Studii şi Cercetări Ştiinţifice 2011, **12** (1), pp. 065 – 076
Chimie şi Inginerie Chimică, Biotehnologii, Industrie Alimentară

Scientific Study & Research
Chemistry & Chemical Engineering, Biotechnology, Food Industry **ISSN 1582-540X**

ORIGINAL RESEARCH PAPER

STUDY ON ALCOHOLIC FERMENTATION IN A STATIONARY BASKET BIOREACTOR WITH IMMOBILIZED YEAST CELLS

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Received: December 16, 2010
Accepted: January 22, 2011

Abstract: The use of a stationary basket bioreactor with immobilized *S. cerevisiae* cells indicated the possibility to extend the number of alcoholic fermentation cycles that can be carried out with the same biocatalysts to over nine. Although the rates of glucose consumption and ethanol production were lower than those recorded for the mobile beds of immobilized yeast cells, the mechanical lysis of the biocatalysts is avoided in the case of basket bed. Due to the substrate and product accumulation inside the basket bed, the fermentation process can be improved by washing out the biocatalysts bed over two or four cycles.

Keywords: *alcohol, basket bioreactor, glucose, immobilized cells, yeast*

INTRODUCTION

The new and attractive applications of the immobilized biocatalysts required the design and use of some proper bioreactors, specific or derived from the "classical" ones. Although these bioreactors are derived from the conventional bioreactors and, therefore, their constructive and functional characteristics are rather similar with the second ones, they offer important advantages, namely as: the increase of the thermal, chemical and to the shear forces resistance of the enzymes or cells, 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 [1 – 3].

Among these bioreactors, those with fixed beds of biocatalysts are intensively used at small and large scale due to their simple construction, lower cost for operation and maintenance, facile automatization and scaling-up, as well as due to the avoidance of the mechanical lysis of the immobilized cells or enzymes. The bioreactors with fixed beds of biocatalysts promote the intimate contact between the phases and their easier separation, the products with inhibitory effect being removed from the reaction zone (fixed bed), this leading to the reutilization of the biocatalyst without its preliminary regeneration.

But, the bioreactors with fixed bed of biocatalysts have some disadvantages [3]. The flow inside the bed is laminar, thus leading to low rates 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 bed, the biocatalysts particles being fixed in an annular cylindrical or conical bed, which is either static around the stirrer [4 – 6], or rotating one [7 – 10]. Owing to their design, these bioreactors avoid 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. Moreover, 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 [11].

Developing our previous studies on alcoholic fermentation using immobilized yeast cells, these studies are dedicated to the investigation of the substrate consumption and alcohol production in a stationary basket bioreactor with immobilized *Saccharomyces cerevisiae* cells. The experimental data are analyzed comparatively to the other fermentation systems with free or immobilized yeast cells.

EXPERIMENTAL

The experiments were carried out in 10 L (8 L working volume) laboratory bioreactor Fermac 310/60 (Electrolab), with computer-controlled and recorded parameters [12, 13]. The bioreactor characteristics are given in Table 1.

Table 1. *Characteristics of bioreactor*

d, m	**d/D**	**H/D**	**w/d**	**l/d**	**h/d**	**No. blades**	**No. baffles**	**s/d**
0.082	0.41	1.95	0.20	0.33	0.50	6	4	0.18

The bioreactor has been provided with a cylindrical bed of basket type having 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). 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 [14]. 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 stirrer rotation speed was of 150 rpm. Any mechanical damage of the biocatalyst due to the shear forces was recorded during the experiments.

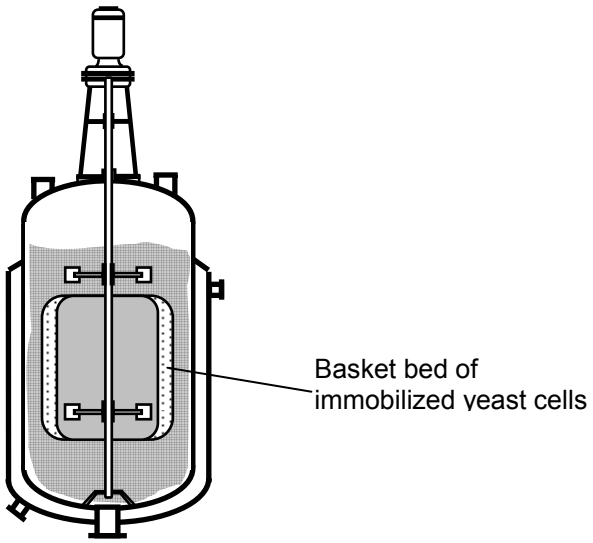

Figure 1. *Experimental stationary basket bioreactor*

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 [15]. The spherical biocatalyst particles having 4 mm diameter were obtained, their volumetric fraction in the basket bed being of 0.56.
The composition of the medium was: glucose 50, 100 or 150 g/L, KH_2PO_4 5 g/L, $(NH_4)_2SO_4$ 2 g/L, $MgSO_4$ 0.2 g/L, yeast extract 2 g/L, tap water to the prescribed volume [16]. The experimental studies presented in this paper have been carried out at

various glucose concentrations into the medium, some of them higher than the level generating the substrate inhibition for the fermentation systems with free yeast cells. The fermentation has been carried out at 28 °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 5×10^{-3} N sulfuric acid with a flow rate of 0.6 mL/min. The analysis temperature was of 65 °C.

RESULTS AND DISCUSSION

Glucose consumption

The bacteria or yeasts posses the ability to convert glucose under anaerobic conditions by Embden-Mereyhof-Parnas metabolic pathway, the main final products being the ethanol and carbon dioxide [17, 18]. 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, 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 [19]. This result confirms that the secondary products contribute to the amplification of the inhibitory phenomenon.
The primary analysis of the variation of substrate concentration during the fermentation process indicated that the basket system can be used for many fermentation cycles (Figures 2 – 4).

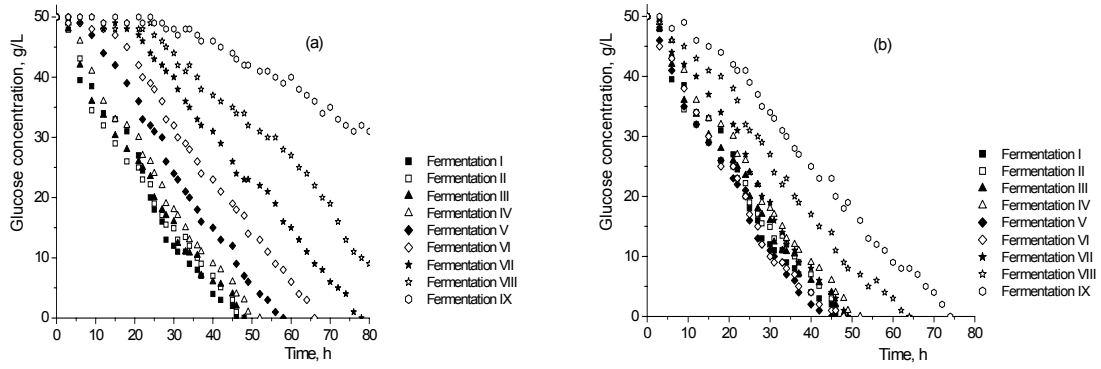

Figure 2. *Variation of glucose concentration during alcoholic fermentation with basket bed of immobilized* ***S. cerevisiae*** *cells for initial glucose concentration of 50 g/L (a) - without bed washing out, (b) - with bed washing out*

Regardless of the glucose concentration, it can be observed that the first fermentation cycles occur 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, while 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) (Figure 2a). 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 [20], 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.

Moreover, the low velocity of liquid circulation inside the basket bed induces 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 minutes after the fourth fermentation cycle, for removing the glucose and alcohol accumulated in the fixed bed. Figure 2b 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.

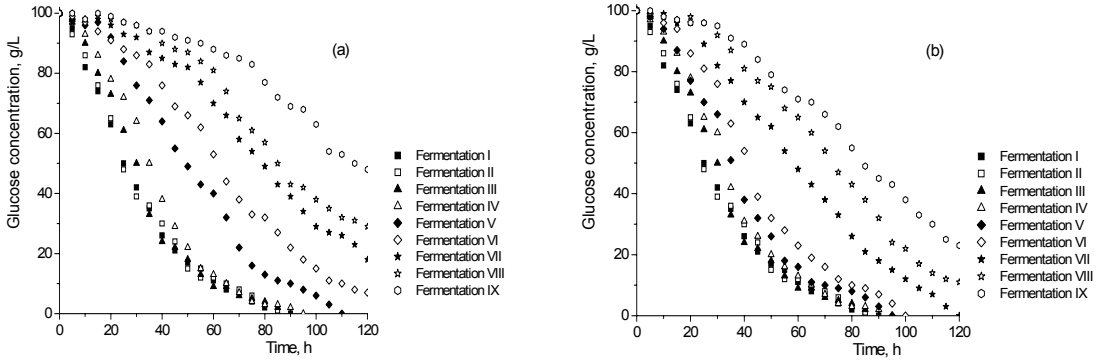

Figure 3. *Variation of glucose concentration during alcoholic fermentation with basket bed of immobilized* ***S. cerevisiae*** *cells for initial glucose concentration of 100 g/L (a) - without bed washing out, (b) - with bed washing out*

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) (Figure 3a). 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 (Figure 3b).
As it can be observed from Figure 4, 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.

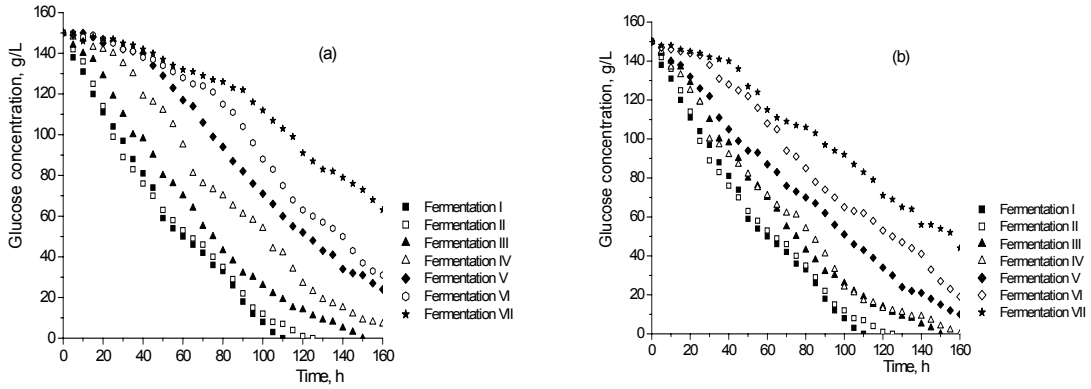

Figure 4. *Variation of glucose concentration during alcoholic fermentation with basket bed of immobilized* ***S. cerevisiae*** *cells for initial glucose concentration of 150 g/L (a) - without bed washing out, (b) - with bed washing out*

According to the previous studies [3, 15], 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 (Figure 4a). 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 minutes after fermentation II and resuming the process it can be seen that the first four cycles occurs almost similarly, although the overall duration of fermentation is slowly diminished (the duration of fermentation VII is reduced with only 20 h) (Figure 4b).
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 do 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 (Figure 5).

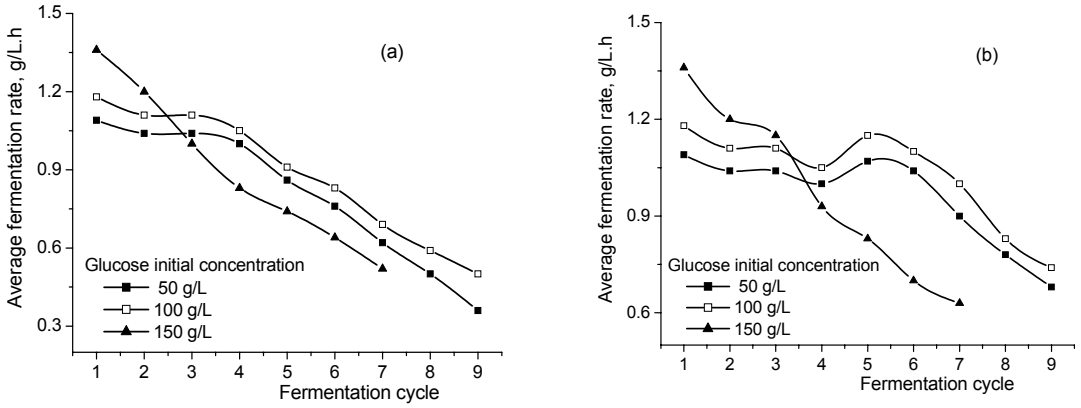

Figure 5. *Variation of average fermentation rate with number of fermentation cycle for different initial glucose concentration*
(a) - without bed washing out, (b) - with bed washing out

The average fermentation rate is calculated using the following relationship:

$$\bar{r}_S = \frac{C_{Si} - C_{Sf}}{t}, \text{ g/(L·h)} \qquad (1)$$

Thus, the results given in Figure 5 indicate 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 (Figure 5a). 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 confirm 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.

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. Therefore, 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.

Ethanol production

Obviously, the variation of ethanol concentration during the fermentation is directly correlated with that of glucose concentration (Figures 6 – 8). Therefore, the first four runs, for glucose concentration up to 100 g/L, respectively the first two runs, for glucose concentration of 150 g/L, occur similarly from the viewpoint of ethanol production rate (Figures 6a – 8a).

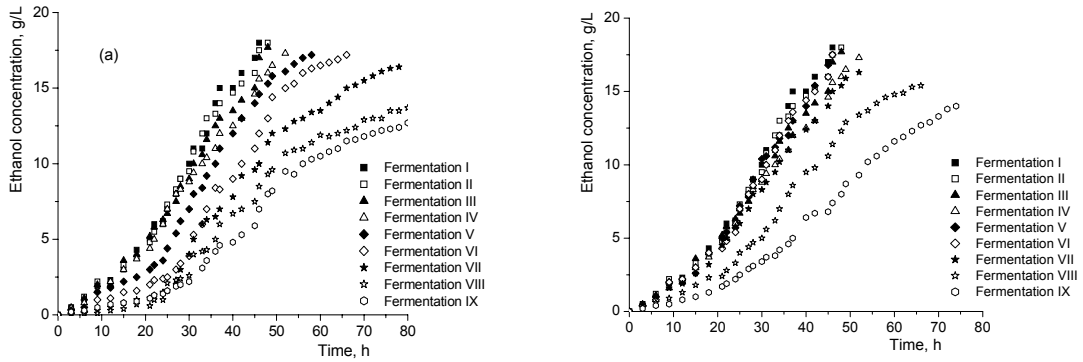

Figure 6. *Variation of ethanol concentration during alcoholic fermentation with basket bed of immobilized* ***S. cerevisiae*** *cells for initial glucose concentration of 50 g/L (a) - without bed washing out, (b) - with bed washing out*

The effect obtained by washing out the basket bed is identical to that observed for the consumption rate of glucose (Figures 6b – 8b).

As it was previously concluded, the biocatalyst particles and the basket bed characteristics influence the glucose internal diffusion velocity and consumption rate [20]. Theoretically, 180 g glucose produces 92 g ethanol, the theoretical yield of substrate conversion being:

$$Y_{P/S} = \frac{C_P}{C_{Sc}} = 0.51 \qquad (2)$$

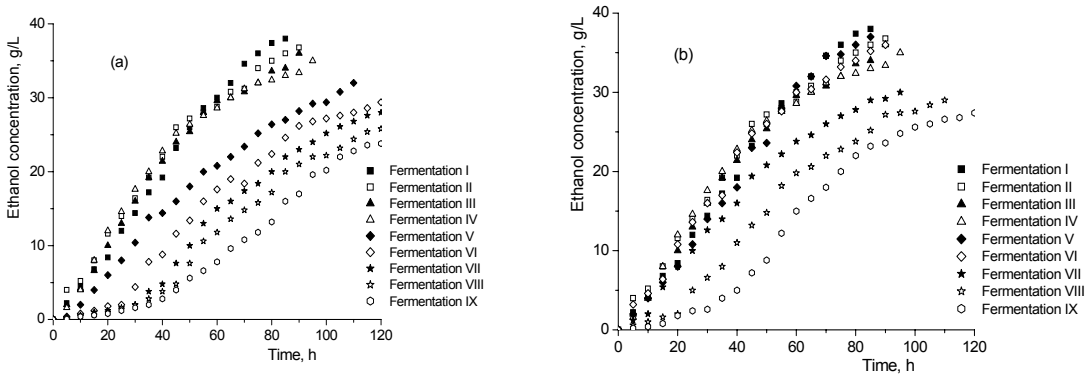

Figure 7. *Variation of ethanol concentration during alcoholic fermentation with basket bed of immobilized* ***S. cerevisiae*** *cells for initial glucose concentration of 100 g/L (a) - without bed washing out, (b) - with bed washing out*

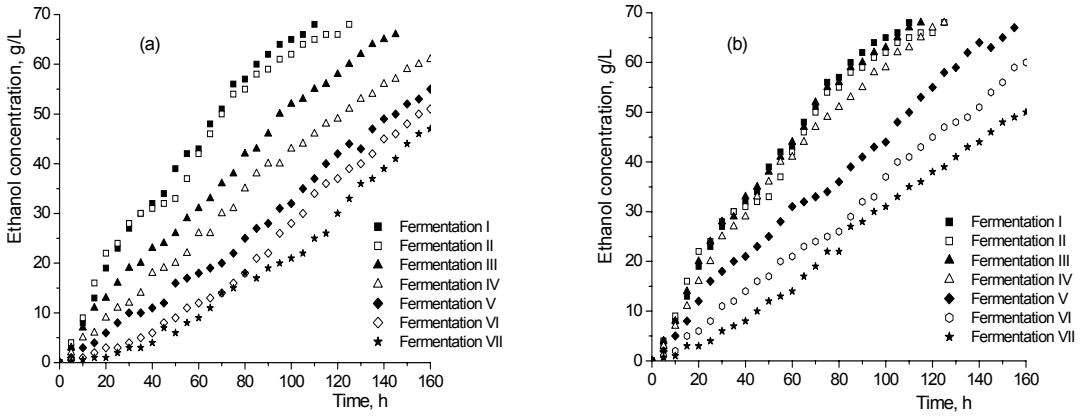

Figure 8. *Variation of ethanol concentration during alcoholic fermentation with basket bed of immobilized* ***S. cerevisiae*** *cells for initial glucose concentration of 150 g/L (a) - without bed washing out, (b) - with bed washing out*

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, Figure 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.

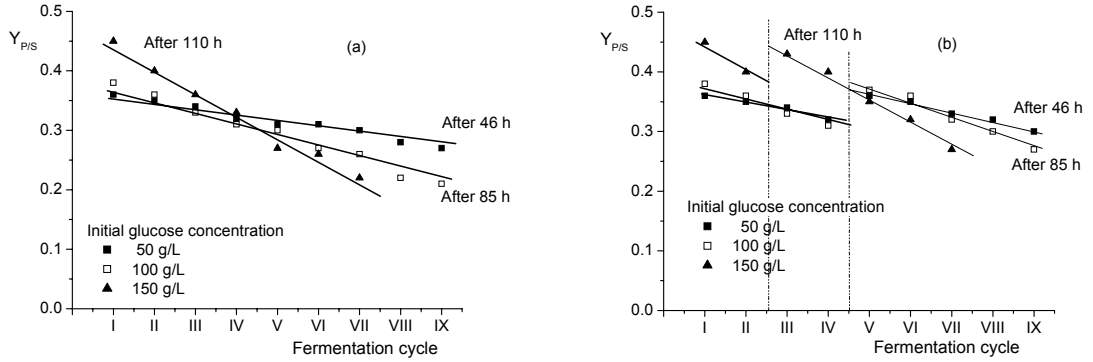

Figure 9. *Variation of ethanol production yield with number of fermentation cycle for different initial glucose concentration*
(a) - without bed washing out, (b) - with bed washing out

Contrary to the fermentation with mobile bed of immobilized yeast cells, Figure 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 increases 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.

CONCLUSIONS

The studies on the substrate consumption and product formation rates during the alcoholic fermentation on glucose using a stationary basket bioreactor with immobilized *S. cerevisiae* cells indicated the possibility to use these biocatalysts for seven to over nine fermentation cycles, in function of the substrate initial concentration. Due to the diffusion inside the biocatalyst particles, the inhibitory phenomenon induced by substrate is avoided, the product inhibition being more pronounced.
The fermentation process can be improved by washing out the basket bed after two or four runs, depending on the initial substrate concentration.

NOTATIONS

C_{Sc} - consumed substrate concentration, g/L
C_{Sf} - final substrate concentration, g/L
C_{Si} - initial substrate concentration, g/L

C_P - product concentration, g/L
d - impeller diameter, m
D - bioreactor diameter, m
H - bioreactor height, m
l - impeller blade length, m
$\bar{r}_S$ - average rate of fermentation, g/(L·h)
s - baffle width, m
t - time, h
w - impeller blade height, m
$Y_{P/S}$ - yield of substrate conversion to alcohol, -.

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