Taylor & Francis
Taylor & Francis Group

JOURNAL OF ENVIRONMENTAL ENGINEERING AND LANDSCAPE MANAGEMENT
ISSN 1648–6897 print/ISSN 1822-4199 online
2011 Volume 19(2): 123–129
doi:10.3846/16486897.2011.579448

PRODUCTION OF ENVIRONMENTALLY FRIENDLY BIODIESEL BY ENZYMATIC OIL TRANSESTERIFICATION

Vida Bendikienė[1], Vita Kiriliauskaitė[2], Benediktas Juodka[3]

Dept of Biochemistry and Biophysics, Vilnius University,
M. K. Čiurlionio g. 21, LT-03101 Vilnius, Lithuania
E-mail: [1]vida.bendikiene@gf.vu.lt (corresponding author)

Submitted 16 Nov. 2010; accepted 31 Jan. 2011

Abstract. In this study, the ability of commercial lipolytic enzyme Lipoprime 50T to catalyze the biotechnologically and environmentally important processes of rapeseed oil hydrolysis and transesterification were investigated and the optimal process conditions were determined using simple and accurate thin layer chromatographic, titrimetric and computer analysis (Micro image 4.0) methods.

Lipoprime 50T lipase-catalyzed transesterification of rapeseed oil with several alcohols for fatty acid alkyl esters (biodiesel) production in n-hexane – a common solvent for lipolytic reactions – and in *t*-butanol – a novel promising solvent for biodiesel production – was investigated. The effects of molar ratio of substrates, reaction temperature and time on the constitution of the methanolysis reaction mixture were analyzed. Lipoprime 50T lipase-catalyzed rapeseed oil methanolysis in n-hexane was determined to be undesirably slow and inefficient process, whereas the lipolytic potential of the studied enzyme was determined to be very high in *t*-butanol (no rapeseed oil left after 1.5 h from the beginning of the reaction under determined optimal reaction conditions).

Keywords: transesterification, methanolysis, biodiesel, lipase, rapeseed oil, *t*-butanol, thin layer chromatography.

1. Introduction

In recent years, attention of the use of alternative forms of energy has increased significantly. Various forms of energy sources are investigated including biodiesel, biolubricants, biogas and *etc.* As one of the biggest environmental problems in Lithuania is large amounts of generated wastes, biogas production from fatty and other organic wastes is widely discussed in literature (Baltrėnas *et al.* 2004; Baltrėnas, Kvasauskas 2008a, b). Other experimental investigations of the use of organic wastes are described in literature as well (Baltrėnas *et al.* 2005; Baltrėnas, Kvasauskas 2009). Biodiesel production from waste cooking oils and other feedstock has become more attractive due to some environmental benefits such as non-toxicity, environmental acceptability, safety to handle and low emission profiles as compared to petroleum-based diesel (Al-Zuhair 2007; Ganesan *et al*. 2009). Since the amount of CO_2 produced during its combustion is the same as absorbed by the vegetable species during their growth, the global balance of CO_2 emission is null, and consequently, a decrease in the greenhouse effect is achieved. Due to its favourable environmental features, biodiesel promises to be the fuel of tomorrow (Al-Zuhair 2007). Different processes are currently available to achieve transesterification of various oils with different alcohols for biodiesel production, which include chemical and enzymatic catalysis (Meka *et al.* 2007). Although biodiesel can be successfully produced by chemical methods in the presence of an acid or an alkaline catalyst, there are several environmental problems associated with this kind of production, such as an excessive energy requirements, difficulties in glycerol recovery, removal of catalyst and undesirable side reactions. Moreover, it is estimated that the amount of wastewater from a traditional biodiesel plant is around 0.2 ton per ton biodiesel produced (Suehara *et al.* 2005). It is a severe problem both from an energy consumption and environmental points of view (Fjerbaek *et al.* 2009). Enzymatic methods involving lipases can be an excellent environmentally friendly alternative to produce biodiesel. Enzymatic processes offer milder reaction conditions as they do not promote secondary reactions, thereby reducing the number of purification steps, and the presence of the enzyme in the glycerol phase can even increase its value as an animal feed (Agarwal, Das 2001; Srivastava, Prasad 2000; Vincente *et al.* 1998). However, this approach has not been used in industrial production due to the high price of lipases, short operational life and low stability of enzyme in the presence of the excess short chain alcohols (Salis *et al.* 2008). One solution of these problems is the selection of a suitable reaction medium, which can preserve both the catalytic activity and the stability of the enzyme during the synthetic process (Al-Zuhair 2007).

It has been reported that the organic solvents can induce significant changes in enzyme activity and specificity by simply changing the physicochemical properties of the reaction medium such as the polarity and hydrophobicity (Lu *et al.* 2008; Türkan, Kalay 2008). *tert*-Butyl alcohol (*t*-butanol, 2-methyl-2-propanol) has been repor-

ted to be a good solvent for biodiesel production due to its ability to eliminate negative effects of excess short chain alcohols on lipases. *t*-Butanol can keep a high catalytic activity of lipase because of its steric hindrance effect. Moreover, both short chain alcohol and glycerol can be dissolved in this organic solvent (Li *et al.* 2006; Royn *et al.* 2007; Wang *et al.* 2006). Thus, it was expected that with *t*-butanol as reaction medium, the negative effect caused by insoluble short chain alcohol and glycerol could be eliminated and enzyme could keep both high catalytic activity and stability.

In the enzymatic process employed for the production of biodiesel from oils, not only the reaction solvent utilized but some other parameters were shown to influence both yield and the rate of the reaction. These parameters include the reaction temperature, the type and concentration of the alcohol, the quantity of the enzyme in the reaction mixture, the water content and mixing rate.

In this study, Lipoprime 50T lipase-catalyzed rapeseed oil hydrolysis and transesterification with different alcohols reactions in n-hexane – a common solvent for lipolytic reactions – and in *t*-butanol – a novel promising solvent for biodiesel production – were investigated. The efficacy of a transesterification process was evaluated using simple and accurate thin layer chromatographic method as the main technology, which allowed a cheap and rapid product analysis during the reaction.

2. Investigation methods

Lipoprime 50T was kindly provided by JSC Biopolis, representative company of Novo Nordisk in Lithuania. All chemicals used in the study were products of guaranteed grade. Silica gel G-25 plates for thin layer chromatography (TLC) were purchased from Merck.

The standard spectrophotometric assay of hydrolytic activity

Hydrolytic activity of lipase upon *p*-nitrophenyl butyrate solution in 2-propanol (Hernaiz *et al.* 1997; Ryu *et al.* 2006) was investigated measuring the change of optical density at 400–410 nm during 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) (Bendikienė *et al.* 2004; Surinėnaitė *et al.* 2002). One unit of lipase hydrolytic activity corresponds to the amount of the enzyme releasing 1 μmol of *p*-nitrophenol per minute under standard conditions.

Reaction system

The transesterification reactions between RO and alcohol were conducted in closed 20 ml batch reactors, with constant stirring, coupled to condensers in order to avoid alcohol loss by volatilization. The water circulating in the condenser was cooled by a thermostatic bath. Reaction progress was monitored by taking duplicate samples of reaction mixture at definite time intervals. For the analysis by TLC method samples were diluted with diethyl ether (v/v ratio 1:1), mixed vigorously and kept at –20 °C until the chromatographic analysis was carried out (Bendikienė *et al.* 2005).

Transesterification reaction

The reaction medium consisted of a mixture of RO, alcohol and enzyme. The alkyl esters synthesis was evaluated as function of type of solvents used (n-hexane or *t*-butanol), type of alcohol used (methanol, 1-butanol, 1-hexanol, 1-octanol or ethane-1,2-diol (ethylene glycol), temperature (30, 40 and 50 °C), alcohol/RO molar ratio (1:4, unless specified otherwise) and in respect to stepwise addition of alcohol (single addition or two or three consecutive additions at different times), (Bernardes *et al.* 2007; Jeong, Park 2008).

Titrimetric assay

The determination of liberated free fatty acids (FFA) in the reaction mixture were carried out by taking samples at definite time intervals and titrating them with sodium hydroxide solution in methanol (50 mM) to the phenolphthalein end point (Hoppe, Theimer 1996; Lim *et al.* 2001).

Chromatographic analysis

The products of RO hydrolysis and transesterification were analyzed by TLC method (Yadav *et al.* 1998) on TLC plates (5×10 cm and 10×10 cm) pre-coated with 0.25 mm Silica Gel 60 (Merck).The samples were applied to the marked start edge of the TLC plate (1.0 cm height from lower edge of the plate) using the specified TLC–Hamilton syringe. The sample volumes for all experiments were 2 μl. The plate was then allowed to be air-dried for 10–15 min before transferring to the TLC tank for development. Chromatograms developments were conducted with solvent system (mobile system) of light petroleum (b.p. 40–60 °C): diethyl ether: acetic acid (85:15:2, v/v) for transesterification reaction and (80:20:2, v/v) for hydrolysis reaction products analysis (Bendikienė *et al.* 2005). The tank with the poured mobile system was covered with a lid and pre-saturated with mobile system vapour for at least 20–30 min at room temperature before use. The sample – loaded TLC plate – was transferred to the TLC tank and then developed for no less than a 4 cm (for 5×5 cm TLC plate) and 8 cm (for 5×10 cm TLC plate) migration distance of the solvent from the start line. The developed TLC plates were air-dried for about 10–15 min. Visualization of spots was developed by using a saturated iodine chamber and spots were identified with reference to standards. Pure methyl oleate, free fatty acid, triacylglycerols (TAG), diacylglycerols (DAG), monoacylglycerols (MAG) and RO solutions in diethyl ether were used as standards.

Quantitative analysis (%) of reaction products separated by TLC (average of 3 assays) was performed using the Micro image 4.0 programme considering the spot area and colour intensity (Bendikienė *et al.* 2008a, b).

3. Investigation results

The effect of the type of alcohol used on the course of RO transesterification reaction in n-hexane was investigated. Five different chain length alcohols were used: methanol, 1-butanol, 1-hexanol, 1-octanol and ethylene glycol (Fig. 1).

The highest yield of FFA was determined for the hexanolysis and octanolysis reactions. However, more

esters were determined for the reactions with methanol and 1-butanol and no synthetic potential was observed in the case of ethylene glycol (Fig. 1). In contrast, Abigor *et al.* (2000) reported PS30 lipase-catalyzed transesterification reaction of some Nigerian lauric oils with methanol as the least effective process in comparison to all their studied alcohols. Since the desirable product was alkyl ester, methanol and 1-butanol were chosen for further experiments.

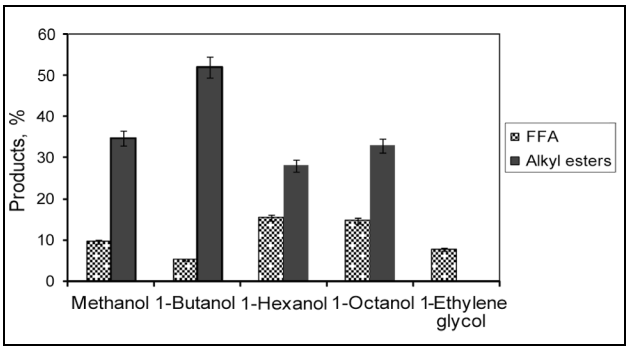

Fig. 1. The effect of type of alcohol used on alkyl esters yield (quantitative analysis, %) and free fatty acids amount (titrimetric results, %) released after 96 h during Lipoprime 50T-catalyzed transesterification of rapeseed oil. Reaction was performed in n-hexane at 30 °C at rapeseed oil and alcohol molar ratio 1:4

In the process of biodiesel production, reaction time is found to be a significant operating parameter, which is closely associated with energy cost from the economic perspective. As it was shown in Fig. 1, the transesterification reaction in n-hexane is undesirably slow and clearly an inefficient process, presumably because of the short chain alcohol inhibitory effect on lipase (Shimada *et al.* 1999; Watanabe *et al.* 2000). It was shown that this problem could be removed by using *t*-butanol as a reaction solvent. Türkan and Kalay (2008) demonstrated that *t*-butanol caused the changes of lipase-catalyzed sunflower oil methanolysis mechanism probably by causing conformational changes of the enzyme structure, that eliminated inhibitory effect of excess methanol. Thus, further experiments were carried out in *t*-butanol instead of n-hexane as a reaction medium (Fig. 2).

Several studies of the minimization of alcohol inactivation have been reported and one of the solutions was a stepwise addition of alcohols, which was introduced and successively performed by Shimada *et al.* (1999). In our study, only a negligible decrease of diacylglycerols yield was observed after the addition of extra portions of alcohols to the reaction mixture (columns 2–4 and 6–8 in Fig. 2). It was determined that the reaction under studied conditions was more effective in the case of methanol (less rapeseed oil left after 1 h from the beginning of the reaction) in comparison to 1-butanol. Furthermore, alcohol used for enzymatic biodiesel production must be cheap and available in large quantities as is methanol (Fjerbaek *et al.* 2009). Due to these results more detailed analysis of RO methanolysis in different solvents were carried out.

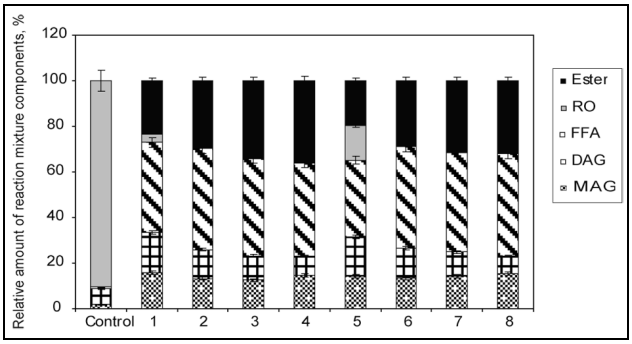

Fig. 2. The effect of reaction time and type of alcohol used (methanol, columns 1–4, 1-butanol, columns 5–8) on Lipoprime 50T-catalyzed rapeseed oil transesterification effectiveness. Control – RO. Quantitative analysis (%) was performed using the Micro image 4.0 programme. Reaction was performed in *t*-butanol at 37 °C after: 1 h – column 1; 15 h – column 2; 1 h after addition of extra amount of methanol – column 3; 11 h after addition of extra amount of methanol – column 4; 1 h – column 5; 15 h – column 6; 1 h after addition of extra amount of 1-butanol – column 7; 11 h after addition of extra amount of 1-butanol –column 8.
Esters – alkyl esters of FA; RO – rapeseed oil; FFA – free fatty acids; DAG – diacylglycerols; MAG –monoacylglycerols. (The contents of MAG and DAG are expressed as the sum of their regioisomers)

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 (Du *et al.* 2007). Lu *et al.* (2008) tested twelve different solvents for transesterification of TO with methanol using immobilized lipase *Candida* sp. 99–125. The general trend is that there is no correlation between yield or conversion with solvents hydrophobicity (log P), dielectric constant (ε) and Hildebrant solubility parameter (δ). The catalytic activity of this immobilized lipase and the product yield are dependent on the different solvents. It was shown that additional water causes the decrease of both, overall conversion and the favourable product yield in the case of the reactions in polar solvents, and the opposite effect was noticed for the processes in non-polar solvents. It should be noted that Lu *et al.* (2008) determined *t*-butanol as an exception as it could keep high conversion of around 96%. These results support wide application of *t*-butanol as a very useful solvent, since it is only moderately polar and is able to stabilize the enzyme, if any; it seems not to be influenced by the polarity of other solvents such as water or some polar components of the reaction mixture like methanol or glycerol.

To optimize reaction conditions for enzymatic methanolysis of RO, two solvents with different log P value (0.79 for *t*-butanol and 3.5 for n-hexane) were compared (Figs 3, 4).

As expected, the highest methyl esters (ME) yield 45% was obtained in n-hexane although the lipase expressed the poor stability (formed agglomerates, which caused the decrease of reaction rate) in this organic medium. Although after 120 h the yield of ME was lower in

t-butanol in comparison to n-hexane, but the hydrolysis of RO process was very fast in this case (no rapeseed oil left after 24 h from the beginning of the reaction).

Another important variable affecting the yield of biodiesel is the molar ratio of oil to alcohol. In order to shift the methanolysis reaction in toward direction, it is necessary to use an excess amount of methanol as methanolysis is a reversible reaction. Using more than 3–4 moles alcohol per mole triglyceride added in one step favours more alcohol tolerant enzymes as in many studies lipases-catalyzed biodiesel production increases with increasing methanol-to-oil ratio of 3:1 and then decreases because of the lipase inactivation by insoluble excess short chain alcohol, which exist as droplets in the reaction mixture (Ganesan *et al.* 2009). One of the solutions to avoid this inactivation is to use a suitable organic solvent as a reaction medium.

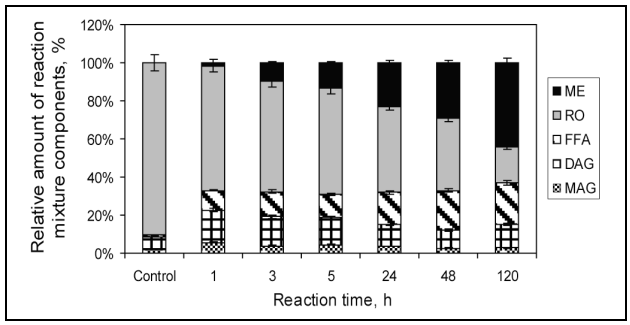

Fig. 3. Effect of reaction time on Lipoprime 50T-catalyzed rapeseed oil methanolysis with 1:4 rapeseed oil to methanol molar ratio at 30 °C in n- hexane. Control – RO. Symbols are the same as in Fig. 2

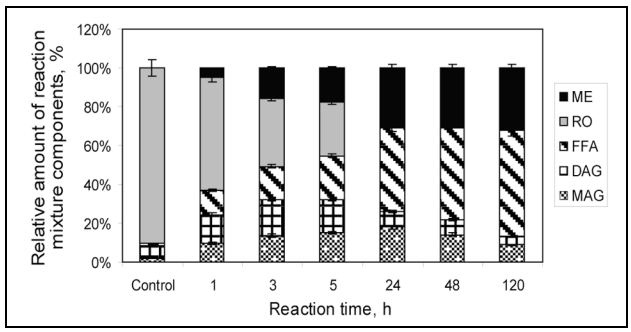

Fig. 4. The effect of reaction time on Lipoprime 50T-catalyzed rapeseed oil methanolysis with 1:4 rapeseed oil to methanol molar ratio at 30 °C in *t*-butanol. Control – RO. Symbols are the same as in Fig. 2

The molar ratios of oil-to-methanol ranged from 1:2 to 1:8 were studied to evaluate the effect of methanol on lipase activity in *t*-butanol. The effect of temperature (30, 40 and 50 °C) on the ME yield was also investigated (Fig. 6). The chromatographic view of control samples is shown in Fig. 5.

As shown in Fig. 6, when the molar ratio of oil-to-methanol was in range of 1:6–1:8, high conversions were achieved and no significant differences were detected with different oil-to-methanol molar ratios within this

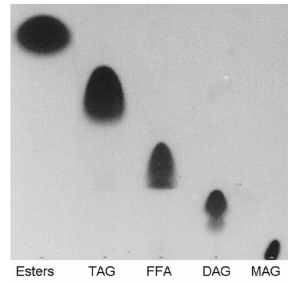

Fig. 5. Thin layer chromatographic view of control samples (TAG – triacylglycerols, FFA – free fatty acids, DAG – diacylglycerols, MAG – monoacylglycerols)

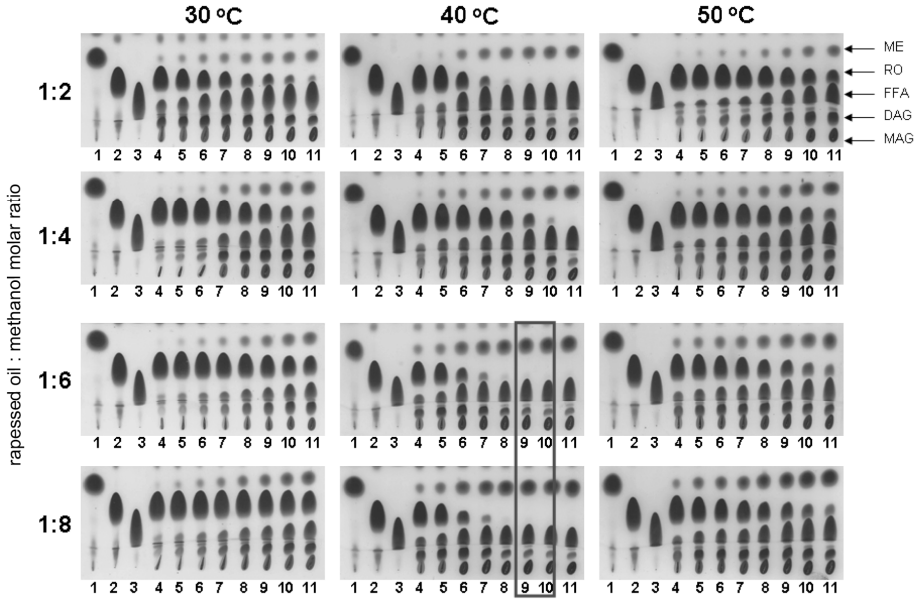

Fig. 6. Thin layer chromatogram of effect of molar ratio of substrates, reaction temperature and time on reaction products of Lipoprime 50T-catalyzed rapeseed oil methanolysis in *t*-butanol. Visualization was by iodine vapour. Identification was by comparison with standards (lane 1 – methyl oleate, lane 2 – rapeseed oil, lane 3 – oleic acid). Solvent system: 85:15:2. The chromatographic view of reaction products after: 15 min – lane 4; 30 min – lane 5; 45 min – lane 6; 1 h – lane 7; 1.5 h – lane 8; 2 h – lane 9; 3 h – lane 10; 4 h – lane 11

range, indicating that under studied conditions, Lipoprime 50T lipase was tolerant to methanol presence within this range and maintained its activity and operational stability. Such high optimal molar ratio of substrates has been reported in other studies. Royon *et al.* (2007) found that the addition of *t*-butanol to the transesterification reaction mixture with the 1:6 oil-to-methanol molar ratio, which normally irreversibly inhibits lipase, resulted in a 90% yield of the product after 10 h from the beginning of the reaction. Noureddini *et al.* (2005) reported the optimal molar ratio of soybean oil to methanol to be 1:8.2 for *P. cepacia* lipase-catalyzed transesterification reaction.

40 °C temperature was determined to be an optimal patrameter for Lipoprime 50T-catalyzed RO methanolysis reaction. Above 40 °C temperature, the process effectiveness decreased, possibly owing to some enzymatic deactivation.

Considering these results, the reaction temperature of 40 °C, the molar ratio of RO to methanol 1:6–1:8 and the reaction duration of 1.5 h were selected as the optimal reaction conditions for the process in *t*-butanol.

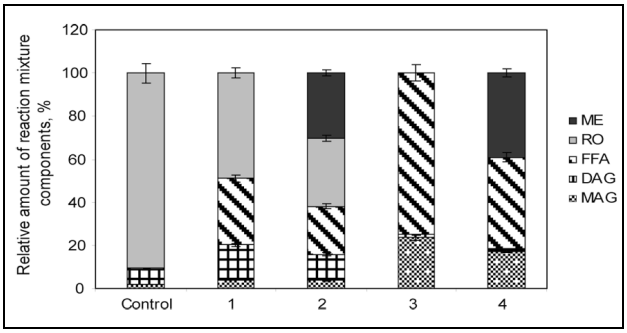

Fig. 7. Lipoprime 50T-catalyzed hydrolysis (columns 1 and 3) and methanolysis (columns 2 and 4) of rapeseed oil in n-hexane (columns 1 and 2) and in *t*-butanol (columns 3 and 4). Reactions were performed at 40 °C with oil-to-methanol molar ratio of 1:6, reaction time 120 h. Quantitative analysis (%) was performed using the Micro image 4.0 programme. Control – RO. Symbols are the same as in Fig. 2

Determined optimal reaction conditions for RO methanolysis in *t*-butanol were used in order to compare the effectiveness of RO hydrolysis and methanolysis in both studied organic solvents (Fig. 7).

As shown in Fig. 7, it is clear that under studied conditions, both the RO hydrolysis and transesterification reactions are more effective in *t*-butanol in comparison to n-hexane. Furthermore, in contrast to results discussed earlier, the ME yields obtained remain higher in *t*-butanol in comparison to n-hexane even after a longer period of time (120 h).

4. Conclusions

1. Thin layer chromatography method was shown to be a promising technology for rapid and cheap optimization of environmentally acceptable biodiesel production.

2. The higher lipolytic activity was determined for transesterification with longer chain alcohols, whereas the higher synthetic potential of Lipoprime 50T lipase was observed for the shorter chain alcohols. The overall process was determined to be the most effective in the case of methanol.

3. Lipoprime 50 T lipase-catalyzed methanolysis in n-hexane was determined to be undesirably slow and inefficient process whereas the lipolytic potential of the studied enzyme was determined to be very high in *t*-butanol. Under determined optimal reaction conditions in *t*-butanol all rapeseed oil was converted to the products after about 1.5 h.

Acknowledgments

We would like to express our gratitude to the Lithuanian State Science and Studies Foundation (Contract No. N-01/2009) for the financial support that made this work possible and to Biopolis Ltd, distributor of Novozymes A/S in Lithuania, for the supply of the enzyme.

References

Abigor, R. D.; Uadia, P. O.; Foglia, T. A.; Haas, M. J.; Jones, K. C.; Okpefa, E.; Obibuzor, J. U.; Bafor, M. E. 2000. Lipase-catalyzed production of biodiesel fuel from some Nigerian lauric oils, *Biochem. Soc. Trans.* 28(6): 979–981. doi:10.1042/BST0280979

Agarwal, A. K.; Das, L. M. 2001. Biodiesel development and characterisation for use as a fuel in compression ignition engines, *J. Eng. Gas Turbines Power* 123: 440–447. doi:10.1115/1.1364522

Al-Zuhair, S. 2007. Production of biodiesel: possibilities and challenges, *Biofuels Bioprod. Biorefin.* 1: 57–66. doi:10.1002/bbb.2

Baltrėnas, P.; Jankaitė, A.; Raistenskis, E. 2005. Natūralių biodegradacijos procesų, vykstančių maisto atliekose, eksperimentiniai tyrimai [Experimental investigation of biodegradation process in food waste], *Journal of Environmental Engineering and Landscape Management* 13(4): 167–177.

Baltrėnas, P.; Kvasauskas, M. 2008a. Anaerobic recycling of organic waste and recovery of biogas, *Ecology* 54: 57–63.

Baltrėnas, P.; Kvasauskas, M. 2008b. Experimental investigation of biogas production using fatty waste, *Journal of Environmental Engineering and Landscape Management* 16(4): 178–187. doi:10.3846/1648-6897.2008.16.178-187

Baltrėnas, P.; Kvasauskas, M. 2009. Research on anaerobically treated organic waste suitability for soil fertilisation, *Journal of Environmental Engineering and Landscape Management* 17(4): 205–211. doi:10.3846/1648-6897.2009.17.205-211

Baltrėnas, P.; Raistenskis, E.; Zigmontienė, A. 2004. Organinių atliekų biodestrukcijos proceso metu išsiskiriančių biodujų eksperimentiniai tyrimai [Experimental investigation of biogas emissions during organic waste biodegradation processes], *Journal of Environmental Engineering and Landscape Management* 12(1 priedas): 3–9.

Bendikienė, V.; Juodka, B.; Surinėnaitė, B.; Dienys, G. 2008a. Lipases in conversion of oils. Experimental screening of enzymes and substrates for biodiesel production, *Biologija* 4: 247-252.

Bendikienė, V.; Šinkūnienė, D.; Kiriliauskaitė, V.; Juodka, B. 2008b. Properties and immobilization of *Enterobacter aerogenes* 13 lipase, *Rom. Biotechnol. Lett.* 13(5) Suppl. 1: 1–8.

Bendikienė, V.; Surinėnaitė, B.; Juodka, B.; Bachmatova, I.; Marcinkevičienė, L. 2005. The specificity of *Pseudomonas mendocina* 3121-1 lipase, *Biologija* 1: 27–30.

Bendikienė, V.; Surinėnaitė, B.; Juodka, B.; Safarikova, M. 2004. Insights into catalytic action mechanism of *Pseudomonas mendocina* 3121-1 lipase, *Enzyme Microb. Technol.* 34: 572–577. doi:10.1016/j.enzmictec.2004.01.006

Bernardes, O. L.; Bevilaqua, J. V.; Leal, M. C. M. R.; Freire, D. M. G.; Langone, M. A. P. 2007. Biodiesel fuel production by the transesterification reaction of soybean oil using immobilized lipase, *Appl. Biochem. Biotechnol.* 136–140: 105–114. doi:10.1007/s12010-007-9043-5

Du, W.; Wang, L.; Liu, D. 2007. Improved methanol tolerance during Novozym 435-mediated methanolysis of SODD for biodiesel production, *Green Chem*. 9: 173–176. doi:10.1039/b613704k

Fjerbaek, L.; Christensen, K. V.; Norddahl, B. 2009. A review of the current state of biodiesel production using enzymatic transesterification, *Biotechnol. Bioeng.* 102(5): 1298–1314. doi:10.1002/bit.22256

Ganesan, D.; Rajendran, A.; Thangavelu, V. 2009. An overview on the recent advances in the transesterification of vegetable oils for biodiesel production using chemical and biocatalysis, *Rev. Environ. Sci. Biotechnol.* 8: 367–394. doi:10.1007/s11157-009-9176-9

Hernaiz, M. J.; Sanchez-Montero, J. M.; Sinisterra, J. V. 1997. Influence of the nature of modifier in the enzymatic activity of chemical modified semipurified lipase from *Candida rugosa*, *Biotechnol. Bioeng.* 55: 252–260. doi:10.1002/(SICI)1097-0290(19970720)55:2<252::AID-BIT2>3.0.CO;2-H

Hoppe, A.; Theimer, R. R. 1996. Titrimetric test for lipase activity using stabilized triolein emulsions, *Phytochemistry* 42: 973–978. doi:10.1016/0031-9422(96)00051-9

Jeong, G-T.; Park, D-H. 2008. Lipase-catalyzed transesterification of rapeseed oil for biodiesel production with *tert*-butanol, *Appl. Biochem. Biotechnol.* 148: 131–139. doi:10.1007/s12010-007-8050-x

Li, L.; Du, W.; Liu, D.; Wang, L.; Li, Z. 2006. Lipase-catalyzed transesterification of rapeseed oils for biodiesel production with a novel organic solvent as the reaction medium, *J. Mol. Catal. B: Enzym.* 43: 58-62. doi:10.1016/j.molcatb.2006.06.012

Lim, L. P.; Ghazali, H. M.; Lai, O. M.; Chu, B. S. 2001. Comparison of transesterified palm stearin/sunflower oil blends catalyzed by *Pseudomonas* and *Mucor javanicus* lipases, *J. Food Lipids* 8: 103–114. doi:10.1111/j.1745-4522.2001.tb00188.x

Lu, J.; Nie, K.; Wang, F.; Tan, T. 2008. Immobilized lipase *Candida* sp 99-125 catalyzed methanolysis of glycerol trioleate: Solvent effect, *Bioresour. Technol.* 99: 6070–6074. doi:10.1016/j.biortech.2007.12.045

Meka, P. K.; Tripathi, V.; Singh, R. P. 2007. Synthesis of biodiesel fuel from safflower oil using various reaction parameters, *J. Oleo Sci.* 56: 9–12.

Noureddini, H.; Gao, X.; Philkana, R. S. 2005. Immobilized *Pseudomonas cepacia* lipase for biodiesel fuel production from soybean oil, *Bioresour. Technol.* 96(7): 769–777. doi:10.1016/j.biortech.2004.05.029

Royn, D.; Daz, M.; Ellenrieder, G.; Locatelli, S. 2007. Enzymatic production of biodiesel from cotton seed oil using t-butanol as a solvent, *Bioresour. Technol.* 98: 648–653. doi:10.1016/j.biortech.2006.02.021

Ryu, H. S.; Kim, H. K.; Choi, W. C.; Kim, M. H.; Park, S. Y.; Han, N. S.; Oh, T. K.; Lee, J. K. 2006. New cold-adapted lipase from *Photobacterium lipolyticum* sp. nov. that is closely related to filamentous fungal lipases, *Appl. Microbiol. Biotechnol.* 70: 321–326. doi:10.1007/s00253-005-0058-y

Salis, A.; Pinna, M.; Monduzzi, M.; Solinas, V. 2008. Comparison among immobilised lipases on macroporous polypropylene toward biodiesel synthesis, *J. Mol. Catal. B: Enzym.* 54: 19–26. doi:10.1016/j.molcatb.2007.12.006

Shimada, Y.; Watanabe, Y.; Samukawa, T.; Sugihara, A.; Noda, H.; Fukuda, H.; Tominaga, Y. 1999. Convertion of vegetable oil to biodiesel using immobilized *Candida antarctica* lipase, *J. Am. Oil Chem. Soc.* 76: 789–793. doi:10.1007/s11746-999-0067-6

Srivastava, A.; Prasad, R. 2000. Triglycerides-based diesel fuels, *Renew. Sustain. Energy Rev.* 4: 111–133. doi:10.1016/S1364-0321(99)00013-1

Suehara, K.; Kawamoto, Y.; Fujii, E.; Kohda, J.; Nakano, Y.; Yano, T. 2005. Biological treatment of wastewater discharged from biodiesel fuel production plant with alkali-catalyzed transesterification, *J. Biosci. Bioeng.* 100(4): 437–442. doi:10.1263/jbb.100.437

Surinėnaitė, B.; Bendikienė, V.; Juodka, B.; Bachmatova, I.; Marcinkevičienė, L. 2002. Characterization and physicochemical properties of a lipase from *Ps. mendocina* 3121-1, *Biotechnol. Appl. Biochem.* 36: 47–55. doi:10.1042/BA20020013

Türkan, A.; Kalay, Ş. 2008. Study of the mechanism of lipase-catalyzed methanolysis of sunflower oil in *tert*-butanol and heptane, *Turk. J. Biochem.* 33: 45–49.

Vincente, G.; Coteron, A.; Martinez, M.; Aracil, J. 1998. Application of factorial design of experiments and response surface methodology to optimize biodiesel production, *Ind. Crops Prod.* 8: 29–35. doi:10.1016/S0926-6690(97)10003-6

Wang, L.; Du, W.; Liu, D.; Li, L.; Dai, N. 2006. Lipase-catalyzed biodiesel production from soybean oil deodorizer distillate with absorbent present in tert-butanol system, *J. Mol. Catal. B: Enzym.* 43: 29–32. doi:10.1016/j.molcatb.2006.03.005

Watanabe, Y.; Shimada, Y.; Sugihara, A.; Noda, H.; Fukuda, H.; Tominaga, Y. 2000. Continuous production of biodiesel fuel fuel from vegetable oil using immobilized *Candida antarctica* lipase, *J. Am. Oil. Chem. Soc.* 77: 355–360. doi:10.1007/s11746-000-0058-9

Yadav, R. P.; Saxena, R. K.; Gupta, R.; Davidson, W. S. 1998. Purification and characterization of a regiospecific lipase from *Aspergillus terreus*, *Biotechnol. Appl. Biochem.* 28: 243–249.

NEŽALINGO APLINKAI BIODYZELINO GAMYBOS FERMENTINIO ALIEJAUS PERESTERINIMO BŪDU OPTIMIZAVIMO TYRIMAS

V. Bendikienė, V. Kiriliauskaitė, B. Juodka

Santrauka

Tirta komercinio preparato *Lipoprime* 50*T*, kuriam būdingas lipolizinis aktyvumas, geba katalizuoti biotechnologiniu ir aplinkos apsaugos požiūriu svarbias rapsų aliejaus peresterinimo bei hidrolizės reakcijas. Optimalios vykdytų procesų sąlygos nustatytos tiksliu bei paprastu plonasluoksnės chromatografijos, titrimetrijos ir kompiuterinės analizės (*Micro image* 4.0) metodais. Optimizuotas biodyzelino – riebalų rūgščių esterių – gavimo procesas bei įvertinta skirtingų alkoholių įtaka *Lipoprime* 50*T* lipazės katalizuojamo rapsų aliejaus peresterinimo efektyvumui dviejuose skirtinguose tirpikliuose – n-heksane (įprastinis lipolizės reakcijų tirpiklis) bei tret-butanolyje (naujas perspektyvus tirpiklis biodyzelino sintezės reakcijoms vykdyti). Ištirta molinio substratų santykio, reakcijos temperatūros bei trukmės įtaka rapsų aliejaus metanolizės veiksmingumui tret-butanolyje. Nustatyta, kad *Lipoprime* 50*T* lipazės katalizuojama rapsų aliejaus metanolizė n-heksane yra lėtas ir ilgai trunkantis procesas, o tret-butanolyje tirti procesai yra ypač spartūs ir efektyvūs (visas rapsų aliejus nustatytomis optimaliomis sąlygomis sureaguoja per apytikriai 1,5 val.).

Reikšminiai žodžiai: peresterinimas, metanolizė, biodyzelinas, lipazė, rapsų aliejus, tret-butanolis, plonasluoksnė chromatografija.

ПОЛУЧЕНИЕ ЭКОЛОГИЧНОГО БИОДИЗЕЛЯ ПУТЕМ ФЕРМЕНТАТИВНОЙ ПЕРЕЭТЕРИФИКАЦИИ МАСЛА

В. Бендикене, В. Кириляускайте, Б. Юодка

Резюме

Была исследована способность коммерческого препарата *Lipoprime 50T*, обладающего липолитической активностью, катализировать биотехнологически и экологически важные процессы гидролиза и переэтерификации рапсового масла с определением оптимальных для них условий доступным и точным методом тонкослойной хроматографии, титрованием свободных жирных кислот и методом компьютерного анализа данных с помощью программы *Micro image 4.0*. Получение биодизеля – эфиров жирных кислот – путем переэтерификации рапсового масла некоторыми спиртами было исследовано в обычном для липолитических реакций растворителе н-гексане и в более приемлемом для синтеза биодизеля растворителе трет-бутиловом спирте (т-бутаноле). Также было исследовано влияние молярного соотношения субстратов, температуры и времени на состав продуктов в реакционной смеси. Процесс метанолиза рапсового масла, катализируемый липазой *Lipoprime 50T*, оказался медленным и малоэффективным в н-гексане, тогда как в т-бутаноле потенциал липолитической активности ферментного препарата был высок (в оптимальных условиях спустя полтора часа от начала процесса в реакционной смеси не оставалось исходного рапсового масла).

Ключевые слова: переэтерификация, метанолиз, биодизель, липаза, рапсовое масло, т-бутанол, тонкослойная хроматография.

Vida BENDIKIENĖ. Dr Habilitation Procedure (HP), Chief Research Fellow of Dept Biochemistry and Biophysics, Vilnius University (VU).
Doctor of Science, Habilitation Procedure (biochemistry), VU, 2008. Doctor of Science (biochemistry), Byelorussian State University (Minsk, Byelorussia), 1980. Publications: author of 1 study-guide, over 40 research papers and 12 patents. Supervisor of PhD projects and diploma projects of B. Sc. and M. Sc. levels. Research interests: biotechnology, applied enzymology, enzymes-catalyzed biotechnologically important processes optimization methods.

Vita KIRILIAUSKAITĖ. PhD student (biochemistry). Dept of Biochemistry and Biophysics, Vilnius University (VU). Publications: co-author of 2 research papers and 4 extended theses of Lithuanian and international conferences. Research interests: biotechnology, enzymology, biodiesel, lipases, environmentally friendly enzymatic catalysis.

Benediktas JUODKA. Rector of Vilnius University (VU). Dr Habil 1981, Moscow State University, Russia; Head of the Department of Biochemistry and Biophysics, Vilnius University, 1971–2002; Docent (Associate Professor) of Biochemistry, Department of Biochemistry and Biophysics, VU, 1971; Professor of Biochemistry, Department of Biochemistry and Biophysics, VU, 1982; Prorector responsible for research of VU, 1991–2001; President of Lithuanian Academy of Sciences, 1992–2002; Adviser for Research and Education to Lithuania government 1993–1994. Member of Lithuanian Biochemical Society; Member of Scientific Council of Institute of Immunology; Member of Scientific Council of VU; Member of Council of World Lab., Lithuanian Division; Vice-president of the Committee on Ethics in Science and Technology (UNESCO, 1994–1996). Member of Lithuanian Academy of Sciences (1987); Foreign member of Latvian Academy of Sciences (1994); Foreign member (Atti della academia dei Georgofili, Italy 2003); Member of European Academy of Sciences and arts (1994); Member of European Academy of Arts, Science and Humanities (2002); Lithuanian State awards in Science (1985, 2003); Awarded Lithuanian Grand Duke Gediminas 4th degree Order (1999); T. Grothus award (2000); Commandeur Order of French Republic (2001); Commandeur Order of Polish Republic (2001); Order of Three Stars of Latvian Republic (2001); Medal of Academies of Sciences of the Baltic States for the merits of promoting collaboration of researchers of Estonia, Latvia and Lithuania (2001); Order of the Cross of the Terra Mariana of Estonian Republic 4th class (2004); Commandeur Order of the Belgian Crown (2006), Commandeur in de Orde van Oranje-Nassau (2008) (Netherlands). Publications: author of 2 monographs, 1 text-book, 1 patent, 3 reviews, over 90 research papers. Research interests: covalent nucleic acid and protein complexes and its chemical modelling; processing of pre-tRNR from eukaryotic cells; mechanism of action of RNA ligase; immobilization of enzymes upon ferromagnetic particles; enzymes-catalyzed biotechnologically important processes; molecular mechanisms of photodynamic oncotherapy.