BIOLOGIJA. 2008. Vol. 54. No. 4. P. 247–252
DOI: 10.2478/v10054-008-0051-3
© Lietuvos mokslų akademija, 2008
© Lietuvos mokslų akademijos leidykla, 2008

Lipases in conversion of oils. Experimental screening of enzymes and substrates for biodiesel production

Vida Bendikienė[1],

Benediktas Juodka[1],

Birutė Surinėnaitė[2]*,

Gervydas Dienys[2]

[1] *Vilnius University,
Department of Biochemistry
and Biophysics,
M. K. Čiurlionio 21,
LT-03101 Vilnius, Lithuania*

[2] *Institute of Biotechnology,
V. A. Graičiūno 8,
LT-02241 Vilnius, Lithuania*

Lipases of different origin were screened for substrates and different reactions from the practical approach to search for the optimal conditions for biodiesel component production. The commercial lipases under analysis were shown to be specific to *p*-nitrophenyl fatty acid esters of medium chain length, although Lipolase100 LEX also showed a relatively high hydrolytic activity on *p*-nitrophenyl palmitate. The soluble *Enterobacter aerogenes* lipase was shown also to be the most active when hydrolysing medium-chain-length *p*-nitrophenyl fatty acid esters. The specificity of immobilized commercial lipases did not change as compared with soluble analogues. The immobilized *Enterobacter aerogenes* lipase showed no significant differences of activity towards *p*-nitrophenyl butyrate and medium-chain *p*-nitrophenyl fatty acid esters versus the attached enzyme on polyurethane and chitosan and also showed the highest hydrolytic activity on *p*-nitrophenyl caprylate as for soluble lipase. *Enterobacter aerogenes* lipase-catalyzed esterification of oleic acid with oleoyl alcohol was a long-lasting process still in progress after 91 hours. The lipase showed the highest catalytic activity when esterifying oleic acid with ethanol and 1.2-ethanediol. The enzyme slightly esterified the acid with 1.3-propanediol and was almost inactive for esterification with 2-hydroxyethyl ether. *Enterobacter aerogenes* lipase more efficiently hydrolysed camelina oil than rapeseed oil, considering the former to be an alternative source to be converted into biodiesel components. Methanolysis of rapeseed oil catalyzed by the lipase adsorbed on chitin was also a long-lasting process still in progress during 124 hours. The optimal glycerol-tricaprylate-to-methanol ratio was 1 : 1 for methanolysis of the substrate catalysed by Resinase HT lipase immobilized on a polyurethane support. The lipase was completely depressed at the ratio 1 : 3.

Key words: biodiesel, transesterification, lipases, immobilized enzymes

INTRODUCTION

The need for alternative fuels is related to both the depleting sources of petroleum and petrodiesel and to unstable costs of traditional fuels, as well as to energy security. Alternative energy sources should be environment-friendly, biodegradable, renewable and of low toxicity. Biodiesel is regarded as one of the new energy sources and chemically consists of monoalkyl esters of fatty acids [1]. It can be produced converting vegetable and microalgal oils, animal fats, waste products of vegetable oil refinery or animal rendering, and used frying oils. The conventional method for biodiesel production involves chemical acid and base catalysis [2]. However, downstream processing costs, environmental problems associated with the processes, and byproducts recovery directed to a search of alternative production methods and alternative substrates. Enzymatic catalysis by lipases can be the most promising alternative to produce biodiesel through alcoholysis or transesterification reactions [3, 4]. The enzymatic approach is environmentally friendly and needs to be explored for industrial production of biodiesel. Furthermore, the possibility to use biodiesel as an additive to mineral diesel can result in a sulfur-free fuel with a higher-cetane number manufactured from a renewable resource. This has motivated the biomodification of traditional and novel oils to reduce environmental investments and import needs.

The use of immobilized lipases or immobilized whole cells for biodiesel production could lower the overall cost and enhance the process efficiency, providing specific conversion of certain oil components and a high yield of essential products [5, 6]. Nevertheless, a careful screening for lipolytic enzymes, for oils as substrates, for biocatalytic reaction conditions and for various factors is the crucial point to ensure a highly effective production of biodiesel components applicable in large-scale biotechnological processes.

The world-wide production of oils and fats is approximately 70 million tons per year, including more than 90% of used as edible products. Rapeseed oil naturally contains glucosinolates and a high percentage (up to 60%) of erucic acid [7]. A variety of rapeseed with reduced levels of both toxic substances was developed, and modified oil as the Canola ("Can.O. L-A.",

* Corresponding author. E-mail: birute@ibt.lt

Canadian Oilseed, Low-Acid) product was supplied to the market. Resources of rapeseed are limited; consequently, a number of other oils are reffered for biodiesel production, including the interest to alternative oil sources, particularly those of high harvest in local areas [8].

The aim of the present report is the screening for soluble and immobilized lipases to convert different oils by hydrolysis and transesterification under various conditions to optimize the production of biodiesel components.

MATERIALS AND METHODS

Materials

Lipases used: from *Enterobacter aerogenes* (provided by the Biocentras, Vilnius, Lithuania); Resinase HT, Lipolase 100 LEX, Lipozyme TL 100L (kindly provided by Biopolis, representative company of "Novo Nordisk" in Lithuania). *p*-Nitrophenyl acetate (*p*-NPA), *p*-nitrophenyl butyrate (*p*-NPB), *p*-nitrophenyl caprylate (*p*-NPC), *p*-nitrophenyl caprate (*p*-NPCa), *p*-nitrophenyl laurate (*p*-NPL), *p*-nitrophenyl myristate (*p*-NPM), *p*-nitrophenyl palmitate (*p*-NPP), Tris, EDTA, 2-mercaptoethanol, SDS, glycerol, glycine, oleic acid, oleyl alcohol, triacylglycerols, diolein, monoolein, methyl oleate, methyl linoleate, molecular markers, metallic iodine and Coomassie Brilliant Blue (CB-R-250) were from Sigma. Gum arabic was from Merck. Polyacrylamide and Bromphenol Blue were from Aldrich. Rapeseed oil was from a local manufacturer as a foodstuff. Camelina oil (*Camelina sativa*) was provided by the Lithuanian University of Agriculture (from experimental fields). Ethanol, propan-2-nol, n-hexane, petrol and diethyl ethers, acetic, boric, hydrochloric and phosphoric acids, and NaOH were from Lachema. Ethylene glycol, propylene glycol and diethylene glycol were kindly provided by Institute of Chemistry, Vilnius, Lithuania. Silica gel G-25 plates for thin-layer chromatography (TLC) were from Merck.

Equipment. The 6405 UV / Vis spectrophotometer with a thermostatically controlled cuvette (Jenway, UK); Titronic 96 titration system (Schott Glass Ltd. UK); pH-720 pH-meter (InoLab); thermostatically controlled magnetic stirrer of 10 points (Ikamag); TS-100 thermal stirrer (Biosan); a vortex mixer (Fisher Vortex Genie 2TM USA); a system for electrophoresis (Merck); a gas chromatographic system (Shimadzu GC 2010A, Japan); automatic micropipettes (Labsystems) and microsyringes (Hamilton).

Methods

Hydrolysis of p-NPB by lipases. The hydrolytic activity of a reaction mixture containing 2.325 ml of 100 mM Britton–Robinson or Universal buffer (Ub), pH 8.5 (composed of acetic, orthoboric and orthophosphoric acids at a ratio of 1 : 1 : 1 which provides a buffering capacity over a wide range of pH), 25 μl of *p*-NPB solution in propan-2-ol and 0.1 ml of lipase solution in the buffer was measured. The final concentrations were: substrate, 0.1 mM, organic solvent, 1% (v/v) and enzyme, 1 μg/ml. The reaction was carried out at 30 °C for 3 min [9]. The absorbance was measured at 400–410 nm. One unit of the hydrolytic activity corresponds to the amount of enzyme that releases 1 μmol of *p*-nitrophenol per min under indicated conditions.

Hydrolysis of p-NP fatty acid esters of various chain lengths by lipases. The hydrolysis reaction of other *p*-NP fatty acid esters was measured under the same conditions as of *p*-NPB. Solutions of *p*-NPA, *p*-NPB, *p*-NPC, *p*-NPCa, *p*-NPL, *p*-NPM and *p*-NPP in propan-2-ol were used as substrates.

Hydrolysis of oils by lipases. The reaction was performed in a mixture containing 0.4 g of rapeseed or camelina oil, 0.1 M Ub pH 8.5 or n-hexane (final volume 4.5 ml) and 0.165 mg or 0.825 mg of lipase. The mixture of this composition was thoroughly selected from a number of special experiments (data not presented). The mixture was incubated at a temperature of 30 °C under continuous stirring. Aliquots of 0.05 ml were extracted from the mixture at 1–128 hours, the reaction was terminated by adding 0.05 ml of diethyl ether, and the samples were frozen. The analysis was made by the TLC method as described below.

Esterification of oleic acid with alcohols by lipases. Reaction between oleic acid and various alcohols – methyl, ethyl, oleoyl, 1.2-ethanediol (ethylene glycol), 1.3-propanediol (propylene glycol) and 2-hydroxyethyl ether (diethylene glycol) (molar ratio 1:2) catalysed by lipase *E. aerogenes* 13 solution in Ub pH 8.0 (final concentration of the enzyme 0.165–0.825 mg/ml) was performed in Ub and n-hexane. This optimal molar ratio was found in a previous series of experiments. The reaction mixture was incubated for 1–74 hours at 30 °C in a thermostated 10 ml glass vessel under constant stirring and temperature control. The reaction progress was followed by extracting 200–500 μl aliquots of reaction mixture at definite time intervals and analysing them. The reaction was terminated by adding 9.5 ml of a methanol : acetone mixture (volume ratio 1 : 1). The residual amount of oleic acid was determined by titration with 0.1 M NaOH solution in methanol at 20 °C. Phenolphthalein solution in ethanol was used as the indicator. For analysis by the TLC method, samples were diluted with diethyl ether (volume ratio 1 : 1) and frozen. The analysis was carried out as described below.

Thin layer chromatography (TLC) method. A modified method of Yadav et al. [10] was used. The reaction mixture containing 0.4 ml of oils, 4 ml of 50 mM Tris-HCl buffer, pH 7.5 (30 °C) or n-hexane and 0.1 ml lipase solution was incubated at 30 °C under intensive continuous stirring for 0–24 h. The reaction was terminated by adding 25 ml of diethyl ether, and the extract was frozen. It was later analysed by TLC (boric acid-impregnated silica gel G-25 plates). The system of solvents for the elution: light fraction of petrol ether / diethyl ether / acetic acid (80 : 30 : 1). The chromatograms were developed with iodine vapour. Solutions of oleic acid, triacylglycerols (TAGs), 1.3 / 1.2-diolein (DO, content of 1.3-DO 85%, of 1.2-DO 15%) and monoolein (MO) in diethyl ether were used as standards. Quantitative analysis (%) of the reaction products (average of 3 assays) from chromatograms was performed employing the microimage 4.0 program considering the spot area and intensity.

Gas chromatography (GC) method. Reaction mixtures of 12 ml containing 20 mmol of glycerol tricaprylate (TC) and methanol at TC-to-methanol molar ratios 1 : 0.5, 1 : 1, 1 : 2 and 1 : 3 were prepared. Immobilized on polyurethane lipase Resinase HT preparations, 200 mg (21 U/g) was mixed with substrates in a solvent-free system, and methanolysis was performed at 30 °C. Samples were extracted at certain time intervals, centrifugated and mixed with n-butanol at a molar ratio 1 : 1 for GC analysis.

Investigation of immobilized lipases. Supports based on polyurethane (PU), chitin (CHT) and chitosan (CHZ) were

tested to select most effective immobilized lipases for desired reactions. Preparations were in store from Department of Polymer Chemistry and from Department of Biochemistry and Biophysics, Vilnius University. They were screened and selected as most efficient in the hydrolysis of *p*-NPB (unpublished data). Most efficient preparations were tested in the hydrolysis reaction for the substrate specificity for *p*-NP fatty acid esters as described above.

Statistics. Up to four measurements were provided in each experiment, and the results are presented as means ± S. E. M.

RESULTS AND DISCUSSION

Enzymatic catalysis is required to be optimized for an effective production of fatty acid and alcohol esters as biodiesel components. Substrate specificity is one of the factors determining an efficient catalysis by lipases. In the present study, we investigated certain lipolytic enzymes that might be attractive for technological processes. Lipase from *Enterobacter aerogenes* is currently under intensive investigation in local areas for a possible application in biodiesel industry. Resinase is a commercially available lipolytic preparation of a fungus which is unidentified in the literature. Resinase HT is a new thermostabilized enzyme. The preparation is relatively not expensive, but it is practically intended for removing pitch components of wood pulp [11–14]. Here, the lipase was screened for conversion reactions practically impotant for biofuel industry. Two other commercial lipases (Lipolase 100 LEX and Lipozyme TL 100L) were used to compare the efficiency of catalysis with Resinase HT.

Substrate specificity to *p*-NP fatty acid esters. *p*-NP fatty acid esters as soluble substrates are convenient for analysing the hydrolytic activity of lipases. The specificity of a number of selected lipases, both soluble and immobilized as a result of the present study, to the esters of four different chain lengths was investigated. Results are presented in Fig. 1–3.

It is evident that all the commercial lipases analysed were specific to fatty acid esters of a medium chain length, although Lipolase 100 LEX showed a relatively high hydrolytic activity on *p*-NPP (Fig. 1). Soluble *E. aerogenes* lipase was shown also to be the most active at hydrolysing medium-chain-length *p*-NP fatty acid esters (Fig. 2). Lipases are usually shown to be specific to substrates of both medium [10] and long [15] chains.

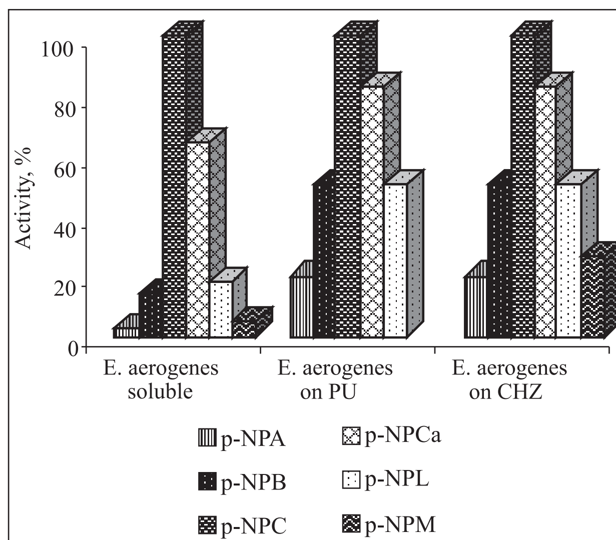

Fig. 2. Substrate specificity of soluble and immobilized *Enterobacter aerogenes* lipase. Solutions of *p*-NPA, *p*-NPB, *p*-NPC, *p*-NPCa, *p*-NPL and *p*-NPM in propan-2-ol were used as substrates

Two of the three commercial lipases immobilized on polyurethane slightly changed the activity to a more efficient hydrolysis of the long-chain *p*-NP fatty acid ester, while covalently bound Lipolase 100 LEX was strongly depressed when hydrolysing *p*-NPP as compared with the activity of the soluble enzyme. On the other hand, the specificity of immobilized commercial lipases did not change as compared with soluble analogues, although differences in the hydrolysis of various chain length substrates were more significant (Fig. 3). The immobilized *E. aerogenes* lipase showed insignificant differences in activity towards *p*-NPB and medium-chain *p*-NP fatty acid esters versus the attached enzyme on polyurethane (PU) and chitosan (CHZ), and also the highest activity was determined to *p*-NPC like for the soluble lipase (Fig. 2). The specificity of certain other lipases has been shown to change after immobilization [16].

Lipases in esterification reactions. The essential reactions of biodiesel component biosynthesis are transesterification of oils or triacylglycerols, alcoholysis, acidolysis and, in certain cases, direct esterification of alcohols by fatty acids [17–19]. Esterification of long-chain oleic acid with oleyl alcohol, which

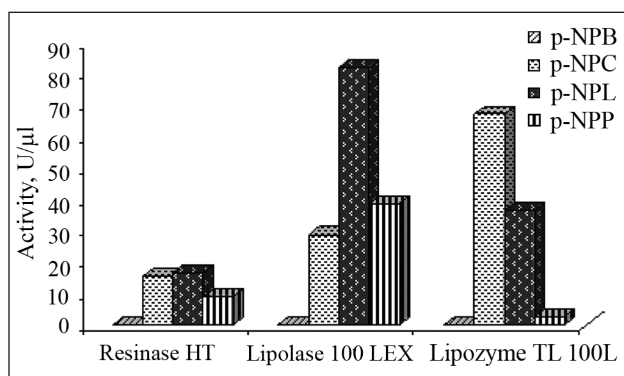

Fig. 1. Substrate specificity of soluble commercial lipases. Solutions of *p*-NPB, *p*-NPC, *p*-NPL and *p*-NPP in propan-2-ol were used as substrates

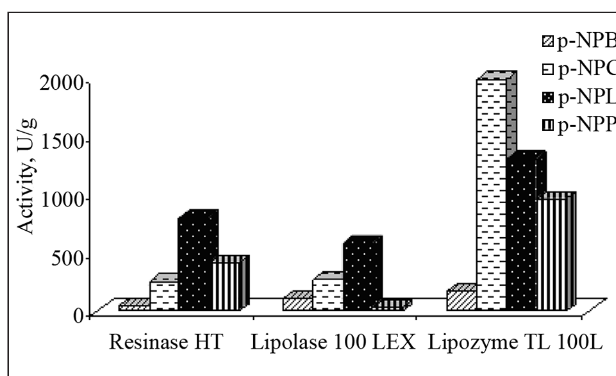

Fig. 3. Substrate specificity of commercial lipases immobilized on polyurethane support. Solutions of *p*-NPB, *p*-NPC, *p*-NPL and *p*- p-NPP in propan-2-ol were used as substrates

is also an attractive reaction for biolubricant production, is not studied enough. Here, the synthetic ability of *E. aerogenes* lipase was investigated. The time course of the reaction is illustrated in a TLC chromatogram (Fig. 4).

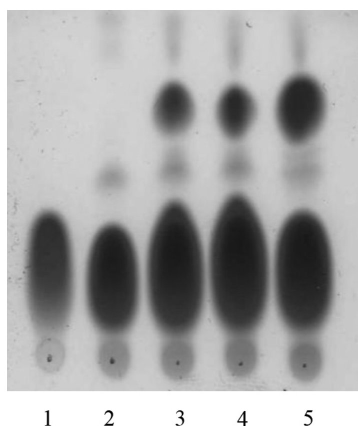

Fig. 4. Time course of *Enterobacter aerogenes* lipase-catalysed esterification of oleic acid with oleyl alcohol. Molar acid-to-alcohol ratio was 1 : 2. *1* – oleic acid (control), *2* – oleyl alcohol (control), *3* – products after 19 hours of the reaction, *4* – products after 22 hours, *5* – products after 91 hours

The esterification reaction of both long-chain fatty acid and alcohol was shown to be a long-lasting process as the reaction was still in progress even after 91 hours. This is not a proper result for large-scale enzymatic catalysis; consequently, the reaction should be further optimized in laboratory conditions.

Also, the esterification reaction of oleic acid with other alcohols including ethanol, 1.2-ethanediol, 1.3-propanediol and 2-hydroxyethyl ether catalyzed by the lipase was investigated. The reaction is illustrated in a TLC chromatogram (Fig. 5).

E. aerogenes lipase was found to show the highest catalytic activity when esterifying long-chain oleic acid with ethanol and 1,2-ethanediol. The enzyme slightly esterified the acid with 1.3-propanediol, and no visible ester was detected for esterification with 2-hydroxyethyl ether under the analysis conditions. It should be noted that the lipase is specific also to a certain structure of the alcohol. This should be accounted for when optimizing the esterification process for the production of biodiesel components. Experimental analysis of a series of other lipases to screen those for an effective esterification is a subject of forthcoming research.

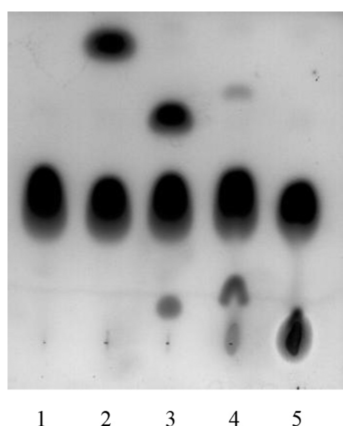

Fig. 5. *Enterobacter aerogenes* lipase-catalysed esterification of oleic acid with various alcohols after 72 hours. The reaction was performed at a temperature of 30 °C. Molar acid-to-alcohol ratio was 1 : 2. *1* – oleic acid (control), *2* – esterification with ethanol, *3* – esterification with 1.2-ethanediol, *4* – esterification with 1.3-propanediol, *5* – esterification with 2-hydroxyethyl ether. System of solvents was petrol ether, ether and acetic acid at volume ratio 75 : 25 : 1

Conversion of oils by lipase-catalyzed hydrolysis and transesterification. Transesterification of oils is an essential reaction to be optimized for the enzymatic production of biodiesel components. The reaction can be performed directly or after the hydrolysis of oils in a two-step process for an efficient conversion of free fatty acids and acylglycerols into fatty acid alcohol esters. *E. aerogenes* lipase-catalysed hydrolysis of rapeseed oil was compared to the reaction using a new resource of camelina oil. Results are presented in a TLC chromatogram (Fig. 6).

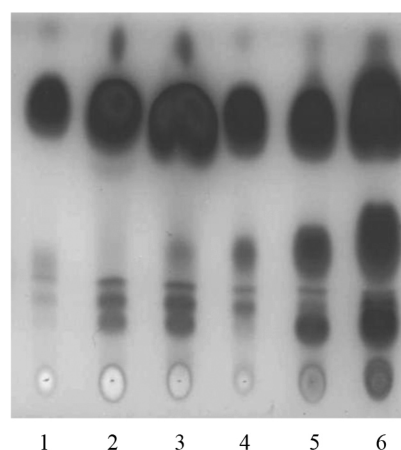

Fig. 6. *Enterobacter aerogenes* lipase-catalyzed hydrolysis of rapeseed (1–3 panels) and camelina (4–6 panels) oils in n-hexane after 48 hours. The reaction was performed at a temperature of 30 °C with different amounts of enzyme in the reaction mixture. 1 and 4 control panels consist of pure oils in n-hexane; 2 and 5 – with 0.165 mg of the enzyme; 3 and 6 – with 0.825 mg of the enzyme

It is worth noting that the lipase more efficiently hydrolysed camelina oil, particulary at a higher amount of the enzyme. According to our results, camelina oil could be an alternative source, next to rapeseed oil, to be converted into biodiesel components in future.

Transesterification of the traditional rapeseed oil with methanol is one of the most common reactions of chemical catalysis for biodiesel, already introduced at the industrial scale. Immobilized lipases should be of particular interest for biotechnological catalysis in this relation [20–22]. We screened a number of immobilized lipase preparations to choose the most effective variants (unpublished data). The time course of the catalysis reaction with *E. aerogenes* lipase adsorbed on chitin (CHT) is illustrated in a TLC chromatogram (Fig. 7).

It is evident that the reaction still in progress after 124 hours confirms the process to be long-lasting as shown by other authors [20, 21]. The main limiting factor for enzymatic catalysis to compete with chemical catalysis in biofuel production is the long duration of the process. The biotechnological transesterification should be shortcut; consequently, screening for most effective lipases, other oils (camelina as the subject), optimal immobili-

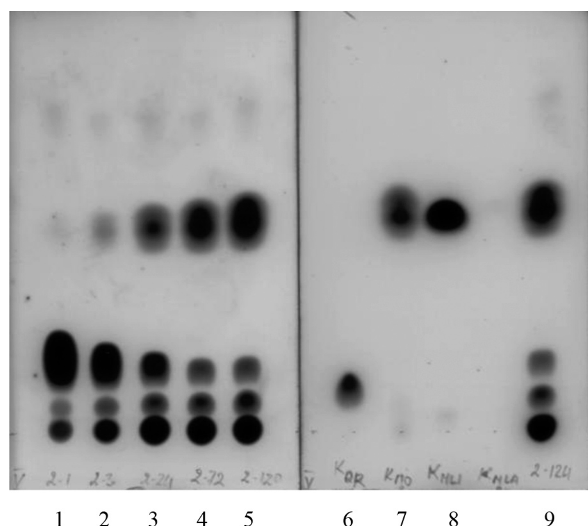

Fig. 7. Time course of rapeseed oil transesterification with methanol catalyzed by *Enterobacter aerogenes* P-3 lipase preparation adsorbed on CHT. Reaction mixture was analysed after: *1* – 1 hour, *2* – 3 hours, *3* – 24 hours, *4* – 72 hours, *5* – 124 hours. Control panels: *6* – oleic acid, *7* – methyl oleate, *8* – methyl linoleate, *9* – reaction mixture after 124 hours (like panel 5)

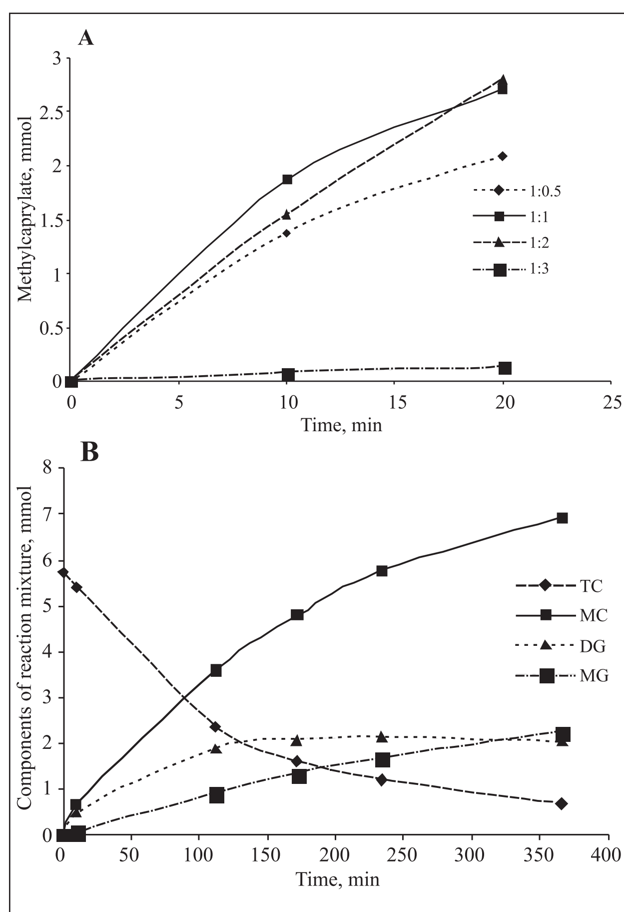

Fig. 8. Methanolysis of glycerol tricaprylate catalysed by lipase Resinase HT immobilized on polyurethane support. A – time course of methyl caprylate formation at four glycerol tricaprylate-to-methanol ratios. B – dynamics of the reaction products formation at optimal ratio (1 : 1). TC – glycerol tricaprylate, MC – methyl caprylate, DG – diglycerol, MG – monoglycerol

zation conditions and other parameters should be provided in forthcoming experiments.

Oils are a mixture of various triacylglycerols to be hydrolysed and transesterifed by lipases. Pure triacylglycerol (TAG) conversion is a convenient process to be processed for optimization. An optimal ratio of triacylglycerol and alcohol is crucial factor in determining the efficiency of alcoholysis and yield of essential products. The ratio of glycerol tricaprylate and methanol was determined for the production of methyl caprylate by the immobilized commercial lipase Resinase HT. As previously noted, the enzyme was shown to be specific to soluble substrates of medium chain length (Fig. 1 and 3), particularly to *p*-NPL. But the available gas chromatographic system could not ensure a complete analysis of glycerol trilaurate methanolysis products. Consequently, glycerol tricaprylate was chosen as the substrate.

Figure 8 illustrates a continuous formation of methyl caprylate (A) and the dynamics of products under an optimal glycerol-tricaprylate-to-methanol ratio (B). It is evident that the optimal ratio was 1 : 1 as the lack of the alcohol most probably caused an uncompleted reaction, and the enzyme inactivation started at the ratio 1 : 2. The lipase was completely depressed at the ratio 1 : 3 (Fig. 8 A).

It has been discussed why enzymatic alcoholysis of TAGs with relatively long chain and branched alcohols proceeds efficiently even in organic solvent-free systems, but the organic solvent-free methanolysis typically does not contribute to a high conversion. This phenomenon may result from the inactivation of the enzyme by methanol [23].

Also, it was shown that the enzyme was more specific to glycerol tricaprylate than to di- and mono-caprylic esters of glycerol as those were not evidently converted by alcoholysis during the time course examined. This should be considered as looking for the optimal yield of final essential products. Analysis in a gas chromatographic system with a mass-spectrometric detector showed a trace of glycerol in the final probe, indicating the alcoholysis of glycerol monocaprylate to start only at approx. 350 min after reaction initiation (Fig. 8 B). Alcoholysis is necessary to be optimized considering the ratio of two lipase substrates for an effective production of biodiesel components [24]. It also should be a subject of screening for new sources of alcohol-tolerant lipolytic enzymes [25].

CONCLUSIONS

Soluble commercial lipases Resinase HT, Lipolase 100 LEX and Lipozyme TL 100 L and their analogues immobilized on polyurethane showed substrate specificity in the hydrolysis of medium-chain *p*-NP fatty acid esters. Those immobilized enzymes must be screened for supports as the catalytic activity highly depended on support origin. The new points of a comparison of commercial preparations and immobilization of the enzymes on supports prepared by our research group should be important for the modern practical recommendations.

E. aerogenes lipase immobilized on two different supports showed not significant differences in the activity towards medium-chain *p*-NP fatty acid esters. The highest activity was determined to *p*-NPC.

E. aerogenes lipase showed the highest catalytic activity when esterifying oleic acid with ethanol and 1.2-ethanediol. Esterification of oleic acid with oleoyl alcohol was a long-lasting process. The lipase hydrolysed more efficiently camelina oil than the traditional rapeseed oil. Transesterification of rapeseed oil with methanol catalyzed by the enzyme adsorbed on chitin was a long-lasting process still in progress during 124 hours. The lipase should be considered as a candidate for biodiesel or biolubricant production, although more experiments are necessary.

The optimal ratio of substrates for alcoholysis catalysed with immobilized Resinase HT was found to be 1 : 1 for glycerol tricaprylate : methanol, and the enzyme was fully inactivated at the 1 : 3 ratio. The enzyme was more specific to glycerol tricaprylate than to di- and mono-glycerol esters of caprylic acid. The lipase should be further investigated to recommend as a biodiesel component.

ACKNOWLEDGEMENT

The study was supported by the Lithuanian State Science and Studies Foundation according to Contracts Nos. N-03/2008 and N-16/2008.

Received 14 April 2008
Accepted 26 November 2008

References

1. Akoh CC, Chang S-W, Lee G-C, Shaw J-F. J Agric Food Chem 2007; 55: 8995–9005.
2. Marchetti JM, Miguel VU, Errazu AF. Renewable Sustainable Energy Rev 2007; 11: 1300–11.
3. Shimada Y, Watanabe Y, Sugihara A, Tominaga Y. J Mol Catal B: Enzym 2002; 17: 133–42.
4. Modi MK, Reddy JRC, Rao BVSK, Prasad RBN. Bioresour Technol 2007; 98: 1260–4.
5. Noureddini H, Gao X, Philkana RS. Bioresour Technol 2005; 96: 769–77.
6. Hama S, Tamalampudi S, Fukumizu T, Miura K, Yamaji H, Kondo A, Fukuda H. J Biosci Bioeng. 2006; 101: 328–33.
7. Shenolikar I. Lipids 1980; 15: 980–2.
8. Pinto AC, Guarieiro LLN, Rezende MJC et al. J Braz Chem Soc 2005; 16: 1313–30.
9. Hernaiz MJ, Sanchez-Montero JM, Sinisterra JV. Biotechnol Bioeng 1997; 55: 252–60.
10. Yadav RP, Saxena RK, Gupta R, Davidson WS. Biotechnol Appl Biochem 1998; 28: 243–9.
11. Kontkanen H, Tenkanen M, Fagerstrom R, Reinikainen T. J Biotechnol 2004; 108: 51–9.
12. Tamerler CB, Martinez AT, Keshavarz T. J Chem Technol Biotechnol 2001; 76: 991–6.
13. Kontkanen H, Saloheimo M, Pere J, Miettinen-Oinonen A, Reinikainen T. Appl Microbiol Biotechnol 2006; 72: 696–704.
14. Kontkanen H, Tenkanen M, Reinikainen T. Enzyme Microb Tech 2006; 39: 265–73.
15. Arreguin-Espinosa R, Arreguin B, Gonzalez C. Biotechnol Appl Biochem 2000; 31: 239–44.
16. Pencreac'h G, Leullier M, Baratti JC. Biotechnol Bioeng 1997; 56: 181–9.
17. Nie K, Xie F, Wang F, Tan T. J Mol Catal B: Enzym 2006; 43: 142–7.
18. Meher LC, Sagar DV, Naik SN. Renewable Sustainable Energy Rev 2006; 10: 248–68.
19. Shaw JF, Chang SW, Liao HF, Shieh CJ. J Sci Food Agric 2003; 83: 1525–30.
20. Samukawa T, Kaieda M, Matsumoto T et al. J Biosci Bioeng 2000; 90: 180–3.
21. Iso M, Chen B, Eguchi M et al. J Mol Catal B: Enzym 2001; 16: 53–8.
22. Selmi B, Thomas D. J Am Oil Chem Soc 1998; 75: 691–5.
23. Kamini NR, Iefuji H. Process Biochem (Oxford, U. K.) 2001; 37: 405–10.
24. Lee GC, Wang DL, Ho YF, Shaw JF. J Am Oil Chem Soc 2004; 81: 533–6.
25. Yang J, Guo D, Yan Y. J Mol Catal B: Enzym 2007; 45: 91–6.

Vida Bendikienė, Benediktas Juodka, Birutė Surinėnaitė, Gervydas Dienys

LIPAZĖS ALIEJŲ PERDIRBIMUI. EKSPERIMENTINĖ KAI KURIŲ FERMENTŲ IR SUBSTRATŲ ATRANKA BIODYZELINO KOMPONENTŲ GAMYBAI

Santrauka

Siekiant optimaliai pritaikyti fermentus biodyzelino komponentų gamybai, iš skirtingų šaltinių išskirtos lipazės buvo išmėgintos katalizuojant įvairių substratų virsmus skirtingose reakcijose. Nustatyta, kad tirtos komercinės lipazės specifiškesnės vidutinio grandinės ilgio riebalų rūgščių *p*-nitrofeniliniams esteriams, tačiau Lipolase 100 LEX taip pat pakankamai aktyviai hidrolizavo ilgagrandį substratą – *p*-nitrofenilpalmitatą. Tirpi *Enterobacter aerogenes* lipazė irgi aktyviau hidrolizavo vidutinio grandinės ilgio riebalų rūgščių *p*-nitrofenilinius esterius. Imobilizuotų komercinių lipazių substratinis savitumas nepakito, palyginus su tirpių analogų savitumu. Lyginant ant poliuretano ir chitozano pagrindu paruoštų nešiklių imobilizuotos *E. aerogenes* lipazės aktyvumą tirpių substratų hidrolizėje, esant trumpagrandžiam *p*-nitrofenilbutiratui ar vidutinio grandinės ilgio esteriams, reikšmingų skirtumų nenustatyta. Kaip ir naudojant tirpų šio fermento analogą, imobilizuotos lipazės aktyvumas buvo didžiausias *p*-nitrofenilkaprilato hidrolizės atveju. Nustatyta, kad *E. aerogenes* lipazės katalizuojama oleino rūgšties reakcija su ilgagrandžiu oleino alkoholiu yra ilgas procesas, vis dar efektyviai vykstantis po 91 valandos nuo reakcijos pradžios. Didžiausiu kataliziniu aktyvumu šis fermentas pasižymėjo oleino rūgštį jungiant su etanoliu ir 1,2-etandioliu, kur kas prasčiau su 1,3-propandioliu, o naudojant 2-hidroksietilo eterį reakcija praktiškai nevyko. *E. aerogenes* efektyviau hidrolizavo judrų aliejų negu rapsų, taigi šis naujas bandomasis aliejus galėtų būti alternatyvus šaltinis biodyzelino komponentų gamybai. Ant chitino adsorbcijos būdu imobilizuoto šio fermento katalizuojama rapsų aliejaus metanolizė nepasibaigė ir po 124 valandų. Ant poliuretaninio nešiklio imobilizuota komercinė lipazė Resinase HT optimaliausiai katalizavo glicerolio trikaprilato metanolizę, kai šio substrato santykis su metanoliu reakcijos mišinyje buvo 1 : 1. Fermentas buvo visai nuslopintas padidinus šį santykį iki 1 : 3.

Raktažodžiai: biodyzelinas, transesterinimas, lipazės, imobilizuoti fermentai