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Application of memberane dispersion for enhanced lipid milking from *Botryococcus braunii* FACHB 357

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ABSTRACT

To improve the mixing efficiency in an aqueous–tetradecane system and thus to increase the lipid milking efficiency, poly (ether sulfones) hollow fiber membrane was applied as dispersion medium to establish an *in situ* lipid extraction process from *Botryococcus braunii* FACHB 357. The lipid location of this microalga was characterized by fluorescence microscope and transmission electron microscopy, respectively. The results showed that *B. braunii* excreted lipids into the outer matrix, which allowed it possible to extract algal lipids *in situ* by organic solvent. Within an aqueous–organic biphasic system, the lipid extraction ratio of tetradecane increased from 38.05% to 50.15% by introducing a microporous membrane as the dispersion medium, mainly because smaller solvent droplets were produced. Under this experimental condition (the volume ratio of tetradecane: 10%, the flow rate: 10 ml min^{-1}), solvent toxicity and shearing stress had not shown significant impact on algal cells viability in 96 h. Within the same time period, the lipid amount extracted by solvent was enhanced with the increase of the solvent flow rate and the initial biomass concentration. These results suggested membrane dispersion was a good choice to improve mixing effect in the algal lipid milking process or other similar cell products extracted processes.

1. Introduction

Algal biodiesel had been considered as one of the most promising renewable transportation fuels due to concerns on recent oil crisis and possible climate change from the greenhouse gases (Chisti, 2007). During the last decade, although significant advances in microalgal biotechnology had been achieved, challenges still remained in the low cost production of microalgal biodiesel. Among the costly downstream processing steps, it was agreed that harvesting/dewatering and the following extraction of fuel precursors from the biomass consists the most energy intensive steps (Radakovits et al., 2010). As a result, to integrate the steps of harvesting and extraction, and thus to allow *in situ* lipid milking, seemed to be a potential solution for cutting the cost of algal oil production.

As shown in literature, *Botryococcus braunii* was one of the most promising algal species for synchronous culture and lipid extraction due to its high lipid content of 25–75% (dry weight biomass) (Mata et al., 2010). The ultrastructural studies about *B. braunii* revealed there was a successive matrix surrounding the basal part of all three races cells. The bulk of *B. braunii* lipids (>50%) were stored in those outer walls, and could therefore be repeatedly extracted by organic solvents from the wet biomass without the usual harvesting and dewatering steps (Frenz et al., 1989). Fox example, the *B. braunii* UTEX 572 could be induced to produce more long-chain unsaturated hydrocarbons within an aqueous–dihexyl ether system, and 32% of those hydrocarbons were extracted into the organic solvent phase after 3 days. Through cycling the solvent into the aqueous phase, the hydrocarbons extraction ratio was enhanced to 60%. Nevertheless, due to the toxic effect of dihexyl ether, the growth rate of *B. braunii* UTEX 572 dropped greatly after adding this solvent into the culture medium (An et al., 2004; Sim et al., 2001). In our previous study, we had screened the biocompatible solvents for *B. braunii* FACHB 357 among 11 kinds of hydrophilic or hydrophobic organic solvents, and found tetradecane could be used to extract *in situ* lipid from this alga and to keep the algal growth rate as the control during a period of 96 h (Zhang et al., 2011a,b). However, the lipid recovery ratio was less than 20% by mixing aqueous and organic phases with simple shaking in our previous work (Zhang et al., 2011a,b). To increase the algal lipid extractability, the intensification of mass transfer in this immiscible liquid–liquid system was necessary.

Traditionally, cycling between organic and aqueous phase (An et al., 2004), or using stirred tanks (Martín et al., 2008) and static mixers (Fang and Lee, 2001) had been applied to improve the mixing efficiency within biphasic system. However, those methods had a number of problems such as wide solvent droplet size distribution and high mechanical stress due to fluctuating forces in the flow field (Zhang et al., 2010). Recently, a novel membrane

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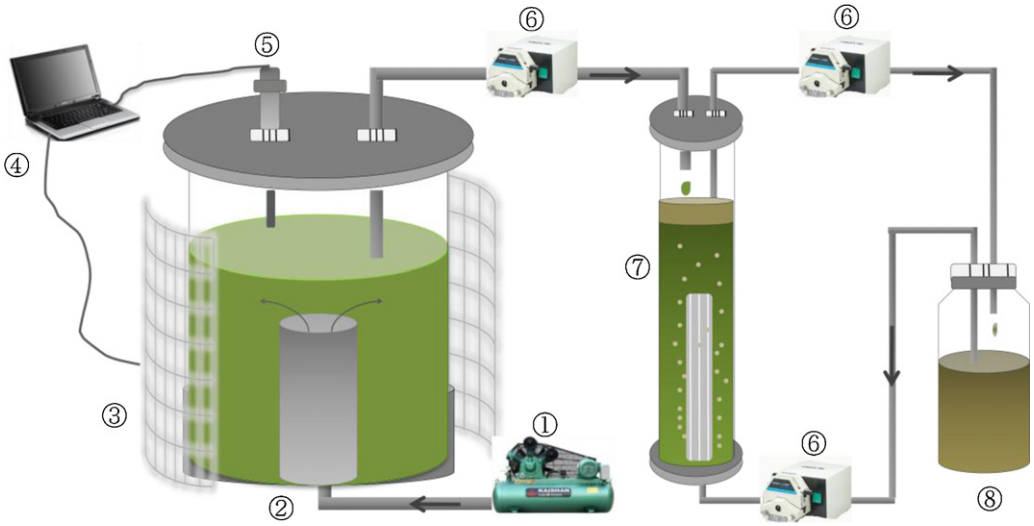

Fig. 1. Schematic diagram of a membrane-dispersed lipid extraction process from *B. braunii*. (1) Air compressor pump; (2) Airlift photo-bioreactor; (3) LED light panel; (4) Computer controller; (5) Electrodes (pH, DO, temperature); (6) Peristaltic pump; (7) PES membrane-dispersed lipid extraction module; (8) Organic solvent tank.

dispersion technique had been developed to produce uniform micrometer sized liquid droplets by dispersing one phase into another through a microporous membrane. For example, a ceramic microfiltration membrane was used to establish a dispersion extractor and its performance was evaluated in a 30% TBP–nitric acid–H_2O system (Chen et al., 2004). So far, membrane dispersion had not been applied in the microalgal lipid extraction system. The most important advantages of using membrane as the dispersion medium were the possibility to produce droplets of a defined size with a narrow size distribution, consequently to increase the contact area between two phases and to shorten the extract time (Chen et al., 2004).

In this study, poly (ether sulfones) (PES) hollow fiber microfiltration membrane was applied due to its good physic-mechanical feature and chemical resistance as dispersion medium to establish lipid extraction *in situ* process by tetradecane from *B. braunii* FACHB 357. The lipid location of this alga was first characterized by fluorescence microscope and transmission electron microscopy (TEM), respectively. The effects of membrane-dispersed process were then evaluated on both the lipid extractability and the cell viability. The solvent flow rate and the initial biomass concentration on the lipid extraction efficiency were further investigated.

2. Materials and methods

2.1. Microalga and culture conditions

The green microalga *B. braunii* FACHB 357 was purchased from Fresh-water Algae Culture Collection of the Institute of Hydrobiology, Chinese Academy of Sciences, China. The strain was cultured in a 7 L photo-bioreactor (BIOTECH, China) with BG11 medium under continuous illumination of 90 μmol photons m^{-2} s^{-1}, and aerated with 1% CO_2 at 25 °C. The biomass concentration was evaluated by the dry weight (*DW*, g L^{-1}), and its relationship with the optical density (*OD*) at 680 nm was shown as

$$DW = 0.26 \times OD_{680}$$

To measure the dry weight of biomass, 5 ml of microalgal culture was taken and centrifuged (1800 × *g*) for 20 min. The supernatant was removed and cells were then washed with distilled water and dried at 80 °C. The dried cells were weighed after 24 h. The optimal density was measured spectrophotometrically at 680 nm in a quartz cuvette with 1 cm light path (LengGuang GS-54, China). To investigate the effect of biomass concentration on the lipid extraction, the algal broth was concentrated by centrifuging and then resuspended into BG11 medium to obtain the proper biomass concentration according its optical density (OD_{680}).

2.2. Algal lipid location by fluorescence microscope and TEM

To locate the lipid of the *B. braunii* FACHB 357, algal cells stained with Nile Red were observed by fluorescence microscope. 1 ml of about 10^6 cells suspension was added with l μl of Nile Red (Sigma, USA) in an acetone working solution to obtain a concentration of 1 mg ml^{-1} for lipid staining. The mixture was gently inverted for mixing and incubated at 37 °C in darkness for 10 min. After the stain reaction, the cells were collected by centrifugation and rinsed with distilled water in triplicates to remove the excess dyes. Afterwards, the cells were resuspended in distilled water and observed by fluorescence microscope (OLYMPUS AX70, Japan) with blue light as the excitation light.

To obtain the TEM image of the *B. braunii* FACHB 357, the algal cells were treated as described by van Lent et al. (1990) and Hejazi et al. (2004). Sections were observed and photographed with a JEM-1230 transmission electron microscope (JEOL, Japan).

2.3. Membrane-dispersed lipid extraction process

As shown in Fig. 1, a certain volume of alga broth cultured in a photobioreactor was pumped into the shell side of the hollow fiber PES membrane module. The organic solvent tetradecane (Sigma, USA) was pumped into the dead-ended membrane tube and dispersed into the algal broth through the micropores of PES membrane. The solvent droplets separated from the aqueous phase and aggregated on the upper layer. The organic phase was then circulated from the membrane module to the solvent tank. The parameter of PES membrane module and the membrane structure were shown in Table 1 and Fig. 2, respectively. The drop size of the

Table 1
The parameters of PES membrane module.

Number	Length (cm)	Area (m^2)	Outer diameter (mm)	Thickness (mm)
150	20	0.0706	1.3	0.2