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Optimization of low quality rapeseed oil transesterification with butanol by applying the response surface methodology

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ABSTRACT

This paper discusses the determination of the optimal conditions for enzymatic transesterification of model waste oil (with 4% acid value) with butanol. It was found that biodiesel synthesis by the enzymatic transesterification of waste rapeseed oil rich in free fatty acid with butanol can be very effective if the catalysis is carried out in two steps. Both transesterification steps were catalysed by Lipozyme RM IM. The optimal conditions for the first step were determined: temperature of 39 °C, butanol and oil molar ratio of 4.5, lipase concentration of 6%, and reaction time of 9.8 h, with an expected yield of 60.1%. The removal of glycerol, enzyme renewal and subsequent addition of 7.8% lipase, with a 1.5 butanol and oil molar ratio and 8-h incubation at 39 °C predicted a 96.7% yield and resulted in a 96.6% butyl ester yield.

1. Introduction

Greenhouse gas emissions are constantly increasing and pose a great risk to environmental stability. Many countries have goals to replace a portion of their energy obtained from fossil fuel with energy from renewable resources, and diesel vehicle fuel is no exception; it is supplemented by biodiesel for certain purposes.

Biodiesel is a mixture of fatty acid and short-chain alcohol esters, and it is obtained from a transesterification process that uses either vegetable or animal fats and an alcohol, most commonly methanol [1] [2] [3], and [4]. However, oil with methanol forms emulsion (during chemical transesterification that causes lower biodiesel yield. In this case, longer-chain alcohols are moore preferable [5]. Longer-chain alcohols such as ethanol and butanol can also be used, and the extended alcohol chain positively influences low-temperature fuel properties and results in higher calorific values [6], [7]. The cloud point of butyl esters is approximately 10 °C lower compared with that of methyl esters, meaning they have better properties under cold conditions. Moreover, the butanol that remains after the transesterification reaction can be mixed with biodiesel or mineral diesel without phase separation [8].

Conventional biodiesel synthesis involves alkaline catalysis, and additional acid catalysis steps are required for fat sources that are rich in free fatty acids [9]. The chemical process could be replaced by lipase enzymatic catalysis which is primarily preferable for environmental reasons: low reaction temperature, pressure, and waste, as well as quality glycerol by-product and easier catalyst removal, etc. Additionally, the transesterification and esterification reactions occur simultaneously during enzymatic catalysis, eliminating the additional free fatty acid esterification step that is obligatory in acid/alkaline catalytic processing [10]. Consequently, enzymatic catalysis is advantageous for processes that use low-quality waste-oils. The greatest disadvantage of enzymatic catalysis is the high price of enzymes that are used in relatively high quantities to obtain high reaction yields: yields greater than 96% are obtained using far more than 10% lipase [11]. Lipases from *Rhizomucor miehei* (Lipozyme RM IM), and *Pseudomonas fluorescens* are commonly used for transesterification with butanol. Rodrigues et al. obtained a 29% yield of butyl ester after 6 h of sunflower oil transesterification with butanol at 30 °C using Lipozyme RM IM, 15% lipase, a 1:7.5 butanol to oil molar ratio, and 4% water [12]. Iso et al. obtained a 90% yield of butyl ester after 10 h at 50 °C using *P. fluorescens* lipase (9.5%) and a 1:3 oil and butanol molar ratio [13]. Dossat et al. obtained a 65% yield from sunflower oil butanolysis at 40 °C using 3.5% Lipozyme RM IM and a 1:3 oil and butanol molar

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ratio [14]. As we can see, high biodiesel yield is depended on temperature, time and amounts of alcohol and enzyme. It is known; that higher temperature intensifies mass transfer in such way affected higher esters concentration [15]. As enzyme is unstable in high temperatures, enzymatic transesterification commonly carried out in moderate (till 60 °C) temperature range (the maximum of the temperature is depended on catalyst thermostability).

To the best of our knowledge, no data concerning the enzymatic synthesis of biodiesel from high acid value rapeseed oil and butanol has been published. Various methodologies could be used to optimize the process of transesterification. Some authors investigated transesterification process by evaluating the mass transfer, reaction kinetics and equilibrium in reaction medium and proposed the model for prediction of oil conversion yield depending on operational parameters. Such methodology can be extended for application in heterogeneous enzymatic catalysis [5,15,16]. The most commonly used methodology for optimization of transesterification process is response surface methodology (RSM). This methodology is a collection of mathematical and statistical techniques for empirical model building. Response (output) is depend on independent variables, so selecting design of experiments could help to develop for given system suitable mathematical model, which allows to determine optimal conditions [17].

Conventionally biodiesel fuel is produced by application of few (two) step transesterification, each of step is performed at different optimal conditions. Taking into account that enzymatic transesterification with higher alcohol - butanol is less effective than chemical transesterification with methanol, optimization of two process steps has to be performed.

This study aimed to apply response surface methodology and optimise the conditions for two-step biodiesel synthesis from high acid value waste oil and butanol.

2. Materials and methods

2.1. Materials

As a model of waste oil that is typically rich in free fatty acids, rapeseed oil from a local distributer was supplemented with oleic acid (Fluka) to a 4% acid value. Butanol (99.5%) was obtained from Sigma–Aldrich (UK), and lipase (Lipozyme RM IM) was kindly donated by a Novozymes representative from Lithuania JSC "Biopolis" (Lithuania). Other analytical grade reagents were obtained from Fluka (UK).

2.2. Experimental design

Response surface methodology central composite design (CCD) was employed to determine the optimal reaction conditions.

Four numeric factors were selected as variables for step I of acidic rapeseed oil transesterification with butanol: enzyme concentration, butanol and oil molar ratio, time, and temperature. Each factor was varied over 5 levels (axial points [$\alpha = 2$]), factorial points and centre point (6 replicates). For the first experimental step, the non-centre points had 2 replicates. The design required a total of 54 experimental runs. The ester yield for each experimental run and the yields from RSM model are listed in Table 1. The variables selected for the transesterification of I step products (step II) were lipase concentration, butanol and oil molar ratio and reaction time. Each numeric factor was varied over 5 levels (axial points [$\alpha = 2$]), factorial points and centre point (six replicates). The temperature was chosen according to step I, and this design had a total of 22 experimental runs. The ester yield for each experimental run and the yields from RSM model are listed in Table 2.

2.3. Enzymatic transesterification

Experiments were carried out in a conical flask connected to a condenser and thermometer with a thermo-controller. A magnetic stirrer was used to mix the reaction (200 min^{-1}). The reaction mixture was equilibrated to the desired temperature and then the lipase was added. The reaction was stopped after step I or II by filtering to remove the lipase catalyst and glycerol, and washing the product 3 times with distilled water. To determine the optimal conditions for the second step, the product of a first step reaction under optimal conditions was supplemented with an additional portion of butanol and lipase and then incubated for an additional 4–8 h at the optimal temperature determined in step I.

Gas chromatographic analysis of the reaction mixture was carried out after the mixture was washed with water and dried using a vacuum rotary evaporator.

2.4. Determination of biodiesel yield

Following the first stage of transesterification, the sample ester contents were expected to be low; consequently, the ester contents were determined from the glyceride contents (glycerol, monoglycerides, diglycerides and triglycerides) in the samples. Glycerides were determined by gas chromatography using a Perkin Elmer Clarus 500 (detector – FID) gas chromatograph according to the requirements of the EN 14105 standard. The ester levels were calculated based on the glyceride levels according to the following formula [18]:

$$\eta E = 100.(1 - (0.2411.MG + 0.1426.DG + 0/1012.TG)/10.441)$$

where ηE is the % of esters; MG, DG, and TG are the % concentrations of mono-, di-, and triglycerides, respectively; 0.2411, 0.1426, and 0.1012 are the respective conversion indicators for the glycerides; and 10.441 is the amount of glycerol obtained from 1 kg of rapeseed oil.

The gas chromatography method specified by the EN 14103 standard was used to determine the ester contents following the second transesterification stage.

2.5. Data analysis

The experimental data were analysed using Design Expert version 8.01 and then interpreted. Analysis of variance (ANOVA), regression analysis and response surface plots were used to investigate the effects of reaction parameters and establish the conditions for optimum reaction yield. ANOVA was used to evaluate the adequacy and fitness of the responses for linear, 2 function interaction (2fi) and quadratic functions of the variables. A model having a P-value ($P > F$) less than 0.05 was regarded as significant. The lack-of-fit test was used to compare the residual and pure errors at the replicated design points. The highest-order significant polynomial with an insignificant lack-of-fit was selected. The predicted residual sum of squares (PRESS) was used as a measure of model fit to the points in the design. Following prediction of the optimal conditions for the synthesis reaction, the experiment was performed in triplicate to verify the reliability of the predicted values and experimental data.

3. Results and discussion

3.1. Step I of the enzymatic transesterification of rapeseed oil with butanol

Results presented by Gog et al. showed that the optimal