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Effect of glucose internal diffusion on alcoholic fermentation in a stationary basket bioreactor with immobilized yeast cells

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Abstract

This paper develops the previous studies on the transfer processes and biochemical reaction kinetics involved in the alcoholic fermentation by immobilized S. cerevisiae cells using a bioreactor with stationary basket bed of biocatalysts. Although the immobilization of cells or enzymes offers a lot of advantages as compared to the free ones, it induces the unfolding of the internal diffusion of substrate or product, with negative effect on the overall biochemical process. In the investigated case, the influences of the substrate internal diffusion on physical and biochemical processes have been quantified by means of the Biot number, Thiele modulus and reduction factor λ. These three parameters vary within the biocatalyst particle centre, as well as with the basket bed width. The reduction of the biochemical rate inside the biocatalyst particle is more important than in the case of the bioreactor with mobile bed of immobilized yeast cells.

Keywords: alcoholic fermentation, immobilized cells, yeast, inhibition, internal diffusion, basket bed bioreactor

Introduction

The bacteria or yeasts posses the ability to converse glucose under anaerobic conditions by Embden-Mereyhof-Parnas metabolic pathway, the main final products being the ethanol and carbon dioxide [1,2]. Glucose represents the substrate with the fastest conversion rate in the bacterial or yeasts metabolic processes for ethanol production. The efficiency of ethanol production by yeasts can be affected by glucose or ethanol concentration, due to the specific phenomenon of substrate or product inhibition. In these circumstances, the viability of *S. cerevisiae* population, the substrate consumption and ethanol biosynthesis rates are directly controlled by the cultivation conditions. An interesting experiment indicated that the addition of ethanol in a culture of *S. cerevisiae* induces less toxic effect than that generated by ethanol biosynthesized during the fermentation, the cells death occurring with lower rate in the former case [3]. This result could be explained by the presence of other metabolic products beside ethanol, these secondary products contributing to the amplification of its inhibitory phenomenon.

As it was previously concluded, the fermentation with immobilized cells could avoid the substrate inhibitory effect, increases the resistance of the enzymes or cells to high temperature, chemical action and to the shear forces, allows an easier recovery of biocatalysts and attenuates the reduction of the microbial activity [4-7]. Therefore, the biocatalysts can be reused for many fermentation cycles.

Most of the experiments on alcoholic fermentation with immobilized cells have been carried out in fixed/packed bed bioreactors in continuous, semicontinuous or fed-batch systems. The fixed beds of immobilized cells have been preferred due to the higher sensitivity of the immobilized cells or enzymes to the shear forces generated by the impellers. But, these have some disadvantages [6]. The flow inside the bed is laminar, thus leading to low rate of mass and heat transfer and inducing the back-mixing of reverse flow phenomenon. The turbulent flow could be reached only at high flow rate inside the bed, but this is less possible due to the resistance to flow induced by the biocatalysts. On the other hand, the solid particles from effluent can clog the biocatalyst bed, thus leading both to the reducing of the flow rate inside the bed, and to the biocatalyst inactivation. Another important undesirable phenomenon is the formation of the preferential flow channels inside the bed at the beginning of the feed with medium or during the bioreactor working. The formation of these channels induces the deviation from the plug flow and the inefficient conversion of the substrate.

The bioreactors of basket type are derived from the bioreactors with fixed beds, the biocatalysts particles being fixed in an annular cylindrical or conic bed, which is either static around the stirrer [8-11], or rotary [12-15]. Due to its design, this bioreactor avoids either the disadvantages of the bioreactors with fixed beds, and the flooding/deposition or the mechanical disruption of the biocatalysts particles, phenomena that are encountered in the bioreactors with mobile beds. In this 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 conversion [16].

The previous studies on alcoholic fermentation performed in a stationary basket bioreactor with immobilized *S. cerevisiae* cells in alginate indicated the possibility to reach an efficient mixing by selecting the optimum impellers position on the stirrer shaft [16]. These experiments have been continued by investigating the kinetics of alcoholic fermentation and the external and internal mass transfer of substrate under glucose inhibitory effect [17].

In this paper, the studies on the influence of glucose diffusion inside the biocatalyst particle on its bioconversion rate are presented, taking into consideration the mechanism of glucose transfer and consumption under substrate inhibition limitation.

Materials and Methods

The experiments were carried out in 10 l (8 l working volume) laboratory bioreactor Fermac 310/60 (Electrolab), with computer-controlled and recorded parameters [18]. The bioreactor characteristics are given in Table 1.

Table 1. Characteristics of bioreactor

d, mm	d/D	H/D	w/d	l/d	h/d	No. blades	No. baffles	s/d
82	0.41	1.95	0.20	0.33	0.50	6	4	0.18

The cylindrical bed of basket type has the inner diameter of 102 mm, height of 100 mm and the bed thickness of 8 mm. The basket was placed centered around the stirrer, at 120 mm from the bioreactor bottom (Figure 1). The basket was filed with *S. cerevisiae* cells immobilized on alginate. The immobilization was carried out by cells inclusion into the alginate matrix, according to the method given in literature [16]. The spherical biocatalyst particles having 4 mm diameter were obtained, their volumetric fraction in the basket bed being 0.56.

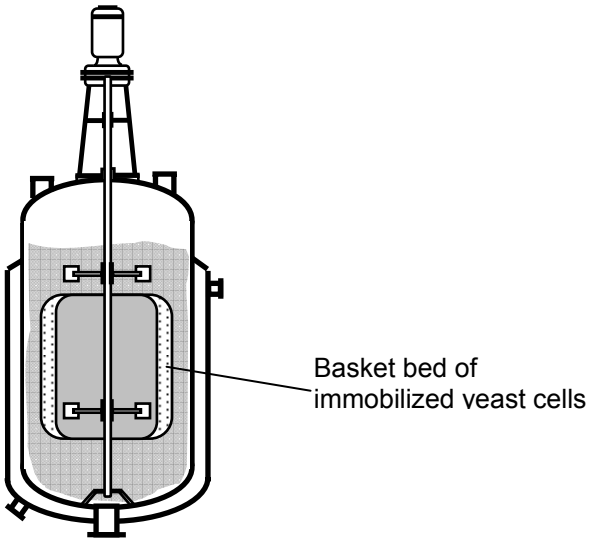

Figure 1. The experimental stationary basket bioreactor

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 [16]. This combination led to the lowest mixing time values and to the most important attenuation of the negative influence of the apparent viscosity increase on the liquid phase circulation. The rotation speed was of 150 rpm. Any mechanical damage of the biocatalyst due to the shear forces was recorded during the experiments.

The composition of the medium was: glucose 150 g/L, KH2PO4 5 g/L, (NH4)2SO4 2 g/L, MgSO4 0.2 g/L, yeast extract 2 g/L, tap water to the prescribed volume [19]. The fermentation has been carried out at 28^{o}C.

The process evolution has been analyzed by means of the variation of glucose concentration in the liquid 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 $5x10^{-3}$ N sulfuric acid with a flow rate of 0.6 mL/min. The analysis temperature was 65^{o}C [17].

Results and Discussion

The kinetics of alcoholic fermentation using a bioreactor with immobilized *S. cerevisiae* cells has been previously analyzed under substrate inhibition conditions [20]. Generally, the rate of the biochemical reactions in heterogeneous systems is lower than that recorded for homogeneous media, due to the radial decreasing of the substrate concentration inside the biocatalyst particles. For the heterogeneous systems, not only the value of the biochemical reaction rate is affected, but also the kinetic model is modified compared to the ideal models describing the substrate consumption or product formation. For these reasons, the kinetic parameters of the biochemical reactions which involve immobilized cells or enzymes differ from those corresponding to the homogeneous media. For the analyzed fermentation systems, the bioethanol formation can be mathematically described by means of a modified Michaelis-Menten equation, which takes into account simultaneously the cells immobilization and the inhibitory effect of substrate [20]:

$$r_S = \frac{V \cdot C_C \cdot C_S}{K'_M + C_S + \frac{C_S^2}{K_I}} \quad (1)$$

Besides the modification of the kinetics of biochemical reaction, the internal diffusion affects also the physical process of transfer and, consequently, the substrate concentration inside the biocatalyst particles. In this case, the rate of the biochemical reaction occurring inside the biocatalyst particle is inferior to that corresponding to the homogeneous system, due to the lower substrate concentration as compared to its value in the liquid bulk.

The external and internal mass flows of glucose for the basket bed of biocatalysts have been calculated by means of the substrate mass balance related to a single particle [17]:

$$\frac{dC_S}{dt} = D_{Se} \cdot \left[\frac{1}{r^2} \cdot \frac{d}{dr}\left(r^2 \cdot \frac{dC_S}{dr}\right)\right] - \frac{V \cdot C_C \cdot C_S}{K'_M + C_S + \frac{C_S^2}{K_I}} \quad (2)$$

Considering the steady-state conditions and the substrate concentration inside the biocatalyst particle, C_{SP}, equation (2) becomes:

$$\frac{d^2C_{SP}}{dr^2} + \frac{2}{r} \cdot \frac{dC_{SP}}{dr} = \frac{1}{D_{Se}} \cdot \frac{V \cdot C_C \cdot C_{SP}}{K'_M + C_{SP} + \frac{C_{SP}^2}{K_I}} \quad (3)$$

and has been solved under the following boundary limits:

1) $r = 0$ (at particle centre), $\frac{dC_{SP}}{dr} = 0$

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

In these circumstances, the glucose concentration profile inside the biocatalyst particle has been described by the solution of equation (3):

$$C_{SP} = \frac{R_p \cdot \sinh(3\varphi \cdot r)}{r \cdot \cosh(3\varphi \cdot R_p)} \cdot \frac{(C_{SL} - C_{Si}) \cdot B_i \cdot k_L \cdot V \cdot C_C}{3\varphi \cdot B_i \cdot D_{Se} - k_L \cdot \tanh(3\varphi \cdot R_p) + K_I} \quad (4)$$

and its concentration at the particle surface by the following relationship:

$$C_{Si} = \frac{\tanh(3\varphi \cdot R_p) \cdot C_{SL} \cdot B_i \cdot k_L \cdot V \cdot C_C}{3\varphi \cdot B_i \cdot D_{Se} + k_L \cdot \tanh(3\varphi \cdot R_p) \cdot [B_i \cdot k_L \cdot V \cdot C_C - 1] + K_I} \quad (5)$$

Expressions (4) and (5) include two parameters which quantify the influence of the internal diffusion either on the transfer process, or on the biochemical reaction rate: the Thiele modulus, φ, and Biot number, Bi. The Thiele modulus indicates the magnitude of the influence of internal diffusion on the biochemical reaction rate [21]. For the studied system, it is defined by the expression:

$$\varphi = \frac{R_P}{3} \cdot \sqrt{\frac{V}{K_M' \cdot D_{Se}}} \quad (6)$$

The Biot number represents the ratio between the resistance to the diffusion in the boundary layer surrounding the particle and that corresponding to the internal diffusion [21]:

$$Bi = \frac{k_L \cdot R_P}{D_{Se}} \quad (7)$$

The variation of the substrate mass flow with the biocatalyst particle radius for stationary basket bed suggested the possibility to reach very low or negligible values of mass

flow near the particle centre [17]. This region could be considered as a "biological inactive region". Taking into consideration the order of magnitude of effective diffusivity, it can be assumed that the extent of biological inactive region varies from 0.34 to 31% from the overall volume of each biocatalyst particle. The extension of this region is important on the radial direction inside the basket bed. Thus, near the outer surface of the cylindrical bed, the volume of the inactive region became for 15 times greater than in the case of mobile bed of immobilized cells. However, as compared to the column bioreactor with packed bed of biocatalysts [22], the extent of this region was lower in the first 75% of the basket bed width from the inner surface.

In this context, the relative importance of the glucose diffusion processes in the liquid boundary layer surrounding the particle and inside the particle, described by the Bi number, is controlled by the size of biocatalyst particles and position inside the basket bed. Therefore, from Figure 2 it can be observed that this parameter is slowly decreased on the radial direction inside the bed of immobilized yeast cells, due to the direct dependence between the external and internal glucose mass flows.

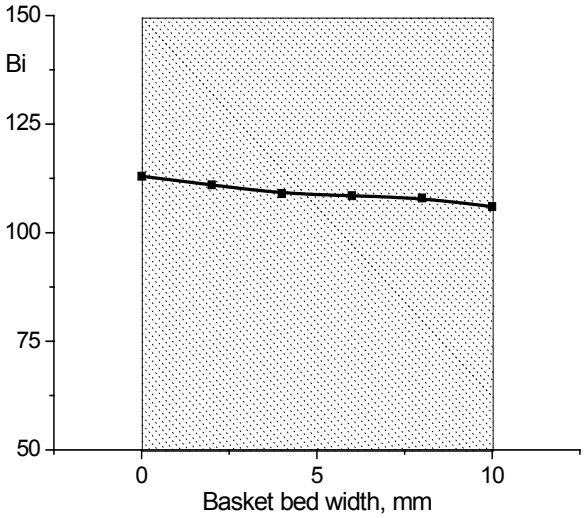

Figure 2. Variation of Biot number inside the basket bed

By comparing the average value of Biot number inside the basket bed, Bi=110, with that obtained for the mobile bed of biocatalysts [21], it can be concluded that the Biot number for the basket bed is approximately 15 times lower. This result is the consequence of the reduction of substrate mass transfer through the boundary layer surrounding the biocatalyst particle in the basket bed, due both to the diminution of the turbulence, and to the increase of the diffusional resistance inside the biocatalysts basket bed.

Indifferent of the position on the radial direction inside the basket bed, the value of Thiele modulus is 0.181, being equal to that corresponding to the mobile bed of the same biocatalysts [22].

For describing more accurately the effect of the internal diffusion on the rate of glucose bioconversion to bioethanol, the reduction factor λ is used. This factor is defined as the ratio between the rates of the biochemical reaction in heterogeneous system and in homogeneous one [21]. Considering the steady-state conditions, it can be assumed that the rate of the internal biochemical reaction is equal with the internal mass flow of glucose. Therefore, for the investigated fermentation system, the following relationship can be used for calculating the λ factor:

$$\lambda = \frac{4\pi \cdot R_P{}^2 \cdot D_{Se} \cdot \left. \frac{dC_{SP}}{dr} \right/_{r=R_P}}{\frac{4}{3}\pi \cdot R_P{}^3 \cdot \frac{V \cdot C_C \cdot C_{Si}}{K'_M + C_{Si} + \frac{C_{Si}^2}{K_I}}} \tag{8}$$

Because, the internal mass flow is described by the expression [17]:

$$n_P = -D_{Se} \cdot \frac{dC_{SP}}{dr} = -D_{Se} \cdot \frac{R_p \cdot (C_{SL} - C_{Si}) \cdot B_i \cdot k_L \cdot V \cdot C_C \cdot \left[r \cdot \varphi \cdot \cosh(3\varphi \cdot R_p) - \sinh(3\varphi \cdot R_p) \right]}{r^2 \cdot \cosh(3\varphi \cdot R_p) \cdot \left[3\varphi \cdot B_i \cdot D_{Se} - k_L \cdot \tanh(3\varphi \cdot R_p) + K_I \right]} \tag{9}$$

the equation (8) can be written as follows:

$$\lambda = \frac{3D_{Se} \cdot (C_{SL} - C_{Si}) \cdot \left(K'_M + C_{Si} + \frac{C_{Si}^2}{K_I} \right) \cdot B_i \cdot k_L \cdot \left[R_P \cdot \varphi \cdot \cosh(3\varphi \cdot R_p) - \sinh(3\varphi \cdot R_p) \right]}{R_P{}^2 \cdot C_{Si} \cdot \cosh(3\varphi \cdot R_p) \cdot \left[3\varphi \cdot B_i \cdot D_{Se} - k_L \cdot \tanh(3\varphi \cdot R_p) + K_I \right]} \tag{10}$$

The variation of λ factor with the particle radius is graphically presented in Figure 3. The analysis of the plotted dependences indicates that this factor varies slowly near the particle surface or centre. In the region vicinal to the biocatalyst surface, the higher concentration of substrate, rather equal with that at the particle surface, leads to the values of λ close to 1.

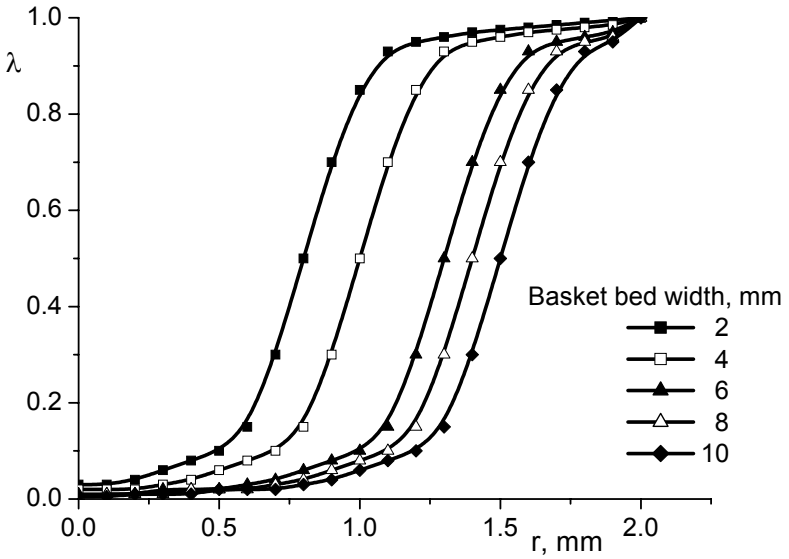

Figure 3. Radial variation of reduction factor λ for different positions inside the basket bed

But, the width of the superficial layer corresponding to the value of λ close to1 depends on the radial position inside the biocatalysts bed. Thus, this width decreases from 1 mm, in the vicinity of the inner surface of the cylindrical bed, to 0.25 mm, in the vicinity of the outer surface of the bed. This variation is the result of the radial reduction of substrate concentration inside the basket bed and, implicitly, of the biochemical process rate which occurs inside the particles of immobilized *S. cerevisiae* cells.

The slow variation of λ factor in the central region is the result of the rather constant low level of substrate concentration near the particle centre. The thickness of the intermediary

region corresponding to the significant reduction of λ from its superficial value to a rather constant one in the particle centre (λ below 0.05) is extended by increasing the width of the basket bed. This result could be attributed also to the diminution of the glucose concentration inside the biocatalyst particle.

In these circumstances, it can be affirmed that by immobilizing the yeast cells the rate of the biochemical reaction of glucose conversion to ethanol is considerably reduced, for 1/λ times, compared to that reached for free yeast cells. The magnitude of this effect has to be correlated with the position of the biocatalyst particle inside the cylindrical bed. Therefore, the value of the ratio 1/λ obtained in the particle centre increases from 33, at the innner surface of the basket bed, to 200, at the outer surface, from the above discussed reasons (Figure 4).

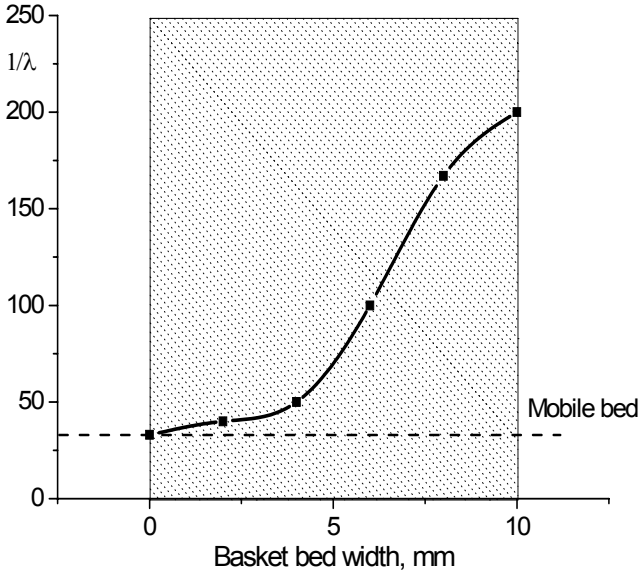

Figure 4. Variation of ratio 1/λ inside the basket bed

The importance of the negative effect of reduction of glucose consumption rate is significantly amplified compared to that recorded for the mobile beds of immobilized yeast cells (the value of ratio 1/λ is for about 6 times higher in the case of basket bed of biocatalysts, using the same fermentation conditions).

Conclusions

The utilization of immobilized biocatalysts offers many advantages, but allows the unfolding of the internal diffusion of substrate or product, with negative effect on the overall biochemical process evolution. In the case of alcoholic fermentation by bioconversing the glucose in a bioreactor with stationary basket bed of immobilized S. *cerevisiae* cells, the influences of the substrate internal diffusion on physical and biochemical processes have been analyzed by means of the Bi number, Thiele modulus and reduction factor λ.

Besides the influences of the biocatalyst particles characteristics (size and volumetric fraction in the medium), the position inside the basket bed exhibits also a significant effect on the above mentioned parameters. Therefore, the increase of the basket bed thickness led to the decrease of Biot number factor λ.

The negative effect of cells immobilization on the biochemical processes is more important in the case of bioreactor with basket bed of biocatalysts, the glucose consumption

rate being for about 200 times lower than that recorded for the homogeneous alcoholic fermentation systems.

Notations

Bi - Biot number, -
C_C - cells concentration, kg/m^3 d.w.
C_S - substrate concentration, kg/m^3
C_{Si} - substrate concentration at the biocatalyst particle surface, kg/m^3
C_{SL} - substrate concentration in the liquid bulk, kg/m^3
C_{SP} - substrate concentration inside the biocatalyst particle, kg/m^3
D_{Se} - effective diffusivity, m^2/s
D_{SL} - liquid phase diffusivity, m^2/s
K_I - inhibition constant, kg/m^3
k_L - liquid phase mass transfer coefficient, s^{-1}
K_M' - apparent Michaelis-Menten constant, kg/m^3
h - distance from the bioreactor bottom to the inferior impeller, m
n_P - internal mass flow, kg/m^{2}s
r_S - rate of glucose consumption, kg/m^{3}s
R_P - biocatalyst particle radius, m
V - maximum biochemical reaction rate, kg/m^{3}s

greek letters
ϕ - volumetric fraction of biocatalyst particles, -
φ - Thiele modulus, -
λ - reduction factor, -

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