data on a substrate's methane yield and digestibility and is also appropriate for small production (Foutch, 2017; Nanda, 2008; Sun, 2015). The batch reactor has the following benefits: 1) ease of design, 2) low operating costs, 3) need for less equipment, and 4) often lower cost of digestion (Jnr, 2011; Teodorita et al., 2010). The disadvantages are high process energy consumption and maintenance costs (Teodorita et al., 2010).

Continuous digestion systems produce biogas continuously by continuously feeding organic matter into the reactor (continuous complete mixing) or adding it to the digester in phases (first in, first out, continuous plug flow). Products are routinely or continuously eliminated at the end of processes, resulting in a consistent biogas yield. (Jnr, 2011; Teodorita et al., 2010). Continuous digesters, as opposed to batch-type digesters, produce biogas continuously while new feedstock is added and digested wastewater is removed. Production of biogas is reliable and consistent. In most cases, continuous digestion is chosen for large-scale production (Nanda, 2008; Teodorita et al., 2010). Vertical, horizontal, or multiple tank configurations are all used for continuous digesters.

Continuous digesters come in two different varieties: plug flow digesters and entirely mixed digesters, depending on the solution used to agitate the substrate (Jnr, 2011; Teodorita et al., 2010).

2.4.4.2. Single-stage and Two-stage AD processes

In single-stage digestive processes, all biological processes take place in a single sealed digester. (C. Akunna, 2019). A one-stage digestive process is less expensive to build and allows for less control over the system's internal reactions. One significant problem is that the system might not be able to appropriately handle changes in substrate and environmental conditions (C.Akunna, 2019; Jnr, 2011).

In a two-stage AD process, the microbiological biodegradation steps are divided into two reactors (C.Akunna, 2019). The first digester experiences both hydrolysis and acidogenesis stages, while the second digestion experiences acetogenesis and methanogenesis stages (Ariunbaatar, 2015; C.Akunna, 2019; Maile et al., 2016; Sun, 2015). Since the optimum pH range for methanogenesis (6.8-7.2) and hydrolysis (5.5-6.5) is different, it has been demonstrated that this separation results in improved hydrolysis of organic molecules.

A two-stage process has been reported to produce a high methane yield. For small-scale domestic digesters, a two-stage process is neither advantageous nor practical (Ariunbaatar, 2015; Maile et al., 2016; Sun, 2015). Figure 2.6 shows single and two-stage AD treatment (C.Akunna, 2019).

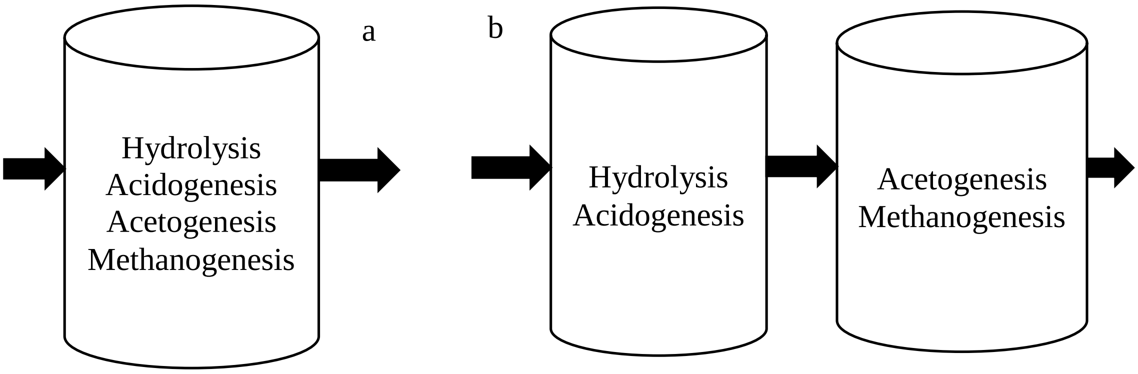


Figure 2. 6: (a) Single-stage and (b) Two-stage anaerobic treatment systems

2.4.4.3. Liquid (wet) and Solid (dry) AD process

Based on the anaerobic digester's total solids (TS) content, two types of anaerobic digestion process can be distinguished: (i) Wet-AD or liquid AD, with a TS concentration of less than 15 percent, and (ii) dry-AD or solid-state AD process with a TS percentage of 15 percent or above. The Liquid AD is more favorable for feedstock with a high MC, such as wastewater streams (Jansson et al., 2019; Sun, 2015). Solid-state AD must be a superior technology over Liquid AD because it requires less water usage. Less water in the digestate (residue) result in a smaller reactor capacity and produce a higher volumetric biomethane yield (Jansson et al., 2019; Sun, 2015).

2.5 Methods to Enhance Biogas Production

The production of biogas can be increased using the following methods (Kumar et al., 2013; Prasad et al., 2017; Yadvika et al., 2004):

i. Use of chemicals or additives

ii. The slurry filtrate and digested slurry recycling

iii. Variations in the substrate's operational characteristics, such as temperature, substrate ratio pH, dilution, retention time, inoculum concentration, particle size, etc.

iv. Operational parameter optimization

2.5.1 Use of additives

To maintain parameters, close to their optimum levels, additives can be used to increase the stability and pace or rate of biogas generation. By stimulating microbial growth and activity with various additives under varied environmental conditions, reactor efficiency and gas production rate can be raised. Additives are frequently employed to accelerate the rate of digestion, stabilize pH fluctuations, lower ammonia and hydrogen sulfide concentrations, and supply nutrients for bacteria (Baredar et al., 2016; Prasad et al., 2017).

The rate of biogas generation was found to be increased by between 10 and 80 percent when crop residues such as cotton stalks, maize stalks, wheat straw, rice straw, and WH were individually treated with digested cow manure (Kumar et al., 2013; Prasad et al., 2017). Numerous inorganic additives, such as iron salt, enhance the production of biogas. It was discovered that adding various salts in the slurry at varying quantities increased the rate at which biogas was produced. Magnesium and calcium salts were added to improve methane production and prevent slurry foaming (Baredar et al., 2016; Prasad et al., 2017).

2.5.2 Biogas improvement through recycling of slurry filtrate or digested slurry

Because the bacteria that are washed away are put back into the digester, increasing the microbial population, it has been demonstrated that recycling biogas digested slurry back into the digester can somewhat increase gas output (Astrid, 2007; Kumar et al., 2013). Slurry recirculation adds moisture to the waste, which simulates the decomposition of organic waste and provides the microorganisms and bacteria needed for the process with the nutrients they need (Astrid, 2007; Jnr, 2011).

2.5.3 Variation in operational parameters

It has been utilized to operate biogas plants efficiently to various factors including pH, temperature, substrate concentration or ratio, particle size, loading rate, dilution, agitation, inoculum concentration, and TS. Controlling and observing certain variables within the desired range can affect the biogas production rate (Kumar et al., 2013). Katima, (2001) studied biogas generation from WH by investigating the impact of substrate concentration (5 to 30 g/l), particle size (1-3mm), and incubation period (1-6 days). The highest methane (72.53%) was generated within 5 days of incubation at a substrate concentration of 25 g/l and particle size less than 1 mm of WH.

Angulo et al., (2018) researched alkaline pretreatment effects on biogas generation from corn crop residue by varying inoculum to feed ratio (1:1, 2:1, and 3:1) and the particle size between (0.5-2 mm). The maximum biogas yield (392.75 mL) was produced from the digestion of corn stalk without pretreatment.

2.5.4 Optimization of biogas production

A system's performance is improved through an optimization procedure to maximize benefits (Araoye et al., 2018). Optimizing anaerobic digestion is the key to maximizing the biogas yield for energy production. It can help prevent or at least lessen the possibility of product failure in addition to being very helpful when anticipating process results. When several variables can be controlled and only one that we wish to maximize, the process is called optimization (Toma et al., 2016).

Response surface methodology (RSM) is regarded to be a useful mathematical and statistical tool to examine the relationships or interactions between variables, estimating the optimum experimental levels for the model and determining the optimal values of the response (Chanathaworn, 2017; Chanathaworn et al., 2018; Sathish, S., 2011).

Research on the biomethane potential of FW digestion and co-digestion has already been presented. Nevertheless, how to maximize the biogas generation from Anco-digestion of FW and WH has not yet been evaluated. The long-term economic benefit of knowing the right parameters to get the best biogas and methane yields will also benefit fish processors. Due to the limited experimental evidence for anaerobic digestion of fish waste, further data must be gathered (Pina et al., 2018). This study investigated the effects of three experimental variables, substrate ratio, inoculum concentration, and dilution.

Design-Expert 13 software, which contains CCD and RSM was used to determine the level of variable inputs and establish the optimum number of experimental runs to maximize biogas yield in batch reactor Anco-digestion of fish waste and water hyacinth.,

ANOVA was utilized for the analysis of the regression coefficient and the prediction equation to show how the variables interacted. The polynomial equation was illustrated in three dimensions using response surface plots.

Optimizing operating parameters for biogas production would be essential for increasing biogas generation and making the process environmentally and economically sustainable (Shahidul et al., 2018; Toma et al., 2016). Araoye et al., (2018) developed an optimization method for the production of electrical energy from biogas. According to the findings, 636.6MW and 889.49MW of total biogas energy were produced before and after optimization, respectively. The biogas yield increased by 39.7%. The results show how the optimization model is applicable and can be fully utilized with the right control approach.

A study on the optimization of biogas from WH and its supplementation was carried out by Rozy, (2016). Incubation temperature, Particle size, moisture content, metal ions, inoculum concentration, and pH were among the various factors that were optimized. Water hyacinth with a diameter of 1 cm, an inoculum concentration of 10%, and a moisture level of 60% produced the most biogas. Dar and Phutela, (2017b) did an investigation on the optimization of biogas from WH (Eichhornia crassipes). Various inoculum concentrations (10–50%) were given to 500 g of water hyacinth to assess the impact on the production of biogas. In their experiment, water hyacinth with an inoculum concentration of 40% and a size of 1 cm produced the most biogas. The choice of inoculum source and the inoculum concentration is among the crucial operational optimization factors of the AD system to evaluate the digestibility of organic matter (Fathya et al., 2014; Kameswari et al., 2011).

It has been proposed that the inoculum concentration used in laboratories should be optimized (Avs, 2016). Sathish, S., (2011) studied the Optimization of various factors affecting Rice straw biogas production such as substrate concentration, temperature, agitation time, and pH by using RSM to estimate the optimum conditions for the production of biogas.

At temperatures of 50°C, pH 7.5, 110.70 kg of the substrate, and 5 seconds of agitation the highest biogas yield of 0.72m^3 was produced. Their results have shown that, temperature, substrate concentration, pH, and agitation time linear model factors had significant interacting impacts on biogas. Usman, (2018) conducted a test on optimum biogas production from sugar cane and rice husk with the cellulolytic fungus by varying factors such as water, fungus concentration, and temperature.

The optimum biogas of 500 cm^3 was produced at the optimal values of 25cm^3 of water, 0.6g of fungus, and a temperature of 33℃. Chanathaworn et al., (2018) studies the optimization conditions for biogas generation from Anco-digestion of sweet corncob waste milling process (WM) with wastewater from the production process (WP) using RSM by investigating three experimental variables, TS of the substrate (TS, 8-16 %), substrate ratio (WM: WP; 0-100), and initial pH (6-8). The optimum conditions were found to be: Initial pH, 7.0; TS of the substrate ,10%; and the WM: MP ratio,50:50. Chanathaworn, (2017) has researched optimization conditions for biogas production from WH and earthworm bedding wastewater by varying particle size (0.3-1.5 cm), TS (4-12%), and pH. Optimum biogas of 35.50% was obtained at 8% of TS, 0.3 cm particle size, and 7.0 initial pH. A study on optimization conditions for Anco-digestion of tannery solid wastes was done by Kameswari et al., (2011). The impact of various inoculum concentrations on the effectiveness of co-digesting was assessed. Toma et al., (2016) investigated agricultural crop and residue optimization for biomethane production. Their result showed that when maize is harvested in the vegetation milk stage to ripeness, the methane yield that can be produced is about 7500–10200 m^3 N ha-1. Shahidul et al., (2018) performed research on the optimization of variables impacting pome biogas generation.

The monitored inputs were hydraulic retention time, ORL, and C/N. By using 3D surface response plots, the optimum conditions were 28 for C/N, 5g/L, and 6.5 days for HRT which resulted in optimum biogas production of 3.8.d-1. The effect of propionic acid and ammonia concentrations on acetate utilizing methanogenesis has been investigated by Shin et al., (2019) using RSM. Their research found that both inhibitors, propionate level of 2–8 g/L, and TAN level of 2–5 g/L had an impact on the lag time.

CHAPTER THREE

RESEARCH METHODOLOGY

3.1 Introduction

The study methodology provides information on the tools and equipment, techniques, and designs used to carry out the research objectives. Figure 3.1 shows the flowchart used for the methodology.

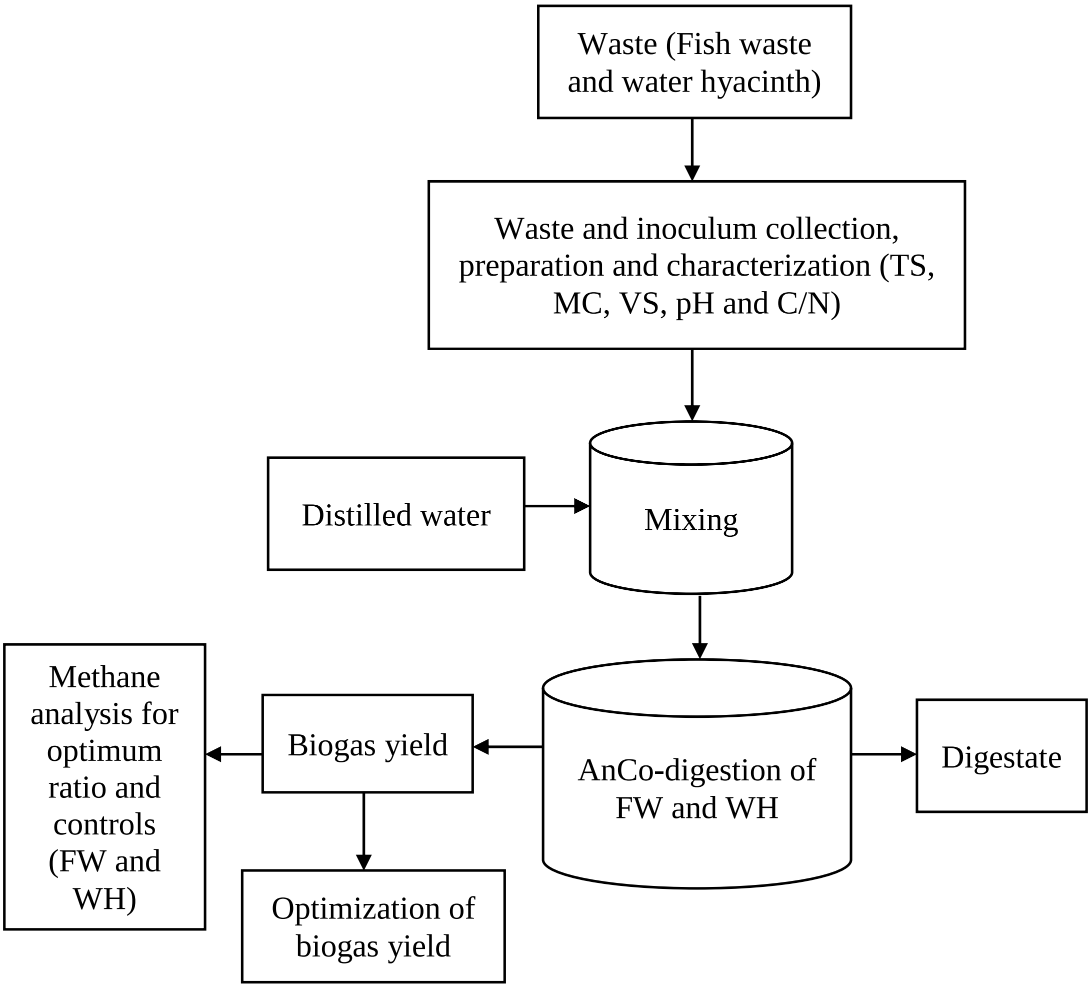


Figure 3. 1: Typical flow sheet for biogas production optimization from AnCo-digestion of FW and WH

3.2 Feed Substrates and Inoculum

WH was collected from Lake Victoria in Kisumu County. Water hyacinth was washed to remove unwanted impurities, cut into small pieces, and mashed using laboratory mortar to increase its biodegradability for microbial activity. Thereafter, they were put in a plastic collector and stored in a refrigerator for further use. Fish waste (fish intestines) used in this experiment was collected from the fish point, Eldoret, Kenya and chopped into small pieces. The inoculum (fresh digested cow dung) used in this research was collected from the Moi University biogas plant, Eldoret, Kenya. Here fresh bio-digested cow dung was used as an inoculum because it contains active bacteria. Figures 3.2, 3.3,3.4, and 3.5 show the procedures for water hyacinth, fish waste, and inoculum collection and preparation.

Figure 3. 2:Bulk Water hyacinth collection and transportation from Lac Victoria to Moi University, main campus

Figure 3. 3: Washing, cutting, and mashing of water hyacinth to be ready for digestion

Figure 3. 4: (a, b, c, d, e, and f). Fish waste sampling, collection, and preparation from fish point Eldoret, Kenya

Figure 3. 5: (a, b, c, d, e, and f) Inoculum collection from Moi University biogas plant, main campus, Eldoret, Kenya

3.3 Equipment

The laboratory equipment used for this study includes: Electronic weighing scale, pH meter, Digesters (Conical flask), Water bath, Rubber Hose, Knife, blender, Crucible, laboratory oven, non-return valves, silicon sealant, multi-gas detector, syringe, gas sampling bag, cocks, gas chromatography and muffle furnace. The summary of equipment and material is shown in table 3.1.

3.3.1 Digester

Conical flasks (250 mL) were used as a digester to carry out the chemical and biological reactions for biogas production as shown in figure 3.6. The sample mixture is poured into the conical flasks, which are then placed in a water bath for maintaining the necessary temperature (37°C) for the AD process.

The rubber hose or rubber piper was used to convey gas from the first conical flask used as a digester to the second one used as a water tank, and then to the third one used as the water collector. The syringe was used to facilitate the passage of water when needed in the water tank.

Figure 3. 6: Prepared digesters with cocks, rubber pipers, non-return valves, and syringe

3.3.2 Water bath

The purpose of the water bath is to maintain the constant temperature for all of the samples that have been immersed in it. It should be operational during the experiment. Therefore, the water bath was utilized to maintain the temperature which settled at 37℃. Figure 3.7 shows the water bath used in this experiment.

Figure 3.7: Water bath

3.3.3 Gas sampling Bag

The gas was collected and put into a gas bag for storage. Figure 3.8 shows the gas sampling bag that was utilized in this experiment.

Figure 3. 8: Gas Sampling Bag

3.3.4 Gas Chromatography (GC)

The biogas generated by the digesters was quantitatively analyzed using a GC-Shimadzu 2010. Table 3.1 lists the specifications and settings of this GC. The Gas Chromatograph utilized in this study is shown in figure 3.9.

Figure 3. 9: GC used for biogas analysis

Table 3. 1: Specifications and settings of GC

GC	**Shimadzu GC 2010**
Injector	Total Flow: 81.2 ml/min, Temperature: 150°C; Purge flow: 3 ml/min, Split ratio: 5.1
Injection volume	2 µL
Nitrogen (Carrier gas)	Total Flow: 81.2 ml/min; Temperature: 150°C; Purge flow: 3 ml/min, Pressure: 227.9 kPa
Column	Inner Diameter: 0.32 mm, Film thickness: 0.25 µm, Length: 30 m, ZB-Wax Column flow: 12.82 ml/min, 40°C to 220°C at 20°C/min,2 min initial hold time, 4 min final hold time (Temperature program); Linear velocity: 120.6 cm/sec
Detector	FID (Flame Ionization Detector)
H_2 (Detector)	Flow: 80 ml/min ,Temperature: 280°C; Make up flow: 20 ml/min
Software	GC Solution

3.3.5 Multi-gas detector

The quantitative evaluation of the digester biogas was conducted using a multi-gas detector, model number SKY200-M4-WH (table 3.2). The multi-gas detector used in this work is shown in Figure 3.10.

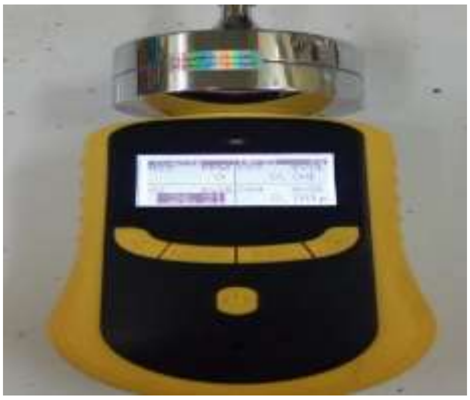


Figure 3. 10: A multi-gas detector used for biogas analysis

Table 3. 2: The specifications and settings of a multi-gas detector

Detector Model	**SKY2000-M4-WH**
Gas Detected	CH4, O_2, CO_2, H_2S
Detection principle	Catalytic combustion, Electrochemistry
Sampling Method	In pumping suction, ten grades of pumping suction for selection, the flow rate can be up to 1L/min
Resolution	CH_4: 1% LEL , O_2: 0.01% VOL ,CO_2: 1 ppm, H_2S: 0.1 ppm
Measure Range	CH_4:0-100%LEL (LEL: Lower Explosive Limit), O_2:0-30%VOL, CO_2: 0-1000 ppm, H_2S: 0-100 ppm
Number	200812B1
Precision	3%~5% F.S. (Full Scare)
Linearity error	≤±1%
Voltage	3.7 V
Range	Standard

3.3.6 pH meter

The pH value of the substrate was determined using a pH meter probe (PH-009(I)A), as specified in table 3.3. The pH meter used is shown in figure 3.11.

Figure 3. 11: A pH meter used for substrate pH range analysis

Table 3. 3: Specifications of the pH meter

Specifications	**Operating Temp.**	**Resolution**	**Range**	**Temp.compensation**	**Accuracy**
Values	0℃ - 50℃	0.1 pH	0.0-14.0 pH	0℃- 50℃	± 0.1 pH

3.3.7 Electronic weighing scale

The electronic scale employed in this experiment can precisely weigh the substrates in both their dry and wet states. The electronic weighing scale used is shown in figure 3.12.

Figure 3. 12: Weighting Balance

3.3.8 Laboratory oven

The purpose of using the oven is to remove the moisture from the FW and WH samples without causing any damage to their constituent parts, the temperature was set at 1050°C. The laboratory oven used is shown in figure 3.13.

Figure 3. 13: Laboratory Oven

3.3.9 Laboratory muffle furnace

The purpose of using the furnace is to remove the volatile solids from the FW and WH without causing any damage to the samples' constituent parts, the temperature was set at 550°C. The laboratory muffle furnace used is shown in figure 3.14.

Figure 3. 14: Laboratory furnace

Table 3. 4: Summary of equipment and materials used in this study

Equipment and Materials	Purpose	Address/source
Conical flask	Act as a digester to carry out the chemical and biological reaction	Chemical and Process Engineering Lab
Water bath	Maintain the required temperature	Chemistry lab
Gas sampling Bag	Gas collection	Chemical and Process Engineering Lab
Gas Chromatograph	Quantitative analysis of biogas	Chemical and Process Engineering Lab
Multi-gas detector	Quantitative analysis of biogas	Chemical and Process Engineering Lab
Electronic weighing scale	Measure the weight of the substrates	Chemical and Process Engineering Lab
Laboratory oven	Moisture determination	Chemistry lab
Muffle furnace	Ash determination	Chemistry lab
pH meter	pH determination	Chemical and Process Engineering Lab
Blender	Blending substrate to reduce particle size	Chemical and Process Engineering Lab
Knife	Cutting substrate into small pieces	Chemical and Process Engineering Lab
Rubber Hose	Convey biogas from the digester to the gas collector and water from the gas collector to the water collector	Chemical and Process Engineering Lab
Cocks	Close the conical flask	Chemical and Process Engineering Lab
Non-return valves	Prevent the passage of biogas back to the digester	Chemical and Process Engineering Lab
Syringe	Gas sample collection and Facilitate the passage of water in the water collector	Chemical and Process Engineering Lab
Silicon sealant	Prevent any leakage to the digester	Chemical and Process Engineering Lab
Crucible	Hold samples for characterization	Chemistry lab

3.4 Characterization of waste

The physiochemical parameters of the organic material are crucial for operating AD system because they affect both biomethane yield and system stability (Kubaská et al., 2010). The parameters were checked before the substrates were put into the digester. Fish waste, water hyacinth, and inoculum were analyzed for moisture content (MC), volatile solids (VS), total solids (TS), ash content, pH, and C/N ratio.

3.4.1 Physical analysis

American Public Health Association (APHA, 2012) standard methods, Methods 2540B and 2540E were used to determine the moisture content (MC), total solids (TS), volatile solids (VS), and ash concentration (APHA, 2000; Cheng & Zhong, 2014; Shen, 2008).

3.4.1.1 Moisture content (MC) and Total solids (TS) determination

The MC is the amount of water content in the material while TS is the amount of dry matter content in the material. Ten grams (10 g) of water hyacinth sample and five grams (5 g) of the fish waste sample were placed in crucibles, which were then baked at 105 °C for 4 hours. The crucibles were cooled down for 10 minutes. The losses were then recorded up until the constant weight was reached. Then, using equations 3.1 & 3.2 respectively, the percentage of moisture content (MC) and total solids (TS) was determined. APHA 2540 B standard method was used (Drosg & Braun, 2013; Jnr, 2011; Maghaydah, 2003; Makhura et al., 2020).

$$\mathrm{MC} = \frac{\mathrm{W2} - \mathrm{W3}}{\mathrm{W2}} \times 100 \qquad \text{eq 3.1}$$

$$\mathrm{TS} = \frac{\mathrm{W3} - \mathrm{W1}}{\mathrm{W2} - \mathrm{W1}} \times 100 \qquad \text{eq 3.2}$$

Where; W1: the weight of crucible, W2: the weight of wet material sample and crucible, and W3: represent the weight of dry material sample and crucible at 105 ℃

3.4.1.2 Volatile solids analysis and Ash determination

To determine how many volatile organic materials were present in the sample, the VS was measured. Ash includes different nutrients in varying amounts that are necessary for microbial metabolism.

Five grams (5g) of fish waste and ten grams (10g) of water hyacinth were weighed into crucibles, dried at a temperature of 105 °C for 4 hours with an oven heater, and then burned for 1 hour in a muffle furnace at a temperature of 550 °C. For around 20 minutes, the crucibles with the ashes were allowed to cool. The percentage of volatile solids (VS) and ash content (% A) was determined using equations 3.3&3.4 respectively. APHA 2540 E standard method was used (Drosg & Braun, 2013; Hasanzadeh et al., 2018; Ismail, 2017; Maghaydah, 2003; Makhura et al., 2020; Twizerimana et al., 2021; Orhorhoro et al., 2017; Rajendran et al., 2011).

$$\mathrm{VS} = \frac{\mathrm{W3} - \mathrm{W4}}{\mathrm{W3} - \mathrm{W1}} \times 100 \qquad \text{eq 3.3}$$

$$\%\mathrm{A} = \frac{\mathrm{W4} - \mathrm{W1}}{\mathrm{W2} - \mathrm{W1}} \times 100 \qquad \text{eq 3.4}$$

Where, W1: the weight of the crucible, W2: the weight of the wet material sample and the crucible, W3: the weight of the dry material sample and the crucible (at 105 °C), and W4: the weight of the material sample and the crucible after ignition at 550 °C.

3.4.2 Chemical analysis

The carbon to nitrogen (C/N) ratio was determined by dividing the total carbon by the total nitrogen. APHA 4500 B standard method was used (Twizerimana et al., 2018).

3.4.2.1 Total carbon determination

The Walkley-Black method was employed to determine the total carbon using H_2SO_4 + $K_2Cr_2O_7$ (Cheng & Zhong, 2014; El-din & Badawi, 1989). The potassium dichromate Walkley-Black technique was employed as explained by (Bakr & El-ashry, 2018; Twizerimana et al., 2021; Myovela, 2018), in which 1 gram of dry materials sample was placed in a conical flask of 250 mL, $K_2Cr_2O_7$ of 10 mL was added, and the mixture was rotated or swirled.

Then, in a fume hood, 15 mL of H_2SO_4 was added and swirled three more times. After 30 minutes, 5 mL of phosphoric acid and 150 mL of distilled water were added. With the solution of ferrous ammonium sulfate (0.5 N), the contents were adjusted until the color changed from blue to green. Equation 3.5 was used to compute the amount of organic carbon.

$$\%\mathrm{C} = ((\mathrm{B} - \mathrm{S}) \times (\mathrm{V} \times 1.3 \times 0.3)) \div \mathrm{W} \qquad \text{eq 3.5}$$

Where, % C: the percentage of total carbon, S: the material sample reading (mL), B: the blank reading (mL), W: the weight of the material sample (g), and 1.3 is a constant for the organic carbon based on a 77 % recovery rate and V: the volume of 1N $K_2Cr_2O_7$ (mL).

3.4.2.2 Total nitrogen determination

Total nitrogen content was determined using the Kjeldahl method (El-din & Badawi, 1989; Elbeshbishy et al., 2010; Twizerimana et al., 2021). The total nitrogen content was calculated using the Kjeldahl method, which required the digestion of the sample and volumetric measurement. 1 gram of the material sample, K_2SO_4 of 5g, and 0.5 g of $CuSO_4$ as a catalyst, and concentrated H_2SO_4 (10 mL) were all weighed into a digestion flask. The mixture was heated to 420 °C in a high-temperature fume hood until the color of the digest turned blue, indicating that the digestion process was complete.

After cooling to room temperature and being filled with distilled water, the digest was moved to a 100 ml volumetric flask. Additionally, an empty digestive tube containing acid and catalysts was produced. The diluted digest (10mL) was placed in a distilling flask and diluted or rinsed with 3mL of distilled water. A solution of 40% NaOH (15 mL) was added and the mixture was also diluted or washed with 3 mL of water (distilled). About 60 mL of distillate was produced after the distillation process. The distillate solution was titrated with HCl (0.02 N) until the color changed to orange (methyl orange). Equation 3.6 was used to compute the total nitrogen.

$$(\%\ \mathrm{N}) = (\mathrm{V}_1 - \mathrm{V}_2) \times \mathrm{N} \times \mathrm{F} \times 100/\mathrm{V} \times (0.014) \times (100/\mathrm{W}) \qquad \text{eq 3.6}$$

Where, % N: percentage of Total nitrogen, V_1: Volume of the sample (mL), N: Normality of standard (HCl), V_2: Volume of the blank (mL), V: Volume for distillation F: Factor of standard (HCl), and W: Weight of the sample (g)

The summary of methods used for the characterization of substrates was shown in table 3.5.

Table 3. 5: Methods used for characterization of substrate

Parameter	Determination
Moisture content (MC)	APHA 2540 B Method (Drying sample in the oven at 105℃ for 4h)
Total solids (TS)	APHA 2540 B Method (Drying sample in the oven at 105℃ for 4h)
Volatile solids (VS)	APHA 2540 E Method (Drying sample in the oven at 105℃ for 4h and burning the sample in a muffle furnace at 550℃ for 1h)
Ash content	APHA 2540 E Method (Drying sample in the oven at 105℃ for 4h and burning the sample in a muffle furnace at 550℃ for 1h)
C/N ratio	APHA 4500 B Method
pH	pH meter

3.5 Biogas production and Methane content analysis

Biogas yield was measured daily and the methane content was determined, using the water displacement method and gas chromatograph respectively.

3.5.1 Biogas production

Biogas production was determined using the water displacement method (measuring the quantity of water displaced in a water collector by the gas generated, in milliliter), as shown in Figures 3.15& 3.16.

Figure 3. 15: Illustration of the experimental setup of anaerobic batch fermenter of FW and WH

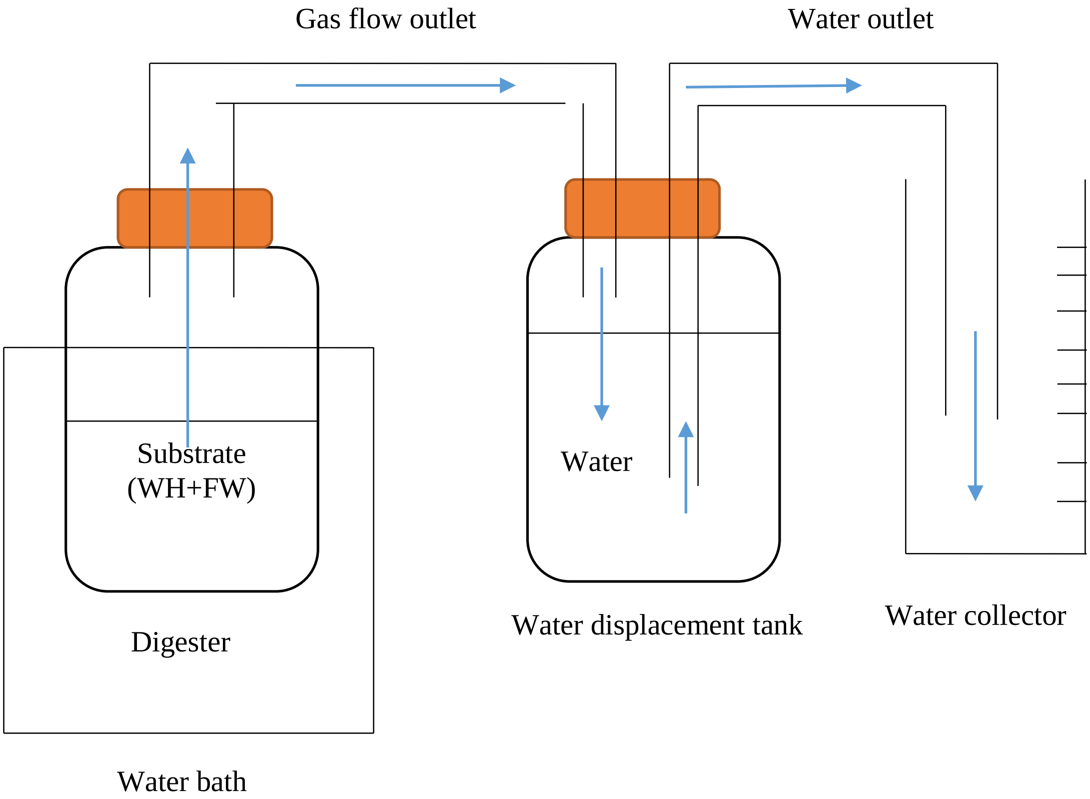


Figure 3. 16: Overview of digester setup

A conical flask of 250 ml with a working volume between 190–210 mL was used for batch digestion of the biogas generation test. According to the experimental plan, the substrates were fed into the reactor at varied substrate ratios (25, 50, and 75 g), IC (5,10, and 15 g), and dilution (85, 90, and 95 mL). Variations to those operating parameters were made to determine the biogas yield and optimum operating conditions for the efficient Anco-digestion of WH and FW. The co-digestion was quantified by substrate ratio based on 100 g. Silicone sealant was used to seal the reactors. Every day, biogas production was measured.

For each run, the batch fermentation took place in the reactor for 20 days. The entire investigation was conducted at mesophilic temperature (37°C), and the organic content was manually slowly shaken for about a minute once a day. The anaerobic digesters were set up in triplicates for each treatment, and the findings were presented as means. As illustrated in figure 3.16, the amount of water that the daily production of biogas displaces in milliliters was used to compute the volume of biogas produced. Table 3.6 provides a summary of the anaerobic digestion experimental setup.

Table 3. 6: Summary of experimental setup for AD

Operational criterion	Value
The volume of the digester	250 mL
The volume of the reaction	190-210 mL
Temperature	Mesophilic (37°C)
Operating time	20 days
Mixing	Daily, Manual shaking

3.5.2 Methane content analysis

The methane content of the biogas produced was determined by Wujick and Jewell procedures (El-din & Badawi, 1989), biogas sample was withdrawn by a syringe and injected into a gas chromatograph through an injection port. The GC has a stainless steel column of 0.32 mm diameter, 30 m length, and 0.25 µm thickness and a dual FID (flame ionization detector). A carrier gas (Nitrogen) was applied at a total flow rate of 81.2 ml/min. 40°C to 220°C were the temperatures used for the column, and the temperature of 280°C and 150°C was used for the detector and injection port respectively. The detector measured the methane gas and the signal displayed on the computer. A gas detector was used to detect methane content from the gas sampling bags. The GC used for biogas is shown in figure 3.17.

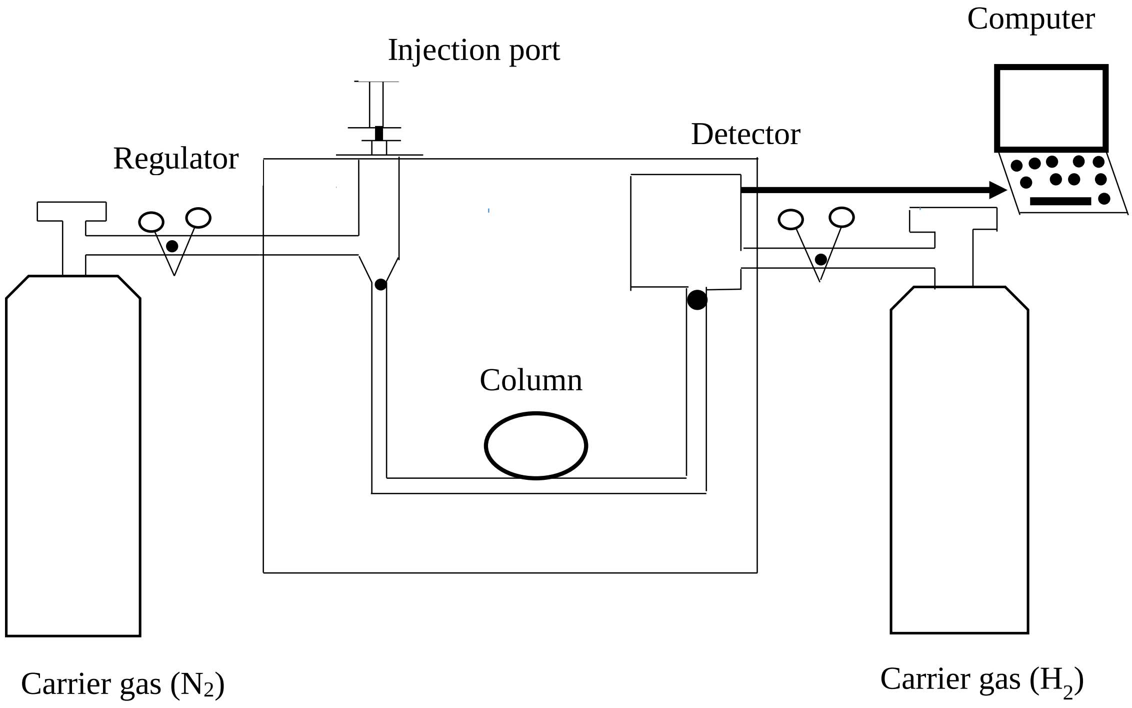


Figure 3. 17: Overview of Gas chromatograph setup

3.6 Optimization of biogas production

3.6.1 Design of Experiments (DOE)

DOE is a technique used to create an experimental model which uses minimum resources. Design Expert 13 software, which contains CCD, ANOVA, and RSM was used to determine the level of variable inputs and establish the optimum number of experimental runs to maximize biogas yield in Anco-digestion of fish waste and water hyacinth. Analysis of variance (ANOVA) was used for the analysis of the regression coefficient and the prediction equation, and to show how the variables interacted.

The polynomial equation was illustrated in three dimensions using response surface plots. For the surface response analysis, RSM was used to examine the relationships or interaction between variables and the response and to estimate the optimum surface area of optimal values of the response. The AnCo-digestion of FW and WH was controlled by three factors: (X_1: Substrate ratio (WH: FW, 25-75g, X_2: Inoculum concentration (IC, 5-15 g), and X_3: Dilution (85-95 mL)). The experimental design levels and AD parameters are shown in table 3.7.

According to the experimental design, 17 runs were carried out with three center point replications. The anaerobic digesters were set up in triplicates for each treatment, and the findings were presented as means. To account for the conditions of the CCD experiment, each independent factor was coded at five distinct levels and given the letters -α, -1, 0, +1, and +α. Biogas yield (mL) was used as the response of the experiment.

Table 3.7: Experimental levels of independent factors for the optimization of biogas production

Factor	**Parameters**	**Levels**				
		-α	**-1**	**0**	**1**	**+α**
X_1	Substrate ratio (g) (WH: FW)	8:92	25:75	50:50	75:25	92:8
X_2	Inoculum concentration (g)	1.6	5	10	15	18.4
X_3	Dilution (mL)	81.6	85	90	95	98.4

3.6.2 Statistical Analysis

For the analysis of the regression coefficient and the prediction equation, ANOVA was used. The second order polynomial regression model (eq 3.7) that follows was used to fit the experimental results of RSM:

$$y = \beta_0 + \sum_{i=l}^{k} \beta_{ii} x_i^2 + \left(\sum_{i=1}^{k-1} \sum_{j=i+1}^{k} \beta_{ij} x_i x_j\right)_{i<j} \qquad \text{eq3.7}$$

Where Y is the predicted response (biogas production), β_i, $\beta_{ii,}$ and β_{ij} are linear, quadratic and interaction coefficients respectively, β_0 is the offset term, Xi, and Xj are independent factors, and k is the number of parameters under investigation. By examining the data using ANOVA to see how variables interacted, the suitability of the constructed model was evaluated. The effectiveness of the second-order polynomial equation fit was expressed using R^2 (coefficient of determination). Model variables or terms were assessed using P-value (Chanathaworn, 2017; Roslina et al., 2014; Sathish, S., 2011; Shahidul et al., 2018).

3.6.3 Response surface plots

The polynomial model was represented in 2D (two-dimensional) contour plots and 3D (three-dimensional) response surfaces to indicate the effects of biogas production variables on the biogas yield and examine the optimum concentration of optimal values.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Characteristics of the Substrates

Anaerobic digestion design and operation heavily depend on the characteristics of the feedstock. The growth rate of microbes and the stability of the anaerobic environment are both significantly impacted by the content of the organic waste supplied to a digesting system (Marchaim, 2007; Phun et al., 2017). The physicochemical properties of the substrate determine its biodegradability (Aslanzadeh, 2014; Nadu & Nadu, 2017).

For maximum quantity and quality of biogas energy, knowledge of the substrate's physicochemical properties is crucial (Makhura et al., 2020). FW, inoculum, and WH were examined for their initial MC, C/N ratio, pH, ash content, and TS. Table 4.1 summarized the results of the feedstock characterization.

Table 4.1: Physicochemical characterization results

No	Substrate	MC (%)	TS (%)	VS (%)	Ash content (%)	Total carbon (%)	Total nitrogen (%)	C/N ratio	pH
1	FW	61.78	38.21	93.94	0.52	54.2	9.2	5.89	6.5
2	WH	94.4	5.6	83.3	16.7	42.7	2.0	21.35	7.2
3	Inoculum	89.67	10.33	74.9	25.1	35.91	1.53	23.47	6.8

From the results above, water hyacinth had a maximum MC of 94.4% and fish waste was the least with a moisture content of 61.78%. The maximum TS was recorded in fish waste (38.21%). The VS in water hyacinth, fish waste, and inoculum was 83.3%, 93.94 %, and 74.9% respectively. The range of biodegradability for the substrate to produce high biomethane yield was found to be 70-95% (Nadu & Nadu, 2017; State et al., 2016, Twizerimana et al., 2021).

The range of biodegradability for inoculum varies from 28 - 70% (Aslanzadeh, 2014; Marchaim, 2007). This shows that fish waste and water hyacinth are biodegradable which makes them potential substrates for the production of biogas. According to published research, organic waste with high VS content has a high potential of being used as a source of biogas energy (Adebayo & Odedele, 2020). The results obtained from water hyacinth are comparable with those reported by Katima, (2001); Nalinga and Legonda, (2016) who reported the Volatile solid in water hyacinth of 80.8% and 80.9% respectively. The VS of inoculum is in a good range with the result reported by Deressa et al., (2015) of 91.56%.

The MC of water hyacinth, fish waste, and inoculum was 94.4%, 61.78%, and 89.67% respectively. According to Alfarjani, (2012), Le et al., (2011), and Khalid et al., (2013) the substrate that includes MC between 60–80% is suitable for the AD process. Therefore, fish waste, water hyacinth, and inoculum are amenable to AD. The results are comparable with Kafle and Kim, (2012); Kandyliari et al. (2020); Maghaydah, (2003) who reported that the MC of fish waste varies between 57.15-73%, and Girisuta, (2014); Katima, (2001) reported that MC of water hyacinth was in the range of 85-95%.

High MC in the substrate facilitates the AD process (Deressa et al., 2015). Consequently, water hyacinth and fish waste are good substrates for the production of biogas. The results for inoculum are in a good range with the value reported by Deressa et al., (2015) of 79.75%. The TS of water hyacinth, fish waste, and inoculum was 5.6%, 38.21%, and 10.33% respectively. According to Muhammad Rashed, (2015) and Yadvika et al., (2004), 7–9% TS is preferable. This shows that TS of fish waste and water hyacinth are out of range.

However, the results for fish waste are in line with the results reported by Kafle and Kim, (2012); Nalinga and Legonda, (2016) who reported that the TS in fish waste is 31.30 % and 32.2 % respectively. The present study agrees also with the values reported on water hyacinth by Katima, (2001) who found the TS of 11.4%, and Chanathaworn, (2017) who found the maximum biogas at 8% of TS of water hyacinth. The TS of inoculum is in a good range, and the result agrees with the value reported by Marchaim, (2007) of 8.16 %.TS measures the overall volume of material that remains after all the moisture has evaporated (Madondo, 2017).

Less than 10% of TS in AD systems are low or wet solids, 15-20% are medium solids, and 22–40% are high or dry solids processes (Jnr, 2011; Madondo, 2017; Muhammad Rashed, 2015). A dry AD system makes the digester's solution more compact, which offers high loading rates, consequently more biogas than a wet AD system because the high loading rate and compactness of by-products increase the level of material digestion (Madondo, 2017; Sun, 2015). Dry AD is advantageous : (i) requires less water, (ii) having less water in the residue (digestate) results in a smaller reactor capacity, and (iii) produces a larger volumetric biomethane yield (Jansson et al., 2019; Sun, 2015).

The C/N ratio for fish waste, water hyacinth, and inoculum was 5.89:1, 21.35:1, and 23.47:1 respectively. According to Jnr, (2011) and Rabii et al., (2019) the optimal C/N ratios for AD is between 20-30. Therefore, the C/N of water hyacinth is in a good range. However, the C/N ratio (5.89) of FW was out of range for biogas generation. Moreover, the results above are within the range of Kafle and Kim, (2012); Ojikutuabimbola & O, (2016); Teodorita et al., (2010) ; Marchaim,(2007) reported the C/N ratio of FW between 3-5, and Katima,(2001); Abbasi et al., (2012); C.Akunna,(2019) reported the

C/N ratio of WH between 24-27. The C/N ratio of inoculum is comparable with the results reported by C.Akunna, (2019) Li et al., (2019); Abbasi et al.,(2012), and Marchaim, (2007) of 14-25 %. The C/N ratio depends on the type of feedstocks. A low C/N ratio can quickly lead to ammonia toxicity and high pH levels, which are poisonous for methanogenic bacteria while a high C/N ratio causes poor buffering capacity, hence lower biogas generation (Avs, 2016; C.Akunna, 2019; Hhaygwawu, 2016; Jnr, 2011; Katima, 2001; Ojikutuabimbola & O, 2016; Rabii et al., 2019; Stojkovi et al., 2018). Nevertheless, the C/N ratio (21.35) of WH and inoculum (23.47) was in a preferable range to keep the AD process stable.

Organic waste with a low C/N ratio can be combined with high C/N ratio organic waste to reduce the concentration of inhibitory substances and achieve the digester's ideal C/N ratio (Abbasi et al., 2012; Hhaygwawu, 2016; Jnr, 2011; Ojikutuabimbola & O, 2016; Owamah, 2010; Rabii et al., 2019). The pH value of each substrate varied between 6.5 to 7.2 which is within the range of acceptable pH values. The optimum pH range for biogas production varies between 6.5 to 7.2 (Deressa et al., 2015; Rabii et al., 2019; Sathish, S., 2011). In the conclusion, the fish waste and water hyacinth used in this research showed that they are good feedstock for biogas production. Table 4. 2 summarizes the results from previous studies.

Table 4. 2: Summary of substrates characteristics from previous studies

No	Substrate	MC (%)	TS (%)	VS (%)	Ash content (%)	C/N ratio	pH
1	Fish waste	57.15-81.43	31.30-32.2	27.50-55.5	2.14-5.7	3-10.1	NA
2	Water hyacinth	85-95	8-27.76	80.9-93.2	15-26	10.1-27.4	NA
3	Inoculum	79.75-92.67	7.14-11.03	88.64-91.56	8.44-14.36	14-25	6.40

Sources : (Abbasi et al., 2012; Bote et al., 2020; C.Akunna, 2019; Chanathaworn, 2017; Deressa et al., 2015; Girisuta, 2014; Kafle & Kim, 2012; Kandyliari et al., 2020; Katima, 2001; Li et al., 2019; Maghaydah, 2003; Marchaim, 2007; Twizerimana et al., 2021; Nalinga & Legonda, 2016; Njogu et al., 2015a; Ojikutuabimbola & O, 2016; Pina et al., 2018; Rozy, 2016; Teodorita et al., 2010; Tham, 2012).

4.2 Production of Biogas from the AnCo-digestion of FW and WH

The results of the 20-day AD of FW and WH at mesophilic temperature (37^0C) are shown below (Figure 4.1). According to Owamah, (2010), co-digestion of feedstock can increase anaerobic digestion process production and quality due to better carbon, nitrogen, and nutrient balance. According to findings, by co-digesting fish waste (FW) with water hyacinth (WH) under optimal conditions, the production and quality of anaerobic digestion of fish waste can be increased. A low methane production will arise from the single substrate's low digestibility and accumulation of inhibitory substances like lipids, potassium, etc., and lipids, as well as its likely lack of buffering and necessary nutritional content (Chanathaworn, 2017).

When compared to the mono-digestion, co-digestion has several benefits, including improved C/N ratio, improved biogas yield, and quality (Maile et al., 2016), good buffering capacity (Chanathaworn, 2017), fewer inhibition impacts (e.g., accumulation of VFA, NH_3, etc.), and system stability due to synergistic effects (Hanghome, 2014;

Madondo, 2017; Maile et al., 2016; Makhura et al., 2020; Owamah, 2010; Rabii et al., 2019; Roslina et al., 2014).

To evaluate the effect of substrate ratio, IC, and dilution on AnCo-digestion of FW and WH, the cumulative biogas, and the daily biogas production was computed. Variations in the substrate ratio, IC, and dilution, as described in table 4.5 from CCD, were tested in batch reactors. The study's results were averaged after being conducted in triplicate. Daily measurement was made for the production of biogas. Within a retention period of 7-12 days, biogas was generated, as shown in figure 4.1 for daily biogas production. Cumulative biogas volume for bio-digesters is shown in figure 4.2. For the first days, biogas production was very high for all digesters and started to decrease rapidly to low levels in 7-12 days.

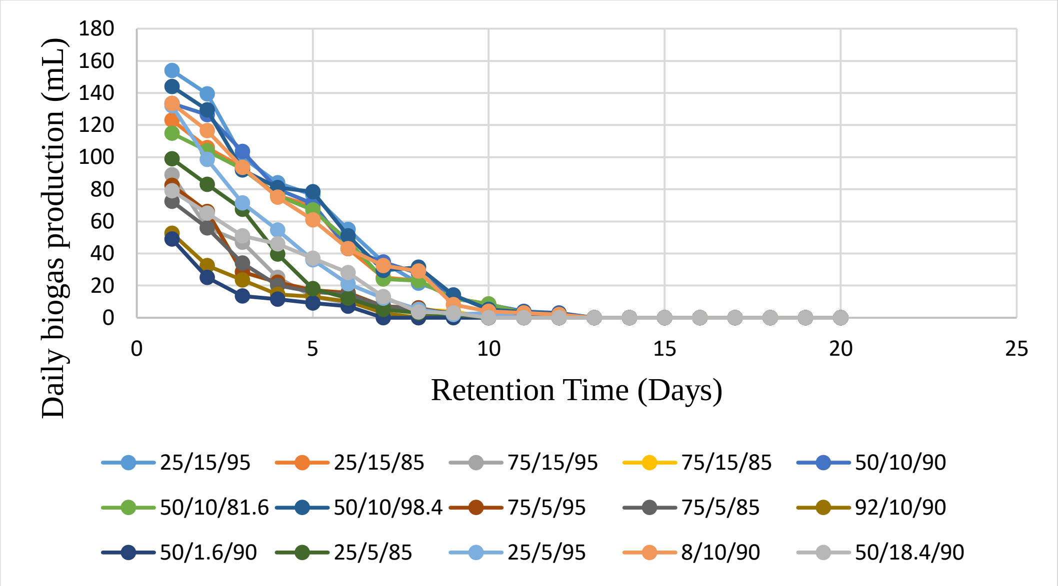


Figure 4.1: Daily production of biogas from AnCo-digestion of FW and WH

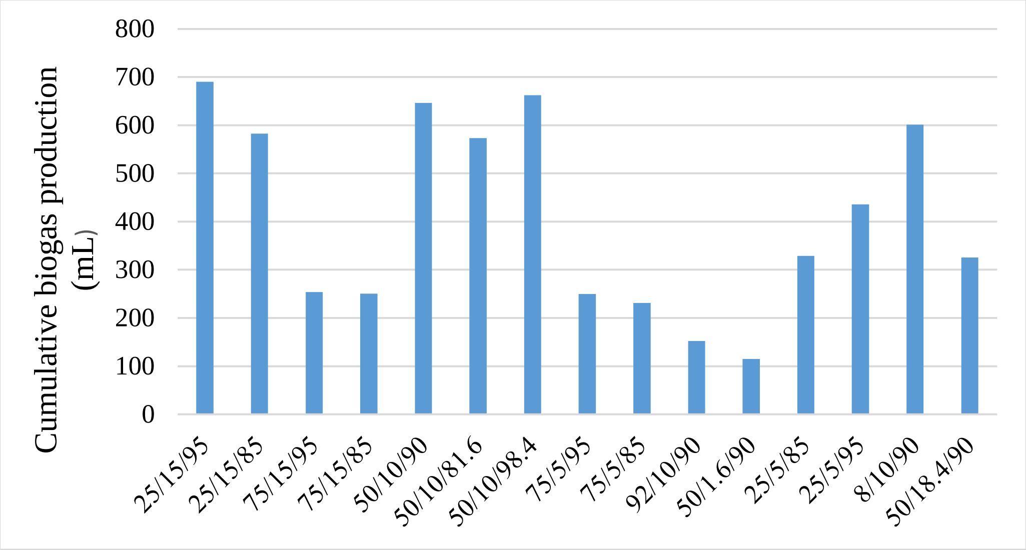


Figure 4. 2: Cumulative biogas volume from bio-digesters

A total of 17 batch bio-digesters (with three replicates for the center point) and three batch bio-digesters for the control (water hyacinth, fish waste, and Inoculum alone) were set up to investigate the impact of co-digesting fish waste with water hyacinth on biogas generation.

4.2.1 The bio-digesters results

The biogas digesters were set up with the following substrate ratio(g) (WH: FW), IC(g), and dilution(mL): 50:50/10/81.6, 50:50/10/90, 50:50/1.6/90, 8:92/10/90, 25:75/15/95, 25:75/5/95, 50:50/18.4/90, 75:2525/5/95, 92:8/10/90, 75:25/15/95, 75:25/5/85, 50:50/10/98.4, 25:75/15/85, 25:75/5/85, and 75:25/15/85 at a mesophilic temperature (37°C) to calculate the biogas yield and look into the best conditions for efficient co-digestion of these substrates. The substrates that are characterized in table 4.1 are the ones that were employed for this study. The experiment's findings are shown in figure 4.1, with the daily biogas production of the biodigesters. As shown in figure 4.2, accumulated biogas quantity from biogas reactors was measured to be 573,646.6, 115, 601, 690, 434.5, 325.5, 249.5, 152, 254, 231, 662, 582.5, 329.5, and 250.5 mL for the substrate ratio (g) (WH: FW) /IC(g) (g)/dilution(mL) of 50:50/10/81.6, 50:50/10/90, 50:50/1.6/90, 8:92/10/90, 25:75/15/95, 25:75/5/95, 50:50/18.4/90, 75:25/5/95, 92:8/10/90, 75:25/15/95, 75:25/5/85, 50:50/10/98.4, 25:75/15/85, 25:75/5/85, and 75:25/15/85. The biodigesters have a retention period of 7-12 days. The retention time is in agreement with literature from other authors as shown in table 2.3 and table 2.4, the retention period for wastes handled in mesophilic conditions ranges between 4 to 30 days. The type of feedstock affects incubation time for biogas production as well (Girmaye et al. 2019). At a ratio of 25:75 g (WH: FW), 15g IC and 95mL dilution, the highest rate of biogas production were attained. In 12 days of digestion at this ratio, 690 mL of biogas were produced. The overall biogas generation from co-digestion increased by 32.4 %.

To increase the biological and nutritive environment for bacteria in the digester and increase the production of biogas, appropriate and selective amounts of AnCo-digestion should be used (Girmaye Kenasa & Ebsa Kena, 2019). According to Makhura et al., (2020) because of its rich nutritional levels, high digestibility, and improved buffering capacity, fish waste can operate as an effective inoculum, and there was an enhanced rate of hydrolysis phase. The ratio of 50:50g (WH: FW),1.5g IC and 90mL dilution, resulted in the lowest biogas yield of 115 mL.

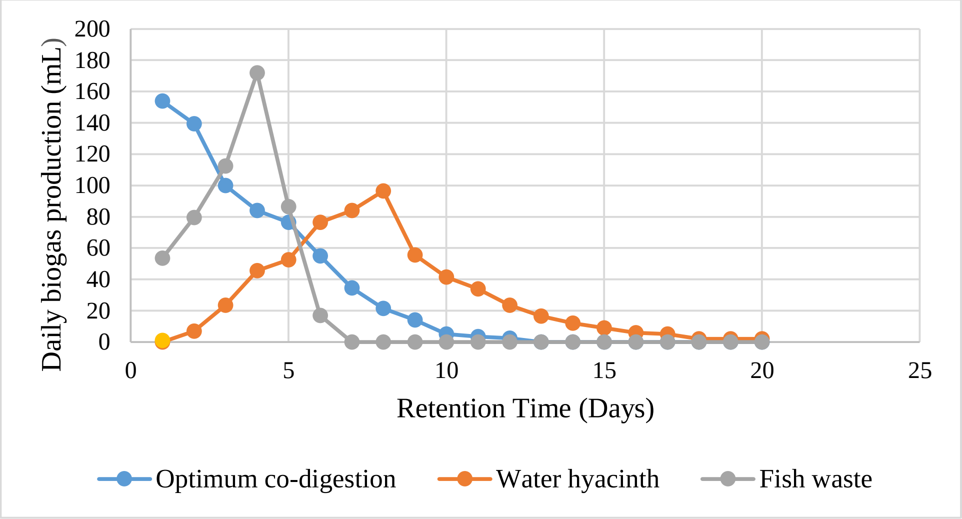
The high amount of substrate compared to low inoculation caused the poor biogas yield of 115 mL from the ratio of 50:50 g (WH: FW),1.5g IC and 90mL dilution, and 152 mL from the ratio of 92:8g (WH: FW), 10g IC and 90mL dilution. Filer et al., (2019) and Girmaye et al., (2019) showed that the quantity and quality of inoculums are crucial to the efficiency, duration, and stability of bio-methanogenesis. This is because a higher ratio of the substrate may cause an acidic environment in the AD system, which leads to methanogenesis inhibition, thus decreasing biogas production (Gooch, 2011; Jnr, 2011; Rabii et al., 2019; Shen, 2008).

If the inoculum in the reactor is low, there is a chance that only small amounts of biogas will be produced because of the microorganisms' low metabolic activity, which leads to low biogas generation. When there is overloading, the production of organic acids increases quickly which results in accumulation of acids, depletion of buffer, and pH lowering which inhibits the methanogenic activity thus, the production of biogas will decline and possibly stop altogether (Gooch, 2011; Jnr, 2011; Rabii et al., 2019; Shen, 2008).

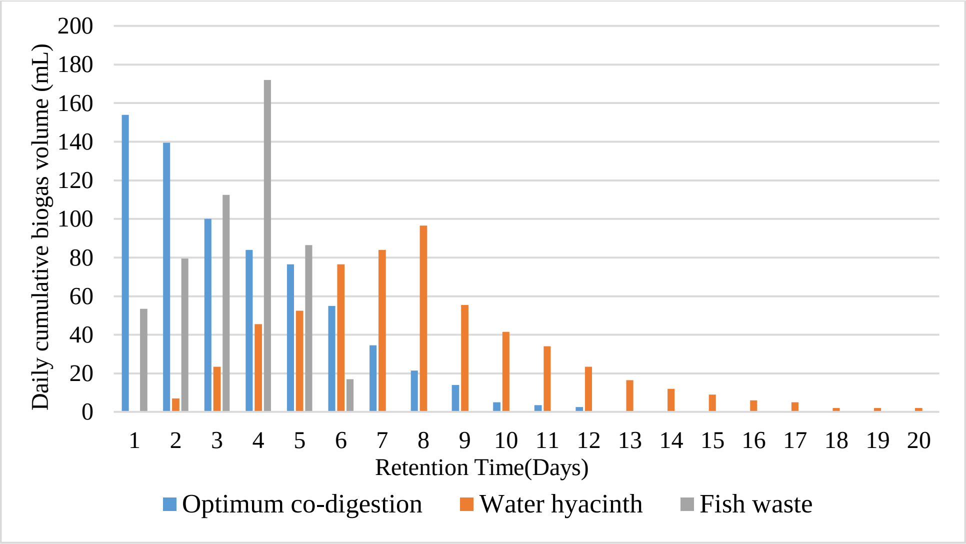
These findings are in line with those of other writers who investigated the biogas production capacity at various inoculum concentrations, the higher the inoculum concentration than the feedstock (1:4), the higher biogas generation and stable the AD process (Armah et al., 2017; Dar & Phutela, 2017b; Jnr, 2011; Madondo, 2017; Owamah, 2010; Yadvika et al., 2004). For every biodigester, the rate of biogas production was high and quick. Due to the fish waste and inoculum's ability to speed up the digestion of water hyacinth, the biogas yield for all reactors was extremely high in the first days of AD and was also quick (ranging from 6 to 12 days).

This might be because fish waste contains a lot of quickly biodegradable organic material (Marchaim, 2007; Pina et al., 2018). This demonstrates how crucial the fish waste ingredient is for accelerating digestion and the creation of biogas in a fermenter. The results from this study also showed that the biogas production increased as the dilution (water content) increased. The anaerobic digestion with lower water content results in low biogas production (Hhaygwawu, 2016). It’s necessary for the survival and movement of microorganisms. Besides, it enhances the breakdown of the organic material (Aslanzadeh, 2014; Jnr, 2011).

According to (Jnr, 2011), the percentage of waste degradation normally increases with increasing dilution, and biogas generation increases with increasing percentage degradation.



(a)



(b)

Figure 4. 3: (a) and (b) Daily and cumulative daily production of biogas from mono-digestion of FW, WH, and optimum co-digestion

4.2.2 Effect of substrate ratio on biogas production

Comparing the optimal co-digestion of FW and WH to the mono-digestion of each substrate, the findings of the experiment indicated an increase in biogas production, as shown in figures 4.3 and 4.4. The optimum values for maximum biogas production of 690mL with the highest methane yield of 68.15% were found to be WH: FW ratio,25:75g, 15g of IC, and 95 ml for dilution. The yield was 16.1% and 32.4% greater than that of WH and FW mono-digestion, respectively.

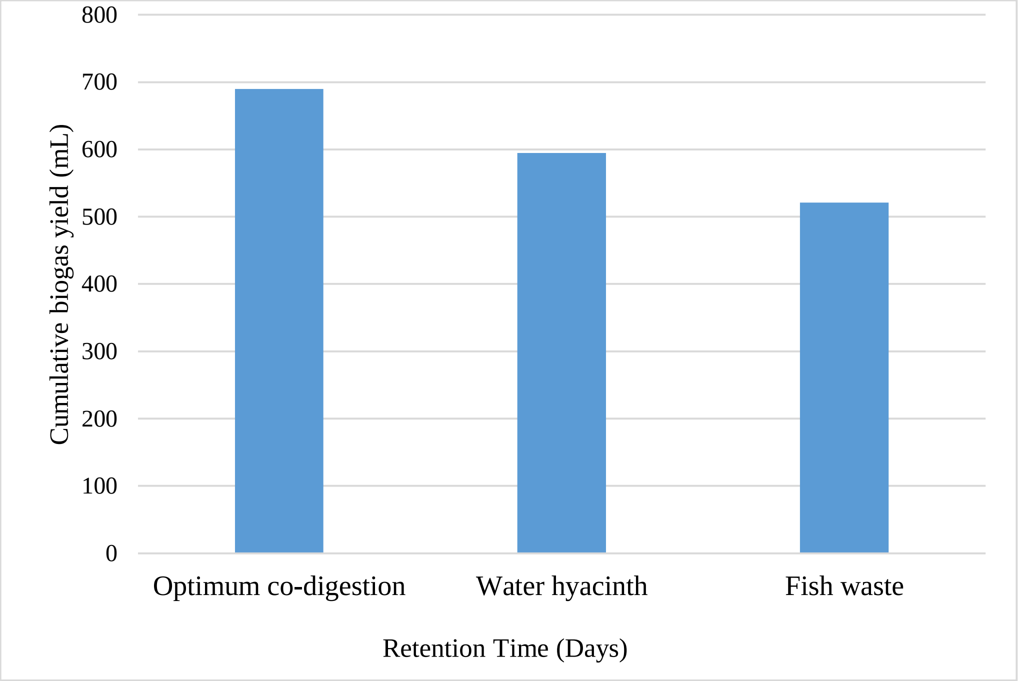


Figure 4. 4: Effect of mono-digestion and optimum co-digestion of FW and WH on cumulative biogas production

4.2.2.1 Effect of Mono digestion of substrate on biogas yield

As indicated in figure 4.5, mono-digestion of water hyacinth, fish waste, and inoculum produced maximum volumes of 594.5, 521, and 111.5 mL respectively. According to figure 4.5, the reduced cumulative biogas generation from mono-digestion of fish waste may be caused by the rapid consumption and depletion of readily biodegradable organic material and toxic compounds accumulation as a result of increased microbial populations, which impeded or inhibited the fermentation process (Zamanzadeh et al., 2017). However, compared to fish waste, water hyacinth has more available biodegradable organic matter that was used as an energy source for microorganisms, which resulted in higher cumulative biogas yield (Zamanzadeh et al., 2017).

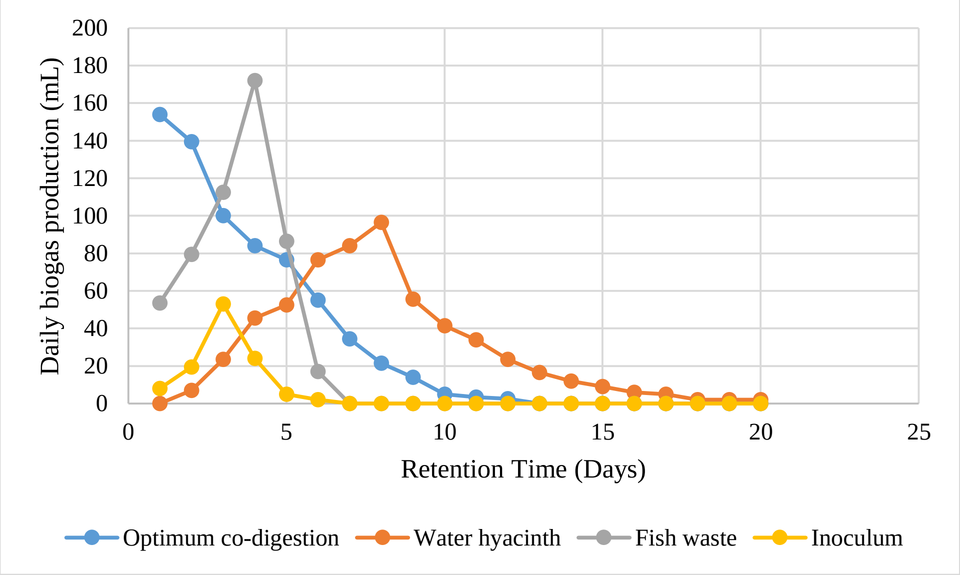


Figure 4. 5: Comparison of biogas production from Mono-digestion of FW, WH, and Inoculum

Low biogas output for mono-digestion of inoculum as shown in figure 4.5 may be caused by more bacteria being present, which reduces the amount of food accessible and leads to less food being converted to biogas (Hhaygwawu, 2016).

4.2.2.2. Effect of AnCo-digestion of FW and WH on biogas production

Mono-digestion of fish waste generated low biogas output (521mL) compared to water hyacinth (594.5 mL). According to Pina et al., (2018), fish waste emits a lot of ammonia when it is digested, which inhibited or reduced methanogenic bacteria activities, consequently, the accumulation of VFAs and lower pH resulted in a decreased biogas yield. To enhance the digestion process, it is advised to mix organic substrates with a low ratio of C/N, such as FW, with a high C/N ratio organic waste, such as WH

(Hanghome, 2014; Madondo, 2017; Maile et al., 2016; Makhura et al., 2020; Owamah, 2010; Rabii et al., 2019; Roslina et al., 2014).

According to Nalinga and Legonda, (2016), AnCo-digestion of FW with WH has been found to increase the materials' biodegradability and biogas yield. The optimum co-digestion was found to be 25/15/95 (690mL) of substrate ratio (25g WH,75g FW), 15g IC and 95mL dilution respectively. According to Katima, (2001) maximum, biogas production was observed at less than 1mm particle size and 25 g/l of WH after 5 days of incubation. The optimum biogas yield was found at 25% maize husk (MH) and 75% food waste (FW) (w/w) during AnCo-digestion of FW and MH for producing biogas (Owamah, 2010).

At a higher concentration of water hyacinth as for the ratio 75:25/15/95 (254mL) and 92:8/10/90 (152mL) of substrate ratio(g) WH: FW , IC(g), and dilution (mL) respectively, the biogas production was reduced. This might be explained by the presence of an insufficient amount of methanogens. When there is overloading (higher ratio of the substrate), the production of organic acids increases quickly, which inhibits methanogenic bacteria activities.

This leads to the accumulation of acids, depletion of the buffer, and pH lowering thus, the production of biogas will decline and possibly stop altogether (Gooch, 2011; Jnr, 2011; Rabii et al., 2019; Shen, 2008). Also, the formation of intermediate products which are inappropriate for conversion by methanogenic bacteria to biogas may have been the cause of this inhibition (Owamah, 2010). This is because a higher ratio of the substrate may cause an acidic environment in the AD system, which leads to methanogenesis inhibition, thus decreasing biogas production

Literature shows that FW and WH are potential feedstock for biogas generation. FW was found to hasten the biodigestibility of organic material as it contains high and readily biodegradable organic matter while water hyacinth has been found to enhance biogas production as it contains a high C/N ratio. The highest biogas (690mL) and methane (68.15%) production were produced from the digester containing substrate ratio(g) WH: FW, IC(g), and dilution (mL) of 25:75/15/95 respectively. An increase in biogas production of 95.5 mL and 169 mL showed substantial enhancement of 16.1 % and 32.4 % over control means WH and FW respectively.

Therefore, nutrients (C/N ratio, etc.) balance needed by microorganisms, optimal MC, increased capacity of the buffer, and reduced effects of toxic compounds or dilution of inhibitory substance is all factors that contributed to an increased biomethane yield from AnCo-digestion of WH and FW. Co-digestion of FW and WH had a higher hydrolysis rate than mono-digestion of each feedstock.

4.2.2.3 Effect of retention time of substrate on the production of biogas

The quality and quantity of inoculums are crucial to the efficacy or performance, the time required, and stability of bio-methanogenesis for the start of an anaerobic digester, (Girmaye et al., 2019).

To decrease the anaerobic digestion period (retention time) and digester volume, it is usually preferable to use an active anaerobic inoculum (C.Akunna, 2019; Kameswari et al., 2011; Owamah, 2010). As shown in figure 4.4, there was a delayed start of water hyacinth mono-digestion. The biogas started to appear on the second day of digestion and continued up to the 20th day with almost constant daily production. This phenomenon was caused by the low inoculum concentration which leads to the increase in the time needed for the microorganisms to adapt to the conditions in the bioreactor.

This could also be due to the more availability of biodegradable material in water hyacinth (Bote et al., 2020; Chanathaworn, 2017; Marchaim, 2007).

While mono-digestion of fish waste started production early (on the first day) at a high rate and continued up to the 7th day as shown in figure 4.4. This decomposition rate is caused by the softness and the presence of a large quantity of quickly degradable organic materials in fish waste (Marchaim, 2007; Oke, 2016; Pina et al., 2018). This demonstrates how crucial the fish waste ingredient is for accelerating digestion and the creation of biogas in a fermenter. As illustrated in figure 4.4, both mono-digestion of fish waste and water hyacinth resulted in low biogas yield after the 4th and 8th days, respectively.

This is because the remaining material or residual has proven to be more resistant to hydrolysis which led to a significant fall in the rate of biogas production. This might be explained also by the presence of an insufficient amount of methanogens in the digester. Also, the formation of intermediate products which are inappropriate for conversion by methanogenic bacteria to biogas may have been the cause of this inhibition (Owamah, 2010). Hhaygwawu, (2016) and Katima, (2001) have made similar observations, an increase or decrease in the rates of fermentation product formation can be related to high or reduced microbial activity.

It can be caused also by an increased number of microorganisms and decreased amount of food available, which leads to less food being converted into biogas. Optimum biogas yield was produced, with the ratio of substrate ratio(g) WH: FW, IC (g), and dilution (mL) of 25:75/15/95, respectively showing the best performance (690mL) within 12 days of incubation. This might be explained by the presence of enough active methanogens, which decreased the time needed for the growth of methanogenic populations. The production was very high on the first days of anaerobic digestion and decreased up to zero on the 12th day of incubation as shown in figure 4.3.

This high decomposition rate was due to the increased hydrolysis rate, thus increasing material biodegradability in the co-digestion (Marchaim, 2007; Oke, 2016; Pina et al., 2018). The observed decrease in biogas production rate on the last day was attributed to the accumulation of acid and consequent lowering of pH value which in turn prevented methanogenesis or inhibit methanogenesis activity. The remaining organic material was essentially inaccessible to the microbes, which may have caused the microbial population to decline over time.

Consequently, the few microbes that survived were those that could degrade the tough materials (Katima, 2001). According to Oke, (2016), when there is a healthy or good substrate present, both bacteria and fungi multiply quickly. As a result, FW was an excellent substrate for the incubation of bacteria and fungus that was used to kick-start the biogas from the Anco-digestion of FW and WH. The biodigesters had a retention period of seven to twelve days as shown in figure 4.1.

4.2.3 Effect of IC on biogas production

As the inoculum concentration increased, a higher biogas yield (690mL) was produced, with the ratio of 25:75g (WH: FW), 15g IC and 95mL dilution. This might be explained by the presence of enough active methanogens. The results showed that the number of active methanogens increased in the biodigesters with more inoculum concentration, which decreased the time needed for the growth of the necessary number of methanogenic populations and enhanced biogas production. In general, adding enough active inoculum concentration is necessary to increase the rate of biogas production.

Previous research also supports the idea that selecting the right inoculum concentration is essential for optimizing biogas output from various substrates (Owamah, 2010). (Owamah, 2010) made similar observations, the highest possible level of biogas generation was positively impacted by an increase in inoculum concentration. IC between 10% and 40% were observed to increase biogas yield. Methane content has increased from 19 to 23% with the use of 10% inoculation as reinforcement of anaerobic digestion (Xu et al., 2019). High biogas yield was observed at 10% IC, 1cm particle size of WH, and 60% MC (Rozy, 2016).

In their experiment, water hyacinth with an inoculum concentration of 40% and a size of 1 cm produced the most biogas (Dar & Phutela, 2017b). There was low biogas produced (115 mL) at the ratio of 50:50g (WH: FW), 1.5g IC and 90mL dilution. This might be explained by the presence of an insufficient amount of methanogens. The formation of intermediate products which are inappropriate for conversion by methanogenic bacteria to biogas may have been the cause of this inhibition (Owamah, 2010). When there is overloading, the production of organic acids increases quickly, then inhibition of methanogens activity.

This leads to acid accumulation, depletion of the buffer, and pH lowering thus, the production of biogas will decline and possibly stop altogether (Gooch, 2011; Jnr, 2011; Rabii et al., 2019; Shen, 2008). At lower IC, there aren't enough bacteria present to start the methanogenesis process (Dar & Phutela, 2017a). However, it should be noted that the generation of biogas was slightly reduced (325.5 mL) as a result of the significant increase in IC for the ratio of 50:50g (WH: FW),18.4g IC and 90mL dilution.

This might have happened as a result of modifications to the substrate's characteristics, which may have had an impact on the bioavailability during hydrolysis (Owamah, 2010). The addition of the required IC in the AD process is very important as it will enhance biogas yield and methane content, speed up the process, and improve the stability of anaerobic digestion (Jnr, 2011; Madondo, 2017; Owamah, 2010; Yadvika et al., 2004).

4.2.4 Effect of Dilution on biogas production

It should be highlighted from this study that the effect of dilution was considered based on the ratios of 50:50/10/98.4 (662ml), 50:50/10/90 (646.5mL), 50:50/10/81.5 (573mL), 25:75/5/95 (434.5mL), 25:75/5/85 (329mL), 75:25/15/95 (254mL), 75:25/15/85 (250.5mL), 75:25/5/95 (249.5mL) and 75:25/5/85 (231mL) of substrate ratio(g) (WH: FW), IC (g) and dilution (mL) respectively. It has been demonstrated that dilution accelerates the production of biogas. Water will lessen the concentration of some elements, such as nitrogen and sulfur, which result in byproducts that hinder anaerobic digestion, such as ammonia and hydrogen sulfide.

According to Hhaygwawu, (2016), anaerobic digestion without or with lower water concentration produced lower biogas yield. Jnr, (2011) made a similar observation, the reactor was operated using various water dilutions of 8, 10, 12, 15, and 20 liters. The 20 L dilution produced the maximum amount of biogas (8.91–3.15 L/day), whereas the 8 L dilution produced the least amount (0.65–1.36 L/day).

The results proved that the increased dilution leads to increased material degradation, consequently improving biogas and methane yield.

4.3 The Biogas Compositions

To assess the stability of the AD system, it is important and necessary to examine the content of the biogas generated in terms of methane (CH_4) and carbon dioxide (CO_2). The substrate mixture had an impact on the production of biogas as the highest methane

(68.15%) yield was observed in the Anco-digestion of FW and WH. Methane content in mono-digestion of FW and WH was 50.12% and 55.67% respectively, as shown in Table 4.3. The results are within the range of Bote et al., (2020); Katima, (2001); Nalinga and Legonda, (2016), and Njogu et al., (2015b) where found that the CH_4 content of WH, FW, and their co-digestion was between 45.18 % to 73.3%.

Co-digestion's objectives are to balance out any imbalances or downsides of mono-digestion, enhance methane yields, improve biogas quality and yield as co-substrates supply missing nutrients, reduce toxic effects, increase organic matter, improve the system stability due to synergic effect, better C/N balance and biodegradability (Hanghome, 2014; Madondo, 2017; Maile et al., 2016; Makhura et al., 2020; Mel et al., 2015; Owamah, 2010; Rabii et al., 2019; Roslina et al., 2014).

Each digester had unique CH_4 content, and all of the tested biodigesters had a low H_2S concentration. Similar observations were reported by Chanathaworn, (2017); Jaroenpoj, (2015); Orhorhoro et al., (2017); Pina et al., (2018), and Rabii et al., (2019). Table 4.3 shows the content of biogas produced.

Table 4. 3: The Biogas Compositions

Compositions	Fish waste	Water Hyacinth	Co-digestion
CH_4 (%)	50.12	55.67	68.15
CO_2 (%)	39.72	34.40	28.85
O_2 (%)	0.34	1.10	0.15
H_2S (ppm)	235	120	105
Others (%)	8.92	8.73	2.75

According to Rabii et al., (2019), the AnCo-digestion of sugarcane press mud and food waste achieved a high methane of 82.36 mL CH4/g VS. The yield was 54% and 22% greater than that of mono-digestion of food waste and sugarcane press mud, respectively. Research on AD and co-digestion of FW was conducted by (Pina et al., 2018), and the generation of biomethane increased from 0.2-0.9 CH_4 m^3/kg VS.

Increased methane (CH_4) content in biogas production, resulted in a decreased carbon dioxide (CO_2) as shown in table 4. 3. This is because the amount of methane increased as the retention time increased while the carbon dioxide level declined at a similar pace. This might be explained that organic matter removal was related to methane production (Chanathaworn, 2017). Because the CO_2 is hazardous to humans and corrodes motors and pipes, its removal is crucial (Okonkwo et al., 2016; Rozy, 2016).

4.4 Optimization of Biogas Production

4.4.1 Statistical analysis and Model fitting

The CCD of experimental variables in the actual and coded values and experimental results of the response (Biogas yield) are shown in table 4. The model equation (eq 4.1) was obtained based on multiple regression analysis for biogas production, and yielded the following full quadratic model:

$$Y = -6129.30019 + 32.47516\,X_1 + 165.91014X_2 + 109.98071X_3 - 0.485000X_1X_2 - 0.191000\,X_1X_3 - 0.065000\,X_2X_3 - 0.157252\,X_1^2 - 6.14100\,X_2^2 - 0.523040\,X_3^2 \qquad \text{eq 4.1}$$

Where, Y: estimated Biogas Yield (response), X_1: Substrate (WH: FW) ratio, X_2: IC, and X_3: Dilution. The significant p-value must be less than 0.05 ($p < 0.05$), Any value above this is considered insignificant and can be removed.

Accordingly, the reduced model equation (eq 4.2) can be rewritten as follows:

$$Y = -6129.30019 + 32.47516\,X_1 + 165.91014X_2 + 109.98071X_3 - 0.485000X_1X_2 - 0.191000\,X_1X_3 - 0.157252\,X_1^2 - 6.14100\,X_2^2 - 0.523040\,X_3^2 \qquad \text{eq 4.2}$$

ANOVA was used for the analysis of the regression coefficient and the prediction of a quadratic equation. The experimental and predicted values from CCD are shown in table 4.4. Table 4.5 shows the analysis of variance (ANOVA).

Table 4.4: Experimental and predicted data

Std	**Run**	**Coded values**			**Actual values**			**Actual values**	**Predicted values**
		X_1	**X_2**	**X_3**	**X_1**	**X_2**	**X_3**		
13	1	0	0	α-	50	10	81.6	573	563.11
12	2	0	α+	0	50	18.41	90	325.5	322.47
14	3	0	0	2	50	10	98.41	662	657.86
11	4	0	-2	0	50	1.591	90	115	104
2	5	1	-1	-1	75	5	85	231	240.09
7	6	-1	1	1	25	15	95	690	690.83
5	7	-1	-1	1	25	5	95	434.5	442.93
4	8	1	1	-1	75	15	85	250.5	251.99
10	9	2	0	0	92.04	10	90	152	147.07
16	10	0	0	0	50	10	90	648	647.47
17	11	0	0	0	50	10	90	648.5	647.47
6	12	1	-1	1	75	5	95	249.5	251.93
9	13	-2	0	0	7.955	10	90	601	591.9
8	14	1	1	1	75	15	95	254	257.33
3	15	-1	1	-1	25	15	85	582.5	589.99
1	16	-1	-1	-1	25	5	85	329	335.59
15	17	0	0	0	50	10	90	643.5	647.47

X_1, X_2, and X_3 are the coded values of Substrate ratio, IC, and Dilution, respectively.

Table 4.5: ANOVA for response surface polynomial model

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	6.67E+05	9	74136.94	807.7	< 0.0001	significant
X_1-Substrate ratio	2.39E+05	1	2.39E+05	2602.33	< 0.0001	
X_2-IC	57612.09	1	57612.09	627.67	< 0.0001	
X_3-Dilution	10835.46	1	10835.46	118.05	< 0.0001	
X_1X_2	29403.13	1	29403.13	320.34	< 0.0001	
X_1X_3	4560.13	1	4560.13	49.68	0.0002	
X_2X_3	21.13	1	21.13	0.2302	0.646	
X_1^2	1.09E+05	1	1.09E+05	1186.38	< 0.0001	
X_2^2	2.66E+05	1	2.66E+05	2894.89	< 0.0001	
X_3^2	1927.55	1	1927.55	21	0.0025	
Residual	642.51	7	91.79			
Lack of Fit	627.34	5	125.47	16.55	0.058	not significant
Pure Error	15.17	2	7.58			
Cor Total	6.68E+05	16				

$R^2 = 0.999$
Adjusted $R^2 = 0.9978$
Predicted $R^2 = 0.9928$
Adeq Precision = 79.8627
C.V = 2.2

The linear model terms X_1, X_2, X_3, interactive model term X_1X_2, X_1X_3, and quadratic model term X_1^2, X_2^2, and X_3^2 are significant ($P<0.05$), whereas interactive model terms, X_2X_3 was not significant ($P>0.05$). As illustrated in table 4.5, the model *F*-value of 807.70 implies that the model is significant. There is only a 0.01% chance that an *F*-value this large could occur due to noise. P- values less than 0.0500 indicate model terms are significant. In this case, X_1, X_2, X_3, X_1X_2, X_1X_3, X_1^2, X_2^2, and X_3^2 are significant model terms. Model terms are not significant if the value is higher than 0.1000. The P-Value measures the relevance of each variable; the lower the P-Value, the greater the significance of that particular variable.

The model terms are significant if the P-Value is less than 0.05 (Shin et al., 2019). The lack-of-fit F-value of 16.55 implies the lack-of-fit is not significant relative to the pure error. There is a 5.80% chance that a lack-of-fit F-value this large could occur due to noise. The p-value for lack-of-fit was greater than 0.05, and therefore, it was not significant. The correction coefficient R^2 0.9990 was used to show how well the model fits the data. The high R^2 of 0.9990 indicates that the relationship between the response and the biogas production factors (substrate ratio, IC, and dilution) is roughly linear and that the model could account for 99.99% of the response variability.

A strong model fit is indicated by an R^2 value between 0.75 and 1.0 for a good statistical model. The quadratic equation could be used to obtain a precise estimate for biogas production because of the high value of R^2 (Shahidul et al., 2018). According to (Chanathaworn, 2017), the adjusted R^2 of 0.9978 indicate that the response surface model created for this study's biogas prediction was completely appropriate. A value greater than 4 is desirable for the "Adeq precision," which measures the signal-to-noise ratio, the ratio of 79.8627 from this study indicated an adequate signal.

The Predicted R^2 value of 0.9928 showed a good agreement between the predicted and observed values as shown in figure 4.6. The low CV (coefficient of variation) of 2.20 showed the high reliability and precision of experimental outcomes. The trustworthiness of experimental results decreases with an increased coefficient of variance (C.V) (Roslina et al., 2014; Sathish, S., 2011). The experimental biogas production results were close to the predicted results as shown in figure 4.6.

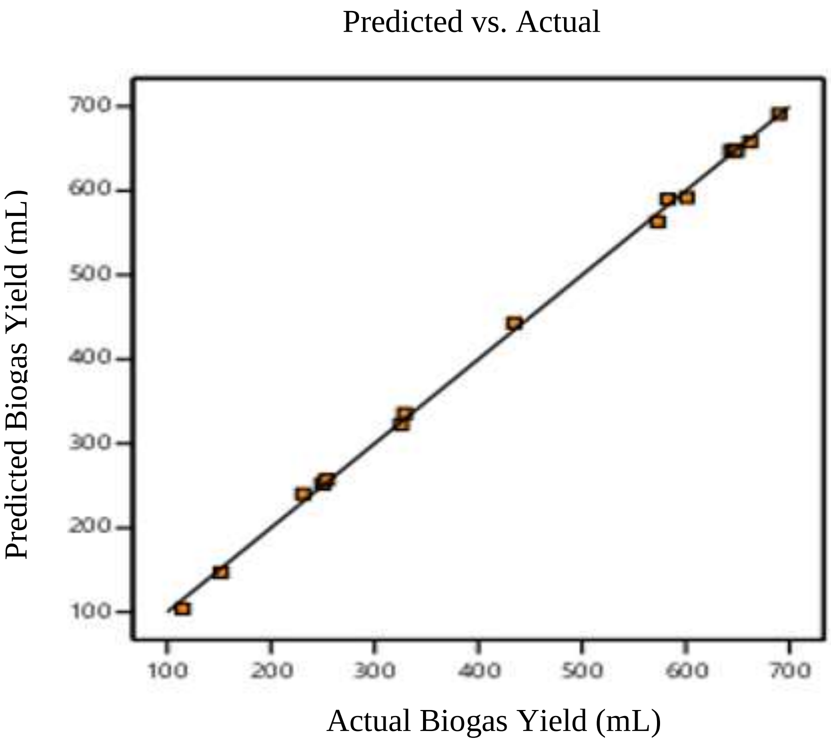


Figure 4.6: Plot of predicted response vs. actual value from response surface

4.4.2 Analysis of response surfaces plots

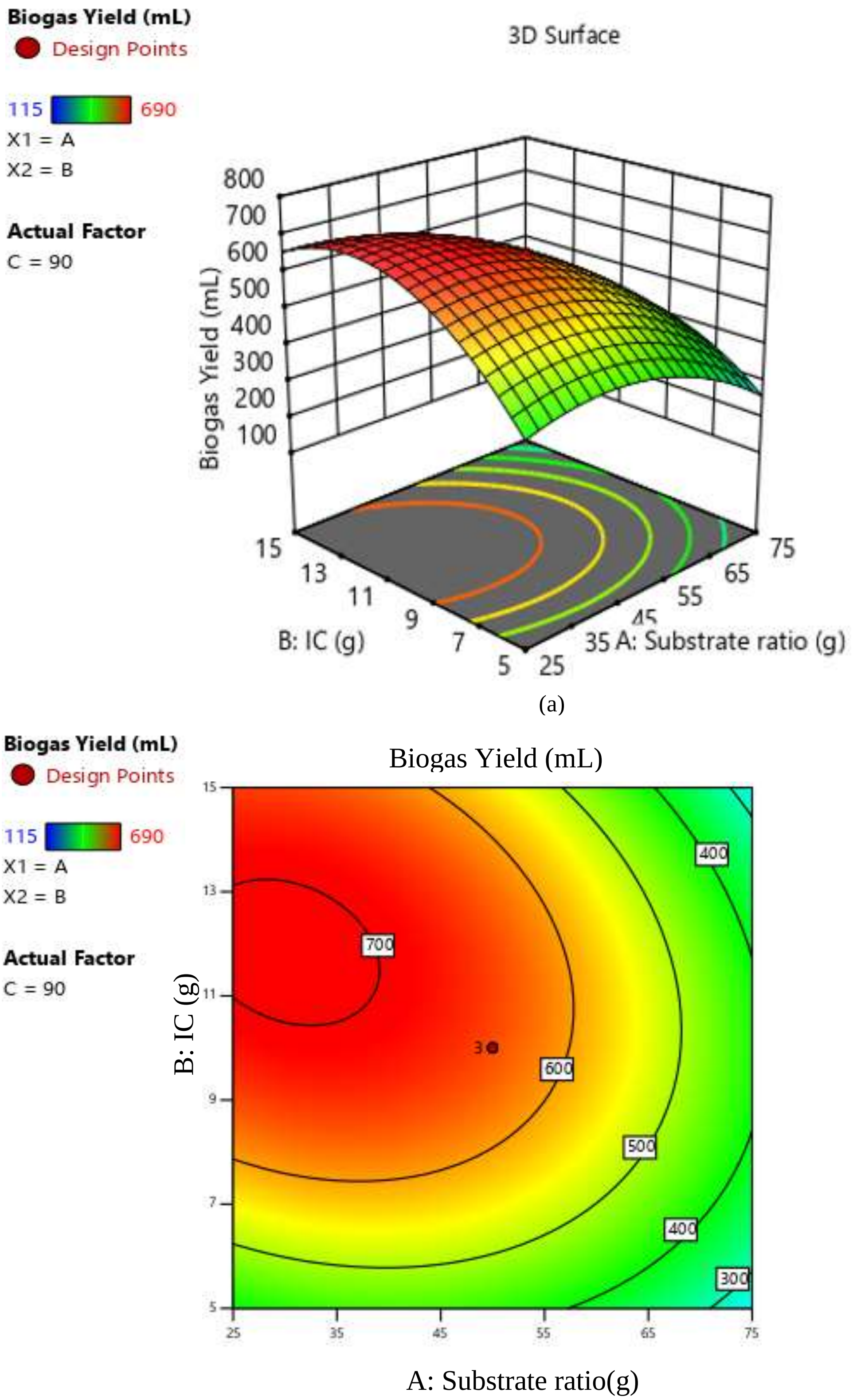
The 2D (two-dimensional) contour and 3D (three-dimensional) response surfaces plots for biogas production optimization were represented using the polynomial model equation 3.5 to show the interaction effect of biogas production variables on the biogas yield. Figure 4.7(a) and (b), figure 4.8 (a) and (b), and figure 4.9(a) and (b) all display the 3D and 2D surface response plots.

4.2.2.1 Effect of substrate ratio and IC on the production of biogas

Effect and relationship between substrate ratio and inoculum concentration on biogas produced were determined as shown in figure 4.7(a) and (b). Biogas yield increased to its maximum when the IC increased. However, biogas production decreased when the substrate ratio increased as shown in figure 4.7 (a) and (b). ANOVA showed that the interaction effect between substrate ratio and IC on biogas production was significant ($P > 0.05$) as shown in table 4.5.

Biogas production increased when the substrate ratio (WH: FW) was 25:75g, however, when the substrate ratio (WH: FW) exceeds 25:75g, respectively the biogas production decreased rapidly as shown in figure 4.7 and also when the substrate ratio (WH: FW) was less than 25:75g, respectively a slight or very little inhibition was observed on the response surface plot. This might be explained by the presence of an insufficient amount of methanogens. This inhibition was due to the formation of intermediate products which are inappropriate for conversion by methanogenic bacteria to biogas and when there is overloading, the production of organic acids increases quickly, then inhibition of methanogens activity (Owamah, 2010).

Similar findings were reported by Gooch, (2011); Jnr, (2011); Rabii et al., (2019), and Shen, (2008), overloading caused microbial activity to be inhibited, which decreased the rate of biogas generation. The optimum biogas production of 690mL with the methane content of 68.15% was obtained at 25:75g of substrate ratio (WH: FW) when IC and dilution were 15g and 95mL, respectively.



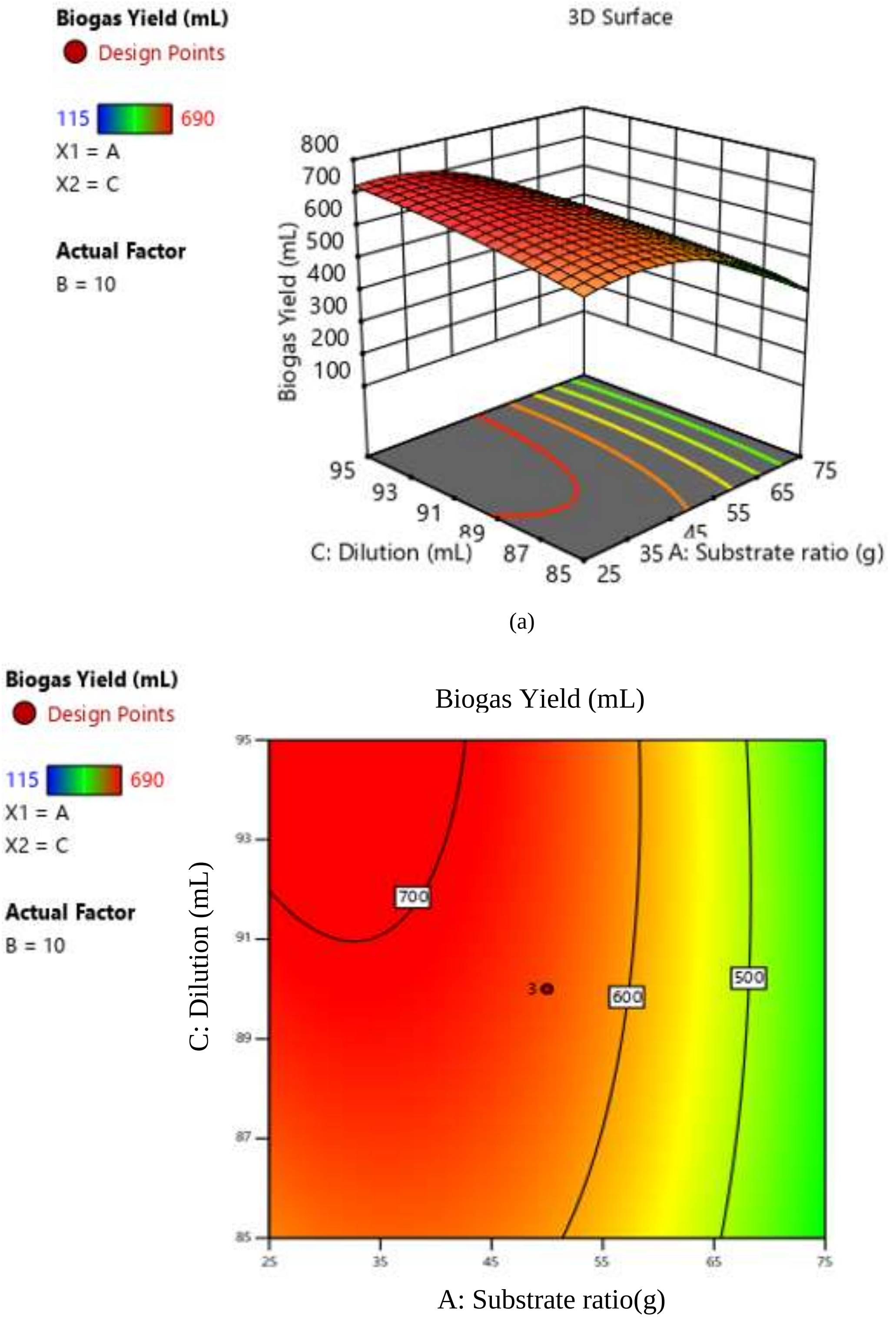
(a)

(b)

Figure 4.7: Effect of substrate ratio and IC on biogas production: (a) response surface and (b) contour plot

4.2.2.2 Effect of substrate ratio and dilution on biogas production

The relationship between substrate ratio and dilution in biogas production is shown in figure 4.8 (a) and (b). The results revealed that the interactive effect of substrate ratio and dilution on biogas production is significant ($P < 0.05$) as shown in table 4.5. The biogas production increased as dilution increased and decreased when the substrate ratio increased. This is because a higher ratio of the substrate may cause an acidic environment in the AD system, which leads to methanogenesis inhibition, thus decreasing biogas production (Gooch, 2011; Jnr, 2011; Rabii et al., 2019; Shen, 2008). The optimum dilution for biogas production was 95mL which obtained the highest biogas production when substrate ratio (WH: FW) and IC were 25:75 g and 15 g, respectively.



(a)

(b)

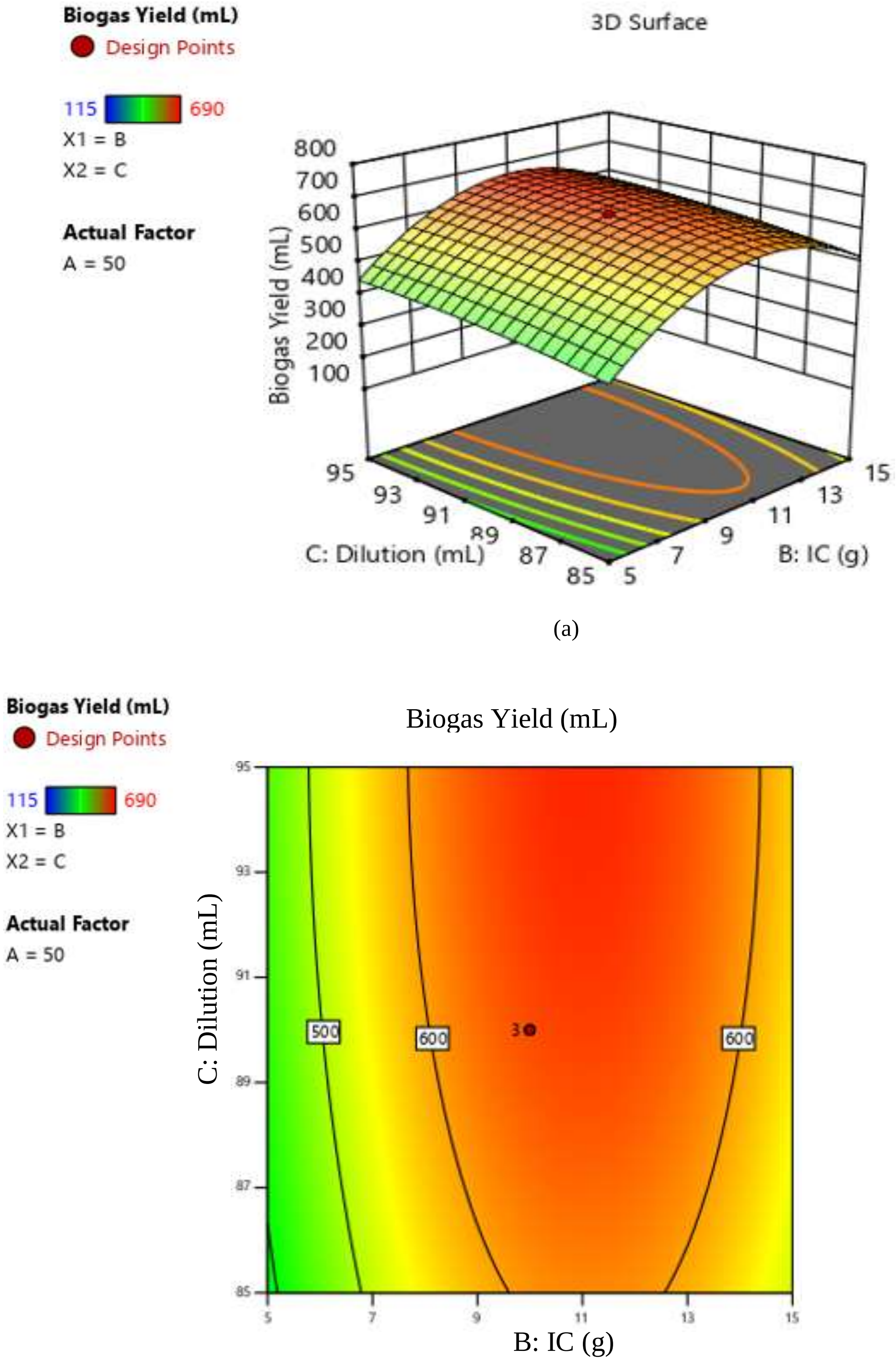
Figure 4. 8: Effect of substrate ratio and dilution on biogas production: (a) response surface and (b) contour plot

4.2.2.3 Effect of IC and dilution on the production of biogas

The interaction effect of IC and dilution on biogas yield was insignificant ($P > 0.05$) as shown in table 4.5. However, ANOVA indicated that the quadratic and linear terms of IC and dilution were significant ($P < 0.05$) as shown in table 4.5. Figure 4.9 (a) and (b) show the relationship between IC and dilution in biogas generation. Biogas production was higher when IC was 11g, however, when IC was less than 11g the biogas production decreased rapidly, and also when IC exceeds 11g a slight inhibition was observed as shown in figure 4.9.

The biogas yield was very low when the IC was 1.5 % and 18.4%. This might be explained by the presence of an insufficient amount of methanogens. Similar observation were reported by Dar & Phutela, (2017a), at lower IC, there aren't enough bacteria present to start the methanogenesis. The results agree with Filer et al., (2019) and Girmaye et al., (2019), the low inoculum concentration in the reactor could result in the microorganisms' low metabolic activity which leads to inhibition of the methanogenesis process resulting in low biogas yield.

It was noted that the generation of biogas was slightly reduced as a result of the significant increase in IC (18.4%). This might have happened as a result of modifications to the substrate's characteristics, which may have had an impact on the bioavailability during hydrolysis (Owamah, 2010). The addition of the required IC in the AD process is very important as it will enhance biogas yield and methane content, speed up the process, and improve the stability of anaerobic digestion (Jnr, 2011; Madondo, 2017; Owamah, 2010; Yadvika et al., 2004).



(a)

(b)

Figure 4. 9: Effect of IC and dilution on the production of biogas: (a) response surface and (b) contour plot

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusion

The substrates were examined for their initial MC, pH, TS, C /N ratio, VS, and ash content before digestion. The substrates characterization results were 61.37, 94.4, and 89.67 % for MC, 38.21, 5.6, and 10.33% TS, 99.48, 83.3 and 74.9% VS, 5.89, 21.35, and 23.47 for C/N, and 6.5, 7.2 and 6.8 for pH for fish waste, water hyacinth, and inoculum respectively. The C/N ratio of FW was 5.89 which was out of range for the accepted C/N ratio (20-30:1) of anaerobic digestion (AD).

However, the C/N ratio of WH and inoculum were 21.35 and 23.47 respectively which are in the good range for AD system stability. Nevertheless, the VS of fish waste was high which was good for this substrate to be easily digested as the sign of producing biogas. Therefore, the results obtained from this study showed that FW and WH exhibited characteristics indicating that they are a potential substrates for biogas generation.

AnCo-digestion of FW and WH feedstock has been shown to enhance biomethane yield.

The optimum values for maximum biogas yield of 690mL with the highest methane yield of 68.15% were found to be substrate ratio (WH: FW),25:75g, 15g of IC, and 95 mL for dilution. The yield was 16.1% and 32.4% greater than WH and FW mono-digestion, respectively. Therefore, nutrients (C/N ratio, etc.) balance, increased biodegradability and capacity of the buffer, and reduced effects of toxic compounds or dilution of inhibitory substance are all factors that contributed to an increased biomethane yield from AnCo-digestion of FW and WH.

The AnCo-digestion of FW and WH had a higher hydrolysis rate than the mono-digestion of each feedstock. WH has been found to improve the biogas yield from AnCo-digestion of FW and WH as it contains a high C/N ratio while FW was an excellent substrate for the incubation of bacteria and fungus that was used to kick-start the biogas production from the AnCo-digestion of FW and WH. The biodigesters had a retention period of six to twelve days. This work investigated the effects of IC, substrate ratio, and dilution on biogas yield to identify the optimal condition.

The biogas yield was expressed as function of operating variables using a quadratic equation. The model was significant ($P<0.05$). All factors had significant linear and quadratic effects on biogas while only the interaction effects of the two factors were significant. The coefficient of determination(R^2) of 99.9% confirms the good fit of the model with experimental variables. Optimum values for RSM were within the range of experimental results. Biogas yield decreased as substrate ratio increased. According to the high value of R^2, the model could be effectively utilized for the prediction of biogas generation from AnCo-digestion of FW and WH.

Design Expert 13 software, which contains CCD, ANOVA, and RSM was used to determine the level of variable inputs and establish the optimum number of experimental runs to maximize biogas yield in Anco-digestion of FW and WH. ANOVA was used for the analysis of the regression coefficient and the prediction of the model and to show how the variables interacted. The polynomial equation was illustrated in three dimensions using response surface plots. For the surface response analysis, RSM was used to evaluate the interaction between biogas production parameters and the biogas yield and to estimate the optimum surface area and optimal values for the production of biogas.

In conclusion, FW and WH were potential substrates for biogas generation. AnCo-digestion of FW and WH feedstock has been shown to enhance biomethane yield. Results revealed that the model was significant ($P<0.05$). All factors were found to have linear and quadratic significant effects on biogas while the interaction effects of the two factors were significant. Biogas yield decreased as substrate ratio increased.

5.2 Recommendations for Further Research

FW had a lower C/N ratio, further study needs to consider co-digestion with other higher C/N ratio substrates. Because the CO_2 is hazardous to humans and corrodes motors and pipes, its removal is crucial. The biogas was not upgraded, research is still needed in purifying or upgrading the biogas for CO_2 removal and improved methane content to be used directly for cooking or as fuel for vehicles.

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APPENDICES

Appendix: The Average Daily Biogas Yield

	The average daily biogas production (mL)														
Days	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Run 6**	**Run 7**	**Run 8**	**Run 9**	**Run 10**	**Run 11**	**Run 12**	**Run 13**	**Run 14**	**Run 15**
1	154	123	89	82.5	133	115	144	82.5	72.5	52.5	49	99	132	133.5	79
2	140	106	56	66	126.5	104	129.5	66	56	32	25	83	98	116.5	65
3	100	93.5	47	28.5	104	92.5	92	28.5	34	23.5	13	67	71.5	93.5	51
4	84	76	25	22	80	76	81	22	20	15	12	40	55	75	46
5	76.5	69.5	13	17	71	67	78.5	17	16	13	9	18	36	61	37
6	55	43	9	15.5	43	48	51	15.5	14.5	10	7	12	21	43	28
7	34.5	25	7	7	34.5	24	29.5	7	7	3	0	5	12	32.5	13
8	21.5	23	5	5	27.6	23	31.5	6	5	0	0	3	5	29	3.5
9	14	12	3	4	12	12	14	2	3	3	0	2	3	8	3
10	5	8.5	0	0	8	8.5	5	1	0	0	0	0	2	4	0
11	3.5	3	0	3	4	3	3.5	2	3	0	0	0	0	3	0
12	2.5	0	0	0	3	0	2.5	0	0	0	0	0	0	2	0
13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TOTAL	**690**	**582.5**	**254**	**251**	**646.6**	**573**	**662**	**249.5**	**231**	**152**	**115**	**329**	**436**	**601**	**326**