Catalysis Communications 11 (2010) 576–580

Contents lists available at ScienceDirect

Catalysis Communications

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

Enhanced catalytic activity of lipase in situ encapsulated in electrospun polystyrene fibers by subsequent water supply

Shinji Sakai [a,*], Tetsu Yamaguchi [a,b], Rie Watanabe [c], Masaaki Kawabe [c], Koei Kawakami [a]

[a] Department of Chemical Engineering, Faculty of Engineering, Kyushu University, 744 Motooka, Nishi-ku, Fukuoka 819-0395, Japan
[b] Biotechnology and Food Research Institute, Fukuoka Industrial Technology Center, 1465-5 Aikawa, Kurume, Fukuoka 839-0861, Japan
[c] Japan Vilene Company, Ltd., 7 Kita-Tone, Koga, Ibaraki 306-0213, Japan

ARTICLE INFO

Article history:
Received 4 November 2009
Received in revised form 15 December 2009
Accepted 16 December 2009
Available online 24 December 2009

Keywords:
Lipase
Electrospinning
Polystyrene
Transesterification
Encapsulation
Electrospun

ABSTRACT

Rhizopus oryzae lipase prepared was immobilized in polystyrene electrospun fibers from a suspension of crude lipase powder in an *N,N*-dimethylformamide solution of polystyrene. The performance of the enzyme was enhanced by supplying water onto the resultant non-woven fabric. The electrospun fibers supplied with water by spraying showed 47-fold faster initial transesterification rate measured as conversion of (*S*)-glycidol to glycidyl *n*-butyrate with vinyl *n*-butyrate compared with a non-encapsulated crude lipase control. Before being moistened with water, the initial transesterification rate was slower than non-encapsulated lipase. The encapsulated and moistened lipase showed 77% of residual activity after 10 cycles of use.

1. Introduction

Electrospinning is a well established method for fabricating non-woven fabric membranes consisting of continuous ultra-fine fibers with diameters from nano- to micrometer scale. The small diameter of individual electrospun fibers results in remarkable characteristics of the resultant non-woven fabric membrane due to the very large surface area-to-volume ratio, high porosity and very small pore size [1–3]. One possible application is as a carrier for immobilizing enzymes. Enzyme immobilization is a well-known strategy for industrial scale applications because the immobilization allows recovery and re-use of the enzymes [4]. The density of immobilized enzymes in a certain volume of catalyst is drastically improved over traditional methods by using ultra-fine fibers.

There are two main types of enzyme immobilization: binding onto the surface of a support, and encapsulating within the support matrix. Surface binding immobilization consists of two separate processes, production of the support material and immobilization of enzymes onto it. Encapsulation is a single stage process, where the enzyme is mixed in with the support raw material prior to manufacture. From the manufacturing perspective, the fewer process steps in encapsulation make it the more attractive method. However, the finished product made by this method may suffer from hindrance of substrate diffusion through the matrix. The small diameter of electrospun fibers and the consequent short diffusion path minimize any hindrance of substrate diffusion. A number of enzymes have been immobilized in or on a variety of electrospun fibers [5–9]. The aim of this study is to develop lipase encapsulated within electrospun polystyrene fibers and to demonstrate the availability of the enzymes for reaction in organic solvent systems.

Lipases are versatile enzymes used in a wide variety of industrial and pharmaceutical applications. They are able to catalyze reactions in organic solvents such as transesterification, esterification, aminolysis, and acyl exchange [10]. It is widely recognized that the catalytic activity of the enzymes in organic solvents can be enhanced by altering the degree of hydrophobicity of the surrounding environment [10–14]. When applying this technique to develop lipase-encapsulating electrospun fibers, a viscous non-aqueous solution of a hydrophobic polymer containing lipase has to be electrospun without denaturing the enzyme. Several papers have reported successful encapsulation of enzymes in electrospun fibers made from water-insoluble materials [15,16]. However, the direct in situ encapsulation in water-insoluble electrospun fibers is still a challenge, and encapsulation of lipases within electrospun hydrophobic polymer fibers to produce effective enhanced enzyme catalytic activity in organic solvents has not been reported elsewhere. In this study, we

* Corresponding author. Tel./fax: +81 92 802 2768.
E-mail address: sakai@chem-eng.kyushu-u.ac.jp (S. Sakai).

1566-7367/$ - see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.catcom.2009.12.023

dissolved polystyrene in *N,N*-dimethylformamide (DMF) and dispersed reagent grade lipase powder in the solution. From this suspension, we prepared non-woven fabric membranes by electrospinning. Polystyrene is known as an effective material for supporting lipases, and catalysts immobilizing lipase onto the surface of the polystyrene resin have been studied [17,18].

2. Experimental methods

2.1. Immobilization of lipase in electrospun fibers

Polystyrene (MW 280,000, Sigma, MO, USA) particles were dissolved in DMF at 30% (w/w). *Rhizopus oryzae* lipase powder containing lipase at 65.0% (crude lipase, Lipase F-AP, Wako Pure Chemicals, Osaka, Japan) was added to the solution and stirred to a homogeneous dispersed solution for about 30 min. The quantity of crude lipase added resulted in a non-woven fabric product containing crude lipase at 10% (w/w) in dry weight. The resultant solution was electrospun from a syringe equipped with a 20-gauge stainless steel needle at 1.0 ml/h under being applied +17 kV.

2.2. Supplying water to catalysts

After 12 h of conditioning in air at room temperature and a subsequent vacuum drying, the electrospun polystyrene non-woven fabric membranes were treated with one of three processes: (1) Put into a desiccator containing a dish of water and maintained at 40 °C for 2 days to expose the material to saturated vapor. (2) Immersed in distilled water for 15 min. The amount of protein leaked to the water was measured using a protein assay reagent (Bio-Rad, Tokyo, Japan). (3) Distilled water was sprayed onto the fabric using an atomizer dampen but not saturate the fabric, these damp fabric specimens were then conditioned in air at room temperature for 30 min.

After treatment, the specimens were lyophilized at 10–20 Pa of vacuum. The existence of water in the lyophilized specimens was determined using Fourier transform infrared spectroscopy equipped with an attenuated total reflection unit (ATR/FTIR, FT/IR-4000, Jasco, Tokyo, Japan).

2.3. Catalytic activity measurement

The catalytic activity of the lipase containing systems was determined from the initial transesterification rate of (*S*)-glycidol (20 mM) with vinyl *n*-butyrate (400 mM) to produce (*S*)-glycidyl *n*-butyrate in isooctane (Scheme 1) during the first 2–8 min of reaction in the same manner with previous study [6] using the lyophilized specimens (50 mg-dry weight containing 5 mg-crude lipase), or non-treated crude lipase (5 mg-dry weight) at 35 °C. The initial transesterification rate was expressed as a rate based on the unit mass of immobilized crude lipase or a unit mass of catalyst, i.e., lipase-encapsulating fibers.

The effect of continuing reaction cycles on catalytic activity was measured using the following method: after the measurement of the initial transesterification rate, the reaction was allowed to proceed up to 60 min. The conversion at 60 min was also measured. Subsequently, specimens were centrifuged and the supernatant was decanted. The amount of solvent remaining (less than 0.5 ml) was calculated based on weighing the specimen and solvent. Fresh reaction solvent was added to the vessels to give a total of 10 ml of solvent. Then, the cycle was repeated as necessary.

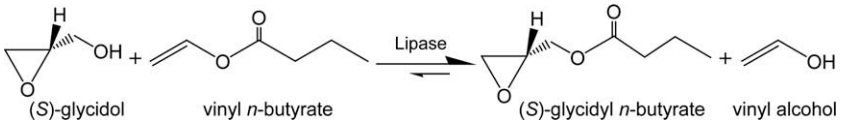
3. Results and discussion

3.1. Catalytic activity directly after electrospinning

DMF is a suitable solvent for electrospinning polystyrene, however, lipase and water-soluble enzyme stabilizing additives such as dextrin are not soluble in DMF. Fig. 1 shows microphotographs of (a) No lipase and (b, c) lipase-encapsulating polystyrene fibers. From the difference in appearance of fibers electrospun from the solutions containing crude lipase at (b) 10% (w/w) and (c) 50% (w/w) in dry weight of final products, we demonstrated dispersion of matrices insoluble to DMF producing electrospun fibers of about 5 μm diameter. First, we measured transesterification properties of the fibers after drying. Based on the previous reports for the effectiveness of polystyrene as matrices for lipase immobilization [17,18], we expected that the catalyst would show higher catalytic activity than non-immobilized lipase, but this expectation was incorrect: the polystyrene fibers encapsulating lipase ("Unmoistened" in Fig. 2) showed lower transesterification activity than non-treated lipase powder ("Lipase powder" in Fig. 2). There were two possible reasons for the lower catalytic activity of the lipase-encapsulating polystyrene fibers. First, the removal of essential water for expressing the enzymatic function of the lipase by DMF: It was reported that catalytic activity of lipases in non-aqueous solutions depended on water activity [19]. In general, a certain amount of water is required for enzyme action because water participates in all non-covalent interactions maintaining the native, catalytically active enzyme conformation [10]. The second reason was the hindrance of substrate supply to the encapsulated lipases from the reaction medium by the polystyrene layer surrounding lipase molecules. In the following experiments, we investigated these hypotheses.

3.2. Catalytic activity after water supply

We exposed lipase-encapsulating polystyrene non-woven fabric membranes to saturated vapor at 40 °C for 2 days and then lyophilized to evaluate the effect of moistening and the potential hindrance of molecular diffusion through polystyrene layer surrounding lipase molecules. No difference was observed for the morphologies of the resultant lyophilized specimens before and after the process (data not shown). However, the initial transesterification rate per amount of crude lipase detected for the resultant catalyst (9.01×10^{-1} μmol/(min mg-crude lipase)) was 100-fold faster than that of the unmoistened control (9.00×10^{-3} μmol/(min mg-crude lipase)), see Fig. 2. The enhanced initial reaction rate revealed that the lower catalytic activity detected for the unmoistened lipase-encapsulating polystyrene fibers was due to the removal of water essential for the expression of the enzymatic function of lipase by the DMF. It was expected that further increasing the period for exposing saturated vapor would increase catalytic activity. However,



Scheme 1. Transesterification of (*S*)-glycidol and vinyl *n*-butyrate to produce (*S*)-glycidyl *n*-butyrate using lipase.

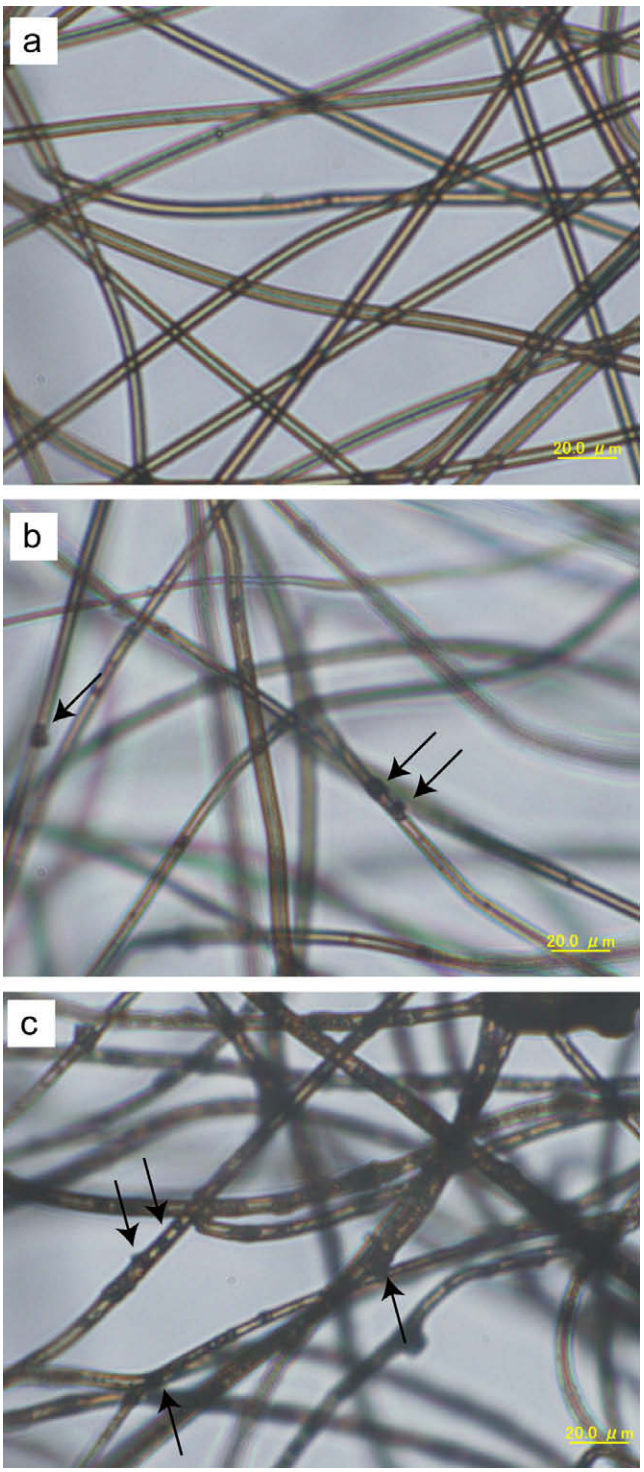


Fig. 1. Electrospun polystyrene fibers (a) without and (b, c) with lipase powder. The increase of visible particles (examples indicated by arrows) with increasing content of lipase powder from (b) 10% (w/w) to (c) 50% (w/w) of resultant fibers demonstrate the insolubility of the powder.

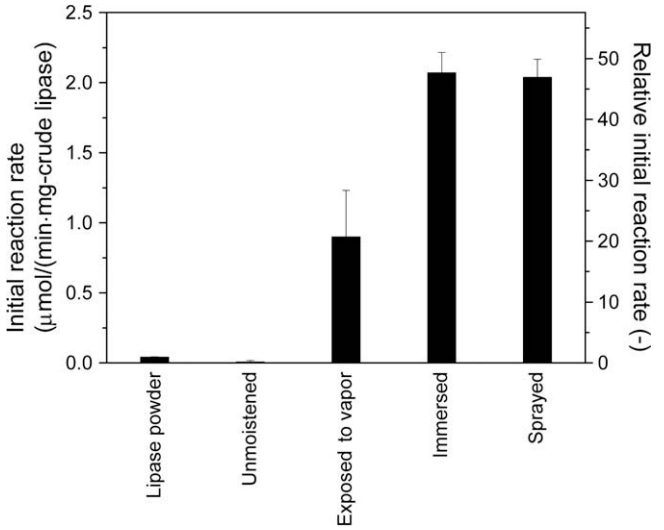


Fig. 2. Initial reaction rates of non-encapsulated lipases (lipase powder), and encapsulated in unmoistened electrospun polystyrene fibers (Unmoistened) and moistened by exposure to saturated vapor (Exposed to vapor), immersion in water (Immersed), and spraying water (Sprayed). The value for immersed was calculated taking into account the 30.4% protein release into the immersion water. Relative initial reaction rate: Measured initial reaction rates were normalized to the corresponding value for non-encapsulated lipase. Data represent mean and S.D. ($n = 3$).

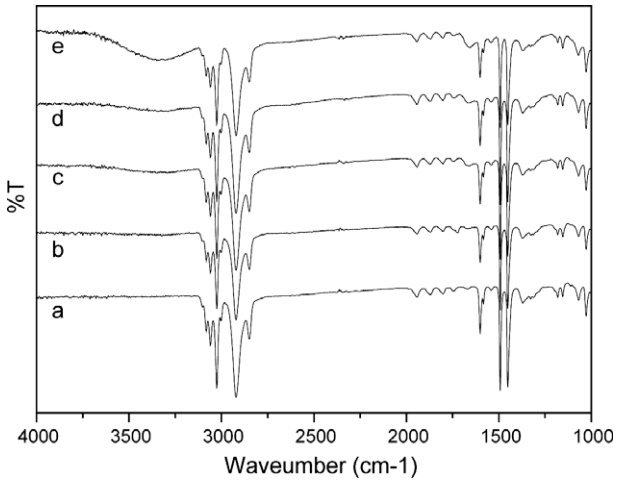


Fig. 3. ATR-FTIR spectrum of polystyrene fibers non-encapsulating lipase (a) unmoistened, (b) immersed in water, and those encapsulating lipase (c) unmoistened, (d) exposed to saturated vapor, and (e) immersed in water. All specimens were lyophilized before measurement.

increasing the exposure period also increases the time necessary for producing the final catalytic material. Therefore, we attempted to supply larger amount of water to lipase in a shorter time by immersing lipase-encapsulating fabrics in water.

The existence of water in the saturated vapor exposed lyophilized specimen was not clearly demonstrated by the ATR/FT-IR spectrum, Fig. 3c and d shows material before and after treatment. In contrast, a lyophilized specimen immersed in distilled water showed an obvious increase in the broad stretching band in the 3500–3000 cm^{-1} region. This increase results from –OH stretching and therefore indicates the existence of water (Fig. 3e). An increase of absorbance in this range was not detected for the electrospun polystyrene fibers without lipase even after immersion in water (Fig. 3a and b). The initial transesterification rate per amount of catalyst (polystyrene + encapsulated lipase) detected for the catalyst immersed in water (1.49×10^{-1} μmol/(min mg-catalyst)) was 165-fold faster than those for the catalyst without subsequent water supply (9.00×10^{-4} μmol/(min mg-catalyst)) and 1.7-fold faster than the catalyst exposed to saturated vapor for 2 days (9.01×10^{-2} μmol/(min mg-catalyst)). SEM examination showed

no difference in the morphologies of individual fiber types before and after immersion in water (data not shown), however, a 30.4% change in protein content in the water is evidence of leakage of protein. Taking into account the leakage of lipase into the water upon immersion of the non-woven fabric membranes, the transesterification rate of the specimen immersed in water, compared with the rate measured from an equivalent amount of crude lipase (2.07 μmol/(min mg-crude lipase)) showed a 48-fold increase (Fig. 2).

To suppress leakage of lipase from the catalyst, we sprayed water using an atomizer and subsequently lyophilized the product. The resultant specimen showed a 47-fold faster initial reaction rate (2.04 μmol/(min mg-crude lipase)) than that measured for non-treated lipase powder. Despite the same initial reaction rate per amount of crude lipase, that for an equivalent amount of catalyst was 39% faster than that detected for a specimen immersed in water. This demonstrates that spraying water onto the lipase-encapsulating non-woven fabric membranes was effective for suppressing leakage of lipase with enhancing catalytic activity of enclosed lipase. From our study of initial reaction rates, it was expected for a given amount of catalyst that the time necessary for achieving an equilibrium conversion state would be the shortest for the catalyst sprayed water (Fig. 4). However, the degree of equilibrium conversion was independent of the water supplying method.

In this study, we used the specimens dried under vacuum after each moistening process. It is known that catalytic activity of lipases in non-aqueous medium strongly depends on the hydration level of them, i.e., catalytic activity can be control by changing the hydration level [19]. Considering it, we predict the superiority of the moistened fibrous catalysts over non-treated lipase powder will be further enhanced under the optimal hydration level, and the study is under investigation.

3.3. Repeated cycles of use

We studied the stability of spray moistened lipase-encapsulating polystyrene fibers during repeated cycles of use. As shown in Fig. 5a, the residual activity of the lipase encapsulated in polystyrene fibers gradually decreased with an increasing number of cycles but showed a level of 77.1% of the original even after ten cycles of use. In addition, the equilibrium conversion was constant, at about 82%, during repeated use (Fig. 5b).

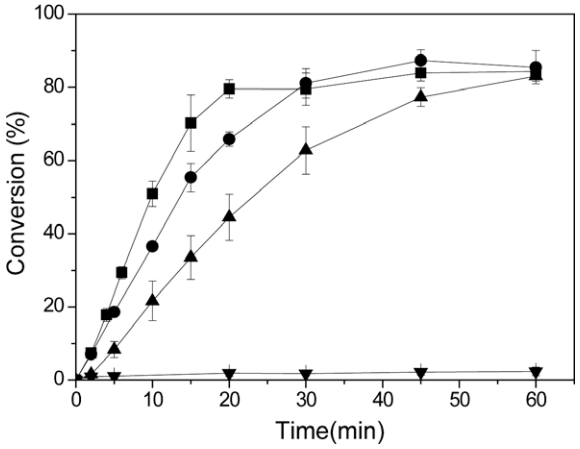


Fig. 4. Time-conversion plots for the transesterification of (*S*)-glycidol to glycidyl *n*-butyrate by reaction with lipase-encapsulating electrospun polystyrene fabrics (▼) unmoistened and moistened by (▲) exposing saturated vapor, (●) immersion in distilled water, and (■) spraying water using an atomizer. Bars represents standard deviations ($n = 3$).

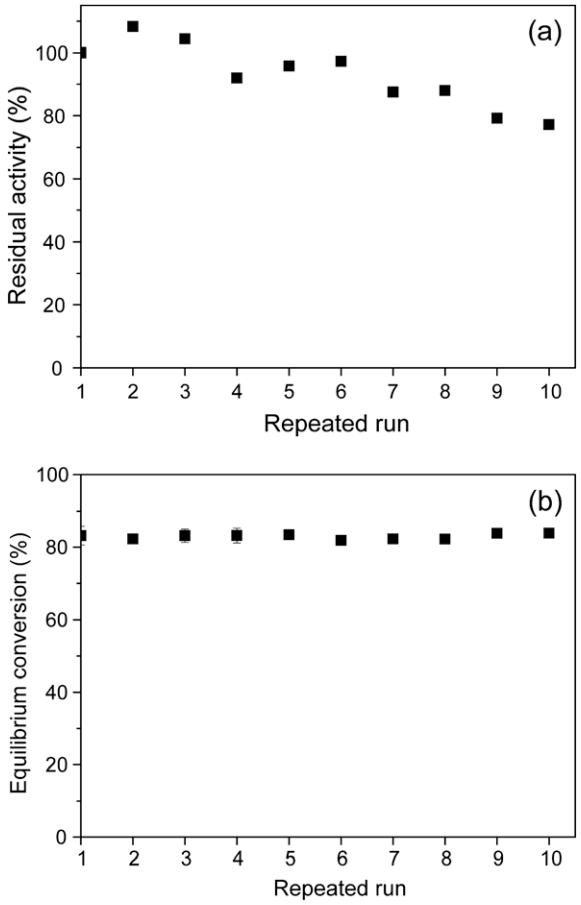


Fig. 5. (a) Transition of transesterification activity of lipase-encapsulating electrospun polystyrene fibers moistened by spraying water during repeated cycles. Residual activity: initial transesterification rates at each run were normalized to the values of the first run. (b) Transition of equilibrium conversion during repeated cycles for the system containing polystyrene fibers moistened by spraying water.

4. Conclusion

In the present study, we fabricated polystyrene non-woven fabric membrane encapsulating lipase in individual fibers for use in organic solvent systems. The fibers were prepared by electrospinning dispersed reagent quality lipase powder in polystyrene dissolved in DMF. Dry specimens showed slower initial reaction rates than non-treated lipase powder for transesterification in isooctane. Lyophilized specimens moistened by exposing to saturated vapor at 40 °C for 2 days, showed a 20-fold faster initial reaction rate compared with non-treated lipase powder. The initial reaction rate was further enhanced to a 47-fold increase by altering the method of moistening to reduce lipase loss. The catalyst kept about 80% of catalytic activity even after ten cycles of repeated use. From these results, we conclude that in situ lipase-encapsulating electrospun polystyrene fibers have a high degree of suitability as a carrier for catalytic activity in organic solvent systems.

Acknowledgments

This research was supported by the “Collaborative Development of Innovative Seeds” grant program from the Japan Science and Technology Agency, a Grant-in-Aid for the Global COE program, “Science for Future Molecular Systems” from the Ministry of Education, Culture, Science, Sports and Technology of Japan, and the Nippon Sheet Glasses Foundation.

References

[1] Z.M. Huang, Y.Z. Zhang, M. Kotaki, S. Ramakrishna, Compos. Sci. Technol. 63 (2003) 2223–2253.
[2] W.E. Teo, W. He, S. Ramakrishna, Biotechnol. J. 1 (2006) 918–929.
[3] S. Ramakrishna, K. Fujihara, W.E. Teo, T. Yong, Z.W. Ma, R. Ramaseshan, Mater. Today 9 (2006) 40–50.
[4] R.A. Sheldon, Adv. Synth. Catal. 349 (2007) 1289–1307.
[5] S. Sakai, K. Antoku, T. Yamaguchi, K. Kawakami, J. Membr. Sci. 325 (2008) 454–459.
[6] S. Sakai, K. Antoku, T. Yamaguchi, K. Kawakami, J. Biosci. Bioeng. 105 (2008) 687–689.
[7] X.J. Huang, D. Ge, Z.K. Xu, Eur. Polym. J. 43 (2007) 3710–3718.
[8] Z. Wang, L. Wan, Z. Liu, X. Huand, Z. Xu, J. Mol. Catal. B: Enzym. 56 (2009) 189–195.
[9] Y. Dror, J. Kuhn, R. Avrahami, E. Zussman, Macromolecules 41 (2008) 4187–4192.
[10] A. Zaks, A.M. Klibanov, Proc. Natl. Acad. Sci. USA 82 (1985) 3192–3196.
[11] M.T. Reetz, A. Zonta, J. Simpelkamp, Biotechnol. Bioeng. 49 (1996) 517–534.
[12] M.S. Antczak, A. Kubiak, T. Antczak, S. Bielecki, Renew. Energy 34 (2009) 1185–1194.
[13] A.C. Pierre, Biocatal. Biotransform. 22 (2004) 145–170.
[14] K. Kawakami, S. Yoshida, J. Ferment. Bioeng. 82 (1996) 239–245.
[15] T.E. Herricks, S.H. Kim, J. Kim, D. Li, J.H. Kwak, J.W. Grate, S.H. Kim, Y.N. Xia, J. Mater. Chem. 15 (2005) 3241–3245.
[16] H. Jiang, Y. Hu, Y. Li, P. Zhao, K. Zhu, W. Chen, J. Control, Release 108 (2005) 237–243.
[17] M. Murray, D. Rooney, M. VanNeikerk, A. Montenegro, L.R. Weatherley, Process Biochem. 32 (1997) 479–486.
[18] B. Chen, M.E. Miller, R.A. Gross, Langmuir 23 (2007) 6467–6474.
[19] X. Xia, C. Wang, B. Yang, Y.H. Wang, X. Wang, Appl. Biochem. Biotechnol. 159 (2009) 759–767.