Characeae and *Hydrilla verticillata* for Enhancing Biogas Production in Landfill Leachate

Wichidtra Sudjarid*†, Pita Jarupunphol**

*Department of Environmental Science, Faculty of Science and Technology, Sakon Nakhon Rajabhat University, 47000

** Department of Information Technology, Faculty of Science and Technology, Phuket Rajabhat University, 83000

(Wichidtra.s@snru.ac.th, p.jarupunphol@pkru.ac.th)

† Wichidtra Sudjarid; Pita Jarupunphol, 47000, Tel: +66 84 128 4421,
Fax: +66 042 970 029, wichidtra.s@snru.ac.th

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**Abstract**- This study investigated the enhancement of biogas production in leachate wastewater under anaerobic digestion process. Characeae and *Hydrilla verticillata* were collected from a natural water resource, then shredded at a ratio of 1:1 (w/v) and used as a co-substrate. The simulated reactors were stabilised within 60 days of incubation. Five ratios of leachate to co-substrate as 10:1, 10:2, 10:3, 10:4 and 10:5 were separately tested. It found that the pH ranged from 5.83 to 7.78 and 5.63 to 7.35 in *Hydrilla* and Characeae, respectively. The COD degradation efficiency was a range of 65 – 81% and 55 – 68% in *Hydrilla* and Characeae. While leachate seems to be retarded. The accumulative biogas production in leachate was significantly improved, while the biogas production in leachate was 2.6 mL. The highest accumulative biogas production was found 5193, 2943, 5654 and 5843 mL in sole and ratio five in *Hydrilla*, 100% and ratio four in Characeae. Moreover, these substrates could generate densely and sustained of H2 and could initiate CH4 after 20 days. This suggests an advantage of using Characeae and *Hydrilla verticillata* as a co-substrate to improve biogas and CH4 production in long operating landfill leachate.

**Keywords** Characeae, *Hydrilla verticillata*, Leachate, Biogas, Anaerobic Digestion.

1. **Introduction**

Leachate wastewater management is one of the most challenging issues for developing countries. It is a consequence of the compost of organic solid waste from the landfill. The municipal solid waste has been reported to be approximately 27 Mt across Thailand [1]. Sanitary landfill with the dumping of 1,000 t/d could produce about 200-300 t/d of leachate and more or less depending on its climate location and moisture content [2]. There are several factors affecting the quality of leachate (i.e. age, type, and composition of waste) [3]. Notably, leachate characteristics were mainly dependent upon the age of landfill [4]. The old leachate (> 10 years) was recalitritan to biodegradation due to the long residue of organic molecules released from the solid waste [5].

The old landfill is, however, characterised by its low biodegradable and relatively high NH3-N, which could be inhibitory to microbial activity [3, 6]. However, high content of N could stabilizes the pH and the isobutyric and valeric acids that increase the biogas production [7]. A lesser measurement and low headspace pressure represented positive effect in biogas production [8].

Nevertheless, anaerobic digestion has the potential to degrade organic contents and convert them to biogas in leachate as renewable energy [9]. Co-digestion is a mix of at least two different wastes digested. Significant benefits of applying the co-digestion have been observed, e.g., improving the degradation process stability, increasing in biogas and methane yield, and utilising of a synergistic effect of microorganisms [10]. Please note that applications of fruit and vegetable waste [11], co-digestion of different mixtures of seaweed, food waste leachate, vegetation, algal biomass, and raw sludge could cause methane production more than using only individual substrates [12, 13, 14]. The mixed silage of seaweed biomass and wheat straw in a long-term semi-continuous could produce biogas effectively [15]. The eutrophic lake contains nutrients dissolved in water lake that
support aquatic weed and algae growth in a tropical climate. In this case, the substrate as co-digestion to enhance biogas production might be applied besides being used as fertilisers or animal feedings. Because of nitrogen-rich biomass in aquatic plants [16], their digestions could produce H₂ and CO₂ and could be used as electron donors in the acetogenesis and methanogenesis step [3]. Due to the increase of energy consumption, renewable energy has become an essential alternative source to fossil fuels that are declining [17] and it could be applied for domestic use [18]. Furthermore, it can address CO₂ emission issues of fossil fuels that can potentially cause global warming.

Anaerobic digestion process, whereas, is a complex process involving microbial consortia in several intermediate steps. Hydrolysis can hydrolyse complex organic compounds into simpler organics. After acetogens are fermented to volatile acids, obligate hydrogens producing acetogens will convert volatile acid longer than two carbons to acetate and hydrogen gas. They will be converted to methane gas by the methanogens in the final process [6]. This study would apply aquatic weeds which are spreading in a natural lake in Sakon Nakon Province, Thailand to improve the biogas production efficiency in old landfill leachate in different ratios of aquatic weeds.

2. Materials and Method

2.1. Sampling Sites

A collection of Characeae and Hydrilla verticillata (Hydrilla) was sampling in Nong Han, a fresh, natural water lake located in the northeast of the provincial capital town of Sakon Nakon. There are two majorities of submerged plants widespread in this lake. The landfill leachate was sampled in the primary wastewater collection leachate pond in Sakon Nakon province’s sanitary operating landfill, which has been operating since 1994.

2.2. Inoculums Preparation

Characeae and Hydrilla were separately mixed with a water lake at a ratio of 1:1 by weight to volume to simulate the natural environmental condition. It was later shredded by two bread turbines to make homogeneously small particle sizes, and they were used as an initiating inoculum. However, leachate wastewater collected from sanitary landfill was directly used without any pre-treatment.

2.3. Biogas Experimental Reactor

The batch reactor of anaerobic digestion was constructed at 7.5 litres with duplication. The schematic of the simulating system was shown in Fig. 1. Inoculums were added directly to leachate wastewater under selected rations into the reactor to make a final volume of 5 litres and kept in the dark at room temperature. Five ratios of leachate to inoculums including 10:1, 10:2, 10:3, 10:4, 10:5 (v/v) were investigated. The control sets were constructed as 100% for each Characeae, Hydrilla and leachate to verify their performance. The accumulative biogas and its compositions were analysed every four days until the system was stabilised.

2.4. Biogas quantitation and qualification

The accumulative biogas production used the fluid displacement of gas to measure the volumetric of biogas (Fig.1). Thermal conductivity detector (TCD) was used to detect the qualitative analysis of biogas production. Twenty millilitres of headspace was injected with valve sampling in the Gas Chromatography System. The identified tool for gas chromatography in this experiment was based on Shimadzu Gas Chromatography System (GC-2014), which is a high performance and reliability for packed column coupled with Polapak-w (50/80), 0.3 m length 0.3 m, 0.25 mm thickness, and 0.25 mm diameter. The column was resistant to the highest temperature at 190 °C. Analytical temperature program was set as an injector at 80 °C and remained in the column at 45 °C for 7 minutes. Helium gas was used as a carrier gas as 170 mL/min. The quantitative measurement was used 1%M of H₂, CH₄ and CO₂ as standard gas to be calculated.

2.5. Statistical Analysis

The accumulative biogas productions under different criteria were used One-way ANOVA, a significant level at .05. It analysed the variance among the ratio of the experiment.

Fig. 1. The schematic of simulating anaerobic digestion reactor. A: Reactor, B: Rubber capped, C: Rubber tube, D: Controlling gas valve, E: Biogas sampling port, F: Measuring cylinder and water bath (adapted from Ramaraj and Unpaprom [19])
3. Results and Discussion

3.1 Substrate characteristics

Table 1 represents tested substrate and inocula characteristics. It could reveal the initiating environment to start up the anaerobic digestion process. The pH ranged from 5.66 to 7.96; it is suitable to initiate biogas production. The inoculum is highly organic solid, which replies to the prevalent use of microorganism consortia. Carbon/Nitrogen (C/N) is a parameter to determine the biogas production. C/N ratios ranging from 20 to 30 were found to be the optimum for biogas production. The C/N of Hydrilla, Characeae and Leachate were 34, 37 and 27, respectively. The study found the C/N ratio was in the range of leachate and upper rage in Hydrilla and Characeae. The C/N ratio of aquatic weeds found relatively high might have occurred due to nutrient contents in Nong Han lake, where the surrounding area is mainly an agricultural land used. Moreover, the Hydrilla is the nitrogen-rich biomass as suggested by Abbasi et. al. [20]. The COD:N:P were 100:3:0.5, 100:3:0.5, 100:4:1 in Hydrilla, Characeae and Leachate, respectively. This ratio would give an adequate nutrient would support the growth of anaerobes suggest as 100:2.2:0.4. Moisture content in both Hydrilla and Characeae was high because of submerged plants. Thus this study did not amend any micronutrient.

Table 1. Characteristics of Hydrilla verticillata, Characeae inoculums and landfill leachate

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Unit</th>
<th>Hydrilla</th>
<th>Characeae</th>
<th>leachate</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>-</td>
<td>6.23</td>
<td>5.66</td>
<td>7.96</td>
</tr>
<tr>
<td>MC**</td>
<td>%</td>
<td>95</td>
<td>94</td>
<td></td>
</tr>
<tr>
<td>Total Solid</td>
<td>mg/L</td>
<td>57,610</td>
<td>61,130</td>
<td>3,945</td>
</tr>
<tr>
<td>COD</td>
<td>mg/L</td>
<td>54,400</td>
<td>80,000</td>
<td>2,000</td>
</tr>
<tr>
<td>Total P</td>
<td>mg/L</td>
<td>293</td>
<td>389</td>
<td>16</td>
</tr>
<tr>
<td>TKN</td>
<td>mg/L</td>
<td>1,588</td>
<td>2,164</td>
<td>84</td>
</tr>
</tbody>
</table>

Note: * no measurement, ** MC: Moisture Content

3.2 Effects of COD to biogas production

This parameter could imply both biodegradable and recalcitrant organic substrates. Organic biodegradability could be converted to biogas. The decrease in COD was dramatically directed to biogas production. The organic compounds could be degraded after four days not only using Hydrilla but also Characeae, except only sole leachate seems to be retarded (Fig. 2 & 3). The degradation efficiency of the use of Hydrilla as a co-substrate could be degraded as 67, 70, 65, 68 and 81 % in 10:1, 10:2, 10:3, 10:4, 10:5 ratios, respectively (Fig 2). Thus the only sole Hydrilla degradation efficiency still reached 67%. Besides, the ability of degradation of Characeae was revealed that 55, 62, 60, 68, 63% in 10:1, 10:2, 10:3, 10:4, 10:5 ratios, respectively (Fig 3) and 62% in a hundred percentages of Characeae.

3.3 Effects of pH for biogas production

The methanogens were near neutral pH, considered optimal in the range of 6.5 to 8.2 [21]. The anaerobic system was operating under a various group of microbes such as acedogens, acetogens and methanogens. The accumulative of volatile fatty acids from this metabolism could affect the potential of biogas production. The pH of the used Hydrilla and Characeae as co-substrates can be found in the range from 5.83 to 7.78 (Fig.4) and from 5.63 to 7.35 (Fig. 5).

The pH dropped from 6.92 to 5.83 in day 16 and from 7.18 to 6.65 in day 36, which might be due to the accumulative function of VFA [22]. However, it increased after methanogens used VFA to produce methane in ratio two of Hydrilla as related to Fig.7E. The impact did not have any effect on biogas production.

In contrast to the Characeae test, it notifies that the pH dropped within the 4th day and would increase after that. The results show that use of Characeae could be spontaneous hydrolysed and used by acedogens; even though the degradation efficiency was lower than Hydrilla. By using leachate alone, however, pH was dropped in the 16th day and increased after that (Fig. 4).
The biogas production from five ratios of Hydrilla (1 to 5) was investigated to enhance the biogas production and compositions. The accumulative biogas productions were 2,292, 2,475, 2,733, 2,634, 2,943 and 5,193 mL in 10:1, 10:2, 10:3, 10:4, 10:5 and 100% of Hydrilla, respectively (Fig.7A-F). The quantitative of accumulative biogas in the incubation of only sole Hydrilla was the highest. This implies the biogas production performance itself. Furthermore, it could enhance its biogas production in leachate as well. The ratio of the use of Hydrilla as co-substrate to biogas production was insignificantly different at .05 (p<.05). The degradation of Hydrilla could begin to produce CH₄ in day 12 and day 16 could detect H₂ and CO₂ in 100% of Hydrilla. The highest CH₄, H₂ and CO₂ concentrations were found at 14.96, 15.95, 16.20 %M in day 44, 24 and 48, respectively (Fig.7A). This was revealed to be effective digestion of Hydrilla, as also suggested by Abbasi et al. [20] and Chen et al. [23]. The water weed like water hyacinth also effectively produces biogas as well [21, 22]. The ratio 1 to 5 of Hydrilla could improve the biogas production and biogas compositions in leachate (Fig.7B-F). This finding suggests the minimum quantity of co-substrate of used was sufficiently promoted the growth of methanogens.

The H₂ could be detected on day 8 and then could measure CH₄ and CO₂. H₂ could be used as an electron donor to support the growth of methanogens [21]. The ratio of 4 and 5 of Hydrilla could provide a high concentration of CH₄ and H₂. It regarding quantitative of co-substrate was used to initiate the system (Fig.7B & 7C), not only accumulative biogas volume but also the composition of biogas. A highly accumulative concentration of hydrogen that might be due to methanogens could not prompt converts to CH₄. This finding indicates the potential to use Hydrilla as a substrate which could alternately produce hydrogen energy and initiate the biogas production in leachate.

The simulated reactors were randomization position and kept in the dark under room temperature approximately 30 – 35 °C during the incubation period. Accumulative biogas production in leachate could be detected only at 2.6 mL within 60th days (Fig.6). Hydrilla and Characeae could be applied as co-substrates to improve the biogas and composition production. These two distinct substances could be tested for the generation capability comparison.

3.5 Enhancement of Biogas Production by Hydrilla verticillata

The biogas production from five ratios of Hydrilla (1 to 5) was investigated to enhance the biogas production and compositions. The accumulative biogas productions were 2,292, 2,475, 2,733, 2,634, 2,943 and 5,193 mL in 10:1,
The on 14.96 %M could be detected on day 40 at 100% then notice CH₄ hundred percent could de 4, 506, 5, 843, 3, 276 and 5, 654 mL in 10:1, 10:2, 10:3, 10:4, 3

Hydrilla Fig: Ratio 4 of Hydrilla

Accumulative biogas compositions(%M)

Biogas compositions(%M)

Incubation time (days)

Accumulative biogas (mL)

CH₄ (%M)

CO₂ (%M)

H₂ (%M)

Accumulation gas (mL)

Fig.7. Accumulative and biogas compositions produced by Hydrilla. A: 100 Hydrilla, B: ratio 5, C: ratio 4, D: ratio 3, E: ratio 2, F: ratio 1

3.6 Enhancement of Biogas Production by Characeae

The accumulative biogas was found at 3,604, 3,038, 4,506, 5,843, 3,276 and 5,654 mL in 10:1, 10:2, 10:3, 10:4, 10:5 and 100% of Characeae, respectively (Fig.8A-F). A hundred percent could detect H₂ at 8.36%M after day 12 and then notice CH₄ at 0.53 %M after day 20. The highest CH₄ at 14.96 %M could be detected on day 40 at 100% (Fig.8A). The only sole and ratio four of Characeae could provide the best performance (Fig.8A&B). Biogas production was varied with a ratio of Characeae due to its diverse set of polymers [26]. Even though the ratio four could give the highest accumulative biogas volume, the ratio did not provide the highest of CH₄. The highest level of CH₄ at 18.65 %M was noticed in ratio five which related to the quantity of co-substrate (Fig.8B). All ratios had been tested and CH₄ occurrences were discovered after 20 days. Please note that the use of Characeae and Hydrilla as co-substrate could continuously produce H₂ and CO₂ along with incubation time. Algae can split water molecules into hydrogen ion and oxygen via photosynthesis, which converts hydrogen ion to H₂ from a hydrogenase enzyme. There is a carbohydrate enrichment substrate [27]. These two properties could support the growth of H₂-producing bacteria, and H₂ could be converted to CH₄ by methanogens. H₂ is an essential intermediate in the metabolism of the substrate of carbohydrates and has not proven to be a reliable indicator of toxicity to the anaerobic process [28]. This study was successful in the case of improvement of biogas production in leachate wastewater but has yet to enhance the CH₄ concentration.
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References


