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

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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, but 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 H_2 and could initiate CH_4 after 20 days. This suggests an advantage of using Characeae and *Hydrilla verticillata* as a co-substrate to improve biogas and CH_4 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 recalcitrant 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 NH_3-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 waterweed 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 H_2 and CO_2 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 CO_2 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 acedogens 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 Nakhon. 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 Nakhon 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 H_2, CH_4 and CO_2 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.

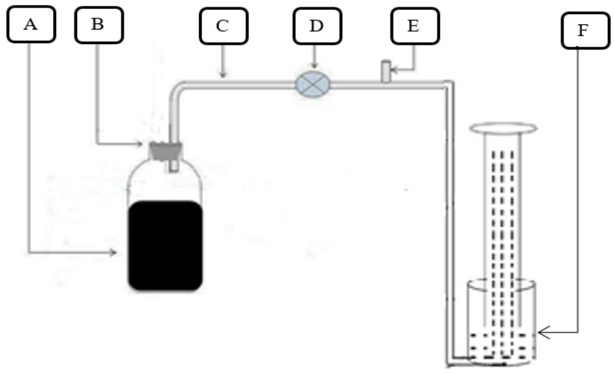


Fig. 1. The schematic of simulating anaerobic digestion reactor. A: Reactor, B: Rubber capped, C: Rubber tube, D: Controlling gas valve, E: Biogas sampling port, F: Measuring cylinder and water bath (adapted from Ramaraj and Unpaprom [19])

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 consortiums. Carbon/Nitrogen (C/N) is a parameter to determine the biogas production. C/N rations 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

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

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).

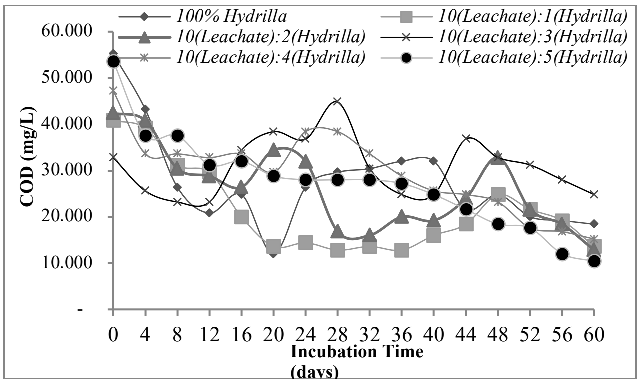


Fig.2. COD degradation of *Hydrilla* to leachate in different ratios during the incubation time.

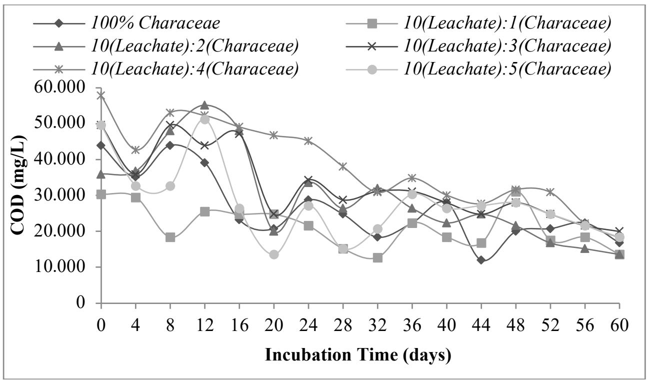


Fig.3. COD degradation of Characeae to leachate in different ratios during the incubation time.

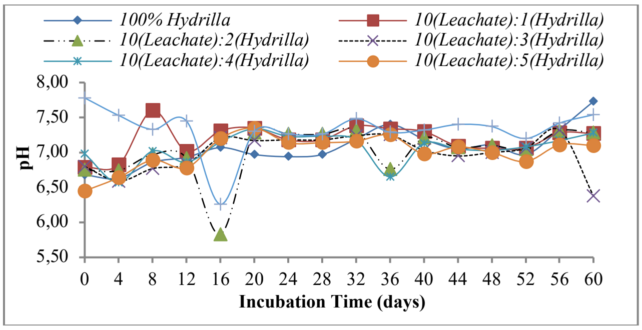


Fig.4. pH regimes under different ratios of *Hydrilla* to Leachate

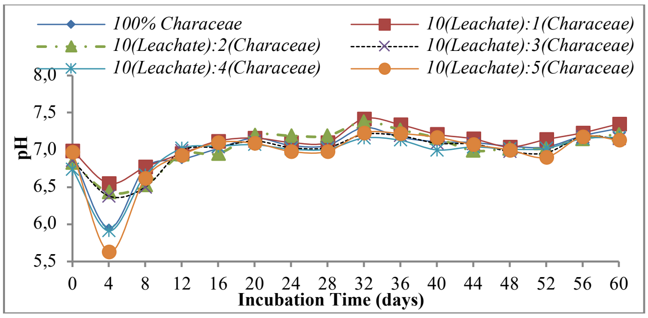


Fig. 5. pH regimes under different ratios of Characeae to Leachate.

Fig. 6. Accumulative and biogas compositions produced by 100 of leachate

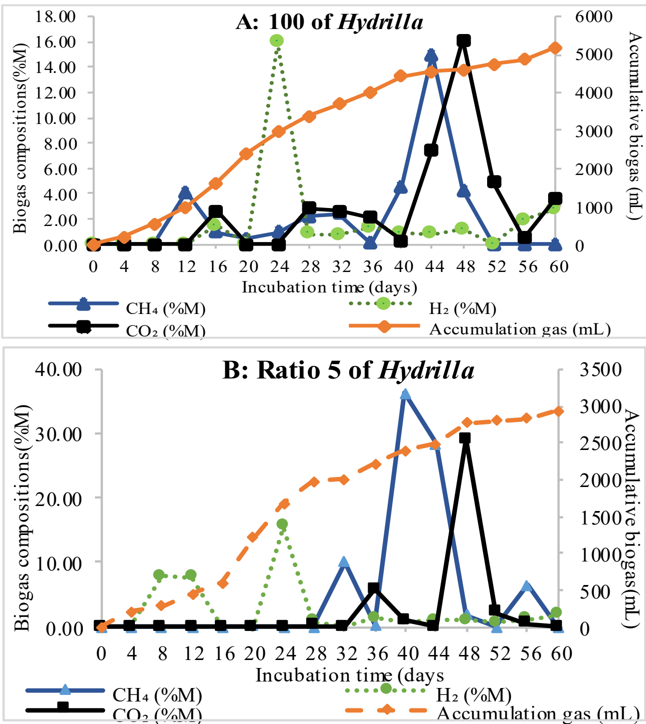
3.4 Enhancement of biogas production in leachate wastewater in simulating anaerobic digestion reactor

The simulated reactors were randomization position and kept in the dark under room temperature approximately 30 – 35 ºC 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 fives 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 CH_4 in day 12 and day 16 could detect H_2 and CO_2 in 100**%** of *Hydrilla*. The highest CH_4, H_2 and CO_2 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 H_2 could be detected on day 8 and then could measure CH_4 and CO_2**.** H_2 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 CH_4 and H_2. 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 CH_4. This finding indicates the potential to use *Hydrilla* as a substrate which could alternately produce hydrogen energy and initiate the biogas production in leachate.



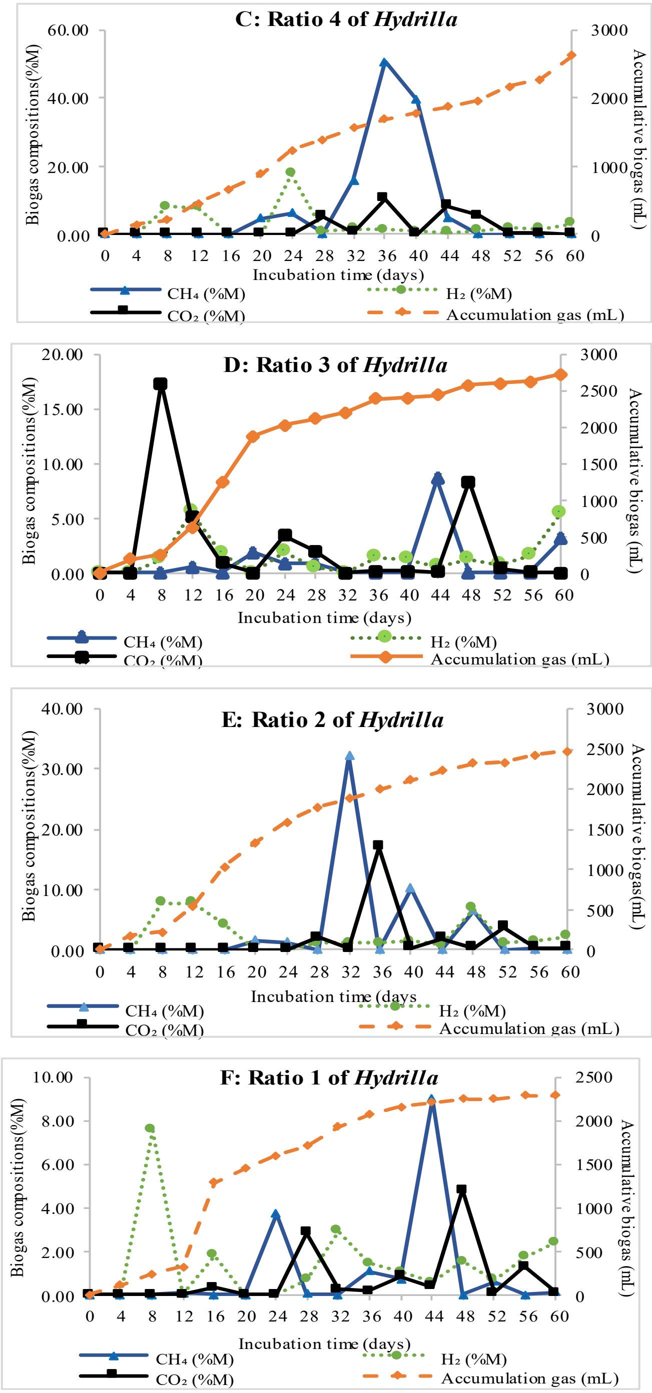
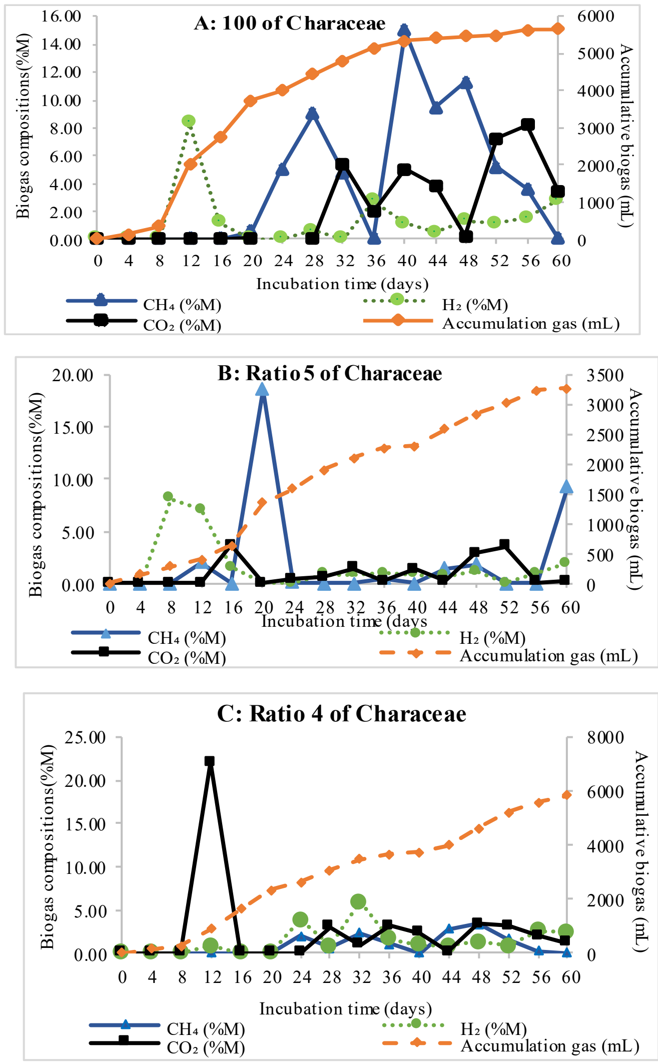
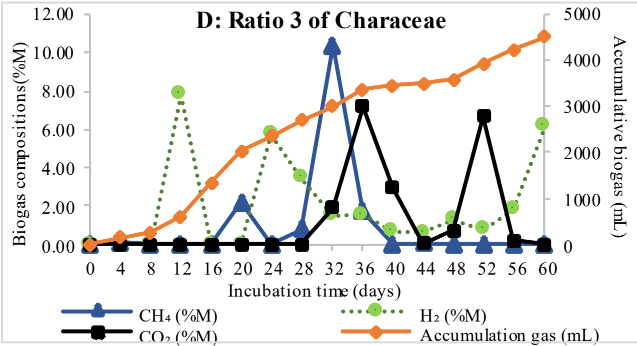


Fig.7. Accumulative and biogas compositions produced by *Hydrilla*. A: 100 *Hydrilla,* B: ratio 5, C: ratio 4, D: ratio 3, E: ratio 2, F: ratio 1

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 H_2 at 8.36%M after day 12 and then notice CH_4 at 0.53 %M after day 20. The highest CH_4 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&8C). 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 CH_4. The highest level of CH_4 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 CH_4 occurrences were discovered after 20 days. Please note that the use of Characeae and *Hydrilla* as co-substrate could continuously produce H_2 and CO_2 along with incubation time. Algae can split water molecules into hydrogen ion and oxygen via photosynthesis, which converts hydrogen ion to H_2 from a hydrogenase enzyme. There is a carbohydrate enrichment substrate [27]. These two properties could support the growth of H_2-producing bacteria, and H_2 could be converted to CH_4 by methanogens. H_2 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 CH_4 concentration.





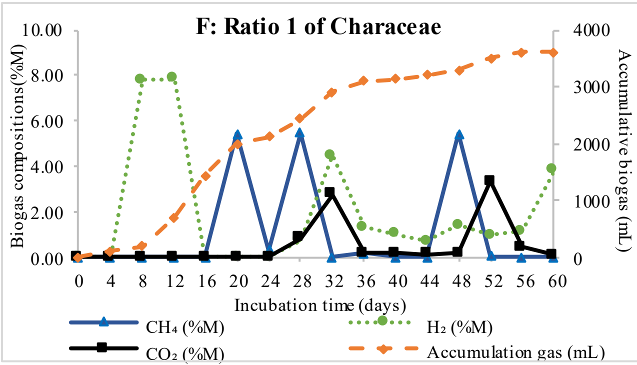


Fig.8. Accumulative and biogas compositions produced by Characeae. **A:** 100 Characeae, **B:** ratio 5, **C:** ratio 4, **D:** ratio 3, **E:** ratio 2, **F:** ratio 1

4. Conclusions

This article has represented techniques for enhancing biogas production and composition by using *Hydrilla* and Characeae in leachate wastewater. The simulated reactors were a randomisation position and kept in the dark under room temperature approximately 30 – 35 °C during the incubation period. Five ratios of co-substrate were experimented including 1, 2, 3, 4 and 5, respectively. Only sole leachate, *Hydrilla* and Characeae were investigated to compare the individual's performance. The pH regimes incubated by *Hydrilla* and Characeae found ranged from 5.83 to 7.78 and 5.63 to 7.35, respectively. The decreasing efficiency of COD was 55 – 81% and maximum in sole Characeae and *Hydrilla*; thus degradation of leachate seems to be retarded. *Hydrilla* and Characeae as co-substrate could enhance accumulative biogas as well as CH_4. These substrates have the potential to generate H_2 because of carbohydrate-rich substrates. However, a further step treatment is still required to improve the CH_4 generation performance. Moreover, it found some potentially of use the aquatic weed as clean energy like H_2 in the future. The enrichment and isolated culture to increase the rate of biogas production and composition will be investigated.

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