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Synthesis of cephalexin in organic medium at high substrate concentrations and low enzyme to substrate ratio

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Abstract

The kinetically controlled synthesis of cephalexin (CEX) in ethylene glycol (EG) was previously optimized at moderate substrate concentrations and high enzyme to substrate ratio, obtaining yields close to stoichiometric. However, substrate concentrations were low and enzyme loads high enough for production purpose. The synthesis of cephalexin in 40% (v/v) ethylene glycol at 20 °C and pH 7.0 with glyoxyl-agarose immobilized penicillin acylase (GAPA) was studied at high substrates concentrations to the point of saturation and beyond. Phenylglycine methyl ester (PGME) was the acyl donor at a molar ratio of 3 with respect to nucleophile. At initially homogeneous conditions with nucleophile concentration close to its solubility and at low enzyme to substrate ratio, productivity increase eight times and specific productivity five times with respect to a control at moderate substrates concentrations and high enzyme to substrate ratio. At initially heterogeneous conditions with partially undissolved nucleophile and low enzyme to substrate ratio, increases in productivity and specific productivity were eleven and seven times, respectively. The biocatalyst was very stable under reaction conditions, so that a very high global productivity is anticipated, making the enzymatic process competitive with existing chemical synthesis.

Keywords: Penicillin acylase; Cephalexin; β-Lactam antibiotics; Enzymatic synthesis; Organic cosolvents

1. Introduction

Penicillin acylase has received considerable attention in recent years because it has surpassed its conventional use as hydrolase for the production of 6-amino penicillanic acid (6APA) from either penicillin G or V, to become a valuable biocatalyst to perform several reactions of organic synthesis [1]. Amongst them [2–4], the synthesis of derived penicillins and cephalosporins from the corresponding β-lactam nuclei and suitable acyl donors is of paramount importance for the pharmaceutical industry [5]. Synthesis can be performed under thermodynamic [6] or kinetic [7] control and in both cases the reduction of water activity in the reaction medium will improve it, by displacing the equilibrium towards synthesis, in the first strategy [8], or depressing the competing hydrolytic reactions, in the second [9,10]. Water miscible organic cosolvents, especially polyols, have proven to be suitable media to perform the synthesis of β-lactam antibiotics with immobilized penicillin acylase [11,12]. Rather harsh conditions for synthesis require robust biocatalysts; in this sense, substantial improvements have been reported recently for penicillin acylase, considering directed immobilization [13–15], aggregation [16–19], derivatization [20] and activation [21], as well as genetic manipulations involving site-directed mutagenesis and protein engineering [22,23].

Biocatalysis is becoming a viable alternative to conventional chemical synthesis of β-lactam antibiotics, but conversion yield and productivity are still to be improved [24]. Several strategies of reactor operation have been proposed to improve conversion yield and/or productivity. In situ product removal [25] or partition to a second phase [26], have been proposed to alleviate product hydrolysis and, in some cases, substantial improvements in yield have been reported

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[27]. Aqueous solution precipitate, consisting in keeping a saturated concentration of the nucleophile throughout the reaction by repetitive additions leading to product precipitation, has been successfully applied to the synthesis of ampicillin [28,29]; conversion yield was over 95%, which is the highest value reported for such antibiotic [30]. Synthesis in solid sate, a broad denomination for systems containing a low amount of water without the presence of any other solvent so that most of the volume of the reaction is occupied by the solid mixture of substrates and products [31], has also been applied for the synthesis of β-lactam antibiotics, although with a moderate success [32–34]. Recently, a 70% conversion yield was reported for the solid sate synthesis of ampicillin [35], which is a very encouraging result. Temperature and pH profiling has also proven to be a good strategy when diffusional restrictions are present and substantial increases in conversion yield have been obtained [36,37]. All these strategies have succeeded in obtaining high yields of synthesis; not much attention has been paid, however, to productivity which is a very relevant process parameter to assess.

We have previously reported the optimization of the kinetically controlled synthesis of cephalexin (CEX) in ethylene glycol (EG) medium at moderate substrates concentrations and high enzyme to substrate ratio, obtaining yields close to stoichiometric both with a commercial and with an in-house immobilized penicillin acylase [38,39]; such high yields were unattainable in fully aqueous medium. However, substrate concentrations were low and enzyme loads high enough, so that productivity and specific productivity were still low for being technologically competitive. Results are presented on the synthesis of CEX from 7-amino 3-desacetoxicephalosporanic acid (7ADCA) and phenylglycine methyl ester (PGME) in 40% (v/v) EG medium at 20 °C and pH 7, with glyoxyl-agarose immobilized penicillin acylase (GAPA) from recombinant *Escherichia coli* at high substrate concentrations to the point of saturation and beyond, under the hypothesis that this will favor enzyme kinetics, so increasing specific productivity.

2. Materials and methods

2.1. Materials

Penicillin acylase from *E. coli*, with 230 ± 30 IU/mL and 22 ± 2 mg/mL protein, was a product from Antibióticos S.A. (León, Spain), kindly provided by Dr. José Manuel Guisán (Instituto de Catálisis, CSIC, Madrid, Spain). The enzyme was centrifuged and dialysed prior to use and remained fully stable for more than a year stored at 5 °C. Agarose 10 BCL was from Iberagar (Coina, Portugal). Penicillin G potassium salt (PGK) was kindly provided by Natsus S.A. (Lima, Perú); 7-amino 3-desacetoxicephalosporanic acid, (*R*)-(−)-2-phenylglycine methyl ester hydrochloride (97% pure) and cephalexin hydrate were from Sigma Chemical Company Inc. (St. Louis, MO, USA); (*R*)-(−)-2-phenylglycine (PG) was from Aldrich (Milwaukee, WI, USA). EG, glycidol and all other reagents were analytical grade either from Sigma–Aldrich or Merck (Darmstadt, Germany).

2.2. Analysis

Hydrolytic activity of penicillin acylase was determined from initial rate data, analyzing 6APA formation as reported by Shewale et al. [40]. One international unit of activity (IU) was defined as the amount of penicillin acylase that catalyzes the hydrolysis of 1 μmol of PGK/min from 134 mM PGK in 0.1 M phosphate buffer pH 7.8 at 30 °C and 250 rpm. Substrates and products of synthesis were identified and analyzed by HPLC using a Shimadzu delivery system LC-10AS with a Shimadzu UV SPD-10AV UV–vis detector and a CBM-101 Shimadzu HPLC/PC integrator. The column used was a μ-Bondapack C_{18} (300 mm × 3.9 mm) from Waters (Milford, MA, USA). Samples were eluted isocratically with a sonicated mixture of 70% (v/v) 20 mM phosphate buffer pH 7.0 and 30% (v/v) methanol at a flow rate of 1 mL/min, and analyzed in the UV detector at 214 nm. Elution times were 2.8, 3.5, 6.3 and 12.7 min for 7ADCA, PG, CEX and PGME, respectively. Concentration of substrates and products were calculated from calibration curves using stock solutions.

2.3. Enzyme immobilization

Glyoxyl-agarose gel was prepared as reported by Guisán [41]. Penicillin acylase was immobilized in glyoxyl-agarose gel beads, based on the procedure described by Alvaro et al. [42], but using phenylacetic acid (PAA) instead of penicillin G sulfoxide as protecting agent during immobilisation; time of immobilization was extended to 20 h, determined as the optimum for biocatalyst stability [43]. The glyoxyl-agarose immobilized penicillin acylase was stored as a wet gel at 5 °C. No enzyme inactivation or leakage has been detected during prolonged storage.

2.4. Solubility of substrates and products

Solubility of 7ADCA, PGME, PG and CEX in 40% (v/v) EG was determined at varying pH and temperature. Substances were added to the point of exceeding saturation and kept under agitation at controlled temperature and pH. After 2 h, solids were removed and supernatant was assayed by HPLC. Solubilization beyond 2 h was in all cases negligible. Controls were run in parallel at the same conditions and for the same time with dissolved substances to determine any chemical degradation.

2.5. Synthesis of CEX with GAPA

Syntheses of CEX were conducted under kinetic control in 40% (v/v) EG medium using PGME as acyl donor and 7ADCA as the limiting substrate at pH 7.0 and 20 °C, at the previously selected PGME–7ADCA molar ratio of 3 [39]

and varying enzyme to substrate ratio (IU/mmol 7ADCA) and substrate concentrations, below and over the solubility limit of 7ADCA. Syntheses were performed batch-wise with temperature and pH control in 50 mL Pyrex glass reactors with a working volume of 30 mL, equipped with a paddle impeller to keep biocatalyst particles in suspension, and a bottom sintered glass filter to recover the biocatalyst after each run. Samples were taken at intervals and were properly diluted prior to be assayed by HPLC. Molar conversion yield (Y) was defined as the maximum molar conversion of 7ADCA into CEX. Productivity was determined as the amount of CEX produced per unit time and unit reaction volume at maximum yield (mM/h). Specific productivity was determined as the amounts of CEX produced per unit time, unit reaction volume and unit mass of biocatalyst at maximum yield (mM/h g). Experiments were done in duplicate and samples assayed in triplicate with variations below 5% among them.

2.6. Operational stability of GAPA

Operational stability of the biocatalyst was determined by measuring residual activity after each batch. The reactor was emptied through its bottom filter, the biocatalyst was thoroughly washed to remove the precipitated products (PG and eventually CEX) and the biocatalyst assayed for residual activity. In one case, a series of 10 consecutive batches was performed to asses the long-term operation of a sequential batch system; in that case, the reactor was again filled with reaction mixture and the operation repeated. Maximum conversion yield and time to attain it was recorded in each batch. Accumulated productivity, P, was defined as:

$$P = \sum_{i=1}^{n} P_i = \sum_{i=1}^{n} \frac{c_i}{t_i}$$

where P_i is the productivity in batch i, n is the number of batches, c_i is the concentration of product at maximum conversion yield in batch i and t_i is the time to attain it. Accumulated specific productivity within one cycle of biocatalyst use is:

$$P_{SP} = \sum_{i=1}^{n} P_{SP,i} = \frac{\sum_{i=1}^{n} c_i/t_i}{m_{cat}}$$

where m_{cat} is the mass of biocatalyst loaded in the reactor.

3. Results and discussion

3.1. Solubility of substrates and products

Solubility of 7ADCA, CEX and PG at 40% (v/v) EG is presented in Fig. 1 at different pHs and temperatures. Solubility of 7ADCA and CEX was highly dependent on pH being much higher at higher pH; a similar trend was reported for 6APA and ampicillin [29]. Solubility of PG was hardly affected by pH as also reported by Youshko et al. [29]. As expected, solubility increased with temperature in all cases, but the effect was mild except for the case of CEX at high pH. Solubility of PGME (not reported) largely exceeds the solubility of 7ADCA; PGME was in all cases completely soluble at a molar ratio of 3 with respect to the saturation concentration of 7ADCA. Solubility of PG was low, between 15 and 30 mM in the whole range of pH (6.5–7.5) and temperature (0–20 °C) tested, which is in agreement with the value of about 35 mM reported by Youshko et al. at varying pH and 25 °C [29]. PG precipitation during synthesis is therefore inevitable, even at moderate substrate concentrations. Solubility of 7ADCA was substantially increased by the presence of PGME, so homogeneous solutions could be obtained at concentrations exceeding its saturation as pure compounds. As an example, solubility of 7ADCA at pH 7.0 and 20 °C in 40% (v/v) EG was increased by 180% when a three times molar concentration of PGME was added. A similar effect has been reported by Youshko et al. for the solubility of 6APA in the presence of PGME [29].

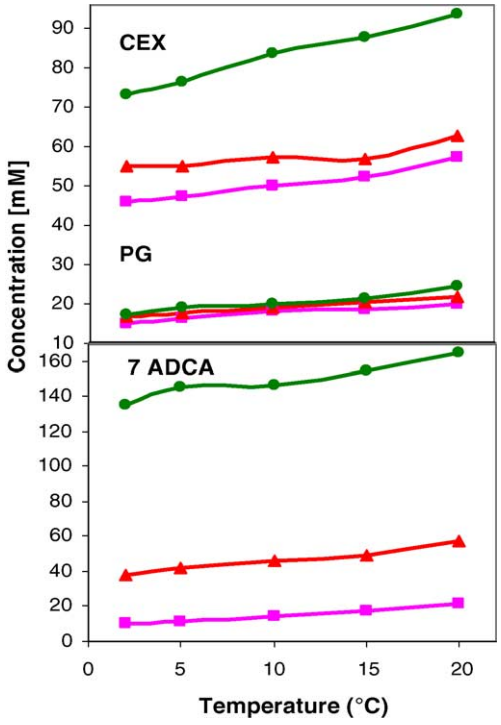

Fig. 1. Solubility of 7ADCA, CEX and PG at different temperatures at pH 7.5 (●); 7.0 (■) and 6.5 (▲) in 40% (v/v) EG medium.

3.2. Synthesis of CEX with GAPA at high substrates concentrations

3.2.1. At initially homogeneous conditions

Considering the conditions determined as optimum for the synthesis of CEX under kinetic control with GAPA at low substrate concentrations [38] and the solubility of substrates above reported, the following conditions were selected for the synthesis at high substrate concentrations: pH 7.0, 20 °C and

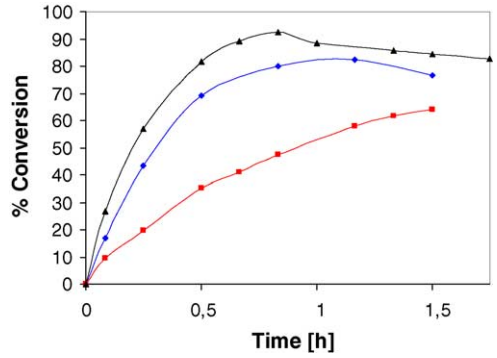

Fig. 2. Synthesis of CEX at 20 °C, pH 7.0 and 40% (v/v) EG and PGME–7ADCA molar ratio of 3 at: 30 mM 7ADCA and 240 IUH/mmol 7ADCA (●); 30 mM 7ADCA and 80 IUH/mmol 7ADCA (■); 150 mM 7ADCA and 80 IUH/mmol 7ADCA (▲).

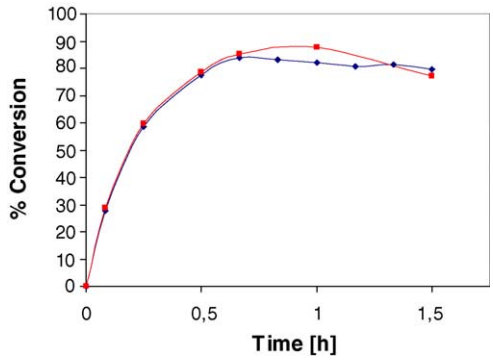

Fig. 3. Synthesis of cephalexin at 20 °C, pH 7.0 and 40% (v/v) EG and 180 mM 7ADCA and PGME–7ADCA molar ratio of 3 at: 80 IUH/mmol 7ADCA (●); 67 IUH/mmol 7ADCA (■).

40% (v/v) EG. All experiments were done at an acyl donor to nucleophile molar ratio of 3.

A control experiment was conducted at low substrate concentrations (30 mM 7ADCA, 90 mM PGME) and high enzyme to substrate ratio (240 IU/mmol 7ADCA). Results are presented in Fig. 2. A maximum conversion yield of 83% was obtained with a productivity of 21 mM/h and a specific productivity of 19 mM/h g. At the same experimental conditions, enzyme load was reduced to 80 IU/mmol 7ADCA and, as seen in Fig. 1, conversion yield was reduced to 64%, productivity decreased to 13 mM/h and specific productivity increased to 36 mM/h g. At that low enzyme load, substrates concentrations were increased to 150 and 450 mM PGME, which is close to the solubility of 7ADCA (165 mM) in such mixture at that conditions. Results are also presented in Fig. 2. As shown, conversion yield was increased to 93%, productivity to 167 mM/h and specific productivity to 93 mM/h g. Therefore, 12% increase in conversion yield, 695% increase in productivity and 390% increase in specific productivity were obtained with respect to the control. The sole effect of increasing substrates concentration at low enzyme loading produced a 45% increase in conversion yield, 1185% increase in productivity and 158% increase in specific productivity. Results are summarized in Table 1.

3.2.2. *At initially heterogeneous conditions*

The system was further challenged by working at 7ADCA concentrations above saturation, meaning an initially heterogeneous system. Concentration of 7ADCA was increased to 180 mM, maintaining the PGME–7ADCA molar ratio of 3, at enzyme loads of 80 and 67 IU/mmol 7ADCA. Results are presented in Fig. 3 and summarized in Table 1. Conversion yields were 84 and 85%, productivities were 226 and 229 mM/h and specific productivities were 105 and 127 mM/h g, respectively. At comparable conditions (80 IU/mmol 7ADCA), productivity was further increase by 35% and specific productivity by 13% when going from initially homogeneous to initially heterogeneous conditions; however this increase was at the expense of a moderate decline in yield. Enzyme load was further reduced to 67 IU/mmol 7ADCA without affecting yield, with a non-significant additional increase of 2% in productivity and a significant additional increase of 21% in specific productivity. At enzyme loads lower than 50 IU/mmol 7ADCA reduction in conversion yield was significant. Comparing the performance at 80 IU/mmol 7ADCA and 180 mM 7ADCA with the corresponding result at low substrates concentrations, 1638 and 192% increases in productivity and specific productivity were obtained, respectively, with an increase in conversion yield of 31%. Comparing the performance at low enzyme loading (67 IU/mmol 7ADCA) and 180 mM 7ADCA with the control experiment at high enzyme loading (240 IU/mmol 7ADCA) and low substrates concentration (30 mM 7ADCA), increases of 990 and 568% in productivity and specific productivity were obtained, respectively without reduction in conversion yield. The system was pressed further by going to initial 7ADCA concentrations over 200 mM, but a paste-like fluid was formed which was very difficult to handle in the reactor configuration used. The system behaved more like a solid state synthesis and was not further studied.

The results obtained in the synthesis of CEX at high substrates concentration at initially homogeneous and heterogeneous conditions are encouraging because, without reduction in yield, both productivity and specific productivity increased several times by forcing the system to work at high substrates concentrations. These values are significantly higher than those recently reported for the kinetically

Table 1
Conversion yield (Y), productivity (P) and specific productivity of CEX synthesis with GAPA at varying substrates concentration and enzyme loads, at pH 7.0, 20 °C and PGME–7ADCA molar ratio of 3

[7ADCA] (mM)	Enzyme load (IU/mmol 7ADCA)	Y (%)	P (mM/h)	P_{sp} (mM/h g)
30	240	83	21	19
	80	64	13	36
150	80	93	167	93
180	80	84	226	105
	67	85	229	127

controlled synthesis of CEX and other β-lactam antibiotics. Wei et al. [44] reported the synthesis of cefaclor with a penicillin acylase from *Bacillus megaterium* immobilized on epoxyacrylic resin: at 50 mM of nucleophile as the limiting substrate, conversion yield was 50% and productivity 4.4 mM/h which were increased to 65% and 8.1 mM/h when using in situ product removal by complexation with 1-naphthol. Trevascio et al. [37] reported the synthesis of CEX in a membrane bioreactor under non-isothermal conditions at 40 mM of nucleophile as the limiting substrate at varying temperature differences across the membrane, obtaining a productivity of 9 mM/h; maximum conversion yield could not be determined because reaction was not conducted to completion. Illanes et al., working with a commercial immobilized penicillin acylase in the synthesis of ampicillin at 30 mM nucleophile concentration as the limiting substrate, obtained a productivity of 13 mM/h at a conversion yield of 60% [45]. Schroën et al. [46] working with a commercial immobilized penicillin acylase (Assemblase®) in the synthesis of CEX obtained a productivity of 45 mM/h at a yield of 60% when working at 100 mM nucleophile concentration; yield increased to 90% when working under heavy excess of acyl donor (500 mM phenylglycine amide) but time course of the reaction was not reported in that case, so that productivity cannot be determined. Shaw et al. [9] obtained a productivity of 18 mM at a conversion yield of 60% in the synthesis of cephalotin at 100 mM nucleophile as the limiting substrate with a commercial immobilized penicillin acylase. All these results come from reactions carried out at moderate substrate concentrations. Using the strategy of aqueous solution precipitate for the synthesis of ampicillin, Youshko et al. [29], working at initially homogeneous conditions, obtained a productivity of 130 mM/h at a conversion yield of 75% with 300 mM 6APA and 500 mM phenylglycine amide; yield increased to 87% at longer reaction time at the expense of productivity. Precipitation of the product ampicillin via supersaturation and a high concentration of nucleophile throughout the synthesis were very important to achieve such high yields. They forced the system to initially heterogeneous conditions at 450 mM nucleophile to ensure saturation with nucleophile during a substantial part of the synthesis; productivity of 167 mM/h was obtained at a conversion yield of 93%. The same authors obtained a productivity of 220 mM/h at a yield slightly over 90% when working under initially heterogeneous conditions with 600 mM 6APA and 900 mM PGME [30]. They forced the system even more by developing a semicontinuous process with repetitive additions of the substrates 6APA and FGME, obtaining a conversion yield of 97%, the highest reported to date for ampicillin, but productivity fell to 83 mM/h [30]. These syntheses were carried out with free penicillin acylase, which precludes enzyme recovery; the use of immobilized enzyme is worthwhile studying in this system, though mass transfer limitations and biocatalyst recovery will hamper process development. Very interesting results have also been reported for the solid sate synthesis of ampicillin, where 70% conversion yield was obtained in a system in which a substantial portion of the reactor volume was occupied by the reacting mixture [35]. Volume of reaction was not reported but, considering that water comes only from the salt hydrate, productivity can be roughly estimated around 300 mM/h. As seen, the results obtained in this work compare quite favorably with those above mentioned. Productivities are orders of magnitude higher than those obtained at moderate substrate concentrations and are comparable to those obtained at high substrates concentrations in homogeneous and heterogeneous systems. Results are hardly comparable in terms of specific productivity because very different types of enzyme preparations are being used and in many cases not enough information is given to calculate it.

3.3. Operational stability of PARI in the synthesis of CEX

The biocatalyst was very stable during reactor operation, with an average of more than 98% recovery of activity after reaction. Operational stability was assessed for the synthesis of CEX at high substrate concentrations and low enzyme to substrate ratio at initially homogeneous conditions (150 mM 7ADCA, 450 mM PGME and 80 IU/mmol 7ADCA). A cycle of 10 consecutives batches was conducted without any biocatalyst supplement between them. Results are summarized in Table 2. Almost 90% of the initial activity was recovered after the cycle; conversion yield remained almost the

Table 2
Operational stability of GAPA and cumulative productivity in sequential batch production of cephalexin at high substrate concentrations (150 mM 7ADCA, 450 mM PGME) and low enzyme to substrate ratio (80 IU/mmol 7ADCA) at pH 7.0 and 20 °C

Batch no.	t_i (min)	Y_i (%)	c_i (mM)	$a_{res,i}$ (%)	P_i (mM/h)	$\sum P_i$ (mM/h)	$\sum P_{SP,i}$ (mM/h g)
1	50	93	139	100	167	167	93
2	50	93	140	102	168	335	186
3	55	94	141	100	154	489	272
4	50	91	136	96	163	652	362
5	60	93	140	94	140	792	440
6	60	90	135	94	135	927	515
7	55	91	137	92	149	1027	598
8	65	92	138	92	128	1155	642
9	70	94	141	90	121	1276	709
10	70	91	136	87	117	1393	774

Y_i: maximum conversion yield in batch i (the rest of nomenclature is in Section 2).

same in all batches with 30% reduction in productivity as a consequence of the longer reaction times required to attain maximum conversion yield. At the end of the cycle, accumulated productivity was 1393 mM/h and accumulated specific productivity was 774 mM/h g. A similar study was previously conducted at low substrates concentration and higher enzyme to substrate ratio (30 mM 7ADCA, 90 mM PGME and 125 IU/mmol 7ADCA). After 10 batches, residual activity was 86% of initial, maximum conversion yield decreased from 98 to 93% and accumulated specific productivity was only 34 mM/h g [47], this is, only 4.4% of the value reported here at high substrates concentrations. After the cycle of 10 batches, residual activity of the biocatalyst here was still 87%; considering first-order inactivation kinetics, projected half-life of the biocatalyst under operation is about 2900 h. This means that the cycle of use of the biocatalyst will be much longer than 10 batches, so that actual global specific productivity within a whole cycle of biocatalyst use will be much higher than the value of 774 mM/h g reported. Long-term operation of the reactor is required to determine it and a definition of a biocatalyst replacement policy to ensure maximum yield remains to be established. This study is underway.

4. Conclusions

At previously optimized conditions at moderate substrate concentrations, the kinetically controlled synthesis of CEX is unable to compete with the existing chemical technology because of the low productivity obtained, so that higher substrates concentrations and lower enzyme to substrate ratios are required. Therefore, syntheses were conducted at high concentrations of substrates under initially homogeneous and initially heterogeneous conditions (partially undissolved 7ADCA).

Solubility of substrates and products at the reaction conditions proved to be a strong function of pH and a moderate function of temperature. Solubility of 7ADCA was greatly enhanced by the presence of PGME, being 165 mM at a PGME–7ADCA molar ratio of 3, 20 °C and pH 7.0.

Synthesis of CEX with GAPA was conducted at progressively higher substrates concentrations, up to and beyond the limit of solubility, and at lower enzyme to substrate ratio. At initially homogeneous conditions and substrates concentrations close to the solubility of 7ADCA, an increase of 12% in conversion yield, 695% in productivity and 390% increase in specific productivity were obtained with respect to the control at moderate substrates concentrations and high enzyme to substrate ratio. At initially heterogeneous conditions, conversion yield was the same and an increase of 990% in productivity and 568% in specific productivity were obtained with respect to the same control. These results highlight the benefit of working at high substrate concentrations even beyond the solubility of the nucleophile. They compare quite favorably with those reported for the kinetically controlled synthesis of CEX and other β-lactam antibiotics in aqueous solution precipitate and solid state mode of operation. The system can still be pressed further to higher [7ADCA] which might allow an additional reduction in enzyme load without affecting yield. At such conditions, the effect of reducing [PGME]/[7ADCA] below 3 remains to be assessed. It might be substantially reduced, which is an important issue for product recovery and process economics.

The biocatalyst was very stable under reaction conditions. At initially homogeneous conditions and high substrate concentrations, after 10 consecutive batches residual activity was still close to 90% with a projected half-life of the biocatalyst of about 2900 h. This means that the cycle of use of the biocatalyst will be much longer than 10 batches so that actual global specific productivity will be much higher than the reported value of 774 mM/h g.

Results are very promising to make the enzymatic synthesis competitive with existing chemical technology.

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References

[1] M. Arroyo, I. de la Mata, C. Acebal, M. Castillón, Appl. Microbiol. Biotechnol. 60 (2003) 507.
[2] C. Ebert, L. Gardossi, P. Linda, Tetrahedron Lett. 37 (1996) 9377.
[3] D. Roche, K. Prasad, O. Repic, Tetrahedron Lett. 40 (1999) 3665.
[4] L. van Langen, N. Oosthoek, D. Guranda, F. van Rantwijk, V. Švedas, R. Sheldon, Tetrahedron: Asymmetry 11 (2000) 4593.
[5] A. Bruggink, Synthesis of β-lactam Antibiotics, Kluwer Academic Publishers, Dordrecht, 2001.
[6] V. Nierstrasz, C. Schroën, R. Bosma, P. Kroon, H. Beeftink, A. Janssen, J. Tramper, Biocatal. Biotransform. 17 (1999) 209.
[7] O. Hernández-Justiz, M. Terreni, G. Pagani, J. García, J. Guisán, R. Fernández-Lafuente, Enzyme Microb. Technol. 25 (1999) 336.
[8] C. Schroën, V. Nierstrasz, P. Kroon, R. Bosma, A. Janssen, H. Beeftink, J. Tramper, Enzyme Microb. Technol. 24 (1999) 498.
[9] S. Shaw, J. Shyu, Y. Hsieh, H. Yeh, Enzyme Microb. Technol. 26 (2000) 142.
[10] C. Aguirre, M. Toledo, V. Medina, A. Illanes, Process Biochem. 38 (2002) 351.
[11] A. Illanes, A. Fajardo, J. Mol. Catal. B: Enzym. 11 (2001) 605.
[12] M. Arroyo, R. Torres-Guzmán, I. de la Mata, M. Castillón, C. Acebal, Biocatal. Biotransform. 20 (2002) 53.
[13] R. Fernández-Lafuente, C. Rosell, L. Caanan-Haden, L. Rodes, J. Guisán, Enzyme Microb. Technol. 24 (1999) 96.
[14] O. Abián, C. Mateo, G. Fernández-Lorente, J. Palomo, R. Fernández-Lafuente, J. Guisán, Biocatal. Biotransform. 19 (2001) 489.
[15] M. Terreni, G. Pagani, D. Ubiali, R. Fernández-Lafuente, C. Mateo, J. Guisán, Bioorg. Med. Chem. Lett. 11 (2001) 2429.
[16] L. Cao, L. van Langen, F. van Rantwijk, R. Sheldon, J. Mol. Catal. B: Enzym. 11 (2001) 665.
[17] L. Wilson, A. Illanes, O. Abián, C.B. Pessela, R. Fernández-Lafuente, J. Guisán, Biomacromolecules 5 (2004) 852.
[18] C. Mateo, J. Palomo, L. van Langen, F. van Rantwijk, R. Sheldon, Biotechnol. Bioeng. 86 (2004) 273.

[19] L. Wilson, A. Illanes, C.B. Pessela, O. Abián, R. Fernández-Lafuente, J. Guisán, Biotechnol. Bioeng. 86 (2004) 558.
[20] D. Özturk, D. Kazan, E. Erarslan, World J. Microbiol. Biotechnol. 18 (2002) 881.
[21] J. Lindsay, D. Clark, J. Dordick, Biotechnol. Bioeng. 85 (2004) 553.
[22] S. Yang, L. Zhou, H. Tang, J. Pan, X. Wu, H. Huang, Z. Yuan, J. Mol. Catal. B: Enzym. 18 (2002) 285.
[23] J. Rajendhran, P. Gunasekaran, J. Biosci. Bioeng. 97 (2004) 1.
[24] M. Wegman, M. Janssen, F. van Rantwijk, R. Sheldon, Adv. Synth. Catal. 343 (2001) 559.
[25] C. Schroën, V. Nierstrasz, R. Bosma, P. Kroon, P. Tjeerdsma, E. de Vroom, J. van der Laan, H. Moody, H. Beeftink, A. Janssen, J. Tramper, Biotechnol. Bioeng. 80 (2002) 144.
[26] D. Wei, J. Zhu, X. Cao, Biochem. Eng. J. 11 (2002) 95.
[27] L. Yang, Z. Wei, Biotechnol. Lett. 25 (2003) 1195.
[28] M. Youshko, V. Švedas, Biochemistry (Mosc.) 65 (2000) 1367.
[29] M. Youshko, L. van Langen, E. de Vroom, H. Moody, F. van Rantwijk, R. Sheldon, V. Švedas, J. Mol. Catal. B: Enzym. 10 (2000) 509.
[30] M. Youshko, L. van Langen, E. de Vroom, F. van Rantwijk, R. Sheldon, V. Švedas, Biotechnol. Bioeng. 73 (2001) 426.
[31] M. Erbeldinger, X. Ni, P. Halling, Enzyme Microb. Technol. 23 (1998) 141.
[32] R. Ulijn, A. Janssen, B. Moore, P. Halling, Chem. Eur. J. 7 (2001) 2089.
[33] M. Diender, A. Straathof, T. van der Does, M. Zomerdijk, J. Heijnen, Enzyme Microb. Technol. 27 (2000) 576.
[34] R. Ulijn, L. de Martin, P. Halling, B. Moore, A. Janssen, J. Biotechnol. 99 (2002) 215.
[35] M. Youshko, V. Švedas, Adv. Synth. Catal. 344 (2002) 894.
[36] C. Schroën, M. Mohy Eldin, A. Janssen, G. Mita, J. Tramper, J. Mol. Catal. B: Enzym. 15 (2001) 163.
[37] P. Trevascio, E. Zito, A. De Maio, C. Schroën, D. Durante, P. De Luca, U. Bencivenga, D. Mita, Biotechnol. Bioeng. 79 (2002) 334.
[38] A. Illanes, Z. Cabrera, L. Wilson, C. Aguirre, Process Biochem. 39 (2003) 111.
[39] A. Illanes, M. Anjarí, C. Altamirano, C. Aguirre, J. Mol. Catal. B: Enzym. 30 (2004) 95.
[40] J. Shewale, K. Kumar, G. Ambedkar, Biotechnol. Tech. 1 (1987) 69.
[41] J. Guisán, Enzyme Microb. Technol. 10 (1988) 375.
[42] G. Alvaro, R. Fernández-Lafuente, R. Blanco, J. Guisán, Appl. Biochem. Biotechnol. 26 (1990) 181.
[43] M. Iturra, C. Zamorano, A. Illanes, Ingenerare 15 (2003) 25.
[44] D. Wei, L. Yang, Q. Song, J. Mol. Catal. B: Enzym. 26 (2003) 99.
[45] A. Illanes, S. Anjarí, R. Arrieta, C. Aguirre, Appl. Biochem. Biotechnol. 97 (2002) 165.
[46] C. Schroën, V. Nierstrasz, H. Moody, M. Hoogschagen, R. Bosma, H. Beeftink, A. Janssen, J. Tramper, Biotechnol. Bioeng. 73 (2001) 171.
[47] Z. Cabrera, C. Altamirano, C. Aguirre, A. Illanes, in: Proceedings of the 12th International Biotechnology Symposium, Santiago, Chile, 17–22 October, 2004, p. 54.