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Microalgae biomass as an alternative substrate in biogas production

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Abstract: The running down of fossil energy sources makes the production of bioenergy an expected need worldwide. Therefore, energy crops have gained increasing attention in recent years as a source for the production of bioenergy because they do not compete with food crops. Microalgae have numerous advantages such as fast growth rates and not competing with food production. Because of the fast growth, many high valuable products are generated, e.g. food, biofuel, etc. Due to the energy crisis, renewable energy becomes a popular issue in this world today and there are several alternatives such as bioenergy, solar, wind, tide, geothermal, etc. For bioenergy, algae are the third generation biofuel crop. There is an increased demand for biogas in the society and one way to meet this is to use cultivated microalgae as fermentation substrate. In the present study, we maintained algae growth process and biomass production in autotrophic condition continuously for over 2 month's period. Growth system (photobioreactor) was setup under room temperature and continuous illumination light through fluorescent lamps; light intensity was average as 48.31 [$\mu mol^{-1}m^{-2}$ per μA]. In reactor, dominant microalgae species were including *Anabaena* sp., *Chlorella* sp., *Oscillatoria* sp., *Oedogonium* sp. and *Scenedesmus* sp. The content of total solids (TS) and volatile solids (VS) in the algae biomass was measured; the results were average as 12500 g/m^3 and 6320 g/m^3, respectively. Furthermore, microalgal biomass is a potentially valuable fermentation substrate, and produce over 60% of methane gas.

Keywords: Microalgae, Biomass, Bio-Reactor, Fermentation Feedstock, Biogas

1. Introduction

Algae are the most important primary producer in aquatic ecosystem [1]. They are a diverse group of photosynthetic organisms with a range of unicellular to multicellular forms that are found in the ocean, freshwater bodies, on rock, soils and vegetation [2]. Algae can be broadly divided into macroalgae, which include multicellular seaweeds, and microalgae, which are small unicellular algae, found in a wide variety of environments and comprising of many evolutionarily distinct organisms. It provides food and oxygen for many species in the aquatic Environment and it's vitally crucial to keep carbon dioxide (CO_2) of carbon cycle via photosynthesis to balance the CO_2 concentration in atmosphere. Through photosynthesis they fix light energy and reduce simple inorganic molecules into complex organic molecules supporting the whole community of living organisms occupying higher trophic levels in the ecosystem [3].

Microalgae are ideal organisms for biological monitoring [4]. Microalgal density, abundance, and diversity are ideal indicators of the health of aquatic ecosystems and water quality. Hence, algal biomass measurement is important in many biological and ecological studies and in algal industry [5]. Therefore, algal community plays critical roles as the primary producer and as a major biotic component in the nutrient/energy cycle in aquatic ecosystems [3]. Microalgae have the ability to fix carbon dioxide, nutrients and store the solar energy into their cells via photosynthesis which makes them interesting as an alternative energy source; some studies had also indicated the importance of algae in carbon dioxide fixation [1–3].

Microalgae growth rate is the highest compared with the other plants. Due to the energy crisis, renewable energy becomes a popular issue in this world today and there are several alternatives such as bioenergy, solar, wind, tide, geothermal, etc. Since it is an excellent biomass producer, the

biomass is broadly extracted to obtain various biochemical used as medicine, nutrition, food etc. For energy crisis, algae provide an excellent biomass as a renewable energy source, so called "bioenergy", and turn algae as the most efficient bio-component (carbohydrates, proteins, lipids and other mineral source) and bio-oil maker [2]. It is one of the most important bio-technological species currently.

For bioenergy, algae are the third generation biofuel [6]. For the reasons of the best energy conversion efficiency of sunlight and the highest growth rate, algae have the best potential among all the energy crops [2, 5]. Regarding biofuel production, microalgae can provide different types of biofuels, including: biodiesel (from algal fatty acids); ethanol (produced by fermentation of starch); hydrogen (produced biologically); and methane (produced by anaerobic digestion of algal biomass). Some authors are more assertive, and suggest that the production of methane via anaerobic digestion (AD) is the most feasible and cost-effective route to an energy product [7,8]. Feasibility analysis of biodiesel production from algae underlined the importance of including AD technology into the overall fuel production process, either by utilizing defatted algae or whole algal biomass for conversion to methane. Economic analysis conducted with respect to the cost of lipid extraction and conversion to biofuels suggests that for algae with lipid content below 40% direct methane production is the most economically feasible approach [9]. This is supported by Harun et al. [10] who demonstrated that more energy could be generated from the production of methane from microalgae (14.04 MJ/kg), rather than biodiesel (6.6 MJ/kg) or ethanol (1.79 MJ/kg) where their unit "kg" is assumed to be "kg of dry weight algae". Furthermore, up to 65% of the chemical energy stored in the algal biomass can be potentially recovered through AD to methane [11].

Recent studies are increasing our knowledge about anaerobic digestion of microalgae. Theoretical calculations [9] as well as bottle and digester experiments [12] have shown the great potential of anaerobically digesting microalgae for methane production which can be further converted into a clean and renewable biofuel [8]. Accordingly, the biofuel feedstock source to be considered is algae. This paper highlights some of the current details of biofuel generation, focusing mainly on microalgae, which may allow biofuel production from these organisms to be economically and sustainably viable in the future. Consequently, the primary objective of this study was to investigate the potential of the freshwater mixed culture microalgae biomass substrate to estimation and production of biogas.

2. Materials and Methods

The methodology is illustrated in Figure 1. In the first stage of reactor setup, we selected the standard bioreactor, "CSTR (continuously stirred tank reactor)". Two bench scale CSTRs were set up in the Energy Research Center (ERC), Maejo University, Sansai, Chiang Mai, Thailand. All the reactors were operated by batch feed conditions and other operational factors were listed in Table 1.

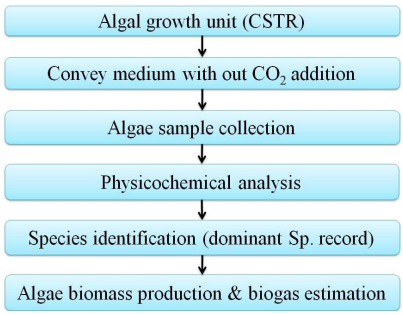

Figure 1. *Flow chart of methodology.*

Table 1. *Operational parameters*

Operational parameter	Photo-bioreactor
Scale	Laboratory
Reactor design	1L flask
Water volume	1L
Feeding	Batch
Filter size	0.45 µm
Mixing speed	Magnetic mixer
Light source	Fluorescent tube panels
Temperature	Room temperature
Algae species	Mixed microalgal culture
Operation period	60 days

2.1. Algae Culture, Medium Preparation and Species Identification

Algae samples were collected by plankton net (20-µm pore size) from solar freshwater fish pond at School of Renewable Energy, Maejo University, Sansai, Chiang Mai, Thailand. Algae medium was prepared using method of Ershad-Langroudi et al. [13] to make the specific medium of Conway. One liter solutions of chemical compounds demonstrated in Table 2 were prepared and then mixed and sterilized. The mixture of chemical compounds was added to filter by 0.2 µ filter and distributed to 1000 ml Erlen Meyer flasks to culture the algae. All experiments were carried out in triplicate. The microalgae were identified microscopically using light microscope with standard manual for algae [14,15].

Table 2. *Chemical compounds utilized in Conway medium preparation Concentration*

Chemical compound	Concentration
KNO_3	116 g
NaEDT	45 g
H_3BO_3	33.6 g
$MnCl_2.4H_2O$	0.36 g
$ZnCl_2$	2.1 g
$CoCl_2.6H_2O$	2 g
$(NH_4)\ 6MoO_7.4H_2O$	0.9 g
$CuSO_4.H_2O$	2 g
Vitamin B1	200 mg
Vitamin B12	100 mg
$NaSiO_3$	20 g
$Na_2H_2PO_4.2H_2O$	20 g
$FeCl_3.6H_2O$	1.3 g

2.2. Analytical Methods

All the physicochemical indexes including pH, chemical oxygen demand (COD); total suspended solids (TSS), total solids (TS), volatile solids (VS), were continuously monitored according to the standard method [16]. Light intensity measured by light meter (LI-COR light meter (LI-250)) and temperature was measured by laboratory thermometer. Biogas estimation method was adopted from von Sperling & Chernicharo [16] and Pavlostathis & Giraldo-Gomez [17].

3. Results and Discussion

3.1. Algae Growth and Measurement

Algae were the polyphyletic, simple microscopic or macroscopic, unicellular to multicellular, motile or immotile organisms which grew in abundance in any water body such as lakes, ponds, rivers, streams, marine etc (before mentioned). Algae varied in size and shape, from microalgae of less than 1μm to macroalgae over 30 m in length. They grew in any aquatic environment and used light and CO_2 to create biomass. Ecologically, algae were the most widespread of the photosynthetic plants, constituting the bulk of carbon assimilation through microscopic cells [1,2,4,5].

There were several main groups of freshwater algae, which differed primarily in pigment composition, biochemical constituents and life cycle. The most important algae in term of known species are classified in four groups: green algae (Chlorophyceae), blue-green algae (Cyanophyceae), brown algae (Chrysophyceae) and red algae (Rhodophyceae), and the brief explanations were shown in Table 3.

Table 3. *The known species of algae species in freshwater.*

Main groups	Class	Known species	Size	Chlorophyll	Carotenes	Storage material
Green algae	Chlorophyceae	5000	3-10 μm	Chl-a, Chl-b and Chl-c1	α, β, γ	Starch and triglycerides
Blue-green algae	Cyanophyceae	2000	0.5-60 μm	Chl-a and Chl-b	β	Starch and triglycerides
Brown algae	Phaeophyceae	1000	up to 30 m	Chl-a and Chl-c1&c2	α, β	triglycerides and carbohydrates
Red algae	Rhodophyceae	164	up to 10 cm	Chl-a and Chl-d	β, ε	Starch

The biomass of microalgae contains many various chemical compounds, which were significant in different aspects such as pharmaceutics, human food and energy. Carbohydrates, proteins, lipids, nucleic acids and pigments were the basic and major components of algae; beside those, Acylglycerides, Glycolipids, phospholipids, fatty acids, methyl esters, polysaccharides and all had important roles. Table 4 gave the cell content of these major fractions with their elemental composition and energetic properties [2].

Table 4. *Elemental composition of algal biochemical components.*

Biochemical component	Characteristic elemental composition	Calculated calorific value/kJ g^{-1}	Range of typical cell content (%)
Lipids	$C_1H_{1.83}O_{0.17}N_{0.0031}P_{0.006}S_{0.0014}$	36.3	15–60
Acylglycerides	$C_1H_{1.83}O_{0.096}$	40.2	—
Glycolipids	$C_1H_{1.79}O_{0.24}S_{0.0035}$	33.4	—
Phospholipids	$C_1H_{1.88}O_{0.173}N_{0.012}P_{0.024}$	35.3	—
Fatty acids	$C_1H_{1.91}O_{0.12}$	39.6	—
Methyl esters	$C_1H_{1.92}O_{0.05}$	43.0	—
Protein	$C_1H_{1.56}O_{0.3}N_{0.26}S_{0.006}$	23.9	20–60
Nucleic acids	$C_1H_{1.23}O_{0.74}N_{0.40}P_{0.11}$	14.8	3–5
Polysaccharides	$C_1H_{1.67}O_{0.83}$	17.3	10–50

Biomass was a critical measurement in the microalgal harvesting process for applications. A number of methods had been developed to estimate and quantify, which were useful in different cases. Different methods were available such as dry weight: Total suspended solids (TSS), volatile suspended solids (VSS) and fixed suspended solids (FSS); wet weight method; chlorophyll (Chl) method: Chl-a, Chl-b and Chl-a+b), epifluorescence microscopy, bioluminescence, photometric, turbidity, packed cell volume and cell count etc [5]. In general, algal biomass measurement indexes were classify into two groups, (1) direct index such as TSS and VSS and (2) indirect index such as chlorophyll, so-called proxy index. They all were the popular indexes [2,5]. In this study, we used gravimetric analysis (by TSS) measurement was called as direct biomass method. Algae biomass was obtained by filtering a sample through 0.45 μm Whatman filter paper followed by drying in oven for 1h at 103°C [15]. The dry weight method is the most widely applied biomass estimation. The dry weight measurement usually gave a much more consistent result than the wet weight and was usually used as a standard method [2,5,6,15]. It was an important parameter for estimating biomass concentration, productivity and percentages of cell components. In this study, for aeration with atmospheric air there is a significant yield in both the growth rate and final biomass concentration (3.04–3.6 g L^{-1}); the average biomass production was 3.6 g L^{-1} with the lowest agitation and aeration conditions used whole study period.

3.2. Microalgal Culture Conditions and System

The batch system of algal cultures of 1000 ml, in duplicate reactor, was grown for 2 months, in continuously-stirred tank reactors (CSTR) under room temperature. All the reactors (1000 ml Erlen Meyer flasks) were continuously illuminated with the fluorescent lamps day and night for the photoautotrophic growth.

The light intensity and temperature were monitored through the study and the average was 48.31 [µmol^{-1}m^{-2} per µA] and 27.5 °C. In reactor, dominant algae species were including *Anabaena* sp., *Chlorella* sp., *Oscillatoria* sp., *Oedogonium* sp. and *Scenedesmus* sp. Dominant algal species microscopic structure shown in Figure 3.

Figure 2. *Photobioreactor*

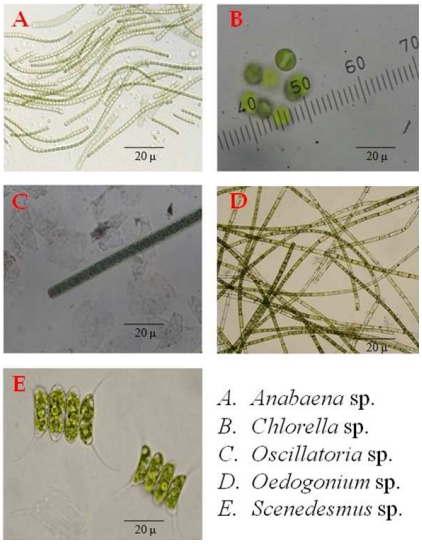

Figure 3. *Light microscopic pictures of dominant microalgae spices (identified before the fermentation stage).*

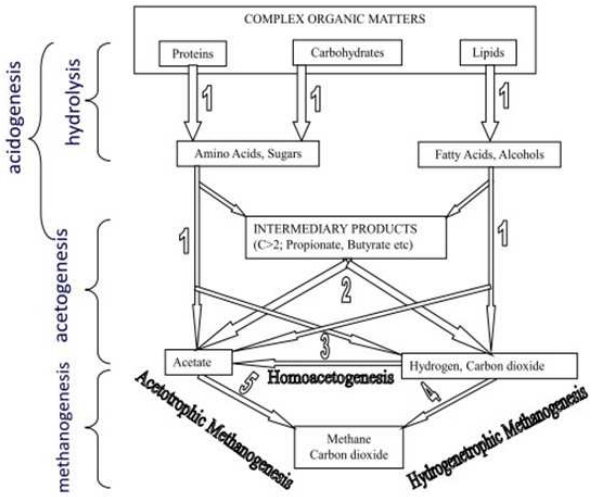

Figure 4. *Organics conversion of microalgae biomass and anaerobic system process.*

The many studies in the literature concerned the maximum CO_2 uptake rate by the artificial photobioreactors used with artificial medium [16–21] and natural water medium [1–6] such as continuously stirred tank reactor (CSTR) which was the standard reactor [2,22]; and this utilized air CO_2 to grow microalgae with Conway medium. In the literature, many studies used Conway Medium to cultivate marine microalgae (23, 24). Higher cell density was achieved by genus *Dunaliella*, *Chlorella* and *Isochrysis* in Conway Medium. There is very few studies applied Conway Medium in freshwater microalgae cultivation [25,26]. However, this study results showed better results (3.04–3.6 g L^{-1} of algae biomass) using Conway medium with mixed culture of freshwater microalgae cultivation.

3.3. Biochemical Methane Potential (BMP) of Microalgae

Biomass can be considered as solar energy collected and stored by plants termed as “energy crops”, such as algae. Algae are an efficient tool to trap solar energy into biomass for later conversion into biogas. Biomethanation is an anaerobic microbiological process by which biomass can be microbiologitally converted into methane. Hence through methane fermentation the chemical energy fixed from solar energy by algae may be converted into the readily available chemical energy in methane gas [27].

The biochemical methane potential (BMP) assay constitutes a useful tool to determine both the ultimate biodegradability and the methane conversion yield of organic substrates [28]. The BMP evaluates the ultimate amount of methane produced by any given waste or biomass under anaerobic conditions. The information provided by the BMP value is important when evaluating potential substrates and for optimizing the design and functioning of an anaerobic digester. Apparently, the BMP of microalgae depends mainly on its composition, which itself depends on the growth conditions and and is specific species [29].

Algal biomass contains considerable amount of biodegradable components such as carbohydrates, lipids and proteins. This makes it a favorable substrate for anaerobic microbial flora and can be converted into methane rich biogas [9]. In spite of the fact that microalgae have high potential for biogas production, there are some studies on anaerobic digestion of microalgal biomass utilizing *Chlamydomonas reinhardtii*, *Scenedesmus obliquus*, *Chlorella vulgaris*, *Dunaliella tertiolecta*, *S. obliquus* and *Phaeodactylum triconutum* biomass. The concentration of substrate in the BMP assay also impacts on the final biodegradability and methane productivity [9].

When the C, H, O and N composition of a wastewater or substrate is known, the stoichiometric relationship reported by Buswell and Boruff [30] and Angelidaki and Sanders [31], and can be used to estimate the theoretical gas composition on a percentage molar basis. However, it must be kept in mind that this theoretical approach does not take into account needs for cell maintenance and anabolism.

In this equation, the organic matter is stoichiometrically converted to methane, carbon dioxide and ammonia. The

specific methane yield expressed in liters of CH_4 per gram of volatile solids (VS) can thus be calculated as:

$$C_aH_bO_cN_d + \left(\frac{4a - b - 2c + 3d}{4}\right)H_2O \rightarrow \left(\frac{4a + b - 2c - 3d}{4}\right)CH_4 + \left(\frac{4a - b + 2c + 3d}{4}\right)CO_2 + dNH_3$$

Equation (1)

$$B_o \frac{4a + b - 2c - 3d}{12a + b + 16c + 14d} * V_m$$ Equation (2)

where V_m is the normal molar volume of methane.

The ratio r_G of methane to carbon dioxide can therefore be computed from

$$n = \frac{-b + 2c + 3d}{a},$$

the average carbon oxidation state in the substrate [32] as follows:

$$r_G = \frac{4}{4}$$ Equation (3)

The biogas composition however also depends on the amount of CO_2 which is dissolved in the liquid phase through the carbonate system, and is therefore strongly related to pH.

The ammonium production yield in the digester can be evaluated using Eq. (1):

$$Y_{N-NH_3}\,(mg\,g\,VS^{-1}) = \frac{d*17*1000}{12a+b+16c+14d}$$ Equation (4)

Eq. (1) is a theoretical approach that allows estimation of the maximum potential yields. Using Eq. (1), it is possible to compute a theoretical specific methane yield associated to a theoretical ammonia release (Table 5).

Table 5. *Theoretical methane potential and theoretical ammonia release during the anaerobic digestion of the total biomass [adopted from 9,33]*

Species	Proteins (%)	Lipids (%)	Carbohydrates (%)	CH_4 (L CH_4 g VS^{-1})	N–NH_3 (mg g VS^{-1})
Euglena gracilis	39–61	14–20	14–18	0.53–0.8	54.3–84.9
Chlamydomonas reinhardtii	48	21	17	0.69	44.7
Chlorella pyrenoidosa	57	2	26	0.8	53.1
Chlorella vulgaris	51–58	14–22	12–17	0.63–0.79	47.5–54.0
Dunaliella salina	57	6	32	0.68	53.1
Spirulina maxima	60–71	6–7	13–16	0.63–0.74	55.9–66.1
Spirulina platensis	46–63	4–9	8–14	0.47–0.69	42.8–58.7
Scenedesmus obliquus	50–56	12–14	10–17	0.59–0.69	46.6–42.2

Gross composition (Table 2) of several microalgae species adopted from Becker, [33]. As expected, the species that can reach higher lipid content (e.g. *C. vulgaris*) have a higher methane yield.

COD is commonly used in the water and wastewater industry to measure the organic strength of liquid effluents. It is a chemical procedure using strong acid oxidation. Organics conversion of microalgae biomass and anaerobic system process shown in Figure The strength is expressed in 'oxygen equivalents' i.e. the mg O_2 required to oxidise the C to CO_2. However, the COD concept could be estimate the methane yield [16, 17]. One mole of methane requires 2 moles of oxygen to oxidise it to CO_2 and water, so each gram of methane produced corresponds to the removal of 4 grams of COD.

$$CH_4 + 2O_2 \rightarrow CO_2 + H_2O$$

$$16 \qquad 64$$

or

1kg COD is equivalent to 250g of methane.

1kg COD ⇒ 250g of CH_4

250g of CH_4 is equivalent to 250/16 moles of gas = 15.62 moles

1 mole of gas at NTP = 22.4 liters

Therefore 15.62 x 22.4 = 349.8 liters = 0.35 m^3.

In our study, the content of total solids (TS) and volatile solids (VS) in the algae biomass was measured; the results were average as 12500 g/m^3 and 6320 g/m^3, respectively. The average pH was 8.2 and average COD 2190 (ml/L). Methane formation takes place within a relatively narrow pH interval, from about 6.5 to 8.5 with an optimum interval between 7.0 and 8.0. The process is severely inhibited if the pH decreases below 6.0 or rises above 8.5. The pH value increases by ammonia accumulation during degradation of proteins, while the accumulation of VFA decreases the pH value. The accumulation of VFA will often not always result in a pH drop, due to the buffer capacity of the substrate [34]. According to the COD estimation, our study shows the mixed culture microalgal biomass is a potentially valuable fermentation substrate, and produce 60.3% of methane gas.

4. Conclusions

Production of biofuels is undoubtedly one of the best solutions for declining the crude oil reserves and global warming due to excessive greenhouse gasses emissions. As fossil fuel prices increase and environmental concerns gain prominence, the development of alternative fuels from biomass has become more important. Biogas is considered a renewable energy carrier. As demonstrated here, microalgal biogas is technically feasible. Microalgae have several advantages over terrestrial plants such as higher photosynthetic efficiencies, lower need for cultivation area, higher growth rates, more continuous biomass production, no direct competition with food production, and possibility to use artificial medium, natural water medium (freshwater/marine water) and wastewater for biomass production. The algae biomass thus produced will constitute an additional source of

organic substrate in the installation for biogas production.

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