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Full Length Article

Investigation of an alternative cell disruption approach for improving hydrothermal liquefaction of microalgae

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HIGHLIGHTS

- An alternative cell disruption approach of microalgae was developed.
- The approach uses low-temperature NaOH/urea solvent.
- Hydrothermal liquefaction of crude and pre-treated microalgae was carried out.
- A maximum bio-crude oil yield (26 wt.%) was obtained using the pre-treated microalgae.
- The bio-crude oil from the pre-treated microalgae has a better flow property.

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ABSTRACT

High-energy and cost-intensive cell disruption processes represent one of the major techno-economic bottlenecks in the microalgae-based bio-refineries. Therefore, a feasible disruption method is required to ensure low energy input and operating cost, as well as high target-product (e.g., lipid) recovery. In this study, several different pre-treatment strategies for the disruption of *Chlorella vulgaris* were investigated, including NaOH/urea, sulfuric acid and ultra-sonication. Experimental results showed that the pre-treatment by NaOH/urea solution resulted in an average mass loss of 33.7 wt.%, and resulted in the removal of 77.2% of carbohydrates and 46.3% of protein (as N) from the original biomass. While these results were comparable to those obtained from the other cell disruption methods, the NaOH/urea method is believed to be more advantageous in terms of energy-efficiency and cost. Afterwards, all pre-treated microalgae samples were subjected to the liquefaction process towards bio-crude oil production. The bio-crude oils obtained from NaOH/urea solvent pre-treated microalgae resulted in higher yields and demonstrated better flow properties.

1. Introduction

In recent years, microalgae have been regarded as a potential source for the sustainable production of various products ranging from biofuels to nutraceuticals due to their high biomass productivities and abilities to be cultured in different environmental conditions and climates [1]. Moreover, microalgae can be used as an option for both CO_2 mitigation and capture and biofuel production. Despite these advantages, microalgal technologies also have a number of limitations. One of the main techno-economic challenges is attributed to the rigid micro-algal cell walls, which can be a complex assembly of carbohydrates and glycoproteins [2]. However, the target intracellular bio-products (e.g., lipid) are usually located in intra-cellular globule bodies or bound within cell membranes [3]. Algaenans, which are insoluble and highly aliphatic structures have also been detected in the cell walls of certain microalgae, making the extraction of intracellular products more difficult. To-date, numerous cell disruption methods, including acid/alkaline hydrolysis, ultra-sonication, bead beating, grinding, and enzymatic lysis, have been reported. Zheng et al. [4] investigated the cell disruption efficiency from *C. vulgaris* via ultra-sonication, bead milling, grinding, and enzymatic lysis. The grinding in liquid nitrogen was found to be the most effective method. Another study by Hernández et al. [5] observed that the acid pre-treatment was the most efficient method to disrupt the cell walls and remove carbohydrates from *C. sorokiniana*. However, none of them are desired for microalgal biomass. For example, the acid/alkali pre-treatment could induce protein denaturation and pigments degradation due to the requirement of high

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temperatures (>120 °C), making this technique less suitable for microalgal cell disruption [6]. Therefore, an energy-efficient and benign microalgae disruption technique should be established.

According to previous studies, the low-temperature NaOH/urea solvent has been regarded as an effective approach to remove cellulose through hydrolyzing inter-molecular bonds and destroying crystalline structures. Kuo and Lee [7] observed that the low-temperature NaOH/urea solvent greatly improved the cellulose dissolution for defatted cotton. Wang et al. [10] investigated the pre-treatment of wheat straw by NaOH/urea solvent at low temperature (−20 °C) and found that this approach could break down the hydrogen bonds in cellulose and simultaneously solubilize hemicellulose. However, to the best of our knowledge, the effect of low-temperature NaOH/urea solvent pre-treatment on microalgal cell disruption has not been investigated, in particular as a pre-treatment for biofuel production.

Microalgae-derived biofuels have been extensively investigated due to the depletion of fossil fuels and the climate change [15]. Recently, various technologies have been developed to convert microalgae into liquid fuels, including pyrolysis, gasification, and hydrothermal liquefaction. Among these techniques, hydrothermal liquefaction (HTL) is more suitable for feedstock with high moisture content (e.g., microalgae) due to its inherent advantage of being a wet processing technique without the requirement of drying the feedstock [8]. Furthermore, oil products produced from HTL have much lower oxygen content and moisture, as compared to pyrolysis oils [9]. The lower oxygen content is related to a higher heating value of bio-crude oil. In addition, the thermal and chemical stability of bio-crude oils is determined by oxygen and water content [20].

In this research, the cell disruption efficiency of NaOH/urea solvent pre-treatment for *C. vulgaris* was investigated and compared with conventional approaches, like dilute acid and ultrasonication. The degree of microalgae disruption was quantified by measuring the release of cellular metabolites, such as carbohydrates and protein. Furthermore, hydrothermal liquefaction studies for both crude and pre-treated microalgae were performed to illustrate the effect of pre-treatment method on quantity and quality of bio-crude oil.

2. Materials and methods

2.1. Materials

Powder *C. vulgaris* was purchased from a health-food store as food-grade material and received as fine powder (Pure Bulk, Inc., Roseburg, USA). Reagent grade sulfuric acid was purchased from Caledon Laboratories Ltd (Georgetown, Canada). Sodium hydroxide and urea were supplied by Sigma Aldrich (Oakville, Canada). The contents of lipid and carbohydrates were determined by the Bligh & Dyer method [21] and the phenol-sulfuric acid method [22], respectively. The content of protein was estimated as% N × 6.25 [23].

2.2. Pre-treatment methods

2.2.1. NaOH/urea solvent

Microalgal cell disruption studies by low-temperature NaOH/urea solvent were carried out according to Kuo et al. [7] and Wang et al. [10]. In each experiment run, 50 g of NaOH/urea aqueous solution was first prepared by mixing NaOH powder, urea, and distilled water (7:12:81 by weight) and this mixture was stored in a freezer for 12 h at −5 to −10 °C. An amount of 5.0 g of dry microalgae was thoroughly mixed with 50 g of the cold NaOH/urea solvent in a shaker at 200 rpm for 2 min.

2.2.2. Dilute acid

An aliquot of 5.0 g of dry algal biomass was thoroughly mixed with 50 mL of distilled water. The H_2SO_4 concentration of the mixture was adjusted to 1%, 2%, and 4% (v/v), respectively. The resultant mixture was then heated to 120 °C for 30 min in a thermostatic oil bath with constant agitation at 60 rpm. After the selected processing time was elapsed, the mixture was cooled down to 22 °C.

2.2.3. Ultrasonication

An amount of 5.0 g of dry microalgae was mixed with 50 mL of distilled water. The mixture was placed into an ultra-sonication apparatus (1510 Branson, Branson Ultrasonics, Danbury, USA) at frequency of 40 kHz and power of 80 W. The ultra-sonication pre-treatment was carried out continuously at room temperature for 10 min, 20 min, and 30 min.

After pre-treatment, all samples were filtered and washed with distilled water till neutralization and then oven-dried at 50 °C for three days. The dry samples were thereafter kept in sealed plastic bags until further analysis. It should be mentioned that the particle size distribution of biomass is considered to be a secondary parameter in HTL. This is due to the fact that sub/supercritical water can both serve as a heat transfer medium and as an effective extractant during liquefaction [27]. As a result, thermal gradients within the biomass particles (due to size distribution) themselves can be neglected. The ash content was determined by heating the dry biomass at 575 °C in a muffle furnace for 3 h to constant weight according to ASTM E1755 standard. The C, H, and N contents were analyzed using an elemental analyzer (Vario EL Cube, Elementar, Hanau, Germany), and the O content was estimated by difference ($O\% = 100\% - C\% - H\% - N\% - Ash\%$). TGA analysis of crude and pre-treated microalgae was performed on a TGA analyzer (PerkinElmer Thermogravimetric analyzer Pyris 1 TGA, Massachusetts, USA) from 50 °C to 800 °C in 20 mL/min N_2 at a heating rate of 10 °C/min. FT-IR spectra of the crude and pre-treated microalgae were recorded on a Nicolet 6700 Fourier Transform Infrared Spectroscopy (Thermo Fischer Scientific, Massachusetts, USA) in the region from 4000 to 550 cm^{-1}.

2.3. Hydrothermal liquefaction studies

The hydrothermal liquefaction experiments were carried out in a 100 mL batch autoclave (Parr 4590, Illinois, USA). In the case of crude microalgae, 5.0 g of dry sample was mixed with 25 g of fresh water and loaded to the reactor. In the case of pre-treated sample, the pre-treated microalgae sludge as described before (without drying) was mixed with additional fresh water to make a total of 30 g and then added to the reactor as a slurry. The reactor was sealed and purged with N_2 for three times to remove air inside the system. Following this, the reactor was heated to a set-point temperature (250 °C) at a heating rate of ~5 °C/min and then the temperature was maintained for 30 min. During the liquefaction process, the temperature was measured by a thermocouple inside the reactor, and the pressure was monitored by a pressure gauge connected to the reactor. After the process, the reactor was cooled to room temperature using tap water. The resulting gaseous products were then released through a control valve. Dichloromethane (DCM) was added to the reaction mixture to extract the bio-crude. The water phase and solid product were separated by filtration. The insoluble fraction remaining on the filter paper was dried in an oven at 105 °C overnight to obtain a solid residue. The remaining reaction mixture was transferred to a separatory funnel. The bio-crude oil phase (lower phase) was recovered by vacuum evaporation at 45 °C under reduced pressure to remove DCM. The upper phase was defined as aqueous phase, which was composed of large amounts of dissolved organics.