Optimization of biodiesel production by transesterification of vegetable oils using lipases

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Abstract

Enzymatic production of biodiesel by lipase transesterification of vegetable oils, requiring mild reaction conditions, tolerating water and free acids into the oil, facilitating the recovery of glycerol and fuel purification and producing low amounts of waste, overcomes the drawbacks of chemically catalyzed reactions. The main parameters influencing the yield of an enzymatic transesterification are amount of enzyme, alcohol/oil molar ratio, temperature, content of water and mixing intensity. In order to develop an efficient process the relation between the variables and yield must be known in detail. Monofactorial experimentation, practiced usually to determine all the independent parameters, is time consuming for complex processes, particularly when they interact with each other. Their designing requires an adequate experimental strategy – statistical experimental design, method that allows determination of the optimum set of experimental conditions. Response surface methodology proved to be a suitable technique to optimize lipase transesterification of vegetable oils. Its application both to some solvent-free and organic solvent systems is discussed.

Introduction

According to the US Standard Specification for Biodiesel (ASTM 6751-02), this is defined as a fuel consisting of mono-alkyl esters of long-chain fatty acids originating in vegetable oils or animal fats. The definition is also accepted by the European Union Specification Concerning Biodiesel (EN 14214). But the very strict regulations define biodiesel as fatty acid methyl esters (FAME). However, the definition is currently extended to the fatty acid ethyl esters [1, 2].

Biodiesel is a substitute or an extender for the conventional petroleum-based diesel and can be used either in its pure form or can be blended in any proportion with diesel fuel.

Compared to the petroleum-based diesel, biodiesel is known as a carbon neutral fuel, the carbon present into the exhaust being originally fixed from the atmosphere [3]. It presents the following main advantages: biodegradability, non-toxicity, low emission profiles [4], its combustion products have reduced levels of particulates, carbon monoxide [5], sulphur oxides, aromatic hydrocarbons and soot particles [6-9], as a renewable resource is environmentally beneficial [7,8] and maintains a balanced carbon dioxide cycle [10]. Moreover, the laboratory studies demonstrated no significant differences between biodiesel and conventional diesel in engine durability or in carbon deposits [11].

However, the production of biodiesel has a number of limitations: it is restricted to certain area and seasons in which the oilseed feedstock is produced, is in conflict with the utilization of oils as food compounds [2] and crystallizes at low temperatures.

Transformation of vegetable oils into biodiesel can be realized using three technologies [8]:

1. Pyrolysis or catalytic cracking, practiced to reduce the viscosity of vegetable oils and increase cetane number. The products contain alkanes, alkenes and carboxylic acids and have acceptable amounts of sulphur, water and particulate matter [12,13]. The method has as disadvantages the expensive equipment and the need to separate the products by distillation, which is energy consuming. Moreover, the products are similar to gasoline containing sulphur that makes them less eco-friendly [8] and are not accepted from the points of view of ash content, carbon residues and pouring point.

2. Microemulsification, practiced also to increase the cetane number and reduce the viscosity. Diesel fuel, vegetable oil, alcohol, surfactant and additive for increasing of cetane number are mixed in suitable proportions and the mixture transformed, under vigorous stirring, into a microemulsion with lower viscosity [8]. Alcohols as methanol, ethanol and propanol are used as additives for reducing of viscosity, higher ones as surfactants and alkyl nitrates as cetane number improvers [3]. But biodiesel microemulsions produce injector needle sticking and formation of carbon deposits due to the incomplete combustion if they are used for a long time [8]; the combustion is incomplete in the 200 h endurance test [14].

3. Transesterification using low molecular weight alcohols. A mixture of monoalkyl-esters of fatty acids and glycerol are obtained as a result of reaction. Glycerol, having high viscosity, must be removed from the reaction product.

The most popular method for biodiesel production is the transesterification technique.

Transesterification of vegetable oils

Transesterification is the chemical reaction between a triglyceride and an alcohol in the presence of a catalyst and consists of three consecutive reversible steps: transformation of triglyceride in diglicerides, of these in monoglicerides, which transform into monoalkyl-esters and glycerol [15]. It reduces the molecular weight to about 1/3 in relation to triglyceride and improves the volatility and is the most viable process for reducing the vegetable oil viscosity. The reaction product is a mixture of fatty acids esters, glycerol, alcohol, catalyst and low amounts of tri-, di- and monoglicerides. Glycerol also has a commercial value.

Stoichiometrically, 1 mol of triglyceride requires 3 mols of alcohol, but generally higher amounts of alcohol are used to obtain high ester outputs, dependent on the type of feedstock, amount of catalyst, temperature, etc. The most used alcohols are C_1-C_4. The biodiesel yield does not depend on the alcohol used and the choice of alcohol is determined by its cost and performance. The preferred alcohol is methanol, due to its low cost [16].

The conventional catalysts used for transesterification are acids and alkali, both liquid and heterogeneous, depending on the oil used for biodiesel production. To these catalysts the free and immobilized lipases were added [6,8,17-22].

Transesterification by conventional techniques

Alkaline transesterification is the current technology used for diesel preparation by transesterification of vegetable oils [6,17,23-25]. But, when the row materials contains large amount of water or free fatty acids, a pretreatment with an acid catalyst is required for the esterification of free fatty acids [17,25,26]. This is necessary to reduce the soap formation and to facilitate the separation of biodiesel and glycerol, at the same time the catalyst and alkaline waters are removed [27,28]. It is 4000 times more rapid than the acid one, [15] but is preferred only when the oil contains less than 2% free fatty acids, to eliminate the pretreatment.

Large scale industrial production of biodiesel by alkaline transesterification is done using the homogenous catalysts sodium or potassium hydroxide. The catalyst and glycerol are removed by 2-3 washings with distilled water. The amount of waste waters is about 0.2 t/t biodiesel [29], which requires their treating and, eventually, reusing.

Sodium methoxid is a better catalyst compared with hydroxides, because it does not produce water, which facilitates soap formation, when dissolved into alcohol [30].

It was found [31] that the alkali catalyzed transesterification is very sensitive to oil purity: when the free fatty acids content is over 0.5% the reaction is affected to some extent.

In the case of virgin oils the amount of catalyst for transesterification is calculated as the sum of the quantity required to neutralize the free fatty acids plus 0.35%, which means about 0.55 w/v [32]. Catalyst in excess produces an emulsion, which results in increasing of viscosity and formation of a gel.

Heterogeneous catalysts, which separate from the final product by filtration and can be reused, were used to overcome the drawbacks produced by the enormous consumption of water and time. It was found [33] that calcium oxide can be used up to eight runs with no significant deactivation. Zeolite, montmorillonite K-10, zinc oxide [34] and magnesium oxide [35] also were used, but the conversion was lower. They have also the advantage that the final product is les corrosive, chipper and less damaging for the environment [35].

Acid transesterification. In spite of its insensibility to the free fatty acids from the row materials, it was ignored, especially due to its low reaction rate [36].

The acid transesterification does not need a low acid value of the raw material. The most used catalyst is sulfuric acid [15,32,37-39].

The main disadvantage of acid transesterification is that the formation of esters is accompanied by that of water, which inhibits the process [40]. At the same time, the high temperature and acid concentration can burn a part of the oil, which reduces the biodiesel yield and the acid – being corrosive – deteriorates the equipments [26].

Transesterification in supercritical alcohol gives almost complete conversion (95%) and the reaction time is very low – only 10 min [12]. This is due to the fact that the oil and the supercritical alcohol form one phase, the solubility parameters of the two components becoming close in the given conditions [41-46]. But the method presents many disadvantages, including a very high ratio methanol/oil, than can attain 40/1, as well as high pressures and temperatures (about 20 MPa and 300°C) [41,47], which imply high costs. The conversion is even higher when supercritical ethanol is used, the solubility parameter of ethanol being closer to that of the oil, which makes their reciprocal solubility higher.

Lipase-catalyzed transesterification

More recent studies [7,48-57] shown that biodiesel can be enzymatically produced by lipase-catalyzed transesterification. The interest for using lipases as biocatalyst for vegetable oil transesterification is due to the high efficiency and selectivity, facilitation of glycerol removal and biodiesel purification, toleration of water into the oil, low energy consumption (reaction requires mild conditions) and low waste amounts (more environmentally friendly) [58]. Moreover, the esterification of free acids also takes place and soap formation is avoided, both reactions producing in the same step and thus the washing step is eliminated.

Compared with chemical methods using alkaline or acid catalysts, utilization of lipases for biodiesel production has the following advantages [22]:
- more compatibility with variations in the quality of the row material and reusability;
- ability to produce biodiesel in a lower number of steps using less energy and with drastically reduced amount of wastewater;
- improving of product separation and glycerol quality [6,14,17,59];
  But the use of enzymes as catalysts presents also some drawbacks [22]:

- low reaction rate [25];
- very high cost for industrial scale use: [6,8,14,60,61] 1,000 USD/kg enzyme compared to 0.62 USD/kg sodium hydroxide [21];
- loss of activity within about 100 days of operation.

Choice of enzymes. Lipases must be nonspecific, so that all the glycerides (tri-, di- and mono-) and free fatty acids can be transformed into alkyl esters. A large variety of lipases has been used for transesterification and esterification [22]. The optimal parameters for the use of a given lipase depend on its origin and formulation.

The most performing lipases are able to produce conversions over 90% at temperatures ranging between 30 and 50°C and reaction times from 8 h for immobilized enzymes to 90 h for the same free enzymes, depending of the oil and the alcohol used [22].

The maximum biodiesel yield depends not only on the origin of lipase, but also on enzyme formulation (immobilized or not), alcohol used, alcohol to oil molar ratio, optimal activity of water, reaction temperature, reaction time and enzyme life time. Elucidation of the above aspects requires a look at the reaction mechanism and kinetics.

Kinetics and mechanism of transesterification. Transesterification of a single ester bond of a triglyceride with an alcohol imply a two-step mechanism: hydrolysis of ester bond and release of alcohol, followed by esterification with the second substrate [17,59-61] .

The widely accepted mechanism for alcoholysis of triglycerides is a ping-pong bi bi one [62,63], each product being released between addition of the substrates [64], but usualy simplified Michaelis-Menten kinetics is applied when fitting the experimental results [63,65].

Steady-state kinetics, to which Michaelis-Menten kinetics belongs, can describe satisfactorily the enzymatic conversion for a series of models having different complexities, but its accuracy is questionable. Fitting of experimental data with models, by its own nature, can not give indications on the intermediate formed or confirm the mechanism, but some other evidence must be brought, that is some other methods for determination of structure must be used, such as spectroscopy [66]. Furthermore, none of the models used consider the formation and conversion of di- and monoglicerides, the influence of temperature on enzyme deactivation or the equilibrium limitation for conversion [22].

Water activity in solvent-free systems. Removal of water surrounding an enzyme produces usually irreversible changes in protein structure [59] and its protection is important for the optimal conformation required by the transesterification. A series of results [49, 67-71] shown that the optimal activity of water for an enzymatic transesterification system is specific for a given lipase. An important factor when deciding on water activity is the way the enzyme was immobilized: in active conformation or water is necessary to stabilize it [22].

Water activity in organic solvent systems. Transesterification in organic solvents serves the following purposes [22]: (a) ensures a homogeneous reaction medium; (b) reduces the viscosity of reaction mixture, increasing the mass transfer around enzyme; (c) non-polar solvents force residual water to stay around immobilized enzyme, increasing locally the water activity and helping thus its stabilization. Conventional diesel can be used as solvent [72,73].

So no correlation of yield or conversion with solvents polarity, solubility parameters or dielectric constants was found [22], for polar solvents addition of water decreases conversion and product selectivity, while for non-polar ones the opposite is true [71].

Large scale industrial production of biodiesel in the presence of solvent is convenient only when it is used blended with conventional diesel and the last one is used as solvent. All the other solvents have to be recovered. Moreover, they are volatile, potentially hazardous and require large reactor volumes to accommodate solvents and reactants. Therefore, the solvent-free process, superior economically and energetically, is preferred. So similar yields were obtained with and without solvent [74], solvent-free biodiesel process has lower reaction rate.

Effect of temperature. An increase of the optimal temperature of transesterification for immobilized enzyme is expected [75], binding to carrier increasing its stability and decreasing thus the effect of thermal deactivation compared to free lipase. The heat of reaction for transesterification is generally small and the equilibrium conversion is expected to be not influenced in the temperature range for production of biodiesel (20–70°C).

The following reliable conclusions on the effect of temperature on enzymatic transesterification were drown until now: (a) immobilized lipases show more temperature resistance than free ones; (b) the initial rate of reaction increases with reaction temperature; (c) some enzymes are partially deactivated at 60°C by methanol or ethanol within the first 24 h, while others are practically not affected during such a short reaction time.

Designing and statistical analysis of complex processes

Designing of complex processes, implying a high number of reciprocally influencing variables, requires the performing of efficient, systematic and economic experimental investigations, which can be done using some programs for their modeling and control. They are structured in an adequate experimental strategy, known as statistical experimental design.

Statistical experimental design is defined as the methodology showing how the experiments have to be conducted and planned to obtain maximum amount of information from a minimum number of experimental trials [76].

One or more factors or process variables are deliberately modified in an experiment in order to observe the effect the changes have on one or more variables or response parameters.

The statistical design is an efficient procedure for planning experiments and the obtained data can be analyzed to get valid and objective conclusions. Thus, it involves the designing of experiments, consisting in determining the experiment objectives and selection of process factors that must be considered, as well as the analysis of experimental data.

Designing of experiments

The first phase of statistical design supposes the laying out of a detailed experimental plan in advance of doing the experiment [77]. Well chosen, the experimental design maximizes the amount of "information" that can be obtained for a given amount of experimental effort.

Obtaining good results from an experimental design involves six steps [77]:

1. Selection of system or technological process – a product to be modeled by pre-formulation or formulation studies can be selected as system, while the optimization of some steps or specific stage, an ensemble of correlations, etc. as technological process.

2. Selection of process variables – experimental design requires two categories of variables: input or influence factors, which the experimenter can control (vary at will), that is the independent variable codified by X, and parameters to be optimized or response parameters, which have the quality of dependent variable, codified by Y.

3. Selection of the levels of variation of independent variables: more levels are attributed to independent variables, codified by numbers (0, ± 1, ± 2); they are selected in such a way that the differences between the variables have a detectable effect on response; when the effect of one factor depends on the level of the other, the two factors interact;

4. Elaboration of experimental matrix or factorial program: the columns represent the factors to be tested and the lines the experiments that must be made. Usually, monofactorial experimentation is practiced, but such a determination of optimal values for all the process variables requires a great number of experiments, that is time consuming. Moreover, the variables may interact with each other. Elaboration of a factorial program considering the interactions between all the variables is necessary to obtain simultaneously the optimal values for all the independent variables. The factorial programs may have different types of levels:

integer, fractionated or composed. The most used is the factorial program with two levels, stipulating 2^k experiments, k designating the number of independent variables. Fractional programs are used to reduce the number of experiments [78].

5. Performing of the experiments from the experimental matrix: just a part of the experiment must be performed, aleatory decided to assure the objectivity of experimentation.

6. Obtaining of experimental results or values of response parameters, which must be analyzed to be optimized. An optimal response means generally a minimal or maximal value.

Analysis of experimental data

The second phase of a statistical design consists of the process model, with several discrete or continuous input factors and one or more continuous output responses, and the analysis of response surfaces given by derived empirical model linking the outputs and inputs.

1. A **process model** supposes establishing of equations describing the experimental results as a function of factors levels. The most common empirical models fit to the experimental data take either a linear or a quadratic form [7]. A linear model with two factors, X_1 and X_2, can be written as:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + experimental\ errors \tag{1}$$

where Y is the response for the given levels of the main effects, X_1 and X_2 and X_1X_2 – the term included to account for a possible interaction effect between X_1 and X_2, b_o – response of Y when both main effects are 0, b_1 and b_2 – the coefficients of regression equation.

For a linear model with three factors the equation is:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{123}X_1X_2X_3 + \\ + experimental\ errors \tag{2}$$

The three terms with single "X's" represent the main effects, the $k(k-1)/2 = 3$ terms of the type X_1X_2 are the two-way interaction ones and $X_1X_2X_3$ – the three-way interaction term, often omitted. When analyzing the experimental data, all the unknown b parameters are estimated and the coefficients of the "X" terms are tested to see which are significantly different from 0.

A second-order (quadratic) model, typically used when model a response giving a response surface with suspected curvature, does not include the three-way interaction term but adds three more terms to the linear model, namely $b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2$

Regression coefficients are calculated using multiple regression programs, their values indicating the way each factor contributes to increase or decrease of the response Y, that is which interactions have synergistic or antagonistic effect on the considered response variable.

The model is validated evaluating the multiple correlation coefficients, examination of residuals and variance analysis. The values of the coefficients give information on the effect of independent variables on the dependent one [79].

2. **Response surface methodology** is a collection of statistical and mathematical techniques useful to develop, improve and optimize processes and products [80] largely applied in industry, particularly in the situations where several input variables influence some process performances or quality characteristics. In the case of a chemical reaction the dependence between the response variable yield and the two inputs, process or independent variable time and temperature can be represented graphically, as Figure 1 shows [80].

A value of yield corresponds to each value of time and temperature. The response values form a surface placed above the plane of the two variables and can be represented by it, as can be seen in Figure 1a. This way of analyzing the response gave the name of methodology [80]. But is convenient that the response surface to be also produced in the plane

of the two variables, as shown in Figure 1b. Such a curve is obtained by connecting all the points with the same yield and such a plotting modality is known as contour diagram. Thus,
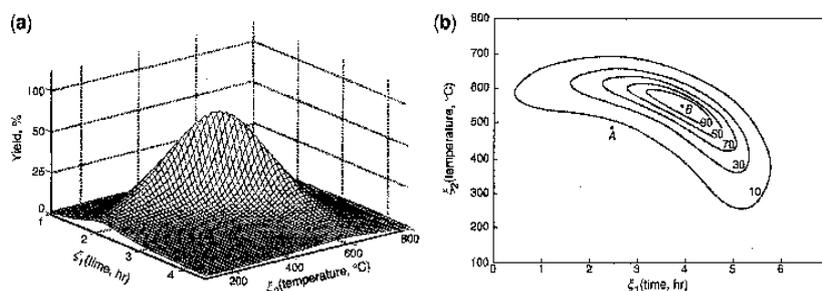
Figure 1. (a) A theoretical response surface showing the relation between the yield of a chemical process and process variable time and reaction temperature; (b) contour diagram of the theoretical response surface [80].

the objective of RSM is the investigation of responses over the entire variation domain of independent variables and the localization of regions of interest, in which the responses are optimal or very close to the optimal ones. It consists on experimental strategy for exploring the process space or independent variables, empirical statistical modeling to establish an adequate approximate relation between response and process variables and the optimizing methods to find the levels or values of process variables producing the desired responses. The method allows the determination of optimum set of experimental conditions which minimize or maximize the response and the changes in response surfaces produced by variation of independent variables. Localization of optimum response is done by graphic analysis, which allows a visual detection of response and determines the robustness of response surface.

Optimizing of enzymatic alcoholysis of vegetable oil in free-solvent systems by response surface methodology

Lipases (E.C. 3.1.1.3.) which transform the triglycerides in fatty acids and glycerol, catalyze also with high specificity some other reactions as esterification, transesterification and regioselective acylation. Such reactions require a given amount of water [81].

Given the advantages: mild reaction conditions, easy recovery of glycerol and simple purification of biodiesel [7, 82], free or immobilized lipases were used for transesterification of vegetable oils and animal fats, both in the absence and presence of organic solvents [17,49,83]. The transesterification in organic solvents presenting the disadvantages of toxicity and flammability, damaging effects on environment and necessity to be recovered, for enabling enzymatic process to be competitive solvent-free systems have been developed [17,83,84].

The relations between the variables important for lipase catalyzed transesterification and the yield of biodiesel must be known in detail to develop an efficient process and the experimental design was used to optimize this parameters [14,57, 83,84,85-89], particularly when the reaction is carried out in the presence of organic solvents [57,86,87].

The most important parameters influencing the reaction are: amount of catalyst, molar ratio alcohol/vegetable oil, water content, temperature, mixing intensity, type of acyl donor and acceptor and quality (purity, free fatty acid content) of starting materials [2,90] but only the first five can be considered when RSM is used. As response variables the reaction rate or biodiesel yield were selected.

Optimizing of transesterification of sunflower oil

To evaluate the effect of each of the first four independent variables and their interactive effects on sunflower oil methanolysis by two immobilized commercial lipases: Lipozyme RM-IM, Novo Nordisk, Denmark) in a solvent-free system, RSM and 5-level-4-factor central composite rotatable design (CCRD) was performed [91]. It allows the

investigation of linear, quadric and cross product effects of the four factors, each varied at five levels [92]: zero (center point), level ± 1 and ± 2 on reaction rate as response variable.

Data were evaluated by response surface methodology, and a second order polynomial equation was tested. The method of least squares was used to evaluate the coefficients of the response function and their statistical significance. For the final reduced model only the significant terms ($p \leq 0.05$) were considered. Fisher test was used to determine the adequacy of the model and Student distribution to evaluate the significance of the coefficients.

The response surfaces of the predicted reaction rates as a function of process variables obtained from RSM analysis are presented in Figure 2a-c and the contour plot only for water content and temperature in Figure 1d.
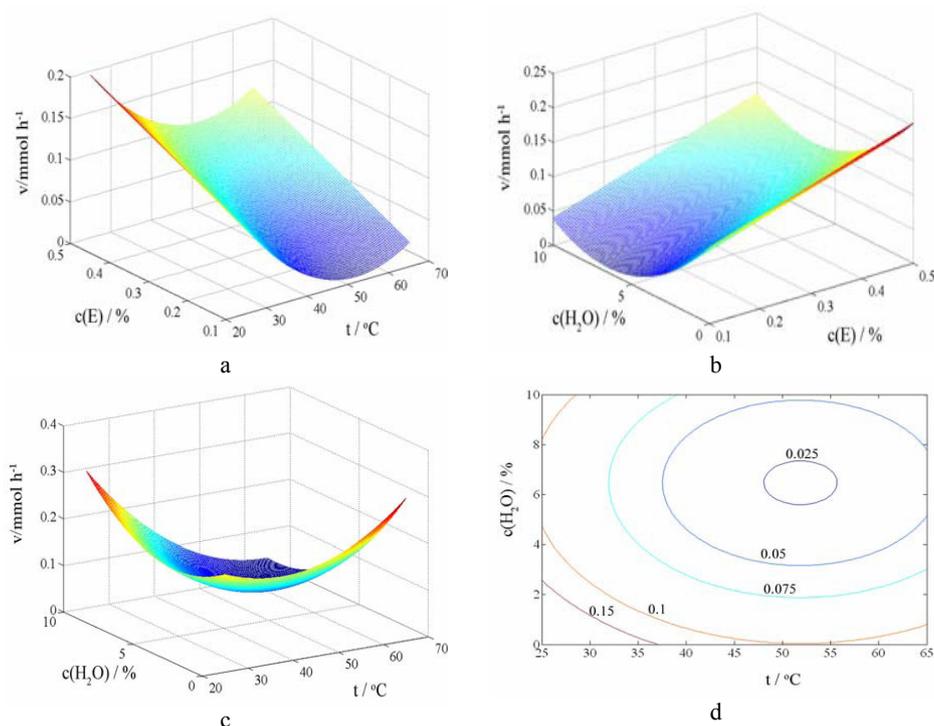
Figure 2. Response surfaces of the reaction rate at different levels of: a) enzyme concentration and temperature; b) water amount and enzyme concentration; c) water amount and temperature. d) Contour plot of the reaction rate at different levels of water amount and temperature

The Figure 2a-d leads to the following conclusions: (a) amount of enzyme has the largest effect on reaction rate, it increasing with enzyme concentration; (b) temperature has a relatively reduced effect, with maximum values at the lowest and highest temperatures and minimum at $52^{o}C$; (c) amount of water has a reduced effect, with minimum value at 6.7% and maximum at 0 and 10%; (d) methanol/oil molar ratio had no significant influence; (e) the only interactive effect between parameters is between temperature and water content, with minimum value at $52^{o}C$ and 6.7% water and high ones at high water content and low temperature or low water content and high temperature; (f) methanol/oil molar ratio ($3/1 \div 9/1$) has no significant effect on the oil reaction rate.

After eliminating the insignificant terms based on the composite rotatable design, a second order model with three significant parameters without interactions was obtained:

$$Y = 0{,}0596 - 0{,}0180\, X_1 + 0{,}0233\, X_2 - 0{,}0181\, X_3 + 0{,}0131\, X_1^2 + 0{,}0154\, X_3^2 \qquad (3)$$

Here Y is rate of reaction, X_1 – temperature, X_2 – amount of enzyme and X_3 – water content.

The equation shows that, indeed, only concentration of enzyme has a positive effect on reaction rate, while temperature and amount of water have a negative effect.

The amount of water should be a compromise between minimizing hydrolysis and maximizing enzyme activity for the transesterification reaction [89], hydrolysis of methyl esters being favoured by high water content and high temperatures.

Optimizing of transesterification of soybean oil

The monofactorial experiments indicated enzyme concentration, temperature, molar ratio methanol/oil and stirring rate as the most important variables in transesterification of soybean oil with lipase NS81006 (Novo, Denmark) [4]. To investigate the effect of the above parameters on the yield of fatty methyl ester, selected as response parameter, RSM was used and 5-level-4-factor central composite design with eight star points, including six replicates at the centre point leading to 30 runs was performed to fit a second-order response surface.

Using the designed experimental data, the polynomial model for the yield of fatty methyl ester, Y, was regressed by considering only the significant terms:

$$Y = 0.90 + 0.049X_1 + 0.023X_3 - 0.027X_4 + 0.033X_1X_4 - 0.011\,xX_3^2 - 0.012\,x_{4X}^2 \qquad (4)$$

where X_1 is the amount of enzyme, X_3 – molar ratio methanol/oil and X_4 – rate of stirring.

The largest coefficient in equation (4) was obtained for amount of enzyme, which means that it has the most significant effect on fatty methyl ester yield. The positive coefficients of X_1, X_3 and X_1X_4 indicate a linear effect to Y increase. The linear term X_4 and the quadratic ones – X_3^2 and X_4^2 – are negative, which signify a decreasing effect on yield.

The 2D contour plots, similar to those in Figure 2d, were used to appreciate the optimal value range of the independent variable, while their form to estimate the significance of the mutual interactions, an elliptical profile contour plot indicating a considerable interaction. The optimal transesterification conditions were calculated with equation (4), according to the limit criterion of enzyme minimization and yield maximization: enzyme amount 50 Ug^{-1} soybean oil, temperature 45°C, molar ratio of methanol/soybean oil 6.6 and rate of stirring 400 rpm. Validation experiments, carried out in triplicate, shown a good agreement with the calculated value: predicted value 92.8%, experimental – 93.6±1.4.

Optimizing of transesterification of residue oil from vegetable oil refining

The above method was also used to obtain the optimal transesterification condition for acid residue oil from vegetable oil refining, representative for poor quality oil and inexpensive stocks, using as catalysts a mixture of enzymes produced by Novozymes, Denmark: NS81006 and NS81020 [93]. The following values were obtained for the optimal transesterification conditions: amount of NS81020 – 40.5 U/g oil and of NS81006 – 20.1 U/g oil, temperature – 28.1^{o}C, molar ratio methanol/oil 2.4, rate of stirring – 600 rpm, for which the predicted yield was 91.5% and the experimental one 88.7±1.61. When used separately, NS81006 has high activity both on triglycerides and free fatty acids, while NS81020 has high activity towards free fatty acid and almost no activity on triglycerides.

Optimizing of transesterification of palmitic acid

Optimizing of esterification of palmitic acid, found in large amounts in vegetable oils, with ethanol using as catalyst the commercial immobilized lipase Lipozyime RM IM supplied by Novozymes, Denmark in a solvent-free system was performed using a 2-level-3-factor central composite design with six central points, selecting ethanol/oil molar ratio, temperature and enzyme concentration as independent variable and initial reaction rate as response variable [88]. The ranges for the independent variables were: ethanol/oil molar ratio – 0.16-1.84, temperature – 65-75°C, enzyme concentration – 0.48-5.52% w/w. Statistical analysis

indicated enzyme concentration and ethanol/oil molar ratio as the most significant factors affecting the initial reaction rate. Conversion values higher than 50% after 1 h were obtained for an ethanol/oil molar ratio of 0.5, temperature of 67°C and enzyme concentration of 4.5%.

Optimizing of enzymatic alcoholysis of vegetable oil in organic solvent systems by response surface methodology

In spite of disadvantages (toxicity, flammability, damaging effects on environment, necessity to be recovered), transesterification in organic solvents assure an intimate contact between the components of reaction system and reduces the viscosity, which increases diffusion and therefore the reaction rate. Thus, the yields of alkyl esters are high in the presence of non-polar solvents such as n-hexane, while it is low in their absence [14,83]. Glycerol liberated in the alcoholysis can inhibit the reaction. By using silica gel or other adsorbents for glycerol adsorption, higher alkyl ester yields were reported [83].

Optimizing of transesterification of soybean oil in n-hexane

The relationships between the independent variables reaction temperature, enzyme amount, and substrate molar ratio and the response parameter methyl ester content, as well as the identifying of the optimal conditions for soybean oil methanolysis with a commercial immobilized lipase – Lipozyme RM IM supplied by Novozymes, Denmark – in n-hexane as solvent at a short reaction time of 30 min were performed using a 3-level-3-factor face-centered cube design [82].

Before RSM was applied, approximate conditions were determined by varying one independent variable at a time while keeping the others constant. Thus an appropriate range for each independent variable was determined for RSM. It was found that methyl ester content increased with increasing methanol/oil molar ratio up to 2.5/1. A higher ratio inhibited the reaction, result in good agreement with the literature, which reported that the stepwise addition of methanol is more effective than a one-step one [7,83,89,94].

The relationships between the responses and process parameters were examined by 2-D contour plots. Critical conditions for a methyl ester content of 76.9% were determined to be 50°C, 2.37 methanol/oil mole ratio, and 0.09 enzyme/oil weight ratio.

Optimizing of transesterification of castor oil in n-hexane[87]

The production of fatty acid ethyl esters from castor oil in *n*-hexane as solvent and two commercial immobilized lipases, Novozym 435 and Lipozyme IM supplied by Novozymes, as catalysts, was optimized using an experimental design with two levels and four variables. The ranges of variation of the independent parameters were: temperature – 35-65°C, water – 0-10% wt/wt, enzyme – 5-20% wt/wt, oil/ethanol molar ratio – 1/3-1/10. The empirical model built, assessing the main and cross-variable effects on the reaction conversion and maximizing biodiesel production for each enzyme, gave the following results: Novozym 435 – maximum conversion was 81.4% at 65°C, enzyme and water concentrations 20% wt/wt, and 0% wt/wt respectively, oil/ethanol molar ratio 1/10; Lipozyme IM – maximum conversion 98% at 65°C, enzyme concentration 20%, water content 0%, and oil/ethanol molar ratio 1/3.

Conclusions

Lipase catalyzed transesterification of vegetable oils for producing of biodiesel could become a viable alternative to the conventional techniques, due to its advantages.

Biodiesel yield depending on lipase origin, its formulation (immobilized or not) and type of acyl donor and acceptor, the optimum condition must be established for each system.

Given the large number of independent variables determining the optimum transesterification conditions: amount of catalyst, molar ratio alcohol/vegetable oil, water

content, temperature, mixing intensity and the probability of their interaction, determination of their optimal values for maximum conversion by monofactorial experimentation is very expensive and time consuming.

The experimental design proved to be a rational means to investigate the effect of independent variables and of their interactions on vegetable oils conversion for production of biodiesel by enzymatic transesterification of vegetable oils.

Response surface methodology was found to be particularly convenient, allowing the determination of optimum set of experimental conditions which minimize or maximize the response, as well as the changes in response surface produced by variation of independent variables and their mutual interactions. Localization of optimum response, done by graphic analysis, allows a visual detection of response.

Acknowledgement

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