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Full Length Article

Biodiesel production from *Mucor circinelloides* using ethanol and heteropolyacid in one and two-step transesterification

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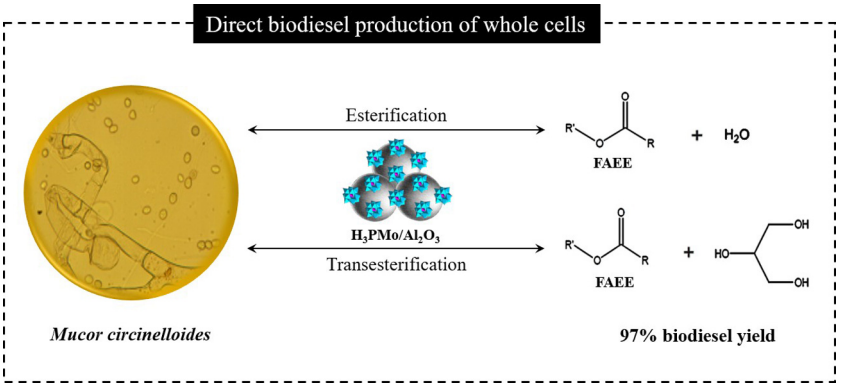
HIGHLIGHTS

- Two approaches for transesterification reaction of filamentous fungus were compared.
- Ethanol was used for lipid extraction and acyl acceptor.
- Reactions were catalyzed by heteropolyacid impregnated on alumina (H_3PMo/Al_2O_3).
- High levels of FAEE (>97%) were attained by both procedures.
- Direct transesterification of biomass is simpler and renewable process.

GRAPHICAL ABSTRACT



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ABSTRACT

Biodiesel production from *Mucor circinelloides* biomass was investigated by conventional method involving the lipid extraction using ethanol followed by transesterification (two-step process) in comparison with directly producing from the whole biomass without extraction (one-step process). Both processes used solid heteropolyacid catalyst (12-molybdophosphoric acid ($H_3PMo_{12}O_{40}$) support on alumina (Al_2O_3) under conditions previously set up (200 °C for 4 h). Either one or two-step process was able to convert the microbial lipids into ethyl esters (FAEE) with high yields (97%), though the former simplifies the production process due to the elimination of an oil extraction step that incurs oil loss. Moreover, to minimize the energy used for dewatering, this method can be also used for transesterification of wet *M. circinelloides* biomass. Direct transesterification of wet biomass greatly simplifies the process of FAEE production by eliminating the drying and oil extraction steps, making this a renewable and environmental friendly process.

1. Introduction

Sustainable development is now a major focus of research worldwide because of the environmental impacts of waste production and greenhouse gas emissions caused by the use of fossil fuels, which contribute to global warming [1,2]. Thus, research has focused on technologies that can replace petroleum-based refineries for biorefineries using renewable raw materials [3], including microbial refineries [4].

Single-cell oils may be defined as oils obtained from microorganisms [5], which have a similar type and composition of oils and fats to that obtained from plants or animals [6]. Advantages

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of single-cell oils are that microorganisms can accumulate high level of lipids and do not require arable land [7]. Few microorganisms are known to accumulate a significant amount of such lipids [8], but species that are able to do so to a level corresponding to more than 20% of their biomass are described as oleaginous [9,10]. Microbial oils are postulated to be an alternative to plant oils, but not all oleaginous microorganisms have ideal lipid profiles for biodiesel production. In this context, filamentous fungi have emerged as a promising resource in the development of new sustainable products. In particular, fungi belonging to the phylum Zigomicete have seen significant use in biorefineries, with special emphasis on the *Mucor* sp. fungus genus [4]. Members of this genus, particularly the species *Mucor circinelloides*, have many relevant features favoring its use for biodiesel production [11–13], including the presence of a high level of lipids in the mycelium (approximately 20% dry mass in wild-type strains) [14], as well as good biomass production during submerged batch cultivation using a wide range of carbon sources [15–17].

In a previous study, the Brazilian strain *Mucor circinelloides* URM 4182 was found to have potential to produce oleaginous biomass (SCO) for using as a feedstock for biodiesel production by enzymatic route [13]. In addition, advanced processes for oil extraction using ethanol coupled with microwave irradiation have been applied to reduce the steps needed to obtain microbial oil. Lipid extraction using ethanol as a solvent meets the concept of sustainable extraction (green extraction), which aims to use extraction processes having low energy consumption, reduced unit operations and which use alternative and renewable solvents. Nevertheless, microwave technology has yet to prove its potential at large scale applications [18]. Hence, it can be advantageous to perform extraction and transesterification reactions simultaneously. This approach designated as direct transesterification [19] has been suggested to simplify the production process and reduce the cost of biodiesel production [12,19]. Thus, the aim of this study was to compare the conventional procedure for ethyl esters (FAEE) production, in which lipid extraction using organic solvent is followed by transesterification of the lipid extract with the direct transesterification of whole cells without extraction. In this investigation the catalyst 12-molybdophosphoric support on alumina (H_3PMo/Al_2O_3) was chosen because acid-catalyzed reactions have been shown to be effective at converting both triacylglycerol and fatty acids into alkyl esters. This is important since free fatty acids (35 mg KOH g^{-1}) are a major constituent of *M. circinelloides* oil [13]. The choice of a solid acid catalyst was based on the potential economic and green benefits of solid acid catalyzed-biodiesel production [20,21], a process in which heteropolyacids (HPAs) have been extensively studied and shown to perform a wide range of reactions [22,23]. The majority of catalytic applications use the most stable and easily available Keggin HPAs, with typical ones including $H_3PW_{12}O_{40}$, $H_4SiW_{12}O_{40}$, $H_3PMo_{12}O_{40}$ and $H_4SiMo_{12}O_{40}$ [24]. HPAs possess very strong Brønsted acidity, much stronger than conventional acids such as H_2SO_4 and HCl. The acid sites in HPAs are more uniform and easier to control than other acid catalysts. Hence, HPAs frequently exhibit superior catalytic activity in acid-catalyzed reactions, including transesterification in both homogeneous and heterogeneous systems and favor the use of feedstocks with any acidity and moisture content.

2. Materials and methods

2.1. Materials

The strain of *Mucor circillenoides* f. griseo-cyanus URM 4182 was obtained from the mycology collection (URM) from the Federal University of Pernambuco (Recife-Pe, Brazil). All chemical reagents and solvents were of analytical grade and used without further purification. 12-molybdophosphoric acid ($H_3PMo_{12}O_{40}$) and anhydrous ethanol (99.8%) were purchased from the Vetec® Sigma-Aldrich. The aluminum oxide (calcined alumina A-1) containing 98.8% Al_2O_3 and surface area of 72 m^2 g^{-1} was supplied by Alcoa Aluminum Company S.A. Anhydrous sodium sulfate, ethyl acetate (99.5%) and hexane (65.0%) were supplied by Cromoline. Methanol (99.95%) and acetonitrile (99.9%) were purchased from J.T. Baker.

2.2. Microbial biomass production

The *Mucor circillenoides* URM 4182 culture was previously grown in PDA (Potato dextrose agar) at 30 °C for 72 h. Single Cell Oil production was performed in bioreactor model BioFlo®/CelliGen® 115, with a capacity of 1 L, equipped with a 6-Blade Rushton Impeller and temperature and pH control, dissolved oxygen monitoring and constant aeration. For biomass oleaginous production, 1×10^5 spores mL^{-1} were inoculated in bioreactor having a volume of 700 mL on culture medium (consisting of: glucose, 40 g L^{-1}; ammonium sulfate, 1.5 g L^{-1}; glutamic acid, 1.5 g L^{-1}; nicotinic acid, 1 mg L^{-1}; thiamine, 1 mg L^{-1} and yeast extract, 0.5 g L^{-1}). Cultivations were carried out aerobically (via supply of air to 1.5 vvm and agitation 250 rpm) at pH 4.5, 26 °C for 120 h and performed at least in duplicate. A pH meter (pH electrode sensor, Ingold, gel filled, Mettler Toledo) was utilized for monitoring the medium pH by addition of NaOH 0.1 mol L^{-1} through peristaltic pump. Dissolved oxygen was measured utilized an oxygen sensor (O_2 Sensors Series 6800 InPro®; Mettler Toledo). The initial oxygen mass transfer coefficient (kLa) was found to be 41.6 h^{-1} as determined by static methodology with a polarographic probe in the abiotic culture medium [25]. After fungal growth period (120 h), biomass was separated from culture medium by centrifugation (1520*g*) for 15 min and then the moisture content determined directly in moisture balance coupled with infrared (Marte ID 50). For comparison purpose, *M. circinelloides* URM 4182 biomass was also grown in cultivations performed in orbital shaker using the same culture medium.

2.3. Microbial oil extraction

Lipids were extracted from *Mucor* biomass using digestion system with radiation in the microwave region (Titan™ MPS PerkinElmer®) as previously established [13]. Each glass vessel was loaded with 5.0 g of wet fungal biomass (moisture 80% w/w) and 50 mL of ethanol (96% w/w). The extract containing lipids was recovered and evaporated in a vacuum rotary evaporator, and the microbial oil was subsequently dried at 60 °C until it reached a constant weight. Washed biomass was dried to calculate the biomass free of lipids and the amount of lipids was estimated as mass difference between the extracted lipids and the biomass before lipid extraction.

2.4. Heterogeneous catalyst synthesis

In the incipient-wetness, a required amount water to fill the pores of the support material (Al_2O_3) was added and then an amount of H_3PMo dissolved in alcohol solution (70%) at ambient temperature to a final concentration of 30 wt% was mixed to the support. The resulted material was dried at 100 °C for 1 h and subsequently calcined at 300 °C for 4 h, and the following properties: surface acidity (6.8 mmol H^+ g^{-1}); surface area (31.7 m^2 g^{-1}), pore diameter (78.0 Å) and pore volume (0.08 cm^3 g^{-1}).

2.5. Biodiesel synthesis

Reactions were performed with either microbial oil or microbial biomass and were codified as methods 1 and 2, as illustrated in Fig. 1.