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An organic solvents free bio-lipids extraction process using non-woven fabric from pretreated fermentation broth

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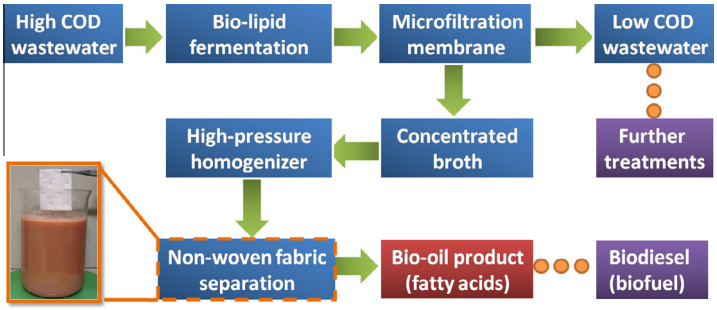
HIGHLIGHTS

- A novel bio-lipids extraction process without organic solvents associated was developed.
- Non-woven fabric was synthesized to separate bio-lipids from pretreated *Rhodotorula glutinis* fermentation broth.
- The non-woven fabric based bio-lipids extraction process has the potential for long terms separation.
- About 81.7% of the theoretical yield of bio-lipids was achieved under the optimized condition.
- The composition of fatty acids separated by the non-woven fabric was similar with the conventional ones.

GRAPHICAL ABSTRACT



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ABSTRACT

In this study, to avoid solvent contamination and decrease the cost of bio-lipids extraction, a novel non-woven fabric (NWF) was synthesized to separate bio-lipids from pretreated *Rhodotorula glutinis* fermentation broth. After fermentation with the wastewater mixture, cells were concentrated by microfiltration and the concentrated cells were disrupted via high pressure homogenization. Bio-lipids was extracted and separated from cell disruption suspension via NWF. The NWF can be used to carry out 25 cycles of oil extraction, which showed it was effective and easily recyclable in bio-oil recovery. The extraction temperature was also optimized, and about 25.5 mL/L of final bio-lipids product with 81.7% of the theoretical yield was achieved at 70 °C. The fatty acid compositions extracted by NMW were similar with those obtained using the conventional organic solvent extraction methods. The results showed that the novel process provided an attractive and promising way to separate bio-lipids from microorganisms.

1. Introduction

Microbial oil, produced by oleaginous microorganisms, is considered to be a superior biodiesel feedstock with the advantages of high productivity, less labor requirement, easier to scale up and non-arable land usage [1,2]. However, up to now, biodiesel from microbial oil is becoming less economically competitive with the traditional feedstock such as vegetable oil and waste oil because of high cost of the feedstock and the extraction process of oil from microorganisms [3–5].

To minimize the feedstock cost, lignocellulosic hydrolysate has been used as the substrate in micro-oil production [6,7]. Besides that, oleaginous microorganisms also showed a great propensity

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to treat the industrial organic wastewater and generated low cost microbial oil at the same time [8–13]. Since the majority of lipid products are synthesized in the microorganism cells, extraction of the lipids from biomass has been identified as a common bottleneck of microorganisms due to the difficulties of cell disruption and the challenges of the lipids collection from the diluted fermentation broth [14–17]. More specifically, it was reported that more than 80% of water needs to be removed by a dewatering process to obtain the dry cells [18]. And more importantly, the bio-lipids downstream separation process is recognized to be energy intensive, less effective, costly and environmentally unfriendly, due to the high pressure and expensive organic solvents at high temperature which are demanded during the conversional physical and chemical lipids extraction processes [18–21].

In current years, the development of hydrophobic and oleophilic materials such as NWF has provided a promising way for oil–water separation and alternative to the current separation methods [22–24]. When the NWF with hydrophobic and oleophilic properties was used for bio-lipids separation, no organic solvents as extractant needs to be contact the cell disrupted mixture. In addition, due to the similar polarity, long chain fatty acid, the main composite of microbial oil, could efficiently transport from the water phase broth into the NWF material. Then, with the mechanical extrusion or squeezing the fabric, the oil product could be obtained by breaking away from the fabric. Obviously, this novel lipid extraction process is environmentally friendly and easy-to-control. More important, the easy repeatability of NWF helped to greatly decrease the lipid extraction cost compared with the traditional solvent extraction [18,25]. However, to our best knowledge, the NWF with hydrophobic and oleophilic properties has not been reported to be applied in microbial oil extraction and separation from oleaginous microorganism fermentation broth.

In this study, a novel bio-lipid separation strategy was established. Emphasizing the economical competitiveness of bio-lipids and the development of 'green' bio-chemistry process, wastewater mixture was used as the substrate in *Rhodotorula glutinis* fermentation to produce bio-lipids. The fermentation broth was then concentrated by microfiltration membrane, and followed by high pressure homogenization (HPH) to disrupt the cell wall of the microorganism. The NWF with hydrophobic and oleophilic properties was used to contact and extract lipids from the mixture. The extraction temperature was optimized and the composition of the fatty acid separated was further analyzed. The novel separation method showed great potential for separating bio-lipids in industrial-scale processes.

2. Material and methods

2.1. Strain, medium and batch fermentation

The mutant strain, *R. glutinis* B 13, was obtained by using atmospheric and room temperature plasma method and stored in our lab. According to literature, the seed medium contained 40 g/L of glucose, 2 g/L of $(NH_4)_2SO_4$, 7 g/L of KH_2PO_4, 2 g/L of Na_2SO_4, 1.5 g/L of $MgSO_4$ and 1.5 g/L of yeast extract [12]. After inoculated, the seed strains were cultured at 30 °C with rotation speed of 150 rpm for 24 h. Then, at an inoculation rate of 10% (v/v), the seed was pumped into a 5 L fermentor which contained 4 L of wastewater mixture from Ruixing Chemical Co. Ltd, China. While the temperature was set at 30 °C, and the rotation speed was set at 150 rpm. The wastewater mixture was consisted of waste syrup, gluten and corn steeped wastewater, with pH 3–5, initial COD of ~53,000 mg/L, and BOD of ~31,000 mg/L. The concentration of sugar in the wastewater mixture was ~25 g/L, and the protein content was ~650 mg/L, which was similar to our previous study [12]. After 36 h of batch cultivation at 30 °C, the lipid content of cells was harvested.

2.2. Microfiltration and disruption of cells

Since the culture provided a dilute aqueous suspension, which resulted in the high cost of downstream processing, the fermentation broth needed to be concentrated before the step of oil extraction [26]. A ceramic microfiltration membrane with 0.36 m^2 of area was used to concentrate the fermentation broth (0.8 μm of pore size, EP 1960, Pall Corporation, France,). Two batches of fermentation broth (about 8 L) were feed. The temperature of the broth was maintained at 30 °C, and the pressure was maintained at 0.1 MPa.

After concentration, the condensed broth was further pre-treated by nano homogenize machine (ATS Eng. Inc, Canada) to release the intracellular lipids to surrounding medium. The pressure of HPH ranges from 40 to 120 MPa and the flow speed of liquor was maintained at 15 L/h. Because of the low efficient of the disruption force by HPH, three cycles of the broth was encouraged to ensure the completely disruption of suspended cells.

2.3. Hydrophobic NWF preparation and application

The hydrophobic and oleophilic NWF was prepared using polypropylene powder as raw material. The melt blown method was slightly modified according to Świątek et al.'s study [27]. In general, pelleting process was firstly performed via twin-screw extruder (BL-6177-A, Bolon Precision Testing Machines Co. Ltd, China) under a screw speed of 350 rpm. The ratio of length to diameter was 40, and the screwed head temperature was set at 180 °C. The barrel temperature profile was set at 175–185–185–185–185–185 °C (from hopper to die). After pelleting, the particles were immersed in hot air from the top and bottom side of the melt blown machine (experimental facilities, TUST, China). Then, fibers with fine diameter (<10 μm) were subsequently blown into a collector screen, and self-bonded to form the entanglement and cohesive sticking structure. Thus, the NWF was formed with randomly oriented fibers connected together. The thickness of the NWF was about 3 mm and the mass of the NWF was 40 g/m^2 of the area.

After disruption of the cells, the liquor consisting of water, lipids and cell debris was contacted with the surface of the mesh. The lipids were adhered on the mesh, and were separated from water and cell debris due to the hydrophobic ability of the NWF [28]. Then, the bio-lipids product was desquamated from the fabric and collected.

2.4. Analytical methods

The measurements of biomass, liquid content, COD concentration and residual sugar during the fermentation process were conducted according to the method of Xue et al. [10,11]. The biomass was measured by weighting the dry biomass with a certain volume of fermentation broth. Lipid content of the biomass was obtained with Soxhlet extraction method [29]. The COD concentration was tested by COD rapid determinator (Twt, Germany). The residual sugar was tested by a glucose biosensor (SBA 40C, Biological Institute of Shandong Academy of Science, China).

The lipids composition of the separated oil was determined by gas chromatograph analysis according to the method of Xue et al. [12]. The GC-2010 gas chromatography (Shimadzu, Japan) was equipped with a DB-1ht capillary column and a flame ionizing.

The structure of NWF was studied by scanning electron microscope (SEM) (JSM-6700F, Japan), and the hydrophobic performance of the textiles was measured by the contact angle for at least three pieces of the mesh using an OCA 20 contact angle system (Dataphysics Instruments Gmbh, Germany).