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Botryococcus braunii cells: Ultrasound-intensified outdoor cultivation integrated with *in situ* magnetic separation

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HIGHLIGHTS

- Outdoor cultivation of algal cells with *in situ* magnetic harvesting was developed.
- Ultrasonic treatments improved algal cell biomass and hydrocarbon productivity.
- Algal cells were harvested efficiently using a magnetic separator.

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ABSTRACT

An integrated system combining ultrasound-intensified outdoor cultivation of *Botryococcus braunii* with *in situ* magnetic harvesting of the algal cells was developed. The algal cells were cultivated in 200 L plastic bag reactors, and seven five-minute ultrasonic treatments at a four-day interval using a fixed frequency of 40 kHz and a total power of 300 W improved algal cell biomass and hydrocarbon productivity. The algal cells were harvested using functional magnetic particles and a magnetic separator, and a recovery efficiency of 90% was obtained under continuous operation at a flow rate of 100 mL/min using the *in situ* magnetic separation system. The overall production cost using the integrated system was US$ 25.14 per kilogram of *B. braunii* dry biomass. The system developed in this study provides a base for the industrial production of *B. braunii*.

1. Introduction

Renewable biofuels have received increasing attention as serious issues arise from the continuous use of fossil fuels. Compared with other potential biofuel sources, microalgae are considered to be a particularly promising feedstock and appear to be the only source that has the potential to replace fossil fuels and meet the global fuels demand (Chisti, 2007). In addition, biofuels from microalgae are more ecologically sustainable and have a high land use efficiency compared with traditional land oil plants (Chisti, 2007). The green alga, *Botryococcus braunii*, is a biofuel source that can grow under various climatic conditions and can produce an unusually high level of liquid hydrocarbons (Metzger and Largeau, 2005). In addition, the long-chain hydrocarbons it produces can be easily converted to transport fuel after catalytic cracking (Yamamoto et al., 2014; Hillen et al., 1982). These characteristics have encouraged numerous studies on the growth and hydrocarbon production of *B. braunii*. The effects of growth conditions, including the levels of nitrate, phosphate, CO_2, light, and salinity on its growth and hydrocarbon accumulation have been studied (Ruangsomboon, 2012; Ranga Rao et al., 2007a,b; Sakamoto et al., 2012; Baba et al., 2012), and culture media and photobioreactor types have also been optimized (Dayananda et al., 2005; Xu et al., 2012a). Although these studies have resulted in improved methods for *B. braunii* growth and hydrocarbon production, it is also necessary to optimize methods for the large-scale outdoor cultivation of *B. braunii* in order to satisfy the demands of industrial applications. The cultivation of *B. braunii* has been scaled-up in outdoor open raceways and circular ponds in the range of 80 L to 2000 L, achieving a maximum biomass of 1.8–2.0 g/L (Dayananda et al., 2010; Ranga Rao et al., 2012; Ashokkumar and Rengasamy, 2012). In addition, a 0.4 m^3 panel was tested for the outdoor cultivation of *B. braunii*; however, the biomass and lipid productivity only

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reached 0.02 g/L/day and 2.5 mg/L/day, respectively (Bazaes et al., 2012). An efficient and economic culture system is still needed for the large-scale outdoor cultivation of *B. braunii*.

For the industrialization of microalgal biofuels, harvesting and dewatering are difficult steps in the process due to the small size of algal cells and the dilute nature of microalgal cultures (Uduman et al., 2010); the operating costs of harvesting are approximately 20–30% of the total production cost (Williams and Laurens, 2010). An electroflocculation method integrated with dispersed-air flotation was developed and tested for harvesting *B. braunii*; however, although a high recovery efficiency of 98.9% was obtained within 14 min, the harvesting process was energy-inefficient (Xu et al., 2010). Flocculation is commonly used in algal harvesting, and different kinds of flocculants, such as metal salts, organic polymers, and biopolymers have been used for the highly efficient harvesting of *B. braunii* (Lee et al., 1998; Zheng et al., 2012; Kim et al., 2013). In addition, several emerging magnetic flocculants, such as naked Fe_3O_4 nanoparticles and cationic polyacrylamide (CPAM) surface functionalized magnetic particles, have been developed and applied for the flocculation of *B. braunii* (Xu et al., 2011; Wang et al., 2014). The principle of this separation process is based on tagging the non-magnetic algal cells with magnetic particle; the particle-attached algal cells can be rapidly isolated from the medium using an external magnetic field (Borlido et al., 2013). These magnetic flocculants show promise because they can effectively recover the algal cells in a relatively short time. However, for large-scale biodiesel production, the magnetic materials would need to be scaled-up and an efficient magnetic separation system would be necessary. Magnetic separation has been widely applied in the steel industry, wastewater treatment, kaolin clay purification, and the paper-coating industry (Ohara et al., 2001). The specific characteristics of the algal medium do not allow these previously developed magnetic separators to be utilized for the harvesting of microalgal cells. The development of a specialized magnetic separator is necessary for the large-scale harvesting of microalgae by magnetic particles.

In the current study, an integrated system for the outdoor cultivation and magnetic harvesting of *B. braunii* was developed. The cultivation of *B. braunii* was studied using plastic bag reactors under outdoor conditions. The biomass concentration, hydrocarbon content, and composition in the full growth stage were measured. In addition, based on a previous study, a new ultrasonic stimulation system was constructed and utilized during the cultivation process (Xu et al., 2014). A magnetic separator was developed, and at the end of the cultivation process it was applied for the *in situ* harvesting of the microalgal cells using CPAM surface modified Fe_3O_4 particles (Wang et al., 2014).

2. Methods

2.1. Microalgae strain and culture systems

B. braunii IPE 001, stored in the Institute of Process Engineering, Chinese Academy of Sciences, was grown in modified Chu 13 agar slants and liquid medium. Algal cells were maintained in 250 mL Erlenmeyer flasks containing 100 mL liquid medium in an orbital shaker at 100 rpm for three weeks, and then transferred to a 2 L airlift bioreactor and cultivated for two weeks (Xu et al., 2012b). All cultures were kept in sterile conditions and grown photoautotrophically at 25 °C under a light/dark cycle of 16/8 h per day with 35 μmol m^{-2} s^{-1} light intensity. The airlift bioreactor cultured cells were used as the inoculum for the outdoor cultivation experiments.

The bubble column reactor has a working volume of 200 L and was constructed of a PE membrane supported by a circular wire mesh (Supplementary material-Fig. 1C). The modified Chu 13 medium was prepared using tap water without any treatment. The indoor airlift bioreactor cultured cells were collected by filtration with a 500 mesh nylon sheet and then transferred to the outdoor culture at an inoculum concentration of 0.17 g/L. CO_2-enriched air (1%, v/v) was bubbled directly into the reactor at a flow rate of 0.1 v/v/min. The outdoor cultivation tests were conducted under the natural light and temperature conditions in the Haidian district of Beijing, China (latitude 39°59′ N, longitude 116°19′ E), in autumn (September 3, 2013 to September 30, 2013).

2.2. Conduction of the ultrasonic treatment

An ultrasonic stimulation system integrated with the culture system was constructed. Three ultrasonic vibrators were enclosed in a sealed stainless steel box (80 cm L × 10 cm W × 10 cm H) with a maximum total power of 300 W (Supplementary material-Fig. 1A and B). The ultrasonic frequency and the power of each vibrator were 40 kHz and 100 W, respectively. A periodic ultrasonic stimulation strategy was utilized, the treatment was initiated the first day after inoculation, and the microalgae were periodically exposed to five minutes ultrasonic treatments at four-day intervals throughout the culture period. The ultrasonic box was placed in the center of the photobioreactor for each treatment and removed following the treatment (Supplementary material-Fig. 1C).

2.3. Analytical methods

2.3.1. Microalgae growth measurement

The samples were collected at 9:00 am every three days. 500 mL of the sample was filtrated through a 500 mesh nylon sheet and then lyophilized using a lyophilizer (ALPHA1–2, CHRIST, Germany). The dry cell weight (DCW, g/L) of the microalgal cells was determined gravimetrically.

The maximum specific growth rate (μ_{max}, day^{-1}) at the exponential stage was calculated using equation (1):

$$\mu_{max}(day^{-1}) = (\ln DCW_2 - \ln DCW_1)/(t_2 - t_1) \quad (1)$$

where DCW_1 and DCW_2 are the dry cell weights (g/L) at time t_1 and t_2,respectively.

The generation time (T, days) was calculated according to the following equation:

$$T(\text{days}) = \ln(2)/\mu_{max} \quad (2)$$

The rate of biomass production (P, mg L^{-1} day^{-1}) was calculated as follows:

$$P(\text{mg}\,L^{-1}\,day^{-1}) = (DCW_x - DCW_0)/t_x \quad (3)$$

where DCW_0 and DCW_x are the initial dry cell weight (mg/L) and the dry cell weight (mg/L) at time t_x, respectively.

2.3.2. Hydrocarbon extraction and analysis

Hydrocarbons were quantified according to the method described by Yonezawa et al. (Yonezawa et al., 2012). 1 mL of H_2O, and 0.2 mg of *n*-triacontane (Sigma–Aldrich, America) as an internal standard were added to 10 mg lyophilized algal cells in a test tube. The mixture was vigorously shaken and 1 mL of a KOH ethanol solution (2 M) was added to the mixture and incubated at 35 °C for 2 h. Next, 1 mL of methanol and 5 mL of *n*-hexane were added and thoroughly mixed. The organic phase was dried using anhydrous Na_2SO_4 and filtered through a 0.22 μm membrane for GC analysis. The samples were analyzed using a gas chromatograph equipped with an FID detector (Agilent, 7890 A, America) and an Agilent HP-5 column (30 m × 0.25 mm). The temperature of the injection port and detector were 240 °C and 250 °C, respectively. The column temperature was set at 130 °C for 5 min, then