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ScienceDirect
Journal of Biotechnology 131S (2007) S74–S97
Journal of BIOTECHNOLOGY
www.elsevier.com/locate/jbiotec

Industrial Biotechnology

BIOCATALYTIC PROCESS DESIGN

1. Kinetically controlled synthesis of cephalexin at very high substrate concentrations in aqueous medium

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Organic cosolvents are necessary to attain high conversion yields in the enzymatic synthesis of β-lactam antibiotics at moderate substrate concentrations. However, at very high substrates concentrations the effect of cosolvent concentration on yield is not significant, so that high yields and productivities could be obtained at such conditions in fully aqueous medium. To test the hypothesis, the kinetically controlled synthesis of cephalexin in aqueous medium was conducted with carrier-bound penicillin acylase (PGA-450). Substrates concentrations up to their solubilities at varying temperature and pH were tested at predetermined enzyme to nucleophile (*E*:*N*) and acyl donor to nucleophile (*A*:*N*) ratios. Conversion yield (*Y*), volumetric productivity (*P*), specific productivity (P_{sp}) and biocatalyst stability were selected as suitable parameters to optimize conditions based on a weighed objective function. Based on such function, pH of 7.4 and 14 °C were selected as the best conditions to further study the effect of *E*:*N* and *A*:*N*. The former could be reduced to one half without any sacrifice in *Y* and P_{sp}; however the latter could not be reduced without compromising *Y*. At the best conditions, 99% *Y* was attained with *P* of 300 mM/h and P_{sp} of 7.8 mmol/(h g_{cat}). These values are higher than those obtained under optimized conditions in organic medium so that substantial process improvement in terms of costs and environment can be attained in such an environmentally friendly process.

doi:10.1016/j.jbiotec.2007.07.716

2. Carrier-bound and carrier-free penicillin acylases as catalysts for cephalexin synthesis in aqueous medium

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The kinetically controlled synthesis of cephalexin with penicillin acylase (PA) is favoured by organic cosolvents that depress water activity so reducing the competing reactions of product and acyl donor hydrolysis. However, a similar effect can be attained at very high substrates concentrations with obvious benefits in terms of cost and environment. At concentrations close to the solubility of nucleophile and at previously determined enzyme to nucleophile and acid donor to nucleophile ratios, three biocatalysts were tested: commercial PGA-450 and in-house produced glyoxyl agarose immobilized (PAGA) and carrier-free cross-linked enzyme aggregates of penicillin acylase from recombinant *E. coli* (PACLEA). Optimum temperature and pH were determined for each biocatalyst based on an objective function considering conversion yield, productivity and enzyme stability as evaluation parameters. Despite the fact that stability was higher with PAGA and specific productivity was higher for PACLEA, best results were obtained with PGA-450. However, the three biocatalysts performed well and differences were rather small when using the objective function to compare them: 5% lower for PAGA and 2.5% lower for PACLEA. Yields were stoichiometric and productivities higher than those previously reported in organic medium, which implies significant savings in terms of costs and environmental protection.

doi:10.1016/j.jbiotec.2007.07.130

0168-1656/$ – see front matter
doi:10.1016/j.jbiotec.2007.07.128