

The role of organic solvent amount in the lipase-catalyzed biodiesel production

O papel da concentração de solvente orgânico na produção enzimática de biodiesel

Clarissa DALLA ROSA¹, Débora de OLIVEIRA¹, José VLADIMIR OLIVEIRA^{1*}

Abstract

This research note addresses the role of organic solvent amount in the production of fatty acid ethyl esters from soybean oil. N-hexane was chosen as solvent and two commercial immobilized lipases as catalysts, Novozym 435 and Lipozyme IM. The reactions were conducted in 6 hours, varying the solvent to oil ratio from zero to 50 (v/wt) and adopting for Novozym 435: 65 °C, enzyme concentration (E, wt%) = 5, oil to ethanol molar ratio (R) = 1:10, water addition (H, wt%) = 0, and for Lipozyme IM: 35 °C, E = 5 wt%, R = 1:3, H = 10 wt%. For Lipozyme IM, an increase in solvent amount is shown to lead to an enhancement of reaction conversion, while a negligible effect was found for Novozym 435. When using 30 mL of solvent the reaction conversions were 88% for Lipozyme IM and 15% for Novozym 435.

Keywords: *biodiesel; lipases; organic solvent; soybean oil.*

Resumo

O objetivo desta pesquisa é reportar o papel da quantidade do solvente orgânico na produção de ésteres etílicos de ácidos graxos do óleo de soja. O n-hexano foi escolhido como solvente e duas lipases comerciais imobilizadas como catalisadores, Novozym 435 e Lipozyme IM. As reações foram conduzidas em 6 horas, variando a razão de solvente de zero a 50 (v/m), adotando para a Novozym 435: 65 °C, concentração de enzima (E, m/m%) = 5, razão mola óleo:etanol (R) = 1:10, adição de água (H, m/m%) = 0, e para a Lipozyme IM: 35 °C, E = 5 m/m%, R = 1:3, H = 10 m/m%. Para a enzima Lipozyme IM, um aumento na quantidade de solvente conduz a uma maior conversão da reação, enquanto um efeito não significativo foi encontrado para a Novozym 435. Com 30 mL de solvente, a conversão encontrada para a Lipozyme IM foi de 88% e 15% para a Novozym 435.

Palavras-chave: *biodiesel; lipases; solvente orgânico; óleo de soja.*

1 Introduction

Biodiesel has progressively become acceptable worldwide due to its beneficial properties (a non-toxic, biodegradable, domestically produced, renewable source) and its potential advantages to be used as diesel fuel additive or replacement (higher cetane number when compared to diesel from petroleum and favorable combustion emissions profile, such as reduced levels of particulate matter and carbon monoxide, and under some conditions, nitrogen oxides) (MA; HANNA, 1999; SRIVASTAVA; PRASAD, 2000; ALTIN; ÇETINKAYA; YUCESU, 2001; FUKUDA; KONDO; NODA, 2001; McCORMICK et al., 2001; ZHANG et al., 2003). As a consequence, there has been much effort over the past years to develop new methods and to improve the existing biodiesel production technologies. Among some of the processes used for biodiesel production, such as pyrolysis and micro-emulsification, transesterification is one of the most common methods to produce biodiesel (MA; HANNA, 1999; FUKUDA; KONDO; NODA, 2001). Transesterification, also called alcoholysis, refers to a catalyzed reaction involving the displacement of an alcohol, preferentially methanol or ethanol, from an ester by another alcohol to yield fatty acid alkyl esters (i.e., biodiesel) and glycerol as a by-product. Conventionally, transesterification can be performed using alkaline, acid or enzyme catalysts in short reaction times but there are several

drawbacks: it is energy intensive; recovery of glycerol may be difficult; the acid or alkaline catalyst has to be removed from the product; alkaline wastewater requires treatment; and free fatty acids and water interfere with the reaction (MA; HANNA, 1999; FUKUDA; KONDO; NODA, 2001; ZHANG et al., 2003).

The use of enzyme-catalyzed transesterification methods has been proposed to overcome the problems arising from the so-called chemical catalysis. Though at present the high cost of enzyme production is the major obstacle to commercializing enzyme-catalyzed processes, advances in enzyme technology, such as the use of solvent-tolerant lipases and immobilized lipases, which enable the catalyst re-utilization, have been made to develop cost-effective systems (FUKUDA; KONDO; NODA, 2001; ISO et al., 2003). In this sense, several research studies have reported an alternative method to produce esters through enzymatic reactions using lipases as catalysts, the majority of them using organic liquid solvents as reaction medium (YAMANE, 1988; ABRAMOWICZ; KUSE, 1989; BARZANA; KAREL; KLIBANOV, 1989; YAMANE et al., 1990; STEVENSON; STORER, 1991; CARTA; GAINER; ZAIDI, 1995; OLIVEIRA; ALVES, 1999, 2000; OLIVEIRA et al., 2004, 2005a). Many lipase-catalyzed reactions have been performed in compressed fluids (mostly carbon dioxide) in an attempt to

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¹ Departamento de Engenharia de Alimentos, Universidade Regional Interaga – URI, Campus de Erechim, Av. Sete de Setembro, 1621, CEP 99700-000, Erechim, RS, Brazil, E-mail: vladimir@uricer.edu.br

*A quem a correspondência deve ser enviada

overcome the alleged drawbacks found in conventional liquids (OLIVEIRA et al., 2005b).

When conducting lipase-catalyzed production of biodiesel in solvent medium, the following natural questions may arise: does the solvent amount affect the conversion of alcoholysis? What is the minimum amount to minimize mass-transfer limitations, i.e., sufficient to give acceptable yields?

Thus, this work aims to answer these questions, since to the best of our knowledge, they have not yet been addressed in the literature. Therefore, the production of fatty acid ethyl esters from soybean oil using n-hexane as solvent and two commercial lipases as catalysts, Novozym 435 and Lipozyme IM, was adopted as a model system.

2 Materials and methods

2.1 Materials

Commercial refined soybean oil (Soya – Brazil) was used as purchased without any pre-treatment. The fatty acid composition used in this work (OLIVEIRA et al., 2005a) is very similar to the typical values reported in the literature (SRIVASTAVA; PRASAD, 2000; ALTIN; ÇETINKAYA; YUCESU, 2001). Ethyl alcohol and n-hexane (both from Merck, 99.9% pure) were used as substrate and solvent, respectively.

2.2 Enzymes

Two commercial immobilized lipases were kindly supplied by Novozymes Brazil (Araucária, PR, Brazil): *Mucor miehei* (Lipozyme IM) immobilized on a macroporous anion exchange resin (0.15 U.g⁻¹ and 4 wt% water) and *Candida antarctica* (Novozym 435) immobilized on a macroporous anionic resin (0.12 U.g⁻¹ and 1.4 wt% water). According to the manufacturer, the optimum activities for both enzymes are achieved at 40 °C for Lipozyme IM and at 70 °C for Novozym 435 (OLIVEIRA; ALVES, 1999).

2.3 Analytical method

The reaction conversion was determined by gas chromatography coupled with a mass spectrometer detector (GC/MS). The analytical procedure used for determining the fatty acid ethyl esters content is fully described by Oliveira et al. (2005a).

2.4 Apparatus and experimental procedure

The experimental procedure adopted to conduct the alcoholysis reactions is described in detail by Oliveira et al. (2004, 2005a). Briefly, the experiments were performed in stoppered 300 mL Erlenmeyers flasks. Lipase was added to the mixture of oil-ethanol-solvent and the flasks were agitated at 200 rpm for 6 hours in a shaker with temperature control. Reactional mixtures were then filtered and submitted to solvent evaporation at mild temperature under moderate vacuum up to constant weight. Based on previous works the following parameters were adopted: for Novozym 435-65 °C, enzyme

concentration (E, wt%) = 5, oil to ethanol molar ratio (R) = 1:10, water addition (H, wt%) = 0, and for Lipozyme IM – 35 °C, E = 5 wt%, R = 1:3, H = 10 wt% (OLIVEIRA et al., 2005a), 1 g of oil was used in the experiments and n-hexane was used as solvent medium, varying its amount from zero to 50 mL.

3 Results and discussion

In order to investigate the effect of solvent amount in the enzymatic biodiesel production, the experimental conditions adopted were those found in the work of Oliveira et al. (2005a). Figure 1 shows the experimental results for Lipozyme IM, which exhibits specificity in the 1.3 triglyceride positions, and Novozym 435, a non-specific lipase, for 6 hours reaction.

It can be observed that much higher yields were achieved in the experiment with 30 mL of n-hexane for Lipozyme IM when compared to Novozym 435, around 88% compared to ~15%, respectively. This specific result obtained for both enzymes with 30 mL of solvent will not be overemphasized, as it does not bring new information nor is it the focus of the present work; i.e., differences found between the performance of Novozym 435 compared to Lipozyme IM are not stressed as they may be considered a “classical result” available in the open literature (OLIVEIRA et al., 2005a; VICENTE et al., 1998). Perhaps, the poorer performance verified for Novozym 435, compared to Lipozyme IM, may be related to substrate inhibition (alcohol and/or oil), solvent denaturing caused by deleterious interaction between the enzyme-solvent.

The interesting point in Figure 1 is that an increase in the solvent amount up to a certain value is not capable of improving the reaction conversion for both enzymes. However, beyond this value reaction the performance becomes very attractive for Lipozyme IM, with a final conversion of about 90%. Thus it seems that an enhancement in the solvent to oil ratio breaks

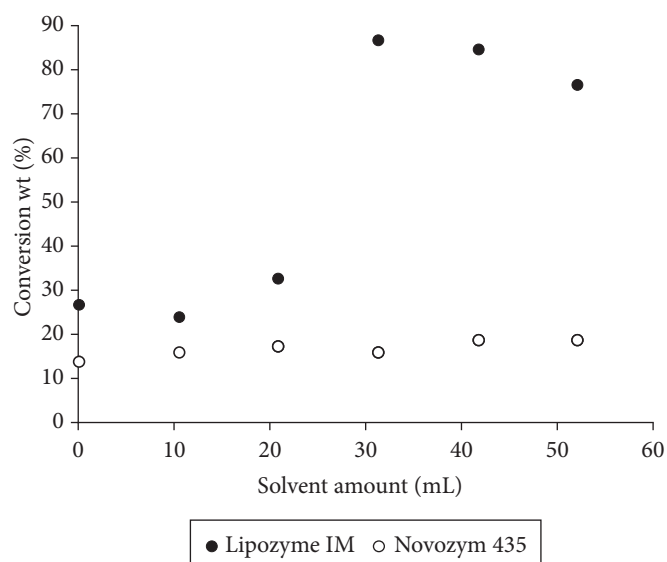


Figure 1. Effect of n-hexane amount on the conversion of lipase-catalyzed alcoholysis of soybean oil using Lipozyme IM and Novozym 435. The experimental conditions are described in the text.

mass-transfer limitations, increasing diffusion of solutes. Nevertheless, rising the organic solvent amount again does not provoke significant changes in the reaction conversion, with a slight decrease in conversion for the last solvent volume tested. This reduction in the reaction performance may be due to the lessened substrates molar fraction in the reaction bulk, i.e., excessive dilution leading to poor interaction between substrates and enzyme particles (LAUDANI et al., 2007).

Undoubtedly, knowing the solvent to oil ratio is of primary importance for industrial applications if we take into account that capital equipment and energy (separation) costs are closely related to operational charges applied to the process. The results obtained in this work corroborate the commonly used 40 mL of solvent for each gram of oil used in alcoholysis reactions (OLIVEIRA; ALVES, 1999, 2000; OLIVEIRA et al., 2004, 2005a; MITTELBAACH, 1990). From an industrial point of view, this seems to be an unacceptable, unfeasible, result when compared to the use of carbon dioxide as solvent medium, for which a ratio of solvent to oil as low as 2:1 has been found to allow complete reaction conversions (OLIVEIRA, D.; OLIVEIRA, J. V., 2000).

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