Chemistry Research Journal, 2016, 1(1):58-66

Available online www.chemrj.org

Research Article

ISSN: 2455-8990
CODEN(USA): CRJHA5

Effect of temperature on the performance of biogas production from Duckweed

Rameshprabu Ramaraj[1], Yuwalee Unpaprom[2]*

[1]School of Renewable Energy, Maejo University, Chiang Mai 50290, Thailand
[2]Program in Biotechnology, Faculty of Science, Maejo University, Chiang Mai 50290, Thailand

Abstract The anaerobic digestion (AD) process is one of the technologies used to produce energy as well as to reduce the organic content of biomass. Aquatic plant biomass has emerged as an alternative for the production of renewable fuels such as biogas from AD. In relation to the biogas production rate, digester temperature setting is one of the most critical factors for an economically viable fermenter operation. In this study, the effect of fermenter temperature on biogas and methane production efficiency during the AD of duckweed was evaluated. Two liter batch fermenters were incubated at room temperature, specific mesophilic (35 °C) and thermophilic (50 °C) conditions for 45 days. The digesters were shaken everyday to prevent the formation of surface crust which may prevent contact between microorganisms and the substrate. The results show that as the temperature was increased the biogas production and methane was also increased, however the high amount of biogas production rate and methane content was observed in the digester operated at 35 °C. During the study period, fermenters incubated at 35 °C produced the highest biogas (10377 ml) and highest methane yields were reached 64.47 %. Thermophilic reactors (50 °C) produced less biogas and less methane, followed by room temperature reactors which produced lesser biogas and methane. Mesophilic temperatures will be used in further studies to examine scaling up of the process.

Keywords biogas, Duckweed, anaerobic digestion

1. Introduction

Biogas, a clean and renewable form of energy could very well substitute for conventional sources of energy which are causing ecological–environmental problems and at the same time depleting at a faster rate. 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 [1]. The quest for alternative sources of energy has evoked the interest in exploring potentials of living biological wastes as new energy materials [2]. Duckweeds are produced abundantly as weeds in freshwater surface bodies and can be a source of biomass for biogas productions.

AD is an application of biological methanogenesis which is an anaerobic process responsible for degradation of much of the carbonaceous matter in natural environments where organic accumulation results in depletion of oxygen for aerobic metabolism. Since AD is a process by which almost any organic waste can be biologically converted in the absence of oxygen [3].

The anaerobic biological conversion of organic matter occurs in three steps. The first step involves the enzyme-mediated transformation of insoluble organic material and higher molecular mass compounds such as lipids, polysaccharides, proteins, fats, nucleic acids, etc [4]. Into soluble organic materials, i.e. to compounds suitable for the use as source of energy and cell carbon such as monosaccharides, amino acids and other simple organic compounds. This step is called the hydrolysis and is carried out by strict anaerobes such as *Bactericides*, *Clostridia* and facultative bacteria such as *Streptococci*, etc. In the second step, acidogenesis, another group of microorganisms ferments the break-down products to acetic acid, hydrogen, carbon dioxide and other lower weight simple volatile organic acids like propionic acid and butyric acid which are in turn converted to acetic acid. In the third step, these acetic acid, hydrogen and carbon dioxide are converted into a mixture of methane and carbon dioxide by the methanogenic bacteria (acetate utilizers like *Methanosarcina* spp. and *Methanothrix* spp. and hydrogen and formate utilizing species like *Methanobacterium*, *Methanococcus*, etc.) [5].

During the anaerobic process, organic waste is biologically degraded and converted into clean biogas [1]. The main product of the anaerobic digestion process is a gas mixture (biogas) mainly composed of methane (CH_4) and carbon dioxide (CO_2) that is used as fuel for power and heat production. The quantity of biogas produced as a function of the quantity of introduced raw material will be variable according to several factors such as the quality of the organic matter and the environmental parameters [6]. The intensity of the microbial activity on which the production of methane depends, is a function of the environment temperature [7]. There are three possible ranges of temperature in which the process can be carried out (psychrophilic 15–25 °C, mesophilic 35–37 °C and thermophilic 50–60 °C).

Temperature and influent substrate may be the most important parameters determining performance and stability of the anaerobic digestion process [8]. Together, they influence the microbial community structure, the biochemical conversion pathways, the kinetics and thermodynamic balance of the biochemical reactions, and the stoichiometry of the products formed [9]. Because formation and consumption of products can occur at different rates, transient accumulation of potentially inhibitory substances is possible, particularly with complex substrates [10]. Consequently, temperature is a critical factor affecting anaerobic digestion because it influences both system heating requirements and methane production. The aim of this study was to comparatively evaluate performance and stability of room temperature, mesophilic and thermophilic anaerobic digestion for biogas production through duckweed.

2. Materials and methods

2.1. Feedstock and inoculums

Duckweed (*Lemna* sp.) was collected from pond at located at the Maejo University, Chiang Mai, Thailand. Subsequently collected duckweed was grown in cement pond (Figure 1) at Faculty of Science (Maejo University) for all experimental analysis. Harvested duckweed was washed manually with tap water and plant were dried at 60°C and then powdered for further analysis and experiments. The anaerobic inoculum was obtained from a working anaerobic digester at Energy Research Center, Maejo University. 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 mgCH_3COOH/L and pH was 6.66 ± 0.03.

Figure 1: Mature duckweed in cement pond

2.2. Experimental conditions

The batch fermenters were as follows: (1) at room temperature (23-28°C), (2) at mesophilic temperature (35°C) and (3) at thermophilic temperature (50°C). The room temperature and specific mesophilic and thermophilic conditions of 2 liter fermenters were set up as shown in schematic Figure 2 and 3. Specification of experimental parameters and biogas measurements were listed in Table 1. For all experiments, prepared duckweed was used as a mono-substrate. Biogas production was received through improvements in the fermentation process using with duckweed and water. The batch digestion system was performed. 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. 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 duckweed 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.

2.3. Analytical Methods and Statistical Analysis

The solids contents, including total solids (TS) and volatile solids (VS), were characterized using the Standard Methods for the Examination of Water and Wastewater (method # 2540) [11]. Metrohm 774 pH-meter was used in all pH measurements. A titration method with sulphuric acid was used to determine total alkalinity (TA) at pH 4.3 as $CaCO_3$ [12]. The chemical oxygen demand (COD) was determined by standard methods # 5220 [11]. The nitrogen concentration, as total Kjeldahl nitrogen (TKN), was determined using the Nessler method # 8075 [13]. Total fat, ash, moisture, fiber contents and volatile fatty acids (VFA) were determined using AOAC official method [14]. The composition of biogas (CH_4, CO_2, H_2S, H_2 and O_2) was measured using a biogas analyzer (GFM 416 series, UK). All the values or readings are the result of mean of three replicates. Data was reported as mean ± standard deviation (SD). Statistical analyses were performed using Microsoft Excel.

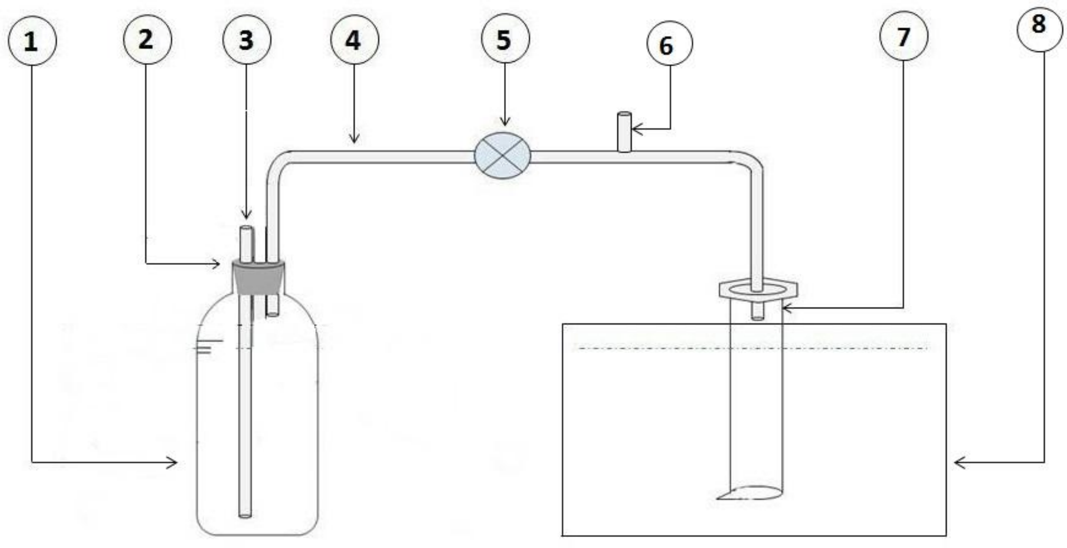

Figure 2: Schematic view of the experimental set up during anaerobic digestion of duckweed. (1) digester (2000ml) 2: rubber stopper, 3: inlet of substrate & inoculums, 4: rubber septum, 5: valve 6: gas sampling port, (7) gas measuring cylinder, (8) water bath

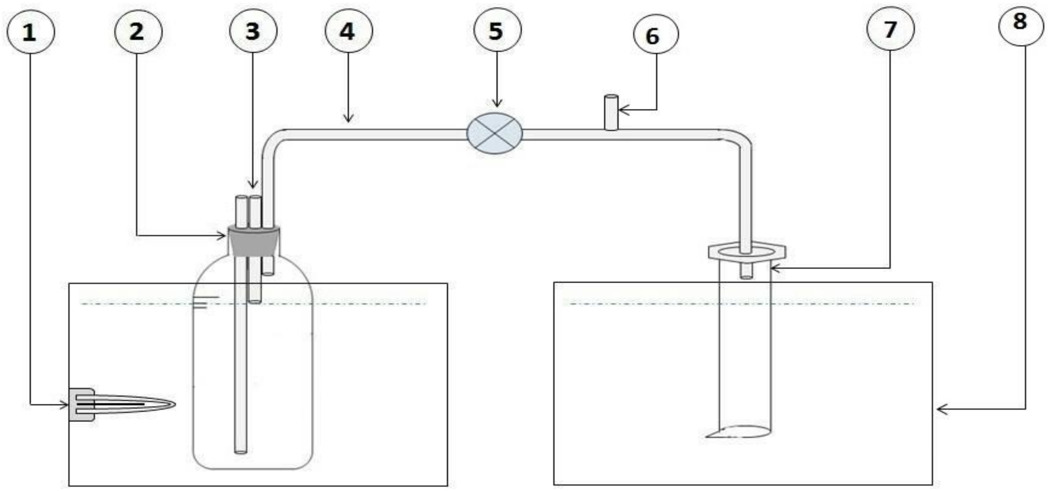

Figure 3. Schematic view of the experimental set up during anaerobic digestion of duckweed. (1) Heater 2: rubber stopper, 3: inlet of substrate and inoculums, 4: rubber septum, 5: valve 6: gas sampling port, (7) gas measuring cylinder, (8) water bath

3. Results and Discussion

Duckweed (*Lemna* sp.) commonly known as classified as the subfamily Lemnoideae belongs to Araceae. Duckweed, a small floating aquatic plant, belongs to the monocotyledonous [15]. The geographic ranges of duckweed span the entire globe and 37 species belonging to five genera (*Lemna*, *Landoltia*, *Spirodela*, Wolffia, and *Wolffiella*) have been identified so far [16].

Table 1: Physical, chemical and composition of duckweed (dry basis)

Parameters	Results
Proximate analysis (%)	
Moisture	12.48 ± 0.21
Ash	19.35 ± 0.57
Volatile matter	71.05 ± 0.01
Fixed carbon	10.11 ± 0.05
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 (%)	25.55 ± 0.47
Protein (%)	32.28± 0.36
Fat (%)	7.42 ± 0.58
Crude Fiber (%)	12.05 ± 1.34
TS (ml/L)	55,712 ± 0.49
VS (ml/L)	32,229 ± 1.02
COD (ml/L)	10,827 ± 1.44
VFA (mgCH_3COOH/L)	2152 ± 0.55
Alkalinity (mg $CaCO_3$/L)	2592± 0.11
pH	7.2 ± 0.41

This plant inhabits in a wide range of aquatic ecosystems from tropical to temperate zones, and from freshwater to brackish estuaries, except arctic and antarctic zones. Duckweed occurs abundantly in still or slightly moving water, but flourishing growth is reported to occur in stagnant ponds, brackish water or ditches rich in organic matter, or near sewer outlets [2, 17].

Duckweed grows faster than most other plants, and under ideal condition [18]. These plants float on the surface of water and have thin leaves attached to a simple root [19. And duckweed exhibits very much higher specific growth rates than other larger aquatic or terrestrial plants, with doubling times of between 48 h and 96 h depending on species [20].

The physico-chemical and compositions properties of duckweed contents were listed in Table 1. The substantial biomass of water lettuce has relatively high levels of chemical compositions such as carbohydrate 25.55 (%), protein 32.28 (%), fat 7.42 (%) and crude fiber 12.05 (%) were demonstrated that as a feedstock of choice for bioenergy production. Excluding the chemically rich, the duckweed is one of the fastest growing weeds which make this as suitable candidate for rich biomass production. Table 2 demonstrated the bioenergy potential of harvested duckweed biomass. A considerable number of studies have been conducted to investigate anaerobic digestion of aquatic weed as feedstock for digestions processes [29, 30]. The production of biogas there are several other advantages to harvesting of aquatic weeds including duckweed.

The duckweed can be a potential source of feedstock in anaerobic digestion process which is utilized as major mechanism in biogas production. The results illustrates that temperature has a significant effect on biogas production. Figure 4 shows the results of biogas production at each temperature. Hence a higher biogas potential which was reflected in the present study as the duckweed 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 5, 6, 7 and 8. Average methane contents were relative to the biogas production.

Table 2: Overview of bioenergy production from different species of duckweed

Species	Treatment	Product	Reference
Lemna gibba	Thermochemical pyrolysis	Bio-oil	[21]
	Hydrolysis and fermentation	Bioethanol	[22]
Lemna minor	Thermochemical pyrolysis	Biochar	[23]
		Gas, bio-oil and char	[24]
	Pre-treatment and fermentation	Bioethanol	[25]
Lemna minuta	Photosynthetic plant fuel cell	Electricity	[26]
Lemna spp.	Thermochemical liquefaction	Bio-oil	[27]
Lemna sp.	Anaerobic digestion	Biogas	
Spirodela polyrrhiza	Hydrolysis and fermentation	Bioethanol	[28]
Wolffia and *Spirodela* species	Thermolysis	Bioleum	[24]

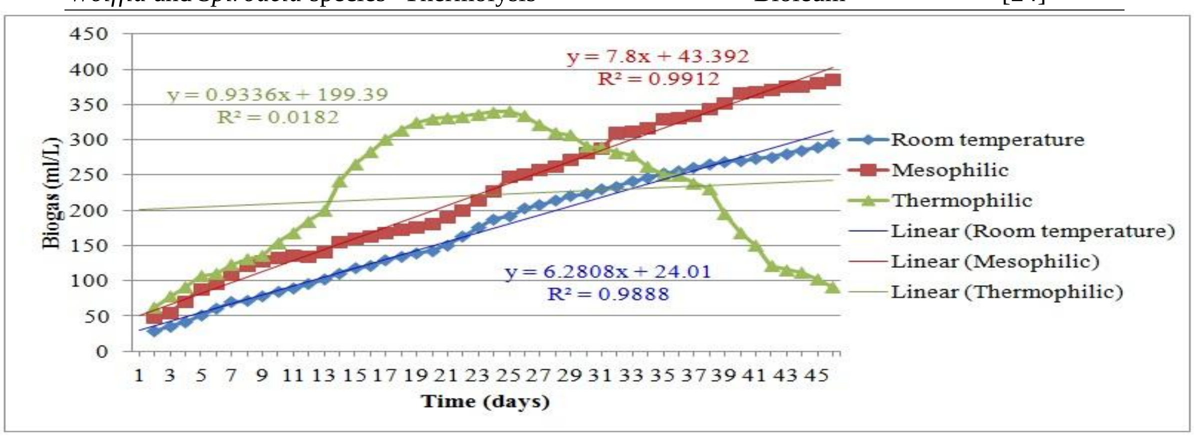

Figure 4: Biogas yield of duckweed with different temperature digesters

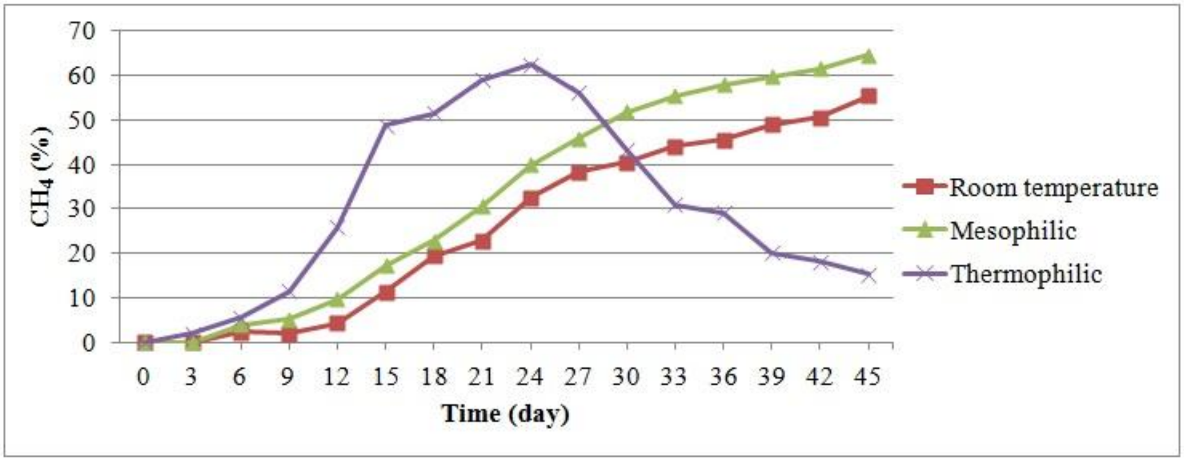

Figure 5: Methane production of duckweed with different temperature digesters

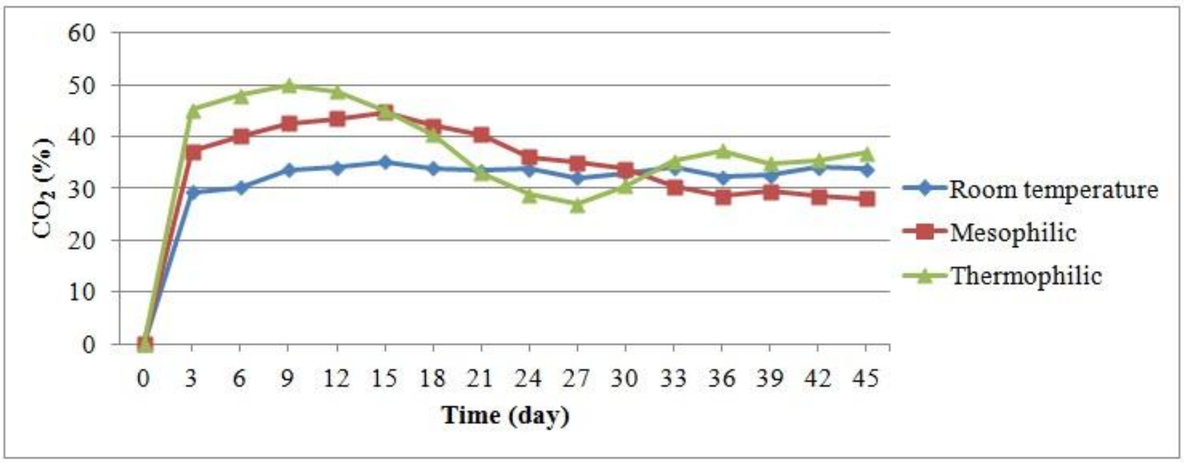

Figure 6: CO_2 level of duckweed with different temperature digesters

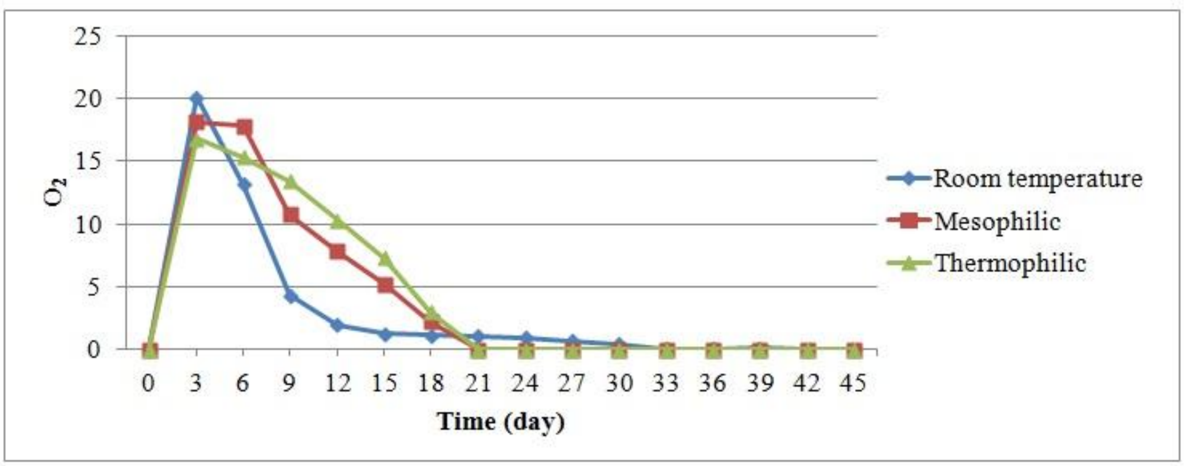

Figure 7: O_2 level of duckweed with different temperature digesters

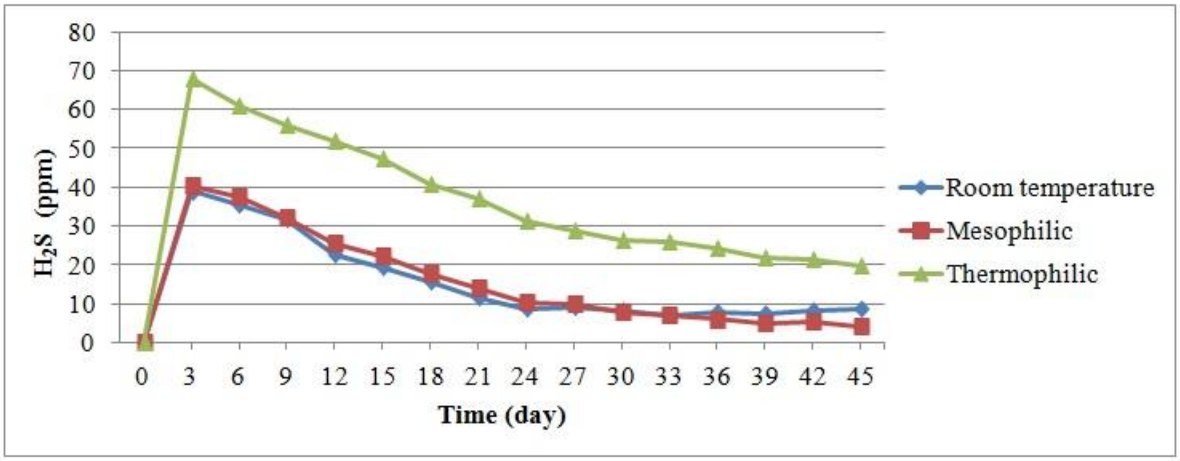

Figure 8: H_2S amount of duckweed with different temperature digesters

The investigations on temperature to indicate that the digester was operated at 35ºC, and had maximum methane content than other digesters (room temperature and thermophilic conditions) in this study. This can be attributed to the volume of a biogas production, which was also the highest at this condition. The total biogas yield was achieved 7863.69 ml/L in room temperature, 10376.59 ml/L in mesophilic temperature and 9981.08 ml/L thermophilic temperature. Since the total biogas yield was reached 10377 mL and maximum methane content was 64.47 %. An efficiency criterion of methane production was explained good performance throughout the study.

For achieving anaerobic digestion several physical and chemical factors must be considered. The most important physical factor is temperature. Temperature and substrate composition are among the main factors affecting performance and stability of anaerobic digestion process [31, 32]. From the experimental results demonstrates that typical of the temperature ranges that at higher temperature decomposition take place quickly. Technically only the mesophilic and thermopfilic range is interesting, since at the room temperature the anaerobic degradation is very slow. However there are disadvantages of thermophilic anaerobic fermentation are the reduced process stability and reduced dewatering properties of the fermented sludge and the requirement for large amounts of energy for heating. In order to start to move toward a potential industrialized cultivation system, preliminary tests on the biogas yield and methane content have been carried in this study. The results indicate that duckweed 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 through mesophilic conditions. Consequently, duckweed can be utilized as a substrate for biogas production further scale up studies.

Conclusion

This study investigated the potential of duckweed biomass as a feedstock for biogas production. Duckweed is a fast growing, high yielding aquatic plant and highly nutritious especially, so it is suitable for use as energy crops for biogas production. These results indicated that, duck contains rich organic substances and these substances are suitable to use in the anaerobic fermentation process to be used to sustain microbial life, and transform nutrients into biogas. In this investigation, the results of batch experimental test on the anaerobic digestion of duckweed in room temperature, mesophilic and thermophilic conditions. The anaerobic digestion process was achieved with temperatures tested. In terms of biogas production, mesophilic fermenter would be more effective than others. This suggested that it is possible to achieve stable operation using duckweed, as a substrate for biogas production in pilot or large scale biogas plant in the future. It was concluded that duckweed as energy crop can be an alternative energy resource.

Reference

1. Pantawong, R., Chuanchai, A., Tipbunrat, P., Unpaprom Y., & Ramaraj, R. (2015). Experimental investigation of biogas production from Water Lettuce, *Pistia stratiotes* L. *Emergent Life Sciences Research*, 1(1):38-45.
2. Ramaraj, R., & Dussadee, N. (2015). Biological purification processes for biogas using algae cultures: A review. *International Journal of Sustainable and Green Energy*, 4(1-1):20-32.
3. Unpaprom, Y., Intasaen O., Yongphet, P., & Ramaraj, R. (2015). Cultivation of microalga *Botryococcus braunii* using red Nile tilapia effluent medium for biogas production. *Journal of Ecology and Environmental Sciences*, 3:58-65.
4. Verma, R, & Suthar, S. (2015). Utility of duckweeds as source of biomass energy: a Review. *BioEnergy Research*, 8(4): 1589-1597.
5. Ramaraj, R., Unpaprom, Y., Whangchai, N., & Dussadee, N. (2015). Culture of macroalgae *Spirogyra ellipsospora* for long-term experiments, stock maintenance and biogas production. *Emergent Life Sciences Research*, 1:38-45.
6. Converti, A., Delborghi, A., Zilli, M., Arni, S., & Delborghi, M. (1999). Anaerobic digestion of the vegetable fraction of municipal refuses: mesophilic versus thrmophilic conditions. *Bioprocess Engineering*, 21:371-376.
7. Kettunen, R. H., & Rintala, J. A. (1997). The effect of low temperature (5-29 degrees C) and adaptation on the methanogenic activity of biomass. *Applied Microbiology Biotechnology*, 48(4):570-576.
8. Chae, K. J., Jang, A., Yim, S. K., & Kim, I. S. (2008). The effects of digestion temperature and temperature shock on the biogas yields from the mesophilic anaerobic digestion of swine manure. *Bioresource Technology*, 99:1-6.
9. Arikan, O. A., Mulbry, W., & Lansing, S. (2015). Effect of temperature on methane
    a. production from field-scale anaerobic digesters treating dairy manure. *Waste Management,* 43:108-113.
10. Labatut, R. A., Angenent, L. T., & Scott, N. R. (2014). Conventional mesophilic vs. thermophilic anaerobic digestion: a trade-off between performance stability? *Water Research*, 53:249-258.
11. APHA-AWWA-WEF. (2005). Standard Methods for the Examination of Water and Wastewater, 21st ed. American Public Health Association/American Water Works Association/Water Environment Federation, Washington, DC, USA.
12. Jenkins, D., Richard, M. G., & Daigger. G. T. (2004). Manual on the Causes and Control of Activated Sludge Bulking, Foaming, and Other Solids Separations Problems. 3rd Ed., IWA Publishing, London, UK.
13. HACH. (2003). DR/2500 Spectrophotometer procedure manual. HACH Company.
14. Official Methods of Analysis of AOAC International. (2012). 19th Ed., AOAC International, 420 Gaithersburg, MD, USA.
15. Yin, Y. H., Yu, C. J., Yu, L., Zhao, J. S., Sun, C. J., Ma, Y. B., Zhou, G. K. (2015). The influence of light intensity and photoperiod on duckweed biomass and starch accumulation for bioethanol production. *Bioresource Technology*, 187:84-90.
16. Appenroth, K. J., Borisjuk, N., & Lam E. (2013). Telling duckweed apart: genotyping technologies for the Lemnaceae, *Chinese Journal of Applied Environmental Biology*, 19:1-10.
17. Hillman, S. W. (1961). The Lemnaceae or duckweeds. A review of the descriptive and experimental literature. *Botanical Review*, 27:221-287.
18. Peng, J. F., Wang, B.Z., Song, Y.H., & Yuan P. (2007). Modeling N transformation and removal in a duckweed pond: model development and calibration. *Ecological Modelling*, 206:147-152.
19. Landolt, E., Kandeler, R. (1987). The family of Lemnacae-a monographic study. Vol 2. Part of the series: biosystematic investigations in the family of duckweeds (Lemnacae), vol 3 of 4. Veroffentlichungen Des Geobotanischen.

20. Zuberer, D. A. (1982). Nitrogen fixation (acetylene reduction) associated with duckweed (Lemnaceae) mats. *Applied and Environmental Microbiology*, 43:823-882.
21. Muradov, N., Fidalgo, B., Gujar, A. C., T-Raissi, A. (2010). Pyrolysis of fast-growing aquatic biomass-Lemna minor (duckweed): characterization of pyrolysis products. *Bioresource Technology*, 101:8424-8428.
22. Ge, X., Zhang, N., Phillips, G. C., & Xu, J. (2012). Growing Lemna minor in agricultural wastewater and converting the duckweed biomass to ethanol. *Bioresource Technology*, 124:485–488
23. Mohedano, R. A., Rejane, H. R., Tavares, F.A., Filho, P. B. (2012). High nutrient removal rate from swine wastes and protein biomass production by full-scale duckweed ponds. *Bioresource Technology*, 112:98-104.
24. Muradov, N., Taha, M., Miranda, A. F., Kadali, K., Gujar, A., Rochfort, S., Stevenson, T., Ball, A. S., Mouradov, A. (2014). Dual application of duckweed and azolla plants for wastewater treatment and renewable fuels and petrochemicals production. *Biotechnology for Biofuels*, 7:30.
25. Bayrakci, A. G., & Koçar, G. (2014). Second-generation bioethanol production from water hyacinth and duckweed in Izmir: a case study. *Renewable Sustainable Energy Reviews*, 30:306-316.
26. Hubenova, Y., & Mitov, M. (2012). Conversion of solar energy into electricity by using duckweed in direct photosynthetic plant fuel cell. *Bioelectrochemistry*, 87:185-191.
27. Xiu, S. N., Shahbazi, A., Croonenberghs. J., Wang, L. J. (2010). Oil production from duckweed by thermochemical liquefaction. *Energy Sources A*, 32:1293-1300.
28. Xu, J., Zhao, H., Stomp, A. M., Cheng, J. J. (2012). The production of duckweed as a source of biofuels. *Biofuels* 3:589-601.
29. O'Sullivan, C., Rounsefell, B., Grinham, A., Clarke, W., & Udy, J. (2010). Anaerobic digestion of harvested aquatic weeds: water hyacinth (*Eichhornia crassipes*), cabomba (*Cabomba caroliniana*) and salvinia (*Salvinia molesta*). *Ecological Engineering*, 36:1459-1468.
30. Mathew, K., Bhui, I., Banerjee, S. N., Goswami, R., Chakraborty, A. K., Shome, A., Balachandran, S., & Chaudhury S., (2015). Biogas production from locally available aquatic weeds of Santiniketan through anaerobic digestion. *Clean Technology Environmental Policy*, 17: 1681-1688.
31. Labatut, R. A., Angenent, L. T., & Scott, N. R. (2014). Conventional mesophilic vs. thermophilic anaerobic digestion: a trade-off between performance and stability? *Water Research*, 53:249-258.
32. Ziganshin, A. M., Liebetrau, J., Pröter, J., & Kleinsteuber, S. (2013). Microbial community structure and dynamics during anaerobic digestion of various agricultural waste materials. Applied Microbiology Biotechnology, 97:5161-5174.