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One-step enzymatic production of fatty acid ethyl ester from high-acidity waste feedstocks in solvent-free media

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ABSTRACT

This work aims to demonstrate the enzymatic production of fatty acid ethyl ester biodiesel from highly acidic feedstock in a single-step reaction, without co-solvents and avoiding the inhibition of the enzyme by ethanol and glycerol. Additionally, an empirical equation is proposed to predict the kinetics of the production reaction as a function of the used feedstock and catalyst concentration. Biodiesel production from highly acidic feedstock perform via simultaneous esterification of free fatty acids and transesterification of triacylglycerols. Enzymatic catalysis is one of the most promising alternative technologies for the biodiesel production. Increasing of the enzymatic bioactivity is crucial for the success of such process in industrial scale. Currently, stepwise addition of the alcohol or the use of co-solvents have been proposed to avoid enzyme inhibition, such strategies add downstream processes to the production. These results can be applied to the development economical-viable enzymatic production of biodiesel in industrial scale.

1. Introduction

Biodiesel has become an important renewable fuel as an alternative to petroleum-based energy. Biodiesel is considered a carbon-neutral fuel because the carbon present in its exhaust originally comes from the plant taking in atmospheric carbon and fixing it. Considering the existing restrictions on greenhouse gas emissions, biodiesel provides significant advantages compared with petroleum-based fuels. Biodiesel consists either of fatty-acids-methyl-esters (FAME) from methanolysis reactions or fatty-acids-ethyl-esters (FAEE) when using ethanolysis reactions. Industrial sources routinely produce biodiesel from vegetable oil using alkaline transesterification of triacylglycerols (TAG), which generates glycerol as a by-product and requires additional purification steps. Additionally, the presence of even a small amount of contaminating free fatty acid (FFA), less than 4%, impairs the transesterification reaction by forming soaps when alkaline catalyst is added (Ma and Hanna, 1999; Fukuda et al., 2001).

The high cost of feedstock for biofuel production can represent up to 95% of the total cost of production and acts as one of the main bottlenecks to its universal production and consumption (Kulkarni and Dalai, 2006; Zhang et al., 2003). Most Biodiesel plants are currently using refined edible vegetable oils as their main feedstock; therefore, the cost of refined vegetable oils contributes to most of the overall production cost. Thus, the price of biofuel feedstock controls the global price of biodiesel (Lam et al., 2010). Using low cost feedstocks, such as waste cooking oil and other high-acidity fats, can reduce this cost (Lou et al., 2008; Watanabe et al., 2005; Zafiropoulos et al., 2007), but these sources can form soap during alkaline-catalyzed transesterification, which inhibits the biofuel production reaction (Zhang and Jiang, 2008). Furthermore, large amounts of soap can aggregate and prevent the separation of glycerol from biodiesel (Demirbas, 2009; Zhang and Jiang, 2008). Acid-catalyzed transesterification has been proposed as a method for biodiesel production from high-acid oils. Many chemical methods have been developed to produce biodiesel by esterification of FFAs (Berchmans and Hirata, 2008; Lou et al., 2008; Zafiropoulos et al., 2007; Zhang and Jiang, 2008). Both processes provide the opportunity to use low-cost, high-acidity substrates for biodiesel production (Cavalcanti-Oliveira et al., 2011; Kusdiana and Saka, 2004).

Recently, researchers have proposed methods to produce biodiesel by enzymatic transesterification using immobilized lipases as the catalytic agent. Lipases (E.C.3.1.1.3) have become popular in the last 10 years because they are active in mild reaction conditions

Abbreviations: FA, fatty acid; FFA, free fatty acid; FAEE, fatty acid ethyl ester; FAME, fatty acid methyl ester; TAG, triacylglycerol; HAWF, high-acidity waste feedstock; POFA, palm oil fatty acid; CRO, chicken residual oil; TLC, thin layer chromatography; HPLC, high performance liquid chromatography.

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while also generating fewer undesirable by-products. These enzymatic reactions make the recovery of biofuel products easy and are insensitive to the presence of FFA (Kulkarni and Dalai, 2006). The catalysts can also be recovered after reaction, which lowers the cost of biofuel production. Although lipase-catalyzed biodiesel production has certain advantages, the process has not been fully implemented on an industrial scale due to the high cost of enzymes, slow reaction rates and the deactivation of enzymes due to reaction by-products (Bajaj et al., 2010). The large amount of enzyme needed for each reaction makes this process extremely expensive. Paired with long reaction times of about 24 h, these systems are cost-prohibitive to industrial producers. Commercial lipases are inhibited by the polar coating effect that short-chain alcohols, such as methanol and ethanol, and glycerol have on the surface of the enzyme, which decrease their interaction with TAG (Fu and Vasudevan, 2010; Lara and Park, 2004; Tan et al., 2010). To avoid this inhibition, strategies such as the use of co-solvents like *t*-butanol (Ferrão-Gonzales et al., 2011; Fu and Vasudevan, 2010; Lara and Park, 2004; Li et al., 2006; Royon et al., 2007), the use of longer-chain alcohols (for instance, *n*-butanol) as acyl acceptors (Iso et al., 2001) and the stepwise addition of short-chain alcohols, as methanol and ethanol (Hama et al., 2007; Matassoli et al., 2009; Wang et al., 2010) have been suggested; however, such strategies require additional downstream processing that makes production more expensive and time-consuming. Presently, there are no processes that produce biodiesel in an industrially-viable manner and with a one-step addition of short chain alcohols.

This work presents a new method of biodiesel production by simultaneous enzymatic esterification of FFA and transesterification of TAG, two products that are present in high-acidity waste feedstocks (HAWFs) from palm oil (*Elaeis guineensis*) refinement and the chicken processing industry, called chicken residual oil (CRO). The HAWFs used in this work have different ratios of FFA and TAG. The results suggest that the FFA present in HAWFs can act as a surfactant to avoid the coating effect that causes lipase inhibition. Additionally, an empirical equation is proposed, based on the rate constants for the esterification and transesterification reactions, that predicts the yields of the enzymatic FAEE production from HAWFs with high efficiency. Our results may help develop enzymatic processes for biodiesel production that have the potential to be both economically and technically viable for industrial applications.

2. Methods

2.1. Enzymatic reactions

FAEE Biodiesel synthesis was performed in a Tec-Bio-1,5 reactor (Tecnal, Piracicaba, SP) attached to a reflux condenser. Temperature and agitation were controlled automatically by the reactor software interface. HAWFs, including palm oil fatty acid (POFA) obtained from distilling oil palm from (*E. guineensis*) oil or chicken residual oil (CRO) obtained from broiler chicken processing in the food industry, were equilibrated with ethanol at a ratio of 1:5 at 60 °C. The molar excess of ethanol was calculated using the average fatty acid composition and density of the used feedstocks. The fatty acid (FA) compositions of the HAWFs were obtained from Dubois et al. (2007) and from Feddern et al. (2010). Immobilized lipase from *Candida antarctica* (Novozym 435) was then added at the weight-to-volume concentrations described in Figures caption. The system was reacted at 60 °C at a constant stirring speed of 400 rpm. A filter was installed in the bottom sampler of the reactor flask to separate the catalyst from the collected aliquots during sampling. Aliquots were collected using a 1 mL syringe through the bottom sampler of the reactor without stopping the agitation. The thin layer chromatography (TLC), high performance liquid chromatography (HPLC) or titrimetric analysis were performed using 200 μL of the samples, as described below.

2.2. Titrimetric analysis of free fatty acid

For titrimetric analysis, reaction aliquots were diluted in 15 mL of ethanol and 6 drops of 0.1% phenolphthalein. Titration of free fatty acid was then performed against a standardized 0.05 N KOH solution until the phenolphthalein changed color. Blank titrations of 15 mL of ethanol were performed to measure the basal acidity of the alcohol. All titrations were conducted in triplicate. All reagents used were of analytical grade of purity.

2.3. Measurement of fatty acid methyl esters and triacylglycerols

To measure the FAEE and TAG contents, 800 μL of hexane was added to each sample and mixed vigorously, and then, 150 μL of this mix was diluted in 850 μL of methanol and analyzed by chromatography in a Hitachi Elite Lachrom HPLC equipped with a 20 μL sample loop. HPLC analyses were performed using a C18 Lichrosphere column (Merck) equilibrated at 40 °C. UV absorption was measured at 210 nm. Chromatographic runs were performed by isocratic elution using a mixture of methanol:hexane (85:15) for FFA, FAEE and TAG analysis (Royon et al., 2007). Yields of FAEE production were determined by the fraction of FAEE in the samples measured by the absorbance at 210 nm using FAEE standards from Supelco. All solvents and standards used were of chromatographic grade.

Immobilized lipase from *C. antarctica* (Novozym 435) was kindly given by Novozymes Latin America. POFA was donated by Petrobras (Biodiesel Production Unity, UBC Candeias, BA). CRO was provided by Biotank Gestão de Resíduos LTDA, Lauro de Freitas, BA. All other reagents were of analytical grade.

3. Results and discussion

This work demonstrates an enzymatic process for the production of FAEE-based biodiesel from HAWF. Two HAWFs were used in this work: FFA from palm oil (POFA) and chicken residual oil (CRO). A 50–50 blend of the two feedstocks was also used to evaluate the effect of the feedstock acidity on the kinetics of the enzymatic production of FAEE. Both feedstocks were used in raw form, with no pre-treatment. The acidities and TAG content of HAWF were determined as described in the Section 2. Table 1 summarizes the main properties of POFA, CRO and the blend of these two HAWFs, hereafter referred to as Blend. Acidity is expressed as acidity index (I_a) what reflects the concentration of FFA in fraction of equivalent mols of oleic acid. TAG content is expressed as TAG index (I_{TAG}) what reflects the fraction of FA in TAG form.

Although the oil to alcohol molar ratio is one of the parameters that usually affect the production yields of biodiesel, there is no agreement on the influence of this parameter on the rate constant of the lipase activity in different reaction media, mainly if high-acidity feedstocks are used (Fjerbaek et al., 2009). Thus, each

Table 1
Acidity index (I_a), fraction of TAG (I_{TAG}) and density (D) of feedstocks.

Feedstock	I_a[a]	I_{TAG} (mol/mol)[b]	D (g/ml)
POFA	0.805 ± 0.002	0.120 ± 0.008	0.8991[c]
CRO	0.089 ± 0.002	0.824 ± 0.016	0.9112[d]
Blend	0.455 ± 0.004	0.479 ± 0.028	0.9009[d]

[a] In equivalent mol of oleic acid.
[b] Considering the average fatty acid composition (see Material and Methods).
[c] At 40 °C.
[d] At 25 °C.