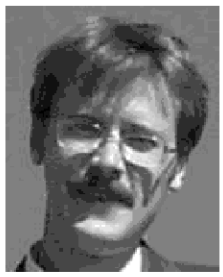
Ultrasound and beads with a liquid core used for the mass transfer between encapsulated cells and nutrient medium acceleration



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Introduction

Encapsulated cells have some advantages: an opportunity realization of continuous process, etc. The disadvantage of these systems is the decrease of mass transfer between cells and nutrient medium (Sun Y., Furusaki S. (1990), Krischke W. et. al. (1991). In this work the experiments on the study of influence of ultrasonic treatment of sodium alginate on the rate of saccharose fermentation by encapsulated yeast cells were carried out. The experiments with the use of alginate beads with a liquid core were carried out. The results showed that ultrasonic treatment of sodium alginate and use of such beads accelerates process of fermentation in comparison with solid beads.

Material and methods

As a model microorganism for the approbation encapsulation method yeast cells (*Saccharomyces cerevisiae*) were chosen, they are characterized by a short incubation period and a high speed of biocatalysis.

Sodium alginate was obtained from ZAO "ArialBio" (Moscow, Russia). All other reagents used were of analytical grade.

In this work ultrasound generator IKASONIC U50 control (Germany) was used.

Result and Discussion

Experiments with the beads with a liquid core

The nutrient medium, which was used in the experiments, consisted of:

- distilled water - 100 ml;
- saccharose - 3 g;
- K_2HPO_4 - 0.5 g;
- $MgSO_4$ - 0.3 g;
- yeast (free cells or cells immobilized in a matrix) - 3 g.

The temperature of the nutrient medium was 32^0 C. To eliminate of influence of outside diffusion braking on mass transfer between immobilized cells and nutrient medium, stirring of bioreactor medium at the rate of 100 - 150 revolutions per minute was carried out. The concentration of the initial sodium alginate solution used for the beads obtaining was 0.75 % (w/v). The experimental setup used for alginate beads preparation is presented in Fig. 1. The alginate solution (0.75 %, w/v) was added dropwise from a separating funnel *1* equipped with metal needle to flask *2* under vacuum and gentle stirring *3*. The beads formed were incubated in 2.0 % calcium chloride solution, rinsed with distilled water and used in further studies.

The diameter of the beads obtained on the average was equal to 3.5 mm. The research was carried out with the use of two groups of beads. The first group (I) was kept in 2.0 % solution of $CaCl_2$ within 25 minutes, till the formation of homogeneous internal structure. The second (II) - within 5

minutes that resulted in the formation of on a surface of beads of a calcium alginate layer 0.8 mm thick (Fig 2.). Diameter of a liquid core was on the average was equal 1.9 mm.

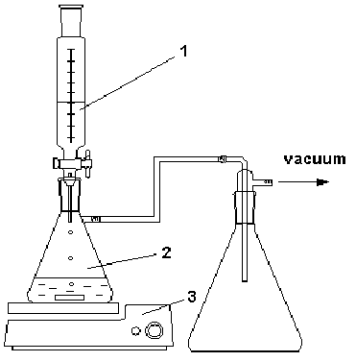

Fig. 1 Setup used for the alginate beads preparation

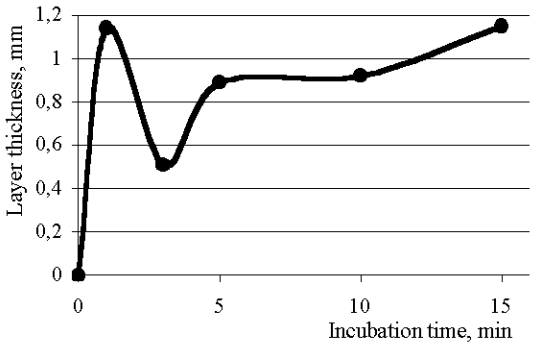

Fig. 2 Layer thickness vs incubation duration

The beads containing a certain quantity of cells were brought in a nutrient medium preliminary heated up to optimum temperature. Then stirring was turned on. The kinetics of the process of fermentation supervised on volume of the allocated carbonic gas collected in an eudiometer. As control experience experiment with free cells has been carried out.

The results of the experiments are represented in Fig. 3. The change of weight of saccharose contained in a nutrient medium, calculated on the basis of the values of allocated CO_2 volume is shown on the graph.

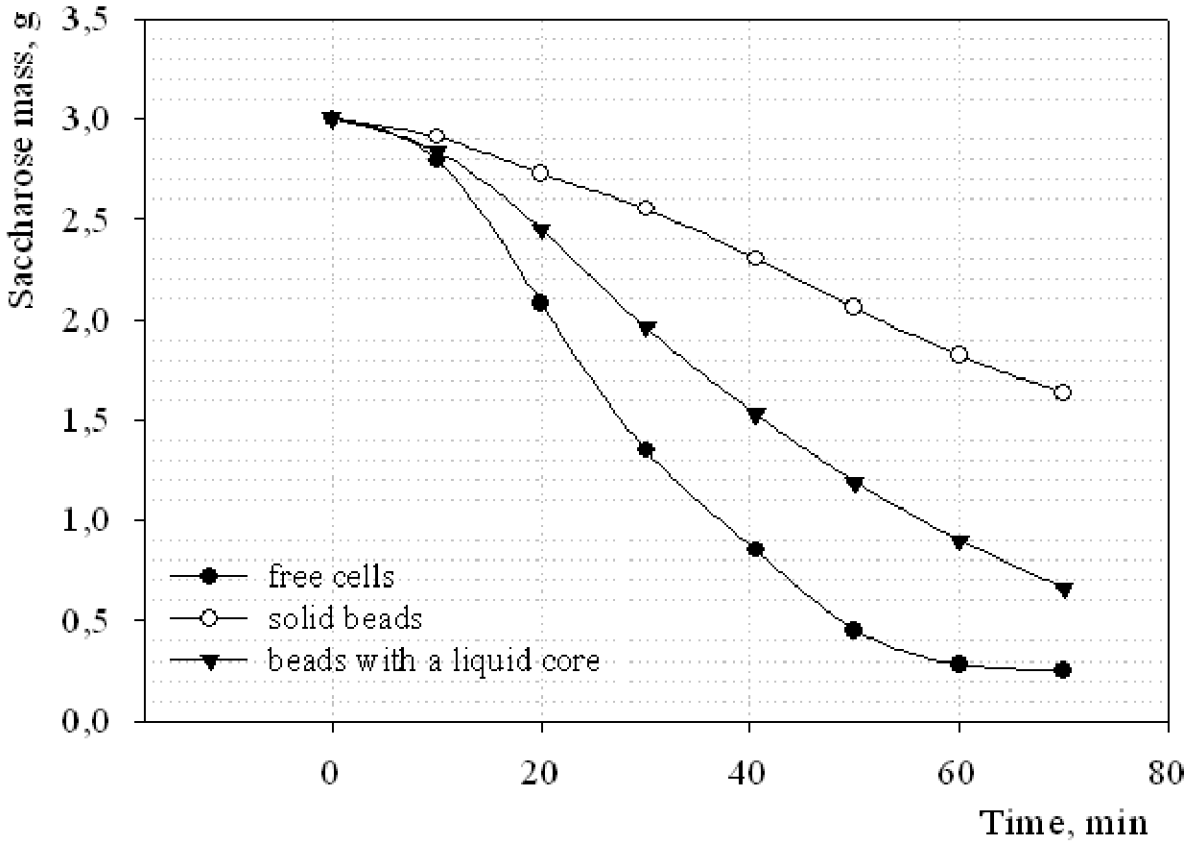

Fig. 3

The results of experiments showed, that immobilization of the yeast cells in solid beads increases saccharose fermentation time approximately by 2 times (~ 120 min). The use of beads with a liquid core results in the increase only by 1.3 time (from 60 up to 80 min). The use of such beads, from the point of view of fermentation process rate, will be preferable.

Experiments with sodium alginate with different molecular weight

To carry out the experiments two groups of beads with a liquid core were prepared. The first group was prepared on a basis sodium alginate treated with ultrasound at the intensity of 460 W/sm^2 within 30 minutes. The alginate molecular weight obtained was 500000. The second group of beads was prepared on the basis of raw sodium alginate with molecular weight 1500000. The results of the experiments are presented in Fig. 4.

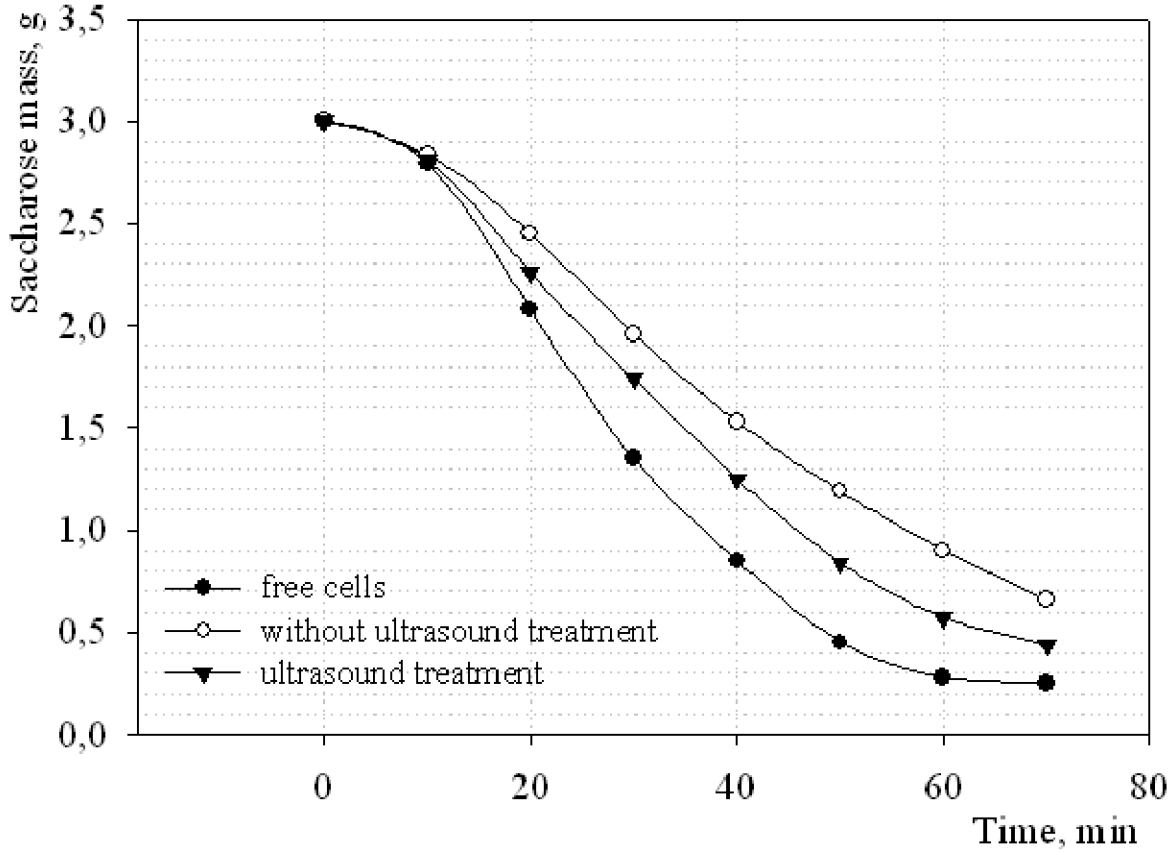

Fig.4

Experiments with the coated beads

The main disadvantage of the use alginate beads without coat is the output of encapsulated cells in a nutrient medium. Besides it makes the separation of a target product from the biomass difficult, this fact brings corrective amendments in to the kinetics of fermentation process which could be hardly estimated. For the estimation of influence of a coat on the fermentation process a number of experiments have been carried out. The initial sodium alginate solution (concentration 0.75 % (w / v) used for the beads obtaining and formation of a coat was treated by ultrasound (460 W/sm^2, 30 min). The conditions of the experiments corresponded to the ones discussed earlier. The results are presented in Fig. 5.

The presence of coat as an additional barrier for mass transfer, promoted the increase in time of fermentation process by 1.4 times (up to 80 - 85 min). However microscopic researches showed that the output of cells from capsules into the bioreactor medium did not occur.

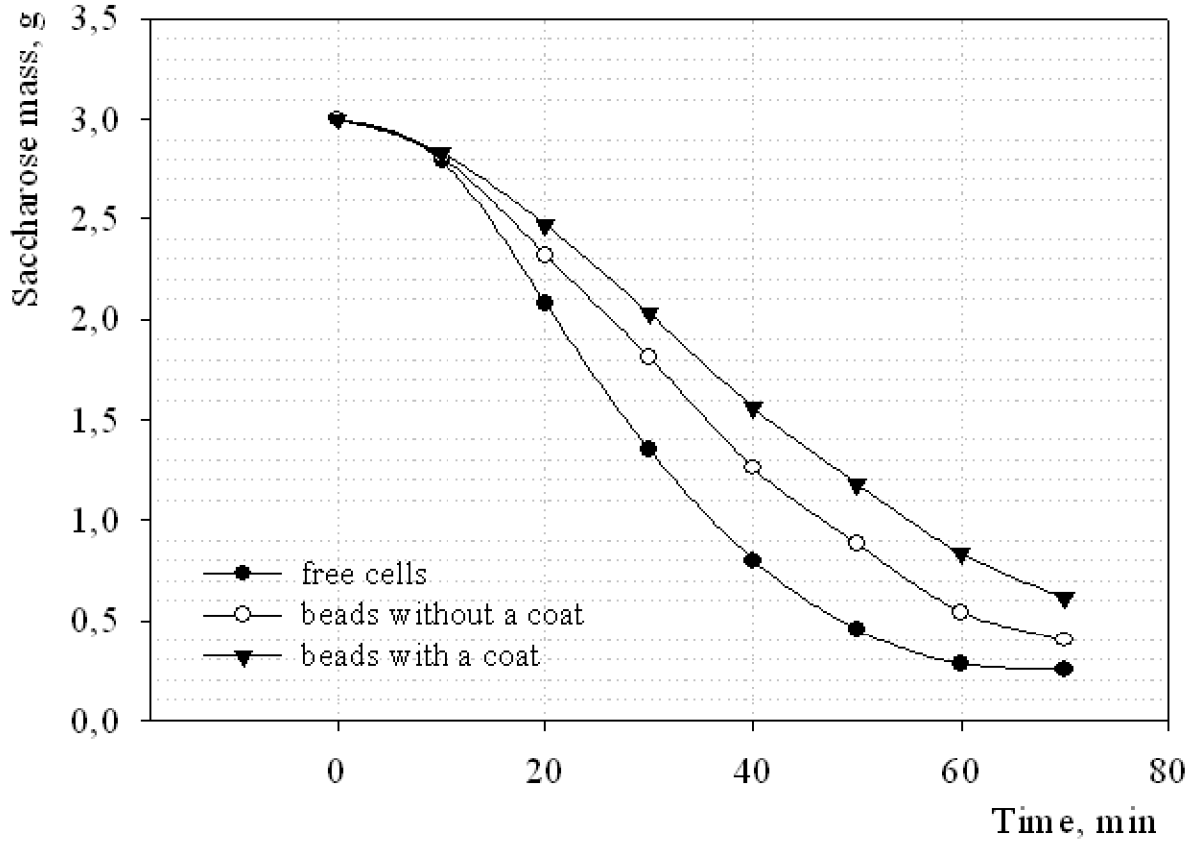

Fig. 5

Conclusions

Thus, the use of the combination of sodium alginate ultrasonic treatment with the cells encapsulation in the beads with a liquid core and with a coat allows speeding up appreciably the fermentation process in comparison with solid beads, thus slowing down fermentation the process only by 1.4 times in comparison with free cells.

Acknowledgement

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References

Sun Y., Furusaki S. (1990) *Continuous production of acetic acid using immobilized Acetobacter aceti in a three-phase fluidized bed bioreactor*. J. Ferment. Bioeng., 69, p. 162 - 167.

Krischke W. et. al. (1991) *Continuous production of L-lactic acid from whey permeate by immobilized Lactobacillus case subsp. casei.* Appl. Microbiol. Biotechnol., 34, p. 573 - 578.