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Fed-batch fermentation and supercritical fluid extraction of heterotrophic microalgal *Chlorella protothecoides* lipids

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ABSTRACT

Lipids obtained from *Chlorella protothecoides* in heterotrophic cultivation are considered a suitable feedstock for biodiesel production. In this study, glucose fed-batch fermentation was performed to increase final biomass and lipid production. The biomass productivity and lipid productivity were 6.28 and 2.06 g/L day, respectively. Biomass/glucose conversion and the lipid/glucose conversion were 43.3% and 14.2%, respectively. Extraction of lipids from algae has been identified as a key bottleneck in bioprocessing operations. Supercritical carbon dioxide (SC-CO_2) was applied for neutral lipids extraction and the SC-CO_2 kinetics was investigated by the Goto et al. model. The modeling showed a good fit with experimental data. Additionally, neutral lipids extracted by SC-CO_2 displayed a suitable fatty acid profile for biodiesel [mainly C18:1 (60.0%), C18:2 (18.7%) and C16:0 (11.5%)]. Our study demonstrated the ability to produce high levels of neutral lipids through heterotrophic algal culture and subsequent extraction of lipids with SC-CO_2 method developed.

1. Introduction

Biodiesel, a mixture of fatty acid methyl/ethyl esters (FAMEs/FAEEs), made by transesterification of triacylglycerols, is recognized as a promising strategy for producing sustainable biofuel (Chisti, 2007). Compared to the petroleum-based diesel, biodiesel exhibits favorable environmental properties, such as biodegradability, lower sulfur content and less carbon monoxide emission (Huang et al., 2010). Microalgal biodiesel is regarded as a second-generation of biofuel because it will not compromise food resources and other products derived from energy crops such as soybean (Cheng et al., 2009). In addition, lipids obtained from *Chlorella protothecoides* in heterotrophic cultivation have been recommended as a suitable feedstock for sustainable biodiesel production because of the algae's shorter growth cycle, less need of land, proper fatty acid composition for biodiesel production (Chisti, 2007; Li et al., 2007; Xiong et al., 2008), and ability to utilize different carbon sources, such as glucose (Xu et al., 2006), fructose (Gao et al., 2009), sucrose (Gao et al., 2009) and crude glycerol (Chen and Walker, 2011).

Microalgal lipids are generally obtained by mechanical pressing or solvent extraction (Fishman et al., 2010; Mercer and Armenta, 2011); however, the application of mechanical pressing is limited because the slow recovery rate for microalgae (Mercer and Armenta, 2011). In addition, solvent extraction can result in poor product quality and requires intensive use of volatile and toxic organic solvents (Mercer and Armenta, 2011). Supercritical fluid extraction (SFE) is considered an important alternative technique to traditional separation methods because the solvent's density and diffusivity can be controlled by changes in pressure and temperature for better solvent power (Machmudah et al., 2007). Supercritical carbon dioxide (SC-CO_2) is the most commonly used fluid for SFE, as it is non-flammable, non-toxic, inexpensive and easily separated from the product (Crampon et al., 2011). In addition, SC-CO_2 is a suitable solvent for extracting neutral lipids (triglycerides), which are an adequate feedstock for biodiesel applications. Moreover, SC-CO_2 does not solubilize polar lipid, such as phospholipids, which eliminates the need for degumming. The use of SC-CO_2 has already been investigated for extraction of β-carotene from *Dunaliella salina*, *Skeletonema costatum*, *Spirulina pacifica* and *Synechococcus* sp. (Crampon et al., 2011), astaxanthin from *Haematococcus pluvialis* (Krichnavaruk et al., 2008), lutein from *Chlorella pyrenoidosa* (Wu et al., 2007), γ-linolenic acid from *Spirulina platensis* (Sajilata et al., 2008) and lipids from *Chaetomorpha linum*, *Crypthecodinium cohnii*, *Chlorococcum* sp., *Chlorella vulgaris*, *Nannochloropsis* sp. and *Ochromonas danica* (Couto et al., 2010; Crampon et al., 2011; Halim et al., 2011). Mathematical models have been proposed to correlate the experimental overall extraction curve (OEC) during the SFE process (Sousa et al., 2005; Carvalho et al., 2005). The mass transfer behavior related to the rate of solute transferred from solid particles to the supercritical phase is useful for project and process design. The model by Goto et al. (1993) was

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