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Simultaneous cell disruption and lipid extraction in a microalgal biomass using a nonpolar tertiary amine

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HIGHLIGHTS

- Simultaneous cell disruption and lipid extraction of microalgae is accomplished.
- The cell surfaces are significantly disrupted by the triethylamine/methanol system.
- 92.5% of the total lipids are extracted from the wet microalgal biomass.

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ABSTRACT

A simultaneous cell disruption and lipid extraction method is developed for microalgal biodiesel production using a triethylamine/methanol solvent system. Individually, the pure solvents, i.e. triethylamine and methanol, do not exhibit significant enhancement in lipid extraction, but a 3:7 (v/v) triethylamine/methanol mixture exhibits the highest lipid extraction, corresponding to 150% of the conventional chloroform/methanol (2:1, v/v) solvent extraction. This extraction is equivalent to 92.5% of the total lipids, even when extracted from a wet microalgal biomass with a water content of 80%. The cell surfaces of the microalgae are significantly disrupted without using additional cell disruption reagents and without requiring energy-intensive equipment. The lipid mass transfer coefficient is 1.6 times greater than that of the chloroform/methanol solvent system. It is clearly demonstrated that triethylamine and methanol cooperate well for the cell disruption and lipid extraction.

1. Introduction

The accumulation of greenhouse gas in the atmosphere due to the use of fossil fuels has resulted in increased interest in renewable fuels (Ahmad et al., 2011). Among the various types of renewable fuel sources, microalgae have been considered as one of the most promising biofuel sources due to their advantages, including high photosynthetic efficiency, high lipid content, and high growth rates (Ahmad et al., 2011; Lam and Lee, 2012; Lee et al., 2015).

Producing biodiesel from microalgae typically includes four steps: microalgae cultivation, harvesting, lipid extraction, and biodiesel conversion (Ahmad et al., 2011; Huang et al., 2017; Park et al., 2014; Yoo et al., 2014). Among these four steps, the operational cost of the lipid extraction process is particularly high, because the microalgal cells are protected by cell envelopes (cell walls and cell membranes) that severely hamper the solvent accessing the cytoplasm (Bai et al., 2014; de Boer et al., 2012). Furthermore, another challenge is the presence of moisture that significantly reduces the lipid extraction efficiency through limiting the contact between the hydrophobic solvents and negatively charged microalgal cells (Huang and Kim, 2013; Kim et al., 2013). Thus, the recovery process of microalgal lipids requires cell disruption and drying steps.

The cell disruption methods developed thus far include ultrasonic cavitation, bead milling, microwave heating, and osmotic shock, and microalgae drying has been achieved through freeze drying, oven drying, and spray drying (de Boer et al., 2012; Halim et al., 2012; Steriti et al., 2014). Despite the high performance, improving the recovery process has remained limited due to its energy-intensive or cost-ineffective operations (de Boer et al., 2012; Yoo et al., 2014). Moreover, microalgal pretreatment including cell disruption and drying results in the lipid extraction process being economically unfeasible (de Boer et al., 2012; Kim et al., 2013; Lam and Lee, 2012; Seo et al., 2016). Therefore, it has become imperative to develop a lipid extraction process without a pretreatment.

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In this study, a novel solvent extraction method is developed for microalgal lipid extraction using a nonpolar tertiary amine. Various volume ratios (0:10, 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1, and 10:0) of triethylamine and methanol mixtures are used to extract lipids from microalgae. The extracted lipids are analyzed using gas chromatography–mass spectrometry. The lipid extraction kinetics of a wet biomass is analyzed in order to investigate the rate of the overall process.

2. Materials and methods

2.1. Materials

The materials used in this study were resourced as follows: Triethylamine, Sigma–Aldrich (USA); chloroform, Merck (Germany); sulfuric acid, Junsei (Japan); and methanol, OCI (South Korea). PVDF syringe filter was purchased from Sigma-Aldrich (USA), and *Chlorella* sp. was purchased from Daesang Wellife (South Korea).

2.2. Lipid extraction using a triethylamine/methanol solvent system

In order to extract the lipids from the microalgae, 10 mL of microalgal culture (1 wt%) was centrifuged at 7000 rpm for 5 min. Then, pellets were collected without drying. The volume ratio of triethylamine and methanol for the microalgal lipid extraction was varied to create ratios of 0:10, 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1, and 10:0. The total volume of the triethylamine/methanol mixtures were fixed at 12 mL. Two identical triethylamine/methanol mixtures were prepared for each ratio. Then, the triethylamine/methanol mixtures were added to the harvested microalgae, and the two microalgae-solvent mixtures were shaken vigorously in a vortex mixer(Vortex Genie 2, Scientific Industries) at room temperature; one mixture was shaken for 1 h and the other was shaken for 2 h. Next, the cell debris was separated from the liquid phase via centrifugation at 7000 rpm for 7 min. After collecting the supernatants, the triethylamine and methanol were evaporated in order to obtain crude lipids.

The chloroform/methanol mixture (2:1, v/v) is one of the most frequently used organic solvent mixtures for lipid extraction from microalgal biomasses (Halim et al., 2012; Huang and Kim, 2013, 2016). For comparison, the extraction was also tested using the chloroform/methanol solvent system. For harvesting, 10 mL of microalgal culture (1 wt%) was centrifuged at 7000 rpm for 5 min. Two identical chloroform/methanol mixtures (12 mL) were prepared. Then, the chloroform/methanol mixtures were added in order to extract the lipids from the harvested microalgae, and they were shaken in a vortex mixer(Vortex Genie 2, Scientific Industries) at room temperature; one mixture was shaken for 1 h and the other for 2 h. After the extraction, crude lipids were obtained via evaporating the chloroform and methanol.

2.3. Conversion and lipid analysis

The extracted lipids were converted to fatty acid methyl esters (FAMEs) via transesterification (Huang and Kim, 2013, 2016). Each isolated lipid sample was dissolved in 4 mL of chloroform/methanol mixture (1:1, v/v) in Pyrex tubes. Heptadecanoic acid dissolved in 1 mL of chloroform was used as an internal standard, and 0.5 mL of sulfuric acid was added to each tube. Then, the mixture was mixed vigorously using a vortex mixer(Vortex Genie 2, Scientific Industries) at room temperature for 10 min. Next, the mixture was reacted in a heating block at 100 °C for 1 h. After the reaction, the mixture was cooled to room temperature, 10 mL of distilled water was added to the reaction mixture, and the mixture diluted with water was gently mixed using a vortex mixer(Vortex Genie 2, Scientific Industries) for 10 min. Subsequently, the organic phase was separated from the water mixture phase via centrifugation at 5000 rpm for 10 min, and then the separated organic phase was filtered through a 0.2 μm PVDF syringe filter. After conversion, the FAMEs were analyzed using gas chromatography–mass spectrometry (GC HP5890/MSD HP5989B, Agilent).

2.4. Total lipid extraction

The lipid extraction efficiency of the triethylamine/methanol solvent system was evaluated from the total lipids contained in the microalgal biomass. The total lipids were extracted from the microalgae using Folch method with a slight modification (Folch et al., 1957). Briefly, 10 mL of microalgal culture (1 wt%) was taken and disrupted using an ultrasonic processor. Then, the disrupted cells were dried using a freeze dryer. Next, microalgal lipids were extracted from the disrupted microalgae through adding 12 mL of chloroform/methanol mixture (2:1, v/v). Subsequently, the extracted lipids were converted to FAMEs via transesterification. The experimental procedures for the transesterification are the same as those described in Section 2.3.

The content and composition of the fatty acid extracted from the microalgae were determined using gas chromatography–mass spectrometry (GC HP5890/MSD HP5989B, Agilent).

2.5. Scanning electron microscopy (SEM) observations

The surface morphologies of the microalgal cells before and after the solvent treatment were observed using scanning electron microscopy (Sirion FE-SEM, FEI). Prior to the SEM observation, the samples were immersed in a liquid nitrogen bath and freeze-dried in a freeze dryer. Then, the samples were coated with a thin platinum film (Pt coating) and attached to a carbon tape stick on a metal stub. All SEM images were recorded at an acceleration voltage of 10 kV.

2.6. Kinetic analysis of lipid extraction

The internal pathway of the microalgae lipid extraction using an organic solvent is not simple, but it was assumed to follow an overall first-order kinetics, which is a widely used model in analyzing the kinetics of lipid extraction with organic solvents (Bai et al., 2014). The first-order kinetics leading to the recovery of lipids is represented using the following equation:

$$Y(t) = Y(1 - e^{-kt}),$$

where Y(t)(%) is the amount of microalgal lipids recovered at time t, Y is the total lipids extracted from the microalgal cells (Y was fixed at 100%), k(h^{-1}) is the lipid mass transfer coefficient, and t(h) is the extraction time.

The lipid mass transfer coefficient k governs the speed of the overall process of the organic solvents, which diffuse into the microalgal cell, interact with the lipids in the cytoplasm, and diffuse out of the microalgal cell (Halim et al., 2014). The lipid mass transfer coefficient was determined through fitting the experimental data with the first-order kinetics using Matlab.

3. Results and discussion

3.1. Lipid extraction efficiency of the triethylamine/methanol solvent system

The extraction efficiencies of the triethylamine/methanol solvent system and chloroform/methanol solvent system are depicted in Fig. 1. The extraction efficiency continuously improved when the