METHYL ESTERIFICATION OF CARBOXYLIC ACIDS PRESENT IN BIO-OIL USING COMMERCIAL LIPASES

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ABSTRACT

The triglyceric biomass has been investigated in recent years as an alternative source for the production of fuel through thermal cracking, which generates solid, liquid, and gaseous products with high energy contents. The liquid product, known as bio-oil, has characteristics similar to those of fossil fuels. It contains mainly hydrocarbons and carboxylic acids, the latter limiting the use of bio-oil as fuel. In this study, commercial soybean oil was thermally cracked, resulting in a bio-oil with a relatively high acidity index, with the carboxylic acids being subsequently methyl-esterified, through enzymatic catalysis, to reduce the acidity index of the bio-oil. Reactions were done at 40 °C for 6 h, using lipase B from *Candida antarctica* (CALB) and lipase from *Rhizomucor miehei* (Lipozyme RM-IM) under different mass ratios of 1:1 and 1:3 (methanol:bio-oil). The best result was obtained using lipase CALB at a concentration of 5 % (w/w) relative to the substrate mass, achieving a 96 % reduction in acidity. The lipases remained active, indicating the possibility of reuse in subsequent reactions under these conditions.

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

1 INTRODUCTION

The bio-oil obtained from the thermal cracking of biomass has potential for use as a traditional fuel, such as gasoline, diesel, and heating oil, derived from triglycerides which results in the production of charcoal (solid), bio-oil (liquid) and gaseous fuel products, has a low water content, high calorific value. However, bio-oil from triglycerides also has several disadvantages: it is corrosive due to its acidity, it is unstable due to a high concentration of olefins, and it sometimes contains high levels of benzene, a carcinogen.[1,2] The acidity of bio-oil is due to the presence of carboxylic acids, which form during the breakdown of the C-C bonds in the fatty acids of the triglycerides. To reduce its acidity, the carboxylic acids in the bio-oil can be esterified, using acid, basic, or enzymatic catalysis.[3]

The acidity index (AI) of bio-oil 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}. Therefore, it is necessary to study suitable operational conditions and use subsequent reactions, such as esterification, to reduce the acidity index to acceptable quality levels. Lipases are renewable biocatalysts with significant potential for use in esterification of bio-oil to reduce its AI, however, their use for this purpose is not well studied.[4] Their potential is mainly due to their selectivity and specificity for their substrates, leading fewer by-products, higher purity of the desired products, under mild conditions (ambient temperature and pressure, neutral pH), reducing energy consumption and minimizing the need for harsh chemicals.[5,6,7]

2 MATERIAL & METHODS

The bio-oil was produced through the thermal cracking of refined soybean oil (SOYA - Bunge Brasil) at 525 °C, with a residence time of 4.5 s. In order to identify the carboxylic acids in the crude bio-oil, gas chromatography mass spectrometry (GC–MS) was used with a Restek Stabilwax capillary column and the compounds were identified by comparison with the NIST 08 Mass Spectral Database.[3,8]

Two commercial immobilized lipases were used as catalysts: *Candida antarctica* lipase B (CALB, Novozyme 435) and lipase from *Rhizomucor miehei* (Lipozyme RM-IM). All reactions were done at 40 °C. Bio-oil was used at different mass ratios: 1:1 and 1:3 (methanol:bio-oil), using CALB and RM-IM. The enzyme amount was kept fixed at 5% (w/w) relative to the mass of bio-oil. The amount of alcohol was chosen according to previous studies.[3,4] The reactions were done in hermetically sealed 10 mL test tubes. The lipases were weighed and placed in glass vials. Methanol and bio-oil were then added, and the vials were incubated on an orbital shaker at 180 rpm and 40 °C for 6 h. Aliquots taken hourly for AI measurement. AI measurements were also done for the crude bio-oil from thermal cracking. 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 residual activity post-reaction were determined with ethyl-oleate synthesis reactions, undertaken 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.[10,11]

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 results of the bio-oil esterification using CALB and Lipozyme RM-IM. With the mass ratio of 1:1 (methanol:bio-oil), CALB reduced the AI from an initial value of 65.1mg KOH g^{-1} to 2.3 mg KOH g^{-1} at 6 h, while Lipozyme RM-IM reduced the AI from an initial value of 68.1 to 53.3 mg KOH g^{-1} at 6 h. With the mass ratio of 1:3 (methanol:bio-oil), CALB reduced the AI from an initial value of 101.3 mg KOH g^{-1} to 9.3 mg KOH g^{-1} at 6 h, while Lipozyme RM-IM reduced the AI from an initial value of 103.9 mg KOH g^{-1} to 86.4 mg KOH g^{-1} at 6 h. The Brazilian legislation requires the maximum IA for bio-oil to be around 0.5 mg KOH g^{-1},[9] so further work is needed to reach this value. The carboxylic acids that were not esterified by CALB are likely to be short-chain carboxylic acids that are not esterifiable by these two lipases.

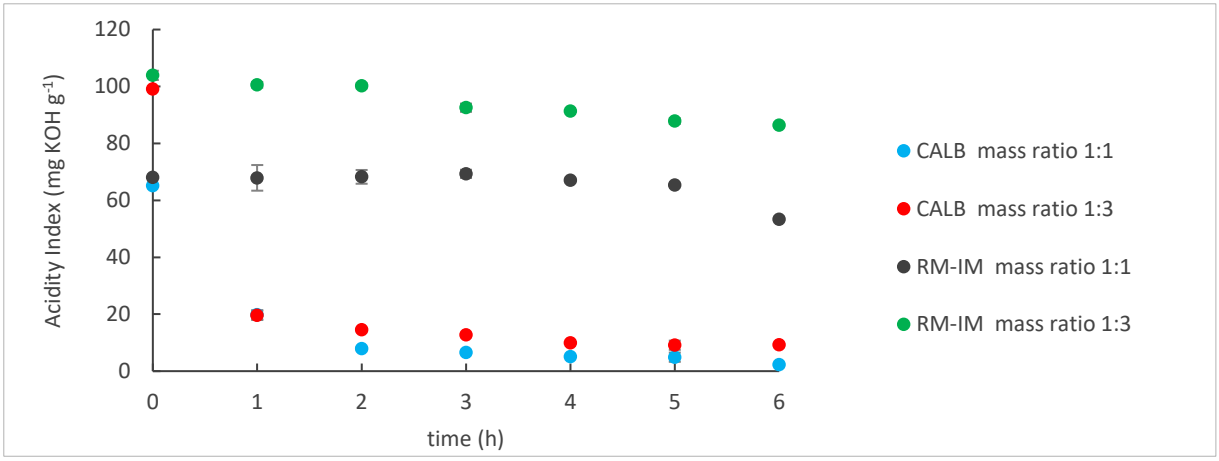

Figure 1 Reduction of bio-oil acidity index via lipase-catalyzed methylic esterification reactions in two mass ratio (1:1 and 1:3, bio-oil:methanol)

The residual activities of CALB and RM-IM after their use in bio-oil esterification were determined to assess the stability of the enzymes during the reaction. Both lipases lost significant activity, especially at the mass ratio (methanol:bio-oil) of 1:3, with RM-IM losing 86% activity and CALB losing 70% activity (Table 1). This loss of activity is probably caused by the methanol in the reaction medium, as it is known to inhibit lipases.[12] These results suggest that the concentration of methanol in the reaction medium should be kept to a minimum, using the lowest mass ratio of methanol to bio-oil possible.

Table 1 Enzyme activities before and after the esterification reactions

Lipase	Mass ratio (methanol:bio-oil)	Initial Activity (U g^{-1})	Residual activity (U g^{-1})	Loss of activity (%)
CALB (Novozyme 435)	1:1	208.9 ± 1.3	129.0 ± 1.8	38
	1:3		61.1 ± 1.2	71
Lipozyme RM-IM	1:1	183.8 ± 1.6	32.6 ± 1.7	82
	1:3		25.8 ± 1.4	86

Reaction conditions: Bio-oil esterification: 40 °C; 6 h; at 180 rpm in an orbital shaker;
Activity determined by ethyl-oleate synthesis [10].
CALB: *Candida antarctica* lipase B; Lipozyme RM-IM: *Rhizomucor mieihei* immobilized lipase

4 CONCLUSION

The commercial lipases from *Candida antarctica* (CALB, Novozyme 435) and *Rhizomucor miehei* (Lipozyme RM-IM) both reduced the acidity index of the bio-oil by methyl esterification of the carboxylic acids. CALB was the best catalyst, producing a 96% acidity reduction and was relatively stable during reactions conducted at a mass ratio 1:1. CALB will be used in future studies aimed at further reducing the acidity index of the bio-oil under different conditions.

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