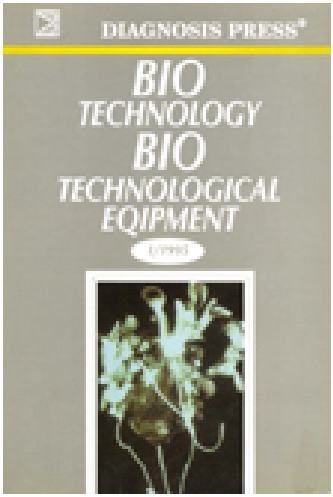

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INVERTASE BIOSYNTHESIS BY SACCHAROMYCES CEREVISIAE USING CANE MOLASSES

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ABSTRACT

Invertase biosynthesis in Saccharomyces cerevisiae 01K32 cells after alcohol fermentation using cane molasses has been carried out. At TRS concentration of 4%, a maximum degree of intracellular invertase production (140-150 E/g) has been reached for 6 hours. The sugars induction effect in molasse medium is registered to the 3% concentration in case of using for invertase biosynthesis the strain only. A two-stage culture of the strain examined has been carried out in a bioreactor and a four times increase of intracellular invertase activity has been reached.

Introduction

The enzyme invertase (β-D-fructofuranoside-fructohydrolase, E.C.3.2.1.26.) is widely applicated in food industry (1, 2), as well as in apiculture (3). The yeast invertase has been best studied, the enzyme being synthesized from natural strains, as well as from mutant strain-producers obtained by means of a physical and chemical mutagenesis (4, 5). As far as the influence of mutagenic factors upon invertase biosynthesis has not been sufficiently efficient, the role of physiological regulation of this process through culture medium composition and other factors has acquired a primary importance.

Data have been published concerning various substrates for the invertase production: glucose (6), saccharose and some semiproducts (7), malt wort (8), sugar-containing waste products from the food industry. Cane molasses has been used as a substrate for invertase biosynthesis from *Saccharomyces cerevisiae* in continuous culture (9).

The invertase is an inducing enzyme and its biosynthesis is directly dependent on inductor concentration in the medium that should be determined accurately for each strain-producer of the respective nutrient medium: the molasse medium.

In our previous investigations (10) it has been determined that cane molasses can be successfully used for invertase synthesis in waste beer's yeasts, at sugars concentration in the medium max 3%.

The purpose of this study is to examine the process of invertase biosynthesis from the selected by us alcohol yeasts strain in two directions:

1. To develop the possibilities for invertase synthesis in the yeasts cells after cane molasses alcohol fermentation, the investigations being directed towards the influence of sugars concentration in molasse medium upon the processes of induction and repression of enzyme biosynthesis.

2. To investigate the influence of sugars concentration in molasse medium upon the process of enzyme biosynthesis, as well as in the process dynamics at two-stage strain cultivation in the bioreactor, when the strain has been used for invertase biosynthesis only.

Materials and Methods

Strain producer. The strain *Saccharomyces cerevisiae* 01K32 from the collection of the Higher Institute of Food and Flavour Industries, Department of Biotechnology has been used.

Culture medium and cultivation conditions. In all experiments, cane molasses has been used

TABLE

Chemical composition of cane molasses

Name	Contents %
Dry substances	82.30
Saccharose	30.50
Invert sugar	25.30
Total reducing substances	57.00
Anine nitrogen	0.19
Betaine	0.234
Glutamic acid	0.0055
Ash substances	5.20
Calcium	0.72
Sodium	0.18
Magnesium	0.1610
P_2O_5	0.235
pH	5.89

as the primary carbon source, having a composition indicated in the **Table**. The quantity of total reducing sugars (TRS) as invert has been determined by the method of I.C.U.M.S.A. recommended as universal one (11). The molasses can be clarified by water dilution 1:3 and heating by the method of Walter Borzani (12). The molasse solution is sterilized at 0.06 MPa, temperature 115°C for 30 min., pH before sterilization 7, after sterilization 4.5.

When studying the invertase synthesis conditions in waste alcohol yeasts only molasses has been used, the TRS concentration at the separate experiments varies from 1 to 10%. After the alcohol fermentation the culture liquid has been centrifugated and the biomass thrice rinsed with water. 20 g of centrifugated yeasts with 75% of moisture have been weighed up, 40 cm^3 of molasse solution with varying concentration of total reducing sugars as invert ones have been added. The cultivation has been carried out in flasks of 750 cm^3 each on a shaker for 6 hours, at 30°C.

In the investigations in which the selected strain has been used for biosynthesis of invertase only, the medium contained salts besides molasses in the following quantities :

$(NH_4)_2SO_4$ – 5.1 g/dm^3

$Na_2HPO_4.12H_2O$ – 2,4 g/dm^3

$MgSO_4.7H_2O$ – 0.075 g/dm^3

In order to obtain inoculum pure yeast culture has been used developed upon a solid culture medium (agar-malt wort) and having a composition of (g/dm^3) : glucose - 10; peptone - 5; yeast extract - 3. Incubation 48 hours at 29°C.

When determining the concentration influence of TRS upon the level of enzyme biosynthesis the fermentation has been carried out in Erlenmeyer flasks (750 cm^3) containing 150 cm^3 molasse culture medium on a shaker with 220 min^{-1} revolutions at 30°C and inoculum 10 vol.% for 48 h. TRS concentration in the medium varied in the separate experiments 1 – 12%.

When studying the dynamics of enzyme biosynthesis in two-stage strain culture the experiments have been carried out in a laboratory type bioreactor (MBR) with a total volume of 2.0 dm^3. 24 h's inoculum has been used in a quantity of 10 vol.% obtained in Erlenmeyer flasks (750 cm^3) containing 150 cm^3 molasse culture medium, inoculated with 10 vol.% of yeast suspension obtained in test-tubes. The flasks developed on a shaker having 220 min^{-1} revolutions, for 24h, at 30°C.

The aeration biosynthesis during, in the bioreactor has been 100 $dm^3/dm^3/h^{-1}$; pH – 4.5; periodic process duration – 48 h.

Methods for analysis. In order to determine the invertase activity the polarimetric method has been used. Per unit invertase activity such a quantity of enzyme has been accepted that can catalyze the transformation of 1 μmol saccharose per 1 min at 30°C and pH 4.6. The measurement of activity should be carried out with 10 % saccharose solution at initial ratio enzyme/substrate, limiting polarization velocity of 4.2°/h according to the international sugar scale in a cuvette of 200 mm that corresponds to the inversion of 0.2 g saccharose per 1 h (13).

The extracell invertase has been determined in the supernatant after centrifugation of yeast cells and is presented in E/cm^3 culture medium.

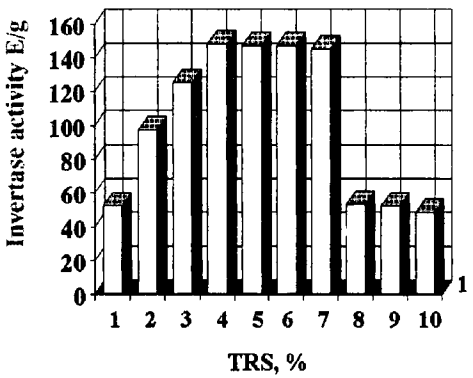

Fig. 1. Influence of TRS concentration in molasse medium upon the level of intracell invertase biosynthesis in waste alcohol yeasts at 6 hours of culture.

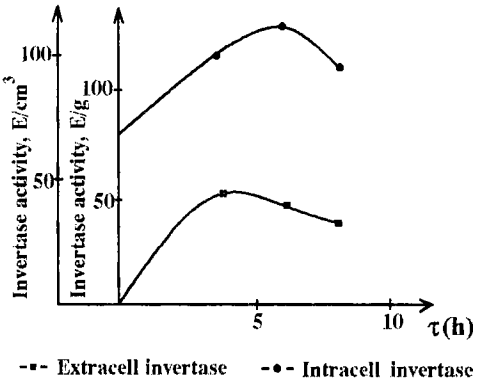

Fig. 2. Dynamics of biosynthesis of intracell and extracell invertase in alcohol yeasts after alcochol fermentation on cane molase medium with TRS concentration - 4%.

The intracell invertase has been determined after a preliminary homogenization of a definitely weighed up yeast cell quantity and is presented as E/g yeasts with 75% moisture.

The residual sugars in the culture medium have been determined after the ebuliostic method (14), and the yeast quantity after the weighing method.

Results and Discussion

Invertase biosynthesis in waste alcohol yeasts

It has been established that after the alcohol fermentation, invertase activity of 90 E/g has been registered in the cells of *Saccharomyces cerevisiae* 01K32. The results from investigating the influence of TRS concentration in molasse medium upon invertase biosynthesis level in waste alcohol yeasts have been presented on **Fig. 1** and show maximum additional accumulation of enzyme at TRS concentration 4%. The interesting in this type of cells unlike beer's yeast (10) is to preserve the possibilities for a comparatively maximum additional invertase synthesis when increasing the TRS concentration. It is only at TRS concentration of 8 % in the medium that the activity of the accumulated intracell invertase decreases abruptly below 50% of the maximum.

When changing culture conditions as follows: aeration and fresh culture medium (only molasses), the anaerobic type of cells of *Saccharomyces cerevisiae* 01K32 strain have been readjusted comparatively quickly and start synthesizing the invertase as a part of the enzyme has separated extracellularly, as well. For a short period of time 4-6 hours the enzyme activity increases to 150 E/g (**Fig. 2**).

A certain enzyme secretion has been registered, as well, corresponding to the 6-th hour of 30 E/cm^3. The quick increase of total enzyme activity has shown that the cane molasses can be used for induing of invertase biosynthesis in waste alcohol yeasts.

The optimum sugars concentration in the medium (4-6%) has been established at which a maximum invertase synthesis has been registered. These values are far lower that those cited by other authors - 15-20% at waste beer's and breadmaking yeasts (7, 13).

Parallel with this, these results have confirmed the expectations of a biological potential of *Saccharomyces cerevisiae* 01K32 strain as regards the biosynthesis of invertase enzyme.

Invertase biosynthesis from *Saccharomyces cerevisiae* 01K32 on cane molasse substrate

When studying the influence of sugars concentration upon the processes of induction and catabolytic repression in invertase biosyn-

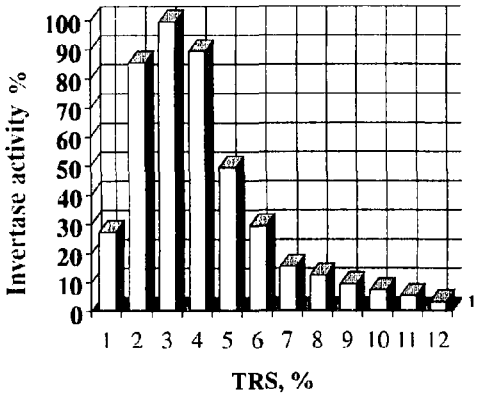

Fig. 3. Influence of TRS concentration in molasse medium upon biosynthesis in intracell invertase by using the yeasts for enzyme biosynthesis only.

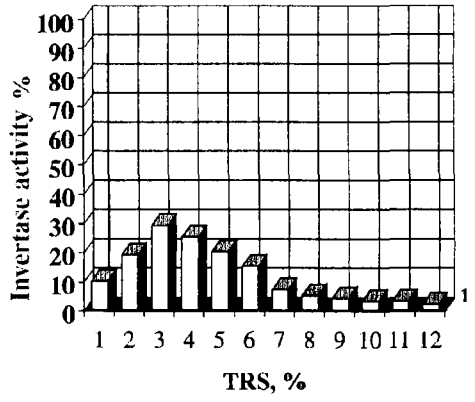

Fig. 4. Influence of TRS concentration in molasse medium upon biosynthesis in extracell invertase by using the yeasts for enzyme biosynthesis only.

thesis from *Saccharomyces cerevisiae* 01K32 it has been established that the induction effect of the sugars contained in the molasses upon intracellular invertase biosynthesis has been strongly expressed at 2.0 – 4.0% concentration. A maximum enzyme cell activity can be registered at TRS 3%. TRS concentration increase leads to an abrupt decrease of enzyme activity. At TRS 5% it is 50% from the maximum, at the higher TRS concentration the activity being insufficient (**Fig. 3**).

It makes impression that the formation of extracell invertase correlates with that of the intracell biosynthesis (**Fig. 4**). Maximum secretion of extracell invertase is also registered at 3% concentration of TRS. At a concentration above 6% the formation of extracell invertase is insufficient - 22% of the maximum.

Probably at TRS concentration increase above a definite level (4%) the effect of catabolytic repression starts dominating above the effect of induction, as a result of which the invertase biosynthesis from *Saccharomyces cerevisiae* 01K32 of a medium with cane molasses is significantly supressed.

On the basis of the determined optimum TRS concentration - 3% cultivation of *Saccharomyces cerevisiae* 01K32 in a laboratory bioreactor has been carried out with the purpose of revealing some special peculiarities of biosynthetical process of invertase and assimilation of the substrate in dynamics. **Fig. 5** presents the dynamics of accumulation of extracell and intracell invertase at yeasts development in a bioreactor at the indicated in Materials and Methods conditions.

It should be noted that in the production of the inoculum still, the yeast cells have biosynthesized about 120 E/g of invertase.

In the process of biosynthesis initially there is a decrease of intracell invertase activity which is probably connected with yeasts adaptation to the new conditions, i.e. hydrolysis of saccharose in the microzones around the cells and easening its transportation and quicker metabolyzation to biomass. This process of intracell invertase transformation to extracell one is completed at the 10-th to the 12-th hour approximately where there lies the maximum of extracell invertase accumulation; after the 12-th hour, biomass accumulation continues to the 16-th hour; and together with this there is a decrease starting of extracell invertase activity. The TRS adoption completes at the 24-th hour. The intracell invertase biosynthesis continues to a stationary phase of development of biomass to the 32-36-th hour after which no in-

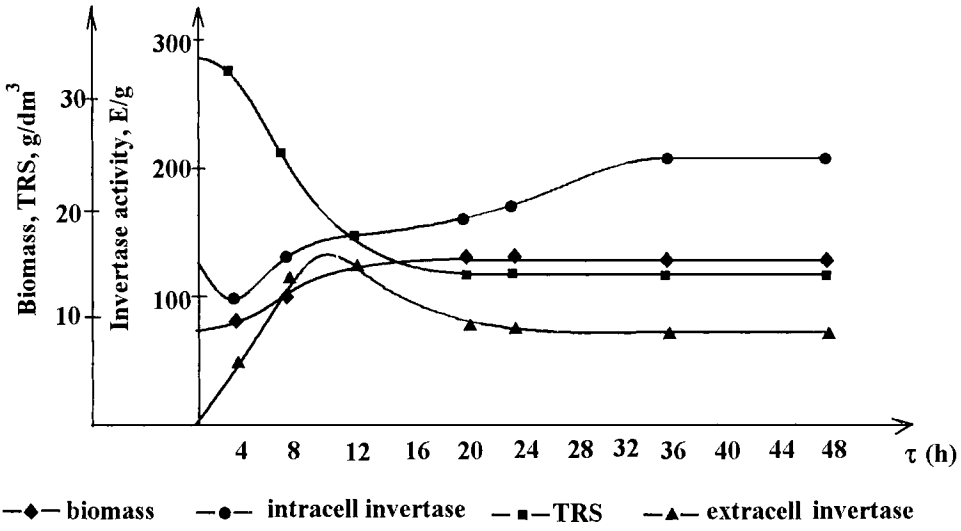

Fig. 5. Dynamics of biosynthesis of intra- and extracell invertase from *Saccharomices cerevisiae* 01K32 at I-st degree of culture in a bioreactor.

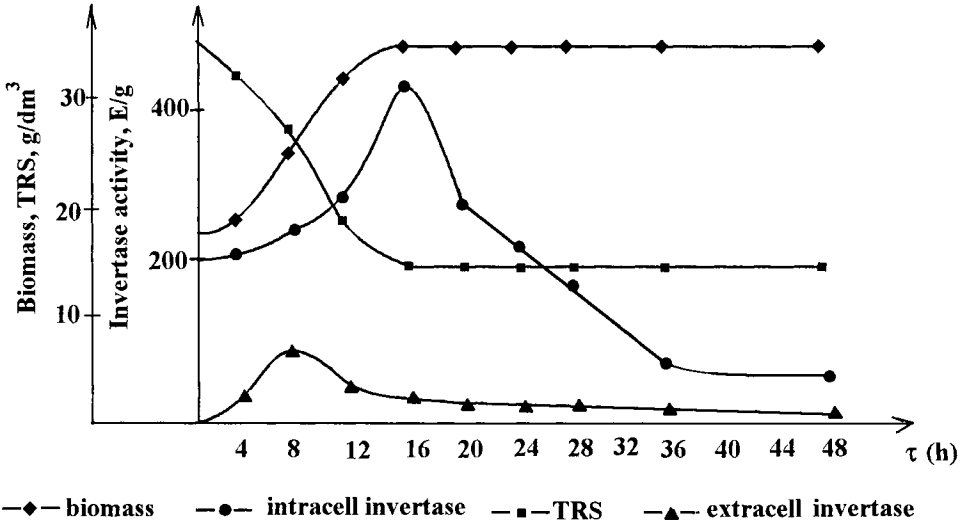

Fig. 6. Dynamics of biosynthesis and additional accumulation of overquantities of invertase from *Saccharomices cerevisiae* 01K32 at II-nd degree of culture in a bioreactor.

tracell invertase accumulation can be found (Fig. 5).

Following the logics of the experimental results obtained, the experiments have been carried out at which the yeast cells developed in a bioreactor, have synthesized intracell invertase to 200 E/g, gave been transformed upon a fresh culture medium containing TRS 3 % (II degree of culture). The purpose has been to study more completely the yeast cell biological

abilities to accumulate additional higher quantities of intracell invertase which could have a significance for the industrial enzyme production. The data have been reflected on **Fig. 6**. At this statement, the effect of induction has still been more clearly expressed. The yeast cells for a very short time can additionally biosynthesize intracell invertase, whose maximum of 440 E/g of yeasts can be reached to the 16-th hour.

The active enzyme biosynthesis correlates with the active cell reproduction that goes out of the exponential phase also at approximately the 16-th hour. To this corresponds the quick TRS adoption, as well, whose value decreases from 35 g/dm^3 to 15 g/dm^3 and in this respect the separation of extracellular invertase can be registered whose activity is the highest at the 8-th hour, namely 75 E/cm^3.

The character of the curves gives the grounds to suppose that when reaching the maximum of intracell invertase accumulation, the biological abilities of yeast cells in the experiment carried out in that way, are exhausted.

Most probably, the proteolytic systems of the cell are activated that leads to a sharp decrease of intracell invertase activity. These results give the grounds to suppose that invertase biosynthesis realization in semicontinuous or continuous mode of yeast cells culture would have been the best solution possible.

At conditions of invertase biosynthesis at two-stage culture of *Saccharomyces cerevisiae* 01K32 on molasse medium, a considerable intracell invertase activity can be reached (above 400 E/g) that is almost four-times increase of activity and give good reason to state that *Saccharomyces cerevisiae* 01K32 strain can be utilized successfully for industrial enzyme production.

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