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Research article

Prediction of hydrocarbon yield from wet *Botryococcus braunii*: The influences of colony surface charge and diameter on the amount of extractable hydrocarbon

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ABSTRACT

A colonial microalga, *Botryococcus braunii* synthesizes extracellular polysaccharides (EPS) forming a sheath around its colonies, which limits the extraction of hydrocarbons from this microalga. As thermal or mechanical pretreatment of *B. braunii* improves the hydrocarbon yield, the EPS removal and degree of algal colony disruption before and after pretreatment were evaluated using zeta potential and particle size distribution (PSD) to predict hydrocarbon yield from the alga. Since zeta potential can evaluate the ionizable state of particles, the zeta potential of *B. braunii* would be able to evaluate the removal degree of EPS and this feature had the potential to determine hydrocarbon yield quickly. The zeta potential of algal samples without pretreatment was −30.5 mV at pH 9, whereas that with pretreatment ranged from −29.7 to −18.8 mV. The elevation of zeta potential and EPS removal had a linear relationship (R-squared value = 0.96). The hydrocarbon yields exponentially increased in proportion to the degree of colony disruption after mechanical treatments (R-squared value = 0.81). These findings suggest that the zeta potential and PSD of *B. braunii* may be used as indicators for EPS removal and hydrocarbon yield.

1. Introduction

The use of biofuels, especially liquid fuels, is expected to serve as an alternative to fossil fuel as biofuels can decrease the risks of global warming and cover the increasing global demand of liquid fuels for the transportation sector [1–3]. Among biofuel sources, microalgae can be utilized to produce next-generation biofuels because of their non-competitive use of food supply, high growth rate, high lipid content, and CO_2 fixation capacity [3–10].

In general, microalgae are converted to biofuel via algal cultivation, harvesting, and the extraction of crude oil that contains hydrocarbons or lipids. The algal hydrocarbons and lipids are usually extracted by organic solvents such as *n*-hexane, based on its advantages for large-scale extraction [2,4,11]. Against the high moisture content (averaging 99.9 wt%) of algae, which affords poor affinity to organic solvents, the elimination of water from algal suspensions is used to improve hydrocarbon yields. However, the drying of algal suspensions, a common intensive dewatering method, consumes 30 to 90% of total energy input [12,13]. To reduce this requirement, techniques for extraction of hydrocarbons or lipids from wet microalgae have been widely investigated [14–18].

Botryococcus braunii is a microalga that has a cell size of approximately 10 μm and forms colonies sized in the same order of magnitude. This alga, particularly race B, constitutes an attractive candidate as a biofuel feedstock because of its ability to generate a high C30–34 triterpenoid hydrocarbon content (up to 75 wt%) depending on the conditions of cultivation [1,19–22]. Notably, hydrocarbon extraction using organic solvents such as *n*-hexane from *B. braunii* is expected to be possible without pretreatment, even in wet conditions, because unlike other alga it secretes produced hydrocarbons outside of its cells, thus accumulating almost all the hydrocarbons into the extracellular colony matrix [23,24]. However, in practice, it has not yet been possible to extract sufficient hydrocarbons using *n*-hexane without any pretreatment [25–29].

High yields of hydrocarbons using *n*-hexane or *n*-heptane have been extracted from wet *B. braunii* pretreated by several methods. Atobe et al. [30] Furuhashi et al. [31] and Kita et al. [25] utilized thermal treatment of wet *B. braunii* by heating a cell suspension to 90 °C for 20 min; Eroglu and Melis [32] used a vortex mixer on a cell suspension with glass beads; and Tsutsumi et al. [27–29] conducted mechanical cell disruption using a high-pressure homogenizer, a bead mill, and/or a circular centrifugal pump. Thus, thermal and mechanical

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Nomenclature

$D_{50,\ num\%}$ [μm]	median diameter of each sample in the number distribution
$D^{NT}_{50,\ num\%}$ [μm]	median diameter of the untreated sample in the number distribution
$D_{50,\ vol\%}$ [μm]	median diameter of each sample in the volume distribution
$D^{NT}_{50,\ vol\%}$ [μm]	median diameter of the untreated sample in the volume distribution
$F(k_a)$	Henry's constant
U_E [μm·cm/(V·s)]	electrophoretic mobility of particles
Y_{HC} [%]	hydrocarbon yield extracted from each sample
Y^{NT}_{HC} [%]	hydrocarbon yield extracted from the untreated sample
ε_0 [F/m]	permittivity of a vacuum
ε_r	relative permittivity
ζ [mV]	zeta potential
η [mPa·s]	viscosity of the solvent

pretreatments have been shown as effective in achieving hydrocarbon extraction using organic solvent from wet *B. braunii*. Therefore, factors preventing the hydrocarbon extraction without any pretreatment are considered as including the presence of (i) amphiphilic extracellular polysaccharides (EPS) on the algal surroundings and/or (ii) cells within the colonies that isolate hydrocarbons from external regions.

Previous studies have reported that EPS were removed and algal hydrocarbons successfully extracted following algal suspension heating at 90 °C for 20 min [13,19,22,23,25]. Furuhashi et al. [31] evaluated the degree of such EPS removal through observation with an electron microscope and extraction of EPS in a cell suspension. However, as electron microscopy requires complex pretreatments for biotic samples, this method is not convenient. Alternatively, Uno et al. [33] visualized the EPS surrounding *B. braunii* cells and colonies using negative staining with India ink and an optical microscope. Unfortunately, as the algae in the samples were deformed by the weight of the cover glass over time, a quantitative evaluation of the amount of EPS was difficult.

Ideally, an in-situ and quantitative method to evaluate the degree of removal of EPS is required as an effective indicator of the efficacy of pretreatment methods for hydrocarbon extraction. For example, Eroglu and Melis [32], Moheimani et al. [34] Moheimani et al. [35] and Tsutsumi et al. [27] disrupted, dispersed, or deformed colonies of *B. braunii* and obtained high hydrocarbon yields from wet biomass. Although these studies demonstrated that the disruption or dispersion of colonies affects hydrocarbon yields, the degrees of efficacy of algal colony disruption or dispersion remain unknown owing to the lack of a suitable evaluation method. For evaluating the degree of cell disruption, quantification by intact cell count using microscopy and/or quantification of the release of intracellular substances such as proteins or chlorophyll have been performed [5,36–38]. However, because colonies of *B. braunii* exhibit varying diameters and shapes and do not contain releasable internal substances, such evaluation methods cannot be applied toward evaluating the degree of colony disruption for this alga.

In general, the EPS surrounding *B. braunii* are comprised of monosaccharides including glucose, galactose, arabinose, mannose, and uronic acids [30,39]. Moreover, the EPS that contain carboxyl, amino, and hydroxyl groups are ionized in the cell suspension [40–43]. Therefore, the algae have a charge depending on the pH of the cell suspension; accordingly, several authors have reported the generation of an electric potential around the cells [42,44]. In particular, as the pKa value of the carboxyl groups in EPS is low [40], *B. braunii* carries a negative charge in cell suspension.

The measurement of zeta potential can be used as an indicator of the surface charge of particles and can be used for the practical evaluation of their aggregability or dispersion. Here, the zeta potential constitutes the electric potential of the slipping plane, which is located near the particle and used to quantify the surface potential of the particle. Because the zeta potential value represents the electrical state of the particle surface, several studies have applied zeta potential measurements to microalgae. For example, Hadjoudja et al. [40] Ozkan and Berberoglu [42] and Xia et al. [44] measured the zeta potential of various microalgal species to evaluate adhesiveness to materials such as metals. Ozkan and Berberoglu [42] and Xia et al. [44] also measured zeta potential to propose more effective algal harvesting technologies such as flocculation. Moreover, Henderson et al. [45] evaluated the influence of flocculant dosage on the zeta potential of algae to evaluate algal harvesting methods. Notably, zeta potential is proportionate to the surface charge density of particles. As *B. braunii* is composed of cells, colonies, and EPS, with the latter covering the whole surface of algal colonies, when the EPS are removed from the colonies, the charge density of the algal surface would thus be changed. Therefore, evaluation using zeta potential is expected to be useful for evaluating the degree of EPS removal.

However, although the zeta potential is effective for evaluating the physicochemical characteristics of algae, there is no precedence for measuring the zeta potential as an indicator of the degree of removal of EPS. Use of the zeta potential as an in-situ and quantitative indicator of the degree of EPS removal and the predicted hydrocarbon yields from wet *B. braunii* might therefore allow the efficient determination of optimal pretreatment methods and other operating conditions for hydrocarbon extraction. Furthermore, identification of the degree of colony disruption necessary for hydrocarbon extraction may facilitate the proposal and optimization of novel and effective mechanical treatments. The aim of the present study was therefore to investigate the relationships of zeta potential and particle size distribution with the hydrocarbon yield from wet *B. braunii* to identify an indicator that could be used for predicting hydrocarbon yield. In the present study, the EPS sheath surrounding the *B. braunii* cells and colonies was removed, and colonies were disrupted by applying different physical and thermal pretreatments to the cell suspension. Subsequently, the zeta potential of the samples, particle size distribution, EPS removal, and hydrocarbon yield were measured, and their values correlated.

2. Materials and methods

2.1. Microalgae

B. braunii BOT-22 (race B), provided by Prof. Makoto M. Watanabe from University of Tsukuba, was used for the algal samples. The details of the culture medium and cultivation method are described in Shimamura et al. [22]. The algal samples were cultured in a 10-L polycarbonate carboy (Nalgene ClearBoy, Thermo Fisher Scientific, USA) for one month. The cell concentration was measured by filtering the cell suspension with pre-weighed glass fiber filters and drying in a vacuum dryer at 30 °C for 24 h. The cell concentrations were found to range from 0.56–0.58 g/L.

2.2. Mechanical and thermal treatment of the algal samples

In the present study, mechanical cell disruption or thermal heating of a cell suspension was conducted to disrupt the algal colonies and to remove the EPS surrounding the *B. braunii*. A high-speed JET PASTER® (Nihon Spindle Manufacturing Co, Ltd., Japan), a bead mill (LMZ015, Ashizawa Finetech Ltd., Japan), or a high-pressure homogenizer (LAB2000, APV, USA) was used as the disruption device. The mechanism of the JET PASTER was described in our previous studies [27–29]. In the JET PASTER treatment, the rotational speed of the