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KINETIC PARAMETERS STUDY OF HETEROGENEOUS FERMENTATION SYSTEMS WITH IMMOBILIZED YEAST

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Abstract

Among the tested used biofuels, the bioethanol represent an attractive alternative to the conventional fossil fuel, its production by converting various substrates by free or immobilized cells of yeasts *S. cerevisiae* being intensively studied. In the experiment, immobilized cells in alginate have been used with the method of entrapment. The process evolution has been analyzed by means of the variation of glucose and ethanol concentration during the fermentation.

The rate of the biochemical reactions in heterogeneous systems is lower than homogeneous systems, as the result of decreasing of the substrate concentration inside of 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 with immobilized cells differ from those of homogeneous environments. For the analyzed fermentation systems, the ethanol formation can be mathematical described by Michaelis - Menten equation. During the fermentation with immobilized yeast cells observed the inhibitory effects. This induce the modification of Michaelis - Menten equation for immobilized yeast. The kinetic paramters, μmax, KM', and KI, are estimated by Linewever - Burk linearization.

The apparent Michaelis - Menten constant is not influenced by the size and concentration of biocatalyst particles. The maximum rate of ethanol production is favorably influenced by the increase of the biocatalysts concentration, and but is affected by their size enlargement. Besides the positive exhibited by the attenuation of the inhibitory phenomena, the immobilization of *S. cerevisiae* cells affects the fermentation rate compared to the systems without inhibition containing free yeast cells.

The studies on the substrate consumption and product formation rates during the alcoholic fermentation with immobilized cells and glucose substrate indicated the possibility to use these biocatalysts for many fermentation cycles.

***Key words**: Immobilized yeast, Kinetic parameters, Heterogeneous system, Fermentation, Inhibition constant.*

1. Introduction

The "White Biotechnology", te new concept, proclaims that the use of renewable raw materials for biofuels production by low-expensive and ecofriendly biotechnologies constitutes one of the priority of industrial activities.

Among the tested and currently used biofuels, the bioethanol represents an attractive alternative to the conventional fossil fuels, its production by converting various substrates by free or immobilized cells of yeast *Saccharomyces cerevisiae* being intensively studied.

The fermentation with immobilized cells avoids the substrate inhibition. Therefore, the biocatalysts can be reused for many fermentation cycles.

The experiment on alcoholic fermentation with immobilized cells of yeast *Saccharomyces cerevisiae* in alginate natrium have been carried out in batch bioreactor. These studies have been dedicated to the analysis for different biocatalysts particles sizes.

The experiments are continued by investigating the kinetics of alcohol fermentation of glucose under substrate inhibition limitation and by developing a mathematical model describing and internal diffusion of the substrate.

2. Materials and Methods

The experiments have been carried out in 2 L laboratory bioreactor.

The equipment consists in eight conical bioreactors (vessels), where was placed the immobilized yeast with the entrapment method and free yeast, in respective amount 2.5 g and 5 g.

On average, every four hours was carried out measurements for determining the amount of glucose consumed and the amount of yeast which was released for each sample. Measurements were carried out through the refractometer and the spectrophotometer (in 550 nm).

In the experiments, immobilized *S. cerevisiae* cells in alginate natrium have been used [1]. The immobilization has been carried out by cells inclusion into the alginate matrix, respecting the entrapment method [3]. The following diameters of the biocatalyst spherical particles have been obtained: 4, 5.3 and 7 mm.

The fermentations have been carried out until a constant amount of glucose was taken, at the ambient temperature.

3. Results and Discussion

3.1 Glucose consumption

The yeast posses the ability to converse glucose under anaerobic conditions and the main final products being the ethanol and carbodioxide. The efficiency of the ethanol production by yeasts can be affected by glucose or ethanol concentration, due to the specific phenomenon of substrate product inhibition [1].

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 results will be analyzed in relation with the size of the particle: 4, 5.3 and 7 mm.

The initial analysis of the results plotted in Figures (1 - 3) suggest that the immobilized yeast cells can be used for several fermentation cycles, the magnitude of the modification of biocatalyst activity being correlated with the particles characteristics and substrate initial concentration.

The amounts of converted substrate for a certain time interval are rather equal, indifferent of its concetration in medium. This result suggests that the substrate inhibition is avoided by cells immobilization, owing to the internal diffusion of the substrate inside the biocatalyst particles which reduces the inner glucose concetration below that inducing the inhibition.

The utilization of the smallest particles increases the time required for the entire glucose consumption. This inversion is the consequence of the more important resistance to the diffusion of glucose inside the biocatalyst for the particles with 5.3 mm diameter. The magnitude of resistance to the internal diffusion is directly related to the particle and controls the glucose concentration gradient between the inner outer regions of the particle. Thus, this gradient is increased by reducing the internal diffusion velosity and consequently, the internal concentration of substrate could be lower than that inducing the inhibition phenomenon for the biocatalyst particles with diameter of 5.3 mm.

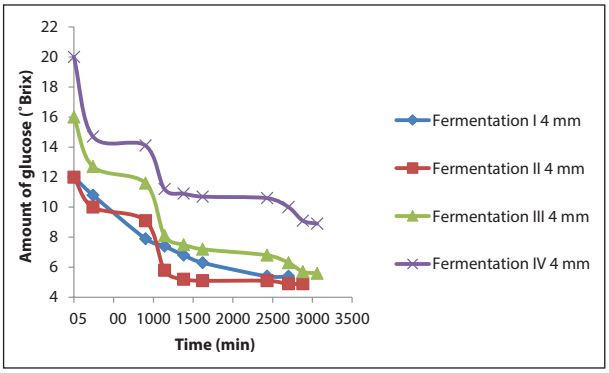

Figure 1. Variation of amount of glucose for biocatalyst particles - diameter 4 mm

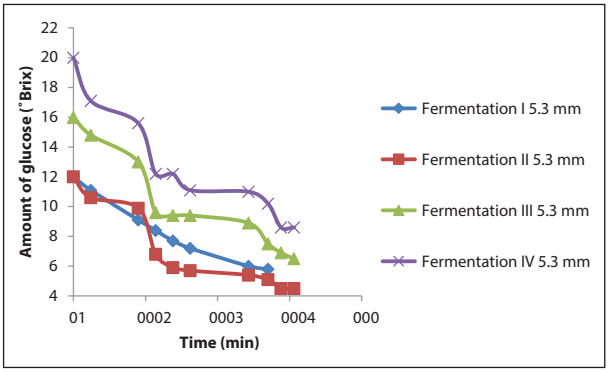

Figure 2. Variation of amount of glucose for biocatalyst particles - diameter 5.3 mm

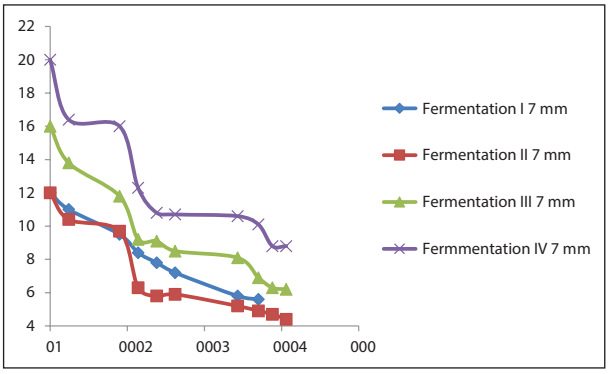

Figure 3. Variation of amount of glucose for biocatalyst particles - diameter 7 mm

If the biocatalyst particles with 7 mm diameter are used, the time needed for total glucose consumption is increased.

The internal diffusion contributes decisively to the diminution of the inhibitoty effect of glucose. But, in this case, the product inhibition could become important, due to the low diffusion rate of ethanol towards the outer medium and to its accumulation inside the particle. However, the experiments indicate that the substrate inhibition represents the main phenomenon affecting the fermentation performance compared to the product inhibition.

3.2 Kinetic Parameters

The rate of biochemical reactions in heterogeneous systems is lower than that recorded for homogeneous media, owing to the radial decreasing of the substrate concentration inside of 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 differ from those of homogeneous environments. For the analyzed fermentation systems, the ethanol formation can be mathematically described by Michaelis - Menten equation [3]:

$$\mu = \mu_{max} \frac{1}{[s] + K_M} \quad (1)$$

The equation (1) can be used for fermentation systems without inhibitory phenomena. But the inhibitory effects occur also in the case of alcoholic fermentation with immobilized yeast cells, the most important being that induced by glucose. Therefore, taking into account the substrate inhibition, the Michaelis - Menten equation [3] can be written for the immobilized cells as follow as:

$$\mu = \mu_{max} \frac{1}{1 + \frac{K_M}{[s]} + \frac{[s]}{K_I}} \quad (2)$$

The kinetic parameters, μ, K_M and K_I can be estimated by Lineweaver - Burk linearization method adapted to the studied conditions:

$$\frac{1}{\mu} = \frac{K_M}{\mu_{max}} \frac{1}{[s]} + \frac{1}{\mu_{max}} \quad (3)$$

Thus, by plotting the dependence between $\frac{1}{\mu}$ and substrate concentration, the straight line have the slope equal with $\frac{K_M}{\mu_{max}}$ is obtained. Its intercept from the ordinate is $\frac{1}{\mu_{max}}$. The Michaelis - Menten constant can be calculate using its definition.

For respecting the accuracy of the results and for avoiding the negative influence of biocatalyst particles, the calculations have been only for the first and the second fermentation cycle.

The obtained straight lines are plotted in Figures 4 and 5.

Obtained value are given in Tables 1 and 2.

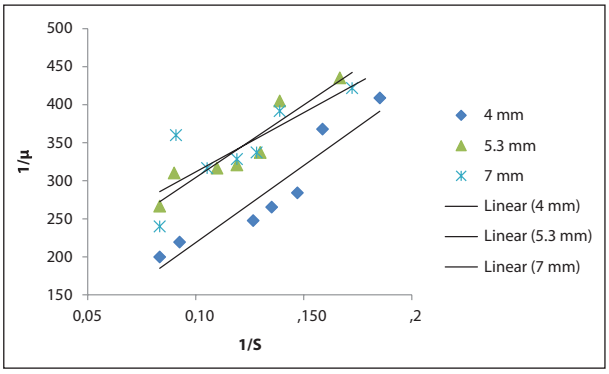

Figure 4. Lineweaver - Burk plot for the first fermentation

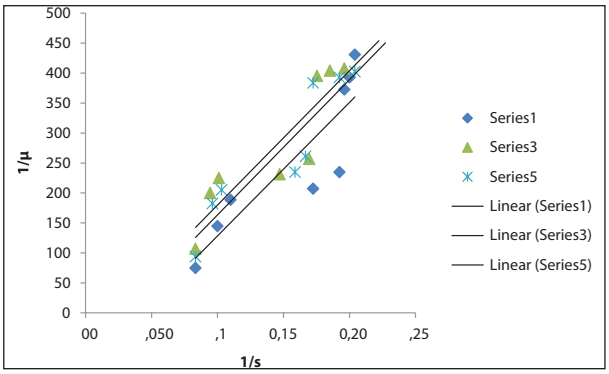

Figure 5. Lineweaver - Burk plot for the first fermentation

Table 1. The values of kinetic parameters from Figure 4

Biocatalyst diameter	μ_{max} (1/min)	K_M (° Brix)
4 mm	0.0629	127.5423
5.3 mm	0.0088	16.7965
7 mm	0.0064	9.9744

Table 2. The values of kinetic parameters from Figure 5

Biocatalyst diameter	μ_{max} (1/min)	K_M (° Brix)
4 mm	0.0106	23.6136
5.3 mm	0.0225	50.4562
7 mm	0.0161	36.3344

The results indicate that the inhibition constant has an unique value indifferent of the biocatalyst particles size, but depending on their concentration. The Michaelis - Menten constant is influenced by the size and concetration of biocatalyst particles. The maximum rate of ethanol production is favorably influenced by the increase of the biocatalysts concentration, and but is affected by their size enlargement.

The Michaelis - Menten constant is greater compared with homogeneous systems fermentation by the internal diffusion [2]. Besides the positive exhibited by the attenuation of the inhibitory phenomena, the immobilization of *S. cerevisiae* cells affects the fermentation rate compared to the systems without inhibition contationing free yeast cells.

4. Conclusions

- The studies on the substrate consumption and product formation rates during the alcoholic fermentation with immobilized *S. Cerevisiae* cells and glucose substrate using a batch bioreactor indicated the possibility to use these biocatalysts for more than six fermentation cycles, in function of the substrate concentration and biocatalyst characteristics. Moreover, due to other diffusion inside the biocatalyst particles, the inhibitory phenomena are avoided, the microbial activity being preserved.

- Using a specific mathematical model for ethanol formation in the investigated systems, the kinetic parameters μ, K_M and K_I have been estimated and compared with their values previously reported for alcoholic fermentation in homogeneous media with or without inhibitory effects.

5. References

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