Anaerobic co-digestion of cocoa husk with digested swine manure: evaluation of biodegradation efficiency in methane productivity

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Abstract: The purpose of this study was to investigate the potential of methane production from anaerobic co-digestion of coco husk with digested swine manure. The experiment was set up in the batch system. Inoculum utilized in this experiment was derived from semi-continuous reactor run at steady state condition, with 25 days of hydraulic retention time and mesophilic condition. The temperature applied in this experiment was maintained under mesophilic condition, which was 35oC. The highest methane productivity generated from anaerobic co-digestion of cocoa husks with digested swine manure(CH) was 345.8±7.82 ml/d, which was higher compared with the anaerobic digestion of digested swine manure alone (286.97±16.8 ml/d). CH reactors had less methane yield (60.3±1.6 ml CH_4/g VS added) compared with control reactors (104.1±4.4 ml CH_4/g VS added). However, chemical oxygen demand (COD) removal and volatile solids (VS) reduction of CH reactors were 45.1%±4.3%, 19.9%±0.5%, respectively, which were higher compared with control reactors (20.3%±5.0%, 14.7%±1.0%, respectively). Based on the results, a lower biodegradation efficiency of anaerobic co-digestion of cocoa husks with digested swine manure was affected by the high cell wall content of cocoa husks that may hinder the anaerobic microbes to convert cocoa husk into methane.

Keywords: anaerobic co-digestion, cocoa husk, methane production, biodegradation efficiency

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1 Introduction

Anaerobic digestion is an ideal bioprocess technology in terms of handling and treatment of waste. Anaerobic digestion has some benefits to be applied and developed in the field of waste management technologies. Thus, the application of anaerobic digestion technology is also potential to be developed in the future for the purpose of processing technology to generate renewable energy (McCarty, 1964). Anaerobic digestion consists of several steps that are responsible for converting waste materials into methane that occurs naturally in anaerobic condition (Verma, 2002). Anaerobic digestion is a natural process converting biomass into energy, and recovers organic nutrients into soil conditioner (Burke, 2001).

Environmental benefits obtained by applying anaerobic digestion technology include minimizing odor, reducing pathogens, and cutting greenhouse gas emissions. Economic advantages derived from running anaerobic digestion technology include producing biogas or bio-methane production that can be utilized for generating electricity, producing bio-fertilizer containing a significant amount of nutrients that can be used for soil conditioner in land application (Burke, 2001). Biogas is a major product derived from the degradation process of organic materials where a consortium of microorganisms was involved. Thus, an understanding of microbiological process is extremely required to know the process stages occurred in anaerobic digestion (Waishet al., 1988). Microbial activities involved in the fermentation process of biological wastes can produce a biogas. Thus, biogas is considered a final product produced from microbial

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fermentation performed by methanogenic bacteria (Nagyand Szabó, 2011).

Anaerobic digestion operated in a controlled reactor can generate biogas containing a significant amount of methane compared with anaerobic digestion process occurred in a landfill. Methane produced from anaerobic digestion process in the controlled reactor is extremely dependent on the feedstock or organic materials loaded into the digester. Some potential agricultural wastes that can be used as substrates in anaerobic digestion include waste from cattle manure poultry, pigs and other livestock. Other wastes such as food scraps, woods, forest wastes, rice straw, and other agricultural residues can be used as a co-substrate in the anaerobic digestion process (Monnet, 2003; Steffenet al., 1998).

Anaerobic digestion process generates methane along with other substances including carbon dioxide, hydrogen sulfide and small amount of nitrogen (Kelleheret al., 2002). Study conducted on the methane productivity of manure, straw and solid fractions of manure, revealed that the volumetric methane yield of agricultural residues is higher than the yield from both manure and solid fraction of manure. This indicated that anaerobic digestion using agricultural residues as a co-substrate may generate more methane yield compared with anaerobic digestion that utilized manure as a single substrate. This condition occurred since adding agricultural residues to the anaerobic digestion of manure; it may enhance carbon content in the culture. Thus, it may enhance carbon to nitrogen ratio in the culture as anaerobic digestion using manure only may accumulate ammonia in the digester (Callaghan et al., 2002).

A lot of ammonia derived from manure may generate an inhibition in the process of anaerobic digestion leading to the reduction of methane production. Thus, adding agricultural residues in the anaerobic digestion of manure can enhance a buffer capacity in the digester for preventing the failure of anaerobic digestion process (Banksand Humphreys, 1998). Anaerobic digestion using agricultural residues as a co-substrate may enhance methane production as it may enhance volatile solids content in the culture. Volatile solid is regarded as an indicator of organic matter that can be converted to biogas during the process of anaerobic digestion (Schmidt, 2005). Methane production can be increased by 10% when there is an addition of a kilogram of agricultural residues to the digester containing a hundred kilogram of manure (Moller et al., 2004).

Some studies found that agricultural residues added to anaerobic digester may significantly cut the total concentration of ammonia that may inhibit the methane production (Cuetos et al., 2011; Angelidaki and Ahring, 1994; Henze, 1995; Hansen et al., 1998). It is revealed that free ammonia concentration is considered as a major factor that contributes to the inhibition of anaerobic digestion process. Some studies had found that there are some different threshold values for free ammonia concentrations that may be acceptable for the life of anaerobic microorganisms. For microorganisms that have not been adapted with the condition of free ammonia content in the digester yet, the concentration of free ammonia that is acceptable for their life is about 200 mg of ammonia nitrogen ($N\text{-}NH_3$)/L while the microorganisms that have been adapted previously in the condition where there is any ammonia content in the digester, the free ammonia concentration which is acceptable for their life is around 700 to 1100 mg of ammonia nitrogen ($N\text{-}NH_3$)/L (Henze, 1995; Hansen et al., 1998; Mata-Alvarez et al., 2000). Based on the literature review, it is extremely crucial to utilize agricultural residues as co-substrate in anaerobic digestion of manure in order to enhance the carbon to nitrogen ratio (Mata-Alvarez et al., 2000; Molinuevo-Salceset al., 2010). Thus, by operating anaerobic co-digestion composed with different substrates, the production of biogas and the stability of the process can be enhanced. The purpose of this study is to evaluate and assess potential methane production of cocoa husk under mesophilic conditions. Cumulative methane production over digestion time was examined, and the effects of an addition of cocoa husks on methane production were also evaluated through biodegradation efficiency assessment.

2 Materials and methods

2.1 Preparation of substrates

Batch experiment was conducted to evaluate and assess potential methane production of cocoa husk (CH). Dried cocoa husk was milled by using a laboratory grinder with the particle size of 1.5 and 2 mm before loading it into the digesters. Cocoa husk used in this experiment was not given any pretreatment as the purpose of this experiment was to assess the potential of cocoa husk as a co-substrate for generating methane. This research was conducted in triplicates where three reactors with a working volume of 500 ml were loaded with cocoa husk and inoculums, and other three reactors of 500 ml were control reactors or without adding cocoa husk. Inoculum used in this research was taken from an effluent of semi-continuous reactors operating in steady state condition at mesophilic temperature (35^{o}C). The effluent culture taken from semi-continuous reactor was stored in the fridge with the temperature of ±5^{o}C until required for use (Jorge et al., 2012). Details of the running procedure as well as the operating conditions for this anaerobic reactor can also be found elsewhere (Jorge et al., 2012; Gómez et al., 2009; Darwin et al., 2014; Darwin et al., 2016).

2.2 Experimental procedure

The culture in the batch reactors was continuously stirred at 270 r/min in order to prevent cocoa husks particles settled at the bottom of the reactor. The batch reactors were stirred at 270 r/min since at this speed, the cocoa husk particles and digested swine manure was mixed homogeneously; thus it may enhance the contact between the anaerobic bacteria and substrates. This experiment was operated under 2% total solids (TS) concentration to prevent acid accumulation in the batch digesters that may lead to a failure of anaerobic digestion process due to overloading of solid substrates in the batch reactors; thus, the effect of adding cocoa husks as a co substrate for methane production can be assessed.

Total solids of inoculums were measured in order to determine the proportion of biomass that should be added to each batch reactor. The mixture of biomass and inoculums loaded into each reactor as an influent was prepared homogeneously. In this study, 5.463 g of the ground cocoa husk (93.4% TS) was added to the batch digester, and mixed with 500 ml of digested swine manure (0.98% TS).

During the measurement of the bio-methane production test, first there was no addition of any other nutrient including chemicals as well as enzyme in order to know how much methane that can be produced by substrate loaded. Furthermore, this batch experiments were performed to evaluate and determine the bio-methane (CH_4) potential from cocoa husk (Darwin et al., 2014).

The temperature for this batch experiment was maintained under mesophilic condition at 35^{o}C. This temperature was selected as mesophilic temperature was considered as a feasible condition used for anaerobic digesters worldwide due to less energy consumption for biogas production. Mesophilic temperature used in anaerobic digesters also can generate a stable anaerobic digestion process since mesophilic bacteria typically are more tolerant to changes in environmental conditions compared with thermophilic bacteria (Kardoset al., 2011). Furthermore, the mesophilic temperature was applied in this batch experiment since the inoculums used for this experiment were derived from semi-continuous anaerobic reactors operated in mesophilic condition. Thus, it can reduce the time for anaerobic digestion process to acclimate where mesophilic bacterial population did not need the adjusting temperature and environment for their growth.

Five hundred ml of 0.4 N sodium hydroxide solutions was prepared and filled into filter flasks. The filter flask containing 0.4 N of sodium hydroxide solutions was connected from each reactor to the gas meter. Sodium hydroxide solutions used in this experiment was utilized for purifying biogas that contains some amount of CO_2 and H_2S; thus, biogas which appeared in the gas meter was methane gas only. In addition, study revealed that sodium hydroxide can be used to purify biogas generated from anaerobic digestion process since it can react with both carbon dioxide and hydrogen sulfide while it does not react with methane. Sodium carbonate will be formed once the carbon dioxide reacts with the sodium hydroxide (Zhao et al., 2010).

In addition to this experiment, before starting to run an anaerobic digestion process, each reactor was purged with nitrogen gas for about five minutes to get rid of oxygen traces to ensure anaerobic condition in the reactor. To prevent 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 para film. The duration of the experiment was determined by the point at which biogas production stopped completely, which was around 27 days of anaerobic digestion process. See Figure 1.

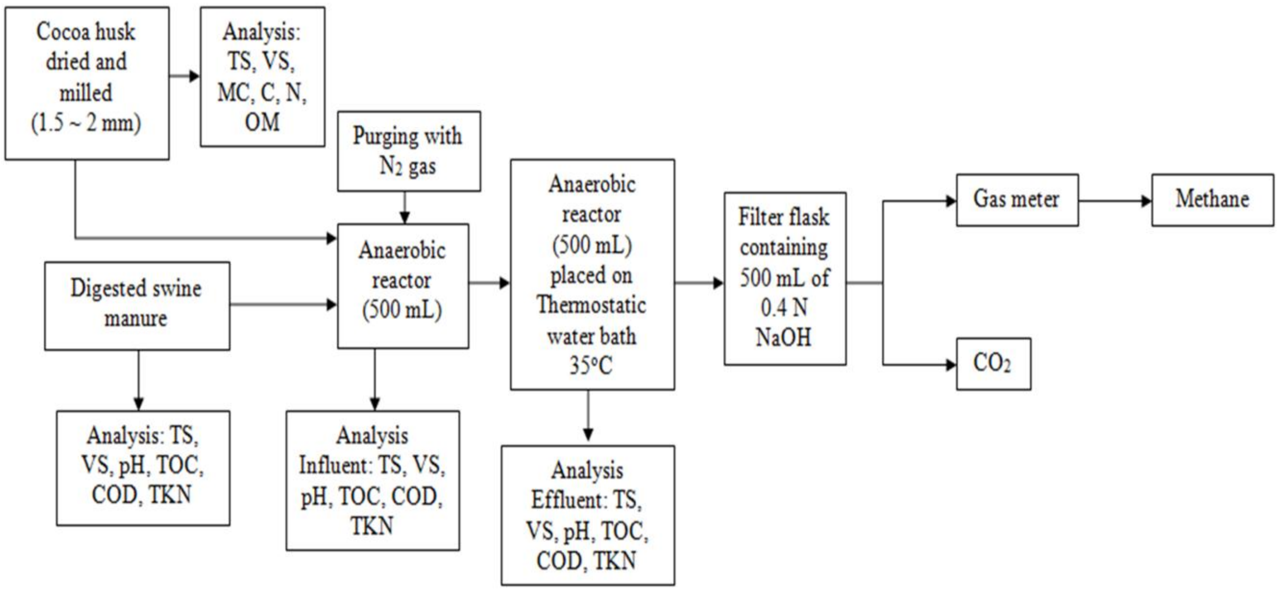

Figure 1 Process flow diagram for the experiment

2.3 Analytical methods and statistical analysis

The parameters analyzed for the characterization include moisture content (MC), organic matter (OM), carbon and nitrogen content of each substrate, total solids (TS), volatile solids (VS), pH, Total Kjeldahl nitrogen (TKN), total carbon (TC), total organic carbon (TOC), and chemical oxygen demand (COD). Methane production rates and methane yield measurements were conducted by using the method that has been developed in the previous studies (Loet al., 1984; Parawiraet al., 2008) where the rate of methane production was calculated based on the volume of bio-methane produced per day. Furthermore, methane yield was determined based on the cumulative methane produced per gram volatile solids added (Parawiraet al., 2008). Influent as well as effluent samples derived from anaerobic digestion process were also analyzed for pH, TS, VS, COD and TKN. TS samples were dried in an oven at 105^{o}C, and VS samples were burnt in the furnace at the temperature of 550^{o}C. All analytical assessments were measured based on the "Standard Methods" (APHA, 1998). Experimental data obtained while performing an anaerobic digestion process were statistically analysis with single factorial of analysis of variance (ANOVA) in triplicate at steady state conditions. In addition, data analyzed by using ANOVA test within 5% (α = 0.05) level of significance also assessed the influence of substrates loaded in the reactors with digestion parameters of batch experiment.

To analyze the effectiveness of the digestion process, some parameters including volatile solids reduction as well as COD removal were measured. The percent of volatile solids reduction was determined according to the formula developed by previous study (Joanne, 1991).

The Percent Volatile Solids Reduction Equation 1 is as follows:

$$\%VS\,Reduction = \frac{(\%\,VS\,In - \%VS\,Out)}{\%VS\,In - (\%VS\,In \times \%VS\,Out)} \times 100 \quad (1)$$

For the COD removal, it was measured by using Equation 2:

$$\%COD\,removal = \frac{(Initial\,COD - Final\,COD) \times 100\%}{Initial\,COD} \quad (2)$$

3 Results and discussion

3.1 Anaerobic co-digestion performance

The study was programmed to investigate methane potential of cocoa husk through anaerobic digestion process. This anaerobic digestion process was operated in mesophilic condition at 35°C. The physical-chemical characteristics of substrate are revealed in Table 1. The characteristic values mentioned in Table 1 show the abundance of organic matter of cocoa husk allowing the substrate to be feasible for anaerobic co-digestion with digested swine manure. Methane production can be enhanced as cocoa husk used contained a significant amount of organics solids as well as organic carbon that can be converted to methane. Initial characteristics of cocoa husk included volatile solids of 88% w/w, total solids of 93.4% w/w, organic matter of 58% w/w, carbon content of 45% w/w, and 1582.4 mg/L of COD. Total solids (TS) and volatile solids (VS) content are considered as a vital factor when substrate is loaded into anaerobic digester (Darwin et al., 2016) as the two parameters represented the amount of solid content as well as organic solid content that can be converted to volatile fatty acids and followed with methane formation during anaerobic digestion process. Further, the total solids is utilized to determine whether the digester has been sufficient for the amount of substrate coming in, and the volatile solids may be considered as a measure of the organic matter in the digester that can be converted into methane. In addition, the volumetric methane yield obtained from anaerobic digestion using agricultural residues was higher due to high volatile solids content per unit mass of feedstock (Asam, 2011). Also see Table 2.

Table 1 Characteristics of cocoa husk (wet basis)

Parameter	Unit	Quantity
Total solids, TS	% w/w	93.4
Volatile solids, VS	% w/w	88
Moisture content, MC	% w/w	6.7
Organic matter, OM	% w/w	58
Carbon content, C	% w/w	45
Nitrogen content, N	% w/w	1.4
Chemical Oxygen Demand, COD	mg/L	1582.4
C:N Ratio	-	33.1

Table 2 Characteristics of Inoculum

Parameter	Inoculum
TS, % w/w	0.98 ±0.04
VS, % w/w	78.2 ±1.64
COD, mg/L	13853 ±2962
TOC, mg/L	860 ±121.2
TKN, mg/L	566.7 ±92.4
pH	7.3 ±0.3

Cocoa husk has a high percentage of both total solids and volatile solids (Table 1). The percentage of carbon content of cocoa husk (CH) is also pretty high indicating that the substrate should be feasible for co-digestion with swine manure. Carbon to nitrogen ratio of cocoa husk is 33.1. However, this C:N ratio is still not appropriate to enhance methane production through anaerobic digestion as the optimum C:N ratio for anaerobic digestion is about 20 to 25:1 (Yen and Brune, 2007). Therefore, by co-digesting this substrate with animal manure, it may enhance the performance of anaerobic digestion process to generate methane production.

Table 3 shows the influent data derived from the anaerobic co-digestion of cocoa husk with digested swine manure. All anaerobic digesters were operated in the optimum pH between 6.5 and 8.0. This condition may support the anaerobic microorganisms for converting organic wastes into methane as a major product of anaerobic digestion process (Cheng, et al., 2010). This result is in agreement with the previous study revealing that the anaerobic digestion process performed in pH between 7 and 8 was found to be effective for breaking volatile suspended solids as well as total suspended solids during the anaerobic digestion (Dinamarca et al., 2003).

Table 3 Influent data

Parameters	Control	Coco husk co-digested with swine manure, CH
Total organic carbon, mg/L	994	793
Chemical oxygen demand, mg/L	13500	36450
Total Kjedahl nitrogen, mg/L	575	830
Volatile solids, % w/w	77	78
Total solids, % w/w	0.95	1.88
pH	7.16	7.28

As shown in Table 3, COD of CH reactors (36450 mg/L) were higher compared with COD of control reactors (13500 mg/L). However, total organic carbon represented in TOC of CH reactors was lower compared with TOC of control reactors. It occurred as cocoa husk containing a significant amount of protein may generate higher in nitrogen content. It can be noticed that total organic nitrogen represented in TKN of cocoa husk is extremely higher compared with control reactor. Thus, this condition leads cocoa husk reactors to have a lower TOC compared to the control reactors. Based on the experimental results, CH reactors containing manure co-digested with cocoa husk had C:N ratio of 7.74:1. This C:N ratio is lower than an optimum C:N ratio which is about 20 to 25:1. The low C:N ratio of CH culture may potentially inhibit the anaerobic digestion process as it may indicate an accumulation of ammonia in the digester, which is toxic to methanogenic bacteria. Further, another study also revealed that C:N ratio less than 10:1 was susceptible to being inhibitory (Kimchie, 1984).

CH digesters operated in mesophilic condition performed well compared to control reactors (Figure 2). A lag phase occurred at the beginning of the anaerobic digestion process. It can be noticed that CH digesters generated 60.7±7.5 ml CH_4 at the first day of the digestion process. This result was higher compared with control reactors where at the first day of digestion process they started to generate methane at 29±10.2 ml CH_4.

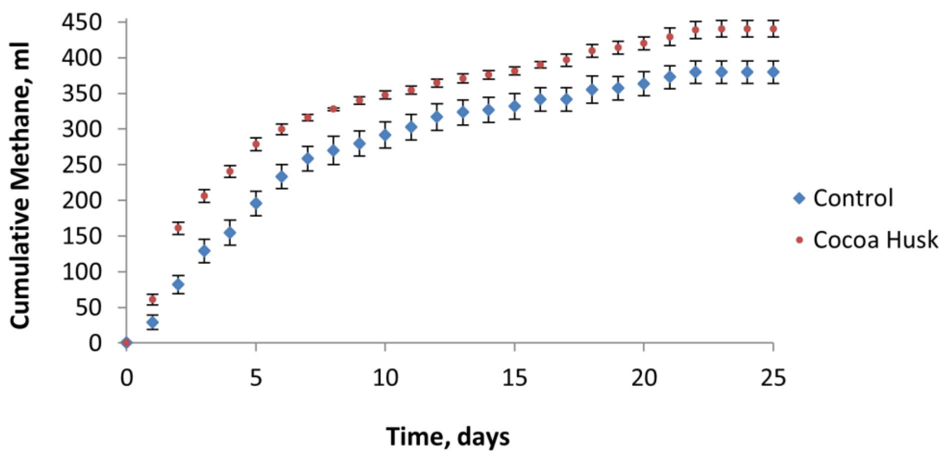

Figure 2 Cumulative methane production of cocoa husk and control reactor

A considerable increase of methane production between two and six days of digestion process occurred in cocoa husk reactors (Figure 2). They continuously produced methane until reaching a peak at 23 days of digestion process (440.3±10.2 ml CH_4). This condition was different from control reactors where methane production reached an asymptote at 21 days of digestion process (372.7±15.9 ml CH_4). This phenomenon indicated that adding cocoa husks as co-substrate in anaerobic digestion can enhance methane production compared with animal manure alone.

Each reactor still performed in the optimum pH range for anaerobic digestion process (Table 4). Even though CH reactors produced more methane compared with control reactors, they only produced about 16% higher compared with control reactors (Figure 2). The performance of each reactor during anaerobic digestion process also can be known where TS reduction of CH reactors was almost double (15.1%±0.5%) compared with control reactors, where control reactors only had TS reduction at about 8.3%±0.7% (Table 3 and Table 4). This phenomenon also may be understood by referring to Table 2, where cocoa husk virtually contained a significant amount of nutrients required for biogas and methane production such as high amount of carbon content, high volatile solids and total solids content. Therefore, it may be believed that lignin content of cocoa husks was considered as a source of barriers that hindered this substrate for being converted into biogas as well as methane (Alemawor et al., 2009). Statistical analysis by applying ANOVA test with 5% level of significance showed that there is significant difference between substrate loaded and effluent digestion parameters (pH, TKN, COD, TOC, VS, TS, and methane production) within anaerobic digestion process of cocoa husk (p value=2.76×10^{-17}; F_{test}=467.5; F_{crit}=2.66; df=7).

Table 4 Effluent data

Parameters	Control	CH
Total organic carbon, mg/L	482 ±48	536.21 ±19
Chemical oxygen demand, mg/L	10767 ±677.2	20020 ±1583.4
Total Kjedahl nitrogen, mg/L	621.2 ±7.0	840 ±3.23
Volatile solids, % w/w	73.8 ± 0.23	73. 6 ± 0.13
Total solids, % w/w	0.87 ± 0.01	1.6 ± 0.01
pH	6.81 ± 0.2	6.92 ± 0.15

3.2 Biodegradation efficiency of anaerobic digestion process

Biodegradation is a process to convert organic (carbon-based) materials from complex or insoluble molecules into simpler or soluble molecules through chemical as well as biological process. Some researches revealed that methane production is extremely influenced by biodegradation and availability of the major components contained in biomass, such as carbohydrates, protein, and lignin contents (Contreraset al., 2012; Darwin et al., 2014; Kalra and Panwar, 1986). Low methane production obtained in this experiment indicated that the process of co-digestion of cocoa husk with swine manure did not perform very well. High cell wall content of cocoa husk was also believed as a source of inhibition during anaerobic digestion process (Darwin et al., 2016; Tuah and Orskop, 1987).

Methane yield presented in terms of ml CH_4/gVS added indicates the biodegradation efficiency. The digestibility and composition of the substrates was the major determinant of maximum methane yield (Wilkie, 2005). The study also revealed that several factors that influence methane yield include temperature, loading rate, biodegradability, and retention time (Wilkie, 2005).

In addition, ANOVA analysis revealed that there is statistically significant difference between substrates loaded and biodegradation efficiency parameters (VS reduction, COD removal, methane yield) within anaerobic digestion process (p value = 1.29×10^{-22}; F_{test}=427; F_{crit}=2.75; df=4). Results showed that CH reactors still produced more methane daily compared to control reactors and they still operated in the optimum pH range for anaerobic digestion. This indicated that co-digestion process still benefit to stabilize the digester by maintaining optimum pH and enhance methane production.

CH reactors generated more methane production compared with control reactors. However, CH reactors had less methane yield compared with control reactors (Table 5). It indicated that cocoa husk was not degraded completely during anaerobic digestion process. This condition also can be understood by evaluating other biodegradation parameters including COD removal and VS reduction. As presented in Table 5, CH reactors only had slightly higher of COD removal and VS reduction (45.1%±4.3%, 19.9%±0.5%, respectively) compared with control reactors (20.3%±5.0%, 14.7%±1.0%, respectively).

Table 5Biodegradation efficiency

Parameters	Control	Cocoa husk
Methane yield, ml CH_4/g VS added	104.12 ±4.42	60.31 ±1.58
COD removal, %	20.3±5.03	45.11 ±4.37
Total methane accumulated, ml	379.7±16	440.32 ±11.61
VS reduction, %	14.7±1.0	19.9±0.5

Although CH reactors produced more methane compared with control reactors, they still experienced any inhibition during anaerobic digestion process leading to low biodegradation efficiency. 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. Further, as lignocellulosic biomass has a complex structure it provides a major protective barrier that may prevent cell destruction by biological as well as chemical process. This condition may cause a lower digestion rate that will reduce biogas production. To deal with this issue, in the future research, pretreatment should be taken into consideration and applied in order to enhance digestibility of lignocellulosic biomass. The study also revealed that by pre-treating biomass, it may enhance the hydrolysis process leading to an increase of total methane yield (Hendriks and Zeema, 2009). Another study added that the chemical composition as well as physical structure of lignocellulosic biomass may be converted by applying several pretreatments. Thus, it can induce the composition in lignocellulosic biomass to be more readily biodegradable and more accessible to microorganisms during the anaerobic digestion process (Panget al., 2008).

4 Conclusions

This study has shown that adding cocoa husk as co-substrates in anaerobic digestion may enhance methane production, and stabilize the process through maintaining optimum pH between 6.9 and 7.2. The maximum methane productivity of CH and control reactors within 25 days of digestion process was 345.8±7.82 and 286.97±16.8 ml/d, respectively. Biodegradation efficiency evaluated for CH and control reactors revealed that CH reactors had lower methane yield compared with control reactors where methane yield of CH and control reactors was 60.31±1.58 and 104.12±4.42 ml CH_4/g VS added, respectively. This indicates that there is any inhibition occurred in CH digesters that lead to lower biodegradation efficiency. High cell wall content or lignin content of cocoa husk was still believed as the source of barrier during anaerobic digestion process, where lignin cannot be degraded during anaerobic digestion process.

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