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Biochemical Methane Potential (BMP) of Cattle Manure, Chicken Manure, Rice Straw, and Hornwort in Mesophilic Mono-digestion

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Abstract: Biochemical Methane Potential (BMP) assay was used to investigate the potential of methane production from agricultural wastes and weed. The objective of the study is to investigate the methane production potential from cattle manure, chicken manure, rice straw, and hornwort (*Ceratophyllum demersum*). The result of the study can be used to choose the most suitable substrate for renewable energy generation as well as to prioritize waste treatment to reduce Greenhouse Gases (GHGs) emission from waste. Cattle manure (CWM), chicken manure (CHM), rice straw (RSW), and hornwort (HNW) were used as substrates for batch anaerobic digestion under mesophilic condition at 35 °C using 500 mL glass bottles and working volume of 350 mL with substrate at inoculum to substrate ratio (ISR) of 1:1 based on Volatile Solids (VS) weight (g VS). Parameters observed including biogas production, biogas composition, methane production, and specific methane yield. Results showed that among four substrates, RSW had the highest total biogas production of 3773.33 mL, while the lowest was CHM with 1443.00 mL. In term of the biogas composition, HNW had the highest methane proportion of 66.68% among all substrates used, while RSW had the lowest proportion (62.50%). Furthermore, the highest methane production was from RSW at 2135.52 mL and the lowest was from CHM at 736.28 mL. In addition, the highest specific methane yield was obtained from RSW with a total of 331.99 NmL CH_4/g VS, while CHM had the lowest yield with 114.55 NmL CH_4/g VS. From results of the study, RSW was found to be a very promising substrate for a potential source of renewable energy with a high methane yield.

Keywords: BMP, livestock manure, poultry manure, paddy straw, *Ceratophyllum demersum*

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1. Introduction

Biomass is one of the most important potential sources for bioenergy generation. Nowadays, renewable energy generation from biomass is merely focused on the use of waste or residual materials. Manure from livestock farming and residual material from rice cultivation (e.g. rice straw and husk) are examples of viable options for waste utilization for bioenergy. Livestock farming produces manure which naturally emits methane and may become good sources of bioenergy if processed in a sustainable way. Rice straw is an abundant resource and may be an example of agricultural waste that suitable for bioenergy generation. Fast growing weeds in the aquatic ecosystem (e.g. hornwort - *Ceratophyllum demersum*) are also abundant and may also become a resource for bioenergy generation. However, information about the methane production from mono-digestion of cattle manure, chicken manure, rice straw, and hornwort in a mesophilic condition is limited.

The objective of the study is to investigate the methane production potential from cattle manure, chicken manure, rice straw, and hornwort (*Ceratophyllum demersum*) in a mesophilic condition. The result of the study can be used to choose the most suitable substrate for renewable energy generation as well as to prioritize waste treatment to reduce Greenhouse Gases (GHGs) emission from waste.

Nomenclature	
GHG	: Greenhouse Gas
BMP	: Biochemical Methane Potential
AD	: Anaerobic Digestion
OLR	: Organic Loading Rate
CWM	: Cattle Manure
CHM	: Chicken Manure
RSW	: Rice Straw
HNW	: Hornwort
INOC	: Inoculum
TS	: Total Solids
VS	: Volatile Solis
SMY	: Specific Methane Yield

2. Literature Review

Biochemical Methane Potential (BMP) is a method to determine the potential methane production from an organic matter through the anaerobic digestion process. Specific Methane Yield (SMY) reported in the volume of CH4 per gram VS added describes the methane production or the yield of a specific substrate. Therefore, BMP can be conveniently determined using the SMY. The method was recommended by Angelidaki, et al. [1] for solid organic wastes and energy crops biodegradation.

Agricultural wastes were among the most promising substrates for biogas production through anaerobic digestion due to availability in amount, distribution, continuity, low price, as well as its status as a waste or byproduct. For example, the global population of cows and chicken as the two most common and popular livestock are 1,474,526,581 and 21,409,683,000 heads respectively [2]. Furthermore, with an annual liquid manure production of 19.8 m^3 for cow and 0.07 m^3 for chicken [3], it can be estimated that cow manure and chicken manure are produced globally at 29 billion m^3 and 1.5 billion m^3 annually. Moreover, rice straw is estimated to be produced annually at 150 million tons in Southeast Asia only [4]. On the other hand, aquatic plant like hornwort (*Ceratophyllum demersum*) may serve as a potential substrate for biogas due to its vast geographical distribution and rapid growth [5]. Cattle manure is a common substrate for anaerobic digestion with methane productivity of 0.17 NL/g VS [6]. Chicken manure has been investigated as a potential feedstock for anaerobic digestion system with a result of 126.9 mL/g VS [7]. In a recent study, rice straw showed methane productivity at 178.3 mL/g VS using manure inoculum [8]. Another study showed methane productivity at 136.3 L/kg VS using wet thermophilic process, while in the dry mesophilic process the productivity was 123.5 L/kg VS [9]. A study in Japan reported a methane productivity of hornwort at 249 mL/g VS [10].

The anaerobic digestion process consists of four stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis [11]. The hydrolysis stage in lignocellulosic biomass is mostly comprised of degradation of cellulose and hemicellulose into smaller monomers and oligomers, while lignin is slowly and only partially degraded [12]. Factors affecting biogas and methane production including Organic Loading Ratio (OLR), C/N ratio, temperature, pH, retention time [13], and inhibitors [14]. Higher temperature (55-70 °C) contributes to higher methane production than lower temperature (37 °C). However, higher temperature has been found to have lower process stability compared to those of lower temperature. The ideal pH range for anaerobic digestion (AD) is around 6.8 - 7.4. The optimum C/N ratio for the AD process is found to be in the range of 20 - 30 or 20 - 35 [13]. On the other hand, ammonia or ammonium ion, sulfides, heavy metals, phenols, LCFA, and lignin have been found to have inhibitory effects on the AD process [14].

3. Methodology

This study used a proposed BMP protocol [1] with small modifications. Cattle manure (CWM), chicken manure (CHM), and inoculum (INOC) were obtained from Prince of Songkla University (PSU) Demo Farm in Pattani, Thailand. Rice straw (RSW) was obtained from Pattani Rice Research Center (PRRC) in Pattani, Thailand. Hornwort - *Ceratophyllum demersum* (HNW) was obtained from BioMEC laboratory collection in Pattani, Thailand. The CWM and CHM were put in the plastic bags and then stored in a refrigerator at 4 °C after collection until ready for use. Rice straw and hornwort were dried at 40 °C for three days to be suitable for grinding and then kept in plastic bags at ambient temperature until ready for use. An electronic grinder (Retsch, SM100) equipped with 1 mm sieve was used to grind the rice straw and the hornwort. The inoculum was obtained from the effluent of a running anaerobic digester treating cattle manure and put in a sealed container at ambient temperature after collection. The inoculum has been tested for methane production by putting the inoculum into empty batch digesters and observing the gas production and composition. The inoculum was considered active when the gas produced contains methane. Moreover, to achieve a sustainable process, all required nutrients must be fully supplied by the substrate, and thus in the experiment, this assumption was followed and deionized water was used to mix the substrate.

The inoculum, cattle manure, chicken manure, rice straw, and hornwort were analyzed for Total Solids (TS), Volatile Solids (VS), ash content as well as C, H, O, N, S, and P content. The C, H, O, N, and S content were determined using CHNS-O Analyzer (Thermo Quest, CE Instruments Flash EA 1112 Series for cattle manure, chicken manure, rice straw, and hornwort; and ThermoScientific, Flash 2000 for the inoculum) using dynamic flash combustion. The P content was determined using ICP-OES method (Perkin Elmer, Optima 8000). Next, 250 mL of inoculum was put on 500 mL clean glass bottles. Cow manure, chicken manure, rice straw, and hornwort was added in separate bottles for each substrate at 1:1 ratio to inoculum in a VS basis as the 1:1 ratio was considered as an optimum ratio [15]. Each bottle was then filled with deionized water to adjust to 350 mL and then stirred thoroughly. Bottles were then sealed with rubber septa and secured with aluminum clamp caps. Every bottle was flushed with pure nitrogen for 5 minutes and then put into incubator maintaining a constant temperature of 35 °C. Bottles were shaken after gas measurement. All treatments were done in triplicate. The diagram of the batch digester is given in Fig. 1.

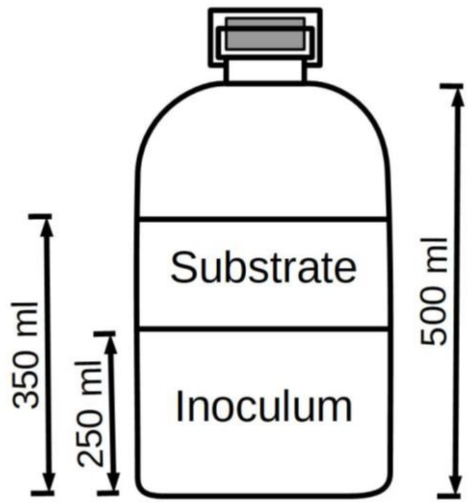

Fig. 1 - Schematic diagram of the batch digester

Parameters observed including biogas production, biogas composition, methane production, and specific methane yield until biogas ceased to produce or the biogas production reached the stationary phase. Biogas production (gas volume) and biogas composition were measured daily in the first 20 days, then measured once in two days on day 20 to day 62, and measured once in three days on day 65 to day 74. Gas volume was measured using gas displacement column (expressed in mL). Gas composition was measured using GC-TCD (Shimadzu, GC14A). Gas composition was expressed in percent (%) and focused on the methane fraction. The methane production was calculated from the percentage of methane in the biogas multiplied by the gas volume, expressed in mL. Specific Methane Yield (SMY) was calculated from methane production divided by the organic fraction (VS) added and was expressed in mL CH_4/g VS.

4. Results and discussions

Substrates used in this study were first analyzed for its physical and chemical characteristics. The results of the characterization were provided in Table 1 and Table 2.

Table 1 - Physical characteristics of CWM, CHM, RSW, HNW, and INOC

Substrates	Total Solids (%)	Moisture Content (%)	Volatile Solids (%)	Ash (%)
CWM	20.07	79.93	86.14	13.86
CHM	23.30	76.70	77.29	22.71
RSW	89.54	10.46	85.95	14.05
HNW	85.57	14.43	77.05	22.95
INOC	3.77	96.23	61.52	38.48

Table 2 - Chemical characteristics of CWM, CHM, RSW, HNW, and INOC

Item	C (%)	H (%)	O (%)	N (%)	S (%)	P (mg/kg)	C/N Ratio
CWM	40.48	5.29	29.66	1.37	0.211	5312.11	29.55
CHM	37.25	5.01	28.56	3.09	0.609	19035.30	12.06
RSW	37.45	5.10	33.81	0.412	0.061	637.49	90.90
HNW	33.81	4.93	28.42	3.37	0.381	12716.07	10.03
INOC	0.46	10.46	80.51	0.12	NA	212.25	3.83

The CWM and CHM both had low dry matter content, while RSW and HNW had higher dry matter content due to the drying process for grinding to 1 mm in size during preparation. The CWM and RSW showed high VS content at 86.14% and 85.95% while CHM and HNW had lower VS content at 77.29% and 77.05% respectively. The INOC was liquid in form and had very low dry matter content, whereas the ash content was very high. In addition, the chemical analysis showed that the C, H, O, N, S and P content of the substrates had a different characteristic. For example, the CWM had the highest C element and if compared with the N component, the ratio was 29.55. The RSW had a very high C/N Ratio at around 90.90 due to a limitation of the N. The CHM and HNW had a low C/N ratio due to high Nitrogen content at around 12.06 and 10.03 respectively. Moreover, the INOC had a C/N ratio of only 3.83 and was the lowest compared to substrates.

The characteristics of CWM were different from another study with TS at 9.3% and VS at 81.7% [16] but very similar to a study in Nigeria with TS at 20.62% and VS at 89.70% [17]. The total solids content of the CHM was similar to a study at 25%, while the volatile solids content was far higher compared to result in the same study at 58% [18]. Furthermore, the C/N ratio in this study was also higher compared to the result of that study at 4.37 [18]. Moreover, results of the experiment showed that RSW characteristics were quite different from a recent study [8] which reported TS at 97.3±0.3%, VS at 84.0±0.5%, ash at 13.6±0.3%, and C/N ratio at 58.6 respectively. The characteristics of hornwort had a small difference from another study which reported the total solids at 5.11% and volatile solids at 78.30% [19]. Metal cations are present in the substrate as the ash content and may be released during the biodegradation process [14]. The high content of alkali/metal cations in the ash of the substrates may provide buffering capacity to stabilize the AD process. In addition, the alkali in the ash may provide a slightly basic condition in favor of methanogenesis. It may also reduce the ammonia toxicity and also known to stimulate biogas generation and methane yield as shown by a previous study [14].

From the results of chemical characterization, RSW may be suitable for co-digestion with other substrates having low C/N ratio, due to its high C content which will balance the high N content. The CWM already had suitable C/N ratio for the AD. On the other hand, CHM and HNW had a low C/N ratio and may not be suitable for mono-digestion using the INOC which had even lower C/N ratio. The optimum C/N ratio ranged from 20 to 35 [13].

From BMP experiments in this study, results showed that CWM, CHM, RSW, and HNW had the potential for biogas production and renewable energy generation. The biogas production of four substrates is shown in Fig. 2.

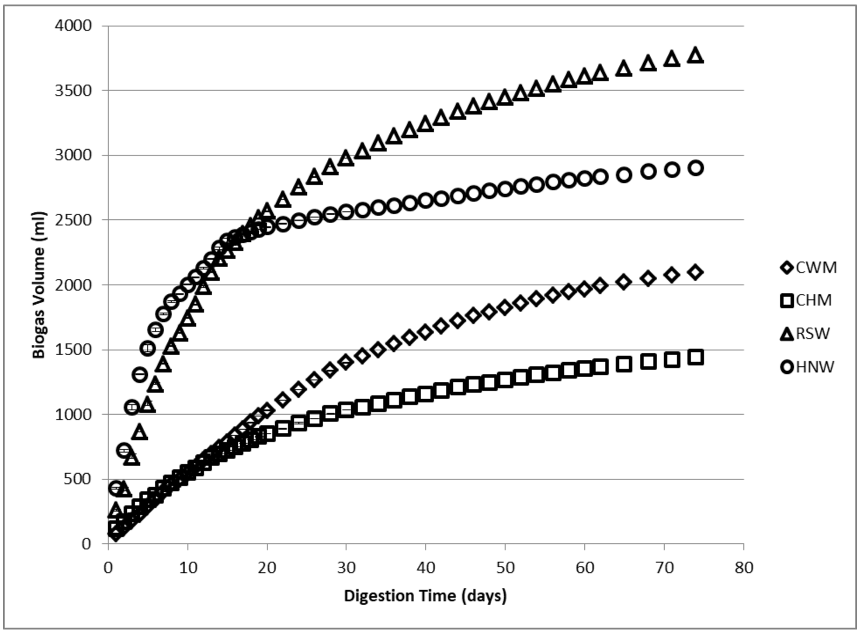

Fig. 2 - Cumulative Biogas Production of CWM, CHM, RSW, and HNW

The cumulative biogas production of four substrates in Fig. 2 shows that RSW and HNW had high biogas potential. The biogas productions of the two substrates were far higher than the productions of CWM and CHM respectively. At the end of 74 days period, RSW had the highest cumulative biogas production. It was followed by HNW with a slight difference and tailed by CWM and CHM with 3773.33 mL, 2907.00 mL, 2096.33 mL, and 1443.00 mL respectively.

Fig. 2 shows that the biogas has been produced from day 1. The HNW was very quickly and easily digested in the first 17 days and then reached stationary phase afterward. The RSW was also digested quickly and the biogas production continued until day 35 where the process finally slowed down. The CHM was digested faster than the CWM in the first 10 days and then quickly slowed down until reaching stationary phase. The CWM was digested slowly and consistently until slowing down on day 35. These patterns indicate that substrates with low C/N ratio were digested relatively faster in the beginning and then reached stationary phase earlier compared to substrates with high C/N ratio. This phenomenon may be caused by ammonia inhibition due to the high content of N in substrates. The ammonia is produced during anaerobic digestion from N-rich substrates, mainly proteins and urea [14]. Ammonia inhibition has been recognized to affect anaerobic digestion process from reduced performance to severe cases [20]. Moreover, the RSW and CWM were digested slower compared to HNW and CHM respectively. This phenomenon perhaps due to RSW and CWM contain lignocellulosic complex which was harder to break down, where hydrolysis is the most critical and become the rate-limiting step during the anaerobic digestion process [21]. Regarding the process rate, it is possible to have an anaerobic digestion process without lag phase as shown in a recent study using fruit and vegetable waste co-digested with domestic primary sewage sludge [22]. Furthermore, RSW and HNW had higher biogas production compared to CWM and CHM. This could be due to the fact that both CWM and CHM were animal wastes where the digestible portions have been absorbed in the animal digestive tract, and the hardly-digestible portions remain in the wastes, which is similar with findings from a recent study [23]. Furthermore, the biogas composition in this study was determined and the result is shown in Fig. 3.

Fig. 3 shows that the biogas composition, in regard to methane content, was slightly varied among four substrates. The CWM, CHM, RSW, and HNW had stabilized methane content in the biogas at 62.51%, 64.88%, 62.50%, and 66.68% respectively from day 17 onwards. This result is in accordance with the literature, where methane content in the biogas is usually in the range of 55 - 65% [6]. Interestingly, the methane content in the biogas produced from HNW and CHM which has low C/N ratio was slightly higher compared to those from RSW and CWM with higher C/N ratio. This phenomenon could be attributable to the possibility of high pH in the system due to high ash content, the presence of ammonium ions from degradation of N-rich substrates, or combinations thereof. A pH between 6.5 and 8.2 was known to be in favor of and most efficient for methanogenesis [13]. Another possible explanation is that in high pH, the CO2 in the biogas was absorbed by alkali ions [6], hence the CO2 proportion in the biogas was reduced and thus, the methane proportion was increased.

The methane production from CWM, CHM, RSW, and HNW was calculated from the methane portion of the biogas produced. The cumulative methane production is provided in Fig. 4.

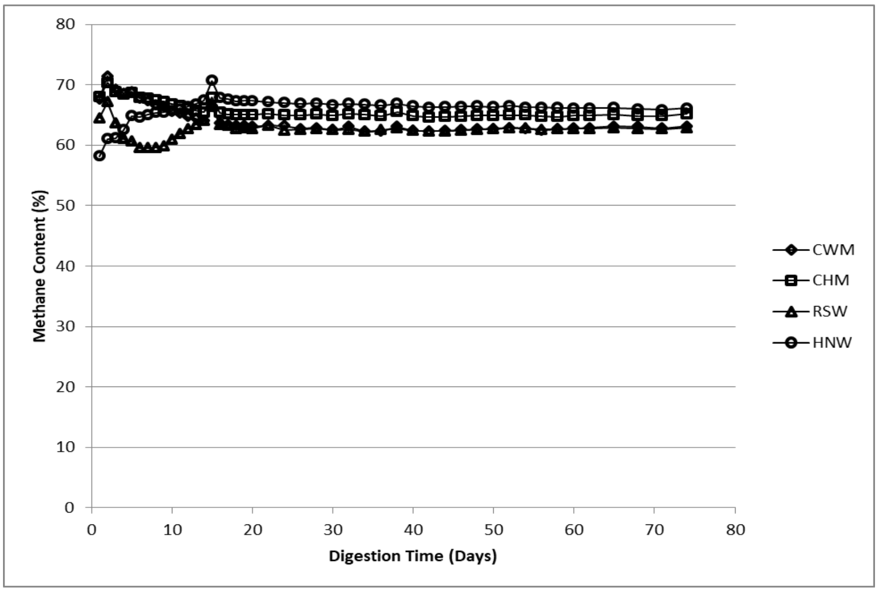

Fig. 3 - Biogas Composition of CWM, CHM, RSW, and HNW from BMP experiments

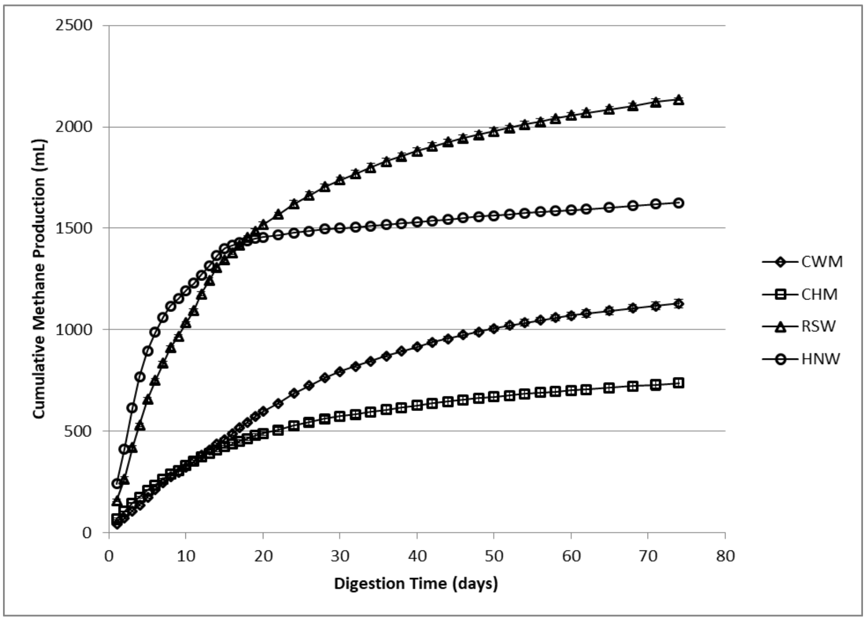

Fig. 4 - Cumulative Methane Productions of CWM, CHM, RSW, and HNW

The methane production was tightly related with biogas production and its composition. The methane production of RSW and HNW were at 2135.52 mL and 1625.83 mL respectively, much higher than CWM and CHM at 1128.96 mL and 736.28 mL respectively. The methane production values were used to calculate the SMY by dividing the values with the amount of substrate added (in VS basis). The highest SMY was obtained from RSW with 331.99 NmL CH4/g VS while the lowest one was from CHM with 114.55 NmL CH4/g VS. The SMY of HNW was less than RSW with a little difference at 255.23 NmL CH4/g VS. On the other hand, the CWM had a little higher SMY than CHM with 175.79 NmL CH4/g VS.

Results of this study showed that the methane yield of CWM was similar to a recent study at 0.17 NL CH4/g VS [6]. Moreover, the methane yield of HNW was comparable to another study in Japan with a result of 249.2 mL CH4/g VS [10]. Furthermore, the methane yield of CHM from the experiment was found to be slightly lower than a recent study at 126.9 mL/g VS [7]. This is probably due to different inoculum used in the experiment, the correction for gas measurement at STP condition, or combinations thereof. In addition, a higher methane yield from RSW compared to another study at 178.3 mL/g VS [8] was observed in this study. This could be caused by several factors. First of all, the particle size of RSW in this study was smaller (1mm) compared to 8 mesh (2.38 mm) in the former study [8]. A smaller particle size is more advantageous in the anaerobic digestion process compared to bigger particle size. Small particle size has the bigger surface area, and it has a lower degree of cellulose crystallization. Both factors may improve the substrates' biodegradability and thus, biogas production [24]. Secondly, the characteristics of RSW used in this study were slightly different from the former study. In this study, it had a higher C/N ratio compared to the former study at 58.6 [8]. The high C/N ratio prevents ammonia inhibition [13] and promotes the growth of methanogens [25], and could possibly increase the methane production.

5. Conclusion

From results of the study, RSW was found to be a very promising substrate for a potential source of renewable energy with a high methane yield. In addition, HNW is also attractive given that the supply could be guaranteed for its availability and continuity. On the other hand, CWM and CHM were less attractive due to lower methane production compared with the other two substrates. Therefore, RSW can be used to enhance the biogas/methane production by co-digestion with manures or other N-rich substrates.

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