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# 6-Aminopenicillanic acid production in stationary basket bioreactor with packed bed of immobilized *penicillin amidase*—Penicillin G mass transfer and consumption rate under internal diffusion limitation

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ABSTRACT

The external and internal mass transfers of Penicillin G in the process of its enzymatic hydrolysis to 6-Aminopenicillanic acid under competitive and non-competitive inhibitions using a bioreactor with stationary basket bed of immobilized *penicillin amidase* have been analyzed. By means of the Penicillin G mass balance for a single particle of biocatalysts, considering the specific kinetic model proposed by Warburton et al., mathematical expressions have been developed for describing the profiles of Penicillin G concentrations and mass flows in the outer and inner regions of biocatalyst particles, as well as for estimating the influence of internal diffusion on its hydrolysis rate. The results indicated that very low values of internal mass flow could be reached in the particles centre. The corresponding region was considered an "enzymatic inactive region", its extent varying from 0 to 51% from the overall volume of each biocatalyst. By enzyme immobilization and using the basket bed, the rate of enzymatic reaction is reduced over 160 times compared to the process with free enzyme



## 1. Introduction

6-Aminopenicillanic acid is the main intermediate for the synthesis of semi-synthetic beta-lactamic antibiotics (amoxycillin, ampicillin, etc.) [1]. This acid can be obtained by *Penicillium sp.* fermentation without precursor, but the yield of substrate conversion to 6-Aminopenicillanic acid is low. For this reason, the method applied at industrial scale for 6-Aminopenicillanic acid production consists on the deacylation of natural Penicillins (Penicillins G and V) with immobilized *penicillin amidase* [1]. This enzyme is produced by various microorganisms (*Escherichia coli*, *Bacillus megatherium*, *Arthrobacter viscosus*, *Streptomyces sp.*), the main commercial producer being *E. coli* [1]. For increasing its stability, as well as for facilitating its recovery and re-using in many hydrolysis cycles, several supports have been studied for *penicillin amidase* immobilization (polyacrylamide, agarose, chitosan, epoxy-activated, etc.) [1–3]. Among them, the covalent immobilization of *penicillin amidase* in the epoxy-activated support Eupergit C is efficiently applied at industrial scale [2].

Generally, the use of immobilized enzymes offers the advantages of the increase of the thermal, chemical and to the shear forces resistance of the biocatalysts. Other advantages consist on the attenuation of the substrate inhibition processes, the easier recovery of the biocatalysts from the final medium, and, consequently, the increase of number of the repeated enzymatic reaction cycles re-using the same particles of biocatalysts [4].

The bioreactors of basket type are derived from the bioreactors with packed beds, the biocatalyst particles being fixed in an annular cylindrical or conic bed, which is either static around the stirrer [5–7], or rotary [8–10]. Owing to its design, this bioreactor avoids either the disadvantages of the bioreactors with packed beds, and the flooding/deposition or the mechanical disruption of the biocatalysts particles, phenomena that are encountered in the bioreactors with mobile beds widely used for 6-Aminopenicillanic acid industrial production. In the basket bioreactor, the liquid phase flow combines the perfect mixed flow around the basket with plug flow inside the biocatalysts bed. Thus, the hydrodynamics of the medium around the basket exhibits an important influence on the transfer processes involved in the substrate enzymatic conversion.

The previous studies indicated that the immobilization of microbial cells in alginate and their utilization in systems with basket bed of biocatalysts can represent a viable alternative to the fermentation with free cells [11]. By selecting the optimum operating

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**Nomenclature**

| | |
|---|---|
| $C_S$ | Penicillin G concentration (mol m$^{-3}$) |
| $C_{Si}$ | Penicillin G concentration at biocatalyst particle surface (mol m$^{-3}$) |
| $C_{SL}$ | Penicillin G concentration in liquid phase (mol m$^{-3}$) |
| $C_{SP}$ | Penicillin G concentration inside the biocatalyst particle (mol m$^{-3}$) |
| $C_{SPo}$ | Penicillin G concentration at the biocatalyst particle centre (mol m$^{-3}$) |
| $C_{S0}$ | initial Penicillin G concentration in liquid phase (mol m$^{-3}$) |
| $d$ | impeller diameter (m) |
| $d_P$ | biocatalyst particle diameter (m) |
| $D$ | bioreactor diameter (m) |
| $D_{Se}$ | Penicillin G effective diffusivity (m$^2$ s$^{-1}$) |
| $D_{SL}$ | Penicillin G liquid phase diffusivity (m$^2$ s$^{-1}$) |
| $H$ | basket bed height (m) |
| $k_L$ | liquid phase mass transfer coefficient of Penicillin G (m s$^{-1}$) |
| $K_{iF}$ | phenylacetic acid inhibition constant (mol m$^{-3}$) |
| $K_{iP}$ | 6-Aminopenicillanic acid inhibition constant (mol m$^{-3}$) |
| $K_{iS}$ | Penicillin G inhibition constant (mol m$^{-3}$) |
| $K_M$ | Michaelis–Menten constant (mol m$^{-3}$) |
| $N$ | rotation speed (s$^{-1}$) |
| $R$ | basket bed radius (m) |
| $R_P$ | biocatalyst particle radius (m) |
| $v_S$ | liquid superficial velocity (m s$^{-1}$) |
| $V$ | maximum enzymatic reaction rate (mol g$^{-1}$ s$^{-1}$) |

*Greek symbols*

| | |
|---|---|
| $\phi$ | volumetric fraction of biocatalyst particles |
| $\eta_L$ | liquid phase viscosity (N m$^{-2}$ s$^{-1}$) |
| $\rho_L$ | liquid phase density (kg m$^{-3}$) |
| $\lambda$ | effectiveness factor |

regime of the bioreactor, the activity and physical integrity of the biocatalysts remain unaffected for many fermentation cycles, even if the process is carried out under substrate or product inhibition conditions [11].

Because there is no previous information concerning the use of basket bioreactor for 6-Aminopenicillanic acid production, this work investigates the external and internal mass transfer of Penicillin G, under substrate and products inhibitory effects, in 6-Aminopenicillanic acid enzymatic synthesis by *penicillin amidase* covalently immobilized in Eupergit C using a stationary basket bioreactor. Based on the experimental results, new mathematical models quantifying the implication of internal diffusion on the profile of Penicillin G concentration in the outer region and inside the biocatalyst particle, as well as on the enzymatic hydrolysis rate have been established.

## 2. Materials and method

The experiments were carried out in batch system in 10 L (8 L working volume) laboratory stirred bioreactor Fermac 310/60 (Electrolab) [11]. The bioreactor has been provided with a cylindrical bed of basket type having the inner diameter of 100 mm, height of 100 mm and the bed thickness of 10 mm. The basket was made by plastic mesh and placed centered around the stirrer, at 100 mm from the bioreactor bottom. According to the previous studies, the optimum impellers combination was found to be of two Rushton turbines, the superior one placed outside the basket and the other inside the basket at its inferior extremity [12]. The impeller rotation speed was of 300 rpm.

The basket was filed with immobilized *penicillin amidase* from *E. coli* (Fluka). The enzyme was immobilized in Eupergit C, according to the covalent binding method described by Torres-Bacete et al. (2000) [13]. The specific activity of the immobilized biocatalysts was 180 UI g$^{-1}$. The following sizes of the biocatalysts have been used: 1.0, 1.5 and 2.0 mm, respectively. The corresponding volumetric fractions of the biocatalyst inside the basket bed were as follows: 0.88 for biocatalysts particle with 1.0 mm diameter, 0.83 for biocatalysts particles with 1.5 mm diameter, and 0.80 for the largest biocatalyst particles. Any mechanical damage of the biocatalysts due to the shear forces was recorded during the experiments.

The medium was a solution of 80 mol m$^{-3}$ Penicillin G potassium salt (Merck), maintained at pH 8 with phosphate buffer. The enzymatic hydrolysis has been carried out at 30 °C.

The experimental values of the external mass transfer rate have been calculated and analyzed by means of the variation of Penicillin G concentrations in the liquid bulk volume and biocatalysts particle surface during the enzymatic conversion. For the experimental determinations, the location of each sampling points were at 150 mm × 100 mm from the bioreactor bottom, as follows: one in the inner region of the cylindrical bed, one at the inner and other at the outer surfaces of the cylindrical bed, and three inside the cylindrical bed (two sampling points each at 25 mm from the two surfaces of cylinder and one in the middle of the bed thickness). The Penicillin G concentration has been measured by high performance liquid chromatography technique (HPLC) using an UltiMate 3000 Dionex system with a Acclaim 120 C18 column (4.6 mm diameter, 150 mm length), provided with the Variable Wavelength RS Detector at 220 nm. The mobile phase was a mixture 28% acetonitrile and 72% solution of 0.64 g L$^{-1}$ $KH_2PO_4$ with a flow rate of 0.7 ml min$^{-1}$. The analysis temperature was of 30 °C.

The internal values of Penicillin G concentration or mass transfer have been calculated using only the proposed mathematical model.

The hydrolysis end has been considered when the Penicillin G conversion degree reached minimum 90–95%. The samples have been taken at 10, 20, 45 and 60 min from the process beginning.

Each experiment has been repeated for two or three times for identical conditions, the average value of the considered parameters being used. The average experimental error was of ±4.55%.

## 3. Theory and calculations

The values of Penicillin G concentrations at the biocatalyst particle surface and inside the particle can be obtained by means of its mass balance related to a single biocatalyst particle. In this purpose, the following assumptions have been considered:

- the kinetics of 6-Aminopenicillanic acid production can be described by the Warburton et al. (1973) model, verified by Bryjak and Noworyta (1993) and Illanes (2008) [14–16]:

$$v_P = \frac{V \cdot C_S}{\left[K_M \cdot \left(1 + \frac{C_{S0} - C_S}{K_{iF}}\right) \cdot \left(1 + \frac{C_{S0} - C_S}{K_{iP}}\right) + C_S \cdot \left(1 + \frac{C_{S0} - C_S}{K_{iP}} + \frac{C_S}{K_{iS}}\right)\right]} \quad (1)$$

This kinetic model was proposed for enzymatic processes in batch system and takes into consideration the inhibitory effects, namely the non-competitive inhibitions induced by Penicillin G and 6-Aminiopenicillanic acid, and the competitive inhibition due to phenylacetic acid [17].

- the biocatalyst particle is spherical;
- the enzyme is uniformly distributed inside the particle;
- there are no interactions between the substrate or products and support;