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Research article

Investigation of colony disruption for hydrocarbon extraction from *Botryococcus braunii*

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ABSTRACT

Botryococcus braunii is a colonial microalga that produces hydrocarbons. While the microalga stores almost all the amount of hydrocarbons in its colony matrix, the amount of the extracted hydrocarbon without any pretreatment is typically very small. We performed mechanical cell disruption using a JET PASTER® and a bead mill as the pretreatment ways to facilitate hydrocarbon extraction from *B. braunii*. After the disruption, the size and shape of colonies changed. In the JET PASTER treatment at 4800 rpm, the concentration of removed polysaccharides increased 146 to 173 μg/mL and the hydrocarbon yield increased 2.7 to 82.8%. In the bead mill treatment, the concentration of removed polysaccharides increased 146 to 210 μg/mL and hydrocarbon yield increased 2.7 to 42.3%. Therefore, the disruption of colonies and polysaccharides around algal colonies would affect the hydrocarbon extraction. In addition, the apparent photosynthetic activity of the sample treated by the JET PASTER was 0.71 that is almost the same value as that of the untreated sample, whereas that of the sample treated by the bead mill was 0.64. Therefore, the JET PASTER treatment did not affect the photosynthetic function of *B. braunii*.

1. Introduction

The use of biofuels, especially liquid fuel, attracts attention in the social condition where the rapid depletion of fossil fuel, the global growth of transport sector, and the countermeasures against global warming are becoming serious global problems in recent years [1–3]. In the conventional biofuel produced from corn, soybeans or sugar cane, the source of crops has the problem of the competition of demand against food [1,3]. On the other hand, microalgae are extremely expected to be used as a source of next-generation biofuel because the production of energy from microalgae is not competitive to food [4]. In addition, the microalgae contain high levels of hydrocarbons or lipid (up to 75 wt%) which can be used as sources of biofuels, and have high CO_2 fixation abilities compared to conventional plants [1,3,5–12].

In general, microalgae are converted to biofuel through the following processes: algal cultivation, algal harvesting, and extraction of crude oil that contains the hydrocarbons or lipids with microalgae [13]. The hydrocarbons and lipids are usually extracted by organic solvents such as *n*-hexane [2,14]. However, as microalgae are generally cultivated at low concentrations (i.e., about 1 g/L), the intensive dewatering of algal suspensions is critical in progressing the contact of hydrocarbons and organic solvents. Thus, the conventional dewatering method of thermal drying consumes 30–90% of the total energy production [15,16]. The energy consumption for biofuel production was larger than the energy production from biofuel, and the conventional system with thermal drying would not use energy effectively [15]. To reduce energy consumption, researchers have attempted to extract hydrocarbons or lipids without using thermal drying [17–21]. In other words, a truly effective extraction method of hydrocarbons from wet microalgae is required.

Green algae, *Botryococcus braunii*, is a microalga which has cells with a diameter of about 10 μm and forms colonies with sizes of dozens of micrometers due to the aggregation of the cells.

Especially as *B. braunii* race B, which is one of the species of *B. braunii*, contains high hydrocarbon level, producing C30–34 hydrocarbons according to a culture condition (e.g., 45–80% of total dry cell weight [22]), the microalga is expected to become one of available biofuels [23]. Unlike another microalga, *B. braunii* has the specific features such as the storage of produced hydrocarbons into both the inside and within its colonies. Therefore, the hydrocarbons can be extracted easily even in wet conditions, because the microalga secretes almost all the hydrocarbons (i.e., > 90%) within its colonies. In fact, a small quantity of hydrocarbons can be extracted without any pretreatment for solvent extraction from wet *B. braunii* [24,25]. Atobe et al., Furuhashi et al., and Kita et al. showed that most of the hydrocarbons were extracted when the amphiphilic polysaccharides were

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removed from the colony surrounding by heating [24,26,27]. Thus, the hydrocarbon yields were poor unless the polysaccharides were removed. Furuhashi et al. performed the alternative cultivation using seawater added culture for wet extraction [28]. Sodium chloride in seawater prevented the production of polysaccharides, and Furuhashi et al.'s method achieved quite high hydrocarbon yield, which extracted without any pretreatment. However, the method took over 70 days for cultivation to extract the sufficient amount of hydrocarbon. Since the small growth rate of *B. braunii* is one of the major bottlenecks for utilizing the species as the energy feedstock [29,30], the sea-water cultivation is not the best feasible way for biofuel production.

As *B. braunii* has the above feature, the intensive cell disruption is unnecessary for hydrocarbon extraction. Actually, Eroglu and Melis suggested that increases in solvent extraction of the amount of hydrocarbon were proportionate to the degree of the division of colonies into smaller ones, which were accomplished by intensively mixing samples with glass beads [31]. Generally, the intensive cell disruption has the drawback that is the generation of fine cell fragments [32]. The fine cell fragments raise the costs of downstream processes such as a solid-liquid separation [32] and energy consumption because cell fragments would prevent the conductance of external forces to undisrupted cells [33]. Therefore, the mild cell disruption of algal cells would be expected to reduce processing cost and energy consumption. For *B. braunii*, the mild disruption would be achieved by disrupting algal colonies and/or removing the polysaccharides around colonies. While Eroglu and Melis suggested the importance of colony disruption from the extracted hydrocarbon yields from *B. braunii* disrupted by a bead mill, the bead mill is generally intensive cell disruption method [32]. Furthermore, hydrodynamic cavitation is thought to be an energy efficient method [34]. The cells are disrupted from peripheral region by the hydrodynamic cavitation, whereas bead mill disrupts whole cells [34]. Therefore, as hydrodynamic cavitation would be able to remove polysaccharides from algal colonies without whole cell disruption, the hydrodynamic cavitation would be applied to a colony disruption.

The polysaccharides around algal colonies displayed water absorption characteristics [35], which may prevent the hydrocarbon extraction when hydrocarbons are extracted using hydrophobic solvent. Therefore, to improve extracted hydrocarbon yields from *B. braunii*, it is important to observe and evaluate the existence of the polysaccharides. Negative staining using India ink gives information on the colony surrounding and colony interior (which was not stained) that the polysaccharide locates on the colony surface [36]. Soot particles in India ink cannot penetrate the interior of colonies through the fibrillar polysaccharides, resulting in the negative stain. As this method easily classifies whether the polysaccharides are removed from the colony surroundings, it can be applied to evaluate changes in the polysaccharide presence before and after mechanical treatments.

In the present study, the colonies of *B. braunii* were disrupted using two mechanical methods (i.e., a bead mill and a hydrodynamic cavitation device) and the amount of hydrocarbon extracted by *n*-hexane from *B. braunii* was evaluated. The degree of cell and colony disruption was evaluated by measuring the diameter and shape of cells and colonies using image analysis with in-situ images of *B. braunii* before and after treatments. In addition to evaluating the size and shape of cells and colonies, the effects of the removal of polysaccharides around colonies on hydrocarbon extraction was investigated to analyze the morphology of polysaccharides using India ink. Moreover, the apparent photosynthetic activities of samples before and after treatments were measured to evaluate the viability of cells. In previous studies, because photosynthetic activity is sensitive to cell damage, it has generally been used as the metric of cell viability [30]. From above experimental, we discuss the colony disruption methods that improve the extraction of hydrocarbons from *B. braunii*. We also discuss the factors to enhance hydrocarbon yields using colony disruption on the basis of particle size/shape properties and the morphology of polysaccharide.

2. Materials and methods

2.1. Microalgae cultivation

Botryococcus braunii BOT-22 (race B) was used as a sample strain. This microalga was kindly provided by Prof. Makoto M. Watanabe in the University of Tsukuba. The microalga was cultivated using a modified KAI medium. The details of the culture medium and cultivation method are described by Shimamura et al. [23]. The KAI medium does not contain salt water. Hence, hydrocarbons were not easily extracted from wet microalgae as with shown by Furuhashi et al. Air with a CO_2 concentration of 4800 ppm was supplied to the culture medium. The medium was exposed to light with intensity of 50 μmol/m^2/s for 24 h daily, and the cultivation was conducted for a month. The cell concentration used as the cell disruption samples for the particle size analysis and hydrocarbon analysis was measured by filtering a cell suspension with pre-weighed glass fiber filters and drying with a vacuum dryer at 80 °C under 0.05 MPa pressure for 24 h. The determined concentrations ranged from 0.82 ± 0.024 kg/m^3.

2.2. Cell disruption

Two disruption devices were used in this study: a bead mill (LMZ015, Ashizawa Finetech Ltd., Japan) and a circulating particle disruption device called a JET PASTER® (JP-SS, Nihon Spindle Manufacturing Co, Ltd., Japan). The detailed image of disruption devices were shown in our previous study [37]. The internal flow channel of JET PASTER is shown in Fig. 1. The bead mill disrupted samples in a grinding chamber by high shear forces caused by the acceleration of beads which formed stream layer of different velocity [38]. The JET PASTER was composed of a centrifugal pump with a recirculation pipe, and an impeller rotating at high speed was present inside the device; the sample supplied to the casing was agitated, and then the stirred sample was recirculated through the recirculation pipe. The samples were disrupted by shear force between the impeller and sample, which were generated by the transfer of sample due to rapid impeller rotation. Besides, hydrodynamic cavitation occurred at some operation conditions because the fluid in the device was accelerated by the impeller rotation, and low-pressure spaces subsequently appeared in a part of casing. Thus, the samples were also disrupted by impulsive forces caused by the collapse of cavitation bubbles. Here, as the exhaust liquid

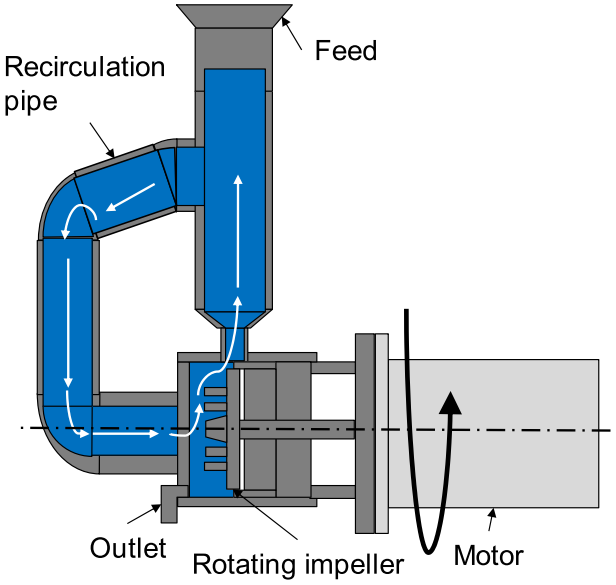

Fig. 1. Images of internal flow channel of the JET PASTER.