

A Study on the Biochemical Reaction Engineering of Biodiesel Production

Alberto Gallifuoco, Laura Cantarella^a, Agata Spera, Isa Venti, Maria Cantarella

Department of Chemistry, Chemical Engineering and Materials, University of L'Aquila,
Monteluco di Roio, 67040, L'Aquila, Italy

^aDepartment of Industrial Engineering, University of Cassino, Via Di Biasio 43, 03043
Cassino (FR), Italy

Corresponding e-mail: cantarel@ing.univaq.it

The bioesterification of ethyl alcohol with palmitic acid catalyzed by lipase from *Candida rugosa* was studied. This key model reaction of biodiesel production was assayed in two different organic solvents, n-hexane and iso-octane. The activity was studied with respect to the effect of enzyme loading, and linearity conditions were determined. Substrates concentrations were varied both at stoichiometric ratio (1:1) and keeping alternatively one substrate fixed at 100 mM and varying the other one. The presence of an optimal condition (50 mM, stoichiometric ratio) was detected. The system behaves differently depending on the organic solvent, and ethanol demonstrated to have a rate depressing effect at excess concentration. These findings confirm that the kinetic pattern is complex. Finally, the effect of temperature was investigated and the activation energies in the two different organic solvent media were determined, resulting 7268 and 8763 cal/mole respectively with iso-octane and n-hexane.

Introduction

Biodiesel (mono-alkyl esters of primary alcohol and long-chain fatty acids derived from vegetable oils or animal fats) is nowadays an important alternative, biodegradable renewable fuel for diesel engines. Although its use with internal combustion engines is technologically well assessed, the industrial production is not yet completely optimized. Most processes for making biodiesel are based on esterification reactions and require the use of a catalyst (strong mineral bases) to initiate the reaction, to promote the solubility of the reactants and to allow the process to proceed at a significant rate (Vicente et al, 2004). Several aspects are still under investigation aiming to improve the process efficiency, reduce the amount of by-products, and mitigate the ecological impact. The key chemical reaction involved in the process are esterifications and transesterifications (Fukuda et al, 2001). The primary raw materials used for making biodiesel are from agricultural sources: energy crops, such as sunflowers and rapeseed, or waste oils from the food industry. Till now methyl alcohol has been used in the esterifications, although green ethanol could represent a valid alternative owing to the lower toxicity and the higher yield on a weight basis (Masaru et al, 2001).

A totally green production of biodiesel should be performed in a bioprocess using lipases (EC 3.1.1.3) as catalyst (Shimada et al, 2002).

The present study deals with a model reaction, esterification between palmitic acid and ethyl alcohol catalyzed by lipase from *Candida rugosa*. This system is complex, ruled by a two-substrate kinetics, and possibly controlled by physical phenomena, such as interfacial tension between the liquid phase and the insoluble enzyme. Furthermore solvent polarity, and product and substrate solubility superimpose their effects

This contribution reports on the effect of substrate concentration and enzyme loading on the reaction rate, investigating in order to determine the optimal conditions which maximize the yield of esterification. The kinetic runs were performed in batch reactors using two different solvents, n-hexane and iso-octane. The driving force for the reaction to proceed is represented by the overall concentration of the two different substrates, but alcohol and acid play different roles, and these have been isolated performing runs at constant concentration of one substrate and varying the concentration of the other. An optimal ratio of substrate concentration has been detected. The effect of enzyme loading was also investigated maintaining the system at the best substrate concentration. Finally, the effect of temperature has been studied.

The body of the results so far obtained provides essential information to address further on the future research to ascertain the best reactor configuration and feeding policy in order to perform the quantitative continuous bioconversion, and to lessen the amount of solvent to be removed at the end of the reaction to produce a biodiesel suitable as commercial fuel.

Materials and methods

Lipase (triacyl glycerol lipase, EC 3.1.1.3) from *Candida rugosa* was the type VII lyophilized powder from Sigma-Aldrich (USA). The enzyme powder was directly suspended in the reaction media. Ethyl alcohol (EtOH) was from Fluka Chemika (Germany). Palmitic acid (PA), ethyl palmitate (EP), iso-octane and n-hexane were from Sigma-Aldrich (Germany). All the chemicals were laboratory grade pure reagent.

Two different stirred reactors were employed in the kinetic runs: one of 4 ml volume, thermostated in a Reacti-Therm model 18971 module (Pierce, USA) by insertion in an aluminium plate controlled for stirring speed and temperature; the second one is a water-jacketed glass reactors, 10 ml volume, designed on purpose, whose temperature and stirring control is assured by a thermostatic bath (Haake D8, Denmark) and a magnetic stirrer (Heidolph, Germany). The analytical determination of residue substrates and products was performed using a HP 5890 series II gas chromatograph equipped with a flame ionization detector, a capillary column HP-FFAP, and an autosampler HP 6890. The whole equipment was from Hewlett-Packard (USA).

Unless otherwise specified, all the kinetic runs were performed at 40 °C under 300 rpm stirring conditions and according to the following procedure. Substrates were dissolved carefully into the appropriate solvent, iso-octane or n-hexane, until a clear solution was obtained. The mixture was introduced into the reactor and allowed to equilibrate at the set temperature. The reaction was started adding the amount of enzyme powder. The reactor was then sealed to reduce losses by evaporation. At fixed time intervals, samples of negligible volume (50 µl) were withdrawn using a syringe through a Teflon cap, diluted with 150 µl of solvent to adjust the concentration range of analytes, filtered with a syringe microfilter for removing traces of enzyme powder, and injected in the column. Two different blanks were prepared for each run: just before the enzyme addition and a few seconds after. The peak areas of the two blanks resulted similar in all the

experiments and the average was taken as reference. The samples, prepared in the autosampler vials and where then analyzed.

The standard temperature program of analysis was the following. Injector: 220 °C; column: initial, 80 °C (2.5 min); up to 120 °C (2 min); hold at 120 °C (9.5 min); up to 230 °C (6 min); hold 230 °C (5 min). Carrier gas was helium at 16 ml/min. Under these conditions, total analysis time resulted 25 min and retention times were: ethanol, 2 min; ethyl palmitate, 12 min; palmitic acid 21 min. Peak resolution was fair, and the concentration of each chemical was easily estimated using as reference calibration lines performed with internal standards expressly prepared. In order to reduce the errors, all the analyses were performed at least in duplicate.

Results and discussion

In preliminary runs, the enzyme activity was assayed with both solvents with 100 mM of the two substrates at stoichiometric ratio. Enzyme loading varied from 4 to 20 mg/ml. Linearity conditions were verified with both solvents and the specific activities were 0.0056 and 0.0049 EU/mg respectively for iso-octane and n-hexane, resulting the activity 14% higher in iso-octane. This activity ratio should be considered a reference datum, since varying the concentrations of the two substrates different results could be obtained. The bioesterification was assayed in the jacketed reactor performing kinetic runs at fixed amount of enzyme (10 mg per ml of iso-octane) and different concentration of the two substrates (stoichiometric ratio $[\text{EtOH}]/[\text{PA}] = 1$). The reaction was followed up to 240 min withdrawing samples at regular time intervals and assaying for substrates and products. Results are reported in Figure 1 as substrate conversion vs process time.

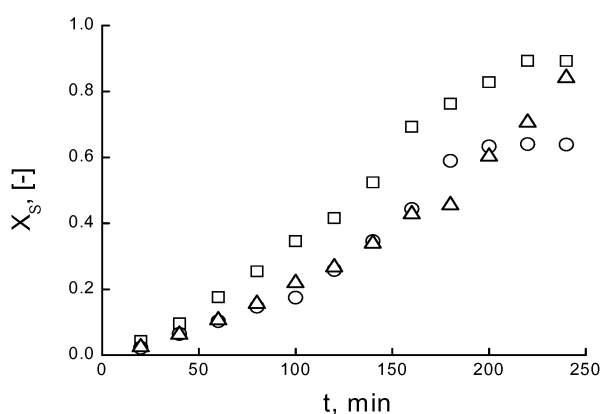


Figure 1. Substrate conversion as a function of process time. $[S]_0$ (mM): \square , 20; \triangle , 40; \circ , 60.

The enzyme activity increases with substrate concentration, but not linearly, as evident from the initial derivatives of the curves (respectively 0.055, 0.065, and 0.066 min^{-1}). As the reaction proceeds, substrate concentration diminishes, causing initially an acceleration then, at longer process time, the rate depletes: consequently, s-shaped

curves are generated. The higher the initial substrate concentration, the more evident this behaviour. This indicates that substrate(s) act as driving force for the reaction to proceed but that an excess could depress the kinetics. Data of Figure 1 also show that conversion as high as 90% could be attained.

In order to sustain the bioprocess, substrate concentration should be carefully maintained close to the optimal value. Experiments performed in 4 ml reactors aiming to detect this optimal substrate concentration are shown in Figure 2.

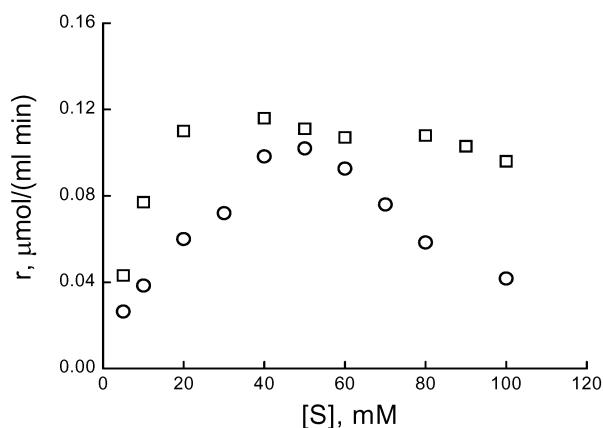


Figure 2. Reaction rate as a function of substrate concentration. Solvent: □, iso-octane; ○, n-hexane.

The amount of enzyme powder per ml of reaction medium was the same of the previous experiments while substrate concentrations varied from 5 to 100 mM, at constant ratio $[\text{EtOH}]/[\text{PA}] = 1:1$. The obtained data show a rather different behaviour with both the solvents. The rate depression effect is more evident using n-hexane as solvent, with the existence of a maximum at roughly 50 mM. Besides, whatever the substrate concentration, the reaction rate in the n-hexane medium is lesser than that in iso-octane. These results indicate that solvent plays an important role, presumably modifying forces acting at the enzyme/liquid interface. To reduce costs of production, the industrial bioprocess should be performed in scarcity of solvent and this should be selected considering both the enzyme activity and the easiness of separation. On the other hand, the kinetic pattern of this reaction is complex, and several equations are available in the literature for modelling the biocatalytic system. As a general rule, these equations contain too more parameters and non linear terms to be easily handled. The preliminary results here reported could help to address further on the research aiming to get a lumped kinetic equation simple but predictive with respect to the effect of operational parameters variations.

To meet this goal, further experiments were performed varying alternatively one substrate concentration from 15 to 180 mM, while keeping the other one constant at 100 mM. Results obtained with both solvents are reported in Figure 3 (constant $[\text{PA}]$) and Figure 4 (constant $[\text{EtOH}]$).

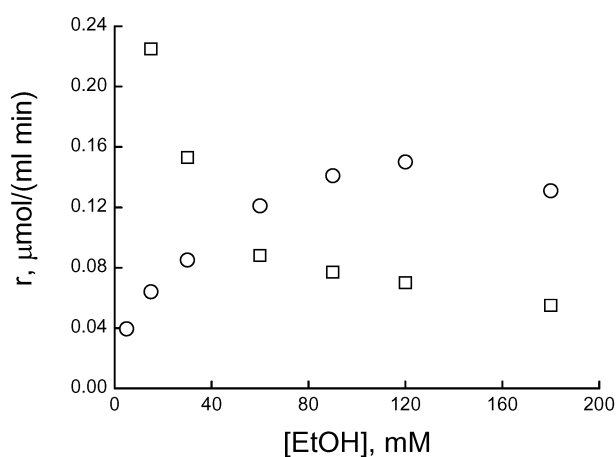


Figure 3. Reaction rate as a function of ethanol concentration.

Solvent: □, iso-octane; ○, n-hexane.

Observing Figure 3, the effect of solvent is dramatically evident. In the iso-octane medium, the higher the ethanol concentration, the lower the reaction rate in the whole range explored. Using n-hexane, the reaction rate passes through a maximum. Obviously, if no ethanol was present, the esterification could not occur at all, so that also with iso-octane there should be a maximum, but this evidently occurs at a concentration below 15 mM. The other substrate, PA, acts similarly in both solvents. In fact, as PA concentration increases, the reaction accelerates and, at the experimental conditions adopted in Figure 4, the bioconversion occurs at higher rate using n-hexane.

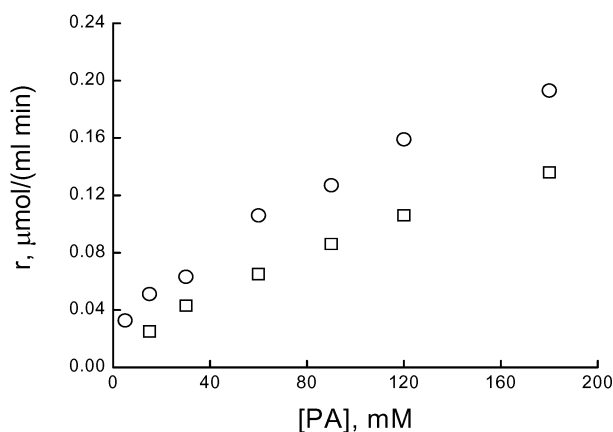


Figure 4. Reaction rate as a function of palmitic acid concentration.

Solvent: □, iso-octane; ○, n-hexane.

The last part of the research was devoted to determine the effect of temperature on the bioesterification rate (see Figure 5). Experiments were performed at 100 mM stoichiometric substrate ratio and demonstrated that in both solvents the reaction follows the Arrhenius law.

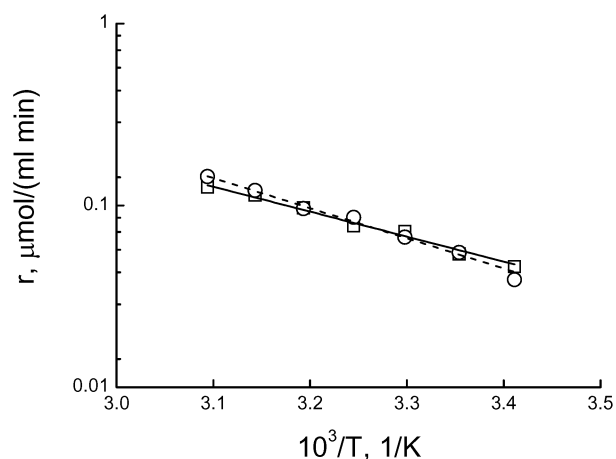


Figure 5. Arrhenius plot. Solvent: □, iso-octane; ○, n-hexane.

The activation energies detected were 7268 and 8763 cal/mole respectively for iso-octane (dashed line) and n-hexane (full line), thus indicating that the kinetics is not masked significantly by physical phenomena, such as mass transport or interfacial interactions, which could superimpose their effect to the biocatalytic act.

Conclusions

The body of the experimental findings here discussed constitutes a good starting point for extending the experiments on this model reaction. Useful information has been obtained for addressing further research. The main points to be studied appear to be the optimal substrate feeding policy, the strategies to get 100% bioconversion lessening the amount of organic solvent employed.

Acknowledgments

The authors would like to acknowledge the support of University of L'Aquila Research Fund, and MIUR-PRIN 2002095553_002

References

- Masaru K, S. Taichi, K. Akihiko, H. Fukuda, 2001, Effect of methanol and water contents on production of biodiesel fuel from plant oil catalyzed by various lipases in a solvent-free system. *J. Biosci. Bioeng.*, 91, 1, 12.
- Fukuda H., A. Kondo, H. Noda, 2001, Biodiesel fuel production by transesterification of oils. *J. Biosci. Bioeng.*, 92, 405.
- Vicente G., M. artinez, J. Aracil, 2004, Integrated biodiesel production: a comparison of different homogeneous catalysts system. *Biores. Technol.*, 92, 297.
- Shimada Y., A. Watanabe, Y. Sugihara and, T. Tominaga, 2002, Enzymatic alcoholysis for biodiesel fuel production and application to the reaction to oil processing. *J. Mol. Catal. B-Enzym.*, 17, 133.