

BIOMETHANE PRODUCTION FROM POTATO
AND PSEUDOSTEM BANANA WASTE BY
ANAEROBIC DIGESTION

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BIOMETHANE PRODUCTION FROM POTATO AND PSEUDOSTEM BANANA
WASTE BY ANAEROBIC DIGESTION

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ABSTRAK

Tujuan kajian ini adalah untuk menghasilkan gas metana daripada campuran sisa makanan melalui proses penghadaman anaerobik. Pencernaan anaerobik mampu mengubah sisa makanan menjadi tenaga boleh diperbaharui yang bermanfaat melalui proses semula jadi kerana proses ini menghasilkan gas metana yang tinggi yang boleh digunakan untuk menjana tenaga elektrik. Proses ini memerlukan reaktor untuk mengawet sisa makanan dalam kaedah sistem tertutup kerana pencernaan anaerobik tidak memerlukan oksigen untuk pertumbuhan. Oleh itu, dalam penyelidikan ini, mereka bentuk dan fabrikasi reaktor adalah salah satu tujuan untuk menghasilkan gas metana. Beberapa pengiraan diperlukan seperti jumlah gas yang dihasilkan di mana oleh gas piawai yang dihasilkan dalam AD diperlukan untuk menganggar pengeluaran biogas maksimum. Masa pengekalan juga diperlukan dalam mereka bentuk reaktor. Tempoh pengekalan kajian ini ialah 28 hari dan sementara itu, pH dan isipadu biogas sehari diperlukan untuk dipantau bagi mengesan perubahan proses dalam reaktor yang boleh menyebabkan kekurangan pengeluaran metana. Untuk menghasilkan gas metana, pra-rawatan diperlukan kerana sebahagian daripada sisa tersebut terdiri daripada hemiselulosa yang mempunyai struktur ikatan berganda. Untuk memecahkan ikatan berganda, rawatan fizikal telah digunakan dalam kajian ini kerana ia berkesan untuk meningkatkan luas permukaan yang boleh diakses, memecahkan kompleks lignin-hemiselulosa, dan bahan selulosa yang boleh digunakan. Keputusan yang ditunjukkan dalam kajian boleh digunakan untuk penyelidikan lanjut untuk meminimumkan pengeluaran sisa makanan.

ABSTRACT

The purpose of this study is to produce methane gas from food waste mixture by anaerobic digestion process. Anaerobic digestion is able to turn the food waste into a beneficial renewable energy through a natural process as this process highly produces methane gases which can be used to generate electricity. This process requires a reactor to preserve the food waste in a closed system method as anaerobic digestion doesn't need oxygen for growth. Thus, in this research, designing and fabricating the reactor is one of the purposes to produce methane gas. A few calculations are needed such as the amount of gas produced where the standard gases produced in AD are needed to estimate maximum biogas production. Retention time is also required in designing the reactor. The retention time of this study is 28 days and meanwhile, the pH and volume of biogas per day are needed to monitor in order to trace the changes of the process in the reactor which could lead to a lack of methane production. In order to produce methane gas, pre-treatment is needed as some of the waste consists of hemicellulose which has a double bond structure. In order to break down the double bond, physical treatment was applied in this study as it is effective for increasing accessible surface area, breaking down lignin-hemicellulosic complexes, and the usable cellulosic material. The results shown in the study can be used for further research in order to minimize food waste production.

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LIST OF SYMBOLS

%	Percentage
°C	Celcius
C	Carbon
C/N	Carbon nitrogen
CO ₂	Carbon dioxide
C/N	Carbon nitrogen
H	Hydrogen
N	Nitrogen
ml	Milliliter
O ₂	Oxygen gas
pH	Potential of hydrogen
Q	Flowrate
S	Sulphur
So	Solid concentration
V	Volume
w/w	Weight/weight

LIST OF ABBREVIATIONS

AD	Anaerobic Digestion
ATSDR	Agency For Toxic Substances and Disease Registry
COD	Chemical Oxygen Demand
GC-TCD	Gas Chromatograph with Thermal Conductivity Detector
HRT	Hydraulic Retention Time
MHLG	Ministry Of Housing and Local Government
OLR	Organic Load Retention
TS	Total solid
TVS	Total Volatile Solid

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Food waste is refer as an edible pieces of food that are produced or harvested for human consumption but are not eaten by humans and usually occurs during the retail and consumption periods as a result of a decision to dump food, regardless of the reason .Food waste come from many ways such as from new produce that deviates from what is considered desirable is often withdrawn from the supply chain, items that are close to or beyond their expiration date are frequently discarded by manufacturers and customers, and food is often left over or unwanted in food service industries or homes, and is ultimately discarded.(Patra et al., 2020). Food waste is one of the most daunting problems that the environment has had to face lately. This is due to its many negative effects on food sustainability, fair utilization of natural resources, and environmental deterioration. Food that is never consumed necessitated a significant amount of farm land to cultivate crops and rear cattle, as well as a massive amount of water for field irrigation and animal drinking. Furthermore, consistent energy sources are needed at various stages of food production, such as manufacturing, transportation, and storage, resulting in substantial greenhouse gas emissions. (Harun et al., 2019) (Adelodun et al., 2021)..

According to the Food and Agriculture Organization (FAO), one-third of all edible pieces of food produced for human consumption are discarded worldwide, amounting to 1.3 billion tonnes of food each year and according to research, 40–60% of a typical consumer's annual household waste is food waste (Flanagan & Priyadarshini, 2021) According to research, food deficiency per capita in Central and West Asia and North Africa is 6–11 kg per year, compared to 95–115 kg per year in North America and Europe. (Mirmohamadsadeghi et al., 2019) (Mirmohamadsadeghi et al., 2019).

In Malaysia, about more than 1000 tonnes/day of food waste produced every. According to the Ministry of Housing and Local Government (MHLG), household food waste volume stands at 8,745 tonnes/day, which is 3,192,404 tonnes/year, comprising more than 38.32 percent of all total waste produced, more than restaurants, which produce 941,608 tonnes/year, 23.35 percent (Jereme, 2017). Malaysia is known for their culturalism where there about more 10 festivities celebration occur every year. For the Muslim in Malaysia, every year they will celebrate Ramadhan festivities where they need to fast and only eat when the time has come. SW Corp conducted a survey that examined 8,861 kg of food leftovers in ten market bazaars during the festive Ramadhan season and the total of 170 waste disposal sites were registered by 2016, with only 14 having the status of sanitary landfill (Sciences et al., n.d.). The reasons for this increasing amount of food waste was due to improvements in dietary patterns when people's living conditions have changed over time, allowing them to afford more food items than previously as well as steady population growth (contributed to the huge rise in food waste) and urbanization (Lim et al., 2016).

In the manufacture of carbonaceous adsorbent, any carbonaceous substance might be employed as a raw material. The high carbon concentration of palm oil sludge makes it perfect for making carbonaceous adsorbents. Biochar generated from POME sludge may be used to minimise a number of pollutants in water. Biochar is one of the most studied compounds for dye adsorption in textiles. Biochar is both a cost-effective and a highly efficient material (Li, 2019).

Food waste can be reduced in a variety of ways such as anaerobic digestion process. Anaerobic digestion is a method to turn the food waste into a beneficial renewable energy through natural process. This method is one of the oldest technologies found in this world. This method was developed via the development of the septic tank system around 1870, the idea of anaerobic digestion was introduced. The first anaerobic digestion plant was installed in the United States in 1939 to process the organic fraction of urban solid waste, while many anaerobic digestion plants have been built in Europe during the last few decades (Pramanik et al., 2019). Anaerobic digestion process occurs in 4 phases starting from hydrolysis, acidogenesis, acetogenesis and

methanogenesis. This phases occur naturally and this method doesn't need any oxygen during the process.

1.2 Problem Statement

Grocery store produced food waste. There are many explanations for food waste production in grocery retail. The key reason, according to Norden (2011), is the difficulty in marketing food that is about to become unsellable. Unsalable food includes items with an outdated "best by" date, unlabeled fresh fruits and vegetables, and items with minimal packaging or cosmetic harm (Norden, 2011; Papargyropoulou et al., 2014). Furthermore, Norden (2011) claims that imperfect market forecasting is a major problem for supermarket retail stores, which may result in significant food waste. Customers' purchasing decisions are influenced by a variety of influences, including temperature, season, fashion patterns, celebrity endorsements, competitor deals, and personal mood. Developing and implementing modern, more sophisticated demand forecasting strategies is critical for supermarkets of all sizes (Mena et al., 2010). There are many types of food waste produced from grocery store such as vegetable waste and fruit waste where about not able to be sell. One of the vegetable wastes is from potato. According to the Waste and Resources Action Programmed (WRAP), the potato is the second most wasted food product, with around 5.8 million potatoes thrown away every day and the key sources of loss and waste in the fresh potato supply chain include field loss (1–2 percent), grading loss (3–13 percent), storage loss (3–5 percent), packaging loss (20–25 percent), and retail waste (1.5–3%) (Jagtap et al., 2019). Potatoes were the only food in the top eight most discarded in the FFV (fresh fruit and vegetables) department that had the bulk of the waste in the in-store waste division. Rejections are the leading source of waste in the other materials. Potatoes were available in both bagged (with a best-before date) and lose weight form. The bulk (83 percent) of potato in-store waste came from items sold in containers, despite the fact that processed potatoes accounted for just 27 percent of sales. Owing to the higher waste for packaged potatoes relative to potatoes delivered piecemeal, it is apparent that packets are a possible aim for reducing in-store waste of potatoes. The best-before date of 10 days on packets could be the primary issue, since it is determined by the estimated shelf-life of

the potato in each bag with the shortest shelf- life. This also ensures that if one potato is unsellable due to poor consistency, the whole bag is thrown away. When sold in halves, buyers choose the potatoes based on their visual appearance, and any potatoes that do not meet the criteria can be picked out and discarded as singles. As a result, removing packets and their related best-before dates may potentially reduce potato in-store waste.

There is other waste that very highly produced in agriculture especially in plantation which is banana stem. The banana plant's stem, also known as the pseudo stem, grows a single bunch of bananas before dying and being replaced by a new pseudo stem (Anhwange et al., 2009). According to reports, the banana is the world's second most grown fruit in terms of size, accounting for about 16% of overall fruit production (Mohapatra et al., 2010). Since each plant grows only one bunch of bananas, this crop produces a considerable amount of residue. Following harvest, the bare pseudo-stem is removed and then left on the plantation or burnt, potentially causing environmental problems (Cordeiro et al., 2004) such as contaminating water bodies and threatening the atmosphere and the health of living microorganisms (Aziz et al., 2011, Hossain et al., 2011). If banana tree waste is not well handled such as if it spilled in wet weather, it can emit greenhouse gases and also, farmers dumped banana tree waste in river sand wetlands, where it eventually dissolved and produced methane and other gases that dispersed putrid odors and harmed the surrounding environment (Ahmad & Danish,2018).

1.3 Objectives

The purpose of this research is:

- To design and fabricate anaerobic digestion reactor
- To check the performance of the methane production from potato with pseudo stem banana waste by anaerobic digestion.

1.4 Scope of Project

The scope of this project is identifying the best materials, dimension and size of the anaerobic reactor by designing the anaerobic reactor using AutoCAD modelling. Besides, fabricating the anaerobic reactor based on the requirement by parameter and the factor that effect the methane gas production. The requirement of the anaerobic digestion is such as temperature. The temperature that will be used is on mesophilic temperature where will be around 30 to 40 degree Celsius (Kothari et al., 2014). In this project, pseudo stem banana waste from agricultural waste will be used as the second co digestion while for the first is from grocery waste like potato and fruit waste. The potato waste will add and mix with pseudo stem banana while the fruit waste will add mix with pseudo stem banana. Based on previous study from Chen year 2021 title “Methane production and characteristics of the microbial community in the co-digestion of potato pulp waste and dairy manure amended with bio char”, it showed that 3:1 ratio is quite balanced for the production of methane gas due to different pH of the wastes. Those study showed that the pH from anaerobic digestion process have neutral and acidic For this study, the pH of the potato waste was 6.5 which suit for methanogenic micoorganisms to produced methane while for the pH of the pseudo stem banana was 5.99 to 6.00 which a little acid which might affect the production of methane gas. Next, recording the temperature from anaerobic reactor every day to maintain the optimum value which is 6.5-7.5. Last but not least, analyzing the production of bio-methane by collecting using gas chromatography (TCD) and the data obtained based on the pH, chemical oxygen demand (COD) and total solid (TS) from potato, fruit waste and pseudo stem banana by anaerobic digestion for 28 days.

1.5 Significant of Study

This study will introduce the method of renewable energy from biomass where used the waste from grocery and agricultural waste. Methanogenic microorganisms are the last in a chain of microorganisms, which degrade organic material and return the decomposition matters to the environment. In this process biogas is generated, a source of renewable energy. This process produces a combination of gases, mostly methane. Methane can be use as to generate electricity as the source of fossil is getting decrease every year. This study can help to ensure clean and healthy surroundings.

Biomethane can be reduces greenhouse gas emissions by preventing additional fossil fuel burning and it is therefore a great way to combat global climate change. The other significant is able to control the excessive pseudo stem banana waste from dumped in the river and also from burned that can cause the pollution such as air pollution and water pollution.

CHAPTER 2

LITERATURE REVIEW

2.1 Potential sources of organic biomass for biomethane production

2.1.1 Food waste

Food waste is a source of energy contained mainly in landfills, where it rots and emits greenhouse gases into the atmosphere. Food waste is difficult to treat and recycle since it contains high levels of sodium, salt, and moisture and is mixed in with other waste during processing.

Since the load of food waste in industry is increasingly rising, an effective food waste management system must be developed in order to ensure its eco-friendly and safe disposal. Consequently, there is a pressing need to explore more viable recycling solutions. Anaerobic digestion has been used effectively in Europe and Asia to stabilize food waste and develop beneficial end-products (Zhang et al., 2007).

2.1.2 Potato waste

Whole potatoes, peels, cooking oil, and rotten meat are also examples of potato waste. It's all thrown into big bins and transported by digesters, pumps, and other machinery that breaks it down and converts the organics into gas. Potato waste is of little quality to be used as animal feed, and it is therefore disposed of as a slurry (Parawira et al., 2004). Potato contains biodegradable components such as starch and proteins, which could be used for biogas production through anaerobic digestion (Chen et al., 2021). Potato contains high levels of nutrients and decomposing (Zhang, Caldwell, Zealand, et al., 2019) and it also consist C/N ratios from 12.1/1 to 30.0/1

which promising the feedstock for anaerobic co-digestion with other low carbon substrates (Zhang, Caldwell, Zealand, et al., 2019).

2.1.2.1 Characteristics of potato waste

(Jacob et al., 2016)

Parameter	Unit	Potato waste
Moisture	(% , w/w) ^a	84 ± 4.5
Total solid		16 ± 5.5
Total volatile solid	(% , w/w) ^b	90 ± 2.3
Ash		10 ± 7.7
Carbohydrate		43 ± 2.2
Protein		8.75 ± 0.4
C		40 ± 2.2
N		1.4 ± 0.71
H		6.9 ± 0.06
S		0.17 ± 0.07
C/N	-	ranges from 12.1/1 to 30.0/1
COD	(g/L)	14.85 ± 0.75
Phosphorous (P)	(% , w/w) ^b	14.85 ± 0.75
Potassium (K)		1.05 ± 0.045

Table 2.1 Characteristics of the potato wastes

Potato waste has a high percentage of moisture (84%) and volatile solids (90%). Further biochemical analysis reveals that it contains 43–45 percent (w/w) total carbohydrates, 1.4 percent (w/w) nitrogen, 8.75 percent (w/w) protein, and COD 14.85 g L⁻¹, with a C: N ratio of 28.57. Potato it is easy to infer that the chosen potato waste is extremely biodegradable and demonstrates not only the capacity for biological treatment but also the quantity of usable resources that remain unutilized. The pH for the potato waste is 6.5.

2.2 Food waste from agricultural residues

Agricultural residues, which are largely composed of lignocellulose wastes, are a cost-effective and sustainable source of second generation carbon-neutral biofuels. This constitute plant biomass waste, which is typically composed of photosynthesis-derived cellulose, hemicellulose, and lignin. Agricultural residues are generated when commercially valuable crop products are harvested and residues such as straw, stoves, peelings, cobs stalks, and bagasse are left over. The total annual output of agricultural residues in 2010 was approximately 5.1 billion dry tonnes. The waste produced by the agriculture, forestry, and aquaculture industries is increasing as the population grows; hence, waste from this sector would increase (Ghimire et al., 2015)

2.2.1 Pseudo stem banana

Banana is a large perennial herbaceous flowering plant of the genus *Musa*. It is a tall arborescent monocotyledon with a false stem (pseudo stem) made up of leaf sheaths and an underground true stem (corm) capable of producing suckers through vegetative reproduction (Mukhopadhyay et al., 2008). The pseudo stem is a cylindrical aggregation of leaf stalk bases that is clustered (Tock et al., 2010; Mukhopadhyay et al., 2008). It bears banana fruit only once in its life cycle, and waste is produced four times for each cycle of processing. Rotten fruit, peels, fruit-bunch stem, leaves, pseudo stem, and rhizome are all examples of banana waste (Abdullah et al., 2014). Following the crushing of the banana fruit, banana pseudo stem becomes abundant as waste, and is either left to rot at the nearby dumpsite, polluting the natural area, or left to decompose at the plantation, serving as organic soil fertilizer.

2.2.1.1 Composition of pseudo stem banana

New banana pseudo stem has a moisture content of 94 – 96 percent (Farjana Begum et al., 2014 – 2015; Li et al., 2010). The pseudo stem is a lignocellulose substance composed primarily of cellulose, hemicellulose, and lignin. The cellulose content ranges from 31 to 44 percent, hemicellulose from 12 to 33 percent, and lignin from 6 to 14 percent, as seen in the table below. BPS has a higher holocellulose (cellulose + hemicellulose) content than other agricultural residues used for bioethanol

processing, such as rice straw, wheat straw, corn stove, and so on, and it also has a lower lignin content than woody biomass, as seen in the table below. The sum of each polymer in each plant varies in various parts of the same plant and can be affected by a plant's cultivar age and geographic place of cultivation (Jahn et al., 2011).

Table 2 . 2 Chemical composition of lignocellulose biomass (adapted from Sun and Cheng 2002)

Plant biomass	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Hardwood stems	40 – 55	25 – 40	18 – 25
Softwood stems	45 – 50	25 – 35	23 – 35
Nut shells	25 – 30	25 – 30	30 -40
Corn cobs	45	35	1 5
Grasses	25 – 40	35 – 50	10 – 30
Paper	85 – 99	0	0 – 15
Wheat straws	30	50	1 5
Sorted refuse	60	20	2 0
Leaves	15 – 20	80 – 85	0
Cotton seed hairs	80 – 95	5 – 20	0
News papers	40 – 50	25 – 40	18 – 30
Waste paper from chemical pulps	60 – 70	10 – 20	5 – 10
Solid cattle manure	1.6 – 4.7	1.4 – 3.3	2.7 – 5.7

Coastal Bermudagrass	25	35. 7	6 . 4
Switch grass	45	31. 4	1 2
Swine waste	6.0	28	N / A
Primary wastewater solids	8 – 15	N/ A	24 – 29
*<u>Banana pseudostem</u>	31 – 44	12 – 33	6 – 13

Pseudo stem banana contains 31-44% of cellulose, 12-33% hemicellulose and 6-13% lignin. Pseudo stem fiber are biodegradable with high degree of crystallinity with a spiral angle of about 15° but will be decreased after 3 months due to low strength (Subagyo & Chafidz. Achmad, 2018).

2.2.2 Lignocellulose biomass

Lignocellulose cell walls are made up of various amounts of cellulose, hemicellulose, and lignin. This ratio, however, varies depending on the type of plant, such as hardwood, softwood, and herbaceous plants. Aside from the three main elements, lignocellulose contains a trace of pectin, nitrogenous compounds, and ash (Chen, 2014). The cellulose micro fibrils in plant cell walls are encrusted in lignin and hemicellulose in a complex architecture, as seen in Figure below (Mussatto & Teiseira, 2010). This, along with cellulose crystallinity, allows untreated cellulose biomass resistant to enzymatic hydrolysis to release fermentable sugars (Wu et al., 2011a; Sánchez, 2009).

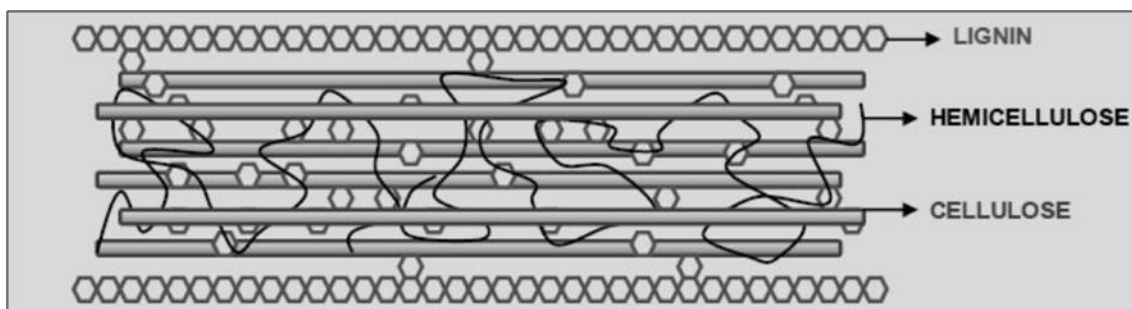


Figure 2.1: Schematic of lignocellulosic plant cell wall structure (Mussatto & Teiseira, 2010).

2.2.2.1 Composition of lignocellulosic materials

The essence of the feedstock determines the composition of biomass. (Mosier et al., 2005). Cellulose is the most abundant, accounting for 30–70 percent of lignocellulosic biomass; hemicelluloses and lignin account for 15–30 percent and 10–25 percent of the biomass, respectively. (Monlau et al., 2011).

2.2.2.2 Cellulose

Cellulose is a fibrous, tough, water-insoluble substance found in plant cell walls, especially in stalks, stems, trunks, and all woody portions of plant tissues (Sullivan, 1997). Cellulose is a linear polymer composed of glucose units joined together by β -1,4-glycosidic bonds to form cellobiose. This is shown by the mathematical formula $(C_6H_{10}O_5)_n$ and its chemical structure in Fig. 1.2 (Sandgren et al., 2005)

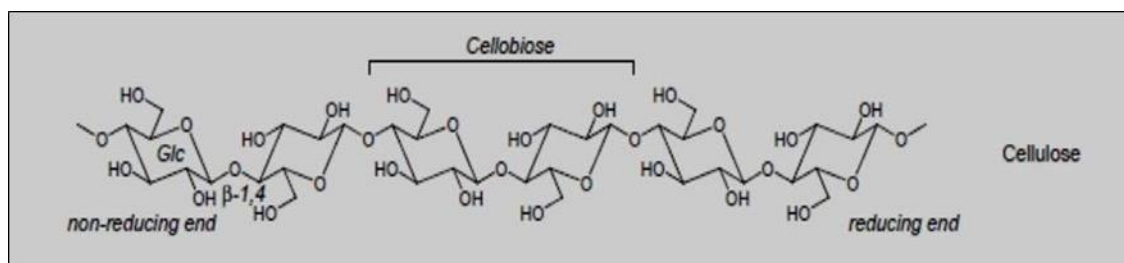


Figure 2.2 Chemical structure of cellulose (Sandgren et al., 2005).

The reducing end of the polymer is the end of the glucan chain with anomeric carbon that is not attached to the next glucose. The other end of the polymer is referred to as the non-reducing end (Sandgren et al., 2005). The length of the cellulose chains, i.e. the degree of polymerisation (DP), ranges from 2000 glucose units or less in the primary wall to 15000 or more in the secondary walls (Quiroz-Castaneda & Folch-Mallol, 2013; Sandgren et al., 2005; Cowling, 1958). As seen in Figure 1.3 (Sánchez, 2009; Cowling, 1958), hydrogen bonds or van der Waals forces tie cellulose molecules together, forming microfibrils. This hydrogen bonding within the cellulose chains can play a role in determining the chain's "straightness." Depending on the regularity of the bonds, inter-chain hydrogen bonds can insert order or disorder into the structure (Sánchez, 2009; O'sullivan, 1997).

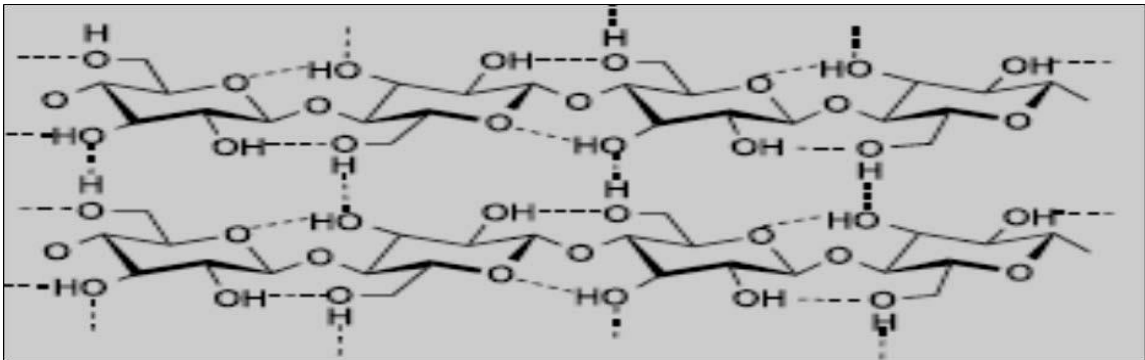


Figure 2.3 Cellulose chains held together by hydrogen bonds (Sánchez, 2009)

As seen in Figure above, chain regions that are strongly oriented (ordered) are referred to as crystalline, whereas those that are less ordered are referred to as amorphous. These areas are scattered throughout the cellulose chains (Lynd et al., 2002; Cowling, 1958).

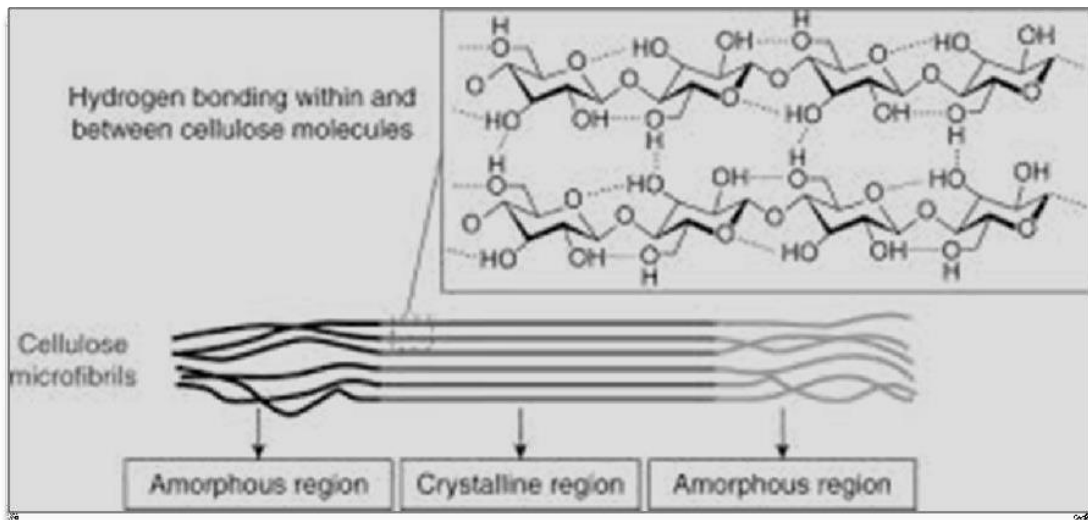


Figure 2.4 Homopolymer structure of crystalline and amorphous cellulose

Since cellulose is crystalline, it means a structural order in which all of the atoms are fixed in distinct positions with respect to one another. Specific micro-fibrils that are closely packed block enzymes, or small molecules of water, from penetrating (Lynd et al., 2002). Twists and turns in the cellulose fibrils preclude an ordered organisation of the cellulose fibrils in the amorphous areas (Quiroz-Castaneda & Folch-Mallol, 2013).

2.2.2.3 Hemicelluloses

Hemicellulose is the second most available natural resource capable of being turned into usable end goods (Satyanarayana et al., 2012). Hemicelluloses are heterogeneous polymers that can be readily hydrolyzed by acids to their monomeric bases, which are composed of pentoses (D-xylose and D-arabinose), hexoses (D-glucose, D-mannose, and D-galactose), and sugar acids (Pérez et al., 2002; Sjöholm et al., 2000; Sjöstrom, 1993). Hardwood hemicellulose differs from softwood hemicellulose. Hardwood is primarily composed of glucuronoxylan, while softwood is composed primarily of glucomannans (Chen, 2014; Kumar et al., 2008; Pérez et al., 2002; Jeffries, 1994). Hemicelluloses are categorised based on the primary sugar

residue in the backbone, such as xylans, mannans, and glucans, with xylans and mannans being the most common (Saha, 2003; Pérez et al., 2002). Xylan is the main ingredient of hemicellulose in most plant cell walls, accounting for about one-third of overall plant biomass; it has a β -1,4-D-xylopyranose backbone with a number of side chains (Prade, 1996). The side chain structure and linkages define the form of xylan (Gielkens et al., 1997). Linear homoxylan, arabinoxylan, glucuronoxylan, and glucunoarabinoxylan are examples of xylan forms (Saha, 2003). The precise chemical composition and structural features of hemicellulose vary depending on plant type, subcellular area, and developmental stage (Saha, 2003).

Arabinoxylans are grasses (graminaceous plants) with a high concentration of β -L-arabinofuranosides and acetyl groups connected to the β -1,4-D-xylan backbone through β -(1,2,3) linkages. The esterified acetyl groups are connected to the hydroxyl groups of carbon 2 or carbon 3. Since the xylans of hardwood (Angiosperm) are heavily substituted with 4-O-methyl-glucuronic acid and acetyl groups, they are referred to as glucuronoxylans (Fig. 2.8a). β -(1-2) glycosidic bonds connect 4-O-methyl-glucuronic acid to the xylan backbone, while ester connected acetyl groups bind to hydroxyl groups of carbon 2 or 3 (Pérez et al., 2002; Sjöholm et al., 2000; Jeffries, 1994). Softwood (Gymnosperm) xylans, on the other hand, are not acetylated, but the xylan backbone is connected at carbon 2 with 4-O-methyl-D-glucuronic acid and carbon 3 with β -L-arabinofuranosyl moiety. Softwood hemicellulose is dominated by galactoglucomannan, which accounts for 15 – 20% of the dry weight, and xylan, which accounts for 7 – 10% of the biomass dry weight (Sjöholm et al., 2000). Fig. below shows a galactose side branch connected to the main mannose chain by β -(1-6) linkages in softwood glucomannan (Pérez et al., 2002; Jeffries, 1994).

2.2.2.4 Lignin

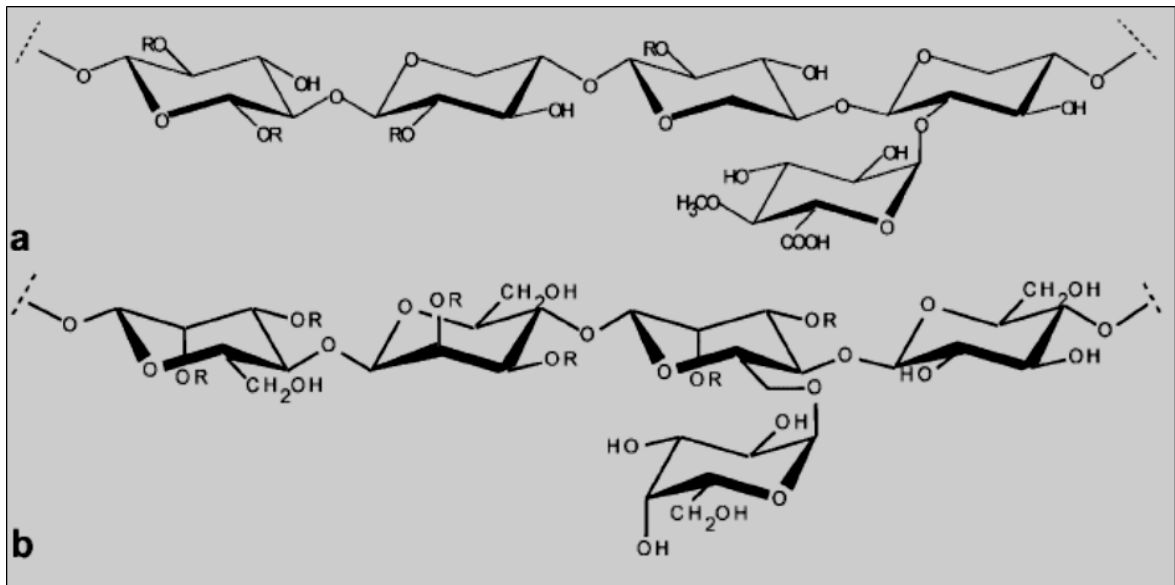


Figure 2.5 A structure of O-acetyl-4O-methylglucuronoxylan from angiosperms and O-acetyl-galactoglucomanna from gymnosperms (Perez et al., 2002).

Lignin is the third plant polymer which offers the stability and strength that plants need. As seen in Figure 1.6 lignin is an aromatic and hydrophobic polymer derived from one, two, or three related phenyl-propanoids: -coumaryl alcohol (-hydroxyphenyl propanol), sinapyl alcohol (syringyl propanol), and coniferyl alcohol (quaiacyl propanol) (Boerjan et al., 2003), which are isolated from the amino acid phenylalanine through an enzymatic process (Leisola et al., 2012; Jeffries, 1994). These phenyl-propane units are joined together by an unusual C-C and C-O coupling (Chen, 2014). The proportions of the three structural monomers differ between plant families (Chen, 2014; Leisola et al., 2012).

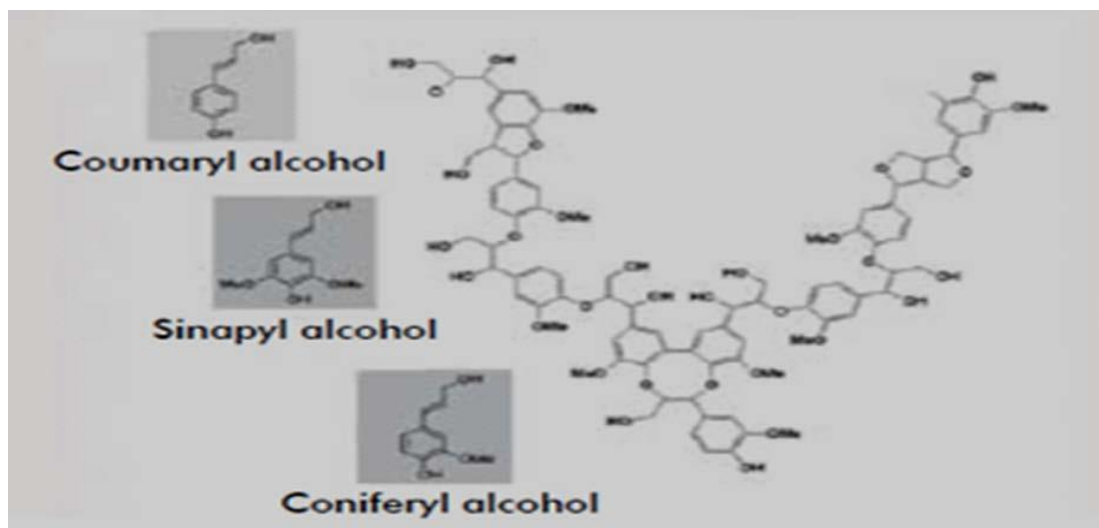


Figure 2.6 Structure of lignin formed by the polymerization of the three phenolic alcohols,(Boerjan et al., 2003).

Coniferyl alcohol is the main constituent of lignin in softwood, while coniferyl and sinapyl alcohols are found in hardwood lignin (Chen, 2014; Perez et al., 2002; Jeffries, 1994). All three phenolic alcohols are found in grass lignin. Both softwoods, hardwoods, and grasses have common lignin inter-monomer linkages (Jeffries, 1994). The polymerization of phenolic alcohols results in a heterogeneous configuration, as seen in Figure 1.6, in which the basic units are joined by C-C and aryl-ether linkages, with the aryl-glycerol -aryl ether being the dominant structure (Perez et al., 2002).

2.2.2.5 Chemical characteristics of pseudo stem banana

Type of biowaste	DS	VS	COD	Cellulose	Hemi-cellulose	Glucose	Holo-cellulose	Lignin	N-tot	P-tot	K	C/N-ratio	pH	Reference
	%	% of DS	g/L	% of DS	% of DS	% of DS	% of DS	% of DS	% of DS	% of DS	% of DS			
Banana pseudo stem	6.6	83.2											5.9	Velmurugan and Ramanujam, 2011
Dried banana steam	92	83						15						Kalia et al., 2000
Banana pseudo stem		98.5		63.9			65.2	18.6						Khalil et al., 2006
Banana pseudo stem	8.3	81		42.2				13.3	0.3	0.2	4.1			Velásquez Arredondo et al., 2009
Banana pseudo stem				44			16.5	8.1						Goncalvo Filho, 2011
Banana pseudo stem									2.8	0.4	4.2			Rahman, 2012
Banana pseudo stem	4	91.8		39.1		52.2	72.7	8.9						Li et al., 2010
Banana pseudo-stem		86		34-40		74	60-65	12						Mohapatra et al., 2010
Banana pseudo-stem		97.0		42.1	18.6			5.1						Aziz et al., 2011
Banana pseudo-stem		86.4					57.5	20.3						El-Zawawy et al., 2011
Banana pseudo-stem	5	90		52.3	9.9			11.2	0.6			66.0		Romero-Anaya et al., 2011
Banana pseudo-stem		84.6		55.5				22.3	1.4			27.5		Rosal et al., 2012
Banana pseudo-stem		85.4					65.2	12.7						Cordeiro et al., 2004
Average	7.5	87.9	48.9	47.0	13.0	63.1	55.4	13.4	1.5	0.4	4.3	38.2	6.0	
Banana stem and leaves			46.9						0.9	0.2	4.1	53		Formowitz et al., 2007
Banana pseudo-stem pith	13.6	21.6		10.8		11.7		3.3						Deivanai and Kasturi Bai, 1995
Banana pseudo-stem core		89.9		27.4		11.0		4.6						Aziz et al., 2011
Banana pseudo-stem juice	2.3-2.6	27-38	10.5-14.3										5.3-6.1	Calzada et al., 1988

Table 2.3 Characteristic of pseudo stem banana (adapted from Saraiva et al., 2012)

Sl. No	Parameters	Values
		Pseudo stem
1.	Total Solids (TS), %	15
2.	Moisture Content, %	85
3.	Volatile Solids (VS), %	11.37
4.	Ash content, %	2.4
5.	Fixed Carbon, %	1.2
6.	Total Kjeldahl Nitrogen (TKN), mg/l	34.2
7.	Total Organic Carbon (TOC), mg/l	941.5
8.	C : N Ratio	27.53

Table 2.4 Characteristics of pseudo stem banana (Divyabharathi et al., 2017)

The chemical oxygen demand (COD) is estimated about 10.5 to 48.9 g/L with pH about 5.9 to 6.0 pH.

2.3 Anaerobic digestion

AD is a biochemical mechanism in which microorganisms convert organic substrate into biogas in the absence of oxygen. It is the mechanism that not only produces renewable biogas energy (methane 50-75 percent and carbon dioxide (CO₂) 25-50 percent), but also leads to the elimination of greenhouse gas emissions, eutrophication, dissolved oxygen depletion, and so on. Temperature, pH, C/N ratio, alkalinity, organic loading rate, hydraulic holding time, and VFA concentration all have an impact on AD (Mussoline et al., 2017). The composition and heating value of the emitted biogas are determined by the substrate used and the digestion conditions given.

The AD mechanism consists of four stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis, all of which are carried out by various types of microorganisms such as acidogens and methanogens. The hydrolytic bacteria convert complex biopolymers such as lipids, carbohydrates, polysaccharides, proteins, and nucleic acid into soluble compounds such as monomers, starch, amino acids, fatty acids, purines, and pyrimidines in the first phase of AD. Acidogenesis is the process by which fermentative bacteria convert condensed monomers, starch, amino acids, and fatty acids into intermediate propionic, butyric acid, and other acids.

Acetogenesis is the mechanism by which acidogenesis products are converted into acetate by homoacetogens. Methanogenesis occurs at the end of the process, in which methane (biogas) is formed from acetate and carbon dioxide by two types of methanogens: acetoclastic (acetate consumers) and hydrogen using methanogens (carbon dioxide reducing methanogens). Acetoclastic methanogens use acetate to produce methane and carbon dioxide, while hydrogen-utilizing methanogens use carbon dioxide and hydrogen as electron acceptor and donor, respectively, to produce methane (Shah et al., 2015). The various functional microbial associations are complex, and a mismatch between microbial groups influences reaction rate and allows inhibitory substances to accumulate. Methanogens are thought to have the slowest growth rate of any microbial community, so their growth is thought to be the rate limiting stage in the AD phase. In the case of lignocellulosic substances, however, hydrolysis is the rate limiting stage. The AD process is divided into solid and liquid-state AD (LS-AD) based

on the TS content; AD with a TS content greater than 15% is categorised as solid-state AD (SS-AD), while AD with a TS content less than 15% is classified as LS-AD (Zhao et al., 2014; Zheng et al., 2018).

AD systems typically have greater reaction speed and shorter retention periods, whereas SS-AD systems have a smaller reactor capability and need less energy to stabilise the floating of lignocellulosic material.

2.3.1 Landfill gases process

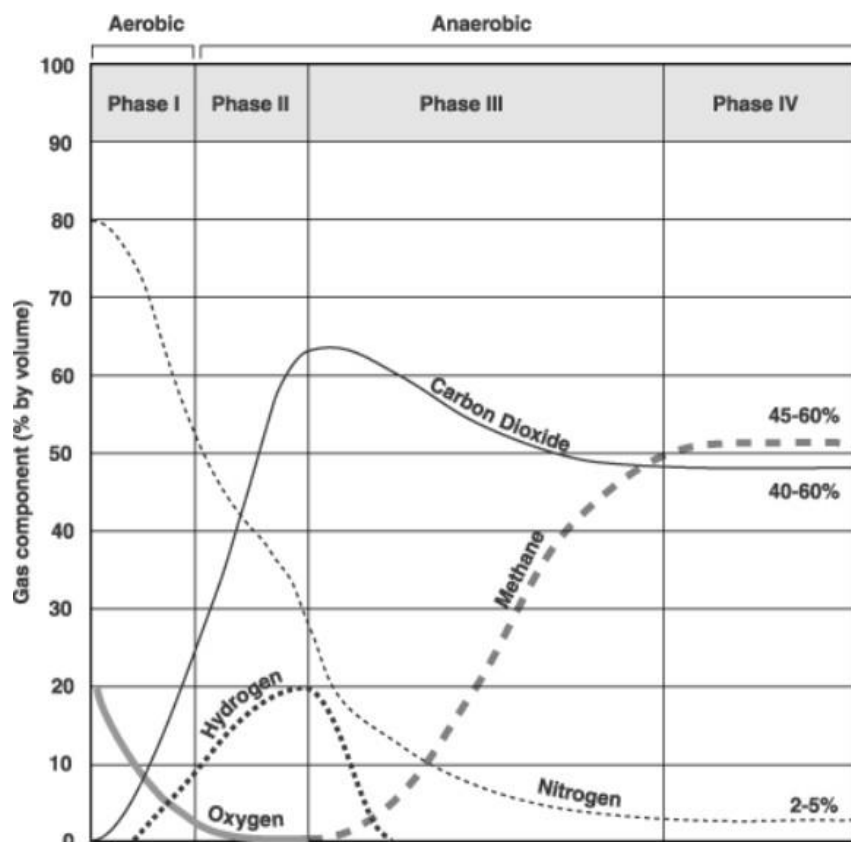


Figure 2.7 Landfill Gas Basics. In Landfill Gas

Bacteria break down landfill debris in four stages. With each of the four stages of decomposition, the makeup of the gas produced varies. Landfills frequently receive garbage over a 20- to 30-year period, therefore waste at a landfill may be in many stages of decomposition at the same time. This implies that older garbage in one region

will be recycled. may be in a different stage of decomposition than newly buried rubbish in another location.

In phase 1, aerobic bacteria that only exist in the presence of oxygen, consume the oxygen while breaking down the lengthy molecular chains of complex carbohydrates, proteins, and lipids that compose organic waste during the initial phase of decomposition. Carbon dioxide is the principal output of this process. The nitrogen level is high at the start of this phase, but it decreases as the landfill progresses through the four phases. Phase I lasts until all available oxygen is gone. Phase I decomposition might take days or months, depending on the amount of oxygen available when the garbage is disposed of in a landfill. The amount of oxygen in the soil will vary depending on factors such as how loose or compacted the trash was when it was buried.

In phase 2, after the oxygen in the landfill has been depleted, the decomposition begins. Bacteria use an anaerobic process (one that does not require oxygen) to transform chemicals produced by aerobic bacteria into acetic, lactic, and formic acids, as well as alcohols such as methanol and ethanol. The landfill deteriorates into a very acidic environment. As the acids react with the moisture in the waste, some nutrients dissolve, making nitrogen and phosphorus accessible to the landfill's increasingly diversified species of bacteria. Carbon dioxide and hydrogen are the gaseous byproducts of these activities. If the landfill is disturbed or oxygen is injected into it, the microbial activities will revert to Phase I.

In phase 3, Decomposition begins when anaerobic bacteria devour the organic acids generated in Phase II and produce acetate, an organic acid. As a result of this process, the landfill becomes a more neutral environment in which methane-producing bacteria may establish themselves. Bacteria that produce methane and acid have a symbiotic, or mutually beneficial, connection. Acid-producing bacteria produce chemicals that methanogenic bacteria ingest. Methanogenic bacteria eat carbon dioxide and acetate, both of which are poisonous to acid-producing bacteria if consumed in excess.

For last phase, when the composition and production rates of landfill gas stay essentially stable, decomposition occurs. Phase IV landfill gas typically comprises 45

percent to 60 percent methane by volume, 40 percent to 60 percent carbon dioxide by volume, and 2 percent to 9 percent other gases such as sulphides. Gas is generated at a constant rate in Phase IV, normally for around 20 years; however, gas will continue to be vented for 50 years or more after the trash is disposed of (Crawford and Smith 1985). Gas production might remain longer, for example, if there are more organics in the trash, such as at a landfill that receives more than typical volumes of domestic animal waste (Atsdr, 2001).

2.3.2 Anaerobic digestion process

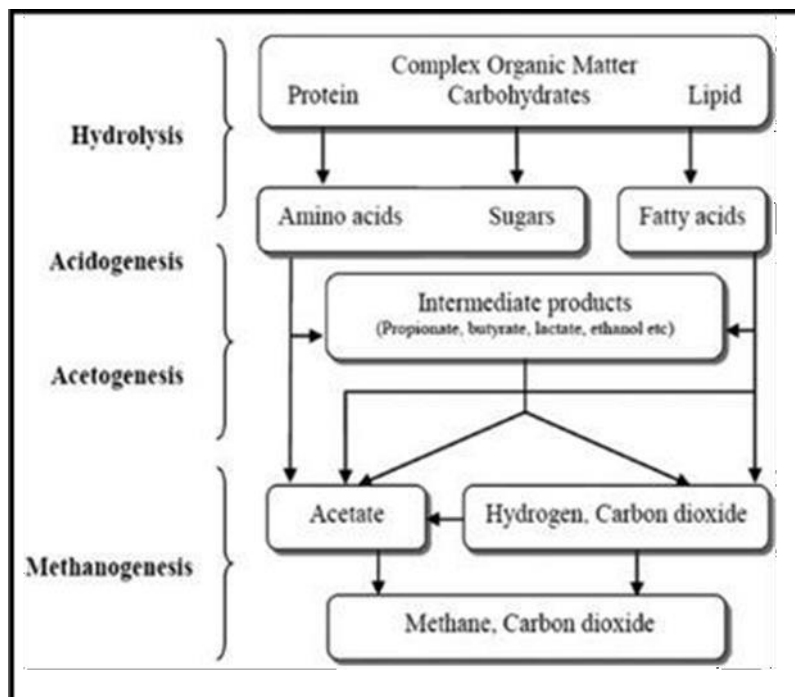


Figure 2.8 AD process

2.3.2.1 Hydrolysis

The initial phase in anaerobic digestion processes is hydrolysis. During the hydrolysis process, extracellular enzymes such as cellulase, amylase, protease, or lipase hydrolyze complex organic materials such as carbohydrates, proteins, and lipids into soluble organic molecules such as sugars, amino acids, and fatty acid (2005) (Parawira et al.) Hydrolytic bacteria are bacteria that hydrolyze the substrate. These facultative anaerobes produce extracellular enzymes. If the substrate comprises big molecules

(particulates) with a low surface-to-volume ratio, hydrolysis may be the rate-limiting step (Vavilin et al., 1996). If the substrate is easily degradable, acetogenesis and methanogenesis will be the rate-limiting steps (Björnsson et al., 2001). When the substrate is hydrolyzed, it becomes accessible for cell transport and can be destroyed by fermentative bacteria during the acidogenesis stage that follows.

2.3.2.2 Acidogenesis

The soluble organic compounds from hydrolysis are used by fermentative bacteria or anaerobic oxidizers in the acidogenesis stage (Garcia-Heras, 2003). These bacteria are obligatory as well as facultative anaerobes. The major breakdown pathway in a stable anaerobic digester produces acetate, carbon dioxide, and hydrogen. Intermediates such as volatile fatty acids and alcohols play just a tiny influence. This breakdown pathway provides a greater energy output for bacteria, and the products can be directly consumed by methanogenic microbes (Schink, 1997). When the concentration of hydrogen and formate is high, however, the fermentative bacteria will change their course to generate more reduced metabolites (Angelidaki et al., 2002). The results of the acidogenesis stage include roughly 51% acetate, 19% H₂/CO₂, and 30% reduced compounds such as increased VFA, alcohols, or lactate (Angelidaki et al., 2002). The acidogenesis phase is commonly regarded as the quickest stage in the anaerobic digestion of complex organic materials (Vavilin et al., 1996).

2.3.2.3 Acetogenesis

Acidogenesis intermediates include fatty acids with more than two carbon atoms, alcohols with more than one carbon atom, and branched-chain and aromatic fatty acids. These products cannot be utilized directly in methanogenesis and must be oxidized to acetate and H₂ in the acetogenesis phase by obliged proton reducing bacteria in a syntrophic connection with hydrogen users. Low H₂ partial pressure is required for thermodynamically advantageous acetogenic processes (Schink, 1997). Acetogenesis products are subsequently used as substrates for the final phase of anaerobic digestion, known as methanogenesis.

2.3.2.4 Methanogenesis

Methanogenesis is the final stage of anaerobic digestion that produces methane gas from acetate, hydrogen, and carbon dioxide. Archaea that are strictly obligate anaerobic perform methanogenesis. (Hinds et al. 2016). Methanogens contribute to the maintenance of a stable pH by metabolising acetate, which increases alkalinity and enhances buffering ability. Methanogenic bacteria are extremely infectious and need extremely complex operating survive and to ensure uninterrupted methane gas supply Methanogens are particularly sensitive to pH changes, and grow at neutral pH levels, but can live at pH levels as low as 6.5. (Meegoda et al. 2018). Methanogens are also temperature sensitive and do not react positively to temperature shock. (Rittmann and McCarty 2001). They still have the longest doubling time of any microorganism in the AD phase, so plants must account for their sluggish growth by not overloading digesters until there are adequate methanogenic microorganisms' archaea is a kind of algae. The process chemistry is based on the assumption that carbon dioxide is the primary electron acceptor in anaerobic digestion. Although acetate fermenting methanogens do not use carbon dioxide as an electron acceptor in operation, it does help to stabilise the stoichiometric equation. Coefficients based on the assumption of carbon dioxide (Rittmann and McCarty 2001)

2.3.3 Bacteria in AD process

2.3.3.1 Hydrolytic microorganisms

The most complex particulate organic matter (polymers) is converted into dissolved basic materials during the first step of the AD procedure. The behaviour of hydrolytic microorganisms (Clostridia, Micrococci, Bacteroides, Butyrivibrio, Fusobacterium, Selenomonas, Streptococcus) drives this conversion mechanism and Exoenzymes (cellulase, cellobiase, xylanase, amylase, protease, lipase) excreted by fermentative bacteria are needed to break down complex compounds such as proteins, amino acids, and carbohydrates into mono- and disaccharides and to allow the conversion of lipids are broken down into long-chain fatty acids and glycerin

(Chernicharo 1997; Merlin Christy et al. 2014). Due to the creation of poisonous or undesirable chemicals, this is the most time-consuming stage in the AD method (Ariunbaatar et al. 2014). If the substrate is in the form of particles, hydrolysis often restricts the AD speed (Bouallagui et al. 2005). Then, an intensification of the hydrolysis mechanism leads to an improvement in digestion efficiency (Yu et al. 2016). Since they can induce lysis or disintegration of the substrate and enable the release of intracellular matter, biological, chemical, and mechanical pretreatments, or a combination of these, can be used to accelerate hydrolysis, allowing better access to anaerobic bacteria microorganisms, reducing the time spent in the digester (Ferrer et al. 2008).

2.3.3.2 Acidogenic microorganisms

Acidogenic fermentative microorganisms (Streptococcus, Lactobacillus, Bacillus, Escherichiacoli, Salmonella) convert soluble hydrolysis materials into compounds such as fatty acids, alcohols, lactic acid, carbon dioxide, hydrogen, ammonia, and urea as well as hydrogen sulphide (Chernicharo 1997; Merlin Christy et al. 2014). The pH of the system decreases as organic content is converted into organic acids. This acidic environment promotes the growth of acidogenic and acetogenic microorganisms, all of which prefer a mildly acidic pH (4.5–5.5). Since acetic and butyric acids are the desired precursors for methane production, which occurs in the final stage of the organic material degradation process, the acetic and butyric acids formed in this step are critical in the overall performance of the AD (Hwang et al. 2001).

2.3.3.3 Acetogenic microorganisms

Acetogenic bacteria process the compounds produced during the acidogenic period in the third phase, forming hydrogen, carbon dioxide, and acetate. A significant number of hydrogen ions are produced during the synthesis of acetic and propionic acids, allowing the pH of the aqueous medium to decrease (Chernicharo 1997; Mes et al. 2003; Gkamarazi 2015). The optimal pH for acetogenic microorganism action is about 6. This microorganism evolves slowly and are vulnerable to changes in organic loadings and environmental conditions (Merlin Christy et al. 2014).

2.3.3.4 Methanogenic microorganisms

Methanogenesis is the final stage of the AD process. Methanogenic archaea facilitate the degradation of organic compounds originating from the acetogenic process during this phase. Methanogenic archaea are classified into two types: acetoclastic, which degrades acetic acid or methanol to produce methane, and hydrogenotrophic, which produces methane using hydrogen and carbon dioxide (Chernicharo 1997; Mes et al. 2003; Gkamarazi 2015). Hydrogenotrophic methanogens outperform acetoclastic methanogens in terms of resistance to environmental change. Methanogens, unlike other microorganisms, prefer a mildly alkaline atmosphere (Merlin Christy et al. 2014) around 6.5–8 (Kothari et al. 2014). A well-balanced AD mechanism happens as all of the chemicals produced in one metabolic stage are transformed in the next without the aggregation of intermediate products, resulting in the full breakdown of organic matter in final products of interest such as methane, carbon dioxide, hydrogen sulphide, and ammonia (Bouallagui et al. 2005). However, if the goal is to produce hydrogen by fermentative processes, it is critical to block or inhibit methanogenic bacteria by regulating temperature, pH, or by using additives, since certain bacteria absorb hydrogen for methane production (Wang and Zhao 2009).

2.3.4 Types of Bacteria in AD process

Phase	Microorganism	Role
Hydrolysis	Bacteriodes, Clostridium,and Acetivibrio	Breaks down complex substrates to monomers and dimers
Acidogenesis	Clostridium, Peptococcus, Selenomonas, Campylobacter, and Bacteroides	Converts monomers and dimers into volatile fatty acids, short chainorganic acids and alcohols.
	Enterobacterium, Acetobacterium andEubacterium	Converts monomers and dimers into volatile fatty acids, short chainorganic acids and alcohols.
Acetogenesis	Syntrophomonas, Syntrophus, Clostrid- ium,and Syntrobacter	Converts the volatile fatty acids and the alcohols to acetates,hydrogen, and car- bonic anhydride
Methanogenesis	Methanosarcina spp. AndMethanothrix spp.	Converts acetates to methane
	Methanobacterium, Methanococcus,	Converts hydrogen to Methane
	Methanogenium and Methanobrevibacter	

Table 2.5 Bacteria in AD process

Bacteria that take part during mesophilic temperature in anaerobic digestion is such as Clostridium, and Methanosarcina spp.

2.3.5 Microbial community with cellulolytic abilities

(Adapted from Khan et al., 2016)

Microorganisms	Examples of cellulase producers
Fungi	<p>Soft-rot fungi</p> <p><i>Aspergillus niger</i>; <i>A. nidulus</i>; <i>A. oryzae</i>; <i>A. terreus</i>; <i>Fusarium solani</i>; <i>F. oxysporum</i>; <i>Humicola insolens</i>; <i>H. grisea</i>; <i>Trichoderma longibrachiatum</i>; <i>T. harzianum</i>; <i>T. reesei</i>;</p> <p><i>T. atroviride</i>; <i>Chaetomium cellulyticum</i>; <i>C. thermophilum</i>; <i>Neurospora crassa</i>; <i>Penicillium fumigosum</i>; <i>P. occitanis</i>; <i>P. brasilianum</i>; <i>P. decumbens</i>; <i>P. echinulatum</i>;</p> <p><i>Melanocarpus albomyces</i>; <i>Thermoascus aurantiacus</i>; <i>Mucor cernelloides</i></p>
	<p>Brown-rot fungi</p> <p><i>Coniophora puteana</i>; <i>Lanzites trabeum</i>; <i>Poria placenta</i>; <i>Tyromyces palustris</i>; <i>Fomitopsis sp.</i></p>
	<p>White-rot fungi</p> <p><i>Phanerochaete chrysosporium</i>; <i>Sporotrichum thermophile</i>; <i>Trametes versicolor</i>; <i>Agaricus arvensis</i>; <i>Pleurotus ostreatus</i>; <i>Phlebia gigantean</i></p>
Bacteria	<p>Aerobic bacteria</p> <p><i>Acinetobacter junii</i>; <i>A. anitratus</i>; <i>Acidothermus cellulolyticus</i>; <i>Anoxybacillus sp.</i>; <i>Bacillus subtilis</i>; <i>B. pumilus</i>; <i>B. amyloliquefaciens</i>; <i>B. licheniformis</i>; <i>B. circulans</i>;</p> <p><i>B. flexus</i>; <i>Bacteriodes sp.</i>; <i>Cellulomonas biazotea</i>; <i>Cellvibrio gilvus</i>; <i>Eubacterium cellulosolvens</i>; <i>Geobacillus sp.</i>; <i>Microbispora bispora</i>; <i>Paenibacillus curdlanolyticus</i>;</p> <p><i>Pseudomonas cellulose</i>; <i>Salinivibrio sp.</i>; <i>Rhodothermus marinus</i></p>
	<p>Anaerobic bacteria</p> <p><i>Acetivibrio cellulolyticus</i>; <i>Butyrivibrio fibrisolvens</i>; <i>Clostridium thermocellum</i>;</p> <p><i>C. cellulolyticum</i>; <i>C. acetobutylium</i>; <i>C. papyrosolvens</i>; <i>Fibrobacter succinogens</i>; <i>Ruminococcus albus</i></p>
Actinomycetes	<p><i>Cellulomonas fimi</i>, <i>C. biazotea</i>, <i>C. uda</i>; <i>Streptomyces drozdowiczii</i>; <i>S. lividans</i>;</p> <p><i>Thermomonospora fusca</i>; <i>T. curvata</i></p>

Table 2.6 Microbial community with cellulolytic for anaerobic digestion

2.4 Operating parameters

The microorganisms involved in anaerobic digestion collaborate in a symbiotic relationship, and if one microbe fails, the whole digester can go bad. Because of this symbiotic relationship, different operational conditions are needed to keep the digester stable and each organism operating as efficiently as possible

2.4.1 Organic load rate and solids concentration

The amount of influent entering a given sized continuous or semi-continuous digester over a unit of time is referred to as the loading rate. The following equation can be used to measure the organic loading rate (OLR):

$$\text{OLR} = \frac{S_0 \times Q}{V}$$

where S_0 refers to the influent solids concentration (kg/m³ VS or COD), Q refers to the flow rate (m³ /time) and V (m³) refers to the volume of the reactor. The optimal organic loading rate is often determined by the type of reactor and the substrates used in the digester. According to the Water and Environment Federation, the average wastewater anaerobic digester runs at a rate of 1.6 to 6.4 kgVS/ m³.day (Roos et al. 2004). Loading concentrations should be kept constant to prevent startling the bacteria in the digester. Loading shocks are particularly dangerous to methanogenic bacteria (Meegoda et al. 2018). Loading rates can increase in the presence of higher flow rates or higher solids concentrations. As a result, flow rates in larger operations such as wastewater treatment plants can be stabilised through holding tanks (Labatut and Pronto 2018). To avoid an excessive organic loading volume, solids concentrations must also be maintained at acceptable amounts. Anaerobic digestion can also take place at different solids concentrations. AD can be classified as low solids (15 percent TS), medium solids (15-20 percent TS), or heavy solids (>20 percent TS) depending on the total solids (TS) concentration (Kothari et al. 2014). Low solids AD takes more water but is easier to stabilise the structure. High solids AD reduces added water consumption but can be more difficult to keep intact due to the need for higher quantities of

inoculum, longer retention periods, and a greater propensity for VFA aggregation (Kothari et al. 2014).

2.4.2 Temperature

Temperature has a significant impact on enzyme production, microbial development, methane output, and fertilizer efficiency. In anaerobic digestion, there are three major operating conditions: psychrophilic (10-30 °C), mesophilic (30-40°C), and thermophilic (50-60°C). Raising the temperature in the reactor would increase the output of methane gas up to around 60 degrees Celsius (Labatut and Pronto 2018). Most digesters, however, function in the mesophilic range. Methanogens that thrive in the mesophilic spectrum are more resistant to temperature fluctuations, lowering the chance of the reactor sour. Thermophilic environments can also reduce food waste solubilization (Labatut and Pronto 2018). Psychrophilic environments are often seen only in small anaerobic digesters, which are commonly used in homes and small farms. This range is not recommended for large- scale applications due to the high expense of larger reactors needed for psychrophilic environments, which is not always commercially feasible or sustainable at industrial scales. The operation of two-phase anaerobic digestion, where the first phase operates in thermophilic conditions with a shorter retention period and the second phase operates in mesophilic conditions with a longer retention time, is one method for achieving the advantages of both mesophilic and thermophilic digesters.

2.4.3 pH value

One of the most influential parameters on the process of anaerobic digestion is pH as it can affect the equilibrium between most chemical species. The anaerobic digester contains a consortium of microorganisms with different optimal pH ranges. Specifically, the acid- producers favour a pH range of 5.0- 8.5, whereas methanogens prefer a pH range of 6.5-8.0. Optimally, anaerobic digesters are run within a pH range of 7.0-8.5, outside this range imbalance can occur. In addition, methane production is reported to cease once the pH drops below 6.0. In order to maintain a stable pH within the digester, it is vital that the alkalinity is kept high and steady. Alkalinity can be considered the quantity of basic compounds within the bioreactor. At high alkalinity

values, the buffering capacity is higher thus contributing to the stabilisation of the pH. Alkalinity is predominantly based upon carbonate (CO_3^{2-}) in equilibrium with dissolved carbon dioxide (CO_2). Substrates which are protein rich may also contribute to the alkalinity as ammonia is released as the proteins are broken down. Specifically, carbon dioxide produced during anaerobic digestion solubilises due to the partial pressure of gas within the digester, and reacts with water reversibly to form carbonic acid.

2.4.4 C/N ratio

The carbon-to-nitrogen ratio of an organic substrate is denoted by the C/N ratio. It is important to keep the C/N ratio in the optimum range for effective AD. A C/N ratio of 16- 25, 20-30, or 20-35 has been found to be optimal for healthy AD. It exposes the nutrient levels of the digestive process, making the system vulnerable to the C/N ratio. Poor protein solubilisation happens as a result of a high C/N ratio, which results in low FA and overall ammonia nitrogen in the environment. A high C/N ratio also reduces the supply of nitrogen, which is needed to sustain the desired microbial flux in the reactor, resulting in lower biogas production and vice versa. Feedstock with an exceedingly low C/N ratio increases the likelihood of ammonia inhibition in the system; high stored ammonia is harmful to methanogens and reduces biogas output (Gupta et al., 2012; Kondusamy and Kalamdhad, 2014). Few experiments have shown that the C/N ratio varies with temperature. Ammonia inhibition was detected at temperatures of 35°C and 55°C, with C/N ratios of 15 and 20, respectively (Chen et al., 2016). Co-digestion of straw and manure at 25 C/N ratios resulted in a net methane yield of 341 L/(Kg of VS added). C/N ratios of 25 and 30 delivered the maximum combined methane output volumes, which were three times those of a C/N ratio of 15 (Mao et al., 2015; Mussoline et al., 2017).

2.4.5 Mixing and agitation condition

In the AD method, mixing or agitation is needed to promote efficient interaction of active microbes and organic matter, to prevent coarser material settling, to achieve slurry homogeneity, to avoid the formation of temperature gradients, and so on (Rizwan et al., 2015). The level of agitation is also affected by the solid concentration in the reactor. The formation of anaerobic granules has been found to be the cause of low biogas yield. The efficiency of a reactor's mixing is calculated by its hydrodynamic test report, which decides whether or not a reactor is operating at maximum capacity. The hydrodynamic analysis employs Li^+ as a visible tracer in AD; at low concentrations, this detector is non-toxic to the system and is likely to be present in the majority of the feedstock (Ward et al., 2008). Recirculation of digestate or biogas through the bottom of the digester with the pump can also be used to achieve the optimal mixing in the reactor.

2.4.6 Toxicity

Toxic compounds are either pre-existing in the environment or are formed through substrate degradation. Non-dissociated hydrogen sulphide concentrations are toxic to sulphate reducers and methanogens (Naji et al., 2016; Zhou et al., 2016). Since this type can quickly diffuse through cell membranes, it is regarded as the most toxic. Toxic compounds are either naturally occurring in the atmosphere or are produced as a result of substrate degradation. Concentrations of non-dissociated hydrogen sulphide are toxic to sulphate reducers and methanogens (Naji et al., 2016; Zhou et al., 2016). This form is considered the most harmful because it can easily disperse across cell membranes. It induces protein denaturation and interferes with bacteria's assimilatory metabolism. Concentrations of sulphur and H_2S less than 0.003 and 0.002 mole/L, respectively, are called inhibitory. According to some research, toxicity is attributed to the unionised concentration of sulphide in the pH range of 6.8-7.2 (Weiß et al., 2010; Anjum et al., 2016). Such radioactive agents that hinder the development of microbes in the reactor include detergents and mineral ions in heavy metals. Smaller concentrations of these substances stimulate development, while higher concentrations can inhibit growth. The digester's recovery strategy is to stop feeding in the reactor and

flush the contents to eliminate the concentration of certain compounds below the toxic limit (Appels et al., 2008).

2.4.7 Chemical Oxygen Demand (COD)

COD is typically used as an indication of the strength (in terms of concentration of pollutants) of a sample of sludge or wastewater (Gerardi, 2003). It can be defined as the total oxygen necessary to oxidize all organic material into carbon dioxide and water and the oxidation of inorganic chemicals such as ammonia and nitrate. Therefore, COD can be considered a measure of the total amount of organic matter in a particular substance (Watershed Protection Plan Development Guidebook, 2001). The amount of substrate or COD of the digester feed sludge may be used to determine the quantity of nitrogen and phosphorus that is necessary for optimal digester performance. Although nutrient requirements differ according to the organic loading rates, COD: N: P ratios of 1000:7:1 and 350:7:1 are typically used for high-strength wastes and low loading rates respectively (Gerardi, 2003). When using either of the COD: N: P ratios, the assumption is made that 12% of the dry weight of bacterial cells consist of nitrogen and 2% of phosphorus.

2.4.8 Effect of trace elements on performance

Trace elements in the digestion mechanism, such as iron (Fe), cobalt (Co), nickel (Ni), zinc (Zn), and others, have an effect on the multistage AD method. Trace elements must be provided in sufficient quantities to sustain the metabolism of the microbial population; otherwise, the AD mechanism would fail (Choong et al., 2016). Trace elements Co, Fe, Cu, Zn, and Ni concentrations less than 30, 1.32, 0.12, 1.13, and 4.8 g/L prevented the development of methanogens in the environment, according to research (Zhang et al., 2003). Fe was discovered to be important in stimulating heme protein ferroxins (Fd) and cytochromes, which are responsible for energy metabolism in cells (Yenigün and Demirel, 2013). Trace factor incidence and supplementation are linked and have different effects at different stages of the AD process. The addition of micronutrients affects the methanosarcina and archeal populations, which improves AD production. The availability of optimal trace element dosages results in effective organic matter dissolution, low fatty acid aggregation, and higher digester stability, which

contributes to increased biogas output. The addition of several elements improves the digestibility of the substrate (Shitophyta and Fuadi, 2016).

2.5 Enhancement techniques

It is critical to be aware of substrate characteristics and potential metabolic mechanisms during AD in order to enhance the degradation process effectively. Lower methane yield is observed as a result of the inaccessibility of lignocellulose substrates, which also increases the concentration of inhibitory compounds in the process. As a result, various optimization methods, which are discussed further below, are used to increase the degradability of the substrate while also improving the hydrolysis rate and methanogen metabolism.

2.5.1 Physical pre treatment

Grinding (size reduction) is a tentative method of breaking the lignocellulosic structure. Sieving aids in the collection of the desired fine powder. Grinding has a significant impact on the polymerization degree, porosity, rising surface area, and decreasing crystallinity of the substrate. High moisture content (M.C.) of cellulose increases machine power consumption, while low M.C. of it is ideal for size reduction by grinding. Maryanty et al. (2017) investigated the impact of particle size on RS biogas generation. It was discovered that smaller particle sizes had a greater propensity for RS degradation. In a lab scale reactor, particle sizes of 0.038, 0.053, and 0.112 mm of RS revealed cellulose degradation rates of 71.96 percent, 50.15 percent, and

24.03 percent, respectively (100 mL). Menardo et al. (2012) discovered that when the size of the reactor was reduced to less than 50 mm in a lab scale reactor (2 L). It was discovered that particle sizes up to 5 mm resulted in a high methane yield and an improved electric energy balance. The pre treatment result shows that physical pre treatment is effective for increasing accessible surface area, breaking down lignin-hemicellulosic complexes, and the usable cellulosic material.

2.5.2 Chemical Pre-treatments

Chemical agents are used as catalyst for delignification and disrupting the bond of lignocellulosic matrix (Boonterm et al., 2016). Acid pre treatment involves usage of H_2SO_4 , H_2O_2 , HCl , HNO_3 etc. Acid pretreatment enhances the biogas production by altering the biodegradability of the waste such as steam pre-treatment, with notable biodegradation enhancements, for example, investigated H_2SO_4 as a catalyst for steam treatment at 155 °C and found that the CH_4 yield improved by 67%. Alkali pretreatment involves usage of $NaOH$, Na_2CO_3 , lime, ammonia, etc. Alkali pretreatment methods can increase the cellulose accessibility for the microorganisms by disrupting the lignocellulosic structure with enhanced surface area and porosity and decreased cellulose crystallinity (Boonterm et al., 2016).

2.5.3 Thermo-physical and Thermo-chemical pre treatment

Accelerated temperature pretreatment is widely used to increase the porosity of the surface and improve delignification. The lignocellulosic contents of the substrate are hydrolyzed by liquid at high temperatures. Water molecules dissociate into H_3O^+ and OH^- ions at elevated temperatures and pressures, which aids in the catalytic conversion of lignocellulosic structures. Pre-treatment of lignocellulose at higher temperatures and shorter reaction times (e.g., dilute sulfuric acid, ammonia recycle percolation (APR), or steam explosion) could increase porosity and delignification efficiency significantly. The cellulosic content of the processed biomass is higher. Any hemicellulosic material is also eliminated, increasing the surface area available for enzymatic attack. Ma et al. (2009) demonstrated that at an optimum microwave strength of 680W and irradiance time of 24 minutes, cellulose and hemicellulose reduction efficiencies of

30.6 percent and 43.3 percent were obtained, respectively (100 mL). Zhu et al. (2005) discovered that a microwave pretreatment of 700 W (30 min) removed 6% of the lignin, which increased the hydrolysis rate. However, these processes produce certain inhibitory compounds (for example, furan derivatives, phenolic substances, and so on) that reduce the efficiency of anaerobic fermentation. As a result, removing anaerobic fermentation inhibitors is an essential step in the bioconversion of lignocellulosic

biomass to biogas. The selection of an effective pre treatment method is critical for the long-term conversion to renewable energy sources.

2.5.4 Biological pre treatment

There is a paucity of literature on the impact of ensiling pre-treatment. It consists of anaerobic lactic fermentation, which converts sugars into lactic acids, acetic acids, and other acids. As a result, lignin is not digested, and is usually less than 6% without the addition of NaOH, and large losses of holocellulose can be observed, ranging from 1 to 13%. (Herrmann et al., 2011). These losses are affected by the length of ensiling, which can range from 10 to 365 days, the feedstock and chemicals such as sodium nitrite (NaNO₂) and methenamine, as well as biological agents such as *Lactobacillus buchneri* and *Lactobacillus plantarum*. While a 17 percent increase in methane yield was achieved, it resulted in a very small increase in real methane yield (per g VS). Any researchers have used diverse microbial consortia for pre-treatment purposes, such as Zhong et al. (2011), who used a 0.01 percent complex consortium consisting of lignin-degrading white-rot fungus i.e *Pleurotus florida* sp., cellulolytic bacteria such as *Bacillus licheniformis* sp., *Pseudomonas* sp., and *Bacillus subtilis* sp., yeasts, and other acid degrading bacteria *Lactobacillus deiliehii* sp. for 15 days' pre-treatment on corn straw resulted in a 75% increase in methane yield. Taha et al. (2015) found that using a fungal consortium increased saccharification rate sevenfold. When the influence of fungal pre-treatment (*Trametes versicolor*) on corn silage was compared to ensiling, a 41.3 percent rise in methane production was obtained, along with a favourable effect on pH stability (Ti et al., 2018). The most frequently studied biological pre-treatment of lignocellulosic biomass is fungal pre-treatment prior to AD, which consumes less resources and has less environmental impacts. The number of steps involved in this method is minimal, and it does not need any additional feedstock for fungi processing. Furthermore, since the pre-treatment is carried out under mild conditions, the waste streams are minimised, the downstream costs are minimal, and the by-products created during fungal pre-treatment do not normally obstruct subsequent AD processes. Fungi that can degrade lignocellulosic biomass are known as soft-, brown-, or white-rot fungi. When compared to white-rot and brown-rot fungi, little is known about the lignin degradation activity of soft-rot fungi. However, Sánchez (2009)

confirmed that soft-rot fungi can degrade lignin and that they normally target materials with lower lignin content and higher moisture.

Soft-rot fungi are classified as Ascomycetes, while brown-rot and white-rot fungi are classified as Basidiomycetes. Brown-rot fungi quickly depolymerize holocellulose while only changing the lignin; thus, lignin persists as a key component after fungal pre-treatment (Sánchez, 2009). However, white-rot fungi, which are classified into over 1500 different groups, have a remarkable ability to destruct the structure of lignin, the most recalcitrant portion of lignocellulosic biomass, to CO₂ (Miiller and Trfisch, 1986; Wan and Li, 2012). White-rot fungi have a remarkable degradative capacity due to their lower substrate utilisation specificity and robust oxidative action of their ligninolytic enzymes. Apart from lignin, white-rot fungi can degrade a wide range of environmental contaminants, including heterocyclic aromatic hydrocarbons, chlorinated aromatic compounds, various dyes, and synthetic polymers (Bermek et al., 1998). As a result, white-rot fungi are used in a variety of soil and water remediation strategies. However, free phenoxy and aromatic radicals produced by peroxidases are small enough to reach the cell wall, creating an initial cracking reaction that begins the decomposition of lignin polymers and radicals. The presence of radicals in lignin degradation suggests that it is not a highly specific procedure. The activities of ligninolytic enzymes are not always interconnected with lignin degradation, indicating a lack of understanding of the ligninolytic enzyme complex; additionally, these phenolic compounds have an inhibitory effect on the AD process, indicating that fungal pre-treatment is appropriate prior to AD of lignocellulosic biomass.

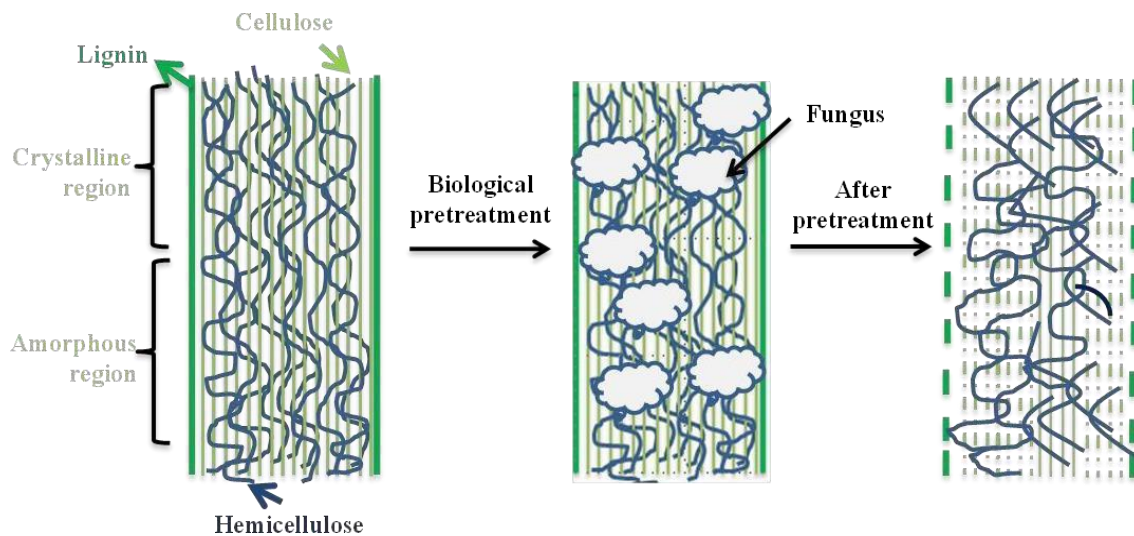


Figure 2.9 Schematic diagram of biological pre-treatment of lignocelluloses

Lignocellulosic biomass extremely recalcitrant by limiting enzyme accessibility to holocellulose, thus limiting biomass conversion into biofuels. White- rot fungi have tremendous capacity to increase methane yield due to delignification and the resulting increase in biomass digestibility. While biological pre-treatments with white- rot fungi have many advantages, they are also fraught with difficulties such as long incubation times (weeks to months), substrate colonisation by the inoculum, and a scarcity of studies on selective delignification of lignocellulosic biomass using white-rot fungi. Fungal pre- treatment during storage will alleviate the problem of long incubation times but further research is needed to improve fungal pre- treatment.

2.6 Different Pre-treatment Modules used for Banana waste

Banana biomass	Pretreatment method	Pretreatment condition	Product yield	Ref.
Peel	Physical	Chopping (0.5 × 1 cm), and grinding	Methane 439 mL g ⁻¹ TVS	(Pisutpaisal et al., 2014)
Peel	Physical	Drying at 60 °C, and grinding (2 mm mesh)	Methane 0.321 L g ⁻¹ VS	(Gunaseelan, 2004)
Peel	Physical	Chopping (0.5 × 1 cm), and blending	Methane 64% (w/w)	(Housagul et al., 2014)
Pseudostem	Physical	Grinding, and blending	Methane 0.387 L CH ₄ g ⁻¹ VS	(Velmurugan and Ramanujam, 2011)
Pseudostem	Alkali	55 °C for 54 h,	Methane 232.4 mL g ⁻¹	(Zhang et al., 2013)
Peel	Steam and acid pretreatment Physical and acid	Chopping (2–4cm), drying(60°C,1day), autoclaved at 15psi pressure for 30min, and 0.5–2.5% (v/v) H ₂ SO ₄ at 70 and 110 °C for 10–30min	Ethanol 45.088% (w/w)	(Gebregergs et al., 2016)
Peel	Physical and acid pretreatment	Chopping (4-5 cm), drying (65 °C, 24 h), and 4 N HCl for 75 min at 80–100 °C	Glucose 0.81% (w/w)	(Bhatia and Paliwal, 2016)
Pseudostem	Steam and acid	177 °C, 5 min and 2.2% H ₂ SO ₄ (v/v)	Glucose 91.0% (w/w)	(Guerrero et al., 2017)
Rachis	Steam and acid	198 °C, 5 min and 1.5% H ₂ SO ₄ (v/v)	Glucose 87.1% (w/w)	(Guerrero et al., 2017)
Peel	Biological (<i>Aspergillus niger</i>)	7 days, pH 6, 30 °C	Ethanol 6.287% (w/w)	(Singh et al., 2014)
Peel	Biological (<i>A. niger</i>)	30 °C at 300 rpm for 5 days	Reducing sugar 0.82 mg/cm ³	(Itelima et al., 2013)
Pseudostem	Biological (cellulolytic bacteria)	30 °C at 100 rpm for 72 h	Glucose 0.87 g/h	(Meena et al., 2015)
Rotten banana	Biological (cellulase)	0.3ml of pectinases at 40 °C in a water bath for 2h & then treated with 0.3 ml of cellulase at 60 °C for 2 h	Glucose 0.537% (w/v)	(Alshammari et al., 2015)

Table 2.7 different pre-treatment for pseudo stem banana waste

2.7 Different mixture of wastes in anaerobic digestion

Title	pH	Methane yield	Reactor	References
Anaerobic co-digestion of microalgae <i>Chlorella vulgaris</i> and potato processing waste: Effect of mixing ratio, waste type and substrate to inoculum ratio	<ul style="list-style-type: none"> pH for <i>Chlorella vulgaris</i> is 7.96 for algae growth pH for methanogenic microorganisms of potato waste is 6.5 to 7.2 	22–47%	BMP : glass bottles with a capacity of 160 mL An addition of 10% v/v (9 mL) of NaHCO ₃ (5 g/L) (27 days)	(Zhang, Caldwell, Zealand, et al., 2019)
Semi-continuous anaerobic co-digestion of marine microalgae with potato processing waste for methane production	<ul style="list-style-type: none"> pH for microalgae is 8.10 pH for methanogenic microorganisms of potato waste is 6.5 to 7.2 	328 – 374 (mL CH ₄ /g VS)	Semi-continuously fed reactor	(Zhang, Caldwell, & Sallis, 2019)
Methane production and characteristics of the microbial community in the co-digestion of potato pulp waste and dairy manure amended with biochar	<ul style="list-style-type: none"> pH for the Biochar is 8.7 pH for methanogenic microorganisms of potato waste is 6.5 to 7.2 pH for dairy manure is acidity. 	200 mL/g TS	BMP	(Chen et al., 2021)

Table 2.8 Different mixture of wastes in anaerobic digestion

2.8 Anaerobic Bio-Reactors used for Methane Production

2.8.1 Batch systems

In batch systems, digesters are finished with or without seed materials and allowed to go through all degradation stages sequentially. The pure distinction between the first step, where acidification occurs even faster than methanogenesis, and the second phase, where acids are processed into biogas, is the hallmark of batch processes.

Converti et al. (2008) investigated anaerobic batch digestion under mesophilic and thermophilic environments. The findings showed that the mixture of vegetable wastes could be digested in mesophilic and thermophilic environments. At 5% total solid concentration, anaerobic batch digestion of mixed vegetable waste was efficient. After 47 days, the waste was digested, yielding 0.16 m³ biogas /kg TS applied, with a maximum gas output on day 26. Two other experiments (Bouallagui et al., 2003, and Marouani et al., 2003) showed that anaerobic therapy at 8 percent TS in a batch digester was accomplished by VFA aggregation and immutable decreasing pH issues. Batch systems have previously failed to capture a significant market share (Naik et al., 2009).

However, the characteristics of batch processes, such as easy architecture and process management, robustness against coarse and heavy pollutants, and lower investment costs, make them more attractive to developing countries. SBR technology is worth considering for anaerobic treatment because of its operational stability, which is distinguished by three factors: a high degree of process versatility in relation to cycle time and order, the absence of separate clarifiers, and the presence of a higher concentration of slow-growing anaerobic bacteria inside the reactor. According to research into the ASBR technique, it can achieve relatively high solid material waste degradation and suspended solid removal (90–93 percent) using the ASBR (Naik et al., 2009).

2.8.2 Continuous One-Stage systems

Approximately 89 percent of waste treatment facilities in Europe that perform anaerobic digestion of the organic portion of municipal solid wastes and bio wastes rely on continuous one-stage systems (Mata-Alvarez et al., 2000). Nonetheless, a substantial amount of research has been conducted on waste management in two stages, primarily an acid producing process followed by a methanogenic stage. This is because a two-stage structure has more potential and has more opportunities to explore the intermediate steps of the digestive process. Alternatively, because of their simpler architectures and lower investment costs, one-stage systems are preferred by industrialists.

Mata-Alvarez et al. (2000) discovered that a mesophilic one-stage procedure would adequately stir a reactor during the treatment of organic waste from a vast food industry. The overall OLR attempted was less than 3 kg TVS/h(m³day). A limit condition for comparable waste digestion was discovered to be an OLR of 6 kg TVS/(m³day). Furthermore, as quoted by Mata-Alvarez et al., (2000), this waste appeared to be more biodegradable, allowing for greater and faster VFA processing, highlighting the validity of this OLR cap. Lane examined overloading digesters by more than 4 kg TVS/m³day and discovered a decrease in pH and gas yield as well as an increase in the CO₂ content of gas produced while using a CSTR (Naik et al., 2009). It was attempted to use a semi-continuously mixed tubular digester. The best results were

obtained after 20 days of using a HRT with an OLR of 2.8 kg TVS/m² (m³day). The pH may decrease to 6.1 during the hydrolysis, but it remains at 7.2 for the majority of the time. When the HRT was reduced to 10 days, the pH fell to 5 and inhibition was observed. The tubular reactor's most significant feature was its ability to differentiate acidogenesis and methanogenesis longitudinally down the reactor, allowing it to function as a two-phase mechanism (Naik et al., 2009).

Problems may arise if the substrate is simply degradable in one-step anaerobic digestion of solid wastes because there is no choice for the accumulation/retention of biomass inside the reactor in solid waste digestion; therefore, the slower growing methanogens are overfed at higher loading speeds.

In a one-stage procedure, acidogens and methanogens are combined in one tank, and hydrogen formed by acidogenic metabolism is incorporated by the methanogens to reduce carbon dioxide to methane and water. When acidogenic activities such as acetate, carbon dioxide, and hydrogen production are active, the feeding rate of the substrate improves while the methanogenic population cannot increase to the same level. At a loading rate where the hydrogen-consuming reactions become soaked, hydrogen accumulation partly prevents its extra production, and further organic electron sinks are formed accordingly. This causes imbalances and a halt in methane activity (Naik et al., 2009).

2.8.3 Continuous Two-Stage systems

Both groups of acidogenic and methanogenic species differ in terms of nutritional resources, physiology, pH optimal, development, nutrient uptake kinetics, and ability to withstand environmental stress factors. Two-phase anaerobic digestion, on the other hand, denotes a process design that employs isolated reactors for acidification and methanogenesis connected in sequence, allowing for optimization of both processes (Naik et al., 2009). Several researchers have considered two-phase anaerobic digestion of a mixture of fruit and vegetable wastes (Rajesh Wari et al., 1999). Rajesh Wari et al. (1999) used a two-step technology that allowed them to convert more than 94 percent of vegetable market waste into biogas. In a solid bed reactor, the untreated waste was acidified. Following the acidification process, the leachate was processed in a UASB

reactor for biogas processing. The hydrolysis–acidification process was completed in an reactor, and methane fermentation was carried out in an up-flow fixed film reactor. The global depletion yield was greater than 87 percent, and the biogas output yield was approximately 0.29 L/g of the initial TCOD. At a volumetric loading rate of 5.65 g VS/L d, over 95 percent volatile solids were converted into methane using a two-stage procedure involving a thermophilic liquefaction CSTR reactor and a mesophilic anaerobic filter. The methane yield was approximately 420 L/kg VSadded (Naik et al., 2009)

CHAPTER 3

METHODOLOGY

3.1 Introduction

In this chapter, it discussed regarding the parameter and analysis used in this study such as water displacement method, pH, temperature and GC-TCD analysis. For water displacement method, the gas occupied the measuring cylinder, then water will flow out from the measuring cylinder. The pH and temperature were monitored every day to see any changes that can affect the anaerobic digestion. For GC-TCD, the collected gas in the gas bag that produced in anaerobic digestion process was ran for analysis testing as to determine the concentration of gas and also type of gases produced. In this chapter also discussed about the type of pre-treatment used for the wastes. In this study, physical treatment was used to treat the wastes before isolated it in the reactor. Next is it discussed about the designation of the reactor based on the volume, weight and the gas produced of by wastes and also equipment that used to make the reactor.

3.2 Parameter and analysis

3.2.1 Water displacement method

Water displacement method is a method to get the amount of gas produced in the reactor. This method involved gas and liquid matter. For the gas, it come out from reactor while the water was used as medium of displacement for the gas. In this method, the gas will flow in into the water.in the measuring cylinder As the gas occupied in the measuring cylinder, the water will flow out of the measuring cylinder. The volume was calculated based on the amount of water flow out of the measuring cylinder.

3.2.2 pH

pH is a way to express the concentration of hydrogen ions in a solution. Since solution acids and bases dissociate to yield hydrogen ions $[H^+]$ and hydroxyl ions $[OH^-]$ respectively, pH is used to indicate the intensity of a solution's acid or alkaline state. Alkalinity is a measure of dissolved substances' acid-neutralizing potential in water which compares the amount of strong acid needed to reduce the solution from the original pH to around 4.5. Many of the materials may contribute to water alkalinity. It is primarily attributed to the presence of salts with low acids (primarily bicarbonate and carbonate) and hydroxide (at high pH) for most practical purposes.

3.2.3 Gas chromatography (GC-TCD)

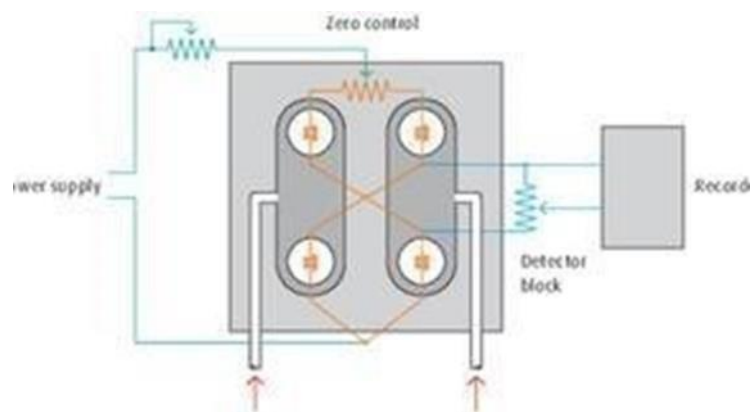


Figure 3.1 GC-TCD process

Gas chromatography (GC or GLC) is a standard analytic method used in many research and commercial facilities for quality control, as well as the identification and quantification of substances in a mixture. Because it allows for the detection of extremely minute amounts, GC is also a popular method in many environmental and forensic laboratories. As long as the chemicals are sufficiently thermally stable and adequately volatile, a wide range of samples can be examined.

This technique, like all other chromatographic procedures, requires a mobile and a stationary phase. The mobile phase (carrier gas) is made up of inert gases such as

helium, argon, or nitrogen. The stationary phase is made up of a packed column in which the packing or solid support functions as the stationary phase itself or is covered with the liquid stationary phase (high boiling polymer). The stationary phase covers the walls of a small-diameter tube directly (i.e., 0.25 μ m film in a 0.32 mm tube) in most analytical gas chromatographs.

The separation of the components is influenced by a variety of factors such as vapor pressure, the polarity of components versus the polarity of stationary phase on column, column temperature, carrier gas flow rate, column length and the amount of material injected.

First, for the vapor pressure, a compound's boiling point is frequently connected to its polarity. The lower the boiling point, the higher the vapour pressure of the compound and, typically, the shorter the retention period since the chemical spends more time in the gas phase. Second is polarity of components. When the polarities of the stationary phase and compound are identical, the retention duration rises because the compound interacts with the stationary phase more strongly. As a result, at the same temperature, polar compounds have long retention durations on polar stationary phases and shorter retention times on non-polar columns. Chiral stationary phases based on amino acid derivatives, cyclodextrins, and chiral silanes can separate enantiomers because one enantiomer interacts with the stationary phase somewhat stronger than the other, frequently due to steric effects or other extremely specific interactions. Next is column temperature. In this factor, a high column temperature results in a very short retention period but also in a very poor separation since all components mostly remain in the gas phase. However, for the separation to take place, the components must be able to interact with the stationary phase. The retention period will decrease if the chemical does not interact with the stationary phase. At the same time, the separation quality deteriorates since the disparities in retention durations are no longer as significant. Temperature gradients often provide the best separations because variations in polarity and boiling points are exploited. The fourth is carrier gas flow rate. For this factor, a high flow rate minimises retention durations, but it also results in poor separation. As previously stated, the components have little opportunity to interact with the stationary phase and are simply propelled through the column. For the fifth factor

which is column length, a longer column enhances separation. The trade-off is that the retention period grows proportionately to the column length, and there will be substantial peak widening due to enhanced longitudinal diffusion inside the column. It is important to remember that the gas molecules are not only moving in one direction, but also sideways and backwards. The flow rate is inversely proportional to this widening. Broadening is also noticed due to the limited rate of mass transfer between the phases and the fact that the molecules take distinct courses through the column. The last factor is the amount of material injected. In this factor, the chromatogram's peaks should ideally have a symmetric form (Gaussian curve). When too much material is injected, the peaks exhibit excessive tailing, resulting in a worse separation. Most detectors are relatively sensitive and do not require a large amount of material to give a detectable signal. Because most GC instruments work in split-mode to prevent overloading of the column and detector, only 1-2 percent of the substance put into the injection port travels through the column under ordinary circumstances. The splitless mode will only be employed if the analyte concentration in the sample is exceedingly low. High temperatures and flow rates shorten the retention duration but degrade the separation quality while lower temperature and flowrate will increase the retention time

3.2.4 Temperature


Temperature has a significant impact on enzyme production, microbial development, methane output, and fertilizer efficiency. In anaerobic digestion, there are three major operating conditions: psychrophilic (10-30 °C), mesophilic (30-40°C), and thermophilic (50-60°C). Raising the temperature in the reactor would increase the output of methane gas up to around 60 degrees Celsius (Labatut and Pronto 2018). Most digesters, however, function in the mesophilic range. Methanogens that thrive in the mesophilic spectrum are more resistant to temperature fluctuations, lowering the chance of the reactor sour. Thermophilic environments can also reduce food waste solubilization (Labatut and Pronto 2018). Psychrophilic environments are often seen only in small anaerobic digesters, which are commonly used in homes and small farms. This range is not recommended for large- scale applications due to the high expense of larger reactors needed for psychrophilic environments, which is not always commercially feasible or sustainable at industrial scales. The operation of two-phase




anaerobic digestion, where the first phase operates in thermophilic conditions with a shorter retention period and the second phase operates in mesophilic conditions with a longer retention time, is one method for achieving the advantages of both mesophilic and thermophilic digesters. This study used mesophilic temperature where the reactor was placed in the room temperature.

3.3 Physical pre-treatment

The pre treatment that was applied towards the wastes was physical pre-treatment where the potato and pseudo stem banana wastes were chopped and grinded using knife and electronic blender. First, the food wastes was chopped then weighted with ratio 3:1 where the 3 ratio was potato with weight 600 gram while for the 1 ratio was pseudo stem banana with weight 200 gram. Then, the wastes were grinded using blender with water to ease the grinding process. The water ratio used was 1:1 where the water had same weight as the wastes weight. Then, the wastes were transferred into reactor.

3.4 The equipment used in AD reactor

Equipment	Parameter	Descriptions
<p data-bbox="341 1270 715 1301">pH and temperature meter</p> 	<p data-bbox="745 1270 1011 1406">To monitor the performances of the wastes</p>	<p data-bbox="1024 1270 1326 1406">To monitor the temperature and pH value of the mixture</p>

<p style="text-align: center;">Silicon tube</p> 	<p>For the methane gas to flow to the tyre tube</p>	<p>The silicone tube was connected to the flat tyre tube and the biogas that were produced from the waste was flow through the silicone tube.</p>
<p style="text-align: center;">Gas bag</p> 	<p>To collect the methane that were produced from the mixture</p>	<p>The methane gas that were produced from the wastes was collected from the gas bag sampling</p>
<p style="text-align: center;">Container</p> 	<p>To store the waste</p>	<p>The wastes that had been grinded using mixer will be store into this container and will be lock to ensure the gas doesn't flow out. The volume of reactor used was 2000 ml</p>



<p style="text-align: center;">Aquarium heater</p> 	<p>To heat the water</p>	<p>The heater was use to heat the water in the box to maintain the temperature of the reactor about 35 degree celcius</p>
<p style="text-align: center;">Valve</p> 	<p>To control the outflow gas</p>	<p>The valve was used to control the outflow gas into the gas bag.</p>

Table 3.1 Equipment for reactor

3.5 Design and calculation of volume in reactor

3.5.1 Potato waste

3.5.1.1 Feedstock

Using ratio 3:1 from Chen et al.(2021) and the volume of reactor with lab scale size (2 L) by Menardo et al. (2012) the amount in gram for potato is

$$200\text{g} \times 3 = 600 \text{ g} = 0.6 \text{ kg} \text{ Thus, the amount of feedstock per day is } 0.6 \text{ kg}$$

Using ratio 1:1 (waste : water);

$$= (1 \times 0.6 \text{ kg}) + (1 \times 0.6 \text{ kg})$$

$$= 1.2 \text{ kg, } 1 \text{ kg approximate } 1 \text{ Liter Thus,}$$

$$= 1.2 \text{ L} \times 1\text{m}^3 / 1000 \text{ L}$$

$$= 0.0012 \text{ m}^3/\text{day} \text{ (flowrate)}$$

3.5.1.2 Retention time

The retention time of this project is 28 days. Since there is no volume changing of the potato waste for the 28 days, thus:

$$= 0.0012 \text{ m}^3 \text{ (reactor volume)}$$

$$= 0.0012 \text{ m}^3 \times 28 \text{ days}$$

$$= 0.0336 \text{ m}^3$$

3.5.1.3 Feedstock quality

$$\text{Total solid, TS for potato waste} = 16\% = 0.16$$

$$= 0.6 \text{ kg wet weight} \times 0.16$$

$$= 0.096 \text{ kg dry matter}$$

Volatile solid, VS for potato waste = 90% = 0.90

= 0.096 kg dry matter x 0.90 VS

= 0.0864 VS kg / day per 1.8 L

= 0.0864 VS kg/day /1.8 L x 1000

= 48 kg VS / m³

3.5.1.4 Organic load retention (OLR)

OLR = [Flowrate (m³/day) x concentration (kg VS / m³)] / (m³)

OLR = [0.0012 m³/day x 48 kg VS / m³] / 0.0336 m³

OLR = 1.7143 kg VS / m³ day

3.5.1.5 Amount of gas produced

Based on journal, the amount of CH₄ produced was 0.39 m³/kg VS in 50 days for 2 stages reactor (Parawira et al., 2005). Assume that 1 scale is half of 0.39 m³/kg VS, thus 0.195 m³/kg VS. For per day methane production is 0.195 m³/kg VS / 50 equal to 0.0039 m³/kg VS. Per 28 days is 0.1092 m³/kg VS Based on figure below:

Composition of Biogas Produced via Anaerobic Digestion	
<i>"Major" Components</i>	
Methane (CH ₄)	50-75% ^[15,16]
Carbon Dioxide (CO ₂)	25-45% ^[15,16]
Water Vapor (H ₂ O)	2-7% ^[15,16]
<i>"Minor" Components</i>	
Hydrogen Sulfide (H ₂ S)	0-1% ^[15,16]
Hydrogen (H ₂)	0-1% ^[16] , 1-2% ^[15]
Ammonia (NH ₃)	0-1% ^[15,16]
Carbon Monoxide (CO)	0-2% ^[16]
Oxygen (O ₂)	0-2% ^[16]
Nitrogen (N ₂)	0-2% ^[16]

CH₄

$$0.1092 \text{ m}^3/\text{kg VS} (\text{CH}_4) = 50 \%$$

$$0.1092 \text{ m}^3/\text{kg VS} (\text{CH}_4) = 0.50$$

CO₂

$$0.1092 \text{ m}^3/\text{kg VS} (\text{CH}_4) / \text{CO}_2 = 0.50 / 25 \%$$

$$0.1092 \text{ m}^3/\text{kg VS} (\text{CH}_4) / \text{CO}_2 = 0.50/0.25$$

$$\text{CO}_2 = 0.0546 \text{ m}^3/\text{kg VS}$$

H₂O

$$0.1092 \text{ m}^3/\text{kg VS (CH}_4) / \text{H}_2\text{O} = 0.50 / 2 \%$$

$$0.1092 \text{ m}^3/\text{kg VS (CH}_4) / \text{H}_2\text{O} = 0.50/0.02$$

$$\text{H}_2\text{O} = 0.00437 \text{ m}^3/\text{kg VS}$$

Minor gas component

$$0.1092 \text{ m}^3/\text{kg VS (CH}_4) / \text{minor components} = 0.50 / 2 \%$$

$$0.1092 \text{ m}^3/\text{kg VS (CH}_4) / \text{minor components} = 0.50/0.02$$

$$\text{Minor components} = 0.00437 \text{ m}^3/\text{kg VS}$$

Total of biogas yield produced from potato waste

$$= \text{CH}_4 + \text{CO}_2 + \text{H}_2\text{O} + \text{Minor components}$$

$$= 0.1092 \text{ m}^3/\text{kg VS} + 0.0546 \text{ m}^3/\text{kg VS} + 0.00437 \text{ m}^3/\text{kg VS} +$$

$$0.00437 \text{ m}^3/\text{kg VS}$$

$$= 0.1725 \text{ m}^3/\text{kg VS}$$

$$= 0.1725 \text{ m}^3/\text{kg VS} / 28$$

$$= 0.00616 \text{ m}^3/\text{kg VS per day}$$

OLR x biogas production per day x reactor volume

$$= 1.7143 \text{ kg VS} / \text{m}^3 \text{ day} \times 0.00616 \text{ m}^3/\text{kg VS} \times 0.0012 \text{ m}^3$$

$$= 0.0000127 \text{ m}^3/\text{day}$$

$$= 0.0127 \text{ L} \times 28 \text{ days}$$

$$= 0.3556 \text{ L gas} / 28 \text{ days}$$

Total volume for reactor

$$= 0.3556 \text{ L gas} + 1.2 \text{ L (potato waste + water)}$$

$$= 1.5556 \text{ L}$$

3.5.2 Pseudostem banana waste

3.5.2.1 Feedstock

Using ratio 3:1 from Chen et al,(2021) and the volume of reactor with lab scale size (2 L) by Menardo et al. (2012) the amount in gram for pseudo stem banana is

$$200\text{g} \times 1 = 200 \text{ g} = 0.2 \text{ kg} \text{ Thus, the amount of feedstock per day is } 0.2 \text{ kg}$$

Using ratio 1:1 (waste: water);

$$= (1 \times 0.2 \text{ kg}) + (1 \times 0.2 \text{ kg})$$

$$= 0.4 \text{ kg, } 1 \text{ kg approximate } 1 \text{ Liter Thus,}$$

$$= 0.4 \text{ L} \times 1\text{m}^3 / 1000 \text{ L}$$

$$= 0.0004 \text{ m}^3/\text{day (flowrate)}$$

3.5.2.2 Retention time

The retention time of this project is 28 days. Since there is no volume changing of the pseudostem waste for the 28 days, thus :

$$= 0.0008 \text{ m}^3 \text{ (reactor volume)}$$

$$= 0.0008 \text{ m}^3 \times 28 \text{ days}$$

$$= 0.0224 \text{ m}^3$$

3.5.2.3 Feedstock quality

Total solid, TS for pseudostem waste = 15% = 0.15

= 0.2 kg wet weight x 0.15

= 0.03 kg dry matter

Volatile solid, VS for pseudostem waste = 11.37% = 0.1137

= 0.03 kg dry matter x 0.1137 VS

= 0.003411 VS kg / day per 0.4 L

= 0.003411 VS kg/day /0.4 L x 1000

= 8.5275 kg VS / m³

3.5.2.4 Organic load retention time (OLR)

OLR = [Flowrate (m³/day) x concentration (kg VS / m³)] / (m³)

OLR = [0.0004 m³/day x 8.5275 kg VS / m³] / 0.00224 m³

OLR = 1.5228 kg VS / m³ day

3.5.2.5 Amount of gases produced

Based on journal, the amount of CH₄ produced is 0.43 m³/kg VS or 0.387 L/g VS or 0.387 m³/kg VS (Velmurugan and Ramanujam, 2011) Based on figure below:

Composition of Biogas Produced via Anaerobic Digestion	
<i>"Major" Components</i>	
Methane (CH ₄)	50-75% ^[15,16]
Carbon Dioxide (CO ₂)	25-45% ^[15,16]
Water Vapor (H ₂ O)	2-7% ^[15,16]
<i>"Minor" Components</i>	
Hydrogen Sulfide (H ₂ S)	0-1% ^[15,16]
Hydrogen (H ₂)	0-1% ^[16] , 1-2% ^[15]
Ammonia (NH ₃)	0-1% ^[15,16]
Carbon Monoxide (CO)	0-2% ^[16]
Oxygen (O ₂)	0-2% ^[16]
Nitrogen (N ₂)	0-2% ^[16]

CH₄

$$0.387 \text{ m}^3/\text{kg VS (CH}_4 \text{)} = 75 \%$$

$$0.387 \text{ m}^3/\text{kg VS (CH}_4 \text{)} = 0.75$$

CO₂

$$0.387 \text{ m}^3/\text{kg VS (CH}_4 \text{)} / \text{CO}_2 = 0.75 / 45 \%$$

$$0.387 \text{ m}^3/\text{kg VS (CH}_4 \text{)} / \text{CO}_2 = 0.75 / 0.45$$

$$\text{CO}_2 = 0.23 \text{ m}^3/\text{kg VS}$$

H₂O

$$0.387 \text{ m}^3/\text{kg VS (CH}_4 \text{)} / \text{H}_2\text{O} = 0.75 / 7 \%$$

$$0.387 \text{ m}^3/\text{kg VS (CH}_4 \text{)} / \text{H}_2\text{O} = 0.75 / 0.07$$

$$\text{H}_2\text{O} = 0.036 \text{ m}^3/\text{kg VS}$$

Minor gas components

$$0.387 \text{ m}^3/\text{kg VS (CH}_4) / \text{ minor components} = 0.75 / 11 \%$$

$$0.387 \text{ m}^3/\text{kg VS (CH}_4) / \text{ minor components} = 0.75 / 0.11$$

$$\text{Minor components} = 0.057 \text{ m}^3/\text{kg VS}$$

Total of biogas yield produced from potato waste

$$= \text{CH}_4 + \text{CO}_2 + \text{H}_2\text{O} + \text{Minor components}$$

$$= 0.387 \text{ m}^3/\text{kg VS} + 0.23 \text{ m}^3/\text{kg VS} + 0.036 \text{ m}^3/\text{kg VS} + 0.057 \text{ m}^3/\text{kg VS}$$

$$= 0.71 \text{ m}^3/\text{kg VS}$$

OLR x biogas production per day x retention time

$$= 0.71 \text{ kg VS} / \text{m}^3 \text{ day} \times 0.02536 \text{ m}^3/\text{kg VS} \times 0.0004 \text{ m}^3$$

$$= 0.0000072 \text{ m}^3/\text{day} \times 1000$$

$$= 0.0072 \text{ L} / \text{day}$$

$$= 0.0072 \text{ L/day} \times 28 \text{ days}$$

$$= 0.2016 \text{ L} / 28 \text{ days}$$

Total volume for reactor

$$0.2016 \text{ L gas} + 0.4 \text{ L (pseudostem banana + water)}$$

$$= 0.6016 \text{ L}$$

Total volume potato waste + total volume pseudostem banana

$$= 1.5556 \text{ L} + 0.6016 \text{ L}$$

$$= 2.1572 \text{ L}$$

Total volume gas potato waste + volume gas pseudostem banana

$$= 0.3556 \text{ L} + 0.2016 \text{ L}$$

$$= 0.5572 \text{ L}$$

3.6 Flowchart of the study

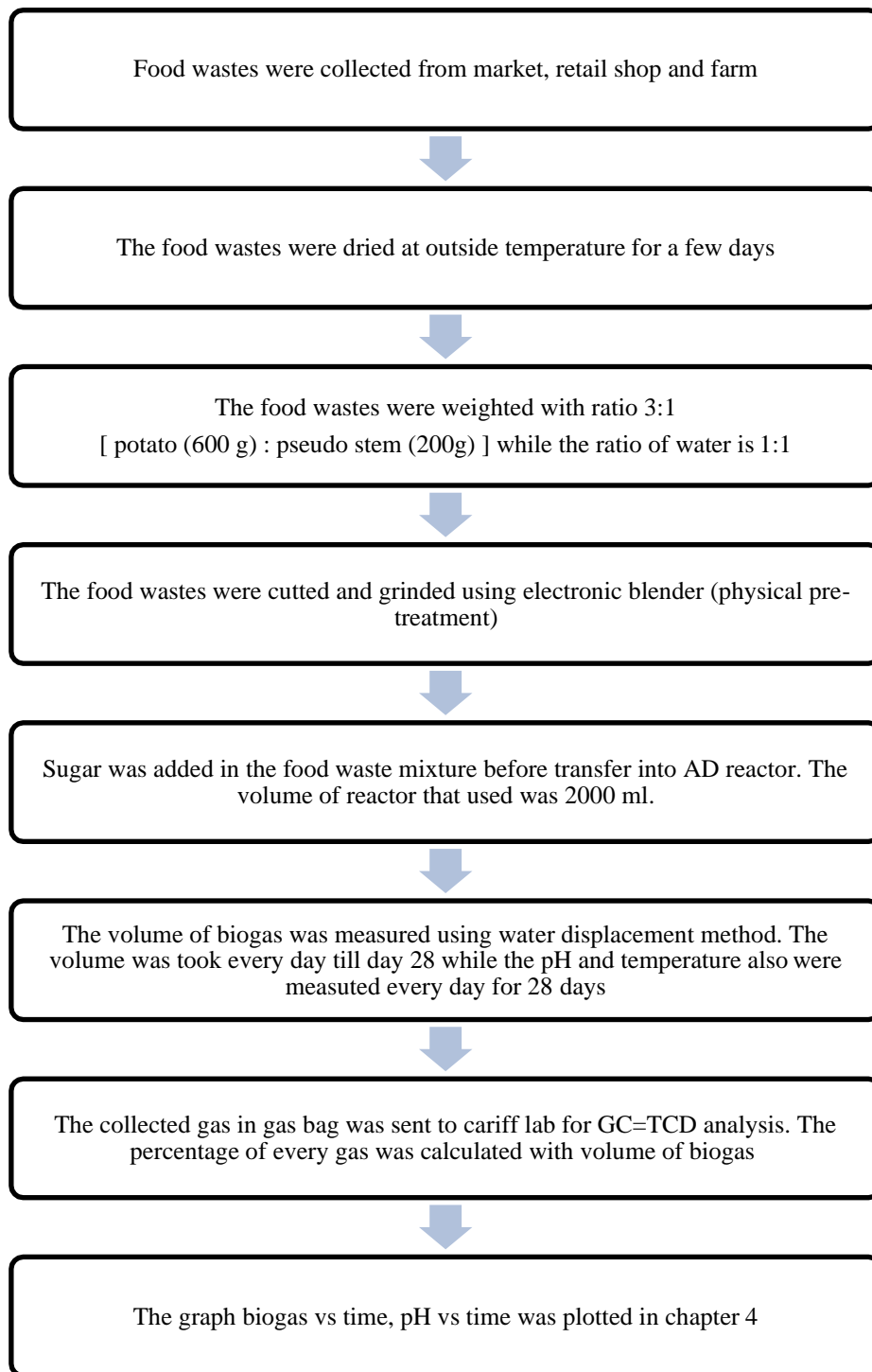


Figure 3.2 Flowchart process

]

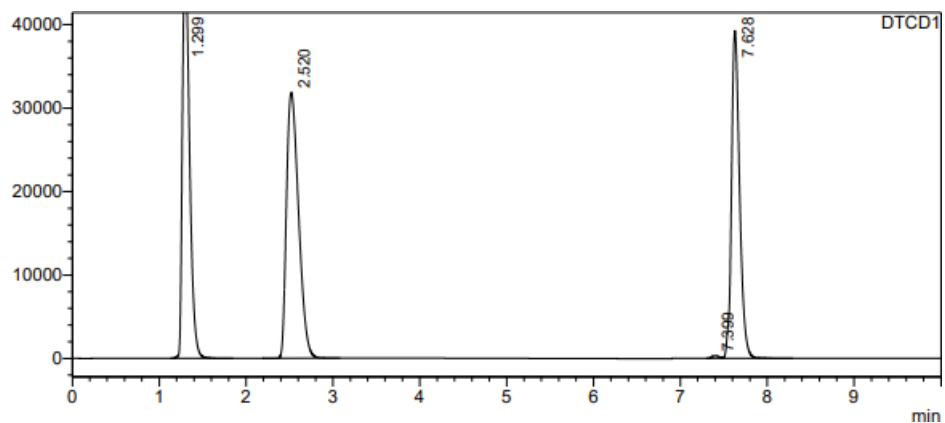
CHAPTER 4

RESULTS AND DISCUSSION

4.1 Introduction

The results below will be explained about the biogas produced from potato and pseudo stem banana wastes, volume of biogas and gases produced after 28 days through water displacement method and also the changes of the pH value in the anaerobic digestion process.

4.2 Determination of biogas produced from potato and pseudo stem banana wastes after 28 days by gas chromatography



Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name	Area%
1	1.299	278301	49077	0.000				33.087
2	2.520	307604	31810	39.388	%		CO2	36.570
3	7.399	1855	312	0.253	%		O2	0.221
4	7.628	253369	39217	34.582	%	SV	O2	30.123
Total		841128	120416					100.000

Figure 4.1 Chromatogram of biogases concentration for potato and pseudo stem waste

Figure 4.1 shows the result obtained by gas chromatography from laboratory-scale experiments where the potato and pseudo stem banana waste were mixed together

with ratio 3:1, where the weight of potato waste thrice of the weight pseudo stem banana. The weight of the potato waste was 600g while the pseudo stem banana waste was 200g.

In this figure 4.1, it shows the 3 types of gases produced after 28 days where carbon dioxide, methane and oxygen produced. The chromatogram shows that CO₂ has highest gas concentration compared to 2 others gases. The CO₂ concentration was 39.388% while for the O₂ was 34.582%. and 0.253%. The concentration of every gas appeared on it's peak. Based on the figure 4.1, the peak was determined by the retention time of the gases.

In this gas chromatography analysis, the boiling point of gases will affect the retention time. CO₂ has highest boiling point compared to other gases where by the boiling point of the CO₂ is -78.46 °C while for the O₂ is -183 °C. Based on the result of chromatogram data, it shows that the decreasing of boiling point gases will increasing the retention time. This happened due to carrier of gas flow rate in the GC-TCD is lower. Thus, it increasing the retention time of the gases to appear by its peak.

4.3 Biogas generation from potato and pseudo stem banana wastes.

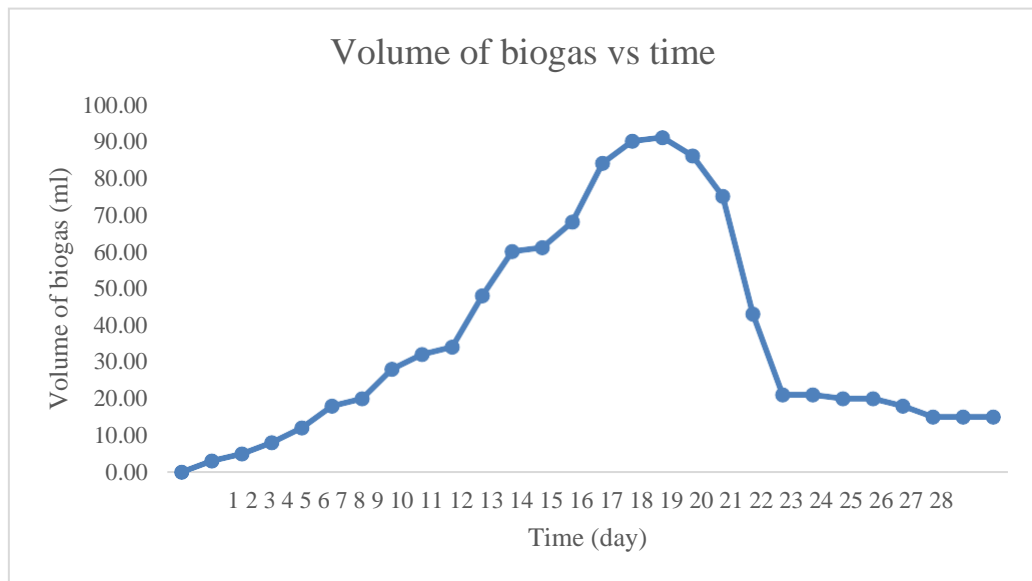


Figure 4.2 Volume of biogas by potato and pseudo stem banana wastes

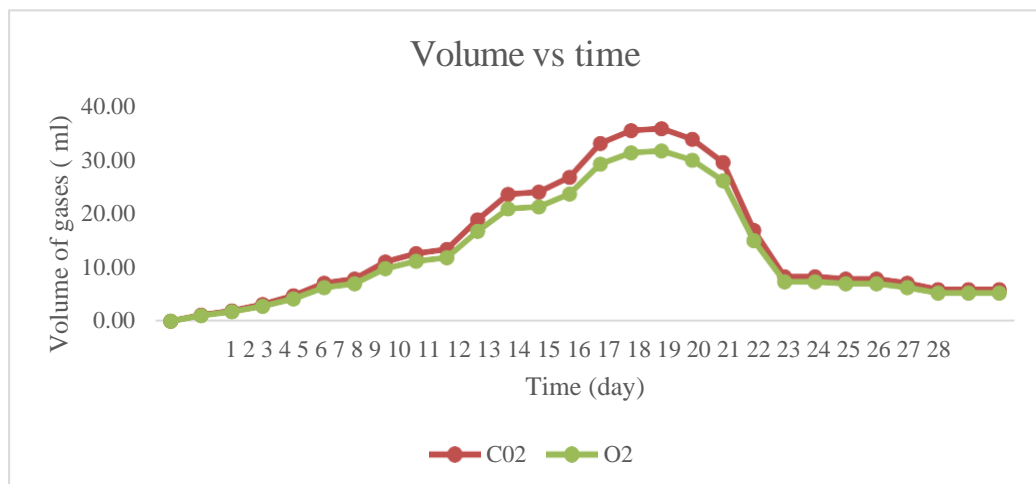


Figure 4.3 Volume of CO₂ and O₂ from potato pseudo stem banana waste

Time (day)	Volume biogas (ml)	Volume of CO₂ (ml)	Volume of O₂ (ml)
1	0.00	0.00	0.00
2	3.00	1.18	1.05
3	5.00	1.97	1.74
4	8.00	3.15	2.79
5	12.00	4.73	4.18
6	18.00	7.09	6.27
7	20.00	7.88	6.97
8	28.00	11.03	9.75
9	32.00	12.60	11.15
10	34.00	13.39	11.84
11	48.00	18.91	16.72
12	60.00	23.63	20.90
13	61.00	24.03	21.25
14	68.00	26.78	23.69
15	84.00	33.09	29.26
16	90.00	35.45	31.35
17	91.00	35.84	31.70
18	86.00	33.87	29.96
19	75.00	29.54	26.13
20	43.00	16.94	14.98
21	21.00	8.27	7.32
22	21.00	8.27	7.32
23	20.00	7.88	6.97

24	20.00	7.88	6.97
25	18.00	7.09	6.27
26	15.00	5.91	5.23
27	15.00	5.91	5.23
28	15.00	5.91	5.23

Table 4.1 volume of biogas produced after 28 days

Figure 4.2 shows the volume of biogas produced by potato and pseudo stem banana wastes from day 1 until day 28. The volume of biogas was determined through water displacement method. The volume of biogas produced for whole 28 days was 1011 ml. Based on the graph or figure 4.2, it shows that the volume of biogas from day 1 until day 17 was increased significantly. But, at day 18, the volume of biogas started to decrease until day 28. This happened due to the amount of carbon dioxide reduced at day 18. From day 1 until day 17, the aerobic digestion occurred in the anaerobic digestion reactor. This can be proven from landfill gases process at phase 1 in chapter 2 literature review. In this process, the amount of carbon dioxide increased due to the aerobic process that consumed oxygen and released the carbon dioxide (breathing concept). Thus, the amount of carbon dioxide increased until day 17. On day 18, anaerobic digestion took over the process and thus, the amount of carbon dioxide decreased and also the amount of oxygen decreased as the anaerobic digestion process doesn't consume any oxygen in its process. The reduction of CO₂ and O₂ volume can be seen at figure 4.3.

For the figure 4.3, the volume of every gas can be calculated with the concentration of gas from GC-TCD data with volume of biogas:

$$\text{Volume of CO}_2 = \text{volume of biogas (ml)} \times \frac{39.388\%}{100}$$

$$\text{Volume of O}_2 = \text{volume of biogas (ml)} \times \frac{34.852 + 0.253\%}{100}$$

As the decreasing of the CO₂ and O₂, the anaerobic digestion process will occur and the production of methane will occur. The reason why there is no methane concentration produced was due to late of anaerobic digestion process as the aerobic digestion process occurred longer compared to anaerobic digestion process. In this experiment, the aerobic digestion process occurred about 17 days while anaerobic digestion process occurred around 11 days. This happened due to higher moisture content of the potato and pseudo stem banana wastes which can slow down the anaerobic digestion process. Water or H₂O exist in the moisture content and water also consist of oxygen gas which can increasing the aerobic digestion process and also increasing the amount of carbon dioxide. Thus, the retention time of the aerobic digestion process occurred longer than the anaerobic digestion process. Although the potato and pseudo stem banana wastes had been dried at outside temperature and been left for 2 months, it still consist higher moisture content. Other reason why the aerobic digestion process took longer than anaerobic digestion is due to outside gas entering the reactor through the gas bag valve. At the top of gas bag valve, there is a overflow outlet where by it used to remove the exceeded gases in gas bag. During loose the valve, the overflow outlet will opened too. Thus, the surrounding gas from the outside will possible to enter the inside reactor. The second is at the control valve from reactor. During installing the control valve with the bottle cap, the hot glue chemical was used to seal and connect the items. The last reason could happen was a leakage between the sealer.

4.4 The changes in the pH value during anaerobic digestion for the potato and pseudo stem banana wastes

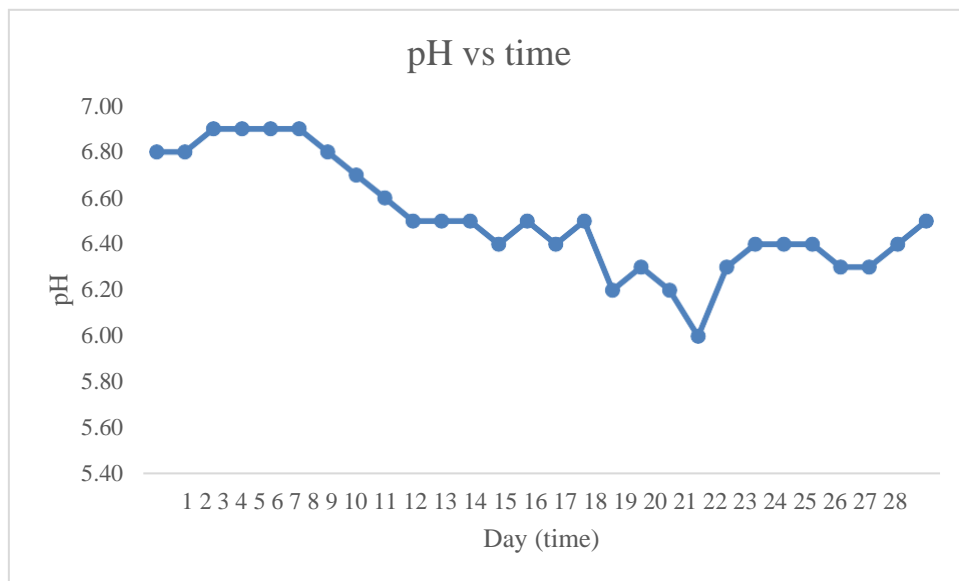


Figure 4.4 pH value of the anaerobic digestion for 28 days

Time (day)	pH
1	6.80
2	6.80
3	6.90
4	6.90
5	6.90
6	6.90
7	6.80
8	6.70
9	6.60
10	6.50
11	6.50
12	6.50

13	6.40
14	6.50
15	6.40
16	6.50
17	6.20
18	6.30
19	6.20
20	6.00
21	6.30
22	6.40
23	6.40
24	6.40
25	6.30
26	6.30
27	6.40
28	6.50

Table 4.2 pH changes for 28 days

Based on the figure 4.4, the pH started to decrease from day 1 until day 20 due to decomposition of wastes by the acidogenic microorganisms. In the acidogenesis process, the product from hydrolysis was converted into smaller size such as acetic acid which affected the pH in the anaerobic digestion reactor. Thus, the pH decreased significantly. Based on figure 4.2, the carbon dioxide started to reduce at day 18 as the anaerobic digestion took over the process. Based on figure 4.4 at day 18, the pH started to increase due to hydrolysis process of anaerobic digestion took over the process, but at day 19 to 20, the acidogenesis process and acetogenesis process started again but in anaerobic digestion process. On day 21 until day 24, the methanogenesis process started to occur due to increasing of pH where the optimum pH for methanogenesis process

around 6.5 to 8.0. Thus, the pH started to increase as to reach the pH optimization for methane gas production. At day 25 until day 26, acidogenesis and acetogenesis process occurred again due to the conversion of hydrolysis product to smaller size. At day 27 until 28, methanogenesis process started to occur as the pH increasing and reached the optimum pH at day 28.

CHAPTER 5

CONCLUSION

5.1 Introduction

The objective of this research is to fabricate the anaerobic digestion reactor and analyse the methane production from potato and pseudo stem banana waste. The fabrication and analyzation were achieved but need some improvement in term of calculation of the reactor and the improvement of the methane production..

5.2 Conclusion & recommendation

The designation of the reactor is needed some improvement especially for the calculation of the volume of the gases Based on the calculation of the volume in the reactor, the volume of gases that can produced was below 600 ml, but in the result of analysis by gas chromatography, The total volume of gases produced was around 1011 ml. Based on the ideal gas law $pV=nRT$, the volume of gas is directly proportional to the temperature. As the temperature increase, the pressure will increase too. If the volume of gases exceeded from the calculation, the reactor might be broken which also can lead to accident and injury to towards the user. To avoid the accident, the designation should be recalculated based on the total of moisture content of the wastes too and also the volume of the gases in the silicon tube and the gas bag. The maximum of the gas bag can content is around 1 L of gas. The reactor also should be included with overflow pipe if the gas in the reactor and gas bag reached the maximum volume.

For the analyzation of methane production, based on the result, the retention time for the reduction of the carbon dioxide and the oxygen gas were quite longer where by the reduction was started at day 18. The maximum retention time for the mesophilic process is 40 days. If more than that, the methane production will stop. To avoid the late production of methane gases, the pre treatment of the wastes should be improvise especially for the hemicelluloses waste which consist of double bond

structure. The double bond structure can be broke down using low pH or in other word using acid. The acid can break the double bond structure into single bond and the extra single bond can be react with the other charge to convert it into glucose. This chemical pre treatment process able to reduce the retention time and can increasing the methane production. The temperature of the pre-treatment should be increased in order to remove the moisture content of the waste. In this study, the method of removing moisture content was only dried at outside temperature which around 30 degree celcius and below and also only been left for 2 months. Plus, the humidity of the outside temperature also will effect the moisture content removing. To avoid this, the temperature should be increased using furnace around 60 degree celcius based on the Alshammari (2011) study. The method of physical pre-treatment in this study also need some improvement where by in this study, it used water to ease the grinding process. This water has increased the moisture content of the waste. To avoid it, the best way is by drying it using furnace around 60 degree celcius to remove the moisture content of the wastes. In this study, there is some error in plotting the graph for the volume versus time graph. In this graph, at day 18, the line of the oxygen gas graph supposed has stopped at day 17, but in this graph, the oxygen started to decline along with carbon dioxide . This was based on the landfill gases process (atdsr 2008) where by there will be 2 process occurred starting from aerobic than anerobic. The anaerobic process in this study was started at day 18 which the methane production supposedly produced. This has lead to error of the plotted graph and this study. To avoid and minimize the error, gas detection sensor should be implemented in the reactor to over see the methane production. This sensor must be included with coding to display the output reading. Last but not least, to improve the methane gas production, agitator should be included in the reactor as to promote efficient interaction of active microbes and organic matter, to prevent coarser material settling, to achieve slurry homogeneity, to avoid the formation of temperature gradients (Gómez et al.,2006; Rizwan et al., 2015). Finally, to detect the leakage of the reactor, pressure gauge should be installed at the reactor in other to trace the leaking by monitor the reduction of pressure. Using formula $pV = nRT$. as the pressure decrease, volume increase. This happened due to outside gases entering the reactor.

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APPENDICES

Appendix A:

Potato waste from super market



Appendix B:

Pseudo stem banana waste from agricultural farm



Appendix C:

Chopping process at Environmental laboratory



Appendix D:

Grinding process at Environmental laboratory.



Appendix E:

Weighing the food waste using analytical balance



Appendix F:

Preservation of the food waste in the anaerobic digestion reactor



Appendix G:

Water displacement method



Appendix H:

pH and temperature monitoring of the food waste mixture in the reactor



Appendix I :

Expanded of the gas bag after 28 days



Appendix J :

Arrangement of the anaerobic digestion reactor

