Anaerobic digestion of cyanobacteria and chlorella to produce methane for biofuel

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Abstract: The methane potentials of cyanobacteria and chlorella have been investigated in eight different lab scale reactors at 25℃ for three-day Hydraulic Retention Time (HRT). Autoclavation pre-treatment was applied to the cyanobacteria to aid digestion, while the Chlorella was obtained and digested in powdery form. The organic loading rates were 1g VS, 2 g VS, 3 g VS, 4 g VS, 5 g VS, 6 g VS, 7 g VS, 8 g VS and 9 g VS. Methane production rates increased with increasing loading rates and started declining at loading rate higher than 7 g VS, while the HRT was kept constant. The highest methane production rates for cyanobacteria and chlorella were (78±25) mL/(L·d) and (100±25) mL/(L·d), respectively, at loading rate of 7 g VS. Digester instability occurred at loading rates of 8 g VS and 9 g VS with higher accumulation of methane concentrations. Lipid compositions of both feeds were close and the methane production potentials of both biomasses were also close and followed the same trend.

Keywords: anaerobic digestion, biofuel, carbon dioxide (CO_2), Hydraulic Retention Time (HRT), methane (CH_4), chemical oxygen demand (COD), volatile fatty acids (VFAs)

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1 Introduction

Production of biofuels as alternative energy sources to conventional fuels is becoming important because of the diminishing petroleum reserves and the environmental effects of combustion of petroleum based fuels. Biofuels have huge potentials to mitigate greenhouse gases (GHG) and to limit the dependence on hydrocarbon resources[1]. Nevertheless, the deforestation of some carbon-rich forests for the purpose of producing biofuels might lead to the emissions of GHG if compared with emissions from the combustion of petroleum products[2]. Furthermore, global food supply could be negatively affected if cultivable land is used for the biofuel production[3].

The ability of microorganisms with photosynthetic capabilities to serve as other sources to energy crops, coupled with their carbon dioxide (CO_2) absorption quality is a topic attracting strong interests. Algae could be described as various groups of photosynthetic, aquatic organism, categorized generally either as macro-algae, e.g. sea weed, or as micro-algae typically unicellular organism. Green micro-algae which are eukaryotic belongs to the chlorophyceae class and the most widely usage for commercial purposes belongs to the genera, chlorella, dunalialla, chlamydomonas and haematococcus. The investigation of these marine and fresh water algae has created large amount of information about their biochemistry cultivation and physiology[4].

Microalgae are divided into several autotrophic organisms. They have unicellular structure which enables them to transform solar energy to chemical energy. Microalgae have many commercial applications

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ranging from pharmaceutical and cosmetic to food purposes. Because of their high protein, polysaccharides and vitamins contents, they are used as nutrient supplements for humans' consumption. Some microalgae species are most suitable for biodiesel production because of their high lipid contents[5].

A group of micro-algae that is rich in silicon are called diatoms and cyanobacteria (prokaryotic) offering huge opportunities for biotechnology and metabolic engineering. Microalgae flourish in oxygenated liquid cultures where they have access to CO_2, light and some other nutrients[6]. Micro-algae are grown primarily photoautotrophically, but some other species can thrive heterotrophically by disintegrating organic matter like sugar[7]. Algae do not need fertile land to grow and survive compared with terrestrial plants. In addition, because micro-algae make use of CO_2, large scale breeding might be used for CO_2 sequestration.

Cyanobacteria and Chlorella were used in this study because of their availability and ongoing studies on them at the Chemical Engineering Department, Heriot Watt University. The blue-green algae usually called cyanobacteria are ancient photosynthetic microbes group that exist in inland waters and they have possibilities of having major impacts on the water quality as well as the aquatic ecosystems. There are about 2000 species of cyanobacteria in 150 genera, with various sizes (typically 2-5 μm) and shapes. They have a variety of cellular structures, physiological strategies and cell types that added to their success ecologically in the metaphyton or plankton[8]. Cyanobacteria usually obtain their food by oxygenic photosynthesis. Since they have more than one form of chlorophyll, they are able to absorb light in form of energy in conjunction with phycobillus and caroteres. Based on this structure, they have the capability to interact with photon energy, receive and transport energy among molecules by resonance. The occurrences of these chlorophylls make cyanobacteria different from the remaining photosynthetic bacteria[9].

Chlorella belongs to the *Chorophyta*- phylum and a genus of algae with a single cell. The sizes are usually around 2 μm to 9 μm in diameter, posse a spherical shape and have no flagella. Its chloroplast consists of two photosynthetic pigments, the chlorophyll-a and chlorophyll-b. Through the process of photosynthesis, chlorella can multiply very fast with water, CO_2, some minerals and sunlight[10].

Microalgae composition meets the requirement of the bacteria for anaerobic digestion process. Apart from nitrogen, phosphorus and carbon, which are regarded as the main elements in algae composition, some elements such as cobalt, iron and zinc which are oligo nutrients were found[11], and they are capable of exciting methanogenesis[12]. Microalgae usually contain carbohydrates (5%-23%), protein (6%-52%) and lipids (7%-23%) and these compositions vary in species[13]. The variations in composition may have impact on the anaerobic digestion of micro-algae.

There are two factors that can make reasonable impact on the methane output causing restrain on some bacteria population for anaerobic digestion. The protein content in micro-algae, which is usually high, leads to high NH_4 release, thereby inhibiting the anaerobic micro-flora and the high amount of sodium in micro-algae may disturb the anaerobic digestion process. One of the most determining factors for methane yield is the composition of the organic matter. The cell walls of micro-algae could prevent the enzymes generated during anaerobic digestion to digest the micro-algae substrate, thereby creating resistant to hydrolysis. To make this recalcitrant portion lower and to increase methane yield, pre-treatment could be applied. Some of the pre-treatment methods that could be used are thermal treatment, chemical treatment (bases, acids, ozonation) and ultrasonic pre-treatment. In addition to theoretically increase the methane output, a metabolic approach could be applied since most algae have the ability to absorb a large amount of carbon under some conditions in the form of lipids and starch[14].

Batch anaerobic digestion of chlorella-*scenedesmus*[15] at 35-50℃ with hydraulic retention time (HRT) of 30 days and loading rate of 1.45-2.88 g $VS \cdot L^{-1} \cdot J^{-1}$ yielded 0.17-0.32 L CH_4 g VS^{-1} and the methane percentage was between 62% and 64%. This result is similar to the previous study[16], where algal biomass was digested in a batch reactor at 35℃ with HRT of 28 days and loading

rate of 1 g VS L^{-1} J^{-1} produced 0.42 L CH_4 g VS^{-1} and methane concentration of 72%. Using a continuously stirred reactor (CSTR)[17] performed biomethanation using fresh *Tretraselmis*, dry *Tretraselmis* and dry *Tretraselmis* with NaCl at 35℃, HRT of 14 days and a loading rate of 2 g $VS{\cdot}L^{-1}{\cdot}J^{-1}$. The methane concentration of 72%-74% were constant for the three forms of substrate, however, the methane volume differs slightly. From the result, the addition of NaCl to dry *Tretraselmis* made no significant impact on methane yields. At temperature between 28-31℃ and with HRT of 64 days, with using Chlorella-*vulgaris* as feedstock the study of Sanchez and Travieso 1993[18] yielded 0.31-0.35 L CH4 g VS-1 and 68%-75% methane concentration were achieved. This outcome is however similar to experiments conducted by Yen and Burne[19], using Chlorella-scenesmus as substrate at 35℃ in CSTR for 10 days with loading rate of 2-6 g $VS{\cdot}L^{-1}{\cdot}J^{-1}$, the methane were concentration low compared to previous study[18] and 69% methane concentration was achieved. Spirulina maxima was digested by Samson et al.[20] in a semi-continuous reactor at 35℃ in 33 days with loading rate of approximately 1 g $VS{\cdot}L^{-1}{\cdot}J^{-1}$. The methane yield was 0.26 L CH_4 g VS^{-1} and concentration between 68% and 72% was achieved.

The percentage of methane in the biogas is in the range of 69%-75% for most studies, regardless of operating conditions and species, however most at 35℃ and above. This exhibits a good attributes of conversion of algae into methane. Micro-algae digestion releases small amount of H_2S than other organic matter, because they don't usually have sulphurated amino acids[21].

This study aimed to investigate the biomethane potentials of cyanobacteria *(Gloethece Membranacea)* and Chlorella at temperature lower than those reported in previous studies (25℃). Also to access the methane potential of cyanobacteria which are not readily reported in literature and would be compared with chlorella as single substrates at psychrophilic temperature (25℃).

2 Materials and methods

2.1 Anaerobic digestion experiment

The anaerobic digestion of Cyanobacteria (*Gloethece membranacea*) and chlorella were carried out using 8 laboratory scale digesters made of glass bottles with volume of 330 mL, working volume of 300 mL, placed inside incubators (Stuart Scientific, Orbital incubators S150) at 25℃. The digesters were connected to 120 mL gas storage bags with a sample port. The digesters were operated in semi-continuously mode. The digesters were fed every three days, after the removal of the same quantity of effluent. Mixing of the digesters was done automatically by the shaker inside the incubator at 60 r/min. The reactors were fed with homogenous mixt ure of cyanobacteria and chlorella. The mass of substrate added varied from 1 g VS to 9 g VS. The digestion experiments were carried out for 35 days. The pH before digestion was recorded and adjusted with sodium hydrogen carbonate dissolved in water. Trace metals and selenite solutions were added to provide nutrients for the anaerobic digestion process and the details are: $FeCl_2.4H_2O$, 2; H_3BO_3, 0.05; $ZnCl_2$, 0.05; $CuCl_2.2H_2O$, 0.038; $MnCl_2.4H_2O$, 0.05; $(NH_4)_6Mo_7O_{24}.4H_2O$, 0.05; $AlCl_3$, 0.05; $CoCl_2.6H_2O$, 0.05; $NiCl_2.6H_2O$, 0.092; ethylenediaminetetraacetate, 0.5; concentrated HCl, 1 mL; $Na_2SeO_3.5H_2O$, 0.1. Biogas production was monitored by the displacement of acidified water and gas analyses were done daily.

2.2 Characteristics of substrate

Cyanobacteria -*Gloethece membranacea* were utilized as feeding substrates in this investigation. The Cyanobacteria-*Gloethece Membranacea* used in the experiment were harvested from the bio-processing laboratory, Chemical Engineering Department, Heriot-Watt University. Chlorella used in this study was supplied by Aqua Supplies, London, United Kingdom in powdery form. The inoculum used for the experiments was obtained from a food anaerobic digester. The inoculum was kept at 4℃ before use. The cyanobacteria were pre-treated by autoclavation at about 110℃ for 10-20 min before anaerobic digestion.

2.3 Analytical methods

2.3.1 pH measurement

In this study, the pH meter used is made by Thermo Orion, Fisher brand model Hydrus 400. The pH values of the contents of the digesters were measured at the

beginning and end of the batch digestion process.

2.3.2 Nutrients (Nitrogen and Phosphorus) measurements

There are some basic levels of nutrients required by anaerobic micro-flora for anaerobic digestion and the basic ones are nitrogen and phosphorus. In addition, digestate usually contains some important nutrients, such as Mg, N, P, Na, K, which make it applicable for use as organic fertilizer. Nitrogen and phosphorus elements were determined using HARCH spectrophotometer DR/4000 series, manufactured by Camlab. The measurements were conducted following standard methods in HACH DR/4000 Spectrophotometer handbook.

2.3.3 Lipid extraction

The extractions of lipids were completed by chloroform/methanol extraction technique described by Rezanka et al.[22]. This was based on the idea of the composition of the microalgae. Lipids were extracted because lipid content is a major determinant to know the methane potential of micro-algae as shown in the previous study[14]. From all the studies reported, the species with the highest lipid content produced the highest methane volume.

2.3.4 Chemical Oxygen Demand (COD)

The COD of a system is a tool to estimate the equivalent oxygen in an organic matter of a substrate that is capable to oxidize by a chemical oxidant that is strong. The CODs of the contents of the reactors were measured before and after anaerobic digestion process following standard methods as described by HMSO[23].

2.3.5 VFAs and alkalinity

Gas chromatography was used to determine volatile fatty acids (VFA) as explained by Goodwin and Stuart, (1994). Alkalinities were estimated as stipulated by HMSO[24]. One hundred milliliters of sample was titrated to pH 4.5 with 0.2 M hydrochloric acid.

2.3.6 Gas sampling and analysis

Biogas production was determined by displacement of water and adjusted to standard pressure and temperature (101 325 Pa, 0℃). The gas samples from the reactors were analyzed basically for methane concentrations and CO_2, by Gas Chromatograph (GC) 600 series manufactured by GOW-MAC Instruments Co., with a thermal conductivity detector (TCD), and helium gas was used as the carrier gas. The oven temperature was 50℃ and the column length is 100 μL. The gas chromatograph was calibrated with standard 55% methane and 45% CO_2 from Cryoserve. The standard curves area from the GC was integrated to give the standards for the methane and the CO_2. Five milliliters glass syringe with 0.4×25 mm was used to collect gas sample from the gas storage and injected to the GC column. About 0.5 mL was usually injected into the GC. Gas sampling and test were done daily after methane was detected.

3 Results and discussion

The major aim of this study was to investigate the anaerobic digestion potentials of cyanobacteria and chlorella at 25℃ (mesophilic condition) to effectively manage and reduce energy demand of anaerobic digesters. All the anaerobic digestion experiments in this study were carried out at the same temperature (25℃).

Autoclaved cyanobacteria and chlorella were digested in 8 digesters each at loading rate of 1 g VS and 2 g VS, 3 g VS and 4 g VS, 5 g VS and 6 g VS, 7 g VS, 8 g VS and 9 g VS. Methane was detected on day 20 for all the digesters, HRT was 3 days and the digesters were operated for a period of 35 days. The cyanobacteria were autoclaved at 110℃ for approximately 10 min. The digesters responded well to loading rate throughout the most period of the study, however, the digester became slightly unstable during loading rate between 7 g VS and 9 g VS of 3 days HRT. Throughout the study the general performance of the reactors was good, with COD removals of 75%-85% and pH values were mostly between 6.5 and 7.4. For most of the experiments, methane production rates increased with increasing organic loading rates from 1 g VS to 7 g VS with both feedstock.

Methane was first detected in all digesters with loading rates of 1 g VS, 2 g VS, 3 g VS and 4 g VS on the 20^{th} day. The delay could be attributed to the operating temperature (25℃) and the organic loading rate. For digesters with loading rates of 5 g VS, 6 g VS, 7 g VS, 8 g VS, and 9 g VS methane gas was first detected on the

9th day. The delays could also be attributed to the operating temperature of the digesters. This might have prolonged methanogenesis thereby prolonged the growth rate of methane producing bacteria.

Methane production rate was (5±3) mL/(L·d) at 1 g VS loading rate and increased to a maximum of (12±4) mL/(L·d) at 2 g VS. For digester two, methane production rate was (15±6) mL/(L·d) at 3 g VS loading rate and increased to (29±4) mL/(L·d) at 4 g VS loading rate. The VFAs increased with increasing loading rate as shown by the results (Table 1). A toxicity concentration would be achieved if the loading rate remained increasing.

This trend also continued in digester three, however, in digester four keeping the HRT at three days, methane production decreased from (78±25) mL/(L·d) at 7 g VS loading rate to (67±20) mL/(L·d) at 8 g VS loading rate and further to (50±24) mL/(L·d) at 9 g VS loading rate.

Table 1 Methane production rate, loading rates, COD added and removed, VFAs, and three-day HRT

Feedstock	Loading rate VS/L day	Average CH_4 (mL/L day)	COD (mg/L) added	COD (mg/L) removed	Digester	VFAs (mg/L)
Cyanobacteria	1	5±3	1 196±134	415±154	1	709±58
	2	12±4	2 475±235	440±185	1	1 400±78
	3	15±6	3 375±310	980±210	2	2 189±37
	4	29±4	5 425±398	1 917±369	2	2 968±46
	5	29±15	7 825±479	3 250±120	3	3 900±78
	6	49±11	10 075±678	4 016±789	3	5 689±120
	7	78±25	14 500±567	5 678±648	4	5 900±36
	8	67±20	18 967±345	13 858±356	4	7 089±245
	9	50±24	23 690±456	18 700±457	4	9 145±198
Chlorella	1	4±3	1 308±230	473±80	5	607±43
	2	7±2	2 925±389	513±200	5	1 230±88
	3	15±8	3 750±501	1 217±275	6	2 289±39
	4	40±8	5 875±678	2 233±875	6	3 068±40
	5	50±18	8 850±824	3 583±125	7	3 890±75
	6	66±10	10 400±134	4 317±345	7	6 089±128
	7	100±25	17 467±654	6 879±587	8	6 900±89
	8	80±34	24 678±456	1 600±359	8	9 089±100
	9	55±28	30 897±345	2 1897±200	8	1 2145±300

The decrease in methane production could be attributed to the higher loading rates with three-day HRT. At these loading rates, digesters (D4 and D8) generated more VFAs. At 8 g VS and 9 g VS loading rates the VFAs concentrations increased to (7 089±245) mg/L and (9 145±198) mg/L, respectively, in digester 4, (9 089± 100) mg/L and (12 145±300) mg/L in digester 8, these are relatively higher compared to the VFAs levels at lower loading rates used in this study. VFAs is very important in the CH_4 metabolic chain, Hill et al.[25] suggested the monitoring of VFAs concentrations to know the performance of anaerobic digesters. VFAs levels could therefore be used to forecast digesters effectiveness and performance by checking overloading as shown in this study. The higher level of VFAs in digesters 4 and 8 as observed in this study could be as a result of increase in activities at the acidogeneses phase and slower acid intermediates consumption by methanogenic bacteria[26]. Apparently, a longer HRT would have resulted in an increase in methane production rate. The use of three days HRT was considered, because a higher organic loading rate and relatively shorter HRT could reduce the cost of digester volume, which could make the economics of biogas reactors more competitive. In the anaerobic digestion process, the cost of building a biogas reactor is considered as a major part of investment[27].

The drop of the methane production rates in digesters 4 and 8 were at 8 g VS and 9 g VS loading rates, compared to other digesters with lower loading rates and same HRT, COD removal reduced to approximately 35%. The COD added increases with increasing loading rates as expected, and the COD removal in digesters, 1, 2, 3, 5, 6 and 7 were between 65% and 85% (Table 1). The

drop in the efficiency could be attributed by the accumulation of VFAs, thereby leading to the drop in methane production rate. The comparison of the digestate COD and VFA concentrations indicated that during the loading rates of 8 g VS and 9 g VS, the overall performance may be limited by the conversion of VFAs to methane.

The pH of all the digesters in respective of the loading rate and feedstock were between 6.5 and 7.4. The pH value of the reactors did not fall below 6.5 during the reactor instability and low methane production rates. The pH value was around 7.0 even during the accumulation of VFAs and reduction in the COD removal efficiency. The reason for such pH value could be the present of pockets of clog feedstock at the bottom of the reactor producing high concentrations of VFAs and moderately pH values. This activity might have led to the deactivation of methanogenic bacteria at the bottom of the reactor which created a zone with less methanogenic activities which advance upwards gradually until the digester became slightly unstable.

In an anaerobic digestion process, two main products were generated: digestate and biogas. If these two products are well managed, the process can have good economy. Apart from the organic matter content, the digestate also has nutrients that can affect the soil quality positively, stimulate microbial activities and also help in improving the water-holding potential if applied as fertilizer. Some of the nutrients are N, K, P and Mg[28]. The application of the digestate as fertilizer will also increase crop yields, soil quality and grain quality[29]. However, there are possibilities of heavy metals concentration in the digestate, which is the reason why digestate, which must be used as organic fertilizer, must be screened and certified. In this study only Nitrate and Phosphate concentrations were determined and compared in the feed and digestate for all the digesters as detected using spectrophotometer; however, this is not one of the main objectives of the study.

Comparing with the other studies, methane production yield in this study is lower than the methane yields of 100-140 mL CH_4/g VS introduced at ten-day HRT[19] and much lower than the yield of 269 mL CH_4/g VS introduced at 30-day HRT investigated by Oswald and Golueke[30]. One of the major reasons for the low methane production in this study is the operating temperature compared to other studies. Because, the anaerobic digestion process, especially methanogenesis phase, are temperature dependent. In addition, since the temperature is at mesophilic range, a longer HRT than three days might have yielded a higher methane production rates. On the other hand, it could be argued that the methane yields at 25℃ is competitive with methane yields at 35℃, if the energetic cost of increasing the energy input from 25℃ to 35℃ is properly evaluated.

The anaerobic digestion of cyanobacteria and chlorella at the same operating conditions and HRT yielded similar results and trends; although the methane production rates from chlorella digestion was slightly higher than cyanobacteria. These results could be attributed by the lipid contents of the micro-algae. From the extraction experiments performed in this study, the lipids content of cyanobacteria is (24%±8%) and of chlorella is (28%±6%). The methane production rates of chlorella digestion experiments from this study are higher than that of cyanobacteria due to the difference in the lipids contents.

4 Conclusions

Anaerobic digestion of chlorella and cyanobacteria at 25℃ has been investigated. The methane production rates increased with increasing loading rates for both feedstock. However, methane production decreased, while the accumulation of VFAs concentrations increased, with both feedstock at loading rates of 8 g VS and 9 g VS. During the decline of the methane production rates COD removal efficiency dropped below 50%, indicating digester instability. However, pH value remains at approximately 7.1. The methane production rates observed in this study were lower compared to other previous studies, which could be attributed by the operating temperature. Cyanobacteria and chlorella used in this study have close lipids contents, and therefore produced close methane volume. The pH values measured from all the digestion experiments were moderate and were between 6.5 and 7.4.

The methane potential of the anaerobic digestion of chlorella and cyanobacteria at 25℃ could be competitive compared to biomethanation of algae at higher temperature if energetic cost is properly evaluated. Cyanobacteria and chlorella may therefore have similar potential of methane production rates.

[References]

[1] Wiley P, Campbell J E, McJuin B. Production of biodiesel and biogas from algae: a review of process train options. Water Environ. Res., 2011; 83: 326-338.

[2] Fargione J, Hill J, Tilman D, Polasky S, Hawthorne P. Land clearing and the biofuel carbon debt. Science, 2008; 319: 1235-1238.

[3] Johansson D, Azar C. A scenario based analysis of land competition between food and bioenergy production in the US. Climatic Change, 2007; 82(3): 267-291.

[4] Harris E H. Chlamydomonas as a model organism. Annual Review Plant Physiology Plant Mol Biology, 2001; 52: 363-406.

[5] Harun R, Singh M, Forde G M, Danquah M K. Bioprocess engineering of microalgae to produce a variety of consumer products. Renewable and Sustainable Energy Reviews, 2010; 14: 1037-1047.

[6] Anderson R A. Algal culturing techniques. Elsevier Academic Press, 2005 (Ed).

[7] Chen F, Chen G Q. Growing phototrophic cells without light. Biotechnol Lett, 2006; 28: 607-616.

[8] Vincent W. Cyanobacteria, Encyclopedia of Inland Waters. Elsevier, 2009; 226-232.

[9] Graham L E, L W Wilcox. Algae. Prentice- Hall, 2000; 112p.

[10] Scheffler J. Underwater Habitats, Ilumin; A Review of Engineering in Everyday Life, 2009; 9(4): 1-4.

[11] Grobbelaar J U. Algal nutrition. In Richmond A, editor. Handbook of microalgal culture: Biotechnology and applied phycology. Wiley-Blackwell. 2004.

[12] Speece R E. Anaerobic biotechnology for industrial wastewaters. Nashville: Archae press. 1996.

[13] Brown M R, Jeffrey S W, Volkman J K, Dunstan G A. Nutritional properties of microalgae for mariculture. Aquaculture, 1997; 151: 315-31.

[14] Sialve B, Bernet N, Bernard O. Anaerobic digestion of microalgae as a necessary step to make microalgal biodiesel sustainable-Review. Biotechnology Advances, 2009; 27: 409-416.

[15] Golueke C G, Oswald W J, Gotaas H B. Anaerobic digestion of algae. Appl. Microbiology, 1957; 5(1): 47-55.

[16] Chen P H. Factors influencing methane fermentation of micro-algae. PhD dissertation. California: University of California, Berkeley, CA, USA, 1987.

[17] Asinari Di San Marzano C M, Legros A, Naveau H P, Nyns E J. Biomethanation of the marine algae tetraselmis. Int J Sustain Energy, 1982; 1: 263-272.

[18] Sanchez E P, Travieso L. Anaerobic digestion of chlorella *vulgaris* for energy production. Resour Conserv Recycle, 1993; 9: 127-132.

[19] Yen H, Burne D E. Anaerobic co-digestion of algal sludge and waste paper to produce methane. Bioresource Technology, 2007; 98: 130-134.

[20] Samson R, LeDuy A. Biogas production from anaerobic digestion of *spirulina maxima* algal biomass. Biotechnol Bioeng, 1982; 24: 1919-1924.

[21] Becker E W. Micro-algae forhuman and animal consumption. In: Borowitzka M A, Borowitzka L J, editors. Micro-algal technology. Cambridge University Press, 1988: 222-56.

[22] Rezanka T, Zahradnik J, Podojil M. Hydrocarbons in green and blue green algae. Folia. Microbiol, 1982; 27: 450-454.

[23] HMSO. Chemical oxygen demand (Dichromate value) of polluted and waste water. Second Edition, HMSO, London UK. 1986.

[24] HMSO. The Determination of alkalinity and acidity in water, HMSO, London, UK. 1981.

[25] Hill D T, Cobb S A, Bolte J P. Using volatile fatty acid relations to predict anaerobic digester failure. Trans ASAE, 1987; 30: 496-501.

[26] Ehimen E A, Sun Z F, Carrington C G, Birch E J, Eaton-Rye J J. Anaerobic Digestion of microalage residues resulting from biodiesel production process. Applied Energy, 2011; 88: 3454-3663.

[27] Rivard C J. Anaerobic bioconversion of municipal solid waste using a novel highosolids reactor design. Applied Biochemistry and Biotechnology, 1993; 39/40: 71-82.

[28] Marinari S, Masciandaro G, Ceccanti B, Grego S. Influence of organic and mineral fertilizers on soil biological and physical properties. Bioresource Technology, 2000; 72: 9-17.

[29] Odlare M. Organic residues-A Resource for arable soil. Doctoral thesis, Department of Microbiology. Swedish University of Agricultural Sciences. Uppsala. 2005.

[30] Oswald J W, Golueke C G. Biological transformation of solar energy. Advance Microbiology, 1960; 2: 223-261.