THE STUDY OF ALCOHOLIC FERMENTATION OF THE *SACCHAROMYCVES CEREVISIAE* TYPE CELLS WHICH ARE IMMOBILIZED ON ACRYLIC TYPE COPOLYMERS

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Abstract

The paper presents the immobilization of Saccharomyces cerevisiae cells through entraping on an acrylamide - maleic anhydride - bisacrylamide copolimer. The immobilized cells ensure a 31% alcoholic fermentation process companing with free cells fermentation process.
***Keywords**: Saccharomyces cerevisiae, alcoholic fermentation, immobilization*

Introduction

The chemical or biochemical transformations represent the basis for the obtaining of many products of economic importance. In the context of the power crisis, the superiority of the biological synthesis methods, compared to the chemical methods, has led to the use on a larger scale of the biotechnological processes.

On this subject, the expert literature indicates the use of different organic or inorganic, synthetic or natural substratum for the immobilization of the enzymes and entire cells (Chibata, 1978; Andrés et al., 2003). The usage of immobilized enzymatic products has a series of advantages in comparison with the classic biocatalyse (Kötter and Ciriacy, 1993; Meena and Raja, 2006). However, a series of enzymes become highly unstable after extraction and purification, while for another series of enzymes the purification raises problems of economic or technical nature (Mosbach, 1988). Because of this fact, the usage of these enzymes in their immobilized form becomes non-efficient. For this reason, the idea of immobilizing the cellular organelles or even the entire cells on different insoluble base has appeared lately.

Using the cells or the cellular organelles in immobilized form eliminates the previous mentioned disadvantages. At the same time, it secures the obtaining of several compounds which require multiple

successive biochemical transformations, which are catalyzed by the enzymatic equipment of those cells (Oztop et al., 2003).

Experimental

As immobilizing base it has been used the acryl amide – maleic anhydride – methylene-bis-acrylamide copolymer. The copolymerization has been realized at 25°C in the presence of ammonium persulfate as a polymerization initiator and N,N,N',N' – tetramethyl-ethylen-diamine (TEMED) as a polymerization accelerator. It has also been used a suspension of *Saccharomyces cerevisiae* cells in an isotonic sodium chlorine solution. The cells have been immobilized by gel inclusion, adding the cellular suspension in the monomer mixture, which was composed of: 10% acryl amide, 3,5% N,N'-methylene-bis-acrylamide and 10% maleic anhydride. After homogenization, the mixture has been introduced into a reactor and has been saturated with nitrogen. Afterwards, 5 ml of ammonium persulfate solution 0,14% and 1 ml of N,N,N',N'-tetramethyl-ethylen-diamine have been added. When stirred up, the copolymer crumbles resulting grains which contain immobilized yeast cells.

In order to separate the solid phase from the liquid one, a solution of polymerization stabilizers (SAM) is added during the copolymerization, so the final concentration will be 2%.

The obtained grains have been washed and put into a column, where a glucose solution 0,5 M is added in a buffer solution with pH = 4,5, in order to test the alcoholic fermentation process.

The process parameters (the glucose, the carbon dioxide, and the ethylic alcohol) have been determined every 24 hours. The glucose concentration dynamics has been determined using the o-toluidine method. The resulted carbon dioxide has been dosed using gas chromatography, with the help of a chromatographic gas type GCHF-183, using Porapak Q columns and nitrogen as carrier gas. The ethanol has been dosed iodometric, using the Cordebard method in the Banciu and Droc modification.

Classical fermentations were performed in parallel using cells from the same suspension, in order to determine the fermentation output of the immobilized cells compared to the output of the free cells. The dried substance of the biomass has been determined for every charge (Cojocaru et al., 2007).

Results and Discussions

The obtained experimental results, that represent alcoholic fermentation parameters for a whole cycle, are systematized in table I and figures 1-4. The ethanol production increases in both types of fermentation maintaining its maximum value for the first 120 days (1,07 and, respectively, 3,57 ml/mg S.U./24 hours). The ulterior decrease is due to the reduction of the substratum concentration in the fermentation environment. The same aspect appears in the carbon dioxide forming dynamics (maximum values for the first 120 hours: 0,58 and, respectively, 1,93 ml/mg S.U./24 hours) and in the glucose consumption dynamics (maximum values for the first 120 hours: 2,49 and, respectively, 9,31 ml/mg S.U./24 hours).

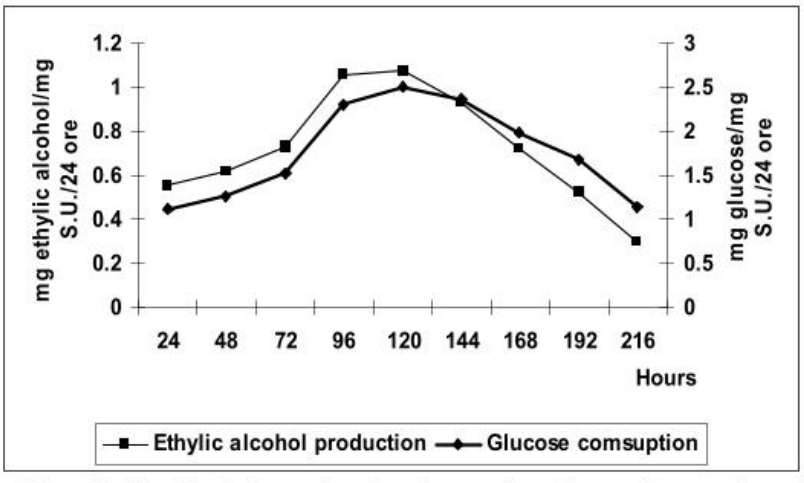

Fig.1: The ethylic alcohol production dynamics, depending on the glucose consumption level in the case of the immobilized *Saccharomyces cerevisiae* cells

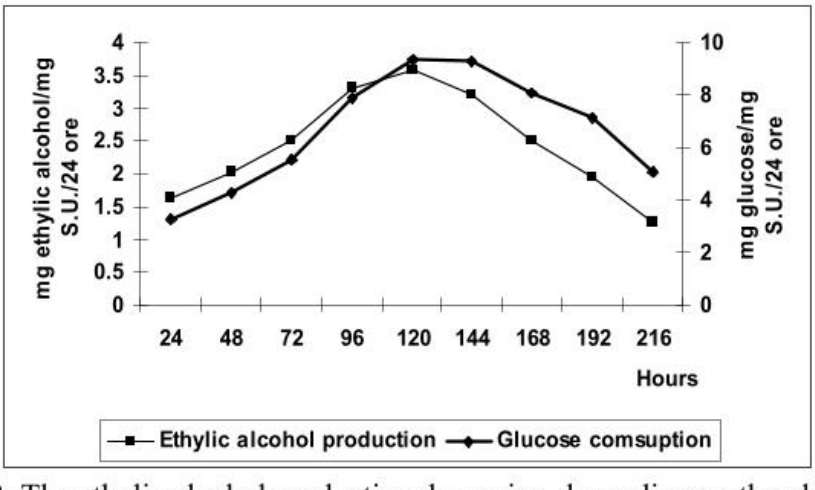

Fig.2: The ethylic alcohol production dynamics, depending on the glucose consumption level in the case of the free *Saccharomyces cerevisiae* cells

The comparative analysis of the fermentation parameters of each phase reveals stoichiometric inconsistencies. Thus, the formed carbon dioxide volume is bigger than the carbon dioxide volume that theoretically corresponds to the ethanol quantity resulted in the respective phase, but it is smaller than the carbon dioxide volume that could result from the corresponding quantity of glucose. This fact shows that a quantity of glucose turns into carbon dioxide and ethanol, the rest being used in the cells biosynthesis processes.

Table 1: Dinamics on the parameters of alcoholic fermentation of immobilized (a) and free cells (b) of *Saccharomyces cerevisiae*

Parameters	Age	24h	48h	72h	96h	120h	144h	168h	192h	216h
Gucose comsuption (mg/mg S.U. / 24h)	a	1,11± 0,40	1,26 ± 0,32	1,52 ± 0,36	2,30 ± 0,48	2,49 ± 0,50	2,36 ± 0,30	1,99 ± 0,26	1,68 ± 0,12	1,14 ± 0,09
	b	3,24 ± 0,47	4,26 ± 0,41	5,56 ± 0,42	7,88 ± 0,56	9,31 ± 0,63	9,24 ± 0,59	8,08 ± 0,47	7,12 ± 0,32	5,10 ± 0,19
Ethanol production (mg/mg S.U. / 24h)	a	0,55 ± 0,9	0,62 ± 0,11	0,73 ± 0,11	1,06 ± 0,17	1,07 ± 0,15	0,93 ± 0,17	0,72 ± 0,11	0,52 ± 0,08	0,30 ± 0,08
	b	1,62 ± 0,11	2,03 ± 0,10	2,51 ± 0,19	3,31 ± 0,23	3,57 ± 0,27	3,20 ± 0,18	2,51 ± 0,17	1,95 ± 0,09	1,25 ± 0,13
Carbon dioxide comsuption	a	0,27 ± 0,10	0,32 ± 0,12	0,37 ± 0,12	0,55 ± 0,15	0,58 ± 0,13	0,53 ± 0,08	0,43 ± 0,11	0,33 ± 0,07	0,20 ± 0,03
	b	0,79± 0,20	1,27 ± 0,38	1,27 ± 0,38	1,72 ± 0,26	1,93 ± 0,37	1,81 ± 0,02	1,49 ± 0,32	1,23 ± 0,11	0,84 ± 0,09

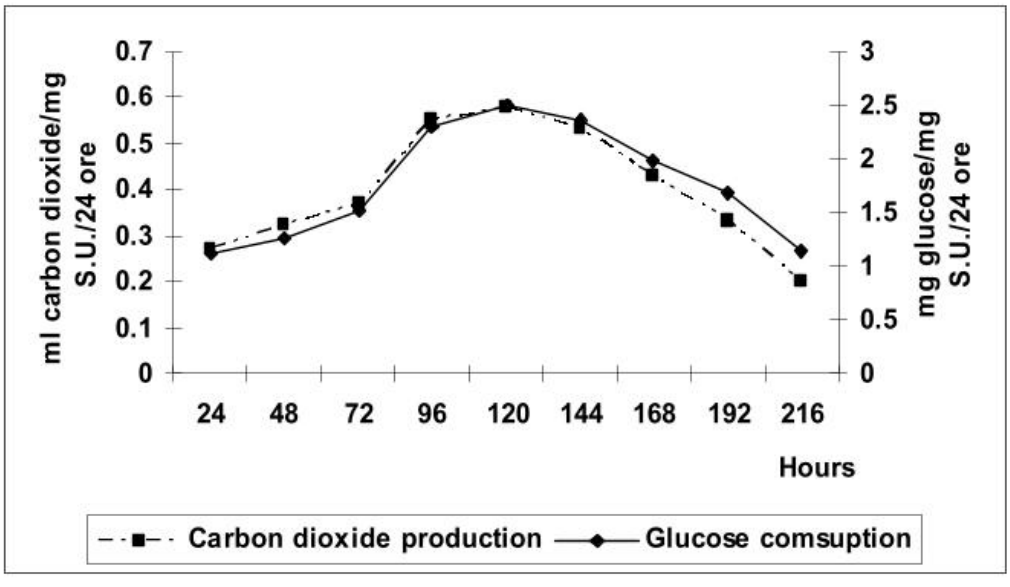

Fig.3: The carbon dioxide production dynamics, depending on the glucose consumption level in the case of the immobilized *Saccharomyces cerevisiae* cells

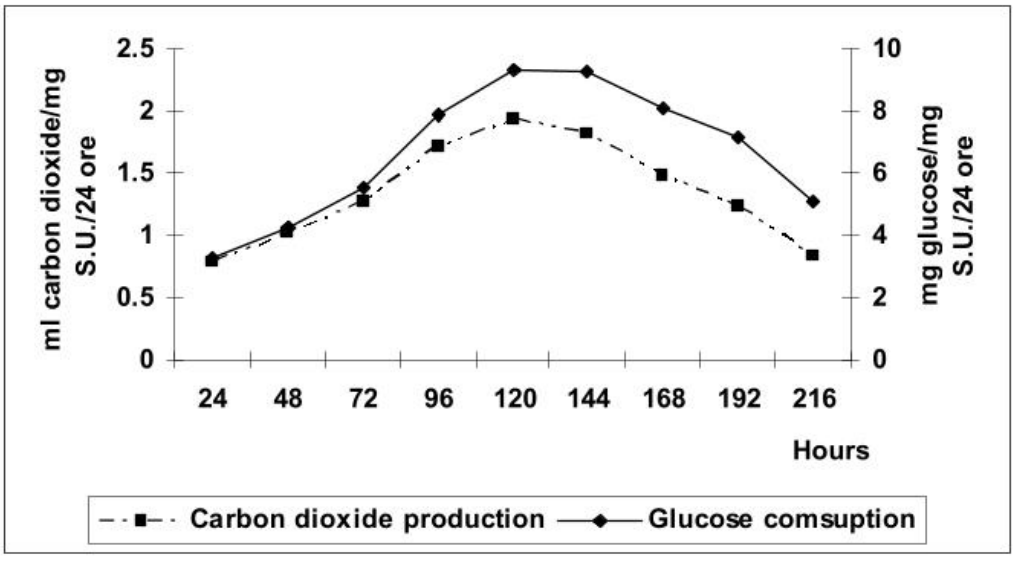

Fig.4: The carbon dioxide production dynamics, depending on the glucose consumption level in the case of the free *Saccharomyces cerevisiae* cells

The increase of the glucose consumption level is much higher in the free cells case than in the immobilized form case, which demonstrates that the multiplying process is present and relatively strong in the first case. The use of the same quantity of biomass in both fermentation types reveals that the immobilized form prevents proliferation, in this case the cells being contained in the copolymer grains.

By analyzing the ethanol production dynamics of the immobilized cells in comparison with the ethanol production dynamics of the free cells, we obtain an output of the fermentation by immobilization process of approximately 31%.

The alcoholic fermentation parameters maintain the same dynamics in the following fermentation cycles, in which, however, an evaluation of the biomass growth in the case of the free cells fermentation is necessary, knowing that in this case the proliferation relatively strong. In our future researches, we will analyze the fermentation cycles in order to determine the halving period, the number of fermentative cycles in which the immobilized cells can be implicated, the regeneration process, as well as other aspects. Another interesting subject is the choosing of the optimal porosity and conditions for polymerization in order to increase the output of the fermentative process using immobilized cells.

Conclusions

1. The acryl amide – maleic anhydride – methylene-bis-acrylamide copolymer, resulted from monomers in certain concentrations, has porosities and physical-chemical properties which are favorable for the immobilization of the microbe cells.
2. The immobilized Saccharomyces cerevisiae cells from this copolymer are securing an alcoholic fermentation process with an output of approximately 31% in comparison with the free cells.
3. The alcoholic fermentation process that uses immobilized cells can become a continuous process, if an optimal speed is provided for the substratum's passing through the column.

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