Appl Biochem Biotechnol (2009) 154:253–267
DOI 10.1007/s12010-008-8444-4

Biodiesel Synthesis via Esterification of Feedstock with High Content of Free Fatty Acids

Marcella S. Souza · Erika C. G. Aguieiras · Mônica A. P. da Silva · Marta A. P. Langone

Received: 21 May 2008 / Accepted: 13 November 2008 /
Published online: 9 December 2008

Abstract The objective of this work was to study the synthesis of ethyl esters via esterification of soybean oil deodorizer distillate with ethanol, using solid acid catalysts and commercial immobilized lipases, in a solvent-free system. Three commercially immobilized lipases were used, namely, Lipozyme RM-IM, Lipozyme TL-IM, and Novozym 435, all from Novozymes. We aimed for optimum reaction parameters: temperature, enzyme concentration, initial amount of ethanol, and its feeding technique to the reactor (stepwise ethanolysis). Reaction was faster with Novozym 435. The highest conversion (83.5%) was obtained after 90 min using 3 wt.% of Novozym 435 and two-stage stepwise addition of ethanol at 50°C. Four catalysts were also tested: zeolite CBV-780, SAPO-34, niobia, and niobic acid. The highest conversion (30%) was obtained at 100°C, with 3 wt.% of CBV-780 after 2.5 h. The effects of zeolite CBV 780 concentration were studied, resulting in a conversion of 49% using 9 wt.% of catalyst.

Keywords Esterification · SODD · Biodiesel · Ethanol · Immobilized lipase · Zeolite

M. S. Souza · M. A. P. da Silva
Escola de Química, Universidade Federal do Rio de Janeiro, Rio de Janeiro CEP 21949-900, Brazil

M. A. P. da Silva
e-mail: monica@eq.ufrj.br

E. C. G. Aguieiras
Instituto de Biologia Roberto Alcântara Gomes, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, Brazil

M. A. P. Langone (✉)
Instituto de Química, Universidade do Estado do Rio de Janeiro, Rua São Francisco Xavier, 524, PHLC, IQ, sl. 427, Rio de Janeiro CEP 20559-900, Brazil
e-mail: langone@uerj.br

Introduction

The growing interest for renewable sources of energy is responsible for the worldwide efforts towards the development of biofuels. Biodiesel in particular is synthesized via transesterification of triglycerides from vegetable oils with ethanol or methanol [1]. Biodiesel is a mixture of mono-alkyl esters of higher fatty acids. The use of biodiesel as an alternative fuel has a promising potential since it is based on renewable resources like vegetal oils and animal fats, does not present sulfur and aromatic chemicals in its composition, reduces the life cycle of carbon dioxide besides being biodegradable, and is non-toxic [2].

The industrial process of biodiesel production is usually carried out by heating an excess of alcohol with vegetables oils, called transesterification or alcoholysis, in presence of an inorganic catalyst (NaOH, KOH). A disadvantage of alkali-catalyzed processes is that catalysts are lost to the glycerol layer and cannot be reused. Furthermore, neutralization to prevent toxic wastes is necessary and the purification of glycerol is more difficult when large amounts of catalyst are present. Besides, the use of more expensive refined oils is necessary so as to have low free fatty acids content (inferior to 1%).

Thus, the current production costs of biodiesel are not competitive with diesel due to a relatively high cost of lipid feedstocks, usually edible-grade refined oils. An alternative route to biodiesel is based on esterification of free fatty acids present in high concentrations in the by-product obtained from vegetable oil refining [3, 4].

Soybean oil deodorizer distillate (SODD) is an important by-product in the soybean oil refining process. About 80 wt.% of SODD corresponds to free fatty acids plus triglycerides [3]. Biodiesel production via esterification of fatty acids present in SODD is undoubtedly a promising alternative, since the unit price of this material is significantly lower than that of refined oils [5].

In conventional esterification processes for production of biodiesel, strong acids are used as catalysts. The process is highly energy consuming and fluids are difficult to handle, causing problems of corrosion in equipment and producing waste acid, with serious environmental impact. Also, synthesis schemes are such that poor reaction selectivity and undesirable side reactions often occur [3].

The current demand for cleaner and more selective processes has motivated the development of solid catalysts for organic synthesis in general as well as for biodiesel synthesis. The possibility of catalyst reuse is also an important factor, considering the reduction in the operational costs.

The use of heterogeneous catalysts, less polluting and more selective, has led to the development of enzyme supported catalysts in organic synthesis. Lipases are a class of enzymes known as triacylglycerol ester hydrolases. Most of these enzymes show high selectivity including stereo-selectivity, work on mild operation conditions, and give products of high purity. Esterification reactions between alcohols and free fatty acids can also be catalyzed by lipases in non-aqueous or microaqueous media. The production of esters can be achieved either by synthesis with free fatty acids and alcohols or by transesterification [6–9].

Motivated by the fact that Brazil is the largest world producer of ethanol and the second largest producer of soybeans, the objective of this work was to study the synthesis of mono-alkyl esters (biodiesel) from esterification of SODD with ethanol, using solid acid catalysts and commercial immobilized lipases in a solvent-free medium. This work studied the effects of ethanol concentration and its feeding technique to the reactor, temperature,

enzyme concentration, and type of immobilized lipase as well as to esterification reactions using solid acids catalysts (CBV-780, SAPO-34, niobia, and niobic acid).

Experimental

Materials

SODD from soybean oil refining process was provided by Piraquê S. A. (Rio de Janeiro, Brazil). Commercial lipases used were: Lipozyme RM-IM (lipase from *Rhizomucor miehei*, immobilized on macroporous anion exchange resin), Lipozyme TL-IM (lipase from *Termomyces lanuginosus*), Novozym 435 (lipase from *Candida antarctica*, immobilized on acrylic macroporous resin), all kindly donated by Novozymes Latin America Ltda (Araucária, Brazil).

Two commercial catalysts, CBV 780 (zeolite type SDUSY-ZEOLYST TM) and niobic acid (CBMM) and two synthesized catalysts, SAPO-34 (molecular sieve-type alumino-silicate-phosphate) and niobia, were tested. The choice of these catalysts is related to the fact that among various catalysts tested previously CBV 780 presented the highest activity in esterification of palmitic acid and ethanol [10]. Niobic acid (CBMM) is the commercial catalyst used in a biodiesel process by AGROPALMA/Brazil and niobia is very similar. Regarding SAPO-34, it was used because of its high density of acid sites. SAPO-34 was obtained according to the procedure reported by Prakash and Unnikrishnan [11] and calcination of the solid was carried out by a procedure adapted from Gomes et al. [12]. Niobia was synthesized according to a methodology adapted from Chuah et al. [13]. Synthesis of niobia consisted of addition of NH_4OH to an aqueous solution of ammonia complex of niobium (CBMM-AD 2698). The preparation was carried out in a rotavapor during 96 h to 95°C. The pH was maintained at 9.0. Then the material was filtered and dry in oven at 100°C for 24 h. The calcination was performed under flow of air at 500°C (1°C/min) for 12 h.

Ethanol P. A., acetone P. A., and sodium hydroxide were supplied by Vetec Química Fina Ltda (Rio de Janeiro, Brazil).

SODD Characterization

Acidity and acid value of SODD were determined according to AOCS Te 1a-64 [14]. Iodine index and humidity were determined according to AOCS Cd 1d-64 [14] and Ca 2e-84 [14], respectively.

Fatty acids present in SODD were determined according to AOCS Ce 1f-96 [14], in a HP 6890N GC, equipped with flame ionization detector and capillary column SP 2340.

Measurement of Lipase Activity

The esterification activity of commercial lipases Lipozyme RM-IM, Lipozyme TL-IM, and Novozym 435 was measured by the consumption of oleic acid at 45°C in the esterification reaction with butanol (oleic acid/butanol molar ratio of 1) with the enzyme concentration of 3 wt.%. One unit of enzymatic activity (U) in this process was defined as 1 μmol of oleic acid consumed/min under the experimental conditions described herein. The option for butanol instead of ethanol is related to the fact that ethanol promotes a quick deactivation of the enzyme due to dehydration effects.

The activity of commercial lipases Lipozyme RM-IM, Lipozyme TL-IM, and Novozym 435 was 1,510, 454, and 2,960 (μmol acid/min g), respectively.

Solid Acid Catalyst Characterization

The chemical composition of catalysts was determined by X-ray fluorescence (XRF), using a spectrometer Rigaku, Rix 3100 model equipped with a rhodium X-ray generator tube. X-ray diffraction (XRD) analyses were carried out in a Rigaku Miniflex diffractometer using CuKα radiation (α=1.5417 Å) operating at 30 kV and 15 mA. Textural analyses were carried out according to the BET method in equipment Micromeritics ASAP model 2000, using N_2 at 196°C. The degree of hydration and the thermal stability of the catalysts were determined in a thermogravimetric balance (RIGAKU-TAS100) under flow of nitrogen from 25 to 600°C and heating rate of 10°C/min.

Temperature-programmed desorption of ammonia (TPD) analyses were carried out in a multipurpose unity coupled with a Balzers QUADSTAR 422 QMS 200 mass spectrometer. For the TPD analyses, NH_3 adsorption was carried out at 100°C, by submitting the samples to a 4% NH_3/He mixture flow of 60 ml/min during 30 min; desorption was carried out by heating the samples from 100 to 550°C at 10°C/min, under He flow of 60 ml/min.

Before each TPD analysis, CBV-780 and SAPO-34 were dried under He flow of 30 ml/min simultaneously with heating from ambient temperature to 500°C at 10°C/min. Niobia and niobic acid were dried at 250°C using a similar procedure.

Reaction System

Esterification reactions took place in a closed 15-ml batch reactor magnetically stirred and coupled to a condenser in order to avoid alcohol loss. Water circulating in the condenser was cooled by a thermostatic bath. Reacting medium temperature was kept constant by circulating hot ethylene glycol through the reactor jacket. A thermostatic bath (HAAKE D10) allowed a close control over the process temperature. The mass of SODD used was 8 g in all experiments.

Quantification of Free Fatty Acids

Reaction progress was monitored by taking duplicate samples (100 μl) each 30 min until 2.5 h of reaction. Free fatty acids present in reaction medium samples were analyzed by titration with NaOH 0.02 M using a Mettler DL 25 autotitrator.

Results and Discussion

Characterization of SODD

Acidity, acid value, iodine index, and humidity of SODD are shown in Table 1.

The chemical composition of SODD regarding fatty acids is shown in Table 2.

The concentration of linoleic and palmitic acids are atypical most probably due to partial hydrogenation of the original soybean oil as well as blending with palm oil.

Table 1 Physicochemical analysis of SODD.

Acidity (%)	72.6
Acid value (mg KOH/g)	144.4
Iodine index	63.8
Humidity (%)	0.173

Esterification Reactions using Lipases

Effects of Ethanol Concentration

A generic esterification process mediated by a biocatalyst is represented by the following stoichiometry:

$$R_1COOH + R_2OH \xrightleftharpoons{\text{Lipase}} R_1COOR_2 + H_2O$$

fatty acid *alcohol* *ester* *water*

Since the reaction is reversible, one reagent should be in excess so as to displace the equilibrium towards products. Thus, the effects of ethanol concentration on the fatty acid conversion were investigated. The amount of SODD was always 8 g while the ethanol mass ranged between 1 and 4 g. Based on the SODD composition shown in Table 2, we can admit that the proportion 8 g of SODD:1 g of alcohol corresponds to a molar ratio 1:1 of fatty acids to ethanol. Figure 1 shows that an increase in ethanol concentration leads to a decrease in fatty acid conversion. The highest conversion observed was 59%, using 3 wt.% Lypozyme RM-IM and 1 g of ethanol at 50°C after 2.5 h. Notice that triglycerides present in SODD can react directly with ethanol-producing ethyl esters for immobilized commercial lipases such as Lipozyme RM-IM and Novozym 435, which also catalyze transesterification reactions. However, in this work, only the consumption of free fatty acids was measured.

Table 2 Chemical composition of SODD.

Fatty acid	Number of carbon atoms	Insaturation number	Composition (%)
Capric acid	10	–	0.1
Lauric acid	12	–	0.1
Myristic acid	14	–	0.7
Palmitic acid	16	–	30.9
Palmitoleic acid	16	1	0.2
Stearic acid	18	–	23.7
Oleic acid	18	1	23.8
Linoleic acid	18	2	6.4
Linolenic acid	18	3	0.3
Arachidic acid	20	–	0.4
Behenic acid	22	–	0.3
Other fatty acid	–	–	1.0
Total trans isomer	–	–	12.1

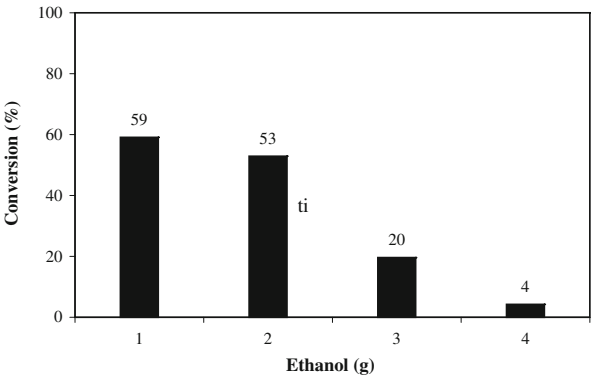

Fig. 1 Effects of ethanol concentration on fatty acids conversion after 2.5 h, using Lypozyme RM-IM 3 wt.% and 8 g of SODD at 50°C

Proteins are generally unstable in media containing short chain alcohols like methanol and ethanol [15]. The lipase inhibition by alcohol was also investigated by Ghamgui et al. [16] in butyl oleate synthesis. When butanol is the major component (ratio oleic acid/butanol of 1:3), the conversion is systematically lower than the one observed when the acid is the major component. This could be due to the fact that butanol (polar substrate) may be accumulated in the aqueous microenvironment surrounding the enzyme, reaching a concentration level sufficient to cause a denaturation of the protein. The authors obtained the best conversion in ester synthesis with an equimolar concentration of substrates either in a solvent-free system or in *n*-hexane.

Denaturation of lipase caused by polar substrate (alcohol) was also observed elsewhere [8, 17], suggesting a stepwise addition of ethanol to avoid lipase deactivation due to the high initial concentration of alcohol. As shown in Fig. 2, the highest fatty acid conversion was obtained with 4 g of ethanol in four steps of 1 g each, equally spaced by 30 min (T_0= 1 g, $T_{30\ min}$=1 g, $T_{60\ min}$=1 g, and $T_{90\ min}$=1 g).

The stepwise ethanolysis in shorter time intervals or in greater amounts was shown to deactivate the enzyme more rapidly, resulting in smaller conversions.

Shimada et al. [18] studied the inactivation of immobilized *C. antarctica* lipase by ethanol in tuna oil ethanolysis. Conversion in the two-step ethanolysis did not decrease up to the 37th cycle, and then decreased rapidly. However, the three-step reaction maintained a

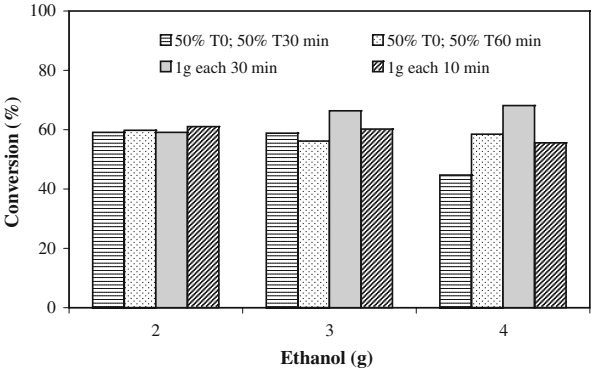

Fig. 2 Effects of stepwise addition of ethanol on fatty acids conversion after 2.5 h, using Lypozyme RM-IM 3 wt.% and 8 g of SODD at 50°C

conversion of over 95% up to the 54th cycle (108 days). The significant decrease in enzymatic activity in the two-step reaction could be related to the addition of 2/3 molar equivalent of ethanol for starting the second-step reaction, interfering with the lipase stability. The amount of ethanol added in the three-step reaction was only 1/3 molar equivalent though.

Temperature Effects

Effects of temperature were studied in the range 45–78°C for reactions carried out with 2 g of ethanol (added at the beginning of reaction) and 3 wt.% of Lipozyme RM-IM. The minimum and maximum temperatures were defined as that of SODD fusion and ethanol boiling point, respectively. Results are shown in Fig. 3.

The fatty acid conversion increased when temperature was increased from 45 to 50°C. However, at temperatures higher than 50°C, the conversion decreased due to enzyme thermal deactivation. Since ethanol is highly hydrophilic, enzyme denaturation and loss of activity could also have been enforced. Vieira et al. [19] observed the same effect on the ethyl palmitate synthesis using Lipozyme RM-IM. After 2 h, palmitic acid conversion decreased 16% when temperature was increased from 70 to 75°C.

Trubiano et al. [8] also verified that rates and conversion at equilibrium were improved increasing the temperature from 25 to 65°C in oleic acid esterification with ethanol catalyzed by *Candida antarctica* (Novozym 435).

Effects of Enzyme Concentration

Effects of enzyme concentration on the fatty acids conversion were studied using 1.5, 3, 4.5, 6, 7.5, and 9 wt.% of Lipozyme RM-IM at 50°C, with 2 g of ethanol (added at the beginning of reaction). Results are shown in Fig. 4.

Higher conversions of fatty acids were obtained with enzymatic concentrations around 3 wt.%. Loading of Lipozyme RM-IM above 3 wt.% did not cause significant conversion increase. It was concluded that 3 wt.% of enzyme was enough to saturate the reacting system under tested conditions. Similar results were obtained by Vieira et al. [19] in ethyl palmitate synthesis and by Wang et al. [17] in isopropyl oleate synthesis.

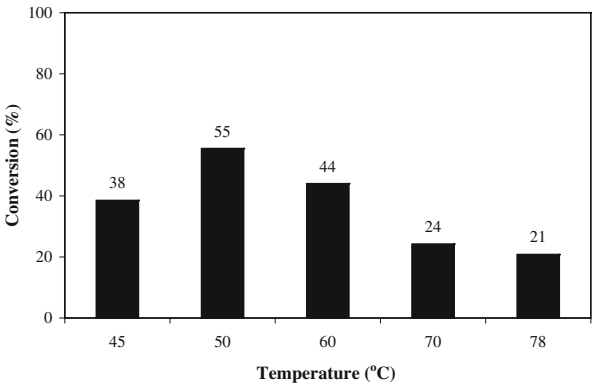

Fig. 3 Effects of temperature on fatty acid conversion after 2.5 h, using Lypozyme RM-IM 3 wt.%, 8 g of SODD, and 2 g of ethanol

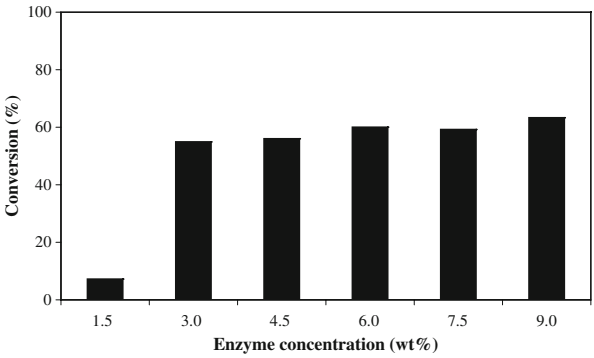

Fig. 4 Effects of Lipozyme RM-IM concentration on fatty acids conversion after 2.5 h, using 8 g of SODD and 2 g of ethanol at 50°C

Type of Lipase

Three commercial immobilized lipases were compared regarding conversion of fatty acids from a SODD. Reactions were carried out at 50°C using 3 wt.% of enzyme and 2 g of ethanol (T_0=1 g; $T_{30\ min}$=1 g). According to the results shown in Fig. 5, Novozym 435 presented the highest conversion (83.5%). These results are expected considering esterification activity values obtained for the three commercial lipases.

Wang et al. [3] also found Novozym 435 to exceed the catalytic performance of Lipozyme TL-IM regarding conversion of fatty acids present in a SODD using *t*-butanol. However, Novozym 435 is more expensive that Lipozyme TL-IM. The authors used a mixture of the enzymes (3% of Lipozyme TL-IM and 2% of Novozym 435 based on the oil weight), obtaining a yield of 84% in methyl esters after 12 h.

Characterization of Solid Acid Catalysts

Composition and Crystalline Structure

Table 3 presents the composition of SAPO-34 calcined and obtained by XRF.

The oxide contents observed for the SAPO-34 synthesized are similar to the relative contents verified by Prakash and Unnikrishnan [11] for the non-calcined solid (20.8% SiO_2,

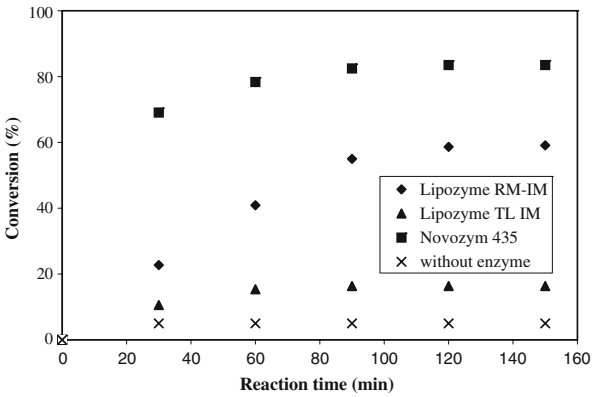

Fig. 5 Time profile of fatty acids conversion in esterification of SODD with ethanol using commercial lipases

Table 3 Composition of SAPO-34.

SiO_2 (% *w*/*w*)	Al_2O_3 (% *w*/*w*)	P_2O_5 (% *w*/*w*)	C (% *w*/*w*)	Fe_2O_3 (% *w*/*w*)
14.1	38.3	40.0	7.6	0.03

40.7% Al_2O_3, 38.5% P_2O_5). There is also the presence of a significant impurity carbonic not eliminated during the calcination of solid, probably due to template (morpholine). Figure 6 presents the X-ray patterns of catalysts.

Only peaks corresponding to the crystal structure of SAPO-34 were observed, 2θ=9.6°, 18°, 20.8°, 25.2°, and 30.8°, indicating high purity [11]. There was no observed presence of cristobalite ($AlPO_4$), suggested by Prakash and Unnikrishnan [11] as a competitor phase during the SAPO-34 synthesis.

The CBV-780 zeolite presented the characteristic peaks, situated at 2θ=16, 19–21°, 24°, 27°, and 32° [20]. For niobia, we observed characteristic peaks of Nb_2O_5 phase (2θ=22.6°, 28.5°, and 36.6°), while the niobic acid was almost amorphous [20].

The textural properties of catalysts are shown in Table 4.

The specific area and micropore volume values observed for SAPO-34 are consistent with typical values observed in the literature [21,22], while the observed mesopore volume is significantly lower than the value reported by Aguayo et al. [22]. It can be observed that the structure of SAPO-34 is predominantly microporous, since the mesopore area and volume are relatively small.

The CBV-780 zeolite presents high area and microporous structures predominantly because of the shape their adsorption isotherms, is characteristic of microporous solid [23]. The mesopore volume is typical of dealuminated zeolite [24]. Niobia presents the lowest specific area among catalysts studied and is essentially mesoporous because the micropore volume is insignificant.

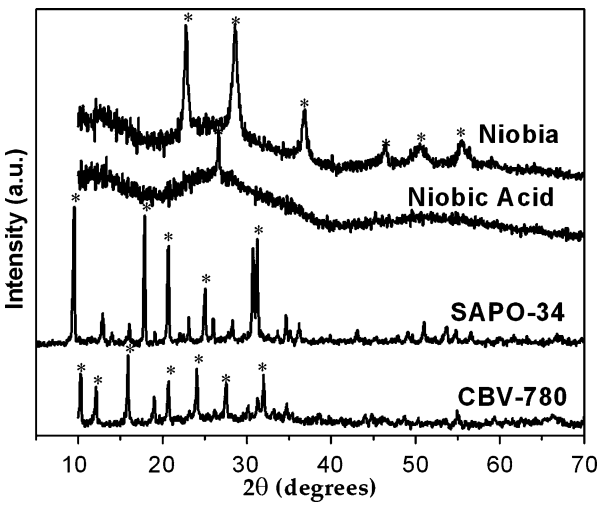

Fig. 6 XRD patterns of catalysts

Table 4 Textural properties of catalysts.

Catalyst	Specific area (m^2/g)	Micropore area (m^2/g)	Micropore volume (cm^3/g)	Mesopore area (m^2/g)	Mesopore volume (cm^3/g)
SAPO-34	536	570	0.27	0.2	0.002
CBV-780	678	536	0.25	183	0.27
Niobia	92	–	–	88	0.10
Niobic acid	95	2.6	0.0001	–	–

Thermal Properties

Thermal analysis of catalysts indicated processes related to the loss of mass due to desorption of water (Fig. 7). The SAPO-34 showed the greatest loss about 20%.

Acid Properties

A typical NH_3 TPD profile from an acid type zeolite has two peaks, termed l- and h-peaks (low and high temperatures, respectively) [25]. These peaks are related to two types of acid

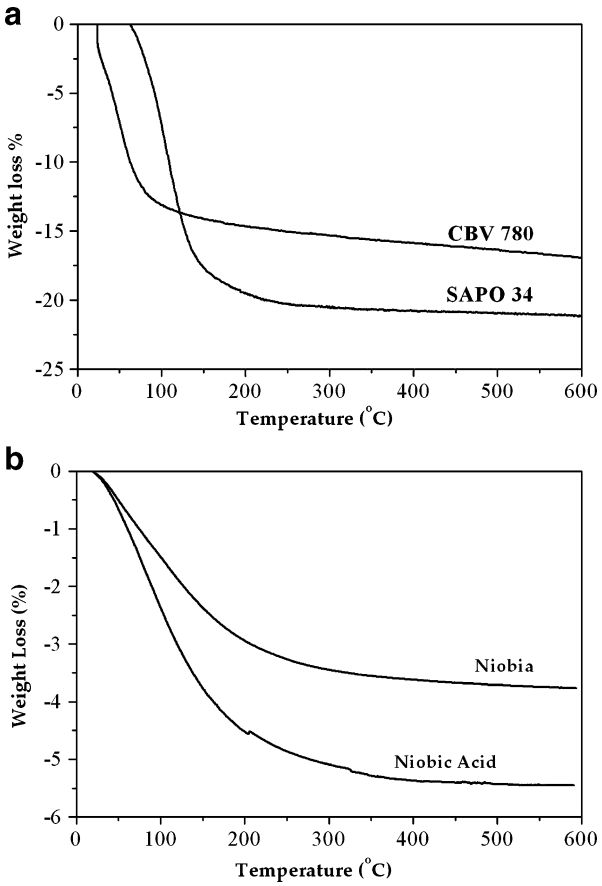

Fig. 7 Thermal profiles of catalysts

sites with different acidity strengths, i.e., weak and strong, according to the peak maximum temperature. The above characterization of the acid sites is a qualitative indication of how strongly the ammonia molecules are connected to the acid sites. The temperature range for the peaks' maxima of the weak acid sites is set between 150 and 220°C and for the strong acid sites above 350°C.

Based on the area under each desorption profile, the total desorbed NH_3 amount by mass unity of each catalyst was calculated and results are shown in Table 5.

The NH_3 TPD profiles for SAPO-34, CBV-780, niobia, and niobic acid are shown in Fig. 8. Each desorption profile was well fitted by three Gaussian curves: the first, at lower temperatures, relates to low acid strength sites; the second, at intermediate temperatures, relates to moderate acid strength sites; the third, at higher temperatures, corresponds to stronger acid sites. The temperature of this third Gaussian (shown in Table 5) is an indication of the acid strength of the catalysts in order: CBV 780>SAPO-34>niobia> niobic acid.

The area under each profile for desorption was related to the fraction of weak acid site temperatures lower than 340°C present in each catalyst, the rest of the area was related to the fraction of strong acid sites. The global acid site density decreased in the sequence: SAPO-34>CBV-780>niobia>niobic acid. However, CBV-780 zeolite is the catalyst that presents the stronger acid sites as the maximum temperature above of 550°C is already in the stage of isothermal desorption of NH_3. For SAPO-34, the strong acid site density obtained is consistent with results reported in the literature [22, 26, 27], within the range of 0.18–1.3 mmol/g.

Aguayo et al. [22] also observed two peaks in NH_3 TPD profile for SAPO-34: one at approximately 230°C attributed to weak acid sites, or Brönsted of Lewis acid sites, and the other at approximately 400°C related to strong Brönsted acid sites.

The acidity of niobium oxide (CBMM) was evaluated by infrared spectroscopy using pyridine as a probe molecule. Results showed a small interaction between niobia and pyridine indicating the presence of weak acid sites. These results are in good agreement with the analysis of desorption of NH_3 for niobic acid and niobia, suggesting the predominance of weak acid sites. Typical deviations from the mean for triplicate experiments in the range 3–7% as shown in Tables 3, 4, and 5.

Esterification Reactions using Solid Acid Catalysts

Esterification is a classic reaction of organic chemistry that can be catalyzed by Brönsted acids [28]. All the experiments used a molar ratio (ethanol/fatty acids) of 2:1 (8 g of SODD and 2 g of ethanol), 3 wt.% of catalyst, and reactions were carried out at 100°C.

Figure 9 shows the conversion obtained in esterification between SODD and ethanol after 2.5 h of reaction. It can be noted that the fatty acid conversion was similar, 30% and

Table 5 Acid properties of catalysts.

Catalyst	Weak acid site density (mmol/g)	Fraction of strong acid site (%)	Global acid site density (mmol/g)	Highest desorption temperature (°C)
SAPO-34	2.72	37	4.32	383
CBV-780	0.48	44	0.86	>550
Niobia	0.43	29	0.60	341
Niobic acid	0.41	33	0.61	345

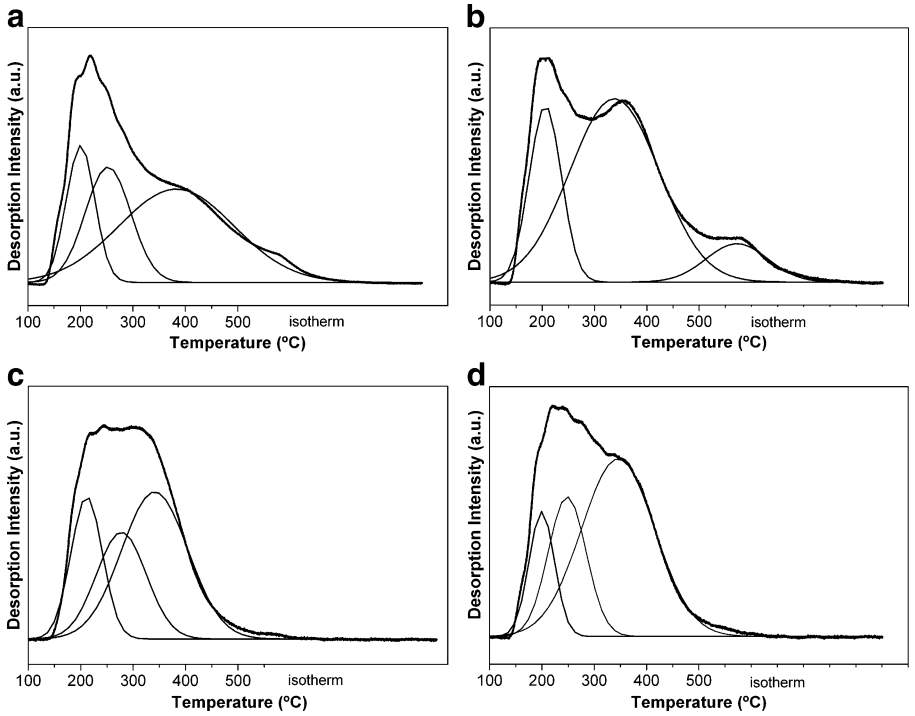

Fig. 8 NH_3 desorption profile of SAPO-34, CBV-780, niobia, and niobic acid

28%, respectively, for CBV-780 and SAPO-34. The low level of conversions was probably related to the low temperature used (100°C). The other catalysts showed similar conversions to the blank test (without catalyst).

The catalytic activity is related to the strong acid site density. Although the SAPO-34 presents the greatest density of sites, its strong performance was slightly less than the CBV-780. The catalyst's hydrophobicity, related to ratio Si/Al, is another important characteristic that influences their activity because it reduces the inhibition caused by the formation of water explaining the higher conversion obtained with the CBV-780 [29].

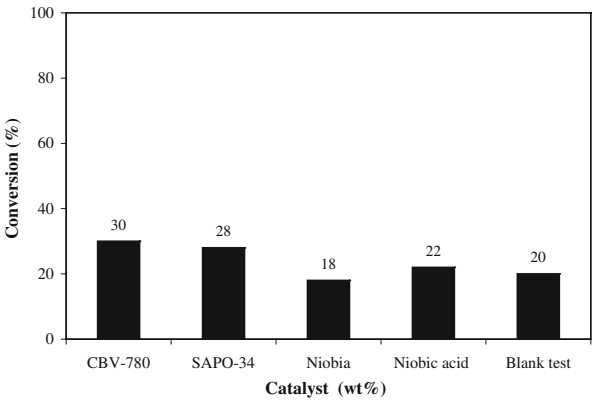

Fig. 9 Conversion of fatty acids in esterification of SODD with ethanol, using 3 wt.% of catalyst at 100°C

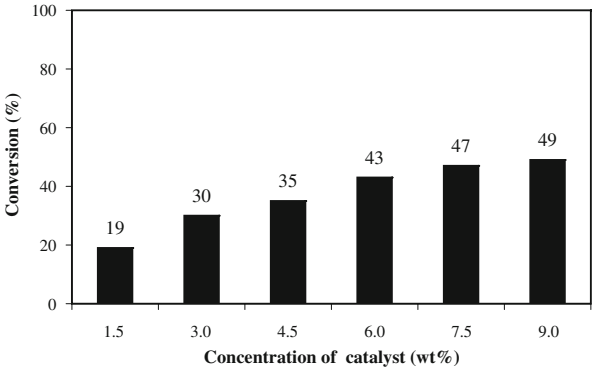

Fig. 10 Effects of CBV-780 concentration on fatty acids conversion after 2.5 h, using 8 g of SODD and 2 g of ethanol at 100°C

Vieira et al. [10] studied the synthesis of ethyl hexadecanoate via esterification between palmitic acid and ethanol catalyzed by zeolites HZSM-5, H-modernita, CBV-760, and CBV-780. The results are similar to those obtained in this work. The best results were obtained using CBV-780, which converted 23% of fatty acids. Vieira et al. [10] explained this result in terms of greater hydrophobicity of zeolite CBV-780, which has a high value of the ratio SiO_2/Al_2O_3. This effect decreased the water concentration inside the catalyst's pores and in the vicinity of the acid sites, which allowed a shift of the reaction of esterification towards the product formation. According to the authors, another explanation for the best performance of CBV-780 was the strength of their Brönsted acid sites.

Effects of Catalyst Concentration (CBV-780)

Considering that the best results of fatty acids conversion were obtained in the reaction using the zeolite CBV-780, experiments with different concentrations of this catalyst were carried out at 100°C and using 8 g of SODD and 2 g of ethanol. Concentrations of catalyst tested were 1.5, 3, 4.5, 6, 7.5, and 9 wt.%. Results are shown in Fig. 10.

It was observed that an increase in catalyst concentration in the reaction medium promoted an increase in the fatty acid conversion. The highest conversion (49%) was obtained using 9 wt.% of catalyst. This finding is expected because catalyst loading increment is proportional to availability of acid sites, which favor the accessibility of a large number of reactant to catalyst active sites. However, there is no significantly catalytic enhanced when the concentration of catalyst increased from 7.5 to 9 wt.%.

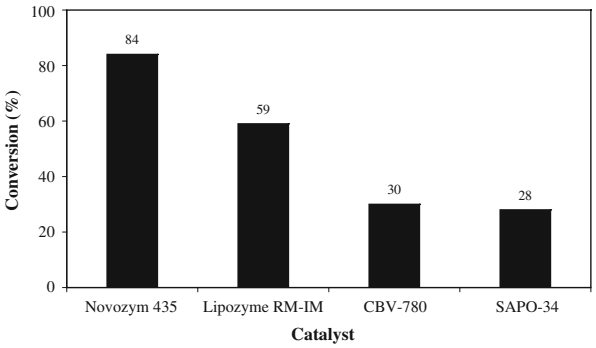

Fig. 11 Comparison of Novozym 435, Lipozyme RM-IM, CBV 780 zeolite, and SAPO-34 regarding conversion of fatty acids after 2.5 h

Ramu et al. [30] studied the effect of catalyst amount (5% WO_3/ZrO_2) on the esterification of palmitic acid with methanol. Their results are similar to those obtained in this work. The authors observed a significant increase in the conversion to varying the concentration of catalyst from 30 to 50 wt.%. However, a larger increase in concentration of catalyst to 75 wt.% produced only a slight increase on conversion.

Comparison of Results

The synthesis of ethyl esters using SODD as raw material was investigated in the presence of biocatalysts (immobilized enzymes Lipozyme RM-IM, Lipozyme TL-IM, and Novozym 435) and solid acid catalysts (zeolite CBV 780, SAPO-34, niobia, and niobic acid). As shown in Fig. 11, conversion for reactions catalyzed by lipases was around three times greater than that obtained with solid acid catalysts, for a reaction time of 2.5 h. Thus, immobilized lipases seem to be a much more attractive alternative.

Conclusions

This work shows the viability of conversion of fatty acids present in a SODD (soybean oil deodorizer distillate) into ethyl esters using commercial immobilized lipases and solid acid catalysts in a solvent-free medium.

By comparing results obtained by chemical and enzymatic route, it was observed that immobilized lipase offered better conversions that solid acid catalyst for the same reaction time. Although ester synthesis is usually catalyzed by acids, these systems are difficult to deal with, causing corrosion of equipment and produce acid effluent a common cause of serious environmental problems. The use of immobilized lipases offers environmental advantages and a reduction in energy consumption. The results of this work illustrate the technical feasibility of enzymatic biodiesel synthesis using a by-product of oil industry at mild conditions with high yields. However, it is important to emphasize the need for a study of economic viability of the lipase used in this process as well as to investigate other parameters involved in the synthesis of biodiesel using solid catalysts.

Acknowledgements The authors would like to acknowledge CENPES–Petrobras for financial support and Novozymes Latin America Ltda for kindly providing the enzyme for this research. Dr. Marta A.P. Langone would also like to thank the Programa Prociencia/UERJ. Marcella S. Souza is thankful to CENPES–Petrobras for the scholarship.

References

1. Meher, L. C., Sagar, D. V., & Naik, S. N. (2006). *Renewable and Sustainable Energy Reviews*, *10*, 248–268. doi:10.1016/j.rser.2004.09.002.
2. Bournay, L., Casanave, D., Delfort, B., Hillion, G., & Chodorge, J. A. (2005). *Catalysis Today*, *106*, 190–192. doi:10.1016/j.cattod.2005.07.181.
3. Wang, L., Du, W., Liu, D., Li, L., & Dai, N. (2006). *Journal of Molecular Catalysis. B, Enzymatic*, *43*, 29–32. doi:10.1016/j.molcatb.2006.03.005.
4. Chongkhong, S., Tongurai, C., Chetpattananondh, P., & Bunyakan, C. (2007). *Biomass and Bioenergy*, *31*, 563–568. doi:10.1016/j.biombioe.2007.03.001.
5. Appleby, D. B. (2005). *The biodiesel handbook*. New York: American Oil Chemists Society.
6. Kraai, G. N., Winkelman, J. G. M., de Vries, J. G., & Heeres, H. J. (2008). *Biochemical Engineering Journal*, *41*, 87–94. doi:10.1016/j.bej.2008.03.011.

7. Hasan, F., Shah, A. A., & Hameed, A. (2006). *Enzyme and Microbial Technology*, *39*, 235–251. doi:10.1016/j.enzmictec.2005.10.016.
8. Trubiano, G., Borio, D., & Errazu, A. (2007). *Enzyme and Microbial Technology*, *40*, 716–722. doi:10.1016/j.enzmictec.2006.06.003.
9. Rocha, J. M. S., Gil, M. H., & Garcia, F. A. P. (1999). *Journal of Chemical Technology and Biotechnology*, *76*, 607–612. doi:10.1002/(SICI)1097-4660(199907)74:7<607::AID-JCTB74>3.0.CO;2-N.
10. Vieira, A. P. A. (2005). Master's Thesis. Escola de Química/UFRJ, Rio de Janeiro, Brazil.
11. Prakash, A. M., & Unnikrishnan, S. (1994). *Journal of the Chemical Society, Faraday Transactions*, *90*, 2291–2296. doi:10.1039/ft9949002291.
12. Gomes, A. C. L., Nunes, M. H. O., Silva, V. T., & Monteiro, J. L. F. (2004). *Studies in Surface Science and Catalysis*, *154*, 2432–2440. doi:10.1016/S0167-2991(04)80508-1.
13. Chuah, G. K., Jaenicke, S., & Chan, K. S. (1996). *Applied Catalysis A General*, *145*, 267–284. doi:10.1016/0926-860X(96)00152-4.
14. Official Methods and Recommended Practices of The American Oil Chemists' Society.5th, AOCS: Champaign (1998).
15. Villeneuve, P., Muderhwa, J. M., Graille, J., & Hass, M. J. (2000). *Journal of Molecular Catalysis. B: Enzymatic*, *9*, 113–148. doi:10.1016/S1381-1177(99)00107-1.
16. Ghamgui, H., Karra-Chaâbouni, M., & Gargouri, Y. (2004). *Enzyme and Microbial Technology*, *35*, 355–363. doi:10.1016/j.enzmictec.2004.06.002.
17. Wang, J.-X., Huang, Q.-D., Huang, F.-H., Wang, J.-W., & Huang, Q.-J. (2007). *Chinese Journal of Biotechnology*, *23*, 1121–1128. doi:10.1016/S1872-2075(07)60067-3.
18. Shimada, Y., Watanabe, Y., Sugihara, A., & Tominaga, Y. (2002). *Journal of Molecular Catalysis. B: Enzymatic*, *17*, 133–142. doi:10.1016/S1381-1177(02)00020-6.
19. Vieira, A. P. A., Silva, M. A. P., & Langone, M. A. P. (2006). *Latin American Applied Research*, *36*, 283–288.
20. Joint Committee on Powder Diffraction Standards, International Center for Diffraction Data, Pennsylvania (1998).
21. Dahl, I. M., Wendelbo, R., Andersen, A., Akporiaye, D., Mostad, H., & Fuglerud, T. (1999). *Microporous and Mesoporous Materials*, *29*, 159–171. doi:10.1016/S1387-1811(98)00328-X.
22. Aguayo, A. T., Gayubo, A. G., Vivanco, R., Olazar, M., & Bilbao, J. (2005). *Applied Catalysis A General*, *283*, 197–207. doi:10.1016/j.apcata.2005.01.006.
23. Tonetto, G., Atias, J., & Lasa, H. (2004). *Applied Catalysis A General*, *270*, 9–25. doi:10.1016/j.apcata.2004.03.042.
24. Katada, N., & Niwa, M. (2004). *Catalysis Surveys from Asia*, *8*, 161–170. doi:10.1023/B:CATS.0000038534.37849.16.
25. Triantafillidis, C. S., Vlessidis, A. G., Nalbandian, L., & Evmiridis, N. P. (2001). *Microporous and Mesoporous Materials*, *47*, 369–388. doi:10.1016/S1387-1811(01)00399-7.
26. Lee, Y.-J., Baek, S.-C., & Jun, K.-W. (2007). *Applied Catalysis A General*, *329*, 130–136. doi:10.1016/j.apcata.2007.06.034.
27. Yoo, K. S., Kim, J.-H., Park, M.-J., Kim, S.-J., Joo, O.-S., & Jung, K. D. (2007). *Applied Catalysis A General*, *330*, 57–62. doi:10.1016/j.apcata.2007.07.007.
28. Bhatt, N., & Patel, A. (2005). *Journal of Molecular Catalysis A Chemical*, *238*, 223–228. doi:10.1016/j.molcata.2005.05.019.
29. Corma, A. (2003). *Journal of Catalysis*, *216*, 298–312. doi:10.1016/S0021-9517(02)00132-X.
30. Ramu, S., Lingaiah, N., Devi, B. L. A. P., Prasad, R. B. N., Suryanarayana, I. P. S., & Prasad, P. S. S. (2004). *Applied Catalysis A General*, *276*, 163–168. doi:10.1016/j.apcata.2004.08.002.