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Direct transesterification of wet *Cryptococcus curvatus* cells to biodiesel through use of microwave irradiation

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HIGHLIGHTS

- Direct transesterfication of wet yeast cells using methanol and microwave irradiation is feasible.
- Methanol to biomass ratio, stirring speed and KOH concentration were critical to biodiesel yield.
- Under optimal conditions, the crude biodiesel contained 64% of FAMEs and was 92% of yeast lipids.

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ABSTRACT

Cryptococcus curvatus is a highly promising oleaginous yeast strain that can accumulate intracellular lipids when grown on renewable carbon sources. In order to convert yeast lipids to biodiesel in a simple but cost-effective way, we aim to react whole yeast cells with methanol to produce biodiesel eliminating the step of drying and lipid extraction while adopting microwave energy for heating and disrupting cell walls. Through use of a screening test followed by response surface methodology, optimal parameters leading to the highest yield of crude biodiesel and FAMEs were identified. Under optimal conditions of reaction time (2 min), methanol/biomass ratio (50/1, v/m), stirring speed (966 rpm), KOH concentration (5%), and water content (80%), the yield of crude biodiesel (% of total lipids) was 56.1% after the first round reaction. A second round reaction using the residual yeast cells increased the total yield to 92%. Among the crude biodiesel, 63.88% was FAMEs as revealed by GC analysis. Results from this study indicated that it is feasible to produce biodiesel from wet microbial biomass directly without the steps of drying and lipid extraction. With the assistance of microwave, this process can be accomplished in minutes with good process efficiency.

1. Introduction

During recent years, interest in producing microbial oils by using oleaginous microorganisms has been on the rise. In particular, oleaginous microorganisms that can grow on either CO_2 (e.g. microalgae) or renewable carbon sources, such as sugars from lignocellulosic materials [1–4] or crude glycerol [5,6] from the biodiesel industry have attracted significant attentions. The resulting microbial lipids which are similar to vegetable oils can be converted to biodiesel easily or to other liquid transportation fuels, biogasoline, green diesel or jet fuel through hydrotreatment, decarboxylation and other upgrading approaches.

But unlike vegetable oils that can be pressed out of oil seeds at commercial scales, extracting lipids out of microbial cells is not a simple effort. Traditionally, microbial lipids are obtained from microorganisms through steps of breaking cells open if cell walls are present and then use of organic solvents to extract lipids out of other cellular components. Apparently, this approach suffers from several drawbacks: (1) long extraction time, (2) large volume of solvents, (3) intensive energy and cost, and (4) nearly impossible for scale-up. Another strategy is to skip the lipid extraction step and conduct the transesterification reaction using whole microbial cells instead of lipids for the purpose of biodiesel production. Toward this end, researchers have attempted to produce fatty acid methyl esters (FAMEs) by: (1) reacting dried algal cells, *Schizochytrium limacinum* SR21 with methanol and sulfuric acid with or without an organic solvent using conventional heat [7]; (2) heating dried oleaginous yeast and fungal biomass with methanol and a mineral acid (sulfuric or hydrochloric acid) for an extensive time of 20 h [8]; (3) using microwave energy to heat the mixture of dry algal biomass with methanol and KOH [9]; and (4) heating the mixture of dried algal cells with methanol/chloroform (v/v, 1:2) and a SrO catalyst by microwave irradiation or sonication [10].

Among these studies, Liu has reported excellent yield of FAMEs of 98% [8]. However, the reaction time of 20 h at 70 °C is just too

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long. Using microwave irradiation, however, is very fast. Within 4 min, a FAME yield of 80% can be achieved [9] and microwave has been demonstrated to be more effective than sonication [10].

Microwaves are extremely high frequency radio waves that can cause certain molecules to vibrate around a billion times per second. The friction of molecules rubbing against each other converts mechanical energy into heat. Among different materials, such as proteins, fibers, and oils, water is most strongly excited by microwaves. Water excitement can then lead to pressure build-up within the processed materials at the microscopic level. In terms of yeast cells, the rapid heating rates and pressure increase can disrupt the yeast cell walls, which is critical for releasing intracellular lipids. Thus, compared to conventional lipid extraction, microwave-assisted extraction (MAE) offers several advantages. First, it can be accomplished in minutes versus hours for some lipid extraction methods, such as Soxhlet. Second, it requires less energy due to the short reaction time. Third, unlike supercritical methanol or CO_2 extraction and advanced solvent extraction which necessitate a completely dry biomass, MAE can deal with a wet biomass since the presence of water is beneficial for the extraction process. Fourth, MAE could be scaled-up as commercial microwave systems are available.

Microwave irradiation has been widely used in several areas such as chemical synthesis, solvent extraction, and solid state reactions. Recently, it has been adopted to: (1) pretreat lignocellulosic biomass include sweet sorghum bagasse [11], wheat straw [12], rice straw [13], and switch grass [14]; (2) extract lipids from microalgae [15,16] and (3) produce biodiesel from microalgal biomass [9,10]. Among several approaches to disrupt microbial cell wall structures, such as autoclaving at 125 °C with 1.5 Mpa for 5 min, bead-beating, sonication, and osmotic shock in a 10% NaCl solution, microwave at 100 °C and 2450 MHz for 5 min had the highest lipid extraction efficiency for *Botryococcus* sp., *Chlorella vulgaris*, and *Scenedesmus* sp. In addition to effectiveness, microwave is also credited as the most simple and easy process to operate [15].

Simultaneous extraction and transesterification using microwave for the purpose of producing biodiesel from microbial lipids have also been proven feasible. Under the optimum experimental conditions: dry algae (*Nannochloropsis* sp. CCMP 1776) to methanol ratio of 1:12 (w/v), KOH concentration of 2%, reaction time of 4–5 min and reaction temperature of 60–64 °C, 80% of intracellular lipids were converted to fatty acid methyl ether (FAME) [9]. In another study of direct transesterification of *Nannochloropsis* sp. but with the aid of SrO catalyst, microwave irradiation (60 °C and 5 min) resulted in the 99.9% conversion of TAG to crude biodiesel [10]. Therefore, microwave energy can play an important role in converting microbial lipids to FAMEs.

However, all of the researches aforementioned above used dry microbial biomass as the study material. Considering the energy and equipment cost related to drying microbial cells, it would be cost-effective if wet cells can be used directly for biodiesel production. Thus, this study aimed to investigate the feasibility of converting wet cells of *Cryptococcus curvatus* (ATCC 20509) to biodiesel through direct transesterification using microwave irradiation. Compared to other oleaginous yeast strains, *C. curvatus* seems to be one of the best. On one hand, like other lipid-producing yeasts, this strain accumulates more than 40–70% of dry biomass as lipids depending on cultural conditions. On the other hand, it is so far the best yeast strain for accumulating lipids on hydrolysates developed from lignocellulosic feedstocks. Unlike *Rhodosporidium toruloides* which does not tolerate the inhibitors in the hydrolysates of wheat straw [1] and corn stover [17] and *Yarrowia lipolytica* whose preferred substrates are those hydrophobic, such as fatty acid and alkaline [18–20], *C. curvatus* grows well on unconditioned hydrolysates of sweet sorghum bagasse based on our own observation [3,4] and tolerates inhibitory compounds well according to other researchers' report [1]. In particular, among several oleaginous yeast species, *Rhodotorula glutinis, R. toruloides, Lipomyces starkeyi,* and *Y. lipolytica, C. curvatus* had the highest lipid yield on both the detoxified and non-detoxified hydrolysates of wheat straw after dilute sulfuric acid pretreatment [1]. In addition, this yeast has good lipid productivity on effluent from hydrogen production from food waste [21], crude glycerol [22], and wheat straw pretreated by ozonation and alkaline peroxide [23].

To obtain the maximum yield of FAMEs, this study first evaluated the effects of five factors: time, solvent/biomass ratio, stirring speed, catalyst concentration, and water content. Parameters that were critical to FAME yield were then further investigated to identify the optimal condition for conducting direct transesterification using microwave energy.

2. Materials and methods

2.1. Direct transesterification reaction

C. curvatus cells obtained from our previous fed-batch study [6] were used for the work reported here. Briefly, the cells were grown on crude glycerol for 12 days. The resulting biomass had a lipid content of 42%. The cell pellet obtained after centrifugation of the yeast culture was freeze dried and stored in a −80 °C freezer until use. A domestic microwave oven (900 W) was modified for this study. The roof of the oven was drilled with three holes to pass through a digital thermocouple (Omega 2160A, Omega Engineering Inc., Stamford, CT, USA), a water-cooled reflux condenser and a motor driven stirring bar, which could ensure uniform mixing of the reaction mixture. Microwave-transparent, three-neck round-bottom flasks (100 ml) were used as sample vessels.

To these flasks, freeze dried *C. curvatus* (0.5 g dry weight) was added. Different volume of distilled and deionized water (DDW) was also supplemented to create slurries with different moisture contents. Methanol as a solvent and a reactant and KOH as a catalyst were then added to the reaction vessel followed by start of microwave irradiation. During reaction, the stirring bar was controlled to have different rotating speed for different time intervals. After completion of the reaction, the mixture was centrifuged at 4500*g* for 5 min for phase separation. The lighter phase which contained biodiesel dissolved in methanol was filtered further through a 0.45 μm filter to remove the fine particles that floated on top of the liquid. The solution of methanol was transferred into a 50 ml round-bottom flask where methanol was evaporated in a Rotovap. The remaining product was added with hexane followed by centrifugation at 4500*g* for 3 min. The upper layer was transferred to a pre-weighed glass tube where hexane was evaporated under a gentle nitrogen stream. Mass of crude biodiesel was then determined gravimetrically. To the crude biodiesel, an internal standard, methyl heptadecanoate (C17:0) was added quantitatively together with known volume of hexane. The mixture was then analyzed by gas chromatography (GC).

2.2. Experimental design for screening significant factors

The Plackett–Burman design [24] was used to screen significant factors among five with respect to their effects on the crude biodiesel yield. The five factors are: time, solvent/biomass ratio (v/m), stirring speed, catalyst concentration, and water content. Each variable was represented at two levels, i.e., high (+) and low (−). The highest and lowest levels of each factor were given in Table 1. According to the Plackett–Burman design developed by using Design Expert System (version 7.1.6, Stat-Ease Inc., Minneapolis, MN, USA), twelve trials were performed with the crude biodiesel yield in terms of percentage of yeast lipids as the response (Table 2).