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Full Length Article

Direct transesterification of *Mucor circinelloides* biomass for biodiesel production: Effect of carbon sources on the accumulation of fungal lipids and biofuel properties

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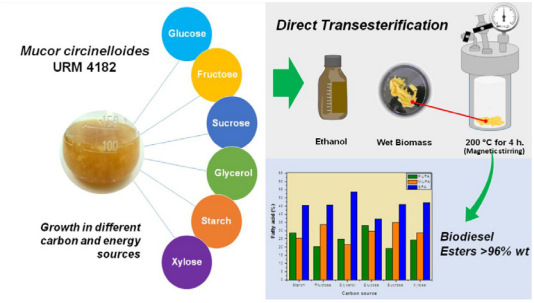
GRAPHICAL ABSTRACT



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ABSTRACT

The wild strain of *Mucor circinelloides* URM 4182 from a Brazilian culture collection was previously recognized as potential oleaginous microorganism that supplied single cell oil (SCO) with suitable properties for biodiesel synthesis. This work focused on assessing the accumulation of storage lipid by this strain grown in various media containing different carbon sources and the subsequent conversion of the microbial lipids into biodiesel. The chosen carbon sources can be obtained from several agro-industrial residues such as sucrose and fructose (sugarcane molasses), xylose (hydrolysate of lignocellulosic materials like sugarcane bagasse), starch (corn milling), ethanol and glycerol (byproducts from biodiesel production). The carbon source was found to influence the obtained fatty acid profile of *M. circinelloides* oil, realizing important attributes that favor its use for biodiesel production, including good levels of saturated (Cn: 0) and monounsaturated (Cn: 1) fatty acids. Polyunsaturated fatty acids with two (linoleic acid) or three double (linolenic acid) bonds were also found, but their concentrations decreased from 33.2 to 19.8% when glucose was replaced by a substrate based on sucrose. The feasibility of using low-cost feedstocks in the synthesis of microbial lipids was demonstrated, with the exception of ethanol that inhibited fungal growth. The lipid-bearing biomass was then subjected to direct transesterification using a robust solid acid catalyst (12-molybdophosphoric acid supported on alumina) in a high-pressure reactor, producing ethyl esters as biodiesel material with very high conversion yields (98.5%) and minor levels of byproducts.

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1. Introduction

The production of biodiesel using single cell oils (SCOs) has recently been highlighted as a potential source of renewable energy, which is concurrently compatible with the biorefinery concept [40]. SCOs mainly represent the triacylglycerols of microorganisms, which are similar to those found in oils and fats from animal and vegetable sources. Microorganisms capable of producing and accumulating more than 20% on their biomass in lipids are designated oleaginous and may belong to the group of bacteria, yeast, filamentous fungi and algae [12].

Oleaginous filamentous fungi present several biotechnological advantages for biodiesel production such as convenient and diverse fatty acid profiles, cost-effective downstream processing, and the ability to degrade a range of renewable carbon sources. Among the oil-producing fungi, those of the genus *Mucor* have great biotechnological importance because they accumulate high levels of triacylglycerol in their mycelium [40]. Concerning the lipid-producing Mucorales fungi, several previous studies have demonstrated that fungus *Mucor circinelloides* presents lipids rich in polyunsaturated fatty acids that can be used as alternative feedstocks for obtaining third-generation biodiesel [5,4,20,40,38,39].

Microbial lipid production does not depend on a particular season, climate, or location and does not use arable land. It can be achieved using a wide range of carbon sources such as waste streams from food industry or renewable carbon sources. This production is generally high-yielding and can also be accomplished with genetically modified organisms, which change the fatty acid composition and further enhance the yields [29,31].

The choice of the carbon source in SCO growth is a major factor in deciding the total production costs. Generally, conventional carbon sources such as glucose are used for the cultivation of fungi with concomitant accumulation of lipid. However, the introduction of alternative substrates as agricultural and industrial residues is advantageous due to their abundance and low costs [21].

In this context, the aim of the current research was to study the production of high-value oil obtained from the cultivation of *M. circinelloides* in different substrates and to convert the resulting microbial biomass into alkyl esters by direct transesterification using a solid acid catalyst. A wild strain of *M. circinelloides* URM 4182 was selected based on its potential feasibility to supply SCO with suitable properties for biodiesel synthesis when cultured in glucose-based substrate [4,5].

Our interest was focused on assessing the accumulation of storage lipid during the growth of this fungus for the production of SCO in various carbon sources such as sucrose, xylose, fructose, starch, ethanol, and glycerol. These sources were chosen because of their known potential for microbial lipid production as described in the literature in addition to their availability in large amounts in agro-industrial residues such as sugarcane molasses and lignocellulosic materials [27,9]. In addition, the approaches to quantify the influence of the carbon source on the fatty acid profile and biodiesel properties will be presented in this paper. In order to transform the lipids present in the microbial biomass into alkyl esters, an alternative procedure to replace the conventional method of biodiesel synthesis was proposed based on previous work developed by our research group [5]. This method combines in one-step the conversion of oil-bearing biomass to ethyl ester using ethanol (solvent extractor –acyl acceptor) and a solid acid catalyst, thereby avoiding the steps of cell disruption and oil extraction from biomass. The acid-catalyzed transesterification is better placed when compared to the base-catalyzed reaction because of its suitability for processing feedstocks even in the presence of moisture and/or free fatty acids [5,10].

2. Materials and methods

2.1. Microorganism

The culture of an isolate strain of *Mucor circinelloides* f. griseo-cyanus URM 4182 was obtained from the mycology collection (URM) from the Federal University of Pernambuco (Recife-PE, Brazil), maintained on potato dextrose agar (PDA) slants at 4 °C, and subcultured every month. The fungus was resubcultured onto PDA plates at 30 °C for 72 h before use as a source of inoculum.

2.2. Reagents

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 was supplied by Alcoa Aluminum Company S.A and used as a support to prepare the catalyst 12-molybdophosphoric acid supported on alumina (H_3PMo/Al_2O_3). Anhydrous sodium sulfate, ethyl acetate (99.5%) and hexane were supplied by Cromoline. Methanol (99.95%) and acetonitrile (99.9%) were purchased from J.T. Baker. Lewatit® GF202, a macroporous cation-exchange acid resin, was kindly donated by Lanxess (São Paulo, Brazil).

2.3. Catalyst preparation

The catalyst H_3PMo/Al_2O_3 was prepared via incipient-wetness impregnation. In a typical synthesis, the heteropolyacid mass was dissolved in an alcoholic solution 70% at room temperature, transferred to a ceramic crucible containing the support (Al_2O_3) and then mixed. The solid formed was then dried at 100 °C for 30 min, followed by calcination at 300 °C for 1 h in a muffle furnace, this step was repeated two additional times with a final calcination at 300 °C for 3 h. H_3PMo/Al_2O_3 catalyst was prepared using 30% wt. H_3PMo relative to the support. The resulted material H_3PMo/Al_2O_3 was dried at 100 °C for 1 h and subsequently calcined at 300 °C for 4 h achieving 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 $cm^3 g^{-1}$). Further properties concerning this catalyst are shown in Supplementary data.

2.4. Culture media

Different carbon sources purchased from Vetec (Rio de Janeiro, Brazil), namely, glucose, fructose, sucrose, soluble starch, xylose, ethanol and glycerol were tested for their impact on lipids production. The carbon sources were added individually to batches of culture medium to give a substrate mass level of 40 g L^{-1} supplemented with following nutrients: ammonium sulfate, glutamic acid, yeast nitrogen base without amino acids; nicotinic acid and thiamine at concentrations that give the carbon to nitrogen ratio 55:1 as previously established [5]. Culture media were adjusted to pH 4.5 with NaOH (1 mol L^{-1}).

2.5. Fungi cultivation

Cultivations were performed in 250 mL Erlenmeyer flasks contained 50 mL of culture medium and inoculated by aseptically transferring an aliquot of spore suspension to achieve a final concentration of 10^5 spores mL^{-1}. Flasks were incubated at 26 °C on an orbital shaker at 250 rpm for 96 h. During cultivation pH was measured every 24 h and manually adjusted to 4.5 by the addition of NaOH (1 mol L^{-1}). Samples obtained at 96 h incubation were processed for growth and lipid production was assayed.