METHANE POTENTIAL FROM ANAEROBIC CO-DIGESTION OF SEWAGE SLUDGE AND FOOD WASTE IN A STIRRED BATCH REACTOR

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ABSTRACT

Anaerobic digestion is a promising method for organic waste stabilization, including food waste and sewage sludge. Anaerobic digestion can be completed either in mono digestion or co-digestion, respectively; no longer limited to waste stabilisation method but also towards renewable energy production in the form of methane. The co-substrate, a mixture of two or more organic waste, was used as a substrate in anaerobic co-digestion. The effect on co-digestion of municipal sewage sludge and food waste has been reported previously. However, there is less information on the digestibility of co-substrate, specifically a mixture of domestic sewage sludge and food waste. Hence, this study was conducted to examine the characteristics of the mixture of domestic sewage sludge and food waste, to compare the methane yield from a batch test of mesophilic anaerobic co-digestion and to determine the best fit between laboratory and modelling analysis of methane production's kinetics. Two sets of batch biochemical methane potential (BMP) test were conducted using Automatic Methane Potential Test System (AMPTS II); each for digestion of co-substrate; 1) a mixture of primary sewage sludge and food waste (PSS+FW) and 2) a mixture of secondary sewage sludge and food waste (SSS+FW). The results showed that the addition of food waste to domestic sewage sludge improved volatile solids to total solids (VS/TS) ratio. Between two co-substrates, PSS+FW showed better digestibility shown by 530.4mL methane accumulated, ultimate methane yield of 1233.57 mL CH_4/g VS and methane production rate of 625.18 CH_4/gVS day. Modified Gompertz modelling found fit well to the laboratory data indicatedby R^2 of 0.997. In conclusion, co-digestion improved the synergy effect between organic substrates, indicated by improved volatile solid, VS to total solid, TS (VS/TS) ratio; and increased the efficiency of the anaerobic process shown by high methane production at early stage of the digestion inline with no lag phase.

ABSTRAK

Pencernaan anaerobik ialah kaedah kestabilan sisa organik seperti sisa makanan dan enapcemar kumbahan. Pencernaan anaerobik tidak lagi terhad sebagai kaedah penstabilan sisa tetapi juga bagi penghasilan tenaga boleh diperbaharui dalam bentuk gas metana; boleh dilakukan secara pencernaan tunggal mahupun pencernaan bersama. Substrat bersama merupakan campuran dua atau lebih sisa organik yang digunakan dalam pencernaan bersama anaerobik. Kesan terhadap pencernaan bersama enapcemar kumbahan dan sisa makanan telah dilaporkan, namun terdapat kekurangan maklumat. Oleh itu, kajian ini telah dilaksanakan bagi menguji ciri-ciri yang terdapat pada substrat bersama iaitu campuran enapcemar kumbahan domestik dan sisa makanan, seterusnya membandingkan hasil metana terkumpul daripada ujikaji pencernaan bersama anaerobik mesofilik dan menentukan model kinetik penghasilan metana terpaling sesuai diantara ujikaji makmal dan analisis. Dua set kumpulan ujikaji potensi metana biokimia (BMP) telah dilaksanakan menggunakan Automatic Methane Potential Test System (AMPTS II) bagi setiap substrat bersama; 1) ialah campuran enapcemar domestik primer (EDP) dan sisa makanan (SM), (EDP+ SM) dan 2) ialah campuran enapcemar domestik sekunder (EDS) dan sisa makanan (SM), (EDS +SM). Hasil dapatan menunjukkan di antara dua substrat bersama, EDP+SM mempunyai kelebihan prestasi pencernaan yang ditunjukkan dengan 530.4 mL metana terkumpul, hasil titik alah metana tertinggi ialah 1233.57mL CH_4/g VS , kadar penghasilan metana harian ialah 625.18 CH_4/g VS. Modified Gompertz menunjukkan keputusan memuaskan melebihi First Order ditunjukkan oleh nilai R^2 bersamaan 0.997. Kesimpulannya, pencernaan bersama meningkatkan kesan sinergi organik antara substrat , ditunjukkan oleh peningkatan nisbah pepejal meruap (PR) terhadap pepejal jumlah (PJ) (PR/PJ); dan meningkatkan kecekapan proses anaerobik yang ditunjukkan oleh penghasilan banyak metana pada masa -masa awal pencernaan yang selaras dengan tiadanya masa ketinggalan

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LIST OF SYMBOL AND ABBREVIATION

AD	Anaerobic digestion
AMPTS II	Automatic Methane Potential Test System II
BMP	Biochemical Methane Potential
BSA	Bovine Serum Albumin
COD	Chemical Oxygen Demand
FAO	Food and Agricultural Organization
FW	Food Waste
MSW	Municipal Solid Waste
OFMSW	Organic Fraction Municipal Solid Waste
POME	Palm Oil Mill Effluent
PSS	Primary Sewage Sludge
SSS	Secondary Sewage Sludge
TS	Total Solids
UTHM	Universiti Tun Hussein Onn Malaysia
VS	Volatile Solids
WWTP	Wastewater Treatment Plant

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CHAPTER 1

INTRODUCTION

1.1 Background of study

Malaysia, as a developing country, experienced exponential social and economic growth. This rapid development also comes with an increase in population, leading to a significant environmental problem with the increase of waste generation. According to National Solid Waste Management Department (2016), waste composition fraction in Malaysia was dominated by municipal solid waste (MSW) (64%), followed by industrial waste (25%), commercial waste (8%) and construction waste (3%). According to Ghafar (2017), about 50% of MSW were dominated by food waste. Due to the improper separation of food waste from municipal solid waste, food waste contributes to serious environmental issues in the landfill. Once it is disposed in landfills, food waste naturally biodegrades and release harmful environmental elements such as leachates and gases (e.g., methane) (Kaur et al., 2019).

In addition, sewage sludge generated in Malaysia is also disposed of in a landfill (National Solid Waste Management Department, 2016). Implementing sewage sludge treatment has been challenging for most countries due to the lack of expertise and fund (Kaur et al., 2019). About 39% of sewage sludge were used in agriculture in the European region. Besides, high-income countries such as the United States of America (USA), Germany, and Canada treated sewage sludge by adapting the anaerobic digestion treatment (Fijalkowski et al., 2017). Anaerobic digestion for sewage sludge has a long history. This approach purposely aimed to get rid of and

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focused on recycling sewage sludge. Practically, the anaerobic digestion of sewage sludge comprises a mixture of primary and secondary sewage sludge (Girault et al., 2012). Afterwards, the focus was slowly changed as methane gas could generate electricity (Sembera et al., 2019). The anaerobic digestion is also actively developed for food waste management sectors (Dai et al., 2013). Unfortunately, the anaerobic digestion of food waste is inefficient when used as the sole substrate (Xiaofeng et al., 2014).

Therefore, researchers studied the approach to improve the mono digestion and found that the co-digestion is a promising method. Co-digestion's main purpose is to improve the anaerobic digestion process in terms of economic viability and stability Co-digestion is the combination of different substrates mixed and anaerobically digested (Astals et al., 2015). The co-digestion process lies in balancing several parameters of co-substrate mixture include macro and micronutrients, pH, inhibitors/toxic compounds, biodegradable organic matter, and dry matter (Alvarez et al., 2010). Co-digestion shows better performance and provide better nutrient balance and biogas production than mono-digestion (Lv et al., 2021). In addition, large amount of biogas production rate and yield along with higher process stability were also obtained through co-digestion (Hosseini Koupaie et al., 2014).

As a result, the interest of the researcher in co-digestion has been increased. Several laboratory scales for studying co-digestion of sewage sludge and food waste have been conducted (Koch et al., 2015; Nielfa & Cano, 2015; Cabbai et al., 2013; Dai et al., 2013). Almost all these studies show positive improvement. For instance, Dai et al., (2016) found significant improvement of the methane yield of 471.1 mL CH_4/g VS with the supplementary of food waste compared to 385.9 mL CH_4/g VS from digestion sewage sludge alone. The addition of food waste as co-substrate into sewage sludge significantly improved the methane yield about 24% of methane yield (368.7 mL CH_4/g VS) compared to 280.4 mL CH_4/g VS mono-digestion (sewage sludge alone) (Gu et al., 2020).

Not only limited in laboratory scale, co-digestion of sewage sludge and food waste also applied at full-scale. East Bay Municipal Utility District Wastewater Treatment Plant (WWTP) is the first plant in the USA to implement co-digestion of sewage sludge and food waste. Other than that, the Rovereto plant and Treviso plant in Italy also implemented co-digestion of sewage sludge and food waste (Nghiem et al., 2017).

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Methane is estimated through the biochemical methane potential test (BMP) (Achu et al., 2016; Fonoll et al., 2015). BMP is a reliable and well-known test to evaluate the ultimate methane potential per mass of substrate. According to Valero et al. (2016), by adapting the BMP test, the matter removal (in terms of total solid (TS), volatile solid (VS) or chemical oxygen demand (COD)) and the kinetics studies of organic substrate in the anaerobic digestion process could be observed. BMP test was done normally using bottle reactor or serum bottle either at mesophilic or thermophilic conditions (Braguglia et al., 2017; Kumaran et al., 2016; Fonoll et al., 2015).

BMP test could be conducted through several methods such as continuous, semi-continuous, or batch study (Zahedi et al., 2018; Nielfa & Cano, 2015; Dai et al., 2013). However, methane's manual sampling could be the greatest human error for the BMP test (Himanshu et al., 2017; Kleinheinz & Hernandez, 2016). Therefore, it is appropriate to use the automatic machine to reduce human competency. Automatic Methane Potential Test System II (AMPTS II) was used to minimise human errors and reflects the major processes involved in allowing users to determine the true biochemical methane potential and dynamic degradation profile of any biomass substrate (Himanshu et al., 2017). AMPTS II is the most advanced system due to its precise predictability and strong generality for the BMP test (Himanshu et al., 2017; Kleinheinz & Hernandez, 2016; Valero et al., 2016).

Besides the cumulative methane yield, the methane production rate and the lag phase's duration are also crucial indicators reflecting the efficiency of the anaerobic digestion (Zhen et al., 2016). The methane production rate of particulate organic matter and the cumulative methane yield can be calculated using the Modified Gompertz and First-Order kinetic. In this sense, these two models were adapted, and findings were compared to truly elucidate the kinetics of methane production during the co-digestion of sewage sludge with food waste.

1.2 Problem statement

Since the mid-eighties, waste generated in urban areas has increased year by year due to the rapid urbanisation and diversity of Malaysian lifestyles. As a result of rapid urbanisation, rising waste management costs and securing landfill sites have arisen.

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Malaysia, a country with a population, increased by 1.10 million for three years (2011-2014), shows a proportionally increment in the generation of sewage sludge with approximately 0.25m^3/day for each person (Kumaran et al., 2016). According to the Ministry of Housing and Local Government's annual report, about 25,000 tons of solid waste was generated daily in 2011, and these amounts were dominated by food waste with 47% (Jereme et al., 2016).

Currently, about 165 landfills are operated in Malaysia. The dependency on the landfill as the main disposal method was expected to increase 50% of the greenhouse effect in 2020 (Zainu & Songip, 2017). Wastes in the form of municipal solid waste (MSW) (including food waste) and sewage sludge were dumped into landfills for final disposal. Unfortunately, food waste and sewage sludge, which is organic waste, will easily turn into contaminants, causing serious effects to the environment; contribute to greenhouse emissions and health problems (Syed Ismail & Abd Manaf, 2013).

In Malaysia, incineration cannot be implemented because of high moisture content from an organic compound in waste, especially food waste, which needs additional costs to cover the auxiliary fuel in burning processes (Syed Ismail & Abd Manaf, 2013). Therefore, anaerobic digestion emerged as an alternative to landfilling. It stabilises organic materials, destroys pathogens, and produces by-products, i.e. methane gas that can be used to generate electricity (Pan et al., 2019; Iacovidou et al., 2012). As the anaerobic digestion showed a positive effect, studies of this treatment using various substrate was actively conducted. Food waste is also a suitable substrate for anaerobic digestion (Cabbai et al., 2013). Gu et al., (2020) conducted a study of co-digestion of sewage sludge and food waste in batch reactor with mesophilic and thermophilic condition. The results found that the temperature is not significantly influence the methane yield but relatively accelerated the methane production rate. However, as predicted, the co-digestion enhanced the methane yield and methane production rate by about 24% increase compared to mono-digestion (food waste) (Gu et al., 2020). Dai et al., (2013) also used to combined sewage sludge and food waste for anaerobic co-digestion.

Hence, on BMP study, this research aims to estimate the ultimate methane yield from the co-digestion of domestic sewage sludge and food waste. In addition, the kinetic analysis using the modelling was also included to assess the biodegradability of the co-digestion.

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1.3 Objective of study

The objectives of this study are as follows:

i. To examine the characteristics of the co-substrates (a mixture of domestic sewage sludge and food waste).
ii. To compare the methane yield from the batch test of mesophilic anaerobic co-digestion of co-substrates.
iii. To determine the best fit methane production's kinetics between laboratory and modelling analysis for the co-digestion.

1.4 Scope of study

The co-substrate is a mixture of sewage sludge and food waste that was prepared accordingly to wet mass. Two co-substrates were prepared; each was differentiated by the types of domestic sewage sludge, either primary or secondary. The samples of sewage sludge were taken from the UTHM sewage treatment plant. Meanwhile, food waste was collected from the cafeteria in UTHM main campus. The inoculum for the anaerobic co-digestion test is the active anaerobic biomass taken from an existing anaerobic digester treating palm oil mill effluent (POME). The characteristics such as chemical oxygen demand (COD), protein, carbohydrates, total solids (TS) and volatile solids (VS) were determined following Hach-Method 8000, Lowry Method, Phenol-Sulphuric Method and APHA Standard Method 2540, respectively. The AMPTS II was used for monitoring methane production during the co-digestion via BMP. Modified Gompertz and First-order modelling were applied to describe the kinetics from the co-digestion of co-substrates.

1.5 Significance of study

Knowing that deposition of food waste in landfills is related to the higher emission of greenhouse gas (including methane) in Malaysia, National Solid Waste Management Department provided Food Waste Management Development Plan for Industry, Commercial and Institution Sector (FWMDP IC, 2016-2026) to achieve an efficient and effective food waste management. FWMDP IC is developed in line with the Solid

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2 Waste Management Policy (2016) and Strategic Plan of the National Solid Waste Management Department (2016-2020) (National Solid Waste Management Department, 2016). This research meets the FWMDO IC inspiration for food waste treatment at source includes possible conversion of food waste into useful resources generating electricity from food waste or making alternative fuel from food waste.

This study improved the understanding of local organic wastes, including food waste and domestic sewage sludge, particularly the digestibility of co-substrate. The result from the digestability test, particularly the kinetic parameters such as ultimate methane yield, the lag phase, and methane production rate, could be used to design the laboratory scale anaerobic digester to treat organic waste with similar characteristics as observed in this study.

This study also supports the Malaysia Government policy in managing waste holistically to promote anaerobic digestion in managing organic waste as an alternative to divert the waste from being dumped in landfills.

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CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

This chapter starts with explanation on the anaerobic digestion al to provide the overview on how anaerobic digestion is used to manage the food waste for mono digestion and co-digestion. The fundamental on the anaerobic process and the factors that affecting the anaerobic digestion also described. This chapter also describe the kinetics analysis of methane production . The discussion on anerobic digestion of foodwaste at batch mode was comprehensively included.

2.2 Anaerobic digestion

Sewage sludge and food waste conventionally was sent into landfill for disposal. However, a diversion of this method of landfilling are needed in order to reduce the emission of greenhouse gases. As an alternative, many options have been adapted for the waste management including incineration and composting (Iacovidou et al., 2012). However, incineration of food waste consisting high moisture content results in the release of dioxins which leads to several environmental problems. In addition, incineration reduces the economic value of the substrate as it hinders the recovery of nutrients and valuable chemical compounds from the incinerated substrate (Kunwar et al., 2017).

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Hence, appropriate methods are required. Anaerobic digestion can be an alluring option to strengthen world's energy security by employing food waste to generate biogas while addressing waste management and nutrient recycling (Kunwar et al., 2017). Anaerobic digestion technology was basically an environmental-friendly with lower CO_2 emission and fewer fossil energy cost. In addition, anaerobic digestion also beneficial for synergistically disposal organic wastes and resources recovery including volatile fatty acids (VFAs), hydrogen and methane (Silva et al., 2021). Anaerobic digestion can be directed to the production of a specific resource such as VFAs, hydrogen and methane) or all of them simultaneously. The production of only one specific resource can make the control of the operational parameters simpler, maximizing the product yield (Jiang et al., 2022). The simultaneous production allows to obtain different products with high added value, potentiating the economic benefits of the process (Silva et al., 2021).

Anaerobic digestion is a well-proven and mature technology for producing methane-rich biogas from organic waste decomposition (Rubia et al., 2018). Anaerobic is a complex biochemical process that undergoes hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Nayak & Bhushan, 2019). Anaerobic digestion of a single substrate (mono-digestion) presents some drawbacks linked to the characteristics of the substrate. Simultaneous digestion of two or more substrates is known as co-digestion. Co-digestion also enhanced the productivity of renewable energy, the possibility of nutrient recycling and the reduction of wastes (Maragkaki et al., 2017). Recently, co-digestion was realised to be more stable when a variety of substrates were applied. The most common situation is when many main basic substrates such as sewage sludge are mixed and digested with a minor amount of as single; a variety of additional substrates (Maragkaki et al., 2017).

Europe has implemented a full-scale digester for anaerobic digestion with over 10,000 plants in Germany, followed by Italy and France with 7,000 plants. The majority of these biogas plants are treating agricultural resources, and the remainder uses mainly organic waste substrates and sewage sludge. Major Greece cities operate their anaerobic digestion in full-scale treating sewage sludge. Not only treating sewage sludge, agro-industrial waste such as wineries, cheese factories and livestock units also treated by anaerobic digestion process (Maragkaki et al., 2017). Co-digestion of sewage sludge and food waste in wastewater treatment plants (WWTPs) are widely demonstrated in Italy, Germany, Denmark, and Switzerland (Chakraborty et al., 2017).

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Mono-digestion and co-digestion processes could be conducted in either batch mode, semi-continuous, or continuous study (Zahedi et al., 2018; Nielfa & Cano, 2015; Dai et al., 2013). Table 2.1 shows the reactor types of anaerobic digestion and co-digestion process takes placed by recent research. Various of substrate ranging from agricultural waste, domestic waste and municipal waste has been used as substrate for anaerobic digestion and co-digestion. Most study for the mono-digestion were conducted at batch mode under mesophilic temperature (37^0C). Batch reactor is a traditional method and mainly used in determining the maximum methane potential and kinetic measurement of a substrate (Tsapekos et al., 2018). Batch digestion was widely used for comparison and evaluation, since many tests can be conducted simultaneously (Tsapekos et al., 2018). On the other hand, semi-continuous digestion was conducted to examine the performance and stability of the digester in the long-term. The advantage of semi-continous over the batch method is the ability to detect inhibitory compounds at low levels. However, this method is labor intensive and requires operating experience. The most widely used of the semi-continuous reactor is the continuously stirred tank reactor (CSTR), since it is available from lab scale to commercial scale (Wikandari, 2014).

Gu et al., (2020) conducted a study of co-digestion of sewage sludge and food waste in batch reactor with mesophilic and thermophilic condition. Result shows that both temperature used does not significantly influence methane yield although higher temperature resulted in accelerated methane production. However, short lag phase was found in mono-digestion of food waste, which likely related to accelerated hydrolysis due to its readily available degradable content in both condition.

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Table 2.1 Reactor used for the anaerobic and co-digestion study

Substrate	Setup	Reactor type	Temperature	References
Decanter cake, Palm oil mill effluent	Mono digestion	Batch	Mesophilic	(Lim et al., 2021)
Sewage sludge, food waste		Batch	N.A	(Zhang & Wang, 2021)
Sewage sludge, food waste		Batch	35 °C	(Gu et al., 2020)
Sewage sludge, food waste		Batch	37°C	(Pan et al., 2019)

Table 2.1 Continued

Food waste		Batch	37°C	(Seswoya et al., 2018)
Sewage sludge, Food waste		Batch	37°C	(Li et al., 2018)
Sewage sludge, food waste		Batch	37°C	(Cabbai et al., 2013)
Slaughterhouse waste		Batch	N.A	(Pages-diaz et al., 2018)
Slaughterhouse waste, manure, various crops, municipal solid waste	Co-digestion	Semi-continuous	N.A	
Sewage sludge, grease trap sludge and organic fraction of municipal solid waste		Semi-continuous	Mesophilic	(Grosser et al., 2017)

*Note: N.A-not available

Biomethane potential (BMP) test is a useful and inexpensive assay used to estimate the digestibility and maximum methane production of various organic substrates in anaerobic digestion process (Ohemeng-ntiamoah & Datta, 2019). Traditionally, BMP test was used to estimate methane potential of organic substrates (Ohemeng-Ntiamoah & Datta, 2021). The BMP can be achieved by adding a known quantity of organic substrate to an active anaerobic inoculum in an air-tight serum bottle where the methane produced is measured and determined in unit of mL CH_4/g VS (Ohemeng-ntiamoah & Datta, 2019). Besides giving information on methane production, BMP also provide results of the rate of degradation process and level of biodegradability of the substrate used (Stromberg et al., 2014).

Typically, BMP was manually set up to keep it simple. However, the time and labor required for manually experimental setup, coupled with potential inaccuracies that may occur during daily gas measurement, have made it worthwhile for some studies to conducted the BMP with automated systems. Wang et al. (2014) assessed the methane potential of cellulose by conducting BMP tests with three different conventional manually operated experimental setups (i.e., pressure-based gas measuring system aided bymanometer, water-column based measuring system, gas-bag-based measuring system) and an automated BMP setup (i.e., automatic methane

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potential test system (AMPTS II) (Bioprocess Control, Sweden). Table 2.2 shows the BMP for digestability study of mono-substrate and co-substrates.

Table 2.2: BMP for digestability study of various substrate and co-substrates

Substrate	Temperature	Duration (days)	Reference
Sewage sludge	Mesophilic	45	(Arelli et al., 2021)
Sewage sludge	Mesophilic	30	(Gu et al., 2020)
Sewage sludge	Mesophilic	30	(Gaur & Suthar, 2017)
Sewage sludge	Thermophilic	45	(Arelli et al., 2021)
Sewage sludge	Thermophilic	20	(Gu et al., 2020)
Sewage sludge and food waste	Mesophilic	45	(Arelli et al., 2021)
Sewage sludge and food waste	Mesophilic	30	(Gu et al., 2020)
Sewage sludge and food waste	Mesophilic	30	(Gaur & Suthar, 2017)
Sewage sludge and food waste	Thermophilic	45	(Arelli et al., 2021)
Sewage sludge and food waste	Thermophilic	20	(Gu et al., 2020)

2.3 Fundamental of anaerobic mono- digestion and co-digestion process

Anaerobic digestion, either mono-digestion or co-digestion, was completed at four (4) stages, including hydrolysis, acidogenesis, acetogenesis and methanogenesis, as shown in Figure 2.1. The anaerobic digestion process starts with the hydrolysis process. In the hydrolysis process, the substrates with a complex organic polymer such as carbohydrates, protein, and fats were disintegrated by bacteria producing simple sugar, amino acids, and fatty acids. Next, the acidogenesis process takes place. This

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stage occurs by fermenting the hydrolysis product into short-chain acids. The by-products of this stage, such as acetate, hydrogen and carbon dioxide, can be utilised directly for methane. Acidogenesis is known as the fastest stage in the anaerobic digestion process. The short-chain acids will undergo the acetogenesis process under strict conditions. Lastly, the methanogenesis process in which the acetate and hydrogen can be utilised directly for methane generation. Biogas as a final product of anaerobic digestion comprises methane, carbon dioxide and other gases. Biogas produced contained about 50 to 65% of methane, 40 to 50% carbon dioxide, and the rest are hydrogen sulphide, nitrous dioxide and other gases (Mehariya et al., 2018). Each process conversion of organic occurs with different types of bacteria, as tabulated in Table 2.3.

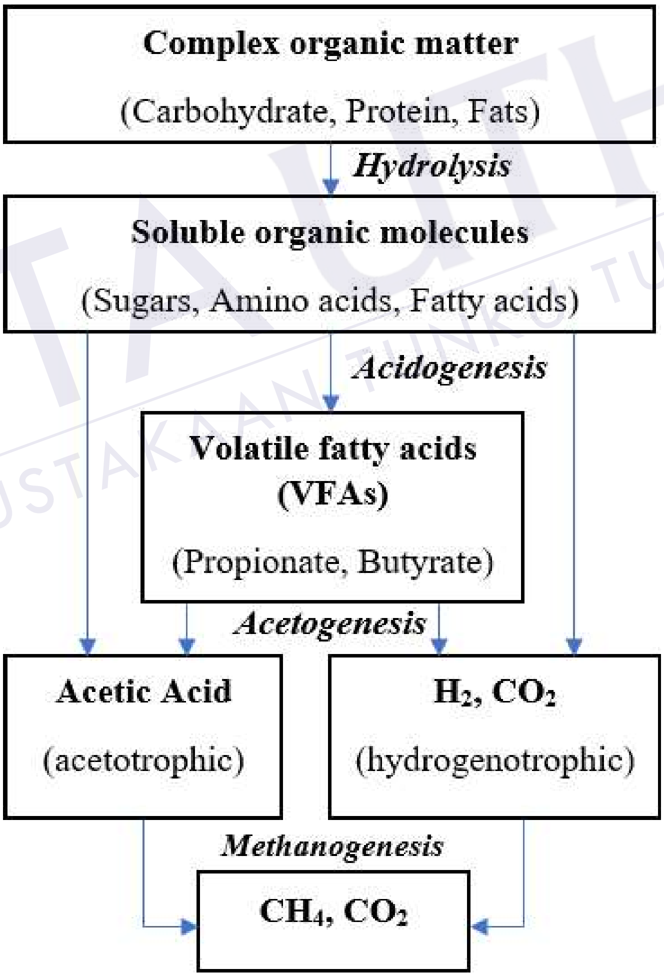

Figure 2.1 Anaerobic digestion process (Paritosh et al., 2017)

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Table 2.3 Chemical reaction and bacteria involved in the anaerobic digestion (Deepanraj *et al*., 2014)

Stage	Type of Conversion	Bacteria Involved
Stage I (Hydrolysis) $(C_6H_{10}O_5)n + nH_2O = n(C_6H_{12}O_6)$	Protein to soluble peptides and amino acids Carbohydrates to soluble sugars Lipids to fatty acids or alcohol	*Clostridium, Proteus Vulgaris, Vibrio, Bacillus, Peptococcus, Bacteroides* *Clostridium, Acetovibriocelluliticus, Staphylococcus,* *Clostridium, Micrococcus, Staphylococcus*
Stage II (Acidogenesis) $C_6H_{10}O_6 + 2H_2O \rightarrow 2CH_3COOH + 4H_2 + CO_2$ $C_6H_{10}O_6 + 2H_2 \rightarrow 2CH_3CH_2COOH + 2H_2O$ $CH_6H_{10}O_6 \rightarrow 2CH_3CH_2CH_2COOH + 2H_2 + 2CO_2$ $C_6H_{10}O_6 \rightarrow 2CH_3CH_2OH + 2CO_2$ $C_6H_{10}O_6 \rightarrow 2CH_3CHOHCOOH$	Amino acids to fatty acids, acetate and NH_3 Sugars to intermediary fermentation products	*Lactobacillus, Escherichia, Bacillus, Staphylococcus, Pseudomonas, Sarcina, Desufovibrio, Selenomonas, Streptococcus, Veollonea, Desulfobacter, Desulforomonas* *Clostridium, Eubactriumlimosum, Streptococcus*
Stage III (Acetogenesis) $CH_3CH_2 + 2H_2O \rightarrow CH_3COOH + 2H_2$ $2CH_3CH_2OH + 2CO_2 \rightarrow CH_4 + 2CH_3COOH$ $CH_3CH_2COOH + 2H_2O \rightarrow CH_3COOH + 3H_2 + 2CO_2$ $CH_3C\,H_2CH_2COOH + 2H_2O \rightarrow CH_3COOH + 2H_2$ $CH_3CHOHCOOH + H_2O \rightarrow CHCOOH + 2CO_2 + 2H_2$	Higher fatty acids or alcohol to hydrogen and acetate Volatile fatty acids and alcohols to acetate or hydrogen	*Clostridium, Syntrophomonaswolfeii* *Syntrophomonaswolfei, Syntophomonaswolinii*
Stage IV (Methanogenesis) $CH_3COOH \rightarrow CH_4 + CO_2$ $CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$	Acetate to methane and carbon dioxide Hydrogen and carbon dioxide to methane	*Methanobacterium formicicum, Methanobrevibacterium, Methanoplanus, Methanospirilum*

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2.3.1 Hydrolysis stage

Hydrolysis is defined as the reaction in which a molecule split, and the hydrogen and hydroxide ions from a water molecule are attached to the different products. Hydrolytic bacteria will hydrolyse the complex organic matter into monomeric units (Braguglia et al., 2017; Zhang et al., 2014). The hydrolysis stage converts the complex organic polymers like carbohydrates, protein and fat degraded by bacteria to form sugar, amino acid and long fatty acid before undergoing the next phase (Braguglia et al., 2017). The hydrolysis rate is relatively slower than the rate of acid formation. The hydrolysis rate mainly depends on the nature of substrate, bacterial concentration, pH and the bioreactor temperature. Other important parameters, such as the size of substrate particles, pH, production of enzymes, and adsorption of enzymes on the substrate particles also affect the hydrolysis rate (Paritosh et al., 2017).

2.3.2 Acidogenesis stage

Acidogenesis of fermentation is the second phase in anaerobic digestion. This stage is also called as acid formation stage. Unlike hydrolysis, this stage normally occurs in a fast reaction. The mixture of organic acids was formed in the acidogenesis stage, such as lactate, butyrate, ethanol, and propionate (Matheri et al., 2016). The main product produce from this stage depends on the substrate present and culture conditions. As the acidification process occurs, facultatively anaerobic bacteria utilise oxygen and carbon, creating an anaerobic condition for the methanogenesis stage. At low partial pressure of hydrogen, acetate and /or hydrogen dominate the product, while ethanol or organic acid is produced at a high partial pressure of hydrogen. Acidogenesis occurs in an acidic environment created by ammonia, H_2, CO_2, H_2S, shorter volatile fatty acids, carbonic acids, alcohol, and other trace amounts from another by-product. Acetate, hydrogen and carbon dioxide can be utilised directly for methane production. The syntrophic acetogenic bacteria will degrade the propionate, butyrate, valerate and isobutyrate to form acetate and hydrogen (Paritosh et al., 2017).

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