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Microalgal cell disruption via extrusion for the production of intracellular valuables

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ABSTRACT

The objective of this study was to evaluate the effectiveness of extrusion on cell disruption of a marine alga Nannochloropsis oceanica with a single-screw extruder for the production of intracellular valuables. The effect of operating parameters including feed moisture $(15-24%)$ and screw rotation speed (300 e450 rpm) was also determined. Experimental results demonstrated that extrusion was effective in algal cell disruption indicated by direct observation of cell break-up through scanning electron microscope (SEM) and significant increases up to 94.3% and 68.7% in lipid and sugars yield, respectively between treatments and the control (no treatment). It was found that feed moisture (15%) generally improved cell disruption efficiency due to longer residence time of biomass in the extruder. Increasing screw rotation speed from 300 to 450 rpm tended to enhance cell disruption because of stronger shear forces on algae. However, the effectiveness was restricted by residence time of biomass in the extruder because a faster screw rotation speed resulted in a higher biomass flow rate. Thus, the optimal cell disruption was not always achieved at the fastest screw rotation speed; instead, it appeared at the levels depending on specific evaluation indicators (around 350 rpm), therefore the optimal conditions for algal cell disruption were obtained at about 15% of feed moisture and 350 rpm of screw rotation speed. This study also confirmed that extrusion was beneficial for the production of more valuables including polyunsaturated fatty acids (PUFA) and essential amino acids (EAA) from microalgae, for example up to 74.3% increase of PUFA and 20.5% increase of EAA after extrusion compared to the control.

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1. Introduction

In review of renewable energy, microalgae have been considered as one of the most promising biofuel feedstocks that can potentially address the twin challenges of energy security and environmental protection [\[1\]](#page--1-0). However, at present the production of algal biofuels is not economically sustainable due to the cost of production and the low cost of conventional fuels [\[2\]](#page--1-0) and [\[3\].](#page--1-0) To overcome it, the attempts have been carried out by researchers either by increasing productivities or producing more high value products to make up for the low economic potential of algal

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biofuels [\[4\]](#page--1-0) and [\[5\]](#page--1-0). Generally five steps are involved in algae biodiesel manufacturing including algae culture, harvesting, drying, oil extraction, and biofuel conversion [\[6\].](#page--1-0) Though the concentration of microalgae is very low for current large-scale culture methods and the size of algae is only a few micrometers, which make the harvesting and further concentration of algae difficult and therefore expensive [\[7\]](#page--1-0) and [\[8\]](#page--1-0), algae harvesting has been extensively studied since 1990s, and tremendous improvements in the development of the techniques of centrifuge filtration, screening, flocculation sedimentation etc. were achieved by many researchers $[8]$ and $[9]$. Similarly, different from other terrestrial oil crops, the extracellular coverings of algae vary significantly, ranging from multiple layers of elaborate scales to highly mineralized coats to complex cell walls consisting of structural fibrils enmeshed in complex matrices [\[10\].](#page--1-0) Therefore, most microalgal cells are resistant to disintegration, it Γ mail advantument of the space of the space of Γ mail advantument of the space of Γ mail advantument of the space of Γ mail advantument of t

makes the extraction of intracellular products difficult. More attempts and achievements have also been gained in cell disruption process, which meets the goals of not only assisting lipid extraction by removing cell wall barriers, but being able to increase mass transfer and simplify downstream processing as well $[11–13]$ $[11–13]$.

Current algal cell disruption methods can be classified into two categories, non-mechanical and mechanical. Some examples of non-mechanical cell disruption are chemical (e.g. using detergents, solvents, antibiotics), physical (e.g. electric or osmotic shock, pressure, supercritical $CO₂$), and enzymatic treatments. Chemical additions are potentially toxic towards humans and are environmentally unfriendly. Enzymatic degradation methods are restricted by not only the high cost but also low effectiveness [\[14\].](#page--1-0) Physical treatments are more economical, but most of these methods are either not effective for treating tiny algae cells or difficult to scale up [\[15\]](#page--1-0) and [\[16\].](#page--1-0) Mechanical methods include bead/grind milling, high-shear mechanical processing, high-pressure homogenization, ultrasonication, etc. [\[17\]](#page--1-0). Unfortunately, these methods can only be effective at the lab scale or can not handle microalgal cells in an effective way [\[18\].](#page--1-0) Recently Topare et al. [\[19\]](#page--1-0) used a screw type machine that press the filamentous algae through a cage barrel, which recovered 75% of the oil from algae. However, this method was reported less effective due to comparatively longer extraction time [\[20\].](#page--1-0)

Extrusion pretreatment is a physical method in which biomass is processed by means of heat, compression, mixing and shear forces, leading to physical disruption and chemical modifications of biomass during the passage through the extruder, and forming steam-expanded pellets at the system outlet [\[21\]](#page--1-0) and [\[22\]](#page--1-0). The application of continuous oil extraction process using extrusion technology has received more interest since last century owing to its specific advantages including: increased recovery of oil by increasing the bulk density and the porosity of the material to be extracted; more readily accessible of the freed oil to the solvent; opportunities to deactivate undesirable enzymes such as lipase or phospholipase; as well as its versatility, high productivity, and low cost etc. [\[23\]](#page--1-0) and [\[24\].](#page--1-0) Extensive studies on extrusion processes applied to oil seeds to generate oil and fatty acid ester have been successfully carried out. For example, it was found that when extracted oil from dehulled sunflower seeds in a contra-rotating or co-rotating twin-screw press, the highest oil recovery of 85% -

93.6% with best cake meal quality was achieved [\[25,26\]](#page--1-0). Similarly, Sriti et al. [\[27\]](#page--1-0) obtained the maximum oil extraction yield of 74% from coriander fruits by single screw extruder with an optimized configuration, and the oil quality was proved very good with the acid value below 1.8 mg of KOH/g of oil and tolerable iodine values (44 mg of iodine/100 g of oil). Though compared with traditional oil crops, microalgae have advantages of rapid growth, high lipid content, non-occupation of arable land etc., it still cannot ignore that microalgae owns the unique characteristics of very small cell size (mostly micron-scale), relatively thin but mechanically strong cell wall, cell wall disrupted firstly for liberating intracellular valuables, and also large amounts of water in culture etc., it is thus worth to investigate the efficiency of extrusion on microalgal cell disruption for the production of intracellular valuables.

The objective of this study was to evaluate the effectiveness of extrusion on cell disruption of microalgae N.oceanica for the production of intracellular valuables. Operating parameters including feed water content and screw rotation speed were individually examined. Algal cellular components including lipid, protein and sugars as well as fatty acids, amino acids, sugar constituents were also individually determined.

2. Materials and methods

2.1. Algae sample preparation

N.oceanica (fresh spray dried powder) was bought from Yantai Hairong Microalgae Breeding, China. The water content of dried microalgal biomass was 2.43 ± 0.2 %. The microalgal biomass was conditioned to $15-24\%$ moisture by spraying with an amount of water and mixing continuously at medium speed in a mixer, and then allowed 3 h to equilibrate at room temperature prior to extrusion. This preconditioning procedure was employed to ensure uniform mixing and hydration and to minimize variability in the state of the feed material.

2.2. Experimental setup

The extrusion experiments were carried out in a single-screw extruder with a screw diameter of 34 mm, a length/diameter ratio of 4.7:1 and a die opening of 3 mm (Fu Xuxiang Machinery Manufacturing Co., Hebei, China). The inner barrel was grooved to ensure zero slip at the wall. The diagram of extruder is shown in Fig. 1. A General Electric 7.5 kW DC motor was used to drive the extruder. The screw rotation speed of the extruder was adjusted by a frequency converter (Model HB-H6, Hongbao Enterprise Development Co., Shanghai, China).

2.3. Experimental and analytical procedures

The processing parameters including water content (15%, 18%, 21%, 24%, displayed as W) and screw rotation speed (300, 350, 400 and 450 rpm, displayed as R) were individually evaluated. Here 21% of water content was set up as the control, since preliminary experiment (data not shown) displayed that so much water in microalgae can result in much higher cell disruption efficiency and an acceptable handling productivity for the extruder design in this study. It also should be noted that the algal culture without predewatering or the dry algal biomass without much water was not applicable in extrusion induced microalgal cell disruption, because their applications could resulted in either very low cell disruption efficiency or the associated stuck operation of the extruder. Similarly, the range of screw rotation speed was set up based on preliminary experiment (data not shown), as microalgal cells were Fig. 1. Single-screw extruder used in this study. disrupted to the greatest extent at the selected rotation speeds

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