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Research Article

Experimental Investigation of Biogas Production from Water Lettuce, *Pistia stratiotes* L.

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Abstract

Biogas production through anaerobic digestion (AD) has emerged as one of the renewable energy production technology of choice because through AD biogas as a renewable fuel. The current study focused on the feasibility of biogas production from water lettuce, *Pistia stratiotes* L. water lettuce contains 49.4% carbohydrates, 16.5% protein, lipid 3.6%, fiber 17.8% and ash 23.8%, and furthermore these compositions are an ideal feedstock for biogas production. The fresh water lettuce was harvested after that dried then powdered before fermentation in mesophilic batch reactors. Experiments were carried out as batch runs in laboratory-scale digesters with the addition of inoculums. Total gas yield was 9667.33 mL, during 45 days digestion time. The maximum methane content was reached 66.35%. Based on these results, water lettuce is very suitable as a substrate for biogas production.

Keywords aquatic plant, biogas, fermentation, lake, renewable energy

Introduction

The running down of fossil energy sources makes the production of bioenergy an expected need worldwide (Ramaraj et al., 2015a); with increasing prices of oil and gas the world looks towards alternative and green energy resources. The utilization of renewable energy is significantly increasing, together with energy security concerns, efforts to mitigate the environmental impact of fossil fuels, and upgrading in living standards and renewable technologies (Unpaprom et al., 2015a). Biogas is a renewable gaseous fuel. It is generated by anaerobic digestion (AD) of organic wastes (Unpaprom et al., 2015b).

AD has gained increasing attention in recent years, and is considered an efficient and low cost approach for renewable energy production (Ramaraj et al., 2015b). And AD process contains four main steps, namely hydrolysis, acidogenesis, acetogenesis and methanogenesis. These mechanisms occur with different groups of bacteria in four steps or reactions (Figure 1). During the hydrolysis step, the large organic compounds are broken down into small ones, i.e., amino acids, long chain fatty acids, sugars etc. by hydrolytic bacteria, which hydrolyse the substrate by secreting extracellular enzymes.

For example, cellulase can decompose cellulose into glucose, while starch is broken down into glucose by amylase enzymes. When microorganisms are able to produce suitable enzymes, the hydrolysis step is going to speed up, however, in some other conditions; hydrolysis may become a rate-limiting step if the substrate is hardly accessible by the enzymes because of some rigid structural composition (Unpaprom et al., 2015b).

Biodegradability of a substrate and its potential to produce methane via AD can be studied preliminarily by biochemical methane potential test (Nielfa et al., 2015) and batch digestion system. Various forms of organic substrates such as food waste, organic fraction of municipal solid waste,

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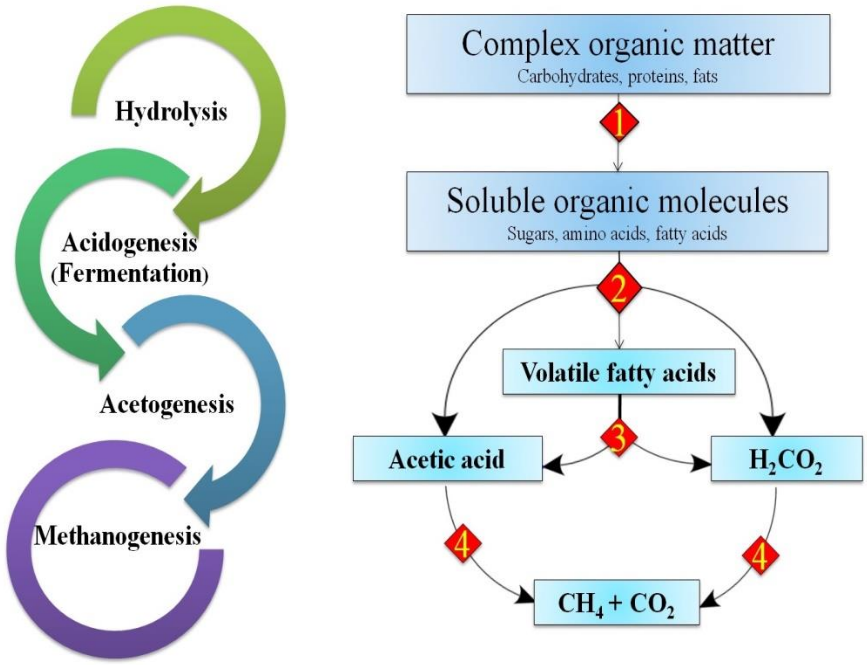

Figure: 1 Flow diagram of the anaerobic digestion process.

sewage sludge and agricultural and forest industries such as corn cobs, sugar cane bagasse, wheat straw, and grasses can be use as feedstock for biogas production (Ramaraj et al., 2015b). Instead of terrestrial plants, aquatic plants are the next promising renewable energy resource. Since, aquatic biomass is regarded as a significant contributor to future biomass production as it can be cultivated with high biomass yields per area (Mishima et al., 2008; Wilkie and Evans, 2010). Aquatic macrophytes have many advantages such as growing on and in waterbodies without competing against most grains and vegetables for arable land; they are also used for water purification to extract nutrients and heavy metals.

In addition, aquatic macrophytes play an important role in the structural and functional aspects of aquatic ecosystems by altering water movement regimes, providing shelter to fish and aquatic invertebrates, serving as a food source, and altering water quality by regulating oxygen balance, nutrient cycles, and accumulating heavy metals (Li, 2014).

The use of aquatic macrophytes, such as water lettuce with hyper accumulating ability is known to be an environmentally friendly option to restore polluted aquatic resources. However, the water lettuce is an aquatic weed, infestation of which causes great problem in the aquaculture system. It is a free-floating freshwater macrophyte that is very abundant in tropical and subtropical regions (Sanchez-Galvan et al., 2013). Moreover, the vegetation form of free-floating aquatic plants especially water lettuce has facilitates their movement and harvest. It is therefore important to utilize the biomass to develop a feasible conversion process. In this study, water lettuce (*Pistia stratiotes* L.) was selected as an aquatic biomass for biogas production.

Material and Methods

Plant Material and Inoculum

The water lettuce, *Pistia stratiotes* L. was collected from the fresh water lake at San Sai district (19° 4' 16" N; 98° 57' 9" E), Chiang Mai province, Thailand and transport to the Energy Research Laboratory at Maejo University, Chiang Mai, Thailand. The plant was identified by a taxonomist (Dr. Yuwalee Unpaprom, Lecturer) at Botany unit where voucher specimen was kept for reference. The arial photograph of location of water lettuce grown lake was illustrated in (Figure 2 a). The plant structure was shown in (Figure 2 b). Harvested water lettuce was washed manually with tap water and leaves and roots were separated. The leaves and

Figure 2. (a) Mature water lettuce in a lake (19° 4' 16" N; 98° 57' 9" E) (b) Water lettuce, *Pistia stratiotes* L.

roots were dried at 60°C and then powdered to 350 μm-mesh for further analysis and experiments. The mesophilic anaerobic inoculum was obtained from a working mesophilic anaerobic digester Energy Research Center, Maejo University.

Batch experimental setup and design

The batch digestion system was performed as was previously reported by Ramaraj et al. (2015a; 2015b). The tests were performed under mesophilic conditions for 45 days. The tests were conducted in triplicate 2 L capacity of Duran glass bottles with working volume of 1 L (Figure 3). The bottles were flushed with nitrogen gas to generate anaerobic conditions. The anaerobic assays were containing 80 mL of inoculums and 400 g of powdered water lettuce and remaining make up with double distilled water.

The substrate (anaerobic assays) was prepared at the start of the experiment and mixed for 1 h using a magnetic stirrer before being added to the fermenters. All the experiments were carried out in triplicates. The bottles were shaken manually twice a day. During the experiment, total gas volume and composition were periodically monitored by gas counters and gas analyzer, respectively. The produced biogas was measured daily basis. And the compositions of biogas contents were measured three days once during the remaining incubation period.

Analytical Methods

Samples were analyzed for total solids (TS), volatile solids (VS), chemical oxygen demand (COD) and alkalinity using standard methods (APHA-AWWA-WEF, 2005). Soluble parameters were determined after filtering the samples through 0.45 μm filter paper. Metrohm 774 pH-meter was used in all pH measurements. Total fat, ash, moisture, fiber contents, volatile fatty acids (VFA) and protein and carbohydrates were determined using AOAC official method (AOAC, 1990). Elemental composition analysis was carried out using a Perkin–Elmer 2004 element analyzer, to determine the carbon (C), hydrogen (H), nitrogen (N), sulfur (S) contents of the sample. The oxygen (O) content was subsequently calculated as the difference. The composition of biogas (CH_4, CO_2, H_2S and O_2) was measured using a biogas analyzer (GFM 416 series, UK).

Figure 3. Batch digester

Statistical Analysis

All the values or readings are the result of mean of three replicates. Data is reported as mean ± standard deviation (SD). Statistical analyses were performed using Microsoft Excel.

Results and Discussion

Water lettuce (*Pistia stratiotes* L.) commonly known as belongs to Araceae. It also known as, water cabbage, Nile cabbage, or shellflower is a free floating aquatic plant of streams, lakes and ponds (Sanchez-Galvan et al., 2013). Taxonomical classification is following:

Kingdom: Plantae

Division: Magnoliophyta

Class: Liliopsida

Order: Alismatoles

Family: Araceae

Genus: Pistia L.

Species: *P. stratiotes*

The leaves are light green, with parallel veins, wavy margins and are covered in short hairs that form basket-like structures that trap air bubble. Leaves are approximately 10-20 cm long and 10-17cm wide and of fan-shaped having parallel venation, blunt apex, and entire margin. Around 7-15 veins run parallel from the base. The lower surface is covered with whitish hairs. The plant forming rosettes up to 15 cm across that may resemble ordinary lettuce (Coelho et al, 2005). It is known for its efficient nutrient uptake ability and something coupled to its fast-growing capacity that in many places has turned water lettuce into a strongly invasive plant (Šajna et al, 2007; Lu et al, 2010). The plant forms dense mats on the water surface and grows at a rate of 60-110 t DW ha^{-1} yr^{-1} (Mishima et al, 2008).

The inoculums characteristics including TS, VS, COD were 296.1 ± 0.05 mg/L, 158.5 ± 1.15 mg/L and 1241.6 ± 2.01 mg/L, respectively; along with alkalinity of 136.4 ± 0.04 mg/L as $CaCO_3$, VFA of 136.4 ± 0.25 mg CH_3COOH/L and pH was 6.66 ± 0.03. The quality of inland waters in Chiang Mai province (Northern part of Thailand) is

Table 1. Physical, chemical and composition of water lettuce

Parameters	Results
Proximate analysis (wt.%)	
Moisture	91.25 ± 0.06
Ash	23.75 ± 0.42
Volatile matter	66.06 ± 0.01
Fixed carbon	12.74 ± 0.07
Ultimate analysis (%)	
Carbon (%)	44.19 ± 0.02
Hydrogen (%)	5.22 ± 0.06
Oxygen (%)	39.18 ±0.02
Nitrogen (%)	0.44 ± 0.02
Composition and others	
Carbohydrate (%)	49.45 ± 1.21
Protein (%)	16.47± 0.48
Fat (%)	3.56 ± 1.55
Crude Fiber (%)	17.81 ± 2.14
TS (ml/L)	36,000 ± 1.36
VS (ml/L)	81,650 ± 1.02
COD (ml/L)	11,379 ± 1.72
VFA (mgCH3COOH/L)	1360 ± 0.43
Alkalinity (mg CaCO3/L)	557± 0.61
pH	7.5 ± 0.03

characterized as a potentially good resource for biomass production. The selection of macrophyte biomass for experiments raises some issues related to naturally grown water lettuce collection and use for biogas production. The physico-chemical and compositions properties of water lettuce contents were listed in Table 1. The substantial biomass of water lettuce has relatively high levels of chemical compositions such as carbohydrate 49.45 (%), protein 16.47 (%), fat 3.56 (%) and crude fiber 17.81 (%) were demonstrated that as a feedstock of choice for biogas production.

A considerable number of studies have been conducted to investigate anaerobic digestion of

Table 2. Comparison of methane yield

Aquatic weed	Digestion time	Methane (Maximum)	Reference
Lemna minor L. (common duckweed)	32 days	41 %	Ström, 2010
Eichhornia crassipes (water hyacinth)	60 days	62%	Mathew et al., 2015
Salvinia (watermoss)	60 days	63%	Mathew et al., 2015
Pistia stratiotes L. (water lettuce)	45 days	66.35%	This study

aquatic weed such as *Cabomba* (fanwort), *Salvinia* (watermoss), and *Eichhornia crassipes* (water hyacinth) as feedstock for digestions processes (O'Sullivan et al., 2010; Mathew et al., 2015). The production of biogas there are several other advantages to harvesting of aquatic weeds including water lettuce. Dalal et al., 2008 stated that removal of plant biomass from the waterways could lead to decreases in the organic carbon load to the anaerobic bottom waters and sediments of the reservoirs. Water lettuce had a higher carbohydrate and protein contents (Table 1). Hence a higher biogas potential which was reflected in the present study as the water lettuce produced much higher biogas yield. The total biogas production and composition of biogas such as CH_4, CO_2, O_2 and H_2S results were presented in Figure 4a and Figure 4b. The total biogas yield was reached 9667.33 mL and maximum methane content was 66.35 %. An efficiency criterion of methane production was explained good performance throughout the study.

The maximum methane content of water lettuce was achieved higher compared with other aquatic weeds such as *Lemna minor* L., *salvinia* and water hyacinth (Table 2). In order to start to move toward a potential industrialized cultivation system, preliminary tests on the biomethane potential yield have been carried in this study. A greater understanding of the AD process and the input material characteristics would be useful. The chemical composition analysis of both inoculum and biomass was provided data for better understanding of the materials as well as the overall process. These results indicate that water lettuce can be successfully converted using AD and while further investigation into the techno-economics is required it is expected that this process is economical and scalable. Consequently, water lettuce can be utilized as a substrate for biogas production further scale up studies.

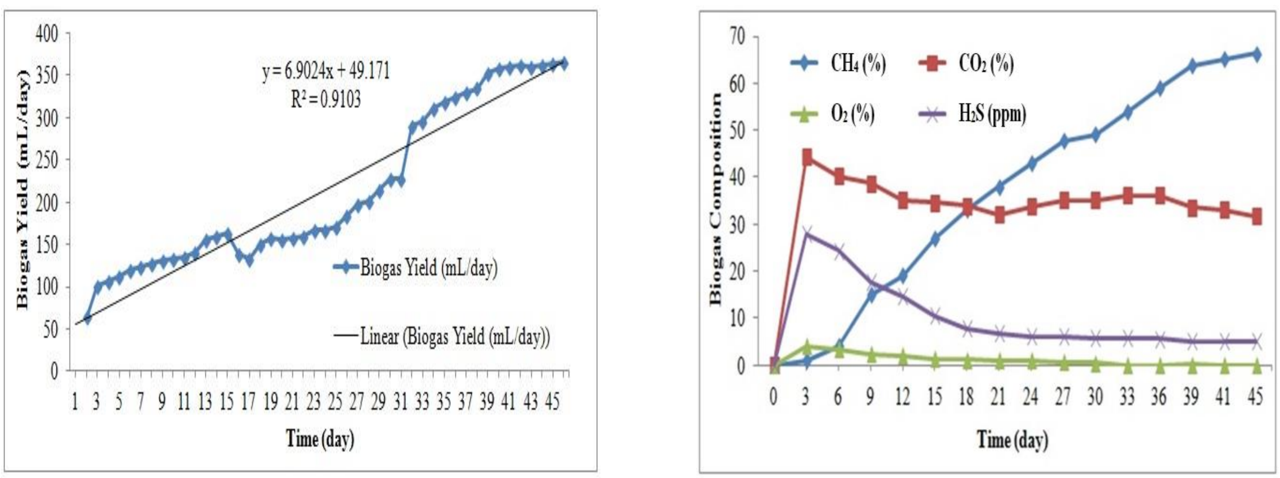

Figure 4. (a) Biogas yield of water lettuce (b) Biogas composition of water lettuce

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