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Effect of bed configuration of immobilized biocatalysts on penicillin G hydrolysis efficiency

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Abstract–The external and internal mass transfer of Penicillin G in the process of its enzymatic hydrolysis to 6-Aminopenicillanic acid under competitive and non-competitive inhibitions have been comparatively analyzed for a bioreactor with mobile bed *vs.* a stationary basket bioreactor, both with *Penicillin amidase* immobilized in Eupergit C. The Penicillin G mass transfer and hydrolysis enzymatic rates have been analyzed by means of the ratios' values between the oxygen mass transfer coefficients, effectiveness factors, external mass flows and Penicillin G concentrations at the biocatalyst particle surface for the considered bioreactors. The results indicated that the bioreactor with mobile bed is more efficient especially for biocatalyst particles with diameter under 1.5 mm. For larger particles the performances of the two bioreactors become similar. Moreover, taking into consideration the external mass flow of Penicillin G and the number of enzymatic hydrolysis cycles, the basket bioreactor is recommended. The mathematical equations proposed are in good concordance with the experimental results, the average deviations varying from ±4.11% for the bioreactor with mobile bed of immobilized *Penicillin amidase* to ±5.03% for the basket bioreactor.

Keywords: Bioreactor, Diffusion, Immobilized Enzymes, Mass Transfer, Penicillin, *Penicillin amidase*

INTRODUCTION

6-Aminopenicillanic acid is the key intermediary for the synthesis of semi-synthetic beta-lactamic antibiotics (amoxicillin, ampicillin, etc.) [1]. The method currently applied at industrial scale for 6-Aminopenicillanic acid production is the hydrolysis of natural penicillin (penicillins G and V) with *Penicillin amidase* biosynthesized by various microorganisms (*Escherichia coli*, *Bacillus megatherium*, *Arthrobacter viscosus*, *Streptomyces* sp.) [1]. For increasing its stability, as well as for facilitating its recovery and reuse in many hydrolysis cycles, the enzyme was immobilized using several supports (polyacrylamide, agarose, chitosan, epoxy-activated, etc.) [1-3]. Among them, the covalent immobilization of *Penicillin amidase* in the epoxy-activated support of Eupergit C type is efficiently applied at industrial scale [2].

As previously stated, 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 are the attenuation of the substrate inhibitory effect, the easier recovery of the biocatalysts from the final medium, and, implicitly, the increase of the number of the repeated enzymatic reaction cycles which use the same particles of biocatalysts [4].

Most of the industrial processes for 6-Aminopenicillanic acid production involve bioreactors with partially or completely stirred beds of immobilized *Penicillin amidase* [5]. Because of their constructive and technological characteristics, which are similar to the well-known stirred bioreactors, these bioreactors allow reaching higher rates of heat and mass transfer. But, in these bioreactors the biocatalyst physical integrity could be affected by the shear forces, thus leading to the reduction of the number of successive hydrolysis cycles [6].

One of the most attractive bioreactors with immobilized enzymes or cells is the basket bioreactor type. The basket bioreactors are derived from the bioreactors with packed beds, the biocatalyst particles being fixed in an annular cylindrical or conic bed, which is either static and placed around the stirrer [7-9], or rotary [10-12]. This type of bioreactor avoids the disadvantages of the bioreactors with packed beds, the flooding or deposition, as well as the mechanical disruption of the biocatalysts particles, phenomena that are encountered in the bioreactors with mobile beds.

The previous studies indicated that the use of *Penicillin amidase* immobilized in Eupergit C represents a viable alternative to the hydrolysis process with free enzyme or to those using other supports for immobilization [5,13]. Regardless of the bioreactor type, by selecting the optimum operating regime, the activity and physical integrity of the biocatalysts remain unaffected for many hydrolysis cycles, even if the process is carried out under substrate or product inhibitions [5,13].

Developing the previous studies on penicillin G mass transfer in 6-Aminopenicillanic acid production for the bioreactors with mobile and basket beds of biocatalysts [5,6,13], this work analyzes the relative rates of external and internal diffusion of antibiotic for

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these two bioreactors types, under substrate and products inhibitory effects, for the purpose to establish the influence of biocatalyst bed configuration and operating conditions on the efficiencies of transfer and conversion processes.

EXPERIMENTAL

The experiments were performed in batch system in two bioreactor types. The bioreactor with mobile bed of biocatalysts was a 10 *l* (8 *l* working volume) laboratory stirred bioreactor (Fermac 320, Electrolab), with computer-controlled and recorded parameters [14]. The mixing equipment consisted of two pitched bladed turbines of 64 mm diameter and three baffles. The inferior impeller was placed at 64 mm from the bioreactor bottom. The superior impeller was placed on the same shaft at a distance of 32 mm from the inferior one. The rotation speed was maintained at 250 rpm, this value avoiding the "cave" formation at the broths surface, solid phase deposition at the bioreactor bottom, and mechanical disruption of the biocatalysts particles. According to the previous results, this impeller combination and rotation speed were found to be the optimum ones for the investigated fermentation system [15].

The stationary basket bioreactor was designed by modifying the above presented stirred bioreactor (Fig. 1). In this case, the bioreactor was 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 consists of plastic mesh and was placed centered around the stirrer, at 100 mm from the bioreactor bottom. The optimum impeller combination was found to be two Rushton turbines, the superior one being placed outside the basket and the other inside the basket at its inferior extremity [15]. The impeller rotation speed was of 250 rpm.

In both cases, the enzymatic hydrolysis of penicillin G was carried out using *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. [16]. The specific activity of the immobilized biocatalysts was 180 UI g^{-1}. The following sizes of the biocatalysts were used: 1.0, 1.5 and 2.0 mm. The volumetric fraction of the immobilized enzyme into the whole medium

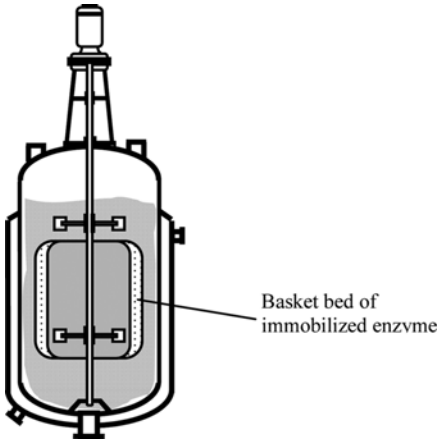

Fig. 1. The experimental basket bioreactor.

Table 1. Parameters used for Penicillin G concentration calculations

Parameter	Value
D_{SL} (m^2 s^{-1})	$4.0 \cdot 10^{-10}$
D_{Se} (m^2 s^{-1})	$8.27 \cdot 10^{-12}$
K_M (mol m^{-3})	13
K_{iF} (mol m^{-3})	313
K_{iP} (mol m^{-3})	132
K_{iS} (mol m^{-3})	25
V (mol g^{-1} s^{-1})	$2.83 \cdot 10^{-2}$

was 0.28. Any mechanical damage of the biocatalysts due to the shear forces was recorded during the two series of 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 was at 30 °C.

The experimental values of the external mass transfer rate were calculated and analyzed by means of the variation of penicillin G concentrations in the liquid bulk volume and at the biocatalysts particle surface during the enzymatic conversion. The penicillin G concentration was 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 30 °C.

The internal values of penicillin G concentration or mass transfer were calculated using only the proposed mathematical model. The values of the parameters used for calculations are given in Table 1 for the batch system with immobilized *Penicillin amidase* [13].

The hydrolysis end was considered when the penicillin G conversion degree reached minimum 90-95%. For both bioreactors, the samples were taken at 10, 20, 45 and 60 minutes from the process beginning.

Each experiment was repeated two or three times with identical conditions, the average value of the considered parameters being used. The average experimental error was of ±4.11% for the bioreactor with mobile bed of immobilized *Penicillin amidase*, and ±5.03% for the basket bioreactor, respectively.

RESULTS AND DISCUSSION

Internal diffusion is an important limiting step for the biocatalysts immobilized inside of an inert matrix, its importance depending especially on the substrate and support characteristics. In the case of 6-Aminopenicillanic acid production by enzymatic hydrolysis of penicillin G, the substrate has to migrate to *Penicillin amidase* through non-linear channels, its diffusion being described by the effective diffusivity. Thus, the rate of the enzymatic hydrolysis occurring inside the biocatalyst particle could be inferior to that corresponding to the homogeneous system, due to the lower penicillin G concentration compared to its value in the liquid bulk. However, due to the reduced rate of inhibitor internal transfer to and from the active centre, the inhibitory phenomena could be dimin-

ished or amplified, respectively.

The enzymatic hydrolysis of penicillin G occurs under inhibitory effects induced by products, as well as by substrate [13]. Therefore, according to literature, to penicillin G and 6-aminiopenicillanic acid generate non-competitive inhibition, while phenylacetic acid is responsible for the competitive inhibitions [17]. For the ideal immobilization process (uniform distribution of enzyme inside the biocatalyst, no interactions between the substrate or products and support, spherical shape of the biocatalyst particle), the following model was established for the steady-state conditions of penicillin G mass transfer and conversion inside the biocatalyst particle [5,13]:

$$\left(\frac{d^2C_{SP}}{dr^2}+\frac{2}{r}\cdot\frac{dC_{SP}}{dr}\right)=\frac{V\cdot C_{SP}}{D_{Se}\cdot\left[K_M\cdot\left(1+\frac{C_{S0}-C_{SP}}{K_{iF}}\right)\cdot\left(1+\frac{C_{S0}-C_{SP}}{K_{iP}}\right)+C_{SP}\cdot\left(1+\frac{C_{S0}-C_{SP}}{K_{iP}}+\frac{C_{SP}}{K_{iS}}\right)\right]} \quad (1)$$

This model represents the expression for the mass balance of penicillin G related to the biocatalyst particle, respecting the hydrolysis kinetics proposed by Warburton et al. [18] and the Bird equation [17-20]. Eq. (1) was solved under the following boundary limits [5,13]:

$$1)\ r=0\ \text{(at particle centre)},\ \frac{dC_{SP}}{dt}=0 \quad (2)$$

$$2)\ r=R_P\ \text{(at particle surface)},\ -D_{Se}\cdot\frac{dC_{SP}}{dr}=k_L\cdot(C_{SL}-C_{Si}) \quad (3)$$

and describes the penicillin G concentration profile inside the biocatalyst particle with radius R_P:

$$C_{SP}=\frac{(C_{Si}-C_{SL})\cdot R_P^{\,3}\cdot K_{iF}\cdot C_{SL}^{\,2}\cdot\left(Bi\cdot e^{\frac{36\cdot D_{Se}\cdot r}{R_P^{\,2}\cdot K_{iP}\cdot K_{iF}\cdot C_{SL}^{\,2}}}+K_{iP}\right)-18\cdot D_{Se}\cdot\left(Bi\cdot C_{SL}+e^{\frac{36\cdot D_{Se}\cdot r}{R_P^{\,2}\cdot K_{iP}\cdot K_{iF}\cdot C_{SL}^{\,2}}}\right)}{36\cdot D_{Se}\cdot B_i\cdot C_{SL}\cdot V\cdot\sinh\left(\frac{D_{Se}}{R_P\cdot K_{iP}\cdot K_{iF}\cdot C_{SL}^{\,2}}\right)\cdot e^{\frac{18\cdot D_{Se}}{R_P\cdot K_{iP}\cdot K_{iF}\cdot C_{SL}^{\,2}}}}$$
$$-\frac{k_L\cdot[\varphi\cdot(K_M+C_{SL})-K_M\cdot R_P]\cdot\left(e^{\frac{36\cdot D_{Se}\cdot r}{R_P^{\,2}\cdot K_{iP}\cdot K_{iF}\cdot C_{SL}^{\,2}}}+1\right)}{36\cdot D_{Se}\cdot C_{SL}\cdot V\cdot\sinh\left(\frac{D_{Se}}{R_P\cdot K_{iP}\cdot K_{iF}\cdot C_{SL}^{\,2}}\right)\cdot e^{\frac{18\cdot D_{Se}}{R_P\cdot K_{iP}\cdot K_{iF}\cdot C_{SL}^{\,2}}}}$$
$$+\frac{e^{\frac{D_{Se}\cdot(K_{iF}-K_{iP}+2\cdot C_{S0})}{R_P\cdot K_{iP}\cdot K_{iF}\cdot C_{SL}^{\,2}}}}{R_P^{\,2}\cdot K_{iP}\cdot K_{iF}\cdot C_{SL}^{\,2}}\cdot\{9\cdot D_{Se}-Bi\cdot k_L\cdot[\varphi\cdot(K_M+C_{SL})+R_P]\}\cdot\left(e^{\frac{36\cdot D_{Se}\cdot r}{R_P^{\,2}\cdot K_{iP}\cdot K_{iF}\cdot C_{SL}^{\,2}}}+1\right) \quad (4)$$

and the substrate concentration at the particle surface:

$$C_{Si}=C_{SL}+\frac{R_P^{\,3}\cdot K_{iF}\cdot C_{SL}^{\,3}-18\cdot D_{Se}-Bi\cdot k_L\cdot[\varphi\cdot(K_M+C_{SL})-K_M\cdot R_P]}{k_L\cdot B_i\cdot C_{SL}\cdot V\cdot\sinh\left(\frac{D_{Se}}{R_P\cdot K_{iP}\cdot K_{iF}\cdot C_{SL}^{\,2}}\right)\cdot e^{\frac{18\cdot D_{Se}}{R_P\cdot K_{iP}\cdot K_{iF}\cdot C_{SL}^{\,2}}}}$$
$$+\frac{36\cdot D_{Se}^{\,2}\cdot e^{\frac{D_{Se}\cdot(K_{iF}-K_{iP}+2\cdot C_{S0})}{R_P\cdot K_{iP}\cdot K_{iF}\cdot C_{SL}^{\,2}}}}{k_L\cdot R_P^{\,4}\cdot K_{iP}^{\,2}\cdot K_{iF}^{\,2}\cdot C_{SL}^{\,4}}$$
$$\cdot\{9\cdot D_{Se}-Bi\cdot k_L\cdot[\varphi\cdot(K_M+C_{SL})+R_P]\}\cdot e^{\frac{36\cdot D_{Se}}{R_P\cdot K_{iP}\cdot K_{iF}\cdot C_{SL}^{\,2}}} \quad (5)$$

Eqs. (4) and (5) are valid for both bioreactor types.

Therefore, the penicillin G external and internal mass flows can be calculated by means of its concentrations at the biocatalyst surface and inside it. Regardless of the bioreactor type, for avoiding the errors that could appear as the result of the substrate and products accumulation inside the biocatalyst particle, the experiments and related calculations were carried out for the first cycle of enzymatic hydrolysis of penicillin G. Moreover, for all parameters analyzed for the basket bioreactor, the average values reached in the cylindrical bed were considered in the calculations.

For both considered systems, the penicillin G mass flux from the liquid phase to the particle surface was:

$$n_L=k_L\cdot(C_{SL}-C_{Si}) \quad (6)$$

where k_L is calculated with the expression adequate either for the mobile bed or for the packed one [21]. To quantify the influence of the design of biocatalyst bed and associated diffusional phenomena on the substrate mass transfer rate in the liquid phase surrounding the biocatalyst particle, the variation of the ratio between the penicillin G mass transfer for the bioreactor with mobile bed, k_{LM}, and that for the basket bioreactor, k_{LB}, with the particle size is plotted in Fig. 2.

Although the diminution of k_L from the smallest biocatalyst particles to the largest ones can be observed, as the result of the increase of the boundary layer thickness, the k_{LM} values are significantly higher than those for k_{LB} (the k_{LM}/k_{LB} ratio varies from 4, for the largest biocatalyst particles, to 5.5, for the smallest ones). Obviously, this variation is due to the amplified turbulence that can be induced in the system containing mobile bed of immobilized *Penicillin amidase*.

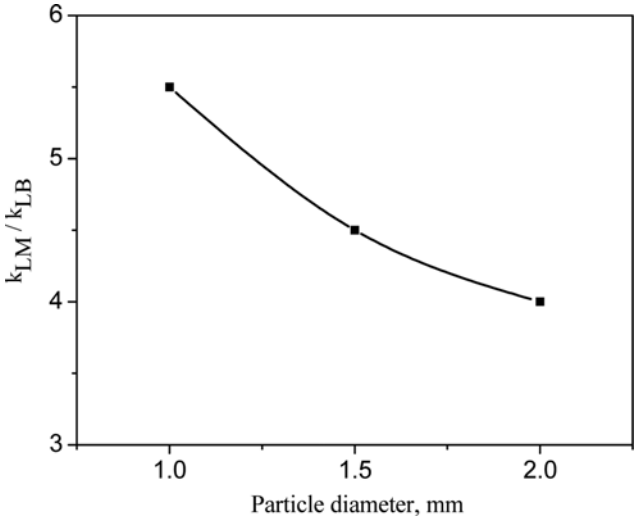

Fig. 2. Variation of ratio between the mass transfer coefficients, k_L, for the mobile and basket beds with the biocatalyst particle diameter.

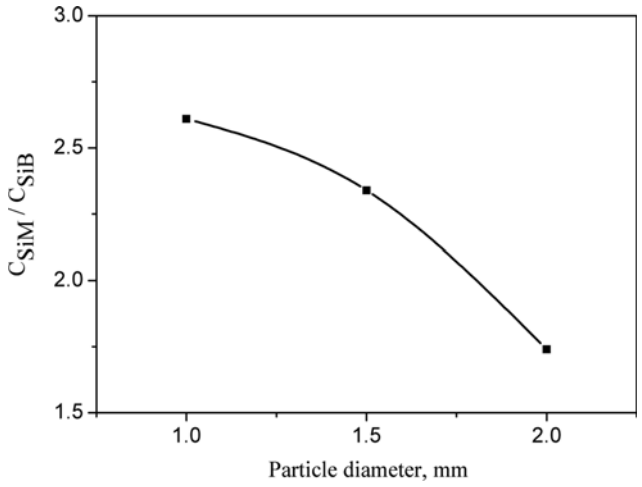

Fig. 3. Variation of ratio between the Penicillin G concentrations at the biocatalyst particle surface for mobile and basket beds with the biocatalyst particle diameter.

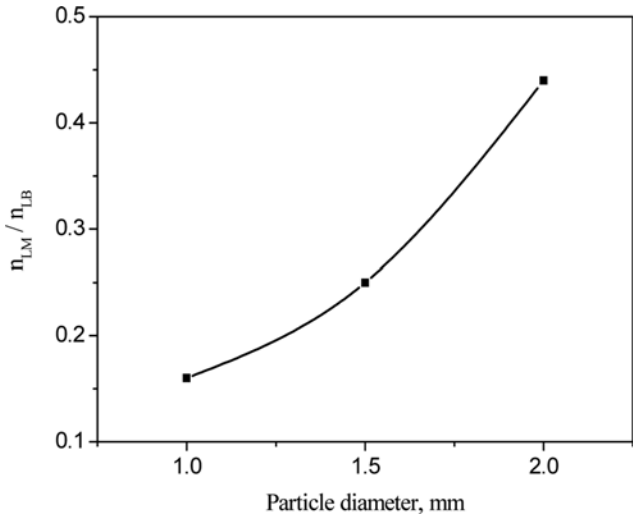

Fig. 4. Variation of ratio between the external mass flows for mobile and basket beds with the biocatalyst particle diameter.

As previously concluded, the variation of the ratio between the penicillin G concentrations at the particle surface and in the liquid bulk with the biocatalyst particle size depends on the biocatalyst bed type. Thus, for the mobile bed, the lowest superficial concentration of penicillin G is reached for the intermediary size of the particles, due to the equilibrium between the antagonistic processes of internal diffusion and inhibition induced by substrate [5]. For a packed bed of basket conformation, this ratio decreases radially towards the outer surface of the biocatalysts bed, this variation being the consequence of the decreasing of penicillin G mass transfer rate through the liquid boundary layer, as well as of its consumption by enzymatic hydrolysis [13].

Fig. 3 indicates the decreasing of the ratio between the superficial concentrations of penicillin G corresponding to the mobile bed, C_{SiM}, and that for the basket bed, C_{SiB}, by increasing the biocatalyst particle diameter. This dependence suggests the more pronounced increase of the superficial concentration of substrate for the basket bed containing larger biocatalyst particles, owing to the highest void fraction of the packed bed and, consequently, of the lowest resistance to the diffusion inside the cylindrical bed.

The variation of external mass flows through the liquid boundary layer surrounding the biocatalyst particle is contrary to that of mass transfer coefficients for both bioreactor types, especially due to the amplification of the substrate concentration gradient between the liquid phase and the particle surface from the smallest biocatalysts to the largest ones [5,13]. The ratio between the external flow for the bioreactor with mobile bed, n_{LM}, and that for the basket bioreactor, n_{LB}, is lower than 1 for all considered biocatalyst sizes (Fig. 4).

This result suggests that the mass flow of penicillin G is accelerated if the particles of immobilized *Penicillin amidase* are included in the packed bed. In this case, the acceleration of the hydrolysis rate of substrate by increasing the basket bed thickness leads to the amplification of penicillin G concentration gradient and, consequently, to the increase of its external mass flow. The reduction of external mass flow recorded for the basket bioreactor becomes more important by decreasing the biocatalyst particle size and by increasing the cylindrical bed thickness.

The preliminary experiments on these two bioreactors indicated that it is possible to reach very low or negligible values of penicillin G internal mass flow near the particle center for both bioreactor types [5,13]. This central region was considered an "enzymatic inactive region," its extent being estimated by considering the order of magnitude of penicillin G effective diffusivity. Therefore, it was assumed that the values of internal mass flow lower than 10^{-11} mol m^{-2} s^{-1} can be associated with this inactive region [5,13].

According to Fig. 5, the extent of the inactive region increases with the size of the biocatalyst particle. But, a less extended inactive region is reached for the bioreactor with mobile bed of immobilized *Penicillin amidase*, being below 9.8% from the overall volume of each biocatalyst particle. For the bioreactor with basket bed, the extent of the inactive region varies between 4.4 and 17.4%, due to the lower values of penicillin G mass flows reached in the biocata-

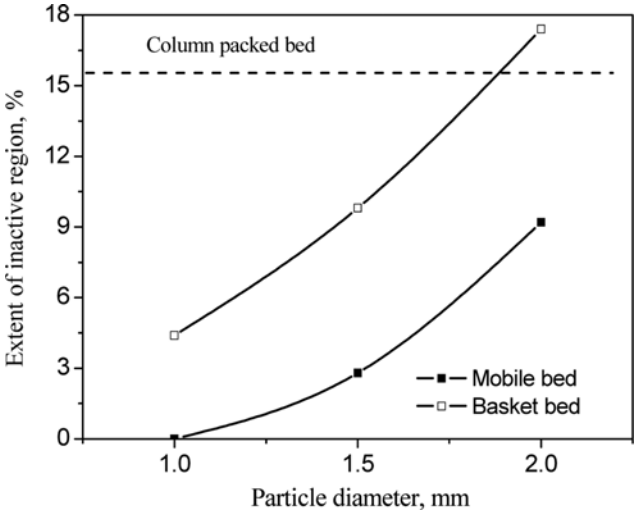

Fig. 5. Extent of inactive regions with the biocatalyst radius.

lyst particle.

On the basis of the extent of the enzymatic inactive region, the comparative analysis of the bioreactors with mobile, basket and column packed bed underlines the superior efficiency of the first two bioreactors. Only for the largest biocatalyst, the extent of the inactive region for the basket bioreactor exceeds that for the column packed bed.

The influence of the internal diffusion on overall enzymatic process can be quantified by means of the Thiele modulus, φ, and Biot number, Bi. The Thiele modulus indicates the magnitude of the influence of internal diffusion on the enzymatic reaction rate, being defined for the studied system by the following expression [5,13]:

$$\varphi = \frac{R_P}{3} \cdot \sqrt{\frac{V}{2K_M \cdot D_{Se} \cdot \left[\frac{C_{SL}}{K_M} - \ln\left(1+\frac{C_{SL}}{K_M}\right)\right]}} \cdot \frac{C_{SL}}{K_M + C_{SL}} \quad (7)$$

The values of the Thiele modulus are specific for a given size of immobilized enzyme particles, depending on the concentration of penicillin G in the liquid phase, but not on the conformation of the biocatalysts bed [5,13]. Thus, the values of this parameter for the mobile and basket beds are similar. Because values of Thiele modulus over 0.3 indicate an important limitation of the process of penicillin G enzymatic hydrolysis by immobilized *Penicillin amidase*, induced by the internal diffusion of substrate [20], it was previously found that the relative magnitude of resistance to the internal diffusion becomes more pronounced only at lower penicillin G concentration in the liquid phase and larger biocatalyst particle size, for both types of bioreactors [5,13].

The Biot number represents the ratio between the resistance to the internal diffusion and that corresponding to the boundary layer surrounding the particle:

$$Bi = \frac{k_L \cdot R_P}{D_{Se}} \quad (8)$$

From Fig. 6, the relative importance of resistance to the penicillin G diffusion inside the particle compared to that opposite to its diffusion through the liquid boundary layer surrounding the particle is considerably higher in the case of mobile bed of immobilized *Penicillin amidase*. This result is the consequence of the increased turbulence in the liquid phase for the bioreactor with mobile bed of biocatalysts compared to that reached in the basket bioreactor, this leading to higher external diffusion rate in the boundary layer. The reduction of the ratio Bi_M/Bi_B with the increase of biocatalyst particle size could be associated with the more pronounced amplification of the resistance to the penicillin G internal diffusion for the basket bed, owing to the lower substrate concentration inside the cylindrical packed bed.

The effect of the internal diffusion on the rate of penicillin G conversion during the enzymatic process can be described more accurately by the effectiveness factor λ, defined as the ratio between the rates of the enzymatic reaction in heterogeneous system and in homogeneous one. Considering the steady-state conditions, it can be assumed that the rate of the internal enzymatic hydrolysis equals the internal mass flow of penicillin G, the relationship for calculating the factor λ becoming:

$$\lambda = \frac{4\pi \cdot R_P^{\,2} \cdot D_{Se} \cdot \left.\frac{dC_{SP}}{dr}\right/ r = R_P}{\frac{4}{3}\pi \cdot R_P^{\,3} \cdot \frac{V \cdot C_{SP}}{\left[K_M \cdot \left(1+\frac{C_{S0}-C_{SL}}{K_{iF}}\right) \cdot \left(1+\frac{C_{S0}-C_{SL}}{K_{iP}}\right) + C_{SL} \cdot \left(1+\frac{C_{S0}-C_{SL}}{K_{iP}}+\frac{C_{SL}}{K_{iS}}\right)\right]}} \quad (9)$$

For both bioreactor types, the factor λ varies slowly near the particle surface or center. In the region vicinal to the biocatalyst surface, the higher concentration of penicillin G, rather equal with that at the particle surface, leads to the values of λ close to 1. The slow variation of factor λ in the central region is the result of the constant low level of penicillin G concentration near the particle center [5,13].

The effect of internal diffusion on the enzymatic hydrolysis rate is more important for the basket bioreactor. Therefore, the variation of the ratio between the effectiveness factors for the bioreactors with mobile and basket beds, respectively, with the biocatalyst

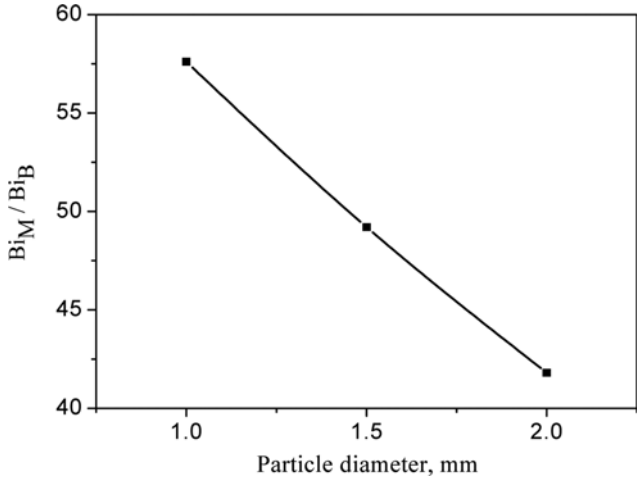

Fig. 6. Variation of ratio between the Biot numbers for mobile and basket beds with the biocatalyst particle diameter.

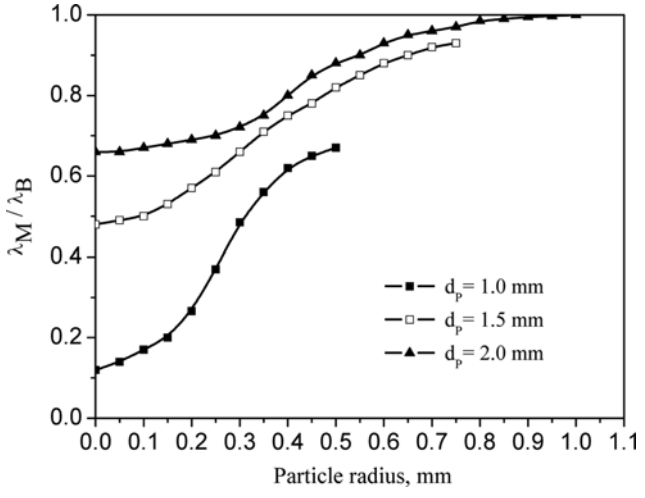

Fig. 7. Variation of ratio between the effectiveness factors for mobile and basket beds with the biocatalyst particle diameter.

particle radius, plotted in Fig. 7, suggests that the decreasing of the penicillin G conversion rate compared to the system containing free *Penicillin amidase* is more pronounced for the basket bed, due to the lower substrate concentration inside the biocatalyst particle.

The relative magnitude of this phenomenon is amplified towards the particle center, but is attenuated with the increase of particle size. Because the ratio λ_M/λ_B is close to 1 for the immobilized enzyme particles with 2 mm diameter, it can be concluded that the bed conformation exhibits less significant influence on the enzymatic process rate for larger biocatalysts.

Thus, by using both the mobile bed and the basket bed containing immobilized *Penicillin amidase*, the rate of the enzymatic production of 6-Aminopenicillanic acid is considerably reduced compared to the system containing free enzyme, but this reduction is for up to 1.5-8.3 times higher in the case of basket bioreactor.

CONCLUSIONS

6-Aminopenicillanic acid production by enzymatic hydrolysis of penicillin G using immobilized *Penicillin amidase* in Eupergit C was comparatively analyzed for two types of bioreactors: with mobile and basket beds of biocatalysts. The studies focused on the external and internal mass transfer and, implicitly, on the influence of the internal diffusion on the transfer and enzymatic processes rates. The kinetic model took into consideration the substrate (penicillin G) and products (6-Aminopenicillanic acid and phenylacetic acid) inhibitory effects.

Excepting the criterion of penicillin G external mass flow, the bioreactor with mobile bed of immobilized *Penicillin amidase* was found to be more efficient, but the difference between the performances of the two bioreactors was attenuated by increasing the biocatalyst particle size.

Depending on the desired characteristics of biocatalyst, operating conditions and desired number of enzymatic hydrolysis cycles, the selection of the optimum configuration of immobilized enzyme bed has to involve all the discussed aspects regarding the relative diffusion and hydrolysis rates.

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LIST OF ABBREVIATIONS

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}]
D_{Se} : Penicillin G effective diffusivity [m^2 s^{-1}]
D_{SL} : Penicillin G liquid phase diffusivity [m^2 s^{-1}]
k_L : liquid phase mass transfer coefficient of Penicillin G [m s^{-1}]
K_M : Michaelis-Menten constant [mol m^{-3}]
V : maximum enzymatic reaction rate [mol g^{-1} s^{-1}]
ϕ : volumetric fraction of biocatalyst particles
η_L : liquid phase viscosity [Pa s]
ρ_L : liquid phase density [kg m^{-3}]

Subscripts
B : for basket bioreactor
M : for bioreactor with mobile bed

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