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ENVIRONMENT-FRIENDLY BIODIESEL PRODUCTION BY TRANSESTERIFICATION OF RAPESEED OIL: EFFECT OF REACTION PARAMETERS

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Abstract. The huge energy demand in the industrialized world and the pollution problems caused due to the worldwide consumption of fossil fuels has made it necessary to develop alternative sources of energy. It is estimated that pure biodiesel provides over 90% reduction in unburned hydrocarbons and 75–90% reduction in aromatic hydrocarbons. This results in almost total reduction in sulphur dioxide (which causes acid rains), 40–60% reduction in soot particles, 80% and 10–15% reduction in carbon dioxide and carbon monoxide emissions respectively. Reduction of poly-aromatic hydrocarbons is extremely important as many of these hydrocarbons are cancer causing and ozone-forming compounds. Biodiesel does not interfere with the carbon cycle or cause climate change (vegetables from which oils are extracted for biodiesel production remove carbon dioxide from the atmosphere to grow).

Due to the global drive towards renewable, non-toxic and environmentally acceptable products the ability of the commercial lipolytic enzyme Lipoprime 50T to catalyze the biotechnologically important process of biodiesel (fatty acid methyl esters) production via rapeseed oil transesterification (methanolysis) was investigated. The optimal reaction conditions were determined using titrimetric and simple and accurate thin-layer chromatography and computer analysis methods that enable to follow the changes of all reaction mixture components simultaneously. The effects of molar ratio of substrates, reaction temperature and time, lipase dosage and water content in different organic medium on the composition of the reaction mixture were analyzed.

The hydrolytic activity of lipase directly depends on the reaction solvent and the water content required for high yields of the product increases with increasing the reaction medium hydrophobicity. Under the optimal process conditions identified (n-hexane, 40 °C temperature, 66 mg/ml enzyme, water content of 26%, rapeseed oil to methanol molar ratio of 1:16 and reaction time 24 h) the highest biodiesel conversion yield of around 85% was obtained.

Keywords: biodiesel, transesterification (methanolysis), rapeseed oil, lipase, organic solvent, thin layer chromatography, environmental strategy.

Introduction

The Earth faces a variety of environmental problems that are causing long-term damage to the earth's ecosystem. The only way to control current environmental issues is to create sustainable development strategies and continue to instill conservation methods. For this reason bioeconomy is seen as the main future economy that is based on environmentally friendly products and services produced by the use of biotechnology and renewable energy sources as the basic building blocks for industry. The development of the biotechnological industry and its application to agriculture, health, chemical or energy industries are the examples of bioeconomic activity.

The major part of all energy consumed worldwide comes from fossil sources. Diesel fuels have an essential function in the industrial economy of a country as they are used in heavy trucks, city transport buses, locomotives, electric generators, farm equipment, underground mine equipment, etc. (Ganesan *et al.* 2009). The huge energy demand in the industrialized world and the pollution problems caused due to the widespread use of fossil fuels has made it necessary to develop alternative sources of energy to resolve these problems. Moreover some recent estimates of the world-wide petroleum reserves predict them to last only for 50 years (Barnwal, Sharma 2005).

An increasing environmental awareness in society gave rise to intensified efforts to bring the products of the conversion of fat-rich feedstock like various oils and waste fats into the market as an additional source of bioenergy (Rass-Hansen *et al*. 2007). Such products are defined as being renewable, non-toxic and environment-friendly components of biodiesel (Bendikienė *et al.* 2011) and biolubricants (Kiriliauskaitė *et al.* 2011). Use of bioenergy feedstocks could not only provide significant environmental benefits, but could also help to reduce reliance on foreign oil. Biofuels also offer a promising road to enhance development since they use local materials, can provide local jobs, and do not require the import of expensive equipment and expertise.

In recent years various important experimental and theoretical environmental investigations and developments of technologies for the protection of environment

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have been conducted in Lithuania, including the studies of ability to use fatty waste for biogas production during organic waste biodegradation process (Baltrėnas, Kvasauskas 2008; Baltrėnas *et al.* 2004), investigations of biodegradation processes in food waste with different amounts of moisture (Baltrėnas *et al.* 2006).

The global biodiesel industry grew very fast in the last years and even a faster growth rate is foreseen in the near future as it shows clear advantages over traditional fuel from an economical and environmental point of view as it is a biodegradable, renewable, and non-toxic fuel (Fjerbaek *et al.* 2009).

Biodiesel can be produced by transesterification of fat-rich feedstock like various oils and waste fats. Vegetable oils present higher viscosity than diesel, normally 25 times. For this reason these mixtures of free fatty acids (FFA) and triacylglycerols (TAG) need to be chemically altered to fatty acid alkyl esters to be used as biodiesel fuel for currently used diesel engines. Catalysts investigated for transesterification are either acids, bases, both liquid and heterogenous, as well as free or immobilized enzymes (Haas *et al*. 2006; Meher *et al.* 2006). Enzymes are an interesting prospect for industrial-scale production for the development of environmentally friendly production process. The number of enzymes commercially available and the range of applications are gradually increasing. Of all known enzymes, lipases are gaining more attention due to their ability to catalyze a wide range of reactions. This makes lipases the enzymes of choice for potential applications in various fields of industry. Lipolytic enzymes work at low temperature, atmospheric pressure and are not aggressive to reactors and accessories which are closely associated with energy cost from the economic perspective (Al-Zuhair 2007).

Industrial production of biodiesel today is based on vegetable oils (Fjerbaek *et al.* 2009). The choice of alcohol has some influence on the properties of the biodiesel produced, though this does not draw as much attention as the price of the alcohol used for biodiesel production (Fjerbaek *et al.* 2009). This makes methanol and ethanol the alcohols of choice for biodiesel production.

The main problem to overcome is an inactivation of the biocatalyst due to the inhibitory effect of excess of alcohol. Short chain alcohols, especially methanol, have a low solubility in oils; therefore a new liquid phase appears in the system leading to an inactivation of the enzyme and decreased yields of alkyl ester. This problem could be removed by selection of a suitable reaction medium for lipase (Adamczak *et al.* 2009; Lu *et al.* 2008). Thus the main parameters that cause the reaction effectiveness are organic solvent and water content. Although water is not involved as a reactant or a product in transesterification reactions, water content is very important as it favors the expression of the full enzymatic activity (Salis *et al.* 2008). The use of solvents in the enzymatic production of biodiesel is mentioned as being inconvenient, but solvent recovery is a common practice in the chemical production of biodiesel and on an industrial scale, solvents can be recovered together with methanol after the enzymatic reaction (Adamczak *et al.* 2009; Royn *et al.* 2007). In the enzymatic oil transesterification (alcoholysis) other factors can influence the yield of the product and the reaction rate as well. These parameters include the reaction time and temperature, molar ratio of oil to alcohol, type of alcohol, nature and amount of enzyme, purity of reactants and mixing intensity (Fjerbaek *et al.* 2009; Adamczak *et al.* 2009).

In the present study, Lipoprime 50T-catalyzed transesterification (methanolysis) of rapeseed oil (RO) in n-hexane and in *t*-butyl alcohol was investigated in order to obtain high degrees of transesterification and high yields of environment-friendly biodiesel-fatty acid methyl esters. The effects of molar ratio of substrates, reaction temperature and time, lipase dosage and water content in different organic medium on the composition of the reaction mixture were analyzed. Methyl ester (ME) production and RO disappearance were followed by thin-layer chromatography (TLC).

1. Investigation methods

LipoPrime® 50T (hereafter Lipoprime) was kindly provided by Biopolis Ltd, the distributor of Novozymes A/S in Lithuania. Lipoprime is classified in the Chemical Abstracts Service Registry as "Lipase, triacylglycerol, CAS no. 9001- 62-1". Lipoprime is a protein-engineered lipase produced by submerged fermentation of a genetically modified *Aspergillus* microorganism (the product sheet of Novozymes). The enzyme has a broad substrate specificity. The product is produced by a non-pathogenic microorganism and is classified as non-toxic. The product preparations are biodegradable. The components of Lipoprime are listed in the relevant inventories, e.g., in EINECS and TSCA.

All chemicals used in the study were products of analytical grade.

1.1. The standard spectrophotometric assay

The hydrolytic activity of lipase on *p*-nitrophenyl butyrate solution in 2-propanol was investigated measuring the change of optical density at 400–410 nm within 3–6 min at 30 °C and pH 7.0–10.0, 100 mM universal buffer (Britton – Robinson buffer; composed of acetic, *ortho*-boric and *ortho*-phosphoric acids at a ratio of 1:1:1 providing buffering capacity over a wide range of pH) (Surinėnaitė *et al.* 2002; Bendikienė *et al.* 2004). One unit of lipase hydrolytic activity corresponds to the amount of the enzyme releasing 1 μmol of *p*-nitrophenol per minute under standard conditions.

1.2. Procedure for enzymatic transesterification of oil

Enzymatic processes are more environmentally friendly than traditional chemical methods as enzymatic reactions require milder conditions, less solvent, and give significantly cleaner products. Reactions were carried out in closed 20 ml batch reactors at a constant stirring (180 rpm), coupled to condensers in order to avoid the loss of reaction mixture components by volatilization. RO and methanol were mixed in a molar ratio of 1:4 unless specified otherwise. Enzyme was used with an amount equivalent to 44 mg/ml unless specified otherwise. Lipoprime 50T de-

clared activity is 50 KLU/g. The activity is determined relative to an enzyme standard under the assay conditions: hydrolysis of tributyrin, 30 °C, pH 7.0 (the product sheet of Novozymes). 1 LU is the amount of enzyme, which releases 1 pmol butyric acid per minute under the given standard conditions. 1 KLU = 1000 LU. The reaction progress was followed by extracting 50 μl of reaction mixture aliquots at definite time intervals and analyzing by TLC. For analysis by the TLC method, samples were diluted with diethyl ether (v/v ratio 1:1), mixed vigorously and kept at –20 ºC until chromatographic analysis (Bendikienė *et al.* 2008a).

1.3. Chromatographic analysis

TLC analysis was carried out on silica gel G-25 plates. System of solvents for the elution was chosen according to methods described by Yadav (1998) and Bendikienė (2008a). Chromatograms were developed by iodine vapor. Pure methyl oleate (MO), FFA, TAG, diacylglycerols (DAG), monoacylglycerols (MAG) and RO solutions in diethyl ether were used as standards.

1.4. Quantitative analysis

Quantitative analysis (%) of reaction products separated by TLC (average of 3 assays) was performed with a Uvitec Cambrige Fire-reader imaging system and Uvitec Fire-reader software by photodensitometry assessing spot area and color intensity. Verification of the products yield was performed also using Micro image 4.0 program which had been successfully applied in our previous experiments. The accuracy of this method was tested by determination of released FFA content by titration against sodium hydroxide using phenolphthalein as an indicator. The yields determined by TLC and titrimetry were in good agreement. The validation of the analysis had been described elsewhere (Bendikienė *et al.* 2008b; Kiriliauskaitė *et al.* 2011). Each experiment was carried out in triplicate. The results are presented as means ± S.E.M.

2. Investigation results

It was well known that enzyme activity has a relationship with the log P value of the organic solvent used as the reaction medium and that much higher enzymatic activity could be obtained in hydrophobic organic solvent that has a high log P value (Lu *et al.* 2008; Janssen *et al.* 1993). The octanol-water partition coefficient, P, is a measure of the differential solubility of a neutral substance between these immiscible liquids and thereby, an indicator of hydrophobicity of this substance. It is typically used in its logarithmic form, log P. It has been estimated that for the production of monoesters during enzymatic acylglycerol synthesis, a polar solvent with log P value below 1 is favorable, while for the production of triesters it is better to choose a non-polar solvent with a high log P value. Only the group of tertiary alcohols is an exception. Lu *et al.* (2008) studied twelve different solvents for triolein transesterification (methanolysis) catalyzed by immobilized lipase from *Candida* sp. 99–125 and investigated the effect of water content added to the reaction mixture in the range of 0–10%. They noticed that there is no correlation between the MO yield or conversion efficiency with solvents hydrophobicity (log P), dielectric constant (ε) and Hildebrant solubility parameter (δ). However, the water content needed to obtain the maximum yield of the products and the highest activity of lipase depends on solvents hydrophobicity significantly. More hydropilic solvents needed relatively less water to keep its maximum activity, while the hydrophobic solvents needed more water to maintain higher transesterification (methanolysis) yield (Lu *et al.* 2008).

To optimize reaction conditions for enzymatic RO transesterification, two solvents *t*-butanol and n-hexane with different log P value (0.79 and 3.5 respectively) were compared. n-Hexane is one of the most commonly used solvents in synthetic reactions using lipases. Bernardes *et al.* (2007) reported that the use of n-hexane helped the biodiesel production. It has been shown that there are many benefits in using *t*-butanol as a solvent in comparison to other organic solvents in lipase-catalyzed processes (Royn *et al.* 2007). *t*-Butanol dissolves both methanol and the glycerol and is not a substrate for the lipases because it does not act on tertiary alcohols. Moreover, *t*-butanol is a non-toxic solvent of relative low cost.

2.1. Effect of different reaction medium

The hydrolysis was more favorable in *t*-butanol, while transesterification reactions were more effective in n-hexane. Optimal conditions for the methanolysis in *t*-butanol were determined (40 °C, rapeseed oil to methanol molar ratio 1:6 and 1:8 and reaction time 1.5 h) in our earlier investigations (Bendikienė *et al.* 2011), and since the reaction manner was investigated to be dramatically different in studied organic solvents (Fig. 1), analogous experiments were carried out in n-hexane (Fig. 2, a) and in the mixture of *t*-butanol and n-hexane (1:1, v/v) (Fig. 2, b). At least three moles of methanol are required for the transesterification (methanolysis) reaction to accomplish a complete conversion of RO into its ME. An alcohol to oil molar ratio of six is commonly used in industrial processes to obtain higher yields of esters (Barnwal, Sharma 2005; Van Gerpen 2005). Considering the reports of inhibitory effect of excess alcohol (Adamczak *et al.* 2009), methanol to RO molar ratios of 2, 4, 6 and 8 were tested in the reactions conducted at different temperatures.

As shown in Fig. 2, changing the reaction conditions no significant differences were observed in n-hexane, indicating that Lipoprime 50T lipase retained its activity and stability within the studied range of temperatures. However, it was shown that a higher amount of methanol in the reaction mixture causes the decrease of the hydrolysis effectiveness. This could be due to the inhibition of lipase with excess methanol (Jeong, Park 2008).

Although the reaction in *t*-butanol and n-hexane mixture (1:1, v/v) was slower as compared with the processes in *t*-butanol (the traces of RO were still noticed after 48 h), the highest ME yield of about 30% was obtained under the same optimal conditions in both cases (40 °C temperature and RO to methanol molar ratio 1:6). Above 40 °C temperature, the process effectiveness decreased, possibly owing to some enzymatic deactivation.

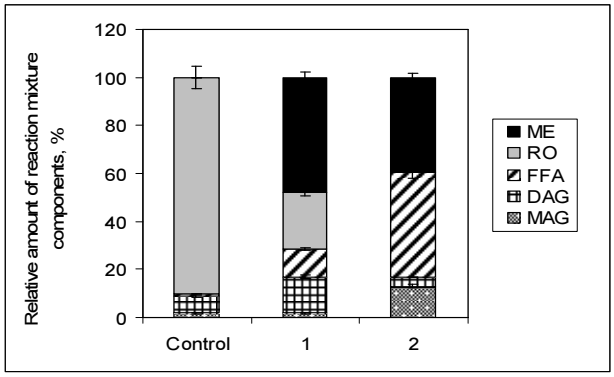

Fig. 1. Effect of organic solvent on Lipoprime 50T-catalyzed rapeseed oil transesterification. Reaction was performed at 30 °C with 44 mg/ml enzyme, RO to methanol molar ratio 1:4 and reaction time 120 h in n-hexane (1) and in *t*-butanol (2). Control – rapeseed oil. ME – methyl esters of FA; RO – rapeseed oil; FFA – free fatty acids; DAG – diacylglycerols; MAG – monoacylglycerols (MAG and DAG contents are expressed as the sum of their regioisomers)

2.2. Effect of solvent quantity

As shown in Fig. 3, the hydrolytic activity directly depends on the quantity of *t*-butanol in the reaction mixture.

The highest yields of FFA were determined in the reaction mixture when n-hexane and *t*-butanol volumetric ratio was in range of 1:2 to 1:4 (Fig. 3, 6–8 columns) and in pure *t*-butanol (Fig. 3, 9 column). As it was noticed earlier, the hydrolysis was more favorable in *t*-butanol in comparison to n-hexane and the processes in n-hexane are much slower (ME yields are higher in pure n-hexane rather than in pure *t*-butanol after 120 h from the reaction beginning, Fig. 1). After 24 h not only hydrolysis effectiveness but also the ME yields were determined to be lower in the reaction mixtures with the bigger portion of n-hexane (Fig. 3, 1–4 columns) than in the ones where *t*-butanol dominated (Fig. 3, 5–9 columns).

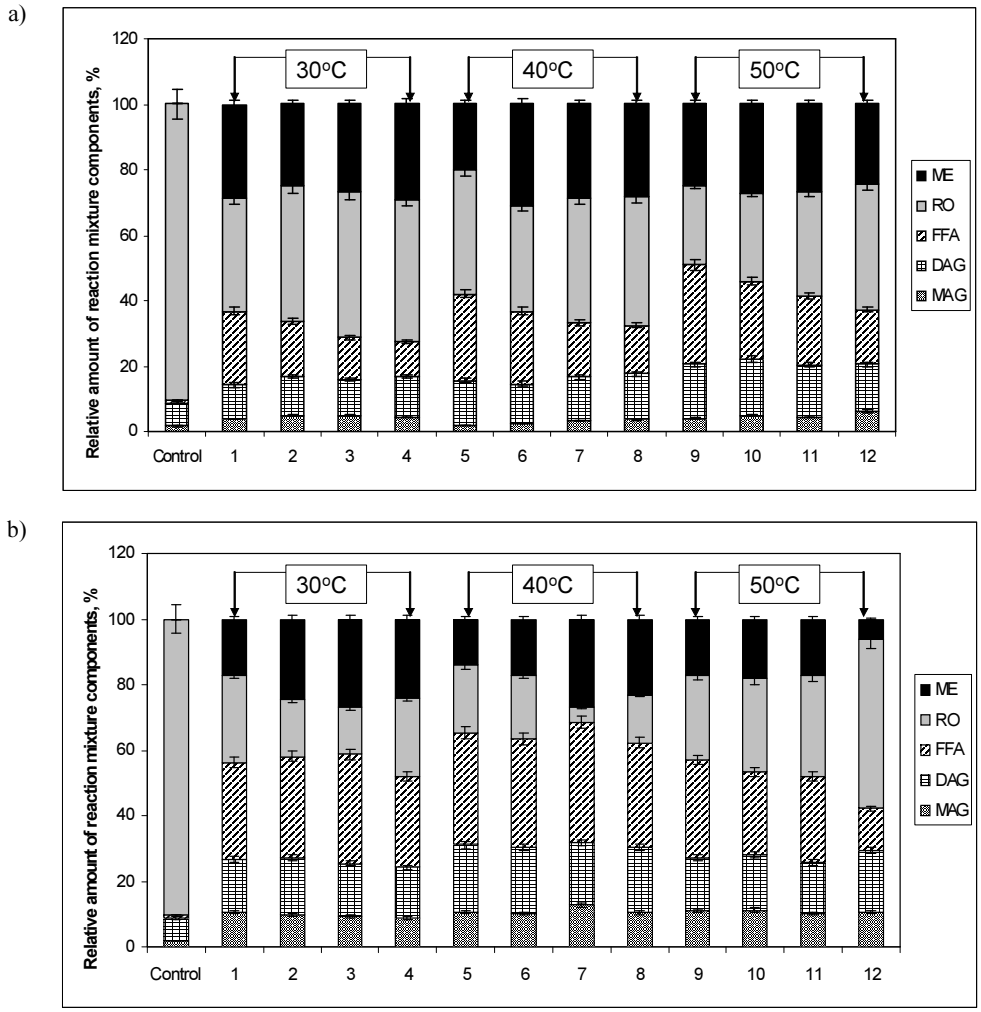

Fig. 2. Effect of molar ratio of substrates and reaction temperature on Lipoprime 50T-catalyzed rapeseed oil transesterification in n-hexane (a) and in n-hexane:*t*-butanol mixture (1:1, v/v) (b). Reaction was performed with 44 mg/ml enzyme, RO to methanol molar ratio **1:2** (1, 5, 9), **1:4** (2, 6, 10), **1:6** (3, 7, 11), **1:8** (4, 8, 12) and reaction time 48 h. Symbols are the same as in Fig. 1

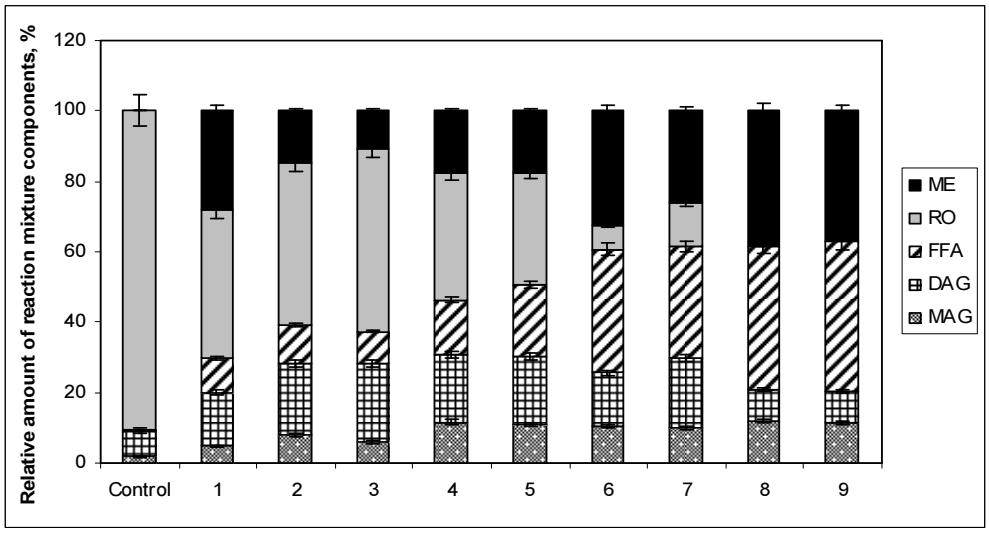

Fig. 3. Effect of solvents on Lipoprime 50T-catalyzed rapeseed oil transesterification in n-hexane:*t*-butanol mixture of different molar ratio. Reaction was performed at 40 °C with 44 mg/ml enzyme, and RO to methanol molar ratio 1:6 and reaction time 24 h. 1 – n-hexane (pure); n-hexane:*t*-butanol (v/v) ratio in the reaction mixture: 2 – 4:1; 3 – 3:1; 4 – 2:1; 5 – 1:1; 6 – 1:2; 7 – 1:3; 8 – 1:4; 9 – *t*-butanol (pure). Symbols are the same as in Fig. 1

2.3. Effect of `water content

Water content is known to be one of the main factors that affect the activity of lipase in non-aqueous medium (Adamczak *et al.* 2009). A small amount of water is necessary to keep the active conformation of the enzyme (Al-Zuhair 2007; Noureddini *et al.* 2005). However, excessive water promotes the hydrolysis of substrate and decrease the yield of the ester production. To obtain high RO conversions, the aqueous phase should be reduced to avoid ME hydrolysis and aggregation of enzyme molecules (Iso *et al.* 2001). On the other hand, the water content must be large enough to prevent enzyme inactivation by the methanol (Oliveira, Rosa 2006).

In this study, the effects of water content on the lipase-catalyzed transesterification (methanolysis) of RO was investigated via the addition of a definite quantity of water to the reaction solution of methanol and RO as substrates, *t*-butanol, n-hexane or the mixture of these solvents (1:1, v/v) as a solvent, and Lipoprime 50T as the enzyme. The reaction was conducted with the addition of water in a range from 0 to 18% (v/v) based on reaction mixture volume with RO to methanol molar ratio of 1:6, a constant temperature of 40 °C, and reaction time of 24 h (Fig. 4 a, b).

It was observed (Fig. 4 a, b) that lipase-catalyzed transesterification of RO in *t*-butanol had a clear water content dependence. The ME yield increased with increasing the water content from 0 to 9% (v/v). When the water content used was 9%, the highest ME yield of 38% was obtained. However, the ME yield sharply decreased when water content used was above 9%. This is because a higher water content shifts the reaction toward hydrolysis of substrate rather than the synthesis of ester.

Lipoprime 50T-catalyzed transesterification of RO in n-hexane had a clear water content dependence as well. However, the optimal conditions could not be determined due to too narrow range of concentrations. Moreover analogous reactions in *t*-butanol and n-hexane mixture had no clear water content dependence in this range of concentrations. Thus, these reactions were repeatedly conducted with the addition of water in a wider range from 0 to 40% (v/v) based on reaction mixture volume with RO to methanol molar ratio of 1:6, a constant temperature of 40 °C, and reaction time of 24 h.

It was observed (Fig. 5) that lipase-catalyzed transesterification (methanolysis) of RO in n-hexane had a clear water content dependence within the range of concentrations investigated.

The ME yield increased with increasing the water content from 0 to 26% (v/v). When the water content used was 26%, the highest ME yield of 68% was obtained. However, the ME yield sharply decreased when water content used was above 26%. As well as in the case of *t*-butanol, the higher content of water shifts the reaction toward hydrolysis of substrate rather than the synthesis of ester.

These results support widely discussed dependence of organic solvent used for the transesterification reaction and water content required for maximum conversion. The water content required for high yields of the product increases with increasing the reaction medium hydrophobicity (Al-Zuhair 2007).

The investigation of effect of water content on Lipoprime 50T-catalyzed RO methanolysis in hexane: *t*-butanol mixture (1:1, v/v) is presented in Fig. 6 (a-c).

The lowest (4%, Fig. 6, a, b and lane 3, Fig. 6, c) and the highest (35% and 40%, Fig. 6, a, b and lanes 10, 11, Fig. 6, c) water contents have the similar effect on the production of esters during the reaction in *t*-butanol and n-hexane mixture as a reaction medium, when the other reaction conditions are the same. When the water content was increased from 9 to 30% not only the ME yield decreased significantly (Fig. 6, a, b and lanes 4–9, Fig. 6, c), the rate of RO hydrolysis decreased as well, especially in the case of water content of 30% (v/v).

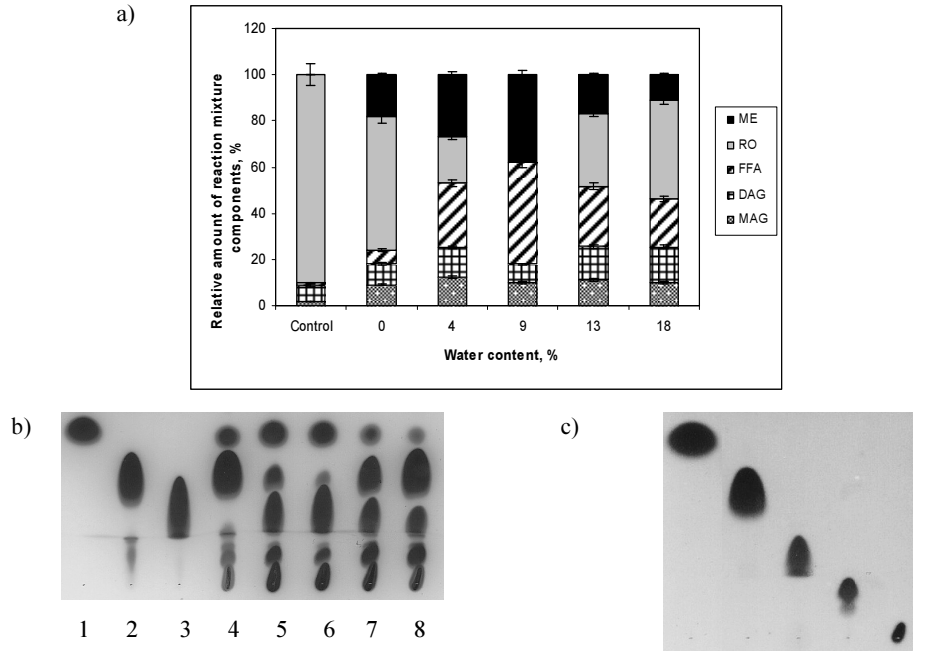

Fig. 4. Effect of water content on Lipoprime 50T- catalyzed rapeseed oil transesterification in *t*-butanol (a). TLC plate showing the products of lipase–catalyzed rapeseed oil methanolysis in *t*-butanol (b): controls: 1 – MO; 2 – RO; 3 – oleic acid (OA); 4 – components of reaction mixture without water; 5 – 4% (v/v) addition of water; 6 – 9% (v/v) addition of water; 7 – 13% (v/v) addition of water; 8 – 18% (v/v) addition of water. Reaction was performed at 40 °C with 44 mg/ml enzyme, RO to methanol molar ratio 1:6 and reaction time 24 h. The view of control samples (c). Symbols are the same as in Fig. 1

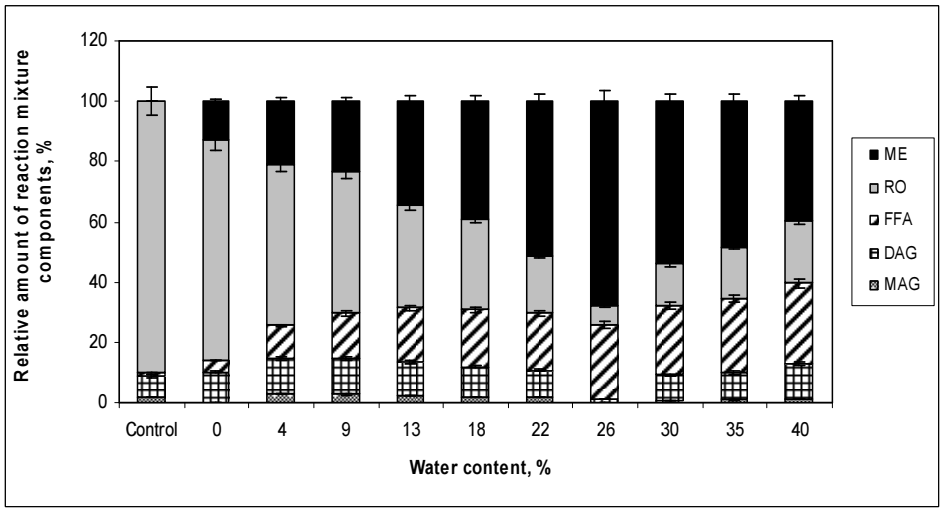

Fig. 5. Effect of water content on Lipoprime 50T-catalyzed rapeseed oil transesterification in n-hexane. Reaction was performed at 40 °C with 44 mg/ml enzyme, RO to methanol molar ratio 1:6 and reaction time 24 h. Symbols are the same as in Fig. 1

It should be noted that when the content of water was the highest (35 and 40%), only ME and FFA were observed in the reaction mixture after 168 h from the reaction beginning. Considering these results analogous reactions with water content of 35 and 40% were carried out in *t*-butanol in order to obtain a higher process rate as in earlier experiments reaction in *t*-butanol was found to be faster in comparison to n-hexane and *t*-butanol mixture. However, no significant increase of the reaction effectiveness was observed (data not presented).

2.4. Effect of substrate molar ratio

When the reactions were carried out in n-hexane with a water content of 26%, the highest ME yield of about 68% was obtained. Therefore, new transesterification (methanolysis) reactions were carried out to determine the optimal molar ratio of substrates in n-hexane in order to obtain even higher yield of the desired product (Fig. 7).

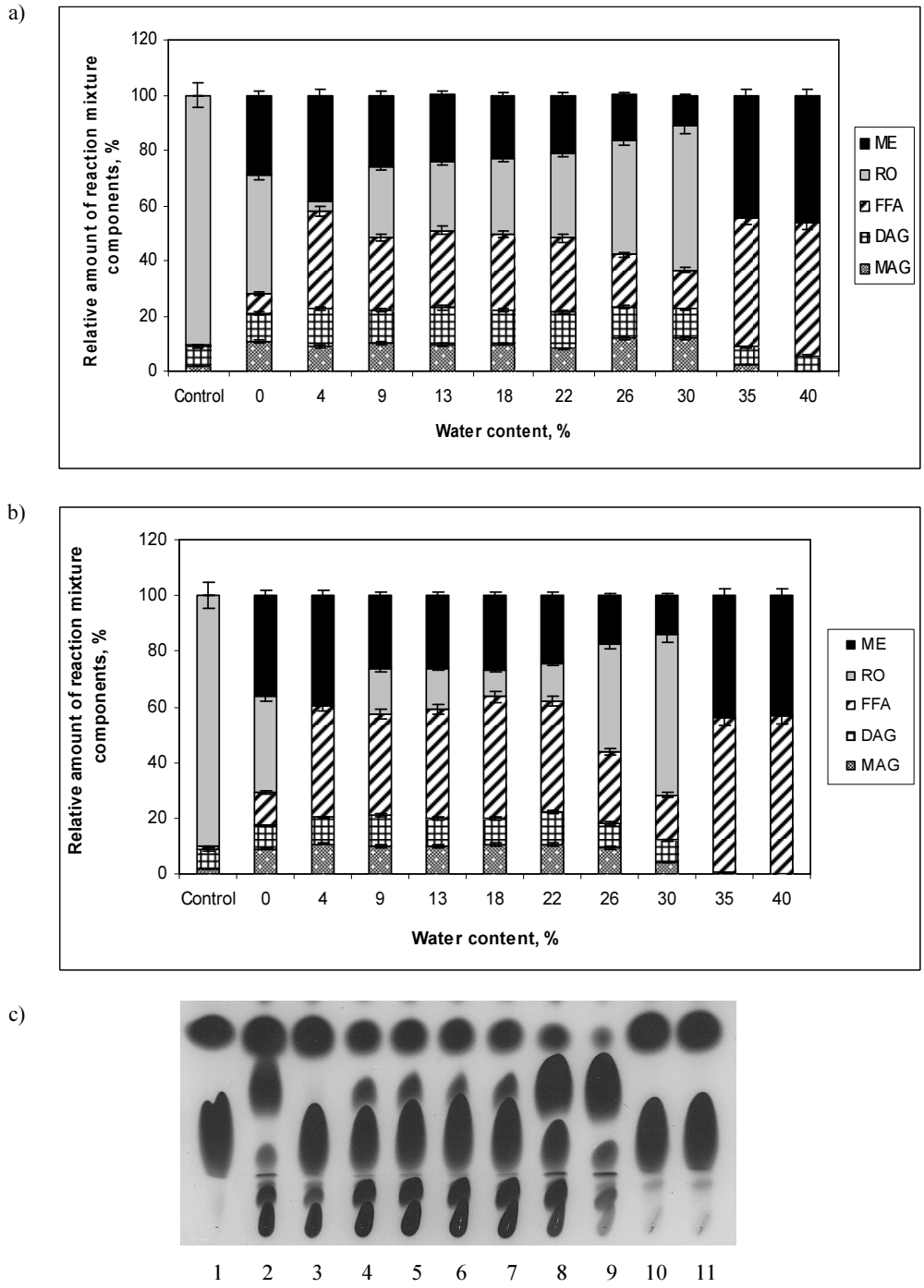

Fig. 6. Effect of water content on Lipoprime 50T-catalyzed rapeseed oil transesterification in hexane:*t*-butanol mixture (1:1, v/v). Reaction was performed at 40 °C with 44 mg/ml enzyme, RO to methanol molar ratio 1:6 and reaction time 24 h (a) and 168 h (b). TLC plate showing the products of lipase–catalyzed rapeseed oil methanolysis n n-hexane:*t*-butanol mixture (1:1, v/v), (c): control: 1 – MO and OA mixture (1:1); 2 – components of reaction mixture without water; 3 – 4% (v/v) addition of water; 4 – 9% (v/v) addition of water; 5 – 13% (v/v) addition of water; 6 – 18% (v/v) addition of water; 7 – 22% (v/v) addition of water; 8 – 26% (v/v) addition of water; 9 – 30% (v/v) addition of water; 10 – 35% (v/v) addition of water; 11 – 40% (v/v) addition of water. Symbols are the same as in Fig. 1

The molar ratio of RO to methanol ranged from 1:2 to 1:16 was studied to evaluate the effect of methanol on lipase activity in n-hexane. From the results of RO and methanol molar ratio study, it was determined that when the molar ratio of oil to methanol was in range of 1:10 – 1:16, high conversions were achieved (ME yields of around 75%) after 24 h, and no significant differences were detected with different oil to methanol molar ratios within this range, indicating that Lipoprime 50T lipase was tolerant to methanol presence within this range and maintained its activity when the water content was 26%. Considering the substantial operational stability of lipase, a RO to methanol molar ratio of 1 to 16 was utilized in the rest of the study in order to obtain higher ME yields.

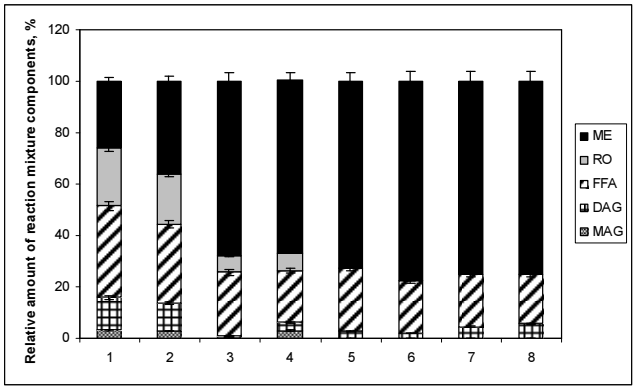

Fig. 7. Effect of rapeseed oil:methanol molar ratio on Lipoprime 50T-catalyzed rapeseed oil transesterification (methanolysis) in n-hexane. Reaction was performed at 40 °C with 44 mg/ml enzyme, the water content of 26% (v/v) and reaction time 24 h. RO : methanol molar ratio: 1 – 1:2; 2 – 1:4; 3 – 1:6; 4 – 1:8; 5 – 1:10; 6 – 1:12; 7 – 1:14; 8 – 1:16. Symbols are the same as in Fig. 1

2.5. Effect of lipase dosage

Considering earlier determined optimal conditions for the reaction the effects of Lipoprime 50T lipase quantity on transesterification (methanolysis) of rapeseed oil for biodiesel production with n-hexane used as a solvent has been evaluated. Fig. 8 depicts the effect of lipase dosage on rapeseed oil transesterification (methanolysis) at 40 °C at rapeseed oil and methanol molar ratio of 1:16 at water content of 26% (v/v).

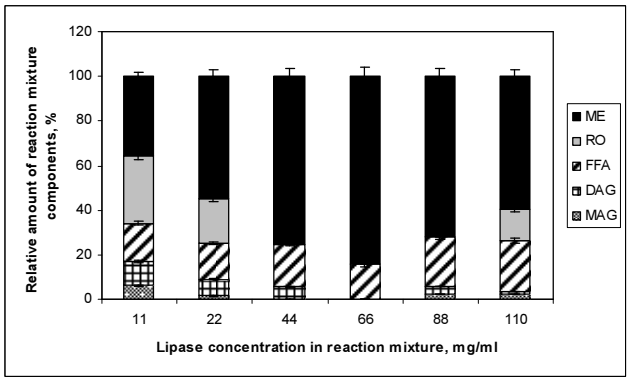

Fig. 8. Effect of enzyme amount on Lipoprime 50T-catalyzed rapeseed oil transesterification (methanolysis) in n-hexane. Reaction was performed at 40 °C with 44 mg/ml enzyme, water content of 26% and RO to methanol molar ratio 1:16 and reaction time 24 h. Symbols are the same as in Fig. 1

It was found that lower dosage of enzyme led to lower ME yield. The ME yield increased with increasing the lipase dosage from 11 to 66 mg/ml. When the lipase dosage reached 66 mg/ml, the ME yield of around 85% was obtained within 24 h. However, further increase in lipase dosage above 66 mg/ml was not capable of enhancing the ME yield, possibly owing to some enzymatic deactivation because of the formation of enzyme excess agglomerates. In enzyme-catalyzed transesterification (methanolysis), the optimal lipase and its usage concentration should be selected in terms of optimal concentration rather than high conversion conditions, as a consequence of the significant cost associated with higher enzyme amounts. Therefore, Lipoprime 50T lipase at 44 mg/ml of reaction volume could be defined as the optimal parameter for the reaction under studied conditions as the yield of ME was about 75% in this case. However, when the lipase dosage reached 66 mg/ml, the reaction mixture consisted only of ME and FFA. Taking into account the fact that a certain amount of FFA is permissible as an impurity of biodiesel, these results are really promising for the production of biodiesel in the future.

Conclusions

1. The ability of commercial lipolytic enzyme Lipoprime 50T to catalyze the biotechnologically important process of environment-friendly biodiesel production by rapeseed oil transesterification (methanolysis) was studied, the optimal process conditions were determined and the effects of water content and different organic solvent were analyzed. The reaction manner was investigated to be dramatically different in studied organic solvents. It was shown that the hydrolytic activity directly depends on the quantity of *t*-butanol in the reaction mixture and the water content required for high yields of the product increases with increasing the reaction medium hydrophobicity. When the water content is large enough the problem of the enzyme inactivation by the methanol excess can be removed in n-hexane.

2. Under the optimal conditions identified (n-hexane, 40 °C temperature, 66 mg/ml enzyme, 26% water content, rapeseed oil to methanol molar ratio 1:16 and reaction time 24 h) the highest biodiesel conversion yield of around 85% was obtained. These results indicate a great potential of producing biodiesel during Lipoprime 50 T lipase-catalyzed methanolysis under relatively mild and environment-friendly conditions.

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