

ABSTRACT

DARWIN. Potential of Methane Production from Anaerobic Co-digestion of Swine Manure with Rice Straw and Cocoa Husk. (Under the direction of Dr. Jay J. Cheng.)

The production of methane through anaerobic digestion process highly depends on substrates loaded into the digester. This research aimed to evaluate the methane production potential of anaerobic co-digestion of swine manure with rice straw (RS) and cocoa husk (CH). To evaluate the stability of anaerobic co-digestion process of swine manure with rice straw and cocoa husk, batch reactors utilized were operated in triplicate. The process was carried out in 500 mL batch reactors under constant temperature and agitation. Temperature was set under mesophilic condition at 35°C and agitation speed was set at 270 rpm. Culture utilized as inoculums for the batch experiment was taken from a semi-continuous reactor operated in steady state conditions, with 25 days of hydraulic retention time under mesophilic conditions.

The results showed that in terms of methane production and biodegradation efficiency within each percentage of total solids concentration tested, RS had better performance compared with CH. RS had the highest methane production at 3% TS, where after 30 days of digestion, total methane production reached 1814 ± 47.43 mL. RS also obtained highest methane yield at 3% TS, which was 141.4 ± 3.70 mL CH₄/g VS added. However, RS had a slightly poor performance at 4% TS due to solid accumulation, where it only obtained methane yield of about 55.44 ± 2.69 mL CH₄/g VS added.

However, CH showed low biodegradation efficiency during anaerobic digestion that lead to lower methane production compared to RS. CH generated highest methane production at 4% TS, which was 661 ± 27.87 mL but it was still 45.64% lower compared with RS that generated 962.6 ± 46.70 mL CH₄ at 4% TS. Although CH produced less methane and showed poorer performance compared with RS, it still generated more methane production than control reactors. Thus, this still indicates that adding agricultural residues as co-substrates may potentially enhance methane production. Based on this research, it was also found that even though cocoa husk contains sufficient amount of nutrient, which is feasible for anaerobic digestion, it did not produce much methane due to high cell wall content. In addition, although adding rice straw into digester can increase methane production, adding more rice straw which is more than 3% TS is not recommended due to solid accumulation that leads to lower biodegradation efficiency.

Potential of Methane Production from Anaerobic Co-digestion of
Swine Manure with Rice Straw and Cocoa Husk.

by
Darwin

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DEDICATION

I want to dedicate this thesis to my beloved parents Margani and Pariem, and my supportive friends, they always have been encouraging me to achieve my goal.

BIOGRAPHY

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CHAPTER 1

INTRODUCTION

1.1 Background

Anaerobic digestion (AD) is a series of processes that convert waste materials to methane (Verma, 2002). Monnet (2003) mentioned that AD is a naturally occurring process of decomposition and decay. In the past, anaerobic digestion was related to the treatment process of animal manure and sewage sludge from wastewater treatment plant. Since there is an increasing awareness in terms of renewable energy and environmental concerns, the development of advanced technology related to anaerobic digestion is inevitable. Anaerobic digestion can help to cut the emission of greenhouse gases that may contribute to global warming. This process is widely used for domestic and industrial purposes to manage waste and to generate energy.

Steffen et al. (1998) revealed that AD also can produce biogas from industrial wastes, wastewater, as well as municipal bio-wastes. Furthermore, there is a research which revealed that the disposal of animal carcasses can be processed in anaerobic digestion; thus, it can deal with the problems of the environment such as minimizing odor derived from those carcasses, and reducing pollution of greenhouse gases emissions (Erickson et al., 2004). Since AD produces biogas that consists of methane, carbon dioxide and traces of other gas contaminants, this process is considered as a source of renewable energy.

Biogas produced by AD also can minimize the use of fossil fuels and reduce the emission of landfill gas to the atmosphere (Schievano et al., 2009). In AD, organic matter is broken down to simpler chemical components in the absence of oxygen and in the presence of anaerobic microorganisms (Verma, 2002). Moreover, raw biogas particularly consists of methane (60%), carbon dioxide (40%), hydrogen sulfide, and trace elements. About 90% of biodegradable organic compound of manure can be converted to methane gas through anaerobic digestion (Wilkie, 2005). The main advantages of anaerobic digestion for livestock farming include energy production, odor control, waste stabilization, pathogen reduction, nutrient conservation and mineralization, reduction of surface water and groundwater contamination potential, stable liquid fertilizer and high-quality solids for soil amendment (Ogejo et al., 2009; Wilkie, 2005).

In order to produce methane (CH_4) in a large proportion during anaerobic digestion, it is extremely important to know the type of feedstock or substrates added to the digester. Monnet (2003) mentioned that the production of biogas is not the same when different feedstock is added to the digester; composition of feedstock highly determines biogas production. Furthermore, feedstock added to the digester also takes an important role to support the activity of microorganisms in order to enhance the production of biogas and methane. This occurs since feedstock can range from readily biodegradable organic wastewater to complex high solid waste (Steffen et al., 1998). On the other hand, feedstock that is not typically water-soluble will degrade slowly (Burke, 2001).

Agricultural or botanical wastes are also suitable for use as feedstock for anaerobic digestion. Since these wastes contain a high component of woody lignin, these wastes require a kind of pretreatment (such as shredding) before adding these wastes to the digester. However, not all components derived from wastes can be converted to methane; there are several constituents that cannot be degraded during anaerobic digestion including lignin and some other hydrocarbons such as aromatic hydrocarbon like benzene (Steffen et al., 1998; Karthikeyan and Bhandari, 2001). Moreover, Burke (2001) revealed that toxic concentrations of hydrogen sulfide and ammonia can be generated in AD if substrates added to the digester contain high concentration of nitrogen and sulfur. Steffen et al. (1998) investigated feedstock for anaerobic digestion, and they revealed that one of the most important things for generating methane and CO₂ in anaerobic digestion is the feedstock must contain sufficiently essential amount of organic matter.

Wilkie (2005) revealed that the most substantial parameters for characterizing substrates for anaerobic digestion are total solids (TS) content and volatile solids content (VS). The author also mentioned that when establishing biogas production, the VS content of material is as vital as the TS content since it indicates the solid compound that may be converted into methane. In addition, Erickson et al. (2004) stated that the most prevalent method of determining organic matter is to utilize the VS parameter. The anaerobic digestion process can be performed in batch as well as continuous system. In a batch system, organic material is loaded in the reactor and digested for the period of retention time. Some factors such as volume and temperature may influence the retention time. Once the digestion is

completed the effluent will be removed and the operation may be restarted again (Erickson et al., 2004). The authors also revealed that a batch digester is cheaper, as well as, easier to design and set up compared with a continuous reactor.

Furthermore, a batch system is more potent against an inhibitor than a continuous system. However, it has lower loading rates that lead to generating less biogas. In terms of a continuous system, organic material should be regularly or constantly loaded into the reactor. The continuous system may be more suitable for large scale operation, and generate more biogas (Erickson et al., 2004). Even though anaerobic digestion is a natural biological process, controlling and managing the reactor in order to reach the appropriate degradation of organic matter and generate the maximum amount of biogas may be complicated. This is because the reactor needs thorough control to keep optimal pH, temperature, and feedstock composition.

Furthermore, it is extremely important to assess any potential toxic elements derived from specific feedstock, or which are generated during an anaerobic digestion process (Ogejo et al., 2009; Monnet, 2003). In addition, it is extremely important to provide an appropriate environment as well as food for microorganisms to develop and grow. Several factors that affect the quality as well as quantity of biogas production include temperature, retention time, loading, toxicity, manure quality, and composition (Erickson et al., 2004; Ogejo et al., 2009; Monnet, 2003; Verma, 2002). Cuetos et al. (2011) mentioned that anaerobic co-digestion is a process that combines different types of wastes with the purpose of enhancing biogas production and methane yield.

Recently, co-digestion of animal wastes with agricultural residues has attracted researchers in many parts of the world. Anaerobic co-digestion of bio-waste with agricultural residues will also generate some benefits to the stakeholders when they establish this technology. First, it will enhance the benefit for biogas/methane plants, and generate bio-fertilizers that can be used for farming. It also can be implemented as a waste treatment plant for the community. Furthermore, this technology can effectively minimize environmental issues since it will integrate management of some kinds of waste, such as manure and municipal solid waste into a single system plant (Bozano et al., 2012). Hills and Roberts (1981), and Hashimoto (1983) revealed that by co-digesting animal manure with agricultural wastes, it will generate synergetic effects that can stabilize the system, improve the carbon to nitrogen (C/N) ratio of the feedstock, and minimize the risk of ammonia inhibition to the digestion process.

1.2 Objectives

The aim of this research was to determine the methane potential of agricultural residues (rice straw and cocoa husk) under mesophilic conditions. Previous studies had revealed that adding agricultural residues to the digester can enhance methane production. This study also aimed to investigate whether cocoa husk, as lignocellulosic material, can produce a considerable amount of methane and perform properly like rice straw during an anaerobic digestion process within a different percentage of total solids concentration. Cumulative methane production over digestion time was examined, and the effects of an addition of agricultural residues on methane production were evaluated.

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CHAPTER 2

LITERATURE REVIEW

2.1 Feedstock

Feedstock is a comprehensive term that correlates with many aspects in anaerobic digestion, such as biogas production as well as end-product quality (Steffen et al., 1998). Feedstock added to the digester can be derived from grass, wood, crop residues, forest waste, animal wastes, industrial and municipal sources. Moreover, Johnson (2007) stated that in order to be considered as a feedstock for biofuel or bioenergy production, it has to be guaranteed that the raw materials will be abundantly and continuously available. Steffen et al. (1998) stated that the feedstock for anaerobic digestion process is very different in terms of biodegradability, homogeneity, fluid dynamics, qualitative and quantitative composition. The authors also mentioned that the degradation rates of waste organic matter can be varied depending upon the substrate composition, e.g. carbohydrate, protein, and fat content; and they revealed that proteins and carbohydrates show the fastest conversion rates.

Esposito et al. (2012) revealed that biomass containing carbohydrates, proteins, lipids, cellulose, and hemicelluloses was considered as the primary substances, which are feasible to be utilized as substrates for biogas production. Animal manure, sewage sludge from wastewater treatment, dairy and food waste, fish and meat industry wastes are the substrates typically utilized for feeding anaerobic digesters. Furthermore, the authors mentioned that lipids may generate the highest biogas yield; however, these require long retention time since

it has slow biodegradability. On the other hand, proteins and carbohydrates generate low biogas yield but these have faster conversion rates compared to lipids.

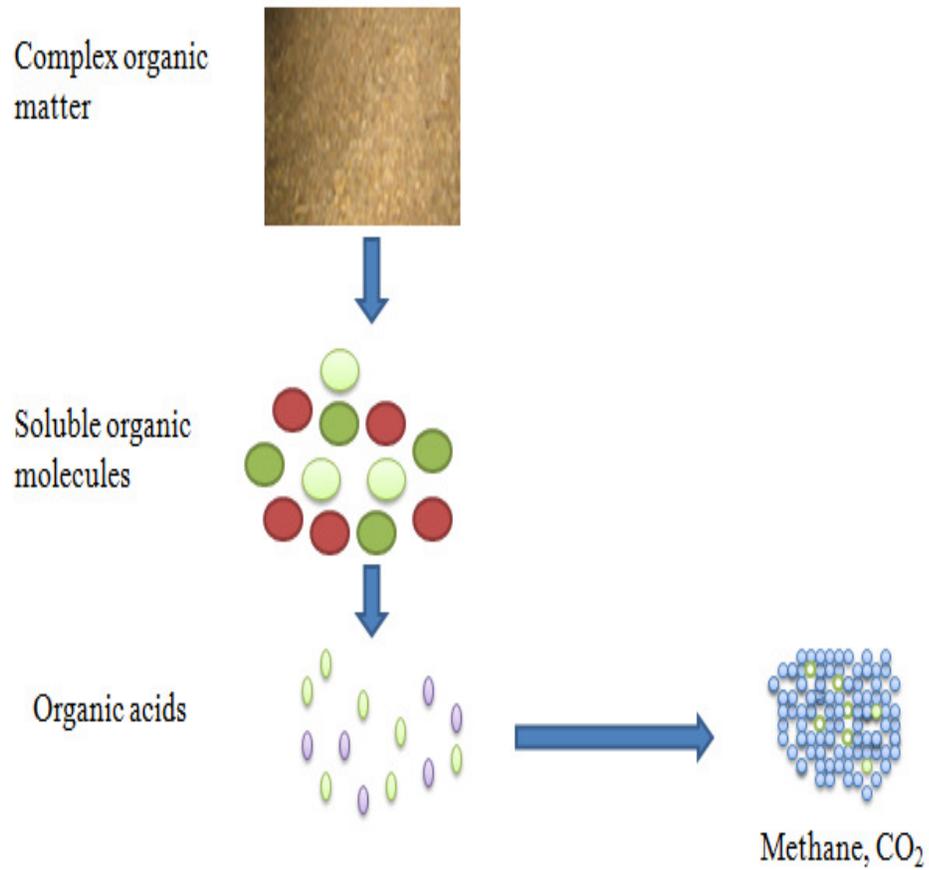


Figure 1. Conversion of biomass into methane gas

In addition, before choosing wastes for anaerobic digestion, it is crucial to consider the total solids content, the percentage of volatile solids, the carbon to nitrogen (C:N) ratio and the biodegradability. Schanbacher (2009) revealed that biogas production and its quality depends heavily on digestible nutrients in feedstock biomass. Furthermore, Zhang et al. (2006) mentioned that the biodegradability of a feedstock is determined by biogas, methane yield, and percentage of solids (total solids as well as volatile solids) that are degraded in anaerobic digestion. The authors stated that the biogas as well as methane yield is measured by the amount of biogas or methane that can be generated per unit of volatile solids contained in the feedstock after subjecting it to anaerobic digestion for a sufficient amount of time under a specific temperature.

Monnet (2003) mentioned that the production of biogas is not the same between one anaerobic digestion and other digestions. The condition highly depends on the kind of feedstock added into the digester. Furthermore, the author mentioned that animal waste in anaerobic digestion is an inhomogeneous material which contains total solid between 2 and 12 percent total solids, and it is also the predominant waste material in anaerobic digestion. Therefore, it will require a continually stirred tank reactor in order to mix the substrate in the digester thoroughly. There are several farm wastes which can be used as substrates in anaerobic digestion; e.g., manure from cattle, poultry, and pigs or swine. Other farm wastes are farmyard manure and harvest wastes. Furthermore, wood shavings and straw used as bedding are usually mixed with farmyard manure. In this case, straw can absorb the water in

the manure which generates dry matter content between 10 and 30% (Monnet, 2003; and Steffen et al., 1998).

Swine manure (SM) is an abundant source of organic matter that can be used as feedstock in anaerobic digestion. SM is also considered a good co-substrate due to its high nitrogen content, high buffering capacity and the wide range of nutrients needed by methanogenic bacteria (Chen et al., 2008; Cuetos et al., 2011; Moral et al., 2008; Weiland, 2000). Furthermore, Ogejo et al. (2009) stated that quality and characteristics of manure should be taken into consideration before running anaerobic digestion. They mentioned that anaerobic digestion is usable for manure that is collected in fresh condition, or typically less than seven days old. The old manure will not generate biogas as much as fresh manure. Manure should be free from contaminants including fibrous bedding material, sands, soil, and stones. The quality of manure highly depends on animal diet, storage method, and manure handling. Manure derived from animal fed with higher energy crops may generate more biogas compared with manure from animals on a roughage diet (Ogejo et al., 2009).

A literature review by Hansen et al. (1998) shows that running anaerobic digestion with pure manure resulted in a low methane production. This occurred since there was an accumulation of ammonia in the digester that led to an increasing level of volatile fatty acid (VFA) in the effluent. Research conducted by Gumisiriza et al. (2009) showed that animal manure as well as waste water containing ammonia and lipids may inhibit AD to produce biogas. Although there is some research that revealed that adding lipids to anaerobic digestion can increase methane production, it is still necessary to conduct further research

(Cirne, 2006; Gumisiriza et al., 2009). Moreover, Pereira et al. (2004) revealed that substrates containing high concentration of lipids may cause some problems in anaerobic digestion. This occurs since lipids may clog and interrupt the mass transfer during the process of anaerobic digestion.

However, compared to carbohydrate and protein, lipids are a good feedstock for running anaerobic digestion since they are able to generate higher methane yield. Therefore, substrates containing lipids should be considered for potential methane production as a renewable energy (Ahring et al., 1992; Hansen et al., 1999). Moreover, Ahring (2003) stated that a manure digester using substrates containing lipids will be able to increase the production of methane between 25 and 50 m³ biogas/ m³ cattle waste; thus, it is very beneficial to add substrates containing lipids since it can enhance methane production. Therefore, conducting further research in this area will be more attractive in order to find appropriate feedstock in the different kind of wastes that can increase methane production.

Agricultural residues such as wheat straws, rice straws, and corn stalks are produced annually in large quantities in both the US and other countries in the world. Since agricultural wastes are a plentiful source of organic matter, these can be used as a valuable alternative feedstock for biogas production (Li et al., 2009). Furthermore, these wastes also have considerable amount of carbon content that may be beneficial for anaerobic co-digestion with animal manure. According to Milbrandt (2005), agricultural residues include the crop residues and processing residues. A million tons of agricultural residues are generated every year. Because of their abundance characteristic, they have a great potential in many areas,

especially in anaerobic co-digestion process. Moreover, Wang and Schmidt (2010) stated that the structure of agricultural residues primarily consist of cellulose, hemicelluloses, and lignin (Figure 2). Corn stover, wheat straw, cocoa husk and rice straw are examples of crop residues.

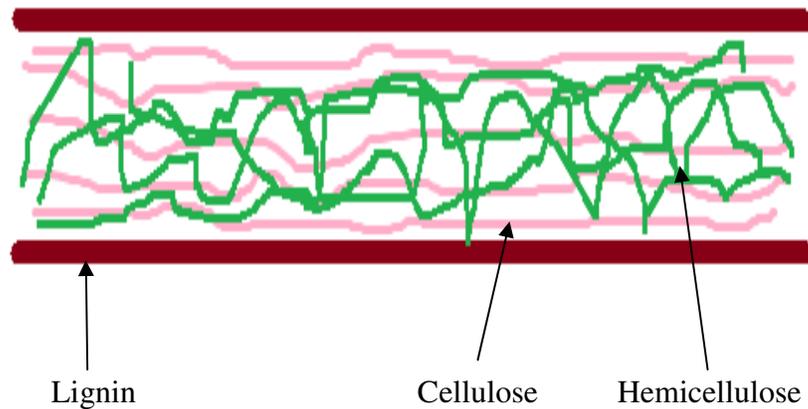


Figure 2. Lignocellulose agricultural residues structure

Cocoa husk is an agricultural by-product largely available in cacao plantations in large cacao producing countries such as Indonesia, Ghana, Nigeria, and Brazil. Cocoa husk generated in plantations is mostly discarded as waste (Falaye and Jauncey, 1999). Cocoa husk is also considered as a good source of dietary fiber (Bonvehi and Coll, 1999). In addition, cocoa husk is considered as a highly fibrous biomass containing substantial amount of cell wall components including cellulose (35%, w/w), hemicellulose (11%, w/w) and lignin (14%, w/w) (Alemawor et al., 2009).

In the developing world, there is a tendency that rice straw is either disposed or utilized to provide energy for household cooking and heating. Usually it is burned in the field after harvesting, and this will result in severe environmental pollution such as greenhouse gases and nitrogen oxide (Cao et al., 2008; and Yang et al., 2008). Moreover, in rice-producing countries, an important source of agricultural waste is derived from rice crop residues. Contreras et al. (2012) reported that although there are methods available to utilize rice straw (animal feedstock, fuel etc.), significant amounts still remain unused and some of them are burned in the open field. This practice may lead to serious environmental damage due to the air pollution.

Therefore, advanced anaerobic digestion may be a promising alternative approach to deal with imminent rice straw disposal problems in concentrated rice production regions (Zhang, 1999). Rice straw is also considered as one of the most abundant lignocellulosic waste materials, and it has great potential in terms of biofuel production. Culms, leaf sheaths, panicle remains are the main parts of rice straw (Juliano, 1985). Like corn stover and wheat straw, rice straw also contains three components: cellulose, hemicelluloses, and lignin (Nigam et al., 2009). Mussatto and Teixeira (2010) mentioned that rice straw is lignocellulosic biomass containing cellulose (36.2% w/w), hemi-cellulose (19% w/w), and lignin (9.9% w/w). Furthermore, Hills and Roberts (1981) reported that rice straw has C/N ratio at around 75, and contains about 0.4% nitrogen. The authors mentioned that in order to enhance the anaerobic digestion of rice straw, it is necessary to add animal manure which

contains a high amount of nitrogen; thus, it can meet an optimum C/N ratio between 25 and 35.

However, the main issues when co-digesting agricultural wastes with animal manure is the lignin content that cannot be degraded during anaerobic digestion process (Hendriks and Zeeman, 2009; Wang and Schmidt, 2010). This occurs during hydrolysis process, penetration by microorganisms, or extracellular enzymes will be inhibited by crystalline structure of cellulose. Furthermore, Rubia et al. (2011) revealed that because of the chemical and physical construction of lignocellulosic materials, bacterial hydrolysis degrades the substrate slowly during digestion. They mentioned that in order to avoid clogging of the digester and because of the difficulty for microorganisms to break down the substrate, it is necessary to reduce the size of the feedstock.

Yadvika et al. (2004) revealed that a size reduction of the particles can enlarge specific surface area that can support the biological activity in the digester to improve biogas production. Therefore, it will lead to a decreased amount of agricultural residues to be disposed of and to an increased quantity of biogas (Palmowski and Müller, 2000). According to Contreras et al. (2012), there are two main factors that need to be considered for applying methane fermentation to lignocellulosic biomass; they are the rate and extent biodegradability of degradation. They mentioned that high biodegradability not only means that there is more methane and biogas production per unit feed mass but also less residues resulted for the subsequent disposal. The authors also stated that fast biodegradation rates

may reduce the required size for a reactor; therefore, it can make the process more attractive economically.

Moreover, Tong et al. (1990) had revealed that the main factors related to methane production from lignocellulosic biomass are functions of the intrinsic properties of the lignocellulosic material itself and microorganism activities. It is necessary to do pretreatment (Figure 3) before adding substrate to the digester in order to degrade the polymer chains to a smaller molecule or a more easily accessible soluble compound (Wang and Schmidt, 2010).

Furthermore, Hendriks and Zeeman (2009) revealed that several pre-treatments that can be done include mechanical pre-treatment, chemical pre-treatment, and biological pre-treatment. The authors stated that various pre-treatments that have been applied are to improve the biodegradability of agricultural wastes. Moreover, research conducted by Wang et al. (2009) showed that the low biodegradability of the lignocellulosic biomass will limit the efficiency of methane production during anaerobic digestion.

Therefore, by pre-treating lignocellulosic biomass before loading it into the digester, it will enhance methane yield. This happens since during pretreatment, the structure of lignocelluloses will be destructed and the sugar content will be released (Wang et al., 2009). Zhang (1999) mentioned that mechanical size reduction may help biodegradation by breaking cell walls and causing the biodegradable components to be more accessible to bacteria.

Moreover, Palmowski and Müller's studies (2000) regarding the influence of the size reduction of organic wastes on their anaerobic digestion, they concluded that a reduction in particle size by conducting mechanical pre-treatment leads to an increase in the specific surface available and the release of intra cellular components. Therefore, this condition will improve biogas production in the digester and reduce the technical digestion time.

In addition, Mata-Alvarez et al. (2000) revealed that size reduction of particles may also help enhance biological processes in the digester since it is known that the smaller the fibers, the higher the biogas production. The authors also mentioned that research of biodegradation of soluble carbohydrates and proteins had revealed that a slowly degradable fraction of carbohydrates were removed through disintegration.

Research conducted by Rubia et al. (2011) regarding the influence of particle size and chemical composition on the performance and kinetics of anaerobic digestion process of sunflower oil cake in batch mode revealed that by optimizing the size reduction of sunflower oil cake, it could potentially improve the methane yield of anaerobic digestion process of this substrate. In addition, Sharma et al. (1988) investigated the effect of particle size on biogas generation from biomass residues under mesophilic condition (37°C). The authors concluded that biogas production increased when particle size was decreased. However, they found that there was no significant difference in terms of biogas yield between the smallest particle sizes tested (0.088-0.4 mm); thus, they stated that grinding below 0.4 mm would seem to be uneconomical.

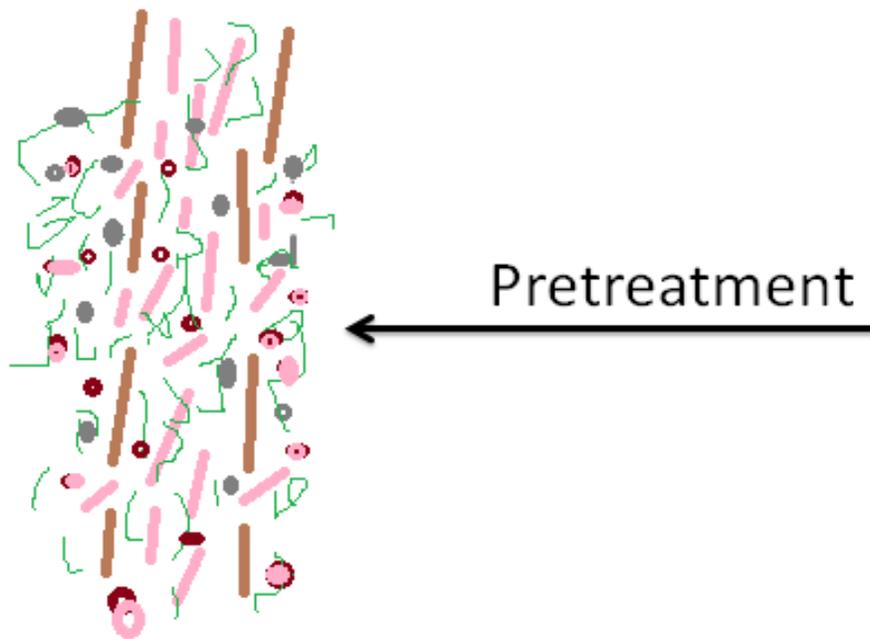


Figure 3. Pretreated lignocellulosic biomass

2.2 Inoculums

Before running anaerobic digestion process, it is extremely important to consider the use of inoculums since it can speed up the fermentative process in anaerobic digestion. Using inoculums is not only important for starting up anaerobic digestion but it is also crucial for running long biochemical processes as it has been known that the production of biogas will be increased if there is an addition of significant amount of inoculums (Mateescu and Constantinescu, 2011; Lopes et al., 2004). Lopes et al. (2004) revealed that the amount of inoculums used could substantially enhance the performance of anaerobic digestion.

Moreover, in dry-thermophilic digestion, the inoculum source and the total solid percentage chosen are responsible to achieve a rapid beginning of a balanced bacterial population (Carneiro et al., 2007). Moreover, research conducted by Carneiro et al. (2008) regarding the influence of total solid and inoculum contents on the performance of anaerobic digestion of food waste had revealed that the best performance for food waste biodegradation and methane production was accomplished with 20% TS and 30% of inoculums source derived from mesophilic digested sludge from a wastewater treatment plant.

The research also revealed that the food waste digestion showed the typical waste degradation pattern with a fast start up phase beginning at 0-5 days, an acclimation phase (acidogenesis/acetogenesis steps) between days 5 and 20-30, and a subsequent stabilization stage (Carneiro et al., 2008). Therefore, it is also crucial for selecting inocula before deciding to start up anaerobic digestion process. Usually it may be taken from the wastewater treatment. Furthermore, anaerobic sludge and digested sewage sludge also can be used as potential inoculums (Muxi et al., 1992). For the present study, culture used as an inoculum for the batch experiment was taken from an effluent of anaerobic digestion of semi-continuous reactor.

2.3 Process of anaerobic digestion for biogas and methane production

Biogas is the product derived from the degradation process of microbiology (Waish et al., 1988). Osho (2010) mentioned that the fermentation of biological wastes and livestock manure without air in closed containers that produce gas as the result of microorganism

activity is called biogas. This means that the biogas is the end product of the microbiological fermentation or metabolic product of the methanogenic bacteria (Nagy and Szabó, 2011).

Biogas is also the fuel that is very combustible, it is composed of several different compounds which are different. Moreover, the type of substrates or feedstock will significantly determine the percentage of compound in the biogas. Waish et al. (1988) stated that biogas that is produced from digesters is higher in methane content than biogas generated from landfill.

During anaerobic digestion, there are several kinds of gases produced. About 60 to 70% of the gas contained in biogas is methane, and there is about 30 to 40 percent of carbon dioxide produced during anaerobic digestion; other gases are ammonia and hydrogen sulfide (Hansen, 2003). The volatile solids (VS) loading of the digester and the percentage of VS reduction usually can be utilized for estimating potential biogas production. In addition, the level of biological activity in the digester and the volatile solid content of the substrates will determine the rates of gas production (Waish et al., 1988). In order to maximize the methane production, it is extremely important to maintain constant pH, temperature, and fresh organic matter added into anaerobic digester. Hansen (2003) revealed that if there is a decrease in temperature of -6.7°C (20°F) during anaerobic digestion, it will reduce almost half of the biogas production. Moreover, maintaining constant temperature is very crucial during anaerobic digestion, if there is a fluctuation as little as -15°C (5°F), it will inhibit the methane production. If this happens, it may cause the accumulation of acid in the digester as well as failure of the digestion process. The production of biogas also depends on the environmental

condition in the digester, such as organic matters digested and the loading rate of the digester. Therefore, it is suggested to maintain temperature at 35°C for mesophilic condition (Hansen, 2003).

During anaerobic digestion, it is extremely important to take appropriate safety precautions since methane is classified as an explosive gas. Nagy and Szabó (2011) mentioned that the biogas is a gaseous matter that is similar to natural gas, and it can be utilized on many applications. Under normal temperature, biogas or methane cannot be converted to liquid. Liquefaction of methane is not practical since it requires pressures of about 34.47×10^6 Pascal. Since this gas cannot be converted easily to liquid, this gas will only be beneficial for fueling stationary use, such as heating water and building, drying grain, cooking, and air conditioning (Hansen, 2003).

Crolla et al. (2011) mentioned that anaerobic digestion is an effective process to reduce pathogen. The authors revealed that pathogens can be inactivated during exposure to heat and competition with other microorganisms within the reactor. Temperature and the length of exposure significantly affect pathogens destruction.

Furthermore, the authors also stated that anaerobic digestion can effectively minimize odors. This occurs since some parameters such as ammonia, volatile fatty acids (VFAs) and phenolic compounds are responsible for releasing odors. Thus, by running proper anaerobic digestion, it can reduce almost 96% of the VFAs in the digestate and eventually cutting the main odor related to manure and other wastes (Crolla et al., 2011).

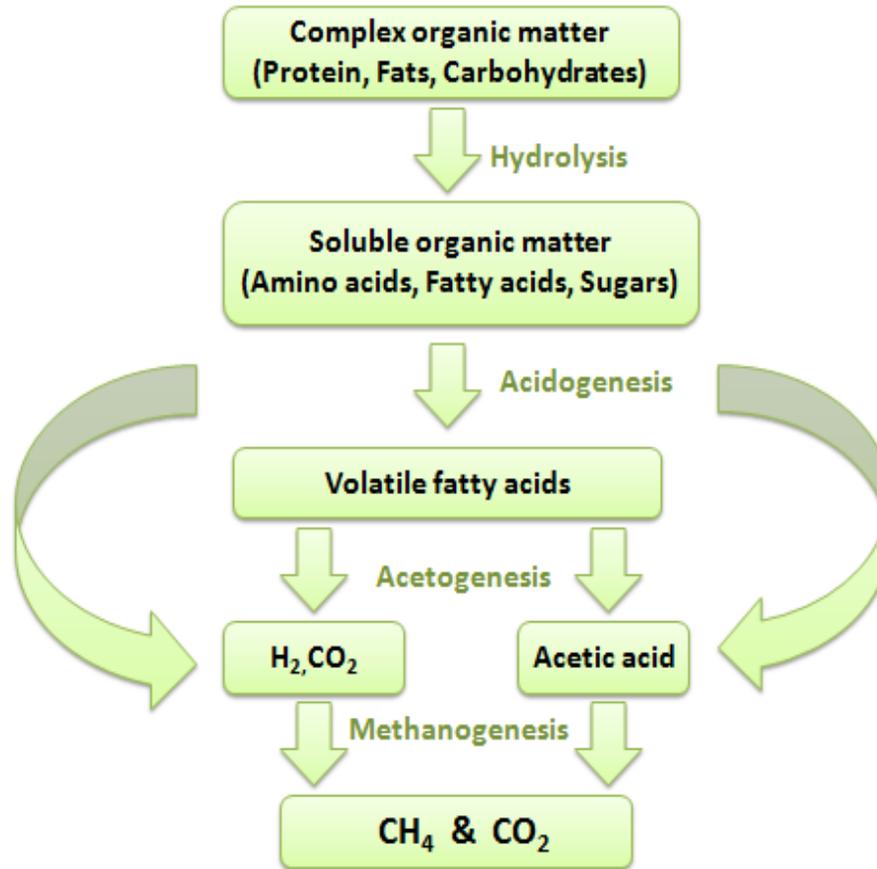


Figure 4. Schematic diagram of anaerobic digestion process steps

There are four main stages in anaerobic digestion process. They are hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Figure 4). Biochemical reactions with different microorganisms and substrate are involved in each of the steps (Cheng, 2010). *Hydrolysis* is the first step in anaerobic digestion. This process converts plant and animal matters containing biopolymers and organic matter into usable sized molecules. For

instances, the conversion of polysaccharides into monosaccharide, protein into amino acid, nucleic acid to amino acids, and fatty acid derived from lipids (Erickson et al., 2004). In this stage, anaerobic bacteria change complex organic matter which is insoluble into soluble molecules. For example, cellulose is converted into soluble compounds such as sugar. Microbes release hydrolytic enzymes such as amylases, proteases, lipases, and cellulases in order to hydrolyze the complex polymeric matter into monomer such as protein to peptides or amino acids and cellulose to sugars or alcohols (Cheng, 2010; Verma, 2002). Vindis et al. (2009) stated that a slight increase of hydrolysis and fermentation rate does not cause an increasing in methane production during the AD process.

Acidogenesis is the process where the anaerobic bacteria ferment the sugar, amino acids, peptides, and fatty acids into volatile fatty acids (VFAs) such as butyric and propionic acids. During this process, hydrogen is produced within 10 percent of energy released, acetic acid is 35 percent and the rest is reserved in the form of the VFAs (Cheng, 2010). *Acetogenesis* is the process where anaerobic bacteria convert VFAs into acetic acids (CH_3COOH), hydrogen and carbon dioxide. In this stage, ethanol ($\text{C}_2\text{H}_5\text{OH}$), propionic acid ($\text{CH}_3\text{CH}_2\text{COOH}$) and butyric acid ($\text{CH}_3\text{CH}_2\text{CH}_2\text{COOH}$) and other products are generated as well (Cheng, 2010; Erickson et al, 2004; Verma, 2002).

In addition, microorganisms involved in the hydrolysis, acidogenesis, and acetogenesis are facultative as well as obligate anaerobic bacteria such as *Clostridium spp.*, *Antinomyces*, *Peptococcus anaerobes*, *Escherichia coli*, and *Lactobacillus*. These bacteria are typically fast growing and more flexible to temperature, pH, and inhibitory chemicals,

compared with methanogenic bacteria or methanogens where they can grow and develop in pH between 4.5 and 8.0 (Cheng, 2010).

Methanogenesis is the last process during anaerobic digestion where there is the formation of methane in large proportion. Hydrogen and acetic acid produced in the previous steps are completely converted to form methane and carbon dioxide (Cheng, 2010). This stage in the anaerobic digestion process is extremely fragile because microorganisms involved are obligate anaerobes that are very slow growing as well as sensitive to inhibition.

Furthermore, they develop and grow in pH between 6.5 and 8.0. Typical methanogens involved in this phase are *Methanosarcina* spp (sphere) and *Methanothrix* spp (filament). These bacteria have a significant role in completing the anaerobic digestion process and accumulating hydrogen that may inhibit acetogens. Hydrogen-using methanogens are also very crucial to maintain low levels of atmospheric hydrogen (Cheng, 2010; Thauer et al., 1977).

2.4 Process parameters

2.4.1 Temperature

Anaerobic digestion can be run at three ranges of temperature. There are psychrophilic (10-20 °C), mesophilic (30-40 °C), and thermophilic (50-60 °C). Erickson et al (2004) stated that a selecting temperature for running anaerobic digestion is extremely crucial, since the effectiveness of the process and pathogens eradicated will be varied based on the temperature selected; at higher temperature, destruction of pathogens is more efficient

(Varel et al., 1980). Furthermore, Ogejo et al. (2009) mentioned that uniform temperature is extremely important throughout the reactor and should be constantly kept to avoid localized zones of depressed temperature and undesired microbial activity. The methane-forming bacteria may be significantly affected by temperature fluctuations in the reactor.

Even though thermophilic conditions may create many benefits for running anaerobic process, mesophilic condition are preferred due to being tolerant to the environmental parameters compared to thermophilic process. Furthermore, setting up thermophilic condition will require much more heat energy. However, an anaerobic digester conducted using thermophilic condition will be able to increase biogas production, and this condition is also more effective in the terms of loading rate as well as retention time, minimizing the risk of pathogen (Monnet, 2003; Verma, 2002; Cheng, 2010). Hashimoto (1982) reported that the temperature between 20 and 60°C may increase the maximum growth rate in anaerobic digestion with manure, and there is a significant decrease if the temperature is above 60°C.

Furthermore, Angelidaki and Ahring (1994) found that there is a relationship between temperature increased and ammonia concentration in digester. It has been revealed that once temperature and pH go up, it will increase the concentration of free ammonia in the digester. Therefore, even though an increasing of the temperature during AD will cause the bacteria to speed up their metabolism, it also will lead to the free ammonia concentration to increase. This phenomenon may cause a negative effect in anaerobic digestion since high concentration of ammonia in AD will inhibit the AD process and biogas production.

Angelidaki and Ahring (1994) mentioned that, when the ammonia load is very high, reduction of the temperature will have a positive net result. They also found that once temperature was decreased below 55°C, it increased biogas and methane yield and generated better process stability; it was shown by a lowering of the concentration of volatile fatty acids in the effluent. Hashimoto et al. (1981) reported that the fermentation temperatures ranging between 30 and 60°C do not show a significant change in terms of the total methane yield.

Furthermore, most of the biogas will be produced in the first ten days if it is run under the mesophilic temperature range, and the anaerobic digestion usually will be finished after two weeks. The amount of biogas produced in anaerobic digestion will decline after 35 days. However, under thermophilic conditions, biogas production will be produced in the first week of experiment, and after 20 days, it is completed. The amount of biogas in the anaerobic digester will be low after 35 days.

Research conducted by Usman et al. (2012) regarding temperature effect on biogas generation from lignocellulosic substrate revealed that digestion rate was pretty fast when anaerobic digestion process was run at 60°C; however, slower digestion rate occurred when anaerobic digestion was operated in 30°C. Better performance was accomplished when anaerobic digestion was performed under 45°C. The authors mentioned that the range of temperature between mesophilic and thermophilic (45°C) was preferable to produce more biogas compared with 30°C and 60°C operations. Furthermore, the authors also found that the percentage reduction of volatile solids derived from anaerobic co-digestion of maize

chaff blended with poultry droppings were 63.64%, 69.09%, and 36.40% at 30, 45 and 60°C, respectively.

In addition, some research conducted by Ahn and Foster (2002), and Kim et al. (2002) revealed that anaerobic digestion operated under thermophilic condition will generate higher biogas production, faster reaction rates, and lead to higher rates of destroying pathogens than mesophilic condition. However, the thermophilic process tends to be more sensitive to environmental changes than the mesophilic process. Bouallagui et al. (2009) investigated thermophilic anaerobic co-digestion of abattoir wastewater and fruit and vegetable waste in anaerobic sequencing batch reactors. Based on the research, the authors stated that anaerobic co-digestion performed under thermophilic condition (55 °C) with the short hydraulic retention time (10 days) resulted in overloading and generated low biogas production.

2.4.2 pH

The pH parameter is a vital factor that has to be considered carefully during running anaerobic digestion. It is one of several parameters in anaerobic digestion that can determine the biogas and methane production since it is related to the activity of microorganisms in the digester. Monnet (2003) revealed that there are some differences between the optimum pH in the acidogenesis and methanogenesis phases. During the acidogenesis phase, pH tends to decline since in this phase, there is the formation of several acids including acetic, lactic, and propionic. Moreover, acidogenesis stage can be inhibited if pH is low, and it also may harm the life of the bacteria that is responsible for forming methane.

A pH between 6.6 and 7 is the optimum range for the methanogenesis phase (Monnet, 2003). Dinamarca et al. (2003) studied anaerobic digestion at a pH level between 7 and 8, and they found it will be able to degrade total suspended solid (TSS) and volatile suspended solid (VSS) better than other pH values at around 75 % degradation of TSS and 85 % degradation of VSS. A pH value in the range of 6.4 to 7.2 is considered as the optimal pH for all stage in anaerobic digestion. Research conducted by Lay et al. (1997) regarding the influence of pH and moisture content on the methane production in high-solids sludge digestion, revealed that the rate of methane production can be maximized with a moisture content of 90 to 96% along with pH of 6.6 to 7.8, and an optimum pH was reached at 6.8. Furthermore, the authors also found that a minimum lag-phase time for methane production can be reached within pH 6.8. They also revealed that methane production may be failed when pH less than 6.1 or more than 8.3.

2.4.3 Hydraulic Retention Time

In anaerobic digestion, the time required for degrading organic materials completely is known as the retention time. Burke (2001) stated that the time when materials remain for a fixed number of days in the digester is called hydraulic retention time. Process parameters such as waste composition and temperature affect the hydraulic retention time of anaerobic digestion (Monnet, 2003). Hydraulic retention time (HRT) can be measured by dividing the volume of the reactor with the rate of daily flow. In terms of gas production, there is a relationship between the hydraulic retention time and volatile solids. Therefore, HRT is an

important parameter used to determine the time required for microbes to grow and the time required for converting organic material into biogas (Burke, 2001).

Moreover, a study conducted by Kaosol and Sohgrathok (2012) regarding the influence of biogas production on anaerobic co-digestion of frozen seafood wastewater with decanter cake from palm oil mill industry, which was operated in continuously stirred tank reactor (CSTR), had revealed that HRT of 20 days was very feasible for this anaerobic co-digestion with the maximum methane production rate at 1.86 L/d and the average maximum methane production at 64.6%. In addition, research conducted by Rani et al. (2003) regarding methane generation from corncobs treated with xylanotic consortia, found that HRT of 20 days with 6% loading rate also generated the maximum biogas and methane production where the maximum biogas yield obtained was 0.59 m³/kg volatile solids with the methane content of 62%.

2.4.4. Ratio of Carbon to Nitrogen (C:N)

The ratio of carbon and nitrogen in anaerobic digestion is a parameter that needs to be considered in order to encourage the production of methane. This ratio should be maintained at an optimum value. The optimum carbon to nitrogen ratio may generate a synergistic effect when different substrates are co-digested. This phenomenon enables some researchers to conduct further study related to C:N ratio in anaerobic digestion process that may enhance methane production (Esposito, 2009).

Hills and Roberts (1981) mentioned that the optimum C:N ratio in anaerobic digester is between 25 and 30. It should be noted that a lower C:N ratio will cause an increasing of pH and accumulation of ammonia in the digester. As a result, it will interrupt the activity of the microbes since this condition can be toxic for microorganisms. On the other hand, a high carbon and nitrogen ratio in the digester will lower the biogas and methane production since the methanogenic bacteria consume a lot of nitrogen in anaerobic digester.

Therefore, anaerobic co-digestion should be considered in order to reach and maintain the optimum C:N ratio in the digester by mixing animal manure with agricultural residues (Monnet, 2003; Verma, 2002). Moreover, Straka et al. (2007) mentioned that the cheapest and most preferable method for biomethanation of substrates containing a lot of nitrogen is co-fermentation with high-carbon substrates or low-nitrogen substrates in order to reach an optimum C:N ratio.

In addition, Yen and Brune (2007) investigated anaerobic co-digestion of algal sludge and waste paper to produce methane; and the authors revealed that the low C:N ratio of algal sludge as a result of its unbalanced nutrients was considered as the major limitation factor to anaerobic digestion. The authors also found that an optimum C/N ratio for co-digestion of algal sludge and waste paper was about 20 to 25:1. Furthermore, Yen and Brune (2007) also revealed that by adding 50% of waste paper (based on volatile solid) into algal sludge may enhance methane production rate to 1170 ± 75 ml/l day, which was higher compared with algal sludge digestion alone (573 ± 28 ml/l day). They also concluded that algal sludge co-digested with waste paper may be beneficial to reach a balance of C:N ratio in the digester.

2.5 Inhibitory factors

Before adding feedstock to the digester, it should be known that there are some unwanted materials and inhibiting substances that may prevent anaerobic digestion. Burke (2001) revealed that toxic materials such as fungicides and antibacterial agents may inhibit anaerobic digestion process. Furthermore, Steffen et al. (1998) reported that pig slurry contains some unwanted materials such as straw, sand, bristles, and wood shavings. This slurry also has inhibiting substances such as antibiotics and disinfectants. There are also some problems derived from using this feedstock in anaerobic digestion; during the operation, sediments and scum layers will usually be found in the digester.

Hernandez and Edyvean (2008) researched on inhibition of biogas production and biodegradability by substituting phenolic compounds in anaerobic sludge, revealed that phenolics may inhibit the degradation of readily biodegradable organic fractions as well as their own biodegradation. The authors found that around fifty percent inhibition was in the range between 120 and 594 mg of compound/g VSS. An initial increase followed by an inhibition of biogas formation was also discovered. They also mentioned that biogas production was affected by concentration rather than any pH change. Velsen (1979) and Farina et al. (1988) mentioned that running anaerobic digestion of swine manure alone as a substrate has shown to be unsuccessful, mainly due to the high content of ammonia in this waste. Research conducted by Angelidaki and Ahring (1993) revealed that ammonia inhibition is especially distinct when digesting poultry or swine manure, which often have total ammonia concentrations higher than 4 g-Nitrogen/liter. However, it is known that an

ammonia concentration of 4 g-Nitrogen/Liter was shown to be inhibitory during digestion of cattle manure.

Hansen et al. (1998) stated that the free ammonia concentration in the digester depends heavily on three factors including the total ammonia concentration, temperature and pH. This means that the free ammonia concentration may increase with increasing temperature. Moreover, several authors have revealed that methane fermentation of waste which contains high ammonia concentration tends to be more easily inhibited at thermophilic condition than at mesophilic condition (Angelidaki and Ahring, 1994; Braun et al., 1981; Parkin and Miller, 1983; Velsen, 1979).

Koster (1986) has found that anaerobic digestion process tends to be more sensitive towards ammonia when the pH value increases; thus it leads to an increasing of the free ammonia concentration. Schnürer and Jarvis (2010) mentioned that sometimes microorganisms in anaerobic digestion may recover from disruption; however sometimes the inhibition is unalterable. This means that the bacteria may not recover from inhibition even though the inhibitory components disappear. Thus, the operation should be restarted again, or fresh microorganisms should be added. The authors also revealed that sometimes a lag phase period also occurred when inhibitory effects appeared in the digester. In order to prevent a complete failure of the process during the lag phase, it is highly crucial to increase the retention time as well as decrease the load in the continuous operation; otherwise there is obvious risk that microorganisms will be washed out. In addition, the level of inhibitory components also can be minimized by changing the composition of the substrate; thus, it may

contain lower inhibitory components that remove inhibitory substances during the decomposition process (Schnürer and Jarvis, 2010).

2.6 Anaerobic co-digestion

An anaerobic digestion process using only one substrate will generate some issues in terms of biogas and methane production. This occurs since there are no sufficient nutrients available in the digester. Therefore, by adding other substrates, it will enable microbes to grow faster to produce biogas and methane. Cuetos et al. (2011) stated that the stability of the process and biogas productivity in anaerobic digestion system can be enhanced by treating anaerobic digestion with different feedstocks.

Conducting anaerobic co-digestion will also be more effective in terms of cost spent and equipment use (Banks and Humphreys, 1998). Mondragón et al. (2006) stated that running co-digestion also can benefit the anaerobic process since it can improve the digestion process of some wastes containing protein and fat that may not be degraded easily. Research conducted by Crolla et al. (2011) regarding anaerobic digestion of dairy manure with various co-substrates (corn silage, whey, switchgrass, and waste grease), revealed that the co-digestion had successfully enhanced biogas and methane production. They mentioned that the addition of 30% co-substrates may increase methane yield by around 1.2 to 1.6 times compared with the anaerobic digestion of dairy manure alone. Based on the research, they found that corn silage and waste grease generate higher methane yield compared with other co-substrates (switchgrass and whey).

Several studies have been focused on the co-digestion of animal manure with straw. Somayaji et al. (1994) focused their study on the biomethanation of wheat straw with rice. They found that with the addition of rice and wheat straw into the cattle manure, the biogas production increased, and higher organic biodegradability was achieved. Moreover, Tosun et al. (1989) investigated co-digestion of cow manure with wheat straw which was pretreated with an alkali treatment, and the authors concluded that the methane yield increased significantly once the pretreated wheat straw was added into cow manure. It happened since the results of the digestion of alkali treated slurry could decrease the association of lignin with carbohydrate portion; thus, it increased the anaerobic biodegradability.

Li et al. (2009) studied the co-digestion process of cattle manure with sodium hydroxide pretreated corn stover. Four different cattle manures to corn stover (CM/CS) ratios-1:1, 1:2, 1:3, 1:4 and three feeding concentrations (FC) 50, 65, 80g L⁻¹ were used. The result showed that when the FC was 65 g L⁻¹ and CM/CS ratio is 1:3, highest biogas production is obtained. In addition, the authors found that due to the synergistic effect of co-digestion, 4.9-7.4% more biogas were produced.

In addition, a research study in co-digestion of pig slurry and organic wastes from food industry under mesophilic and thermophilic conditions was conducted by Campos et al. (1999), where the swine slurry used is derived from a fattening pig farm, and the oil bleaching earth (OBE) waste added came from an olive oil bleaching and filtering factory. Based on their study, it has been revealed that methane production was 2.4 times greater than using only slurry as a substrate. Furthermore, Campos et al. (1999) stated that the anaerobic

co-digestion run in mesophilic temperature performed pretty well for producing biogas compared to anaerobic co-digestion operated in a thermophilic condition that did not perform well due to the inhibition of free ammonia.

Banks and Humphreys' studies (1998) regarding the anaerobic treatment of a lignocellulosic substrate offering little natural pH buffering capacity indicated that during anaerobic digestion, there can be some problems that can interrupt the production of biogas such as unbalanced nutrients and high concentration of toxic compounds. They mentioned that another issue which comes up during anaerobic digestion is an accumulation of volatile fatty acids, and maintaining pH in an optimum range (6.4 to 7.2), which is related to its poor buffering capacity.

In addition, Banks and Humphreys (1998) concluded that by combining several kinds of wastes that contain a high buffer capacity, it will overcome negative effects appeared in anaerobic digestion process. Callaghan et al. (2002) stated that compared to digesting manure only, co-digesting animal manure that has low C/N ratio along with feedstock containing low levels of nitrogen (high C/N ratio) can generate operational performance which is more stable and it also can generate higher methane production. Anaerobic co-digestion also was studied by Ferreire (2008) when swine manure was co-digested with fruit wastes; it is revealed that the biogas production rate was increased when swine manure in the digester was co-digested with fruit wastes. Moreover, co-digestion also enables stabilization of the system during the anaerobic process.

Saev et al. (2009) studied on anaerobic co-digestion of wasted tomatoes and cattle dung for biogas production, and they found that volatile fatty acids (VFAs) in the digestion was low; this means that the co-digestion method is effective to generate the stability of anaerobic process. In addition, it is extremely risky if solely manure is utilized in anaerobic digestion as it contains a lot of nitrogen. Thus, it will inhibit the growth of bacteria during anaerobic digestion that lead to the reduction of methane production.

Therefore, it will be better if manure is co-digested with any other agricultural residue (Chen et al., 2008; Hansen., 1998). In addition, Xiao et al. (2010) studied on co-digestion of swine manure with several crop residues as an external carbon source; they reported that adding crop residues (corn stalks, wheat straw and oat straw) to the co-digestion process with pig manure significantly increased the volumetric biogas production.

Even though there are many studies showing that co-digestion of some substrates may increase methane production, there are also some cases of co-digestion that do not improve production. Therefore, before co-digesting manure with other substrates, it is extremely important to consider substances contained in the feedstock or substrates that will be added to the digester since some substances also can lower the production of biogas. Those substances include nitrogen, lignin, cellulose, and hemicelluloses. It has been known that lignin cannot be degraded during anaerobic digestion process; thus, it may hinder the hydrolytic enzyme to access cellulose and hemicelluloses to convert those into sugar. Moreover, substrates containing a lot of nitrogen may potentially generate high ammonia in the digester; thus, it can inhibit anaerobic digestion process to generate methane production.

In addition, there is research conducted in anaerobic co-digestion where adding organic materials into digester containing manure does not increase the biogas and methane production.

Elijah et al. (2009) studied on cow dung as co-substrate with rice husk for biogas production. They reported that cow manure co-digested with rice husk at the temperature between 26 and 29°C did not improve the biogas production. This occurs since rice husk contains fairly high lignin at about 21.40 to 46.97 percent (Elijah et al., 2009; Pillaiyer, 1988). In addition, a study on co-digestion of organic wastes was conducted by Esposito et al. (2012). They revealed that anaerobic co-digestion may generate a significant increase in methane production when the substrates mixture is loaded with appropriate percentages of the different organic substrates.

The major advantage when co-digesting nitrogen rich substrates with carbon rich substrates is to optimize the nutrient balance in the digester. The authors also mentioned that several pre-treatments that may be applied to enhance methane production include solid liquid separation, bacterial hydrolysis, ensilage, alkaline pretreatment, thermal and ultrasonic pretreatments. However, there are some pretreatments that may lower methane production efficiency when they are applied; these include wet explosion and wet oxidation pretreatment.

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CHAPTER 3 MATERIALS AND METHODS

3.1 Introduction

According to a previous study Kalra and Panwar (1986) revealed that rice straw may provide an alternative feedstock, or a feasible component of a composite feedstock for biogas production. Studies are often primarily focused on the co-digestion process of manure with rice straw. Based on the research conducted by Contreras et al. (2012), they revealed that rice straw and rice residues from the drying process are a potential carbon source to generate biogas through anaerobic digestion. The authors mentioned that rice residues do not have an optimum carbon to nitrogen (C/N) ratio even when the anaerobic digestion runs properly. Based on the results, they reported that rice straw and rice residues from the drying process generated the highest values of biogas and methane yield. Thus, it is necessary to do co-digestion of rice straw with swine manure in order to stabilize the process during digestion, and to enhance methane production. Rice straw used for this research was derived from *Japanese short grain heirloom variety* called *Koshihikari*. The rice straw was taken from Chatham County, Moncure Pittsboro Rd which was harvested during 2012.

Research conducted by Bonvehi and Coll (1999) showed that cocoa husk contains all the essential amino acids, which represented 44.57% (W/W) of the total identified amino acids. Therefore, the total protein content and amino acid score obtained from their research may enable cocoa husk to be utilized as a source of feedstock for biogas production. Cocoa husk used for this research was derived from Kenya.

All agricultural residues (rice straw and cocoa husk) used for this experiment were milled by using a laboratory grinder to an average particle size between 1 and 1.5 mm. The start up phase is considered the most crucial step in the process of anaerobic digestion (Carneiro et al., 2008). In addition, Obaja et al. (2003) mentioned that the use of a highly active anaerobic inoculums or animal inoculum waste will considerably minimize the experimental time, and it also reduces the amount of inoculums required in full scale batch digesters. Culture utilized as inoculums for the batch experiment was taken from an effluent of the semi-continuous digester.

3.2 Reactors utilized

3.2.1 Semi Continuous Reactor

A semi-continuous reactor was operated in steady state condition where the temperature was maintained at 35⁰C by an electrical blanket controller, and the culture was continuously stirred with about 120 rpm. The working volume of the reactor was 14 liters, and the hydraulic retention time of the system was 25 days. Furthermore, wastewater as well as sludge was taken from the swine unit at the Lake Wheeler Road Field Laboratory (LWRFL) of North Carolina State University, this manure was stored in a refrigerator at 5⁰C. Corn stover was collected from a farm in Franklinton, North Carolina. The corn stover was air dried and milled by using a Thomas Willey laboratory mill fitted with a 2 mm screen. Before running the experiment, the reactor was purged with Nitrogen gas for about 10 minutes to get rid of the oxygen. To ensure the semi-continuous system run properly, pH of the culture was measured periodically during feeding period, and gas composition was

measured on a regular basis (once a week) by using a gas chromatographer Shimadzu GC 17-A. Biogas production was measured utilizing a gas meter based on water displacement. Influent as well as effluent samples were analyzed for TS, VS, COD and TKN based on the “Standard Method” (APHA, 1998).

3.2.2 Batch Reactors

Three experiments were carried out in this study. They all were run in bioreactors with the working volume of 500 mL. Each reactor containing 500 mL culture was set on thermostatic water bath computerized respirometer. For agitation purposes, a magnetic bar was put into each reactor. The temperature for this experiment was maintained under mesophilic condition at 35°C. The culture was continuously stirred at 270 rpm. Experiment 1 was operated under 2% total solids (TS) concentration. In experiment 2 and 3, anaerobic co-digestion processes were carried out with 3% TS and 4% TS concentration, respectively. Total solid of inoculums were measured in order to determine the proportion of biomass that should be added to each reactor. All experiments of each substrate were performed in triplicate; and 3 reactors without adding agricultural residues as controls (C) were performed in order to determine the background of biogas productivity of the inoculums.

The mixture of biomass and inoculums loaded into each reactor as an influent was prepared homogeneously. Thus, there were nine reactors utilized to operate the batch experiment. During measurement of the biomethane production test, first there was no addition of any other nutrient including chemicals as well as enzyme. Five hundred mL of

0.4 N NaOH solutions in filter flasks were connected from each reactor to each gas meter for entrapping CO₂ as well as H₂S.

Zhao et al. (2010) revealed that sodium hydroxide can be used to purify biogas generated by anaerobic digestion since it can react with both carbon dioxide and hydrogen sulfide; however, it does not react with methane. Sodium carbonate will be formed once the carbon dioxide reacts with the sodium hydroxide. Moreover, the authors also added that the sodium hydroxide system utilized in anaerobic digestion may enhance methane concentration from around 60% to 94%. Persson et al. (2006) revealed that hydrogen sulphide contained in biogas also can be removed by applying sodium hydroxide scrubbing method where a water solution of sodium hydroxide will react with hydrogen sulfide to form sodium sulfide or sodium hydrogen sulfide.

In addition to this experiment, before starting to run an anaerobic digestion process, each reactor was purged with nitrogen gas for about 5 minutes to get rid of oxygen traces to ensure anaerobic condition in the reactor. To avoid any gas loss due to high pressure in the digester and to ensure completely anaerobic condition, each reactor and filter flask were sealed properly using parafilm. The duration of the experiment was determined by the point at which biogas production stopped completely.

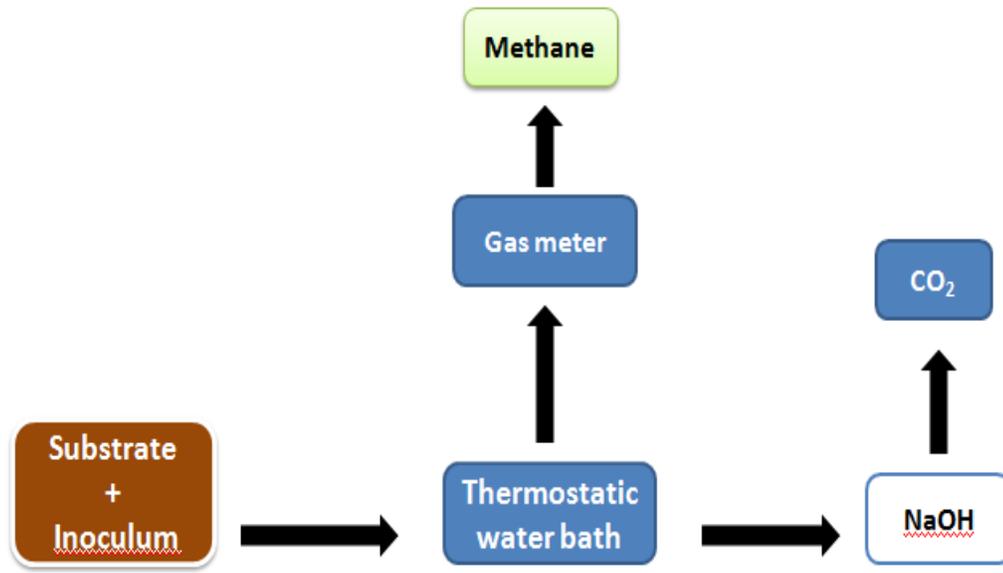


Figure 5. Process Flow Diagram for Batch Experiment

3.3 Analytical methods

The parameters analyzed for the characterization include moisture content (MC), organic matter (OM), carbon and nitrogen content of each substrate, total solids (TS), volatile solid (VS), pH, total kjeldahl nitrogen (TKN), total carbon (TC), total organic carbon (TOC), and chemical oxygen demand (COD). All analytical measurements were performed based on the “Standard Methods” (APHA, 1998). TS samples were dried in an oven at 105°C, and for VS samples were burnt into a furnace at 550°C.

Methane production rates were measured as ml biomethane per day, and methane yield was determined based on the cumulative methane produced per gram volatile solids

added (Lo et al., 1983; Parawira et al., 2008). Furthermore, batch experiments were performed to evaluate and determine the bio-methane (CH₄) potential from each substrate (cocoa husk and rice straw).

In addition, the strength of the waste usually can be known by determining the amount of solids mixed in the culture. To analyze the effectiveness of the digestion process, the percent of volatile solids reduction was determined according to the formula mentioned by Joanne, 1991:

Equation 1. The Percent Volatile Solids Reduction Equation

$$\%VS\ Reduction = \frac{(\%VS\ In - \%VS\ Out)}{\%VS\ In - (\%VS\ In \times \%VS\ Out)} \times 100$$

For the COD removal, it was calculated by using following formula:

Equation 2. COD Removal

$$\%COD\ removal = \frac{(Initial\ COD - Final\ COD) \times 100\%}{Initial\ COD}$$

3.4. Statistical analysis

Experimental data obtained while performing an anaerobic digestion process were statistically analyzed with two factors of analysis of variance (ANOVA) in triplicate at steady state conditions; the main effect and interaction of each factor (substrates and percent

total solids) with digestion parameters were analyzed. Descriptive statistics of two-way ANOVA with replication were conducted to specify the influence between methane production and investigated parameters. Furthermore, the data analyzed by utilizing ANOVA test within 5 percent ($\alpha = 0.05$) level of significance also evaluated the influence of percent total solids, substrates loaded and digestion parameters of the batch experiment.

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CHAPTER 4 RESULTS AND DISCUSSION

4.1. Semi-continuous reactor performance

Anaerobic co-digestion of swine manure and corn stover for biogas production was operated under steady state conditions. The semi-continuous reactor had a working volume of 14 liters, and a hydraulic retention time of 25 days. The reactor was run under mesophilic condition (35°C), and continuously stirred with the speed of 120 rpm. Furthermore, 560 ml of swine manure and 14 g of corn stover were loaded into the digester to maintain the C:N ratio at 24.3:1 as it was obtained that C:N ratio of swine manure, and corn stover were 0.93:1 and 59.6:1, respectively.

The C:N ratio of swine manure co-digested with corn stover derived from this study was close to the optimum C:N ratio ranged between 20 and 30 (Monnet, 2003; Verma, 2002). Thus, it is very feasible to perform anaerobic digestion to generate biogas production without any inhibition. A study conducted by Hills (1979) regarding the effect of C:N ratio on anaerobic digestion of dairy manure revealed that the highest methane production per unit loading rate happened when the C:N ratio of the feed was 25:1. In addition, Tarbaghia (1993) found that an optimum rate of digestion can be accomplished when C:N ratio in the digesters is close to 30:1.

Table 1 summarizes performance of anaerobic co-digestion of swine manure and corn stover using a semi-continuous reactor. As can be observed, the semi-continuous reactor performed pretty well during anaerobic digestion process, where pH influent as well as effluent was in the range of optimum pH to generate methane between 6.5 and 8.0 (Burke, 2001; Cheng, 2010). There was also no negative effect of pH on the biogas production from co-digestion between swine manure and corn stover; it only turned from 6.79 to 7.23. In a well-operated digester, a slight increase of pH in an effluent culture is expected, since microorganisms generate alkalinity while they consume organic matter containing a lot of protein (Labatut and Gooch, 2012). This also means that the anaerobic digestion process was stabilized by applying co-substrates.

Table 1. Anaerobic co-digestion performance operated in semi-continuous reactor

| Parameter | Unit | Influent | Effluent |
|------------------|-------------|-----------------|-----------------|
| COD | mg/L | 38953 ± 6911 | 14593 ± 1239 |
| TKN | mg/L | 536 ± 125.5 | 563 ± 153 |
| pH | - | 6.79 ± 0.09 | 7.23 ± 0.13 |
| TS | % | 2.49 ± 0.21 | 0.93 ± 0.07 |
| VS | % | 85.92 ± 1.81 | 76.26 ± 1.17 |

Based on this experiment, biogas generated was about 5070 ± 338 mL per day. Furthermore, previous research conducted by Jorge et al. (2012) revealed that anaerobic digestion of swine manure operated in semi-continuous reactor produced 274 mL of biogas per day. This means that swine manure co-digested with corn stover in this study can produce biogas about 18.5 times higher compared with anaerobic digestion of swine manure alone.

Cuetos et al. (2011) found that the addition of energy crop residues to the co-digestion system generated a considerable increase in the amount of biogas produced daily. The increase of biogas may be related to the synergistic effects of the enhanced alkalinity and buffering capacity and the structural changes of swine manure fibers in the co-digestion process (Li et al., 2009). They also mentioned that the energy contained in biogas is determined by the volume of biogas and methane content.

In addition to this experiment, GC analysis conducted to evaluate methane production from anaerobic co-digestion of swine manure and corn stover, had revealed that methane content in biogas produced was $56.63 \pm 1.18\%$. This result is in agreement with previous study that found that anaerobic co-digestion of pig slurry and chopped maize performed under mesophilic condition obtaining an average of methane content from 56.9% to 57.7% (Vindis et al., 2009). As shown in Table 1, this study of anaerobic co-digestion operated in 120 days and revealed that there was a considerable decrease in some parameters including COD, TS, and VS. Moreover, percent reductions of COD and TS for this experiment were 62.54% and 62.65%, respectively. This indicates that digestion process performed sufficiently well, where biodegradation efficiency represented in organic matter reductions

reached more than fifty percent during anaerobic digestion process. In addition, VS reduction obtained from the experiment was 47.36%, which was almost fifty percent reduction during digestion process.

Therefore, it was shown that co-digestion of swine manure with corn stover may help convert waste organic materials to energy sources. Moreover, co-digestion of corn stover with swine manure may also generate several other benefits such as enhancing the fertilizer value of the swine manure and agricultural wastes, recycling of nutrients back into the field and environmental protection against pollution (Ozturk, 2013). This experiment also showed that a stable condition during the anaerobic process can be accomplished by establishing co-digestion technology. Mata-Alvarez et al. (2000) mentioned that biogas production can be enhanced by the use of co-substrates as it can generate positive synergisms, which may substitute missing nutrients required for performing anaerobic digestion.

Synergetic effect is principally related to more balanced nutrients and enhanced buffering capacity in anaerobic co-digestion. Thus, more balanced nutrients in the system may support microbial growth for running anaerobic digestion process properly; then, enhanced buffering capacity also may help stabilize anaerobic digestion process (Li et al., 2009). Moreover, several other factors that helped to achieve successful anaerobic digestion include temperature and mixing, which should be set at a constant condition during the process (Vindis et al., 2009).

4.2. Characteristics of substrates and inoculums for batch experiment

The study was programmed to investigate methane potential of agricultural residues including rice straw (RS) and cocoa husk (CH) at three different concentrations of total solids in the process, 2%, 3%, and 4% TS concentration. This process was operated in mesophilic condition at 35°C. The physical-chemical characteristics of substrates are shown in Table 2. The initial characteristics of rice straw were: volatile solids of 84.4%, organic matter of 72.82%, carbon content of 36.42%, and COD of 1950 mg/l. Characteristics of cocoa husk also include volatile solids of 87.5%, organic matter of 57.61%, carbon content of 44.97%, and 1582 mg COD/L.

The characteristic values indicate the abundance of organic matter in both cocoa husk and rice straw, which allowed them to be suitable for anaerobic co-digestion with digested swine manure to generate higher methane production. It is known that total solids (TS) and volatile solids (VS) are also crucial factor when it is loaded into anaerobic digester. Furthermore, the TS is utilized to define whether the digester has been sufficient for the amount of substrate coming in, and the VS may be regarded as a measure of the organic matter in the digester that can be converted into biogas (Schmidt, 2005). In addition, Asam et al. (2011) revealed that the higher volumetric methane yield derived from agricultural residues is due to higher VS content per unit mass of feedstock. The authors also mentioned that by applying agricultural residues as co-substrates in biogas plants, it will enhance the volumetric methane productivity as compared with a swine slurry facility. Even though the VS content of substrate is considered as the determinant of potential methane production, the

methane yield on VS basis is not always fixed. This occurs since there is any variation in terms of VS composition which consists of both readily degradable organic materials (carbohydrates, proteins, and lipids) and refractory organics such as lignocellulosic materials. Therefore, it can be known that all volatile solids of organic materials are not always the same; this condition may generate different rates and extents of biodegradation during anaerobic digestion (Wilkie, 2005).

Moreover, inoculum taken from the semi-continuous reactor also showed that it still had a considerable amount of nutrients, which was available to be co-digested with agricultural residues (rice straw and cocoa husk) to enhance methane production. It can be seen in the characteristics of inoculums. Based on Table 3 showing total organic carbon and TKN of inoculums, it was obtained that C:N ratio of inoculums utilized for the batch experiment was around 1.52:1. The inoculum also contained high VS, TOC and COD, which were $78.19 \pm 1.64\%$, 860 ± 121.2 mg/L and 13853 ± 2962 mg/L, respectively. It also had a neutral pH, which was feasible for running anaerobic digestion process.

A study by Lopes et al. (2004) revealed that inoculums applied into anaerobic digestion process may significantly enhance the performance of the process. The authors also mentioned that the better performance of the inoculated digesters can be associated with the potential increase in a number of anaerobic microorganisms that significantly assisted to break down the organic material in the digester. Furthermore, Pandey et al. (2011) mentioned that inoculum has a substantial role for starting up anaerobic digestion process since it is able

to balance the populations of some bacteria that include *syntrophobacter* which is responsible for degrading propionate as well as butyrate, and *methanogens*.

Table 2. Substrates characterization (wet basis)

| Parameter | Rice straw | Cocoa husk |
|------------------|-------------------|-------------------|
| TS (%) | 91.4 | 93.35 |
| VS (%) | 84.4 | 87.5 |
| MC (%) | 8.6 | 6.65 |
| OM (%) | 72.82 | 57.61 |
| C (%) | 36.42 | 44.97 |
| N (%) | 0.71 | 1.36 |
| COD (mg/L) | 1950 | 1582 |
| C:N | 51.3 | 33.07 |

Table 3. Inoculum characterization

| Parameter | Mesophilic inoculums |
|------------------|-----------------------------|
| TS (%) | 1.02 ± 0.08 |
| VS (%) | 78.19 ± 1.64 |
| COD (mg/L) | 13853 ± 2962 |
| TOC (mg/L) | 860 ± 121.2 |
| TKN (mg/L) | 566.7 ± 92.4 |
| pH | 7.29 ± 0.28 |

4.3 Batch digester performance

4.3.1 Performance under different solid concentrations

The data presented in Table 2 show that cocoa husk and rice straw have a high percentage of both total solids and volatile solids. The percentage of carbon content of both substrates is also sufficiently high, which indicates that both substrates should be decent for co-digestion with swine manure. Rice straw (RS) and cocoa husk (CH) had high carbon to nitrogen ratio (51.3 and 33.07 respectively). As revealed in Table 2, nitrogen content of CH is higher than RS; thus, it leads C:N ratio of CH to be lower than RS. However, it still does

not reach an optimum range for anaerobic digestion between 20:1 and 30:1 (Monnet, 2003; Weiland, 2006).

To optimize the co-digestion performance and to compare anaerobic co-digestion of different substrates (cocoa husk and rice straw) combinations, and to achieve maximum productivity of methane, three experiments were conducted. The first experiment operated in 2% TS, reactors containing 500 mL of inoculum were loaded with substrates including 5.58 g of rice straw and 5.46 g of cocoa husk. In this experiment, methane production stopped completely at 25 days of digestion. Influent data of the process were summarized in Table 4. As revealed in Table 4, it is known that all reactors run in the range of optimum pH (6.5 to 8.0), that allowed them to operate in proper anaerobic digestion for generating methane (Cheng, 2010). This condition is in agreement with a previous study that mentioned that anaerobic digestion ran with pH between 7 and 8 was found to be effective for degrading total suspended solid as well as volatile suspended solid during an anaerobic digestion process (Dinamarca et al., 2003).

Table 4. Influent data operated under 2% total solids concentration

| Analysis | C | CH | RS |
|-----------------|----------|-----------|-----------|
| TS (%) | 0.95 | 1.88 | 2.06 |
| VS (%) | 76.8 | 77.65 | 79.56 |
| COD (mg/L) | 13500 | 36450 | 31700 |
| TOC (mg/L) | 994 | 792.8 | 801 |
| TKN (mg/L) | 574.7 | 829.6 | 821.8 |
| pH | 7.16 | 7.26 | 7.19 |

As presented in Table 4, it can be seen that COD of CH digesters (36450 mg/l) were higher compared with COD of RS and control reactors (31700 and 13500 mg/l, respectively). However, total organic nitrogen and total organic carbon represented in TKN and TOC of both substrates (CH and RS) were almost the same. Moreover, C:N ratio of CH and RS operated in 2% TS were quite low (6.4:1 and 7.74:1, respectively). The low C:N ratio of each culture may potentially inhibit the anaerobic digestion process since it may lead to ammonia accumulation in the digester, which is toxic to methanogenic bacteria (Monnet, 2003). In addition, research conducted by Kimchie (1984) also revealed that C:N ratio less than 10:1 was susceptible to being inhibitory.

As illustrated in Figure 6, control reactors run at 2% TS began to produce methane at the first day of digestion process (29 ± 10 mL). The shape of the curve looks like a sigmoid curve, represented by cumulative methane production within 25 days of digestion process. Maximum production was reached at 22 days of digestion process, which was around 379.6 ± 16 mL CH₄. As shown in Figure 6, there was no lag phase happened at the beginning of digestion process in CH reactors. When the experiment was set up, it was found that CH reactors directly produced methane at 60.67 ± 7.5 mL. At the second day of digestion process, CH reactors had generated 132.7 ± 8.4 mL CH₄. This was quite high compared with CH reactors run at 4% TS, which produced only 44 ± 4 mL CH₄ at the second day of digestion (Figure 8, and 10). However, the results were almost the same as CH reactors run at 3% TS, where they produced 133 ± 12 mL CH₄ at the second day of digestion process (Figure 6, and 10). This phenomenon indicated that CH run using 2% TS were easily digested since culture was mixed properly during digestion process.

As illustrated in Figure 6, maximum methane production was reached at 22 days of digestion process (438 ± 11.8 mL). The anaerobic digestion operated in 2% TS stopped completely after 25 days of the digestion process. Furthermore, CH run at 3% and 4% TS continuously produced methane until 28 days, and required a retention time of 31 days to complete the process. However, CH reactors operated in 2% TS showed a poor digestion process as they only produced methane effectively within 22 days, and a retention time of 25 days was required to complete digestion process. This may occur due to high lignin content

of cocoa husk (Alemawor, 2009), and lack of nutrients available to be digested by microbes at 2% total solids concentration.

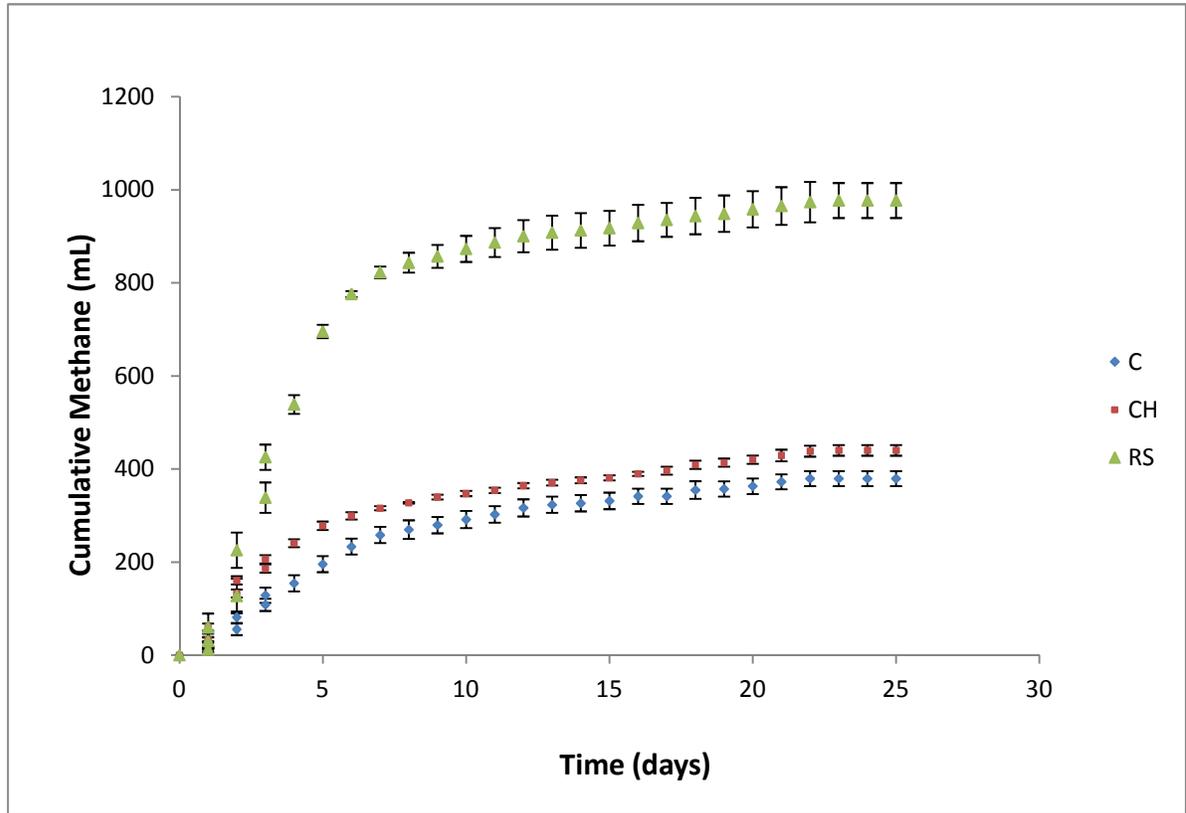


Figure 6. Comparison of cumulative methane under 2% TS

As depicted in Figure 6, RS digesters run at 2% TS performed very well compared to CH and control reactors. There was a little lag phase that occurred at the beginning of the anaerobic digestion process. It can be seen that RS reactors operated in 2% TS produced only 60 ± 30 mL CH_4 at the first day of the digestion process. This result was lower compared with RS reactors run at 3% and 4% TS where at the first day of the digestion process they

started to generate methane at around 68 ± 2.6 mL and 72 ± 12.8 mL, respectively (Figure 6, 7, 8, and 9).

As shown in Figure 6, there was a considerable increase of methane production between two and five days of digestion process. It continuously produced methane with slow increase until reaching a peak at 21 days of digestion process (965.7 ± 40.5 mL). This condition is extremely different from RS reactors run at 3% TS, where at 21 days of digestion, they had produced methane at about 1734 ± 66 mL. Furthermore, RS reactors operated in 3% TS also produced methane until 29 days of digestion, which were around 1814 ± 47.4 mL (Figure 7). This was almost two times higher than RS run at 2% TS. However, RS reactors performed at 4% TS, only produced 764.3 ± 15.6 mL CH₄ at 21 days of digestion, which was lower than RS run at 2% TS. This indicated that RS reactors operated in 4% TS experienced a difficulty to digest at the beginning of the process due to the poor homogeneity of the mixture. In addition, RS run at 4% TS also needed a longer retention time to digest substrate and complete digestion process (Figure 8) compared with RS run at 2% and 3% TS (Figure 9).

Table 5 summarizes the effluent data performed at 2% TS. As can be observed, it is known that each reactor still performed in the optimum pH range for anaerobic digestion. This may indicate that the low gas production generated by some reactors (CH and control reactors) may not be caused by acid accumulation in the digesters. As illustrated in Figure 6, each reactor loaded with different substrates (RS, CH and control) completed their digestion process with a retention time of 25 days. This condition is very different from other

experiments (3% and 4% TS), where they completed their digestion process within 31 days (Figure 7 and 8).

Table 5. Effluent data of 2% total solids concentration

| Analysis | Means and standard deviations | | |
|-------------------------|-------------------------------|---------------|---------------|
| | C | CH | RS |
| TS (%) | 0.87 ± 0.01 | 1.6 ± 0.01 | 1.44 ± 0.01 |
| VS (%) | 73.84 ± 0.23 | 73.57 ± 0.13 | 71.45 ± 0.77 |
| COD (mg/L) | 10766 ± 677.1 | 20020 ± 1583 | 15826 ± 336 |
| TOC (mg/L) | 481.6 ± 47.62 | 536.2 ± 18.5 | 542.8 ± 24.07 |
| TKN (mg/L) | 621.2 ± 6.48 | 840 ± 3.23 | 757.4 ± 10.29 |
| pH | 6.80 ± 0.20 | 6.90 ± 0.13 | 6.73 ± 0.03 |
| Methane production (mL) | 379.6 ± 15.95 | 440.3 ± 11.59 | 977 ± 37.58 |

Based on Table 5, it is revealed that RS reactors operated in 2% TS had the highest total methane production (977 ± 37.58 mL) among other digesters (CH and control). As shown in Figure 6, compared with CH, RS performed better at 2% TS, where RS produced methane at almost two times higher than CH. The poor homogeneity of mixture in RS reactors may lead to more variations in the daily methane production. This phenomenon also

occurred with the previous study conducted by Silvestre et al. (2013) that co-digested rice straw with cattle manure, where during the co-digestion periods the daily biogas production was less stable due to solid accumulation. Furthermore, CH reactors run at 2% TS produced methane at only about 16 percent higher compared with control reactors. This indicates that CH did not perform very well under 2% TS concentration.

Based on the total solids data presented in Table 4 and 5, the performance of each reactor during anaerobic digestion process also can be known where TS reduction of RS reactors operated in 2% TS was double ($30.1 \pm 0.3\%$) compared with CH reactors, where CH run at 2% TS concentration only had TS reduction at around $15 \pm 0.5\%$. High cell wall content in cocoa husk may still be believed as the cause of poor digestion in CH reactors (Tuah and Orskop, 1987). Furthermore, this phenomenon also may be understood by referring to Table 2, where CH virtually contained a significant amount of nutrients required for methane production such as high amount of carbon content, TS and VS which is almost the same as RS had. Therefore, it may be believed that high lignin content of CH was considered a source of barriers that hindered this substrate for being converted into biogas as well as methane (Alemawor, 2009).

In the second experiment performed under 3% TS, RS and CH added to each reactor containing 500 mL of inoculums were 10.67 g and 10.44 g, respectively. Under steady state conditions, the duration of digestion process was 31 days when methane production stopped completely. Table 6 shows initial conditions of co-digestion process ran at 3% TS. It is revealed that total organic carbon of CH and RS were almost the same (1163 mg/L and 1157

mg/L respectively). However, it can be seen that total organic nitrogen represented in TKN of both substrates (CH and RS) were very different, where TKN of CH is higher (1017 mg/L) than TKN of RS (622.5 mg/L). It is known that TKN is the sum of ammonia-nitrogen and organic nitrogen (US-EPA, 2001). This may indicate that CH culture contained higher ammonia-nitrogen than RS culture that potentially may have lowered the rates of anaerobic digestion process.

Moreover, C:N ratio of CH culture run at 3% TS was almost the same as C:N ratio of RS culture. C:N ratio of CH and RS performed at 3% TS were 11:1 and 10.6:1 respectively. Furthermore, study conducted by Kimchie (1984) on high-rate anaerobic digestion of agricultural wastes, revealed that C:N ratios lower than 10:1 were found to be inhibitory during anaerobic digestion process. Based on the current study, although C:N ratio of each substrate was quite low, it still performed very well during digestion process. It can be noticed that pH values of both substrates CH and RS (7.25 and 7.57, respectively), were still in the optimum pH range for anaerobic digestion between 6.5 and 8.0 (Boyer, 2010; Cheng, 2010).

Table 6. Influent data operated under 3% total solids concentration

| Analysis | C | CH | RS |
|-----------------|----------|-----------|-----------|
| TS (%) | 0.99 | 2.81 | 2.98 |
| VS (%) | 77.78 | 85.17 | 86.14 |
| COD (mg/L) | 15850 | 33900 | 48300 |
| TOC (mg/L) | 757 | 1163 | 1157 |
| TKN (mg/L) | 655 | 1017 | 622 |
| pH | 7.62 | 7.25 | 7.57 |

Figure 7 depicts the performance of control reactors ran at 3% TS. As can be observed, there is no a big difference among control reactors ran at 2%, 3% and 4% TS (Figure 6 and Figure 8). This condition happened since each of control reactors were loaded with the same culture derived from semi-continuous reactors operated in steady state conditions. There was a small lag phase that occurred a few hours after running experiment. Each of the control reactors ran at 3% TS had started to produce methane by the second day of digestion process, which were around 32 ± 6 mL. The maximum methane production of control reactors performed at 3% TS was reached on the 28 day of digestion (384.3 ± 8 mL).

In terms of methane production, CH ran at 3% TS showed a slightly lower level compared with CH operated under 4% TS (Figure, 10). Based on Figure 10, it can be seen

that the methane production had started by the second day of digestion (133 ± 12 mL). Compared with CH reactors ran at 4% TS, CH reactors performed at 3% TS produced more methane at the beginning of digestion process. However, they started to produce methane more slowly during the middle of the digestion process. Methane production reached a peak at 27 days of digestion, which was around 531.7 ± 20.1 mL. This result was higher compared with CH performed under 2% TS that reached a maximum methane production at 22 days of digestion process (438 ± 11.8 mL CH₄). However, methane produced by CH reactors operated in 3% TS was lower compared with methane produced by CH reactors run at 4% TS where at 27 days of digestion, CH operated in 4% TS had produced 648.7 ± 23.9 mL CH₄ (Figure 8 and 10).

As shown in Figure 7, RS digesters operated in 3% TS still performed better compared with CH and control reactors. Furthermore, compared with RS performed under 4% TS, cumulative methane generated from RS at 3% TS is double (Figure, 9). Methane production had begun by the first day of digestion process (68 ± 2 mL) after having a lag phase during the first few hours of the experiment. Even though at the beginning of digestion process it produced methane the same as RS reactors operated in 4% TS, it produced more methane significantly at 5 days of digestion (901 ± 22 mL). The result showed that on the 5th days of digestion process, RS reactors run at 3% TS produced methane almost three times higher compared to RS performed at 4% TS (366 ± 19 mL), and almost thirty percent higher than RS performed under 2% TS (695.7 ± 14.36 mL).

In addition, RS reactors performed at 3% TS reached a maximum methane production at 29 days of digestion process (1814 ± 47.4 mL), which was almost two times higher compared with RS operated in 4% TS (954.7 ± 45.4 mL) at 29 days of digestion process (Figure, 9). As illustrated in Figure 7, RS reactors performed at 3% TS also produced methane more than three times greater compared with CH reactors. Moreover, based on Figure 7, it is also known that CH reactors only produced methane about 40% higher than control reactors. This indicates that CH reactors run at 3% TS did not perform very well at producing methane compared with RS reactors. This occurred as cocoa husk cannot be degraded easily during digestion process. It can also be known by observing TS reduction within 30 days of digestion, where TS reduction of CH and RS reactors were $23.06 \pm 2\%$ and $35.5 \pm 0.6\%$, respectively. Thus, it is pretty obvious that there were issues in breaking down and digesting cocoa husk during anaerobic digestion process.

It may be believed that the high lignin content of CH hindered this substrate for being easily converted into biogas as well as methane. Furthermore, Alemawor et al. (2009) revealed that as fibrous biomass, cocoa husk contained a significant amount of cell wall components including high lignin content (14% w/w). The lignin content of cocoa husk is sufficiently high when it is compared to lignin content of rice straw which is around 9.9% w/w (Mussatto and Teixeira, 2010). Thus, it is quite evident why CH produced lower methane compared with RS even though it performed at an optimum pH (Table 6 and 7). This condition also revealed that there was no indication of inhibition formed by acid accumulation during anaerobic digestion process that may lead to lower pH.

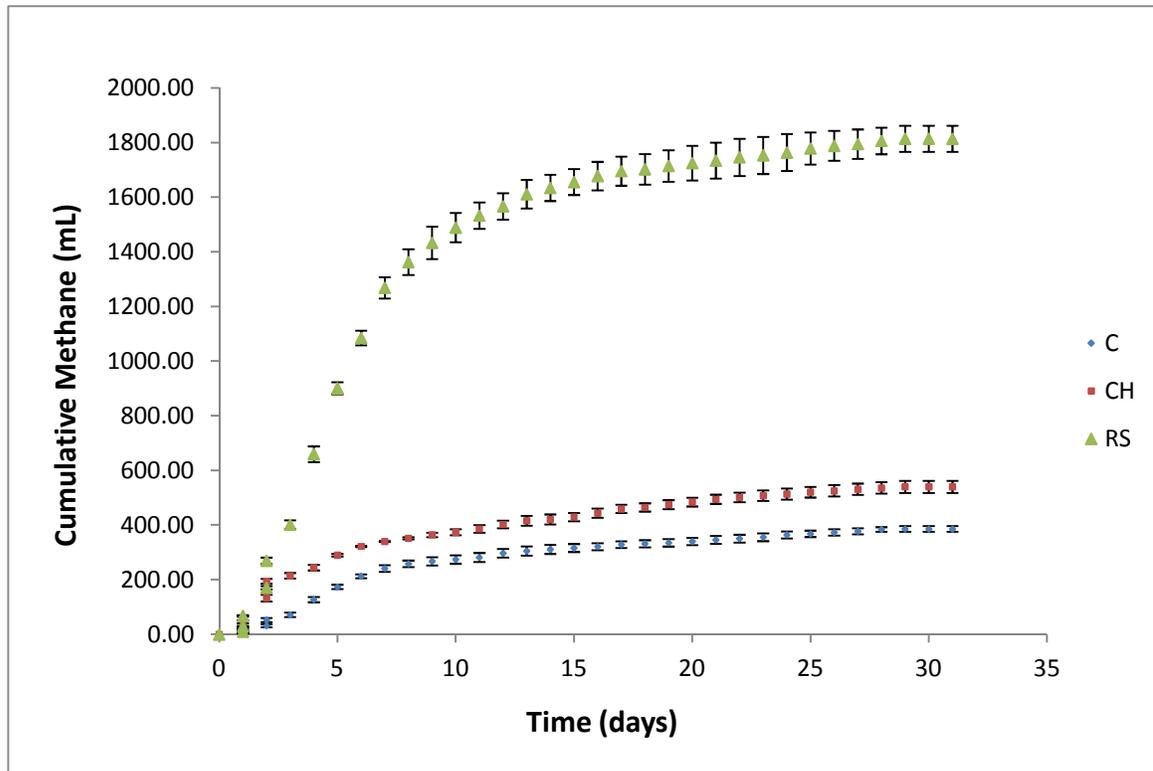


Figure 7. Comparison of cumulative methane under 3% TS

Table 7 summarizes the effluent data of anaerobic digestion operated in 3% TS. As can be noticed, pH values of each reactor were still in the optimum range required for anaerobic digestion. This indicated that each reactor was highly stable during anaerobic digestion without any significant inhibition. Even though some reactors (CH and control) did not produced large amount of methane, they still kept producing small amount of methane until 30 days of digestion (Figure 7). Based on Table 7, it is revealed that RS produced the highest methane (1814 ± 47.43 mL) at 3% TS. This means that RS reactors operated in 3%

TS produced methane almost five times higher than control reactors (386 ± 10.54 mL) with a retention time of 31 days.

Table 7. Effluent data of 3% total solids concentration

| Analysis | Means and standard deviations | | |
|-------------------------|-------------------------------|-------------------|-------------------|
| | C | CH | RS |
| TS (%) | 0.87 ± 0.01 | 2.16 ± 0.05 | 1.92 ± 0.02 |
| VS (%) | 73.93 ± 0.71 | 79.52 ± 0.16 | 70.35 ± 0.56 |
| COD (mg/L) | 10326 ± 336.1 | 28366 ± 838.6 | 22716 ± 709.5 |
| TOC (mg/L) | 493 ± 25.62 | 973 ± 38.41 | 901 ± 21.67 |
| TKN (mg/L) | 604.8 ± 16.30 | 861.4 ± 13.49 | 734.1 ± 41.08 |
| pH | 6.9 ± 0.04 | 7.00 ± 0.13 | 6.92 ± 0.04 |
| Methane production (mL) | 386 ± 10.54 | 540.3 ± 22.19 | 1814 ± 47.43 |

The experiment performed under 4% TS, RS and CH loaded into each reactor were 16.96 g and 16.6 g, respectively. When inoculums were co-digested with rice straw and cocoa husk, C:N ratio of these culture were 18.5 and 17.1, respectively. These C:N ratio values were in agreement with optimum C:N ratio ranges mentioned by Sievers and Brune

(1978). The authors mentioned that the C:N ratio range of 15.5 to 19 was discovered to be the optimum range in terms of maximum methane production.

Table 8 shows the influent data derived from batch digestion under 4% TS. Based on Table 8, it is known that all reactors had favorable conditions for generating methane during anaerobic digestion since they ran at neutral pH. This is in agreement with the previous reports that during anaerobic digestion, methanogenic bacteria require a neutral pH between 6.8 and 8.5 in order to produce methane (Burke, 2001).

Based on Table 8, it is also revealed that RS had higher total organic carbon and volatile solids compared with CH. The high organic content is commonly related to the high biodegradability that enables the substrate to be highly preferred for anaerobic digestion (Zhang et al., 2011). However, CH influent had COD concentration at around 44000 mg/L, which is higher than COD of RS. This condition also indicates that CH operated in 4% TS had a significant amount of organic compounds in the culture, that may be feasible for anaerobic digestion to generate methane (APHA, 1998).

Table 8. Influent data operated under 4% total solids concentration

| Analysis | C | CH | RS |
|-----------------|----------|-----------|-----------|
| TS (%) | 1.10 | 3.83 | 3.95 |
| VS (%) | 80 | 87.75 | 88 |
| COD (mg/L) | 15260 | 44000 | 30150 |
| TOC (mg/L) | 830 | 1181 | 1312 |
| TKN (mg/L) | 470.5 | 1033 | 768 |
| pH | 7.1 | 7.4 | 7.58 |

Figure 8 shows evolutions of cumulative methane production for control reactors operated in 4% TS. It can be seen that there was a lag phase at the beginning of anaerobic digestion process where microbes start to acclimate with the anaerobic condition. Methane production was started at the second day of digestion process, which was around 23 ± 3 mL. It continuously produced methane daily, and reached a peak at 23 days of digestion that produced 391 ± 23 mL CH₄.

Figure 8 shows that the shape of CH curve obtained from 4% TS digestion test looks almost the same as control curves. However, at the second day of digestion, methane produced from CH reactors was double the amount (44.3 ± 4.04 mL) compared with control reactors. Maximum methane production from CH reactors operated under 4% TS was reached at 27 days of digestion (648.7 ± 24 mL).

However, in the case of RS reactors showed in Figure 8, the shape of curve generated is quite different from CH and control reactors operated in 4% TS. It started to generate methane production at the first day of digestion which was about 72 ± 12 mL. This condition represents a high degradation rate from rice straw compared with cocoa husk where the material is highly biodegradable that lead to being easily accessed by microbes. After 29 days of digestion, RS reactors operated under 4% TS, produced methane two times higher than control reactors where it produced methane at around 954.7 ± 45.4 mL.

Table 9. Effluent data of 4% total solids concentration

| Analysis | Means and standard deviations | | |
|-------------------------|-------------------------------|------------------|-------------------|
| | C | CH | RS |
| TS (%) | 0.89 ± 0.00 | 2.96 ± 0.09 | 3.06 ± 0.02 |
| VS (%) | 75.56 ± 0.04 | 81 ± 0.18 | 74.71 ± 0.09 |
| COD (mg/L) | 12700 ± 1040 | 38483 ± 4369 | 27936 ± 1628 |
| TOC (mg/L) | 753.2 ± 223.3 | 1092 ± 108.7 | 1971 ± 193.3 |
| TKN (mg/L) | 538.6 ± 66.8 | 917.6 ± 22.9 | 711.4 ± 19.13 |
| pH | 6.77 ± 0.10 | 6.98 ± 0.03 | 6.83 ± 0.08 |
| Methane production (mL) | 400.7 ± 21.59 | 661 ± 27.87 | 962.67 ± 46.7 |

Table 9 summarizes the values of effluent data and total methane production running with a retention time of 30 days, which was operated in 4% TS. As can be observed, there was a slight decrease of pH values from each influent to effluent culture. However, pH values of each effluent culture performed in 4% TS were still in neutral range between 6.6 and 7 required for proper anaerobic digestion (Monnet, 2003).

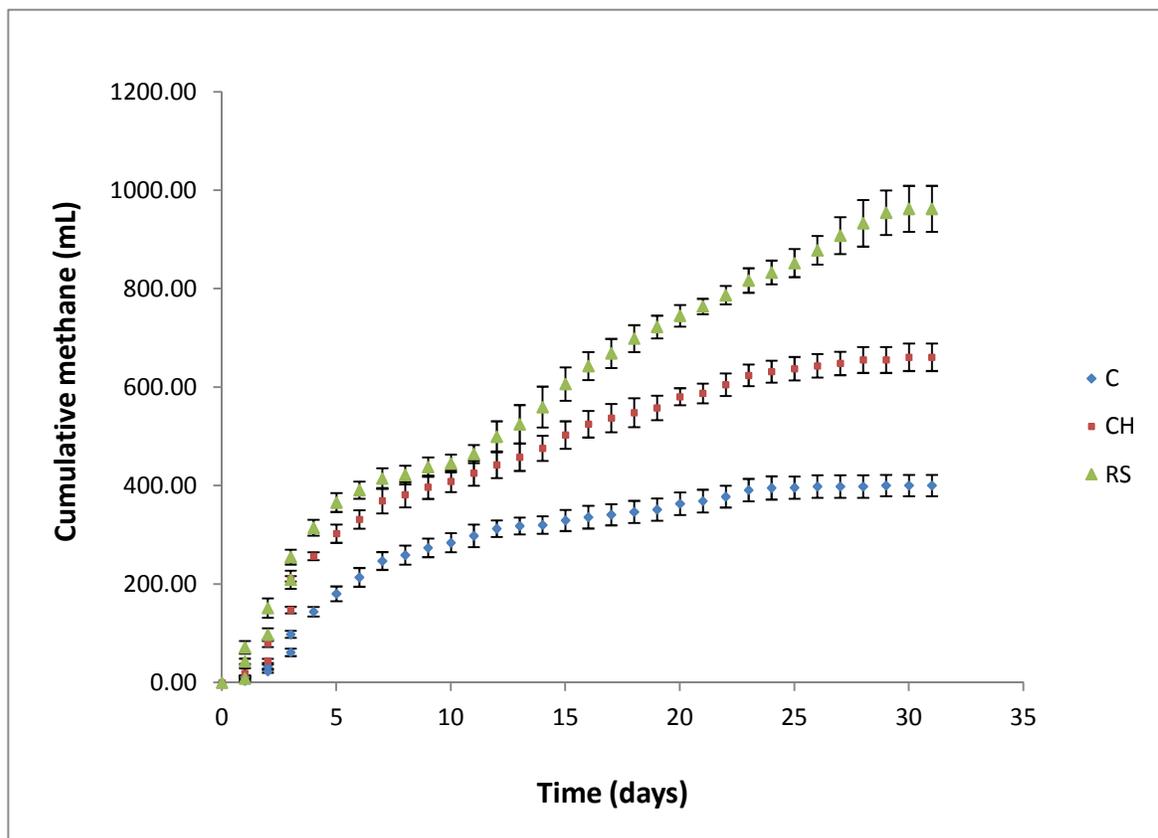


Figure 8. Comparison of cumulative methane under 4% TS

Moreover, based on Table 9, it is revealed that RS generated the highest methane production. Figure 8 depicts that RS reactors produced methane more than a hundred percent higher compared with control reactors, and almost fifty percent higher than CH reactors. According to Table 8 and Table 9, total solid reductions of control, CH, and RS reactors obtained were $19 \pm 0.34\%$, $22.7 \pm 2.23\%$, $22.6 \pm 0.6\%$, respectively. In terms of VS reduction obtained under 4% TS concentration, control, CH, and RS reactors had $22.69 \pm 0.16\%$, $40.51 \pm 0.69\%$, $59.71 \pm 0.19\%$, respectively. These results showed that RS reactors operated under 4 % TS performed very well compared with CH and control reactors (Figure 8).

Compared with RS reactors, CH reactors had less stable during anaerobic digestion even though they run in the range of optimum pH during anaerobic digestion. This phenomenon occurred due to high cell wall content or high lignin content of CH residues (Tuah and Orskop, 1987) that showed micro-organisms were not able to break down lignin for obtaining nutrients to convert into methane. It was also revealed by Wang and Schmidt (2010), which stated that the lignin content of biomass cannot be degraded during anaerobic digestion. Furthermore, it was also mentioned that lignin content of biomass may lower the efficiency of methane production during anaerobic digestion (Wang et al., 2009). In addition, it also can be seen that TKN influent and TKN effluent of CH operated in 4% TS is extremely higher than RS (Table 8 and 9). This indicates that CH ran at 4% TS contained substantially higher amount of ammonia-nitrogen compared with RS (US-EPA, 2001). This condition also may contribute to lower digestion rates of CH that lead to less methane

generated than RS since it is known that a substantial amount of ammonia in the digester may inhibit anaerobic digestion process (Angelidaki and Ahring, 1994; Braun et al., 1981). Based on Table 5, 7 and 9, by utilizing ANOVA test, it is revealed that there is a statistically significant difference between percent total solids and effluent digestion parameters (pH, TKN, COD, TOC, VS, TS, methane production) in anaerobic digestion of rice straw (p value = 2.07×10^{-18} ; $F_{\text{test}} = 124.97$; $F_{\text{crit}} = 3.22$; $df = 2$).

Furthermore, ANOVA test also showed that there is also statistically significant difference between percent total solids and effluent digestion parameters (pH, TKN, COD, TOC, VS, TS, methane production) within anaerobic digestion process of cocoa husk (p value = 4.17×10^{-10} ; $F_{\text{test}} = 37.7$; $F_{\text{crit}} = 3.22$; $df = 2$). In addition, the ANOVA analysis also revealed that there is significant difference between percent total solids applied and methane gas production where this may also indicate that there is any relationship as well as influence between TS and methane production in anaerobic digestion process of cocoa husk and rice straw (p value = 3.77×10^{-14} ; $F_{\text{test}} = 270$; $F_{\text{crit}} = 3.55$).

Furthermore, statistical analysis by applying ANOVA test with 5% level of significance showed that there is an interaction between factors (substrates and percent total solids) with methane gas production (p value = 5.6×10^{-6} ; $F_{\text{test}} = 282$; $F_{\text{crit}} = 2.928$; $df = 4$). This condition may indicate that there is a relationship as well as influence between percent total solids applied and substrates loaded into the digesters with methane production.

4.3.2 Biodegradation efficiency

Biodegradation is a process to convert organic (carbon-based) materials from complex molecules into simpler molecules through chemical as well as biological process. Furthermore, anaerobic biodegradation known as anaerobic fermentation, is a complex process where microorganisms convert carbon into energy and generate methane as well as carbon dioxide (Bio-Tech, 2011). Anaerobic digestion performance also may be known by evaluating biodegradability efficiency.

Some studies had revealed that methane production is extremely influenced by biodegradation and availability of the primary constituents contained in biomass, such as carbohydrates, protein, and lignin contents (Kalra and Panwar, 1986; Contreras et al., 2012). In addition, study conducted by Tong et al. (1990) about methane fermentation of selected lignocellulosic materials, revealed that biodegradability is influenced by lignocellulosic biomass and also restricted by some factors including lignin content, the availability of surface area and cellulose characteristics inside the biomass. The authors also mentioned that lignin may restrict the availability of holocellulose by generating a physical barrier to holocellulolytic enzymes, or by forming chemical bonds that cannot be hydrolyzed. They also revealed that the limiting step in methane fermentation of lignocellulosic materials is to break the lignin barrier in order to get access to the holocellulose. Methane yield presented in terms of mL CH₄/g VS added indicates the biodegradation efficiency (Lo et al., 1984). Wilkie (2005) mentioned that the digestibility and composition of the substrates was the major determinant of maximum methane yield. The author also revealed that several factors

that influence methane yield include temperature, biodegradability, loading rate, and retention time.

In addition, ANOVA analysis revealed that there is an interaction between factors (substrates and percent total solids applied) with methane yield (p value = 2.48×10^{-13} ; $F_{\text{test}} = 141$; $F_{\text{crit}} = 2.9277$; $df = 4$). As presented in Table 10, it is known that RS reactors performed in 2% TS had the highest methane yield (119.3 ± 4.59 mL CH₄/g VS added), which was almost fifteen percent higher than control reactors (104.1 ± 4.37 mL CH₄/g VS added).

RS reactors run at 2% TS also had the highest percentage of VS reduction ($35.66 \pm 2.41\%$), which was two times higher compared with control reactors ($14.73 \pm 1.02\%$). Moreover, good performance of RS reactors operated in 2% TS was also shown in the percentage of COD removal, where they obtained $50.07 \pm 1.06\%$ reduction, which was 147.26% higher than control reactors. These phenomena allowed RS reactors to generate higher methane production within 25 days of digestion process compared with CH and control reactors. ANOVA test also revealed that there is an interaction between factors (substrates and percent total solids) with COD removal (p value = 2.44×10^{-7} ; $F_{\text{test}} = 26.5$; $F_{\text{crit}} = 2.927$; $df = 4$). This condition may indicate that there is relationship as well as influence between TS applied in the digesters and COD removal.

Furthermore, as shown in Table 10, it can be understood that CH reactors operated in 2% TS did not perform very well. It can be seen by observing methane yield of CH, which was the lowest among other reactors (RS and control). In this case, CH reactors only

generated methane yield, which was around 60.25 ± 1.6 mL CH₄/g VS added. This methane yield result was lower than control and RS reactors (42 and 49.5%, respectively).

In addition, CH digesters only had $19.87 \pm 0.52\%$ of VS reduction, which were only 25.87% higher than VS reduction generated by control reactors. ANOVA test also showed that there is an interaction between factors (substrates and percent total solids loaded into the digesters) with VS reduction (p value = 7.95×10^{-10} ; $F_{\text{test}} = 54.65$; $F_{\text{crit}} = 2.927$; $df = 4$). This condition indicated that VS reduction may be influenced by substrates and percent total solids applied to the digesters.

Table 10. Efficiency of digestion at two percent total solids concentration

| Analysis | 2% TS | | |
|--|------------------|------------------|-----------------|
| | C | CH | RS |
| VS reduction (%) | 14.73 ± 1.02 | 19.87 ± 0.52 | 35.67 ± 2.4 |
| COD removal (%) | 20.25 ± 5.02 | 45.08 ± 4.34 | 50.07 ± 1.1 |
| Methane yield (mL CH ₄ /g VS added) | 104.1 ± 4.37 | 60.25 ± 1.6 | 119.3 ± 4.6 |
| Total methane production (mL) | 379.7 ± 15.9 | 438.7 ± 11.9 | 977 ± 37.6 |

Furthermore, performance of CH reactors ran at 2% TS was also shown in the percentage of COD removal, where they gained $45.08 \pm 4.34\%$, which was higher than control reactors by around 122.6%. However, COD removal and VS reduction of CH reactors performed under 2% TS were 11.07% and 79.52%, respectively lower than RS. This condition indicated that CH ran at 2% TS had a low efficiency of digestion even though they still generated higher cumulative methane production compared with control reactors.

Table 11 summarizes anaerobic digestion efficiency operated in 3% TS. As can be observed, RS reactors had the highest methane yield (141.4 ± 3.7 mL CH₄/g VS added), which was 41.8% higher than control reactors. Furthermore, good performance of RS reactors ran at 3% TS was also shown in the percentage of COD removal, where they gained $52.97 \pm 1.46\%$, which was higher than control reactors by 52 %.

In addition, RS reactors also had the highest VS reduction ($61.81 \pm 1.04\%$), which was three times higher compared with control digesters. These phenomena enabled RS reactors to reach the highest cumulative methane production within 31 days of digestion process (Figure 7), where in terms of total methane production, they gained 1814 ± 47.43 mL CH₄, which was extremely higher compared with CH (540.3 ± 22.19 mL CH₄) and control reactors (386 ± 10.54 mL CH₄), where RS generated methane at around 235.8% higher than CH and 370% higher than control reactors. As presented in Table 11, CH ran at 3% TS had the lowest methane yield (45.10 ± 1.85 mL CH₄/g VS added), which was about 121.3% lower compared with control reactors.

Furthermore, CH reactor also had the lowest COD removal ($16.32 \pm 2.47\%$), which represented in 113.5% lower than control reactors. However, CH reactors produced more cumulative methane compared with control reactors. This means that there were a lot of nutrients available in CH reactors represented in high total organic carbon as well as total organic nitrogen compared with control reactors (Table 6). This condition also indicates that CH ran at 3% still did not perform very well.

Table 11. Efficiency of digestion at three percent total solids concentration

| Analysis | 3% TS | | |
|--|------------------|-------------------|-----------------|
| | C | CH | RS |
| VS reduction (%) | 18.90 ± 3.00 | 32.41 ± 0.68 | 61.81 ± 1.0 |
| COD removal (%) | 34.85 ± 2.12 | 16.32 ± 2.47 | 52.97 ± 1.5 |
| Methane yield (mL CH ₄ /g VS added) | 99.79 ± 2.72 | 45.10 ± 1.85 | 141.4 ± 3.7 |
| Total methane production (mL) | 386 ± 10.54 | 540.3 ± 22.19 | 1814 ± 47.4 |

Moreover, methane yield obtained from CH reactors was 213.6% lower compared with RS reactors. CH reactors ran at 3% TS also showed poor performance of digestion, where they had lower COD removal and VS reduction compared with RS reactors. These phenomena revealed that CH reactors faced the difficulty to digest and degrade substrates

during anaerobic digestion process since the experiment was run under steady state conditions, where there was no problem with culture mixture that means substrates and inoculums were mixed very well during anaerobic digestion.

Furthermore, CH reactors still can produce more methane daily compared with control reactors and they also still run in the optimum pH range for anaerobic digestion (Table 7). Thus, high cell wall content of cocoa husk may still be presumed as the source for low digestion efficiency of CH reactors. Furthermore, it also indicates that co-digestion process still benefits to stabilize the digester by maintaining optimum pH and enhance methane production.

Table 12. Efficiency of digestion at four percent total solids concentration

| Analysis | 4% TS | | |
|--|--------------|-------------|--------------|
| | C | CH | RS |
| VS reduction (%) | 22.7 ± 0.2 | 40.51 ± 0.7 | 59.71 ± 0.2 |
| COD removal (%) | 16.8 ± 6.8 | 12.54 ± 9.9 | 7.34 ± 5.4 |
| Methane yield (mL CH ₄ /g VS added) | 90.9 ± 4.9 | 38.99 ± 1.6 | 55.44 ± 2.7 |
| Total methane production (mL) | 400.7 ± 21.6 | 661 ± 27.9 | 962.7 ± 46.7 |

Table 12 shows digestion efficiency obtained from 4% TS process. As can be observed, both CH and RS reactors had lower methane yield compared with control reactors (38.99 ± 1.57 and 55.44 ± 2.69 mL CH₄/g VS added, respectively). RS reactors had the highest VS reduction ($59.7 \pm 0.2\%$); however, they had the lowest COD removal ($7.34 \pm 5.4\%$), which was about 128.61% lower than control reactors ($16.78 \pm 6.8\%$).

However, it is known that RS reactors still generated higher cumulative methane production within 31 days of digestion process compared with CH and control reactors (Figure 8); however, low methane yield as well as COD removal revealed that they experienced issues in the digestion process. Moreover, it also can be known by comparing total methane production of RS operated in 4% TS with 2% and 3% TS concentration (Table 10 and 11).

In terms of total methane production, it was revealed that RS reactors operated in 4% TS, generated methane that was 1.49% lower than total methane production under 2% TS, and they also produced methane that was 88.47% lower compared with RS run at 3% TS. Furthermore, ANOVA analysis also showed that at the 5% level there was a statistically significant difference between percent total solids applied and biodegradable parameters (COD removal, methane yield and VS Reduction) in anaerobic digestion of rice straw (p value = 9.94×10^{-20} ; $F_{\text{test}} = 447.96$; $F_{\text{crit}} = 3.403$; $df = 2$). This is very obvious that RS run at 4% TS did not perform very well due to solid accumulation that lead to lower digestion efficiency. This condition occurs since higher total solids as well as volatile solids loaded

into the digester may generate a lot of volatile solids in the digester that may affect the alkalinity of the digester.

Higher total solids concentration applied to the reactor also can influence the volatile loading rate in the available detention or retention time period. Thus, sufficient time (retention time) should be allowed for the micro-organisms to degrade the organic materials and convert it into biogas (Cantrell et al., 2008; Chandra et al., 2012). In addition, a study conducted by Wilkie (2005) also revealed that there is an upper limit for TS content applied in anaerobic digestion, above which the material was not considered slurry that may generate some issues such as mixing.

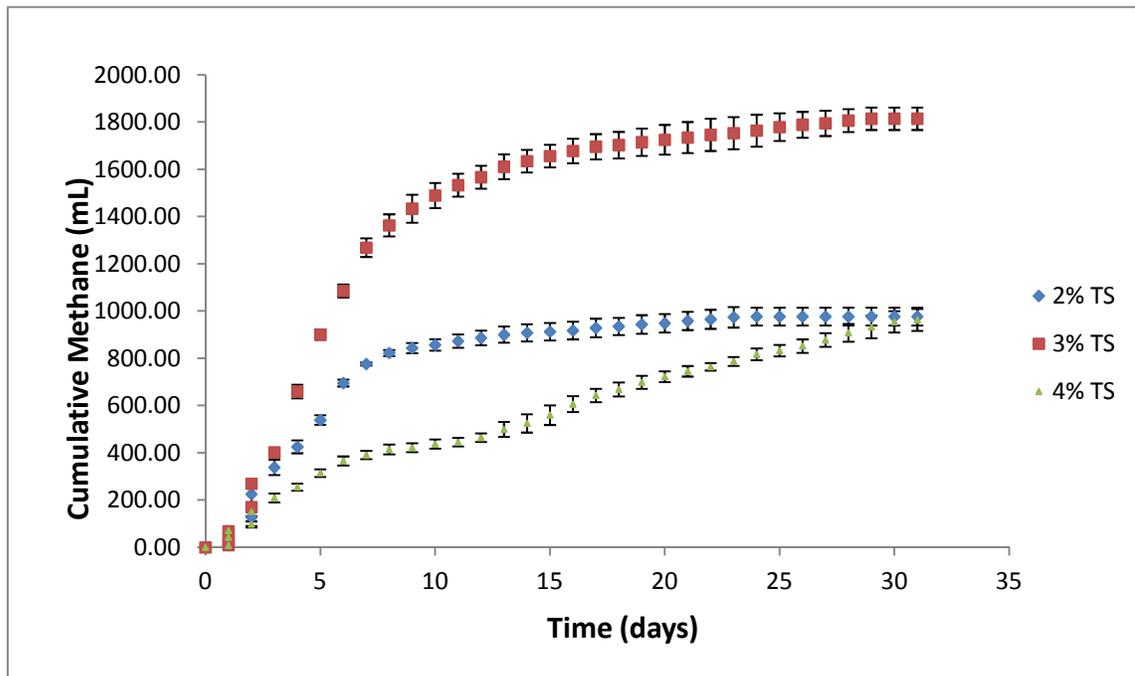


Figure 9. Cumulative methane production of rice straw with different total solids

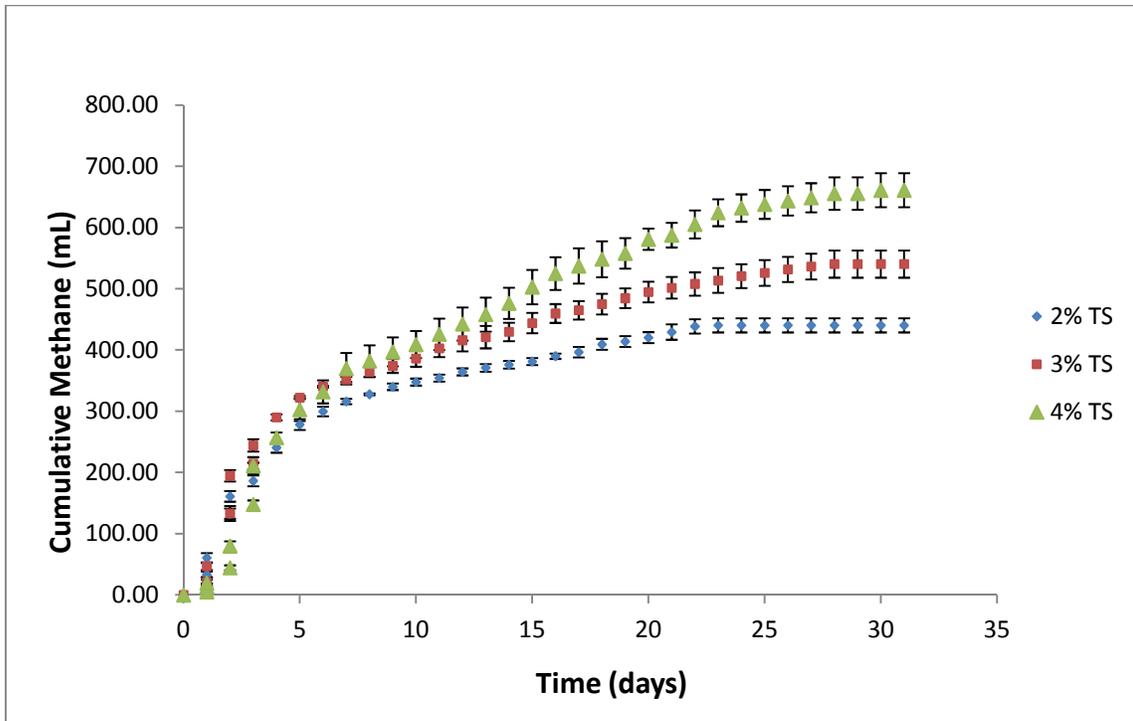


Figure 10. Cumulative methane production of cocoa husk with different total solids

As shown in Table 12 and Figure 10, CH reactors operated in 4% TS had the lowest methane yield (38.99 ± 1.57 mL CH₄/g VS added). However, they gained higher VS reduction compared with control reactors. This also indicated that at 4% TS, digestion efficiency of CH represented in VS reduction was slightly better when compared with CH operated in 2% and 3% TS (Table 10, 11, 12, and Figure 10). This condition lead CH reactors run at 4% TS to produce more methane within 30 days of digestion, where in terms of total methane production they generated about 50.68% higher compared with total methane produced under 2% TS (Table 10), and around 22.33% higher compared with total

methane produced in 3% TS (Table 11, and Figure 10). High cell wall content of CH was also still believed to be a barrier that led to lower biodegradation efficiency of CH.

ANOVA test also indicated that there is a statistically significant difference between percent total solids applied and biodegradable parameters in anaerobic digestion of cocoa husk including COD removal, methane yield and VS reduction (p value = 1.66×10^{-9} ; $F_{\text{test}} = 52.7$; $F_{\text{crit}} = 3.4$; $df = 2$). However, CH ran at 4% TS still performed worse compared with RS. It can be known by observing methane yield obtained by CH in 4% TS, where they generated 42.19% lower compared with methane yield achieved by RS. Compared with RS, CH reactors run at 4% TS generated higher COD removal but they still had lower VS reduction compared with RS reactors (Table 12). This still showed that CH reactors had lower digestion efficiency compared with RS. These phenomena also revealed that CH reactors had problems in the digestion process, where high lignin content was still believed to be the barrier during anaerobic digestion (Tuah and Orskop, 1987).

Furthermore, it has been known that lignocellulosic biomass had a complex structure that provided a major protective barrier that may have prevented cell destruction by biological as well as chemical process. This condition may cause a lower digestion rate that will reduce biogas production (Pang et al. 2008). To deal with this issue, pretreatment should be taken into consideration and be applied in order to enhance digestibility of lignocellulosic biomass (Hendriks and Zeeman, 2009). The authors also mentioned that by pre-treating biomass, it will enhance the hydrolysis process that leads to an increase total methane yield. In addition, Pang et al. (2008) added that the chemical composition as well as physical

structure of lignocellulosic biomass may be converted by applying several pretreatments, which may induce the composition in lignocellulosic biomass to be more readily biodegradable and more accessible to microorganisms during the anaerobic digestion process.

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CHAPTER 5 CONCLUSION AND FUTURE WORK

5.1 Conclusions

Anaerobic co-digestion of swine manure with agricultural residues can improve biogas production. The addition of agricultural residues to swine manure can enhance the carbon to nitrogen balance that enables to a stabilized anaerobic digestion process in the digester. In the present study, swine manure co-digested with corn stover was investigated with a semi-continuous reactor. An optimum C:N ratio was accomplished when corn stover was loaded into the digester, which was about 24.3:1. This condition generates positive synergisms that may help balance the nutrients and effectively improve buffering capacity for stabilizing anaerobic digestion to generate biogas production.

The effect of corn stover addition on the rate of biogas production had been detected. Biogas produced about 5070 ± 338 mL per day which was around 18.5 times higher compared with biogas production generated by anaerobic digestion of swine manure alone (274 mL per day). This demonstrates that utilizing corn stover as co-substrate in anaerobic digestion system may significantly enhance biogas as well as methane production, where in this present study, methane content in biogas obtained was around $56.63 \pm 1.18\%$.

Anaerobic co-digestion of swine manure with corn stover also showed highly biodegradable efficiency where high COD removal, TS and VS reduction were accomplished, which were around 62.54%, 62.65% and 47.36%, respectively. In addition,

this study reveals that anaerobic co-digestion of swine manure with corn stover is a decent method to enhance the stability of digestion process and biogas production.

A methane potential study of agricultural residues from rice straw (RS) and cocoa husk (CH) was carried out under batch conditions. A number of total solids concentration (2%, 3% and 4% TS) had been tested to assess methane potential of individual substrates. Compared with CH, RS showed better performance for all types of total solids concentration tested. RS generated highest methane production in 3% TS which was around 1814 ± 47.43 mL where at 3% TS, RS had C:N ratio at 10.6:1. RS still produced more methane at 2% TS than 4% TS even though at 2% TS methane production was completed in 25 days of digestion. This occurred since the mixture with 4% TS, RS had issues of solid accumulation in the digester that lead to improper mixing during digestion process and required a longer retention time to convert biomass into methane. Moreover, CH showed poorer performance compared with RS in all types of total solids concentration tested. CH produced highest methane at 4% TS (661 ± 27.87 mL). As mentioned by literature, CH had a substantial amount of protein represented in high amino acid that lead to an increase ammonia-nitrogen represented in higher TKN in CH culture. This indicates that the higher ammonia-nitrogen of CH showed in TKN may inhibit digestion rates that enable lower methane production.

Biodegradation efficiency was evaluated for each substrate. RS had the highest methane yield at 3% TS, which was around 141.4 ± 3.70 mL CH₄/g VS added. RS also had highest COD removal and VS reduction at 3% TS which were around 52.97 ± 1.46 and 61.81

$\pm 1.04\%$, respectively. These results may indicate that 3% TS is an optimum condition for RS to produce methane with a stable anaerobic digestion process.

Furthermore, CH reached the highest methane yield at 2% TS which was around 60.25 ± 1.6 mL CH₄/g VS added, and the lowest methane yield was obtained at 4% TS which was around 38.99 ± 1.57 mL CH₄/g VS added. CH also had highest COD removal at 2% TS which was $45.08 \pm 4.34\%$. However, at 2% TS, CH generated less cumulative methane production compared with 3% and 4% TS. This showed that CH experienced difficulty in the anaerobic digestion process even though it was run at an optimum pH range.

As mentioned in the literature review that revealed that CH had higher lignin content compared with rice straw. Thus, it may be believed that higher lignin content of CH was also considered as a source of inhibition during the digestion process. Furthermore, even though CH showed poor performance during the digestion process, it still produced more methane compared with control reactors. This condition indicates that performing co-digestion is still potential to enhance methane production compared with anaerobic digestion of manure alone.

5.2 Suggestions for Future Work

This research showed that utilizing agricultural residues (rice straw and cocoa husk) as co-substrates can increase methane production compared with manure alone. However, digestion efficiency represented in methane yield, COD removal and VS reduction still showed lower biodegradability. In the future, pretreatment of each substrate should be carried

out before loading it into the digester in order to improve biodegradability and enhance methane production.

As revealed in this research, RS experienced issues when it was run at 4% TS that lead to poor digestion efficiency and lower methane production; thus, in the future, anaerobic co-digestion of rice straw and manure is suggested to run at less than 4% TS to reach stable condition and higher methane production. In addition, as revealed in this research that CH performed extremely poor compared with RS; thus, in the future study it is suggested that to analyze the composition of cocoa husk in order to know whether this substrate contains any inhibition substances and a sufficient amount of nutrient required to generate more methane production. Even though pH is also the parameter that shows the performance of anaerobic digestion, some other parameters such as volatile fatty acids (VFA) and total ammonia may also be required to assess any inhibitory factor that occurs in the digester.