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Energy efficient process for microalgae cell disruption for oil recovery using triiodide resin

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ABSTRACT

Oil recovery from microalgal cells is a crucial step in microalgal biodiesel production. Cell disruption is an essential step for lipid extraction from algal biomass. Performance of a new un-conventional disruption method for microalgae cell lysis is reported. Triiodide resin was used for cell disruption for oil recovery from microalgae liquid culture. Cell disruption efficiency depended on contact time and resin quantity along with microalgal strain characteristics. Microalgal strains with a rigid cell structure and small cell size required long contact time and more resin. Time required for cell lysis varied from 30 to 180 min. Cell disruption efficiency and oil extraction ranged from 50 to 100% and 50 to 80% respectively. Cell lysis and oil recovery were dependent largely on microalgal strains and its oil content. During cell disruption process with the triiodide resin, oil was distributed into three different phases. Water phase contained about 20% of total oil, 40–50% remained within the ruptured cells and the remaining 20–30% was adsorbed onto the resin. Triiodide resin-assisted microalgae cell lysis is a low energy process as it is operated at ambient conditions.

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1. Introduction

Potential of microalgae as an alternative source of feedstock for biofuels has initiated a renewed interest in microalgae related research and development [1]. Special characteristics of microalgae that distinguish it from other biofuels feedstock are its high aerial productivity, higher growth rates and high lipid content [2,3]. Notably, microalgae are capable of producing 2–10 times more biomass per unit land area than any terrestrial crop, primarily due to the fact that they can be cultivated year round with continuous harvest cycles [4] and their high photosynthetic efficiency. Moreover, characteristics such as photosynthetic ability of microalgae to fix CO_2 from the environment, production of diverse energy molecules such as lipids, carbohydrates and protein, and wastewater treatment make it a unique feedstock for renewable energy production and an agent for sustainable environment.

Despite all the advantages of microalgal biomass over the land crops, economic viability of microalgal biofuels is still under question. Most of the work has been done only in laboratory scales, with limited pilot scale demonstration while real large-scale applications are very scarce. Therefore, the real potential of microalgae as an industry is still unclear and cannot be predicted [5]. Based on various techno-economic and life cycle analysis, harvesting and oil extraction are very expensive and energy intensive processes, and it has been visualized that the microalgal biofuel is economically unfeasible unless the cost related to these three steps is reduced [6]. Drying of microalgae biomass prior to oil extraction requires a substantial amount of energy. Therefore, wet extraction process for oil recovery is preferred.

Lipids are located inside the microalgae cells in the form of fatty acids, triacylglycerides, phospholipids and glycolipids, with *Botryococcus braunii* being the only known strain which releases some amount of oil extracellularly. The presence of these molecules and other metabolites are integral part of cell wall and cell membrane. Cell disruption increases lipid extraction efficiency as solvent transport is no longer restricted across the cell membrane. During cell disintegration, lipids are released from cellular compartments and move to surrounding medium [7]. Thick and rigid cell wall reduces the penetration of solvent for oil extraction, and poses a significant chemical resistance to most of the organic solvent [6]. The most common pretreatment applied to microalgae cell prior to oil extraction is drying of biomass for water removal. Biomass drying consumes a significant amount of energy [8]. High-pressure homogenization is also reported as a pretreatment option without drying of biomass. But it is also energy intensive due to harsh operating conditions [9]. Due to these various challenges, oil extraction from microalgal cells is not proven economically feasible. Cell disruption improves lipid extraction 3–4 times higher [10]. Various techniques have

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been employed to rupture the microalgal cells in order to reduce the extraction cost. These techniques can be briefly divided into three main categories; mechanical, chemical and biological methods.

Mechanical methods make use of physical forces to disrupt the cell membrane. These methods have the advantage of being applicable to any microalgal strain. Mechanical methods [11–14] include bead beating, microwave [13], ultrasound application and high-pressure homogenization [15] and autoclave. Although they are quite effective for cell lysis, these mechanical methods require harsh condition such as high pressure and temperature. These methods consumed a significant fraction of energy stored in microalgae lipids [16] due to harsh operating conditions. Moreover, some of these methods are difficult to scale up, and hence there is no documented use of some of these techniques on a large scale. It has been assumed that microalgae cell disruption can be achieved via the same process employed in edible oil industry. Small microalgae cell size (3–15 μm), rigid, but elastic type cell walls help the microalgae cell to squeeze through a mechanical device without complete cell disruption. Drying of biomass is required before the use of mechanical press and drying of microalgae is a very energy intensive process [8].

Chemical methods use chemicals to weaken the cell membrane to release the metabolites, and increase the penetration of solvent that can extract the oil out of the cell. Chemical methods include the use of detergent, polymers, ionic liquids [17], cationic surfactant [18], acid and alkali [19], ozone [20] and osmotic shock [14]. These methods can be scaled up easily, but require special types of reaction system. Moreover, further processing of the product stream is essential for the removal and neutralization of the chemicals from extracted oil. The possibility of product damage due to these chemical is also a concern. These concerns have led researchers to investigate the use of biological methods such as enzymes and autolysis [21]. These methods can be effective but finding the enzyme with high activity is essential. New innovative way of microalgae cell rupture using membrane supported cell lysing agent is reported [22].

In this study, a new low energy method for microalgae cell lysis is reported. This is a hybrid process that makes use of mild mechanical operating conditions and non-toxic chemical cell disruption agent. A polymeric resin loaded with iodine-based disinfectant known as Triiodide resin was used to disrupt the microalgal cell. Triiodide is commonly utilized for disinfection purposes in the water and wastewater treatment industry [23]. Triiodide-based resin is commercially available at low cost and has been reported for various other applications as well [24].

There is no report on the use of triiodide resin for microalgal cell lysis for oil extraction. This study investigates the various parameters such as the time required for cell disruption efficiency, effects of resin on different strains of microalgal cells, quantity of resin required, and reusability of resin, and oil recovery efficiency. Four different microalgal strains include fresh water *Chlorella vulgaris*, *Chlamydomonas reinhardtii*, and marine algae *Tetraselmis striata*, and heterotrophically grown *Aurantiochytrium* sp., were tested for the degree of cell lysis by triiodide resin.

2. Material and methods

2.1. Preparation of triiodide-loaded resin

Triiodide resin was prepared as described [23]. Solid iodine was dissolved in 1 M KI aqueous solution and was heated at 80 °C. Triiodide solution was cooled down to room temperature. Cooled triiodide was passed through quaternary ammonium (strong base) resin (500–700 μm) packed in a column multiple times for complete loading of triiodide. After passing the triiodide through the resin bed, column was washed multiple times with distilled water to remove any unbound triiodide. During the preparation of triiodide loaded resin, following reaction takes place:

$$KI + I_2 \rightleftarrows K^+ + I_3^- \quad (1)$$

2.2. Microalgae strains cultivation

Microalgae *Chlorella vulgaris* (UTEX-265) was cultivated in standard BG-11 medium, *Tetraselmis striata* was cultivated in artificial seawater medium (ASW), *Chlamydomonas reinhardtii* was grown in Tris-acetate phosphate medium (TAP) and *Aurantiochytrium* sp. KRS101 was grown in complex glucose medium under heterotrophic condition. *C. vulgaris* and *T. striata* were grown phototrophically in the presence of 2% CO_2 at flow rate of 0.5 vvm under light intensity of 100 μmol/m^2·sec. All the microalgal cells used in this study were in the early stationary phase. The biomass content for microalgae *C. vulgaris*, *T. striata*, *Aurantiochytrium* sp. and *C. reinhardtii* were measured to be 2.5 g/L, 3.0 g/L, 10 g/L and 1.5 g/L under the given growth conditions, respectively.

2.3. Microscopic analysis and cell number measurement

Microalgal cells images were obtained before and after pre-treatment with the triiodide resin using optical microscope equipped with DFC 425C camera (Leica microsystem). Cell number was counted using hemocytometer.

2.4. Microalgal cell rupture using triiodide resin

A known volume (5 ml) of microalgae cell culture was mixed with various quantity of triiodide resin in 20 ml glass vials. Microalgal cells were mixed with the triiodide resin using magnetic stirrer at 150–200 rpm at 25 °C. After every 15 min, cell samples were taken and analyzed under microscope to check the cell rupture. Cell rupture efficiency was calculated based on the number of intact cells before and after treatment using the following formula;

$$\%\text{ of cell rupture} = \left(\frac{N_{t0} - N_t}{N_{t0}}\right) * 100 \quad (2)$$

where, N_{t0} and N_t represent the number of whole cells before treatment and ruptured cells after pre-treatment for a specific period of time t.

2.5. Oil extraction and quantification

Microalgae culture of know volume (5 ml) was mixed with the triiodide resin. Ruptured cells were separated from the resin using stainless-steel 200-μm mesh. Liquid culture with ruptured cells were homogenized with known volume of chloroform/methanol solution (2:1) to separate the oil from the aqueous phase. Lipids bound to resin after treatment were eluted by passing chloroform/methanol (2:1) as well. Same amount of microalgae culture (5 ml) was harvested via centrifuge and was freeze dried to be used for estimation of the lipid as control.

After removing the oil from the ruptured cells in liquid culture, residual biomass was also subjected to transesterification. Solvent from extracted oil was evaporated under nitrogen stream, and dried oil was converted to FAME in order to measure the total amount of oil extracted using gas chromatography [22]. Briefly, 2 ml of chloroform–methanol (2:1) was mixed with the extracted oil for 5 min. After mixing, 1 ml of methanol and 0.3 ml of 98% H_2SO_4 was added and the mixture was heated at 100 °C for 20 min. The sample was cooled down to room temperature after the reaction, and 1 ml of water was added for phase separation. The chloroform phase was transferred to an analysis vial and was analyzed by gas chromatography (GC). Heptadecanoic acid (C-17) and nonadecanoic acid (C19) were used as internal standard for fatty acid quantification. GC system (Agilent 6890) was equipped with flame ionized detector (FID) and HP-INNOWax polyethylene glycol column. Each peak area was divided by the peak area of internal standard, multiplied by internal standard original concentration and

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