Available online at www.sciencedirect.com
ScienceDirect
Journal of Molecular Catalysis B: Enzymatic 47 (2007) 72–78
JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC
www.elsevier.com/locate/molcatb

Synthesis of cephalexin with immobilized penicillin acylase at very high substrate concentrations in fully aqueous medium

Andrés Illanes [a,*], Lorena Wilson [a], Octavio Corrotea [a], Luigi Tavernini [a], Francisca Zamorano [a], Carolina Aguirre [b]

[a] *School of Biochemical Engineering, Pontificia Universidad Católica de Valparaíso, P.O. Box 4059, Valparaíso, Chile*
[b] *Faculty of Sciences, Universidad Católica Ssma, Concepción, P.O. Box 297, Concepción, Chile*

Received 5 January 2007; received in revised form 21 March 2007; accepted 9 April 2007
Available online 13 April 2007

Abstract

The presence of organic cosolvents was previously considered necessary to obtain high conversion yields in the synthesis of β-lactam antibiotics with immobilized penicillin acylase, and it is so when working at moderate substrate concentrations. Conversion yields close to stoichiometric and high productivities were recently reported for the synthesis of cephalexin at high substrate concentrations in ethylene glycol medium. Under such conditions, the effect of cosolvent concentration on yield is not significant so we raised the hypothesis that stoichiometric yields and high productivities are attainable at very high substrate concentrations in fully aqueous medium leading to substantial process improvement in terms of costs and environment. To test the hypothesis, the kinetically controlled synthesis of cephalexin with immobilized penicillin acylase was conducted in aqueous medium at substrates concentrations up to and beyond their solubilities at varying temperature, pH, enzyme to substrate and acyl donor to nucleophile ratios. At the best conditions, 99% conversion yield was attained with volumetric productivity of 300 mM/h and specific productivity of 7.8 mmol/h g_{cat}. These values are slightly higher than those previously obtained under optimized conditions in organic medium so that the hypothesis has been confirmed, which opens up the possibility of efficiently produce the antibiotic through an environmentally friendly process.

Keywords: Penicillin acylase; Cephalexin; β-lactam antibiotics; Kinetically controlled synthesis; Immobilized enzymes

1. Introduction

6-Aminopenicillanic acid and 7-amino 3-desacetoxicephalosporanic acid are industrially produced mainly by hydrolysis of penicillin G and cephalosporin G with immobilized penicillin acylase [1,2], which has replaced the former cumbersome chemical processes [3,4]. Penicillins and cephalosporins, mostly semi-synthetic derivatives, are the most relevant members of that family accounting for 60% of the total antibiotic market [5–10]. Traditionally, these molecules have been synthesized chemically from the corresponding β-lactam nuclei and suitable side chain precursors [9,10]. Enzymatic synthesis is becoming an attractive option since penicillin acylase can be used also as a catalyst for the reverse reactions of synthesis [11–14], either by thermodynamic [15–17] or kinetic control [18,19], the latter being a better strategy when conversion yield is the main issue [20–22]. In both strategies conversion yields can be improved by using organic solvents [23] precipitation-driven [24] and biphasic systems [25,26]. Reduction of water activity by the use of organic cosolvents [27–29] or very high substrates concentrations [30–33] is beneficial to the reaction of synthesis and has been thoroughly evaluated to make enzyme synthesis competitive. Synthesis in the presence of organic cosolvents can lead to conversion yields not attainable in aqueous medium [27,34] and, in fact, we have reported stoichiometric yields in the synthesis of cephalexin at high substrates concentrations in ethylene glycol medium [35]. However, the presence of organic solvent contradicts the concept of clean technology associated to biological processes. We have observed though, that at very high substrates concentrations, cosolvent concentration is no longer a key variable with respect to conversion yield. Therefore, in this study we present results on the kinetically controlled synthesis of cephalexin with immobilized penicillin acylase at very high substrate concentrations in fully aqueous medium to test

* Corresponding author.
E-mail address: aillanes@ucv.cl (A. Illanes).

1381-1177/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.molcatb.2007.04.003

the hypothesis that under such conditions stoichiometric yields and high productivities are attainable, representing substantial process improvement in terms of costs and environment.

2. Experimental

2.1. Materials

Polyacrylamide gel surface bound penicillin acylase (PGA-450) from *Escherichia coli* with 380 ± 20 IU/g was from Roche Molecular Biochemicals (Mannheim, Germany). Immobilized biocatalyst spherical particles were around 0.1 mm in diameter. The biocatalyst was stored wet at 5 °C with no loss of activity during the whole working period.

(*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); 7-amino 3-desacetoxicephalosporanic acid (7ADCA) was kindly provided by Antibióticos S.A. (León, Spain); penicillin G potassium salt (PenGK) was donated by Natsus S.A. (Lima, Perú). All other reagents were analytical grade either from Sigma–Aldrich or Merck (Darmstadt, Germany).

2.2. Analysis

Enzyme activity was measured using a pHstat (Mettler Toledo, DL50), which recorded the evolution of NaOH consumption to keep pH constant as the protons coming from phenyl acetic acid were produced. Initial slope of the reaction was automatically provided by the equipment and, from that slope, a straightforward stoichiometric calculation allowed to convert the rate of equivalents of base consumed to the rate of phenylacetic acid production and then to the rate of penicillin G hydrolysis.

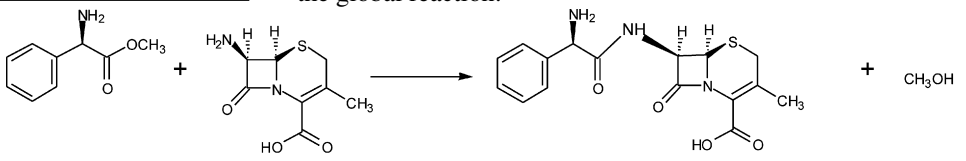
One international unit of activity (IU) of penicillin acylase was defined as the amount of enzyme that hydrolyzes 1 μmol of PenGK per minute from a10 mM PenGK solution in 0.1 M phosphate buffer pH 7.8 at 30 °C.

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 under gradient with a sonicated mixture of methanol and 10 mM phosphate buffer pH 7.0 at a flow rate of 1 mL/min, and analyzed in the UV detector at 220 nm. Elution times were 2.9, 4.5, 5.7 and 7.4 min for 7ADCA, PG, cephalexin and phenylglycine methyl ester (PGME), respectively. Concentration of substrates and products were calculated from calibration curves using stock solutions. HPLC samples were always assayed in triplicate, differences among them never exceeding 3%.

2.3. Synthesis of cephalexin

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, working at a stirring speed of 200 rpm to keep biocatalyst particles in suspension. Samples were taken at intervals and were properly diluted prior to be assayed by HPLC. At high substrate concentrations the system is highly heterogeneous and a fraction of the substrates and the products are in solid state. Samples with solids in suspension were diluted prior to assay so that the solids were dissolved except for the biocatalyst particles that were filtered out (the volume occupied by the biocatalyst was insignificant).

Syntheses of cephalexin with PGA-450 proceed according to the global reaction:



PGME 7ADCA Cephalexin Methanol

Syntheses were performed under kinetic control, whose reaction mechanism has been described elsewhere [36–38].

The study was conducted in a pH range from 6.5 to 7.4 and a temperature range from 10 to 20 °C, at 125 IU/mmol 7ADCA, 200 mM 7ADCA and 600 mM PGME. A 200 mM 7ADCA was close to the solubility limit at the lowest temperature and lowest pH of the range, so that the substrates were soluble at all the conditions tested (solubility of 7ADCA increases both with temperature and pH). Temperature and pH ranges were determined from previous results obtained in water–ethylene glycol medium. Temperatures higher than 20 °C produce a substantial decrease in yield, while temperatures below 10 °C produce a substantial decrease in enzyme activity, which reflects in a low volumetric productivity; a similar trend occurred over pH 7.5 and below pH 6.5 [35]. Once the best pH and temperature conditions were established, the effect of increasing the substrate concentration beyond the solubility limit, the effect of reducing the enzyme to substrate ratio and the effect of reducing the excess acyl donor were studied. Syntheses were evaluated in terms of molar conversion yield (*Y*), volumetric productivity (*P*) and specific productivity (P_{sp}). *Y* was determined as the maximum molar conversion of limiting substrate (7ADCA) into product (cephalexin); *P* was defined as the moles of cephalexin produced per unit time and unit reaction volume at maximum *Y*; P_{sp} was defined as the moles of cephalexin produced per unit time and unit of biocatalyst mass at maximum *Y*. Experiments were done in duplicate and samples assayed in triplicate with variations below 5% among them; data points in figures represent the average of such measurements. Reactions were monitored up to the point in which product concentration levelled off or began to decline and *Y* was evaluated at its maximum value.

3. Results and discussion

Syntheses of cephalexin were conducted at high substrates concentrations below (initially homogeneous) and over (initially heterogeneous) the solubility limit of the nucleophile.

3.1. Effect of temperature and pH on the synthesis of cephalexin at high substrate concentrations at initially homogeneous conditions

The effect of pH and temperature on the kinetically controlled synthesis of cephalexin was studied at high substrate concentrations close to the limit of solubility of the nucleophile within the range of pH and temperatures considered. A 200 mM 7ADCA and 600 mM PGME were selected to conduct this study for being the best conditions previously determined for the synthesis in 30% (v/v) ethylene glycol medium [39]. Experimental design is presented in Table 1. A range of pH from 6.5 to 7.4 and temperatures from 10 to 20 °C were chosen based on previous experience in the synthesis of cephalexin with PGA-450 in organic cosolvent medium [39,40]. Other experimental conditions (125 IU/mmol 7ADCA and a PGME:7ADCA molar ratio of 3) were as suggested from previous work in organic cosolvent medium [35]. Time course of cephalexin syntheses at different pHs and temperatures are presented in Fig. 1 and results summarized in Table 1 for 10, 14 and 20 °C, respectively.

A decrease in Y after reaching its maximum was only observed at pH 7.4 at 14 and 20 °C and even in such cases decreases were less pronounced than those observed when using moderate substrate concentrations, whether in aqueous medium or at low cosolvent concentration [23,41]. For the rest of the cases, Y remained at its maximum without variation for several hours after attaining it, which is a definite advantage of working at high substrate concentrations.

Temperature had a mild effect on Y at pH 6.5 and 7.0 but a definite maximum was obtained at 14 °C at pH 7.4. Y increased with pH but only significantly at 14 °C. As expected, P and P_{sp} increased with temperature at all pHs tested.

From the above results, temperature of 14 °C and pH 7.4 were selected for further studies mainly because the highest Y was obtained at those conditions at a reasonably high P (and P_{sp}). Even though P was higher at 20 °C, as expected, Y decreased significantly. Besides, enzyme stability at 20 °C (see section on enzyme stability) was significantly lower than at 14 °C so that global productivity at 20 °C after a certain number of sequential batches within the cycle of biocatalyst use will most certainly be surpassed by that obtained at 14 °C. The selection can be quantitatively justified in terms of an objective function (F) considering Y, P and stability (evaluated as enzyme half-life) as cost-related parameters, so that:

$$F = \frac{w_Y f_Y + w_P f_P + w_S f_S}{w_Y + w_P + w_S} \quad (1)$$

where f_Y, f_P and f_S are the relative values of the respective parameters (fractions of the highest value) and w_Y, w_P and w_S are

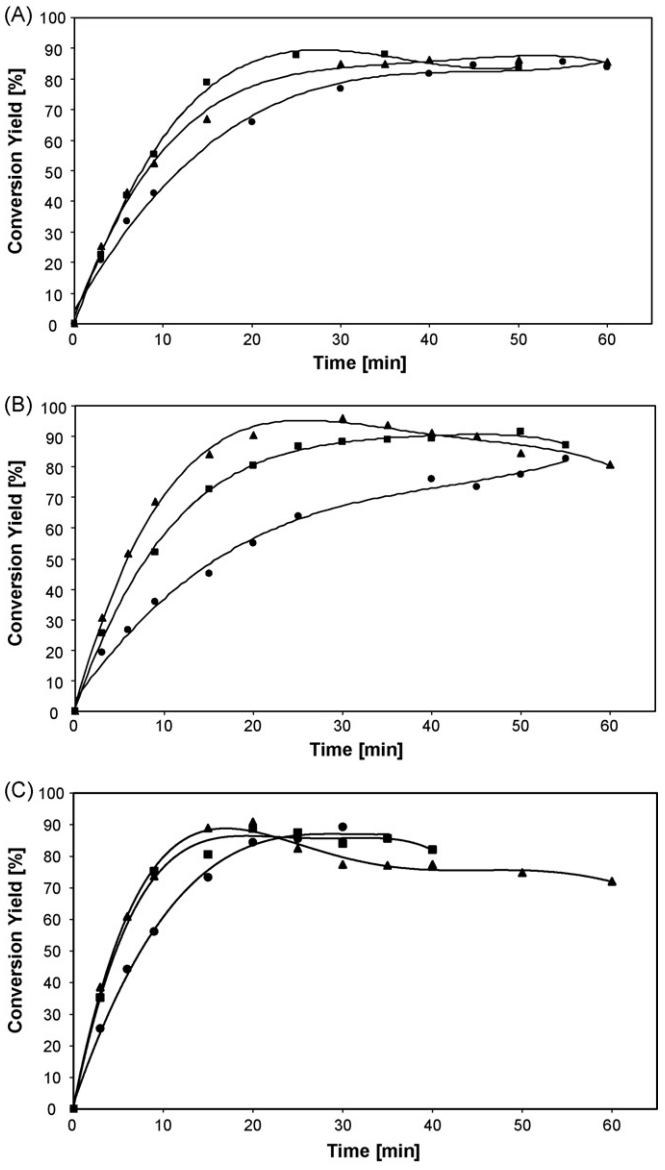


Fig. 1. Effect of pH on the synthesis of cephalexin at 200 mM 7ADCA, 600 mM PGME and 125 IU/mmol 7ADCA. (A) At 10 °C; (B) at 14 °C; (C) at 20 °C. (●) pH 6.5; (■) pH 7.0; (▲) pH 7.4.

Table 1
Experimental design and results of the effect of pH and temperature on the synthesis of cephalexin at 200 mM 7ADCA, 600 mM PGME and 125 IU PGA/mmol 7ADCA

Temperature (°C)	pH	Y (%)	P (mM/h)	P_{sp} (mmol/h g_{cat})
	6.5	84.4	225	2.91
10	7.0	87.8	422	5.45
	7.4	84.8	339	4.39
	6.5	76.1	228	2.95
14	7.0	86.8	417	5.39
	7.4	96.0	384	4.92
	6.5	89.3	357	4.62
20	7.0	88.7	533	6.89
	7.4	90.9	545	7.05

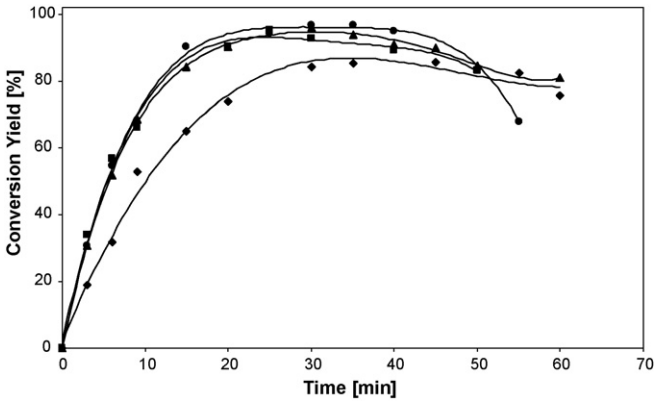


Fig. 2. Effect of 7ADCA concentration on the synthesis of cephalexin at initially homogeneous conditions at pH 7.4, 14 °C, 125 IU/mmol 7ADCA and PGME to 7ADCA molar ratio of 3. (●) 100 mM; (■) 150 mM; (▲) 200 mM; (♦) 250 mM.

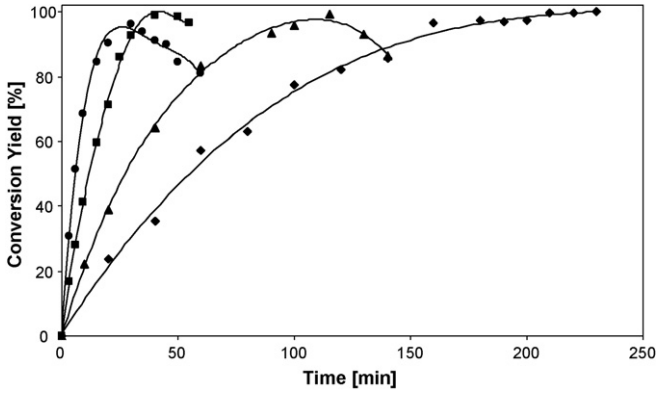


Fig. 3. Effect of enzyme to substrate ratio on the synthesis of cephalexin at pH 7.4, 14 °C, 200 mM 7ADCA and 600 mM PGME. (●) 125 IU/mmol 7ADCA; (■) 62.5 IU/mmol 7ADCA; (▲) 31.25 IU/mmol 7ADCA; (♦) 15.6 IU/mmol 7ADCA.

the corresponding weight coefficients. If these parameters are equally weighed (its effect on process cost is complex and hard to determine a priori), the objective function at 14 °C (0.863) is 15% higher than at 20 °C (0.753); if Y is more heavily weighed the difference at 14 and 20 °C is slightly reduced but always significantly higher at 14 °C.

The best pH and temperature conditions are quite similar to those obtained for the synthesis of cephalexin in 30% (v/v) ethylene glycol medium at high substrates concentrations [39].

3.2. Effect of substrates concentration at 14 °C and pH 7.4 at initially homogeneous conditions

At those previously selected conditions of pH and temperature, and always at an enzyme to substrate ratio of 125 IU/mmol 7ADCA and a PGME:7ADCA molar ratio of 3, the effect of substrates concentration below the solubility limit (250 mM 7ADCA and 750 mM PGME in this case) was studied. Results are presented in Fig. 2 and summarized in Table 2. As expected, decrease in Y after attaining its maximum was more pronounced at the lower substrates concentrations, as seen in Fig. 2. Y were not affected below 200 mM 7ADCA but were substantially reduced at 250 mM 7ADCA. At such conditions (750 mM PGME) copious amounts of PG were precipitated, which has been postulated to decrease the rate of synthesis and reduce product precipitation so that more product is subjected to hydrolysis [42,43], which may explain the reduction in Y attained. P increased steadily with substrate concentration but less variation in P_{sp} was observed. A 200 mM 7ADCA and 600 mM PGME were selected as the best conditions for initially homogeneous systems and further studies were conducted at such substrate concentrations. Values of Y were close to stoichiometric, which is not attainable at moderate substrates concentrations in aqueous medium. However, at very high substrate concentrations, water activity is reduced to the point in which competing hydrolytic reactions are depressed in favour of synthesis.

3.3. Effect of enzyme to substrate ratio at 14 °C, pH 7.4, 200 mM 7ADCA and 600 mM PGME at initially homogeneous conditions

The systems was then challenged to lower enzyme to limiting substrate ratio. These experiments were conducted at the previously selected conditions: pH 7.4, 14 °C, 200 mM 7ADCA and 600 mM PGME. Results are presented in Fig. 3 and summarized in Table 3. Y was close to stoichiometric at all enzyme to substrate ratios within the range studied. As expected, P decreased at lower enzyme to substrate ratios. However, P_{sp} had a clear maximum at 62.5 IU/mmol 7ADCA, being considerably reduced at lower enzyme to substrate ratios. Therefore, 62.5 IU/mmol 7ADCA was selected as the best condition to conduct further experiments. Results in terms of Y and P are close to those obtained at similar conditions in 30% (v/v) ethylene glycol medium [39], with the benefit of avoiding the use of organic solvent which is a drawback in terms of costs and environment. Besides, the enzyme is more stable in aqueous than in organic medium (see section on enzyme stability) so that global productivity will be consequently higher.

Table 2
Effect of substrates concentration on the synthesis of cephalexin at pH 7.4, 14 °C, acyl donor to nucleophile molar ratio of 3 and 125 IU PGA/mmol 7ADCA

7ADCA (mM)	PGME (mM)	Y (%)	P (mM/h)	P_{sp} (mmol/h g_{cat})
100	300	96.8	194	4.96
150	450	92.9	279	4.76
200	600	96.0	384	4.92
250	750	84.2	421	4.28

Table 3
Effect of enzyme to substrate ratio on the synthesis of cephalexin at 14 °C, pH 7.4, 200 mM 7ADCA and 600 mM PGME

PGA-450/7ADCA (IU/mmol)	Y (%)	P (mM/h)	P_{sp} (mmol/h g_{cat})
15.6	99.5	57	5.69
31.2	99.2	104	5.31
62.5	98.9	297	7.61
125.0	96.0	384	4.92

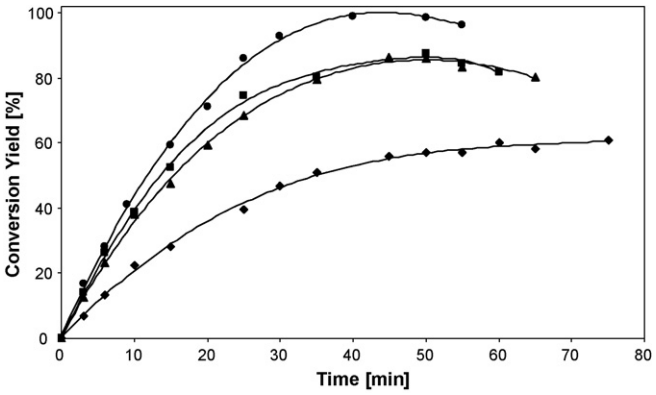


Fig. 4. Effect of acyl donor to nucleophile molar ratio on the synthesis of cephalexin at pH 7.4, 14 °C, 200 mM 7ADCA and 62.5 IU/mmol 7ADCA. (●) PGME/7ADCA = 3; (■) PGME/7ADCA = 2.5; (▲) PGME/7ADCA = 2 IU/mmol 7ADCA; (♦) PGME/7ADCA = 1.

3.4. Effect of acyl donor to nucleophile ratio at 14 °C, pH 7.4, 200 mM 7ADCA and 62.5 IU/mmol 7ADCA at initially homogeneous conditions

Excess PGME is a drawback of the kinetically controlled synthesis of β-lactam antibiotics. However, it has proven to be necessary to attain high *Y*, mainly because penicillin acylase can also act as an esterase and hydrolyze PGME to the side product PG [44]. Lower PGME to 7ADCA molar ratios were then tested. Results are presented in Fig. 4 and summarized in Table 4. As seen, *Y*, *P* and P_{sp} were severely reduced below a molar ratio of 3, which is unfortunate because higher amounts of acyl donor are required and higher amounts of PG are produced making downstream operations for the recovery of product and biocatalyst cumbersome. Similar behaviour has been reported for the synthesis of cephalexin in cosolvent medium at moderate substrate concentrations [40]. An acyl donor to nucleophile ratio of 3 was then considered the best value and was used in further experiments. Ratios higher than 3 reported no benefit, since *Y*, *P* and P_{sp} were not increased any further; results at a ratio of 4 were the same as in 3 within the margin of experimental error.

Results obtained in the synthesis of cephalexin at initially homogeneous conditions at high substrate concentrations compare quite favorably with those reported in similar systems, as can be appreciated in Table 5. *Y* approaching stoichiometric values have been reported by using very high substrates concentrations, in many cases beyond the solubility limit of the nucleophile, and using fed-batch and substrate supersaturation strategies [19,31,42–43]. However, in those cases high *Y* have been obtained at the expense of reduced *P*. By working close to nucleophile saturation in an initially homogeneous system we have obtained almost stoichiometric *Y* at high values of *P*.

Table 4
Effect of acyl donor to nucleophile ratio at 14 °C, pH 7.4, 200 mM 7ADCA and 62.5 IU/mmol 7ADCA

PGME/7ADCA (mol/mol)	*Y* (%)	*P* (mM/h)	P_{sp} (mmol/h g_{cat})
3	98.9	297	7.61
2.5	86.6	231	5.92
2	86.5	231	5.92
1	56.1	150	3.84

Table 5
Conversion yield and productivities of synthesis of β-lactam antibiotics at high substrates concentrations in aqueous medium

Antibiotic	Nucleophile (mM)	Acyl donor (mM)	*Y* (%)	*P* (mM/h)	Ref.
Cephalexin	100[a]	150	60	45	[19] [c]
Cephalexin	100[a]	500	90	ND	[19] [c]
Cephalotin	100[a]	300	60	18	[47] [c]
Cephalexin	180[a]	540	85	229	[35] [d]
Cephalexin	200[a]	600	99	297	This work[c]
Ampicillin	300[a]	500	75	130	[42][c]
Ampicillin	450[b]	600	93	167	[42][c]
Ampicillin	600[b]	900	91	220	[43][c]
Ampicillin	600 + FB[b]	900 + FB	97	83	[43][c]
Ampicillin	SSS	SSS	98	ND	[31]
Amoxicillin	SSS	SSS	91	60	[31]
Cephalexin	SSS	SSS	92	ND	[31]

FB: fed-batch addition of substrates; ND: not determined; SSS: substrate supersaturation.
[a] Initially homogeneous conditions.
[b] Initially heterogeneous conditions.
[c] Reaction medium: aqueous buffer.
[d] 40% (v/v) ethylene glycol.

3.5. Synthesis of cephalexin at initially heterogeneous conditions

Syntheses at nucleophile concentrations over the solubility limit were conducted at 14 °C, pH 7.4, 62.5 IU/mmol 7ADCA and PGME/7ADCA molar ratio of 3. Results are presented in Fig. 5 and summarized in Table 6. For the sake of comparison, results at 200 mM 7ADCA (initially homogeneous condition) are included in Table 6. As seen, *Y* was dramatically reduced at very high substrate concentrations under initially heterogeneous conditions. At 450 mM 7ADCA and 1.35 M PGME the system was hardly tractable because of the semi-solid nature of the

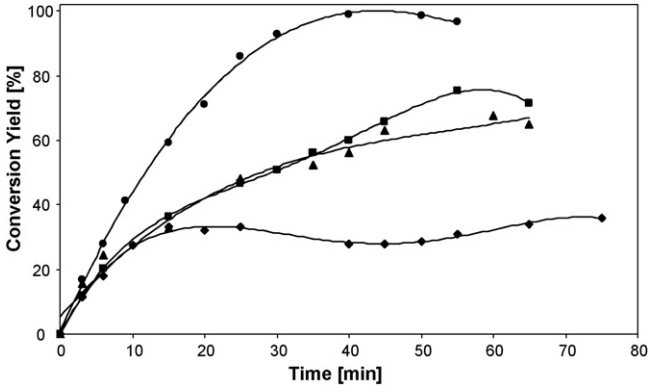


Fig. 5. Effect of 7ADCA concentration on the synthesis of cephalexin at initially heterogeneous conditions at pH 7.4, 14 °C, 62.5 IU/mmol 7ADCA and PGME to 7ADCA molar ratio of 3. (●) Control at 200 mM; (■) 300 mM; (▲) 350 mM; (♦) 450 mM.

Table 6
Synthesis of cephalexin at initially heterogeneous conditions at 14 °C, pH 7.4, 62.5 IU/mmol 7ADCA and acyl donor to nucleophile molar ratio of 3

7ADCA (mM)	PGME (mM)	Y (%)	P (mM/h)	P_{sp} (mmol/h g_{cat})
200	600	98.9	297	7.61
300	900	75.3	246.4	4.25
350	1050	67.5	202.6	2.95
450	1350	33.1	594.9	6.76

reaction system. Even though P was very high at that condition because of the high initial reaction rate at high substrate concentrations, the reaction levelled off at a low Y. When working in 30% (v/v) ethylene glycol medium, Y was not reduced when forcing the system to initially heterogeneous conditions and productivity increased further [39]; however, solubility of 7ADCA in that medium was only 150 mM, while in water was 250 mM, so that conditions are not comparable since lower substrates concentrations were tested.

3.6. Stability of PGA-450 at the selected conditions for cephalexin synthesis

Stability of PGA-450 at pH 7.4 and 14 °C was determined under non-reactive conditions. Time-course of biocatalyst inactivation at the same concentration used in the synthesis of cephalexin at the selected conditions is presented in Fig. 6. A projected half-life of 2480 h at 14 °C and 1090 h at 20 °C was determined, which means that the biocatalyst is very stable at the conditions of cephalexin synthesis. At the same pH and 14 °C, but in 60% (v/v) ethylene glycol medium, the stability of PGA-450 was much lower, with a half-life of only 825 h [40], which highlights the benefit of working in solvent-free medium. Operational stability of PGA-450 at the selected conditions remains to be studied, but stability is usually higher under reactive conditions [40,45–46], so that global specific productivity of the system (amount of product per unit time and unit of biocatalyst mass during a whole cycle of biocatalyst use) should be very high.

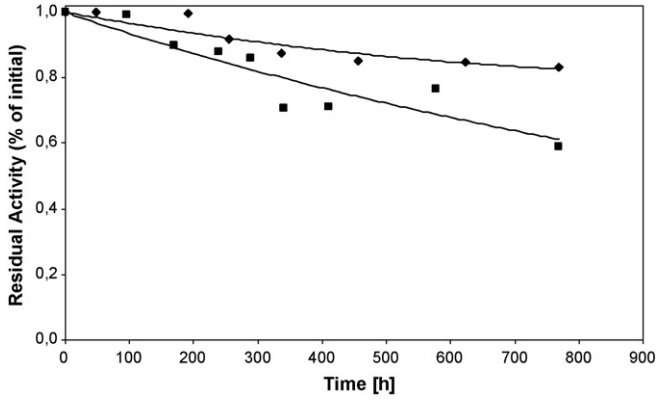


Fig. 6. Time-course of biocatalyst inactivation at pH 7.4 and 30 mg/mL of PGA-450. (♦) 14 °C; (■) 20 °C.

4. Conclusions

The kinetically controlled synthesis of cephalexin with PGA-450 was studied at very high substrates concentrations. Best conditions of temperature and pH were determined considering Y, P (P_{sp}) and biocatalyst stability ($t_{1/2}$) as evaluation parameters. At such conditions, the effect of substrate concentration was studied up to the limit of nucleophile solubility (250 mM); best results were obtained at 200 mM 7ADCA and 600 mM PGME. The system was then forced to lower enzyme to limiting substrate ratio; the enzyme load could be reduced to one-half without reduction in Y and with a 55% increase in P_{sp}. Then the system was again forced to lower acyl donor to nucleophile ratio. Unfortunately, at ratios below 3 Y, P and P_{sp} were significantly reduced. Finally, the system was forced to initially heterogeneous conditions by working at nucleophile concentrations exceeding its solubility. Y were severely reduced at 7ADCA concentrations over 300 mM. At 450 mM 7ADCA P and P_{sp} were higher but Y was quite low. Over such concentrations the system was hardly tractable due to the semi-solid condition of the reaction medium.

Best conditions for cephalexin synthesis were pH 7.4, 14 °C, 200 mM 7ADCA, 600 mM PGME and 62.5 IU/mmol 7ADCA. At such conditions, yield was almost stoichiometric, productivity was 300 mM/h and specific productivity was 7.6 mmol/h g_{cat} which are better values than previously reported. The enzyme catalyst was very stable at those conditions of pH and temperature with a projected half-life close to 2500 h. Operational stability of the biocatalyst should be even higher and will be determined under sequential batch operation. If so, the enzymatic synthesis of cephalexin with PGA-450 at the selected conditions in a fully aqueous medium should be a highly competitive system for its production under a clean technology concept. Results reported here are comparable in terms of Y, P and P_{sp} with those previously obtained in 30% (v/v) ethylene glycol medium, but with the additional benefit of using only water as solvent, which has a strong impact in terms of costs and environmental protection.

Acknowledgements

This work was funded by Grants 1060428 from Fondecyt, Chile. The authors wish to thank Ms. Rosa Arrieta for her valuable analytical support.

References

[1] A. Bruggink, Synthesis of β-Lactam Antibiotics, Kluwer Academic Publishers, Dordrecht, 2001.
[2] A. Parmar, H. Kumar, S. Marwaha, J. Kennedy, Biotechnol. Adv. 18 (2000) 289.
[3] J. Verweij, E. de Vroom, Recl. Trav. Chim. Pays-Bas 112 (1993) 66.
[4] E.J. van de Sandt, E. de Vroom, Chim. Oggi 18 (2000) 72.
[5] R.P. Elander, Appl. Microbiol. Biotechnol. 61 (5/6) (2003) 385.
[6] http://www.biz-lib.com/ZKC78659.html.
[7] M.H. Janssen http://repository.tudelft.nl/file/200584/169747, ISBN: 90-9020754-6, 2006.
[8] M. Marín, F. Gudiol, Enferm. Infec. Microbiol. Clin. 21 (1) (2003) 42.

[9] A. Bruggink, P. Roy, in: A. Bruggink (Ed.), Synthesis of β-Lactam Antibiotics, Kluwer Acad Publ, Dordrecht, 2001, p. 13.
[10] M. Wegman, M. Janssen, F. van Rantwijk, R. Sheldon, Adv. Synth. Catal. 343 (2001) 559.
[11] M. Arroyo, I. de la Mata, C. Acebal, M. Castillón, Appl. Microbiol. Biotechnol. 60 (2003) 507.
[12] J. Rajendhran, P.J. Gunasekaran, Biosci. Bioeng. 97 (2004) 1.
[13] M. Gavrilescu, Y. Chisti, Biotechol. Adv. 23 (2005) 471.
[14] A. Kallenberg, F. van Rantwijk, R. Sheldon, Adv. Synth. Catal. 347 (2005) 905.
[15] R. Fernández-Lafuente, C. Rossell, J.M. Guisán, Biotechnol. Appl. Biochem. 24 (1996) 139.
[16] C.G. Schroën, V.A. Nierstrasz, P.J. Kroon, R. Bosma, A.E. Janssen, H.H. Beeftink, J. Tramper, Enzyme Microb. Technol. 24 (1999) 489.
[17] V.A. Nierstrasz, C.G. Schroën, R. Bosma, P.J. Kroon, H.H. Beeftink, A.E. Janssen, J. Tramper, Biocatal. Biotransform. 17 (1999) 209.
[18] O. Hernández-Jústiz, M. Terreni, G. Pagani, J.L. García, J.M. Guisán, R. Fernández-Lafuente, Enzyme Microb. Technol. 25 (1999) 336.
[19] C.G. Schroën, V.A. Nierstrasz, H.M. Moody, M.J. Hoogschagen, P.J. Kroon, R. Bosma, H.H. Beeftink, A.E. Janssen, J. Tramper, Biotechnol. Bioeng. 73 (2001) 171.
[20] M.B. Diender, A.J. Straathof, J.J. Heijnen, Biocatal. Biotransform. 16 (1998) 275.
[21] C.G. Schroën, V.A. Nierstrasz, R. Bosma, P.J. Kroon, P.S. Tjeerdsma, E. de Vroom, J.M. van der Laan, H.M. Moody, H.H. Beeftink, A.E. Janssen, J. Tramper, Biotechnol. Bioeng. 80 (2002) 144.
[22] E. Baldaro, in: U. Pandit, F. Alderweireldt (Eds.), Bioorganic Chemistry in Healthcare and Technology, Plenum Press, New York, 1991, p. 237.
[23] A. Illanes, S. Anjarí, R. Arrieta, C. Aguirre, Appl. Biochem. Biotechnol. 97 (2002) 165.
[24] L. Yang, Z. Wei, Biotechnol. Lett. 25 (2003) 1195.
[25] D. Wei, J. Zhu, X. Cao, Biochem. Eng. J. 11 (2002) 95.
[26] M. Terreni, D. Ubiali, G. Pagani, O. Hernández-Jústiz, R. Fernández-Lafente, J.M. Guisán, Enzyme Microb. Technol. 36 (2005) 672.
[27] A. Illanes, A. Fajardo, J. Mol. Catal. B: Enzym. 11 (2001) 587.
[28] C. Aguirre, M. Toledo, V. Medina, A. Illanes, Process Biochem. 38 (2002) 351.
[29] M. Arroyo, R. Torres-Guzmán, I. de la Mata, M. Castillón, C. Acebal, Biocatal. Biotransform. 20 (2002) 53.
[30] R.V. Ulijn, L. de Martin, P.J. Halling, B.D. Moore, A.E. Janssen, J. Biotechnol. 99 (2002) 215.
[31] M.I. Youshko, H. Moody, A. Bukhanov, W. Boosten, V.K. Švedas, Biotechnol. Bioeng. 85 (2004) 323.
[32] M.I. Youshko, V.K. Švedas, Adv. Synth. Catal. 344 (8) (2002) 894.
[33] A. Basso, P. Spizzo, M. Toniutti, C. Ebert, P. Linda, L. Gardossi, J. Mol. Catal. B. Enzym. 9 (2006) 105.
[34] M. Arroyo, R. Torres-Guzmán, I. de la Mata, M.P. Castillón, C. Acebal, Biotechnol. Prog. 16 (2000) 368.
[35] A. Illanes, C. Altamirano, M. Fuentes, F. Zamorano, C. Aguirre, J. Mol. Catal. B. Enzym. 35 (1–3) (2005) 45.
[36] D.H. Nam, C. Kim, D.D.Y. Ryu, Biotechnol. Bioeng. 27 (1985) 953.
[37] A. Illanes, F. Rodríguez, C. Bahamondes, C. Altamirano, Biochem. Eng. J. 24 (2005) 209.
[38] R.C. Giordano, M.P.A. Ribeiro, R.L.C. Giordano, Biotechnol. Adv. 24 (2006) 27.
[39] A. Illanes, L. Wilson, C. Altamirano, Z. Cabrera, L. Alvarez, C. Aguirre, Enzyme Microb. Technol. 40 (2007) 195.
[40] A. Illanes, S. Anjarí, C. Altamirano, C. Aguirre, J. Mol. Catal. B.: Enzym. 30 (2004) 95.
[41] C. Aguirre, P. Opazo, M. Venegas, R. Riveros, A. Illanes, Process Biochem. 41 (2006) 1924.
[42] M.I. Youshko, L. van Langen, E. de Vroom, H. Moody, F. van Rantwijk, R. Sheldon, V.K. Švedas, J. Mol. Catal. B: Enzym. 10 (2000) 509.
[43] M.I. Youshko, L. van Langen, E. de Vroom, F. van Rantwijk, R. Sheldon, V.K. Švedas, Biotechnol. Bioeng. 73 (2001) 426.
[44] R. Fernández-Lafuente, C. Rosell, B. Piatkowska, J.M. Guisán, Enzyme Microb. Technol. 19 (1996) 9.
[45] A. Illanes, C. Altamirano, M.E. Zúñiga, Biotechnol. Bioeng. 50 (1996) 609.
[46] A. Illanes, L. Wilson, E. Caballero, R. Fernández-Lafuente, J.M. Guisán, Appl. Biochem. Biotechnol. 133 (2006) 189.
[47] S. Shaw, J. Shyu, Y. Hsieh, H. Yeh, Enzyme Microb. Technol. 26 (2000) 142.