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Production of succinic acid in basket and mobile bed bioreactors — Comparative analysis of substrate mass transfer aspects☆

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ABSTRACT

The glucose mass transfer in the biosynthesis of succinic acid with immobilized *Actinobacillus succinogenes* cells has been comparatively analyzed for a bioreactor with mobile bed *vs.* a stationary basket bioreactor. The process has been considered to occur under substrate and product inhibitory effects. The results indicated that the bioreactor with mobile bed is more efficient for biocatalyst particles with a diameter over 3 mm, while the basket bioreactor is more efficient for smaller biocatalyst particles and basket bed thickness below 5 mm. The performances of both configurations of immobilized *A. succinogenes* cell beds were found to be superior to the column packed bed bioreactor.

1. Introduction

Succinic acid has numerous applications in chemical industry (reagents, synthetic resins, biodegradable polymers, electroplating, green solvents, inks), agriculture (pesticides, growth regulators, and stimulators), and pharmaceutical and food industries (amino acids, antibiotics, vitamins, surfactants, additives) [1–3]. At industrial scale, this acid is produced by chemical synthesis using butane *via* maleic anhydride, but this technology raises important problems concerning the environmental protection [1,3]. Depending on the product required purity, this technology cost could reach 6.3 EUROs·kg^{-1} succinic acid, the contribution of raw material to the final cost varying between 16% and 24% [1,3].

The "white biotechnology", concept promoted since 2007, sustains the priority of the use of renewable sources for chemical production by low-expensive and eco-friendly biotechnologies [4]. These premises lead to the increasing of the interest in producing succinic acid by fermentative low-cost technologies. Therefore, a large number of microorganisms have been tested as potential producers of succinic acid (Table 1).

These strains can convert various carbon sources (glucose, saccharose, molasses, glycerol, starch, cellulosic hydrolysates or milling by-products) under anaerobic conditions into succinic acid and secondary acids (formic, acetic, pyruvic acids). However, either due to the low productivity, or to the non-Newtonian rheology and complex composition of the final broth, only the strains *Actinobacillus succinogenes* and *Actinobacillus succiniproducens* have been considered as important producers with potential application at larger scale [8,10]. Among these two strains, *A. succinogenes* allows reaching higher concentration of succinic acid during mixed-acid fermentation [11].

Table 1
Microorganisms used for succinic acid biosynthesis [4–9]

Type of microorganism	Strain
Bacteria	*Veillonella parvula*, *Selenomonas ruminatum*, *Succiniclasticus ruminis*, *Corynebacterium glutamicum*, *Enterococcus fecalis*, *Actinobacillus succinogenes*, *Actinobacillus succiniproducens*, *Mannheimia succiniproducens*, *Escherichia coli*
Yeast	*Saccharomyces cerevisiae*
Fungus	*Aspergillus niger*, *Aspergillus fumigatus*, *Byssochlamys nivea*, *Lentinus degener*, *Paecilomyces varioti*, *Penicillium viniferum*

Most of the fermentations for succinic acid production have been carried out using free *A. succinogenes* cells, the processes being affected by the substrate and product inhibition phenomena [8,10]. Although the utilization of immobilized microorganisms or enzymes offers the advantages of the increase of the thermal, chemical, and to the shear forces resistance of the biocatalysts, as well as the diminution or avoidance of

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the substrate inhibition processes, easier recovery of the biocatalysts from the final broths, and, consequently, the increase of number of the biosynthesis cycles re-using the same particles of biocatalysts, there are only few reports in literature concerning the succinic acid fermentation with immobilized *A. succinogenes* cells [10].

Generally, the bioreactors with immobilized cells or enzymes can be designed as column, stirred, gas-lift or membrane bioreactors, being operated in batch, continuous or semicontinuous systems, with fixed, mobile/stirred, expanded or fluidized bed. The bioreactors with fixed bed of biocatalysts are widely preferred. However, these equipments have some major disadvantages [11,12]. Thus, due to the laminar flow inside the bed, the rates of mass and heat transfer are low and the back-mixing or reverse flow phenomenon could be induced. Furthermore, the solid particles from effluent can clog the biocatalyst bed, the effect consisting on the reducing of the flow rate inside the bed and on the possibility to inactivating the biocatalysts. On the other hand, the formation of the preferential flow channels inside the bed leads to the deviation from the plug flow and to the inefficient conversion of the substrate.

For the above reasons, the previous studies on succinic acid fermentation with immobilized *A. succinogenes* cells have been carried out in a bioreactor with mobile bed of biocatalysts and in a stationary basket bioreactor [10,13]. Due to the bioreactor with mobile bed of immobilized bacterial cells constructive and operational characteristics, which are similar to the well-known stirred bioreactors, higher rates of heat and mass transfer have been reached. In the same time, the biocatalyst physical integrity could be affected by the shear forces, this leading to the reduction of the number of successive fermentation cycles [10].

The design of the bioreactor of basket type is derived from the bioreactors with fixed beds, the biocatalyst particles being fixed in an annular cylindrical or conic bed, which is either static and placed around the stirrer, or rotary [13–17]. This bioreactor avoids both the disadvantages of the bioreactors with fixed beds and the flooding/deposition or the mechanical disruption of the biocatalysts particles, phenomena encountered in the bioreactors with mobile beds. As the consequence of the combination between the perfect mixed flow around the basket and the plug flow inside the biocatalysts bed, the hydrodynamics of the medium around the basket exhibits an important influence on the transfer processes involved in the substrate conversion.

The previous studies on fermentations with yeast or bacterial cells immobilized in alginate indicated that their utilization in systems with stirred bed or basket bed of biocatalysts can represent viable alternatives to the processes involving free cells [10,13]. By selecting the optimum operating regime of the two types of bioreactors, the activity and physical integrity of the immobilized cells remained unaffected for many fermentation cycles, even if the fermentation is carried out under substrate inhibition conditions.

In this context, on the basis of the previous results on glucose mass transfer into the liquid phase and inside the particles of immobilized *A. succinogenes* cells [10,13], as well as on its consumption in succinic acid fermentation, the aim of this work is to establish the influence of bioreactor design and operating conditions on the efficiencies of transfer and conversion processes. In this purpose, by assuming that the glucose consumption respects the kinetic model including the substrate and products inhibitions, the rates of external and internal diffusions of the substrate, and their influences on substrate conversion have been comparatively analyzed for two types of bioreactors, with mobile bed and, respectively, basket bed of biocatalysts.

2. Materials and Methods

2.1. Bioreactors

The experiments have been carried out in batch system in two types of bioreactors: a mobile bed bioreactor and a stationary basket bioreactor, both with immobilized *A. succinogenes* cells.

The bioreactor with mobile bed of biocatalysts was a 10 L (8 L working volume) laboratory stirred bioreactor (Biostat A, B. Braun Biotech International), with computer-controlled and recorded parameters [18]. The mixing equipment consisted on two pitched bladed turbines of 64 mm diameter and three baffles. The inferior impeller has been 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 $r \cdot min^{-1}$, this value avoiding the "cave" formation at the broth surface, solid phase deposition at the bioreactor bottom and mechanical disruption of the biocatalysts particles. According to the previous results, these impellers combination and rotation speeds were found to be the optimum ones for the investigated fermentation system [19]. Any mechanical damage of the biocatalyst due to the shear forces was recorded during the experiments.

The stationary basket bioreactor was designed by modifying the above presented stirred bioreactor. 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 (Fig. 1). The basket was made by plastic mesh and placed centered around the stirrer, at 100 mm from the bioreactor bottom. The mixing system consisted on two Rushton turbines on the same shaft, the superior one placed outside the basket and the other inside the basket at its inferior extremity [13]. The impeller rotation speed was of 250 $r \cdot min^{-1}$.

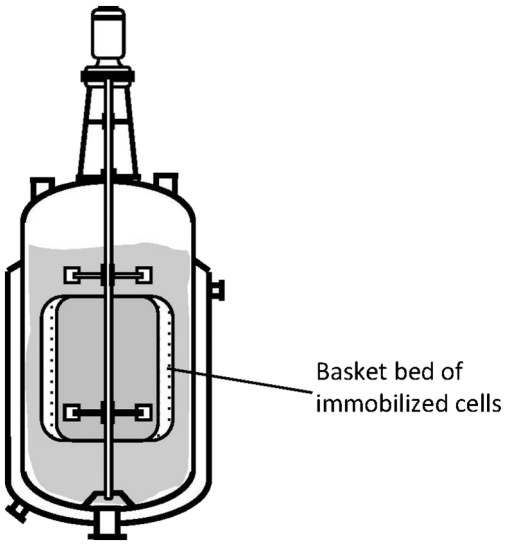

Fig. 1. Experimental stationary basket bioreactor.

2.2. Strain, growth conditions, and cell immobilization

In both cases, the medium composition was (per liter): glucose 30 g, yeast extract 5 g, $NaH_2PO_4 \cdot H_2O$ 1.16 g, Na_2HPO_4 0.31 g, NaCl 1.0 g, $MgCl_2 \cdot 6H_2O$ 0.2 g, $CaCl_2 \cdot 2H_2O$ 0.2 g, vitamin B_{12} 1 μg, biotin 20 μg, folic acid 20 μg, thiamine 50 μg, riboflavin 50 μg, niacin 50 μg, pantothenate 50 μg, *p*-aminobenzoate 50 μg, lipoic acid 50 μg, vitamin B_6 100 μg, $MgCO_3$ 30 g, silicone antifoam 1 ml [20]. The fermentation temperature was 37 °C.

A. succinogenes ATCC 55617 cells immobilized in alginate have been used in the experiments. The microorganism was provided by the American Type Culture Collection and was preserved at −70 °C. The inoculum has been prepared by incubating *A. succinogenes* at 30 °C in 100 ml Duran bottles each containing 50 ml of trypticase soya broth. The bottles were stirred at 100 $r \cdot min^{-1}$ on a rotary shaker for 48 h.

The immobilization has been carried out by bacterial cells inclusion into the alginate matrix, respecting the method given in literature [21]. The biocatalysts were prepared separately for each bioreactor in

aseptical conditions. In this purpose, 6 ml of inoculum was mixed with 20 ml of 5% aqueous solution of sodium alginate. The biocatalyst particles have been obtained by dripping this suspension through a capillary into a solution of 0.2% $CaCl_2$ under constant pressure. Capillaries with three different diameters have been used and the obtained particles of immobilized *A. succinogenes* cells had the following diameters: 3.0, 3.6 and 4.2 mm, respectively. In all cases, the volumetric fraction of the immobilized cells related to the medium phase was 0.23.

2.3. Calculations of parameter values

The experimental values of the external mass transfer rate have been calculated and analyzed by means of the variation of glucose concentrations in the liquid bulk volume and biocatalyst particle surface during the fermentation. The glucose concentration has been measured by high performance liquid chromatography technique (HPLC) with a Phenomenex Rezex ROA column (7.8 mm diameter, 300 mm length), provided with the refractive index detector RID-10A. The mobile phase was a solution of 2.5×10^{-3} mol·L^{-1} sulfuric acid with a flow rate of 0.6 ml·min^{-1}. The analysis temperature was of 65 °C.

The internal values of glucose concentration or mass flow have been calculated using only the proposed mathematical models. The values of the parameters used for calculations are given in Table 2.

Table 2
Parameters used for calculations

Parameter	Value	Reference
D_{SL}/m^2·s^{-1}	6.47×10^{-10}	[22]
D_{Se}/m^2·s^{-1}	4.39×10^{-10}	[23]
K_{iS}/kg·m^{-3}	80	[7]
K_{iP}/kg·m^{-3}	48	[7]
V/kg·kg^{-1}·s^{-1}	1.75×10^{-4}	[7]
$Y_{P/S}$/kg·kg^{-1}	1.10	[20]

The fermentation end has been considered when either the glucose was completely consumed or its concentration remained constant for 12 h.

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 ±5.31% for the bioreactor with mobile bed of immobilized bacterial cells, and ±6.22% for the basket bioreactor, respectively.

3. Results and Discussion

The internal diffusion is an important limiting step for the enzymatic or biochemical processes using enzymes or cells immobilized inside of an inert matrix, its magnitude depending especially on the substrate and support characteristics [10]. In the case of succinic acid production by *A. succinogenes* fermentation, the substrate has to migrate to the immobilized bacterial cells through non-linear channels. Thus, the rate of the substrate conversion occurring inside the biocatalyst particle could be inferior to that corresponding to the homogeneous system, due to the lower glucose concentration compared to its value in the liquid bulk. However, due to the lower rate of substrate internal transfer towards the active centre, the corresponding inhibitory phenomenon could be diminished or avoided.

For the ideal immobilization process (uniform distribution of cells inside the biocatalyst, no interactions between the substrate or products and support, spherical shape of the biocatalyst particle, internal diffusion described by Fick law), and considering the inhibitions induced by substrate and product, the kinetics of glucose transfer and conversion can be described by the following model adapted to the immobilized *A. succinogenes* cells [7]:

$$\frac{dC_{SP}}{dt} = D_{Se} \cdot \left[\frac{1}{r^2} \cdot \frac{d}{dr}\left(r^2 \cdot \frac{dC_{SP}}{dr}\right)\right] - V \cdot C_C \cdot \left(\frac{K_{iS}}{K_{iS} + C_{SP}}\right) \cdot \left(\frac{K_{iP}}{K_{iP} + Y_{P/S} \cdot C_{SP}}\right) \quad (1)$$

This model represents the expression for the mass balance of glucose related to the biocatalyst particle, being based on the Jerusalimsky kinetics and Bird equation [7,24]. Eq.(1) was solved under the following boundary limits [10,13]:

1) $r = 0$ (at particle center), $\frac{dC_{SP}}{dr} = 0$
2) $r = R_P$ (at particle surface), $-D_{Se} \cdot \frac{dC_{SP}}{dr} = k_L \cdot (C_{SL} - C_{Si})$

and describes the glucose concentration profiles inside the biocatalyst particle:

$$C_{SP} = \frac{Bi \cdot (C_{SL} - C_{Si}) \cdot \cosh(3\phi \cdot R_P)}{{R_P}^2} \cdot \left[\frac{3\phi}{R_P} - R_P \cdot \tanh(3\phi \cdot R_P)\right] \cdot \frac{\sinh(3\phi \cdot r)}{r} \quad (2)$$

and at the particle surface:

$$C_{Si} = \frac{Bi \cdot C_{SL} \cdot \cosh(3\phi \cdot R_P) \cdot \left[3\phi - {R_P}^2 \cdot \tanh(3\phi \cdot R_P)\right] \cdot \sinh(3\phi) - C_{SL} \cdot {R_P}^4}{Bi \cdot \cosh(3\phi \cdot R_P) \cdot \left[3\phi - {R_P}^2 \cdot \tanh(3\phi \cdot R_P)\right]} \quad (3)$$

Eqs. (2) and (3) are valid for both bioreactors types.

The influence of the internal diffusion is quantified by the Thiele modulus, φ, and the Biot number, *Bi*. The Thiele modulus indicates the magnitude of the influence of internal diffusion on the biochemical reaction rate. For the succinic acid fermentation system, it is defined by the modified expression [10]:

$$\phi = \frac{R_P}{3} \cdot \sqrt{\frac{V \cdot C_C}{D_{Se}} \cdot \frac{Y_{P/S} \cdot K_{iS} + 1}{K_{iP} \cdot K_{iS}}} \quad (4)$$

The Biot number represents the ratio between the resistance to the diffusion in the boundary layer surrounding the biocatalyst particle and that corresponding to the internal diffusion [10]:

$$Bi = \frac{k_L \cdot R_P}{D_{Se}} \quad (5)$$

Therefore, the glucose external and internal mass flows can be calculated by means of its superficial concentrations at the biocatalyst surface and inside it.

For both bioreactors containing immobilized *A. succinogenes* cells, the glucose flux from the liquid phase to the particle surface is:

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

where k_L is calculated using the expressions adequate either for the mobile bed or for the packed one [25]. In order to quantify the influence of the conformation of biocatalyst bed and related diffusional phenomena on the glucose mass transfer rate in the liquid phase surrounding the biocatalyst particle, the variation of the ratio between the substrate mass transfer coefficient for the bioreactor with mobile bed, k_{LM}, and that for the basket bioreactor, k_{LB}, with the particle size has been plotted in Fig. 2.

Regardless of the conformation of the biocatalysts bed, the previous results indicated the diminution of k_L from the smallest biocatalyst particles to the largest ones, as the result of the increase of the boundary layer thickness [10,13]. Contrary, the ratio between k_{LM} and k_{LB}

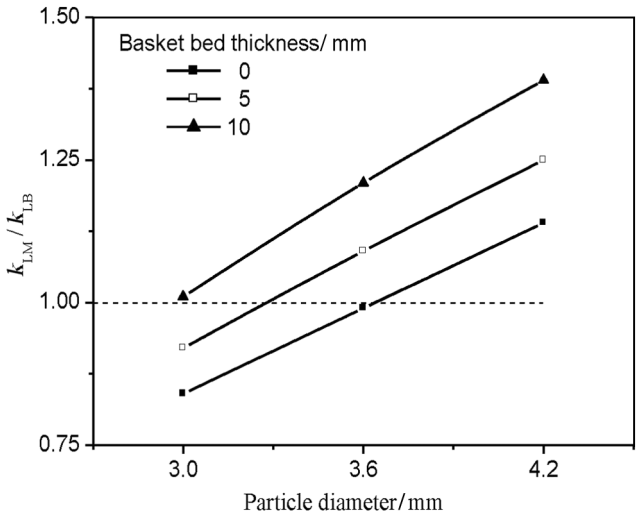

Fig. 2. Variation of ratio between the glucose mass transfer coefficients for the mobile and basket beds with the biocatalyst particle diameter.

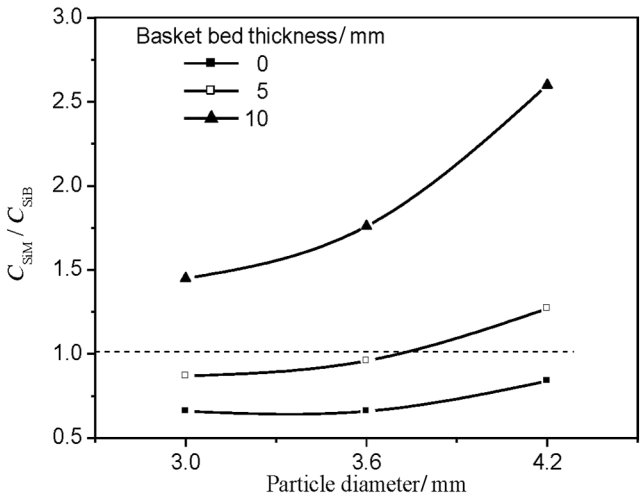

Fig. 3. Variation of ratio between the glucose concentrations at the biocatalyst particle surface for the mobile and basket beds with the biocatalyst particle diameter.

increases with the increase of biocatalyst particle size, due to the turbulence that can be intensified mainly in the bioreactor containing mobile bed of immobilized bacterial cells.

However, the value of k_{LM}/k_{LB} ratio has to be analyzed in direct relation with the position inside the basket bed. Thus, for all particle sizes, this ratio increases with the basket bed thickness, from the basket inner surface to the outer one (Fig. 2). Moreover, for the smallest biocatalyst particles, the k_{LM}/k_{LB} values are below 1 for any position inside the basket bed, while for the intermediary size particles the ratio values less than 1 are recorded only at the basket inner surface. In the other cases, the values of ratio k_{LM}/k_{LB} are over 1. These variations are the consequence of the combined influences of the thickness of boundary layer surrounding the particles and of the liquid phase velocity on substrate diffusion rate towards the biocatalyst surface. According to the previous results, the fluid phase superficial velocity is higher for the basket bed, owing to the reduction of the flow section inside the bed [13]. For the biocatalyst particles with 3 mm diameter, the glucose mass transfer rate for the basket bioreactor exceeds that reached in the bioreactor with mobile bed. The flow section increases inside the basket bed from the inner surface to the outer one and induces the reduction of the superficial velocity. This effect cumulated with the increase of the boundary layer leads to the diminution of the substrate diffusion rate in the basket bioreactor for the larger biocatalyst particles. Consequently, for the biocatalyst particles with diameters over 3 mm, the substrate mass transfer coefficient inside the basket bioreactor becomes inferior to that obtained for the mobile bed of biocatalysts.

The correlation between the glucose concentration at the particle surface and the biocatalyst size depends on the biocatalyst bed type. Thus, for the mobile bed, the lowest superficial concentration of substrate is reached for the intermediary size of the immobilized cells particle, due to the equilibrium between the antagonistic processes of internal diffusion and inhibition induced by substrate [10]. In the case of packed bed of basket conformation, the glucose superficial concentration decreases radially towards the outer surface of the biocatalysts bed, this variation being the consequence both of the reduction of substrate diffusion rate through the liquid boundary layer by increasing the basket bed width, and of its conversion inside the basket bed by the immobilized bacterial cells [13].

The variation of the ratio between the superficial concentrations of glucose corresponding to the mobile bed, C_{SiM}, and that for the basket bed, C_{SiB}, with the particle diameter is indicated in Fig. 3. Due to the superior superficial velocity reaching at the inner surface of the basket bed, the glucose diffusion rate in the boundary layer surrounding the biocatalyst particles in the basket bioreactor is higher than that corresponding to the mobile bed. Therefore, regardless of the particle size, in this region the value of ratio C_{SiM}/C_{SiB} is inferior to 1. By increasing the distance from the inner surface inside the basket bed, the superficial concentration of glucose is reduced for the basket bioreactor and becomes lower than that obtained for the bioreactor with mobile bed. This variation is the result of the diminution of the liquid phase superficial velocity inside the packed bed and, implicitly, of glucose diffusion rate. Due to the higher resistance to the diffusion through the boundary layer, this effect is more important for the larger particles of immobilized *A. succinogenes* cells.

On the basis of the above results, the influence of particle size on the ratio between the glucose external mass flow for the bioreactor with mobile bed, n_{LM}, and that for the basket bioreactor, n_{LB}, is plotted in Fig. 4 considering different positions inside the basket bed. In all cases, the maximum value of this ratio is reached for the intermediary biocatalyst particles. This variation is controlled mainly by the dependence between the external flow for the mobile bed and the particle size. Thus, for the bioreactor with mobile bed of immobilized *A. succinogenes* cells, it was previously concluded that the intermediary size of the particles allows to reaching the highest rate of glucose consumption, due to the equilibrium between the processes of internal diffusion and

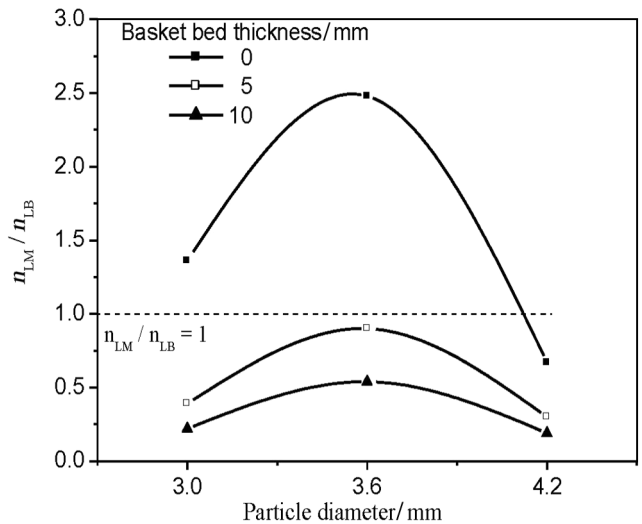

Fig. 4. Variation of ratio between the glucose external mass flows for the mobile and basket beds with the biocatalyst particle diameter.

substrate inhibition [10]. The substrate inhibition is more pronounced for the smaller immobilized cell particles, while the internal diffusion becomes the main limiting step for the larger ones. Consequently, the lowest superficial concentration of substrate and, implicitly, the highest glucose concentration gradient inside the boundary layer correspond to the biocatalyst particles with 3.6 mm diameter. In these circumstances, the most intense mass flow of glucose towards the particle surface is reached for the intermediary biocatalysts [10].

Similarly, for the basket bed bioreactor it was stated that the glucose transfer through the boundary layer surrounding the biocatalyst particles is controlled not by its diffusivity, but by its concentration gradient between the liquid phase and the particle surface [13]. The faster consumption rate of substrate with the increase of the basket bed thickness leads to the amplification of its concentration gradient and, implicitly, to the continuous acceleration of substrate mass transfer towards the particle surface by varying the position inside the cylindrical bed from the inner surface to the outer one.

The above discussed phenomenon generates higher values of glucose external mass flow inside the basket bed compared to those recorded for the mobile bed of immobilized bacterial cells. For this reason, the values of the ratio between the external mass flows for the mobile bed and for the basket one, respectively, are lower than 1, excepting those calculated for the basket inner surface (Fig. 4). However, for the largest biocatalyst particles, because of the higher resistance to the substrate diffusion through the boundary layer, the external mass flows ratio is under 1 also at the basket bed surface.

In the previous works, it was considered that the ratio between the internal and superficial glucose concentrations, C_{SP}/C_{Si}, with the particle radius could describe more accurately the influence of the internal diffusion, owing to the strong dependence between the substrate concentration inside the biocatalyst particle and its concentration at the particle surface [10,13]. In this context, from Fig. 5 it can be seen that the relative increase of this ratio value towards the biocatalyst particle centre is more pronounced for the mobile bed bioreactor. The magnitude of this effect becomes more important for the largest biocatalyst particles. Thus, due to the rather similar values of substrate concentrations in the superficial region of the immobilized bacterial cell particles for the two bioreactors, the values of these ratios are close to 1 in the vicinity of the particle surface, increasing then to 1.66 in the centre of the smallest particles, 2.02 in the centre of the intermediary ones, and to 2.64 in the centre of the largest particles, respectively This variation is not affected by the modification of glucose concentration in the medium.

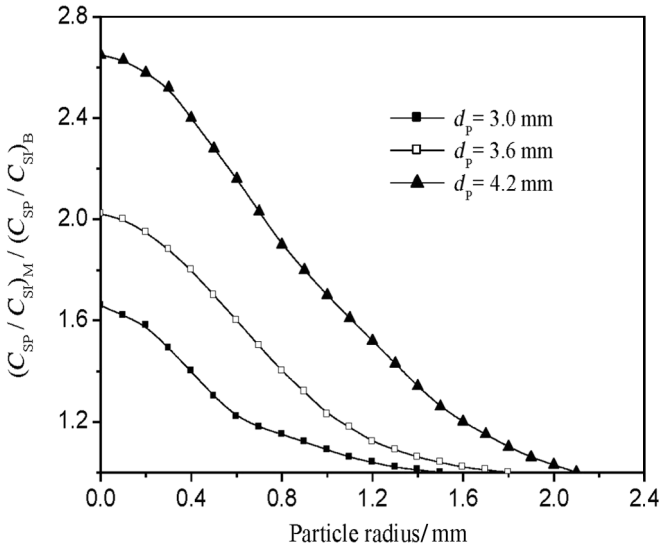

Fig. 5. Variation of ratio $(C_{SP}/C_{Si})_M/(C_{SP}/C_{Si})_B$ with the biocatalyst particle radius.

For both biocatalyst bed configurations, the internal mass flow is calculated by combining the Fick law with expression (2) for C_{SP}, the following relationship adequate for glucose conversion to succinic acid being obtained:

$$n_P = D_{Se} \cdot \frac{Bi \cdot (C_{SL} - C_{Si}) \cdot \cosh(3\phi \cdot R_P)}{{R_P}^3} \cdot \left[3\phi - {R_P}^2 \cdot \tanh(3\phi \cdot R_P)\right] \cdot \left[\frac{3\phi \cdot \cosh\left(\frac{3\phi \cdot r}{R_P}\right)}{R_P \cdot r} - \frac{\sinh\left(\frac{3\phi \cdot r}{R_P}\right)}{r^2}\right] \quad (7)$$

Obviously, due to the direct correlation between the internal mass flow and substrate concentration inside the biocatalyst particle, both parameters are significantly reduced towards the particle centre, an effect that is more important with the increase of the size of immobilized cells particles. As it was discussed before, the reduction of internal mass flow from the biocatalyst particle surface to its centre is more pronounced for the bioreactor with basket bed of immobilized bacterial cells. For these reasons, Fig. 6 suggests similar behavior of internal mass flow ratio, n_{PM}/n_{PB}, with that of internal glucose concentrations ratio. Thus, the ratio between the internal mass flows for the mobile and basket beds, respectively, is increased from 1.02, at the biocatalyst surface, to 2.2–3.8 at its centre (the value of this ratio in the particle centre increases from the smallest particles to the largest ones).

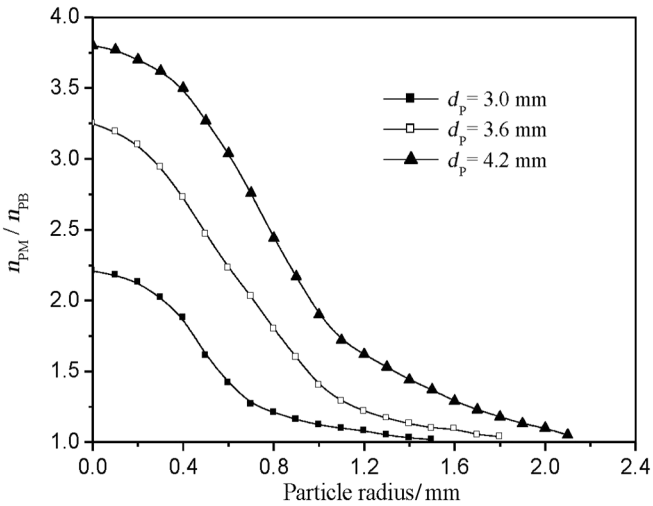

Fig. 6. Variation of ratio between the glucose internal mass flows for mobile and basket beds with the biocatalyst radius.

The previous experiments using these two bioreactors for succinic acid fermentation indicated that it is possible to reach very low or negligible values of glucose internal mass flow near the particle centre for both bioreactor types [10,13]. This central region was considered a "biological inactive region", its extent being estimated by considering the order of magnitude of substrate effective diffusivity. Therefore, it was assumed that the values of internal mass flow lower than 10^{-10} kg·m^{-2}·s^{-1} can be associated with this inactive region, its extent being increased for the larger biocatalyst particles [10,13].

As it can be seen from Fig. 7, for the first half of the basket bed thickness, the extent of the inactive region is inferior to that corresponding to the mobile bed. But, the extent of the inactive region becomes important on the radial direction inside the basket bed. Consequently, near the outer surface of the cylindrical bed, the volume of the inactive region for the basket bioreactor becomes for about 2.2–5 times greater than in the case of mobile bed of immobilized cells, the closest values being reached for the largest biocatalyst.

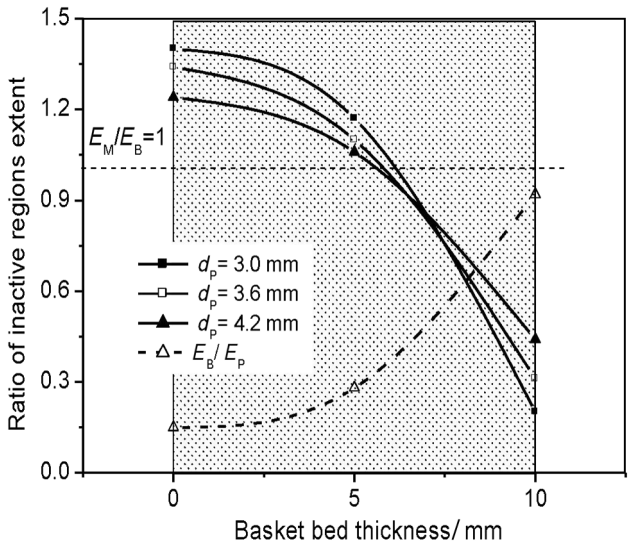

Fig. 7. Variation of ratio between the extent of inactive regions with the biocatalyst radius.

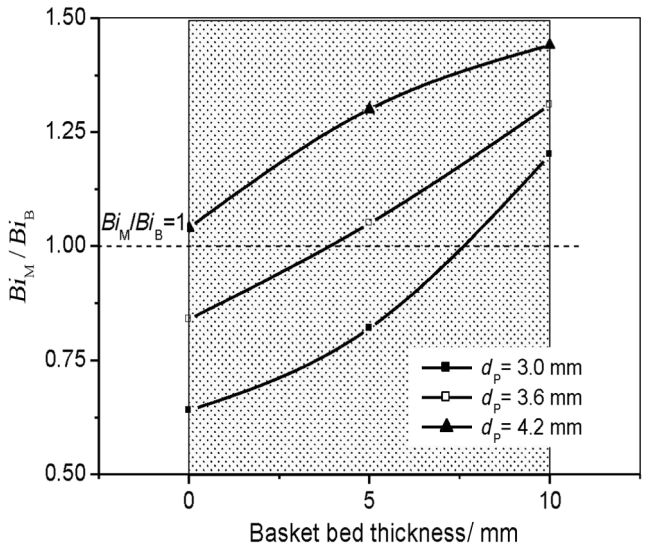

Fig. 8. Variation of ratio between the Biot numbers for mobile and basket beds with the biocatalyst particle diameter.

In the same time, the values of the ratio between the extent of the inactive region corresponding to the basket bed, E_B, and that for the column packed bed, E_P, are under 1 for any position inside the basket bed (the ratio was calculated for the biocatalysts particles with 3 mm diameter [26]) [Fig. 7]. Therefore, the comparative analysis of the bioreactors with mobile, basket and column packed bed underlines the superior efficiency of the first two bioreactors.

As it was above mentioned, the Thiele modulus indicates the magnitude of the influence of internal diffusion on the glucose transport and conversion rate, being defined for the succinic acid fermentation system by the Expression (4). The values of Thiele modulus are specific for a given size of immobilized bacterial cells particles, depend on the glucose concentration in the liquid phase, but not on the conformation of biocatalysts bed ($\varphi = 0.026$ for biocatalysts with 3 mm diameter, $\varphi = 0.032$ for biocatalysts with 3.6 mm diameter, and $\varphi = 0.037$ for biocatalysts with 4.2 mm diameter, respectively). Because the values of Thiele modulus over 0.1 indicate important limitation of the process of glucose conversion by immobilized bacterial cells, due to the internal diffusion of substrate [26], for both types of bioreactors it can be concluded that the relative magnitude of resistance to internal diffusion could become more pronounced only at particle size larger than those considered in the experiments.

By comparing the Biot numbers for the two biocatalyst bed conformations, from Fig. 8 in can be observed that the relative importance of resistance to the glucose 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 basket bed of immobilized *A. succinogenes* cells. Because the variation of the ratio between the Biot numbers corresponding to the mobile bed bioreactor, Bi_M, and, respectively, to the basket bed bioreactor, Bi_B, is similar to that of the ratio between the mass transfer coefficients for the two types of bioreactors, this difference between the two bioreactors could be attributed to the significant reduction of substrate mass transfer coefficient in the liquid boundary layer at the particle surface for the larger biocatalyst particles, especially for the basket bioreactor. This phenomenon is the result of the turbulence diminution from the inner to the outer surface of the basket bad, as well as of the appearance of the supplementary resistance induced by substrate diffusion inside the basket bed.

The effect of the internal diffusion on the rate of glucose conversion during the fermentation process can be described more accurately by the effectiveness factor λ, defined as the ratio between the rates of the biochemical reaction in heterogeneous system and in homogeneous one. Assuming the steady-state conditions, the rate of the internal biochemical reaction equals the internal mass flow of glucose. Thus, the following relationship can be used for calculating the factor λ for the succinic acid fermentation with immobilized bacterial cells [13]:

$$\lambda = \frac{3 \cdot k_L \cdot (C_L - C_{Si}) \cdot \cosh(3\phi \cdot R_P) \cdot \left[\frac{3\phi}{R_P} - R_P \cdot \tanh(3\phi \cdot R_P)\right]}{{R_P}^4 \cdot V \cdot C_c \cdot \left(\frac{K_{iS}}{K_{iS} + C_s}\right)} \cdot \frac{\cosh(3\phi) \cdot [3\phi - \tanh(3\phi)]}{\left(\frac{K_{iP}}{K_{iP} + Y_{P/S} \cdot C_P}\right)} \quad (8)$$

For both bioreactor types, the factor λ varies slowly near the particle surface or centre [10,13]. In the region vicinal to the biocatalyst surface, the higher concentration of glucose, 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 substrate concentration near the particle centre.

The variation of the ratio between the effectiveness factors for the bioreactors with mobile and basket beds, λ_M/λ_B, with the biocatalyst particle radius, plotted in Fig. 9, suggests that the decrease of the glucose

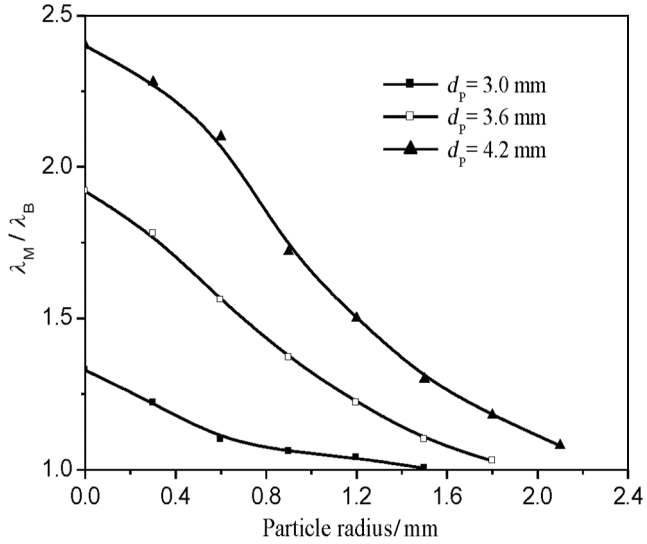

Fig. 9. Variation of ratio between the effectiveness factors for mobile and basket beds with the biocatalyst particle diameter.

conversion rate inside the biocatalyst compared to the system containing free *A. succinogenes* cells is more important for the basket bioreactor. Therefore, this ratio value is over 1 at the biocatalysts surface and increases significantly towards the particle centre. This variation indicates that the negative effect of internal diffusion on the substrate conversion rate is more important for the basket bioreactor, due to the lower substrate concentration inside the biocatalyst particles placed in the basket bed. Although the relative magnitude of this phenomenon is amplified towards the particle centre, it is attenuated for the smallest particles of biocatalyst. Because the ratio λ_M/λ_B is closer to 1 for the immobilized bacterial cells particles with 3 mm diameter, it can be concluded that for this size of biocatalysts the bed conformation exhibits lower influence on the glucose conversion rate inside the immobilized cells particles (in the particle centre, the value of the ratio λ_M/λ_B is reduced λ from 2.4 to 1.3 by decreasing the particle diameter from 4.2 to 3 mm).

Thus, the rate of the biochemical conversion of glucose inside the mobile or basket bed containing immobilized *A. succinogenes* cells is reduced for $1/\lambda$ times compared to that reached for free bacterial cells, phenomenon that becomes more important in the biocatalyst particle centre. The relative magnitude of this reduction is described by means of the ratio $(1/\lambda)_M/(1/\lambda)_B$, which is correlated to the influence of the biocatalysts bed conformation. For the particle centre, according to Fig. 10, the reduction of glucose conversion rate by bacterial cells immobilization is similar for the two types of beds at the basket bed inner surface, but it becomes pronounced for the basket bioreactor as the position inside the basket bed is moved towards its outer surface. From the above discussed reasons, this effect is amplified for the larger biocatalyst particles.

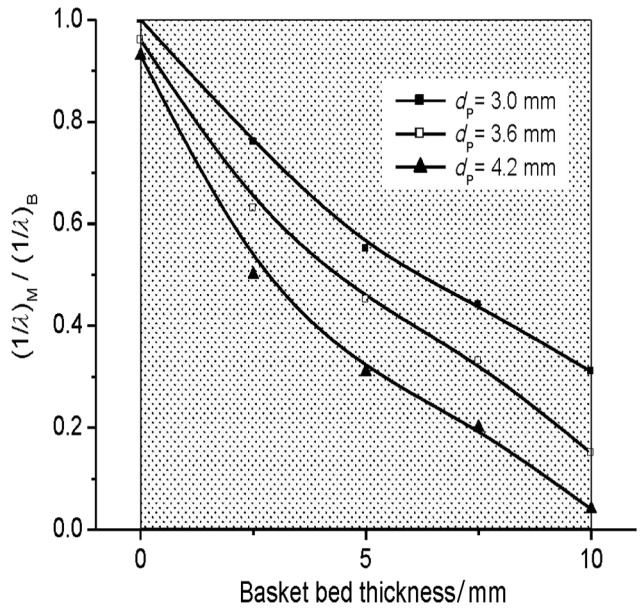

Fig. 10. Variation of ratio between the reduction degree of glucose conversion rate for mobile and basket beds with the biocatalyst particle diameter.

Thus, by using both the packed mobile and the basket beds containing immobilized *A. succinogenes* cells, the rate of the succinic acid production is considerably reduced compared to the fermentation with free bacterial cells. This reduction is higher in the case of basket bioreactor and becomes more important with the increase of the basket bed width.

4. Conclusions

Succinic acid production by glucose conversion using immobilized *A. succinogenes* cells has been comparatively analyzed for two types of bioreactors: bioreactors with mobile and basket beds of biocatalyst, respectively. The studies have been focused on the glucose external and internal mass transfer and, implicitly, on the influence of the internal diffusion on the transfer and biochemical processes rates, assuming the kinetics controlled by substrate and product inhibitory effects.

Considering the criteria of substrate external and internal mass flows, and of the extent of biological inactive region, the basket bioreactor was found to be more efficient for the biocatalyst particles with 3 mm diameter and basket bed thickness up to 5 mm. For larger immobilized bacterial cell particles and for higher width of basket bed, the bioreactor with mobile bed offers superior performances of glucose mass transfer and conversion rates.

Depending on the desired characteristics of biocatalyst, operating conditions and desired number of fermentation cycles, the selection of the optimum configuration of immobilized *A. succinogenes* cells bed has to take into consideration all the aspects regarding the relative diffusion and biochemical reaction rates. Consequently, for establishing the optimum type of bioreactor for succinic acid fermentation, the impact of the inhibitory effect induced by product on the fermentation rate, especially for the larger biocatalyst particles, will be analyzed in the future experiments in correlation with the above discussed ones.

Nomenclature

Bi	Biot number
C_C	cells concentration, $kg\cdot m^{-3}$
C_{Si}	substrate concentration at the biocatalyst particle surface, $kg\cdot m^{-3}$
C_{SL}	substrate concentration in the liquid bulk, $kg\cdot m^{-3}$
C_{SP}	substrate concentration inside the biocatalyst particle, $kg\cdot m^{-3}$
D_{Se}	effective diffusivity, $m^2\cdot s^{-1}$
D_{SL}	liquid phase diffusivity, $m^2\cdot s^{-1}$
K_{iP}	product inhibition constant, $kg\cdot m^{-3}$
K_{iS}	substrate inhibition constant, $kg\cdot m^{-3}$
k_L	liquid phase mass transfer coefficient, $m\cdot s^{-1}$
n_L	external mass flow, $kg\cdot m^{-2}\cdot s^{-1}$
n_P	internal mass flow, $kg\cdot m^{-2}\cdot s^{-1}$
R_P	biocatalyst particle radius, m
V	maximum biochemical reaction rate, $kg\cdot m^{-3}\cdot s^{-1}$
$Y_{P/S}$	yield of substrate conversion to product, $kg\cdot kg^{-1}$
λ	effectiveness factor
φ	Thiele modulus

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