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NEW CONFIGURATIONS OF IMMOBILIZED BIOCATALYSTS BEDS – BIOETHANOL PRODUCTION IN BASKET BIOREACTORS

BY

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Abstract. The basket bioreactors represent a new generation of equipments which offer the possibility to reach high mass and heat transfer rates inside the broths, as well as superior rates of substrates consumption and products biosynthesis. These advantages are due to the complex hydrodynamics around and inside the basket bed (a combination between the perfect mixing and plug-flow) and to the diminishing of the influence of inhibitory effects.

In this context, the review presents the studies on the optimization of broths circulation and the applications of the bioreactor with basket bed of immobilized yeast cells for bioethanol production.

Key words: basket bioreactors, immobilized biocatalysts, alcoholic fermentation.

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1. Introduction

The bioreactor is assimilated with the heart of the biotechnological process, being the equipment in which the substrates are converted to the desired products under the microorganisms, cells or enzymes action. This comparison is due to the fact that the bioreactor "aspirates" the nutritive media and the biocatalysts through the upstream routes, "pumping off" the biosynthetic products through the downstream routes (Caşcaval *et al.*, 2002).

Although the bioreactors with immobilized biocatalysts are derived from the "classical" bioreactors and, therefore, their constructive and operating characteristics are rather similar with the second ones, the place of bioreactors with immobilized biocatalysts is privileged. The top position is the result of the advantages offered by the use of the immobilized microorganisms, cells or enzymes, especially the increase of the thermal, chemical and to the shear forces resistance of the biocatalysts, the increase of the number of the repeated biosynthesis cycles using the same particles of biocatalysts, the easier recovery of the biocatalysts from the final broths, the diminution or avoidance of the inhibition processes. Other advantages consist on the diminution or avoidance of the inhibition processes, the easier recovery of the biocatalysts from the final broths, and, consequently, the increase of number of the repeated biosynthesis cycles re-using the same particles of biocatalysts.

The bioreactors using immobilized biocatalysts can be designed as column, stirred, gas-lift or membrane bioreactors, being operated in batch, continuous or semicontinuous systems, with fixed/packed, mobile/stirred, expanded or fluidized bed (Galaction *et al.*, 2011).

The bioreactors with packed bed of biocatalysts are widely preferred, but they have some major disadvantages (Galaction *et al.*, 2011). The flow inside the bed is laminar, thus leading to low rates of mass and heat transfer and inducing the back-mixing or reverse flow phenomenon. 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 biocatalysts 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 biocatalyst particles being fixed in an annular cylindrical or conic bed, which is either static around the stirrer (Gamarra *et al.*, 1986; Kolagerakis & Behie, 1997; Pitault *et al.*, 2007; Galaction *et al.*, 2011), or rotary (Teshima & Ohashi, 1977; Warna *et al.*, 2002; Sheelu *et al.*, 2008). Owing 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.

Commonly, the stationary basket bioreactor has been provided with a cylindrical bed of basket type placed centered around one or more impellers (Fig. 1). The basket could be filled with immobilized cells or enzymes.

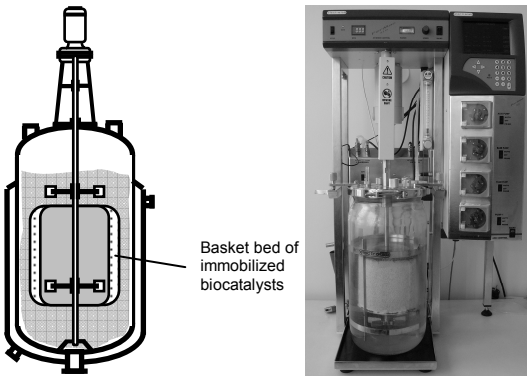

Fig. 1 − Stationary basket bioreactor.

The cylinder could be of porous glass or ceramics and plastic or steel/wire mesh (Kolagerakis & Behie, 1997; Magnico & Fongarland, 2006; Fadnavis *et al.*, 2007; Pitault *et al.*, 2007). The media circulation in the outer and inner region of basket bed, or even inside the packet bed, could become more effective when rotary basket beds are used. Sheelu *et al.* (2008) performed the rice bran oil degumming in a spinning basket bioreactor with immobilized lecitase. The rotary cylindrical basket was made of stainless steel mesh, its optimum rotation speed being of 350 - 400 rpm.

The basket bioreactors have been used in food industry for ethanol and carboxylic acids production or for various enzymatic transformations (Gamarra *et al.*, 1986; Staniszewski *et al.*, 2007; Meanwell & Shama, 2008), in pharmaceutical industry for antibiotics production (Zakaria *et al.*, 1988), and for cells or tissue cultures (Kolagerakis & Behie, 1997; Suehara *et al.*, 1998; Luczkiewicz & Kokotkiewicz, 2005). Because the potential applications of the basket bioreactors recommend them as one of the most promising type of equipments for biotechnologies developed both at laboratory and industrial-scale, this chapter presents some engineering and technological aspects of pharmaceuticals and chemicals biosynthesis, as well as of wastewater treatment using stationary basket bioreactors with immobilized microbial cells and enzymes.

2. Optimization of Media Hydrodynamics Around the Basket Bed

The mixing systems provided with a cylinder around the stirrer are known to offer superior mixing efficiency, due to the extending of the turbulence, respectively to the increase of the velocity of medium flow streams (Caşcaval *et al.*, 2011). In the case of stationary basket bioreactor provided with the cylindrical packed bed around one impeller of Rushton turbine type, the studies on the hydrodynamics of medium with apparent viscosity between 1 and 75 cP indicated the significant intensification of medium circulation compared to a similar stirred bioreactor without the basket bed (Galaction *et al.*, 2011). Moreover, the optimum position was found to be inside the cylindrical bed, at the superior extremity of the basket. This position leads to the lowest mixing time values and to the most important attenuation of the negative influence of the apparent viscosity increase on the medium circulation.

The utilization of double impellers leads to the intensification of medium circulation in the outer region of the basket compared to the previously studied case. But, the induced effect is directly related to the positions of the stirrers on the shaft. For establishing the optimum positions of the Rushton turbines on the shaft, two mixing systems have been considered (A and B) (Galaction *et al.*, 2011). One of these turbines (inferior or superior, respectively) is fixed and the other is placed successively in four positions on the stirrer shaft (Fig. 2).

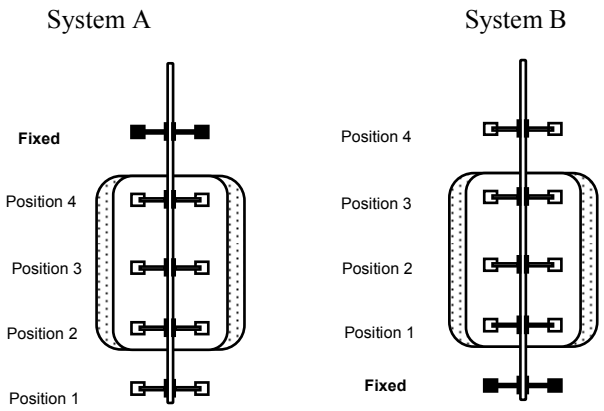

Fig. 2 − The considered positions of the impellers on the shaft.

The mixing efficiency was analyzed by means of the mixing time values, t_m, obtained for various operating conditions, using the tracer method (van't Riet & Tramper, 1991).

For system A, with the inferior Rushton turbine mobile, the lowest values of mixing time have been reached for position 2, respectively inside the basket at its inferior extremity, opposite to the fixed stirrer (Fig. 3). Contrary, the less efficient mixing was recorded for the closest turbines positions (position 4). Intermediary values of mixing time have been obtained for positions 1 and 3, but they differ from the viewpoint of the dependence on the rotation speed.

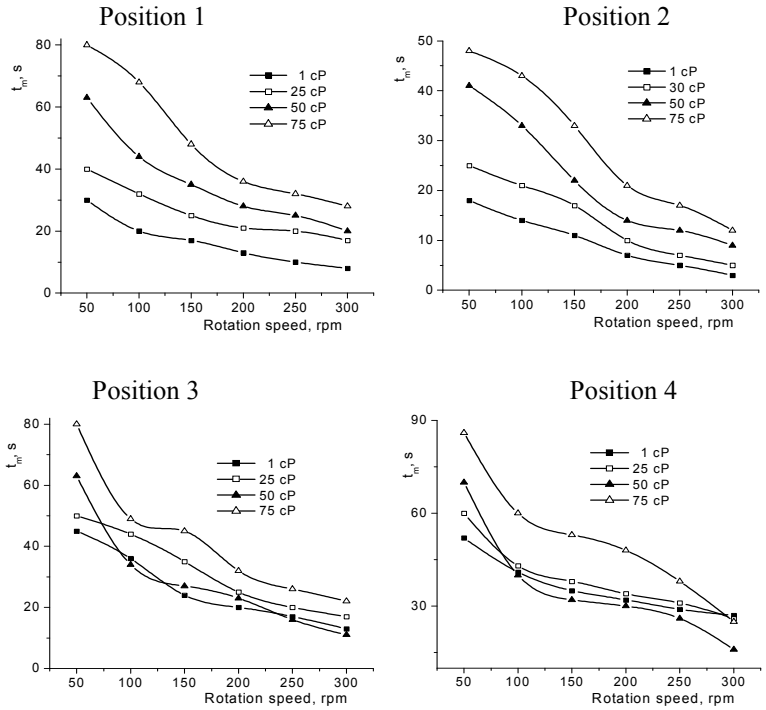

Fig. 3 − Influence of rotation speed and apparent viscosity on mixing time for system A.

Similar to the basket bioreactor with a single Rushton turbine, these results could be the consequence of the acceleration or deceleration of the flow streams induced by the impeller, due either to the basket wall, or to the streams interference. Owing to the presence of two stirrers on the shaft, these phenomena are amplified, thus modifying either the magnitude of the above mentioned effects or the optimum position of the mobile stirrer, compared to the previously studied basket bioreactor with immobilized yeasts cells. Therefore, because the presence of the cylindrical bed intensifies the medium circulation, the reduction of mixing time appears as evidently for the positions inside the basket, respectively for the positions 2, 3 and 4.

But, according to the experimental results, the values of mixing time lower than those corresponding to the position 1 can be reached only if the mobile stirrer is situated in the inferior region inside the basket (position 2). By placing the turbine at the basket inferior extremity, the inferior flow regions induced by the impeller is generated outside the cylindrical bed, this avoiding the flow streams interference and leading to the increase of flow velocity of the medium.

For position 3 of the mobile turbine on the stirrer shaft, both flow regions generated by the Rushton turbine are situated entirely inside the inner region of the basket, therefore the flow streams are accelerated and interfere, thus leading to the hindrance of the medium circulation compared to the position 2. Furthermore, due to the presence of the second stirrer, the magnitude of the streams interference is increased.

The lowest efficiency of mixing has been reached for position 4, as the result of the interference of the flow streams generated by the two vicinal Rushton turbines, phenomenon that is amplified by reducing the apparent viscosity. Thus, by increasing the rotation speed, the values of mixing time recorded for water and media with viscosity of 25 cP exceed those obtained for more viscous media.

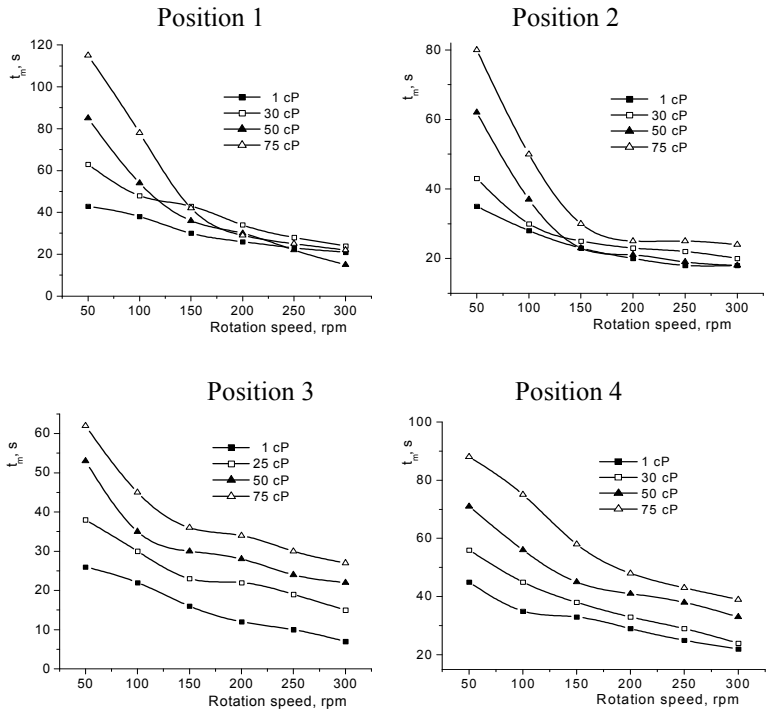

Fig. 4 − Influence of rotation speed and apparent viscosity on mixing time for system B.

The above discussed phenomena constituted the cause also for the variation of mixing time with rotation speed observed for the system B (Fig. 4). For the above mentioned reasons, the most efficient mixing is reached if the mobile stirred is placed inside the basket at its superior extremity (position 3). But, owing to the more important "bottom effect" which hinders the flow of medium promoted by the inferior fixed turbine, the turbulence generated by this stirrers combination is less intense comparatively to that corresponding to the optimum position for the system A (position 2).

The interference of the flow streams for positions 1 and 2 leads to higher values of mixing time for lower viscous medium. Likewise the system A, the most inefficient mixing is recorded for the closest positions of the Rushton turbines on the shaft. On the other hand, owing to the "bottom effect", the medium circulation for position 1 becomes less intense than that observed for the similar position 4 for the mixing system A.

The increase of the apparent viscosity led to the significant reduction of the mixing intensity. Thus, for the rotation speed of 200 rpm, compared to water, the mixing time recorded for media with the apparent viscosity of 75 cP was for about 1.5 - 2.4 times greater in the case of system A, and for about 1.8 - 2.3 times greater for system B, respectively. For both systems, the influence of the apparent viscosity was lower than in the previous studied case of a single Rushton turbine on the shaft. Besides the most intense circulation of the medium, position 2 for system A and position 3 for system B, respectively, offer the most important attenuation of the influence of apparent viscosity on the mixing time. By comparing this magnitude effect for the two positions, and taking into consideration also the induced mixing intensity, the position 2 for the system A can be recommended as the optimum stirrers combination.

The intensification of the medium circulation promoted by the presence of the basket inside the bioreactor can be quantified by means of the ratio between the mixing time obtained in the absence of the basket (Oniscu *et al.*, 2002), t_{m0}, and that corresponding to the studied basket bioreactor, t_{mB}. The variation of this ratio with the apparent viscosity, plotted in Fig. 5 for 200 rpm and both analyzed mixing systems, indicated that the cylindrical bed of immobilized yeast cells amplifies considerably the turbulence inside the medium, t_{m0} being for several times greater than t_{mB}.

For the system A, the ratio t_{m0}/t_{mB} varied between 1.2 and 30, and for the system B between 1.2 and 19. The lowest effect of turbulence intensification has been reached for water and CMC solutions with apparent viscosity up to 25 cP (the ratio t_{m0}/t_{mB} was of 1.2 - 7), whilst the most important effect for apparent viscosity over 50 cP. Among the two possible optimum stirrers combinations (position 2 for system A and position 3 for system B, respectively), the favorable effect induced for the position 2 system A was the most important.

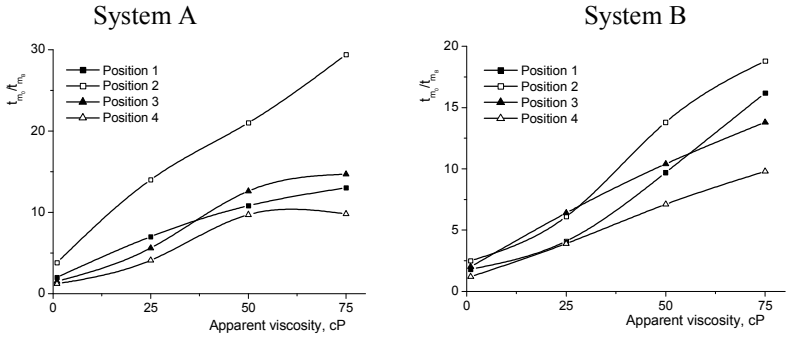

Fig. 5 − Influence of apparent viscosity on ratio t_{m0}/t_{mB} for the two considered mixing systems at 200 rpm.

By means of the experimental data, the mathematical correlation which describes the influence of rotation speed, N (s^{-1}), and apparent viscosity, η_a (Pa·s), on the mixing time has been established for the most efficient position, position 2 system A. The explicit values of the coefficients were calculated by the multiregression method using MATLAB software. Thus, the following correlation has been obtained:

$$t_m = e^{\left(-0.0238\cdot N^3 + 3.4359\cdot e^{\eta_a}\cdot N + 0.2684\right)}, \text{ [s]} \quad (1)$$

The proposed equation offers a good concordance with the experimental data, the average deviation being of ±7.42%. On the basis of the analyzed results, it can be concluded that the media circulation is significantly intensified in a stationary basket bioreactor, the mixing time becoming for about 1.2 to 30 times lower than that reached in the similar conventional stirred bioreactor and allowing to reaching high rates of substrate transfer and consumption.

3. Alcoholic Fermentation in the Basket Bioreactor with Immobilized *Saccharomyces Cerevisiae* Cells

Included in the biofuels class currently tested and used, bioethanol represents an attractive alternative to the conventional fossil fuels, its production by converting various substrates by free or immobilized cells of bacteria (*Clostridium spp.*, *Zymomonas spp.*) or yeast (*Saccharomyces spp.*), being intensively studied in the last two decades (Takamitsu *et al.*, 1993; Flinckinger & Drew, 1999; Najafpour *et al.*, 2004; Staniszewski *et al.*, 2009). In this case, as it was above discussed, the fermentation with immobilized cells

could avoid the substrate inhibitory effect, the use of higher concentration of carbohydrates becoming possible.

Most of the experiments on alcoholic fermentation with immobilized cells have been carried out in packed bed bioreactors in continuous, semicontinuous or fed-batch systems (Williams & Munnecke, 1981; Takamitsu *et al.*, 1993; Flinckinger & Drew, 1999; Najafpour *et al.*, 2004; Rivaldi *et al.*, 2008; Pacheco *et al.*, 2009; Staniszewski *et al.*, 2009; Nikolić *et al.*, 2009; Singh *et al.*, 2009). 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, higher conversion rate and construction simplicity.

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 (Bailey & Ollis, 1986; Ingram *et al.*, 1998). 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 result has been obtained by Nagodawithana and Steinkraus (1976), the authors concluding 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. This result confirms that the secondary products contribute to the amplification of the inhibitory phenomenon.

In the case of alcoholic fermentation carried out in a bioreactor with stationary basket bed with immobilized *S. cerevisiae* cells in alginate, the primary analysis of the variation of substrate (glucose) concentration during the fermentation process indicated that the basket system can be used for many fermentation cycles (Fig. 6).

Irrespective of the glucose concentration, it can be observed that the first fermentation cycles occurred similarly, the substrate variation being rather identical. Thus, for glucose concentrations of 50 and 100 g/L, the similar variations are recorded for the first four cycles, whilst for glucose concentration of 150 g/L for the first three ones. For the initial glucose concentration of 50 g/l, the durations of the first four fermentation cycles are of 46 - 50 h (time necessary for the total consumption of glucose).

Any important modification of the yeast activity has been observed during the first four fermentation cycles, but the glucose consumption rate decreases and, implicitly, the process duration increases significantly from the fermentation V to IX. Therefore, the duration of fermentation IX becomes of 140 h. Compared to the alcoholic fermentation with mobile bed of immobilized yeast cell (Galaction *et al.*, 2010a), the duration of the fermentation using the basket bed system becomes double. This increase of the time needed for the

total consumption of glucose is due mainly to the plug flow of liquid phase inside the biocatalysts bed, the promoted low turbulence leading to low rate of substrate diffusion towards the yeast cells.

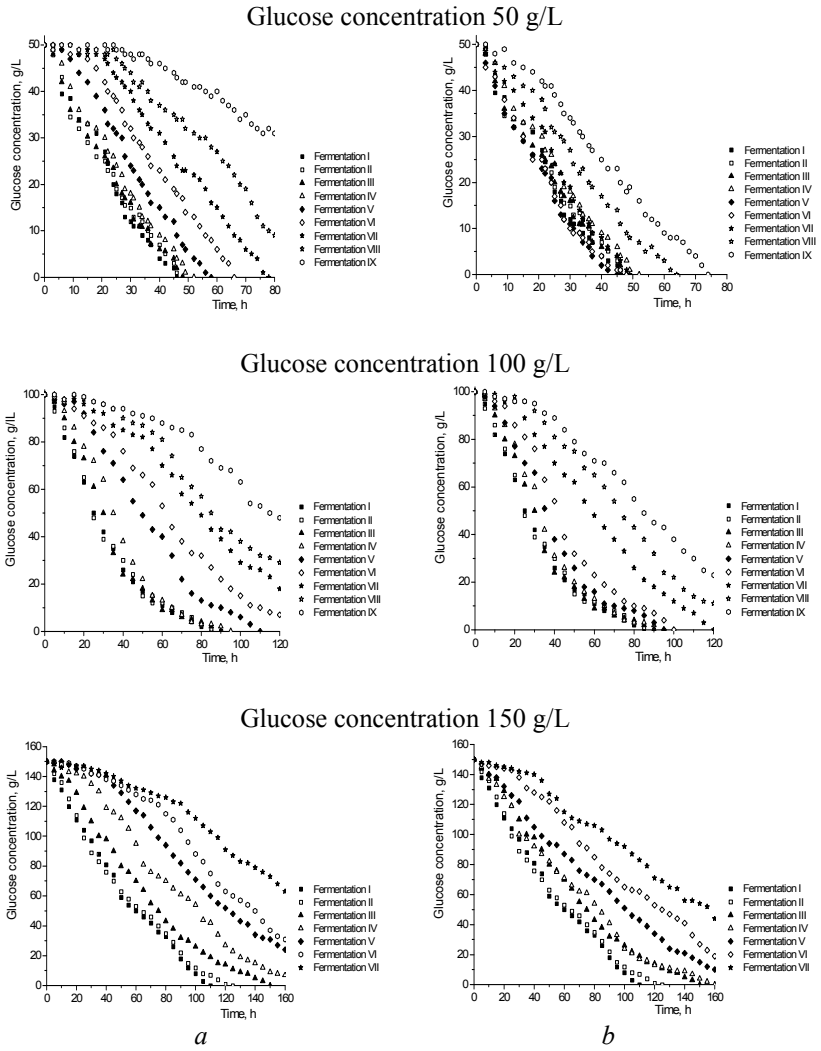

Fig. 6 − Variation of glucose concentration during alcoholic fermentation with basket bed of immobilized *S. cerevisiae* cells
(*a* − without bed washing out, *b* − with bed washing out).

Moreover, the low velocity of liquid circulation inside the basket bed induced both the glucose accumulation inside it, thus reducing significantly the gradient of glucose concentration between the outer and the inner regions of the

biocatalysts bed, and the appearance of the substrate inhibitory effect. Similarly, the ethanol is accumulated inside the basket bed, generating the product inhibition phenomenon. For verifying the above assumptions, the basket bed has been washed out with tap water for 60 min after the fourth fermentation cycle, for removing the glucose and alcohol accumulated in the fixed bed.

Fig. 6 indicates that the variations corresponding for the fermentation cycles V to IX, after the basket bed washing out, are superimposed on those recorded for the first fourth ones. Furthermore, the duration of the fermentation for the last cycles is significantly reduced, becoming of 75 h for fermentation IX. These results underline the importance of the negative effect of increase of substrate and product concentrations inside the basket bed after several fermentation cycles and the necessity of this bed renewal by washing out.

The increase of glucose initial concentration to 100 g/L does not change the system behavior. Thus, the variation of substrate concentration for the first four fermentation cycles is also similar, but the time needed to the total consumption of substrate is longer (the duration of fermentation IX is 190 h). The number of runs corresponding to the similar variations of glucose concentration is extended to six and the duration of the ninth fermentation is reduced to 140 h after the basked bed was washed out.

As it can be observed from Fig. 6, the use of higher glucose concentration, 150 g/L, associated with the appearance of the substrate inhibition phenomenon, does not inhibit the activity of immobilized *S. cerevisiae* cells. According to the literature (Galaction *et al.*, 2010b), the substrate inhibition is avoided by cells or enzymes immobilization, due to the internal diffusion which reduces the substrate concentration inside the biocatalyst particles below to that inducing this negative effect. But, at this value of glucose concentration, the duration of fermentation is considerably increased, varying between 110 h for the first cycle to 220 h for the seventh one, and the differences between the glucose consumption rates are amplified from one fermentation cycle to another. Practically, only for the first two runs the substrate concentration variations could be considered similar.

By washing out the basket bed with tap water for 60 min after fermentation II and resuming the process it can be seen that the first four cycles occur almost similarly, although the overall duration of fermentation is slowly diminished (the duration of fermentation VII is reduced with only 20 h). For all above discussed cases, the reduction of glucose consumption rate with the increase of number of fermentation cycles can be attributed to the accumulation of ethanol inside the biocatalyst particles, implicitly to the product inhibitory effect, and to the cells death, induced by natural causes or by inhibitory phenomena. But, the reduction of the process rate is not the result of the mechanical lysis of the biocatalyst particle as for the bioreactors with mobile beds of immobilized yeast cells.

Due to the diminution of the turbulence in the region around the basket bed, the rate of glucose transfer and, consequently, the rate of its consumption are diminished in this bioreactor. For the same reason, the diffusion rate of ethanol from the inner region of basket to the liquid bulk is also low, thus leading to its accumulation inside the biocatalysts bed and to the appearance of the product inhibition. But, these phenomena seem to not affect the system ability to perform more fermentation cycles than in the case of mobile bed of immobilized yeast cells. This difference is the result of the avoidance of biocatalysts mechanical lysis in the stationary basket bioreactor.

The above conclusions are also suggested by plotting the dependence between the average fermentation rate and substrate initial concentration for each considered fermentation cycle (Fig. 7). The average fermentation rate was calculated using the following relationship:

$$\bar{r}_S = \frac{C_{Si} - C_{Sf}}{t}, \ [g/L \cdot h] \tag{2}$$

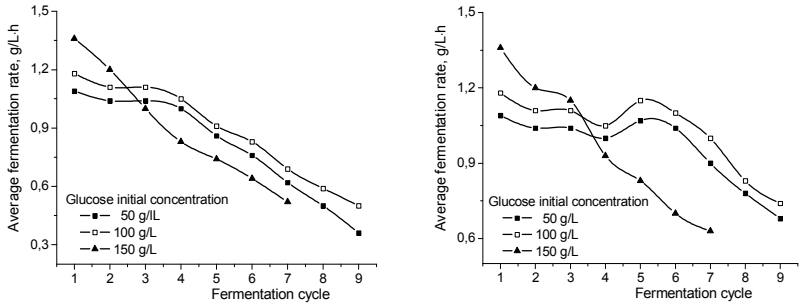

Fig. 7 − Variation of average fermentation rate with number of fermentation cycle (*a* − without bed washing out, *b* − with bed washing out).

Thus, the results given in Fig. 7 indicated that the average fermentation rates in the basket bioreactor are inferior to those recorded for the bioreactor with mobile bed of biocatalysts. In the same time, the variations obtained for glucose initial concentrations of 50 and 100 g/L, respectively, are similar. Because the rate of glucose consumption is directly depended on the glucose concentration, the substrate consumption is faster at a glucose concentration of 100 g/L.

By increasing the substrate initial concentration to 150 g/L its consumption rate is increased only for the first two fermentation cycles (Fig. 7). Starting with the third run, the fermentation rate becomes lower than those for the other two experimented glucose concentrations, due to the substrate or product accumulation inside the basket bed.

The results obtained after the biocatalysts bed renewing confirmed the above conclusions. Thus, the average fermentation rates corresponding to the

renewed basket bed are higher than the value recorded without washing out, indifferent of the initial substrate concentration in the liquid phase. For the lower glucose concentrations, of 50 and 100 g/L, the rates of substrate consumption for fermentations IV become equal to those for fermentations I. In these circumstances, the substrate consumption rates for fermentations IX are for about 1.9, respectively, 1.5 times greater than those obtained without bed renewing.

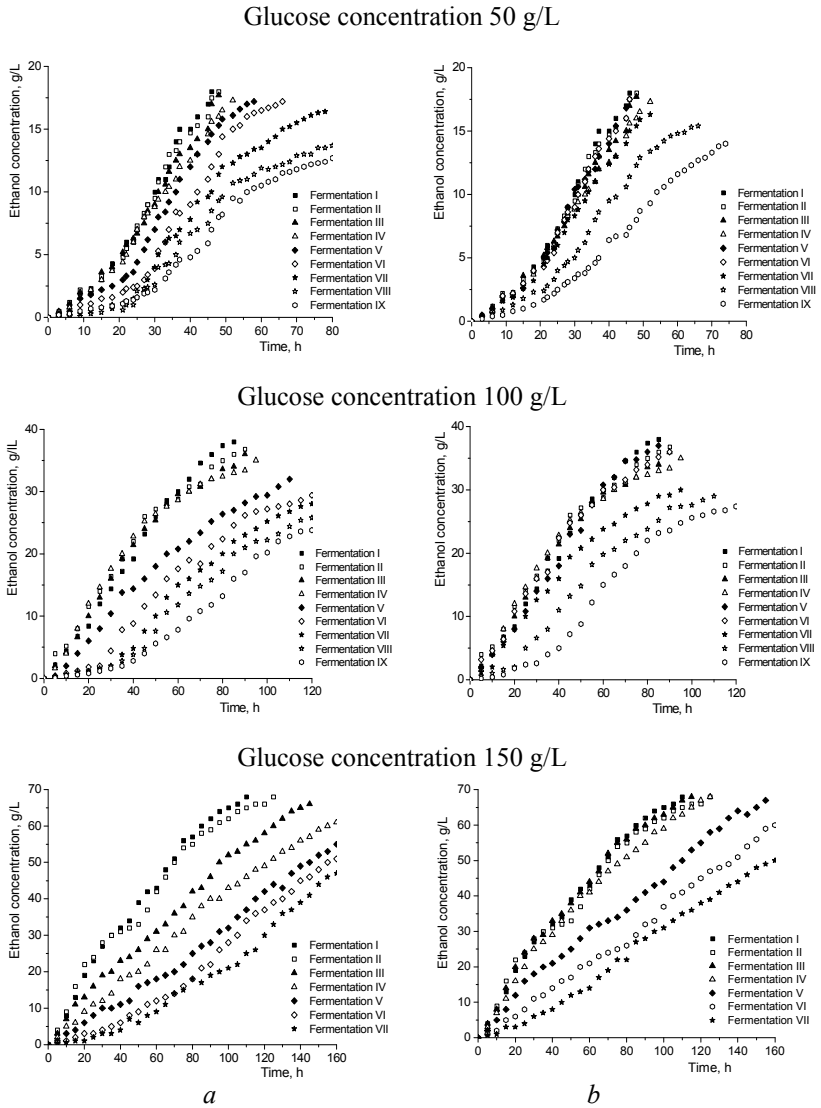

Fig. 8 − Variation of ethanol concentration during alcoholic fermentation with basket bed of immobilized *S. cerevisiae* cells
(*a* − without bed washing out, *b* − with bed washing out).

The favorable effect of basket bed washing out is less evident in the case of 150 g/L glucose concentration, the fermentation VII rate increasing for only 1.2 times after the bed renewing.

These experiments have been carried out also at rotation speeds of 200, 250 and 300 rpm, but any important modification of the above variations have been observed. Because the experiments have been carried out also at 200, 250 and 300 rpm with any important modification of the observed variations, it can be concluded that the diffusion of glucose through the biocatalysts bed and inside the biocatalysts particles represent the limitative step of the substrate consumption process.

Obviously, the variation of ethanol concentration during the fermentation is directly correlated with that of glucose concentration (Fig. 8).

Therefore, the first four runs, for initial glucose concentration up to 100 g/L, respectively the first two runs, for glucose concentration of 150 g/L, occurred similarly from the viewpoint of ethanol production rate. The effect obtained by washing out the basket bed is identical to that observed for the consumption rate of glucose.

The biocatalyst particles and the basket bed characteristics influence the glucose internal diffusion velocity and consumption rate (Galaction *et al*., 2010a). Theoretically, 180 g glucose produce 92 g ethanol, the theoretical yield of substrate conversion being:

$$Y_{P/S} = \frac{C_P}{C_{Sc}} = 0.51 \tag{3}$$

The analysis of the variation of substrate conversion yield offers more suggestive information regarding the effect of cells immobilization on the ethanol production efficiency. In this context, Fig. 9 indicates the dependence of this parameter on the substrate initial concentration from one run to another, related to the duration of the first fermentation cycle.

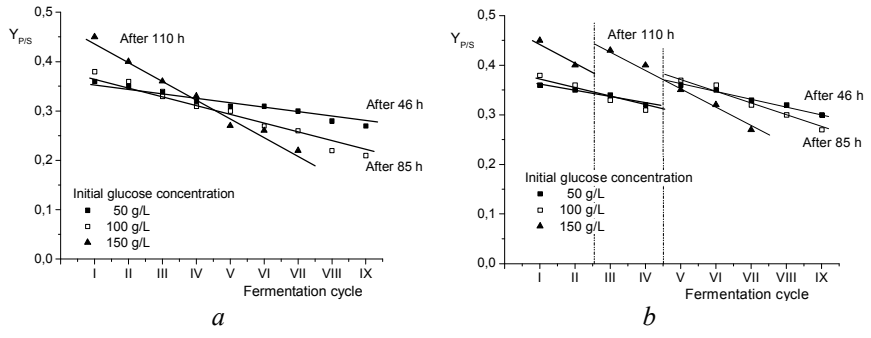

Fig. 9 − Variation of ethanol production yield with number of fermentation cycle (a − without bed washing out, b − with bed washing out).

Contrary to the fermentation with mobile bed of immobilized yeast cells, Fig. 9 indicates the reduction of $Y_{P/S}$ from one fermentation cycle to another, indifferent of the initial value of substrate concentration. This difference between the two systems containing immobilized biocatalysts is due to the ethanol accumulation inside the basket bed which induces the associated inhibitory effect. This effect becomes more important with the increase of substrate concentration. Thus, after the first seven fermentation cycles, $Y_{P/S}$ decreased from 0.36 to 0.30 for 50 g/L glucose, from 0.38 to 0.26 for 100 g/L glucose, respectively from 0.45 to 0.22 for 150 g/L glucose. Moreover, it can be observed that the ethanol production yield increased with the increase of glucose concentration for the first two fermentation cycles, decreasing from the third cycle to the final one. These results underlined the pronounced inhibitory effect generated by the ethanol.

4. Conclusions

Due to the combination between the perfectly mixed flow and plug-flow, the hydrodynamics of media inside the basket bioreactor is complex and allows reaching superior mass transfer and conversion rates compared to other types of bioreactors with immobilized biocatalysts.

Although the production of ethanol by fermentation is subject of important inhibitory processes, induced by substrate or product, the utilization of this bioreactor for ethanol biosynthesis leads to higher productivity and diminishes the magnitude of inhibition effects. Therefore, the alcoholic fermentation with immobilized yeast cells can be carried out at high concentration of glucose, for over seven cycles, but the basket bed has to be periodically washed out.

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NOI CONFIGURAȚII ALE STRATURILOR DE BIOCATALIZATORI IMOBILIZAȚI - PRODUCEREA BIOETANOLULUI ÎN BIOREACTOARE DE TIP „BASKET”

(Rezumat)

Bioreactoarele de tip „basket” constituie o generație nouă de bioreactoare, care oferă posibilitatea atingerii unor viteze ridicate ale proceselor de transfer de masă şi de căldură în interiorul lichidului de fermentație, precum şi viteze superioare ale conversiei substratului şi biosintezei. Aceste avantaje sunt rezultatul hidrodinamicii compleze a mediului în jurul şi în interiorul stratului de tip „basket” (o combinație între curgerea cu amestecare perfectă şi cea cu deplasare totală) şi al diminuării efectelor de inhibiție.

În acest context, lucrarea prezintă studiile privind optimizarea circulației mediului şi aplicațiile acestui bioreactor pentru producerea bioetanolului cu celule de drojdie imobilizate.