Journal of Biotechnology 154 (2011) 281–284

Contents lists available at ScienceDirect

Journal of Biotechnology

journal homepage: www.elsevier.com/locate/jbiotec

Short communication

Mechanism of lipid extraction from *Botryococcus braunii* FACHB 357 in a biphasic bioreactor

Fang Zhang[a], Li-Hua Cheng[b,*], Wang-Lei Gao[c], Xin-Hua Xu[b], Lin Zhang[a], Huan-Lin Chen[a]

[a] Department of Chemical and Biochemical Engineering, Zhejiang University, Hangzhou 310027, PR China
[b] Department of Environmental Engineering, Zhejiang University, Hangzhou 310058, PR China
[c] School of Biosystems Engineering and Food Science, Zhejiang University, Hangzhou 310058, PR China

ARTICLE INFO

Article history:
Received 16 January 2011
Received in revised form 11 April 2011
Accepted 18 May 2011
Available online 26 May 2011

Keywords:
Botryococcus braunii
Lipid extraction
Biocompatible solvent
Biphasic system

ABSTRACT

Algal lipid of *Botryococcus braunii* could be produced continuously and *in situ* extracted in an aqueous-organic bioreactor. In this study, the cell ultra-structure and cell membrane permeability of *B. braunii* FACHB 357 were investigated to understand the mechanism of lipid extraction within the biphasic system. The results showed that biocompatible solvent of tetradecane could induce algal lipid accumulation, enable the cell membrane more active and the cell wall much looser. The exocytosis process was observed to be one of the mechanisms for lipid cross-membrane extraction in the presence of organic solvent.

Algal biodiesel has 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; Greenwell et al., 2010; Hu et al., 2008). During the last decade, although significant advances in microalgal biotechnology have been achieved, challenges still remain in the low cost production of microalgal biodiesel. Among the costly downstream processing steps, it is 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, thus to allow *in situ* lipid milking, seems to be a potential solution for cutting the cost of algal-oil production. In this integrated process, algal cells can be cultured in the biphasic bioreactors for lipids production continuously while the lipids are extracted simultaneously from the aqueous phase into the organic phase. This kind of *in situ* extraction process, also named "milking", has been found successful in the pigment production from algae (Hejazi and Wijffels, 2003, 2004; Mojaat et al., 2007).

As shown in the literature, *Botryococcus braunii* is 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 lipid (including hydrocarbons) of this alga could be repeatedly extracted from the wet biomass without the usual harvesting and dewatering steps. By exposing *B. braunii* to organic solvent of hexane, a substantial fraction of hydrocarbons was obtained (Frenz et al., 1989). When an aqueous-dihexyl ether system was adopted, the *B. braunii* UTEX 572 could be induced to produce more long-chain unsaturated hydrocarbons, and part of those hydrocarbons were extracted into the organic solvent phase after an incubation of 3 days. By recycling of this two-stage system and hence the improvement of mixing, the lipid extractability could be increased from 32% to 60% (An et al., 2004; Sim et al., 2001). Since the organic solvents of both hexane and dehexyl ether were toxic to the algal growth, we had screened the biocompatible solvents for *in situ* extraction of lipid from *B. braunii* FACHB 357 (to be published elsewhere). It has been proven that *B. braunii* FACHB 357 can survive of the 10% (*v/v*) tetradecane even after 45 days. However, to our best knowledge, the understanding of the extraction process at cell level has not been available in open literature. In this work, we will focus on the effect of tetradecane on cell structure of *B. braunii* FACHB 357, including the variation of cell membrane, cell wall and lipid accumulation, in order to know more about the mechanism of lipid extraction in the biphasic reactor.

In current study, *B. braunii* FACHB 357 (obtained from Freshwater Algae Culture Collection of the Institute of Hydrobiology, Chinese Academy of Sciences, China) had been cultured in BG 11 medium for 3 weeks. 250 ml Erlenmeyer flask containing 100 ml algae culture was used as bioreactor to perform biphasic experiments. The biocompatible organic phase of tetradecane (Sigma, USA) was added into culture system at the beginning of algal stationary stage. The volume ratio of tetradecane and algal culture was

* Corresponding author. Tel.: +86 571 87953802; fax: +86 571 87953802.
E-mail address: chenglihua@zju.edu.cn (L.-H. Cheng).

0168-1656/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jbiotec.2011.05.008

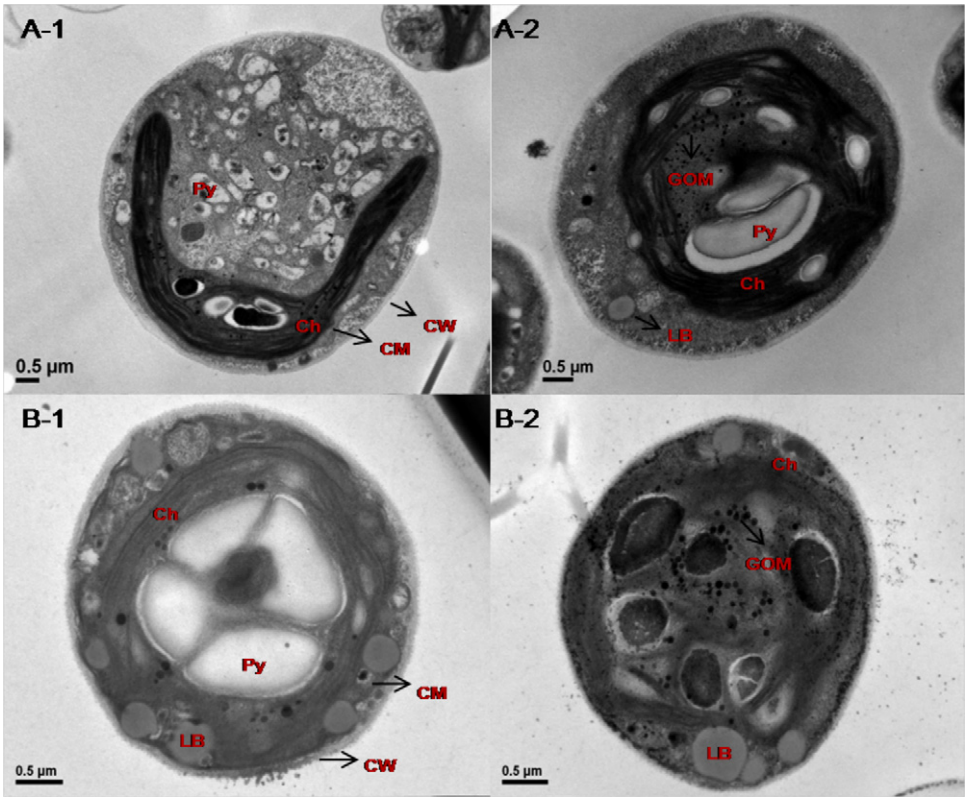

Fig. 1. The location and accumulation of lipid body in *Botryococcus braunii* FACHB 357 cells in the absence (A) and presence of 10% (*v*/*v*) tetradecane (B) cultured under continuous illumination at 60 μmol photons m^{-2} s^{-1} and 25 °C for 8 days. A-1, 2 (and B-1, 2) are different cells of the same sample (Py, pyrenoid; Ch, Chloroplast; LB, lipid body; GOM, granular osmiophilic material; CM, cell membrane; CW, cell wall).

10:1. The flask was put in a shaker with 100 rpm under fluorescent light at 25 °C. In this batch culture and the following extraction process, the lipid recovery ratio obtained was about 20% for 96 h of culture time. To locate the lipid in the cell and its accumulation, the cell ultra-structure was observed and photographed with a JEM-1230 transmission electron microscope (JEOL, Japan) after a biphasic culture of 8 days.

As shown in Fig. 1, several large dark-staining lipid bodies (LB) were observed at the inner side of the cell membrane of *B. braunii* FACHB 357 cells while granular osmiophilic material (GOM, Fig. 1A-2 and Fig. 1B-2), possible as precursor for lipid biosynthesis, were found near the thylakoid membranes. This indicated that algal lipid was synthesized in chloroplasts and then exported into cytoplasm for energy storage and eventual metabolism (Radakovits et al., 2010). Those cytoplasmic lipid bodies were observed without evident membrane-like structure, although it was reported that some eukaryotic cells produced lipid body consisting of a hydrophobic core of neutral lipids (typically containing TAGs and sterol esters or wax esters) and an envelope of phospholipid–protein membrane (Murphy, 2001).

Similar to the effect of environmental changes such as nitrogen limitation, high salinity or high temperature on the lipid accumulation, we found that organic solvent as a stress condition enhanced lipid synthesis for *B. braunii* FACHB 357. Both the number and size of lipid bodies increased in the presence of tetradecane compared to the control (Fig. 1). Only 1–2 small lipid bodies (diameter <0.3 μm) were observed in the control cells (Fig. 1A), while the number of lipid bodies increased to 4–6 and the diameter of LB was around 0.5 μm in the algal cells within the biphasic bioreactor (Fig. 1B).

The effect of organic solvent on algal cell membrane permeability was further characterized by Evans Blue method (Hamer et al., 2002), as shown in Fig. 2. For unicellular microalga *B. braunii* FACHB 357, the cell membrane permeability increased with culture time under both experimental conditions due to the natural aging (Fig. 2). By contrast, this alga which cultured in the presence of tetradecane was apt to absorb more macromolecule Evans Blue into cells. Since the substance cross-membrane transport depended mainly on mechanisms including endocytosis/exocytosis and diffusion (Battey et al., 1999), we deduce that algal cell membrane was stimulated to be more active for exocytosis or more permeable for substance diffusion within the aqueous-organic bioreactor.

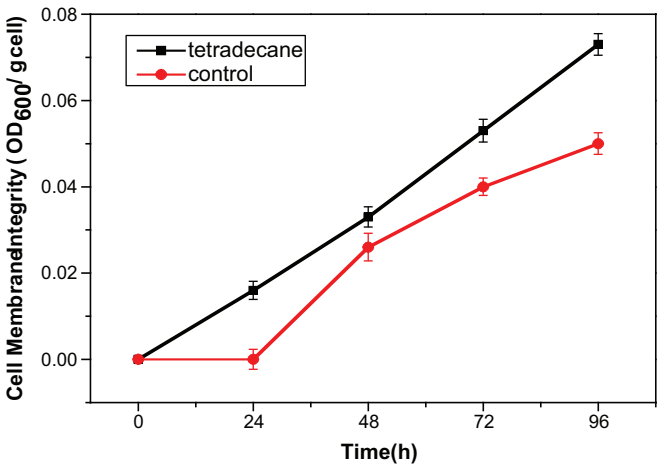

Fig. 2. The effect of 10% (*v*/*v*) tetradecane on *Botryococcus braunii* FACHB 357 cell membrane integrity in an aqueous-organic system under continuous illumination at 60 μmol photons m^{-2} s^{-1} and 25 °C for 96 h.