Biogas Production from Co-Digestion of Livestock Manure with Lignocellulosic Biomass

Alam Surya Wijaya

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of
Master of Science in Sustainable Energy Management
Prince of Songkla University
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I hereby certify that this work has not been accepted in substance for any degree, and is not being currently submitted in candidature for any degree.

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ABSTRACT

The research aimed to investigate how to improve the effectivity and efficiency of biogas (methane) production system from livestock manure using co-digestion with lignocellulosic biomass. The treatment includes co-digestion of cattle manure, chicken manure, rice straw, and hornwort (*Ceratophyllum demersum*) in mesophilic (35 °C) temperature with used frying oil as an additive. The feedstock was determined according to their availability in the environment and conflict of interest. Natural microbial consortium in existing digester processing cattle manure was used as inoculum. Potential feedstock for co-digestion was characterized for their physicochemical properties before being used as substrates. The feedstock was mixed in 14 experiments for Biochemical Methane Potential (BMP) test for 74 days at mesophilic temperature. The BMP result showed that a mixture of Cattle Manure (M), Chicken Manure (P), Rice Straw (R), and Oil (L) at proportion of M:P:R:L 45:15:38:2 had the highest methane yield at 246.02±2.14 NmL CH_4/g VS, increasing 40.25% compared to that of cattle manure single digestion. From the result of the BMP test, a decision analysis was performed to determine the appropriate substrate for continuous digestion considering the BMP methane yield, sustainability, and applicability in Continuously Stirred Tank Reactor (CSTR) reactor. Due to consideration of applicability and sustainability, the M:P:R:L 45:15:38:2 could not be used, and therefore, the second highest yield substrate, the M:P:R 45:15:40 mixture which had a

methane yield of 233.25±0.81 NmL CH_4/g VS in the BMP test or an increase of 32.97% compared to that of cattle manure mono-digestion, was used for continuous digestion instead. A single-stage CSTR digester was used in the study to determine the methane production in continuous production. The Hydraulic Retention Time (HRT) was 35 days and determined from the result of BMP test. The Organic Loading Rate (OLR) was 2 g VS/L · day. The result of continuous digestion test showed that M:P:R 45:15:40 substrate mixture had a stable performance with a yield of 135.30±21.41 NmL CH_4/g VS in the continuous digestion or 71.28% of its BMP value. Therefore, it can be concluded that cattle manure, chicken manure, and rice straw in a proportion of 45%, 15%, and 40% respectively could be used for sustainable biogas production through anaerobic digestion process.

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LIST OF ABBREVIATIONS

BMP	:	Biochemical Methane Potential
CSTR	:	Continuously Stirred Tank Reactor
CH_4	:	Methane
CO_2	:	Carbon dioxide
H_2	:	Hydrogen
N_2	:	Nitrogen
VFA	:	Volatile Fatty Acids
TS	:	Total Solids
VS	:	Volatile Solids
CNR	:	Carbon to Nitrogen Ratio
AD	:	Anaerobic Digestion
STP	:	Standard Temperature and Pressure (0 °C, 1 bar)

CHAPTER 1

INTRODUCTION

1.1.Background

The world demand for energy is increasing every year and there is a global concern to fulfill the energy demand of the human population. Currently, the energy requirement is mostly supplied by energy generated from fossil fuels. However, concerns are raised about the use of fossil fuels, including its availability in the future and its impact on the environment. Greenhouse Gases (GHGs) including CO_2 are produced from combustion of fossil fuels and believed to contribute to global warming, and thus climate change (FAO, 2013).

Livestock wastes have been identified as a source of GHG emission as well as a potential feedstock for energy resources. Livestock sector contributes to 14.5% of total human-made GHG emission or around 7.1 Gigatons CO_2 equivalent every year (FAO, 2013). Cattle are the biggest contributor with 61% share, while pig and poultry contribute to about 9% and 8% respectively. Manure storage and processing count to 10% share of the emission from the livestock sector (FAO, 2013).

Nevertheless, one kilogram of fresh livestock manure can produce 0.03 m3 of biogas daily (Kumar, 2012) which is considered as a big potential to be developed further. Livestock manure has high efficiency of energy yield compared to another type of biomass (Deublein & Steinhauser, 2011). The methane resulted from the biogas production serves a good source of energy with an energy content of 55,525 kJ/kg at 25 °C and 1 atm of air pressure, or equivalent to electricity generation of 5.14 kWh/kg CH4 using heat to electricity conversion at the efficiency of 33% (Khanal, 2008). One animal unit of livestock (abbreviated AU, LU, or GVE) weighted 500 kg can provide 550 m3 of biogas or about 3,500 kWh electricity annually (Deublein & Steinhauser, 2011). This fact is making the biogas production from livestock manure very potential to fulfill our energy needs. However, as the demand for energy is constantly increasing, efforts to increase the productivity are still necessary.

The co-digestion of several substrates have been proven to increase the yield of biogas compared to mono-digestion of a specific substrate (Avicenna, Mel, Ihsan, & Setyobudi, 2015). Energy crops have been identified to produce more methane when co-digested with manure (Cavinato, Fatone, Bolzonella, & Pavan, 2010). The increase is possible because of the nutrient balance resulted from the mixing of the different substrate, particularly the C to N ratio (CNR) (Ward, Hobbs, Holliman, & Jones, 2008). The substrate with low CNR could be co-digested with the substrate with high CNR to obtain the balance. Cattle manure has N content around 6.0 – 6.4 g/kg of dry matter, while poultry manure has N content around 21.8 – 40 g/kg of dry matter (IAEA, 2008). The CNR of cattle manure was 24, and that of chicken manure was 10 (Sawatdeenarunat, Surendra, Takara, Oechsner, & Khanal, 2015). Therefore, the cattle manure can be used as the main substrate, with poultry manure can be used to adjust CNR when co-digested substrates with a high CNR. Rice straw is one of the potential biomass for co-digestion because it has abundant availability (Lianhua et al., 2010) and has a high CNR of around 47 (Sawatdeenarunat et al., 2015), which is suitable for co-digestion with livestock manure. However, the lignin content of rice straw at 13% (Sawatdeenarunat et al., 2015) may reduce its biodegradability/digestibility (Chandra, Takeuchi, & Hasegawa, 2012).

1.2.Objectives of the Study

The significance of the research is that the result of the research would enable optimization of biogas (methane) production from livestock manure, which in turn, would widen choices for biogas industry and commercialization through effective and efficient production as well as reducing GHG emission from the agricultural operation and wasted biomass. The objectives of the research are: (a). to determine the effect of substrate proportion on methane productivity from co-digestion of livestock manure with lignocellulosic biomass in a batch experiment using Biochemical Methane Potential (BMP) assay; (b). to investigate the methane productivity in continuous biogas production using Continuously Stirred Tank Reactor (CSTR) by feeding with optimum mixing substrates obtained from the batch investigation. The hypothesis of the research is: adding lignocellulosic biomass and oil additive at proper portion into livestock manure is expected to favor positive interaction, i.e. macro- and micronutrient equilibrium and/or dilute inhibitory or toxic compounds during anaerobic co-digestion. Consequently, methane produced under these circumstances of co-digestion could be higher than methane produced in single digestions.

1.3.Theoretical Framework

Livestock manure, including cattle manure and chicken manure, are waste from livestock / agricultural production sector that is always produced from every livestock farm. It is an important source of Greenhouse Gases (GHGs) emission, air pollution related to malodor / bad smell, water pollution, and eutrophication, as well as imposes health risks of respiratory diseases in human and animal. Yet it has a big potential to be exploited as an energy source due to its natural methane production. The increasing number of livestock production to supply food for the population also means an increase in the potential of energy source. Therefore, livestock manure would be a good source for bioenergy production as biogas.

Similarly, agricultural production, in particular, rice cultivation has provided more rice for the increasing world demand for food. However, the rice which is used for human consumption is only a small part of the biomass produced during rice production. The bigger part of the biomass produced is being wasted. The biomass produced in the rice production (rice straw) can be used as a feedstock for biogas production.

In addition, hornwort (*Ceratophyllum demersum*) is abundant in the natural body of water. In the water system, it can grow very fast, especially when the nutrients (nitrogen) are abundantly available in the water system. The fast-growing species may outnumber other species, become invasive, and therefore the ecological balance is disturbed. However, the species can be a potential biomass source for biogas production.

Biogas production is a biological process that incorporates mechanisms of organic material degradation by specific microbes. Biological process is highly dependent on environmental conditions, including pH, temperature, and nutrient availability, among others. Therefore, maintaining these conditions within the favorable zone of the microbes is an essential part of the biogas production process.

In conclusion, the biogas production may be increased by setting up favorable condition for anaerobic digestion to take place. This can be done by adding livestock manure (cattle manure and chicken manure) with rice straw and hornwort as lignocellulosic biomass, used frying oil as an additive, and maintaining the pH and temperature in the comfort zone.

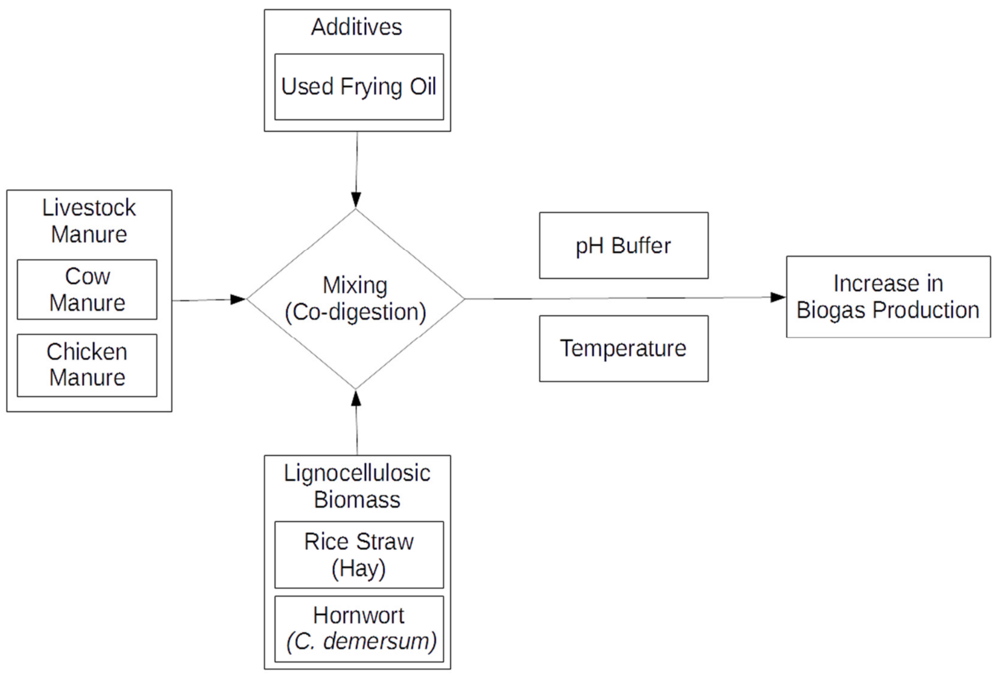


Figure 1.1 Theoretical / Conceptual Framework

CHAPTER 2

LITERATURE REVIEW

2.1. Anaerobic Digestion (AD)

Biogas and particularly methane are produced by anaerobic degradation of organic compounds by specific microbes. Biogas is naturally produced in anaerobic environments where oxygen is not present such as in the marine and freshwater sediments, sewage sludge, mud (Ahring, 2003b), wetlands, swamps, and in the rumen of animals (Vertes, 2010). The process is also called anaerobic digestion (AD) in which the anaerobic respiration process happens and CO_2 as electron receptor reduced to CH_4 by the microbes (Khanal, 2008). The overall biochemical reaction in AD process of methane formation from biomass is mainly consisted of 4 stages (hydrolysis, acidogenesis, acetogenesis, and methanogenesis), and can be described as follows:

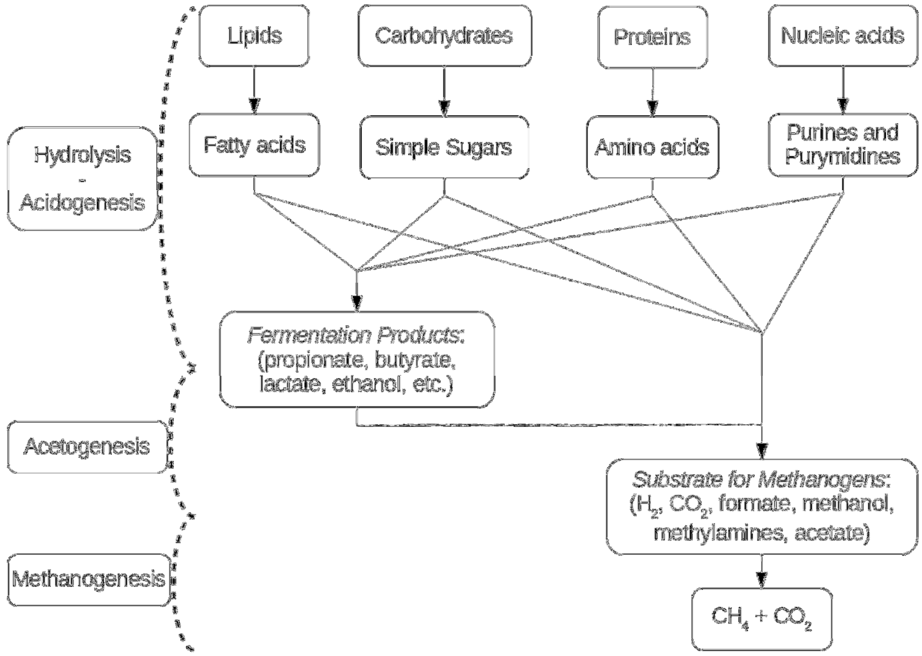


Figure 2.1 Organic matter conversion through Anaerobic Digestion process (Ahring, 2003a)

The biochemical process can also be explained by the following equation (Deublein & Steinhauser, 2011):

$$C_cH_hO_oN_nS_s + y\ H_2O \rightarrow x\ CH_4 + n\ NH_3 + s\ H_2S + (c\text{-}x)\ CO_2 \qquad (1)$$

where

$x = \frac{1}{8}\,(4c + h - 2o - 3n - 2s)$

$y = \frac{1}{4}\,(4c - h - 2o + 3n + 2s)$

Hydrolysis is the breakdown of large polymers into simple monomers. Complex organic materials consisting of carbohydrates, proteins, lipids, and the genetic materials (DNA and RNA) are degraded to simple sugars, amino acids, simple acids, and ketones (Scragg, 2009). In the lignocellulosic biomass, the hydrolysis incorporates the breakdown of cellulose and hemicellulose from the feedstock biomass. On the other hand, lignin is degraded slowly and incompletely (Deublein & Steinhauser, 2011). The cellulose in the feedstock is mainly degraded by microbial glycoside hydrolases (GHs) enzymes. The hemicellulose is composed of heterogeneous sugars and thus needs a various enzyme to be degraded, including xylanases, mannanases, mannosidases, galactosidases, and arabinofuranosidases (Gupta & Tuohy, 2013).

Acidogenesis or fermentation is the degradation process of simple organic materials produced by the hydrolysis (monomers) to Volatile Fatty Acids (VFAs) / Short-Chain Fatty Acids (SCFAs). The products of the fermentation process are formate, acetate, propionate, butyrate, isobutyrate, and succinate with acetate being the majority of the products (Vertes, 2010). Some amounts of alcohol (ethanol) may be produced in the process (Ahring, 2003a). The VFAs produced in the acidogenesis contributes to the lowering of pH of the AD system (Mudhoo, 2012).

The third stage in the AD process is acetogenesis which converts the VFAs and ethanol into acetate, H_2, and CO_2. Acetogenesis is performed by syntrophic acetogens which are a strictly anaerobic bacteria that heavily depend on the hydrogen-consuming methanogens to remove the hydrogen in the environment to make the process thermodynamically possible (Ahring, 2003a). This is the reason why acetogenesis will only occur at the low hydrogen pressure (Mudhoo, 2012). The acetogens grow very slowly with a generation time of more than one week (Vertes, 2010).

The last stage is the methanogenesis with the conversion of CO_2, H_2, and acetic acid into methane and carbon dioxide. The methanogenesis is performed by archaea which are sensitive to environmental conditions including the presence of oxygen (Gerardi, 2003), narrow band of pH around 6.6 – 7.0 (Evans & Furlong, 2003) and maximum temperature of 60 °C for thermophilic microbes (Ahring, 2003a). These organisms grow slowly and can only use a limited type of substrates such as acetate, H_2, CO_2, methanol, formate, methylamines, and methyl sulfides (Vertes, 2010). About 70% of the methane produced from the acetate degradation and the other 30% comes from the reduction of CO_2 by H_2 (Deublein & Steinhauser, 2011).

2.2. Important Factors

All process regarding biological systems has to maintain the stability of the system in regard to the living organisms involved with. Anaerobic digestion is one example that incorporates the use of microbes during the whole process. As living organisms, these microbes must be provided with a suitable condition for their growth and production. Some conditions have been recognized to have a significant relationship with the microbial growth and production in the AD process.

2.2.1. Type of Microbes

The most important biological factors affecting the AD process are the type of microbe. Some microbes are present in the AD system including bacteria, archaea, and possibly some fungi or protozoa (Vertes, 2010). Microbial analysis using 16S rRNA gene clones found that various microbes are present in the anaerobic digestion process including Proteobacteria (*Deltaproteobacteria*), Bacteria (*Chloroflexi, Firmicutes, Spirochaetes, Bacteroidetes*), and Archaea (*Methanomicrobia, Methanobacteria, Thermoplasmata*) (Narihiro & Sekiguchi, 2007).

2.2.2. Temperature

The optimal temperature for anaerobic digester process ranges from 20 °C to 60 °C. The temperature range, however, is separated between below 20°C for psychrophilic reaction, 30 °C to 40 °C for optimum mesophilic reaction, and 50 °C to 60 °C for optimum thermophilic reaction (Ahring, 2003a). The acid-forming microbes have the optimum temperature of 30 °C and the mesophilic methanogens have an optimum temperature of 35 °C (Chandra et al., 2012) also for solid AD process (Gerardi, 2003). The ammonia inhibition is lower in the mesophilic operation because of a lower content of free ammonia. Overall, the energy balance in mesophilic is better than in the thermophilic operation (Deublein & Steinhauser, 2011). At temperature 35C with CNR 15 and at temperature 55C with CNR 20, ammonia inhibition can occur (Wang, Lu, Li, & Yang, 2014).

2.2.3. Alkalinity, pH, and Buffer

The AD system can only operate in a specific range of pH and thus this pH range must be maintained constantly. The optimum pH for anaerobic digestion is between 6.8 – 7.2 (Chandra et al., 2012). Alkalinity is the quantitative capacity of a solution to neutralize acids (particularly carbonate and bicarbonate) and is measured as the strength of the bases in the solution. Alkalinity acts as a buffer to maintain the pH stability and to prevent pH fluctuation shock. The VFA produced in the acidogenesis and acetogenesis will reduce the pH. The most dominant VFA are acetate and butyrate (Boe, Batstone, Steyer, & Angelidaki, 2010). The methanogens will then consume the VFA and produce alkalinity; therefore, the pH will increase and will be stabilized again. Furthermore, citrate or ferric chloride can be used to treat excess alkalinity, because, at a concentration of more than 1500 mg/L, these cations will be toxic to the microbes (Gerardi, 2003).

Table 2.1 Common chemicals for buffer (Gerardi, 2003)

Common Name	Chemical Formula	Cation
Natrium Bicarbonate	$NaHCO_3$	Na^+
Kalium Bicarbonate	$KHCO_3$	K^+
Natrium Carbonate (soda ash)	Na_2CO_3	Na^+
Kalium Carbonate	K_2CO_3	K^+
Calcium Carbonate (lime)	$CaCO_3$	Ca^{2+}
Calcium Hydroxide (quick lime)	$Ca(OH)_2$	Ca^{2+}
Anhydrous Ammonia (gas)	NH_3	NH_4^+
Natrium Nitrate	$NaNO_3$	Na^+

The favorable pH for AD process is in the range of 6.8 to 7.2 (Gerardi, 2003). The methanogenesis required to be in pH range of 6.6 to 7.0 (Evans & Furlong, 2003) and will fail in pH below 6.5 (Vertes, 2010). The fermentation process in the AD system can be monitored by pH value and the CO_2 content in the biogas. If the pH value drops and CO_2 rise, it clearly indicates that the process is disturbed (Deublein & Steinhauser, 2011).

2.2.4. Volume Load

The digester operation highly depends on the volume load. The volume load is highly related with the microbe density in the reactor. Higher volume load will need more microbes to degrade the material. A volume load of 3.2 – 7.2 kg VS/m^3/day is recommended for mixed/heated anaerobic digesters, while 0.5 – 0.6 kg VS/m^3/day is the most common load in a typical system (Gerardi, 2003). Another study recommends maximum loading rate of 6 kg VS/m3/day for co-digestion of rice straw and cattle manure (Xie et al., 2016). In addition, a maximum load of 10.5 kg VS/m^3/day has been determined, whereas a load of 9.2 kg VS/m^3/day still result in a stable process (Nagao et al., 2012).

2.2.5. Mixing

Mixing is very important in the AD process. It is necessary to promote contact between microbes, substrates, and nutrients. Slow and gentle mixing would increase the contact and thus increase the biochemical reactions and biogas production. Cautions should be given because the methanogens can be washed out by rapid mixing. In addition, mixing will make the temperature distributed more evenly throughout the digester. Mixing also can reduce the scum formation and grit settling. Mechanical mixing or fluid recirculation (gas, liquid, or sludge) can be used for mixing. Instead of continuous mixing, routine mixing of 3 – 6 times per day for 1 – 3 hours each may be an efficient alternative (Gerardi, 2003).

2.2.6. Retention Time, Microbial Density, and Inoculum to Substrate Ratio

Retention time in the AD system may refer to Hydraulic Retention Time (HRT) or Solid Retention Time (SRT). The HRT is the average time of the feedstock in the digester, while the SRT is the average time of the microbes in the digester. The SRT value less than 10 days may cause the methanogens to be leached out because of the long generation time and therefore is not recommended (Deublein & Steinhauser, 2011). The recommended SRT is 10 – 15 days (Chandra et al., 2012). High SRT value is advantageous because it means that the microbes will stay longer in the digester and will ensure the maximum AD process. High SRT also means that the digester volume can be reduced, and more buffering capacity against shock loading (Gerardi, 2003).

Microbial density/concentration is closely related to SRT value. High SRT value means that the microbes can stay longer in the digester and thus the concentration/density of the microbes will increase. Normal AD system may contain up to 10^{10} cells/ml of digester content (Vertes, 2010).

In addition, the ratio between inoculum and substrate (I/S ratio) has been observed to be optimal at 1:1 (Sri Bala Kameswari, Chitra Kalyanaraman, Porselvam, & Thanasekaran, 2012). Different study reveals that the I/S ratio is also related with the substrate type, for example I/S ratio of 1.0 – 1.5 is suitable for maize silage, while I/S ratio of 0.5 – 2.5 is suitable for wheat straw (Moset, Al-zohairi, & Møller, 2015).

2.2.7. Hydrogen partial pressure

Hydrogen presence and pressure is a very vital factor in the AD process. Hydrogen is necessary for hydrogenotrophic methanogens to produce methane. On the other hand, the acetogenesis will be prohibited because the process is thermodynamically unfavourable in the presence of / high pressure of hydrogen (Deublein & Steinhauser, 2011).

2.2.8. Type of substrate

The type of substrate will determine the methane production pathway and the amount of CH_4 produced. The substrate rich in sugar or starch, for example, will yield biogas with CO_2 and CH_4 proportion of 50:50. In addition, type of substrate also determines the limiting step in the AD process. Lignocellulosic biomass will need a lot of time to be broken down to simple sugar, and therefore the hydrolysis stage is the limiting factor and needs higher priority (Yang, Xu, Ge, & Li, 2015). A single stage AD system will be sufficient for substrate rich in protein, because the hydrolysis, acidogenesis, acetogenesis, and methanogenesis phase run at the same speed. The acetogenesis is the limiting step if the substrate has high-fat contents because the hydrolysis is very fast, therefore the thermophilic condition is preferred for the acetogenesis (Deublein & Steinhauser, 2011).

2.2.9. Material surface area

The surface area highly corresponds to the speed of the AD process. The smaller particle of the substrate has more surface area. The higher surface area will provide more opportunity for the microbe to attach to the substrate and increase the reaction rate. Increased reaction rate means that less time is needed to complete the AD process (Deublein & Steinhauser, 2011).

2.2.10. Redox potentials

The redox potentials or also known as Oxidation-Reduction Potentials (ORP) determine the possibility of the biochemical reactions of the AD process. The overall AD process needs the redox potential of -300 mV to take place (Vertes, 2010) with the optimum value between -300 mV to -330 mV (Deublein & Steinhauser, 2011). Nitrate ions (NO_3^-) and sulfate ions (SO_4^{2-}) are stronger electron acceptor than carbonate ions (CO_3^{2-}). In the digester, these ions can increase the ORP and will drive the reaction toward formation of H_2S and N_2 instead of CH_4 and therefore will inhibit the AD system (Gerardi, 2003).

2.2.11. Nutrients

Nutrients are necessary to support all biological process in the AD system. Lack of nutrients will prevent the microbial growth and also prevent some important process to occur. Macronutrients and micronutrients must be available in the system either from the feedstock or through addition. One parameter heavily used in the AD system is the carbon to nitrogen ratio (CNR), with the ratio between 20 and 30 is reported to increase the methane production (Chandra et al., 2012). The substrate with very high CNR will have a low methane yield due to N insufficiency. On the other hand, substrates with very low CNR will rise risk of system failure due to excess nitrogen will be converted to NH_3 and become process inhibitor (Ahring, 2003b). The nutrient requirement for AD system of COD:N:P at 1000:7:1 and 350:7:1 has been used in high-load and low-load waste processing AD system. The CNR of 25 is considered optimal for gas production (Gerardi, 2003), while other study suggest CNR of 20 (Álvarez, Otero, & Lema, 2010). The nutrient requirements for AD is given in Table 2.2.

Table 2.2 Nutrients requirement for AD (Gerardi, 2003)

Nutrient	Type	Recommended Minimum (% COD)
Nitrogen	Macronutrient	3 – 4
Phosphorus	Macronutrient	0.5 – 1
Cobalt	Micronutrient	0.01
Iron	Micronutrient	0.2
Nickel	Micronutrient	0.001
Sulfur	Micronutrient	0.2

2.2.12. Oxygen

Most of the microbes involved in the AD systems are facultative, obligate, or strictly anaerobe and some are very sensitive to free molecular oxygen. The presence of oxygen may cause a negative impact on their performance or even death (Vertes, 2010). Oxygen may also increase the ORP and drive the reaction to aerobic respiration that produces only CO_2 and the digester will stop producing CH_4 as a consequence (Deublein & Steinhauser, 2011). Therefore the oxygen must be prevented by airtight insulation.

2.2.13. Sulfur

Sulfur is an important micronutrient that governs AD process. The methanogens contain 2.5% sulfide in its cells and must be obtained from the environment in the form of H_2S. It is normally high in concentration at pH around 6.8 – 6.9 which is within the optimum range for AD process (Gerardi, 2003). However, high sulfuric concentration on the digester also causes an inhibitory effect. Sulfur is toxic to the microbes in high concentration. The total concentration of H_2S of 100 – 300 mg/L will inhibit AD process totally and stop the biogas production completely (Ahring, 2003b).

2.2.14. Organic acids

Organic acids, including VFA, will lower the pH and will drive the system to acidic conditions. This condition will lead to the death of some microbes in the AD system, including the methanogens. A substantial amount of VFA is necessary to run the process because some methanogens consume acetate to produce methane, with around 75% of methane is produced from acetate (Evans & Furlong, 2003). However if it accumulates and the amount exceeds the limit, it will cause excess and drop in pH. The drop in pH will inhibit the methanogens as their consumer and will make the accumulation even worse and eventually lead to system failure (Vertes, 2010). Long Chain Fatty Acids (LCFA) particularly oleate and stearate are found to be inhibitory because of VFA accumulation in the process (Ahring, 2003a). Other LCFA such as capric, caprylic, lauric, myristic, and oleic acids are also inhibitory. Lauric acid is the most toxic and together with other LCFA will exhibit the synergistic effect of inhibition (Gerardi, 2003). The inhibitory effect of LCFA is related with the adsorption to the cell wall and interference with the transport system and protective system (Chen, Cheng, & Creamer, 2008).

2.2.15. Ammonia (NH_3) and ammonium (NH_4^+)

Ammonia and ammonium are important factors in the AD system. Free ammonia is known to be toxic, while ammonium is the nitrogen source for the microbes. Ammonia and ammonium are in a dynamic equilibrium depending on the pH of the system. In low pH (less than 7.2) more ammonium will be favored, while in high pH (more than 7.2) more ammonia will be favored. Previous studies show different results about the inhibitory level of ammonia. Most of the studies, however, state that free ammonia concentration of 400 – 1000 mg/L is inhibitory (Ahring, 2003a), although some microbes can be acclimatized to free ammonia concentration up to 800 mg/L (Ahring, 2003b). Free ammonia of more than 1500 mg/L in high pH, or more than 3000 mg/L will lead to digester failure (Gerardi, 2003). A study of co-digestion of chicken manure with cattle slurry and fruit and vegetable waste shows that an increase of chicken manure portion in the mixture leads to ammonia inhibition (Callaghan, Wase, Thayanithy, & Forster, 2002). A concentration of 150 mg/L N may cause inhibitory effect, although acclimatization can raise this limit to 345 mg/L or even more, depend on the time for acclimatization (Bujoczek, Oleszkiewicz, Sparling, & Cenkowski, 2000). However, the inhibition from ammonia can be recovered by feeding the digester with cattle manure only (Nielsen & Angelidaki, 2008).

2.2.16. Heavy metals

High concentration of heavy metals is found to have the inhibitory effect on AD process, although some of the heavy metals are necessary for the AD process as micronutrients. The heavy metals include cobalt (Co), copper (Cu), iron (Fe), nickel (Ni), zinc (Zn), and molybdenum (Mo). Copper, nickel, and zinc are the most toxic metals to methanogens (Gerardi, 2003). The concentration of 10^{-3} to 10^{-4} are found to be toxic to the anaerobic organisms and thus will inhibit the biogas production (Ahring, 2003b). The heavy metal is a major cause of digester failure because it is not biodegradable (Chen et al., 2008).

2.2.17. Aromatic hydrocarbon compounds

Aromatic hydrocarbons or compounds with benzene ring have been observed to have the inhibitory effect on the AD system. These compounds consist of phenol, phenolic compounds (chlorophenols, nitrophenols, and tannins), benzene, pentachlorophenol, and toluene. The toxicity of the compound comes from the reactions with enzyme and disturbance in the metabolic pathways (Chen et al., 2008). Tannins are toxic at a concentration of 700 mg/L (Gerardi, 2003). The effect of phenolic compounds is not permanent. Thus an AD system which is inhibited by phenolic compounds can be effectively restarted (Ahring, 2003b). Dissolved lignin at concentration between 0.5 to 5 g/L is also known to have inhibitory effect to methanogenesis and acidogenesis at a rate between 7 – 15%, while in hydrolysis, a higher rate of inhibition up to 35% has been observed (Koyama, Yamamoto, Ishikawa, Ban, & Toda, 2017). Essential oils, for example, citrus essential oils, contain compounds that have antimicrobial properties and thus can also inhibit the AD process (Ruiz & Flotats, 2014).

2.3. Co-Digestion

Co-digestion is the use of two or more substrate in an anaerobic digestion process. Co-digestion is believed to have several advantages including for feedstock reliability improvement, toxic substance treatment, balancing the nutrient requirement, increasing biodegradable organic matter, giving synergistic effect, and increasing methane yield (Shah et al., 2015). However, the substrate for co-digestion has to be considered carefully since some of the substrates produce an intermediate that has an inhibitory effect (Q. Zhang, Hu, & Lee, 2016). For example, the use of grease waste up to 37% will decrease the methane production because of inhibition from LCFA (Xie et al., 2016).

The co-digestion of several substrates has been proven to increase the yield of biogas compared to mono-digestion of a specific substrate. The synergistic effect between the substrate used for co-digestion has been identified so that the result of biogas from co-digestion is higher than the sum of the biogas resulted from the substrates digested separately (Xie et al., 2016). The increase is because of the nutrient balance resulted from the mixing of the different substrate, reduction in the toxicity of the certain compound in the substrate, and minimized inhibitor compound accumulation by mixing with a different substrate (Mata-Alvarez et al., 2014).

Substrate properties and the proportion of the substrate used in the co-digestion is a very important factor that affects the anaerobic digestion process (Xie et al., 2016). The proportion must be adjusted to fulfill the nutrient balance of the substrate mixture including its CNR, macro- and micronutrients, toxic/inhibitor contents, pH, dry matter, and biodegradable organic matter (Álvarez et al., 2010). In addition, a substrate for co-digestion should have characteristics including (a) pH buffering capacity; (b) sufficient nutrients balance; (c) high contents of biodegradable organic materials (Xie et al., 2016).

The biomass for co-digestion must have advantageous characteristics because the characteristics and composition of the substrates affect the biogas yield. Some important consideration for selecting co-substrates are high buffering capacity, sufficient nutrient (balanced CNR), high biodegradable organic matter, and low Sulfur (Xie et al., 2016). Other important characteristics of the biomass are: have high biogas potential, low detention time, low moisture content, and financially feasible (Shah et al., 2015). A balanced ratio of C/N is the main characteristics for co-digestion, with the optimum value of the ratio is 20:1 (Álvarez et al., 2010).

Several substrates have been used for co-digestion in the anaerobic digestion process. For example, in Denmark, co-digestion of livestock manure with other organic matter is common. This is due in part to the abundance of livestock manure as a biomass source, which counts to 1.5 million ton each year. This amount of manure can produce biogas up to 39,000,000 m^3/year. In Sweden, manure is commonly co-digested with food processing, restaurant waste, or crop residues (Cavinato et al., 2010). Sewage sludge, fruit and vegetable waste, energy crops, glycerin, and organic portion of municipal solid waste have been used as co-substrate in the anaerobic digestion process (Álvarez et al., 2010).

2.3.1. Livestock Manure

Livestock manure is commonly used in anaerobic digestion as the main substrate. Manure is abundantly available, for example in the UK alone, 34,000 tons of dry solids are produced daily from livestock industry (Callaghan et al., 2002). It is also readily available and very suitable for AD due to high nitrogen content which is favored by microorganisms for growth (Appels et al., 2011). Germany, Sweden, Denmark, and Italy have a lot of running biogas plants utilizing manure as the main substrate (Mata-Alvarez et al., 2014). Biogas production from livestock manure can be increased using co-digestion with other substrates. Some co-substrates rich in C and thus have a high CNR have been identified as suitable for co-digestion with manure (Mata-Alvarez et al., 2014).

Cattle manure has been considered as potential and suitable feedstock for the AD and has been used in many AD plants. Cattle manure production is about 810 million tons in China in 2011 (Yangyang Li et al., 2016). The biodegradability of cattle manure is about 32% (Møller, Sommer, & Ahring, 2004), with CNR 19.8 (Yangyang Li et al., 2016), and 22.2 (Wang et al., 2014).

Chicken manure is a potential substrate for co-digestion. The characteristics of chicken manure are highly biodegradable, high nitrogen content, low CNR, and high solid content around 20-25% (Bujoczek et al., 2000). Pretreated chicken manure has been proven to be potential for biogas production with production up to 230.58 mL/g COD with a composition of 60.2% methane, 38.8% carbon dioxide, and 0% hydrogen (Elasri & El Amin Afilal, 2016). Methane productivity of 31 mL/g VS was observed in another study using chicken manure (Abouelenien, Nakashimada, & Nishio, 2009). When co-digested with wheat straw, chicken manure can produce methane up to 0.12 m^3/kg VS with methane proportion of 53 – 70.2% (Babaee, Shayegan, & Roshani, 2013). Another study observed methane productivity of 218.8 mL/g VS from co-digestion of chicken manure with corn stover in proportion 1:3 (Yeqing Li et al., 2013).

Cattle manure and chicken manure are usually co-digested with other biomass. For example, cattle manure and chicken manure were co-digested with rice straw with CNR of 25 resulted in 272 mL/g VS at 35°C and 286 mL/g VS with CNR of 30 at 50°C (Wang et al., 2014). Other experiment use cattle dung and rice husk as substrates in different OLR with very good results of 67.6 mL/min with a concentration of 63.4% at 43.6 g/VS/L/day (Avicenna et al., 2015).

2.3.2. Rice Straw

Agro-industrial wastes are frequently used for co-digestion with livestock manure. Rice straw is one of the most abundant agro-industrial residues, with production in China alone counts to 109 million ton in 2002 (Lianhua et al., 2010), and in Asia totals 667.6 million tons produced annually or about 91% of total world production (Bajaj, Sharma, & Rao, 2014). The characteristics of rice straw fulfill the requirement of high CNR to balance the nutrient in the manure and maintaining stable pH due to high buffering capacity and reduced ammonia (Mata-Alvarez et al., 2014). A recent study of rice straw used as a feedstock in batch anaerobic digestion with digested cattle manure as inoculum shows that it has a good specific methane productivity (178.3 mL/g VS) with a methane content of 54.8% (Gu, Chen, Liu, Zhou, & Zhang, 2014). Another study using rice straw as feedstock for AD shows that in wet thermophilic and dry mesophilic condition the methane productivity is 136.3 L/kg VS and 123.5 L/kg VS respectively. A trial in the ambient pilot reactor with circulated leachate in that study yields methane productivity of 239.7 L/kgVS (Lianhua et al., 2010).

2.3.3. Hornwort

Aquatic plant is a potential source of biomass that can be used for feedstock in the anaerobic digestion process. These plants are mostly underutilized and neglected and sometimes considered as weeds. These macrophytes, particularly Hornwort (*Ceratophyllum demersum*), have high potential as a feedstock for the AD. Hornwort has a high protein with CNR of 10.4 (Kobayashi, Wu, Lu, & Xu, 2014). In one experiment resembling natural environment, hornwort produces 0.4 g CH_4/kg dry mass/day with the mass loss of 64% due to biodegradation, higher than other submerged macrophytes (Zak et al., 2015). A batch anaerobic digestion experiment of hornwort resulted in CH4 production of 249 mL CH_4/g VS and methane recovery (57.1%) and thus concluded that hornwort has a high potential to be used as feedstock for the AD (Koyama, Yamamoto, Ishikawa, Ban, & Toda, 2014). In another study, methane productivity of 554 L CH_4/kg VS has been obtained from the batch experiment of C. demersum for AD feedstock (Pastare, Romagnoli, Lauka, Dzene, & Kuznecova, 2014).

2.3.4. Used Frying Oil

Fats, oils, and greases (FOG) are now considered as potential substrates for co-digestion. Oily biomass has high COD and thus can produce more methane (Irini Angelidaki & Sanders, 2004). The FOG has high methane potential, ranging from 0.7 – 1.1 m3 CH_4/kg VS, and therefore, become an interesting co-substrate (Mata-Alvarez et al., 2014). The use of FOG, however, must be limited due to the tendency to produce Long Chain Fatty Acid (LCFA) that may inhibit the AD process in the proportion of 30% FOG or more (Mata-Alvarez et al., 2014). There is an evidence that co-digestion of waste activated sludge with greasy sludge can increase the methane productivity from 264 $NLCH_4$/kg VS without greasy sludge addition to 546 $NLCH_4$/kg VS at 60% COD greasy sludge addition (Girault et al., 2012). When 10 – 30% of greasy traps sludge was added to sewage sludge, the methane yield increased 9 – 27% (Davidsson, Lövstedt, la Cour Jansen, Gruvberger, & Aspegren, 2008). A similar study also confirmed these result that fat, oil, and grease (FOG) addition at a proportion of 48% VS can increase methane yield 2.95 times higher at 35°C and 2.6 times higher at 52°C (Kabouris et al., 2009).

2.4. Biochemical Methane Potential (BMP)

Biochemical Methane Potential (BMP) is used to measure the potential of a feedstock to produce biogas, or specifically methane. It is also being used to measure the biodegradability of a substrate in an anaerobic digestion process. Some procedures have been developed to measure the biodegradability of an organic compound as well as measuring the biogas/methane production (I. Angelidaki et al., 2009; Irini Angelidaki & Sanders, 2004). Although theoretical methane production can be determined, however, the real methane production should be obtained from experiments.

Some studies about the BMP testing for manure and co-digestion with other substrate have been done (Labatut, Angenent, & Scott, 2011). In general, solid organic substrate biodegradability and methane production have also been studied using BMP (Raposo et al., 2011). The effort to minimize the error from BMP has also been developed to make BMP more accurate and reliable (Strömberg, Nistor, & Liu, 2014).

2.5. Bioreactor

The anaerobic digestion process needs to be done in a bioreactor to provide effectivity and efficiency on the process control. Some type of reactors has been designed and used in AD research and development including conventional reactors, sludge retention reactors, and anaerobic membrane reactors. In addition, the high-rate digester is increasingly being used recently. Furthermore, tubular digester can be used in a psychrophilic condition (Shah et al., 2015). Novel reactor designs are still being developed, for example, the four-chambered multi-phased anaerobic baffled reactor (Q. Zhang et al., 2016). However, the Continuous Stirred Tank Reactor (CSTR) is one of the most widely used bioreactors for treatment of high suspended solids wastes, for example, animal manures and industrial wastes (Mata-Alvarez et al., 2014; Mao, Feng, Wang, & Ren, 2015).

2.6. Methane Productivity

Methane productivity is used to indicate the methane yield per unit of the variable. It can be expressed in terms of Volatile Solids (VS) destroyed, VS loaded, volume, or animal production (Møller et al., 2004). Furthermore, for the organic compound $C_aH_bO_cN_dS_e$, the theoretical methane yield (B_{OTh}) could be predicted as follow:

$$B_{OTh} = \frac{\left[\left(\frac{a}{2}\right)+\left(\frac{b}{8}\right)-\left(\frac{c}{4}\right)\right] \cdot 22400}{(12a+b+16c)} \tag{2}$$

In addition, for the formulas to be more accurate, a biodegradability/ digestibility factor should be taken into account. The formula is:

$$BD\ (\%) = (B_{OExp} - B_{OTh}) \cdot 100 \tag{3}$$

With

$$BOExp = \frac{Methane\ Volume\ (at\ STP)}{Sample\ Weight\ (VS\ or\ COD\ basis)} \tag{4}$$

Where:

B_{OExp} = the methane yield based on experiment

B_{OTh} = the theoretical methane yield

(Raposo, De la Rubia, Fernández-Cegrí, & Borja, 2012)

2.7. Kinetics and Synergism

Kinetic analysis is useful for predicting the biodegradability rate of the substrate as well as to predict the methane yield at a specific time. Kinetic analysis was done using first-order hydrolysis model (I. Angelidaki et al., 2009):

$$\frac{dS}{dt} = -kS \quad (4)$$

where *S* is the substrate concentration, *t* is the digestion time (days), and *k* is the first-order kinetic constant (day^{-1}).

However, in the case of biogas, it is inconvenient to use substrate concentration as it may be inefficient to measure, and the gas yield is easier to measure instead. Therefore, a model using gas measurement is more preferable (Raposo et al., 2011):

$$B = B0[1 - exp^{(-kt)}] \quad (5)$$

this model can be described in another way as in previous study (I. Angelidaki et al., 2009):

$$\ln\left[\frac{B0 - B}{B0}\right] = -kt \quad (6)$$

Where *B* represents cumulative methane yield (NmL CH_4/g VS), *B0* is the ultimate or maximum methane yield (NmL CH_4/g VS), *k* is the first order hydrolysis constant ($days^{-1}$), and *t* is the time (days).

Mixing two or more substrates may result in an internal reaction between substrates which may result in higher or lower production compared to the result of mono-digestion experiments. If the result of the experiment shows higher values compared to the sum of mono-digestion experiments, a synergistic effect (α) is considered to be present. The evaluation of synergistic effect was done by comparing the experimental yield with the theoretical yield (sum of mono-digestion experiments) as given in Equation 7 (Nielfa, Cano, & Fdz-Polanco, 2015).

$$\alpha = \frac{experimental\ yield}{theoretical\ yield} \tag{7}$$

with possible values of α means as follows:

$\alpha > 1$: the substrate mixture has a synergistic effect

$\alpha = 1$: the substrate mixture is independent of each other

$\alpha < 1$: the substrate mixture has a competitive / antagonistic effect

CHAPTER 3

RESEARCH METHODOLOGY

3.1.Materials and Methods

3.1.1. Feedstock preparation

Livestock manures, agricultural residues, and aquatic plant were used in this research as substrates for anaerobic digestion. Cattle manure, chicken manure, rice straw hay and hornwort (*Ceratophyllum demersum*) were used in the research. The cattle manure and chicken manure were collected from PSU demonstration farm in Pattani. Both cattle manure and chicken manure were used in the substrate mix. The proportion of cattle manure and chicken manure used in the co-digestion treatments was 3:1, except for the mono-digestion. The combined manure altogether would consist minimum 60% of the substrate mix in co-digestion treatments. All proportions used were based on Volatile Solids weight. Rice straw was obtained from Pattani Rice Research Center (PRRC), then dried and chopped for storage. The rice straw hay was milled into mesh 10 – 20 in size. Hornwort was obtained from BioMEC laboratory culture collection and Somdet Phra Sinakarin Pattani Park, then dried and milled into mesh 10 – 20 in size.

3.1.2. Inoculum

The inoculum used in this research was obtained from a working anaerobic digester at PSU Pattani Demo Farm processing cattle manure. This inoculum was used in both BMP test and continuous test.

3.1.3. Characterization

Feedstock and inoculum characterization including Total Solid (TS), Volatile Solid (VS), Ash, Carbon (C), Hydrogen (H), Nitrogen (N), Sulfur (S), Oxygen (O), and Phosphorus (P) content were done according to AOAC 985.29 (1995) and AOAC 955.04 (2000)

3.1.4. Substrates

There were two substrate settings used in this study. For the BMP test, a total of 14 experiments with different substrates were designed with several restrictions, such as 1) The cattle manure and chicken manure were used in a proportion of 3:1 (VS basis) in co-digestion treatments, 2) The total of manure content in co-digestion treatments was minimum 60%, and 3) Two percent (2%) of used frying oil was used as additive and tested for effect on the biogas production. The substrate used in continuous digestion was determined by the result of BMP and further analysis.

3.1.5. Performance monitoring

The biogas volume and composition were monitored and measured during the BMP. The characterization of VFA was done after the biogas ceased to produce. The hydrolysis constant (*k*) was calculated from the result of BMP experiment to be used as a parameter in continuous digestion.

For the continuous digestion, the biogas volume and composition, pH, and alkalinity were monitored during this study. The pH and alkalinity were measured every day during this study.

3.1.6. BMP determination

The Biochemical Methane Potential (BMP) followed a standardized protocol (I. Angelidaki et al., 2009) with minor modification. Three replicates of each experiment used 500 ml bottle as the assay vessel, with a working volume of 350 ml. Firstly, 250 ml of inoculum was put into each bottle. Then, substrates were put into bottles at inoculum to substrate ratio of 1:1 (VS basis), except for experiment number 14 (negative control). The mixture was stirred well and then adjusted with de-ionized water to 350 ml. Subsequently, bottles were then insulated into airtight condition by sealing with rubber septa and aluminum clamps and were flushed with N_2 for 5 minutes. Next, bottles were put into an incubator at mesophilic temperature (35 °C). For sampling, 50 μL of biogas samples were obtained using an airtight syringe for data collection every day until day 20, then every two days until day 62, and then every three days until day 74.

3.1.7. Continuous digestion

A single stage Continuously Stirred Tank Reactor (CSTR) with water blanket heated to 35 °C was used in this study. The reactor was 10 L in volume, with a working volume of 7 L, and made of stainless steel. Automatic stirring was provided from the top of the reactor. The gas outlet was connected to a balloon with a volume of more than 7 liters of gas storage using silicon tubing. A three-way connector was installed between bioreactor and gas storage with a rubber septum as the terminus for gas sampling. Inlet and outlet ports were sealed due to inappropriateness for the substrate, while a reserve hole was used as an inlet, and the bottom drain was used as an outlet. The inoculum used in this experiment was tested for microbial activity before being used in the reactor by putting 5 g VS/L of substrate mixture into the bioreactor and the gas was analyzed for methane content. The substrate mixture was prepared in liquid form with a concentration of 70 g VS/L for feeding. Organic Loading Rate (OLR) of 2 g VS/L of working volume was used. The Hydraulic Retention Time (HRT) was determined by calculating the k value and adjusted to a few days higher than the result of the calculation.

The rundown step consisted of gas sampling, gas volume measurement, draining, feeding, and effluent sampling. Gas sampling was done before feeding, while the balloon was still inflated. The gas was sampled at a sampling port between bioreactor and gas storage. Three samples of 0.5 mL biogas were taken each day for GC analysis. The volume of gas was measured using a 50 mL syringe. The gas was drained from the balloon using the syringe until the balloon was fully flattened. The gas tube was then clamped with clipper after gas volume measurement, before opening the feeding port in the bioreactor. The draining was done manually from the bottom drain port to remove 200 mL of effluent from bioreactor prior to feeding. The feeding port was opened in this procedure to allow the effluent to be withdrawn manually. After finished draining, the bottom drain port was closed and 200 mL of substrate mixture was fed into the bioreactor. The feedstock was given once a day in a form of liquid/diluted slurry. Due to the characteristics of the feedstock, a manual pouring method was done for feeding. The feeding port was then closed and sealed again after feeding finished. The effluent was collected for the sampling of pH, alkalinity, and TS/VS. Soda / Natrium hydro carbonate ($NaHCO_3$) was used as a buffer when the system pH falls below 6.5.

The reactors basic configuration is as shown below:

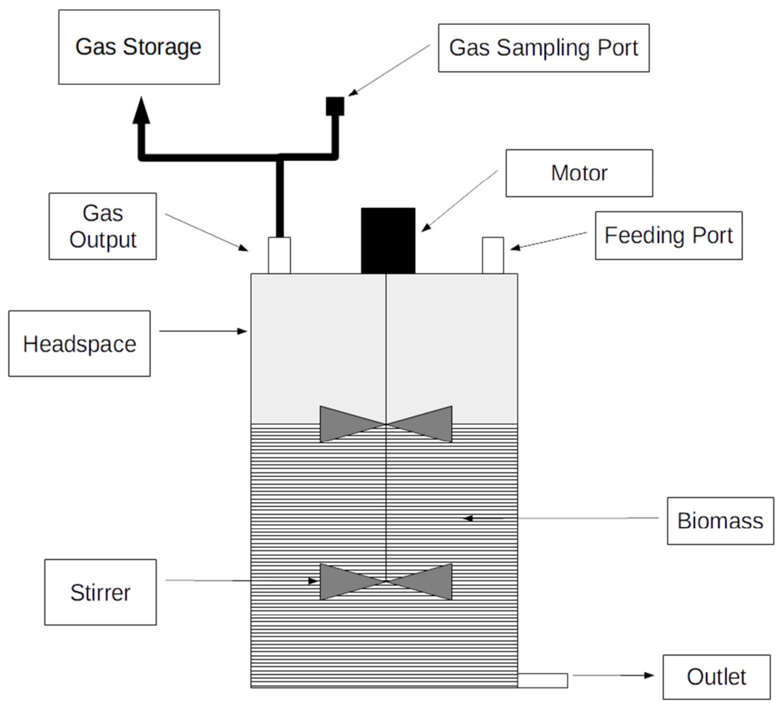


Figure 3.1 A single-stage Continuously Stirred Tank Reactor (CSTR)

3.2. Procedure

3.2.1. Experimental setup

The research was conducted from March 2017 to April 2018. Firstly, all materials for this study, including inoculum, feedstock, and used frying oil were prepared. Secondly, these materials were characterized for TS, VS, Ash, C, H, N, S, O, and P contents. Thirdly, for the BMP test, substrates were mixed according to a pre-defined proportion (Table 3.1). Subsequently, the BMP test was carried out and its biogas production was analyzed. The most appropriate substrate from the BMP test was selected according to the yield, applicability, environmental, socio-economical, as well as scalability measures for future applications. This selected substrate was then used in the continuous AD test. The biogas production in the continuous AD test was then further analyzed. The experimental design for the BMP test was as follows:

Table 3.1. Experimental design for BMP determination

Substrates	Cattle Manure-M (% VS)	Chicken Manure-P (% VS)	Rice Straw-R (% VS)	Hornwort -D (% VS)	Used Frying Oil-L (% VS)
Cattle manure	100	0	0	0	0
Chicken manure	0	100	0	0	0
Rice straw	0	0	100	0	0
Hornwort	0	0	0	100	0
M:P:R 45:15:40	45	15	40	0	0
M:P:R 60:20:20	60	20	20	0	0
M:P:D 45:15:40	45	15	0	40	0
M:P:D 60:20:20	60	20	0	20	0
M:P:R:L 45:15:38:2	45	15	38	0	2
M:P:R:L 60:20:18:2	60	20	18	0	2
M:P:D:L 45:15:38:2	45	15	0	38	2
M:P:D:L 60:20:18:2	60	20	0	18	2

Substrates	Cattle Manure-M (% VS)	Chicken Manure-P (% VS)	Rice Straw-R (% VS)	Hornwort -D (% VS)	Used Frying Oil-L (% VS)
Control(+)	Positive control: Avicell®				
Control(-)	Negative control: de-ionized water				

Notes:

- Each configuration was in a triplicate experiment.
- Substrate mixing was done based on gram VS

The startup of continuous digestion experiment was consisted of setting up bioreactor, inoculum preparation, substrate preparation, and Organic Loading Rate (OLR) and Hydraulic Retention Time (HRT) determination. The gas storage was made of a plastic balloon with a volume of 7 liters and connected to the bioreactor using silicon tubing. The inoculum used in this experiment was obtained on 24 April 2017 and degassed and stored in room temperature until being used on 9 December 2017. It had a pH of 8.02. It was put into the bioreactor at a volume of 5 liters. It had been tested for microbial activity using a sugar, substrate mixture of M:P:R:L 45:15:38:2, and then M:P:R 45:15:40. The M:P:R 45:15:40 substrate mixture was chosen for the substrate in continuous test and then prepared in liquid form with a concentration of 70 g VS/L for feeding. The Hydraulic Retention Time (HRT) of 35 days with an Organic Loading Rate (OLR) of 2 g VS/L/day was used for the continuous digestion, following the result of *k* constant with adjustment to provide better system performance.

3.2.2. Analysis

Gas volume, gas content, VFA content, biodegradability, and hydrolysis constant analysis were done during and after the BMP, while gas volume, gas content, VFA content, and alkalinity were done during the continuous digestion process. A decision analysis was done after BMP results obtained to select the most appropriate substrate for continuous digestion.

The result after BMP test was analyzed for Volatile Fatty Acids using Gas Chromatography (GC-FID). The sample for BMP test was obtained after the BMP test was finished on day 74.

The biogas produced from BMP and continuous digestion was sampled at 0.5 ml using airtight syringes and was tested for its composition using Gas Chromatography (GC-TCD) technique. The biogas volume from the BMP was measured using gas displacement technique, while biogas volume in continuous digestion (CSTR) were measured using a 50 mL syringe.

3.2.2.1. Kinetic analysis

Kinetic analysis was performed from the result of BMP to obtain hydrolysis (*k*) constant using following formula (I. Angelidaki et al., 2009):

$$\ln\left[\frac{\mathrm{B0-B}}{\mathrm{B0}}\right] = -\mathrm{kt} \tag{12}$$

where

$B0$ = ultimate methane production

B = methane yield at given time t

k = hydrolysis constant

t = time (days)

3.2.2.2. Data analysis

The data obtained in this study were analyzed using parametric statistics using $\alpha = 0.05$.

CHAPTER 4

RESULT

4.1.Characterization of Materials

The inoculum, substrates, and used frying oil were characterized for Total Solids (TS), Volatile Solids (VS), ash, as well as elemental C, H, O, N, S, and P. Physicochemical characteristics of inoculum, substrates, and additive were shown in Table 4.1. The CNR were calculated from characterization result and presented in Table 4.1.

Cattle manure and chicken manure contained a high amount of moisture and had low TS contents, with 20.07±0.55% and 23.30±2.77%, respectively. Rice straw and hornwort, on the other hand, have been dried for storage, and thus, their TS contents are relatively high with 89.54±0.09% and 85.57±0.14%. Since TS contents of all substrates are higher than 15%, these substrates were diluted to be suitable for wet anaerobic digestion process (Yeqing Li et al., 2013). The oil had a very high TS content with 99.87±0.03%.

All substrates had a high proportion of organic matter and ash. The VS ranged from 77.05±0.50% of hornwort to 86.14±0.82% of cattle manure, while the ash ranged from 13.86±0.82% of cattle manure to 22.95±0.50% of hornwort. A high VS value indicates that the substrate has a good prospect for anaerobic digestion, due to the availability of organic C sources. Moreover, high ash content reflects the number of cations in the substrate, which represent the buffering capacity of the substrate (Lo et al., 2009).

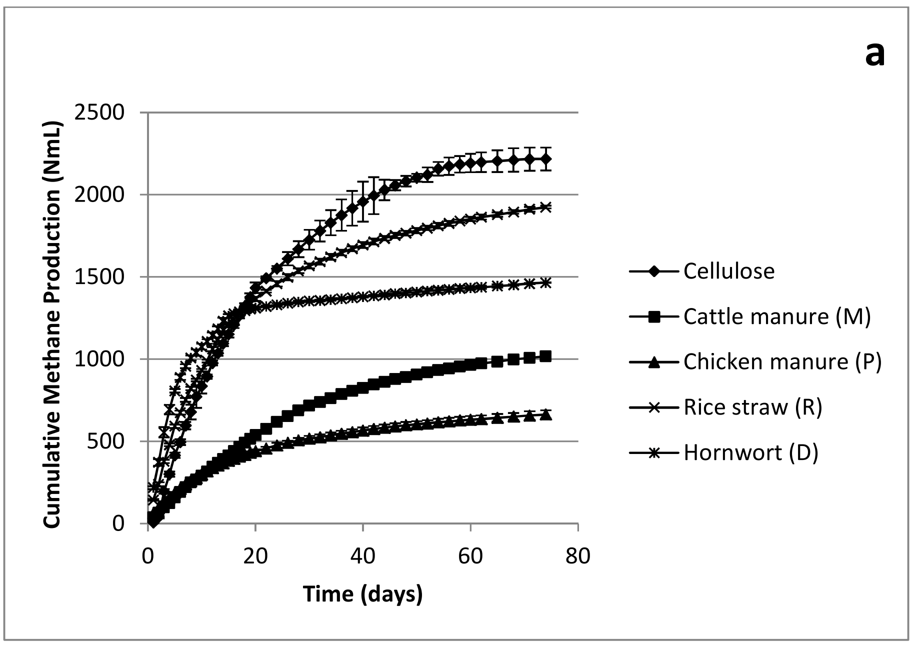
Table 4.1. Physicochemical characteristics and Theoretical Specific Methane Yield (SMYth)

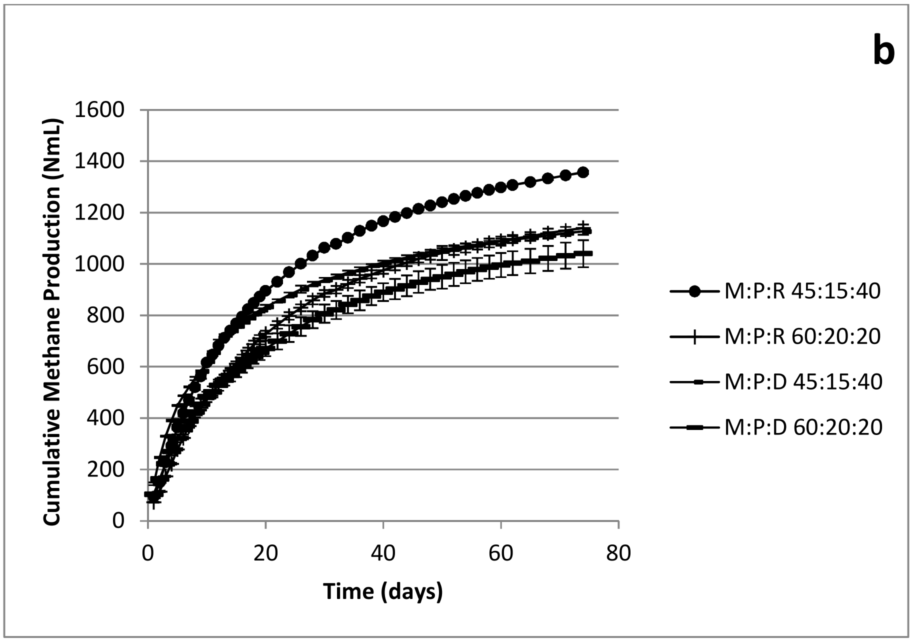
Items	Mix Ratio (VS basis)	TS (%)	VS (%TS)	Ash (%TS)	C (%TS)	H (%TS)	O (%TS)	N (%TS)	S (%TS)	P (mg/kg)	SMYth (NmL CH_4/g VS)
Inoculum	N/A	3.77±0.38	61.52±2.09	38.48±2.09	0.46	10.46	80.51	0.12	ND	212.25*	N/A
Cattle manure (M)	N/A	20.07±0.55	86.14±0.82	13.86±0.82	40.48	5.29	29.66	1.37	0.211	5312.11	478.29
Chicken manure (P)	N/A	23.30±2.77	77.29±2.03	22.71±2.03	37.25	5.01	28.56	3.09	0.609	19035.30	474.78
Rice straw (R)	N/A	89.54±0.09	85.95±0.07	14.05±0.07	37.45	5.10	33.81	0.41	0.061	637.49	430.44
Hornwort (D)	N/A	85.57±0.14	77.05±0.50	22.95±0.50	33.81	4.93	28.42	3.37	0.381	12716.07	430.69
Used frying oil (L)	N/A	99.87±0.03	99.99±0.01	0.01±0.01	77.63	13.32	8.63	ND	ND	1.25	1063.73
M:P:R	45:15:40	48.34	84.74	15.26	1.24	38.78	5.17	0.21	31.16	5500.74	458.62
M:P:R	60:20:20	34.61	84.33	15.67	1.52	39.23	5.20	0.26	30.27	7121.82	468.01
M:P:D	45:15:40	46.75	81.18	18.82	2.43	37.33	5.10	0.34	29.00	10332.17	458.72
M:P:D	60:20:20	33.82	82.55	17.45	2.11	38.50	5.16	0.32	29.19	9357.54	468.06
M:P:R:L	45:15:38:2	48.55	85.02	14.98	1.24	39.59	5.34	0.21	30.65	5488.02	471.29
M:P:R:L	60:20:18:2	34.82	84.61	15.39	1.51	40.03	5.36	0.26	29.77	7109.10	480.68
M:P:D:L	45:15:38:2	47.04	81.64	18.36	2.36	38.20	5.27	0.33	28.60	10077.88	471.38
M:P:D:L	60:20:18:2	34.10	83.01	16.99	2.05	39.38	5.33	0.32	28.80	9283.24	480.73

Notes:
ND = Not Detected
N/A = Data Not Available
* = unit is in mg/L

4.2.BMP Experiment

During this study, biogas production and methane content of the biogas was recorded from the BMP experiment to calculate the methane production of each treatment. The methane production of the BMP experiment was then used to calculate Experimental Specific Methane Yield (SMYexp) and the first order hydrolysis constant (*k*). The methane production was plotted into a cumulative methane production graph and is provided in Figure 4.1.





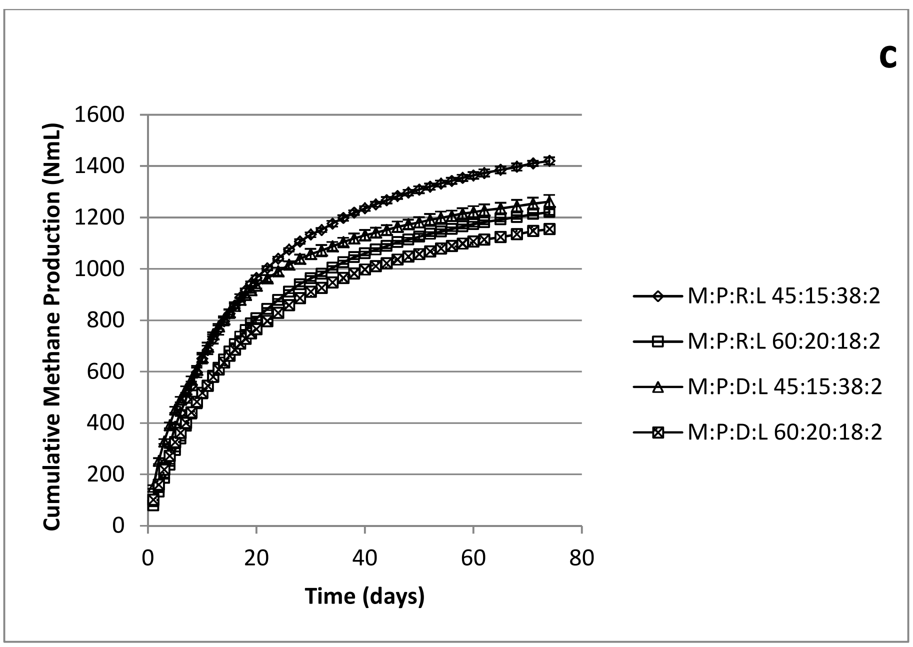


Figure 3.1. Cumulative Methane Production. (a) mono-digestion, (b) co-digestion without oil addition, and (c) co-digestion with oil addition

4.2.1. Specific Methane Yield (SMY)

Theoretical Specific Methane Yield (SMYth) was obtained from calculation of elemental characterization using Equation 2. Values of SMYth for substrates and additive used were presented in Table 3.1. The experimental specific methane yield (SMYexp) was calculated from the total methane produced during experiment divided by the amount of substrate addition in VS basis. The SMYexp was presented in Table 4.2.

In addition, SMYth values for all treatments were calculated according to their proportion in the substrate mixture. A complete set of SMYth values of all treatments were presented in Table 4.1. The highest SMYth value for mono-digestion was 478.29 NmL CH_4/g VS of cattle manure, while the lowest was 430.44 NmL CH_4/g VS of rice straw. For co-digestion treatments, the highest SMYth was 480.73 NmL CH_4/g VS of M:P:D:L 60:20:18:2, with M:P:R 45:15:40 being the lowest at 458.62 NmL CH_4/g VS. The value of SMYth from cellulose as a positive control was at 414.48 NmL CH_4/g VS.

The highest SMYexp was obtained from cellulose with 384.53±5.37 NmL CH_4/g VS, which was the positive control. Among all treatments, rice straw and hornwort had the first and second highest SMYexp with 331.52±1.47 NmL CH_4/g VS and 254.86±2.01 NmL CH_4/g VS respectively, while chicken manure and cattle manure had the first and second lowest SMYexp with 114.19±4.50 NmL CH_4/g VS and 175.42±3.27 NmL CH_4/g VS respectively. Cattle manure, chicken manure, rice straw, and hornwort were all mono-digestion treatments. With due respect to co-digestion treatments, M:P:R:L 45:15:38:2 and M:P:R 45:15:40 had the first and second highest

SMYexp with 246.02±2.14 NmL CH_4/g VS and 233.25±0.81 NmL CH_4/g VS respectively, while M:P:D 60:20:20 had the lowest SMYexp with 180.43±9.07 NmL CH_4/g VS.

Table 4.2. Experimental parameters of BMP test

Treatments	Substrate CNR	Systemic CNR	SMYexp (NmL CH_4/g VS)	Biodegradability (%)	[1]Increase (%)	k (day^{-1})	[2]α
Mono-digestion							
Cellulose	NC	NC	378.29±5.37	91.27	NC	0.04	NC
Cattle manure (M)	29.55	26.74	172.57±3.27	36.08	NC	0.03	NC
Chicken manure (P)	12.06	11.67	112.34±4.50	23.66	NC	0.06	NC
Rice straw (R)	90.90	65.75	326.14±1.47	75.77	NC	0.07	NC
Hornwort (D)	10.03	9.77	250.72±2.01	58.22	NC	0.14	NC
Co-digestion without oil addition							
M:P:R 45:15:40	30.43	27.39	229.47±0.81	50.03	32.97	0.06	1.02
M:P:R 60:20:20	25.18	23.15	193.27±3.04	41.30	11.99	0.05	1.01
M:P:D 45:15:40	14.99	14.32	191.31±2.62	41.71	10.86	0.09	0.98
M:P:D 60:20:20	17.81	16.83	177.50±9.07	37.92	2.86	0.07	1.01
Co-digestion with oil addition							
M:P:R:L 45:15:38:2	30.88	27.77	242.03±2.14	51.35	40.25	0.06	1.01
M:P:R:L 60:20:18:2	25.59	23.50	208.17±1.00	43.31	20.63	0.05	1.01
M:P:D:L 45:15:38:2	15.69	14.96	214.36±4.02	45.47	24.22	0.08	1.02
M:P:D:L 60:20:18:2	18.75	17.68	197.16±1.88	41.01	14.25	0.06	1.02

Notes:
ND = Not Detected
NC = Not Calculated
[1]Increase = Methane yield (SMYexp) of a treatment compared to that of cattle manure mono-digestion (M)
[2]α = synergistic effects

4.2.2. Carbon to Nitrogen ratio

The Carbon to Nitrogen Ratio (CNR) in the treatments was calculated from the C and N content in the substrates multiplied by the composition of the substrate in the treatments. A complete presentation of CNR in treatments was available in Table 4.2.

The result showed that among the CNR values of mono-digestion treatments, only cattle manure that was suitable for anaerobic digestion at

29.55. Other substrates simply fall beyond the range of 20 to 35 which was considered as optimum condition (Mao et al., 2015). Hornwort and chicken manure fell below the range with 10.03 and 12.06 respectively, while rice straw had a very high CNR of 90.90. This result confirmed that cattle manure should be the main component of substrates in treatments, while chicken manure, rice straw, and hornwort, might be used as co-substrates. Co-digestion treatments had various CNR ranged from 14.99 to G to 30.88 of M:P:R:L 45:15:38:2, with M:P:R 45:15:40, M:P:R 60:20:20, M:P:R:L 45:15:38:2 and M:P:R:L 60:20:18:2 had CNR within 20 to 35 bracket at 30.43, 25.18, 30.88, and 25.99 respectively.

When CNR calculations took inoculum profile into account, and thus, the systemic CNR instead of substrate CNR was calculated, the result showed a similar pattern with a small difference. In mono-digestion treatments, the systemic CNR were corrected to a range between 9.77 of hornwort and 65.75 of rice straw, while in co-digestion treatments, the range was between 14.32 of M:P:D 45:15:40 and 27.77 of M:P:R:L 45:15:38:2. Similar with calculations without inoculum, only cattle manure, M:P:R 45:15:40, M:P:R 60:20:20, M:P:R:L 45:15:38:2, and M:P:R:L 60:20:18:2 had CNR within a range of 20 to 35, with 26.74, 27.39, 23.15, 27.77, and 23.50 respectively.

4.2.3. VFA concentration

VFAs are intermediate products in anaerobic digestion, which include acetic acid, propionic acid, and butyric acid among others. VFAs were produced in hydrolysis, acidogenesis, and acetogenesis phase, and were consumed in acetogenesis and methanogenesis phase. High VFA concentration might cause an acidification and lead to pH drop. A complete anaerobic digestion process would have a low VFA concentration. The VFA concentration from the experiment was presented in Figure 4.2.

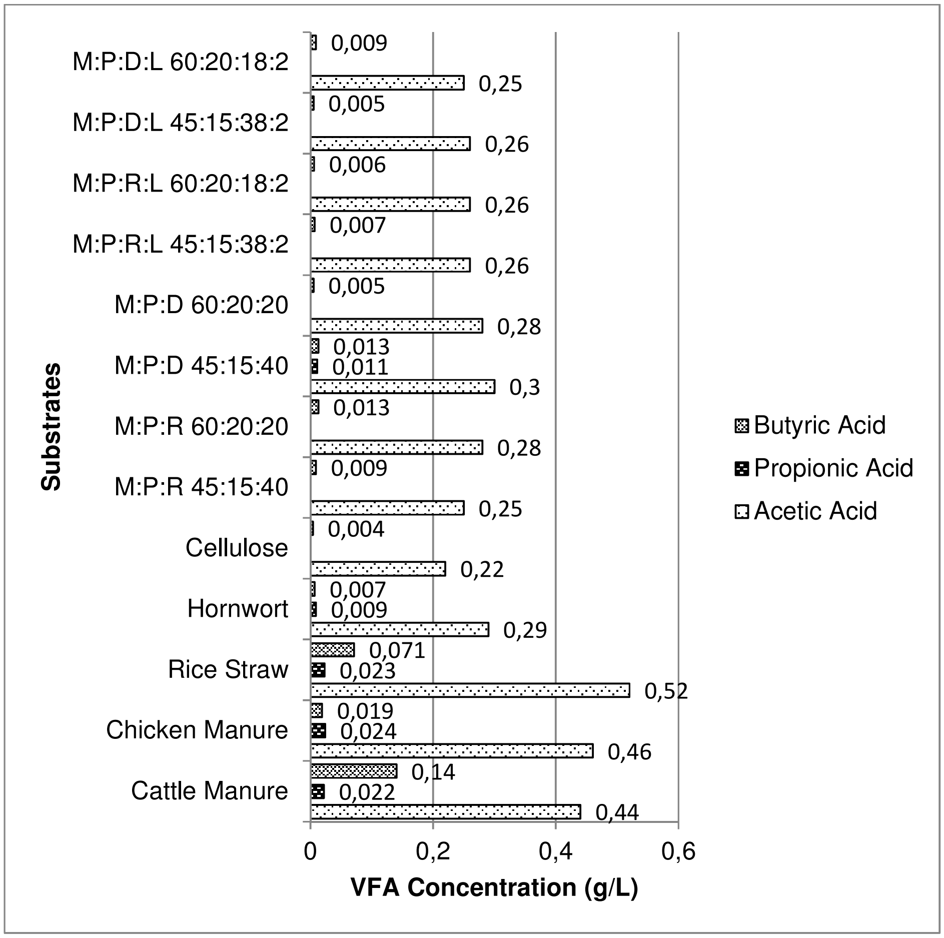


Figure 4.2. VFA concentration

The VFA concentration in mono-digestion treatments was relatively higher than those of co-digestion treatments. In co-digestion treatments, the acetic acid concentration ranged from 0.25 to 0.28 g/L, while in mono-digestion treatments ranged from 0.29 to 0.52 g/L. The propionic acid concentration in co-digestion was not detected except in treatment G with 0.011 g/L, while all mono-digestion treatments had propionic acid content between 0.009 to 0.024 g/L. Butyric acid was detected in all treatments, ranged from 0.005 to 0.013 g/L in co-digestion, and 0.007 to 0.140 g/L in mono-digestion.

4.2.4. Biodegradability/digestibility

Biodegradability or digestibility of the substrate was defined as the fraction of degraded organic matter per total organic matter. The values were calculated from experimental methane yield divided by theoretical methane yield. The biodegradability/digestibility data is presented in Table 4.2.

The biodegradability values of all treatments ranged from 23.66% of chicken manure to 58.22% of rice straw, which both represented mono-digestion. Among co-digestion treatments, the highest biodegradability was 51.35% of M:P:R:L 45:15:38:2, while the lowest was 41.01% of M:P:D:L 60:20:18:2.

4.2.5. Effect of substrates on BMP

The effect of substrate on the BMP was observed by the substrate composition, co-substrate type, additive, and kinetics/synergism.

4.2.5.1. Substrate composition

Methane production was influenced by substrate composition and the proportion of each substrate in the mixture. Substrate mixture with a higher proportion of cattle manure and chicken manure showed a lower methane production compared with the mixture with lower or even without manures. Rice straw and hornwort without manure showed the first and second highest methane production at 331.52±1.47 NmL CH_4/g VS and 254.86±2.01 NmL CH_4/g VS respectively, while both cattle manure and chicken manure without rice straw or hornwort addition had the second and first lowest at 175.42±3.27 NmL CH_4/g VS and 114.19±4.50 NmL CH_4/g VS respectively. The complete data is available in Table 4.2.

4.2.5.2. Co-substrate type

There were two substrates being used as co-substrates in this study: rice straw and hornwort. These two substrates were compared one another to determine which substrate would be the most suitable for co-digestion with cattle manure and chicken manure for methane production. The complete data is available in Table 4.2.

The result showed that in the same proportion, substrate mixtures using rice straw had higher methane yield compared with substrate mixtures using hornwort. For example in a proportion of 40% of rice straw and hornwort added (M:P:R 45:15:40 and M:P:D 45:15:40), the methane yields were 233.25±0.81 and 194.47±2.62 NmL CH_4/g VS for rice straw and hornwort respectively. Other pairs (M:P:R 60:20:20 – M:P:D 60:20:20, M:P:R:L 45:15:38:2 – M:P:D:L 60:20:38:2, and M:P:R:L 60:20:18:2 – M:P:D:L 60:20:18:2) also had a similar result as M:P:R 45:15:40 – M:P:D 45:15:40.

4.2.5.3. Additive

The benefit of additive to improve methane production was investigated in this study. Used frying oil was used as the additive of choice due to availability in each and every household and food processing vendors as well as its continuity of production. This argument was supported by the result of characterization and theoretical methane yield calculation which showed that oil had a superior theoretical SMY compared to other substrates. Results showed that in the same group of substrates, oil addition can improve the methane yield compared to that of the group without oil addition. For example, M:P:R:L 45:15:38:2 with 2% oil addition compared to M:P:R 45:15:40 without oil addition had SMYexp of 246.02±2.14 NmL CH_4/g VS compared to 233.25±0.81 NmL CH_4/g VS respectively. The complete data is available in Table 4.2.

4.2.5.4. Kinetic analysis and synergism

The kinetic analysis of experiment showed that hornwort had a fast reaction rate, while its mono-digestion treatment, had the fastest reaction rate among all substrates, with k=0.14 d^{-1}. In contrary, the slowest reaction rate was of mono-digestion of cattle manure, with k= 0.03 d^{-1}. Among co-digestion substrates, M:P:D 45:15:40 had the fastest reaction with k= 0.09 d^{-1}, while M:P:R 60:20:20 and M:P:R:L 60:20:18:2 had slowest reaction with k = 0.05 d^{-1}. The complete result is presented in Table 3.2.

Synergistic effect (α) in methane production is present when the methane yield of the co-digestion treatment is higher than the sum of methane yield of each component. On the other hand, the antagonistic effect is present when the methane yield of the co-digestion is lower than the sum of methane yield of each substrate in mono-digestion treatments. The synergistic effect of the co-digestion treatments is visualized in Figure 4.3.

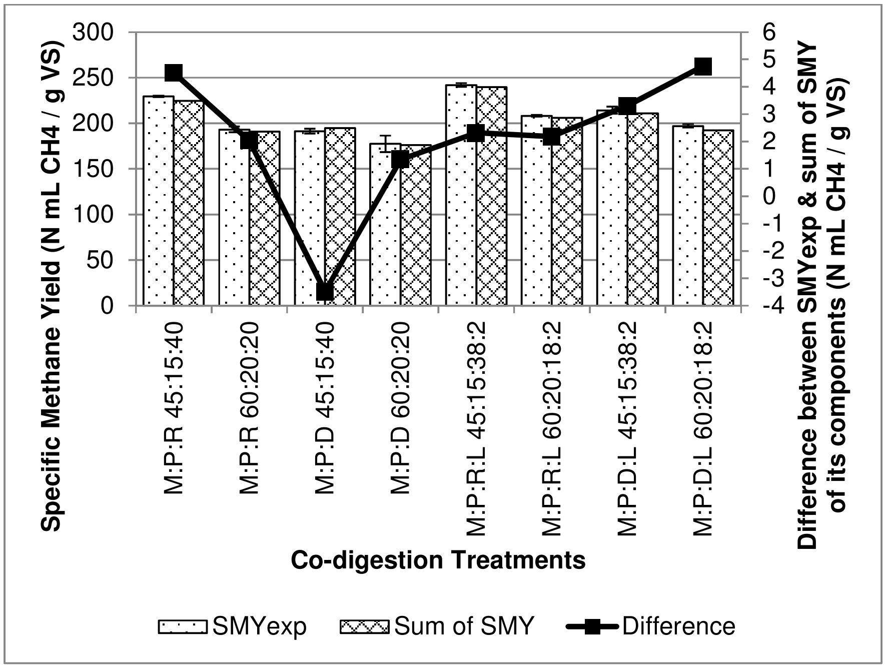


Figure 4.3. Synergistic effects. Sum of SMY is obtained from the sum of mono-digestion of its feedstock components. The difference is obtained from SMYexp subtracted by Sum of SMY.

4.3. Choosing substrate for continuous test

From the result of the BMP experiment, the M:P:R:L 45:15:38:2 mixture showed the highest SMYexp among all co-digestion experiment and thus become a potential substrate for continuous digestion. However, the M:P:R:L 45:15:38:2 had a difficulty in substrate mixing for the continuous feeding. In addition, technical consideration as a sole parameter of production was not sufficient to determine the sustainability of the biogas production scheme. A consideration toward socio-economic, cultural, and management aspect were also involved in the decision making. While cattle manure, chicken manure, and rice straw are available in an abundant amount, the information about the availability and exact amount of used frying oil is limited and may be subject of variations in different locations, seasons, and culture. Therefore, while the M:P:R:L 45:15:38:2 had the highest SMYexp, it was not used in the continuous digestion, and M:P:R 45:15:40 as the second highest substrate in terms of SMYexp was used instead.

4.4.Continuous Digestion

During this study, a continuous digestion was performed using the substrate mixture which was considered as the most suitable mixture according to the result of the BMP experiment and subsequent analysis, which was M:P:R 45:15:40. In the continuous digestion, biogas volume and composition was recorded to determine the methane production. In addition, pH, VFA content and alkalinity test were performed to find out the system stability.

4.4.1. Startup

The startup was initially started using the sugar as the substrate to test the microbial activity after storage. The sugar activated the inoculum and the system produced gas. The gas contained both methane and carbon dioxide. However, the reactor went to an acidic condition and failed to recover. Therefore, the reactor was emptied, and new inoculum from the same stock was used.

Next, the startup was re-started using M:P:R:L 45:15:38:2 which was the substrate with highest methane yield in the BMP. The M:P:R:L 45:15:38:2

was provided at a concentration of 350 g VS/L. The system produced gas and methane was detected in the gas. However, only in few days, the stirrer did not work due to high solid content. In addition, there was a difficulty in using this substrate in the continuous test. Firstly, the oil did not mix well with other substrates. Secondly, the information about used frying oil availability is lacking, and therefore, its sustainability is a matter of concern. Due to these considerations, the substrate was then changed to the substrate which had second highest methane yield in the BMP.

The M:P:R 45:15:40 was chosen for the substrate and was given at a concentration of 350 g VS/L. In this concentration, the system produced methane. However, in few days, the stirrer jammed. Therefore, the concentration was reduced to 125 g VS/L. At this concentration, the stirrer was still jammed, and thus, a recovery strategy by feeding at a concentration of 5 g VS/L was used. After few days, the system was recovered and the stirrer worked. At all concentration, the system yielded methane.

The M:P:R 45:15:40 substrate mixture was then prepared in liquid form with a concentration of 70 g VS/L for feeding. The Hydraulic Retention Time (HRT) of 35 days with an Organic Loading Rate (OLR) of 2 g VS/Ld^{-1} was used for the continuous digestion, following the result of *k* constant with adjustment to provide better system performance.

4.4.2. Specific Methane Yield (SMY)

The biogas volume and methane content of the biogas, as well as the given feedstock, were monitored during the study to determine the SMY of the continuous digestion process. The biogas volume during this study was varied between 1950 mL and 4920 mL with an average of 3116.59 mL/day (SD=500.03) with a methane content between 61.83% and 76.75% with an average of 67.61% (SD=4.15), thus, the methane production was in average 1894.13 N mL/day (SD=299.68) at STP. The SMY from the continuous digestion was varied between 88.58 N mL CH_4/g VS and 210.37 N mL CH_4/g VS with an average of 135.30 N mL CH_4/g VS (SD=21.41), or about 71.28% of the result of BMP at day 34 (189.81 N mL CH_4/g VS). The biogas production and methane content are available in Figure 4.4, while the SMY is available in Figure 4.5.

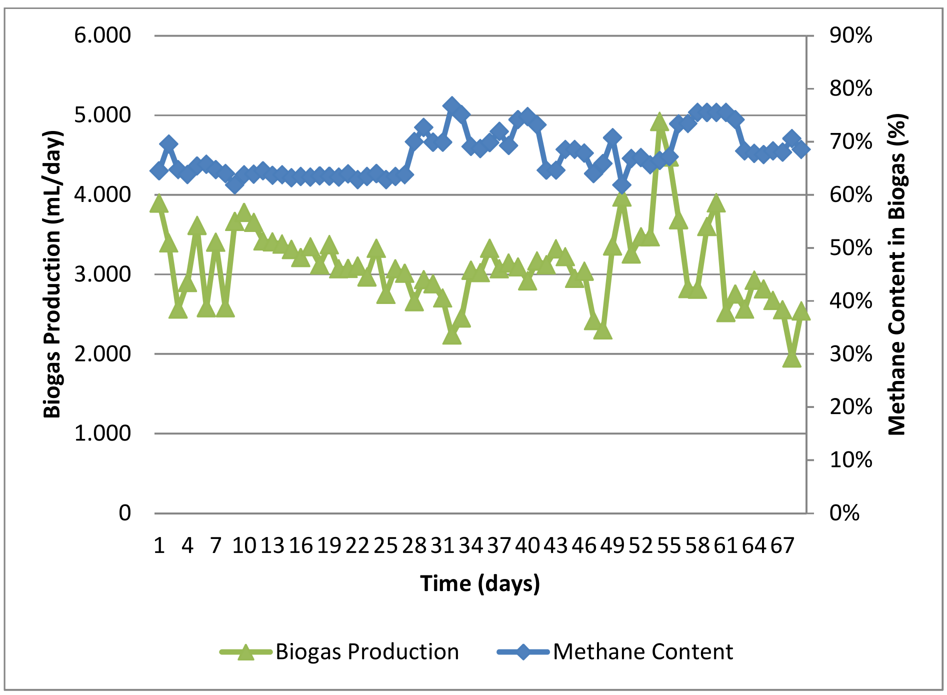


Figure 4.4. Biogas production and methane content in the biogas

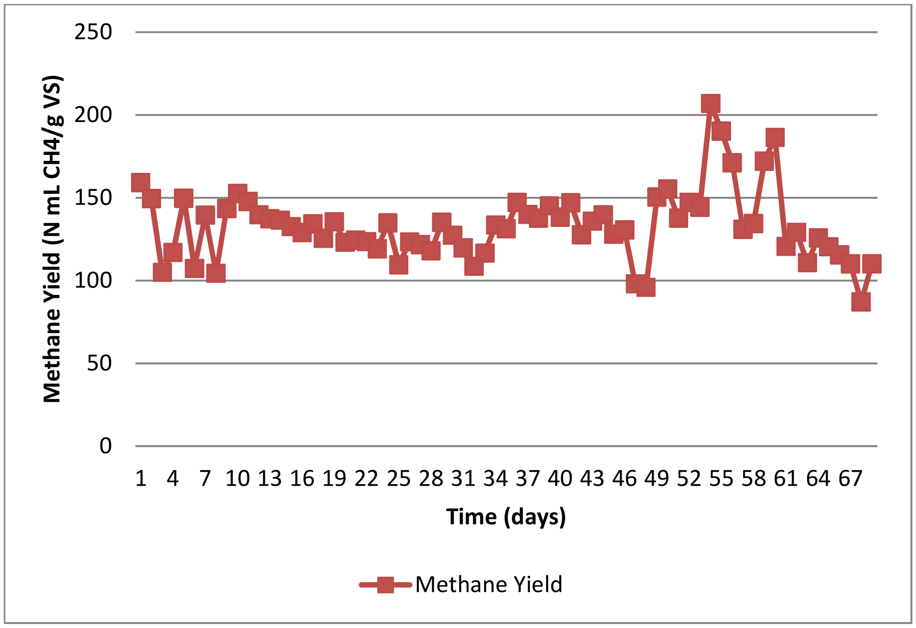


Figure 4.5. Specific Methane Yield (SMY) of the continuous digestion process

The co-digestion of cattle manure, chicken manure, and rice straw altogether in different ratios was a new approach and there is a limited study using co-digestion of this mixture as the substrate for anaerobic digestion. Therefore, a direct comparison to the previous study is difficult. A comparison to similar study using substrates with similar characteristics was then employed. The SMY result of this study was lower to those of the previous study from cattle manure co-digestion with oat straw at 203±25 L CH_4/kg VS added, while was comparable to those of manure mono-digestion at 155±26 L CH_4/kg VS added in the same study (Lehtomäki, Huttunen, & Rintala, 2007). Another study using co-digestion of cattle manure and rice straw had a methane yield of 196.03 L/kg VS (D. Li et al., 2015).

4.4.3. pH

The effluent from bioreactor was monitored for the pH. During the study, the pH of the bioreactor was stabilized between 6.29 and 8.15 with small fluctuations (average=6.94, SD=0.30). The pH data is presented in Figure 4.6.

4.4.4. Alkalinity

The alkalinity of the effluent was monitored during the study. The result of the study showed that the alkalinity of the system was slightly reduced over time. In the beginning, the alkalinity was at an amount higher than 5000 mg/L of $CaCO_3$ and slowly decreased to around 3000 mg/L of $CaCO_3$ at the end of the study. The diagram of alkalinity is provided in Figure 4.6.

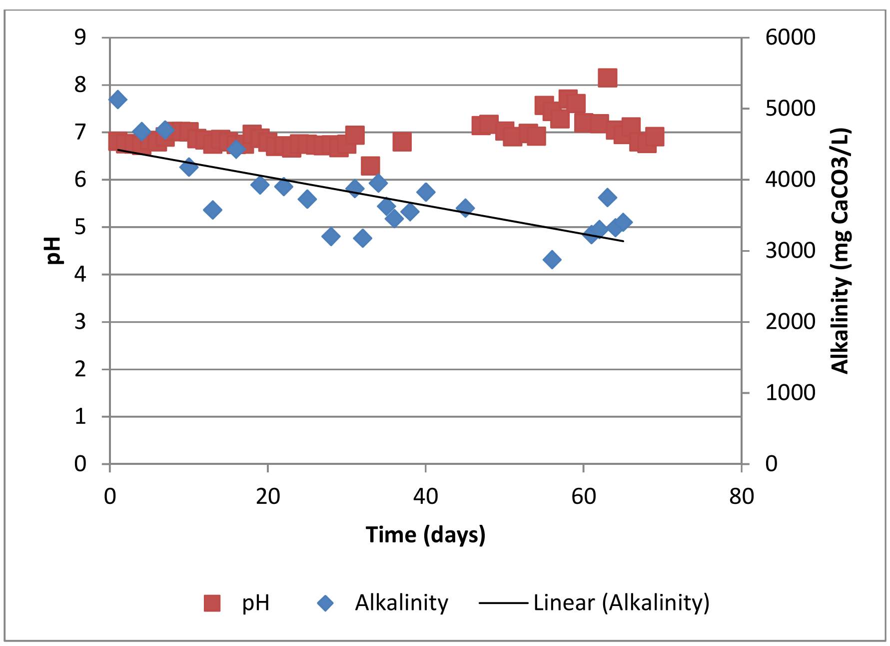


Figure 4.6. The pH and alkalinity of the continuous digestion system

CHAPTER 5

DISCUSSION

5.1.Characterization

Results showed that all substrates could be potentially used for anaerobic digestion, with a co-digestion system to balance the nutrients. Possibilities for using these substrates have been recognized in previous studies. Animal manures are the most frequently used main substrate for anaerobic digestion, with cattle manure being one of the most widely used (Mata-Alvarez et al., 2014), with some efforts using poultry manure (Abouelenien et al., 2009; Elasri & El Amin Afilal, 2016). Recent studies have used co-digestion of animal manures with agro-industrial waste to increase biogas production with positive results (Awais, Alvarado-Morales, Tsapekos, Gulfraz, & Angelidaki, 2016; T. Zhang et al., 2014). In addition, hornwort has also been used for anaerobic digestion by several studies with promising results (Koyama et al., 2014; Pastare, Romagnoli, Rugele, Dzene, & Blumberga, 2015).

5.2.BMP Experiment

5.2.1. Specific Methane Yield (SMY)

According to the result of the calculation, oil has the highest SMYth among substrates with a very big difference with other substrates. Thus, in the calculation, oil showed a very high potential for methane production. Owing to the high potential of oil for methane production, oil could then be added to the anaerobic process to increase methane production. According to the result of characterization, oil had a very high organic content and CNR. Therefore, it was suitable to be added to co-digestion of livestock manure. However, in practice, since degradation of oil would produce Long Chain Fatty Acids (LCFA) that could inhibit methanogenesis process, oil could not be used in a large proportion in the mixture (Chen et al., 2008). Therefore, oil is suitable for use as an additive only, and not as a co-substrate or main substrate. The SMYth value could be sorted from highest to lowest as follows: cattle manure, chicken manure, hornwort, and rice straw.

The SMYth gives an insight of an expected result of a BMP test on a specific substrate. The value of SMYth is an upper limit for the Experimental SMY (SMYexp) in the BMP test. A SMYexp value higher than its SMYth value for the corresponding substrate means that the BMP testing is subject to errors.

In regard to cellulose as a positive control, a similar result was reported by the previous multicenter study (Raposo et al., 2011) with a value ranging from 303 – 412 mL CH_4/g VS. On the other hand, the SMYexp from rice straw mono-digestion was higher than a recent study conducted in China (Gu et al., 2014), while it was lower for chicken manure mono-digestion compared to another study in the same country (Yeqing Li et al., 2013) which might be due to different characteristics of substrates and inoculum used in the experiment. Moreover, the SMYexp of hornwort mono-digestion seems to be lower than a result of a recent study in Latvia (Pastare et al., 2015), although it resembles closely to result of another study in Japan (Koyama et al., 2014).

5.2.2. Carbon to Nitrogen ratio

Results showed that according to calculations of CNR, several co-digestion systems were technically possible. Treatments M:P:R 45:15:40, M:P:R 60:20:20, M:P:R:L 45:15:38:2, and M:P:R:L 60:20:18:2 had suitable CNR values and thus were suitable for anaerobic digestion. On the other hand, treatments M:P:D 45:15:40, M:P:D 60:20:20, M:P:D:L 60:20:38:2, and M:P:D:L 60:20:18:2 had CNR values lower than recommended value. These substrates could be used for anaerobic digestion, yet their yields might be lower than those of in the optimal range. Should these substrates be used for anaerobic digestion, the composition should be adjusted to balance the CNR by mixing with additional carbon sources.

5.2.3. VFA concentration

The result of the study showed that the VFA concentration was very low in all treatments. The VFA concentration in all treatments was below 1 g/L and thus indicated that the system was in a stable condition (Yang et al., 2015). The highest concentration of acetic acid, propionic acid, and butyric acid was detected at 0.52 g/L in rice straw mono-digestion, 0.024 g/L in chicken manure, and 0.14 g/L in cattle manure, respectively. In most co-digestion treatments, propionic acid was not detected. This indicated a stable system and was a good result because propionic acid had the strongest inhibitory effect to AD process compared to other VFAs (Yang et al., 2015). Propionic acid showed inhibitory effects to methanogens activity at a concentration of 2.9 g/L, while total concentration of VFA at 10 g/L also showed the same effect (Xie et al., 2016).

The concentration of acetic acid was the highest among all VFAs due to the process pH during this study at around 6.94. The VFA composition was known to be affected by the pH of AD process. At pH around 7.00, acetic acid would be the major VFA produced, while at pH around 5.00 butyric acid would be the major VFA produced, and at pH around 8.00 propionic acid would be the major VFA produced (Mao et al., 2015).

5.2.4. Biodegradability/digestibility

The biodegradability of cellulose represented a very high biodegradability of 91.27% due to its pure form and microstructure. The values of other treatments corresponded to the fact that lignocellulosic biomasses were rich in lignocellulose complexes which recalcitrant to biodegradation (Sawatdeenarunat et al., 2015). Therefore, the biodegradability of these treatments was relatively low (Yang et al., 2015). Nevertheless, the mechanical preparation of rice straw and hornwort in a mesh form with a size of mesh 10-20 could also increase the biodegradability/digestibility of these substrates. In this size, substrate particles had a bigger surface area compared to those of bigger particles and thus provided a deeper access to microbial degradation. Moreover, mechanical grinding also reduced the crystallinity of the cellulose (Shah et al., 2015). In addition, for treatments with a high amount of manure, their biodegradability was lower compared to those with less or no manure in their mixture. This was due to fact that manures were animal waste which represented the non-digestible part of the consumed feed, thus was considered as low potential for methane production (Awais et al., 2016).

5.2.5. Effects of substrate on BMP

The effect of substrate on the BMP was discussed by the substrate composition, co-substrate type, additives, and kinetic / synergism.

5.2.5.1. Substrate composition

The result showed that the higher proportion of manure in the substrate mix led to a lower methane production. This phenomenon could be explained by the very low biodegradability of manures in this study, which was only 23.66% and 36.08% for those of chicken manure and cattle manure respectively. Therefore, putting more manure in the composition only add a bulk of non-digestible substrates (Awais et al., 2016). On the other hand, the higher proportion of either rice straw or hornwort increased methane production. This effect might be due to an increase of the biodegradable fraction of substrates in the system. Furthermore, regarding the increase in methane yield in co-digestion treatments compared to mono-digestion, the result from the study showed a varied increase from 2.86% of H to 40.25% of M:P:R:L 45:15:38:2. Therefore, it can be confirmed that addition of lignocellulosic biomass as co-substrate to manure had a positive effect of increasing methane production from manure. This result was similar to the result of a recent study (Awais et al., 2016).

5.2.5.2. Co-substrate type

The result of this study showed that rice straw and its mixture had a higher SMY compared to those of hornwort. This is may be due to the fact that rice straw had a high CNR compared to hornwort, and therefore, might provide more C for the AD process. Hornwort was rich in N and thus had a lower CNR. Due to the availability of N from inoculum and manure, an extra C was preferable to N for effective metabolism and thus, methane production.

5.2.5.3. Additive

The effect of oil additive on the methane yield was significant according to the result of this study. The result of BMP confirmed the expectation that oil addition to the substrate mix could increase the methane yield. The effect of oil addition was found to be significant in every substrate proportion. This is similar with a result of previous studies about the use of the oily substrate for anaerobic co-digestion (Davidsson et al., 2008; Girault et al., 2012; Kabouris et al., 2009). However, the increase of the substrate with additive was not so much compared to that of without additive. This could be understood that the additive was only given at 2% on VS basis, and thus could be considered very small amount. Due to the significant effect of oil addition on the methane yield, it could be predicted that addition of more than 2% could give a better yield. However, it must be done carefully, since adding oil in higher amount may lead to inhibitory effect due to Long Chain Fatty Acid (LCFA) accumulation. Previous studies mentioned an inhibitory level of oil addition at an amount of 30% (Mata-Alvarez et al., 2014).

5.2.5.4. Kinetic and synergistic effect

Kinetic analysis showed that hornwort had a fast reaction rate and thus meant that hornwort was easily degradable (Koyama et al., 2014). On the other hand, manure had the slowest reaction rate due to its content that mainly of lignocellulosic complex which was difficult to degrade (Møller et al., 2004). In co-digestion treatments, the addition of hornwort lead to a faster reaction compared to the addition of rice straw, which had a lower *k* constant, 0.14 d^{-1} for hornwort versus 0.07 d^{-1} for rice straw respectively.

From the results of the experiment, synergistic effect existed in the co-digestion which gave an increased methane production compared to the mono-digestion. One treatment, however, showed an antagonistic effect. Treatment M:P:D 45:15:40 had a lower experimental methane yield due to co-digestion compared to the sum of mono-digestion methane yields. It can be seen that the CNR value of M:P:D 45:15:40 was at 14.99 for substrate CNR, and 14.32 for systemic CNR, which was the lowest among all co-digestion treatments and fall far beyond the recommended value of 20 – 35 (Mao et al., 2015). This might be due to an excess of N from a sum of manure and hornwort in the substrate mixture. An excess of N might lead to the production of ammonia or ammonium in the reactor, which acted as an inhibitor to the microbial metabolism (Nielfa et al., 2015). Another possibility is that in M:P:D 45:15:40, the propionic acid was higher compared to other co-digestion treatments. The propionic acid acted as an inhibitor in the AD system and thus, the methane yield was lower compared to other co-digestion treatments. The accumulation of ammonium ions leads to a higher pH, which leads to the production of propionic acid, as mentioned by a recent study (Mao et al., 2015).

The synergistic effect may occur from the complement of nutrients, dilution of inhibitory compounds, counteraction of inhibitory compounds, and pH buffering mechanism of alkalinity. The nutrient shortage may limit microbial growth and productivity (Yang et al., 2015), and providing balanced nutrient may encounter this limiting factor. Furthermore, a specific compound could act as an inhibitor for the anaerobic digestion process. Often, by mixing with another compound, the accumulation of inhibitor compounds could be prevented, or the inhibitor compound could be diluted. For example, propionic acid is mainly produced at higher pH (Mao et al., 2015), which could be triggered by accumulation of ammonium ions from degradation of nitrogenous matter (Chen et al., 2008), which can be potentially prevented by addition of C-rich substrates (Mao et al., 2015). In addition, counteraction of compounds has also been suggested as a mechanism of synergistic effect. A study (Chen et al., 2008) mentioned a mechanism to counteract ammonia inhibition using zeolite. Next, alkalinity could provide a buffering mechanism of the pH and thus, can maintain pH stability (Neshat, Mohammadi, Najafpour, & Lahijani, 2017). Therefore, a balanced nutrient, reduction of the inhibitory compound, and stabilized pH could favor microbial growth and thus, biogas production.

5.2.6. Factors affecting experimental SMY

From results of the study, it was found that the experimental SMY was affected by CNR, biodegradability/digestibility, substrate composition, co-substrate type, additive, as well as synergism. Moreover, the size of substrate particles could also affect the substrate porosity and cellulose accessibility to enzymatic attack and microbial processes and thus, also affect the SMY (Shah et al., 2015). Among these factors, from results of the study, the biodegradability/digestibility of the substrates had the highest impact on the SMY. While the CNR was commonly considered as the most important factor for consideration on choosing substrates for co-digestion (Mata-Alvarez et al., 2014), the impact of biodegradability/digestibility was highly significant and thus could be considered as the decisive factor controlling the SMY. This is similar to a previous study (I. Angelidaki et al., 2009) which highlights the importance of biodegradability. Therefore, the importance of pretreatment has been recognized and emphasized to increase biodegradability and thus, biogas production (Shah et al., 2015).

5.3. Continuous Digestion

From the result of the continuous digestion experiment, biogas production of livestock manure with rice straw was considered viable for continuous production. A discussion of the result is provided in this part.

5.3.1. Startup

The startup of the continuous digestion was characterized by inoculum preparation, equipment preparation, and substrate preparation. The inoculum was tested with M:P:R 45:15:40 at a concentration of 350 g VS/L after 8 months of storage, and was found to be still active and producing methane.

The system produced biogas from day 1 after started. On day 4, the stirrer stopped, the peristaltic pump was jammed, and the biogas volume was dropped due to high viscosity and overload. On day 5, the biogas ceased to produce, and feeding was stopped. After four days of non-feeding, the system was recovered and then fed at a concentration of 125 g VS/L. The reactor worked properly until day 26. On day 27, the stirrer jammed due to high viscosity and overload once again, although biogas was still produced. On day 32, the system was fed a diluted feedstock at a concentration of 5 g VS/L for 8 days for recovery. After fully recovered, the system was then fed at a concentration of 70 g VS/L. The system produced biogas and worked properly using this configuration.

The substrate was composed of cattle manure, chicken manure, and rice straw. Cattle manure and rice straw both had high lignocellulose content and had a complex fibrous structure (Sawatdeenarunat et al., 2015). Even though the rice straw had been milled to small particles, the lignocellulose complex was still difficult to degrade (Neshat et al., 2017). This made the non-soluble solid fibers accumulated in the bioreactor and caused the stirrer jammed because the motor to rotate the stirrer used a specification that was adapted for wastewater. Therefore, a stronger motor was needed for mixing lignocellulosic biomasses. However, to employ such stronger motor would require higher energy consumption and thus made the system less efficient energetically (Sawatdeenarunat et al., 2015).

5.3.2. Specific Methane Yield (SMY)

The biogas production was at a volume between 1950 mL and 4920 mL with methane content between 61.83% and 76.75% with small fluctuations. The biogas volume started at around 4000 mL and fluctuated for about nine days and restored on the 10^{th} day. On the 11^{th} day to the 33^{rd} day, the biogas volume slowly reduced over time with the lowest almost reached 2000 mL. The biogas volume increased to around 3000 mL and stabilized on the 34^{th} day to the 46^{th} day. After that, the biogas volume abruptly fluctuated on the 47^{th} day to the 64^{th} day from slightly over 2000 mL to almost 5000 mL. Next, from the 64^{th} day onward, the biogas volume reduced over time to around 2500 mL at the end of the study on the 69^{th} day.

The methane content of the biogas was started at around 64% and remained stable until the 26^{th} day. On the 28^{th} day, the methane content of the biogas increased to around 70% and fluctuated slightly until the 40^{th} day. It was then lowered to around 67% on the 41^{st} to the 54^{th} day. The methane content jumped again to over 70% from the 56^{th} to 62^{nd} day and finally stabilized at around 70% on the 63^{rd} day onwards.

The methane yield of the continuous digestion was obtained by dividing the methane production by the amount of feeding in the basis of gram Volatile Solids (VS). The methane production itself was obtained by multiplying the biogas volume by the methane content. The methane yield was quite stable during the study with small fluctuations between 100 N mL CH_4/g VS and 150 N mL CH_4/g VS, mainly in the first 10 days and on the 47th to 62nd day.

The result of the study showed that the methane yield was comparable to other similar studies. However, this could be explained that previous studies reported without a reference to STP condition and slightly different substrates, due to the limitation of available study from co-digestion of the same substrate.

5.3.3. pH

The pH of the system during the study showed that the system was in a stable condition. In the beginning, the pH of the system was slightly below 7.00 but stabilized until day 31. On day 33, the pH fell to 6.29, and thus required an addition of buffer. The fall in pH seemed to cause a slight reduction in biogas production, yet the methane content was increased. Therefore, the methane yield remained stable. After $NaHCO_3$ was added on day 37, the pH of the system was then normalized and balanced around 7.00, and the system stability could be restored.

5.3.4. Alkalinity

The alkalinity of the system reflects the amount of cations in the system and was expressed in milligram of $CaCO_3$ per liter of liquid. From the result of this study, the alkalinity at the beginning of the study was more than 5 g/L and thus was adequate to maintain stability from acidification. However, at the end of the study, the alkalinity was around 3 g/L which was lower than the required amount to maintain stability at around 4.2 g/L $CaCO_3$ (Neshat et al., 2017).

This phenomenon might be caused by the lack of cations such as K^+, Na^+, Mg^{2+}, and Ca^{2+} in the substrate which were needed for microbial growth (Chen et al., 2008), due to constant cations drain with the effluent every day. Moreover, the use of deionized water in this study to dilute the solid substrate might also be another cause. Deionized water did not have cations necessary to be used as a buffer. In this condition, the substrate must be added with minerals as buffers to maintain the alkalinity. This cations drain would not happen if tap water was used instead of deionized water. Tap water contains minerals which include cations that could serve as a buffer in the system.

5.3.5. The relationship between VFA, pH, and alkalinity

From the result of the study, the pH remained stable even when the alkalinity was reduced over time. The alkalinity helped to stabilize the pH by acting as a buffer and prevented pH drop due to acidification (Neshat et al., 2017). The accumulation of VFA may lead to acidification and thus, a pH drop. However, a system with high alkalinity may maintain a stable pH by counteracting the effects of acids, including VFAs (Neshat et al., 2017).

The stable pH in the reduced alkalinity condition in this study might indicate that the methanogenesis rate was in accordance with the acidogenesis rate. When methanogenesis is in the same rate as acidogenesis, there would be no VFA accumulation. Therefore, the system would not experience a pH drop and remains stable. This phenomenon was probably caused by the process limitation existed in the hydrolysis stage instead of the methanogenesis stage. It is widely known that the process limitation of degradation of lignocellulosic biomass is in the hydrolysis stages (Neshat et al., 2017).

CHAPTER 6

CONCLUSION AND SUGGESTION

6.1.Conclusion

In conclusion, methane production from anaerobic digestion of cattle and chicken manure was increased by co-digestion with rice straw and hornwort as well as oil addition. The highest methane yield from co-digestion treatments in BMP experiment was obtained from M:P:R:L 45:15:38:2 consisting 45% cattle manure, 15% chicken manure, 38% rice straw, and 2% oil on VS basis at 246.02±2.14 NmL CH_4/g VS, increasing 40.25% compared to that of cattle manure mono-digestion. Due to consideration towards sustainability, the M:P:R 45:15:40 mixture with 45% cattle manure, 15% chicken manure, and 40% rice straw which had the second highest methane yield, was considered as the most suitable for use in continuous digestion, and thus, was used in continuous production. It had methane yield of 135.30±21.41 N mL CH_4/g VS in the continuous digestion or 71.28% of its BMP value, had an increase of 32.97% compared to that of cattle manure mono-digestion, and had a stable process.

6.2.Suggestion

Further research on strategies to increase the energy production, environmental benefit, socio-economic measures, sustainability approach, and the scalability aspect of biogas production from co-digestion of livestock manure with lignocellulosic biomass in pilot / industrial scale is highly recommended and strongly suggested.

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