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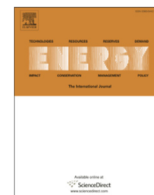

A novel process on lipid extraction from microalgae for biodiesel production

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ABSTRACT

To promote microbial oils as the feedstock for biodiesel preparation, developing an advanced cell treatment technology, especially suitable for treating water-containing microbial feedstock is very important. A novel method based on using formic acid assisted with small amounts of hydrochloric acid, to treat water-containing microalgae and extract lipid subsequently, is proposed in this paper. Effect of several factors on mixed acid treatment of *Chlorella protothecoides* was investigated systematically. It was found that the dosage of formic acid and hydrochloric acid, liquid/solid (l/s) ratio and temperature had a significant influence on lipid extraction from water-containing microalgae. Under the optimum condition of formic acid dosage 5.57 g/g (based on the weight of dried microalgae cell, the same below), hydrochloric acid dosage 0.1 g/g, liquid/solid (l/s) ratio10:1 and temperature 100 °C, the total lipid yield and FAME (Fatty Acid Methyl Ester) yield reached 45.6% and 85.8% respectively. This process allowed water content of wet biomass reaching 82.1%. Further study revealed that the process was suitable for the treatment of different microalgae materials. The results indicate that this mixed acid treatment is very promising for using wet microalgae as the feedstocks for biodiesel production.

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1. Introduction

Microalgae has been considered as a promising feedback for biofuel production because of its high growing rates, short life cycle and diversified cultivation conditions. Besides, the cultivation of microalgae does not take up the agricultural land, which has no deep influence on the global food supply [1–3]. However, the high costs and low lipid yields from algae limit the development of industrialization process for biofuel production based on microalgae oils [4,5]. In fact, most of the lipid from microalgae distribute in the cytoplasm surrounded by the thick cell wall which prevents the release of intra lipid [6,7].

Hence a pre-treatment aimed to disrupt the cell wall of algae is mandatory in order to accelerate the process of lipid extraction and improve the lipid yield from algae. The main cell disruption include ultra-sonication, bead-beating, microwaves, high pressure homogenization, enzymatic hydrolysis and chemical method etc. [3,5]. Previous research showed that there was extensive study related to using ultra-sonication, bead-beating, microwaves and high pressure homogenization to treat different species of microalgae biomass [8–12]. It was found that bead-beating was effective in disrupting *Botryococcus* sp. cell, but has a poor performance in treating *Chlorella vulgaris* [8,10]. A comparative evaluation of different cell disruption methods for the release of lipids from marine *Chlorella vulgaris* cells was investigated and grinding in liquid nitrogen was identified as the effective method in terms of disruption efficiency and time [9]. The sonication-assisted method with n-hexane was demonstrated to be efficient for lipid extraction from dry biomass of *Chlorella minutissima, Thalassiosira fluviatilis and Thalassiosira pseudonana* [11], while laser treatment was found to be the most effective in disrupting *Nannochloropsis oculata* cells [12]. However, for practical application, the energy needs to be considered since the high energy consumption was recognized as the main bottleneck in the lipid extraction from microalgae [13]. It was reported [13] that the energy consumption ranged from 33 MJ/kg to 529 MJ/kg for different mechanical disruption methods, which was far higher than the average energy obtainable 29 MJ/kg through combustion of microalgae. What's more, the above-mentioned methods are only effective for disrupting dry cells, but the drying step consumes a large amount of energy [14]. It would be economically favorable to avoid the drying step while maintaining

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an effective lipid extraction from the wet microalgae biomass.

Some researchers also explored the potential of using lysozyme and cellulase for disrupting wet microalgae cell and the highest lipid extraction yield of 16.6% could be achieved by using lysozyme [14]. But the cost of enzymes should be a serious concern for industrial application. It was reported that using inorganic acid to treat microalgae cells was less energy consumption and very effective in treating different microalgae cells [15,16]. However, the major disadvantages associated with this inorganic acid-treatment method is that the high usage of the strong inorganic acid, which is hard to recycle and subsequently leads to serious environmental problems [17].

Our previous study showed that organic acid such as formic acid was very effective in treating dry microalgae cells and the total lipid yield and FAME yield were far higher than those obtained from conventional cell disruption method and comparable with those obtained from inorganic acid-treatment process [18]. However, this organic acid treatment method was found to be just effective for treating dry microalgae cells. In order to obtain dried microalgae, energy-intensive dehydration process is required [19,20]. Therefore, exploring other effective process suitable for treating water-containing biomass is significant.

A novel method based on using formic acid which is easy to recycle assisted with small amounts of HCl, to treat cells and extract lipid subsequently from wet biomass, is proposed in this paper. The related factors that influence the mixed acid treatment on lipid extraction from microalgae were investigated systematically. Moreover, the quality of lipid extracted and the applicability of this novel method to different microalgae have also been explored.

2. Materials and methods

2.1. Materials

Chlorella protothecoides, *Nannochlorum* sp. and *Nannochloropsis oceanica* were kindly provided by ENN Group Co Ltd. (Langfang, China), South China Sea Institute of Oceanology (Guangzhou, China) and Yantai Hairong Biology Technology Co., Ltd (Yantai, China), respectively. Heptadecanoic acid methyl ester as GC standard and 14% BF_3 (*w/v*) in methanol were purchased from Sigma-Aldrich (St. Louis, MO). Other reagents were obtained commercially of analytical grade.

2.2. Cell disruption

The pretreatment by HCl alone was carried out with 50 mL 13.7% (w/w) HCl solution (HCl dosage equal to 1.5 g/g, based on microalgae weight) and 5 g dry microalgae biomass, heating at 100°Cfor 30 min. The pretreatment process was performed in a 500 mL three-neck round bottom flask equipped with mechanical stirrer at 250 rpm in a water bath (the same below). The disruption treatment was stopped by cooling the flask using the cooling water (the same below).

The pretreatment by formic acid alone was carried out with 30 mL 88% (w/w) formic acid solution (formic acid dosage equal to 6.4 g/g, based on microalgae weight) and 5 g dry microalgae biomass, heating at 100°Cfor 30 min. The microalgae cells was dried in an oven (110 °C) till its weight kept constant.

The pretreatment by mixed acid was carried out by addition of mixed solutions which includes some amount of 88% (w/w) formic acid solution and some amount of 37% (w/w) HCl solution at certain temperature (oil bath is used for temperature over 100 °C). Each experimental conditions were carried out in duplicates.

2.3. Lipid extraction

The total lipid was extracted by using a modified version of Bligh and Dyer's method [21]. After disruption pretreatment, the mixtures (based on 5 g biomass) were transferred into a 500 mL centrifuge tube, followed by addition of 100 mL chloroform-methanol (1:1 v/v) and shaking for 2 min, then centrifuged for 5 min at 5000 rpm. Another 50 mL chloroform was added into the alcoholic layer for further extraction of remaining lipid and then the chloroform layer from the first and second extraction was combined together for further solvent evaporation. The total lipid yield is defined as follows: total lipid yield (%) = the weight of total lipid/ (the weight of dried biomass) × 100%.

2.4. Fatty acid composition analysis

The effective lipid can be defined as “fatty acids and derivatives”, which contain triacylglycerols, phospholipids, glycolipids and free fatty acid [22,23]. It is noted that not all types of lipid extracted can be transformed into fatty acid methyl ester (FAME), such as pigments [24]. Therefore, it is necessary to measure the effective lipid content which can be converted to FAME. FAME content can be measured by the standard procedure AOAC 991.39 (Association of Analytical Communities). The specific procedure is described as follows: 25 mg crude lipid, 2 mg Heptadecanoic acid methyl ester (internal standard) and 1.5 mL NaOH (0.5 mol/L) in methanol were put into glass tube for shaking well, and then the mixture was heated at 100 °C for 15 min. After cooling down, 2 mL 14% BF_3 (*w/v*) in methanol was added into the glass tube. And then the mixture was further heated at 100°Cfor 30min. After being cooling down to room temperature, 5 mL saturated NaCl solution and 1 mL hexane were added into the mixture for agitating thoroughly, followed by standing for being layered. The upper hexane layer of 1 μl was injected for further GC and GC-MS analysis.

The GC analysis was carried out by Agilent 7890A GC system (Agilent Technologies, Santa Clara, USA) equipped with a CP-FFAP CB capillary column (25 m × 0.32 mm × 0.30 μm, Agilent Technologies, USA). The initial column temperature was set at 180 °C and maintained for 0.5 min, then heated to 250 °C at the rate of 10 °C/min and then held for 6 min. Detector and injector were set at 250 °C and 245 °C, respectively.

The GC-MS analysis was carried out on a column AB-5MS(30 m × 0.25 mm × 0.25 μm) and a mass spectrometer (DSQ Thermo Fisher). The oven temperature program is the same as GC analysis and the mass spectrometer was operated in electron impact (EI) mode at 70eV ionization energy. The temperature of ionization source was 250 °C.

Also, the inherent “FAME content” of microalgae can be measured by the standard procedure AOAC 991.39. Based on 1 g biomass, the milligrams of FAME obtained directly from 1 g biomass are defined as the inherent FAME content ($mg/g_{biomass}$) and the milligrams of FAME obtained from the crude lipid extracted from 1 g biomass are defined as the extracted FAME content ($mg/g_{biomass}$). The FAME yield is defined as follows: FAME yield (%) = (the extracted FAME content ($mg/g_{biomass}$)/the inherent FAME content ($mg/g_{biomass}$)) × 100%.

3. Results and discussions

3.1. Comparison of different acid treatment methods

Chlorella protothecoides was chosen as the microalgae feedstock for this study since it was reported that the oil content in this cell is much higher than other species [25]. Our previous study demonstrated that the total lipid yield and FAME yield of *Chlorella*

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