Catalysis Today 196 (2012) 86–90

Contents lists available at SciVerse ScienceDirect

Catalysis Today

journal homepage: www.elsevier.com/locate/cattod

Solvent-free biocatalytic interesterification of acrylate derivatives

Edinson Yara-Varón[a], Jordi Eras Joli[a,*], Mercè Torres[b], Nuria Sala[b], Gemma Villorbina[a], Jonh Jairo Méndez[c], Ramon Canela-Garayoa[a,**]

[a] Department of Chemistry, University of Lleida, Plaça Víctor Siurana 1, Lleida, Spain
[b] Department of Food Science and Technology, University of Lleida, Plaça Víctor Siurana 1, Lleida, Spain
[c] Department of Chemistry, University of the Tolima, Barrio S. Helena, Ibague, Colombia

ARTICLE INFO

Article history:
Received 28 October 2011
Received in revised form 24 February 2012
Accepted 27 February 2012
Available online 8 April 2012

Keywords:
Biocatalytic interesterification
Acrylate derivatives
CALB
Resting cells
Water activity
Solvent-free

ABSTRACT

The ability of diverse commercial lipases and whole cells (fungal resting cells) to synthesise allyl and dichloropropyl acrylate from allyl dodecanoate through an interesterification process is presented. The process was carried out without solvent in a conventional batch system. The best biocatalyst among those studied was the commercial enzyme CALB (*Candida antarctica* lipase B immobilised onto a macroporous acrylic resin). The reaction was sensitive to water activity, and a decrease in the yield was observed at the highest activity studied. CALB could also be applied to diverse acrylic derivatives, although the yields decreased using either ethyl methacrylate or acrylic acid.

1. Introduction

Acrylic acid and its esters are frequently used as monomers for the production of polymers and copolymers of commercial interest. These materials have applications in the production of dyes, paper, textiles, glues, adhesives, binders, paints, dispersants, thickeners, and flocculants. Allyl acrylate is widely produced in addition to methyl, ethyl, *n*-butyl, and 2-ethylhexyl acrylates [1–5]. Chlorohydrin acrylates are putative monomers used to prepare functionalised polyacrylates.

Whereas the synthesis of chlorohydrin acrylates has not been described as far as we know, allyl acrylate is usually prepared by the esterification of acrylic acid or some derivatives with allyl alcohol [6] or allyl bromide [7]. This process can be carried out in different ways, using an alkali or acid catalyst [8–10]. However, chemical catalysis requires the use of special operating procedures and polymerisation inhibitors to avoid undesired reactions. Although these chemical processes are used on an industrial scale, the reaction has several drawbacks: it is energy intensive, the acidic or alkaline catalyst has to be removed from the product, and the non-neutral waste water requires treatment.

On the other hand, the implementation of green chemistry requires the discovery and development of new synthetic pathways using alternative feedstocks, improved reaction conditions, catalysts for improved selectivity and energy minimisation, and the design of environmentally compatible processes. These new techniques are urgently required to overcome many of the bottlenecks associated with conventional organic synthesis.

Enzymatic catalysis presents advantages over chemical catalysts with milder reaction conditions, greater specificity, high stability, high selectivity with fewer by-products, wide sources, and a broad range of substrates [11,12]. Biocatalysis is considered an environmentally benign alternative to chemical transformations. Reactions can be carried out in organic solvents or in solvent-free processes [13–15]. The solvent-free processes are considered more environment-friendly with the additional advantages of a more volume-efficient reactor [16]. The use of hydrolases, especially lipases, has expanded rapidly in recent years. Lipases (EC 3.1.1.3) may catalyse different reactions such as hydrolysis, esterification, transesterification, and interesterification and play an important role in industrial production [17–20]. They have been used to prepare diverse alkyl acrylates by the esterification of acrylic acid or the transacylation of methyl acrylates using diverse alcohols [21].

Recently, we have described the synthesis of chlorohydrin esters using glycerol and carboxylic acids. These esters can be transformed into allyl esters avoiding the use of the harmful allyl alcohol [22]. Crude glycerol from the biodiesel industry and vegetable oils or fat from agrofood wastes can also be used as starting materials to

* Corresponding author. Tel.: +34 973 702589; fax: +34 973 238264.
** Corresponding author. Tel.: +34 973 702843; fax: +34 973238264.
E-mail addresses: eras@quimica.udl.cat (J. Eras Joli), canela@quimica.udl.cat (R. Canela-Garayoa).

0920-5861/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.cattod.2012.02.055

obtain both esters [23]. Consequently, we dispose of a new source of chlorohydrin and allyl derivatives from renewable sources, which may be used to prepare valuable compounds. For instance, they could be used in interesterification reactions, thus diminishing the possibility of enzyme inhibition provoked by diverse alcohols [24,25]. Interesterification processes have been extensively used to modify triacylglycerides [26–28].

The aim of this study was to present the results obtained using diverse commercial lipases and whole cells (fungal resting cells) for synthesising allyl and chlorohydrin acrylates using allyl and dichloropropyl dodecanoates through an interesterification process. The solvent-free process was carried out in a conventional batch system.

2. Experimental

2.1. Enzymes and chemicals

Candida antarctica lipase B immobilised onto a macroporous acrylic resin (CALB), Amano lipase PS-IM immobilised on diatomaceous earth (PS-IM), lipase immobilised on Immobead 150 from *Rhizopus oryzae* (IM-150), lipozyme immobilised from *Mucor miehei* (MmL2), and dodecanoic acid were purchased from Sigma. Ethyl dodecanoate, allyl methacrylate, ethyl methacrylate, acrylic acid, Amano lipase PS (*Burkhloderia cepacia*) (PsL), Amano lipase A from *Aspergillus niger* (AnLA), and Amano lipase AYS from *Candida rugosa* (AYSL) were purchased from Aldrich. Ethyl acrylate, butyl acrylate, lipase from *C. rugosa* (CrL), and Lipozyme immobilised from *M. miehei* (MmL1) were purchased from Fluka (Sigma–Aldrich Quimica, S.A., Madrid, Spain). Allyl acrylate was purchased from Alfa Aesar (Barcelona, Spain) and hexane was purchased from J.T. Baker (Quimega, Lleida, Spain).

2.2. Procedure for obtaining resting cells

The strain of *R. oryzae* 1 used in this work was isolated from *Foeniculum vulgare* as endophytic fungi [29] and the other fungal mycelia (*R. oryzae* 2 and 3, *A. fumigatus*, *A. flavus* and *A. niger*) were isolated from the soil of olive (*Olea europaea*) orchards.

The fungal strains were cultivated in a synthetic liquid medium as previously described [30]. Mycelia were harvested from the whole culture broth by filtration using a Buchner funnel and washed with distilled water followed by acetone. The mycelia were dried under vacuum for 18 h and ground into a powder [31,32]. The enzymatic units (*U*) were determined for each of resting cells considering the enzymatic hydrolysis rate of methyl stearate. *U* corresponds to the amount of enzyme needed to hydrolise 1 μmol of stearic acid per min at 40 °C. The specific activity of fungal resting cells was calculated by U/m, where m is the amount of resting cell measured in g.

2.3. Procedure for the preparation allyl and dichloropropyl dodecanoates

Allyl and dichloropropyl dodecanoates were prepared from dodecanoic acid according to a previously described process [23]. The crude material of allyl dodecanoate synthesis was purified by distillation under vacuum using a Buchi Kugelrohr apparatus (Massó Analítica S.A., Barcelona, Spain). Finally, the crude material of dichloropropyl dodecanoate synthesis was purified by SiO_2 column chromatography.

2.4. Enzymatic interesterification

For the standard reaction, a 1:1 mixture of ethyl acrylate and either allyl or dichloropropyl dodecanoates (1 mmol each) was stirred (1200 rpm) continuously at atmospheric pressure in a reaction vial (1.5 mL) fitted with a PTFE-lined cap. Two sets of experiments were carried out using either 3.3% or 15% (w/w) of a biocatalyst based on the weight of allyl or dichloropropyl dodecanoate. Samples were collected after a given time, depending on the experiment carried out. The reaction temperature was chosen considering the previously described optimum temperature for each commercial enzyme. For resting cells, the temperature used was 40 °C. Once the experiment was concluded, an aliquot of 10 mg of the crude material of the reaction was dissolved in hexane containing an internal standard (typically butyl acrylate). The resulting solution was analysed using gas chromatography (GC-FID). Quantification of each compound was carried out using the corresponding internal standard. Experiments were carried out in triplicate.

Using the same procedure, the reaction was used to study the effect of an excess of either allyl or dichloropropyl dodecanoates on the allyl or dichloropropyl acrylate yields, respectively. The ratio was set at 1:5 ethyl acrylate to dodecanoate esters.

2.5. Equilibration of water activity

The water activity (a_w) in the experiments was set by equilibrating reagents (ethyl acrylate and allyl dodecanoate, independently) and CALB with aqueous saturated solutions of $MgCl_2$. Separate vessels were used for each reactant and enzyme [33]. Equilibration was performed at the reaction temperature (50 °C) for 16 h. The water activity was measured using an Aqua Lab series 3TE from Decagon Devices Inc. (Pullman, WA, USA).

2.6. Gas chromatography analysis

The progress of each reaction was determined by gas chromatography using an Agilent (Barcelona, Spain) HP6890 series GC coupled to a FID detector. The analytical column was a 30 m × 0.25 mm fused silica capillary coated with a 0.20 μm film of poly(80% biscyanopropyl-20% cyanopropylphenyl siloxane) (SP-2330; Supelco, Madrid, Spain). The temperature programme used was 40 °C for 5 min, then the temperature was increased to 225 °C at a heating rate of 20 °C/min, and then to 225 °C at a heating rate of 3 °C/min. A 1:20 split injection ratio was used. Hydrogen was employed as the carrier gas at constant pressure of 620 kPa. The injection volume was 1 μL. The injection system was held at 250 °C and the FID system was held at 280 °C. Quantification was carried out by a conventional internal standard calibration method using the corresponding acrylic ester standards.

3. Results and discussion

The solvent-free reactions were carried out using an equimolar ratio of allyl dodecanoate and ethyl acrylate. Fifteen diverse biocatalysts were used as putative catalysts for the interesterification reaction. Nine of them were commercially available lipases, of which five were immobilised on polymeric supports and four were in powder form. The other six were resting cells. The resting cells were obtained in our laboratory by lyophilising fungal mycelia presenting lipase activity. Two different percentages of biocatalysts, 3.3% and 15% (w/w), were used. Samples were taken at 1 h and the amounts of allyl and ethyl acrylates were determined by GC. Table 1 shows that four of the five supported catalysts were able to catalyse the interesterification reaction to some extent. Among them, IM-150 was the only one which did not show biocatalyst concentration dependence. Curiously, one Lipozyme from *M. miehei* (MmL2) did not present any activity, whereas MmL1 showed yields closer to that of PS-IM. Apart from the supplier, the only difference described by the dealers for MmL1 and MmL2 was the Enzymatic

Table 1
Percentage of allyl acrylate obtained using diverse commercial enzymes and resting cells with lipase activity. Reaction time 1 h.

Biocatalyst	Biocatalyst support	Specific activity[a] (U, g × 10^3)	Temperature (°C)	Yield (%)	
				Biocatalyst concentration (%w/w)[b]	
				3.3	15
CALB	Acrylic resin	111	50	20.5 ± 0.1	45.5 ± 0.1
PS-IM	Diatomaceous earth	0.943	50	4.8 ± 0.1	7.6 ± 0.5
MmL 1	Ion-exchange resin	0.140	50	4.9 ± 0.0	7.1 ± 0.2
MmL 2	Ion-exchange resin	0.110	50	–	–
IM-150	Immobead 150	0.367	40	4.5 ± 0.0	4.5 ± 0.0
AnLA	Powder, free enzyme	129	45	–	–
PsL	Powder, free enzyme	34.5	50	–	7.2 ± 0.0
AYSL	Powder, free enzyme	31600	40	–	–
CrL	Powder, free enzyme	0.0029	45	–	–
R. oryzae (1)	Resting cells	190	40	–	–
R. oryzae (2)	Resting cells	54	40	–	–
R. oryzae (3)	Resting cells	6340	40	–	2.1 ± 0.1
A. niger	Resting cells	234	40	–	–
A. fumigatus	Resting cells	175	40	–	7.6 ± 0.2
A. flavus	Resting cells	118	40	–	7.7 ± 0.1

[a] Specific activity of commercial enzyme provided by the supplier and calculated experimentally for resting cells.
[b] Based on the weight of allyl dodecanoate.

Units, showing that MmL1 was 22% more active than MmL2. Only *R. oryzae* 3, among the three resting cells prepared from different *R. oryzae* strains, was able to transform in a moderate way ethyl acrylate to allyl acrylate (2.13% yield using 15% biocatalyst). Two resting cells, *A. fumigatus* and *A. flavus*, showed better results than *R. oryzae*. These yields were similar to PsL (around 7% using 15% biocatalyst), whereas *A. niger* did not present any activity. Apart from PsL, none of the other powdered commercial enzymes were able to carry out the desired interesterification reaction to any extent. Without any doubt, *C. antarctica* lipase B (CALB) was the best biocatalyst for carrying out this reaction considering that the same amount of catalyst was used in all the experiments. These results are in spite of that the specific activity of the CALB not was the best among all the biocatalysts assayed (see Table 1). These results are similar to those described by Warwel et al. [34] which showed that CALB (also known as *Pseudozyma antarctica* lipase B, EC 3.1.1.3) was a superior catalyst out of 19 enzymes investigated in the biocatalysed transacylation of methyl acrylate with 1-undecanol. This reaction was also carried out in a solvent-free system. Nevertheless, the molar ratio of acrylate ester to 1-undecanol was 90:1 in this case.

Those biocatalysts that showed some activity in transforming ethyl acrylate to allyl acrylate were also used to study the interesterification reaction between dichloropropyl dodecanoate and ethyl acrylate. Table 2 shows that all the tested biocatalysts were able to catalyse to some extent this new interesterification reaction. Although CALB was again the best biocatalysts among those studied, yields were much lower than those obtained in the first assayed reaction. Moreover, in this case, MmL1 gave much closer yields to those obtained with CALB. These results can be explained by the fact that the alkanol residue with dichloropropyl esters is a secondary alcohol whereas allyl alcohol is a primary alcohol.

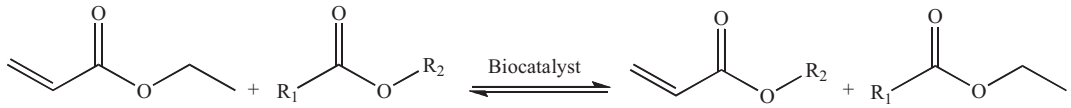
CALB supported on acrylic resin (also known as Novozyme 435) has already been described as the best catalyst to prepare octyl acrylate from acrylic acid and 1-octanol [21]. The reaction was carried out in an organic solvent and the yield was moisture-dependent.

Considering these results, a new set of experiments was carried out using the three commercial supported enzymes showing the best conversion ratio for allyl dodecanoate in the 3.3% and 15% experiments. The new experiments were carried out for 24 h and samples were taken at various time intervals. Fig. 1 shows that CALB was superior in terms of the reaction rate and conversion throughout the 24 h study. Using 3.3% biocatalyst, CALB was able to convert up to 45% of ethyl acrylate to allyl acrylate after 6 h of reaction, whereas the other two biocatalysts were only able to yield conversions around 10% after 24 h of reaction (Fig. 1A). Using 15% (w/w) biocatalyst, CALB gave similar conversions after 1 h of reaction. Although after 24 h of reaction MmL1 showed conversions closer to those of CALB, this enzyme still showed a lower reaction rate than CALB. PS-IM was the worst among the three biocatalysts studied. It yielded conversions up to 30% after 24 h of reaction (Fig. 1, graphic B). Although 45% was the best percentage reached, the final mixture could be useful to prepare copolymers of diverse alkyl acrylates [35].

Table 2
Percentage of dichloropropyl acrylate obtained using diverse commercial enzymes and resting cells with lipase activity. Reaction time 1 h.

Biocatalyst	Biocatalyst support	Temperature (°C)	Yield (%)	
			Biocatalyst concentration (%w/w)[a]	
			3.3	15
CALB	Acrylic resin	50	7.0 ± 0.3	10.4 ± 0.2
PS-IM	Diatomaceous earth	50	6.2 ± 0.2	7.8 ± 0.3
MmL 1	Ion-exchange resin	50	6.7 ± 0.1	9.7 ± 0.2
IM-150	Immobead 150	40	5.1 ± 0.4	5.2 ± 0.3
PsL	Powder, free enzyme	50	5.4 ± 0.7	5.6 ± 0.2
R. oryzae (3)	Resting cells	40	3.4 ± 0.5	4.7 ± 0.1
A. fumigatus	Resting cells	40	5.5 ± 0.6	5.6 ± 0.8
A. flavus	Resting cells	40	6.0 ± 0.2	6.6 ± 0.7

[a] Based on dichloropropyl dodecanoate weight.



R_1= $CH_3(CH_2)_{10}$ -

R_2= $-CH_2CH=CH_2$ or $-CH(CH_2Cl)_2$

Scheme 1. General scheme of the biocatalytic production of allyl and dichloropropyl acrylates.

Once CALB was found as the best biocatalyst among those studied to interesterificate ethyl acrylate, the possible effects of a molar ratio of 1:5 between ethyl acrylate and wither allyl and dichloropropyl dodecanoates were studied. When an excess of allyl dodecanoate was used, the maximum yield of allyl acrylate was 29% using 15% (w/w) biocatalyst, compared to 45% when an equimolar ratio was used. The dichloropropyl acrylate yield also decreased from 10.43% to 6.02%. Considering all of these results, a 1:1 ratio of ethyl acrylate to allyl dodecanoate was used for all subsequent experiments. Fig. 2 shows the influence of water activity in the allyl acrylate conversion. The best conversion was observed using the biocatalyst either as received (a_w = 0.55) or after drying (a_w = 0.33). The biocatalyst with the highest a_w tested (a_w = 0.81) gave the worst conversion. These results are in accordance with those described by Petersson et al. for the synthesis of cetyl palmitate [36].

Finally, CALB was used to interesterificate two different alkyl acrylates and acrylic acid. Table 3 shows that whereas butyl acrylate gave similar yields as ethyl acrylate, ethyl methacrylate and acrylic acid gave worse yields. Diverse authors have already described similar results during transacylation studies using several alcohols and CALB in various solvents [21,37,38]. Syrén and Hult [38] justified the lower conversions found using methyl methacrylate considering the conformomers present in the transition state. According to these authors, only two of these possible conformomers of the bond olefinic were able to be transacylated. They called these the s-cis and s-trans conformations. They determined that 0.003 was the relative probability of forming one of these productive conformomers for methyl methacrylate, whereas for methyl acrylate, this probability increased up to 0.279. Using this approach, they obtained a rationally designed CALB V190A mutant with higher catalytic activity towards methyl methacrylate (Scheme 1).

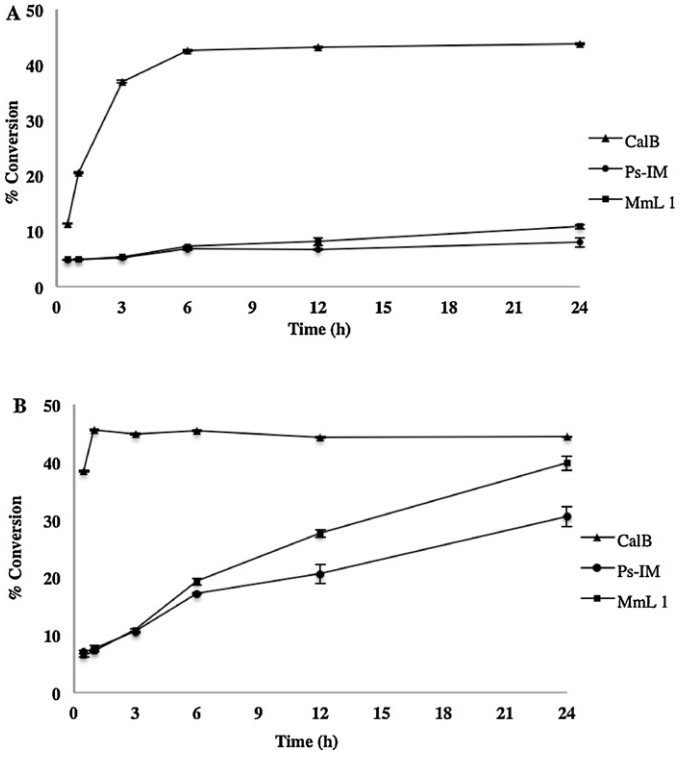


Fig. 1. Influence of the biocatalyst on the conversion of ethyl acrylate to allyl acrylate. The reaction was carried out at 50 °C for 24 h using three different biocatalysts. (A) 3.3% (w/w) biocatalyst based on the weight of allyl dodecanoate. (B) 15% (w/w) biocatalyst based on the weight of allyl dodecanoate.

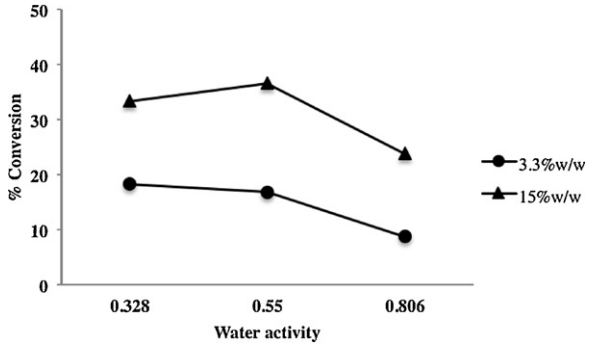


Fig. 2. Influence of water activity on the conversion of ethyl acrylate to allyl acrylate. The reaction was carried out at 50 °C for 24 h using CALB at concentrations of 3.3% (w/w) and 15% (w/w) based on the weight of allyl dodecanoate.

Table 3
Influence of the alkyl acrylate reagent on conversion to the corresponding allyl acrylic ester. The reaction was carried out at 50 °C for 1 h using CALB at 3.3% (w/w) and 15% (w/w) based on the weight of allyl dodecanoate and a_w = 0.55.

Reagents	Yield (%)	
	Biocatalyst concentration (% w/w)	
	3.3	15
Butyl acrylate/allyl dodecanoate	17.0 ± 0.0	45.1 ± 0.1
Acrylic acid/allyl dodecanoate	5.6 ± 0.1	14.7 ± 0.1
Ethyl methacrylate/allyl dodecanoate	12.2 ± 0.3	34.2 ± 1.2

4. Conclusions

A new biocatalytic interesterification reaction between acrylic carboxylates and both allyl and dichloropropyl dodecanoates in a solvent-free system has been described. The reaction allows the preparation of allyl and dichloropropyl acrylates using diverse commercial biocatalysts. The interesterification of dichloropropyl dodecanoate always gave lower yields than using allyl dodecanoate. Some resting cells with lipase-like activity could catalyse the reaction, but always with low to moderate yields. The best biocatalyst among those studied was the commercial enzyme CALB. The reaction using allyl dodecanoate was moisture sensitive, as we observed a decrease in the conversion yield when the highest water activity was used. CALB could be also applied to diverse acrylic compounds, although the yield of allyl acrylates decreased using

both ethyl methacrylate and acrylic acid. Studies are underway to prepare copolymers from the reaction mixtures obtained.

Acknowledgements

This work was supported in part by a Grant-in-Aid for the Secretaría de Estado de Política Científica y Tecnológica of the Spanish Ministry of Education and Culture (contract grant number: CTQ2009-14699-C02-01). The authors are grateful to the Vicerrectorat de Recerca de l'Universitat de Lleida for the UdL grant of Edinson Yara Varón.

References

[1] M.H. Jamróz, M.E. Jamroz, J.E. Rode, E. Bednarek, J.C. Dobrowolski, Vib. Spectrosc. 50 (2009) 231–244.
[2] F. Sugiyama, K. Satoh, M. Kamigaito, Polym. Prepr. Japan 55 (2006) 167.
[3] Z.M.O. Rzaev, Polym. Int. 51 (2002) 998–1012.
[4] D. Avci, L.J. Mathias, J. Polym. Sci. A: Polym. Chem. 97 (1999) 901–907.
[5] D. Avci, L.J. Mathias, Am. Chem. Soc., Polym. Prepr. Div. Polym. Chem. 39 (1998) 484–485.
[6] L. Liangui, M. Yan, C. Juan, A. Wei, S. Xiuhua, China Patent (2011) CN101993371A.
[7] P. Klan, P. Benovsky, Monatsh. Chem. 123 (1992) 469–471.
[8] H. Nakagawa, T. Hirabayashi, S. Sakaguchi, Y. Ishii, J. Org. Chem. 69 (2004) 3474–3477.
[9] T. Le Hanh, Conoco, Inc., USA Patent (1989) US4886520A.
[10] L.E. Trapasso, S.J. Padegimas, P.F. Epstein, P.L. Hung, P. Mukhopadhyay, P.L. Meisel, CPS Chemical Co. Inc., USA Patent (1995) EP646567A2.
[11] M. Escribà, V. Hessel, S. Rothstock, J. Eras, R. Canela, P. Löb, Green Chem. 13 (2011) 1799–1805.
[12] J. West, B. Karamata, B. Lillis, J.P. Gleeson, J. Alderman, J.K. Collins, W. Lane, A. Mathewson, H. Berney, Lab Chip 2 (2002) 224–230.
[13] C. Orellana-Coca, U. Tornvall, D. Adlercreutz, R. Hatti-Kaul, Biocatal. Biotransform. 23 (2005) 431–437.
[14] G.A. Macedo, G.M. Pastore, M.I. Rodrigues, J. Food Agric. Environ. 1 (2003) 59–63.
[15] O. Kose, M. Tuter, H.A. Aksoy, Bioresour. Technol. 83 (2002) 125–129.
[16] U. Törnvall, C. Orellana-Coca, R. Hatti-Kaul, D. Adlercreutz, Enzyme Microb. Technol. 40 (2007) 447–451.
[17] J.H. Liu, Y.Y. Zhang, Y.M. Xia, F. Su, Chem. Biochem. Eng. Q. 24 (2010) 203–209.
[18] R.C. Rodrigues, R. Fernandez-Lafuente, J. Mol. Catal. B: Enzym. 64 (2010) 1–22.
[19] I.G. Rosset, M.C.H. Tavares, E.M. Assaf, A.L.M. Porto, Appl. Catal. A: Gen. 392 (2011) 136–142.
[20] N. Sangeeta, B. Aditya, S. Rakhi, M. Bhawna, P. Pritish, R. Chaterjee, J. Brask, A. Svendsen, J. Mol. Catal. B: Enzym. 65 (2010) 18–23.
[21] M. Nordblad, P. Adlercreutz, J. Biotechnol. 133 (2008) 127–133.
[22] M. Escriba, M. Barbut, J. Eras, R. Canela, J. Avilla, M. Balcells, J. Agric. Food Chem. 57 (2009) 4849–4853.
[23] J. Eras, M. Escriba, G. Villorbina, M. Oromi-Farrus, M. Balcells, R. Canela, Tetrahedron 65 (2009) 4866–4870.
[24] C. Blattner, M. Zoumpanioti, J. Kroner, G. Schmeer, A. Xenakis, W. Kunz, J. Supercrit. Fluids 36 (2006) 82–193.
[25] S. Hari Krishna, S. Divakar, S.G. Prapulla, N.G. Karanth, J. Biotechnol. 87 (2001) 193–201.
[26] E.M. Usai, E. Gualdi, V. Solinas, E. Battistel, Bioresour. Technol. 101 (2010) 7707–7712.
[27] A. Behr, J. Eilting, K. Irawadi, J. Leschinski, F. Lindner, Green Chem. 10 (2008) 13–30.
[28] U. Schuchardt, R. Sercheli, R.M. Vargas, J. Braz. Chem. Soc. 9 (1998) 199–210.
[29] M. Torres, M. Dolcet, N. Sala, R. Canela, J. Agric. Food Chem. 51 (2003) 3328–3333.
[30] M. Torres, E. Barbosa, V. Loscos, R. Canela, Biotechnol. Lett. 22 (2000) 1265–1268.
[31] R.R.M. Paterson, P.D. Bridge, Biochemical Techniques for Filamentous Fungi – IMI Technical Handbooks, CAB International, Wallingford, U.K., 1997.
[32] K.E. Jaeger, B.W. Dijkstra, M.T. Reetz, Annu. Rev. Microbiol. 53 (1999) 315–351.
[33] M. Nordblad, P. Adlercreutz, Biotechnol. Bioeng. 99 (2008) 1518–1524.
[34] S. Warwel, G. Steinke, M.R. Klass, Biotechnol. Tech. 10 (1996) 283–286.
[35] A.B. Hajjar, P.F. Nicks, C.J. Knowles, Biotechnol. Lett. 12 (1990) 825–830.
[36] A.E.V. Petersson, P. Adlercreutz, B. Mattiasson, Biotechnol. Bioeng. 97 (2007) 235–241.
[37] P.O. Syrén, Lindgren, H.W. Hoeffken, C. Branneby, S. Maurer, B. Hauer, K. Hult, J. Mol. Catal. B: Enzym. 65 (2010) 3–10.
[38] P.O. Syrén, K. Hult, ChemBioChem. 11 (2010) 802–810.