Enhancement of Biogas Production in Landfill Leachate by Characeae and *Hydrilla verticillata* (L.f.) Royle

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ABSTRACT

This study investigated the enhancement of biogas production in leachate wastewater under anaerobic digestion process. Characeae and *Hydrilla verticillata* (L.f.) Royle (*Hydrilla*) were collected from natural water resource, then shredded at ratio 1:1 (v/v) and used as co-substrate. The simulated reactors were randomisation position and kept in the dark under room temperature approximately 30 – 35 ^{o}C during 60 days of incubation time. Five ratios of leachate to co-substrate as 10:1, 10:2, 10:3, 10:4 and 10:5 were test. It found that the pH was range 5.83 to 7.78 and 5.63 to 7.35 in *Hydrilla* and Characeae, respectively. There were significantly improved the accumulative biogas production in leachate without any pre-treatment, while the biogas production in leachate was 2.6 mL. The highest of accumulative biogas production was found 2,943 and 5,843 mL in ratio 10:5 and 10:4 in *Hydrilla* and Characeae, respectively. Moreover, these substrates could generate dense of H_2 and seems complete competitive to methanogens. As such, the requirement of using two stages strategy to produce CH_4 is still required.

Keywords: Characeae, *Hydrilla verticillata* (L.f.) Royle (*Hydrilla*), Leachate, Biogas, Anaerobic Digestion

1. INTRODUCTION

Leachate wastewater management is one of the most serious and challenging issues for developing countries. It is a consequence of the compost of organic solid wastes from the landfill. The municipal solid waste has been reported approximately 27 Mt across Thailand [1]. Thai people generate waste as an estimate 1.14 kg/capita/day, which is higher than the middle-income country average usually producing 0.79 kg/capita/day [2]. Moreover, a ton of organic solid waste can produce a monthly leachate up to 130.92 m^3 [3]. The leachate is difficult to be biodegraded, since it contains highly organic concentration, ammonium and hazardous and toxic substrate [4, 5]. However, anaerobic digestion has a potential to degrade organic contents biogas in leachate as a renewable energy [6, 7]. Although the renewable energy is an alternative important source due to the increasing energy consumption, fossil fuels were declined and it can potentially cause global warming by CO_2 emission.

Anaerobic digestion technologies (e.g., UASB and covered lagoon) have been widely operated to produce energy 100-150 kWh/ton treated waste [8]. Anaerobic digestion process is a complex process involving microbial consortia in several intermediate steps. Hydrolysis can hydrolyse complex organic compounds to 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 [9].

Co-digestion is mixed at least two different wastes and digested. The benefit of using the co-digestion application as following; improving the process stability, increasing in biogas and methane yield, and utilising of a synergistic effect of microorganisms [10]. Application of use fruit and vegetable waste [11] or tomato-plant waste to rabbit waste [12] could enhance biogas production and methane proportions. Furthermore, the combination of three substrates: cone silage, cheese whey, and glycerin could result in the biogas production rate of 1.8 L/L/day [13]. This experiment would be used green algae and aquatic plants to enhance the biogas production and methane gas proportion.

2. MATERIAL AND METHODS

2.1 Sampling sites

A collection of Characeae and *Hydrilla verticillata* (L.f.) Royle (*Hydrilla*) were 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 wastewater collecting pond in Sakon Nakhon province's sanitary landfill.

2.2 Inoculums Preparation

Characeae and *Hydrilla verticillata* (L.f.) Royle were separately mixed with water lake at ratio 1:1 to simulate the natural environmental condition. After that shredded by two bread turbines to make homogeneously small particle sizes, and they were used as an initiating inoculum. However, landfill wastewater 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 simulating system was shown in Figure 1. Inoculums were directly added into leachate wastewater under selected rations into the reactor to make a final volume at 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 a 100% of each Characeae, *Hydrilla verticillata* (L.f.) Royle and leachate to verify themselves performance. The accumulative biogas and its compositions were analyzed every four days until the system was stabilised.

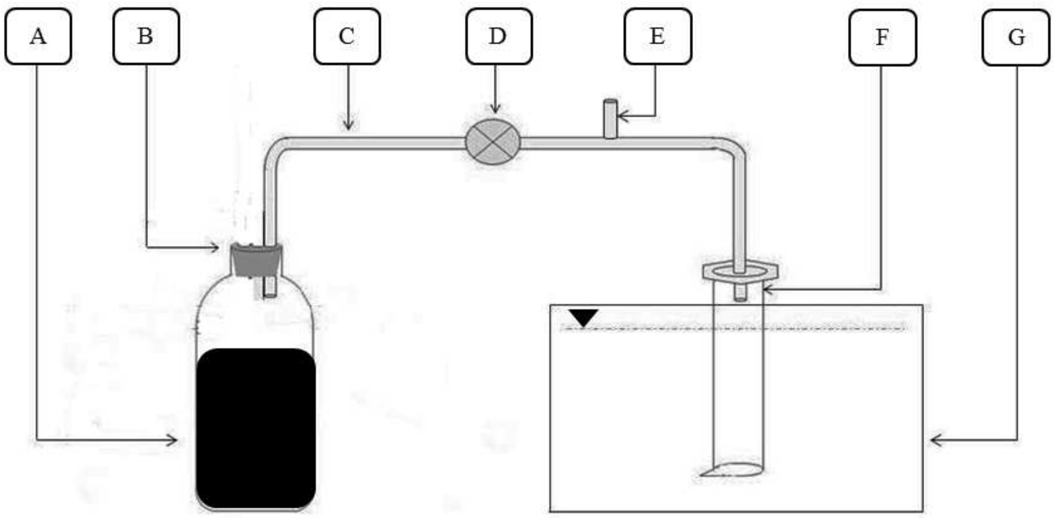

Figure 1. The schematic of simulating anaerobic digestion reactor
A: Reactor, B: Rubber capped, C: Rubber tube, D: Controlling gas valve, E: Biogas sampling port, F: Measuring cylinder, G: Water bath (adapted from [14])

2.4 Biogas quantitation and qualification

The accumulative biogas production used the fluid displacement of gas to measure the volumetric of biogas. Thermal conductivity detector (TCD) was used to detect the qualitative analysis of biogas production. 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 the standard gas to be calculated.

2.5 Statistical Analysis

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

3. RESULTS AND DISSCUSSION

3.1 Substrate characteristics

Table 1 represents tested substrate and inocula characteristics. It could reveal to the initiating environment to start up the anaerobic digestion process. The pH was ranged 5.66 to 7.96; it is suitable to initiate biogas production. The inoculum has highly organic solid which reply to prevalent of microorganism consortiums. COD:N:P were 100:0.5:2.9, 100:0.5:2.7, 100:0.1:0.5 in *Hydrilla*, Characeae and leachate, respectively. There are adequately support growths of anaerobic microorganisms, but nitrogen source seems quite lower than minimum requirement as suggests at 2 [15]. Moisture content in *Hydrilla* and Characeae were high because there are submerged plants. Thus this study did not amend any micronutrient and pre-treatment.

Table 1. Characteristics of *Hydrilla verticillata* (L.f.) Royle, Characeae inoculums and Landfill leachate

Parameters	Unit	*Hydrilla verticillata* (L.f.) Royle	Characeae	Landfill leachate
pH	-	6.23	5.66	7.96
Moisture Contents	%	94.56	94.07	ND
Total Solid	mg/L	57,610	61,130	3,945
COD	mg/L	54,400	80,000	16,800
Total Phosphorus	mg/L	293.14	389.19	15.63
TKN	mg/L	1,588	2,164	84

Note: ND : not detected

3.2 Effects of COD 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 retard (Figure 2 & 3). The degradation efficiency of use *Hydrilla* as co-substrate, it could be degraded as 67, 70, 45, 68 and 81 % in 10:1, 10:2, 10:3, 10:4, 10:5 ratios, respectively (Figure 2). Thus the only sole *Hydrilla* degradation efficiency still reached to 67%. Besides, the efficiency 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 (Figure 3) and 62 % in a hundred percentages of Characeae. However, the degradation efficiency in leachate could be found up to 67%. Among inoculum, *Hydrilla* could degrade better than that Characeae, and this might be due to the physical properties of Characeae quite sticky texture.

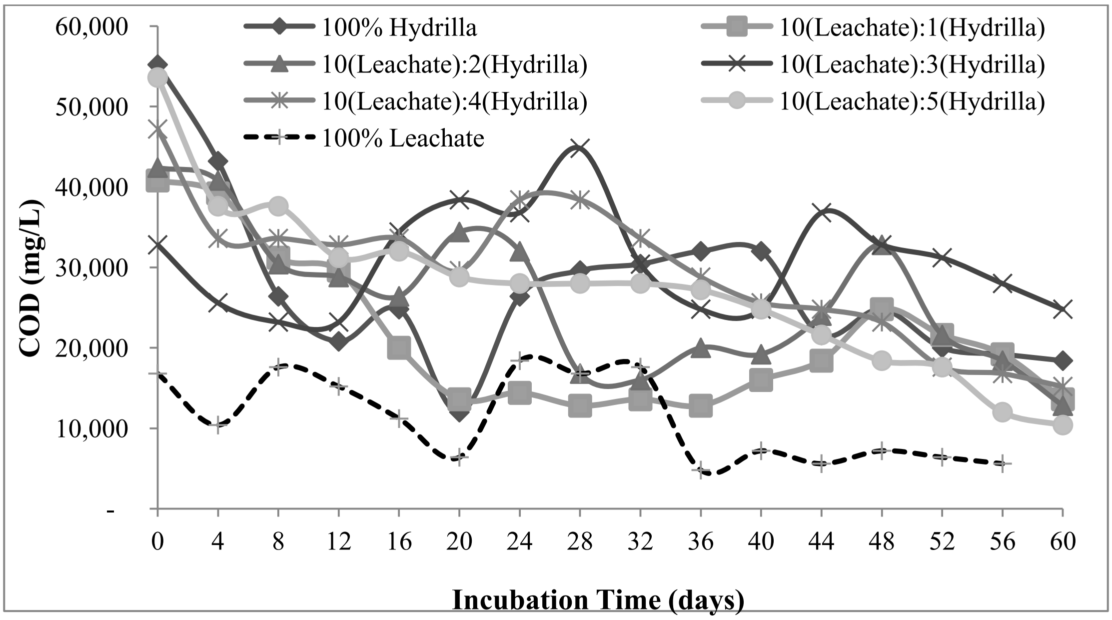

Figure 2. COD degradation of Hydrilla to leachate in different ratio during incubation time

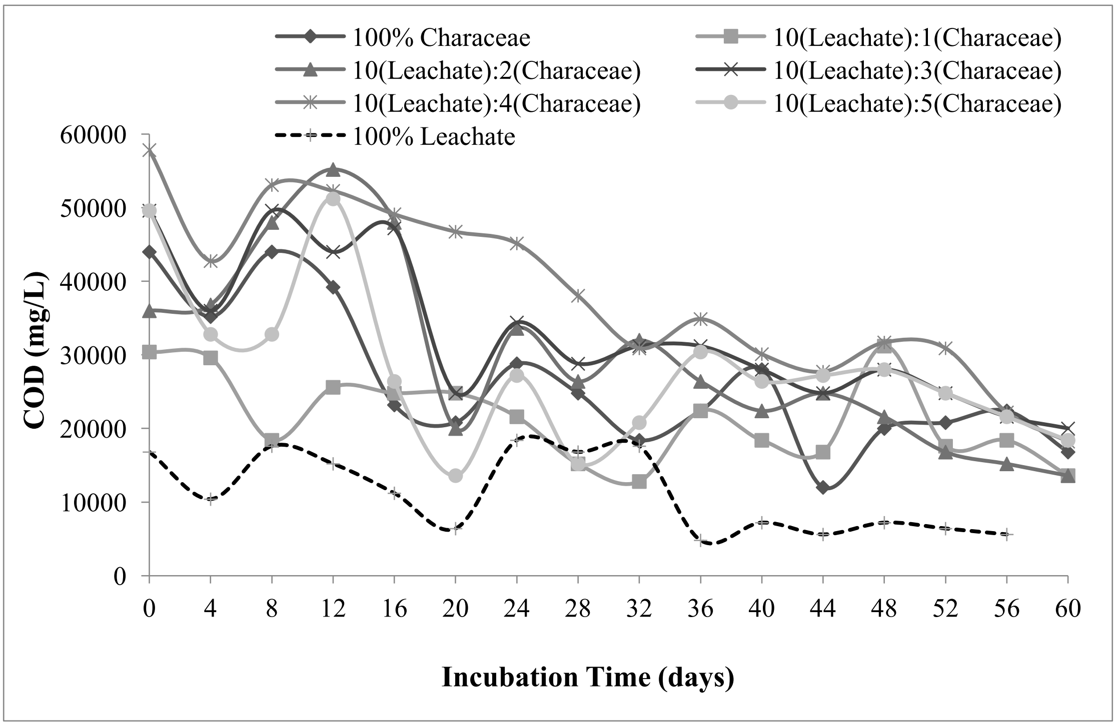

Figure 3. COD degradation of Characeae to leachate in different ratio during incubation time

3.2 Effects of pH to biogas production

The pH of used *Hydrilla* or Characeae as co-substrate could be found ranged 5.83 to 7.78 and (Figure 4) and 5.63 to 7.35 (Figure 5). There were stratified pH operation rage to produce methane and biogas [9]. It found that the pH did not have trends of vary from initial in every ratio of applied *Hydrilla* as co-substrate. However, it notifies some of pH decreased in 16th and 36th days in a ratio of 10(Leachate):2(Hydrilla). It implied to the acedogens could convert the simple organic compound to volatile fatty acid which pH drop. The 36th day found the pH quite drop again that might because of accumulated of acetate or volatile fatty acid. In contrast to Characeae test, it notifies that the pH was drop 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 of leachate alone, however, pH was dropped in the 16th day and could improve after that.

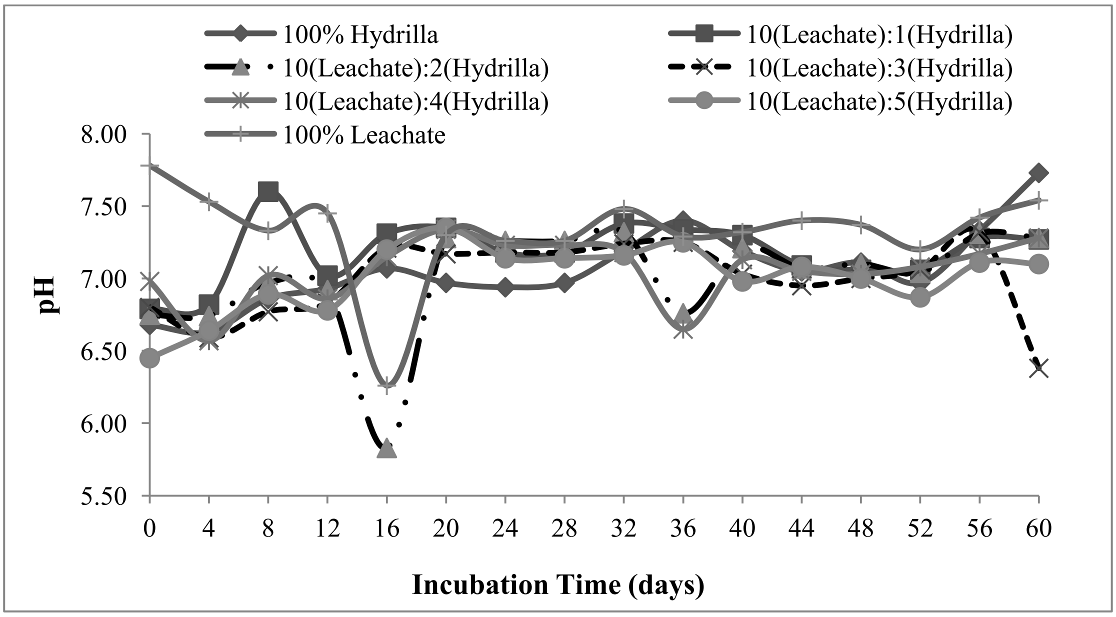

Figure 4. pH regimes under different ratios of Hydrilla to Leachate

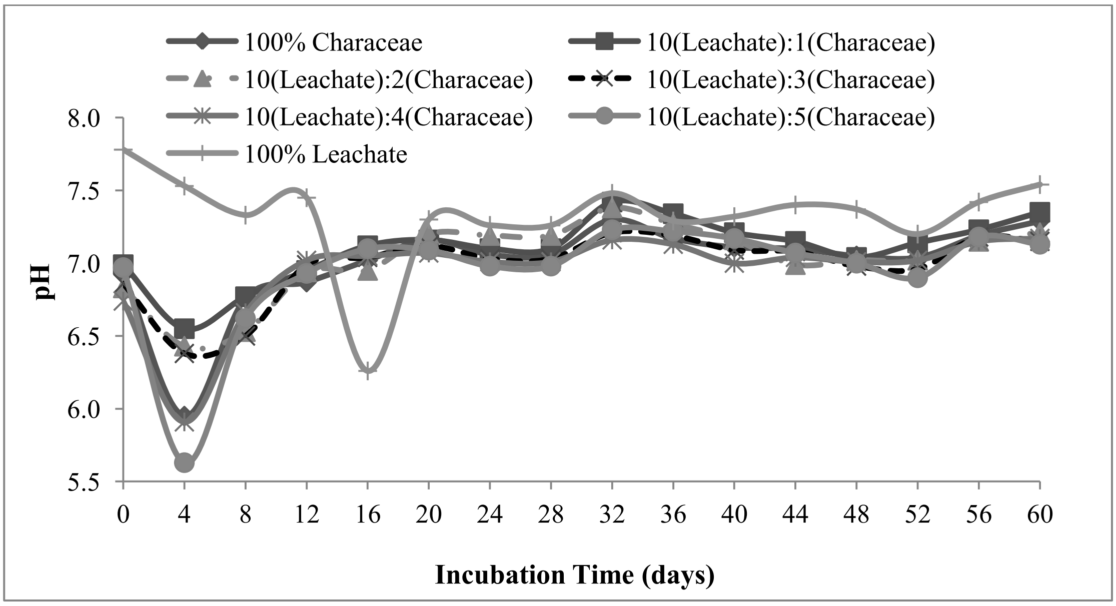

Figure 5. pH regimes under different ratios of Characeae to Leachate

3.3 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 ^{o}C during the incubation period. Accumulative biogas production in leachate could be detected only at 2.6 mL within 60th days (Figure 6). Nevertheless, the degradation of COD could imply potentially to 67 % but it could not be converted to biogas or use of microbial consortiums. *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.

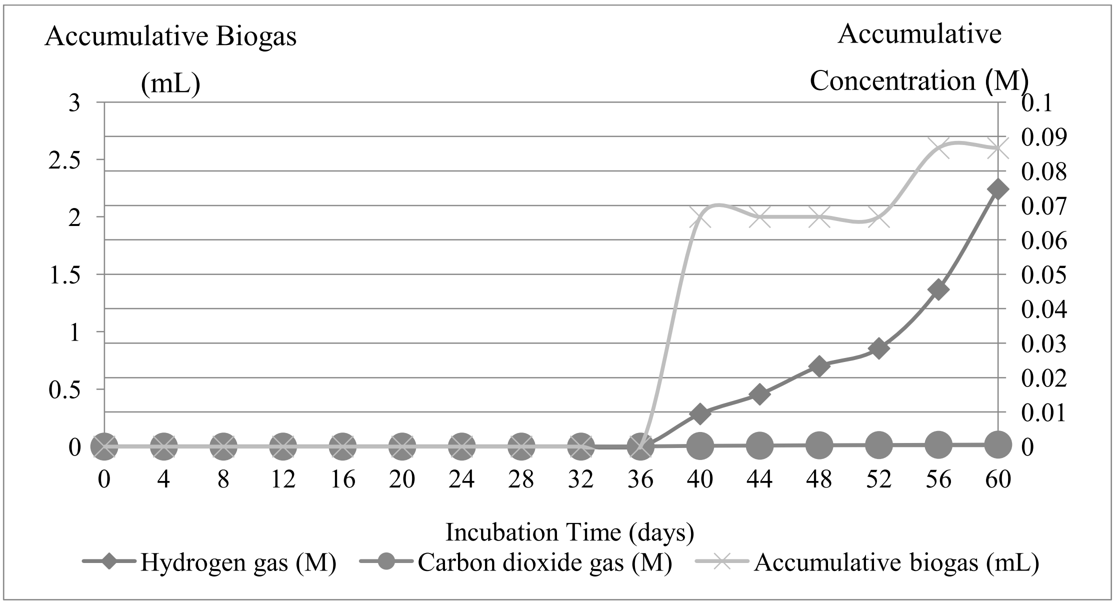

Figure 6. Accumulative biogas and compositions of 100% Leachate

3.3.1 Enhancement of biogas production by *Hydrilla verticillata* (L.f.) Royle (*Hydrilla*)

Fives ratios of *Hydrilla* (1 to 5) were 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. The quantitative of accumulative biogas in the incubation of only sole *Hydrilla* was the highest. This implies the biogas production performance by itself. Furthermore, it could enhance it biogas production in leachate as well. The ratio of use *Hydrilla* as co-substrate to biogas production was insignificantly difference at .05 ($p<.05$). The qualitative of biogas productions were found only two major gas compositions as H_2 and CO_2. The accumulative hydrogen gas was generated at 161, 233, 218, 168, 266 and 479 M in 10:1, 10:2, 10:3, 10:4, 10:5 and 100% of *Hydrilla*, respectively. In addition, the accumulative CO_2 represents 5, 12, 10, 12, 23 and 42 M in 10:1, 10:2, 10:3, 10:4, 10:5 and 100% of *Hydrilla*, respectively. Unfortunately, it could not detect any CH_4.This implies that the *Hydrilla* was not favourable for methanogens to produce methane gas. The results were represented in Figure 7-12. A highly accumulative concentration of hydrogen that might be due to non-exist or less methanogens converts to CH_4. Moreover, *Hydrilla* could generate more H_2 due to there are capable of stocking carbohydrate reserves [15], and it could be a carbohydrate-rich substrate. The hydrogen-producing microorganisms could use them by hydrogenase enzyme to produce H_2 under anaerobic condition. They could complete utilization even methanogens quickly utilised the hydrogen to produce methane [16]. This finding indicates that the potentially of use *Hydrilla* as a substrate which could be an alternately produce hydrogen energy and initiate the biogas production in leachate.

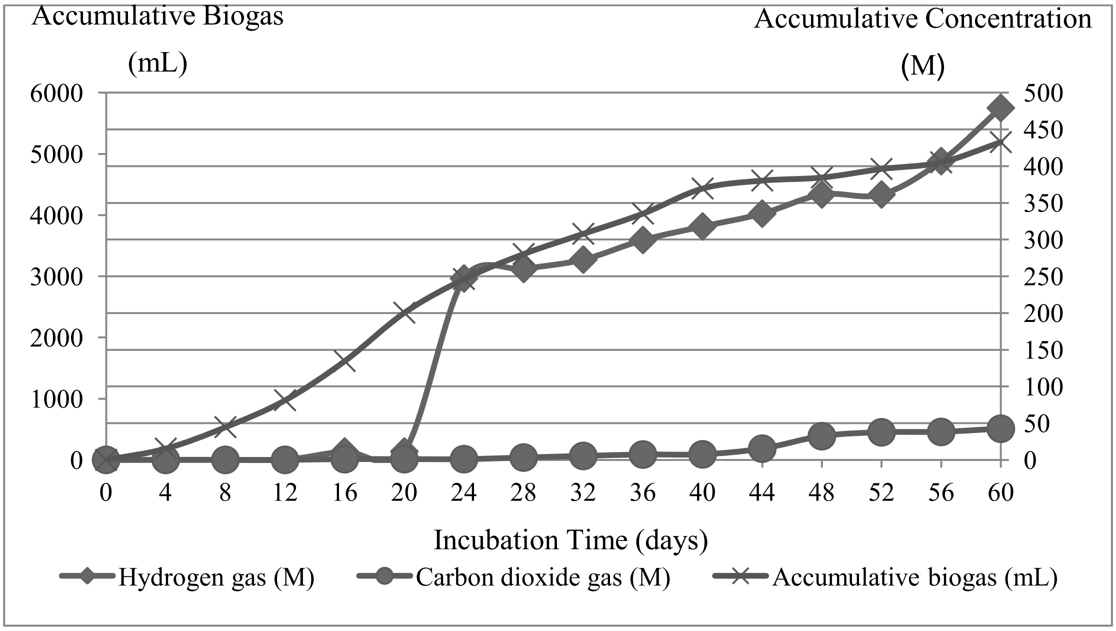

Figure 7. Accumulative biogas and compositions of 100% Hydrilla verticillata (L.f.) Royle

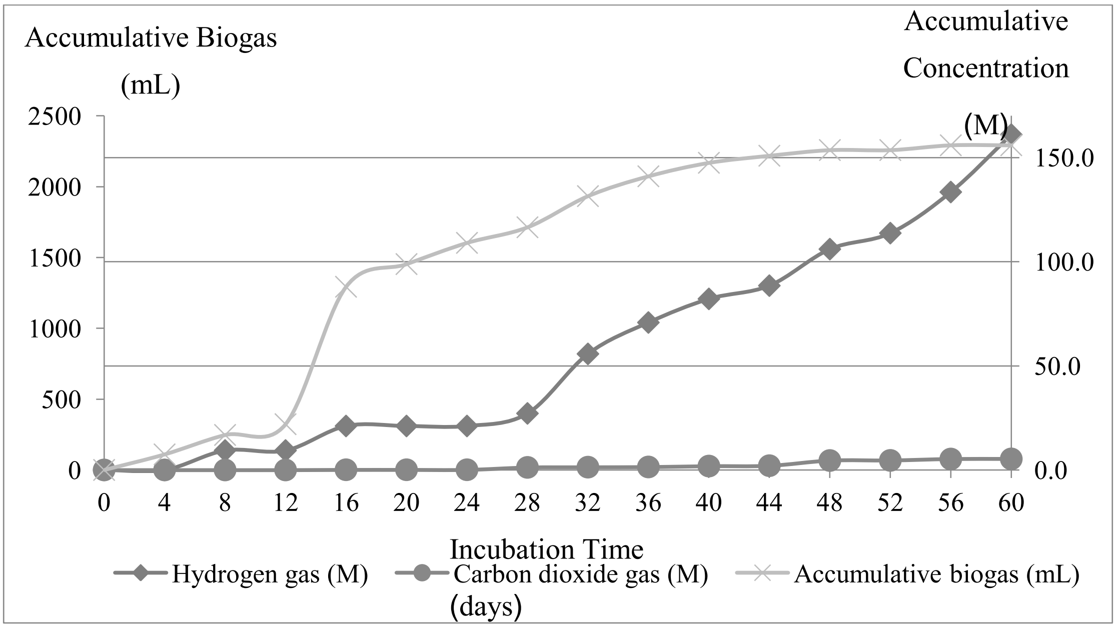

Figure 8. Accumulative biogas and compositions of 10 (Leachate):1(Hydrilla)

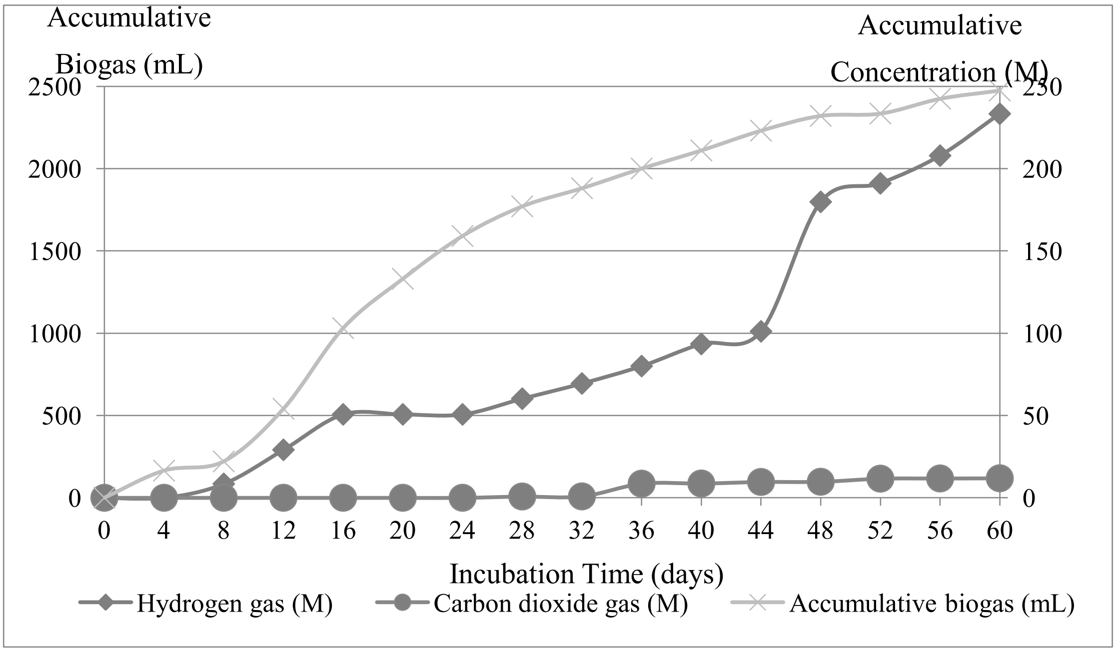

Figure 9. Accumulative biogas and compositions of 10 (Leachate):2(Hydrilla)

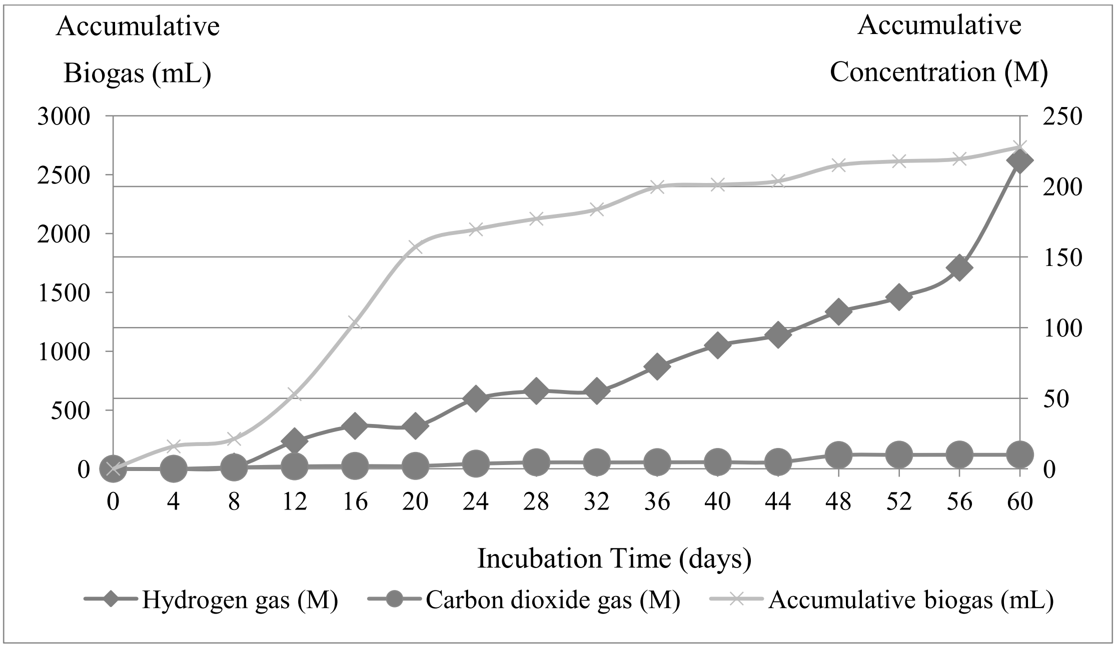

Figure 10. Accumulative biogas and compositions of 10 (Leachate):3(Hydrilla)

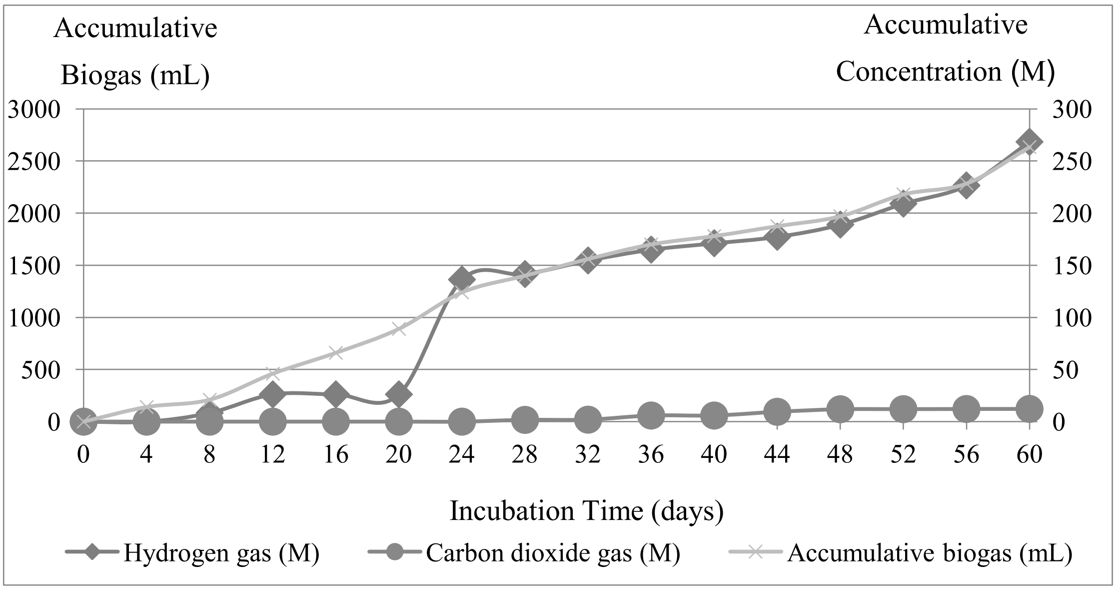

Figure 11. Accumulative biogas and compositions of 10 (Leachate):4(Hydrilla)

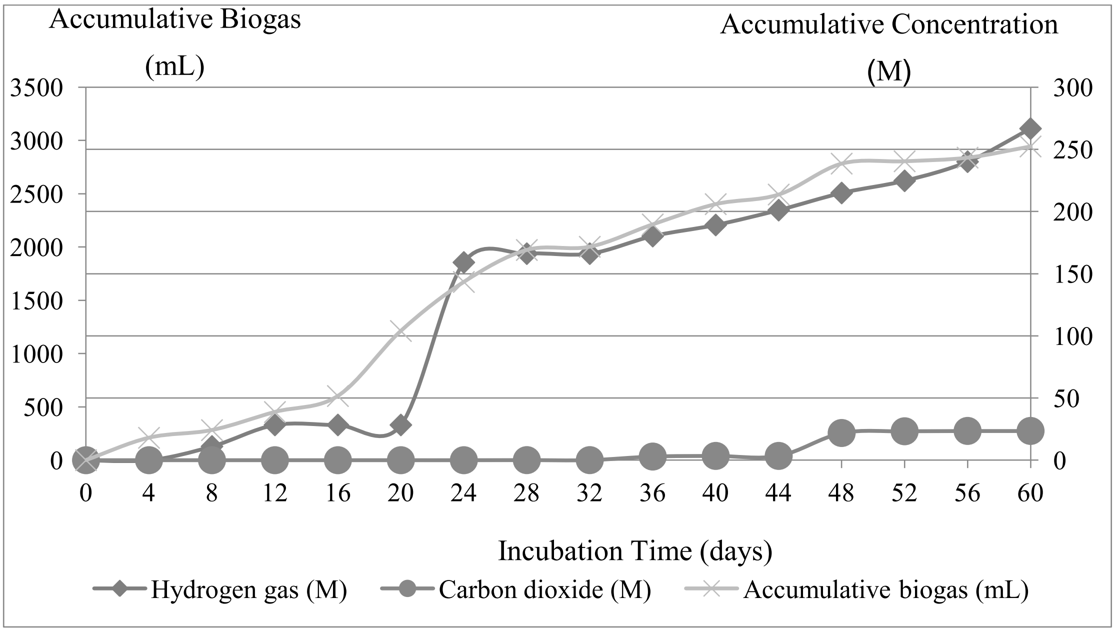

Figure 12. Accumulative biogas and compositions of 10 (Leachate):5(Hydrilla)

3.4.2 Enhancement of biogas production by Characeae

Characeae was used as co-substrate in this part, to compare a potential of using green algae to improve the biogas production and compositions. The accumulative biogas found that 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 (Figure 13-18). The biogas compositions that could be detected were H_2, CO_2 and CH_4, but H_2 was the majority component. The concentration of H_2 production could be measured at 282, 161, 425, 423, 138 and 417 M in 10:1, 10:2, 10:3, 10:4, 10:5 and 100% of Characeae, respectively. Methane was found only in ratio 10:2 at 97 M and 1 M in 100% of Characeae. The analytical of variance among ratio of Characeae notified that 10:2 to 10:4 and 10:4 to 10:5 has a significant difference in statistic at .05 ($p >.05$) in accumulative biogas. Algae could split water molecules into hydrogen ion and oxygen via photosynthesis which converts hydrogen ion to H_2 by hydrogenase enzyme [18]. There is a carbohydrate enrichment substrate. These two properties could make support growth of H_2-producing bacteria, and it could be achieved with methanogens. H_2 is an important intermediate in the metabolism of the substrate of carbohydrates and has not proven to be a reliable indicator of toxicity to anaerobic process [Speech]. This study was successful in case of improvement of biogas production in leachate wastewater but not yet to enhance the CH_4 composition. However, some achievement of using two stages of hydrolysis-acetogenesis for H_2 generation, followed by a methanogenesis stage could apply [19] or establish two stages of hydrogen and methane from organic waste [20].

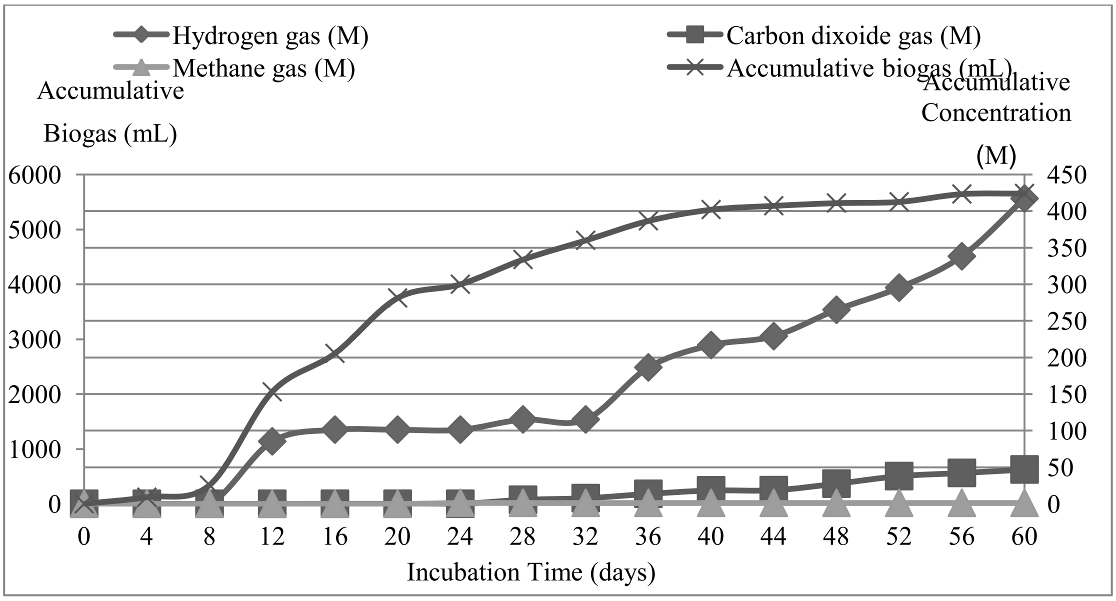

Figure 13. Accumulative biogas and compositions of 100 % Characeae

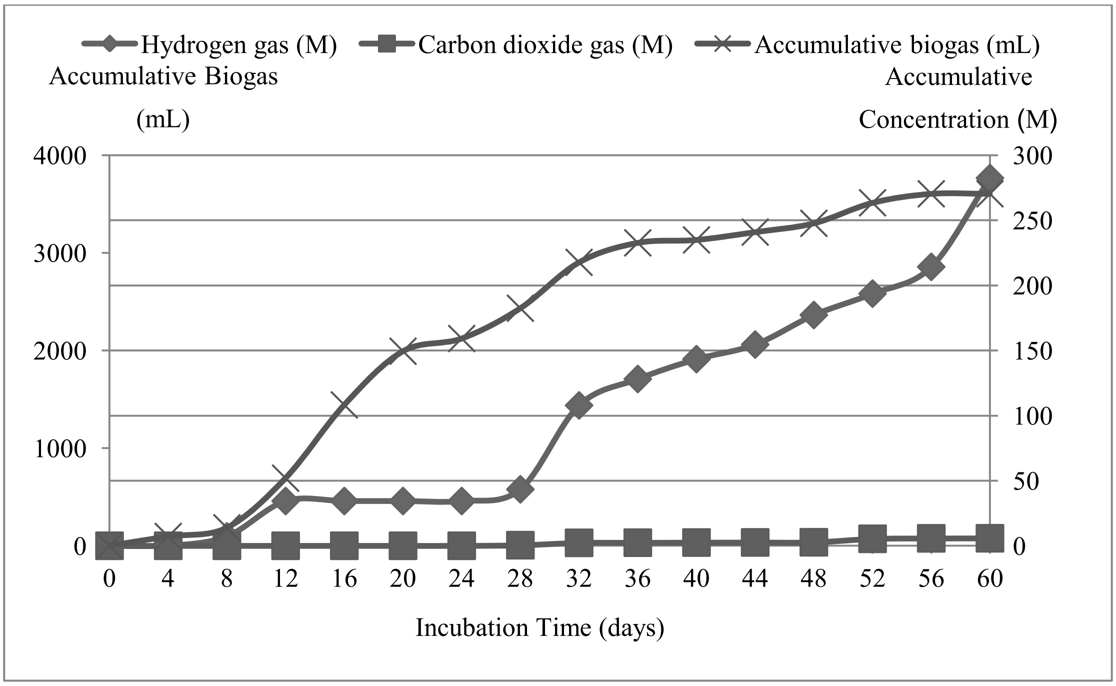

Figure 14. Accumulative biogas and compositions of 10 (Leachate):1(Characeae)

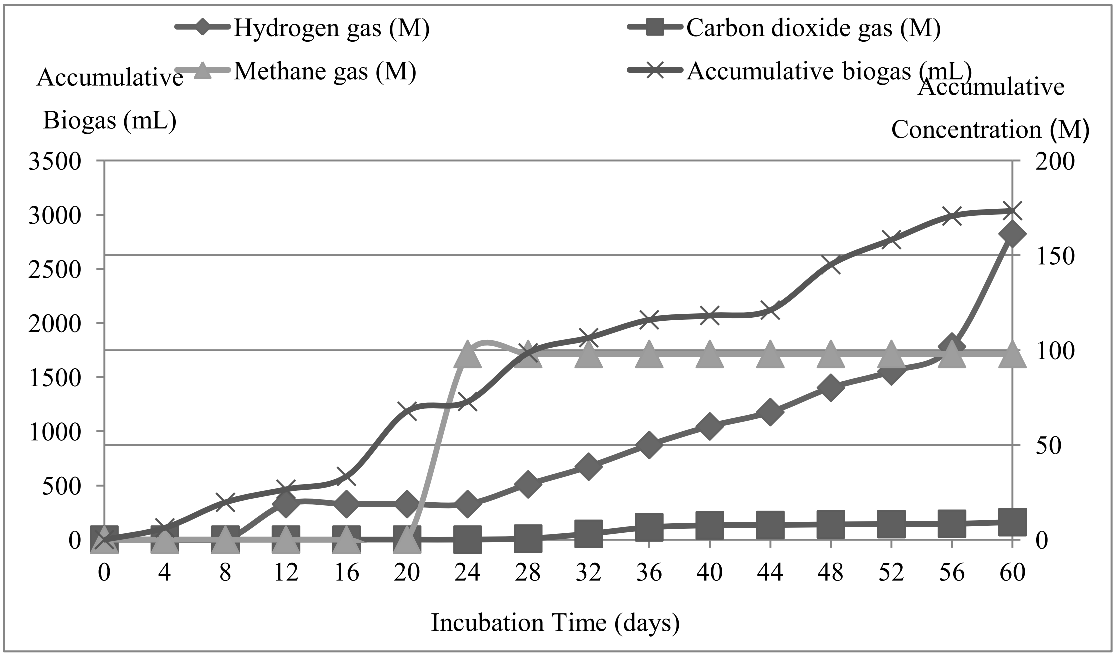

Figure 15. Accumulative biogas and compositions of 10 (Leachate):2(Characeae)

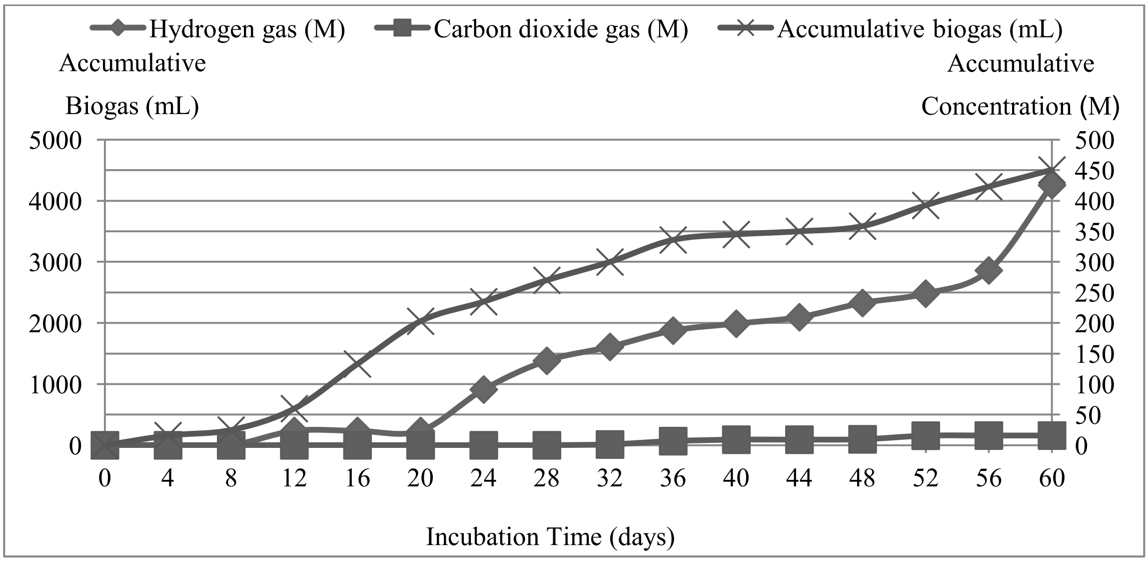

Figure 16. Accumulative biogas and compositions of 10 (Leachate):3(Characeae)

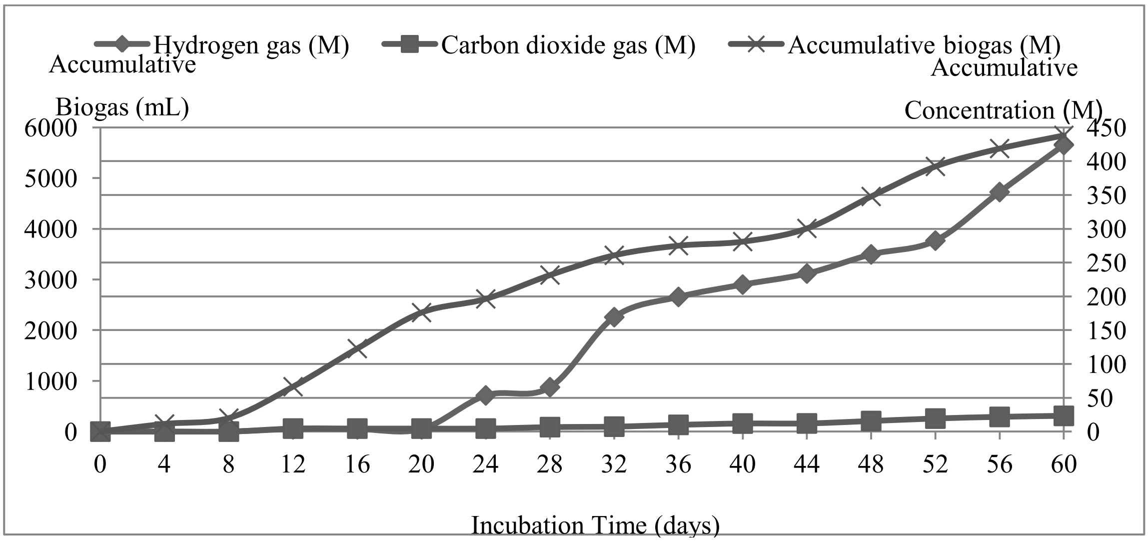

Figure 17. Accumulative biogas and compositions of 10 (Leachate):4(Characeae)

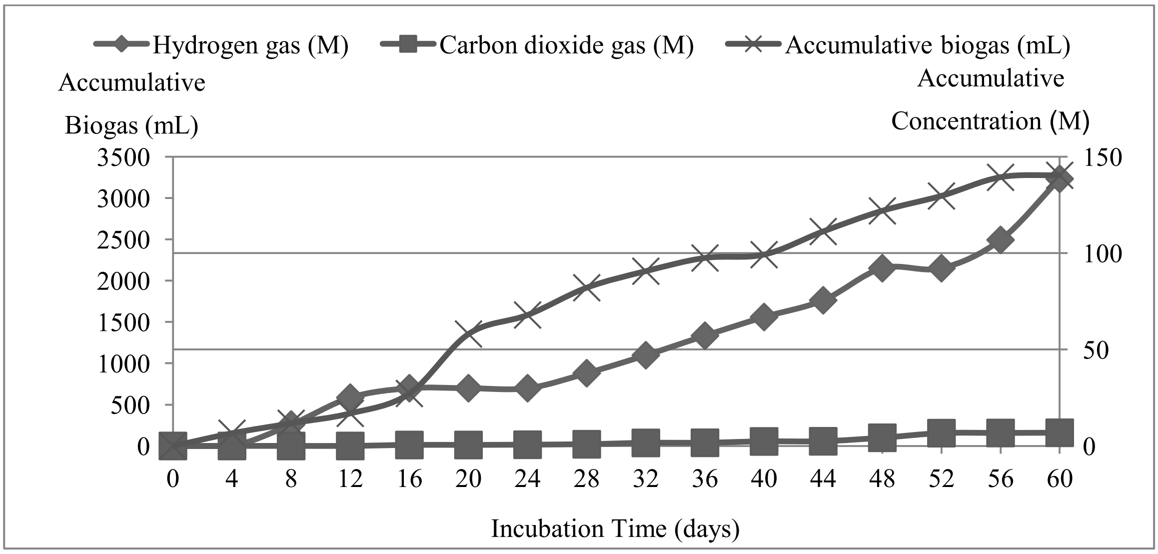

Figure 18. Accumulative biogas and compositions of 10 (Leachate):5(Characeae)

4. CONCLUSIONS

This article has represented techniques for enhancing the biogas production and composition by using *Hydrilla verticillata* (L.f.) Royle (*Hydrilla*) and Characeae in leachate wastewater. The simulated reactors were randomization position and kept in the dark under room temperature approximately 30 – 35 ^{o}C during incubation period. Five ratios of co-substrate were experimented including of 1, 2, 3, 4 and 5, respectively. The only sole leachate, *Hydrilla* and Characeae were investigated to compare the individually performance. The pH regimes incubated of *Hydrilla* or Characeae found ranged 5.83 to 7.78 and 5.63 to 7.35, respectively. The drop of pH on the 4th day of Characeae could increase to neutral after acidogenesis stage, while the pH of use *Hydrilla* was varied. The accumulative biogas production could improve significantly using *Hydrilla* and Characeae. The highest of accumulative biogas production was found in ratio of 10:5 and 10:4 in *Hydrilla* and Characeae, respectively. 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.

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