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Ultrasonication aided biodiesel production from one-step and two-step transesterification of sludge derived lipid

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ABSTRACT

Trichosporon oleaginosus was grown in wastewater sludge to produce lipid. The obtained biomass was employed to study ultrasonication effect on lipid extraction and *in-situ* transesterification. Besides the most utilized solvents (the mixture of chloroform and methanol, 1:1 v/v), other solvents including water, hexane, and methanol were also studied to explore their capacities in lipid extraction with ultrasonication. The maximum lipid recovery was 11.8, 35.3, 62.0, and 95.3% (w/w) with water, hexane, methanol, and the mixture of chloroform and methanol (1:1 v/v), respectively within 20 min. Chloroform and methanol gave the best performance. However, due to the adverse environmental concern related to the utilization of chloroform, *in-situ* transesterification (single step lipid extraction and transesterification to obtain biodiesel) was investigated.

In case of *in-situ* transesterification, biodiesel yield of 95% (w/w) was obtained in 60 min with the assistance of ultrasonication; whereas, 24 h was required to achieve similar yield without ultrasonication.

1. Introduction

Currently, plant seed oils are the major feedstock of biodiesel production. Meanwhile, most of these oils are demanded by food production industry and kitchens. Thus, microbial oil as an alternative for biodiesel production has been attracted considerable attention. It has been observed that some microorganisms could accumulate lipid droplets in the cells as energy source [1–3]. Those with lipid content higher than 25% (w/w) are called oleaginous microorganisms. Some of the microorganisms had the lipid content of 80% of cells dry weight [4,5]. However, it was revealed that microbial oil production cost was too high to be affordable for biodiesel production [6–8]. In microorganisms cultivation, carbon and nutrients are required and they count for a great part of the total microbial oil cost [9–11]. In order to reduce biodiesel production cost, organic waste including wastewater, agriculture waste, and restaurant wastes, which are abundant in carbon and nutrients, have been used as raw material in oleaginous microorganism cultivation for lipid production [12–14]. Wastewater sludge is another promising raw material. In our previous studies [15,16], sludge has been utilized as growth medium for *Trichosporon oleaginosus*, and the lipid accumulation reached 30% w/w dry biomass. In economic analysis of microbial oil to biodiesel, the lipid content assumed was generally in the range of 25%–50% w/w dry biomass, because the value within the range was considered being acceptable to start an industrial scale plant [8,17]. It indicates that *Trichosporon oleaginosus* cultivated with sludge (cheap raw material) could be used to accumulate lipid for biodiesel production in practice.

Ultrasonication has been applied to extract lipid from microbes cultivated with purified glycerol medium in our previous study [18]. It was found that ultrasonication was a promising technology as it largely reduced the lipid extraction time from 12 h (without ultrasonication) to 20 min in achieving similar efficiency. Compared to the biomass (microbial cells) cultivated with purified glycerol, the biomass from sludge medium cultivation contains microbial cells as well as some non-degradable or inert materials [16,19]. The non-degradable materials could be broken down during ultrasonication assisted lipid extraction and some compounds may react or mix with lipid, and hence affect the quality of the lipid [20–23]. Similarly, it may also have an effect on the biodiesel in ultrasonication aided *in-situ* transesterification. Thus, ultrasonication application for the lipid extraction from biomass cultivated with sludge medium should be investigated.

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In the study, ultrasonication was applied for lipid extraction from *Trichosporon oleaginosus* cultivated with sludge. Effect of the parameters including solvent types, temperature, and time on lipid recovery was evaluated. Profiles of biodiesel converted from ultrasonication aided lipid extraction; conventional chloroform methanol extraction, *in-situ* transesterification, and ultrasonication aided *in-situ* transesterification were compared.

2. Materials and methods

2.1. Sludge medium

Secondary sludge was collected from a local municipal wastewater treatment plant (Communauté Urbain de Québec, CUQ), Québec, Canada. The sludge was allowed to settle for 12 h at 4 °C and the supernatant was decanted. The SS (suspended solids) concentration of the resulting solution was measured. Centrifugation would be performed (if required) to finally adjust the SS to 33.3 g/L. Then the pH of the sludge solution was adjusted to 12 and treated at 121 °C for 15 min. After it was cooled down to room temperature, the pH was adjusted to 6.5 and used as the cultivation medium.

2.2. Cultivation and harvesting

Trichosporon oleaginosus (ATCC20509) was used for lipid accumulation in the study. The fermentation was performed in 4 L shake flasks with 1 L working volume incubated in a rotary shaker at 170 rpm and 28 °C [16]. After 72 h, the broth was harvested and dewatered by centrifugation. The obtained suspended solids are *Trichosporon oleaginosus* grown in sludge, which are defined as sludge-biomass. The sludge-biomass obtained from centrifugation was washed several times with distilled water till the supernatant was clear. Part of the wet sludge-biomass was dried by lyophilisation and then used for lipid extraction and *in-situ* transesterification study. The other part was diluted with distilled water to the desired sludge-biomass concentration for lipid extraction with water as solvent.

2.3. Conventional lipids extraction methods

To determine the total lipid of sludge-biomass, the standard chloroform and methanol extraction procedure [24,25] with minor modification was used. One gram of the lyophilized dry biomass was mixed with 15 mL of chloroform and methanol (2:1 v/v, v/v representing volume by volume ratio, which means every 2 mL of chloroform was mixed with 1 mL of methanol), then subjected to 60 °C for 4 h. Thereafter, centrifugation (5000 rpm for 15 min) was conducted and the solvent phase was transferred to a pre-weighed glass vial. The extraction was repeated two more times and the solvents were collected into the same glass vial. After evaporating the solvent at 60 °C, the vial was weighed again. The lipid content was calculated. The lipid content of the original sludge (without fermentation) was considered as control. The experiment was done in triplicates. The extracted lipid was then converted to biodiesel by reaction with methanol (methanol to lipid molar ratio 6:1) in the presence of H_2SO_4 (1% v/v methanol) with 12 h reaction time [16]. The biodiesel was analysed with Gas Chromatography Linked to Mass Spectroscopy (GC–MS) (Perkin Elmer, Clarus 500) [16]. The transesterification efficiency (biodiesel amount obtained from GC–MS divided by the lipid amount calculated from extraction) was estimated based on the GC–MS results.

2.4. Ultrasonication aided lipid extraction

The ultrasonication aided lipid extraction was performed at frequency of 520 kHz with power input 40 W (Fig. 1a) and 50 Hz with power input 2800 W (Fig. 1b). The 520 kHz 40 W ultrasonic system consists of an ultrasonic transducer installed on the bottom of a double-walled (jacket) glass reactor, an amplifier (T&C power Conversion, Inc.) for power control, a Hewlett Packard Model 3300A function generator for frequency control, and a temperature control device (Poly Stat, Cole Parmer), which circulates water in the jacket of the glass reactor. Low frequency high power ultrasonication (50 Hz, 2800 W) extraction was performed in an ultrasonication bath (Fisher Scientific, FB15069).

In the lipid extraction with water as solvent, 500 mL of original sludge or sludge-biomass with a suspended solids concentration (suspended in distilled water) of 30, 50, or 70 g/L, was transferred to the ultrasonication reactor. The extraction was conducted at temperature of 25, 35, 45, or 55 °C. The samples (50 mL) were taken at 5, 10, 15, 20 and 30 min to determine the lipid recovery. 5% (w/v; representing weight by volume) NaCl for demulsifying lipid/water emulsion and few drops of hexane were added to the samples and then centrifuged at 9000 rpm for 15 min. The supernatant was collected and transferred to a pre-weighed glass tube and subjected to 60 °C until a constant weight. The recovered lipid amount was then calculated. The resulting solid of the centrifugation was dried by lyopholization and its lipid content was determined. This amount was considered as non-recovered lipid.

The lipid recovery efficiency was calculated as following:

$$\text{Lipid recovery efficiency} = L/(C \times M) \quad (1)$$

where L is the recovered lipid amount in ultrasonication extraction (g); C is the lipid content of the material (w/w) (w/w; representing weight by weight) (obtained from conventional chloroform and methanol extraction); M is the dry weight of the materials used in ultrasonication extraction (g).

The lipid un-recovered fraction was calculated as following:

$$\text{Lipid un-recovery fraction} = Ln/(C \times M) \quad (2)$$

where Ln is the lipid extracted from the resulting material after ultrasonication extraction (g); C is the lipid content of the material (obtained from conventional chloroform and methanol extraction); M is the dry weight of the materials used in ultrasonication extraction (g).

In the extraction with hexane, methanol, and chloroform and methanol (1:1 v/v) as solvents, the process was similar as that with water as solvent. The solvents were added to achieve a sludge-biomass concentration of 50 g/L. The extraction time was from 5 to 20 min at temperature of 25, 35, 45, and 55 °C.

The lipid obtained from each extraction was finally converted to biodiesel with the similar method used in transesterification of lipid extracted with conventional chloroform methanol approach.

2.5. In-situ transesterification

The *in-situ* transesterification was conducted similarly as reported previously [26]. However, H_2SO_4 (instead of NaOH) was utilized as catalyst due to that the free fatty acid contents of sludge and the sludge-biomass were greater than 0.5% w/w total lipid [15,27]. 1 g of dry original sludge or sludge-biomass was added to methanol containing H_2SO_4. Reactions occurred at 55 °C. The parameters studied were methanol to oil molar ratio (6:1, 60:1, 120:1, 240:1, and 360:1), H_2SO_4 addition (1%, 2%, and 5% H_2SO_4 v/v methanol), and reaction time (2–24 h). 5 mL hexane as co-solvent was added to each experiment. The detailed process of biodiesel recovery after *in-situ* transesterification can be found in Ref. [26]. The biodiesel was analysed with GC–MS.