



## Anaerobic digestion of municipal organic waste by using *Hydrilla verticillata* (Linnaeus f.) Royle as a co-substrate

Wichidtra Sudjarid<sup>1,\*</sup> and Pita Jarupunphol<sup>2</sup>

<sup>1</sup>Department of Environmental Science, Faculty of Science and Technology,  
Sakon Nakhon Rajabhat University, Sakon Nakhon, Thailand, 47000

<sup>2</sup>Department of Information Technology, Faculty of Science and Technology,  
Phuket Rajabhat University, Phuket, Thailand, 83000

### Abstract

This article studied effects of temperature and co-digestion ratios on biogas production under anaerobic condition. The substrate was collected two major sources: 1) municipal organic waste from Sakon Nakhon Rajabhat University and 2) *Hydrilla verticillata* (Linnaeus f.) Royle from Nong Han Lake. The substrate was operated to obtain the initial pH range between 4.73–5.62 and the BOD:N:P ratio 100:4.25–6.27:0.4–1.23 in order to support biodegradation process sufficiently. The BOD:COD of MOW and Aquatic plant was 0.67 and 0.88 respectively. The ratio could imply significant improvement of accumulative biogas production at ratio 1:1-4:1 (MOW:Aquatic plant) at ambient and mesophilic condition. The ratio could imply significant improvement of accumulative biogas production at ratio 4:1 (MOW:Aquatic plant) at mesophilic and thermophilic conditions. The biogas composition concentration could produce the highest rate of CH<sub>4</sub> at 215 M at ambient temperature (18-31°C), 212 M at mesophilic at ratio 4:1. The H<sub>2</sub> could occur maximum at 201M in ratio 3:2 at ambient temperature and 72 M in ratio 2:3 at mesophilic. However, the operation under thermophilic incubation appeared to retard the biogas production.

**Keywords:** biogas, *Hydrilla verticillata* (Linnaeus f.) Royle (Aquatic plant), Municipal Organic Waste (MOW), temperature, co-substrate

### 1. Introduction

His Majesty King Bhumibol Adulyadej initiated several alternative energy sources, e.g., biodiesel, biomass, and biogas. Simplicity, economical concern, community participation, and environmental friendliness are examples of the King's fundamental principles which influence on a considerable number of Thailand's national projects. According to his guideline, several types of renewable energy are considered as clean sources of energy production with minimal environmental impact.

Approximately 61 million ton a year of agricultural material residues were produced in 1997 which can be used for biogas production [1]. Nong Han Lake, locating in Sakon Nakhon province, has confronted eutrophication and widespread of aquatic plant problems for several decades. Domestic wastewater from surrounding area and wastewater from treatment plant were continually discharged to the lake for a long time. Biogas and methane could be produce from algal biomass because of its composition of carbohydrates, proteins, and lipids. In addition to various types of biogas feedstock, municipal organic waste (bio-waste) also has a great potential [2]. This digestion process consists of three different bacterial communities: fermentative, acetogenic, and methano-

genic bacteria. Several environmental factors, including temperature and pH, affect the potential of biogas production. This study aimed to enhance the biogas production in municipal organic waste by using the substrate of *Hydrilla verticillata* (Linnaeus f.) Royle as co-substrate. In addition, the environmental problems caused by these aquatic plants can be reduced.

### 2. Literature Reviews

The evaluation of agricultural wastes for biogas production illustrated that co-digestion of food scraps with agricultural waste could improve the biogas generation from cow dung [3]. Moreover, the duckweed can be a potential source of feedstock in anaerobic digestion process utilized as major production [4]. Besides, DeBere [5] suggested that a small amount of organic solid co-substrate added to manure digesters could improve energy yielding industrial sludge. According to Zong *et al.* [6], the anaerobic digestion of Taihu blue algae using corn straw as a co-substrate could significantly improve the digestion performance of biogas production. Biogas generation was enhanced by adding domestic sewage to municipal solid waste [7]. Solid-state anaerobic digestion was ineffective at mesophilic temperature but thermophilic operation could accelerate the process [8]. However, Speece [9]

\*Corresponding author; e-mail: wichidtra.s@snu.ac.th

**Table 1** Characteristics of substrates used in this study

Parameters	Municipal Organic Waste	Aquatic Plant
pH	4.73	5.62
COD (mg/L)	77,400	25,200
BOD (mg/L)	68,000	17,000
TS (mg/L)	178,500	65,500
VS (mg/L)	81,000	50,500
TKN (g/kg)	3.29	1.58
Total Phosphorus (g/kg)	0.31	0.31

reported that the digestion process could operate at mesophilic (30-37°C) or thermophilic (45°C) conditions and present difficulties at thermophilic 55°C condition. Therefore the capability of biogas production using substrate addition and temperature control should be considered and experimented.

### 3. Methodology

This section contains methodology used in this research.

#### 3.1 Substrates and their preparation

Two substrates used in this study were aquatic plant (*Hydrilla verticillata* (Linnaeus f.) Royle) and municipal organic waste (MOW). The aquatic plant was collected from Nong Han Lake located in Sakon Nakhon Province and MOW was collected from Sakon Nakhon Rajabhat University. Aquatic plant and natural water lake were mixed at ratio 1:1 (w/v), then shredded and used as co-digestion. While the MOW was directly shredded by two-blade turbines. The substrates were those homogeneously into particle smaller than 2 mm and they were used as an initiating substrate.

#### 3.2 Reactor

Seven ratios by weigh to volume of municipal organic waste to aquatic plant were 4:1, 3:2, 1:1, 2:3, 1:4 and a 100% of each substrates were performed. The initiating substrate was used at 50 mL of 120 mL serum bottle with duplication experiment.

#### 3.3 Determination of temperature on biogas production

This study was conducted at ambient temperature (18-31°C), mesophilic (31°C) and 3) thermophilic (55°C) temperatures with duplication were performed.

#### 3.4 Biogas quantitation and qualification

The accumulative biogas production was analyzed by the displacement of fluid by producing gas. The qualitative analysis of biogas production was detected by Shimadzu Gas Chromatography System (GC-2014) equipped with thermal conductivity detector (TCD) and packed column coupled with Polapak-w (50/80), 0.3 m length and 2.00 mm thickness and

diameter. The analytical program was operating temperature was remained in the column at 45 °C and holding for 7 minutes. Helium gas was used as carrier gas as 170 mL/min.

## 4. Results and Discussion

The experimental results are discussed below.

### 4.1 Substrate characteristics

Characteristic substrates were examined and expressed in Table 1. Their pH were ranged 4.73-5.62 which were appropriated for enhancing the efficiency of CH<sub>4(g)</sub> gas production as reported by Latinwo and Agarry [2]. pH is an important factor that affects anaerobic digestion [10]. At beginning the pH quite low, it could be supported fermentative bacteria growth to break down the highly molecular compounds to produce soluble molecules and acetogenic methanogens could break down organic, inorganic and fatty acid compounds [10]. The BOD:COD ratios of these substrates were 0.67 - 0.88. The BOD:N:P ratio of 100:4.25:0.4 was used as a benchmark of carbon and nutrient sources and that of 100:6.27:1.23 was reserved for the aquatic plant. It implied that the substrate was highly biodegradable and adequately nutrient supplement in the anaerobic digestion process [9]. Furthermore, the proportion of VS:TS was quite low; it means that variable microbes were available in fermentative process [11]. The summarizations of the results were represented in Table 1.

### 4.2 Organic compounds degradation

The degradation of organic compounds in this experiment was measured by the reduction of COD value at the initial to the final state. The results showed that the most optimal condition of organic compound degradation was performed at ambient temperature in percentage of 37 and 29 in MOW and aquatic plant respectively. The degradation of MOW was at mesophilic quite slightly better than that at thermophilic temperature. The results were shown in Table 2.

**Table 2** Percentage of organic matter converting by biodegradation under various temperatures

Temperatures	COD of municipal organic waste (mg/L)		MOW degradation (%)	COD of aquatic plant (mg/L)		Aquatic plant degradation (%)
	Initial state	Final state		Initial state	Final state	
Ambient (18-31 °C)	77,400	48,600	37	25,200	18,000	29
Mesophilic (31 °C)		61,200	21		21,600	14
Thermophilic (55 °C)		64,700	16		21,600	14

### 4.3 Biogas production

The experiment was conducted under ambient, mesophilic, and thermophilic temperatures in various MOW and aquatic plant portions. The mesophilic digestion was commonly used in a number of experiments; whereas, thermophilic anaerobic digestion process is applied for accelerating the biochemical reaction. This reaction could stimulate a growth of microorganisms capable of producing hydrogen leading to an increased methanogenic potential in shortening hydraulic retention time [12].

Biogas production was monitored every three days until the simulated anaerobic system became stabilised. The accumulative biogas productions under different temperatures were shown in Figure 1. The experimental results suggested that the accumulative biogas was achieved at 280 mL and the ratio of 1:4 (MOW:Aquatic Plant) was lowest at 151 mL at the ambient temperature. The incubation under mesophilic condition implied that the accumulative biogas was between 144 and 308 mL and the MOW ratio to aquatic plant at 1:4 still gave the smallest production. However, the accumulative biogas was decreased in thermophilic temperature and could be reached to 129 mL at ratio 4:1. These results revealed that the MOW ratio to aquatic plant at 4:1 provided the best performance in supporting biogas production. It might due to the aquatic plant has relatively high composition of carbohydrate, protein, fat, and crude fiber [4]. It could initiate the hydrolysis phase which is a critical rate-limiting step determining the biomass feedstock conversion efficiency [13]. The room temperature could produce biogas in a comparable rate with mesophilic condition. Unfortunately, the biogas production under thermophilic condition was quite retard. The production of biogas in aquatic plant appeared to support the biogas production to other proportions. It was evidenced that the biogas production increased if the aquatic plant could produce some quantitation of biogas dramatically. This finding was quite different from that of Ramaraj and Unpaprom [4], who discovered that the highest biogas production was at mesophilic (10,376.59 ml/L) and thermophilic (9,981.08 ml/L) conditions by using the duckweed as an initial substrate without any co-digestion. This might be due to the microorganisms function within

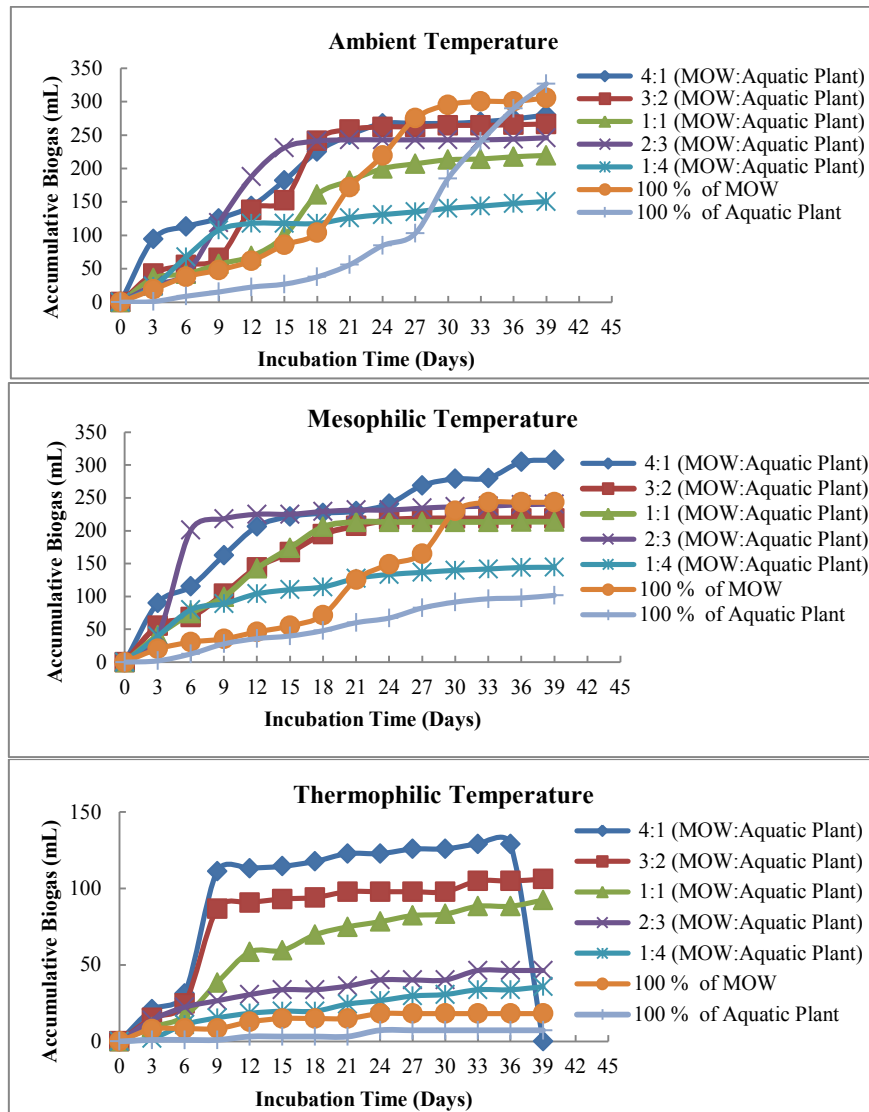
anaerobic digestion process classified as hydrolytic, fermentative, acetogenic, and methanogenic [14]. This result provides an alternative initiating of feedstock for startup anaerobic digester system.

### 4.4 Biogas compositions

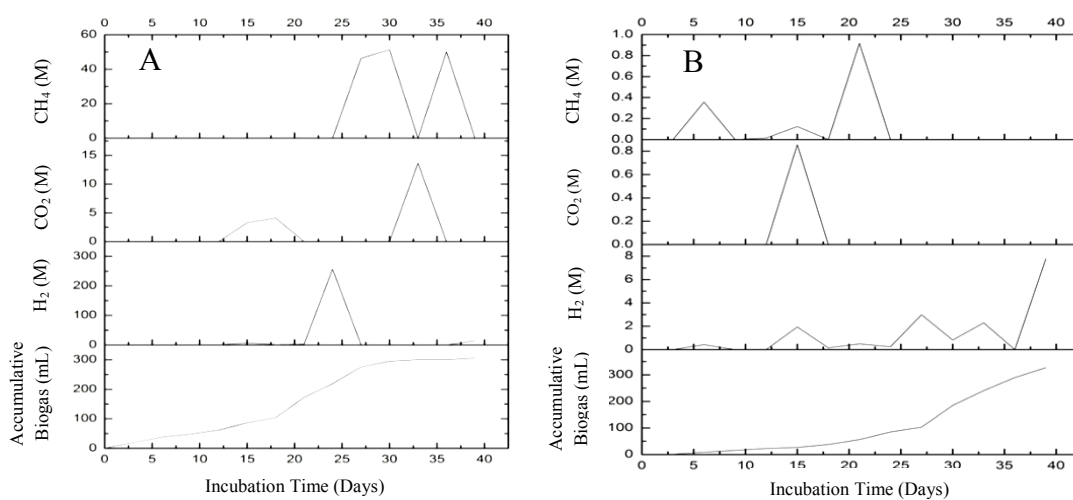
The accumulative concentration of biogas composition such as CH<sub>4</sub>, CO<sub>2</sub> and H<sub>2</sub> were represented in Figure 2-4. In this task, the microorganism consortiums could be simplified by learning from qualitative biogas compositions. The hydrolysis phase occurred by hydrolytic bacteria could reduce complex to simple compounds. Then the fermentation bacteria consumed the simple compounds and produced VFAs, CO<sub>2</sub>, H<sub>2</sub> and alcohols. Acetogens could reduce CO<sub>2</sub> to acetate and acetate, CO<sub>2</sub> and H<sub>2</sub> could be converted to CH<sub>4</sub> by methanogens.

Figure 2 showed experimental results of a 100% of substrate of either MOW (A) or aquatic plant (B) at ambient temperature. The gas composition implied that accumulative CH<sub>4</sub> at 180 M, H<sub>2</sub> at 27 M and CO<sub>2</sub> at 21 M occurred in MOW. The aquatic plant could produce H<sub>2</sub> at 17 M, CH<sub>4</sub> at 1.4 M and CO<sub>2</sub> at 0.9 M. The digestion of MOW caused the emergence of CO<sub>2</sub> and H<sub>2</sub> respectively. The methanogens then could be used to convert to methane gas. However, please note that when the concentration of CO<sub>2</sub> was intense during the day from 30 to 36, CH<sub>4</sub> declined (Figure 2-A). The relation of gas composition in aquatic plant revealed that CH<sub>4</sub> and H<sub>2</sub> were produced within 3 days after substrate degradation. Moreover, it could sustain their H<sub>2</sub> production during incubation (Figure 2-B). This could be another promising evidence of positive effect of using aquatic plant as a co-digestion to support growth of methanogens.

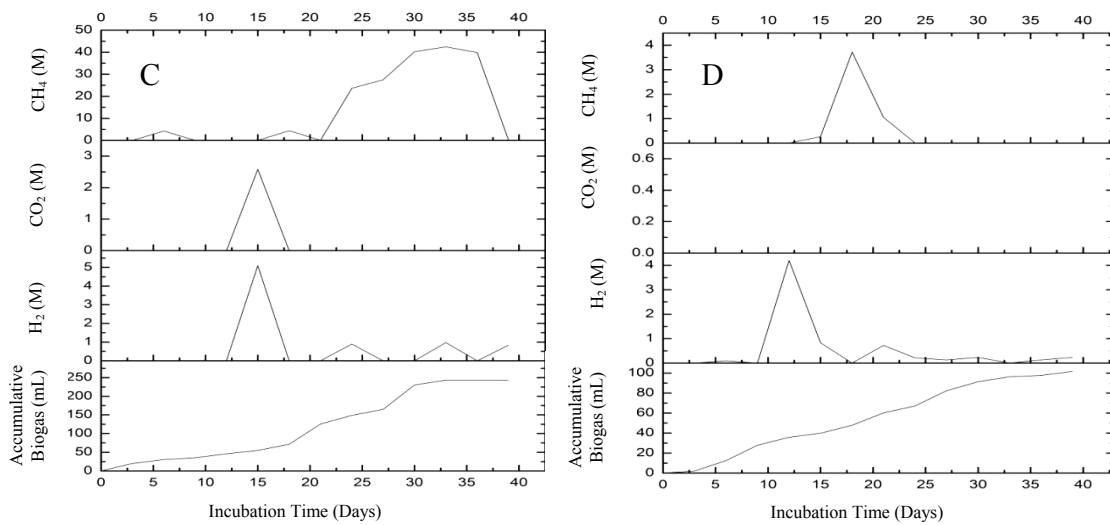
Accumulative biogas and compositions achieved 100 % of substrate under mesophilic (31 °C) condition (Figure 3). The MOW substrate could be produced CH<sub>4</sub> at 182 M, H<sub>2</sub> at 8 M and CO<sub>2</sub> at 3 M (Figure 3-C). In contrast, aquatic plant could only produce CH<sub>4</sub> at 5 M and H<sub>2</sub> at 7 M, but not CO<sub>2</sub> (Figure 3-D). This implied that MOW substrate alone was sufficient to produce CH<sub>4</sub> without co-digestion at mesophilic condition.



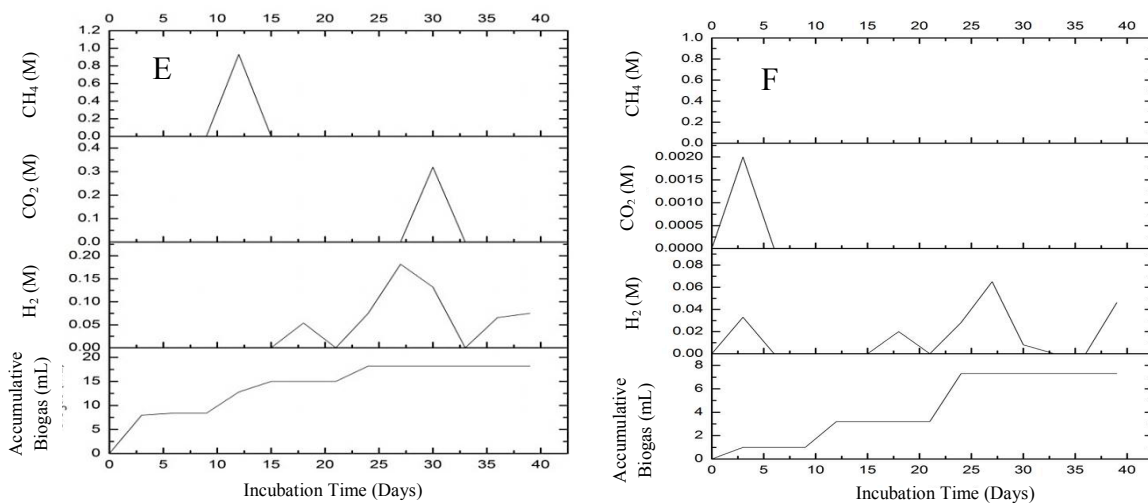
**Figure 1** Accumulative biogas volume under difference temperature and ratio of MOW to aquatic plant



**Figure 2** Accumulative biogas and compositions in 100 % of substrate in ambient temperature; A: Municipal Organic waste, B: Aquatic plant



**Figure 3** Accumulative biogas and compositions in 100 % of substrate under mesophilic (31 °C) condition; C: Municipal organic waste, D: Aquatic plant



**Figure 4** Accumulative biogas and compositions in 100 % of substrate under thermophilic (55 °C) condition; E: Municipal organic waste, F: Aquatic plant

**Table 3** Accumulative biogas composition concentration under various conditions

Accumulative Concentration (M)	Ambient temperature (18-31 °C)					Mesophilic (31 °C)					Thermophilic (55 °C)				
	MOW: Aquatic plant					MOW: Aquatic plant					MOW: Aquatic plant				
	4:1	3:2	1:1	2:3	1:4	4:1	3:2	1:1	2:3	1:4	4:1	3:2	1:1	2:3	1:4
CH <sub>4(g)</sub>	215	28	99	46	54	212	75	125	33	12	15	0	8	5	5
H <sub>2(g)</sub>	67	201	12	51	26	29	30	24	72	24	10	27	9	2	2
CO <sub>2(g)</sub>	13	11	9	9	11	17	0	0	23	11	5	2	5	0	0

The biogas production under thermophilic (55 °C) condition was decreased as shown in Figure 4. The MOW or aquatic plant could produce CH<sub>4</sub>, H<sub>2</sub> and CO<sub>2</sub> less than 1 M under this condition. Please also note that the condition could not enhance the biogas production but inhibit.

The accumulative biogas concentrations under different condition were shown in Table 3. The CH<sub>4</sub> could produce the highest at 215 M and 212 M at ratio 4:1 under ambient and mesophilic condition. Whereas H<sub>2(g)</sub> could occur maximum at 201 at ratio 3:2 at ambient temperature and 72 M in ratio 2:3 at mesophilic. This

might be implied to the microorganism consortium relating to major products production and consumed.

### 5. Conclusions

The municipal organic waste (MOW) and aquatic plant substrates were biodegradable and appropriate to use as feedstock to produce alternative energy. The substrates are adequate to support the growth of anaerobic digestion process in carbon source and nutrient supplement. The simulated anaerobic system could produce biogas within three days and stabilised within thirty-nine days of the incubation period. The ratio could imply significant improvement of accumulative biogas production at ratio 4:1 (MOW: Aquatic plant) at mesophilic and thermophilic condition. The biogas composition concentration could produce the highest rate of CH<sub>4</sub> at 215 M at ambient temperature, 212 M at mesophilic at ratio 4:1. The H<sub>2</sub> could occur maximum at 201M in ratio 3:2 at ambient temperature and 72 M in ratio 2:3 at mesophilic. However, the operation under thermophilic incubation appeared to retard the biogas production. Despite the experimental results, the sustainable approach to disposal and management of municipal organic waste (MOW) and aquatic weed are still required.

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