EFFECT OF THE REACTION MEDIUM ON THE ENZYMATIC ESTERIFICATION OF CARBOXYLIC ACIDS FROM BIO-OIL

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ABSTRACT

The global demand for petroleum fuels and environmental concerns have driven the search for alternatives like biofuels. Triglyceric biomass, when thermally cracked, produces bio-oil similar to fossil fuels but with a high acidity, due to the presence of carboxylic acids. This study aims to reduce the acidity index (AI) of bio-oil using immobilized *Candida antarctica* lipase B (CALB, Novozyme 435). In initial experiments, we tested a solvent-free medium containing bio-oil and ethanol at a mass ratio of 1:3, and CALB concentrations of 3, 5 and 10% (w/w related to bio-oil mass), at 45 °C for 5 h, for 10% CALB the AI values were reduced from 76.2 mg KOH g^{-1} to 15.9 mg KOH g^{-1}. In additional experiments varying amounts of *n*-hexane (5, 15, 50 and 75 % v/v of the reaction medium) were added, while keeping the mass ratio and temperature constant. The AI was reduced from 76.2 mg KOH g^{-1} to 8.9 mg KOH g^{-1} when used 75 % of *n*-hexane in the reaction medium. The CALB remained active, indicating optimal conditions for enzyme efficiency in renewable fuel production, such a biodiesel.

Keywords: Bio-oil. Acidity index. Esterification. Enzymatic catalysis.

1 INTRODUCTION

Industry is increasing its use of renewable energy sources in response to the depletion of natural energy reserves and the need to reduce greenhouse gas emissions, seeking economic and sustainable alternatives to fossil fuels. Bio-oil, obtained by the pyrolysis of plant biomass and distilled to produce petroleum-like fuels, is one such source, with high calorific value and low water content.[1] However, crude bio-oil has high acidity, due to the presence of short-chain carboxylic acids, formed during the breakage of C-C bonds in the fatty acids of triglycerides. To lower this acidity, the carboxylic acids can be esterified using acid, basic, or enzymatic catalysts.[2] The esterification of these acids, catalyzed by lipases, is a specific and sustainable approach.[3]

The acidity of bio-oil is expressed as the acidity index (AI) and can range from 110 to 207 mg KOH g^{-1}, while the acceptable value in petroleum refineries ranges from 0.5 to 3 mg KOH g^{-1}. In previous studies with *Candida antarctica* lipase B (CALB, Novozyme 435), *Rhizomucor miehei* lipase (Lipozyme RM-IM), and *Thermomyces lanuginosus* lipase (Lipozyme TL-IM), CALB was the most effective in reducing the AI of bio-oil from an initial value of 96,8 mg KOH g^{-1} to 30,1 mg KOH g^{-1} at 40 °C for 72 h, with a 1:3 mass ratio of bio-oil to ethanol.[4] However, these studies did not determine the best conditions for esterification. In the current work, we seek to reduce bio-oil acidity to acceptable levels, using different alcohols, substrate ratios, reaction temperatures, and catalyst amounts, in both organic and solvent-free media.

2 MATERIAL & METHODS

The bio-oil used for all esterification reactions was produced after the thermal cracking of refined soybean oil (SOYA - Bunge Brasil) at 525 °C, with a residence time of 4.5 s. The carboxylic acids in the crude bio-oil were quantified by gas chromatography mass spectrometry (GC-MS) with a Restek Stabilwax capillary column and the compounds were identified by comparison with the NIST 08 Mass Spectral Database.[5,6]

Initially, three different concentrations of CALB were added (3, 5 and 10%, lipase mass relative to bio-oil mass), using a solvent-free reaction medium, at a substrate mass ratio of 1:3 (bio-oil:ethanol). The reaction time was 5 h, with aliquots taken hourly for acidity index (AI) measurement. The reactions were done in hermetically sealed 50-mL Erlenmeyer flasks. The lipases were weighed and reserved in test tubes. Ethanol and bio-oil were then added, and the tubes were incubated on an orbital shaker at 180 rpm and 45 °C.

Subsequent experiments investigated the effect of adding *n*-hexane to the reaction medium. Experiments were done with a bio-oil to ethanol mass ratio of 1:3, in 125 mL Erlenmeyer flasks. The bio-oil was dissolved in *n*-hexane at different percentages of the reaction medium (5, 15, 50 and 75% v/v) with ethanol. The enzyme amount was kept fixed at 5 % (w/w) relative to the mass of bio-oil, the reaction time was 2 h at 45°C in an orbital shaker at 180 rpm. Samples were collected at the end of the reaction to determine the AI.

The AI index was determined for crude bio-oil from the thermal cracking, as well as for the bio-oils resulting from the ethylic esterifications reactions done with the addition of *n*-hexane and in solvent-free medium. The method was adapted from the standard method ABNT NBR 14448 [9], using KOH in ethanol (0.1 mol L^{-1}).

The initial activities of lipases and their activity post-reaction were determined with ethyl-oleate synthesis reactions, done in 25-mL Erlenmeyer flasks, using 5 mL of reaction medium containing *n*-hexane, ethanol (210 mmol L^{-1}) and oleic acid (70 mmol L^{-1}). The flasks were placed in an orbital shaker at 40 °C and 180 rpm. The reaction was started with the addition of 110 mg of the immobilized preparation. At each sampling time, a 100 µL sample of the mixture was collected and analyzed for free fatty acids by the Lowry-Tinsley method.[8,9]

3 RESULTS & DISCUSSION

The main carboxylic acids of the crude bio-oil were caproic (2.3%), heptanoic (2.1%), palmitic (1.7%), oleic (1%), caprylic (0.9%), and linoleic (0.6%). The AI of bio-oil was 135.1 mg KOH g^{-1}.

Figure 1 shows the profiles for the AI during the ethyl esterification of bio-oil using three concentrations (3, 5 and 10% w/w related to bio-oil mass) of CALB (Novozyme 435). At 5 h, both 5 and 10% CALB gave similar AI values, 17.9 mg KOH g^{-1} and 15.9 mg KOH g^{-1}, respectively, while 3% CALB resulted in an AI of 21.5 mg KOH g^{-1}.The Brazilian legislation requires the acidity index of bio-oil to be around 0.5 mg KOH g^{-1}, so further work is needed to reach this value.[9] The remaining carboxylic acids are likely short-chain acids that CALB cannot esterify.

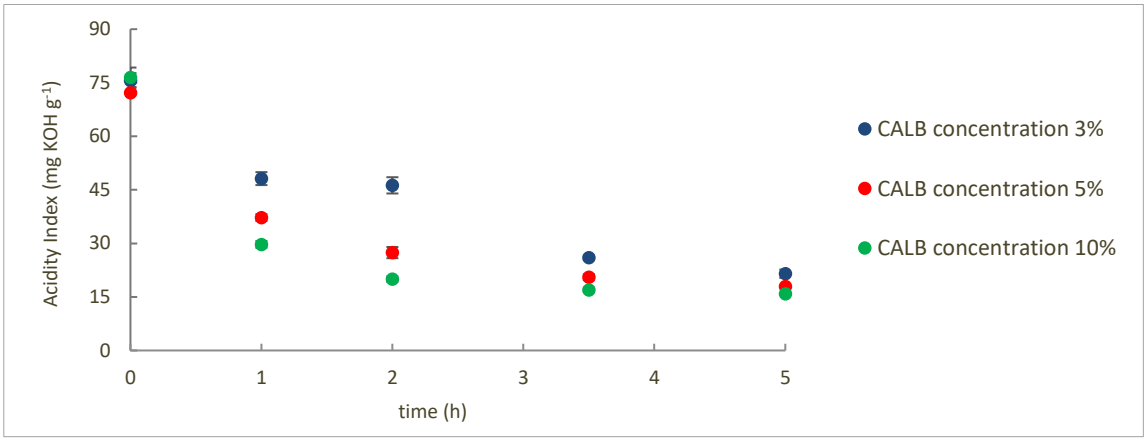

Figure 1 Reduction of bio-oil acidity index via lipase-catalyzed ethylic esterification reactions in three concentration of *Candida antarctica* lipase B (3, 5 and 10 % w/w related to bio-oil mass)

The 5% concentration of lipase was chosen for subsequent reactions with the addition of *n*-hexane to the medium (Table 1). The lowest AI (8.9 mg KOH g^{-1}) was obtained for the reaction medium containing 75 % (v/v) *n*-hexane, while for the other solvent concentrations, the AI varied from 15.8 mg KOH g^{-1} for with addition of *n*-hexane to 54.4 mg KOH g^{-1} in absence of solvent.

Table 1 Acidity index and enzyme activities after esterification reactions with addition of *n*-hexane

n-hexane (%)	Acidity index (mg KOH g^{-1})	Residual activity (U g^{-1})	Loss of activity (%)
0	54.4	38.9	81
5	45.9	42.7	80
15	29.5	43.4	79
50	15.8	77.6	63
75	8.9	83.2	60

Reaction conditions: Bio-oil esterification: mass ratio 1:3 (bio-oil:ethanol), 45 °C; 2 h; at 180 rpm in an orbital shaker; Activity determined by ethyl-oleate synthesis [8].

The residual activities of CALB were determined after the esterification reactions done with the addition of *n*-hexane to assess the stability of the enzymes during the reaction. CALB had an initial activity of 208.9 U g^{-1}. CALB lost over 50 % of its activity under both conditions. The loss of activity was reduced in the presence of n-hexane, relative to the solvent-free medium. These results suggest that it is not due to interaction with the enzyme-substrate but is a consequence of substrate-solvent mixture, such a competitive inhibition.[10]

The reduction in the AI can be attributed the protection of the lipase by *n*-hexane,[11] reducing denaturation caused by ethanol. In another study using a bio-oil derived from triglycerides, the IA was reduced from an initial value of 68.5 mg KOH g^{-1} to 12.7 mg KOH g^{-1} after ethyl esterification for 6 h using an acid catalyst (H_2SO_4) at 75°C and an alcohol/bio-oil mass ratio of 1:1.[5] In this

work, in the presence of 75% *n*-hexane the reduction in the acidity index was higher (from 76.2 mg KOH g^{-1} to 8.9 mg KOH g^{-1}), after two hours of reaction, at 45°C, and a substrate mass ratio of 1:3, making the enzymatic catalytic process more efficient.

4 CONCLUSION

The study demonstrates that CALB can reduce the acidity index of bio-oil by catalyzing the ethyl-esterification of carboxylic acids. The addition of *n*-hexane as a solvent enhances the efficiency of the process, making it faster compared to previous methods using acid catalysts. This enzymatic approach offers potentially economically viable method for bio-oil treatment.

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