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Ultrasonication aided *in-situ* transesterification of microbial lipids to biodiesel

Xiaolei Zhang [a], Song Yan [a], Rajeshwar Dayal Tyagi [a,*], Rao Y. Surampalli [b], Jose R. Valéro [a]

[a] INRS Eau, Terre et Environnement, 490, rue de la Couronne, Québec G1K 9A9, Canada
[b] Department of Civil Engineering, University of Nebraska-Lincoln, N104 SEC, PO Box 886105, Lincoln, NE 68588-6105, USA

HIGHLIGHTS

- Microbial lipid was converted to biodiesel with *in-situ* transesterification.
- Ultrasonication was used to improve feasibility of *in-situ* transesterification.
- Ultrasonication did not impact on the final product composition.

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ABSTRACT

In-situ transesterification of microbial lipid to biodiesel has been paid substantial attention due to the fact that the lipid extraction and transesterification can be conducted in one-stage process. To improve the feasibility of *in-situ* transesterification, ultrasonication was employed to reduce methanol requirement and reaction time. The results showed that the use of ultrasonication could achieve high conversion of lipid to FAMEs (92.1% w lipid conversion/w total lipids) with methanol to lipid molar ratio 60:1 and NaOH addition 1% w/w lipid in 20 min, while methanol to lipid molar ratio 360:1, NaOH addition 1% w/w lipid, and reaction time 12 h was required to obtain similar yield in *in-situ* transesterification without ultrasonication. The compositions of FAMEs obtained in case of ultrasonication aided *in-situ* transesterification were similar as that of two-stage extraction followed by transesterification processes.

1. Introduction

Biodiesel production from microbial oil, also called single cell oil, has grabbed great attention due to unaffordable cost of traditional oils such as vegetable oils, which are used for biodiesel production. Microorganisms grow faster and accumulate higher lipid content (up to 80% w/w) as compared to crops (several months and 30% w/w oil content) (Pimentel and Patzek, 2005; Ruan et al., 2012). Numerous studies have successfully transferred microbial oil to biodiesel (Meng et al., 2009; Gao et al., 2013). The process chain includes microorganism cultivation, harvesting, lipid extraction, and transesterification. Lipid extraction from microorganism requires large amount of organic solvent generally chloroform and methanol (Ruan et al., 2012; Zheng et al., 2012; Zhang et al., 2014). Chloroform has adverse impact on the environment and requires extra attention in manipulation. Hexane/isopropanol has also been applied for lipid extraction but the extraction efficiency is lower as compared to chloroform and methanol as solvent (Ferraz et al., 2004). Terpenes, green solvents obtained from plants, is a great selection to extract microbial oil and yields similar efficiency as chloroform/methanol, yet the cost is high (Dejoye Tanzi et al., 2013). Therefore, extraction becomes an obstacle in biodiesel production from microbial sources.

In-situ transesterification has been reported in biodiesel production from microorganisms. The method simultaneously achieved extraction and transesterification of the lipid in microorganism. It thus eliminated lipid extraction step. In previous studies, *in-situ* transesterification on soy flakes and wastewater sludge have accomplished high yield of biodiesel (up to 97%) (Haas et al., 2004; Haas and Scott, 2007; Mondala et al., 2009). However, methanol addition was around one hundred times higher in one step or *in-situ* transesterification (methanol to lipid molar ratio around 300:1) than two stage conversion (methanol to oil ratio 6:1 to 12:1). Moreover, long reaction time was also required (around 12 h for *in-situ* and 2 h for two stage transesterification).

The objective of this work is to investigate ultrasonication aided *in-situ* transesterification for biodiesel production from oleaginous microorganisms. Ultrasonication was used to investigate its effect on the methanol requirement as well as reaction time. Parameters

* Corresponding author. Tel.: +1 (418) 654 2617; fax: +1 (418) 654 2600.
E-mail address: rd.tyagi@ete.inrs.ca (R.D. Tyagi).

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including ultrasonication time, catalysts concentration, and different methanol to lipid molar ratios were examined. The transesterification without ultrasonication aid was also conducted to compare the results. The impact of ultrasonication aided *in-situ* transesterification on FAMEs composition was also investigated.

2. Methods

2.1. Strain, culture and harvesting conditions

Oleaginous yeast *Trichosporon oleaginosus* (ATCC20509) was grown in a glycerol medium containing (per liter): 1 g $(NH_4)_2SO_4$, 1 g KH_2PO_4, 0.5 g $MgSO_4{\cdot}7H_2O$, 0.2 g yeast extract, 50 g purified glycerol, and minerals 0.04 $CaCl_2{\cdot}2H_2O$, 0.0055 $FeSO_4{\cdot}7H_2O$, 0.0052 citric acid H_2O, 0.001 $ZnSO_4{\cdot}7H_2O$, and 0.00076 $MnSO_4{\cdot}H_2O$ were added (Zheng et al., 2012). The purified glycerol was derived from crude glycerol, which was from an industrial biodiesel production company in Quebec. The purification was performed by lowering the pH of the crude glycerol to 2, and then the free fatty acid (FFA) (on the top) and salt (in the middle) were removed by centrifugation. The experiment was performed in 4 L shake flask with 1 L medium at 200 rpm 28 °C. After 72 h fermentation, biomass was harvested by centrifugation at 5000 rpm for 15 min. The biomass yield was 0.19 g/g glycerol. The biomass was washed twice with distilled water to remove the residual nutrients and glycerol. Part of the biomass was dried by lyophilisation and then stored for further study.

2.2. Lipids extraction methods

The standard chloroform and methanol extraction procedure with minor modification was employed to determine the lipid content in the biomass (Folch et al., 1957; Vicente et al., 2009). 200 mg dry biomass (after lyophilisation) was mixed with 4 mL solvent mixture of chloroform and methanol (2:1 v/v), and then subjected to 60 °C for 4 h. The mixture was then centrifuged at 5000 rpm for 15 min and the solvent phase was withdrawn and transferred into a pre-weighed glass vial (W_1). The extraction procedure was repeated two times. Afterwards, the vial containing the total volume of the supernatant collected from each extraction was subjected to 60 °C in an oven to evaporate the solvents and then weighed (W_2). The lipid amount was calculated by the difference of W_2 and W_1. The lipid content in the biomass is $(W_2 - W_1)/200\ \text{mg} \times 100\%$. The obtained lipid was stored in dark at 4 °C for further transesterification study.

2.3. Lipid transesterification

Lipid obtained from solvent extraction from *T. oleaginosus* was first dissolved in hexane (25 mL hexane per gram lipid), then mixed with methanol. Lipid to methanol molar ratio is 1:6 (0.3 mL methanol for per gram lipid). Sodium hydroxide was used as catalyst with addition of 1% w/w (NaOH/oil). The mixture was then subjected to 55 °C for 2 h. After reaction, 5% w/v NaCl solution was added (100 mL NaCl solution per gram lipid), and then FAMEs was extracted by two times washing with hexane (100 mL per gram lipid). After phase separation by settling, the hexane phase (upper layer) was collected. The FAMEs in hexane was washed with 2% sodium bicarbonate solution (20 mL per gram lipid) and allowed the mixture to stand for 15 min for phase separation, and the top layer was collected and dried at 60 °C in an oven (Halim et al., 2011).

The FAMEs was then re-dissolved in hexane (10 mL/mg lipid) and analyzed using a Gas Chromatograph linked with Mass Spectroscopy (GC–MS) (Perkin Elmer, Clarus 500). The dimensions of the column used are 30 m × 0.25 mm, with a phase thickness of 0.2 μm. The calibration curve was prepared with a mixture comprising 37 FAMEs (47885-U, 37 Component FAME Mix; Supelco, Bellefonte, PA, USA). 1,3-Dichlorobenzene was used as internal standard with a concentration of 50 ppm.

2.4. In-situ transesterification

0.2 g of biomass was mixed with methanol containing NaOH and then the mixture was subjected to 55 °C for 2–12 h. The

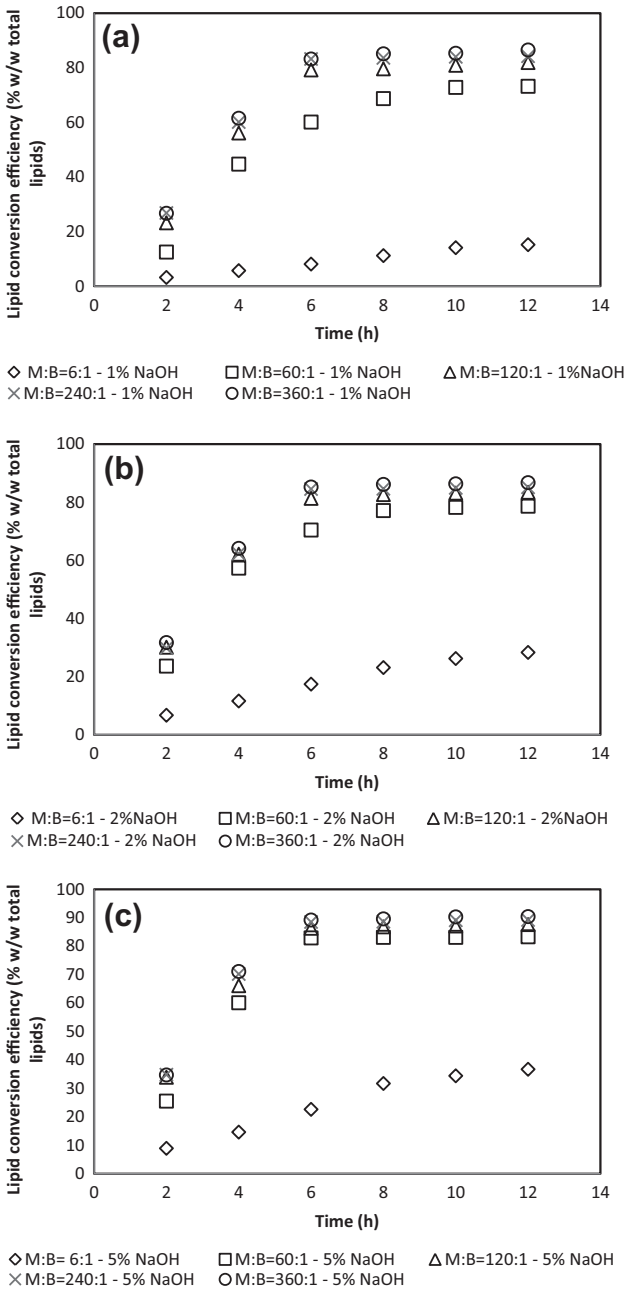

Fig. 1. (a) Variation of lipid conversion efficiency with reaction time for the *in-situ* transesterification at different methanol to lipid molar ratio with 1% catalyst (NaOH) addition; (b) variation of lipid conversion efficiency with reaction time for the *in-situ* transesterification at different methanol to lipid molar ratio with 2% catalyst (NaOH) addition; (c) variation of lipid conversion efficiency with reaction time for the *in-situ* transesterification at different methanol to lipid molar ratio with 5% catalyst (NaOH) addition, standard deviation is less than 5% (M:B = molar of methanol:molar of lipid; 1% NaOH = 1% NaOH w/w lipid).