Amylase's Impact on Co-Digestion for Enhanced Biogas Production from Diverse Food Waste and Napier Grass

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DECLARATION

I declare that this thesis entitled "Amylase play a role between the Co-Digestion Process of Biogas Production from two different food waste with Napier Grass" is the results of my own research except as cited in the reference.

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ABSTRACT

The aim of this thesis is develop a scalable laboratory model to generate sustainable and renewable household energy from organic waste. Production of biogas can utilize high-quality organic fertilizer to improve soil properties, simultaneously reducing pollution from banana waste, onion waste, and Napier grass. This experiment highlight the pressing environmental concern of inefficient waste management in Malaysia due to increasing waste production and inadequate infrastructure and policies. In order to produce methane, the project intends employ Napier grass, onion waste, and banana waste as substrates to produce methane as fuel to make heat and light. Employ a pH meter and a drying oven to measure dry weight, total solids, and pH levels of onion waste, Napier grass, and banana waste substrates. Utilize the HABOTEST Gas Leak Detector HT601B to detect methane presence in the samples, recording a safe 20% LEL (Lower Explosive Limit) or 9999 ppm. The pH values of onion waste, Napier grass, and banana waste substrates (5.9, 6.0, and 5.2, respectively) fall within the ideal range for efficient methane production. Additionally, investigate two substrate combinations: Onion Waste mixed with Napier Grass (OW: NP) and Banana Waste mixed with Napier Grass (BW: NG), with varying ratios before and after the anaerobic codigestion process. The ratios of the mixes both prior to and following the anaerobic codigestion process are different. Isolate Pseudomonas aeruginosa, Bacillus subtilis, and Escherichia coli bacteria from onion waste, Napier grass, and banana waste, respectively, using the streaking and spread culture method on nutrient agar. Report a methane gas detector reading of 20% LEL (Lower Explosive Limit) or 9999 ppm, indicating successful methane production.

Keywords: onion waste, banana waste, Napier grass, methane gas, methane gas detector, anaerobic codigestion process

ABSTRAK

Matlamat tesis ini adalah untuk membangunkan model makmal berskala untuk menjana tenaga isi rumah yang mampan dan boleh diperbaharui daripada sisa organik. Pengeluaran biogas boleh menggunakan baja organik berkualiti tinggi untuk memperbaiki sifat tanah, sekaligus mengurangkan pencemaran daripada sisa pisang, sisa bawang, dan rumput Napier. Eksperimen ini menyerlahkan kebimbangan alam sekitar yang mendesak terhadap pengurusan sisa yang tidak cekap di Malaysia disebabkan peningkatan pengeluaran sisa dan infrastruktur dan dasar yang tidak mencukupi. Untuk menghasilkan metana, projek ini menggunakan rumput Napier, sisa bawang, dan sisa pisang sebagai substrat untuk menghasilkan metana sebagai bahan api untuk membuat haba dan cahaya. Dengan menggunakan meter pH dan Ketuhar pengeringan termostatik elektrik untuk mengukur berat kering, jumlah pepejal dan tahap pH sisa bawang, rumput Napier dan substrat sisa pisang. Pengesan Kebocoran Gas HABOTEST HT601B digunakan untuk mengesan kehadiran metana dalam sampel, merekodkan LEL (Had Letupan Rendah) selamat 20% atau 9999 ppm. Nilai pH sisa bawang, rumput Napier dan substrat sisa pisang (5.9, 6.0 dan 5.2 masing-masing) berada dalam julat ideal untuk pengeluaran metana yang cekap. Selain itu, siasat dua kombinasi substrat: Sisa Bawang dicampur dengan Rumput Napier (OW: NP) dan Sisa Pisang dicampur dengan Rumput Napier (BW: NG), dengan nisbah yang berbeza-beza sebelum dan selepas proses penghadaman anaerobik. Nisbah campuran sebelum dan selepas proses penghadaman anaerobik adalah berbeza. Bakteria Pseudomonas aeruginosa, Bacillus subtilis, dan Escherichia coli daripada sisa bawang, rumput Napier, dan sisa pisang, masing-masing menggunakan kaedah kultur coretan dan taburan pada agar nutrien. Laporkan bacaan pengesan gas metana sebanyak 20% LEL (Had Letupan Rendah) atau 9999 ppm, menunjukkan pengeluaran metana berjaya.

Kata kunci: sisa bawang, sisa pisang, rumput Napier, gas metana, pengesan gas metana, penghadaman anaerobik

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AcoD	Anaerobic co-digestion	9
AD	Anaerobic digestion	10
BW	Banana waste	23
CH_4	Methane	10
CO_2	Carbon dioxide	2
H_2	Hydrogen	12
H_2S	Hydrogen sulphide	12
MSW	Municipal solid waste	12
N_2	Nitrogen	12
NH_3	Ammonia	12
NG	Napier grass	15
OW	Onion waste	15
TS	Total solids	22
WTE	Waste-to-energy	1

LIST OF SYMBOLS

%	Percentage
°C	Degree Celsius
g	Gram
L	Litre
MJ/m3	megajoules per cubic metre

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Global energy consumption has increased in lockstep with economic expansion, putting a strain on the world's supply of renewable energy sources. Municipal solid waste (MSW) has been claimed to have made significant contributions to the enhancement of a secure environment and renewable sources. In developing countries, energy scarcity and traditional MSW disposal methods cause a slew of environmental and economic problems. In light of this condition, scientists have been able to experiment with several waste-to energy conversion devices. This publication highlights and evaluates waste-to-energy (WTE) methods for converting MSW and other feedstock's into power, hydrogen gas, bioethanol, and other value-added goods such as fertilizer(s), platform chemicals, and other environmentally beneficial products. (Vyas et al., 2022)

Agricultural wastes such as leaves, plant parts, and dead plants account for a substantial portion of farm waste. (Ayilara et al., 2020) Nutrients, pesticides, and other pollutants in agricultural waste can leak into waterways and damage water supplies. This can result in hazardous algal blooms, fish fatalities, and other severe consequences for aquatic environments. Improper agricultural waste disposal can cause soil deterioration and erosion, reducing soil fertility and productivity over time. Left to degrade in the open air or in landfills, agricultural waste can emit methane and other greenhouse gases into the environment, contributing to climate change. However, burning agricultural waste also can cause particulate matter and other pollutants to be released into the air, which can harm both human health and the environment. Moreover, Napier grass (Pennisetum purpureum) is a popular energy crop for livestock due to its high nutrient content, including carbohydrates and protein. It is also easy to cultivate. Napier grass can absorb

carbon dioxide and has a high yield. As a result, Napier grass was an appealing candidate for biogas production.

Food waste is defined as any food that is discarded or lost throughout the food supply chain, from manufacturing to consumption. According to the Food and Agriculture Organization (FAO) of the United Nations, almost one-third of all food produced for human use is lost or wasted worldwide. This equates to around 1.3 billion tons of food waste per year. (Global Food Losses and Food Waste, n.d.) Food is wasted at many points of the supply chain in wealthy countries, but a large amount of food waste in nations that are underdeveloped happens during the post-harvest and processing stages due to inadequate infrastructure and storage facilities. Food waste exacerbates food insecurity and hunger by wasting resources that could potentially be used to feed people. Collection of food waste for anaerobic treatment thus provides a waste feedstock for the production of renewable biogas and biofertilizer while eliminating emissions that would otherwise occur during conventional disposal. (Westerholm et al., 2020)

Anaerobic digestion (AD) systems are intended to capture and convert biogas produced during this breakdown into electricity. It is a biological process, with four stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. The first phase is hydrolysis, which is the conversion of organic material into biogas. During this stage, some bacteria break down organic polymers such as carbohydrates into simple sugars, allowing the next set of bacteria to digest the material. Acidogenesis was the second step in the conversion of organic matter to biogas. During this stage, acidogenic bacteria convert simple sugars and amino acids into carbon dioxide, hydrogen, ammonia, and organic acids. In the third stage, acetogenic bacteria convert organic acids to acetic acid, carbon dioxide, and hydrogen. This process is known as acetogenesis. The final phase is called methanogenesis, which is the conversion of organic resources into biogas. In this stage, single-celled organisms known as methanogens transform the intermediate products produced in previous stages into biogas (mainly methane and CO_2). The solid and liquid waste from this process, digestate, contains substances that microorganisms cannot consume as well as dead bacteria. Anaerobic digestion can process a variety of feedstock's, including manure, municipal wastewater, industrial wastewater, municipal solid waste (MSW), fats, oils, and greases, food scraps, and sludge.

A potential substitute for fossil fuels might originate from the biomass, a naturally occurring non-fossil organic substance having intrinsic chemical energy and the ability to reduce emissions from fossil fuels. The diverse components that make up biomass resources derived from agriculture, forestry, and urban waste include wood, crop leftovers, sawdust, straw, manure, paper waste, household wastes, and wastewater. (Antar et al., 2021)

Utilising renewable energy may both ensure that energy needs are met and simultaneously reduce environmental problems like greenhouse gas emissions. One such renewable energy source that could reduce the need for fossil fuels is biogas. Malaysia's energy supply is mostly derived from fossil fuels, which account for 78.0% of the total energy source. Water power and renewable energy sources make up 18.0% and 4.0% of the energy source, respectively. Anaerobic digestion (AD) of solid and liquid biowastes has the potential to produce biogas. Municipal solid waste (MSW), garden waste (GW), landfills, and solid food waste (FW) are examples of solid biowastes; liquid biowastes include animal manure, sewage sludge (SS), palm oil mill effluent (POME), and liquid FW. AD is a biological degradation process that occurs when a broad range of microorganisms break down organic materials in the absence of oxygen or a potent oxidising agent. The primary byproduct of the AD process is biogas, a colourless, odourless gas that is mostly composed of carbon dioxide (30–50%) and methane (50–70%). (Lim et al., 2021) Anaerobic co-digestion (AcoD) is the idea of co-digestion to increase the methane yield utilising various combinations of substrates; it may produce the best synergisms in the biodigester. To increase the methane yield, a variety of studies have been conducted on the AcoD of lignocellulosic feedstocks with other distinct organic biomasses, including animal manure, food waste, aquatic plants, and algal biomass. (Ibro et al., 2022)

Biogas is a promising renewable energy source produced by anaerobic digestion of organic waste materials. Biogas production has the potential to provide a long-term waste management solution while also lowering greenhouse gas emissions and contributing to energy security. Anaerobic co-digestion is an ongoing research topic that tries to improve biogas production efficiency and efficacy by integrating different types of organic waste materials in a single digester. However, there are certain potential environmental consequences of biogas generation that must be properly regulated, such as water contamination and habitat degradation. Overall, continuing research and development in the field of biogas generation will be critical to realising the full potential of this renewable energy source.

Methane and CO_2 contents of biogas range from 30 to 70 percent, depending on the substrate fed into the digester. Small amounts of hydrogen are among the other ingredients in biogas. Heating, lighting, cooking, and the creation of electricity can all be done with the typical heating value of 21–24 MJ/m^3 or 6 kWh/m^3, while a big biomethanation plant can provide enriched biogas into gas supply networks. (Kabeyi & Olanrewaju, 2022)

1.2 Problem Statement

The population of Malaysia is expected to reach 32.8 million in 2021, and as a result, there will be an enormous amount of solid waste produced, amounting to an estimated 38,427 metric tonnes per day (1.17 kg/capita/day). (Shahril, 2022) Due to the growing amount of trash produced and the lack of enough infrastructure and policies to manage it efficiently, waste management in Malaysia is a significant environmental issue. Municipal solid waste (MSW), industrial waste, hazardous waste, and garbage from building and demolition are the main categories of waste produced in Malaysia. MSW, such as food waste, grass, agricultural residues, animal manure, and sewage sludge, can emit harmful greenhouse gases such as methane and contribute to climate change if not managed properly. Additionally, the disposal of MSW in landfills can result in the production of leachate, which can contaminate groundwater and soil. However, MSW is also a significant resource that used to produce biogas, which has the potential to offer renewable energy while reducing greenhouse gas emissions and minimizing environmental degradation.

Some farmers must burn Napier grass in order to clear land or lessen competition for resources such as sunlight, water, and nutrients, which can benefit other plants on the farm. Grass burning can have significant environmental consequences, such as air pollution and greenhouse gas emissions. Furthermore, fire can deplete soil fertility by destroying organic matter. Composting or utilizing grass waste for biogas production may give more sustainable alternatives that benefit the environment while also improving agricultural output. Napier grass has the potential to be a valuable resource in agriculture for renewable energy generation and waste management.

1.3 Objectives

The main objectives of this research are:

1. To study the quantity and quality of onion waste and banana waste with Napier grass used in the anaerobic co-digestion process.

2. To produce the pH, ratio, dry weight, and percentage of total solids before and after the codigestion process.

3. To identify scientific data on the identification of microbial in onion waste and banana waste with Napier grass.

1.4 Scope of Study

The main objective of the study is to determine how Napier grass, onion waste and banana waste can produce methane gas. The quality and quantity of biogas produced depend on the feedstock selection, which is crucial for biogas production. Studies have looked into the use of Napier grass and food waste which is onion waste and banana waste, three different forms of organic waste. The production of biogas and anaerobic co-digestion depend heavily on the microbial identification in these experiments. The efficiency and stability of biogas production from waste can be increased by optimizing the process parameters of pH, ratio, dry weight, and percentage of total solids before and after the co-digestion process. Areas of study geared on optimizing this procedure for waste management and renewable energy production.

1.5 Significances of Study

Proper waste management practices can aid in environmental protection by reducing pollution, conserving natural resources, and reducing greenhouse gas emissions. Recycling and composting are waste management practices that can help to conserve natural resources by minimizing the need for new raw materials and increasing the lifespan of current resources. This

research aids in the identification of methods to optimize the production process and boost the efficiency of biogas generation, which can contribute to the development of a more sustainable energy system. It also aids in identifying solutions to lessen the environmental implications of garbage disposal, such as climate change. To reduce trash in Malaysia, the study used Napier grass and food waste which is banana waste and onion waste as feedstock for anaerobic co-digestion utilizing a digester. The co-digestion technique was used in this experiment because it increased the amount of biogas produced, stabilized the anaerobic digestion process by balancing the nutrient content and pH levels within the digester, and provided a higher-quality digestate.

The residue of Napier grass biogas generation, known as digestate, is a nutrient-rich fertiliser that can be utilised in agriculture. Biogas research can assist uncover strategies to improve digestate quality and quantity for agricultural usage, which can lead to improved soil health and crop yields. Biogas has significant potential benefits for renewable energy production, waste management, and climate change mitigation and agricultural sustainability. Additionally, methane emissions from landfills and manure lagoons that would have otherwise leaked are reduced by the use of biogas. By turning this methane into CO_2, which has a global warming potential of up to 34 times less than methane, using it as fuel significantly lessens its impact on the climate.

CHAPTER 2

LITERATURE REVIEW

2.1 FEEDSTOCK

2.1.1 Napier grass

Napier grass is a perennial grass with rapid growth that is extensively grown in tropical and subtropical climates worldwide. It is native to Sub-Saharan Africa. This adaptable forage crop is mostly utilised in harvest and carry systems to feed cattle. Pennisetum purpureum (PP) and elephant grass, another name for Napier grass, is composed of 46% cellulose, 34% hemicellulose, and 20% lignin in its fibres. It also needs very little in the way of nutrients to flourish. Most investigations used an alkaline treatment, employing sodium hydroxide (NaOH) (4–20%) and 1-2 hours of continuous stirring, to remove contaminants and non-cellulosic debris. After treating the fibres, they were rinsed with distilled water until the pH was balanced, and they were then left to dry overnight at 50°C in an oven. (Radakisnin et al., 2020)

Napier belongs to the Poaceae family and is a C4 perennial grass. It is a very drought-tolerant grass that may reach heights of up to 7.5 m and a penetration depth of up to 4.5 m due to its wide root system. Its leaf blades are long (up to 120 cm) and wide (up to 5 cm), with a thick stem around the base (3 cm in diameter). Compared to other C4 plants, it can sustain radiation use efficiency for a longer period of time due to its robust tillering, big leaf area, high solar radiation interception and radiation use efficiency, towering canopy, and high photosynthetic rate. The average tiller per plant is 35 to 100, depending on season and variety. According to Napier's leaf-to-stem ratio (L: S), dwarf cultivars have more leaves than stems. It can grow in some shade, but it thrives in direct sunlight. (Islam et al., 2023)

Napier grass is well-known for growing quickly and producing a lot of biomass. It is a valuable feedstock for the production of biogas since it may yield significant amounts of biomass per unit area. Anaerobic digestion, which turns organic materials into biogas, has been shown to yield high methane potential from Napier grass. Because grass has a fibrous texture, it breaks down organic matter more easily and releases methane gas, which can be trapped and used as a renewable energy source. It has a comparatively high percentage of organic matter and readily digested carbohydrates, both of which can be effectively transformed into methane during anaerobic digestion. The production of biogas from Napier grass can support waste management and sustainable farming methods.

2.1.2 Onion Waste

The onion, or Allium cepa, is one of the most widely planted plants in the genus Allium and a member of the Amaryllidaceae family. Many chemical substances, including diallyl disulphide and diallyl trisulphide, as well as allicin, quercetin, and fisetin, are found in onions. At certain dosages, onions and their constituent parts have demonstrated numerous health benefits, such as antioxidant and free-radical scavenging qualities, anticholesterolemic, anti-heavy metal toxicity, antihyperuricemia, antibacterial, anti-gastric ulcer, and anticancer effects.

Sugar, carbs, water, proteins, vitamins, fibre, potassium, vitamin C, vitamin B6, and a trace quantity of the mineral schromium are all present in A. cepa. Onions need special growing conditions to thrive, such as loamy, stone-free soil, sunlight, good drainage, and well-irrigated soil. A large amount of nitrogen, phosphate, and potassium are needed for the highest quality product. Temperature can have a significant impact on an onion's nutritional value as hotter weather produces more sulphur and pungent flavour. Other factors that affect onion development include growing onions in dry conditions intensifies their pungent flavours. (Deepthi & Lakshmi, 2021)

The portions of an onion plant that are thrown away or not utilised for processing or consuming are referred to as onion waste. Onion skins, roots, trimmings, and any other portions of the onion that are not usually consumed or used might all be considered waste. Even though these pieces are frequently thrown away as waste, they might have additional uses, like composting, making animal feed, or industrial processes like the creation of biofuel, biogas. The organic materials included in onion waste, such as proteins, lipids, and carbohydrates, can act as substrates for microbes that produce biogas. Consequently, anaerobic digesters that produce biogas can employ onion waste as a feedstock. Improving efforts to

reduce onion waste are being spearheaded by the desire to avoid food waste and environmental concerns. The residual onion waste can be used to make biogas. Due to the high sugar content of onion waste and the presence of metals that boost volatile fatty acids and biogas production, the breakdown of onion residuals can improve CH4 yield to 0.38–0.69 L CH4/g VS. (Tawfik et al., 2022)

Onion waste contains antimicrobial elements that may inhibit the development and activity of the bacteria that produce biogas, decreasing the process's effectiveness. Additionally, the inhibitory effects can be lessened and the overall biogas generation can be increased by combining onion waste with other organic materials that do not contain antibacterial chemicals. The majority of antibiotics have the ability to prevent anaerobic digestion from producing methane. (Wu et al., 2022) Several studies have revealed the antibacterial effects of onion peel. Compared to Gram-negative bacteria (Escherichia coli and Pseudomonas aeruginosa), it has been observed that Gram-positive bacteria (Bacillus cereus and Staphylococcus aureus) were more susceptible to the antibacterial chemicals present in onion peel. Furthermore, an antibacterial panel of enteropathogenic microorganisms was exposed to onion peel extracts. (Joković et al., 2024) the high antibiotic concentrations may have altered the fate of ARGs by introducing them into the AcoD system. (Wang et al., 2022)

2.1.3 Banana Waste

An alternative energy source that can take the place of fossil fuels is biogas. A source of an organic material with a high cellulose, lignocellulose, and lignin content, agricultural residue in the form of fruit and vegetable waste has the potential to be used as a raw material for the manufacture of biogas. (Radware Bot Manager Captcha, n.d.)

Worldwide, banana cultivation is quite prevalent. Of the world's total banana production, India accounts for 14.37%. An acre of banana plantation yields about 220 tonnes of garbage every year. When these bio-wastes are incorrectly disposed of near ponds, rivers, and on land, major health risks arise. Due to its widespread availability, banana waste has recently attracted attention as an alternative energy source. Additionally, a large area for the production of alternative energy has been made possible by the use of biomass and agricultural waste in the creation of bioenergy. The process of converting biowaste into electricity has the potential to significantly reduce greenhouse gas emissions while also opening up job opportunities in rural areas where agriculture is the primary source of income. In order to meet the world's energy needs, banana waste material has been widely used in the manufacturing of ethanol, biogas, and biofuel. However, the high lignocellulosic content of banana peel biowaste plays a mediating role in the

creation of biofuel following hydrolysis. The primary polymers found in lignocellulosic biomass are aromatic (lignin) and carbohydrate (hemicellulose and cellulose). Banana bio-waste is known to contain lignin, cellulose, and hemicellulose at contents of 6-12%, 7.6–9.6%, and 6.4%–9.4%, respectively. Variations in this composition may occur due to the wide range of banana species found worldwide. (Rai et al., 2019)

2.2 ANAEROBIC DIGESTION (AD)

2.2.1 Anaerobic Digestion

In the lack of oxygen, certain biological reactions are known as anaerobic reactions. It is an energy-producing process in which microbes break down organic material, including proteins, carbs, and lipids. Anaerobic respiration is the name given to this process.

Microorganisms produce energy during anaerobic respiration by using different electron acceptors besides oxygen, like nitrate or sulphate. The type of bacterium and the electron acceptor employed determine the final products of anaerobic respiration. For instance, methanogenesis, a process in which microorganisms employ carbon dioxide as an electron acceptor, results in the end products of methane (CH_4) and carbon dioxide (CO_2). (Malyan et al., 2021)

Many naturally occurring and artificial systems, such as landfills, biogas generating facilities, and wastewater treatment plants, depend on anaerobic processes. Anaerobic processes in these systems can aid in the decomposition of organic matter, lower the amount of trash disposed of in landfills, and generate biogas, a renewable energy source. (Srivastava et al., 2020)

AD is one of the most complex biochemical processes, utilising hydrolytic, acidogenic, hydrogen producing, acetate-forming, and methanogenic bacteria as well as a variety of wastes and residuals as substrate and mixed cultures in the bioreactor. (Wainaina, S. et al 2019) Anaerobic degradation occurs in four stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. The earliest and most gradual stage of anaerobic breakdown is hydrolysis. (Qu et al., 2021)

Hydrolytic enzymes made by microorganisms convert complex organic substances like proteins, carbohydrates, and lipids into less complicated ones like amino acids, sugars, and fatty acids throughout this process. The succeeding phases of anaerobic degradation might then further destroy these simpler molecules. Because complex organic matter is broken down into simpler

chemicals that can be utilised by microbes in later phases of anaerobic degradation, hydrolysis is regarded as the stage of anaerobic degradation that occurs most gradually. Temperature, pH, and the kind of organic material being broken down are a few variables that affect how quickly organic matter is hydrolyzed. (Qu et al., 2021)

2.2.2 Differences of Mono and Co-Digestion

Anaerobic digestion is carried out using a single type of organic material, a process known as monodigestion. For instance, a monodigestion system might solely employ grass, food waste and municipal solid waste (MSW) as the feedstock. Monodigestion may not be practical or cost-effective for managing mixed waste streams, but it can be useful for managing some types of organic waste streams.

The method of codigestion, on the other hand, combines various organic components and uses them as the fuel for anaerobic digestion. As an illustration, the feedstock for a codigestion system might consist of a combination of grass, food waste, animal manure, municipal solid waste (MSW) and agricultural leftovers. Codigestion can be a useful method for handling mixed waste streams and has a number of advantages, including higher biogas production and better nutrient balance.

Due to low-cost waste treatment, the methane yield was boosted while costs were kept to a minimum. Anaerobic co-digestion, or the co-digestion of paper waste with other organic wastes such municipal solid wastes, food and vegetable waste, pig manure, and algal sludge, has also been shown to effectively boost the biogas production from waste paper in earlier studies. (Rathaur, et al 2018)

A single type of organic material is utilised as the feedstock in monodigestion, whereas codigestion combines several different types of organic materials and uses them as the feedstock for anaerobic digestion. When it comes to managing mixed waste streams, codigestion can be more advantageous than monodigestion because it can increase the production of biogas and improve nutrient balance. Monodigestion, however, may not be practical or cost-effective for managing certain types of organic waste streams. Codigestion systems need careful management of the feedstock mixture to guarantee ideal conditions for anaerobic digestion, which is another distinction between codigestion and monodigestion. This includes monitoring factors on variables like pH, temperature, and nutrient balance to make sure that microorganisms can break down the organic material and produce biogas effectively. For instance, a monodigestion system

might just utilise food waste or only use animal manure as the feedstock, but a codigestion system might use a combination of food waste, animal manure, and agricultural wastes.

2.3 BIOGAS

Anaerobic digestion of organic materials such food waste, solid municipal waste (MSW) animal manure, agricultural residues, sewage, and industrial waste results in the production of biogas, a renewable energy source. Methane (CH_4) and carbon dioxide (CO_2) make up the majority of biogas' chemical composition, with minor amounts of hydrogen sulphide (H_2S), ammonia (NH_3), and nitrogen (N_2) also present.

In anaerobic digestion, organic material is broken down by bacteria without the presence of oxygen. Complex chemical substances are reduced during this process into less complex ones including amino acids, carbohydrates, and fatty acids. Methanogenic bacteria continue to break down these simpler molecules into methane and carbon dioxide.

Moreover, Biogas is a fuel that can be used to generate energy or heat water for a range of uses, including cooking, lighting, and transportation. Biogas upgrading is a method of converting it to biomethane that cleans out contaminants like CO_2 and H_2S to create a gas with properties akin to natural gas.

However, Landfills have the potential to release noxious odours and poisonous liquids into subsurface water sources. The production of biogas has the potential to enhance water quality. Anaerobic digestion also renders bacteria and parasites inactive, making it a potent tool for lowering the prevalence of waterborne illnesses. Similar to how garbage management and collection greatly improve in places with biogas facilities. Improvements in the environment, sanitation, and hygiene follow from this. HomeBiogas. (2021).

Rice production, agricultural soils, and livestock like cows all contribute to agriculture's greenhouse gas emissions. (US EPA. 2023) In general, biogas is a significant source of renewable energy that can help lower greenhouse gas emissions and offer sustainable energy for a number of uses.

2.4 Methane Gas Detector

Figure 2.1 shows the HABOTEST Gas Leak Detector HT601B+

A methane detector is a tool used to find out whether methane gas is present in the air. It is frequently employed in industrial environments where methane gas might pose a safety risk, such as mines and oil rigs. Methane detectors monitor the level of methane in the air and send out an alert to workers if it rises to risky levels. They are also used in homes to find gas leaks from propane tanks or natural gas lines. Sensors are used by methane detectors to identify the presence of methane gas in the atmosphere. Methane detectors can employ a variety of sensors, however they most frequently use infrared or catalytic bead sensors.

When a tiny bead covered with a catalyst is heated, it reacts with methane gas in catalytic bead sensors. Methane reacts chemically with the bead when it comes into touch with it, producing heat and altering the bead's electrical resistance. In order to calculate the amount of methane in the air, the detector measures this change in resistance. A beam of infrared light is emitted by an infrared sensor into the air, and the amount of that light that is absorbed by methane molecules is then measured. The amount of methane present can be determined by measuring the amount of light absorbed at specific infrared wavelengths, which methane absorbs.

Methane gas can be hazardous if it builds up in an enclosed place since it is very combustible. Methane detectors can help to ensure that workers are informed when harmful levels of methane gas are present, enabling them to take the necessary safety precautions. However, by ensuring that the anaerobic digestion process is working at peak efficiency, methane detectors can aid in optimising the biogas generation process. Operators can optimize biogas

production by modifying the process parameters by monitoring the methane gas concentration. Methane detectors can aid in lowering operational costs and raising profitability by enhancing the biogas generation process. They can also assist in avoiding expensive shutdowns brought on either faulty equipment or safety concerns. Strong greenhouse gas methane plays a role in climate change. Methane detectors can aid in minimising the environmental effect of biogas producing plants by detecting and limiting their methane emissions. In the production of biogas, methane detectors can increase security, efficacy, cost efficiency, and environmental sustainability.

CHAPTER 3

MATERIALS AND METHODS

3.1. Material

3.1. 1 Apparatus and equipment

Glassware	Chemicals	Feedstock
• 500ml measuring cylinder • 100ml measuring cylinder • 10ml measuring cylinder • 250ml erlenyer flask • 250ml conical flask • Petri dish • 2L beaker • L-shaped hockey • Glass slide with cover slip • Container	• Safarin solution • Nutrient agar media • Iodine solution • Crystal violet solution • Diatase •	• Napier grass • Onion waste • Banana waste

Retort stand	Test tube	Distilled water
Pipet	Inoculating loop	2L bottle x4
Test tube rack	Bunsen burner	Tube

3.1. 2 Digester

Digester Setup a 1L batch digester was crafted with designated inlets for feeding and outlets for gas collection. The digester maintained a constant temperature within the range of 23°C - 26°C, ± 2°C. Four digesters were constructed: three received combined waste in ratios of 1:1, 1:2, and 2:1, while the fourth exclusively processed Napier grass.

A 70% operating volume (550 ml) digester, utilizing a 1L container measuring 24 cm by 14 cm by 10 cm, was sealed to maintain anaerobic conditions. Biogas collection employed a water displacement technique with a measuring cylinder attached to the digester's gas exit. This is crucial in ensuring that the digester stays anaerobic. The biogas is collected using a technique called water displacement.

A container is filled with water. A water seal is then created by flipping the measuring cylinder over and partially submerging it in the water. The bottom of the measurement cylinder is attached to the digester's gas exit. The water in the measuring cylinder is replaced by biogas as it flows into the measuring cylinder from the digester. The amount of biogas produced is indicated by the water level in the measurement cylinder. While single trash with 300g of Napier grass mixed with water is fed into the digester for mono-digestion, different ratios of onion waste and Napier grass (OW:NG) are fed into the digester for codigestion process. For the co-digestion of (OW: NG), three distinct ratios were used: 1:1, 1:2, and 2:1. The digester was run simultaneously for 7 days to observe the production of biogas.

3.1. 3 Water Displacement Method

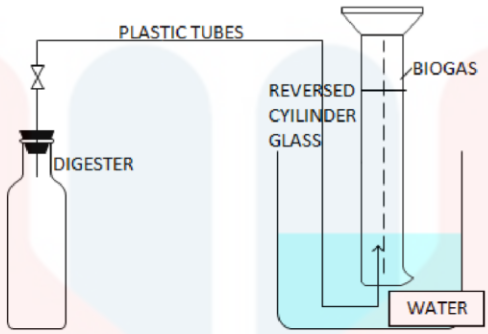


Figure 3.1 shows the anaerobic co-digestion process

Thirty grams each of onion waste and Napier grass formed a slurry in a container half-filled with water. 500mL measuring cylinders, secured by retort stands, were inverted on top of each beaker, connected to conical flasks via a tube system.

3.2 Methods

3.2.1 Sampling Collection

Sampling Collection Representative Samples of feedstock were collected from various locations within the field or storage area. Samples, including different food waste from Pinggiran UMK Jeli, were stored in airtight recycle plastic bags to prevent contamination.

3.2.2 Sampling Identification

The texture or colour of the grass will be used to detect it visually. Visual inspection, based on texture or color for grass and physical properties for food waste, facilitated sample identification. Each sample was labeled with a unique ID, time, date, and location.

3.2.3 Storage

To ensure that food waste and Napier grass is available, samples should be confirmed a week to several days in advance. All the samples will store in airtight recycle plastic bag.

3.3 Microbial identification

3.3.1 Serial dilution

Prepare six sterile test tubes. A 10 ml measuring cylinder is used to add 9 ml of distilled water to the six test tubes. Use a pipet to then transfer 1ml of the slurry into the first test tube. This results in a 10 ml overall capacity for the first test tube. It provides an initial dilution of 10^{-1}. In order to fully mix the sample, a vortex machine is needed. Transfer 1 cc of the mixture sample from the 10^{-1} dilution to the second tube using a pipet. There is now a 10^{-2} dilution factor in the second tube. Repeat the steps for the second test tube using a pipette. Third test tube contains 10^{-3}. The 10^{-4} in the fourth test tube, the 10^{-5} in the fifth, and the 10^{-6} in the sixth test tube has a 10^{-6} dilution factor.

3.3.2 Primary screening

Nutrient agar plates were inoculated with 1 ml of the diluted sample and incubated for 24 hours at 37°C. Agar media with nutrients is made. One millilitre of the sample should be removed from the test tube and placed onto nutritional agar. The test tube sample is then poured onto an agar plate and spread with an L-shaped hockey stick to ensure even distribution. To avoid contamination during the pour plate, some aseptic technique is employed. The plate was then labelled with the identity and dilution factor.

3.3.3 Secondary Screening

Pure colonies were isolated using streak plates and incubated at 37°C for 24 hours. Utilising an inoculating loop to aseptically transfer a single colony, streak it onto a fresh petri plate containing nutritional agar media. A streak plate is used to separate and collect pure colonies of microorganisms from a mixed culture for microbial identification. The petri dish is incubated following inoculation.

3.3.4 Gram Staining

The slide will be heated and fastened by putting it through a flame two or three times. The staining process is improved by this. Cover the bacterial smear with crystal violet (primary stain) for around 30 seconds. Rinse the slide gently to remove any remaining crystal violet stain. Cover the bacterial smear with iodine solution (mordant) for roughly a minute on the slide. A substance that helps with stain retention is created when crystal violet and iodine are combined. Rinse the slide with water slowly to remove any remaining iodine solution. Until the runoff is colorless, add a few of drops of acetone to the slide. This stage divides the bacteria into Gram-positive and Gram-negative groups according to the properties of the cell wall. Safranin (counterstain) should be used to stain the slide, and it should be let about 30 seconds to dry. Safranin, which contrasts with the purple colour of Gram-positive bacteria, stains gram-negative bacteria that have lost their pigment pink or red.

3.4 Experiment setup

Figure 3.2 shows the two different batch with onion waste, banana waste and Napier grass

Two types of digesters (with and without enzyme) were set up using onion waste and Napier grass (OW: NG) without enzyme in batch A and banana waste and Napier grass (BW: NG) with amylase in batch B. Different ratios (1:1, 1:2, and 2:1) of Napier grass and onion waste were used in batch A. Batch B exclusively processed banana waste. The experiment ran for seven days. Biogas production was measured using a water displacement method. Appropriate amounts of feedstocks were used for both mono and co-digestion setups. Amylase enzyme concentration of 18% was used in batch B digesters, and daily biogas production was measured. The purpose of this experiment is to measure the rate at which biogas is produced in the digester by breaking down organic material with the aid of enzymes. Together, the two setups run in seven days. Napier grass and onion waste were combined in ratios of 1:1, 1:2, 2:1 (OW: NG), and Napier grass exclusively (NG), respectively. This will alter the feedstock's content throughout the experiment. For a week, the setup for the experiments with both ratios will be conducted concurrently. The experiment will employ a total of 140g, or 15g with 15g for ration 1:1, 10g with 20g for ration 1:2, 20g with 10g for ration 2:1, and 30g of Napier grass for batch A and banana waste for batch B exclusively for mono digestion.

The water displacement approach was used in an experimental setup. Eight digester with 1 liter conical flasks with the labels 1:1, 1:2, and 2:1 (OW: NG) and Napier grass only (NG) for batch A and 1:1, 1:2, and 2:1 (BW: NG) and banana waste only (BW) will be available. Before starting the experiment, onion waste, banana waste, and Napier grass are broken up into tiny pieces using a mortar and pestle and blender. The conical flask was sanitized after the feedstocks were inserted in order to maintain as anaerobic a working environment as possible before the reaction was started.

On the other hand, the objective of the four digesters equipped with amylase is to investigate the impact of amylase enzyme concentrations on the production of biogas from Napier grass and banana waste, as well as the length of time that the biogas is produced. Subsequently, during the research week, each digester's daily biogas production volume was measured by passing the digester's gas through a tube into a measuring cup that was in advance filled with water. The concept behind this measurement is that the gas in the reactor will move into the measuring cup when the digester valve is opened, allowing for the observation of the

volume difference that results from the gas's nature of pressing in all directions (Boyle's Law). Using an amylase enzyme concentration of 18% in each digester, the study process combined banana waste and napier grass. This is one method of figuring out the necessary enzyme requirements, which is

$$18\% \text{ amylase} = 18/100 \times 1000 \text{ ml} = 180 \text{ mL}$$

The apparatus needed to collect gas over water consists of four measuring cylinders, each with 250 ml of water and placed inside a container that is partially filled with water; eight conical flasks, each holding one litre, are used for the reaction. By connecting one end of a tube to the reaction flask and the other end to an inverted gas collecting measuring cylinder, the gas produced during the reaction is collected. When gas is produced, the water in the measuring cylinder will be driven out. It is possible to estimate the volume of the gas by calculating the amount of water it removes from the system.

The total number of moles of gas can be found by applying the gas laws and the collected gas volume. To keep the pressure in and out of the measuring cylinder constant while data is being gathered, the water level inside the cylinder would rise or fall. Consequently, given the air pressure outside the measuring cylinder, the atmospheric pressure of the gas inside may be computed. Following the digestion process, a methane gas detector was used in the experiment to detect methane.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Results

4.1.1 Substrate Characterization

Characteristics of OW, NG and the mixture of the sample with the ratio of 1:1, 1:2, 2:1 and Napier grass are shown in Table 4.1. 30g of material are utilised in all experimental setups; an additional 10g were used for total solids (TS) and microbial identification.

Parameters	**Before Digestion**				**After Digestion**			
	1:1	**1:2**	**2:1**	**NG**	**1:1**	**1:2**	**2:1**	**NG**
Dry weight	30g	30g	30g	30g	30g	30g	30g	30g
Weigh of dish	0.568	0.570	0.650	0.737	0.995	1.026	0.758	1.193
Weigh of dish and total solid (g)	0.659	0.665	0.781	0.835	1.220	1.317	0.978	1.480
pH	5.9	5.9	5.8	6.0	3.9	3.3	2.3	3.5
Total solid (g)	0.091	0.095	0.131	0.098	0.234	0.291	0.220	0.287
Percentage of total solid (%TS)	9.07%	9.15%	13.08%	9.78%	23.39%	29.11%	21.97%	28.71%

Table 4.1: Characteristics of Onion waste (OW), Napier Grass (NG) and with ratio of 1:1, 1:2, 2:1 and Napier grass

4.1.1 Substrate Characterization

Characteristics of BW, NG and the mixture of the sample with the ratio of 1:1, 1:2, 2:1 and banana waste are shown in Table 4.1. 30g of material are utilised in all experimental setups; an additional 10g were used for total solids (TS) and microbial identification.

Parameters	**Before Digestion**				**After Digestion**			
	1:1	**1:2**	**2:1**	**BW**	**1:1**	**1:2**	**2:1**	**BW**
Dry weight	30g	30g	30g	30g	30g	30g	30g	30g
Weigh of dish	0.763	0.804	1.196	1.300	1.281	0.612	0.712	0.452
Weigh of dish and total solid (g)	0.910	0.895	1.488	1.493	1.585	0.881	1.141	1.366
pH	6.8	6.0	5.1	5.2	3.3	2.2	2.2	2.1
Total solid (g)	0.147	0.091	0.292	0.193	0.304	0.269	0.429	0.914
Percentage of total solid (%TS)	14.67	13.96	29.15	19.30	30.43	26.90	42.94	91.35

Table 4.2: Characteristics of banana waste (BW), Napier Grass (NG) and with ratio of 1:1, 1:2, 2:1 and Banana waste

4.1.2 Microbial Identification

The onion waste municipal solid waste and Napier grass were inoculated on nutrient agar thorough serial dilution and spreading and incubate it for a week to microbes grow for the identification.

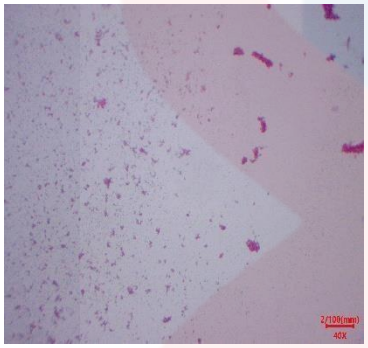
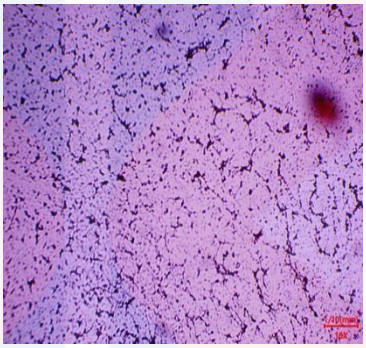
Categories	**Onion waste**	**Napier grass**	**Banana waste**
Morphology of sample			
Technique	Streak plate	Spread plate	Spread plate
Gram stain	Gram-negative bacteria	Gram-positive bacteria	Gram- negative bacteria
Colony	yellow-green fluorescent pigment	Fuzzy white	Circular,smooth, white to grayish colonies
Shape	rod-shaped	Rod-like shape, thick peptidoglycan layer	rod shaped, non-spore forming
Arrangement	singly, in pairs, or in short chains	Singly, clumps and as chains.	Exist in single and pairs.
Motility	Motility	Motile with rotating flagella	Motile
Organism	*Pseudomonas aeruginosa*	*Bacillus subtilis*	*Escherichia coli*

Table 4.3: Microbial Identification of Onion waste, Napier grass and banana waste on Nutrient Agar.

4.1.3 Methane Gas Analysis

Using the HABOTEST Smart Gas Leak Detector, a methane gas detector, the concentration of methane gas and its lower explosive limit (LEL) were measured.
Recorded in a high sensitivity setting, the values are recorded immediately.

Categories	Concentration (ppm)	LEL (%)
Mixture 1:1	9999	20.00
Mixture 1:2	9999	20.00
Mixture 2:1	9999	20.00
Napier grass	9999	20.00

Table 4.4: The Concentration (ppm) and Lower Explosive Limit (LEL) of the mixture of Onion waste and Napier grass with ratio 1:1, 1:2, 2:1 and Napier grass

4.1.4 Methane Gas Analysis

Using the HABOTEST Smart Gas Leak Detector, a methane gas detector, the concentration of methane gas and its lower explosive limit (LEL) were measured. Recorded in a high sensitivity setting, the values are recorded immediately.

Categories	Concentration (ppm)	LEL (%)
Mixture 1:1		
Mixture 1:2	9999	20.00
Mixture 2:1	9999	20.00
Banana waste	9999	20.00

Table 4.5: The Concentration (ppm) and Lower Explosive Limit (LEL) of the mixture of Banana waste and Napier grass with ratio 1:1, 1:2, 2:1 and Banana waste

4.2 Discussion

4.2.1 Substrate Characterization

Table 4.1 displays the characteristics of onion waste with Napier grass with the combination of waste in different ratio and one digester with mono waste which is Napier grass only while Table 4.2 shows the characteristics of banana waste with Napier grass with the combination of waste in different ratio and one also mono waste which is banana waste only. The 10g of each substrate and mixture were utilised for digestion characterization, and 30g of substrates in total were employed for the experimental setup. The pH values of were assessed using distinct digester ratios and a single waste. According to the Table 4.1, there are four digesters with a ratio of 1:1, 1:2, 2:1, and Napier grass which have pH values of 5.9, 5.9, 5.8, and 6.0, respectively while Table 4.2 shows the pH values of 6.8, 6.0, 5.1 and 5.2 before the digestion. After one week of anaerobic digestion, the value of pH on both set up become slump. The pH value on Table 4.1 is 3.9, 3.3, 2.3, and 3.5 while Table 4.2 shows 3.3, 2.2, 2.2 and 2.1.

The pH of anaerobic digestion is a critical component of the process' effectiveness and success. Maintaining the ideal conditions for the action of methanogenic bacteria, which produce methane, is greatly influenced by pH levels. The pH range in the context of anaerobic digestion is normally 6.8 to 7.4. The microbial community responsible for producing methane may suffer if the pH deviates from this range, falling below 6.0 or rising beyond 8.0. While high pH levels can upset the crucial microbial balance for the process, low pH levels can suppress the activity of methanogens, reducing the amount of methane produced. Keeping the pH at the right level encourages the development and activity of bacteria that produce methane, which in turn makes it easier to produce biogas from organic substrates like onion waste, banana waste and Napier grass.

The decrease in pH happen in 8 digester from 5.9, 5.9, 5.8, 6.0 to 3.9, 3.3, 2.3 and 3.5 in combination of OW and NG while from 6.8,6.0,5.1,5.2 to 3.3,2.2,2.2 and 2.1 in combination of BW and NG after anaerobic digestion occurs due to the accumulation of acidic compounds during the digestion process. The initial pH of 5.9 is likely the pH of the substrate of onion waste, banana waste and Napier grass in the different combination ration used for anaerobic digestion. Generally, organic materials used in biogas production, such as agricultural waste, sewage sludge, or food waste tend to have a neutral to slightly acidic pH.

As the organic matter undergoes anaerobic digestion, acidogenic bacteria break down complex organic compounds into simpler molecules through fermentation. This process generates acidic by-products such as volatile fatty acids (VFAs) and organic acids like acetic acid, propionic acid, and butyric acid. These acids accumulate in the digestion system, contributing to a decrease in pH.

The initial pH of 5.9 might be influenced by the buffering capacity of the substrate. Some materials have natural buffering agents that help maintain pH within a certain range. However, as acid production increases during digestion, the buffering capacity may become overwhelmed, leading to a significant drop in pH.

Despite the accumulation of acidic compounds, methanogenic microorganisms continue to convert volatile fatty acids and organic acids into methane (CH_4) and carbon dioxide (CO_2). However, the rate of acid production by acidogenic bacteria may outpace the rate of methane production, causing pH to decrease significantly.

The pH of 3.9 observed after digestion reflects the accumulation of acidic by-products in the digester. This low pH can inhibit microbial activity if not properly managed and controlled. Therefore, monitoring pH levels and implementing strategies such as adding alkaline substances to adjust pH are essential for optimizing biogas production and maintaining a stable digestion process.

The decrease in pH from 5.9, 5.9, 5.8, 6.0 to 3.9, 3.3, 2.3 and 3.5 after anaerobic digestion is primarily attributed to the accumulation of acidic compounds produced during the breakdown of organic matter by acidogenic bacteria.

The organic material breaks down during anaerobic digestion, there be large differences in the total solids (TS) composition before and after the process. The total solids content normally varies in the following ways before and after anaerobic digestion.
Anaerobic digestion's efficiency, especially in producing methane and biogas, can be impacted by the quantity of TS in solid waste.

Before the anaerobic digestion, the total solid of Batch A onion waste with napier grass (OW:NG) in each digester with ratio of 1:1 is 0.091 (9.07%), 1:2 is 0.095 (9.15%) , 2:1 is 0.131 (13.08%) and Napier grass is 0.098 (9.78%) while for after digestion for 1:1 is 0.234 (23.39%), 1:2 is 0.291 (9.15%) , 2:1 is 0.131 (13.08%) and Napier grass is 0.098 (9.78%)

Moreover, for the Batch B banana waste with napier grass (BW:NG) in each digester with ratio of 1:1 is 0.147 (14.67%), 1:2 is 0.1396 (13.96%) , 2:1 is 0.292 (29.15) and banana waste is 0.193 (19.30%) while for after digestion for 1:1 is 0.304 (30.43%), 1:2 is 0.269 (26.90%) , 2:1 is 0.429 (42.94%) and banana waste is 0.914 (91.35 %).

The impact of microorganisms' breakdown efficiency on total solids (Ts) concentration. Total soluble solids (TS) content increased after the digesting process, most likely as a result of microbial activity and the diversity of bacterial structure. Furthermore, it was shown that larger amounts of methane and biogas generation were produced at lower ratios of TS concentration. It is commonly known that, in the context of anaerobic digestion, food waste is not an easily digested substrate.

4.2.2 Microbial Identification

Table 4.3 displays the findings of the identification of the microorganisms that were inoculated from banana waste, onion waste, and Napier grass.

The spread plate method and the streaking culture method were both applied. The aseptic method is used to avoid contamination. After the inoculation, the petri dish is incubated for 24 hours at 37°C. After being incubated on nutritional agar for three days, both plates exhibit colony formation, as well as the active growth of bacteria on the agar surface. Then, the culture cultivated on onion waste displayed a yellow-green fluorescent pigment look, whereas the cultures developed on banana waste and Napier grass waste displayed fuzzy white colonies as well as circular white colonies. Gram-staining is employed on a single colony to determine the type of microbe that plays a part in the anaerobic digestion process in each of the three wastes which is onion waste, banana waste, and Napier grass, respectively.

Moreover, the bacterium Pseudomonas aeruginosa is well-known for its ability to move around. It does this by using a single, rod-shaped polar flagellum that is present in onion waste. Under a component microscope, it is arranged in single and short chains at a 40x magnification while the gram-positive bacteria in the Napier grass, Bacillus subtilis, have a rod-like structure with a thick peptidoglycan covering. They are arranged in chains and clumps. Under a 10x microscope magnification, its flagella are rotating and it is motile. Under a microscope, a facultative anaerobic bacterium identified as Escherichia coli was discovered in the banana waste. It displays purple colour indicating that it is gram-negative and motility with peritrichous flagella. Under a 4x microscope, it is visible as a rod-shaped, non-spore-forming.

4.2.3 Methane Gas Analysis

Table 4.3 displays the methane content along with the percentage of the lower explosive limit (LEL). Methane gas is measured using methane analyzers using the infrared technique. Since methane absorbs infrared radiation in specific spectral bands, infrared technology is perfect for methane detection. Accurate measurements in percent (%) and, in certain cases, parts per million (ppm) ranges are made possible by this technology.

HABOTEST Gas Leak Detector HT601B+, a methane gas detector a flammable gas detector was utilised for the purpose of inspection. Its flexible probe is a 16-inch bending probe that runs on 1.5VX3AAA batteries. It measures temperature and concentration and can detect flammable gas. It also has an audible and visual alarm as well as high and low sensitivity alarms. It has a sensor cage with an indicator of a low battery, a gooseneck, a display screen, and a keypad. In order to make the gooseneck hot, the meter must be turned on in a clean space far from the sample and left on for 30 seconds. It responds in around two seconds and displays a bargraph along with a digit.

HABOTEST HT601B+ Gas Leak Detector The measured concentration of the combustible gas detector was 9999 ppm and 20% LEL, which was the highest value that the installed detector could record. The presence of 20% LEL in the two systems indicates that the generated gas is made up of >99% methane and 1% air. The concentration of the target gas in the surrounding air, which is frequently expressed in parts per million or as a percentage of the volume, determines the LEL.

A significant physicochemical indication is the lower explosion limit (LEL), which is used to evaluate a substance's combustibility and explosiveness. The factory-set default alerts are typically designed to be extremely cautious. Usually, the range of these notifications is from 10% to 20%. 500 ppm of methane is equivalent to 1%. Rather than measuring a substance's toxicity, LEL sensors measure its tendency to explode. Many volatile organic compounds (VOCs) can be harmful even at quantities much below their volatile thresholds and well beyond the range at which LEL sensors can detect them.

Gas detectors are devices used to find out whether gases are present in a given area. The concentration of gas that the detector can measure is referred to as the detection range. A 999 ppm range in this instance indicates that the detector can measure gas concentrations with accuracy ranging from 0 ppm to 999ppm. An environment with a comparatively high concentration of the detected gas would have a gas concentration of 999 parts per million. Depending on the type of gas detector and the intended use, different gases may be detected. For instance, carbon monoxide (CO), carbon dioxide (CO_2), methane (CH_4), hydrogen sulphide (H_2S), oxygen (O_2), and other volatile organic compounds (VOCs) are frequently detected using gas detectors. Gas detectors are frequently used in residential, commercial, and industrial situations where it's necessary to monitor gas concentrations for environmental, health, and safety reasons 999 ppm.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Several crucial factors influence the efficiency of biogas production, including waste surface area, digester water level, enzyme presence, pH levels, temperature, microbial properties, conical flask size, pipe dimensions, waste quantity, and lignin content in Napier grass and total solids. Significant factors that impact biogas quality were identified through the experiment. The experiment faced challenges, particularly with the lignin in Napier grass, which prolonged biogas production. Onion waste, on the other hand, exhibited antibacterial properties, affecting microbial digestion during anaerobic digestion. The use of techniques such as amylase enzymes was implemented to address digestion issues, as enzymes play a crucial role in breaking down complex compounds into smaller organic matter. The primary objective was to generate renewable energy from various food wastes (banana and onion) and agricultural waste (Napier grass) to mitigate greenhouse gas effects, manage county waste, and reduce landfill usage. Utilizing food waste also aids in preventing environmental contamination and associated health issues.

5.2 Recommendations

Lab-scale studies may benefit from being conducted on a pilot scale to address potential variables influencing biogas output. Ongoing issues in anaerobic co-digestion require resolution before extensive commercial use. Further research is recommended, focusing on:

- Incorporating enzymes or isolated microorganisms into the anaerobic digestion process.
- Developing a tool that can reduce the size of the substrate to increase its surface area, making it easier to digest.
- Expanding the application of co-digestion processes to improve future biogas production efficiency.
- Diversifying substrates from agricultural and food waste to analyze methane generation and prevent open burning.

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APPENDIX A

Table A.1: Calculation of each parameter which is percentage of total sample, dry weight and pH in the onion waste and Napier grass (OW: NG) before and after digestion.

Parameter	Before Digestion				After Digestion			
	Mixture	Mixture	Mixture	Mono waste	Mixture	Mixture	Mixture	Mono waste
	1:1	1:2	2:1	Napier grass	1:1	1:2	2:01	Napier grass
Total sample (g)	325	310	225	300	124.978	100.564	133.860	101.532
Dry weight (g)	30	30	30	30	30	30	30	30
Weight of dish (g)	0.568	0.570	0.650	0.737	0.995	1.026	0.758	1.193
Dry weight – weight of dish (g)	30 - 0.568 =29.432	30 - 0.57 = 29.430	30 - 0.65 = 29.350	30 - 0.737 = 29.263	30 - 0.995 = 29.005	30- 1.026 = 28.974	30 - 0.758 = 29.242	30 - 1.193 = 28.807
pH	5.9	5.9	5.8	6.0	3.9	3.3	2.3	3.5
Total Solids:								
Weight of dish + total solids (g)	0.568+0.091 =0.659	0.570+0.095 =0.665	0.650+0.091 =0.781	0.737+0.131 =0.835	0.995+0.0978 =1.220	1.026+0.234 =1.317	0.758+0.220 =0.978	1.193+0.287 =1.480
% TS	$\frac{30-0.568}{325-0.568}\times 100 = 9.07$	$\frac{30-0.5}{30-0.5}\times 100 = 9.1\ \%$	$\frac{30-0.6}{29-0.6}\times 100 = 10.0\ \%$	$\frac{30-0.737}{300-0.737}\times 100 = 9.78\%$	$\frac{30-0.995}{124.977-0.995}\times 100 = 23.39$	$\frac{30-1.02}{15.5-1.02}\times 100 = 21.1\ \%$	$\frac{30-0.75}{18.5-0.75}\times 100 = 29.9\ \%$	$\frac{30-1.19}{15.53-1.19}\times 100 = 27.7\ \%$
TS (g)	$\frac{30-0.5}{30-0.5} = 0.0$	$\frac{30-0.5}{30-0.5} = 0.0$	$\frac{30-0.6}{29-0.6} = 0.1$	$\frac{30-0.737}{300-0.737} = 0.098$	$\frac{30-0.9}{15.9-0.9} = 0.2$	$\frac{30-1.02}{15.5-1.02} = 0.2$	$\frac{30-0.75}{18.5-0.75} = 0.2$	$\frac{30-1.19}{15.53-1.19} = 0.2$

Table A.2: Calculation of each parameter which is percentage of total sample, dry weight and pH in the banana waste and Napier grass (BW: NG) before and after digestion.

Parameter	Before Digestion				After Digestion			
	Mixture	Mixture	Mixture	Mono waste	Mixture	Mixture	Mixture	Mono waste
	1:1	1:2	2:1	Banana waste	1:1	1:2	2:1	Banana waste
Total sample (g)	200	210	100	150	95.672	109.880	68.925	32.795
Dry weight (g)	30	30	30	30	30	30	30	30
Weight of dish (g)	0.763	0.804	1.196	1.300	1.281	0.612	0.712	0.452
Dry weight – weight of dish (g)	30 - 0.763 =29.237	30 - 0.804 =29.196	30 - 1.196 =28.804	30 - 1.300 = 28.7000	30 - 1.281 = 28.719	30 - 0.612 = 29.388	30 - 0.712 = 29.288	30 - 0.452 = 29.548
pH	6.8	6.0	5.1	5.2	3.3	2.2	2.2	2.1
Total Solids:								
Weight of dish + total solids (g)	0.763+0.147 =0.910	0.804+0.091 =0.895	1.196+0.292 =1.488	1.300+0.193 =1.493	1.281+0.304 =1.585	0.612+0.269 =0.881	0.712+0.429 =1.141	0.452+0.914 =1.366
% TS	$\frac{3-0.7}{2-0.7}\times 1 = 1.6$	$\frac{3-0.8}{2-0.8}\times 1 = 1.9$	$\frac{3-1.1}{1-1.1}\times 1 = 2.1$	$\frac{30-1.300}{150-1.300}\times 100 = 19.30$	$\frac{3-1.2}{9.6-1.2}\times 1 = 3.4$	$\frac{3-0.6}{1^{8}-0.6}\times 1 = 26.$	$\frac{3-0.7}{6.9-0.7}\times 1 = 4.9$	$\frac{3-0.4}{3.7-0.4}\times 1 = 9.3$

TS (g)	$\frac{3 - 0.7}{2 - 0.7} = 0.1$	$\frac{3 - 0.5}{3 - 0.5} = 0.13$	$\frac{3 - 1.1}{1 - 1.1} = 0.2$	$\frac{30 - 1.300}{150 - 1.300} = 0.193$	$\frac{3 - 1.2}{9.6 - 1.2} = 0.3$	$\frac{3 - 0.6}{1.8 - 0.6} = 0.2$	$\frac{3 - 0.7}{6.9 - 0.7} = 0.4$	$\frac{3 - 0.4}{3.7 - 0.4} = 0.9$

APPENDIX B

Figure B.1: Napier grass from Pinggiran UMK Jeli

Figure B.2: Onion waste from Oren cafe and Mini Mart

Figure B.3: Banana waste

APPENDIX C

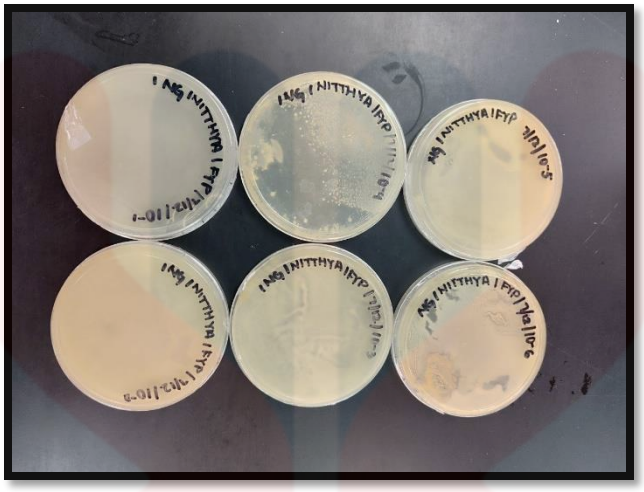

Figure C.1: White colonies grow in a nutrient agar plate with Napier grass sample after incubation

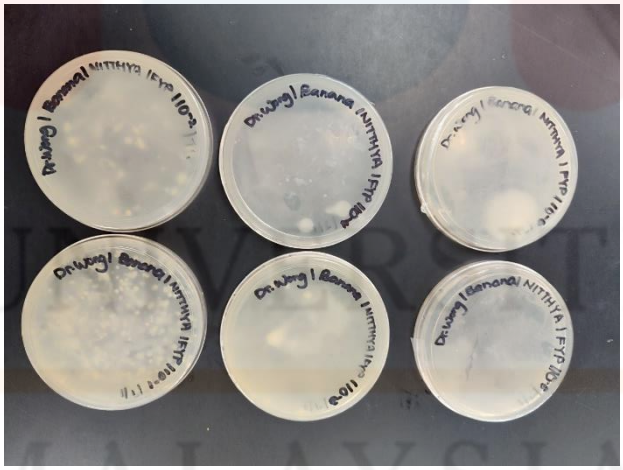

Figure C.2: White colonies grow in a nutrient agar plate with banana waste sample after incubation

Figure C.1: White colonies grow in a nutrient agar plate with onion waste sample after incubation