doi: 10.5505/tjb.2013.30085

Research Article [Araştırma Makalesi]

Yayın tarihi 30 Eylül, 2013 © TurkJBiochem.com

[Published online 30 September, 2013]

Improved Performance of P*seudomonas fluorescens* lipase by covalent immobilization onto Amberzyme

[Amberzyme üzerine kovalent immobilizasyon ile *Pseudomonas fluorescens* lipazın geliştirilmiş performansı]

Yakup Aslan[1],
Nurrahmi Handayani[2],
Erythrina Stavila[3],
Katja Loos[3]

[1]Department of Biochemistry, Faculty of Art and Science, Harran University, Şanlıurfa, Turkey
[2]Chemistry Study Program, Faculty of Mathematics and Natural Sciences, Bandung Institute of Technology, Indonesia
[3]Department of Polymer Chemistry, Zernike Institute for Advanced Materials, University of Groningen, The Netherlands

Yazışma Adresi
[Correspondence Address]

Dr. Yakup Aslan

Department of Food Engineering, Faculty of Engineering and Architecture, Siirt University, Kezer Campus, 56100, Siirt,Turkey
Tel. +90 5064973768
E-mail. dr_yakup@hotmail.com

Registered: 31 March 2013; Accepted: 15 June 2013
[Kayıt tarihi: 31 Mart 2013; Kabul tarihi: 15 Haziran 2013]

ABSTRACT

Objective: In this study, the conditions of covalent immobilization of *Pseudomonas fluorescens* lipase onto an oxirane-activated support (Amberzyme) were optimized to obtain a high activity yield. Furthermore, the operational and storage stabilities of immobilized lipase were tested.

Methods: Optimum conditions for immobilization were determined by changing individually the conditions (pH from 5 to 9; buffer concentration from 0.025 to 2.5 M; amount of Amberzyme from 100 to 500 mg and duration of immobilization from 24 to 120 h). Amounts of protein and the activity of enzyme were determined by UV/Vis (PYE UNICAM SP8-200 UV/Vis spectrophotometer).

Results: Immobilization conditions (pH and molar concentration of immobilization buffer, enzyme/support ratio and immobilization duration) significantly affected the immobilization efficiency. 100% immobilization yield and 145% activity yield were achieved by optimizing the immobilization conditions. Operational and storage stabilities of immobilized lipase were determined as well. The immobilized enzymes retained its activity for 20 consecutive batch reactions. Furthermore, the immobilized lipase showed a high storage stability as no decrease in its activity was observed for 20 days.

Conclusion: Our results obtained in the present study are the best in the covalent immobilization of *Pseudomonas fluorescens* lipase in the literature. Therefore our future studies will focus on using the immobilized *Pseudomonas fluorescens* lipase for the production of biodiesel, hydrolysis of oils and various important esterification reactions.

Key Words: amberzyme, covalent attachment, enzyme immobilization, lipase, *Pseudomonas fluorescens*

Conflict of Interest: The authors declare that there was no conflict of interest in this work.

ÖZET

Amaç: Bu çalışmada, yüksek aktivite verimi elde etmek için *Pseudomonas fluorescens* lipazın oksiran grubu ile aktif bir destek olan Amberzyme üzerine kovalent immobilizasyonunun koşulları optimize edildi ve immobilize lipazın kullanım ve saklama kararlılıkları test edildi.

Yöntem: İmmobilizasyon için optimum şartlar ayrı ayrı değiştirilerek belirlendi (pH 5-9 arası; tampon derişimi 0.025-2.5 M arası; Amberzyme miktarı 100-500 mg arası ve immobilizasyon süresi 24-120 saat arası). Protein miktarları ve enzim aktivitesi UV-visible spektrofotometresi (PYE UNICAM SP8-200 UV/Vis spectrophotometer) kullanılarak tayin edildi.

Bulgular: İmmobilizasyon koşulları (tampon pH'sı ve molar derişimi, enzim/destek oranı ve immobilizasyon süresi) immobilizasyon etkinliğini önemli ölçüde etkilemiştir. İmmobilizasyon koşullarının optimizasyonu sonucu % 100 immobilizasyon verimi ve % 145 aktivite verimi elde edilmiştir. İmmobilize lipazın kullanım ve saklama kararlılığı da test edilmiştir. İmmobilize lipaz, aktivitesini birbirini takip eden yirmi kullanım boyunca korumuştur. Ayrıca immobilize lipaz yüksek bir saklama kararlığı göstermiş ve 20 gün boyunca aktivitesinde herhangi bir azalma olmadığı gözlenmiştir.

Sonuç: Bu çalışmada elde ettiğimiz sonuçlar, *Pseudomonas fluorescens* lipazın kovalent immobilizasyonu ile ilgili literatürdeki en iyi sonuçlardır. Dolayısıyla bizim gelecekteki çalışmalarımız bu immobilize *Pseudomonas fluorescens* lipazın biyodizel üretimi, yağların hidrolizi ve önemli esterifikasyon reaksiyonlarında kullanımı üzerinde yoğunlaşacaktır.

Anahtar Kelimeler: amberzyme, kovalent bağlama, enzim immobilizasyonu, lipaz, *Pseudomonas fluorescens*

Çıkar Çatışması: Yazarlar arasında çıkar çatışması bulunmamaktadır.

Introduction

Lipases (EC: 3.1.1.3) are frequently used for the hydrolysis and transesterication of oils, the production of biodiesel and the synthesis of esters from glycerol and fatty acids in industry [1]. Most of the lipases which are used for industrial purposes are in the immobilized state [2] as an immobilized biocatalyst has more advantages than the soluble lipases, such as repeated use, easy removal from the reaction mixture and the possibility of continuous operation [3]. Therefore, immobilization of enzymes and especially lipases is currently a very attractive research topic.

There are several methods for lipase immobilization, including adsorption, covalent bonding, entrapment, encapsulation, and cross-linking. The most widely used immobilization method for lipases is adsorption [1]. Although the adsorption method is very easy, fast, and cheap, it has drawbacks such as weak interaction between the enzyme molecules and the support, low binding capacity, and loss of activity due to leakage of the enzyme from the support during operation in industrial applications.

Covalent immobilization is one of the ways to overcome these problems [4]. The main advantages of covalent immobilization of enzymes are the achieved high operational and storage stability achieved, since enzyme molecules which are attached to the support via strong interactions, cannot leach from the support during industrial processes. Furthermore, the enzyme is no longer leached out from the immobilization support thereby resulting in purer products.

Oxirane-activated resins are one of the most compatible carriers for the covalent immobilization of enzymes. The immobilization process is quite simple and can be performed in literally any laboratory [5]. Here we used Amberzyme beads as they have strong mechanical stability and provide high immobilization yields and immobilized activities for enzymes. Amberzyme was already used succesfully for the immobilization of various industrially-relevant enzymes such as penicillin G acylase, His-tagged D-amino acid oxidase, and *Candida antarctica* lipase B [6,7].

The lipase from *Pseudomonas fluorescens* is a very interesting heat-resistant biocatalyst that is currently employed in many esterification and transesterification reactions [8]. The methods already used for the immobilization of *Pseudomonas fluorescens* lipase include physical adsorption [9], chemical adsorption [10], entrapment [11], sol-gel encapsulation [12] and covalent attachment [13].

Although the activity of covalently-immobilized enzymes is generally lower than the activity of the soluble enzymes, it was for instance reported that covalent immobilization onto Eupergit C resulted in a higher activity than the activity of soluble enzymes [14]. Here we report the covalent immobilization of *Pseudomonas fluorescens* lipase onto Amberzyme with high immobilization and activity yields.

Materials and Methods

Materials

Pseudomonas fluorescens lipase and p-nitro phenyl acetate (pNPA) were purchased from Sigma-Aldrich Chemical Co. Amberzyme (235 μm particule diameter and 220 Å pore size) was a gift from the Rohm and Haas Company (Philadelphia, USA). 4-nitro phenol (pNP) was acquired from Fluka. Methanol was bought from Lab-Scan and 1,4-dioxane was purchased from Acros Organics. A BCA protein assay kit was bought from Thermo Fisher Scientific Inc (Rockford, USA). This work was carried out at the laboratory of Department of Polymer Chemistry at the Zernike Institute for Advanced Materials at the University of Groningen in The Netherlands.

Immobilization procedure

Immobilization was carried out by reacting Amberzyme (400 mg) with *Pseudomonas fluorescens* lipase (3.87 mg in 200 µL distilled water) in a phosphate buffer (5 mL, 1.0 M, pH 9.0) at 25 °C with gentle shaking (150 rpm) for 24 hours. Beads with the immobilized enzyme were obtained after filtering and washing with phosphate buffer (5 mL, 0.1 M, pH 9.0) and plenty of acetone on a sintered glass filter by vacuum suction. The immobilized enzymes were stored at 4 °C in dried form in a stoppered bottle until used.

Optimization of immobilization procedure

Optimum conditions of immobilization were determined by individually changing the conditions (the amount of Amberzyme from 100 to 500 mg, pH from 5.0 to 9.0, buffer concentration from 0.25 to 1.0 M and immobilization time from 24 to 120 hours).

Determination of protein amounts

Before and after immobilization, the amounts of protein in the immobilization buffer and washing solutions were determined according to the BCA Assay Method [15] by UV/Vis (PYE UNICAM SP8-200 UV/Vis spectrophotometer) at the λ_{max} (562 nm) of a BCA/copper complex. Bovine Serum Albumin (BSA) is used as the protein assay standard in the protein assay.

Determination of activity

pNPA (40 mM) and methanol (80 mM) in 1,4-dioxane (5mL) were reacted with agitation at 150 rpm with 3.87 mg free or immobilized lipase at 25 °C for 30 min in an incubator. The reaction mixture (10µL) was added to 1,4-dioxane (2 mL) and was immediately used for the determination of pNP by UV/Vis (PYE UNICAM SP8-200 UV/Vis spectrophotometer) at λ_{max} (304 nm) of *p*NP. Hydrolytic activities for powdered *Pseudomonas fluorescens* and *Pseudomonas fluorescens* immobilized

on Amberzyme are defined herein as the micromoles of *p*NP produced in 1,4-dioxane per unit of weight of enzyme per time (μmol of *p*NP/min.mg).

Calculation of immobilization and activity yields

Immobilization yields were calculated using the formula:

$$\text{Immobilization Yield} = \frac{\text{mg of bounded lipase}}{\text{mg of powdered lipase used for immobilization}} \times 100$$

Activity yield was calculated using the formula:

$$\text{Activity Yield} = \frac{\text{Relative Activity of immobilized lipase}}{\text{Relative Activity of powdered lipase used for immobilization}} \times 100$$

Operational and storage stability

Operational stability tests were carried out by repeating 20 batch experiments using the method for activity determination outlined above. Storage stability was tested for 20 days by determining the activity every other day with the activity assay method outlined above and the immobilized enzymes stored at 4 °C in a stoppered bottle in dried form until the next usage.

Results

Optimization of immobilization procedure

Effect of the amount of Amberzyme

The effect of the amount of Amberzyme (100–500 mg) on the immobilization efficiency of *Pseudomonas fluorescens* lipase (3.87 mg) was studied. Table 1 shows that the amount of immobilized enzyme is increased with an increasing amount of immobilization support and the highest immobilization yield (57.5±0.3%) was obtained for 500 mg of Amberzyme. Furthermore, the activity yield also increased with an increasing amount of immobilization support. The highest activity yield (84±1.2%) was obtained at a maximum of 400 mg with a decrease in activity yield at higher concentrations of Amberzyme.

Effect of pH of immobilization buffer

Table 2 shows that the highest immobilization yield (70.7±0.6%) and activity yield (103±1.4%) was achieved when immobilizing the enzyme at pH 9.0.

Effect of molarity of immobilization buffer

Table 3 shows that the highest immobilization and activity yields are 80.9±0.2% and 122±1.1% respectively in phosphate buffers of 0.75 M.

Effect of immobilization time

Immobilization time is an important variable in the immobilization process. In Table 4 it becomes obvious that while the immobilization yield changed from 80.9±0.2% to 96.0±0.6%, the activity yield changed from 122±1.1% to 139±1.2% by increasing the immobilization time from 24 to 96 hours. 100±0.4% immobilization yield and 145±1.4% activity yield were obtained at 120 hours. At the end, 3.87 mg *Pseudomonas fluorescens* lipase was immobilized to 400 mg Amberzyme by optimizing the immobilization conditions. Therefore, enzyme loading is 9.68 mg/1g Amberzyme.

Determination of protein amounts

Protein amounts in the buffered lipase solutions (0.1 M, pH 7) before immobilization were found to be 3.87 mg in the 40 mg powdered lipase. According these results, 100 mg powdered lipase preparation contains 9.68 mg protein.

Determination of activity

After pNPA (40 mM) and methanol (80 mM) in 1,4-dioxane (5mL) was reacted with agitation at 150 rpm with same amounts free and immobilized lipase at 25 °C for 30 min in an incubator, pNP amounts with free and immobilized lipase were found to be 23.5 and 34.1 μmol respectively. Therefore, the relative activity of immobilized lipase calculated as 145±1.4%.

Table 1. Effect of Amberzyme Amount on Immobilization and Activity Yields.

Amberzyme (mg)	Immobilization Yield* (%)	Activity Yield** (%)
100	47.4±0.4	71±1.1
200	52.1±0.6	76±1.3
300	55.4±0.7	81±1.5
400	56.4±0.5	84±1.2
500	57.5±0.3	81±1.4

***** 3.87 mg dissolved enzyme in 200 μL 0.1 M phosphate buffer (pH 7.0) were reacted with different amounts of supports in 5mL of phosphate buffer (1.0 M, pH 7.0) at 150 rpm and room temperature in an incubator for 24 hours.

****** Free (3.87mg) and dry immobilized enzymes were reacted with 5 mL substrate solutions (40 mM p-nitro phenyl acetate and 80 mM methanol in 1,4-dioxane) at 150 rpm and room temperature in an incubator for 30 minutes.

Table 2. Effect of pH on Immobilization and Activity Yields.

pH	Immobilization Yield* (%)	Activity Yield** (%)
5	65.7±0.8	55±1.3
6	58.0±0.3	81±1.5
7	56.4±0.5	84±1.2
8	62.6±0.4	73±1.6
9	70.7±0.6	103±1.4

* 3.87 mg dissolved enzyme in 200 µL 0.1 M phosphate buffer (pH 7.0) were reacted with 400 mg support at different pHs of 5 mL phosphate buffer (1.0 M) at 150 rpm and room temperature in an incubator for 24 hours.
** Free (3.87mg) and dry immobilized enzymes were reacted with 5 mL substrate solutions (40 mM p-nitro phenyl acetate and 80 mM methanol in 1,4-dioxane) at 150 rpm and room temperature in an incubator for 30 minutes.

Table 3. Effect of Immobilization Buffer Concentration on Immobilization and Activity Yields.

Concentration of Immobilization Buffer	Immobilization Yield* (%)	Activity Yield** (%)
0.25	78.9±0.7	41±1.6
0.5	77.1±0.4	63±1.3
0.75	80.9±0.2	122±1.1
1.0	56.4±0.5	103±1.5

* 3.87 mg dissolved enzyme in 200 µL 0.1 M phosphate buffer (pH 9.0) were reacted with 400 mg of support at different concentrations of 5mL phosphate buffer (pH 9.0) at 150 rpm and room temperature in an incubator for 24 hours.
** Free (3.87mg) and dry immobilized enzymes were reacted with 5 mL substrate solutions (40 mM p-nitro phenyl acetate and 80 mM methanol in 1,4-dioxane) at 150 rpm and room temperature in an incubator for 30 minutes.

Table 4. Effect of Immobilization Duration on Immobilization and Activity Yields.

Duration of Immobilization (hours)	Immobilization Yield* (%)	Activity Yield** (%)
24	80.9±0.2	122±1.1
48	86.2±0.5	127±1.5
72	92.7±0.2	135±1.3
96	96.0±0.6	139±1.2
120	100.0±0.4	145±1.4

* 3.87 mg dissolved enzyme in 200 µL 0.1 M phosphate buffer (pH 7.0) were reacted with 400 mg of supports in 5mL of phosphate buffer (0.75 M, pH 9.0) at 150 rpm and room temperature in an incubator for 24 hours.
** Free (3.87mg) and dry immobilized enzymes were reacted with 5 mL substrate solutions (40 mM p-nitro phenyl acetate and 80 mM methanol in 1,4-dioxane) at 150 rpm and room temperature in an incubator for 30 minutes.

Operational and storage stabilities

The operational and storage stability of *Pseudomonas fluorescens* lipase immobilized on Amberzyme at pH 9, buffer concentration of 0.75 M and for 120 hours was tested. The immobilized enzyme retained its activity during 20 consecutive batch reactions, each lasting for 30 min at 25 ◦C. The immobilized enzyme was also able to retain its initial activity for 20 days when used every second day.

Discussions

In the optimization of the amount of Amberzyme, the amounts of immobilized enzyme and activity yield were increased with an increasing amount of immobilization support but highest activity yield was obtained for 400 mg of Amberzyme. Similar behavior was previously reported for Eupergit C immobilization supports in which the lower activity yield at higher support amounts was explained by multiple attachments and reactions with groups associated and/or close to the active site and those responsible for the tertiary structure of the enzymes [5].

Immobilized *Pseudomonas fluorescens* lipase showed a maximum activity at pH 9.0. Although oxirane groups can react with various reactive groups of the enzymes in a wide pH range (1–12), immobilization of many enzymes resulted in the highest yield at their respective optimum pH range [5]. It can therefore be expected that the optimum pH condition to immobilized *Pseudomonas fluorescens* lipase is around pH 9 [16]. This is also in accordance with the optimum pH ranges found for the immobilization of *Pseudomonas fluorescens* lipase onto other supports [17,18].

The highest immobilization and activity yields were achieved at 0.75 M immobilization buffer concentration. It is known that the buffer concentration and salts such as ammonium sulphate influence the immobilization efficiency considerably in immobilization protocols using supports containing oxirane groups [19] and this becomes obvious in our experiments, as well. The dependency of enzyme efficiency on the buffer concentration and concentration and type of salts can easily be understood by considering the immobilization mechanism. It is assumed that, in the first step, the enzyme is physically adsorbed on the carrier by hydrophobic interactions. This brings amino and thiol groups on the surface of the enzyme in close proximity to the oxirane groups of the carrier. In the second step they react with the oxirane groups by nucleophilic attack. In this way, very stable C−N and C−S bonds are formed [20].

While the immobilization yield changed from 80.9±0.2% to 96.0±0.6%, the activity yield changed from 122±1.1% to 139±1.2% by increasing the immobilization time from 24 to 96 hours. It can be concluded that an immobilization time of 120 hours is enough to immobilize nearly all lipase molecules from solution onto the Amberzyme support. It is known that immobilization of enzymes with epoxy-activated supports at 20-25 °C and the longer incubation time lead to the improvement of the formation of new covalent linkages between the enzymes and the support. In this way more stable immobilized biocatalysts are produced, leading to a remarkably higher activity as compared to free lipase (145±1.4%), as well as very high operational and storage stability [20]. The current study was performed with just one type of immobilization support. However, it is known that the success of immobilization (yield and activity) strongly depends on variables such as particle size and the pore size of the support [4].

The highest immobilization yields and activity yields were achieved by immobilizing *Pseudomonas fluorescens* lipase onto Amberzyme at pH 9, at room temperature (25 °C) for 120 hours. There are two studies concerning to covalent immobilization of *Pseudomonas fluorescens* lipase in the literature, but immobilization and activity yields lover than that corresponding free lipase. Our resulst obtained in the present study are the best in the covalent immobilization of *Pseudomonas fluorescens* lipase. On the other hand the immobilization method used in other studies in the literature is mostly via adsorption. The adsorption method has some disadvantages such as low enzyme loading to the support, low operational and storage stability and leakage of enzyme from support during reaction and therefore immobilized enzymes quickly lose their activities. Whereas in the covalent immobilization of enzymes, enzyme loading is high than in the adsorption method, operational and storage stability are very high and therefore immobilized enzymes don't lose their activities during long time of operations. For the production of biodiesel, hydrolysis of oils and various important esterification reactions covalently immobilized *Pseudomonas fluorescens* lipase will certainly be more efficient biocatalyst. Our future studies will focus on using the immobilized *Pseudomonas fluorescens* lipase for the production of biodiesel, hydrolysis of oils and various important esterification reactions.

Acknowledgements

The authors acknowledge Rohm and Haas Company for their generous gift by providing Amberzyme. Y.A. also acknowledge The Council of Higher Education of Turkey for their financial support.

Conflict of interest: The authors declare that there was no conflict of interest in this work.

References

[1] Tan T, Lu J, Nie K, Deng L, Wang F. Biodiesel production with immobilized lipase: A review. Biotechnol Adv 2010; 28:628-634.

[2] Ursoiu A, Paul C, Marcu C, Ungurean M, Péter F. Double immobilized Lipase for the Kinetic Resolution of Secondary Alcohols. World Academy of Science, Engeering and Technol 2011; 76:70-74.

[3] Miletic N, Vukovic Z, Nastasovic A, Loos K. Macroporous poly(glycidyl methacrylate-co-ethylene glycol dimethylacrylate) resins – versatile immobilisation supports for biocatalysts. J Mol Cat B 2009; 56:196-201.

[4] Mileti? N, Loos K. Over-stabilization of chemically modified and cross-linked candida antarctica lipase B using various epoxides and diepoxides. Australian J Chem 2009; 69:799-805.

[5] Katchalski-Katzir E, Kraemer DM. Eupergitw C, A carrier for immobilization of enzymes of industrial potential. J Mol Cat B 2000; 10:157-176.

[6] Liu SL, Wei DZ, Song QX, Zhang YW, Wang XD. Effect of organic cosolvent on kinetic resolution of tert-leucine by penicillin G acylase from Kluyvera citrophila. Bioprocess Biosyst Eng 2006; 28:285-289.

[7] Chen B, Hu J, Miller EM, Xie W, Cai M, et al. Candida antarctica lipase B chemically immobilized on epoxy-activated micro- and nanobeads: catalysts for polyester synthesis. Biomacromol 2008; 9:463-471.

[8] Saxena RK, Ghosh PK, Gupta R, Davidson WS, Bradoo S, et al. Microbial lipases: Potential biocatalysts for the future industry. Curr Sci 1999; 77 (1):101-115.

[9] Iso M, Chen B, Eguchi M, Kudo T, Shrestha S. Production of biodiesel fuel from triglycerides and alcohol using immobilized lipase. J Mol Cat B 2001; 16:53-58.

[10] Salis A, Bhattacharyya MS, Monduzzi M, Solinas V. Role of the support surface on the loading and the activity of Pseudomonas fluorescens lipase used for biodiesel synthesis. J Mol Cat B 2009; 57:262-269.

[11] Zarcula C, Croitoru R, Corîci L, Csunderlik C, Peter F. Improvement of lipase catalytic properties by immobilization in hybrid matrices. Proceedings of World Academy of Science, Eng and Technol 2009; 52:179-184.

[12] Salis A, Casula MF, Bhattacharyya MS, Pinna M, Solinas V, et al. Physical and chemical lipase adsorption on SBA-15: effect of different interactions on enzyme loading and catalytic performance. ChemCatChem 2010; 2:322-329.

[13] Gorokhova IV, Ivanov AE, Zubov VP. Coprecipitation of Pseudomonas fluorescens lipase with hydrophobic compounds as an approach to its immobilization for catalysis in nonaqueous media. Russ J Bioorg Chem 2002; 28 (1):38-43.

[14] Aslan Y, Tanrıseven A. Immobilization of Pectinex Ultra SP-L to produce galactooligosaccharides. J Mol Cat B 2007; 45:73-77.

[15] Smith PK, Krohn RI, Hermanson GT, Mallia AK, Gartner FH, et al. Measurement of protein using bicinchoninic acid. Anal Biochem 1985; 150 (1):76-85.

[16] Kulkarni N, Gadre RV. Production and properties of an alkaline, thermophilic lipase from Pseudomonas fluorescens NS2W. J Ind Microb Biotechnol 2002; 28 (6):344-8.

[17] Santos JC, Paula AV, Nunes GFM, de Castro HF. Pseudomonas fluorescens lipase immobilization on polysiloxane-polyvinyl alcohol composite chemically modified with epichlorohydrin. J Mol Cat B 2008; 53 (2):49-57.

[18] Boscolo B, Trotta F, Ghibaudi E. High catalytic performances of Pseudomonas fluorescens lipase adsorbed on a new type of cyclodextrin-based nanosponges. J Mol Cat B 2010; 62 (2):155-161.

[19] Martin MT, Plou FJ, Alcade M, Ballasteros A. Immobilization on Eupergit C of cyclodextrin glucosyltransferase (CGTase) and properties of the immobilized biocatalyst. J Mol Cat B 2003; 21:299-308.

[20] Boller T, Meier C, Menzler S. Eupergit oxirane acrylic beads: How to make enzymes fit for biocatalysis. Org Process Res Develop 2002; 6 (4):509-519.