doi:10.2533/chimia.2009.345 Chimia 63 (2009) 345–348

In situ Product Recovery Integrated with Biotransformations

Matthias Bechtold and Sven Panke*

Abstract: Biocatalysis constitutes an effective tool for the production of fine chemicals. In order to widen the spectrum of applicable reaction types to reactions that are constrained by inhibitions, product toxicity, or degradation, an unfavorable position of the thermodynamic equilibrium, or by kinetic control, *in situ* product removal (ISPR) is an attractive process option to overcome those limitations. To fully exploit the benefits of the ISPR approach, selective removal of the product to an auxiliary phase with high capacity is usually required. Obviously, such an operation becomes increasingly difficult with decreasing differences in the physical properties of substrate(s) and product(s) as it is arguably frequently the case with biotransformations. In this paper we analyze the possibilities to apply ISPR to biotransformations and identify the most promising developments supported by simple model considerations to fully exploit the potential of ISPR.

Keywords: Biotransformations · *In situ* product removal · Product inhibition · Product toxicity · Thermodynamic equilibrium

Introduction

The potential of biocatalysis is unmatched in terms of reaction selectivity and mild reaction conditions, but frequently hampered by interference of an accumulating product with the biocatalyst. A straightforward process option to reduce this problem is the quick removal of formed product from the aqueous phase in which it was produced – *in situ* product removal (ISPR). ISPR is a well-established concept in bioprocessing,[1–5] but rarely applied on industrial scale.[6] One reason for this is the increase in process complexity that is required by the additional processing steps, which is particularly difficult to justify when additional requirements such as mono-septical operation of the bioprocess needs to be ensured.

The complexity argument becomes much less important when looking at biotransformation processes, in which only one or a few enzymes are operated as catalysts and the requirements for mono-septical operation are much less strict.[7] In addition, enzymes as catalysts for a variety of reactions are increasingly easy to obtain from dedicated suppliers. These enzymes can then also be adapted rather efficiently to specific process requirements such as increased stability by directed evolution.[8] As a result, more and more biotransformations can be quickly implemented and operated as very robust industrial processes.

This development is accompanied by a re-evaluation of the role of process design even in the fine chemical and pharmaceutical industry, where the requirement to develop processes rapidly can be paramount. Developments in microfluidics and miniaturization in general and the accompanying enabling of parallelized process research makes it possible to quickly acquire the data necessary to design more complicated processing options, such as continuous processing.[9–11] We argue that these developments, biocatalyst availability and stability and accelerated process research, support a new look at the potential of ISPR in biotransformations.

In this article we present a dedicated overview of the current state of ISPR-biotransformation research. A central problem in ISPR is the frequent lack of selectivity of the applied auxiliary phases. We systematically analyze the effect of selectivity on product yield by basic model considerations. Based on this, we propose a number of process schemes that in principle are generically applicable and provide strong separation capacity even for difficult-to-separate mixtures typical for biotransformations.

*Correspondence: Prof. Dr. S. Panke
ETH Zürich
Department of Biosystems Science and Engineering (D-BSSE)
Mattenstrasse 26
CH-4058 Basel
Tel.: +41 61 387 3209
Fax.: +41 44 632 1993
E-mail: sven.panke@bsse.ethz.ch

Overview of ISPR Activities

A literature survey indicates roughly fifty different biotransformation processes with integrated product removal, most of them using hydrolases (class 3) or oxidoreductases (class 1) as biocatalyst, but also isomerizations (class 5) and C–C bond formations (class 4) have been considered. ISPR was realized in different process configurations that can be schematically represented by the basic cases depicted in Fig. 1. In practice, the applied process schemes can differ significantly and incorporate multiple consecutive separation steps. The applied product removal techniques exploit differences in hydrophobicity (adsorption, extraction, perstraction), volatility (distillation, evaporation, pervaporation), and to a lesser extent differences in charge (reactive extraction, electrodialysis), size (nanofiltration, molecular sieves) or specific binding properties (complexation). Specific process options tend to cluster around specific enzyme classes, as the substrates that are treated with a specific enzyme class share frequently similar physicochemical features.

Class 1, Oxidoreductases

In many cases the required cofactor regeneration for redox-biotransformations, usually NAD(P)H regeneration, is carried out easiest in whole cells, *e.g.* by glucose conversion. For this, it is important that product and/or substrate of the biotransformation are either not toxic to the cell or are rapidly removed from the reaction phase. As many of the substrates to which in particular oxygenases were applied are rather

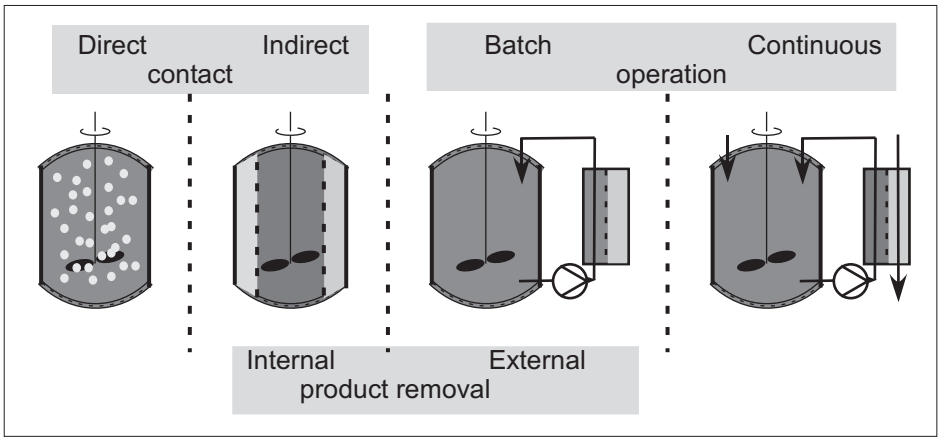

Fig. 1. Basic configuration schemes of ISPR according to direct/indirect contact of auxiliary phase and biocatalyst, internal/external separation loop, batch/continuous operation.

hydrophobic, this can be achieved by adding an apolar phase with a logP roughly higher than 4 which serves as a reservoir for an apolar toxic substrate and as a receiving phase for an apolar toxic product.[12–22] Alternatively, the availability of a toxic substrate can be limited by feeding and a toxic product can be subsequently adsorbed to a solid phase.[12,23–25] In the same context, integration of continuous selective crystallization has been demonstrated successfully for the industrially relevant synthesis of hydroxynicotinate[18] and was proposed as a generic method for various biotransformations.[7,26] In enantioselective reductions, the reduction and then the oxidation reaction for cofactor regeneration are frequently thermodynamically limited, reducing the possible yield. This can be overcome by the removal of the oxidation product. Recently, a very elegant one enzyme-solution for overcoming the yield limitation was suggested: in the required oxidation step, isopropanol was converted to acetone, which could be easily removed by stripping or pervaporation.[27–29]

Class 3, Hydrolases

In biotransformations, hydrolases are applied in both directions, hydrolysis and condensation. Reactions from both directions have benefited from ISPR. It has been applied to overcome product inhibition and solubility problems in the hydrolysis of some bulk compounds such as oligosaccharides or esters[30–32] but also for enantioselective resolution of racemic mixtures.[33–37] In the latter case, ISPR was realized by applying reactive membranes. These membranes incorporate the enzyme and immobilize it. The substrate, usually fed from a hydrophobic organic phase, is converted in the membrane and then partitions into an aqueous phase at the opposite side of the membrane.[34,35,38]

ISPR was also proposed for the hydrolysis of penicillin G to 6-aminopenicillic acid (6-APA) and phenylacetic acid. The removal of phenylacetic acid by several means facilitated the next reaction step, a conversion of 6-APA with an activated substrate to a semisynthetic β-lactam antibiotic, which is usually severely inhibited in the presence of phenylacetic acid.[39–43] ISPR is particularly interesting when hydrolases are used in the synthetic direction, because here the enzymes have to operate against the thermodynamic equilibrium if they are in an aqueous phase. This can be achieved under reverse hydrolysis conditions in a thermodynamically controlled reaction or by exploiting the transferase activity of hydrolases in a process under kinetic control. The latter scenario refers to the situation in which an activated substrate is attached as a covalent intermediate to the hydrolase and then transferred to the second substrate, yielding the condensation product. However, the product is also intrinsically subject to hydrolysis by the same enzyme. If the transferase activity is greater than the hydrolytic activity of the enzyme, the product is formed in excess in the beginning of the reaction. Later, product concentration decreases to the hydrolysis equilibrium value when thermodynamic control becomes more important.

Reverse hydrolysis and kinetic control are typically encountered in the synthesis of esters by lipases, glycosides or oligosaccharides by glycosidases, or the preparation of β-lactams by penicillin amidase.[32,44,45] A large variety of integration schemes were realized for both cases that relied on the typically significant differences in hydrophobicity or volatility between the condensation product and starting materials. Removal schemes include external[46–50] and internal[51,52] adsorption, reactive chromatography,[53,54] extraction,[55] perstraction,[47,49,56] distillation/pervaporation,[6,47,57–59] as well as complexation[52,60,61] and size dependent ultrafiltration.[62]

Class 4 + Class 5 Enzymes, Isomerases, Aldolases, and Transferases

Finally, ISPR was applied to isomerizations and C–C bond formation. Both reactions suffer primarily from an unfavorable position of the thermodynamic equilibrium, but are otherwise very attractive for the synthesis of chiral intermediates.[63,64] For example, the coupling of an enzymatic epimerization of N-acetyl-glucosamine to N-acetyl-mannosamine and subsequent aldolase-catalyzed C–C bond formation with pyruvate constitutes an attractive option for neuraminic acid synthesis. Both reactions show an unfavorable position of thermodynamic equilibrium. Applying pyruvate in excess in order to increase the product yield on N-acetyl-glucosamine results in a difficult-to-separate mixture of neuraminic acid and pyruvate (similar pKa value).[65] Integration of reactive extraction using a pH-shift and two transfer agents (phenylboronic acid and triethylammonium chloride) yielded only a moderate improvement since the substrates are also extracted to a considerable extent.[66,67] Alternatively, separation of pyruvate and neuraminic acid was attempted using a permselective nanofiltration at enzyme-compatible pH yielding promising results – however, not in an integrated operation scheme.[68] Product removal in a direct contact internal batch configuration (Fig. 1) was reported for the synthesis of L-erythrulose by a C–C bond formation from β-hydroxypyruvate and glycoaldehyde[69] and the synthesis of phenylacetylcarbinol from inhibiting benzaldehyde and acetaldehyde or pyruvate.[70] In the former case, a boron-containing resin for selective complexation of the product *cis*-diol was applied as auxiliary phase[69] and in the latter an organic phase for controlled substrate supply and product removal.[71,72]

Another attractive ISPR option applied for isomerization,[73,74] racemization[75–77] or C–C bond formations[78,79] is the integration of the biotransformation step with SMB technology exploiting the unmatched separation capacity of chromatography (see below).

Basic Requirements for Successful ISPR

Many biotransformation-ISPR schemes suffer from a lack of a highly selective auxiliary phase that can efficiently separate starting materials and products.[50,66,69] In order to get a better understanding of how the purity and yield of the product in the auxiliary phase depend on the selectivity of the phase, basic batch process simulations for a typical thermodynamically limited reaction and a typical product inhib-

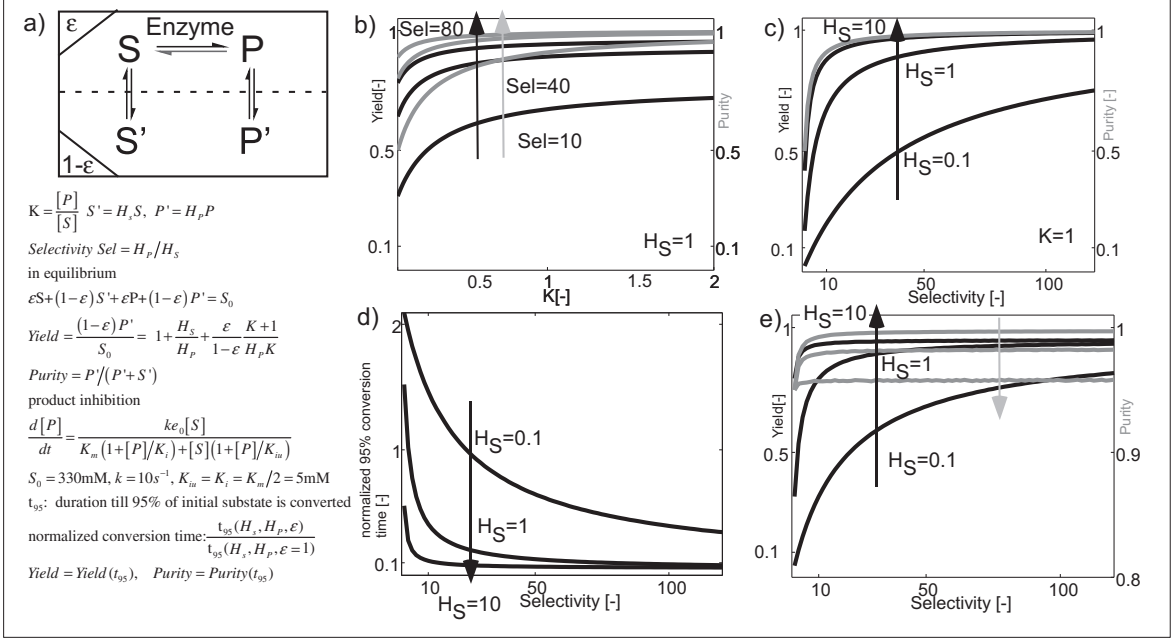

Fig. 2. a) Schematic representation of ISPR combined with batch conversion and equations used. b) Product yield in the auxiliary phase and purity as a function of the equilibrium constant in a thermodynamically limited reaction. c) As a function of the selectivity for a reaction with an equilibrium constant of one. d) Time period till 95% conversion is reached for a typical product inhibited reaction as a function of selectivity. e) Product yield in the auxiliary phase and purity as a function of the selectivity.

ited reaction with simultaneous ISPR were conducted (for details, see Fig. 2). For the sake of simplicity we consider an A to B type reaction that only takes place in the reaction phase which is in equilibrium with an auxiliary phase (Fig. 2a). Partitioning between the two phases is described by simple non-competitive linear isotherms and all non-ideal effects like mass transfer resistance are neglected. Further we assume that for further downstream processing only the auxiliary phase is processed, hence yield and purity refer only to the auxiliary phase.

Yield and purity of thermodynamically limited ISPR batch conversions increase with the equilibrium constant shifting toward the product side (Fig. 2b) and with increasing selectivity of the auxiliary phase (Fig. 2c). At constant selectivity, it is more advantageous to have an auxiliary phase with a rather high affinity for the starting material (Fig. 2c). By reducing residual substrate and product in the reaction phase the product yield in the auxiliary phase is strongly increased indicating an advantage for small reaction phase ratios.

Next, a typical product-inhibited reaction is considered.[80] We compare the situation for 95% conversion of supplied starting material with and without ISPR (auxiliary phase). A significant decrease in conversion time can be expected with increasing selectivity (Fig. 2d). Again, the yield can be significantly increased by using a highly selective auxiliary phase that shows affinity also for the substrate (Fig. 2e). It is worth noting that for product-inhibited reactions feeding strategies allow for increasing the conversion degree in the reactor and hence significantly lessen the demand for selective removal.[23]

Obviously, continuous removal of accumulated product in the auxiliary phase avoids equilibration with the reaction phase and results in higher yield for batch operation – but only in case of the substrate not being simultaneously removed. If the auxiliary phase is not highly selective, then the small differences in selectivity need to be amplified by multi-stage equilibrium operations (Fig. 3). This points clearly in the direction of continuous operation of the product removal, which re-inforces the argument for operating the overall process continuously, which brings many advantages with respect to productivity.

Future Trends

From the analysis it is clear that batch processes need a highly selective auxiliary phase. This need can be circumvented in a number of cases for product inhibition by reaction engineering: for example, feeding strategies, in which the substrate is completely converted before it is transferred to the auxiliary phase,[23] avoid the need for a selective phase. Alternatively, selectivity is also not an issue if the partitioning of both substrates and products into the auxiliary phase is very high (lower left corner of Fig. 2d[81]). Still, as a rule of thumb, for thermodynamically limited reactions the availability of highly selective auxiliary phases becomes crucial. For reverse hydrolysis reactions, this is frequently (but not always[50,60,66]) possible due to the strongly different physicochemical profile of substrates (*e.g.* acid and alcohol) and products (*e.g.* ester and water). However, in the case of isomerases, transferases, and aldolases the physicochemical differences between substrates and products are much smaller and it is very difficult to identify sufficiently selective auxiliary phases for batch operation.

By continuously removing the product, the yield-limitation for batch operation can be overcome and productivity much increased, as it is the case for continuous pervaporation.[57] However, again

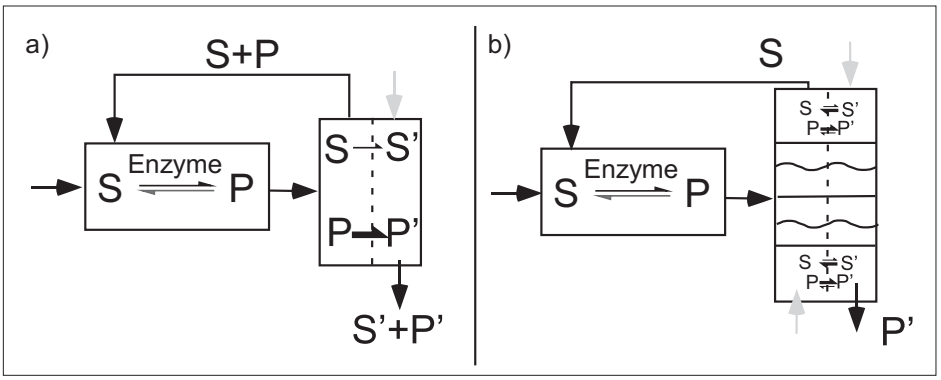

Fig. 3. a) Single-stage partial separation in an external loop configuration. b) Multi-stage complete separation in an external loop configuration.

this is only possible with a sufficiently selective auxiliary phase. The demand for a highly selective phase can be considerably lessened by using advanced technologies that can exploit small differences in selectivity for complete separation. This can be achieved by using multiple equilibrium stages that are characteristic for countercurrent extraction, membrane cascades[82,83] or chromatography.[84] First steps in implementing such technologies for isomerases, transferases, and aldolases have already been made by applying fractionating reactors[39,85] and implementing continuous chromatography namely SMB technology as external separator[76,78] or as SMB reactor.[76,86] These technologies have the potential to be applied as a generic method in ISPR schemes considering the vast selection of existing and the ongoing development of new powerful materials. In particular SMB chromatography is nowadays the industrial workhorse for the most difficult separations *e.g.* the separation of enantiomers using cost-intensive chiral stationary phases,[87] but can also operate with low-cost adsorber material depending on the separation task.[88] Therefore, we argue that the integration of advanced separation technologies opens up a large set of novel biotransformations, in particular in the area of isomerization and C–C bond formation, for industrial exploitation.

Received: March 9, 2009

[1] A. Bruggink, A. J. J. Straathof, L. A. M. van der Wielen, *Adv. Biochem. Eng. Biotechnol.* **2003**, *80*, 69.
[2] A. J. J. Straathof, *Biotechnol. Prog.* **2003**, *19*, 755.
[3] A. Freeman, J. M. Woodley, M. D. Lilly, *Bio-Technology* **1993**, *11*, 1007.
[4] K. Schugerl, J. Hubbuch, *Curr. Opin. Microbiol*. **2005**, *8*, 294.
[5] J. M. Woodley, M. Bisschops, A. J. J. Straathof, M. Ottens, *J. Chem. Technol. Biotechnol.* **2008**, *83*, 121.
[6] A. Liese, K. Seelbach, C. Wandrey, 'Industrial Biotransformations', Wiley-VCH, Weinheim Germany, **2006**.
[7] E. M. Buque-Taboada, A. J. J. Straathof, J. J. Heijnen, L. A. M. van der Wielen, *Appl. Microbiol. Biotechnol.* **2006**, *71*, 1.
[8] G. W. Huisman, D. Gray, *Curr. Opin. Biotechnol.* **2002**, *13*, 352.
[9] D. M. Roberge, B. Zimmermann, F. Rainone, M. Gottsponer, M. Eyholzer, N. Kockmann, *Org. Process Res. Dev.* **2008**, *12*, 905.
[10] T. Laird, *Org. Process Res. Dev.* **2007**, *11*, 927.
[11] G. J. Lye, P. Ayazi-Shamlou, F. Baganz, P. A. Dalby, J. M. Woodley, *Trends Biotechnol.* **2002**, *21*, 29.
[12] D. Stark, U. von Stockar, *Adv. Biochem. Eng. Biotechnol.* **2003**, *80*, 149.
[13] S. Brautigam, S. Bringer-Meyer, D. Weuster-Botz, *Tetrahedron-Asymmetry* **2007**, *18*, 1883.
[14] S. D. Doig, A. T. Boam, A. G. Livingston, D. C. Stuckey, *Biotechnol. Bioeng.* **1999**, *63*, 601.
[15] K. Furuhashi, M. Shintani, M. Takagi, *Appl. Microbiol. Biotechnol.* **1986**, *23*, 218.
[16] S. Harbron, B. W. Smith, M. D. Lilly, *Enzyme Microb. Technol*. **1986**, *8*, 85.
[17] K. Kawakami, T. Nakahara, *Biotechnol. Bioeng.* **1994**, *43*, 918.
[18] H. G. Kulla, *Chimia* **1991**, *45*, 81.
[19] D. J. Leak, P. J. Aikens, M. Seyedmahmoudian, *Trends Biotechnol.* **1992**, *10*, 256.
[20] J. Tramper, M. H. Vermue, *Chimia* **1993**, *47*, 110.
[21] J. T. Vicenzi, M. J. Zmijewski, M. R. Reinhard, B. E. Landen, W. L. Muth, P. G. Marler, *Enzyme Microb. Technol.* **1997**, *20*, 494.
[22] M. G. Wubbolts, J. Hoven, B. Melgert, B. Witholt, *Enzyme Microb. Technol.* **1994**, *16*, 887.
[23] M. Held, A. Schmid, H. P. E. Kohler, W. Suske, B. Witholt, M. G. Wubbolts, *Biotechnol. Bioeng*. **1999**, *62*, 641.
[24] M. Held, W. Suske, A. Schmid, K. H. Engesser, H. P. E. Kohler, B. Witholt, M. G. Wubbolts, *J. Mol. Catal. B Enzym.* **1998**, *5*, 87.
[25] R. M. Lynch, J. M. Woodley, M. D. Lilly, *J. Biotechnol*. **1997**, *58*, 167.
[26] E. M. Buque-Taboada, A. J. J. Straathof, J. J. Heijnen, L. A. M. Van Der Wielen, *Biotechnol. Bioeng*. **2004**, *86*, 795.
[27] K. Goldberg, K. Edegger, W. Kroutil, A. Liese, *Biotechnol. Bioeng.* **2006**, *95*, 192.
[28] K. Schroer, E. Tacha, S. Lutz, *Org. Process Res. Dev.* **2007**, *11*, 836.
[29] T. Stillger, M. Bonitz, M. Villela, A. Liese, *Chem. Ing. Tech.* **2002**, *74*, 1035.
[30] A. Akintoye, G. Ganetsos, P. E. Barker, *TransIChemE* **1991**, *69*, 35.
[31] M. T. Shieh, P. E. Barker, *J. Chem. Technol. Biotechnol.* **1996**, *66*, 265.
[32] A. L. Paiva, F. X. Malcata, *J. Mol. Catal. B Enzym.* **1997**, *3*, 99.
[33] W. J. Choi, C. Y. Choi, J. A. M. De Bont, C. Weijers, *Appl. Microbiol. Biotechnol*. **1999**, *53*, 7.
[34] W. S. Long, A. Kamaruddin, S. Bhatia, *J. Membr. Sci.* **2005**, *247*, 185.
[35] J. L. Lopez, S. A. Wald, S. L. Matson, J. A. Quinn, *Ann. N. Y. Acad. Sci.* **1990**, *613*, 155.
[36] S. L. Matson, J. A. Quinn, *Ann. N. Y. Acad. Sci.* **1986**, *469*, 152.
[37] A. Liese, K. Seelbach, C. Wandrey in 'Industrial Biotransformations', Wiley-VCH, **2006**.
[38] K. Sakaki, L. Giorno, E. Drioli, *J. Membr. Sci*. **2001**, *184*, 27.
[39] J. L. den Hollander, M. Zomerdijk, A. J. J. Straathof, L. A. M. van der Wielen, *Chem. Eng. Sci.* **2002**, 57.
[40] D. Rindfleisch, B. Syska, Z. Lazarova, K. Schugerl, *Process Biochem.* **1997**, *32*, 605.
[41] A. Bruggink, P. D. Roy, 'Synthesis of Beta-Lactam Antibiotics: Chemistry, Biocatalysis & Process Integration', **2001**, pp. 12–54.
[42] C. Schroen, J. L. Van Roon, H. H. Beefink, J. Tramper, R. M. Boom, *Desalination* **2009**, *236*, 78.
[43] Y. Jiang, H. Xia, C. Guo, I. Mahmood, H. Liu, *Biotechnol. Prog*. **2007**, *23*, 829.
[44] V. Kasche, *Enzyme Microb. Technol.* **1986**, *8*, 4.
[45] B. M. de Roode, A. C. R. Franssen, A. van der Padt, R. M. Boom, *Biotechnol. Prog.* **2003**, *19*, 1391.
[46] A. Trusek-Holownia, A. Noworyta, *J. Biotechnol*. **2007**, *130*, 47.
[47] B. M. de Roode, L. Oliehoek, A. van der Padt, M. C. R. Franssen, R. M. Boom, *Biotechnol. Prog*. **2001**, *17*, 881.
[48] B. M. de Roode, S. Peters, M. C. R. Franssen, A. van der Padt, A. de Groot, R. M. Boom, *Biotechnol. Bioeng*. **2001**, *72*, 568.
[49] B. M. de Roode, J. van Beek, A. van der Padt, M. C. R. Franssen, R. M. Boom, *Enzyme Microb. Technol*. **2001**, *29*, 513.
[50] M. A. Boon, K. van't Riet, A. E. M. Janssen, *Biotechnol. Bioeng.* **2000**, *70*, 411.
[51] S. Colombie, R. J. Tweddell, J. S. Condoret, A. Marty, *Biotechnol. Bioeng.* **1998**, *60*, 362.
[52] C. Schroen, V. A. Nierstrasz, R. Bosma, P. J. Kroon, P. S. Tjeerdsma, E. DeVroom, J. M. VanderLaan, H. M. Moody, H. H. Beeftink, A. E. M. Janssen, J. Tramper, *Biotechnol. Bioeng.* **2002**, *80*, 144.
[53] J. P. Meissner, G. Carta, *Ind. Eng. Chem. Res*. **2002**, *41*, 4722.
[54] P. Mensah, G. Carta, *Biotechnol. Bioeng*. **1999**, *66*, 137.
[55] M. Kataoka, M. Hirakata, K. Sakamoto, H. Yamada, S. Shimizu, *Enzyme Microb. Technol.* **1996**, *19*, 307.
[56] Y. Isono, K. Fukushima, G. Araya, H. Nabetani, M. Nakajima, *J. Chem. Technol. Biotechnol.* **1997**, *70*, 171.
[57] L. Gubicza, A. Kabiri-Badr, E. Keoves, K. Belafi-Bako, *J. Biotechnol.* **2000**, *84*, 193.
[58] N. Dörmö, K. Belafi-Bako, L. Bartha, U. Ehrenstein, L. Gubicza, *Biochem. Eng. J.* **2004**, *21*, 229.
[59] A. L. Paiva, D. van Rossum, F. X. Malcata, *Biotechnol. Prog.* **2003**, *19*, 750.
[60] F. Ahmed, A. Stein, G. J. Lye, *J. Chem. Technol. Biotechnol.* **2001**, *76*, 971.
[61] L. Yang, D. Z. Wei, Y. W. Zhang, *J. Chem. Technol. Biotechnol.* **2004**, *79*, 480.
[62] M. Masuda, Y. Kawase, M. Kawase, *J. Biosci. Bioeng*. **2000**, *89*, 119.
[63] W. D. Fessner, C. Walter, *Top. Curr. Chem.* **1996**, *184*, 94.
[64] H. Häusler, A. E. Stütz, *Top. Curr. Chem.* **2001**, *215*, 77.
[65] V. Zimmermann, H. G. Hennemann, T. Daumann, U. Kragl, *Appl. Microbiol. Biotechnol.* **2007**, *76*, 597.
[66] V. Zimmermann, U. Kragl, *Sep. Purif. Technol.* **2008**, *61*, 60.
[67] V. Zimmermann, I. Masuck, U. Kragl, *Sep. Purif. Technol*. **2008**, *63*, 129.
[68] W. R. Bowen, B. Cassey, P. Jones, D. L. Oatley, *J. Membr. Sci*. **2004**, *242*, 211.
[69] R. P. Chauhan, L. W. Powell, J. M. Woodley, *Biotechnol. Bioeng.* **1997**, *56*, 345.
[70] S. Panke, M. Wubbolts, *Curr. Opin. Chem. Biol.* **2005**, *9*, 188.
[71] B. Rosche, M. Breuer, B. Hauer, P. L. Rogers, *Biotechnol. Bioeng*. **2004**, *86*, 788.
[72] B. Rosche, V. Sandford, M. Breuer, B. Hauer, P. L. Rogers, *J. Mol. Catal. B Enzym.* **2002**, 109.
[73] K. Hashimoto, S. Adachi, H. Noujima, Y. Ueda, *Biotechnol. Bioeng.* **1983**, *25*, 2371.
[74] D. C. S. Azevedo, A. E. Rodrigues, *Chem. Eng. J.* **2001**, *82*, 95.
[75] M. Bechtold, M. Heinemann, S. Panke, *J. Chromatogr. A* **2006**, *1113*, 167.
[76] M. Bechtold, S. Makart, M. Heinemann, S. Panke, *J. Biotechnol.* **2006**, *124*, 146.
[77] M. Bechtold, S. Makart, R. Reiss, P. Alder, S. Panke, *Biotechnol. Bioeng*. **2007**, *98*, 812.
[78] S. Makart, M. Bechtold, S. Panke, *J. Biotechnol*. **2007**, *130*, 402.
[79] S. Makart, M. Bechtold, S. Panke, *Chem. Eng. Sci.* **2008**, *63*, 5347.
[80] A. Cornish-Bowden, 'Fundamentals of Enzyme Kinetics', Portland Press Ltd., London, **1995**.
[81] S. Panke, V. de Lorenzo, A. Kaiser, B. Witholt, M. G. Wubbolts, *Appl. Environ. Microbiol*. **1999**, *65*, 5619.
[82] S. S. Gunderson, W. S. Brower, J. L. O'Dell, E. N. Lightfoot, *Sep. Sci. Technol.* **2007**, *42*, 2121.
[83] E. N. Lightfoot, *Sep. Sci. Technol.* **2005**, *40*, 739.
[84] G. Guiochon, A. M. Katti, S. G. Shirazi, 'Fundamentals of Preparative and Nonlinear Chromatography', Academic Press, Boston, **1994**.
[85] J. L. den Hollander, A. J. Straathof, L. A. M. van der Wielen, *J. Chem. Technol. Biotechnol.* **2004**, *79*, 1025.
[86] Y. Zhang, K. Hidajat, A. K. Ray, *Biochem. Eng. J.* **2004**, *21*, 111.
[87] A. Rajendran, G. Paredes, M. Mazzotti, *J. Chromatogr. A* **2009**, *1216*, 709.
[88] H. Schmidt-Traub, 'Preparative Chromatography', Wiley VCH, Weinheim, **2005**.