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Enhancing Biogas Generation Via Anaerobic Co-Digestion of Cow Dung and Durian Peel Waste

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DECLARATION

I declare that this thesis entitled "Enhancing biogas generation via anaerobic co-digestion of cow dung and durian peel waste" is the results of my own research except as cited in the references.

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Date : 1 March 2024

Verified by:

Signature : ____________________

Supervisor's Name : ____________________

Stamp : ____________________

Date : ____________________

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ABSTRACT

This study addresses the pressing challenges of municipal solid waste (MSW) management, particularly in Malaysia, where open dumping landfills are prevalent, leading to severe environmental impacts, including contributions to global warming due to the release of greenhouse gases. The study focuses on leveraging durian biomass waste and cow dung for biogas production through anaerobic co-digestion. This choice of feedstock is motivated by the substantial amount of durian peel waste generated annually (350,000 MT) and the significant increase (50%) in methane emissions from cow dung over the past 15 years. By understanding the composition of these feedstocks and their potential for large-scale utilization, the study aims to address the challenges associated with municipal solid waste management.

The objective of the experiments was to evaluate the efficiency of biogas production, considering various parameters such as feedstock ratios, durian peel sizes, pH levels, and working volumes, to optimize biogas generation. Two anaerobic digestion experiments were conducted at room temperature. The first experiment involved 40g of mixed waste with a 53.3% working volume undergoing a 2-week anaerobic digestion, while the second experiment utilized 100g of mixed waste with a 91.7% working volume and 0.25mm durian peel particles, spanning 21 days. Methane detection was carried out using methane gas detectors, and microbial identification provided insights into biogas composition and microbial roles.

Despite challenges such as pH fluctuations, the study underscores the importance of careful system design for efficient biogas production. Anaerobic co-digestion demonstrated superior efficiency over mono-digestion, particularly with a 2:1 ratio of cow dung to durian peel, which maximized biogas production. Smaller durian peel particle sizes, specifically 0.25mm and 0.5mm, were found to enhance methane generation, highlighting the significance of pre-treatment processes. Overall, the study contributes valuable insights into optimizing biogas production from durian biomass waste and cow dung, offering promising solutions for sustainable MSW management and mitigation of environmental impacts.

Keywords: biogas, durian peel size, anaerobic, working volume and pH.

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Meningkatkan Penghasilan Biogas Melalui Penguraian Anaerobik Bersama Sisa Lembu dan Sisa Kulit Durian

ABSTRAK

Kajian yang revolusioner ini menyentuh cabaran penting pengurusan sisa pepejal bandar (MSW), terutamanya di Malaysia, di mana tapak pelupusan terbuka mendominasi, menyebabkan impak alam sekitar yang teruk, termasuk sumbangan kepada pemanasan global akibat pelepasan gas rumah hijau. Kajian ini memberi tumpuan kepada penggunaan sisa biomass durian dan najis lembu untuk pengeluaran biogas melalui koperasi pencernaan anaerobik. Pilihan bahan baku ini dipacu oleh jumlah besar sisa kulit durian yang dihasilkan setiap tahun (350,000 MT) dan peningkatan signifikan (50%) dalam pelepasan metana dari najis lembu dalam 15 tahun yang lalu. Dengan memahami komposisi bahan baku ini dan potensi penggunaan skala besar mereka, kajian ini bertujuan untuk menangani cabaran yang berkaitan dengan pengurusan sisa pepejal bandar.

Objektif eksperimen adalah untuk menilai kecekapan pengeluaran biogas, dengan mempertimbangkan pelbagai parameter seperti nisbah bahan baku, saiz kulit durian, tahap pH, dan isipadu kerja, untuk mengoptimumkan pengeluaran biogas. Dua eksperimen pencernaan anaerobik dijalankan pada suhu bilik. Eksperimen pertama melibatkan 40g campuran sisa dengan isipadu kerja 53.3% menjalani pencernaan anaerobik selama 2 minggu, manakala eksperimen kedua menggunakan 100g campuran sisa dengan isipadu kerja 91.7% dan zarah kulit durian berukuran 0.25mm, merentasi tempoh 21 hari. Pengesanan metana dilakukan dengan menggunakan pengesan gas metana, dan pengenalpastian mikrob menyediakan pandangan ke dalam komposisi biogas dan peranan mikrob.

Walaupun menghadapi cabaran seperti fluktuasi pH, kajian menekankan kepentingan reka bentuk sistem yang teliti untuk pengeluaran biogas yang cekap. Koperasi pencernaan anaerobik menunjukkan kecekapan yang lebih baik berbanding mono-pencernaan, terutamanya dengan nisbah 2:1 najis lembu kepada kulit durian, yang memaksimumkan pengeluaran biogas. Saiz zarah kulit durian yang lebih kecil, khususnya 0.25mm dan 0.5mm, didapati meningkatkan penghasilan metana, menekankan kepentingan proses pra-rawatan. Secara keseluruhan, kajian ini menyumbang pandangan yang berharga dalam

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mengoptimumkan pengeluaran biogas dari sisa biomass durian dan najis lembu, menawarkan penyelesaian yang menjanjikan untuk pengurusan MSW yang mampan dan pengekalan impak alam sekitar.

Kata kunci: Biogas, saiz kulit durian, anaerobic, isipadu kerja dan pH

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LIST OF ABBREVATIONS

MSW	Municipal Solid Waste
GHG	Green House Gas
CO_2	Carbon Dioxide
CH_4	Methane
AD	Anaerobic Digestion
H_2O	Water
GDP	Gross Domestic Product
DVS	Department of Veterinary Services
DP	Durian Peel
CD	Cow Dung
C	Carbon
N	Nitrogen
P	Phosphorus
K	Potassium
Fe	Iron
S	Sulphur
Mg	Magnesium
Cal	Calcium
Co	Cobalt
Mn	Manganese
Cl	Chlorine
H^+	Hydronium ion
VFA	Volatile Fatty Acid
L	Litre
ml	Millilitre
cm	Centimetre
kg	Kilogram
g	Gram
PP	Polypropylene

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°C	Celsius
Ktoe	Kilo tonne

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CHAPTER 1

INTRODUCTION

1.1 Background of the study

Municipal solid waste (MSW) would be produced at an enormous rate due to the fast urbanization, population increase, economic development, and rise in community living standards (Zamali Tamarmudi 2009). The main environmental issue in Malaysia right now is inadequate solid waste management (Samsudina and 2013). In Malaysia, the preferred method practiced for the disposal of MSW is through landfill (Yusof, Haraguchi et al. 2009) and most of the sites are open dumping areas (Abd Manaf, Samah et al. 2009). Open dumping landfill is preferable due to it is the cheapest cost and most common method to treat solid waste with high percentage of organic components (Ngoc and Schnitzer 2009, Nanda and Berruti 2021). Open dumping gives a lot of severe impacts on environment such as soil pollution, water pollution, uncontrolled GHG emission.

Methane, nitrous oxide, carbon dioxide, and fluorinated gases are all examples of greenhouse gases (Magazzino, Mele et al. 2020). Carbon dioxide (CO_2) is the main greenhouse gas that significantly contributes to global warming because CO_2 emissions from the energy sector account for more than 61% of all GHG emissions. CH_4, which is the second most significant GHG and emits 16% of all global GHG emissions from anthropogenic and natural sources, it is 21 times more potent than CO_2 in trapping heat (Mohajan 2011). The troubling aspect is that human activities like burning fossil fuels, diminishing the quantity of forest cover, the quickening growth of farming and industrial activities causing to increase greenhouse gas concentrations (Thakur and Solanki 2022). In many regions of the world, there is a lot of environmental pressure to figure out the best way to treat animal manure. Anaerobic digestion is a process that convert waste into energy (Ismail2 2012).

Biogas is created when organic matter decomposes in the absence of oxygen. Biogas can be improved to produce biomethane, which can be put into the existing natural gas pipelines, and can be utilized for combined heat and power generation (Tamara Llano and

Finger 2020). The two main substances that make up biogas are methane (CH_4) and carbon dioxide (CO_2) (N A M Hilmi1 2022). Biogas technology, can aid in lessening reliance on non-renewable resources and reducing the negative social effects and environmental concerns related to fossil fuels (Gedefaw 2015).

In this instance, the feedstock for AD is made up of durian peel waste and cow dung. Cow dung is rich in natural microorganisms that help the AD process, and durian peel powder is utilized to boost the biomass availability in the mixed waste slurry.

1.2 Problem statement

According to estimates from the population division of the department of economics and social affairs, the world's population could reach 10.4 billion by the year 2100 and will likely exceed 8.5 billion in 2030. There is a higher demand for energy to meet the needs of individuals, households, and industries. More people require energy for cooking, transportation, and powering various devices and appliances. This leads to increased energy consumption across sectors, including residential, commercial, and industrial.

In Malaysia, fossil fuels are the major energy source that we rely on, which causes GHG emissions. By the end of 2018, approximately 78% of Malaysia's installed capacity and 83% of the country's electricity output were derived from fossil fuels (Administration 2015). Furthermore, there is a greater need for natural gas in peninsular Malaysia to meet the demands of the power and industrial sectors. TNB is the major company that generates electricity in Malaysia. This company has two major plants for the generation of electricity: hydroelectric plants and thermal plants. A thermal power plant generates electricity through the utilization of traditional steam turbines and steam generators, primarily by burning fossil fuels. On the other hand, hydroelectric power is categorized as a renewable energy source since electricity generation primarily relies on the movement of water from a higher elevation, which drives a turbine connected to an electric generator. In this process, the potential energy of the water is converted into electrical energy.

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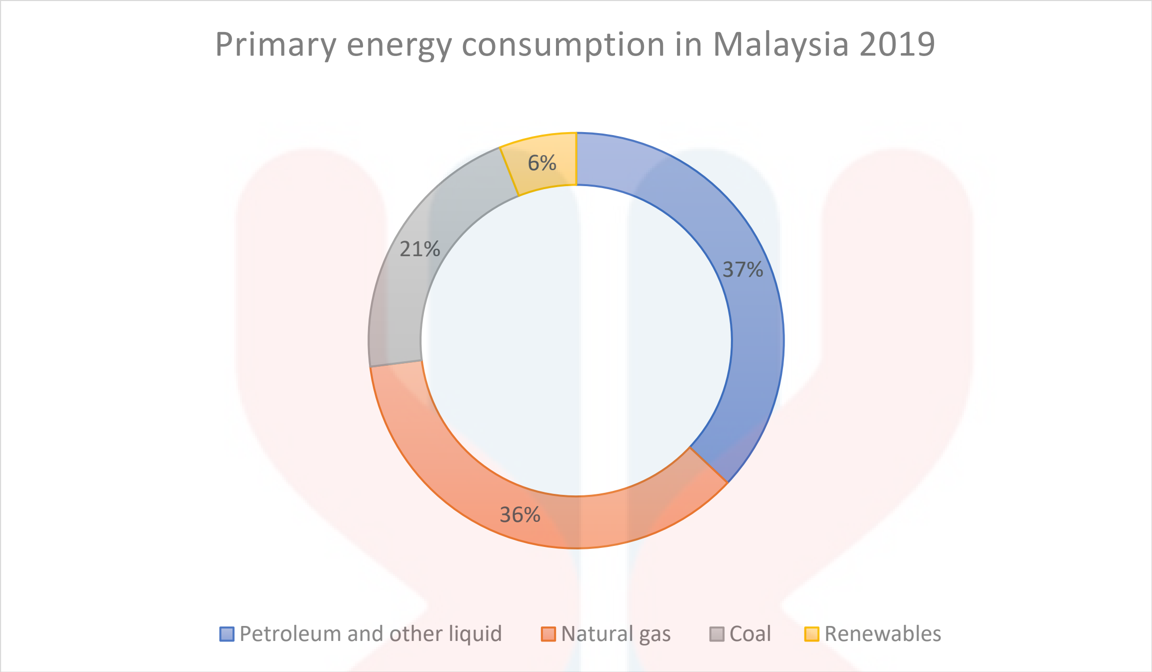

Figure 1.1: Primary energy consumption in Malaysia 2019

Figure 1.1, we can see that the consumption of fossil fuels such as coal, natural gas, and petroleum is high compared to the consumption of renewable energy sources in Malaysia in the year 2019 (by fuel type-Exajoules and Emissions 2006). During the combustion of fuels, the oxygen in the air undergoes reactions with carbon, resulting in the formation of carbon dioxide (CO_2), or with hydrogen, leading to the production of water vapor (H_2O). Among the fossil fuels, coal combustion is particularly impactful in terms of CO_2 emissions due to its higher carbon content compared to oil and gas. Oil and gas, on the other hand, contain a higher proportion of hydrogen relative to water vapor and CO_2. The carbon intensity of each fuel determines the amount of CO_2 generated during its combustion process. Within the United States, coal combustion accounts for 59% of CO_2 emissions, despite only contributing 23% to the electricity generation mix (Gołasa, Wysokiński et al. 2021). It is important to note that even if a greater proportion of oil and gas is burned instead of coal in an effort to minimize CO_2 emissions, all of these fossil fuels still contribute to global warming. Moreover, natural gas predominantly consists of CH_4, which is classified as a short-lived climate pollutant that has an even more pronounced warming effect on the planet.

The livestock sector in Malaysia has grown significantly during the past few years (ROSLAN M.Y.1* 2019). In Malaysia, the livestock industry accounts for 1/10 of agriculture

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sector Gross Domestic Product (GDP) with the whole sector contributing 8.9% of national GDP. Table 1 shows the livestock population in Malaysia based on the 2019/2020 Livestock Statistics by Department of Veterinary Services (DVS) (Zayadi 2021).

Table 1.1: population of livestock in Malaysia for 2016-2020

Type of livestock	2016	2017	2018	2019	2020
Buffalo	119133	114013	106988	101695	100242
Cattle	737827	703832	676686	657407	659317
Goat	416529	385304	359200	312571	320203
Sheep	138479	130658	128298	121677	121173
Swine	1654381	1849351	1967538	1888460	1876029
Chicken	289666002	293301558	259323292	285063636	300145315
Duck	9633185	9283900	9680573	9376456	9628617
Total	302365536	305768616	272242575	297521902	312850896

According to data from the US Environmental Protection Agency, methane emissions from cattle increased by 50% and those from pigs by 37% over the previous 15 years, respectively (Russell 2014). Table 2 shows the manure excreted by the population of livestock in Malaysia for 2016-2020. Livestock makes 85% of global animal faecal waste. Upon observing the table, it becomes apparent that there is a slight decrease in the quantity of manure excretion. However, this decrease is not substantial enough to significantly contribute to environmental issues. The improper waste management of animal manure can cause the emission of GHG and the release of leachate.

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Table 1.2: Manure excreted by the population of livestock in Malaysia for 2016-2020

Type of livestock	**Total daily manure excreted (tons)**				
	2016	**2017**	**2018**	**2019**	**2020**
Buffalo	1999	1913	1795	1706	1682
Cattle	12381	11810	11355	11031	11063
Goat	775	717	668	581	596
Sheep	258	243	239	226	225
Swine	5559	6214	6611	6345	6303
Chicken	26070	26397	23339	25656	27013
Duck	1734	1671	1743	1688	1733
Total	48775	48965	45749	47234	48616

Durian is a tropical fruit known for its distinctive smell and taste. While it is popular for its unique flavour, durian cultivation generates a significant amount of biomass waste, especially in countries like Malaysia where durian production is high. The annual production volume of approximately 350,000 metric tons in Malaysia contributes to the abundance of durian biomass waste. AD help in the reduction of durian peel waste and induce biogas production. One way to address this issue is through the generation of biogas, which serves as a renewable energy source capable of mitigating greenhouse gas (GHG) emissions. Furthermore, biogas is considered carbon neutral since it is derived from renewable energy sources.

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1.3 Objectives

The main objectives of this research are:

1. To analyse the anaerobic parameters, such as pH, feedstock ratio, and feedstock size, in the production of biogas and overall efficiency of the process over a period of 14 working days.

2. To study the quantity and quality biogas generated during a 14-day anaerobic digestion process by employing the water displacement technique and a methane detector.

3. To determine the possible microorganism community that present in the slurry that responsible for enhancing methane production during the 14-day anaerobic digestion process by using gram staining technique.

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1.4 Scope of the study

To analyse the anaerobic digestion parameters, such as the pH, feedstock ratio and size of feedstock in the production of biogas. The pH level of the digester affects the activity and stability of the microbial community. Size of the feedstock help to boost the AD rate. Different feedstock ratios have different composition of organic content it. Balanced organic composition help in the production of high quantity and quality of biogas.

The goal of the study is to research the biogas's quantity and quality. The objective of this study is to determine whether substantial amounts of methane are present in the biogas production. The presence of methane in biogas is highly desirable due to its flammable characteristics, making it suitable for power generation purposes. The quantity of biogas produced is measured using water displacement technique. The volume change in initial and final water level at the end of the experiment shows the volume of biogas produced. Methane detector (HABOTEST) is used to analyse the methane yield constitution in biogas.

Additionally, to identify the microbe community that help in the enhancement of methane yield. 1ml of feedstock slurry were taken by using the pipet and pour into the test tube that contain 9ml of distilled water. Then the test tube mixed thoroughly by using vortex machine. 1ml of the solution is allocated from the first test tube to the next test tube with dilution factors of 10^{-6}. For primary screening pour plate technique were used. 1ml of solution is pipet out from each test tube and pour plate it in a petri dish containing nutrient agar. For secondary screening the single colony is taken by using inoculating loop and streak on to the nutrient agar medium. Gram staining technique used to the bacterial classification. The identification and characterization of specific microorganisms involved in methanogenesis, such as methanogenic archaea, can help understand their metabolic pathways and interactions within the digester.

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1.5 Significance of the study

In Malaysia, 8% of real gross domestic product (GDP) was accounted for transportation in 2018. The transport sector required 23555 ktoe of total final energy demand, which is 36.4%. 96% of greenhouse gases are emitted from this sector because 90% of its energy demand comes from fossil fuels (Solaymani, 2019). Among the transport subsectors, the land transport sector is also one of the leading emitters of CO_2 and have difficulty for decarbonization (Giannakis et al., 2020). The primary goal of this project is to produce biogas. Biogas can be further upgraded into biomethane used as a fuel for the transportation. Biogas is a carbon neutral compound which means its use have a relatively neutral impact on GHG emissions. Utilizing methane from biogas as an alternative means of electricity generation is advantageous due to its positive environmental impact. This is primarily attributed to the fact that methane in biogas is derived from renewable energy sources.

Additionally, this can help the nation's trash management. Majority of the waste will be open dumbed in the landfills. This situation brings serious environmental and social threats like flooding, breeding of insects and rodent vectors and the spread of diseases (Zurbrugg 2002). Anaerobic digestion use waste as a raw material for biogas production. Anaerobic digestion is aid in the development of circular economy (Awogbemi, Kallon et al. 2022).

Table 1.3: Waste treatment method practice in Malaysia

Treatment method	2002	2006	Target 2020
Recycling	5.0	5.5	22.0
Composting	0.0	1.0	8.0
Incineration	0.0	0.0	16.8
Inert landfills	0.0	3.2	9.1
Sanitary landfills	5.0	30.9	44.1
Other disposal sites	90.0	59.4	0.0
Total	100.0	100.0	100.0

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From this table, waste disposal in other disposal sites is higher compare to other methods. In 2002, 90% of waste is disposed in other site while in 2006, 59.4% of waste is disposed in there. The estimation of waste disposal percentages in 2020 does not reflect actual practices during that period. The other disposal site is referred to the open dumbing in landfill that have not proper waste management system.

A sustainable energy source can be produced from this trash. The energy derived from biogas can be utilized locally for heat and power generation, thereby reducing reliance on fossil fuels or minimizing their consumption. Several benefits were offer by utilizing the biogas as a local energy source.

- Unlike fossil fuels, which are finite and contribute to climate change, biogas production provides a sustainable and continuous source of energy.
- The combustion of biogas releases carbon dioxide (CO_2) and water vapor (H_2O) which have lower impact on environment.
- Utilizing biogas locally enhances energy independence by reducing reliance on imported fossil fuels.

In this study, biogas is produced by utilizing fruit waste (specifically durian peel) and animal waste (cow dung) as the primary feedstock. The importance of the research extends to evaluating the scalability and practical applicability of the findings beyond laboratory conditions. This information is essential for the successful implementation of biogas production on a larger, commercial scale, providing valuable insights into system design, feedstock handling, and overall system performance.

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CHAPTER 2

LITERATURE REVIEW

2.1 Introduction to biogas and anaerobic digestion

Anaerobic digestion (AD) is a various microbial-driven process that decomposes organic matter, such as animal manure, food processing waste, and wastewater biosolids, in an oxygen-free environment. This process takes place inside a sealed reactor. The result of AD is the production of biogas and digestate as an end product. This process prevents environmental pollution caused by organic waste (Nkuna, Roopnarain et al. 2022).

The key components of biogas are methane and carbon dioxide, which have a favourable environmental effect (Ukpabi Chibueze [1] 2017). Biogas also consist trace amount of hydrogen sulphide, ammonia, oxygen, carbon monoxide and hydrogen. Biogas is a carbon neutral compound because it is generated from renewable organic materials, unlike non-renewable fossil fuels.

The generation of waste is increasing at a rapid pace. In many developing nations, including Malaysia, the management of solid waste is primarily carried out through open dumping. This is primarily due to the lower costs associated with capital investment, operations, and maintenance compared to other waste disposal methods (Fadhullah, Imran et al. 2022).

Improper waste disposal methods such as non-sanitary and non-engineered approaches contribute to environmental pollution. It is particularly due to the production of methane, a potent GHG that has a greater impact on global warming and climate change compared to CO_2. AD plays a vital role in waste management, renewable energy production, and environmental sustainability. Implementing AD contributes to the development of a circular economy, where waste is effectively utilized and transformed into valuable resources (Ahsan, Awais et al. 2019). The biogas generated through AD can be upgraded into biomethane, which has various applications. Biomethane can be injected into existing natural gas pipelines, to be used as a vehicle fuel (Llano, Arce et al. 2021), providing an alternative to fossil fuels in the transportation sector. By utilizing biomethane, carbon emissions can be reduced, contributing to efforts in mitigating climate change.

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2.2 Microbial community in anaerobic digestion

In this research, cow dung was utilized as an inoculum for AD. Cow dung contains a diverse microbial community that plays a vital role in facilitating the AD process. Previous studies have identified the presence of *Firmicutes, Bacteroides, Proteobacteria,* and *Euryarchaeotic* in cow dung. Within the *Firmicutes* and *Bacteroides* phyla, the dominant classes observed were *Clostridia* and *Bacteroide,* respectively (Christy, Gopinath et al. 2014). Each of these microorganisms contributes to the degradation of organic materials present in cow manure, to the production of biogas. Temperature, pH, retention time, and organic loading rate have a direct effect on the microbial activity (Nguyen, Nguyen et al. 2019). Totally nine bacterial species were isolated from cow dung in an AD process. Out of nine bacterial isolates six were hydrolytic bacteria (*Bacteroides nordii, Clostridium perfringens, Prevotella bivia, Porphyromonas asaccharolytica, Ruminococcus gnavus,* and *Lactobacillus acidophilus*) two were methanogenic archaea (*Methanobacterium formicicum* and *Methanosarcina siciliae*) and one was acetogenic bacteria (*Acetobacter syzygii*) (Sharma, Bano et al. 2023).

2.3 Anaerobic digestion process

The process of anaerobic digestion consists of four distinct stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Njuguna Matheri, Mohamed et al. 2015) .Each stage is facilitated by different groups of microorganisms that work together in consortia, and they have specific environmental requirements for optimal performance. Interactions that occur within a community of microorganisms over time in the utilization of organic matter in the digester to produce biogas, known as microbial dynamics (Li, Chen et al. 2019).

Clostridia and *Bacteroide* present in high numbers at the beginning of the stage because they are responsible for the initial breakdown of complex polysaccharides, proteins, and lipids into simpler compounds during the hydrolysis stage (Malee Suntikunaporn * 2014). Following the hydrolysis stage, acidogenic bacteria convert the simpler monomers derived from organic matter into carbon dioxide, hydrogen, and volatile fatty acids (acetic acid, propionic acid, and butyric acid). These volatile fatty acids are further transformed into acetate and hydrogen by acetogenic bacteria. Methanogenic bacteria, utilize acetate or hydrogen and carbon dioxide to produce methane (Laiq Ur Rehman, Iqbal et al. 2019). In a study, researchers isolated various type of methanogens including *Methanobrevibacter ruminantium, Methanobacterium formicicum, Methanosarcina frisia,* and *Methanothrix soehngenii* (P. Merlin Christy* 2014) from cow dung slurry.

2.4 Pathogen reduction in anaerobic digestion

In the past, there were no restrictions on the spreading of animal manure on agricultural land. However, due to stricter environmental regulations in many countries, the proper treatment and management of manure have become necessary. AD is one of the methods used for this purpose. Animal manure contain various pathogens, including bacteria like *Salmonella spp., Campylobacter spp., Listeria monocytogenes, Yersinia enterocolitica* and *Escherichia coli*. These pathogens typically reside in the intestinal tracts of animals and can be shed without causing symptoms (Doyle and Erickson 2006, Seruga, Krzywonos et al. 2020).

Factors such as temperature, pH, moisture content, nutrient availability, organic content, and time are influence the survival of pathogenic bacteria (Soupir, Mostaghimi et al. 2008). However, AD has been shown to be effective in reducing the amount of pathogenic bacteria in cattle dung (Manyi-Loh, Mamphweli et al. 2013). It appears that proper animal care and adequate management of manure can minimize the introduction of these pathogens into food chains, environment and transmission to humans. Improper treatment of cow dung can lead to health and environmental problems (Manyi-Loh, Mamphweli et al. 2016). The reduction of pathogenic bacteria is influenced by a combination of factors, including pH, time, temperature, and nutrient availability(Jiang, Xie et al. 2020).

2.4.1 Temperature

Anaerobic digestion can be conducted under both mesophilic (35–37 °C) and thermophilic (55–60 °C) conditions. Both temperature ranges have the ability to reduce the pathogenic level. However, thermophilic temperatures have significant effect in reducing pathogenic bacteria (Svoboda and Carcluie 2003). But the AD systems face a lot of challenges

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because of the higher ratio of free ammonia to total ammonium ion resulting from the AD of cattle manure posed instability in digester performance (Nie, He et al. 2021). To ensure the process stability, AD of cattle manure is often performed at mesophilic temperatures (Garcia and Angenent 2009).

2.4.2 Retention time

AD systems with a longer retention time provides the microbial community with more time to efficiently break down organic materials and effectively reduce the population of pathogens present.

2.4.3 pH

The AD process operates within a pH range of 6.5 - 8, which is unfavourable for the survival of pathogenic bacteria(Lu, Zhang et al. 2020). However, the pH alone does not reduce pathogenic bacteria in cow dung. It is important to note that enteric pathogenic bacteria can survive for extended periods in slurry manure due to its high moisture level, low solid content, and alkaline pH resulting from the mixture of faeces, water, and urine (Cools, Merckx et al. 2001).

2.4.4 Nutrient availability

The microbial community within the AD system is characterized by its diversity and complexity. Throughout the process, different microorganisms compete for resources such as organic matter and nutrients. This restricts the growth of pathogenic bacteria by limiting their access to these vital resources.

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2.5 Feedstock

Suitability, availability and digestibility are the main characteristics that need to consider before choosing the feedstock. For this research cow dung and powder durian peel were used as a feedstock.

2.5.1 Cow dung

Cow dung contain various type of pathogenic bacteria that have significant potential to cause various illnesses and infections when ingested or come into contact with humans. Cow dung is chosen as a feedstock because of its unique properties, which are:

High buffer capacity

Cow dung has a natural alkaline pH, ranging from 7.4 - 8.5, which plays a crucial role in maintaining pH stability within the AD system. During the process of AD, the breakdown of organic matter produces acidic by-products, leading to a decrease in pH within the digester. This decrease in pH negatively impacts the activity of methanogenic bacteria, which are responsible for methane production. Methanogenic bacteria thrive within an optimum pH range of 6.8 to 7.8, and their activity is significantly reduced below a pH of 6.6.

However, acidogenic bacteria, are still active at lower pH levels. The accumulation of VFA inhibits the AD process. A study conducted on swine manure without the addition of dairy manure as an inoculum demonstrated that the pH rapidly dropped below 5.9 and remained at inhibitory levels until day 49 due to excessive VFA accumulation. This research suggests that dairy manure, effectively stabilized the pH levels within the AD system (Wi, Lee et al. 2023).

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Natural microbe

Cow dung contains natural microbes (ArthurWellinger 2013) . These microbes include various types of bacteria (such as *Bacteroidetes*, *Firmicutes*, and *Spirochetes*), archaea, and fungi. The anaerobic microbes present in cow dung work together in a complex microbial community to degrade the organic material in cow manure and produce biogas, primarily methane (Behera and Ray 2021).

Rich in nutrients

Cow dung is rich in essential nutrients that are required for the growth of microbes during the AD process. The cow dung contains crude fibre, crude protein, and 24 types of minerals, including nitrogen (N), phosphorus (P), potassium (K), iron (Fe), sulphur (S), magnesium (Mg), calcium (Ca), cobalt (Co), manganese (Mn), and chlorine (Cl) (Behera and Ray 2021). These nutrients provide a favourable environment for microbial activity. Moreover, the digestate of cow manure slurry is rich in nitrogen, potassium, and phosphorus. The digestate can be used as bio-fertilizer.

Cow dung is recognized as an effective feedstock for biogas production due to the presence of a significant amount of anaerobic bacteria that efficiently degrade the organic fraction of cattle manure, even in cases where pH regulation is not implemented (Abubakar and Ismail 2012). However, there are some limitations to using cow dung as a feedstock in the AD system.

One limitation is the low carbon-to-nitrogen ratio of cow dung. The nutrient composition, including the C:N ratio, plays a significant role in the optimal growth and activity of microorganisms involved in the AD process (Yohaness 2010). In the case of the C:N ratio, a range of 25–30:1 is generally considered optimum for biogas production (I. J. Dioha 2013) . This imbalance can result in ammonia accumulation and hinder the performance of methanogenic bacteria.To overcome this issue, cow manure is often co-digested with carbon-rich substrates such as agricultural residues, food waste, or energy crops(Arekemase and Aweda 2021). By adding carbon-rich substrates, the overall C:N ratio of the input substrate can be adjusted to a more favorable range and enhances the overall diversity and composition of

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the microbial community in the digester, leading to improved biogas production (Huda Rosada[1] 2018).

Another limitation is the dry matter content of cow dung. The dry matter content of the feedstock determined the availability of biomass for microbial activity in AD. Animal slurry, including cow manure, often has a low dry matter content. Additionally, cow manure contains crude fibre, which consists of cellulose, hemicellulose, and lignin (Behera and Ray 2021). Lignin and hemicellulose are considered recalcitrant matter as they are not easily degraded during anaerobic digestion and do not contribute significantly to methane production. Cow dung typically has a high moisture content, which can affect the overall efficiency of the anaerobic digestion process. To optimize the biogas production potential, cow slurry is often co-digested with co-substrates. This helps increase the availability of biomass for the microbial degradation and methane production. Studies have shown that the optimum solid content for biogas production typically falls within the range of 7-9%. Below a total solids level of 7%, the process may become unstable, while a level of 10% can overload the fermenter([1] 2010).

To address these limitations, it is often recommended to co-digest cow dung with other feedstocks, helps increase biomass availability and balance the nutritional composition in the feedstock. This is crucial for the efficient biogas production. Durian peel use as co-substrate in this experiment.

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2.5.2 Durian peel waste

Durian, a tropical fruit celebrated for its distinctive aroma and taste, has become a significant agricultural commodity in Malaysia, covering around 41% of cultivated land or approximately 70,000 hectares (Subhadrabandhu and Ketsa 2001). The surging global demand for durian, particularly from regions like China and Hong Kong, is expected to drive a substantial increase in durian production. This surge in production, however, correlates directly with a heightened generation of durian biomass waste. The management of this waste have become a major concern. Currently, the common practice for managing potato peel waste is landfilling, which has adverse effects on the environment due to the release of GHG and leachates (Sadeghi, Fazeli et al. 2013).

Southeast Asia's primary durian producers are Thailand, Malaysia, and Indonesia, collectively contributing to the region's durian cultivation. Table 4, illustrates the cumulative durian biomass waste produced by these countries from 2014 to 2016 (Chua, Pen et al. 2023).

Table 2.1: The yearly production of durian biomass waste in key durian-producing nations in from 2014 to 2016, measured in thousand metric tons.

Country/year	Malaysia	Thailand	Indonesia	Total waste	Waste production ny malaysia based on the total waste (%)
2014	272.43	489.62	665.83	1427.88	19.08
2015	285.41	466.46	771.70	1523.57	18.73
2016	234.55	401.42	569.95	1205.92	19.45

Given that only 15 to 30% of the durian constitutes the edible portion, the majority is designated as durian biomass waste, encompassing both the peel and seed (Ngabura, Hussain et al. 2018). Biorefinery can be used to produce multiple products that not harmful for the environment from durian peel waste(Arekemase and Aweda 2021). In this way the durian peel waste can be reduced and can support the circular economy. Durian peel contain affordable constitution in it which can be used for the production of important substance in various industries such as pharmaceutical and biotech.

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Durian peel is composed of lignin (15.45%), hemicellulose (13.09%), and cellulose (60.45%) (Aimi, Anuar et al. 2014). Moreover durian peel waste contain high cellulose content which make it a good co-substrates for animal slurries in the production of biogas(Muenmee and Prasertboonyai 2021). Durian peel waste is pre-treated before used as a co-substrate in AD system. DPW contains lignocellulosic materials, due to their recalcitrance and complex structure, a suitable pretreatment process is essential to enhance cellulose digestibility.

the International Energy Agency (IEA) anticipates a surge in fossil fuel utilization for energy by 2035 due to escalating global energy demands. This trajectory raises concerns about heightened greenhouse gas (GHG) emissions and environmental degradation, emphasizing the imperative to explore sustainable alternatives, such as harnessing energy from durian biomass waste.

The durian fruit waste biomass, on a dry basis, has a net calorific value of 17.6 MJ/kg (Brunerová, Roubík et al. 2017). To put this into perspective, 1 petajoule (PJ) of energy, equivalent to 1×10^{15} J, can be converted into 46 MW of potential power with an electrical conversion efficiency of 21% (Tock, Lai et al. 2010). As of the end of 2019, Malaysia had a total available capacity of about 32.0 GW, and the estimated power generated by durian biomass waste was 49.7 MW, contributing approximately 0.16% to the total available capacity. In 2020, the energy from durian biomass waste amounted to approximately 5.25×10^{9} MJ, accounting for about 0.13% of Malaysia's primary energy consumption (Chua, Pen et al. 2023).

2.6 Characterization of parameters

There are several important anaerobic digestion process parameters, that have to be controlled in order to optimize the process. Temperature, feedstock ratio, size of the feedstock, carbon nutrient availability and organic loading rate are the important anaerobic parameters. Any slight changes in this parameters have significant effect in the production of biogas. In this research pH, feedstock ratio and size of the feedstock are take into account to observe the quantity and quality of biogas production of biogas.

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2.6.1 pH

The pH value determines the acidity or basicity of an aqueous solution. Its unit is the negative logarithm of the concentration of hydronium ions (H^+) which can be determined by a standard potentiometric electrode. In order to analyze the pH of the feedstock used in this study which is cow dung (semi-solid) and durian peel powder (solid) need to mixed with water.

There are diverse microbial groups taking part in the AD process, which have various optimum pH values for their growth rates. For example, a pH range of 5.0 to 6.0 is suitable for acidogens, while pH from 6.5 to 8.0 is more convenient for the methanogens group. Usually, the biogas plants operate within a pH range of 6.5 to 8 (1, et al. 2020). A slight changes in the pH of the digester can lead to the inhibition of AD process. The pH value is dependent on the VFAs which is the intermediate in the acidogenesis, the ammonium content, and the alkalinity concentrations. pH plays a significant role in the production of biogas (Chibueze, Okorie et al. 2017).

2.6.2 Feedstock ratio

Co-digestion is used to balance the nutritional composition in the feedstock. the optimal carbon-nitrogen ratio on biogas production is in the range of 20:1 to 30:1. Cow manure has very low carbon ratio and it is important to mix it with other substrates that are carbon rich to overcome this deficiency and increase the biogas yield (Bumbiere). durian peel powder is used to increase the carbon content in the digester(Muenmee and Prasertboonyai 2021). The ratio of feedstock is important because variation of feedstock ratio have the variation of the C:N values which have effect on the yield of biogas and pH of a slurry (I. J. Dioha 2013).

A high C:N ratio will reduce the biodegradation rate, whereas a low C:N ratio will tend to produce excessive ammonia and VFAs, which may cause inhibition in AD (Long Lin [a] 2019). Moreover, the mixture of the waste composition correlated with the quantity and quality of biogas yield. Carbon (C) and nitrogen (N) are two fundamental nutrients for microbial growth. Carbon is used as the energy source and nitrogen is used for protein and nucleic acids synthesis.

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2.6.3 Size of the feedstock

The size of the feedstock influence the production of biogas. As the size of the feedstock reduce the anaerobic rate will be increase and so the biogas production. To reduced the size of the feedstock several pre-treatment method were used. Mechanical pre-treatment, thermal pre-treatment, chemical pre-treatment and biological pre-treatment can be used to reduce the size of the feedstock and make the organic content bio-available. In a study shows mechanical pre-treatmet and acid treatment in improving AD efficiency of durian peel and cow dung co-digestion. Both pretreatment methods effectively increased the biogas production yields. R9 (mechanical pretreatment) and R10 (chemical pretreatment) achieved significant high biogas yields of 453.2 mL/g VSadded and 485.4 mL/g VSadded, respectively (Achinas, Li et al. 2019).

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CHAPTER 3

MATERIAL AND METHODS

3.1 Material

Reagents: iodine solution, crystal violet solution, safranin solution and acetone

Feedstock: cow dung and durian peel

Media: nutrient agar

Others: distilled water

3.2 Apparatus

Measuring equipment: 11 units of 250ml measuring cylinder, 100ml measuring cylinder, 10ml measuring cylinder

Container: 2L beaker, 1000ml conical flask, 2 units of 250ml Erlenmeyer flasks, basin

Tools: L-shaped hockey, inoculating loop pipet, 11 unit of retort stand, glass slides, test tubes, test tube rack, bunsen burner, silicone tubing, 12 petri dishes.

Laboratory equipment: microscope, vortex meter, autoclave machine, analytical balance, pH meter, grinder, spatula, oven, and sieves (250 micron and 500 micron).

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3.3 Digester

A series of batch digesters were constructed with a total volume of 1200ml each, featuring appropriate inlets for feeding and outlets for gas collection. These digesters were meticulously designed to maintain a constant temperature at room temperature, ranging between 23°C to 26°C, ± 2°C. In total, three digesters were built in a ratio of 1:1, each incorporating combined waste consisting of different durian peel sizes (0.25mm, 0.5mm, and 6mm). Following the same procedure, another set of digesters was constructed in ratios of 1:2 and 2:1, resulting in a total of nine digesters for combined waste experimentation. Additionally, a tenth digester was exclusively fed with cow dung to serve as a comparative control.

Digester description

Two distinct experiments were conducted to assess biogas production efficiency in different setups. In the first experiment, a digester with a working volume of 53.3% (640 ml) was constructed and utilized. In contrast, the second experiment aimed to increase the total solid content by 2.5 times, resulting in a higher working volume that was 1.7 times larger than the initial experiment, reaching 91.7%.

In this experiment, a 1L conical flask, designed according to the DIN ISO 1773 standard, was employed. This conical flask, measuring 8.5" in height with a 5" base, could accommodate approximately 1.3L of the slurry. Importantly, each digester was intentionally designed to be airtight or sealed to maintain anaerobic conditions crucial for the experiment. The water displacement technique was employed to collect the generated biogas efficiently.

Throughout both experiments, each digester was carefully monitored to assess biogas production efficiency and evaluate the impact of feedstock ratios and durian peel sizes on anaerobic digestion performance. The systematic approach of varying feedstock compositions and ratios in the batch digesters allowed for a comprehensive analysis of biogas generation potential. These experiments highlight the significance of optimized waste management strategies in sustainable biogas production, emphasizing the importance of considering various parameters to maximize biogas yield while maintaining anaerobic conditions.

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Operational method of digester

In the conducted batch mode experiment, a single waste mixture consisting of 40g of cow dung and 600ml of water was introduced into the digester for mono-digestion. In contrast, for co-digestion, various ratios of cow dung and durian peel (CD: DP) were utilized, incorporating different sizes (0.25mm, 0.5mm, and 6mm). Three distinct ratios (1:1, 1:2, and 2:1) of cow dung to durian peel were employed for co-digestion to achieve a balanced nutrient content and optimize biogas production. The digester operated continuously for 14 days,

In the second experiment, the duration was extended to 21 days compared to the 14 days of the initial experiment. This prolonged duration allowed for an extended assessment of biogas production efficiency and performance. Additionally, an increase in total solid waste and working volume was implemented in this experiment. The increment in total solid waste and working volume likely aimed to explore the effects of higher substrate concentrations and volumes on biogas production. This approach allowed for a thorough evaluation of the impact of substrate concentration, volume, and duration on biogas generation, contributing valuable insights to the field of waste management and renewable energy production.

During the operational days of the digester, several important parameters were closely monitored to evaluate the progress and efficiency of biogas production. First, the volume of biogas produced over time was carefully measured, typically on a daily basis or at regular intervals. This helped to understand how well the anaerobic digestion process was working and allowed us to compare different combinations of feedstock to see which ones produced the most biogas. Second, ensuring proper mixing of the waste mixture within the digester was crucial. This helped maintain uniform microbial activity, which is essential for efficient digestion. We adjusted the frequency and intensity of mixing as needed throughout the working period to optimize biogas production. Lastly, biogas was continuously collected throughout the entire working period of the digester. This allowed us to accurately assess the efficiency of biogas production and compare the performance of different feedstock compositions and ratios. By carefully monitoring these parameters, we gained valuable insights into how to improve waste management strategies and promote sustainable biogas production.

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3.4 Water displacement technique

Water is poured into a container. The measuring cylinder are then flipped over and partially submerged in the water creating a water seal. The retort stand was used to secure and clamp the measuring cylinder. The gas outlet of the biogas digester is connected to the bottom of the measuring cylinder via tube. As biogas is produced within the digester, it flows into the measuring cylinder, displacing the water in the measuring cylinder. The change in initial water level and final water level at the end of the experiment shows the volume of biogas produced.

The water displacement technique was chosen for this experiment primarily due to safety concerns, providing a safe and reliable method for collecting and measuring biogas production. Additionally, this method allows for visual confirmation of gas production, ensuring clarity during the experiment. Furthermore, the technique is advantageous because it is simple and inexpensive to implement, requiring only basic laboratory equipment such as measuring cylinders and water. By directly measuring the change in water level, the water displacement technique provides a quantitative measurement of biogas production, facilitating accurate comparisons between different experimental conditions.

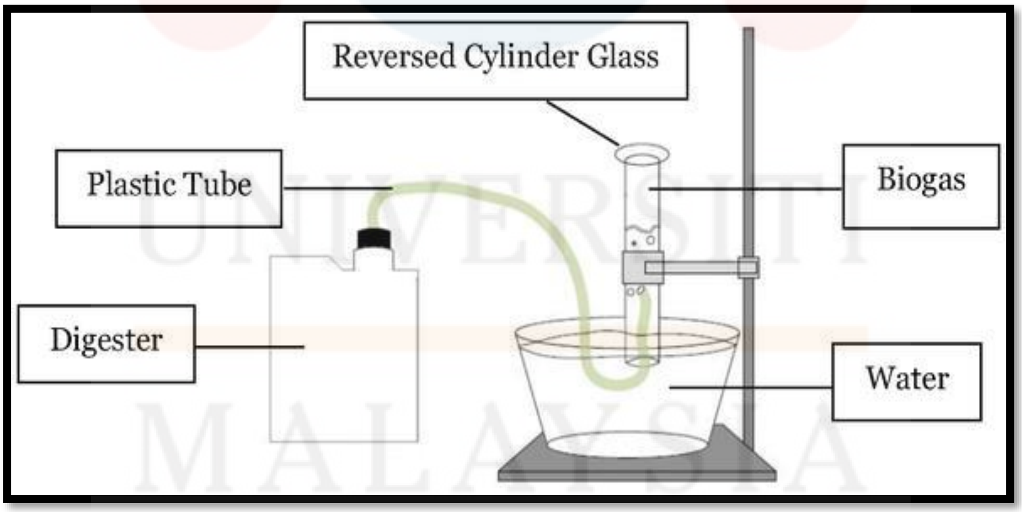

Figure 3.1: Setup of water displacement technique

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3.5 Waste characterization

The objective of the feedstock characterization is to assess and understand the properties of the feedstock materials (cow dung, durian peel) before initiating methane production experiments. This characterization involves testing parameters such as pH, feedstock ratio, and retention time for both substrates.

This characterization contributes significantly to the overall study by providing essential baseline data that informs the design and optimization of the anaerobic digestion process.

3.5.1 pH

The pH of the samples is calculated using a pH metre. The pH and amount of slurry in the digester have an impact on how much biogas is produced. At the start and end of the experiment, the pH is recorded. NaOH is added to the feedstock slurry to neutralise it if it is acidic. The pH of the feedstock should be between 7-8.

3.5.2 Proportion of the feedstock

Table 3.2 contain the quantities of the feedstock in g to prepared the slurry. By using the analytical balance measure the weight of the feedstock according to their ratios. Different proportion of the feedstock is used to analyse the quantity and quality of biogas produced. The quality and quantity of biogas is directly related to the feedstock composition. As mention in 2.5.2 durian peel is rich in carbohydrate while cow dung contains natural microbe that facilitate the AD. Thus, the ideal feedstock ratio for the production of biogas can be observed by employing different ratios of feedstock. Understanding the ideal balance between cow dung and durian peel in terms of their proportions can help maximize biogas yield.

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3.5.3 Size of the feedstock

The durian peel waste is ground into three different sizes to investigate their impact on anaerobic digestion (AD) rates and biogas yield. As the size of the feedstock is reduced, the surface area increases, making it more accessible for microbes in the digester. Durian peel contains a high content of lignin, which can hinder biogas production effectiveness. Mechanical pre-treatments, such as grinding, are employed to overcome this barrier by breaking down the lignin-rich structure of the durian peel into smaller, more digestible fragments. This process facilitates microbial access to the substrate, leading to improved anaerobic digestion rates and higher biogas yields. Ultimately, the varied sizes of the ground durian peel allow for a comprehensive assessment of how particle size influences the efficiency of biogas production from lignocellulosic biomass sources like durian peel.

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3.6 Methods

3.6.1 Sample collection

First experiment

For the initial experiment, durian peel waste was procured from a stall near UMK a week prior. The durian peels, discarded by the stall owner, were collected during the stall's closing hours, cut into small pieces, and washed. Following this, the durian peel underwent pre-treatment, being ground into a powder form, and sifted through sieves with mesh sizes of 250 microns and 500 microns. In this experiment, the residue from sieves with a mesh size of 500 microns was utilized. There were three overall variable sizes of the residue: 0.25mm, 0.5mm, and 6mm. It was observed that the residue predominantly contained particles of 6mm size, which was determined by measuring some particles using a ruler. The image of the residue is attached in the appendix. About 80 grams of durian peel powder for each mesh size were measured and stored in airtight plastic containers. Additionally, 200g of wet cow dung from the UMK agropark was collected using a small shovel and placed in a sterile bag. The experiment involved nine digesters for anaerobic co-digestion, each containing 40g of mixed waste and 600ml of distilled water. A separate digester was dedicated to anaerobic mono-digestion, comprising 40g of cow dung and 600ml of distilled water. The time frame for each of the ten batch digesters was 14 days.

Second experiment

Durian peel waste, ground to a powder, was sifted through a 250-micron mesh sieve and stored in an airtight container. For the experiment, 50g of cow dung was collected using a small shovel, placed in a sterile bag, and later fed into the digester. The waste, comprising durian peel and cow dung, was mixed with 1000ml of distilled water and poured into a conical flask. The batch digester operated over a 21-day timeframe. Extending the duration, is employ to observe any changes or trends in biogas production over time, allowing for a more comprehensive analysis of the anaerobic digestion process

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3.6.2 Sample identification

Based on their outward appearance, cow manure and durian peel waste can be identified visually.

Table 3.1: Visual identification of the cow dung and durian peel waste

Factors	Cow dung	Durian peel
Texture	• has a fibrous and granular texture.	• Hard outer layer with sharp torn
Colour	• commonly brown or dark brown, but it can also have shades of green if the cow has been grazing on fresh vegetation.	• Greenish exterior shell and white soft material under the skin.
Moisture	• Fresh cow manure is moist and may appear wet or damp	• It may appear moist

In wet conditions, a sample of cow dung is collected. The sun-dried cow dung will not use in this study because the microbial activity may be diminished due to the moisture loss which may impair the production of biogas

3.6.3 Storage

The powdered durian peel is stored in a polypropylene (PP) airtight container. The cow dung slurry should be kept chilled, at a temperature of 4 °C to slows the metabolic rate of the bacteria. This is done to preserved the sample and prevent the breakdown of organic matter. Keeping, cow dung at a low temperature will also lessen the possibility of pathogenic microorganism growth, which may happen at higher temperatures. Pathogenic microorganisms can pose a significant risk to public health if used as fertilizer without proper treatment or handling.

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3.6.4 Sample preparation

By wearing a glove sorting the cow dung visually inspecting and feeling any large pieces of thrash or object present in it. Dispose the collected object and large piece of thrash properly. The slurry was made by combining water, durian peel waste, and cow dung in the amounts listed in Table 3.2.

Table 3.2: Quantities of feedstock to prepared the slurry

Ratio (CD: PP)	Weight of cow dung (g)	Weight of durian peel powder (g)	Quantity of water (ml)	Total weight of slurry (g)
1:1	40g	40g	600ml	640g
1:2	13g	27g	600ml	640g
2: 1	27g	13g	600ml	640g

The cow waste was made into a slurry that resembled yoghurt by diluting it with tap water. Before mixed with cow dung, durian peel was ground into various sizes by using grinder. Diverse ratios of the substrates were co-digested with various size of the potato peel. For mono digestion 40g of cow dung were mixed with 600ml of wate o make the slurry. For the second experiment 100g of mixed waste were mixed with 1l of water.

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3.6.5 Pre-treatment

Drying

The durian peel waste is meticulously prepared by eliminating durian peel shells that show visible signs of infection. Infected durian peel shells exhibit slime formation, fungal growth, and the presence of red pigment. Shells displaying these characteristics are carefully removed for the subsequent stages of processing. The durian peel waste was place in the oven at 70°C for 44 hours. The waste should be clean and free from any excessive moisture or other organic materials that may interfere with the grinding process. Drying make it simpler to store the durian peel powder and enhance the biogas production. This is because the content of organic matter in the feedstock will increased through this process.

Mechanical pre-treatments

To reduce the size of the durian peel waste grinding method were employed. This is done by using grinder. durian peel was grinded into fine form to observe the AD rate.

3.6.6 Sieve analysis method

The sieves are stacked on top of each other, with the one with the largest openings on top and the one with the smallest openings at the bottom. Use a scale to weigh a representative sample of the powder. The sample is placed on the top sieve, and the sieves are then mechanically or manually shaken for a specified duration to ensure proper separation of particles. Separate containers should be used for the powder that was retained on each sieve. Note the weight of the particles that each sieve retained. The weight of the material retained and the initial weight of the of the sample were used to comprehend the details on the particle size distribution and particle size ranges.

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3.6.7 Water content

Regardless of the feedstock ratio, water content is kept constant in the experiment because it can affect the gas production and composition within the digester. A balanced water content helps to create an anaerobic environment conducive to the production of biogas. Excessive water content can lead to a decrease in gas production, while insufficient water content can result in the accumulation of volatile fatty acids and may affect the quality of biogas. Throughout the experiment, a fixed 600mL water is measured using a measuring cylinder for first experiment while for second experiment 1000ml of distilled water was used.

3.6.8 Loading rate

Durian peel and cow dung were combined and used as a mixture for anaerobic digestion. By mixing durian peel waste with cow dung, the overall composition of the feedstock is enhanced. Durian peels are rich in carbohydrates and organic matter, while cow dung provides a balanced nutrient profile and acts as a source of beneficial microorganisms. Combining these two materials helps create an optimal environment for the anaerobic digestion process, ensuring efficient for biogas production.

Cow dung and durian peel waste are mixed in proportions of 1:1, 1:2, and 2:1. The slurry was made by combining water, durian peel waste, and cow dung in the amounts listed in Table 5. While for mono-digestion the slurry was made by mixing 40g of cow dung with 500ml of water. Different ratio of feedstock is used to analysed the quantity and quality of biogas. Quantity and quality of biogas is determined by the composition of the feedstock itself. For the second experiment 100g of mixed waste were mixed with 1l of water.

3.6.9 Biogas collection

Water displacement technique is used to collect the biogas. As biogas is produced within the digester, it flows into the measuring cylinder, displacing the water in the measuring cylinder. By subtracting the initial water level and final water level at the end of the experiment, the volume of biogas produced can be calculated.

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3.7 Microbial identification

3.7.1 Serial dilution

Serial dilution is commonly used in microbiology to quantify the number of viable microorganisms present in a sample and to isolate individual microbial colonies for further study. Take 6 sterilised test tubes. 9 ml of distilled water is added to the 6 tests tube by using a 10 ml of measuring cylinder. Then transfer 1ml slurry into the first test tube by using a pipet. This makes the first test tube's total volume 10 ml. It offers a 10^{-1} initial dilution. A vortex machine is required, to mix the sample thoroughly. Using a pipet transfer 1 ml of the combination sample from the 10^{-1} dilution to the second tube. The second tube now has a 10^{-2} dilution factor. Using a pipette, repeat the procedure for the remaining test tube. the third test tube have 10^{-3} the fourth test tube has a 10^{-4}, the fifth test tube has a 10^{-5} and the sixth test tube has a 10^{-6} dilution factor. Repeat this step for cow dung and durian peel waste.

3.7.2 Primary screening

Nutrient agar medium is prepared. Take 1 ml of the sample from the test tube and pour plate it onto a nutrient agar. Then the sample from the test tube is pour plated and spread using L-shaped hockey stick to evenly distributed the sample in the agar plate. Some aseptic technique is used to prevent contamination during the pour plate. Then the plate was label according to the dilution factor to the identification. Then the plate is incubated for 37^{o} C for 24hours.

3.7.3 Gram staining

The bacterial cells will be heated fixed by passing the slide through a flame 2-3 times. This enhances the staining procedure. For around 30 seconds, cover the bacterial smear with crystal violet (main stain). Gently rinse the slide to get rid of extra crystal violet stain. For about a minute, cover the bacterial smear with iodine solution (mordant) on the slide. Iodine and crystal violet combine to generate a compound that aids in stain retention. In order to get rid of extra iodine solution, carefully rinse the slide with water. Add 2-3 drops of acetone to the slide

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until the runoff is colourless. Based on the characteristics of the cell wall, this stage separates the bacteria into Gram-positive and Gram-negative groups. The slide should be stained with safranin (counterstain) and left to dry for around 30 seconds. Gram-negative bacteria that have lost their colour are stained pink or red by safranin, which contrasts with the purple colour of Gram-positive bacteria.

3.7.4 Microscopic observation

When observing microbial cells under a light microscope with an oil immersion objective (100x), various characteristics can be identified to aid in microbial identification. These include cell morphology, such as cocci (spherical), bacilli (rod-shaped), spirilla (spiral), and filamentous forms, which provide initial clues about the microbial species. Additionally, the arrangement of cells, whether in clusters, chains, or pairs, offers further information for identification. Distinctive cellular inclusions, such as granules, vacuoles, or inclusion bodies, may also be observed, providing additional diagnostic features. Moreover, cellular structures like flagella, pili, capsules, and spores contribute to microbial species identification based on their presence, absence, or arrangement.

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3.8 Experiment setup

3.8.1 First experiment

The purpose of conducting a lab-scale experiment is to investigate and evaluate the feasibility of the anaerobic process in the production of biogas by using cow dung and durian peel on a smaller scale before implementing it in a larger-scale system, such as a pilot plant digester. At the beginning, a feedstock ratio of 1:1 (cow dung to durian peel waste) was employed. Three digesters, labelled as A, B, and C, were loaded with a mixture of cow dung and durian peel waste slurry in a 1:1 ratio. However, the size of the potato peel waste varied among the digesters. In digester A, the size of durian peel waste was 0.25mm, in digester B 0.5mm durian peel particle were used, and in digester C 6mm durian peel particle was used. After a period of 14 working days, the biogas production was calculated. The procedure for feedstock ratios of 1:2 and 2:1 remained consistent with the approach utilized in the preceding batch, which had a 1:1 ratio. The procedure for each ratio remained consistent. For each ratio the digester was run for 14 days. Three different types of digesters were employed to comprehensively understand the influence of feedstock composition ratios and durian peel size on biogas yield and production efficiency. The use of different digesters allowed for a more nuanced examination of biogas yield efficiency, enable to assess how factors like digester design, operating conditions, and substrate characteristics interact to influence overall gas production. 40g of cow dung were mixed with 600ml of water is fed into digester tenth. The significance of using 10 digesters with different ratios of cow dung and durian peel is to determine the optimal mixture that yields the highest biogas production and overall process efficiency.

HABOTEST High Accuracy Sensor HT601B Portable Gas Leak Detector. It comes with a 16-inch bendable probe, an HD/LCD screen, an audible and visual alarm, and a high-precision sensor. The probe is inserted into the measuring cylinder that contain biogas and press the appropriate button to initiate the methane detection process. The detector will display the methane concentration reading on its screen. The accuracy level of the instrument toward the methane is high. Figure 4 shows the ranges of detector.

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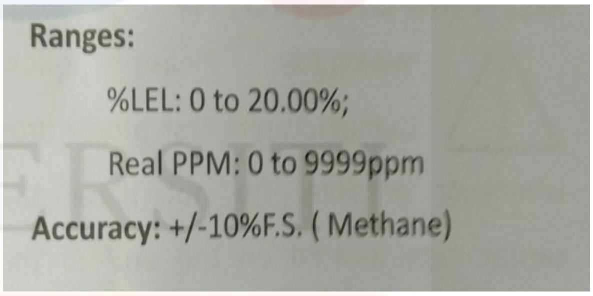
3.8.2 Second experiment: A 2.5-Fold Increase in Co-digested Waste and a 1.7- Fold Expansion in Working Volume

This batch digester, a 1000 ml conical flask, was utilised. In lab scale, the ratio of feedstock is 1:1 (CD: DP). This digester's working volume is 1100ml. In this experiment, 50g of durian peel powder and 50g of cow dung will be combined with 1000ml of water. This slurry was then placed in a 1000 ml conical flask, with the entrance of the flask being sealed with a stopper. The digester runs at room temperature. Using, a water displacement process, the created biogas is collected. By using methane detector measure the quality of the methane. A methane gas detector, HABOTEST High Accuracy Sensor HT601B Portable Gas Leak Detector, was employed for the analysis. Time frame for this experiment is 21days.

Figure 3.2: Methane gas detector



Ranges:

%LEL: 0 to 20.00%;

Real PPM: 0 to 9999ppm

Accuracy: +/-10%F.S. (Methane)

Figure 3.3: Ranges of detector

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CHAPTER 4

RESULT

4.1 Rate of biogas production (Mono-digestion and Co-digestion)

The research conducted a comparative study on the rate of biogas generation through anaerobic digestion using two different feedstocks, namely cow dung and durian peel, as well as a single feedstock, cow dung, over a 14-day period. Various ratios and particle sizes were tested to assess the efficiency of gas production. The results obtained from the anaerobic co-digestion and mono-digestion using different feedstock ratios and particle sizes, as well as the gas production efficiency, were documented in Table 4.1.

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Table 4.1: Rate of biogas production from anaerobic co-digestion and mono-digestion

Type of digestion	**Volume of water (ml)**	**Ratio**	**Size of durian peel**	**No of weeks**		**Differences in biogas production rates between week (w1-w2)**	**Total production (%)**	**Total biogas production varies with each ratio.**
				Week 1 (ml)	**Week 2 (ml)**			
Anaerobic Co-digestion	600 ml	*1:1 (CD20g:DP 20g)*	0.25mm	250ml	100ml	150ml	70%	760ml
			0.5mm	246ml	96ml	150ml	68.4%	
			6mm	50ml	18ml	32ml	13.6%	
		1:2 (CD13g:DP 27g)	0.25mm	214ml	98ml	116ml	62.4%	665ml
			0.5mm	220ml	94ml	126ml	62.8%	
			6mm	24ml	15ml	9ml	7.8%	
		2:1 (CD27g:DP 13g)	0.25mm	280ml	124ml	268ml	80.8%	876ml
			0.5mm	270ml	120ml	150ml	78%	
			6mm	60ml	22ml	38ml	16.4%	
Anaerobic Mono-digestion	**600 ml**	**1**	-	38ml	20ml	18ml	11.6%	**58ml**

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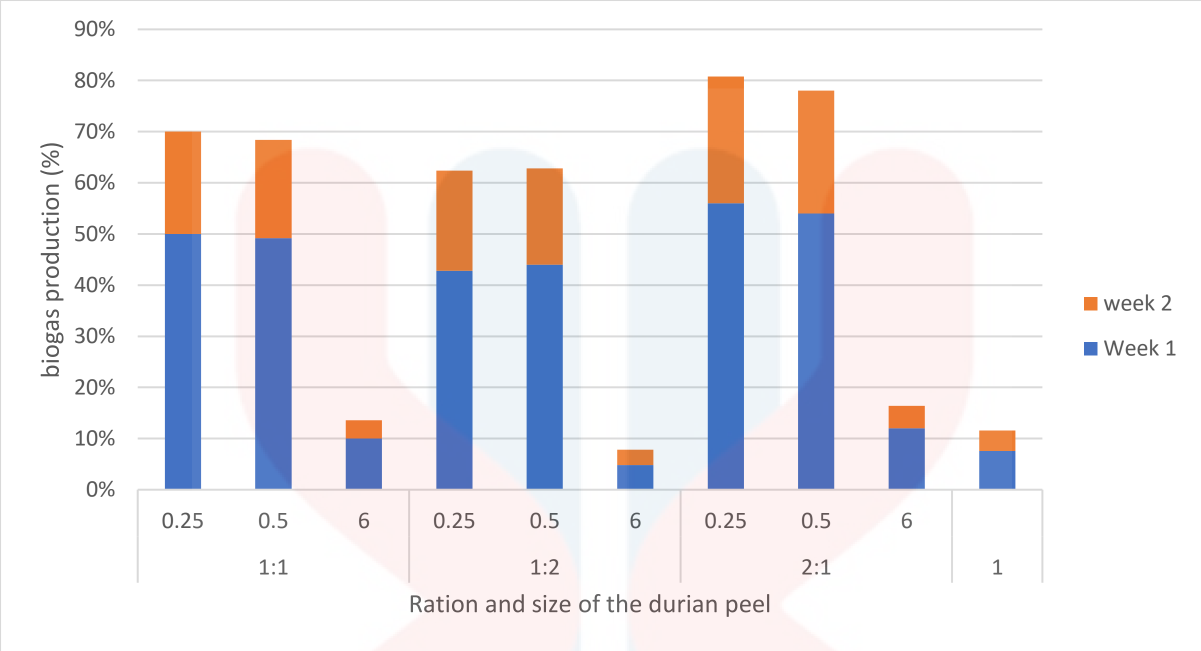

Figure 4.1: Rate of biogas production from anaerobic digestion in 2 weeks

The rate of biogas production was evaluated in anaerobic co-digestion and mono-digestion experiments with varying ratios and durian peel sizes over a two-week period. In the anaerobic co-digestion experiment with a 1:1 ratio (CD20g:DP20g), the use of 0.25mm, 0.5mm, and 6mm durian peel resulted in biogas production rates of 250ml, 246ml, and 50ml in week 1, and 100ml, 96ml, and 18ml in week 2, respectively. Within the corresponding ratio, the total biogas generation varied according to the size of the durian particle: 70% for 0.25mm, 68.4% for 0.5mm, and 13.6% for 6mm. In the 1:2 ratio (CD13g:DP27g), biogas production rates for 0.25mm, 0.5mm, and 6mm durian peel were 214ml, 220ml, and 24ml in week 1, and 98ml, 94ml, and 15ml in week 2, respectively.. According to the ratio, the total biogas generation changed with the durian peel dimension, displaying a 62.4% rise for 0.25mm, a 62.8% increase for 0.5mm, and a 7.8% increase for 6mm.

For the 2:1 ratio (CD27g:DP13g), biogas production rates for 0.25mm, 0.5mm, and 6mm durian peel were 280ml, 270ml, and 60ml in week 1, and 124ml, 120ml, and 22ml in week 2, respectively. The total biogas production varied between the size of the co-substrate, with an 80.8% increase for 0.25mm, 78% for 0.5mm, and a 16.4% increase for 6mm. The incorporation of various sizes of durian peel particles was undertaken to explore how particle size affect the efficiency of biogas production. In anaerobic mono-digestion (40g cow dung),

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the biogas production rates were 38ml in week 1 and 20ml in week 2, resulting in a 11.6% decrease in total biogas production, which was 58ml over the two weeks.

From the table, it is evident that biogas production is more significant in week 1, particularly at the ratio of 2:1, followed by 1:2 and 1:1 ratio. The highest biogas production is consistently observed across all three different types of durian peel size usage digesters. This trend persists across other ratios as well. In week 2, the same trend is followed for the ratio of 2:1, showing the highest biogas production. However, there are slight increase in the production of biogas in the ratios of 1:1 compared to 1:2. Comparatively the production of biogas is lowered in week 2 compared to week 1. This can be obviously seen from the table 4.1. This suggests that the microbial consortia in week 1 for all digesters were working efficiently to break down the substrate and produce biogas. However, in week 2, there are slight changes. In the 1:2 ratio, the microbial consortia are relatively low compared to the substrate concentration. In week 1, the optimal environment and substrate conditions support microbial metabolism, leading to efficient biogas production. However, in week 2, a decrease in biogas production, particularly in the 1:2 ratio, suggests a less favourable environment in the digester. This could result from the accumulation of intermediate products that inhibit microbial activity, disrupting the stability of the microbial community. The lower proportion of cow dung in the 1:2 ratio diminishes its buffering capacity, leading to a less alkaline environment that is conducive to microbial activity. Additionally, cow dung serves as a source of natural microbes that facilitate the anaerobic digestion process. However, as the retention time increases, microbial populations may enter the decline phase, resulting in insufficient microbes for biogas production. Overall, the observed differences in biogas production between weeks and ratios highlight the dynamic nature of anaerobic digestion processes and the importance of maintaining optimal conditions for microbial activity and biogas production. Adjustments to feedstock composition, digester operation, and environmental conditions may be necessary to maximize biogas yields and ensure efficient anaerobic digestion.

The aim of conducting both mono-digestion and co-digestion experiments is to evaluate the efficiency and efficacy of anaerobic digestion processes in producing biogas using various feedstocks. Mono-digestion assesses the biogas production potential and characteristics of a single feedstock under controlled conditions. In contrast, co-digestion investigates the synergistic effects that arise from combining different feedstocks, such as increased methane yield, enhanced process stability, and more effective utilization of organic waste materials. By

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exploring the combination of diverse organic materials, co-digestion aims to optimize biogas production and improve overall process performance.

The biogas production in mono-digestion is notably lower compared to co-digestion, as evident from the data in Table 4.1. Typically, enhanced efficiency in biogas generation is observed when employing a combination of different waste types in the biodigester. This principle boosts methane yield by fostering positive interactions in the digestion medium, leveraging bacterial diversities in various wastes, and supplying essential nutrients through co-substrates (Jin, Liu et al. 2009). This hypothesis seems to hold true as evidenced by the experimental results, where anaerobic co-digestion outperforms mono-digestion in terms of biogas production. The cumulative biogas production in anaerobic co-digestion for the ratios 1:1, 1:2, and 2:1 is 760 ml, 665 ml, and 876 ml, respectively. In contrast, the mono-digestion process only generated 58 ml of biogas.

In this particular digester, the microbial community is deprived of sufficient organic matter for effective breakdown. The inclusion of durian peel serves to increase the total solid content in the slurry, thereby aiding in the efficient breakdown of organic molecules for biogas generation. For cattle, piggery, and poultry wastes, a decrease in total solids (TS) is associated with an increase in specific biogas yield. Therefore, it is suggested to carry out biogas production at lower TS values to attain the highest specific biogas yield (Itodo and Awulu 1999).The slurry consistency is thin, indicating a low total solid waste content. It is crucial to strike a balance, as an excessive amount of durian peel particles can lead to a thick consistency in the slurry, posing an obstacle to optimal biogas generation.

The 2:1 ratio has the largest overall biogas production, followed by the 1:1 and 1:2 ratios. The digester's high total solid content and absence of microbial consortiums are the primary causes of the low biogas production. The chosen ratios determined the proportion of cow dung to durian peel, with the 1:1 ratio comprising equal quantities (20g) of both substrates. In the 1:2 ratio, the durian peel content exceeded that of cow dung (13g cow dung and 27g durian peel), while the 2:1 ratio saw higher cow dung content (27g cow dung and 13g durian peel). When the cow dung content is elevated in the 2:1 ratio digester, it signifies a higher presence of natural microbes that facilitate the breakdown of organic molecules, thereby enhancing biogas generation. maximum gas production per day from the cow dung-containing digester. This is because cow dung has a larger concentration of anaerobic bacteria and a higher carbon to nitrogen (C/N) ratio, both of which promote the anaerobic co-digestion of mixed

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substrates (Hussien, Hamad et al. 2020). The choice of substrate ratio in AcoD depends on optimizing the C/N ratio, although other factors such as pH and alkalinity also play a crucial role. Some studies have suggested that the optimal C/N ratio for AcoD is around 20, with reports indicating that the highest yield is achieved when the C/N ratio is 33, as observed when cassava pulp is co-digested with pig manure. Furthermore, the slurry's consistency is thinner than it was at a 1:2 ratio. Because of the overly high total solid waste load in the 1:2 ratio, microbial activity in the breakdown of organic matter is limited. Several studies have proposed optimal total solids (TS) values, with ranges such as 7%–9% demonstrating improved biogas yield in biogas reactors (Zennaki, Zaid et al. 1996). The increase in total solid can cause in inhibition while decrease in total solid cause instability in the system. The overall biogas generation comes in second in the conventional 1:1 ratio, indicating a balance between the microbial population and organic matter.Both can affect the generation of biogas.

In each digester, notable readings were observed in the digesters employing 0.25mm followed by 0.5mm durian peel, showcasing a significant contrast with the digester utilizing 6mm durian peel (Sharma, Mishra et al. 1988). This clearly indicates that the particle size plays a pivotal role in biogas generation (Hernández-Beltrán, Hernández-De Lira et al. 2019). Durian peel is composed of lignin (15.45%), hemicellulose (13.09%), and cellulose (60.45%) (Aimi, Anuar et al. 2014). Durian peel contains a substantial amount of fiber and lignin. Lignin poses a challenge for microbial degradation, as it forms a protective 'seal' around the cellulose crystalline structure, hindering easy hydrolysis (Khan and Ahring 2019). Before incorporating the durian peel into anaerobic digestion processes, they must undergo suitable pre-treatment, involving mechanical, thermal, chemical, biological, or a combination of these methods (Lin, Ladisch et al. 1981). Pre-treatment plays a crucial role in facilitating the digestion of lignocellulosic substrates and enhancing the overall performance of anaerobic digestion systems utilizing plant matter (Olugbemide, Lajide et al. 2020). In a study focused on enhancing biogas production from anaerobic digestion of lignocellulosic biomass, researchers found that reducing particle size to less than 5 mm in a lab-scale reactor led to increased methane yield and enhanced electric energy balance. They also discovered that physical pre-treatment, such as grinding and sieving, improved surface area accessibility, broke down lignin-hemicellulosic complexes, and increased the amount of available cellulosic content (Kainthola, Kalamdhad et al. 2019). Grinding, on the other hand, leads to smaller particle sizes, increasing the surface area of cellulosic materials. This, in turn, heightens the susceptibility of cellulose to bacterial and enzymatic attack, causing deformation of the crystal lattice, and

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reducing the degree of polymerization (Olatunji, Ahmed et al. 2021). This will lead to higher biogas generation. In simpler terms, a higher lignin content diminishes the biodegradability of the waste and lower the biogas production. A comparison of sizes reveals that 0.5mm is twice the size of 0.25mm, explaining the second-highest biogas generation in the biodigester containing 0.5mm particles. On the other hand, 6mm is twelve times the size of 0.25mm, indicating that larger durian peel particles result in a reduced surface area produced less biogas (Andersen, Parsin et al. 2020).

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4.2 pH value for anaerobic co-digestion

The pH level is a critical factor in biogas production. By evaluating the pH before and after the anaerobic co-digestion period, we can potentially identify the reasons for biogas generation. Table 4.2 displays the pH of the slurry before the experiment commenced and after 14 days.

Table 4.2: pH values for all bio-digesters in 2 weeks of retention time

Type of digestion	Ratio	Size of DP	Initial pH	Final pH
Anaerobic Co-digestion	1:1 (CD20:DP20)	0.25mm	6.8	4.3
		0.5mm	6.7	4.4
		6mm	6.8	4.3
	1:2 (CD13:DP27)	0.25mm	6.8	4.1
		0.5mm	6.8	4.3
		6mm	6.9	4.3
	2:1 (CD17:DP13)	0.25mm	6.5	4.2
		0.5mm	6.5	4.3
		6mm	6.5	4.2
Anaerobic Mono-digestion	**1**		**7.6**	**5.5**

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Table 4.2 outlines the initial and final pH values for both anaerobic co-digestion and mono-digestion. In anaerobic co-digestion, the 1:1 ratio with 0.25mm, 0.5mm, and 6mm durian peel sizes started with an initial pH of 6.8, 6.7, and 6.8, respectively. After the process, the final pH values were 4.3, 4.4, and 4.3. Similarly, in the 1:2 ratio with 0.25mm, 0.5mm, and 6mm durian peel sizes, the initial pH values were 6.8, 6.8, and 6.9, resulting in final pH values of 4.1, 4.3, and 4.3. In the 2:1 ratio with 0.25mm, 0.5mm, and 6mm durian peel sizes, the initial pH values were 6.5 for all the three digester, leading to final pH values of 4.2, 4.3, and 4.2. For anaerobic mono-digestion with a ratio, the initial pH was 7.6, and the final pH was 5.5.

In an article, it was noted that the operational pH directly influences the progress of anaerobic digestion (AD) and the formation of intermediate products. The ideal pH range for the process is suggested to be 6.8–7.4. A decrease in pH typically signifies the generation of higher levels of carbon dioxide (Kainthola, Kalamdhad et al. 2019). Furthermore, the growth rate is notably impacted by changes in pH. The initial pH values for all ten digesters indicated an alkaline condition, influenced by the alkaline properties of cow dung, which is crucial for the anaerobic digestion process. This is because biogas production involves a complex biochemical reaction driven by pH-sensitive microbes, including hydrolytic, acidogenic, acetogenic bacteria, and methanogenic archaea (Schnurer and Jarvis 2010). The pH value influences methanogenic microorganism growth and the dissociation of crucial compounds, such as ammonia, sulphide, and organic acids, in the anaerobic digestion process (Bahira, Baki et al. 2018).

Table 4.2 indicates a decline in pH with an increase in the retention period, signifying a transition in biogas production stages from hydrolysis to acidogenesis. After a two-week period, the pH levels recorded for all ten digesters were in an acidic condition. The nine digesters from anaerobic co-digestion had pH levels ranging around 4.4±1, while the anaerobic mono-digestion digester recorded a pH of 5.5. This acidity is attributed to the ongoing digestion process in the digesters. Determining the optimal mixing ratio of substrates and the inoculum to substrates ratio (ISR) is crucial in preventing the accumulation of volatile fatty acids (VFAs) and other digestion inhibitors (Owamah, Ikpeseni et al. 2022). The selection of the appropriate inoculum is vital in the process because it not only provides trace elements, moisture content, and both macro and micro nutrients, but also contributes to the system's buffering capacity (Kainthola, Kalamdhad et al. 2019).

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High VFAs concentrations and a pH reduction, inhibiting microbial consortia may reduce efficiency and stability of the digester (Xu and Li 2012). Maintaining the pH within the range of 6 to 8 is crucial for a stable anaerobic digestion process, ensuring efficient biogas generation. The biogas yield from cow dung indicated that an alkaline pH of 8.52 resulted in better yield compared to samples with acidic and neutral pH levels (Bahira, Baki et al. 2018). The drop in biogas production in the second week for all digesters could be attributed to the acidic condition of the slurry. This drop of pH in the slurry inhibit the activity of methanogenic bacteria. The production of biogas will deplet due to this.

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4.3 Determining Anaerobic Co-digestion efficiency: A 2.5-Fold Increase in Co-digested Waste and a 1.7-Fold Expansion in Working Volume

In order to gauge the efficiency of biogas generation, the study sought to increase the total solid content within the slurry and expand the working volume of the digester. 2.5-Fold Increase in Co-digested Waste and a 1.7-Fold Expansion in Working Volume. This was undertaken to assess both the quantity and quality of biogas generated. The anaerobic digestion process was carried out for a period of 21 days to determine whether a longer duration and higher total solid content in the digester resulted in a greater quantity and quality of gas produced.

Table 4.3: rate of biogas production through anaerobic co-digestion for 21 days

Feedstock	**Volume of water**	**Initial pH**	**Final pH**	**Days**	**Total gas production**	**Daily gas generation**	**Daily gas generation (%)**
Combination of cow dung and durian peel CD50:DP50	1000ml	6.8	4.3	1	0	0	0%
				2	6	6	1.2%
				3	30	24	4.8%
				4	60	30	6%
				5	120	60	12%
				6	180	60	12%
				7	250	70	14%
				8	268	18	3.6%
				9	272	4	0.8%
				10	280	8	1.6%
				11	284	4	0.8%
				12	292	8	1.6%
				13	300	8	1.6%
				14	306	6	1.2%
				15	308	2	0.4%
				16	318	10	2%
				17	312	-6	-1.2%
				18	310	-2	-0.4%

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				19	314	4	0.8%
				20	316	2	0.4%
				21	318	2	0.4%

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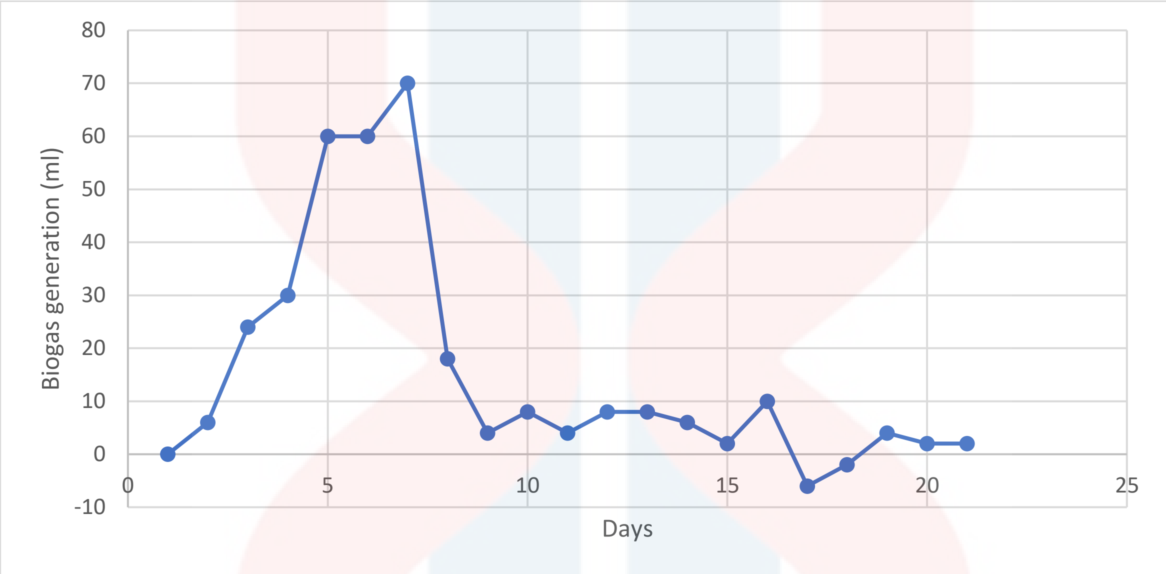

Figure 4.2: rate of biogas production through anaerobic co-digestion for 21 days

To assess anaerobic co-digestion efficiency, the co-digested waste was increased to 2.5 times the previous experiment, total 100g of total solid waste comprising cow dung and durian peel in a 1:1 ratio with 0.25mm durian peel, underwent anaerobic digestion with a volume of 1000ml of water. Untreated agricultural residues and mono-digestion typically yield less biogas due to factors such as a high C/N ratio, lignin content, and potential pesticide contamination. Anaerobic co-digestion (AcoD) is a process wherein two or more substrates are combined to address the limitations of mono-digestion and enhance the economic viability of the AD process (Kainthola, Kalamdhad et al. 2019) . The working volume for this digester increased to 91.7%, 1.7 times more than the previous experiment, aiming to examine the impact of increased total solid waste and working volume on gas generation. The initial pH was 6.8, decreasing to a final pH of 4.3 over a 21-day retention period. The total gas production reached 318ml, with daily gas generation varying throughout the retention period and daily gas generation percentages fluctuating accordingly. The gas is reduced to 2 ml upon daily inspection due to water vapour condensation.

In the first two days, there was no significant biogas production 0ml and 6ml (1.2%). Day 1 to Day, 2 hydrolysis reaction has been occurred. Low biogas production during this period is attributed to the hydrolysis stage. Hydrolysis involves breaking down complex organic substances into simpler structures. The initial slowdown is likely due to the time required for hydrolytic bacteria to effectively break down the substrate into smaller molecules. However, from day 3 to day 7, there was a noticeable increase in biogas generation 24ml (4,8%), 30ml (6%), 60ml (12%), 60ml (12%), and 70ml (14%), resulting in a total of 250ml in the first week. In day 3 and day 4 acidogenesis and acetogenesis process occur efficiently. Biogas production starts to rise as acidogenesis occurs. Acidogenic bacteria convert simple organic compounds from the hydrolysis stage into volatile organic acids, carbon dioxide, and hydrogen. Acetogenesis takes place, and acetogenic bacteria synthesize acetate, hydrogen, and carbon dioxide from the organic acids produced during acidogenesis. This substrate is used as intermediate by other bacteria to produce the biogas. Theis microorganisms work together, relying on each other and forming symbiotic relationships (Murphy and McKeogh 2004). Day 5 to day 7 methanogenesis occur efficiently. Methanogenesis occurs optimally during this period. Methanogenic bacteria convert the intermediate products from the previous stages into biogas, primarily consisting of methane and carbon dioxide. In the first week the biogas shown a notable increment (Chandra, Vijay et al. 2012).

The first week's biogas generation is notable; after that, it declines. The daily gas generation will be 18ml (3.6%), 4ml (0.8%), 8ml (1.6%), 4ml (0.8%), 8ml (1.6%), 8ml (1.6%), and 6ml (1.2%) from day 8 to day 14. This week will generate 56ml of biogas in total. A sudden drop in biogas production on day 8 is attributed to a potential change in pH. pH fluctuations can impact microbial activity, and in this case, a sudden drop may have affected the methanogenic bacteria. The pH decrease explains the shift towards an acidic slurry, forming a substrate that subsequently produces biogas (Otun, Ojo et al. 2015). Factors such as high total solid content, accumulation of volatile fatty acids (VFAs), and the growth of undesirable microbes contribute to low biogas production in the second week. Biogas production stabilizes in week 2, with no significant increase or decrease. This phase suggests a balance in the microbial community and digestion process.

The biogas output in the ensuing week varies since it is not consistent between days 15 and 21, resulting in 2ml (0.4%), 10ml (2%), -6ml (-1.2%), -2ml (-0.4%), 4ml (0.8%), 2ml (0.4%), and 2ml (0.4%) of biogas produced. The entire amount of petrol produced this week will be 12 millilitres. 318 ml of biogas were generated in total over the course of 21 days. In

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week 3 the Biogas Production Decrease (dead Zone). A decrease in biogas production in week 3 is linked to the pH of the slurry. Methanogenic bacteria are pH-sensitive, and the acidic conditions may lead to a decline in microbial activity, impacting biogas production. This does not imply that the methanogenesis process did not occur from day 1 to day 4. Based on the graph, I may conclude that every day the, each stage has been operating at its best. Methanogenesis appears to be an ongoing process, contributing to biogas production daily. However, the efficiency of the methanogenesis process seems to increase significantly after day 4 in week 1.

In the preceding experiment, more biogas was created in the two weeks at a 1:1 ratio using 0.25mm of durian peel dimension. Although there was 2.5 times less solid waste in that experiment—40g—than in the present instance, the experiment was nevertheless able to produce 350 millilitres of biogas. The low generation of biogas can be the cause of the total solid waste, headspace of the digester, pH, and the growth of undesirable microbes (Abbas, Liu et al. 2020).

The production of biogas exhibits differences in total solid waste despite the identical operational conditions. The increase in total solids (TS) levels was associated with a thicker consistency in the slurry, leading to a decrease in specific biogas yield. This decline was attributed to factors such as inhibition, limitations in mixing, and challenges in mass transfer (Jeppu, Janardhan et al. 2022). Hence, the proportion of total solid waste in the slurry plays a pivotal role in the overall process of biogas generation. Increasing total solid waste also raises the carbon-to-nitrogen (C:N) ratio. The C/N ratio, representing the balance between carbon and nitrogen, is a vital determinant of anaerobic digestion (AD) efficiency. Maintaining this ratio within an optimal range, typically between 16–25, 20–30, or 20–35, is crucial for successful AD. This ratio reflects the nutrient levels of the digestion process and significantly influences microbial activity within the reactor. A higher C/N ratio indicates a greater proportion of carbon relative to nitrogen in the substrate. This imbalance can hinder the breakdown of proteins, leading to reduced levels of free ammonia and total ammonical nitrogen, thereby resulting in low biogas production (Wang, Zhang et al. 2015). Conversely, a lower C/N ratio can lead to an excess of ammonia, which can be detrimental to the microbial community responsible for biogas production, also reducing overall production (Kainthola, Kalamdhad et al. 2019).

In anaerobic fermentation, microorganisms thrive in a natural or slightly alkaline environment to ensure effective gas production. The ideal biogas production occurs when the

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pH value of the input mixture in the digester falls within the range of 6.25 to 7.50 throughout the process (Mahanta, Dewan et al. 2004). Biogas production involves a series of reactions where specific bacteria play key roles in creating intermediates for the next stages. Optimal conditions are crucial for this process, and as the reactions progress, there may be pH fluctuations as each microbe works best under specific conditions. Ultimately, the pH tends to return to an alkaline state for efficient biogas production because methanogen sensitive to the pH and do not thrive below a value of 6.5 (Bahira, Baki et al. 2018).

Moreover, the observed decrease in biogas production in this experiment may be attributed to the increase in working volume, which is closely tied to the headspace. In comparison to the last experiment, the working volume has increased by 1.7 times. Throughout fermentation, the generated biogas accumulates in the headspace, playing a vital role in the fermentation process by contributing to the partial pressure within the container. A reduced headspace ratio or an elevated working volume can result in a higher partial pressure because there is less unoccupied space available for the biogas to gather. This limitation in headspace may lead to an increase in partial pressure, potentially suppressing biogas production (Tan, Lutpi et al. 2021). The results show that the rate of biogas production reduces as the headspace pressure increases (Liang 2021).

Certainly, the proliferation of undesirable microorganisms, specifically methanotrophs, could indeed be another contributing factor to the reduced biogas production. Methanotrophic microorganisms have the unique ability to oxidize methane, utilizing various electron acceptors, under both oxic and anoxic conditions (Guerrero-Cruz, Vaksmaa et al. 2021). In a biogas production system, the presence of methanotrophs could divert the methane towards oxidation pathways rather than its conversion to methane-rich biogas. This competition for methane could result in a decreased yield of biogas, as methane is being utilized by the methanotrophic microorganisms for their own metabolic processes.

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4.4 Methane detection for anaerobic co-digestion and mono digestion

Methane detection was conducted to evaluate the quality of the biogas produced when using various feedstock ratios, particle sizes, and feedstock contents. The aim was to determine which combination was most effective in generating high-quality biogas. Methane is particularly valuable in biogas because of its flammable properties, making it a valuable energy source.

Table 4.4: The Concentration and Lower Explosive Limit (LEL) of the anaerobic co-digestion and mono-digestion for 2 weeks

Type of digestion	**Ratio**	**Size of DP (mm)**	**Concentration (ppm)**		**LEL %**	
			Week1	**Week 2**	**Week 1**	**Week 2**
Anaerobic co-digestion	1:1 (CD20:DP20)	0.25mm	9999	9999	20.00	20.00
		0.5mm	9999	9999	20.00	20.00
		6mm	9999	2456	20.00	4.91
	1:2 (CD13:DP27)	0.25mm	9999	9999	20.00	20.00
		0.5mm	9999	9999	20.00	20.00
		6 mm	2141	1571	4.28	3.14
	2:1 (CD27:DP13)	0.25mm	9999	9999	20.00	20.00
		0.5mm	9999	9999	20.00	20.00
		6 mm	9999	3352	20.00	6.70
Anaerobic mono-digestion	1:15 (40CD:DW600)	-	9999	0855	20.00	1.71

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Table 4.5: Methane detection for anaerobic co-digestion: A 2.5-Fold Increase in Co-digested Waste and a 1.7-Fold Expansion in Working Volume for 21 days

Type of digestion	**Ratio**	**Size of DP (mm)**	**Concentration (ppm)**			**LEL (%)**		
			Week 1	**Week 2**	**Week 3**	**Week 1**	**Week 2**	**Week 3**
Anaerobic co-digestion	1:1	0.25mm	9999	1926	0227	20.00	3.85	0.45

A highly sensitive instrument was used to measure gas concentrations, specifically focusing on detecting methane in biogas. Biogas contains different gases, but methane and carbon dioxide are the main ones. The instrument has two sensitivity settings: high and low. We used the low sensitivity mode to accurately detect methane by specifically targeting methane molecules. This is important because methane plays a key role in energy generation.

Methane detection for anaerobic co-digestion and mono-digestion for various digestion ratios and particle sizes were investigated. For anaerobic co-digestion at a 1:1 ratio (CD20:DP20), concentrations of methane remained consistently high at 9999 ppm throughout Week 1 and Week 2, with a corresponding Lower Explosive Limit (LEL) of 20.00%. However, when the particle size was increased to 6mm, a noticeable decrease in concentration to 2456 ppm occurred in Week 2, resulting in a reduced LEL of 4.91%. Similar trends were observed for other digestion ratios (1:2 and 2:1), indicating the influence of particle size on methane concentrations and LEL. In the case of anaerobic mono-digestion (40CD:DW600), exhibited a constant concentration of 9999 ppm in Week 1, decreasing to 855 ppm in Week 2. The corresponding LEL values showed a decline from 20.00% to 1.71%, indicating changes in methane concentrations and flammability. Moving to the investigation involving a 2.5 and 1.7 times increase in solid waste and working volume, the methane levels exhibited stability with a concentration of 9999 ppm in Week 1, followed by a decline to 1926 ppm in Week 2, and a further decrease to 227 ppm in Week 3. Correspondingly, the Lower Explosive Limit (LEL) values showed a descent from 20.00% to 3.85% and 0.45%, indicating fluctuations in methane concentrations and flammability throughout the monitored weeks.

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The changes in methane levels and LEL values highlight shifts in gas production and composition over time. This underscores the significance of closely monitoring and comprehending gas dynamics in anaerobic digestion processes at various scales. Clearly, the 6mm digester has lower methane detection, leading to reduced gas output. This indicates that methane molecules are not effectively utilized, likely due to the larger surface area of the 6mm particle size. Table 1 clearly shows a significant decrease in biogas production in the digester with 6mm durian peel particles, highlighting insufficient gas generation and notably low methane concentration. The study reveals that as total solids (TS) increase, total biogas yield rises, and the anaerobic fermentation cycle extends. However, the efficiency of raw material utilization decreases in the context of biogas production rates (Sun, Huang et al. 2018).

This is further substantiated by the combustion test, where every digester with recorded methane detection of 9999 demonstrated positive results. The test involved the use of a burning wooden splinter. In the presence of sufficient methane gas, a distinct sound is produced during combustion. Conversely, if there is an insufficient amount of methane, the burning wooden splinter will not ignite. This phenomenon indicates a high concentration of carbon dioxide in the biogas. Biogas, being a green energy source, exhibits a pale blue flame that is challenging to see in bright conditions.

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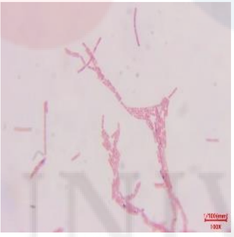
4.6 Microbial identification

Microbial identification was carried out on cow dung, durian peel, and slurry to determine the presence of bacteria and their role in biogas generation. The objective was to identify the bacterial species present and understand their contributions to the process of biogas production.

Table 4.6: Microbial identification for cow dung, durian peel and slurry

	Cow dung	Durian peel	Slurry
Sample picture			
Technique	Spread plate	Spread plate	Spread plate
Shape	Rod shaped	Rods and coccus	Rod and coccus
Gram staining image		 	 
Possible microbe	Methanogenic bacteria	Cellulolytic bacteria	Methanogenic and non-methanogenic bacteria

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Colony	Creamy colour	White colour	Creamy and yellow colour
Gram staining	Presence of gram-negative microorganisms.	Presence of gram-negative and gram positive.	Both gram negative and positive microorganisms exist.

Microbial identification for cow dung, durian peel, and slurry was conducted using the spread plate technique. Cow dung exhibited spherical in shaped microorganisms which is gram negative bacteria. In the article, the bacterial isolation process involved obtaining samples from the anaerobic digester of cow manure, leading to the successful isolation of *methanobacterium*. Methanogenic bacteria play a crucial role in biogas production, and a higher biomass of these bacteria is associated with increased rates of biogas production (Samosir, Anwar et al. 2022). Some examples, of bacteria genus that have methanogenic activity are *Methanospirillum hungatii sp.* and *Methanobacterium formicicum sp.*.

The examination of durian peel revealed the presence of both rod and coccus-shaped microorganisms, including both gram-negative and gram-positive types. It is likely that cellulolytic microbes were isolated from the durian shell waste, which exhibited natural decay (Husnah). Examples of bacteria genera known for cellulotic activity, as mentioned in the article, include *Acetobacter*, *Bacillus*, and *Clostridium* (Lai and Zhou et al., 2021) (Rao 1995).

In the slurry, a variety of microorganisms, including both rod and coccus-shaped ones, were identified using the spread plate technique. Gram staining revealed the coexistence of both gram-negative and gram-positive microorganisms. This diverse microbial community collaborates in the production of biogas. In the slurry, it is expected to have both methanogenic and non-methanogenic bacteria. In Pakistan, research has isolated both methanogenic and non-methanogenic bacteria from biogas slurry, including *Methanobrevibacter ruminantium sp, Methanobacterium formicicum sp, Peptostreptococcus sp, Clostridium difficile sp, Escherichia coli sp, Micrococcus sp, Bacillus subtilis sp, and Streptococcus bovis sp* (Khalid and Naz 2013).

From the discussion, it is evident that both the particle size of the durian peel and the ratio of feedstock components significantly impact biogas production. Smaller particle sizes tend to result in higher biogas yields due to increased surface area for microbial activity, while

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optimal ratios, particularly those with a higher proportion of natural microbes, facilitate greater biogas generation. However, there are limitations to increasing total solid waste and headspace volume, as they can lead to factors such as partial pressures that may inhibit biogas production. Nevertheless, a valuable insight gleaned from the experiments is that co-digestion enhances biogas production efficiency, underscoring the importance of utilizing diverse organic materials to maximize biogas yields.

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CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

In conclusion, our comprehensive investigation into biogas production from durian biomass waste and cow dung has provided valuable insights into sustainable waste-to-energy solutions. We embarked on this study to address the pressing challenges of municipal solid waste management and explore the potential of utilizing organic waste streams for biogas generation. Through anaerobic co-digestion and mono-digestion experiments, we systematically examined various parameters, including feedstock ratios, durian peel sizes, total solid waste, and working volumes.

Our findings underscore the significant potential of durian biomass waste, particularly durian peel, as a valuable feedstock for biogas production. The utilization of durian peel in anaerobic co-digestion with cow dung demonstrated superior efficiency compared to mono-digestion, highlighting the synergistic effects of blending diverse waste types. Notably, we identified the 2:1 ratio of cow dung to durian peel as optimal for maximizing overall biogas production, emphasizing the importance of balanced feedstock proportions.

Furthermore, we observed the influence of durian peel particle size on biogas production, with smaller particles exhibiting higher efficiency in methane generation. Pre-treatment processes such as grinding proved instrumental in reducing particle size and enhancing substrate accessibility for microbial activity. Our investigation into gas dynamics and composition, including methane concentrations and the Lower Explosive Limit (LEL), provided crucial insights into biogas flammability and composition, informing safe utilization practices.

Microbial identification deepened our understanding of the microbial communities involved in anaerobic digestion, highlighting the roles of methanogenic bacteria in cow dung and cellulolytic microbes in durian peel. However, we also encountered challenges such as pH

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fluctuations, headspace pressure, and the potential proliferation of undesirable microorganisms, emphasizing the importance of careful system design and operation.

In summary, our study contributes to advancing knowledge in anaerobic digestion processes and underscores the potential of utilizing organic waste streams for sustainable biogas production and waste management. Through our comprehensive investigation, we aim to inform the development of efficient and effective biogas production systems, paving the way for a greener and more sustainable future.

5.2 Recommendation

In conclusion, the research emphasizes the importance of optimizing various factors to maximize biogas production from durian biomass waste. By understanding the impact of parameters like feedstock ratios and durian peel sizes, we can develop more efficient and economically viable biogas production systems. These insights are crucial for addressing waste management challenges and promoting renewable energy generation, especially in tropical regions where durian biomass waste is abundant.

Moving forward, further research and development in biogas production should focus on refining process optimization and technology innovation. Integrating biogas systems into existing waste management infrastructure can also enhance sustainability efforts. Additionally, policymakers should prioritize the implementation of sustainable waste management policies to incentivize the adoption of renewable energy sources like biogas. Collaboration between researchers, industry stakeholders, and policymakers is essential for driving the adoption of sustainable waste-to-energy solutions and mitigating the environmental impact of waste disposal. Together, we can pave the way for a greener and more resilient future.

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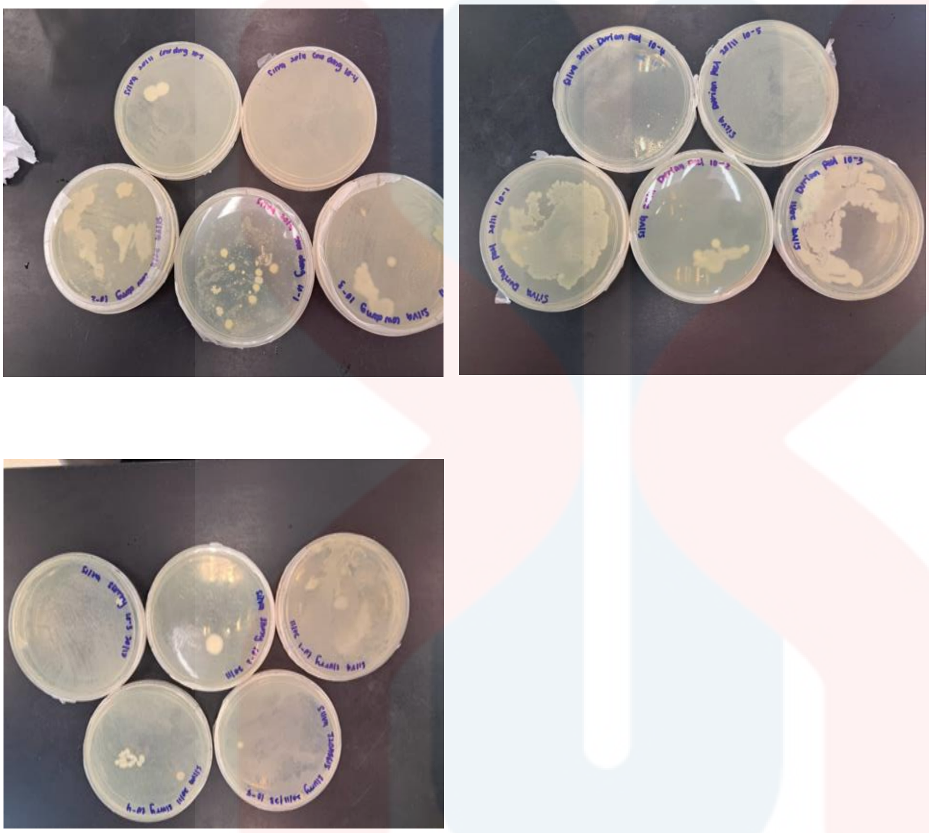
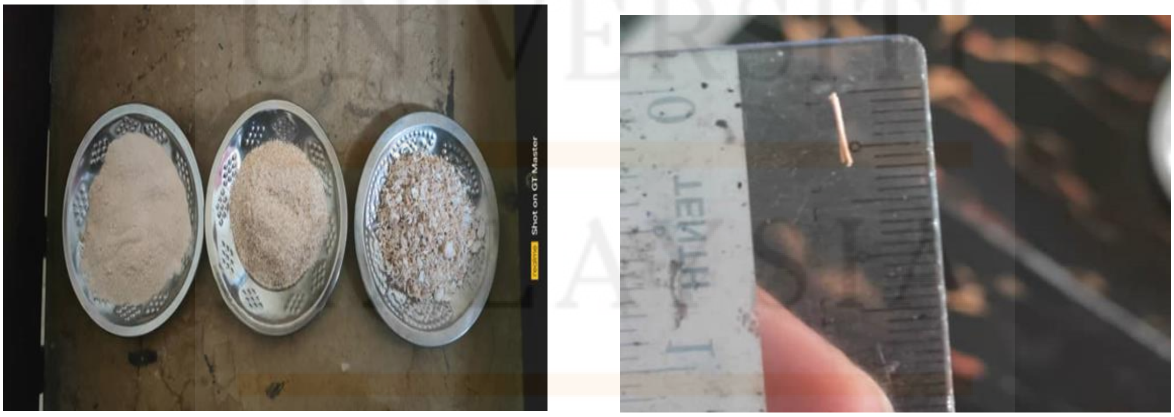
APPENDIX A



Microbial colony growth by using cow dung, durian peel and slurry.



Size of durian peel

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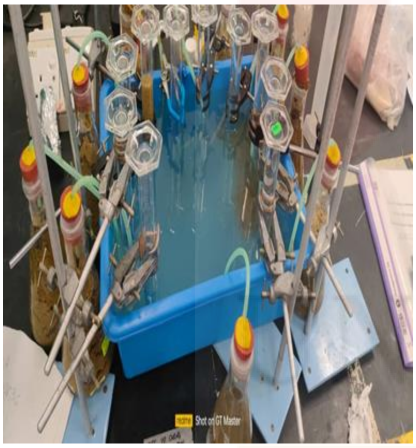
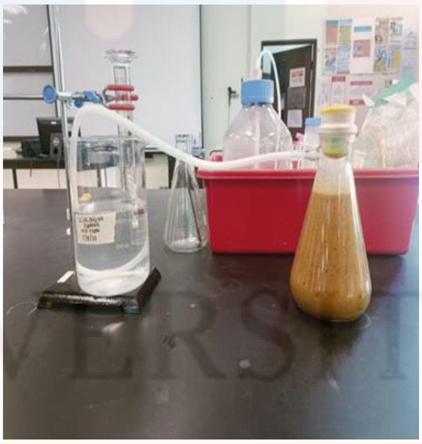
Methane gas reading in low sensitivity





Experimental setup

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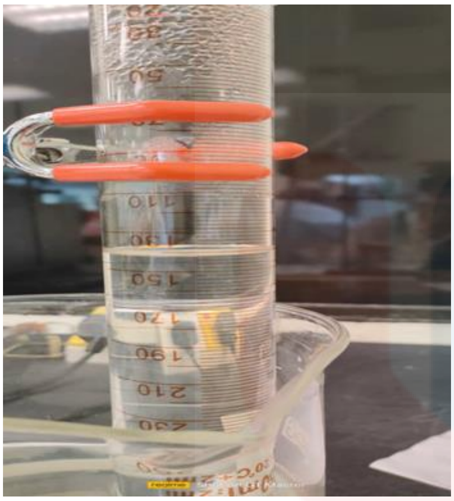

Biogas trapped in measuring cylinder by water displacement method

Growth of undesired microbe

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