Screening of Microorganisms for Transfructosylating Activity and Optimization of Biotransformation of Sucrose to Fructooligosaccharides

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The biotransformation of sucrose to fructooligosaccharides was investigated using the catalytic action of fructosyltransferase originated from several fungi strains in a batch reactor. The microorganisms were selected with respect to the yield and selectivity of fructooligosaccharide production. Two strains of *Aureobasidium pullulans* and *Aspergillus niger* were found as the microorganisms with the highest transferase activity. The effect of pH and temperature on the enzymic production of fructooligosaccharides was investigated using the whole cells of *A. pullulans*. No significant effect of these factors was observed in the range of pH 4—8 and temperature from 50 to 65 °C.

The papers of *Spiegel et al.* [1] and *Yun* [2] review the progress made during the last two decades in the biotechnological production of fructooligosaccharides (FOSs), their applications in various industrial sectors and in the investigation of their properties. The initial interest was stimulated by the sweet taste and low caloric value of fructooligosaccharides which could make them new alternative sweeteners. They furthermore possess a number of desirable characteristics such as noncariogenicity, safety for diabetics, they decrease the levels of serum cholesterol, phospholipid, and triglyceride. The continued interest in fructooligosaccharides stems at present in their beneficial health effects as they stimulate the growth of bifidobacteria in the human colon and suppress putrefactive pathogens.

The term fructooligosaccharides is nowadays preferably used for fructose oligomers, which contain one D-glucose unit and 2—4 D-fructose units bound together by β-(2→1)-glycosidic linkages. FOSs are produced from sucrose by the catalytic action of fructosyltransferase (EC 2.4.1.9) when glucose and small amount of fructose are formed as by-products. The sources of enzyme can be either microorganisms (*Aspergillus* sp., *Aureobasidium* sp., *Fusarium* sp., *Penicillium* sp., *Arthrobacter* sp., *etc.*) or plants. The production yield of FOSs using enzyme originated from plants is low and mass production of enzyme is limited by seasonal conditions. On the other hand, fructosyltransferases derived from fungi provide high yields of FOSs and their mass production is not complicated.

In order to reach the highest possible yield of FOSs, the optimization of conditions of transfructosylating reaction was made by several authors. The sucrose concentration is recommended to be in the range from 600 to 850 g dm^{-3} so that the transferase activity may become dominant over the competing hydrolytic activities [3—7]. All the mentioned studies reported also narrow optimum temperature and pH ranges. Typical values of recommended conditions for the production of FOSs are the pH of about 5—5.5 and temperature of 55—60 °C [3, 5—7]. We consider important to note here that a vast majority of these studies were made in the eastern Asian countries where, in distinction to Europe, also industrial production units exist.

This work was thus aimed at i) the screening of available microorganisms for the production of enzymes with the transfructosylating activity, ii) the study of biotransformation of sucrose to fructooligosaccharides by the fructosyltransferase action of a suitable form of biocatalyst, and iii) the optimization of process conditions of FOSs production in a batch reactor.

EXPERIMENTAL

The cultivation was carried out at the conditions reported by other authors [3, 4]. The microorganisms *Aureobasidium pullulans* (AP) CCY 27-1-94 (AP I), CCY 27-1-93 (AP II), CCY 27-1-89 (AP III), CCY 27-1-56 (AP IV), CCY 27-1-45 (AP V), and CCY 27-1-17 (AP VI), and *Aspergillus niger* (AN I) and (AN II)

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from our own collection were applied for the production of fructosyltransferase. The seed culture medium of both the types of microorganisms consisted of 10 g dm^{-3} sucrose and 2 g dm^{-3} yeast extract, the pH was 5.5. The fermentation medium for isolation and cultivation of AP contained 200 g dm^{-3} sucrose, 10 g dm^{-3} yeast extract, 0.5 g dm^{-3} $MgSO_4 \cdot 7H_2O$, 5 g dm^{-3} K_2HPO_4, and 10 g dm^{-3} $NaNO_3$ and the fermentation medium of AN consisted of 200 g dm^{-3} sucrose, 20 g dm^{-3} yeast extract, 0.5 g dm^{-3} $MgSO_4 \cdot 7H_2O$, 5 g dm^{-3} K_2HPO_4, and 10 g dm^{-3} $NaNO_3$. The value of pH was set to 6.5. The cultivation ran for 92 h at 28 °C in 500-cm^3 Erlenmeyer flasks containing 100 cm^3 of medium placed on a shaker with the rotation rate 180 min^{-1}. After cultivation, the cells were washed with the physiological solution several times and filtered.

The enzymic reactions of the production of FOSs were performed in closed flasks equipped with a magnetic stirrer which were placed in a thermostated bath. The temperatures above 50 °C and the concentration of sucrose in 0.1 M phosphate buffer of 700 g dm^{-3} were used and the enzyme reaction ran for 8 h. The mass ratio of the wet cells and sucrose solution was 1 9. The investigation of the effect of pH was realized in the range of 4—8 at the temperature of 55 °C. The effect of temperature was investigated in the range from 55 °C to 65 °C at pH 6. The reaction was stopped by heating in boiling water for 2 min and the mixture was subjected to the analysis for the determination of the concentration of sugars.

The analysis of sugars was performed by high-performance liquid chromatography (HPLC). The HPLC system consisted of a pump Maxi-Star K-1000, an on-line degasser Model A1050, a refractive index (RI) detector Type 298.00, an injection valve equipped with a 10 mm^3 loop, all from Knauer (Berlin, Germany), and a column thermostat Jetstream Plus II (Thermotechnic Products, Germany). The separation was carried out using a column EuroKat Pb (Knauer, Berlin, Germany) filled with a sulfonated styrene—divinylbenzene copolymer in the lead form (300 mm × 8 mm i.d.) equipped with a guard column of the same filling. Double distilled water filtered through a 0.2 μm membrane filter was used as the mobile phase at the flow rate of 0.8 cm^3 min^{-1}. The column temperature was set to 80 °C. The RI detector was operated at 32 °C. The chromatographic data were recorded and evaluated using an EuroChrom 2000 Integration Package Software (Knauer, Berlin, Germany).

The measure of enzyme activity was the total yield of fructooligosaccharides, $Y(\mathrm{FOS})$, which was calculated from the yields of 1-kestose, $Y(\mathrm{GF_2})$, and nystose $Y(\mathrm{GF_3})$ as follows

$$Y(\mathrm{GF_2}) = \frac{2c(\mathrm{GF_2})}{c_0(\mathrm{S})}$$

$$Y(\mathrm{GF_3}) = \frac{3c(\mathrm{GF_3})}{c_0(\mathrm{S})} \qquad (1)$$

$$Y(\mathrm{FOS}) = Y(\mathrm{GF_2}) + Y(\mathrm{GF_3})$$

where $c(\mathrm{GF_2})$, $c(\mathrm{GF_3})$, $c_0(\mathrm{S})$ are the molar concentrations of 1-kestose, nystose, and initial sucrose, respectively. The selectivity of conversion from sucrose to FOSs was calculated from the following formula

$$S(\mathrm{FOS}) = \frac{2c(\mathrm{GF_2}) + 3c(\mathrm{GF_3})}{2c(\mathrm{GF_2}) + 3c(\mathrm{GF_3}) + c(\mathrm{F})} \qquad (2)$$

where $c(\mathrm{F})$ is the molar concentration of fructose.

RESULTS AND DISCUSSION

The criteria for the evaluation of microorganisms, which were available for the production of enzymes, were the ample growth of mycelia at the cultivation conditions and transfructosylating activities of cells in a batch reaction at pH of 5.5 and temperature of 55 °C. A typical course of saccharide concentrations in such an experiment is shown in Fig. 1. Approximately a 90 % conversion of sucrose was achieved in 4 h of reaction time with a high selectivity to fructooligosaccharide production which follows from the low concentration values of fructose produced through the hydrolytic activity. This figure also demonstrates that a plateau of total mass concentration of FOSs was achieved in about 2 h when the concentration of 1-kestose was decreasing after this time on behalf of the formation of a higher oligomer, nystose.

From the microorganisms used in this study, the cells of the strains of *Aureobasidium pullulans* V, VI grew relatively slowly and the cells of *Aureobasidium*

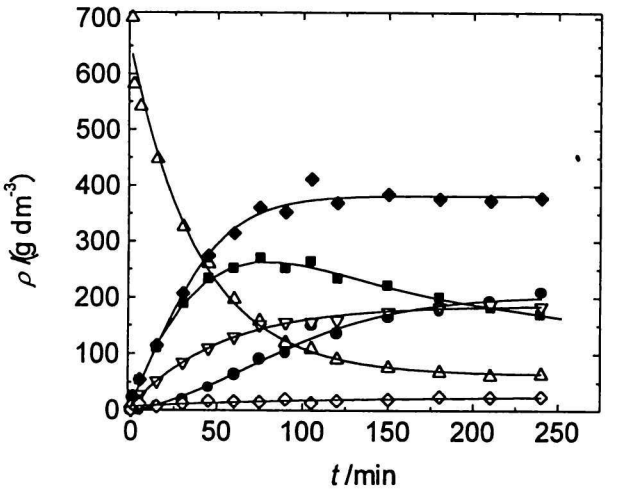

Fig. 1. Time course of the formation of fructooligosaccharides with cells of *Aureobasidium pullulans* II at pH 5.5, 55 °C, and initial sucrose concentration, ρ = 700 g dm^{-3}. ■ 1-Kestose, ● nystose, Δ sucrose, ∇ glucose, ◊ fructose, ♦ total FOSs.

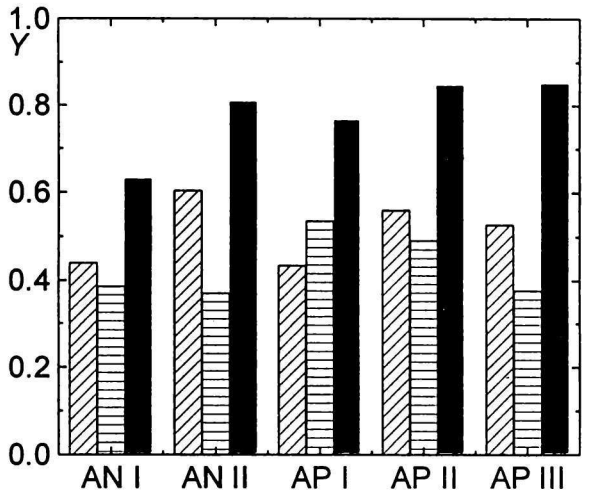

Fig. 2. Comparison of the yields of fructooligosaccharides, Y, achieved using different microorganisms at pH 5.5 and 55 °C (/// 1-kestose, ≡ nystose, ■ total FOSs). Abbreviations: AN = *Aspergillus niger*, AP = *Aureobasidium pullulans*.

pullulans IV possessed a very low transfructosylating activity. The remaining strains exhibited a good transferase activity and the results obtained are presented in Fig. 2. Comparing the yields of 1-kestose, nystose, and total FOSs exhibited by these strains, the best fructosyl transferase-producing microorganisms were found to be *Aureobasidium pullulans* II and III and *Aspergillus niger* II, which provided the yield of FOSs about 80 %. All next experiments were then performed using the mycelium of AP II.

The investigation of the effect of pH and temperature on the enzyme activity was realized in the range pH 4—8 and at the temperature of 55 °C. The results are shown in Fig. 3. In the monitored range, pH had no significant effect on the yield of FOSs. This result differs from the observations published by other authors. *Jung et al.* [3] found the optimum pH of 5.5 using the free cells of *Aureobasidium pullulans*, when the enzyme activity sharply decreased below pH 5 and above pH 6.5. *Cheng et al.* [5] worked with the immobilized mycelium of *Aspergillus japonicus* that had an optimum activity at the pH 4.0—5.5 and above pH 6 the activity rapidly decreased. The selectivity of conversion of sucrose to FOSs was in our case above 95 % in the whole range of pH, as shown in Fig. 3.

The effect of temperature in the range from 55 to 65 °C was determined at pH 6. Fig. 4 shows that the increase of temperature caused an increase of the initial rate of enzyme reaction. Since no noticeable inactivation was observed, the temperature interval of 60—65 °C could be considered as an optimal one. However, already after 60 min of the reaction time, the amount of total FOSs and 1-kestose produced reached the maximum value which was independent of temperature. This diminishes the importance of the observed differences in the rate of transfructosylation reaction in the mentioned temperature interval. Again, our results differ from those of other investigators. For example, *Jung et al.* [3] found that the dependence of enzyme activity on temperature showed narrow maximum at 55 °C, while above this temperature the activity strongly decreased. It is also important to note that in our case the time of duration of the enzyme reaction necessary to reach the final yield of FOSs was much shorter than those reported by other authors [3, 8].

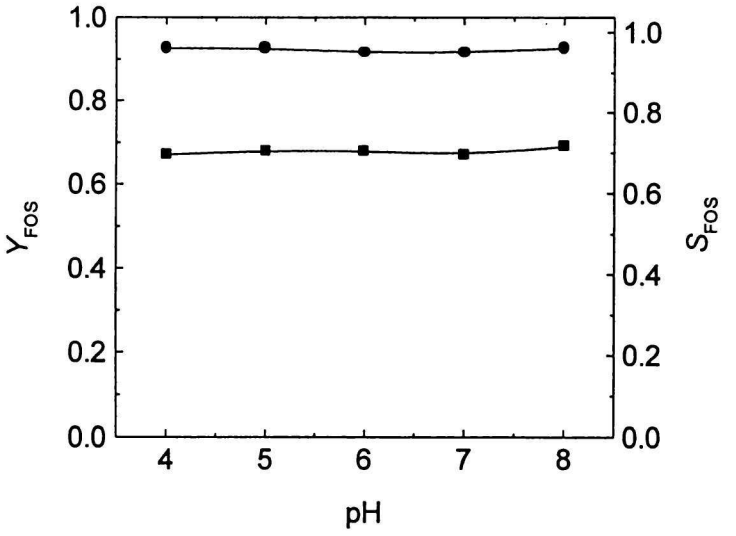

Fig. 3. Effect of pH on the total yield of fructooligosaccharides, ■ Y(FOS), and on the selectivity of conversion from sucrose to FOSs, ● S(FOS), at 55 °C.

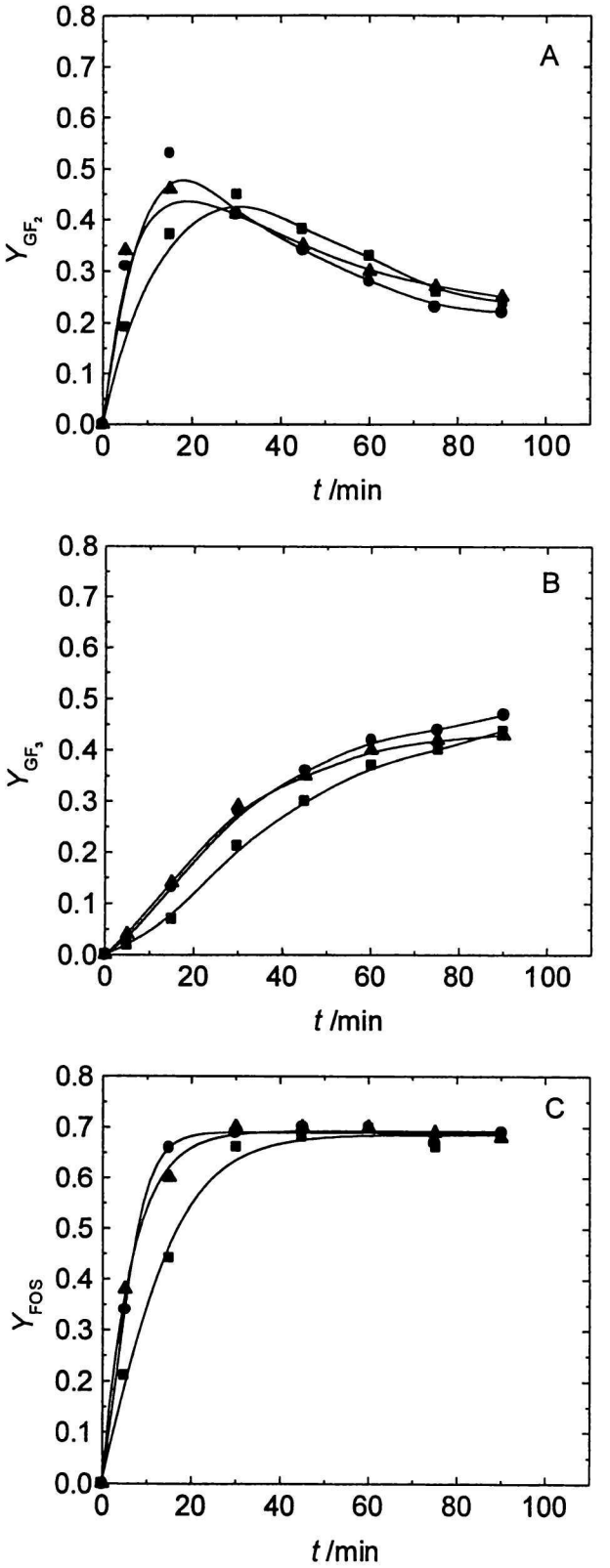

Fig. 4. Effect of temperature on the intermediate yield (Y) of fructooligosaccharides at pH 6. Yield of 1-kestose (A), nystose (B), and total fructooligosaccharides (C) at the temperatures of 55 °C (■), 60 °C (●), and 65 °C (▲).

CONCLUSION

The aim of the present investigation was to optimize the conditions of the production of fructooligosaccharides (FOSs) by the action of enzyme of microbial origin. The screening of microorganisms resulted in the choice of three strains, *Aureobasidium pullulans* II and III and *Aspergillus niger* II, which were the best producers of fructosyltransferase activity. The effect of pH in the range of 4—8 on the production of FOSs was not significant and the yield of total FOSs was about 70 % in all cases. The increase of temperature caused an increase of initial reaction rate, but after 1 h of the reaction time the yield of FOSs reached the maximum value independent of the temperature.

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