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Improved aqueous extraction of microalgal lipid by combined enzymatic and thermal lysis from wet biomass of *Nannochloropsis oceanica*

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HIGHLIGHTS

- Method developed for extracting lipid from wet microalgae with 96.0% moisture content.
- Upto 88.3% of total lipids are extracted from wet algae biomass at optimal conditions.
- Microalgae cells were completely disrupted by combined thermal and enzymatic lysis.
- Excessive extraction of polar lipid was found for wet biomass.

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ABSTRACT

High moisture content in wet algal biomass hinders effective performance of current lipid extraction methods. An improved aqueous extraction method combing thermal and enzymatic lysis was proposed and performed in algal slurry of *Nannochloropsis oceanica* (96.0% moisture) in this study. In general, cell-wall of *N. oceanica* was disrupted via thermal lysis and enzymatic lysis and lipid extraction was performed using aqueous surfactant solution. At the optimal conditions, high extraction efficiencies for both lipid (88.3%) and protein (62.4%) were obtained, which were significantly higher than those of traditional hexane extraction and other methods for wet algal biomass. Furthermore, an excessive extraction of polar lipid was found for wet biomass compared with dry biomass. The advantage of this method is to efficiently extract lipids from high moisture content algal biomass and avoid using organic solvent, indicating immense potential for commercial microalgae-based biofuel production.
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1. Introduction

Biofuels have drawn an increasing attentions in recent years for its inherent advantages, including carbon dioxides sequestration and environmental harmlessly (Hu et al., 2008; Williams and Laurens, 2010). A number of raw materials have been used for biofuel production, of which the oleaginous microalgae are considered as the most promising alternative feedstock in virtue of their fast growth rate, high oil content, high oil productivity and adaptability to live in most of environmental conditions (Hu et al., 2008; Williams and Laurens, 2010).

Nannochloropsis sp. has been considered as one of the most promising reference oleaginous microalgae. Although recent works have studied the entire process of biofuel production of this alga, including omics and physiological aspects (Pal et al., 2011; Wang et al., 2014), mass cultivation (Moazami et al., 2012), as well as oil extraction and conversion (Koberg et al., 2011; Chen et al., 2012a,b), technological barriers for commercial microalgae-biodiesel production are still remained. Among these, lipid extraction from *Nannochloropsis* biomass, in particular wet biomass, is inefficient, energy intensive and costly (Richardson et al., 2014). It should be emphasized that traditional lipid extraction methods for dry biomass encountered very low extraction efficiency when it is applied to wet biomass (Halim et al., 2011; Koberg et al., 2011). Currently, many lipid extraction methods, such as supercritical methanol (Patil et al., 2011), subcritical ethanol/hexane (Chen et al., 2012b) and hydrodynamic cavitation (Lee and Han, 2015), have obtained high oil extraction efficiency for processing wet biomass containing moisture upto 90%. However, an energy intensive step to remove moisture was still required in subsequent desolvation process, since moisture was extracted together with lipid from

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wet biomass. On the other hand, large volumes of organic solvents are consumed in these methods, resulting in environmental risks.

As a method of using water as an extraction and separation solution, aqueous extraction method (AEM) posses an advantage of avoiding the use of organic solvent. Several researchers have demonstrated that aqueous extraction method had been successfully applied to extract oil from oil-bearing seeds/fruits in both laboratory and industrial scales (Zhang et al., 2007; Campbell and Glatz, 2009; Naksuk et al., 2009). However, applying aqueous extraction method in oleaginous microalgae to extract lipid has not been attempted. In addition, cells walls, owing to their robustness, probably represent the biggest barrier to target-compound extraction. Previous studies suggested that cell disruption was necessary before/during aqueous extraction of lipid. But many cell disruption methods for oilseeds such as milling and flaking (Campbell and Glatz, 2009) have been proven ineffective for microalgae cells due to their tiny size and specific property of cell walls (Scholz et al., 2014). Hence, selection of an appropriate cell disruption method is largely dependent on microalgal distinct biology and cell-wall characteristics, and this method will also be applicable for technology scale-up.

In the present work, the feasibility of aqueous extraction for extracting oil from wet microalgae biomass was identified. A primary screen of aqueous solution based on lipid extraction efficiency was investigated firstly, which was followed by optimizing cell disruption processes of thermal and enzymatic lysis, to improve the efficiency of aqueous extraction. In addition, the extraction efficiency and the quality of extracted oil between this proposed method and conventional methods were also compared.

2. Materials and methods

2.1. Materials

The microalgal strain used in this study was *Nannochloropsis oceanica* IMET1, cultivated in a 15 L panel photobioreactor containing 12 L of BG11 medium prepared with sea water, with ambient air and 1% CO_2 agitating. The cultivations were carried out indoor at temperature of 25 °C and illumination intensity of 400 $\mu mol\ m^{-2}\ s^{-1}$ provided by cool white fluorescent for 14 days. Then, all microalgal cells were harvested using a centrifuge for 10 min at 6000 rpm to obtain algal paste, and were kept in refrigerator at −80 °C until use. The obtained algal paste has a moisture content of *ca*. 65% and a total lipid content of 45.6% in dry weight.

2.2. Aqueous extraction method for algal lipid

Microalgal cells in form of paste were resuspended in various aqueous extraction solution including DI water and surfactant aqueous solutions at certain microalgal cell densities of 40–200 $g\ L^{-1}$, and then the algal slurry was kept stirring at 100 rpm until the set time at 50 °C. The blank experiment was carried out with the same procedure, but using silica sand as raw material instead of algal biomass. One anionic surfactant (Sodium dodecylsulphate, SDS), three non-ionic surfactants (Triton X-100, Tween 20 and Polyacrylamide, PAM), and mixtures of anionic and non-ionic surfactants were tested. For surfactants screening test, the final surfactant concentration of SDS was set at 2.5% (w:v) and that of each non-ionic surfactant was set at 0.1% (w:v). All the surfactants at analytical regent were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China).

After extraction, the processed slurry was centrifuged at 8000 rpm for 10 min and separated into three phase, including lipid phase, aqueous phase and solid phase from top to bottom. The lipid phase was carefully collected with assistance of a small volume of hexane for completely collection and transferred into a pre-weighted tube, in which the lipid was further purified to remove residual protein following Bligh and Dyer's method (Bligh and Dyer, 1959). The amount of extracted lipids was determined as the mass difference of tubes. The aqueous phase was also collected for lipids analysis. The lipid yield of each treatment was calculated as dividing mass of extracted lipid by mass of dried microalgae, and was subsequently confirmed based on the lipid content difference between raw algal biomass and residual.

The lipids extraction efficiency was calculated by Eq. (1):

$$\text{Lipids extraction efficiency},\% = \frac{\text{Extracted lipids}}{\text{Total lipids in algae}} \times 100\% \quad (1)$$

2.3. Process for microalgal cells disruption

Thermal lysis and enzymatic lysis for cell disruption of *N. oceanica* were carried out in this study, both singly and in combination. For thermal lysis, microalgal slurry was conducted in a laboratory steam generator (Shanghai Huazheng special boiler manufacture Co., Ltd., China) coupled with twin-channel atomizer. The algal slurry was pumped into atomizer from one channel at a flow rate of 50 $ml\ s^{-1}$, and meanwhile the steam at 0.4 MPa was supplied from the other channel into atomizer. Then, the mixture of algal slurry and steam were released from outlet and collected for further analyses. Alternatively, the thermal lysis of microalgae was conducted in a laboratory autoclave (Hirayama HVE-50) at 121 °C for 20 min. For enzymatic hydrolysis, a concentrated enzyme solution was added into aforementioned algal slurry at a certain concentration and then gently stirring at a moderate temperature (25–50 °C) and pH (4–10) for variable time. The tested enzymes include cellulose from *Aspergillus niger*, lipase (Solarbio Co. Ltd.) and protease (Novozyme Co. Ltd.).

After cell disruption treatment, the processed slurry was separated into a solid phase of algal residue and an aqueous phase by centrifuged at 8000 rpm for 10 min. Both phases were collected for lipids and protein analysis.

2.4. Characterization of cell disruption

The glucose, a main (almost the only) degradation product for cellulose of *Nannochloropsis* cell wall (Scholz et al., 2014; Mirsiaghi and Reardon, 2015), was assayed by a SBA-40 biosensor analyzer (Shandong Academy of Sciences, China) equipped with the glucose oxidase immobilized membrane, to evaluate lysis efficiency of cell walls.

The morphology of microalgal cells were monitored under microscope before and after cell disruption processes. Images were taken with an Olympus BX 51 microscope equipped with DP 72 digital camera.

2.5. Traditional hexane extraction

Hexane extraction was carried out using Soxhlet approach. A 2.0 g of lyophilized algae powder was loaded into Soxhlet extraction thimble, extracted with 100 ml hexane at boiling temperature until the algal color turned gray. Mixture of solvent and extracted lipids was taken out and solvent was evaporated at low pressure to recover lipids.

2.6. Lipids analysis

The triglyceride content in total extracted lipids was determined by the glycerol-3-phosphate-oxidase-p-chlorophenol

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