ACTA ABP BIOCHIMICA POLONICA

Vol. 61, No 1/2014
1–6
on-line at: www.actabp.pl

Regular paper

Screening of lipase carriers for reactions in water, biphasic and pure organic solvent systems*

Zofia Hrydziuszko[1]✉, Agnieszka Dmytryk[1], Paulina Majewska[1], Katarzyna Szymańska[2], Jolanta Liesiene[3], Andrzej Jarzębski[2,4] and Jolanta Bryjak[1]

[1]Wrocław University of Technology, Faculty of Chemistry, Department of Bioorganic Chemistry, Poland; [2]Silesian University of Technology, Department of Chemical Engineering, Poland; [3]Kaunas University of Technology, Faculty of Chemical Technology, Lithuania; [4]Polish Academy of Sciences, Institute of Chemical Engineering, Poland

In bioprocesses lipases are typically used in immobilized form, irrespective of type of reaction systems, to ensure an even distribution of catalysts in water restricted media and/or to facilitate separation and reuse. In these studies we report on the selection of appropriate enzyme-carrier preparation for hydrolysis reaction in aqueous and biphasic systems and transesterification in organic solvent. For this *Candida rugosa* lipase was bound by adsorption or covalent attachment onto various carriers to give 24 preparations. Selection of proper preparation was based on reactivity, thermal stability (4 h at 60°C), possibility of drying and operational stability in 17–23 successive batch processes of 4-nitrophenyl palmitate hydrolysis in water. Activity of preparations varied from 20 to 5100 U·mL^{-1} but the most stable preparations were those of moderate activity: bound by adsorption or covalent attachment to NH_2-Kieselgel or acrylic carrier (retained activity over 90%). Selected preparations were used for hydrolysis of ethyl (1-butyryloxyethyl)-phenylphosphinate in biphasic system, and, after drying, in ethyl (1-hydroxyethyl)-phenylphosphinate transesterification. In this study operational stability was the principal criterion of selection. In water system, lipase covalently bound to NH_2-Kieselgel was the best — preserved 50% of initial activity in consecutive batch processes. In biphasic system and lipase covalently bound to acrylic and NH_2-Kieselgel the values were 90 or 77%, respectively, whereas in organic solvent, when lipase was immobilized on NH_2-Kieselgel by adsorption, it was 50%. Thus, NH_2-Kieselgel appears to be an universal matrix for investigated lipase immobilization and can be used in all reaction systems.

Key words: lipase, immobilization, hydrolysis, transesterification

Received: 09 October, 2013; revised: 21 February, 2014; accepted: 21 February, 2014; available on-line: 17 March, 2014

INTRODUCTION

Lipases (EC 3.1.1.3) are one of the most important classes of hydrolytic enzymes that catalyze both hydrolysis and synthesis of esters (Adlercreutz, 2013). Characteristic feature of these enzymes is stereo-, regio-, and chemoselectivity, a very broad substrate specificity and increase of activity when they act on substrates in contact with an oil-water interface, known as an "interfacial activation". Their unique properties allowed to introduce these enzymes as catalysts for many biotransformations at an industrial scale (cf. a book by Liese *et al*., 2006). For example, lipases are used for production of ibuprofen, isopropyl palmitate, isopropyl myristate, lotrafiban, and intermediates for many pharmaceuticals (e.g. (S)-beta blockers, trinems, diltiazem). Among many lipases, an enzyme produced by *Candida rugosa* seems to be a very attractive candidate for biotransformations at an industrial scale. This enzyme was used in soap production through lipase-based hydrolysis of oils and fats (Miyoshi Yushi, Japan) (Schmid & Verger, 1998) and for production of ibuprofen (Pfizer Inc., USA) (Liese *et al*, 2006). The industrial potential of this enzyme is still exploited and achievements were summarized in several reviews (Domínguez de María *et al*., 2006, and references therein). Currently, *C. rugosa* lipase is readily available on the market.

Esters hydrolysis is the natural reaction of lipases and it is typically conducted in water media. When substrates are sparingly soluble in water, biphasic system can be applied in which an organic phase is treated as substrate reservoir. In both cases lipases can be dissolved at a molecular level in water media (homogenous catalysis). However, esterification and transesterification reactions are normally carried out in organic media in which lipases do not dissolve. In this situation aggregates of protein molecules are formed with mass transfer resistance typical for heterogeneous catalysis. Heterogeneous catalysis is also common for immobilized enzymes. Although other drawbacks of enzymes immobilization, (enzyme deactivation, high costs), lipases immobilization seems to be a prerequisite for their practical applications. In addition to the typical benefits (higher thermal stability, reusability in batch processes or processes in continuous reactors, no protein contamination in a product), most of immobilized lipases were more active in organic solvents than their native counterparts (see an extensive review on lipases immobilization by Adlercreutz, 2013). It is probably caused by profitable conformational changes in the protein molecules when they are in contact with an liquid/solid interface.

✉e-mail: zofia.hrydziuszko@pwr.wroc.pl

*Presented at 5th Central European Congress of Life Sciences "EUROBIOTECH 2013" Kraków, Poland.

Abbreviations: CRL, lipase from *Candida rugosa*; *p*-NPP, *p*-nitrophenyl palmitate; tris, trihydroxymethylaminomethane; EDAC, N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride; DVS, divinyl sulphone; GA, glutaraldehyde; EBPP, ethyl(1-butyryloxyethyl)phenylphosphinate; EHPP, ethyl (1-hydroxyethyl)phenylphosphinate; ZM, silica gel Kieselgel 60; ZS, silica gel with small mesopores; MCF, siliceous mesoporous cellular foams; A, an acrylic carrier; G, cellulose-based Granocel carriers.

Amongst many methods of lipases immobilization, covalent attachment or adsorption onto insoluble carriers are most often applied in practical applications (Liese *et al*., 2006, Adlercreutz, 2013; Knežević *et al*., 2004). Covalent bonding is an expensive method with no enzymes leakage, whereas adsorption is the simplest method but enzymes can desorb, especially when surfactants are applied. Supports used for lipases immobilization are rather hydrophobic, but the high hydrophobicity is not always the best and the choice for proper immobilization procedure and/or carriers' matrix is an compromise between costs and enzyme stability under reaction conditions. Direct comparison of lipase preparation methods is very difficult because different carriers, substrates, solvents, temperatures and *etc*. were used in papers published. The main aim of this work was to screen carriers for *C. rugosa* lipase immobilization, directed to their use in both water and organic solvent systems. For this purpose adsorption and covalent attachment were applied onto several groups of carriers. Selection of the appropriate enzyme-carrier preparation for the hydrolysis of *p*-nitrophenyl palmitate in aqueous medium was based on thermal stability test and the stability of the chosen preparations in about 20 batch processes. Next, selected preparations were tested in biphasic (hydrolysis) and organic solvent (transesterification) systems in the presence of model substrates: ethyl(1-butyryloxyethyl) phenylphosphinate (EBPP) and ethyl (1-hydroxyethyl) phenylphosphinate (EHPP), respectively (Majewska *et al*., 2006). Special attention was paid towards operational stability in consecutive batch processes.

MATERIALS AND METHODS

Chemicals and reagents. Lipase from *Candida rugosa* (CRL) type VII, ≥700 unit/mg solid, *p*-nitrophenyl palmitate (*p*-NPP), 2-propanol, Triton X-100, arabic gum, N- (3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDAC), divinyl sulphone (DVS), diisopropyl ether, vinyl butyrate, trihydroxymethylaminomethane (tris), Lowry reagent and bovine serum albumin were purchased from Sigma-Aldrich (Germany). Glutaraldehyde (GA) was from Merck (Germany). Other reagents, all of analytical grade, were supplied by POCh (Poland). Ethyl(1-butyryloxyethyl)phenylphosphinate (EBPP) and ethyl (1-hydroxyethyl)phenylphosphinate (EHPP) were synthesized according to a procedure described previously (Majewska *et al*., 2006).

Carriers. Two silica gels of different textures were tested: Kieselgel 60 with medium mesopores (Degussa, Germany), named ZM, and silica gel with small mesopores (IE Int. Enzymes Ltd., USA), named ZS. Siliceous mesoporous cellular foams (MCF) were synthesized as described previously (Szymańska *et al*., 2007). MCF, ZM and ZS functionalization with 3-glicydoxypropyl-triethoxysilane (modification of the carrier surface with glycidyl groups; with regard to carriers marked with '-GPT'), 3-aminopropyltriethoxysilane (amino groups on the carrier surface; signature '-APT') or N-(2-aminoethyl)-3-3-aminopropylmethyldimethoxysilane (amino and methyl groups; signature '-APM') according to Jarzębski *et al*., 2007. An acrylic carrier (A) was a copolymer of butyl acrylate and ethylene glycol dimethacrylate, containing –OH, -COOH and -NH_2 functional groups, kindly donated by the Tarchomin Pharmaceutical Plant (Warsaw, Poland). Cellulose-based carriers (trade mark 'Granocel'; signature 'G') were prepared and functionalized (-COOH, -DEAE and -NH_2) according to the procedure previously presented (Bryjak *et al*., 2007).

Immobilization. Functional groups of the carriers were activated as follows: -OH groups with DVS, -COOH with EDAC, -NH_2 with GA whereas protein coupling was done in pH 7.0 (-NH_2) or pH 5.2 (-COOH) or pH 8.2 (-OH and glycidyl moieties) (Jarzębski *et al*., 2007). After activation, the carriers were filtered (centrifuged in the case of MCFs: 8000 rpm, 15 min) and suspended in 5.5 mL of 15 mg mL^{-1} CRL in 0.1 M phosphate buffer with appropriate pH. Excess protein was removed by washing the carrier with buffers of varying pH and ionic strength (Szymańska *et al*., 2007). In all eluates enzyme activity and protein concentration were measured. Finally, the obtained preparations were intensively rinsed with the buffer. Typically, immobilized preparations were stored at 4°C and were washed several times with the buffer prior to experiments. In adsorptive immobilization and in the case of glycidyl-functionalized carriers the step of functional groups activation was omitted.

Before transesterification reactions, selected enzyme-carrier preparations were washed with cold acetone several times and then dried under vacuum. Finally, a sample for activity measurements was taken and the rest was stored at 4°C.

Lipase activity in the presence of *p*-NPP. Activity of the free and immobilized lipase was assayed using 1.65 mM *p*-NPP as the substrate (37°C, pH 8.2, 0.05 Tris-HCl buffer with 0.1% arabic gum and 0.4% Triton X-100). Hydrolysis progress was monitored spectrophotomerically (Cintra 303 spectrophotometer, 410 nm) and the linear dependence of absorbance in time was used for activity calculation (the initial reaction rate). The enzyme activity unit [U] was defined as the amount of the enzyme that caused formation of 1 μmol *p*-nitrophenol in 1 min in the test conditions. Protein concentration was determined by the Lowry's method (Lowry *et al*., 1951) using bovine serum albumin as a standard. CRL preparation from the supplier possessed 0.081mg of protein and 100 U in 1 mg of the powder. The mean analytical error was less than ± 2.5% for protein measurements and less than ± 2.0% for activity assays. Both experiments were carried out in triplicate.

The activity of immobilized lipase was measured in a rotary shaker (Laboplay; 60 rpm, 37.0°C) in batch regime. The preparation was suspended in the buffer, placed into the reactor the temperature of which was maintained at 37°C. Then, preheated substrate was added (1.65 mM end concentration) and several samples were taken from the reactor at one-minute intervals. After absorbance (410 nm) measurement the sample was returned to the reactor. Immobilized enzyme activity was recalculated per 1 mL of freely sedimented carrier. The mean analytical error was less than ± 8.5% (triplicate repeated measures).

Operational stability of selected immobilized CRL preparations was done as above with modifications: after the initial rate measurements, the process was monitored until the substrate was depleted (stable absorbance; on average 1–2 h of the reaction). After that, the enzyme-carrier preparation was washed with the buffer several times and the next batch process was started with the same sample of the carrier. At the night intervals, the washed carrier was stored at 4°C.

Lipase activity in the presence of EBPP. General procedure was described previously (Majewska *et al*., 2006). Briefly, the reaction was carried out in a biphasic system in which organic phase (1.6 mL) consisted of

Table 1. CRL immobilization onto ZM, ZS, MCF, A and G carriers.

No. [-]	Functionality* [-]	Expected activity [U·mL^{-1}]	Measured activity [U·mL^{-1}]	Bound protein [mg·mL^{-1}]	Affinity ratio [-]	Thermal stability** [%]	Activity after drying [%]
Covalent coupling							
1	ZM-GPT	266/1210	273	0.98	0.50	45.3	–
2	ZM-APT	347/1618	399	1.31	0.48	91.9	73.3
3	ZM-ATM	538/1062	553	0.86	1.13	63.5	–
4	ZM-APM	555/716	705	0.58	1.72	51.6	–
5	ZS-ATM	307/1630	777	1.32	0.42	56.9	22.7
6	ZS-APT	409/1124	641	0.91	0.81	62.8	–
7	MCF-GPT	2462/3236	1784	2.62	1.34	82.4	0.9
8	MCF-APT	2433/5706	4234	4.62	0.78	29.0	0.8
9	MCF	3138/4298	5097	3.48	1.29	75.4	1.4
10	A-NH_2	2542/420	210	0.34	5.47	37.4	–
11	A-OH	2450/432	198	0.35	5.48	80.7	98.1
12	A-COOH	1616/358	132	0.29	5.29	91.2	90.2
13	G-OH	1351/1321	48	1.07	1.42	67.9	16.5
14	-OH; (G-DEAE)	1220/1692	25	1.37	1.00	83.3	5.2
15	-OH; (G-NH_2)	1381/1680	37	1.36	1.14	82.8	0.0
16	-OH; (G-COOH)	1374/1173	27	0.95	1.95	30.9	–
17	G-NH_2; (-OH)	1073/729	54	0.59	1.91	38.4	–
18	G-COOH; (-OH)	961/457	36	0.37	2.78	44.0	–
Adsorption							
19	ZM-APT	482/251	145	0.21	4.78	62.2	92.6
20	A	1141/1186	128	0.96	1.95	41.3	69.6
21	G-OH	228/556	31	0.45	1.06	79.0	–
22	G-DEAE	185/618	33	0.50	0.77	75.3	–
23	G-NH_2	60/790	45	0.64	0.20	45.3	–
24	G-COOH	168/605	19	0.49	0.71	77.3	–
–	Native	–	1500	–	–	16.4	–

*Functionalities in parentheses are those accompanied to activated moieties; **relative activities after incubation at 60°C for 4 h; underlined values of expected activities calculated from protein balance.

hexane and diisopropyl ether (3:1) and was composed of 5 mL of 0.05 M phosphate buffer (pH 7.0) with 1 mL of CRL preparation. 70 mg of EBPP was dissolved in organic phase and added to the reactor. The reaction was carried out 24 h at 35°C. Reaction progress was monitored by thin layer chromatography (TLC) using ethyl acetate and dichloromethane (3:5) as eluent and silica TLC Alu foils. After the reaction extraction was done with ethyl acetate and then filtrate was evaporated. The product was dissolved in methanol, then dissolved 50 times in hexane and analyzed by Merck-Hitachi

LaChrom HPLC-system (L-7400 UV-detector, D-7000 interface) using CHIRALPAK AD column (4.6 mm × 250 mm) with hexane:methanol (99:1; gradient 99:4) as a mobile phase at a flow rate 1 mL·min^{-1}.

For selected enzyme-carrier preparations operational stability tests were carried in 7 successive batch processes (as above). After each process carrier was separated from the reaction mixture by filtration and washed with 0.05 M phosphate buffer several times. Substrate and product were extracted as before.

Activity in the presence of EHPP. A general procedure was described previously (Majewska *et al.*, 2006). Briefly, the reaction was carried out in 6 mL of diisopropyl ether with 30 μL vinyl butyrate, 40 mg of powdered molecular sieves (3Å mesh) and 5 mg of substrate. 2 mL of the carrier or 20 mg of lipase powder was added to start transesterification reaction that was carried out in shake-flasks at 37°C. After 24 h of the reaction, the suspension was filtered and filtrate was evaporated. Dry matter was dissolved in 1 mL of hexane:diisopropyl ether (90:10) and analyzed by HPLC as previously.

For the selected immobilized preparations operational stability was tested as described above (7 successive runs). After each reaction carrier was washed out with diisopropyl ether.

Thermal stability of immobilized CRL in a batch reactor. Stability of native and immobilized enzyme was investigated by samples incubation at 60°C and pH 7.0 for 4 h. After incubation the samples were cooled rapidly in iced water-bath and held for 1 h in cold water prior to activity measurement (*p*-NPP as the substrate). Thermal stability was calculated as residual activity in relation

to the activity measured in a control sample, which was kept at room temperature.

RESULTS AND DISCUSSION

CRL immobilization and *p*-NPP hydrolysis

In the experiments five groups of carriers were used on the base of which 24 enzyme-carrier preparations were obtained (Table 1). Majority data are based on the reactivity towards *p*-NPP hydrolysis and in this reaction system the most active preparations were those after covalent coupling onto MCFs carriers, whereas Granocel matrix seemed to be unsuitable for the enzyme. The measured activity of immobilized preparation is influenced by the amount of bound protein, enzyme affinity to the carrier surface and accessibility of the active center for substrates. Affinity of investigated lipase to the carriers can be evaluated on the basis of activity units given for immobilization and that washed out (expected activity from activity balance in Table 1) and expected activity obtained by multiplying bound protein with specific activity of the enzyme (underlined values in Table 1). The most valuable information can be derived from their ratio that approximate affinity of the enzyme molecules to the carrier's surface (affinity ratios in Table 1). The ratio around 1.0 indicates random immobilization of enzyme molecules and ballast proteins, values above 1.0 higher affinity of the enzyme to the surface and opposite effect if lower values are obtained. It was stated that CRL immobilization onto most of the carriers is bound to exclusion of enzyme molecules from carriers surface leading to preferential binding of ballast proteins. In several cases (carrier nos. 3, 4, 7, 9, 13–15, 21) the microenvironment allowed to bind proteins in random manner. In contrast, the microenvironment created by carriers 10, 11, 12 (all acrylic) was more favourable for lipase itself and effectively excluded ballast proteins from the carrier's surface (ratios: 5.47, 5.48, 5.29, respectively). Ratios around to 2 were obtained for carriers 16–18, and 20 (all Granocel-based). This evidences the simultaneous selective immobilization and purification of the crude enzyme preparation in a single process. However, a considerable stress exerted on proteins during the covalent or adsorptive attachment led to their partial inactivation. Thus, about 1–253% of the bound enzyme expressed activity after immobilization (immobilization efficiency calculated as a ratio of measured activity to expected activity from the activity balance; data in Table 1). Generally, all the carriers based on acrylic or Granocel matrixes strongly altered bound lipase reactivity (values below 10%), whereas in all but one case (no. 23) adsorption was less-invasive. Covalent immobilization onto carriers 1-3, 7 and 23 allowed to obtain very good results with retained activities in the range of 70–115%, whereas the use of carriers nos. 4, 5, 6, 8 or 9 represent data that should be viewed in connection with well-known "hyperactivation" phenomenon. Interestingly, lipase immobilized only on siliceous materials (ZM, ZS, MCF) retained high activity or showed hyperactivation. The only exception is the adsorption onto G-NH_2 carrier.

Among the carriers, MCFs-based preparations appeared the most active with high or exceptionally high retained activities. However, also stability of immobilized lipase is one of the most important factors from practical point of view. Thus, thermal stability of all preparations was tested (Table 1) and it was shown that immobilization by adsorption or covalent attachment stiffened

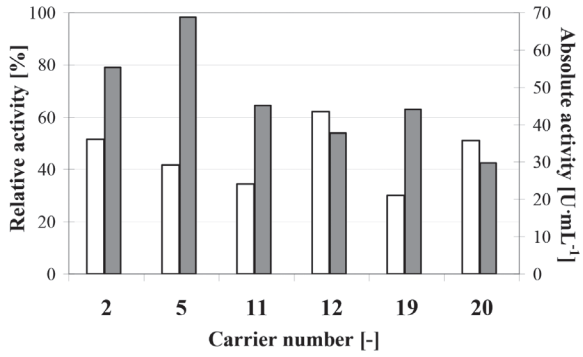

Figure 1. Relative operational stabilities (white) and their corresponding absolute activities (grey) for CRL immobilized by covalent attachment or by adsorption on selected carriers (Table 1).
Reaction conditions: rotary shaker 60 rpm, pH 8.2, 37°C, 1.65 mM *p*-NPP as the substrate, 1–2 h.

lipase structure in all the cases. The worst result was obtained for carrier no. 8 (MCF-APT) that was almost twice more stable than native counterpart; in other cases stabilization was higher. The thermal stability factor was used for selection of preparations used in following experiments on operational stability. First of all, all Granocel-based preparations (nos. 13–18 and 21–24) were excluded because of low activities and moderate increase of thermal stabilities. On the other hand, MCF-based preparations of bead size of about 18-30 μm (Jarzębski *et al.*, 2007) caused considerable separation problems from reaction mixtures resulting in their loss in a microfiltration set-up (see also Rekuć *et al.*, 2009). Moreover, later study on drying of immobilized preparations (Table 1) showed that they can be used in water systems only and thus they were excluded from operational tests. Consequently, preparations nos. 2 (ZM), 5 (ZS), 11 and 12 (A) as well as 19 and 20 (adsorption) were selected for further experiments. Figure 1 presents the results in the form of mean relative stability of immobilized preparations in 17–23 consecutive batch runs. First of all we observed a rapid decrease in activities after the first reaction run. However, after that activity either remained stable (nos. 2, 5, 11), remained so during first 10 runs and declined up to 5% (no. 12), or progressively de-

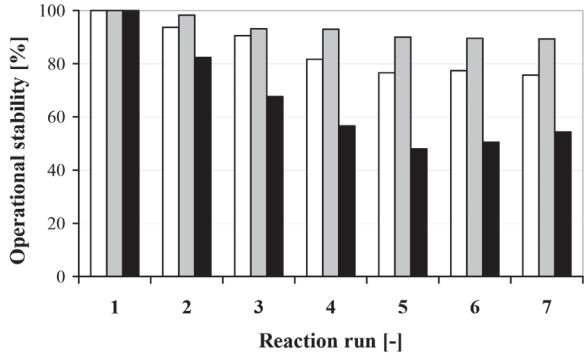

Figure 2. Operational stability of immobilized preparations in biphasic and organic systems in 7 succesive runs.
White bars — preparation 2, grey — 11, black — 20. Process conditions for hydrolysis (white and grey bars): rotary shaker 60 rpm, 35°C, 0.05 M phosphate buffer, pH 7, EBPP in hexane:diisopropyl ether as the substrate, reaction time 24 h. Process conditions for transesterification (black bars): rotary shaker 60 rpm, 37°C, EHPP in diisopropyl ether as the substrate, reaction time 24 h. The conversion values obtained in the first run were set as 100%.

Table 2. Hydrolysis of EBPP in biphasic system and transesterification of EHPP in diisopropyl ether by native and immobilized lipase (ads — adsorption).

Preparation [-]	Reaction [-]	Total activity* [U]	C_P/C_S** [-]	Productivity C_P/U [mg/U]	ee_S*** [%]	ee_P [%]
Native	hydrolysis	190	0.99	0.18	50.0	2.11
2	hydrolysis	65	0.73	0.45	39.3	2.48
11	hydrolysis	29	1.21	1.33	33.4	1.12
Native	transesterification	2000	0.39	0.01	72.5	1.39
2	transesterification	28	0.71	1.06	11.9	4.56
19 (ads)	transesterification	30	1.66	1.46	11.9	0.75
11	transesterification	23	0.14	0.38	11.3	6.40
20 (ads)	transesterification	22	0.19	0.51	12.0	6.35

*Total activity — measured in the presence on *p*-NPP in water system; **C_P and C_S — product and substrate concentrations, respectively; ***ee — enantiomeric excess.

clined up to 10% (nos. 19, 20). Thus, the most promising preparations for water systems are those which are stably active, i.e. samples 2, 5, 11.

Typical medium in transesterification reactions is organic solvent and the enzyme- carrier preparations must be used in a dry state, which is easy to handle. For that reasons selected preparations, used for operational stability tests and all of MCF samples (exceptionally high activity), were dried after several washings with cold acetone and the remaining activity was measured (Table 1; activity after drying). On the base of these data preparations nos. 5, 7–9 were excluded (activity depletion), which left for transesterification reaction tests the carriers nos. 2, 11 and 19. That set was supplemented with no. 20 to obtain a complete view on the properties of catalysts obtained by covalent attachment and adsorption on the same carrier.

Hydrolysis in biphasic system and transesterification

For EBPP hydrolysis in biphasic system only preparations 2 and 11 were used (no. 5 showed low activity after drying). As it is seen from Table 2, both native enzyme and immobilized preparations are enantioselective but not enantiospecific. However, productivity was higher when immobilized preparations were used and that can be attributed to the presence of carrier/water interfacial phase (hyperactivation). Both preparations were subjected to operational stability tests (7 consecutive batch processes, white and grey bars in Fig. 2). It appeared that sample 11 lost only about 10% of initial activity in first three runs and then was quite stable, whereas sample 2 retained 77% of initial reactivity after the four runs.

In pure organic solvent (diisopropyl ether) transesterification of EHPP was carried out using two selected preparations with lipase immobilized by covalent binding (2, 11) and their two counterparts obtained by adsorption (19 and 20). Data in Table 2 show that in this reaction only the native enzyme is enantioselective, yet not enantiospecific, whereas all immobilized catalysts are neither enantioselective nor enantiospecific. The hyperactivation of immobilized lipase is well evidenced by productivity higher even by two orders of magnitude than in the case of the native enzyme. Exceptionally high productivity was obtained in the case of catalyst 20 (adsorption). Therefore, operational stability test (7 runs) was done for this preparation (Fig. 2, black bars); after first 4 runs relative average conversion degree fall was of about 50% but in the following 3 runs the conversion stabilized at over 50%. Despite a decrease in effectiveness, owing to a paramount importance of catalysts stability for industrial applications that result can be considered as very satisfying from an industrial standpoint.

CONCLUSION

The goal of these studies was to find an universal carrier for the lipase-carrier preparation which could be used in various media with similar/acceptable effectiveness. The results of extensive studies allow to single out enzyme-carrier preparations appropriate for hydrolysis reactions in water media or biphasic system and transesterification in pure organic solvent. Using operational stability as an objective criterion the APT-ZM emerges as an optimal carrier suitable for both adsorptive and covalent immobilization of *Candida rugosa* lipase. Moreover, its application in biphasic and non-aqueous system resulted in significant hyperactivity.

Acknowledgements

This study was supported by the project "Biotransformations for Pharmaceutical and Cosmetics Industry" - POIG.01.03.01-00-158/09-00 partly financed by the European Union within the European Regional Development Fund.

REFERENCES

Adlercreutz P (2013) Immobilisation and application of lipases in organic media. *Chem Soc Rev* **42**: 6406–6436.

Bryjak J, Aniulyte J, Liesiene J (2007) Evaluation of mantailored cellulose-based carriers in glucoamylase immobilization. *Carbohyd Res* **342**: 1105–1109.

Domínguez de MP, Sánchez-Montero JM, Sinisterra JV, Alcántara AR (2006) Understanding *Candida rugosa* lipases: An overview. *Biotechnol Adv* **24**: 180–196.

Jarzębski AB, Szymańska K, Bryjak J, Mrowiec-Białoń J (2007) Covalent immobilization of trypsin on to siliceous mesostructured cellular foams to obtain effective biocatalysts. *Catal Today* **124**: 2–10.

Knežević ZD, Šiler-Marinković SS, Mojović LV (2004) Immobilized lipases as practical catalysts. *APTEFF* **35**: 151–164.

Liese A, Seelbach K, Buchholz A, Haberland J (2006) Processes. In *Industrial Biotransformation*, 2nd edn. Liese A, Seelbach K, Wandrey Ch, eds, pp 273–320. Wiley-VCH, Weinheim.

Lowry OH, Rosebrough N J, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**: 265–275.

Majewska P, Kafarski P, Lejczak B (2006) Simple and effective method for the deracemization of ethyl 1-hydroxyphosphinate using biocatalysts with lipolytic activity. *Tetrahedron Asymmetr* **17**: 2870–2875.

Pernas MA, Pastrana L, Fuciños P, Rúa ML (2009) Regulation of the interfacial activation within the *Candida rugosa* lipase family. *J Phys Org Chem* **22**: 508–514.

Rekuć A, Bryjak J, Szymańska K, Jarzębski AB (2009) Laccase immobilization on mesostructured cellular foams affords preparations with ultra high activity. *Process Biochem* **44**: 191–198.
Schmid RD, Verger R (1998) Lipases: Interfacial enzymes with attractive applications. *Angew Chem (Int Ed)* **37**: 1608–1633.
Szymańska K, Bryjak J, Mrowiec-Białoń J, Jarzębski AB (2007) Application and properties of siliceous mesostructured cellular foams as enzymes carriers to obtain efficient biocatalysts. *Micropor Mesopor Mat* **99**: 167–175.
Zynek K, Bryjak J, Szymańska K, Jarzębski AB (2011) Screening of porous and cellular materials for covalent immobilisation of Agaricus bisporus tyrosinase. *Biotechnol Bioproc Eng* **16**: 180–189.