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Biodiesel and biogas recovery from *Spirulina platensis*

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ABSTRACT

The aim of this research was to optimize the energy recovery in the form of biodiesel and biogas from ubiquitous *Spirulina platensis* microalgae. Investigations were undertaken to select appropriate cell disruption technique, organic solvent for lipid recovery and catalyst for transesterification reaction for biodiesel production from *Spirulina platensis*. Based on energy considerations, osmotic shock was the most suitable cell disruption technique which recorded highest lipid recovery yield of 8.9% when chloroform/methanol (1:2 *v/v*) was used as solvent. The extracted lipid from *Spirulina platensis* was esterified into Fatty Acid Methyl Esters (biodiesel) using sulfuric acid as the catalyst with conversion up to 79.5%. Under these conditions, biodiesel yield of 7.1% (based on biomass weight) was obtained. Investigations also brought out that the lipid-extracted residue can be used as feedstock for biogas production with the average methane yield of about 290 ml/gVS.

1. Introduction

Energy is an important resource for mankind. Fossil fuels such as coal, petroleum oil, and natural gas are the major sources of energy. However, global energy demand is increasing at dramatic rate while fossil fuels reserves are rapidly depleting. According to the U.S. EPA data 2015 reported that in 2013 fossil fuel combustion accounted for 82% of CO_2 released into the atmosphere of which 18.8% was from coal combustion, 27.5% from natural gas and 35.7% from petroleum oil (U.S. EPA, 2015). In addition, approximately 35×10^9 million barrels of the crude oil was produced globally whereas the total oil reserves were estimated as about 1700×10^9 million barrel in 2013. Accordingly, with the consumption rate in 2013, these reserves are expected to last for about 53.3 years (IEA, 2014). Furthermore, use of non-renewable energy sources also results in release of greenhouse gases leading to increased CO_2 concentration in the earth's atmosphere; thus impacting the climate change. On the other hand, renewable energy sources such as solar, wind and biomass have much smaller life time carbon emissions. As a result, shift to renewable energy sources for energy generation is imperative in combating climate change.

Algal biomass is an excellent source for recovery of resources as well as energy. Once the petroleum oil extraction is rendered economically unsustainable, algal bio-refineries are expected to replace the current petroleum based refineries for production of resources and energy for mankind. Researchers have extensively used algal biomass for generation of energy in the form of biodiesel (Scott et al., 2010; Krohn et al., 2011; Nautiyal et al., 2014), hydrogen (Das and Veziroglu, 2001; Akkerman et al., 2002) and biogas (Vergara-Fernandez et al., 2008; Mussgnug et al., 2010; Debowski et al., 2013). Biodiesel, which is a fatty acid alkyl ester (FAAE), is produced from renewable resources such as vegetable oil (Issariyakul and Dalai, 2014), animal fat (Encinar et al., 2011), microalgae (Chisti, 2007), etc. Since biodiesel can easily replace the current fossil based fuels, its demand is expected to rise in the near future. However, increased demand for biodiesel will affect the supply of vegetable oil in the food sector. Under such circumstances, use of algae for biodiesel production is preferable since biomass productivity of microalgae is reported to be 30 times higher as compared to terrestrial plants (Sheehan et al., 1998; Petrick et al., 2013) resulting in significant reduction in land requirement. On the other hand, non-edible vegetable oil based biodiesel requires larger land area for plant cultivation as well as longer time for plant growth (Mata et al., 2010). Algal biomass is free of lignocellulose; as a result, its cell disruption requires lower energy input (Habib and Parvin, 2008). On the other hand, use of

Abbreviations: BMP, Biochemical Methane Potential; FAAE, Fatty Acid Alkyl Ester; FAME, Fatty Acid Methyl Ester; FFA, Free Fatty Acid; FID, Flame Ionization Detector; TCD, Thermal Conductivity Detector; VS, Volatile Solids.

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algae for biodiesel production does not affect the food security since microalgae feedstock is not the main source for human food. Algal biodiesel is considered to be net carbon neutral, since the CO_2 produced by burning algae biodiesel is the same as it consumed during photosynthesis. In contrast, CO_2 emission from power plants and industrial processes can be source of carbon for cultivation of microalgae resulting in net carbon capture. Typically, 1 kg of dry algal biomass can capture about 1.8 kg of CO_2 (Chisti, 2007).

Ubiquitous *Spirulina platensis* microalgae is extensively used for human as well as animal feed. Nutritional products such as protein and carotene can be extracted from *Spirulina platensis*. In addition, several medicinal products also can be extracted from *Spirulina platensis* (Habib and Parvin, 2008). Researchers also have investigated biodiesel as well as biogas production from *Spirulina platensis* (El-Mashad, 2013). Production of biodiesel from microalgae involves four different stages, viz., cultivation and harvesting of microalgae, cell disruption, lipid extraction and transesterification (Huang et al., 2010). However, commercial scale production of biodiesel from microalgae is still not practiced since it is uneconomical (Gallagher, 2011). Further research is needed to increase of biodiesel product yield in order reduce the production cost.

Biodiesel yield could be increased by optimizing cell cultivation, disruption, lipid extraction, and transesterification. There are three key factors influencing on biodiesel product yield. Lipid content of microalgae is the first factor which can be increased by cultivation process under proper concentration of nutrients, and suitable light intensity. Second factor is lipid recovery yield which can be influenced by applying appropriate cell disruption technique and using suitable organic solvent in lipid extraction process (Brennan and Owende, 2010). Finally, biodiesel conversion yield (amount of lipid converted into biodiesel) also influences biodiesel product yield. Biodiesel conversion yield depends on transesterification conditions such as alcohol-to-oil ratio, type and concentration of catalyst and reaction conditions.

In an algal bio-refinery, the residue left after extraction of lipid can further be digested anaerobically for the recovery of energy in the form of methane. (Bravo-Fritz et al., 2016). However, limited information is available about anaerobic digestion of the residue left after extraction of lipid from algae. In this research, biodiesel production from ubiquitous *Spirulina platensis* microalgae was optimized by selecting appropriate cell disruption technique, organic solvent for highest lipid recovery yield, and catalyst for transesterification reaction. Furthermore, biogas generation from lipid extracted biomass was investigated using Biochemical Methane Potential (BMP) test in order to maximize the energy recovery.

2. Materials and methods

Spirulina platensis was provided by Royal Chitralada Project, Thailand. Initially, *Spirulina platensis* was grown under fluorescence light in laboratory using Zarrouk's medium (Zarrouk, 1996) as culture media and Na_2CO_3 source of CO_2. This microalgae strain was then grown in raceway ponds under sunlight and ambient conditions.

The schematic of experimental methodology consisted of four distinct phases (Fig. 1). In phase I, algal biomass was subjected to cell disruption using several techniques and the technique with highest crude oil yield was then selected. In phase II, lipid was extracted from the disrupted biomass obtained from phase I as well as from non-disrupted biomass by using different organic solvents. Crude lipid yields from all solvents were then compared. In phase III, crude lipid was converted into biodiesel by transesterification process using various types of catalysts. Finally, in phase IV, lipid extracted residue was used as substrate for Biochemical Methane Potential (BMP) test in order to evaluate its biogas generation potential.

2.1. Cell disruption

Disruption of *S. platensis* biomass was carried out using following techniques:

2.1.1. Ultrasonication

7 g of dry biomass was mixed with 50 ml of distilled water and then subjected to ultrasonication by an ultrasonic bath (Model 3200, Branson Ultrasonic Cleaner, USA) at frequency of 40 kHz for 20 min (Zheng et al., 2011).

2.1.2. Acid and alkali treatment followed by ultrasonication

7 g of dry biomass was treated with 50 ml each of 1 N H_2SO_4, 1 N H_3PO_4, 1 N HNO_3, 1 N NaOH, and 1 N KOH and then subjected to ultrasonication by an ultrasonic bath (Model 3200, Branson Ultrasonic Cleaner, USA) at frequency of 40 kHz for 20 min.

2.1.3. Autoclave

7 g of dry *biomass* was mixed with 50 ml of distilled water and then autoclaved for 30 min at 120 °C and 1.2 bar (Autoclave Model ST510, Yamato Scientific Co. LTD., Japan) (Miranda et al., 2012).

2.1.4. Acid and alkali treatments followed by autoclave

7 g of dry biomass was mixed with 50 ml each of 1 N H_2SO_4, 1 N H_3PO_4, 1 N HNO_3, 1 N NaOH, and 1 N KOH and then autoclaved for 30 min at 120 °C and 1.2 bar.

2.1.5. Osmotic shock

7 g of dry biomass was mixed with 50 ml of 10% *(v/v)* NaCl and then vortexed for 1 min and maintained in solution for 48 h (Kaiwan-arporn et al., 2012).

2.1.6. Osmotic shock with sulfuric acid (H_2SO_4)

7 g of dry biomass was mixed with 50 ml of 1 N H_2SO_4 containing 10% *(v/v)* NaCl and then vortexed for 1 min and maintained in solution for 48 h.

2.1.7. Soaking in sulfuric acid (H_2SO_4)

7 g of dry *biomass* was mixed with 50 ml of 1 N H_2SO_4 for 1 min and maintained in solution for 48 h.

The disrupted biomass was then dewatered by a centrifuge at 5000 rpm for 10 min (Hettich zentrifugen universal 320, Andreas Hettich GmbH and Co. GH, Germany) and dried using a hot oven at 60 °C for 12 h (Model UF110, Memmert GmbH and Co. GH, Germany). The dried biomass was then stored at 5 °C until required for lipid extraction (Halim et al., 2011). Lipid extraction was carried out by using 15:1 *(v/w)* of solvent-to-biomass ratio with hexane/isopropanol 1:1 *(v/v)* followed methods in section 2.2. Efficiency of cell disruption techniques were evaluated by comparing lipid recovery yield as calculated from Equation (1).

$$\textit{Lipid recovery yield} = \frac{\textit{weigth of lipid}\ (g)}{\textit{weigth of dried biomass}(g)} \times 100 \qquad (1)$$

2.2. Lipid extraction

Lipid extraction was carried out in three stages with shaker (Model 75, Wrist action shaker, Borrell Scientific LLC, USA) at room temperature (Chaiklahan et al., 2008). Seventeen different solvents/solvent mixtures were used as presented in Table 1. Solvent

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