Journal of Molecular Catalysis B: Enzymatic 123 (2016) 41–46

Contents lists available at ScienceDirect

Journal of Molecular Catalysis B: Enzymatic

journal homepage: www.elsevier.com/locate/molcatb

Evaluating the kinetics of the esterification of oleic acid with homo and heterogeneous catalysts using in-line real-time infrared spectroscopy and partial least squares calibration

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ARTICLE INFO

Article history:
Received 25 May 2015
Received in revised form
24 September 2015
Accepted 26 September 2015
Available online 30 October 2015

Keywords:
ATR/FT-IR
Enzyme catalysis
Kinetics
Partial least squares
Real time analysis

ABSTRACT

Biodiesel is a mixture of fatty acid alkyl esters with properties similar to petroleum-based diesel. Thus, biodiesel can be used as either a substitute for diesel fuel or, more commonly, in a fuel blend. Biodiesel production can be catalyzed with mineral acids or bases or enzymes. The use of real-time techniques for monitoring the reaction and evaluating the efficiency of the catalyst can be of great use for optimizing the reaction and monitoring the process. In the present work, an in-line real-time methodology was used to evaluate and compare the kinetics of a reaction catalyzed with homo (hydrochloric acid) and heterogeneous (the enzymes Novozym 435, Lipozyme RM, and Lipozyme TL) catalysts. The esterification of oleic acid with ethanol was used as the reaction model. The study used attenuated total reflexion/Fourier transform infrared (ATR/FT-IR) and a single partial least squares (PLS) regression model to evaluate the kinetics of the various catalysts, without multiple calibrations, with validation by GC–MS. Novozym 435, which showed complete conversion after 165 min, was the best catalyst for this reaction. Lipozyme RM and Lipozyme TL had inferior conversion after the same amount of time, in agreement with the literature. All enzymatic catalysts showed higher conversion than hydrochloric acid at the same reaction conditions.

1. Introduction

Biodiesel is a fuel comprised of fatty acid alkyl esters and may reduce soot discharge by one third compared to petroleum-based diesel. Furthermore, biodiesel may reduce CO, SOx, particulate matter, and organic compounds [1–4]. The properties of this renewable biofuel are similar to those of petroleum-based diesel; thus, biodiesel can be used as either a substitute for diesel fuel or, more commonly, in a fuel blend. Several strategies for producing biodiesel have been published in the literature [2,5]. However, the transesterification of triacylglycerides and the esterification of free fatty acids (FFAs) with low molecular weight alcohols are the most important processes [6]. These reactions can be catalyzed by using mineral acids or bases, or by using enzymes. Producing biodiesel using a mineral catalyst has several drawbacks such as difficulty removing the catalyst from the product, high energy requirements for faster kinetics, difficulty recovering the catalyst for reuse, and potential pollution in the environment [7,8]. Today, enzyme-catalyzed biodiesel production has received more attention because of advantages such as nontoxicity, environment-friendly processes, low energy costs, and soft operating conditions compared with chemical-catalyzed methods [9,10]. However, this method is still not favored for industrial use because high costs and low stability of lipases limit potential applications [11,12].

The biodiesel reaction has been monitored with several chromatographic and spectroscopic techniques [13–15]. Among these methods, the use of spectroscopy exhibits great differentiation due to the increase in technology, the advance of real-time analysis, and easy-to-use application. This technique can be applied in- or on-line, eliminating the systematic error of sampling and facilitating data acquisition [16,17]. One of the most frequently used real-time analyses is the attenuated total reflexion–Fourier transform infrared (ATR/FT-IR) spectroscopy, which uses an internal reflection of the radiation beam inside a crystal. The intensity of the signal is attenuated due to multiple reflections along the length of the crystal in contact with the sample [18]. This technique automates the analysis, and the spectra can be acquired in a time resolution, which make the dynamic study of a chemical system possible. ATR/FT-IR

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http://dx.doi.org/10.1016/j.molcatb.2015.09.015
1381-1177/

can rapidly analyze the vibrational bands of the molecules present in the system, which may change during a reaction. Thus, tracking and monitoring of reactions are possible [19].

The reaction system can be more clearly understood by transforming the spectroscopic data into concentration information about the species in the system [15]. This transformation arises from the fact that there is linearity between the spectroscopic quantity (i.e., absorbance) and the concentration, as stated by the Beer–Lambert law. Numerous chemometric strategies can be used for this transformation, from a simple univariate linear regression to a more complicated non-linear multivariate regression, when linearity does not exist [20]. In the case of the infrared spectra of a reaction mixture, there can be much juxtaposition between vibrational bands of different compounds. Therefore, multivariate regressions must be used to quantify the concentrations. The most common regression used in these cases is partial least squares (PLS) [21,22].

In the present work, an in-line real-time methodology was used to evaluate and compare the kinetics of an esterification reaction catalyzed with homo (mineral acid) and heterogeneous (supported enzymes) catalysts. As a model for the reaction, oleic acid was reacted with ethanol and catalyzed with hydrochloric acid, Novozym 435, Lipozyme RM, and Lipozyme TL. ATR/FT-IR and PLS regression modeling were used to easily evaluate the kinetics of the different catalysts without multiple calibrations. Coupled gas chromatography–mass spectrometry (GC–MS) was also used to confirm the final conversion for the best catalyst.

2. Experimental

2.1. Equipment

ReactIR 45 m (Mettler Toledo) equipment was used for monitoring the reactions; it was equipped with an AgX 9.5 mm × 2 m Fiber (Silver Halide), with a 6.35 mm diamond crystal with 6 internal reflections as an ATR element, ZnSe as a support/focusing element and an MCT detector using Happ–Genzel apodization. The spectra were acquired in the range of 2000–650 cm^{-1} with a wavenumber resolution of 8 cm^{-1} in a 15-s interval between each spectrum (average of 25 scans).

The calibration and reactions were carried out in a 100 mL reactor controlled with a EasyMax Workstation (Mettler Toledo). The temperature was regulated by the equipment with a Pt 100 temperature sensor and a Peltier cooling system. The reaction was stirred at 200 rpm by using a propeller stirrer, also controlled by the equipment. The reactor was coupled with a condenser, to prevent loss of ethanol by evaporation, since the reaction temperature was close to its boiling point.

2.2. Multivariate calibration

Thirty-five standard mixtures of oleic acid, ethanol, water, and ethyl oleate were prepared, simulating different conversion times of the reaction. Table 1 shows the concentration of the components in each standard mixture.

The standard mixtures were maintained in the reactor with a stirring rate of 200 rpm and at 57 °C, and the spectra were acquired as an average of 250 scans. Once the spectra were collected, a PLS model was built using the software iCQuanti (Mettler Toledo). The responses of the model were the concentrations of the reaction components (oleic acid, ethanol, and ethyl oleate/water), where each one was calculated in an individual model. A spectral region between 1800 and 1540 cm^{-1} and 1400–1000 cm^{-1} of the first derivative of the spectra with mean centering for the model has used.

2.3. Reaction settings

The reactions started with 0.206 mol L^{-1} of oleic acid, 0.712 mol L^{-1} of ethanol, and the corresponding amount of cyclohexane as solvent. The temperature was set to 57 °C, and after it was stabilized, the catalyst was added. The concentration of the catalyst was 10% w/w in relation to oleic acid. The reactions were monitored by using the ATR/FT-IR probe for 165 min. As the reaction progressed, the PLS model was used for real-time quantification of each component. Reactions were performed in triplicate in order to estimate the experimental variance.

2.4. GC–MS analysis

The reaction conversion was determined by gas chromatography–mass spectrometry (GC/MS) using an internal standard (IS) calibration curve for the quantification of ethyl oleate. Pentadecanoyl propanoate was used as IS [23,24].

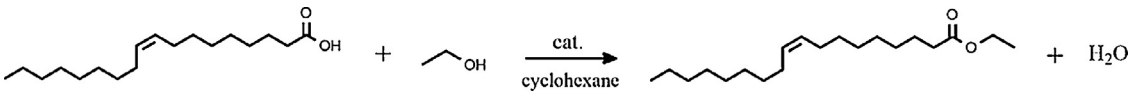
GC/MS analyzes were performed on a Shimadzu GC-QP2010 gas chromatography coupled to a Shimadzu GC–MS-QP2010 mass spectrometer. Electron ionization at 70 eV ionization energy was used. An RXi-1MS (100% methylpolysiloxane) capillary column with 30 m, 0.25 mm i.d., and 0.25 μm df was used. The carrier gas was helium at a flow rate of 2.4 mL min^{-1}. The temperature program was an isothermal period of 3 min at 210 °C, then increased at 20 °C min^{-1} to 290 °C, and final isothermal period of 3 min. Injection volume was 1.0 μL in split mode and with 1:30 split ratio. The injector temperature was 290 °C. The transfer line and ion source were held at 290 °C and 250 °C, respectively. Samples from the reactional media in the reaction catalyzed by Novozym 435 were taken at times: 0, 5, 30, 55, 80, 105, 130 and 155 min. The injection samples were prepared by mixing 500 μL of the 100× diluted reaction medium solution, 50 μL of the IS solution and 450 μL of cyclohexane. Analyses were performed in triplicates.

3. Results and discussion

Scheme 1 shows the esterification of oleic acid with ethanol, which was used in this work as a model reaction for biodiesel production.

The esterification reaction was monitored for 165 min, and an IR spectrum was taken every 15 s (as an average of 25 scans). The 3D infrared surface of the time-dependent spectra throughout the entire course of the reaction was obtained, as shown in Fig. 1 for the reaction catalyzed with Novozym 435. Fig. 1a and b shows the regions with the main variations in the spectra.

In Fig. 1, significant changes are shown in the region around 1750 cm^{-1} (a), which correspond to the carbonyl absorption bands of oleic acid and ethyl oleate, and in the region from 1200 to 1000 cm^{-1} (b), which correspond to the C—O-related vibrations from ethanol and ethyl oleate. As the reaction progresses, the concentrations of the reagents (oleic acid and ethanol) decrease, and



Scheme 1. Esterification of oleic acid with ethanol.

Table 1
Concentration of the different components in the prepared standard mixtures[a].

Theoretical conversion (%)	Concentration (mol L^{-1})			Theoretical conversion (%)	Concentration (mol L^{-1})		
	Oleic acid	Ethanol	Ethyl oleate/water		Oleic acid	Ethanol	Ethyl oleate/water
0	0.206	0.711	0.000	52.5	0.0977	0.602	0.108
2.5	0.201	0.706	0.0052	55	0.0925	0.597	0.113
5	0.196	0.700	0.0103	60	0.0822	0.587	0.124
7.5	0.191	0.695	0.0155	62.5	0.0770	0.582	0.129
10	0.185	0.690	0.0206	65	0.0719	0.577	0.134
15	0.175	0.680	0.0309	67.5	0.0667	0.571	0.139
17.5	0.170	0.675	0.0361	70	0.0616	0.566	0.144
20	0.165	0.669	0.0413	75	0.0513	0.556	0.155
22.5	0.160	0.664	0.0464	77.5	0.0461	0.551	0.160
25	0.154	0.659	0.0516	80	0.0409	0.546	0.165
30	0.144	0.649	0.0617	82.5	0.0358	0.540	0.170
32.5	0.139	0.644	0.0671	85	0.0306	0.535	0.175
35	0.134	0.638	0.0722	90	0.0203	0.525	0.185
37.5	0.129	0.633	0.0774	92.5	0.0151	0.520	0.191
40	0.123	0.628	0.0824	95	0.0100	0.515	0.196
45	0.113	0.618	0.0928	97.5	0.0048	0.510	0.201
47.5	0.108	0.613	0.0980	100	0.000	0.505	0.206
50	0.103	0.608	0.103				

[a] Cyclohexane was added to all standard mixtures, at constant volume, at the same ratio as in the reactions.

thus, the infrared signal decreases, while the product (ethyl oleate) increases. The oleic acid carbonyl band (1735–1700 cm^{-1}), one of the major bands, decreases exponentially, and the ethyl oleate carbonyl band (1770–1735 cm^{-1}) increases in the same manner. In Fig. 1b, a band is observed at around 1180 cm^{-1}, related to the C—C(=O)—O stretch band, as well as the decrease and displacement of the C—O band, from the ethanol to the ester.

The spectra in the infrared region depend on the population in each vibrational state. Therefore, temperature affects the intensities of the absorption bands directly. A calibration model must consider the temperature as a variable or should be constructed for a fixed reaction temperature. In this work, the calibration model was constructed at 57 °C. This temperature was based on previous work developed inside our group, which showed this temperature was the optimal condition for the enzymatic catalysts in the reaction [25]. In addition, since the reaction medium is not homogeneous (ethanol and water are not soluble in the oleic acid and cyclohexane), the mass transfer is important for calibration modeling. Thus, the stirring rate and the stirrer type used to measure the calibration spectra were the same as those used for all reactions, in order to guarantee the similarity of the reaction spectra in relation to the spectra of the standards for the PLS modeling.

After this initial assay, the spectra of 35 calibration standards were measured and were used for the PLS model as training or test points. The training points were used to calculate the calibration model parameters, while the test points were used to check the previously calculated model for accuracy and precision. Fourteen of these standards were used as test points. The responses of the model were the concentrations of the reaction components (oleic acid, ethanol, and ethyl oleate/water), where each one was calculated in an individual model. Fig. 2 shows the spectrum for each standard and the measured vs. predicted graph for the ethyl oleate concentration from the PLS model.

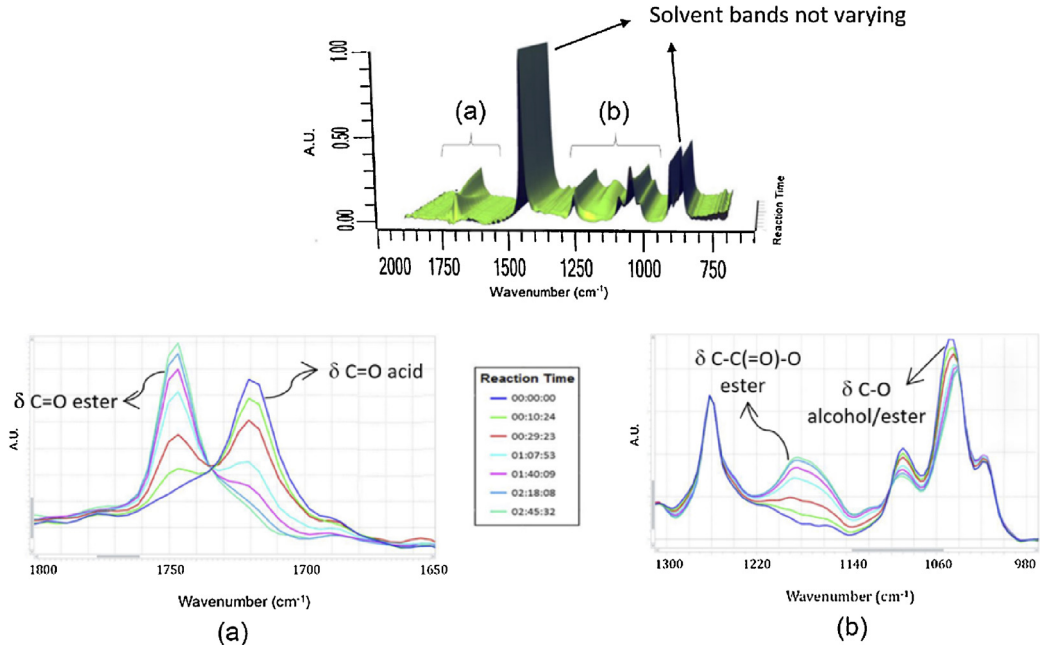


Fig. 1. 3D surface showing the variation in the IR spectra over the course of the reaction and the main regions with significant changes: (a) the carbonyl band region and (b) the C—O-related band region.

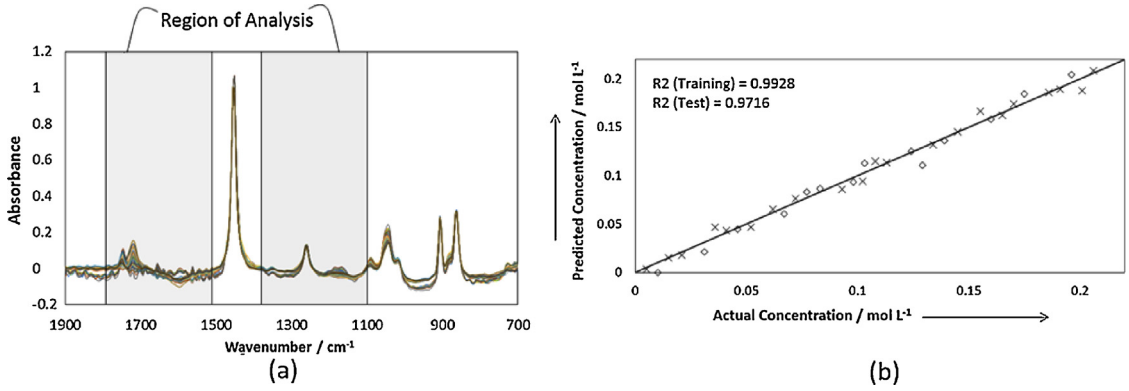


Fig. 2. (a) Spectra taken for each calibration standard and (b) actual vs. predicted graph for the ethyl oleate concentration calculated with the PLS model, where are training points and are test points.

As shown in Fig. 2a, the spectra obtained from the calibration standards present the same profile from the reaction spectra obtained and shown on Fig. 1, which confirms good representativity for the application of the PLS model during the quantification and monitoring of this reaction. The model achieved good linearity, with a coefficient of determination (R^2) close to 1 for the training and test points in the actual vs. predicted graph. This indicates the calibration model is accurate (Fig. 2b). The analytical model was also checked by verifying how well it could be used to predict points that were different from those used for training, by analyzing the error in the prediction of the test set. Table 2 shows the root mean square error of calibration (RMSEC), the root mean square error of cross-validation (RMSECV), and the root mean square of prediction (RMSEP) for the calibration model.

In practical terms, the RMSEP is the most direct error because the RMSEP shows the expected deviation from the predicted response when the calibration model is used. As it can be seen in the table, for the four test points selected, this error was 9.28×10^{-3}, 9.30×10^{-3}, and 9.23×10^{-3} mol L^{-1} for ethanol, oleic acid, and ethyl oleate/water, respectively. This shows that the calibration model has good precision for all responses. The prediction error of the calibration model compared to the reaction data, however, is expected to be larger than the RMSEP, since there could be a lack of correlation between the calibration standard spectrum and the real reaction spectrum and there could be differences in the equipment conditions from one experiment to nother. In order to avoid this type of reproducibility error, an initial correction was made by normalizing the known initial concentration measured and quantified by the ATR/FT-IR, shown in Eq. (1).

$$C_{new} = \left(\frac{C_{known}}{C_{max}}\right) \times C_{old} \quad (1)$$

in this equation, for each of the concentration response of the model (ethanol, oleic acid or ethyl oleate/water), C_{new} is the corrected concentration, C_{old} is the uncorrected concentration calculated directly from the PLS model, C_{known} is the known initial concentration, and C_{max} is the initial concentration calculated directly from the PLS model.

Table 2
Calculated errors for the calibration model.

Error	Response (mol L^{-1})		
	[Ethanol]	[Oleic acid]	[Ethyl oleate/water]
RMSEC	5.63×10^{-3}	5.75×10^{-3}	5.77×10^{-3}
RMSECV	1.31×10^{-2}	1.32×10^{-2}	1.30×10^{-2}
RMSEP	9.28×10^{-3}	9.30×10^{-3}	9.23×10^{-3}

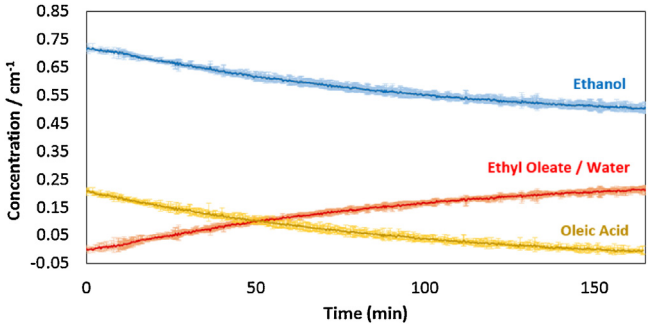


Fig. 3. The average of the components of the esterification reaction calculated with the PLS model using Novozym 435 as catalyst.

The type of catalyst was not considered during the calibration methodology. Thus, the same calibration model can be applied to evaluate different catalysts. With the constructed model, this type of evaluation can be made in real-time, which can be a powerful method for industrial applications and kinetic analyses. Finally, the system was tested for different enzymes and HCl as catalysts to verify the conversion after 165 min in each case. Each experiment was performed in triplicate, and the average response was considered for each catalyst after the same period of time. The reaction profile measured for the reaction catalyzed by Novozym 435 is shown in Fig. 3 with the confidence interval for each response.

To confirm the reliability of the ATR/FT-IR/PLS method, GC–MS was used as a referee quantitative analysis for the reaction catalyzed by Novozym 435, where samples from the reaction medium were taken at different times, diluted and injected in the GC–MS. Quantification was made for the ethyl oleate concentration, using a univariate calibration curve. Ethyl oleate had a retention time of 4.5 min, while oleic acid did not leave the column during the chromatographic analysis. The calibration curve ($y = 0.00621x - 0.1039$) had a coefficient of correlation (R^2) of 0.94. Fig. 4 shows the comparison of the conversion measured with the ATR/FT-IR and with GC–MS.

In order to check if the two averages correspond to the same value statistically, a hypothesis test was performed in the form of a Student's *t*-test. The null hypothesis in the test states the two averages are the same, and if the calculated *t*-value is smaller than the critical *t*-value, then this hypothesis is correct. Table 3 shows the results for the test for each of the points of comparison.

It can be seen that for every sample taken for comparison, the *t*-value was smaller than the critical *t*-value, which means that the two techniques were measuring the same thing, validating the ATR/FT-IR method. The variance of the replicates measured by GC–MS, in these cases, were even greater than the one measured

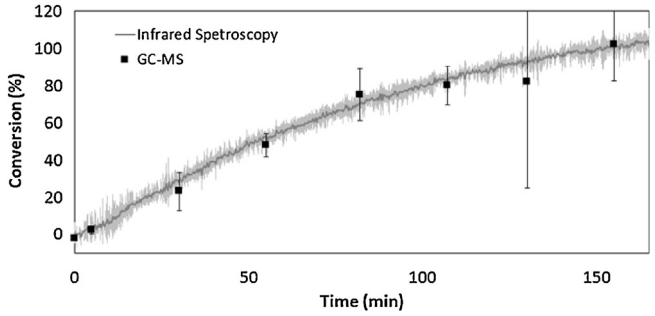


Fig. 4. Comparison of the conversion measured by ATR/FT-IR and GC–MS for the reaction catalyzed by Novozym 435.

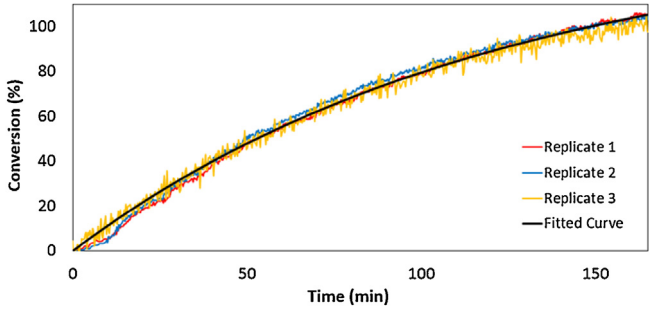


Fig. 5. Fitting of the ethyl oleate concentration as an exponential rise for the reaction catalyzed with Novozym 435.

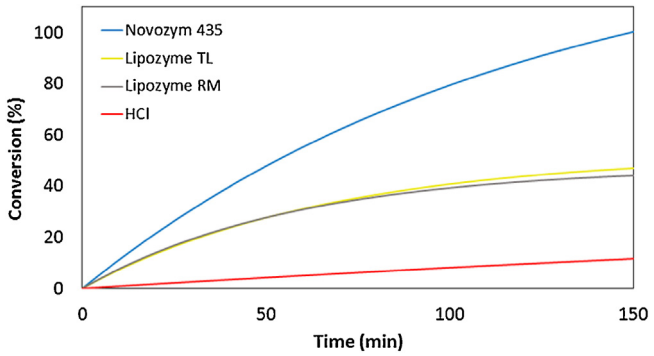


Fig. 6. Trend of the conversion for each catalyst, calculated by exponential fitting of the ethyl oleate concentration.

by ATR/FT-IR. The point for 130 min in the GC analysis showed a disproportional variance, and may be due to an unexplained error. However, validation can still be made. This shows that the ATR/FT-IR model is a good method for quantitatively monitoring these reactions and the evaluation of the efficiency of the catalysts.

A final treatment can be made to evaluate the conversion trend for each reaction. The increase in the ethyl oleate concentration can be assumed to follow an exponential form and can be estimated with Eq. (2).

$$C_{EO} = C_{EO,SS}[1 - \exp(-\lambda t)] \quad (2)$$

where C_{EO} is the time-dependent concentration of ethyl oleate, $C_{EO,SS}$ is the concentration of ethyl oleate in the steady state, λ is the exponential rise coefficient, and t is the time.

With the triplicate of the ethyl oleate concentration measured in each experiment, a non-linear fit was performed to estimate the parameters that best describe the form of the curve, minimizing the sum of squares of error between the modeled response and the response measured in the triplicate. The fitting works as a way to overcome possible systematic errors, such as fluctuation among the three measurements, and to avoid negative values measured at the beginning of the reaction due to the error of prediction. Once the curve is fitted, it can be transformed into conversion and used to compare the catalysts. Fig. 5 shows the fitted curve in the experiment using Novozym 435 as catalyst, and Fig. 6 shows the conversion for each catalyst, calculated with the exponential fitting.

Fig. 6 shows the conversion for all catalysts calculated from the best-fitted curve for all triplicates. Novozym 435, the catalyst with the highest final conversion, also had the best efficiency and the fastest kinetics among the four catalysts. This enzyme is the best because it does not have a "lid" in its structure that covers the active site, while the other two enzymes do. This "lid" has a top hydrophilic side and an interior hydrophobic face, so that the "lid" needs interfacial activation in order to open and expose the active site to reagents. Since Novozym 435 does not have this "lid", the reaction can be catalyzed directly, while the TL and RM lipases require this initial phenomenon, which affects the kinetics.

Table 3
Results of the hypothesis test for the comparison of two techniques.

Time (min)	Conversion by ATR/FT-IR		Conversion by GC–MS		t-value[a]
	Average	Conf. interv.	Average	Conf. interv.	
0	1.13	5.59	-2.33	0.98	2.14
5	4.20	8.35	2.51	2.13	0.69
30	28.2	1.12	23.2	10.3	1.70
55	52.4	2.57	50.3	8.10	0.85
80	70.4	3.10	75.1	14.0	1.15
105	84.0	2.57	80.3	10.5	1.21
130	93.2	1.96	82.0	15.7	1.77
155	101	2.87	102	19.8	0.23

[a] Critical t-value for the degree of freedom between the averages was 4.30.

Table 4
Final conversion measured by the ATR/FT-IR calibration model for enzymatic and HCl catalyzed reactions.

Response	Conversion (%)			
	HCl	Novozym 435	Lipozyme RM	Lipozyme TL
Ethanol (model)	14	101	48	50
Oleic acid (model)	14	102	49	51
Ethyl oleate (model)	16	103	49	51

All enzymes presented higher conversion and fastest kinetic rates than HCl in this reaction condition throughout the entire course of the reaction. The results for the final conversion at time 165 min are presented in Table 4.

Novozym 435 was the best catalyst for the esterification under the experimental conditions, since it had a complete conversion after 165 min of reaction. Lipozyme RM and Lipozyme TL had similar conversions, but their efficiency was almost half that of Novozym 435. These results are in agreement with those found by Corrêa et al. for the esterification of palm oil using the same enzymes [12]. For the reaction catalyzed with hydrochloric acid, under the same experimental conditions as the enzymatic reactions, final conversion almost 4 times lower than Lipozyme RM/TL and almost 6 times lower than Novozym 435.

4. Conclusions

The PLS model in the ATR/FT-IR data was an efficient methodology for real-time quantification of the reaction components in the esterification of fatty acids, here represented by the esterification of oleic acid as a biodiesel reaction. The model showed good precision, with a very low prediction error and good accuracy of the concentrations as responses and was validated statistically in comparison to GC–MS. Novozym 435, which showed complete conversion after 165 min, was the best catalyst for this reaction.

Lipozyme RM and Lipozyme TL showed an inferior conversion after the same time, in agreement with the literature. The enzymatic catalysts showed higher conversion than hydrochloric acid. The advantages of this methodology are the real-time quantitative monitoring and the evaluation of the efficiency of different catalysts for the same reaction, with only one calibration experiment. Furthermore, the treatment shown here makes a future kinetic evaluation of the reactions possible, using the non-linear fitting of kinetic models to the experimental data.

Acknowledgments

Authors thanks FAPERJ, CNPq, CAPES and Petrobras for financial support.

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