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ENZYMATIC BIOPROCESSING OF VEGETABLE OILS FOR THE PRODUCTION OF BIODIESEL

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Abstract

Biodiesel is a renewable, alternative fuel for diesel engines, that has captured the attention of the whole world, as it can be used both alone and mixed with diesel for unmodified diesel engines. It is easily obtained from common raw materials, as well as wastes. Biodiesel obtained through biotechnological procedures (biocatalysis) is of superior quality to chemical synthesis biodiesel. The use of purified lipases, such as pig pancreas lipase, Thermomyces lanuginosus lipase or lipase B from Candida antarctica as a biocatalyst for biodiesel obtainment has shown great results and the optimum control parameters have been studied. The production of biodiesel from vegetable oils using different lipases has been investigated. Results have shown that the type of lipase, reaction media and operational parameters (reaction time, temperature, lipase load, alcohol:oil molar ratio and water concentration) have influenced biodiesel yield. In order to establish the best composition and process conditions, an optimization procedure has been carried out. The enzymatic transesterification was performed in an organic solvent-containing system, in agitated flasks, at various temperatures (40-50ºC) and for different periods of time (10-14 hours). Also, variations of the alcohol:oil molar ratios, enzyme concentrations and added water percent were studied. A statistic evaluation of the results was performed, for the proper optimization of the process parameters in regard to conversion. Under optimal operating conditions, the fatty acid methyl esters (biodiesel) yields were >90%.

Keywords: *biocatalysis, biodiesel, lipase, transesterification*

INTRODUCTION

Fatty acid methyl esters (FAME), commonly known as biodiesel, have received great attention during recent years, due to concerning depletion of fossil fuels, oil price increase and biodiesel benefits towards the environment. Biodiesel can be produced from various animal and plant fats, by transesterification with methanol [4, 10]. Biodiesel obtained through biotechnological procedures (biocatalysis) is of superior quality to chemical synthesis biodiesel [16] and presents many advantages over diesel fuel. The most important are its renewability, biodegradability [18], the emissions of toxic compounds at lower levels [22], and its higher combustion efficiency [5].

Industrial scale production of biodiesel continues to be limited due to undesired by-products obtainment and their hard collection, glycerol recovery, inorganic salts and water, wastewater treatment, and the energy requirement [11]. In order to overcome these impediments, research activities regarding enzymatic catalysis have been carried out [3, 7, 20].

For the production of biofuels, one of the most reported enzyme groups is represented by lipases [12]. The use of purified lipases, such as pig pancreas lipase, Thermomyces lanuginosus lipase or lipase B from Candida antarctica as a biocatalyst for biodiesel obtainment has shown great results and the optimum control parameters have been studied [13, 23, 24]. The process of enzymatic transesterification presents certain advantages over chemical transesterification, along with its environmental benefits [6, 14]. Lipases can catalyze a variety of transesterification and esterification reactions relatively efficiently under mild conditions and in non-aqueous environments [2, 21, 9].

The type of lipase, reaction systems and operational parameters (lipase load, reaction time, temperature and alcohol:oil molar ratio) have a great influence on biodiesel yield [8].

Regarding alcohol to oil molar ratio, the stoichiometric equation requires 3 moles of alcohol and one of triglycerine for the obtainment of 3 fatty acid methyl ester moles and 1 mole of glycerol. Higher molar ratios would lead to higher biodiesel yields. The use of solvents has proven to be necessary to maintain the miscibility between the methanol and triglicerides with the purpose of forming a monophasic system [17]. The water content is also an important parameter [1, 15], and seems to be the subject of dispute. The effect of water in the system depends on the enzyme, immobilization support and the medium (with or without solvent). Probably the main disadvantage in biocatalytic biodiesel obtainment is the cost of the enzyme. Enzymes present different capacities to maintain their activity after recovery and repeated use, probably due to catalyst inactivation in the oil phase, the type of carrier used immobilization or enzyme sensitivity to long-term exposure [17, 19].

The main purpose of this paper was to better understand the relationship between reaction variables (time, temperature, enzyme concentration, substrate molar ratio and added water content) and process response (conversion in mass percentages) in order to optimize biodiesel biosynthesis.

MATERIAL AND METHOD

The substrates used during the enzymatic catalyzed experiments consisted of Olina palm oil, commercially available on the market, and methanol from the National Institute for Chemical-Pharmaceutical Research and Development, Bucharest.

As biocatalyst, pig pancreas lipase (PPL) from Sigma-Aldrich (22,7 U/mg) was employed.

6 mL of n-hexane (Merck Chemical Co. Darmstadt, Germany) were added to the reaction mixture, in order to permit a better solubilization of the mixture and to facilitate enzymatic biosynthesis.

For the optimization of the biodiesel obtainment process, an optimization methodology was employed to determine the interaction of different factors, optimizing one or more experimental responses. To this purpose, a Hadamard experimental matrix has been developed, with elements corresponding to 2 levels of the key factors, -1 and +1. The matrix was built by circular permutation starting from a basic generator, the factors of last experiences being always taken as level -1.

We therefore developed a matrix with 22 experiments and 5 key process parameters at 2 variation levels (chosen as minimum and maximum). Oil to biodiesel conversion was considered as response factor (Table 1).

The matrix was build based on the variation of the 5 essential parameters, for which maximum and minimum levels were chosen. The 5 parameters were: time (x_1), temperature (x_2), enzyme (x_3) (% from weight of oil), alcohol to oil molar ratio (x_4) and water (x_5) (% of oil weight) (Table no 1).

Table 1 – Experimental matrix for the optimization of biodiesel obtainment technology at laboratory level

Experiment no.	Factors					Response: (Y_i)
	X_1	X_2	X_3	X_4	X_5	
1	-	-	-	-	-	Y_1
2	-	-	-	-	+	Y_2
3	+	-	-	-	-	Y_3
4	-	+	-	-	-	Y_4
5	-	-	+	-	-	Y_5
6	-	-	-	+	-	Y_6
7	-	-	-	+	+	Y_7
8	+	-	-	-	+	Y_8

9	+	+	-	-	-	Y_9
10	-	+	+	-	-	Y_{10}
11	-	-	+	+	-	Y_{11}
12	-	-	+	+	+	Y_{12}
13	+	-	-	+	+	Y_{13}
14	+	+	-	-	+	Y_{14}
15	+	+	+	-	-	Y_{15}
16	-	+	+	+	-	Y_{16}
17	-	+	+	+	+	Y_{17}
18	+	-	+	+	+	Y_{18}
19	+	+	-	+	+	Y_{19}
20	+	+	+	-	+	Y_{20}
21	+	+	+	+	-	Y_{21}
22	+	+	+	+	+	Y_{22}

The reaction mixture contained palm oil (2 g) to which 4 portions of methanol were added throughout the process at specific time intervals in order to avoid enzyme inactivation, 6mL *n*-hexane (Merck Chemical Co. Darmstadt, Germany), water (5% and 15% weight of oil) and enzyme, PPL – 45% and 55% weight of oil. The system was stirred (250 rpm) at 40 and 50^{o}C and for 10 and 14 hours. The molar ratios used were 3:1 and 5:1 methanol to oil.

For the obtainment of biodiesel at laboratory level, a Heidolph Unimax 1010 reactor with a stirring unit and Heidolph Inkubator 1000 was used. The samples were vortexed with a Vortex Heidolph Reax Top, for 10 seconds, at the beginning of the experiment and after each methanol aliquot was added.

The sample analysis was performed by injecting a 1mm^3 aliquot in split less mode into a Hewlett Packard 6890 gas chromatograph (Avondale, PA, USA) equipped with a flame-ionization detector (FID), and a CP-Select CB for FAME 50m x 0.25mm x 0.25µm Varian capillary column.

RESULTS AND DISSCUSSIONS

The purpose of the experiments was the study of biodiesel obtainment and the optimization of the process.

The process has been designed using a matrix with 22 experiments to evaluate the effects of five key factors: temperature, time, enzyme concentration, alcohol:oil molar ratio and water concentration. These factors showed a significant influence on biodiesel production, each of them evaluated at two variation levels (Table 2).

Table 2. Biodiesel conversion according to the Hadamard experimental matrix

Experiment no.	Factors					Conversion (%)
	Time (hours) X_1	Temperature (^{o}C) X_2	Enzyme (%) X_3	Alcohol:oil molar ratio X_4	Water (%) X_5	
1	10	40	45	3:1	5	39.8601
2	10	40	45	3:1	15	67.4214
3	14	40	45	3:1	5	41.2884
4	10	50	45	3:1	5	13.7727
5	10	40	55	3:1	5	98.5646

6	10	40	45	5:1	5	12.18309
7	10	40	45	5:1	15	41.475
8	14	40	45	3:1	15	31.6088
9	14	50	45	3:1	5	14.9689
10	10	50	55	3:1	5	19.0209
11	10	40	55	5:1	5	87.8042
12	10	40	55	5:1	15	32.7987
13	14	40	45	5:1	15	18.7578
14	14	50	45	3:1	15	14.9156
15	14	50	55	3:1	5	19.768
16	10	50	55	5:1	5	29.4874
17	10	50	55	5:1	15	79.9573
18	14	40	55	5:1	15	51.943
19	14	50	45	5:1	15	28.2946
20	14	50	55	3:1	15	18.6713
21	14	50	55	5:1	5	78.685
22	14	50	55	5:1	15	26.3884

As it can be observed, experiment no. 8 had the highest yield (98.5646% conversion) after 14 hours, at 40°C, 45% enzyme concentration, 3:1 alcohol to oil molar ratio and 15% water. The lowest rate of conversion was registered for experiment no. 6 (10 hours reaction time, 40°C, 45% enzyme concentration, 5:1 molar ratio and 5% water).

From the obtained results, a classification of the factors with a significant influence on the process response was made, according to linear coefficients (Table 3):

$$b_0 = \sum \frac{y_i}{N} \qquad b_i = \sum \frac{x_i y_i}{N}$$

Where:
b_0, b_i = linear coefficients
x_i = independent variables
y_i = process response (conversion %)

Table 3. Influence of significant factors

b0	**b1**	**b2**	**b3**	**b4**	**b5**
39.43796	-4.58145	-8.17159	0.515073	0.617654	8.552937

Thus, $b_i > 0$ represents a positive influence and $b_i < 0$, a negative influence, obtaining the linear objective polynomial function of the form:

$Y=b_0 + b_1X_1 + b_2X_2+...+b_kX_k = 39.43796 + (-4.58145)\ X_1 + (-8.17159)\ X_2 + (0.515073)\ X_3 + 0.617654\ X_4 + 8.552937\ X_5$

It can thus be observed that enzyme concentration (x_3) (% from weight of oil), alcohol to oil molar ratio (x_4) and water content (x_5) (% weight of oil) had a positive influence on the bioprocess response, while time (x_1) and temperature (x_2), had a negative influence.

CONCLUSIONS

The purpose of this experiment was to achieve the biodiesel process optimization through the use of an experimental factorial plan represented by a Hadamard matrix. By circular permutation of 5 key process parameters, at two variation levels, the significance of their effect was evaluated according to biodiesel conversion yield.

The highest conversion yield was 98.57% after 10 hours, at 40°C, 55% enzyme concentration, 3:1 alcohol to oil molar ratio and 5% water.

According to the determined linear coefficients, enzyme (x_3), alcohol to oil molar ratio (x_4) and water (x_5) had a positive influence on the bioprocess response, while time (x_1) and temperature (x_2) presented a negative influence.

In accordance to the optimization method, in order to obtain a better settlement of the optimal regions, a new experimental plan will be established in which the variable factors will be alcohol to oil molar ratio and water, the rest of the factors remaining unchanged.

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