Bioresource Technology 100 (2009) 5126–5131

Contents lists available at ScienceDirect

Bioresource Technology

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

Factor analysis of transesterification reaction of waste oil for biodiesel production

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ARTICLE INFO

Article history:
Received 16 January 2009
Received in revised form 6 May 2009
Accepted 17 May 2009
Available online 4 June 2009

Keywords:
Lipase
Environmental pollutant
Biodiesel
Immobilized enzymes
Waste conversion

ABSTRACT

In the present paper a factor analysis is presented for the enzymatic transesterification of waste oil for biodiesel production. The experimental data on batch reactor evidence two key variables: enzyme loading and mixing conditions. These variables were subjected to a factor analysis and their combined effect on the reaction performance was determined. Response surface methodology (RSM) was used based on a linear first order model (steepest ascent method) and on a second order one in proximity of the optimal solution. The result was a model able to predict reaction performance within the range of mixing rates and enzyme amount considered for model formulation and outside of it, as shown in the final validation. Best performances were obtained at high stirring and high enzyme loading.

1. Introduction

Biodiesel is a mixture of fatty acids alkyl esters from biological source. It can be obtained by means of transesterification (catalytic or bio-catalytic) of glycerides of fatty acids of vegetal oils with short chain alcohols. As compared to other vegetable oils, biodiesel shows a lower viscosity and it is less polluting with respect to the production of CO_2. It is biodegradable and, during combustion, a reduced level of particulate, carbon monoxide and nitrogen oxides is produced. Moreover, it becomes a major environmental interest when the oil adopted as the main substrate is a polluting waste, as it is the case for frying oil and olive husk oil.

The transesterification reaction can reduce viscosity, because the linear esters, without glycerol, are less viscous than ramified chains of triglycerides (Clark et al., 1984).

To increase the reaction rate a catalyst is normally used: transesterification can be carried out by a mechanism of acid or basic catalysis or by enzymatic catalysis using lipase. The latter has been only recently considered, to overcome the drawbacks of acid catalysis (Ma and Hanna, 1999; Freedman et al., 1984; Srivastava and Prasad, 2000) (kinetics is too slow) and alkaline catalysis (substrate loss due to conversion into soap products, high viscosity, gel formation and difficulty to separate the glycerol that can be entrapped in the soap products) (Fukuda et al., 2001; Formo, 1944; Wright et al., 1944; Eckey, 1956; Calabrò et al., 2002).

The enzymatic conversion is based on the use of lipases that, on one hand, catalyse the hydrolysis of fats and vegetal oils with release of glycerol and, on the other hand, in the presence of short chain alcohols, favour the formation of linear chain esters (Hari Krishna et al., 2001).

Major advantages of enzymatic transesterification with respect to the acid and alkaline process, are: the possibility to carry out the conversion at low temperature conditions; lipases also catalyse re-esterification of free fatty acids (Hamsaveni et al., 2001; Vicente et al., 1998); small quantities of water in the reacting mixture are admitted and, in some cases, necessary (Kaieda et al., 2001); the yield in esters is relatively higher; it is not necessary to purify the produced esters. The reuse of lipases and the recovery of their stability, both thermal and mechanical, are the most significant issues for making the enzymatic process, whose costs are still too high, more competitive for biodiesel production. Immobilized lipases permit to achieve this goal. However, they can be maximally exploited only if operating conditions are optimized; this task requires knowledge of reaction kinetics and, in general, predictions of process performance.

Reaction kinetics can be studied through a fundamental approach based on kinetics modelling, but other factors than kinetics can also affect the reaction progress. An accurate study of the effects and interactions of process operating conditions, such as mixing and reactor fluid-dynamics, on the system performance is necessary to set the bases of bioreactors design. Fluid-dynamics is a relevant aspect in the bioreactor design also because of its influence on mass transport phenomena (Calabrò et al., 2002). However, the effect of mixing on process performance is hardly predictable by means of fundamental modelling; in this regard factor analysis becomes an efficient tool to evaluate the effect of such parameter.

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doi:10.1016/j.biortech.2009.05.027

It is based on experimental design and optimization with the aim of understanding the effect of each significant parameter on the process performance, defined as substrates final conversion or final products concentration (Hari Krishna et al., 2001; Hamsaveni et al., 2001; Vicente et al., 1998).

In this paper a factor analysis has been implemented in order to evaluate the effect of operating parameters on yields of biodiesel production from husk oil. The two variables considered are mixing rate and enzyme loading, since it has been recognized that they affect process performance singularly and through interaction effects. The oil used as substrate is olive husk oil which is a residue of olive oil production whose disposal constitutes an environmental problem for all countries producing olive oil.

2. Background theory

2.1. Experimental design

Factor design is an efficient method to study the combined effect of many factors on one parameter (Hari Krishna et al., 2001; Hamsaveni et al., 2001; Vicente et al., 1998; Lundstedt and Scifert, 1998). The most important is the 2^k factor that allows studying the effect over two levels of k factors on one parameter. These levels can be quantitative, for example two values of temperature or time, or even qualitative, as a lower or higher value of one factor. A special case of 2^k factor model is the one with n central points (consisting of replicated runs positioned at the centre of the 2^k design). Such runs allow the determination of possible quadratic effects (curvature) and an independent estimation of the error.

Response surface methodology (RSM) is a sequential procedure used in modelling and analysis of problems whose response is influenced by many variables and is to be optimized. The relationship between response and independent variables is generally unknown, thus, in the first step of the procedure, it must be approximated. In first approximation a linear model might be used (method of steepest ascent), but close to the optimal solution the second order model has to be used.

2.2. Kinetic model

Transesterification might be schematized as series-side reactions of esterification. The main kinetic models reported in the literature on catalysis by lipases both in hydrolysis and transesterification reactions are based on Ping Pong Bi Bi model (Araujo and Brereton, 1996; Dossat et al., 2002) or an ordered ternary mechanism (Al-Zuhair, 2005). Both of them are characterized by the first stage consisting of enzyme acylation.

In the case of lipase hydrolysis the reaction rate can be also represented by Michaelis–Menten or first order kinetic model (Yadav and Trivedi, 2003), strengthening the hypothesis of a slow acylation controlling step in the first stages of the reaction.

3. Methods

3.1. Materials

The enzyme used is Lipozyme®, 37 U/g (produced by Novozymes), a lipase extracted from *Rhizomucor miehei* (previously *Mucor miehei*) and immobilized on ion-exchange macroporous hydrophilic resins, irregular shape, dimensions ranging between 0.02 and 0.06 cm, bulk density of 0.4 g/cm^3 and porosity between 0.53 ± 0.02.

The substrates are very low quality olive husk oil and ethanol (>99.8%), purchased from Fluka. The solvent was hexane (>95%) from Fluka. The composition in triolein (most relevant triglycerides in the husk oil) was equal to 60%.

Density of husk oil was experimentally found equal to 0.897 g/ml, density of ethanol and hexane was, respectively equal to 0.79 g/l and 0.66 g/l.

3.2. Experimental methods

Reaction were run in a batch reactor of 250 ml, submerged in a thermostatic bath (OLS 200, GRANT) ion order to keep mixing and temperature conditions constant. Oil, enzyme and hexane were weighted and put in the thermostated reactor for 30 min at $T = 37$ °C. At $t = 0$ the reaction was started by adding ethanol to the reacting mixture. Different procedures of substrates loading were tested before choosing the one eventually adopted. In particular, mixing all the reactants and then starting the reaction by adding the enzyme was tried as it seemed a more rational possibility, but it led to enzyme agglomeration and had to be discarded. During all the tests presented in this paper the amount of reactant mixture was the following: 35 g of oil at 60% in triglycerides, 2.72 ml of ethanol and 24 g of hexane, with the aim to have an ethanol/triglycerides molar ratio (Et–TG) equal to 2 and a volumetric hexane/oil ratio (Hex–O) equal to 1. Mixing rates and enzyme amount were changed in the ranges 50–200 rpm and 0.62–3.76 g, respectively; enzyme loading range was chosen on the basis of typical enzyme/substrate concentration ratios for enzymatic bioprocesses (higher values could have been used, but this would imply to work outside from useful values in terms of process economics) while mixing conditions were chosen such that the enzyme could be properly distributed within the reacting mixture (lower limit), without being centrifuged and spread on reactor wall (upper limit). Feed ratios are reported in Table 1. Reactions were run for 24 h; samples of 200 μl were collected periodically, centrifuged for 5 min at 5400 rpm for enzyme separation and analyzed after hexane evaporation.

3.3. Samples analysis

Samples were analyzed by an HPLC system consisting of a pump (Jasco PU-980), a refractive index detector (Jasco RI-930), an autosampler (Jasco AS-1555), a degasser (Gastorr GT-103), a column Alltech Adsorbosphere HS(C18) 5u 250 × 4.6 mm, a guard column Alltech 7.5 × 4.6 mm.

The mobile phase (acetone/acetonitrile 70/30 v/v) was pumped at a flow rate of 1 ml/min; internal normalization has been used as integration method.

Before analyses samples were diluted into acetone.

4. Results and discussion

The preliminary test runs have been carried out at 50, 100 and 150 rpm as mixing rate, with 2.5 g of enzyme.

The time evolutions of mono-glycerides and di-glycerides (data not reported) have the typical behaviour of reaction intermediate during a series-side pattern of reaction, with the mixing rate affecting mono-glycerides production less than di-glycerides that is the first step of the reaction kinetic pattern.

To evaluate the effectiveness of the transesterification in terms of ethyloleate production, the conversion has been calculated with respect to both glycerides (tri-, di- and mono-glycerides) and ethanol (limiting reactant).

The conversion with respect to glycerides has been calculated by preliminarily evaluating the maximum molar concentration of ethyloleate, C_{EOmax}, that might be obtained without ethanol limitation. C_{EOmax} represents the highest possible ethyloleate concentra-