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Microbial biodiesel production by direct methanolysis of oleaginous biomass

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HIGHLIGHTS

- Base catalyzed methanolysis simplified microbial biodiesel production process.
- Base catalyzed transesterification has many advantages over acid catalyzed one.
- Higher FAME yield could be obtained using less catalyst.
- Higher FAME yield could be obtained at lower temperature in shorter time.

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ABSTRACT

Biodiesel is usually produced by the transesterification of vegetable oils and animal fats with methanol, catalyzed by strong acids or bases. This study introduces a novel biodiesel production method that features direct base-catalyzed methanolysis of the cellular biomass of oleaginous yeast *Rhodosporidium toruloides* Y4. NaOH was used as catalyst for transesterification reactions and the variables affecting the esterification level including catalyst concentration, reaction temperature, reaction time, solvent loading (methanol) and moisture content were investigated using the oleaginous yeast biomass. The most suitable pretreatment condition was found to be 4 g L^{-1} NaOH and 1:20 (w/v) dried biomass to methanol ratio for 10 h at 50 °C and under ambient pressure. Under these conditions, the fatty acid methyl ester (FAME) yield was 97.7%. Therefore, the novel method of direct base-catalyzed methanolysis of *R. toruloides* is a much simpler, less tedious and time-consuming, process than the conventional processes with higher FAME (biodiesel) conversion yield.

1. Introduction

Global production and use of biodiesel has increased dramatically in recent years. It represents the most promising alternative fuel for compression-ignition (diesel) engines. Its benefits compared to petroleum derived fuels are that it is produced from renewable resources; is biodegradable and is less toxic (Al-Zuhair, 2007). Biodiesel fuelled engines also produce less carbon monoxide, unburned hydrocarbon, and particulate emissions than petro-diesel fuelled engines (Canakci and Sanli, 2008).

Biodiesel is generally produced from Tri Acyl Glycerides (TAG) in the form of high quality food-grade vegetable oils such as rapeseed, soybean, jatropha and palm oils by a transesterification process using alcohol. Biodiesel has been produced on industrial scale in the European Union (EU) since 1992 and in the USA since 1993 (National-Biodiesel-Board, 2008). In 2010, the world production was approximately 18,000 mL and the EU became the global leading producer of biodiesel with 10,187 mL (Biofuels-Platform, 2010). However, a major handicap in commercial biodiesel production is its high production cost. Utilization of high-quality virgin oils makes biodiesel more expensive than fossil fuels. Also, biodiesel production using conventional technology requires large amounts of arable land, which is expensive and in many regions is not a realistic option. Hence, alternative sources of TAG that are attainable through other routes are considered essential for a sustainable biodiesel industry, especially those that can be operated continuously with less pretreatment process and without extensive land requirement. Low cost feedstocks such as waste or non-edible oils and microbial oils are therefore becoming popular.

Many researchers have investigated lipid production through oleaginous microbial fermentation (Liu and Zhao, 2007; Meng et al., 2009; Shi et al., 2011; Xu et al., 2006). Oleaginous moulds,

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yeasts and algae exhibit the capacity to accumulate intracellular lipids in excess of 70% of their biomass during metabolic stress periods, especially during the stationary growth phase (Li et al., 2007). The major lipid component of oleaginous yeasts and fungi is TAG, composed mostly of C16 and C18 series long chain fatty acids, which are quite similar to those of vegetable oils, such as rapeseed oil and soybean oil (Ratledge and Wynn, 2002). In addition, biodiesel produced from microbial oil has good fuel properties such as low viscosity as well as high cetane number and heating value (Demirbas, 2008) and hence excellent engine performance. Moreover, microorganisms are fast growing and their cultivation is inexpensive compared to that of vegetables. Therefore, microbial lipids have the potential to be used as raw material in biodiesel production. However, little information is available so far on the production of biodiesel using lipids of microbial origin, although there are many recent reports of direct acid transesterification of microbial biomasses (Liu and Zhao, 2007).

In order to produce FAME from microorganisms there are several procedures that must be followed. Firstly, microbial biomass must be cultivated in stationary phase. Then, it must be dried and disrupted mechanically or non-mechanically in order to release the intracellular lipids. Then, the lipids are recovered by liquid–liquid extraction using organic solvents such as hexane, heptane, or either a mixture of hexane–ethanol or chloroform–methanol. Finally, the extracted lipids are esterified using methanol or ethanol as solvent and either strong acid (HCl or H_2SO_4) or base (NaOH or KOH) as catalyst. Then, separation of the organic and inorganic phase of the mixture must occur, usually with hexane, in order to recover the esterified lipids. In direct acid or base-catalyzed methanolysis, after the biomass recovery and drying steps, cell disruption, oil extraction and esterification of the microbial oils occur simultaneously (one-step process), while drying as pretreatment step and FAME recovery using hexane are considered necessary.

Base-catalyzed transesterification is the most commonly used method in commercial biodiesel production processes due to its cost-effectiveness, as the reaction is carried out under a low temperature and pressure environment using low cost materials such as sodium hydroxide and potassium hydroxide (Kumar Tiwari et al., 2007; Van Gerpen, 2005). Moreover, the conversion rate is high with no intermediate steps (Dizge et al., 2009). Acid-catalyzed esterification has not gained as much attention as base-catalyzed transesterification due to the slow reaction rate, need for more extreme temperature and pressure conditions and higher solvent to oil molar ratio (Soriano et al., 2009).

A problem with some inexpensive feedstocks (e.g. waste cooking oils, animal fats) is that they often have a high content of free fatty acids (FFAs) as well as some water. Consequently, alkaline catalysis produces soaps by neutralizing the FFAs and saponifying triglycerides in the presence of residual water (Alcantara et al., 2000). Soap formation leads to partial consumption of the catalyst and reduces biodiesel yield. Thus, sensitivity to FFAs and moisture represents a severe issue for large scale production of biodiesel with such feedstocks. Some researchers have stated that the feedstock should not contain more than 1% FFA for alkaline-catalyzed transesterification reactions (Freedman et al., 1984; Schwab et al., 1987). In order to avoid saponification, strong liquid acid catalysts have been preferred. Moreover, acid-catalyzed esterification of FFAs results in water formation which limits the completion of the reaction (Canakci and Van Gerpen, 1999; ISTC, 2006).

Although many researchers have investigated the potential of microbial lipids for biodiesel production (Gouda et al., 2008; Liu and Zhao, 2007; Meng et al., 2009; Shi et al., 2011; Xu et al., 2012), data for the FFA contents of these microbial lipids are limited. Liu and Zhao (2007) investigated the effects of acid and base catalysts on biodiesel production from oleaginous yeast biomass. They reported that the FAME conversion from *L. starkeyi* biomass was extremely sluggish (with yields lower than 20%) using base catalysts at 70 °C. According to the authors this result might be related to high lysis resistance of the yeast cell wall under anhydrous conditions. Under relatively hydrous conditions, significant amounts of saponification reaction products were observed. Thereby, they discarded the idea of base-catalyst direct transesterification and turned their attention to acid catalyzed methanolysis and optimized the process parameters. They obtained high FAME yields up to 98% with a desired quality biodiesel using acid-catalyzed transesterification.

The effects of base-catalyzed transesterification of microbial lipids on biodiesel production have not been reported in detail, although it can be achieved faster and more efficiently than that of acid catalysis (Freedman et al., 1984). Therefore, in this study the optimal conditions for base-catalyzed transesterification of microbial lipids have been investigated and identified. The findings of this research evince the functionality of this technique as well as demonstrating suitable operation parameters such as catalyst concentration, reaction temperature, reaction time, solvent loading (methanol) and moisture content to obtain the highest efficiency. The results are compared with the current three-step processes and with direct acid-catalyzed methanolysis in terms of their efficiency (FAME yields), total process duration and operation cost. Direct base-catalyzed transesterification resulted in higher fatty acid methyl esters (FAME) yield in lower reaction temperature and shorter time using less catalyst.

2. Methods

2.1. Microbial biomass production

The oleaginous yeast *Rhodosporidium toruloides* Y4 (provided by Professor Zongbao Zhao of the Dalian Institute of Chemical Physics in China), was used throughout this study. Bio-oil production using oleaginous yeast *R. toruloides* Y4 was carried out by using the nutrients solution obtained after the enzymatic pretreatment of rapeseed meal as described in Uçkun Kiran et al. (2012). The microbial oil production medium (1 L) was prepared using 900 mL filtered rapeseed meal hydrolyzate which was diluted to give an initial FAN concentration of 300 mg L^{-1}. The medium was supplemented with 0.4 g L^{-1} KH_2PO_4, 1.5 g L^{-1} $MgSO_4{\cdot}7H_2O$. The stock glucose solution (1000 g L^{-1}) was autoclaved separately and added at the beginning of the fermentation to obtain a concentration of 50 g L^{-1}. The inoculum (100 mL) was transferred aseptically and the fermentation was performed at pH 6, 30 °C and a stirring speed of 1200 rpm for 5 days (Electrolab)".

The biomass was dried at 60 °C overnight then used as feedstock for the transesterification reaction and condition optimization experiments. Lipid content of dried biomass was determined as 59%.

2.2. Transesterification process

Methanol was used as solvent and sodium hydroxide (NaOH) was used as catalyst for the transesterification of the microbial oils. Into a 20 mL lidded test tube, methanol (MeOH) and sodium hydroxide (NaOH) were added. The mixture was stirred vigorously until the NaOH was completely dissolved in the methanol. Then, dry biomass of *R. toruloides* Y4 was added. The mixture was placed in a water bath at the chosen temperature and samples were taken at different reaction times. In order to determine the optimum conditions for the process, some operation parameters were changed while some others were kept fixed. After cooling, the organic phases were collected for GC analysis of FAMEs.