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Effect of amphiphilic polysaccharides released from *Botryococcus braunii* Showa on hydrocarbon recovery

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ABSTRACT

Water-soluble polymers are released from the extracellular matrix of *Botryococcus braunii* by thermal pretreatment prior to hydrocarbon extraction. In this study, hydrocarbon recovery was reduced by adding polymers to algal slurries from which they were previously washed. The water-soluble polymers are amphiphilic and emulsify the water–organic solvent systems used in hydrocarbon extraction. To obtain >90% hydrocarbon yields from high-concentration slurry, the original 10% water-soluble polymer content of dry algal cells had to be reduced to less than 0.5%. The water-soluble polymers were polysaccharides with a molecular weight greater than 2×10^6 and mainly comprised of galactose, arabinose, and uronic acid. We suggest that high-molecular-weight water-soluble polymers are desirable as industrial emulsifiers and thickeners.

1. Introduction

The microalga *Botryococcus braunii* has a high hydrocarbon content and is therefore expected to be a suitable biofuel resource [1–3]. The most striking feature of this microalga is that it forms colonies by connecting cells with an extracellular matrix (ECM) composed of mucopolysaccharides and hydrocarbon-related compounds, most of which accumulate in the ECM rather than in the cells [4,5]. *B. braunii* is classified into three chemical races according to the structure of their hydrocarbons produced: race-A, race-B, and race-L [6]. Race B seems to be the most promising fuel resource because it produces large amounts of branched unsaturated triterpenes with the formula C_nH_{2n-10} (n = 30–37) [7].

The hydrocarbons that accumulate in *B. braunii* can be extracted by soaking dried alga in low-polar solvents such as *n*-hexane [1]; however, the drying process requires an enormous amount of energy and there has been a search for methods of hydrocarbon recovery from wet *B. braunii*. Frenz et al. [5] attempted to recover hydrocarbons by directly mixing the algal slurry with a low-polar organic solvent, but the recovery rate was low. Attempts to extract hydrocarbons without killing the algal cells, "milking" them by using various ionic liquids have been performed, although the selection of appropriate solvents is necessary in order to guarantee culture viability [8]. The ECM has been analyzed by electron microscopy, which revealed a part of the ECM structure named the "retaining wall" sequesters hydrocarbons released from the algal colony [9]. Dote et al. [10] treated *B. braunii* suspended in water at 300 °C under high-pressure (10 MPa) liquefaction and obtained high yields of liquid fuel; however, the liquid fuel fraction obtained by hydrothermal liquefaction contained contaminants.

Hydrocarbons can be obtained with >90 wt.% purity by heating a *B. braunii* slurry with low-concentration *n*-hexane (0.1–0.2%) at temperatures below 100 °C; this method does not require drying [11,12]. We attempted to improve the energy yield by heating a concentrated *B. braunii* slurry (80 g/L), then adding *n*-hexane and centrifuging the mixture. However, the *B. braunii* slurry and hexane formed an emulsion from which the hydrocarbon could not be recovered. Therefore, we added a solid–liquid separation step after heating. By removing the water phase before extraction with organic solvents, we obtained hydrocarbon yields of 90 wt.% from concentrated slurries of *B. braunii* [13,14]. In that study, the energy profit ratios of the wet process by heating at temperatures below 100 °C were approximately twice that of the dry process. Therefore, hydrocarbon can be efficiently recovered from *B. braunii* without drying [13].

Abbreviations: ECM, extracellular matrix; GC, gas chromatography; FID, flame ionization detector; Mn, number-average molecular weight; Mw, weight-average molecular weight; GPC, gel permeation chromatography.

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We conclude that the water-soluble polymers eluted into the water phase of the algal slurry after heat treatment interfere with hydrocarbon recovery. Fig. 1 shows the proposed bioprocess for the recovery of hydrocarbons from highly concentrated slurries of *B. braunii*, which involves a thermal pretreatment. In order to recover the hydrocarbons efficiently, it is necessary to reduce the amount of water-soluble polymers remaining in the solid phase (algal phase) after heating the concentrated algal slurry. However, we do not know the extent to which the water-soluble polymers should be removed from the algal slurries to achieve effective hydrocarbon recovery and the properties and composition of the heat-extracted water-soluble polymers are unclear, although a previous report addressed water-soluble polymers in *B. braunii* culture [15]. We examined the thermophysical properties of the water-soluble polymers at 60, 70, 80, and 90 °C. The water-soluble polymers of the thermally pretreated slurry eluted into the water phase at temperatures above the protein denaturation temperature (64 °C) of algae [16].

In this study, we investigated the interactions between the extraction solvent and water-soluble polymers eluted from heated algae. We also determined which concentrations of water-soluble polymers in the algal slurry interfere with hydrocarbon recovery and characterized the composition and molecular weight of the water-soluble polymer.

2. Material and methods

2.1. Algal strain, culture, and harvest

The Showa strain, a B race of *B. braunii* that produces triterpene hydrocarbons, was grown in a modified Chu 13 medium at 25 °C with a 12-h/12-h light/dark cycle in a 1200-mL Roux culture bottle aerated with air containing 1.0 vol.% CO_2 [17]. The cells were illuminated at a light intensity of approximately 100–150 μmol/m^2 · s. The cells were harvested on day 30 after inoculation by suction–filtration through a nylon plankton net with a mesh size of 20 μm and immediately used for experiments.

2.2. Determination of hydrocarbons and water-soluble polymers from B. braunii

Hydrocarbon content was determined as follows. A wet slurry of *B. braunii* was freeze-dried, then soaked in *n*-hexane. The yellow hexane phase was separated from the extraction residue and collected in a separate flask. This procedure was repeated until the hexane phase became colorless. The hexane extracts were combined and the solvent was removed in a rotary evaporator. The residual oil was separated by silica gel column chromatography (Wako gel C-300; Wako Pure Chemical Industries Ltd.) and eluted with *n*-hexane to remove non-hydrocarbon compounds such as carotenoids. All eluates before the elution of a yellow band corresponding to carotenes were collected as the triterpene hydrocarbon fraction, and the solvent was completely removed by evaporation. The algal hydrocarbon content was determined by weighing the residual oil.

The water-soluble polymers were determined as follows. Algal slurry prepared at a concentration of 50 g/L (dry weight basis, moisture content 95%) was placed in a glass bottle. A 10-mL microalgal slurry was placed in 50-mL plastic centrifuge tubes. The samples were heated at 90 °C for 10 min in a water bath (HWA-50; AS ONE), then centrifuged (TOMY LC-200; 3650 ×*g*, 10 min) to separate the liquid phase. The material in the liquid phase was considered water-soluble polymers extracted from the ECM of *B. braunii*. The liquid phase was freeze-dried and measured gravimetrically.

2.3. Viscosity of water-soluble polymers in a mixture of water and organic solvent

To study the interaction of the organic solvent and water-soluble polymers, the viscosity of a mixture of *n*-hexane and the water-soluble polymer was measured. A 0.5% (wt.%) solution of water-soluble polymers was prepared by dissolving the freeze-dried samples in water. The viscosity of a 1:1 mixture of *n*-hexane and the water-soluble polymer solution was measured with a viscometer (LVDV2T; Brookfield, USA) equipped with a spindle (SC4-18; Brookfield, USA) and sample adapter. Emulsions were formed by combining 4 mL each of *n*-hexane and the water-soluble polymer solution in a glass bottle and mixing vigorously for 1 min. Viscosity measurements were initiated after 2 min of stirring and then every 10 s at 1 rpm and 25 °C.

2.4. Effect of water-soluble polymers on hydrocarbon recovery

To investigate the effect of the water-soluble polymers on hydrocarbon recovery, we prepared algal slurries with varying water-soluble polymer contents by adding known amounts of the polymers to algal slurries that had been washed with water to remove the original polymers. Hydrocarbon recovery from these slurries was determined by preparing polymer-free algal slurries as follows. The microalgal slurry [10 mL, 50 g/L (dry weight basis), moisture content 95 wt.%] was heated at 90 °C for 10 min in a water bath. The thermally pretreated slurries were centrifuged (TOMY LC-200; 3650 ×*g*, 10 min) to separate the solid phase (algae) from the water phase, which was removed by pipetting. The solid phase was washed by adding 10 mL distilled water and stirring with a vortex mixer for 10 s. The water phase and water-soluble polymers were discarded by pipetting. After repeating this washing process twice, the water content of the solid phase (the algal precipitate) was determined using a moisture analyzer (MX-50; AS ONE) and the slurry concentration was adjusted to 15 wt.% (water content: 85 wt.%) [13]. Dried water-soluble polymers were prepared by freeze-drying the aqueous fractions obtained from the wash steps. The dried water-soluble polymers were added to the polymer-free 15 wt.% algal slurry at concentrations of 0, 0.05, 0.1, 0.15, 0.2, 0.3, 0.5, and 1.0 wt.%.

Hydrocarbon recovery was determined as follows. *n*-Decane ($C_{10}H_{22}$, boiling point: 174.2 °C) was chosen as the extraction solvent because it is apolar (similar to *n*-hexane) but nonvolatile (in contrast to *n*-hexane) in gas chromatography (GC). Hydrocarbon recovery was achieved by mixing the algal slurry containing the water-soluble polymers with a 5-fold volume of *n*-decane; the mixture was stirred for 1 h (MMW-1000 W; EYELA) and then centrifuged (LC-200, 3650 ×*g*, 10 min; TOMY), and the upper hydrocarbon-containing decane phase was collected. The hydrocarbon composition in the *n*-decane phase was analyzed by capillary GC (GC-2014; Shimadzu Corporation; Rtx-1; 30 m). The column temperature was programmed as follows: 50 °C for 1 min, from 50 °C to 220 °C at 10 °C/min, 220 °C for 3 min, from

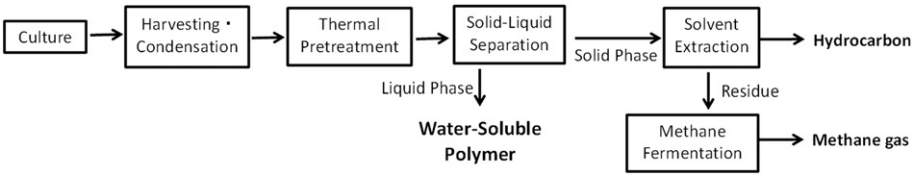

Fig. 1. Proposed bioprocess for the production of hydrocarbons from a high-concentration slurry of *Botryococcus braunii* using thermal pretreatment.