APPLICATION OF ENZYMATIC PROCESS FOR BIODIESEL SYNTHESIS FROM VEGETABLE OIL WITH HIGH FATTY ACID CONTENT USING BUTANOL

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Abstract. Oil produced from spoiled rapeseed has high free fatty acid content. Such oil is not suitable for utilization for food production but could be used for biodiesel production by applying biotechnological methods. It was determined that the most effective biocatalyst for biodiesel synthesis from oil of 4 % acidity for oil transesterification with butanol was Lipozyme RM IM. Biodiesel, which met the standard requirements, was synthesized from rapeseed oil, applying the two-stage transesterification process, which optimal conditions were the following: for the first stage – 39.1 ºC temperature, oil and butanol molar ratio – 1:4.5, 5.96 % of Lipozyme RM IM, 9.75 hours of reaction duration; for the second stage – the same as in the first stage temperature, oil and butanol molar ratio 1:1.5; 7.8 % of lipase, duration – 7.9 h. The physical and chemical properties of the made rapeseed oil butylesters were determined and compared with common biodiesel. According to the obtained results the principal technological design for enzymatic rapeseed oil butylesters production was designed.

Keywords: rapeseed oil, enzyme, biodiesel, butylesters.

Introduction

Usually biodiesel is produced from vegetable oil or animal origin fat by transesterification with methanol and base catalyst [1-3]. However, base-catalyzed transesterification specified with some disadvantages: it was impossible repeatedly to use catalyst or utilize raw materials with high free fatty acid amount (more than 1 %), because free fatty acids react with base and form soaps, therefore product purification is more difficult [4]. These disadvantages could be removed by applying the biotechnological biodiesel production method. In this case biocatalyst (lipase) could be repeatedly used after transesterification and the raw material with bigger free fatty acid content could be involved in the process without soap formation [5; 6].

As the biotechnological method was more and more studied, it was noticed, that methanol could negatively affect lipases, inhibit them [7; 8]. Usage of higher alcohols could reduce such impact. Therefore, in for our investigations butanol was selected for oil transesterification [9]. To avoid problems concerned with utilization in biofuel production the raw materials commonly used in food sector, it was important to look for possibilities to apply non-edible vegetable oil for biodiesel production. Vegetable oil with high free fatty acid content and some toxic components could not be used in food sector. On the other hand, biodiesel could be produced from waste cooking oil. Depending on the cooking process and subsequent storage, the used for frying oil may contain impurities such as water, food residues and free fatty acids [10].

Materials and methods

As waste oil that is typically rich with free fatty acids, rapeseed oil from a local distributer was supplemented with oleic acid (Fluka) to specify of 4 % acid value. Butanol (99.5 %) was obtained from Sigma-Aldrich (UK), and lipase Lipozyme RM IM was kindly donated by a Novozymes, which was represented by JSC "Biopolis" (Lithuania). Other analytical grade reagents were obtained from Fluka (UK). The standards of glycerides and esters were obtained from Sigma Aldrich. The antioxidants IonolBF350,IonolBF100 wereobtained fromRaschig GmbH &Oxiris Chemicals S.A Kerobit – from BASF, and Baynox – from Advanced Industrial Intermediates.

In order to determine the optimal reaction conditions the response surface methodology central composite design (CCD) was employed. The variables selected for step I of acidic rapeseed oil transesterification with butanol were four numeric factors: enzyme concentration (4.50-7.50 %), butanol and oil molar ratio (4.50-7.50), time (5.25-9.75 hours); temperature (27.5-42.50 ºC). Each numeric factor was varied over 5 levels (axial points (α = 2), factorial points and the centre point (6 replicates). For the first step experiment non-centre points had 2 replicates. This design required 54 experimental runs in total. The variables selected for step II (transesterification of step I products) were lipase concentration (4-8 %), butanol and oil molar ratio (1:0.5-1:2) reaction time (4-8 h). Each numeric factor was varied over 5 levels (axial points (α = 2), factorial points and the centre point

(6 replicates). The temperature was chosen according to step I. This design had 22 experimental runs in total.

The experiments were carried out in a conical flask, connected to a condenser and thermometer with a thermo-controller. The reaction volume was mixed by a magnetic stirrer 200 min-1. Reaction mixture was equilibrated in the desired temperature, then lipase was added. The reaction was stopped after step I or step II by filtering for removing lipase catalyst and glycerol and by washing the product 3 times with distilled water. To determine the optimal conditions for the second step, the product of prolonged reaction in optimal conditions for the first step was supplemented with an additional portion of butanol and lipase and incubated for additional 4-8 hours at optimal temperature, determined in step I.

The quality of rapeseed oil butyl esters was determined according to the requirements of the Standard EN 14214. Gas chromatographic analysis of the reaction mixture was carried out after washing with water and drying the mixture by a vacuum rotary evaporator. The conditions used to analyze the glyceride (mono-, di-, triglycerides) content were as follows: a Restek MXT Biodiesel TG capillary column (15 m–0.32 mm–0.10 μm) was used and the initial temperature was maintained at 50 ºC for 1 minute. Next, the temperature was raised by 15 ºC·min^{-1} to 180°C. The temperature was increased by 7 ºC·min^{-1} until it reached 230 ºC and by 30 ºC·min^{-1} until it reached 370 ºC, and this temperature was maintained for 5 minutes. The temperature of the detector (FID) was 380 ºC. The carrier gas (hydrogen) flow was constant -80 kPa. Free and total glycerol and mono-, di-, triglyceridecontent was calculated using the ISTD Response ratio (the ratio of the peak's response to the response of the related internal Standard component). The methyl ester yield was evaluated according to the EN 14103 standard. The conditions for this analysis were as follows: an Alltech AT-FAME capillary column (30 m – 0.25 mm – 0.25 μm) was used, and the initial oven temperature was set at 60 ºC, which was maintained for 2 minutes. Next, the temperature was increased at a rate of 10 ºC·min^{-1} until it reached 200 ºC and by 5 ºC·min^{-1} until it reached 240 ºC, and this temperature was maintained for 15 minutes. Hydrogen was used as a carrier gas. The carrier gas (hydrogen) flow was constant – 70 kPa, split – 1:100. The temperatures of the injector and detector (FID) were set at 250 ºC. The methyl ester content was calculated using the formula from the standard. Standard deviation does not exceed 0.3 %. Oxidation stability was examined applying the Rancimat test according to the requirements specified in the EN 14112. The analyses were done for 2-3 times. The data of the experiments were processed by Excel 2013, according to the methodologies. The standard error of the results did not exceed 5 %, when the reliability was equal to 95 %.

Results and discussion

Step I of the enzymatic transesterification of rapeseed oil with butanol

In order to determine the maximum butyl ester concentration that can be obtained in the range of enzyme concentration (4.50-7.50 %), butanol and oil molar ratio (4.50-7.50), time (5.25-9.75 hours); temperature (27.5-42.50 ºC), response surface methodology and central composite design were used. The analysis of experimental variance (ANOVA) revealed that the reduced quadratic model was the most adequate according the sum of squares, lack of fit and R^2 tests.

The coefficient of determination R^2 (adjusted for the number of parameters in the model relative to the number of points in the design) was 0.7046. The value is high enough and, what is important for the design of the experiment, the predicted R^2 value 0.5719 is in a good agreement with the adjusted R^2 value 0.6521 (the values should differ by no more than 0.2). The final response function to predict the step I esterification yield in terms of actual factors after eliminating insignificant terms was as follows (1):

$$Y = -65.18 + 3.10A + 6.11B + 7.08C + 2.35D - 0.78BC - 0.66BD - 0.04A^2 + 0.23D^2, \quad (1)$$

where A – temperature, ºC;
B – molar ratio of butanol and oil;
C – percent concentration of lipase, %;
D – duration, h.

Visual evaluation of the reaction parameter influences on the yield can be performed by observing graphical images. As choosing the minimum alcohol concentration and maximum reaction time returns the best results, the model in which the butanol concentration and reaction time are set to the preferred lowest and highest values respectively, is shown in Fig. 1.

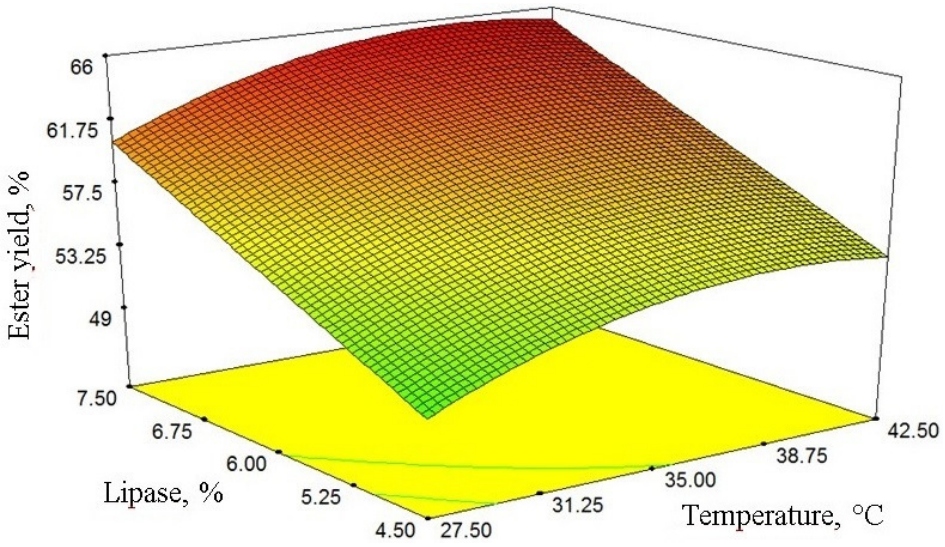

Fig. 1. **Response surface plot showing the mutual effects of time and temperature on ester yield, the butanol and oil molar ratio was 3, and the reaction time was 9.75**

In order to get the maximum ester yield the numerical optimization tool of Design expert software was used to optimize the reaction conditions. The optimization was meant to give preference for lower lipase concentration (lower limit weight 1, upper limit weight 0.5). The optimization used 30 starting points, the simplex fraction was 0.1. The first solution for the preferred parameter values: temperature 39.1 ºC, butanol and oil molar ratio 4.50, lipase concentration 5.96 %, reaction time 9.75 hours.

The predicted ester yield in the given conditions was 60.1 %. The yield is too low to comply with biodiesel requirements according the standard EN 14214. Therefore, a prolonged reaction time with a second reaction step was implemented for a more efficient catalysis. The ester yield in optimal reaction conditions and prolonged reaction time (29 hours) was 79 %.

Step II of the enzymatic transesterification of rapeseed oil with butanol

After removal of glycerol and enzyme from the ester products of step I, the second step of butanolysis was conducted. The reaction mixture was supplemented with an additional portion of butanol, lipase catalyst was refreshed (the first portion of catalyst was filtered-off and a new portion was added) and incubated at 39 ºC for 4-8 hours. The central composite design of the experiment was planned using the design of the response surface methodology. The independent variables were lipase concentration (4-8 %), butanol and oil molar ratio (0.5-2 %), reaction duration 4-8 hours). The percentage of esterification varied from 83 % to 96.6 %.The model equation using Actual Factors is shown below in Eq. 2:

$$Y = 82.69 + 0.37A + 9.92B - 0.55C + 0.55AB + 0.90BC - 6.01B^2, \quad (2)$$

where A – percent concentration of lipase, %;
B – butanol and oil molar ratio;
C – reaction time, h.

Variations in additional butanol:oil molar ratio had a critical impact on the reaction yield. While lower values of additional butanol during step II increased the reaction yield, only up to 88-90 %, butanol:oil molar ratios of approximately 1.25-1.50 % were optimal, and the reaction yield could reach 96 % when 8 % additional lipase was used (Fig. 2).

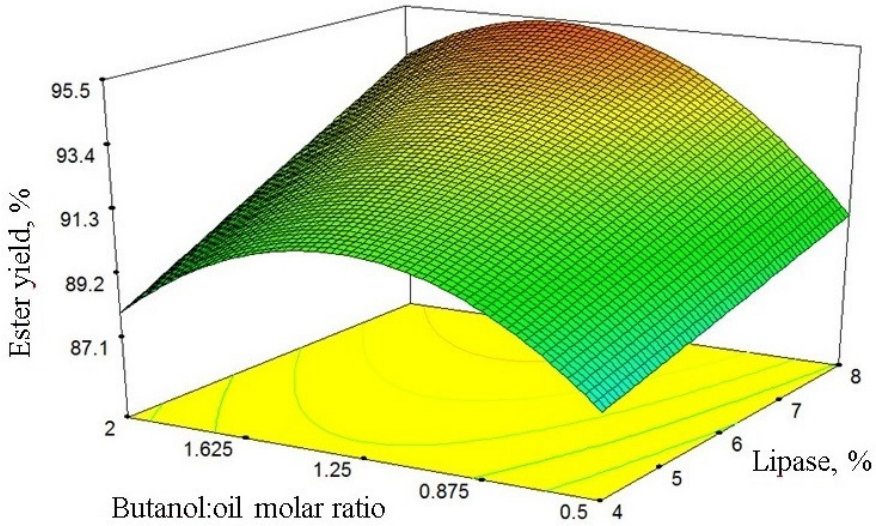

Fig. 2. **Response surface plot showing the mutual effects of the butanol and oil molar ratio and lipase concentration on the ester yield of step II, the step II reaction time was 6 hours**

In order to get the maximum ester yield the numerical optimization tool of Design expert software was used to optimize the reaction conditions. The optimization was meant to give no preference for independent reaction parameters and maximize the yield (butylester concentration). The optimization used 30 starting points, the simplex fraction was 0.1. The first solution for the preferred parameter values: butanol:oil molar ratio 1.50, lipase concentration 7.8 %, reaction time 7.9 hours. The predicted ester yield was 96.7 %. The experimental value was 96.63 %.

In order to determine if chemical and physical properties of rapeseed oil butylesters (RBE) meet the requirements of the standard EN 14214 and to compare them with biodiesel – rapeseed oil methylesters (RME), the chemical and physical properties were determined. The final data are presented in Table 1.

Table 1

Physical and chemical properties of rapeseed oil butyl esters

Parameter EN 14214 requirements	Units	Value		RME	RBE
		Min.	Max.		
Ester content	%	96.5	-	97.00	96.70
Density at 15 ºC	$kg \cdot m^{-3}$	860	900	875.00	880.00
Viscosity at 40 ºC	$mm^2 \cdot s^{-1}$	3.5	5.00	4.60	5.10
Monoglyceride content	%	-	0.8	0.49	0.12
Diglyceride content	%	-	0.2	0.16	0.19
Triglyceride content	%	-	0.2	0.04	0.07
Cetane number		51	-	54.10	59.70
Iodine value	$g \cdot J_2 \cdot (100\ g)^{-1}$	-	120	112.00	111.00
Moisture content	$mg \cdot kg^{-1}$	-	500	150.00	130.0
Oxidation stability, at 110 ºC	h	8	-	8.32	10.40
Cold filter plugging point	ºC	-5 ºC (in summer) -26 ºC, -32 ºC (in winter)		-10.00	-14.00

It was found that the produced RBE meet many requirements of the standard, but viscosity of fuel and oxidation stability do not meet the standard EN 14214 requirements for biodiesel. As we can see, RBE are a little more viscous and denser, comparing with RME. It could be explained by longer carbon chain of fatty acid butylesters. As our data show, RBE have such advantages as higher cetane number, comparing with RME (respectively, 59.7 and 54.1) and lower (-14 ºC) than RME cold filter plugging point. The oxidation stability of RBE is 2.7 h. According to the standard EN 14214

requirements the oxidation stability of biodiesel should be 8 h. In order to achieve this value the commercial antioxidants Ionol, Baynox and Kerobitwere are inserted. The data are presented in Fig. 3.

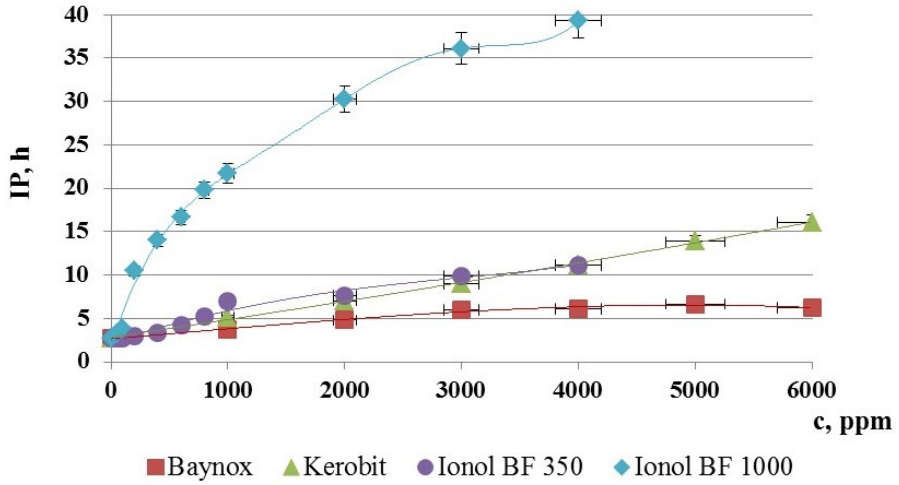

Fig. 3. **Antioxidant impact to oxidation stability of RBE**

As our data showed, the most effective antioxidant for RBE was Ionol. To achieve the required value of oxidation stability only 200 ppm of Ionol BF 1000 was required, while it was necessary to add 2000 ppm of Ionol BF 350, 3000 ppm of Kerobit. As for Baynox, it was the worst antioxidant: even adding 6000 ppm did not allow to reach 8 h of induction period. It is obvious that Ionol BF 350 and Kerobit specified comparable impact to oxidation stability of RBE.

With respect to the results of RBE synthesis from higher acidity oil, the principal biotechnological design for RBE synthesis was prepared (Fig. 4).

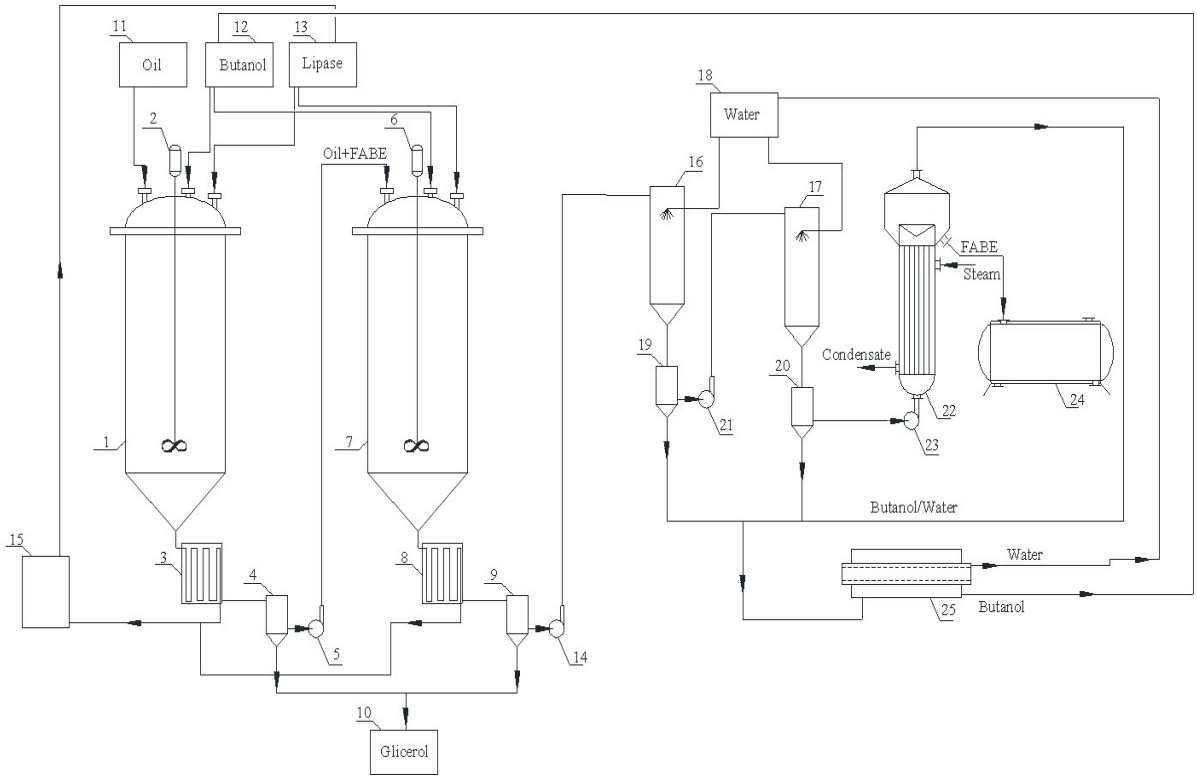

Fig. 4. **Principal biotechnological RBE synthesis scheme:** 1, 7 – transesterification reactors; 2, 6 – mixers; 3, 8 – filters; 4, 9, 19, 20 – settlers; 5, 14, 21, 23 – pumps; 10 – glycerol collection tank; 11 – oil tank; 12 – butanol tank; 13 – lipase tank; 15 – lipase regeneration; 16 – first washing; 17 – second washing; 18 – water tank; 22 – film evaporator; 24 – RBE tank; 25 – butanol/water separator

Oil, butanol, and lipase are supplied from the tanks 11, 12, and 13, correspondingly, to the transesterification reactor 1. The obtained mixture is constantly mixed with the mixer 2. The mixture consisting of RBE, oil, and butanol flows through the filter 3 (for enzyme separation) enters the settler 4, where glycerol is separated. Glycerol goes to the glycerol collection tank 10. After the separation of glycerol, the mixture is supplied to the second transesterification reactor 7 by a pump 5, where new portions of lipase and butanol are fed. The mixture is mixed with the mixer 6, then flows through the filter 8, where lipase is separated, and enters the settler 9, where glycerol is separated and then collected in the tank 10. Used lipase from the filters 3 and 8 enters the lipase regeneration unit 15 and returns to the lipase tank 13. With the pump 14, the mixture cleaned from glycerol is pumped to the first 16 and second 17 wash reactors, to which water is supplied from the water tank 18. Washing is performed twice when supplying esters from one washing reactor to the other. With the pump 23, the mixture obtained after the washing is pumped to the film evaporator 22, where butanol and water are separated from RBE. Butanol and water enter the butanol/water separator 25 and then go into the butanol tank 12 and water tank 18. After the film evaporator 22 RBE is collected in the container 24.

Conclusions

1. When producing rapeseed oil fatty acid butyl esters (RBE) from high free fatty acid oil (acidity 4 %) by the biotechnological method, the double-stage process should be applied. The optimal conditions of the first stage are as follows: 5.96 % of the lipase Lipozyme TL IM (of the weight of oil); molar ratio of oil and butanol – 1:4.5; temperature – 39.1 ºC; duration – 29 hours. The optimal conditions of the second stage are as follows: lipase content – 7.8 %; molar ratio of oil and butanol – 1:1.5; temperature – 39 ºC; duration of synthesis – 8 hours
2. By almost all physical and chemical properties RBE complies with the requirements of the standard EN 14214 and can be used as fuel for diesel engines. In order to enhance the oxidation stability, it is appropriate to use the industrial antioxidant Ionol BF 1000 (optimal dosage – 200 ppm).
3. The results of the investigations of RBE synthesis from high acidity oil allowed to design a principal two-stage biotechnological RBE synthesis scheme.

Acknowledgment

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