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Assessing the potential of non-edible oils and residual fat to be used as a feedstock source in the enzymatic ethanolysis reaction

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ABSTRACT

The focus of this work was to evaluate the potential of non-edible feedstocks to yield biodiesel by an enzymatic route. The ethanolysis of native oils from tropical crops, such as andiroba (*Carapa guianensis*), babassu (*Orbignya* sp.), jatropha (*Jatropha curcas*), macaw palm (*Acrocomia aculeata*), palm tree (*Elaeis guineensis*) and industrial waste (beef tallow) in solvent-free system was studied. All reactions were carried out with the microbial lipase from *Burkholderia cepacia* immobilized on a silica-polyvinyl alcohol matrix in a solvent-free system at 50 °C for a maximum period of 24 h. Under the conditions tested the biocatalyst was efficient in converting all fatty acids in the lipid feedstocks into the corresponding ethyl esters. Viscosity values for biodiesel samples obtained in each reaction (4.3 and 6.0 mm^2/s) showed consistent reduction in relation to their original feedstock material, which also confirms the high conversion of triglycerides to ethyl esters (>94.5%). This comparative study shows that the formation of ethyl esters from different non-edible feedstocks was feasible and can provide a considerable increase in the prospect of attaining an environmental sustainability of the process as a whole.

1. Introduction

Biodiesel is defined as a biofuel derived from renewable biomass for use in internal combustion engines with ignition by compression or for generation of another type of energy that can partially or totally substitute fossil fuel (Takahashi and Ortega, 2010).

Conventionally, the production of industrial biodiesel is based on the chemical transesterification of vegetable oils with methanol, using homogeneous catalysts to promote the cleavage of triglycerides molecules and generate a mixture of fatty acid alkyl esters (biodiesel) (Leung et al., 2010). In this process, high rates can be obtained at short reaction times; however, the energy consumption is very high and requires significant downstream processing steps, such as washing, separation and purification (Ganesan et al., 2009). Alternatively, transesterification of triglycerides to produce biodiesel can be processed by heterogeneous catalysts, including immobilized enzymes (Bajaj et al., 2010). This process has many advantages over its chemical counterpart: it works well with fluctuating raw material quality, it needs fewer processing steps, it produces higher-quality glycerol (a valuable byproduct) and it uses less energy and generates less wastewater (Lam et al., 2010).

The enzymatic transesterification using ethanol instead of methanol has been suggested as a cleaner and more sustainable alternative for biodiesel production. (Stamenkovic et al., 2011). Furthermore, ethanol is a larger and heavier alcohol than methanol, which means a mass yield gain in the enzymatic synthesis of fatty acid ethyl esters (FAEE), resulting in a higher biodiesel per unit of oil (Brunschwing et al., 2012). In some countries, such as Brazil, ethanol is sold at lower prices than methanol, which means that the alcohol component is always significantly cheaper than the oil component. Thus, the extra volume gain when using ethanol instead of methanol could become a major sales argument, particularly for the Brazilian market (Stamenkovic et al., 2011; Brunschwing et al., 2012).

The fundamental advantage of an enzymatic biodiesel process is that triglycerides (and partial glycerides), as well as free fatty acids (FFA), can be efficiently transformed into biodiesel under the same mild conditions (Lam et al., 2010). By selecting the appropriate enzyme, it is possible to make a continuous single-step process, even with very high FFA content in the oil. This allows the use of low-quality and non-edible oils, without causing a negative impact on the environment (Lam et al., 2010; Adlercreutz, 2013).

The choice of feedstock for biodiesel production depends largely on geography, with rapeseed and sunflower oils dominating the European production, soybean oil dominating the USA and Latin American production, and palm oil mainly being used in Asia and

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Table 1
Estimated oil content and yields of the selected tropical crops used in this work.

Feedstock	Oil content (%)	Oil yield (L/ha/year)
Andiroba	43	1420
Babassu	60–65	2689
Jatropha curcas	40–50	1892
Macaw palm	25	4000
Palm	30–60	5950

Sources: Atabani et al. (2012) and Bergmann et al. (2013).

Brazil. The use of edible oils for fuel production is controversial, and with increasing prices, there is a growing interest in alternative feedstocks. It is clear that the search for beneficial biodiesel sources should focus on feedstocks that do not compete with food crops, do not lead to land-clearing and provide greenhouse-gas reductions. These feedstocks include not only high-yielding (Table 1), non-edible tropical crops, such as andiroba (*Carapa guianensis*), babassu (*Orbignya* sp.), jatropha (*Jatropha curcas*), macaw palm (*Acrocomia aculeata*), palm tree (*Elaeis guineensis*), but also waste material as beef tallow. Large amounts of these non-edible oil plants are available in several regions of Brazil (Bergmann et al., 2013) with low cost of exploration and beef tallow becomes a second source of raw material to produce biodiesel in many countries. Saponification number, iodine value and fatty acid profile play an important role in selection of feedstock for biodiesel production. Based on these parameters, the physicochemical properties of several feedstocks (some of which still little explored) were investigated in order to identify their potential as a starting material in the enzymatic transesterification reactions carried out under suitable conditions.

Lipase from *Burkholderia cepacia* was immobilized on epoxy silica-polyvinyl alcohol (SiO_2-PVA) composite; the enzyme was chosen based on its suitability for typical biotransformation applications (Freitas et al., 2009; Da Rós et al., 2010). In addition, to the conventional methods of analysis, information on the quality of the biodiesel samples obtained by ^{1}H NMR, as well as thermogravimetric analysis (TGA), help to attest the efficiency of the transesterification reaction and to select the feedstocks which gave samples having properties in accordance with standard specification to be used as a fuel.

2. Materials and methods

Commercial *B. cepacia* lipase, from Amano Pharmaceuticals (Nagoya, Japan), in a crude form was used without further purification. Tetraethoxysilane (TEOS) was acquired from Aldrich Chemical Co. (Milwaukee, WI, USA). Epichlorohydrin, hydrochloric acid (minimum 36%), ethanol (minimum 99%), polyvinyl alcohol (PVA molecular weight 72,000) and polyethylene glycol (PEG molecular mass 1500) were supplied by Reagen (RJ, Brazil). The feedstocks were obtained from several suppliers as follows: andiroba and babassu oils (Pulcra, Jacarei, SP, Brazil), jatropha oil (IAPAR, Londrina, PR, Brazil), macaw palm oil (Association of Small Farmers D'Antas, Minas Gerais, Brazil), palm oil (Agropalma, Belém, PA, Brazil) and beef tallow (Fertibom, Catanduva, SP, Brazil). Other reagents and solvents were of standard laboratory grade.

2.1. Support synthesis and lipase immobilization

Silica-polyvinyl alcohol (SiO_2-PVA) composite was prepared by the hydrolysis and polycondensation of tetraethoxysilane according to the methodology described by Da Rós et al. (2010). Activation of SiO_2-PVA particles was carried out with epichlorohydrin at 2.5% (w/v) pH 7.0 for 1 h at room temperature, followed by exhaustive washings with distilled water. Epoxy SiO_2-PVA particles were soaked in hexane under stirring (100 rpm) for 1 h at 25 °C. Then, the hexane excess was removed and lipase was added at the ratio of 1:4 grams of enzyme per grams of support. PEG-1500 was added to the enzyme solution at a fixed amount (5 mg g^{-1} support). Lipase and support were maintained in contact for 16 h at 4 °C under static conditions. The immobilized lipase derivative was filtered (nylon membrane 62HD from Scheiz Seidengazefabrik AG, Thal Schweiz, Switzerland) and thoroughly rinsed with hexane. Twelve batches of immobilized lipase were prepared yielding particles with the following properties: average pore diameter (29.42 Å), surface area BET (337 m^2 g^{-1}) and porous volume (0.25 cm^3 g^{-1}). The average measured biocatalyst hydrolytic activity was 1814 ± 281 U g^{-1}, according to the methodology described by Soares et al. (1999). The biochemical, kinetic properties, thermal stability and operational stability of this immobilized lipase are described elsewhere (Da Rós et al., 2010; Freitas et al., 2010).

2.2. Biodiesel synthesis

Reactions were performed in a jacketed cylindrical glass reactor (6 mm high × 4 mm internal diameter and 50 mL capacity, coupled with a reflux condenser) containing 20 g of substrate consisting of feedstock-to-ethanol at molar ratio of 1:9 or 1:7, without addition of solvents. The mixtures were incubated with lipase immobilized on SiO_2-PVA at a fixed proportion of 500 units per gram of lipid raw material. Duplicate reactions were performed for a maximum period of 24 h, under constant agitation (150 rpm) at 45 °C. Aliquots were taken at various time intervals throughout the reaction time and diluted in *n*-hexane for GC-analysis.

2.3. Downstream procedure

When the reaction was completed the lipase was separated from the medium and the organic phase was washed twice with one volume of water to remove both the remaining ethanol and the free glycerol as a by-product. Residual water was removed with a rotary evaporator to attain the final fatty acid ethyl ester product. AKF5000 Karl Fischer (K90365 model, Koehler Instrument Company, Inc) was used to measure the concentration of the water remaining in the purified product.

2.4. Feedstock characterization

Physicochemical properties (iodine value, saponification value, acid value, peroxide value and viscosity) were determined following methods described by the American Oil Chemists' Society (AOCS, 2004). Fatty acid composition was determined by capillary gas chromatography using a CGC Agilent 6850 Series GC System with a capillary column: DB-23 Agilent (50% cyanopropyl) – methylpolysiloxane, size 60 m, Ø int. 0.25 mm, 0.25 mM film. Helium was used as the carrier gas at rate of 1.00 mL/min and linear speed of 24 cm seg^{-1}. The temperatures of the detector and injector were set at 280 °C and 250 °C, respectively. The column temperature was kept at 100 °C for 5 min, heated to 215 °C at 5 °C/min and kept constant for 34 min. The volume injected was 1.0 mL. Degree of unsaturation (DU) and long chain saturated factor (LCSF) were determined according to the fatty acid composition. DU was obtained using (Eq. (1)), taking into account the amount of monounsaturated and polyunsaturated fatty acids (wt.%) present in the feedstock. LCSF parameter was obtained from Eq. (2), based on the composition of saturated fatty acids and lending more weight to the composition of fatty acids with a long chain.

$$DU = (\text{wt.\% monounsaturated}) + 2(\text{wt.\% polyunsaturated}) \quad (1)$$

$$LCSF = (0.1 \cdot C16) + (0.5 \cdot C18) + (1 \cdot C20) + (1.5 \cdot C22) + (2 \cdot C24) \quad (2)$$