Optimization of Cultivation Conditions for Production of Fructosyltransferase by *Aureobasidium pullulans**

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The investigation of optimal conditions for the fructosyltransferase (FTase) production by *Aureobasidium pullulans* CCY 27-1-94 was carried out in shaken flasks. The effect of four medium components was investigated by varying their content compared to the reference medium composition. It was found that none of the investigated components had a very strong effect either on the cell growth or the FTase production. It was, however, disadvantageous to decrease the content of the components below the values in the reference medium. The influence of initial sucrose concentration and cultivation time was investigated in the second stage using a rotatable central composite design where the sucrose concentration was 208—491 g dm^{-3} and the cultivation time was from 26 to 94 h. The sucrose content did not influence the total production of FTase but high sucrose concentrations resulted in high specific activities of the cells. The cultivation time had a positive effect on the fructosyltransferase production up to 40—60 h but it decreased the specific enzyme activity. The effect of the composition of the cultivation medium on the total costs of fructooligosaccharide production was also evaluated.

Fructosyltransferase (FTase) is an enzyme that catalyzes the transformation of sucrose into fructooligosaccharides (FOS's) which have a broad application in food and pharmaceutical industries. Typical sources of FTase are the microbial strains of *Aspergillus niger*, *Aspergillus japonicus*, and *Aureobasidium pullulans* [1—4]. The fermentation production of FTase is strongly dependent on the cultivation conditions, therefore it has been a subject of several studies [1, 3, 5—9]. The comparison of the effect of carbon source at the cultivation of different microorganisms showed that sucrose was by far the best inducer of FTase production [1, 5, 6]. Similarly, yeast extract was declared to be the best nitrogen source [5, 6] although the advantage of yeast extract compared to inorganic nitrogen substrates may lie in the supplementation of some essential growth factors to the microorganism. Another common subject of investigation was the effect of minerals, mainly phosphorus, magnesium, iron, and calcium, on the FTase production and cell growth [1, 5, 6, 9].

The most investigated factor was the initial sucrose concentration in the fermentation medium the effect of which was investigated up to the content value of 45 % [1, 3, 5—9]. In several studies, the optimum of the total FTase produced was found at the sucrose content value of about 25 % [5—8] whereas other authors observed almost a constant value of total enzyme activity above the content of 20 % [1, 3]. Both these observations were caused by the strong inhibition of the cell growth of all above-mentioned strains at higher sucrose concentrations. On the other hand, the specific activity of FTase per cell mass increased with the sucrose concentration in all cases. This means that the high osmotic stress that caused the inhibition of cell growth was accompanied by a significant induction of the production of FTase.

In one of the previous studies of our research group, *Aureobasidium pullulans* CCY 27-1-94 was selected from several microbial fructosyltransferase producers as the best one on the basis of the yield and selectivity of fructooligosaccharide production by the enzyme [10]. *Aureobasidium pullulans* is a dimorphic microorganism the morphology of which is very sensitive to the cultivation conditions [11]. In another study of our research group, the effect of initial sucrose concentration and cultivation time on the FTase production and cell growth was investigated at three levels of these factors [4]. It was found that both total and specific FTase activity was the highest at 350 g dm^{-3}

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sucrose concentration but the maximum of specific activity was achieved after the second day of cultivation whereas the total activity grew all the time.

The principle objective of this work was to study more thoroughly the effect of the sucrose concentration and cultivation time in shaken flask cultivation using the methods of experimental design. The experimental design is a powerful methodology for the optimization of cultivation media composition and has also been applied previously for the optimization of FTase production [6, 8, 9]. We focused on higher sucrose concentrations up to almost 500 g dm^{-3}. The first part of the investigation of the optimal composition of cultivation media dealt with the effect of selected medium components.

EXPERIMENTAL

The analytical standards of 1-kestose, nystose, and 1^F-fructofuranosylnystose were obtained from Wako Pure Chemical Industries (Osaka, Japan); other chemicals used as standards were purchased from Fluka (Buchs, Switzerland) and Sigma (Deisenhofen, Germany). All other chemicals were of anal. grade and were obtained from readily available commercial sources.

Aureobasidium pullulans CCY 27-1-94 (Culture Collection of Yeasts, Bratislava, Slovakia) was maintained at −18 ℃ in sealed plastic microtubes containing the mixture of 0.6 mm^3 of cell suspension and 0.3 mm^3 of 50 % glycerol solution. The content of one microtube was used to inoculate a 100 cm^3 conical flask containing 50 cm^3 of sterile medium. The suspension was subsequently incubated in a rotating shaker at 28 ℃ and 180 min^{-1} for 45 h. The concentrations in the inoculation medium were: sucrose 200 g dm^{-3}; yeast extract 10 g dm^{-3}; K_2HPO_4 5 g dm^{-3}; $NaNO_3$ 10 g dm^{-3}; $MgSO_4 \cdot 7H_2O$ 0.5 g dm^{-3}; chloramphenicol 0.0001 g dm^{-3}. The pH was adjusted to 6.5 before sterilization.

The analysis of saccharides was performed by HPLC (Knauer, Germany) at the column Watrex Polymer IEC Pb form as described previously [4].

Cultivation

One cm^3 of inoculum described above was transferred into 100 cm^3 of cultivation medium in 500 cm^3 flasks and cultivated in a rotating shaker and agitated at the frequency of 180 min^{-1} and temperature of 28 ℃. At the end of cultivation, 10 cm^3 of sample was taken off from the cultivation broth, filtered through a 0.2 μm membrane filter, washed with deionized water and dried to a constant mass to obtain the dry cell mass. The filtrate was used to determine the fructosyltransferase activity in medium. The rest of the suspension was centrifuged at 3000 min^{-1} for 5 min, washed with a physiological solution and used as a source of intracellular enzyme.

Table 1. The Content of the Individual Components at the Optimization of the Medium Composition

Experiment code	ρ_i/(g dm^{-3})			
	Yeast extract	$NaNO_3$	$MgSO_4 \cdot 7H_2O$	K_2HPO_4
Ref.	10	10	0.5	5
YE_+	20	10	0.5	5
YE_-	5	10	0.5	5
N_+	10	20	0.5	5
N_-	10	5	0.5	5
Mg_+	10	10	1	5
Mg_-	10	10	0.1	5
P_+	10	10	0.5	10
P_-	10	10	0.5	0

In all cases, the sucrose and chloramphenicol concentrations were 200 g dm^{-3} and 0.1 mg dm^{-3}, respectively. The codes denote which component was varied compared to the reference (Ref.) composition: YE – yeast extract, N – inorganic nitrogen substrate, Mg – magnesium source, P – source of phosphorus. A plus sign represents an increased content of the substrate and a minus sign a decreased content.

Table 2. Experimental Design. Cultivation Conditions in True and Coded Forms

Trial	Cultivation time	Sucrose concentration	Coded values	
	h	g dm^{-3}		
	z_1	z_2	x_1	x_2
1	84	450	1	1
2	84	250	1	−1
3	36	250	−1	−1
4	36	450	−1	1
5	94	350	1.414	0
6	26	350	−1.414	0
7	60	491	0	1.414
8	60	208	0	−1.414
9	60	350	0	0

The content of other components was the same as in the reference medium (Table 1).

In the experiments aimed at the optimization of the medium composition (except of the carbon source), the cultivation time was 96 h. Different compositions of cultivation media were obtained by the variation of single component content in comparison to the reference composition (Table 1). One set of experiments was conducted at the reference composition, the increased and decreased contents of the component. Three replicates were made at the same conditions. The cultivation time and composition of media in the experimental design part are shown in Table 2. All experiments were performed here in two replicates.

The replicates were conveniently used for the determination of the errors of enzyme activity and dry cell mass. First, the relative variances of individual

sets, s_i^2, were calculated from the replicates. The relative mean variance of the quantity, s_A^2, was obtained using the following formula

$$s_A^2 = \frac{\sum_{i=1}^{k} (n_i - 1)\, s_i^2}{\sum_{i=1}^{k} (n_i - 1)} \qquad (1)$$

where n_i were the numbers of the replicates in individual sets, k was the number of sets ($k = 24$ for FTase and $k = 12$ for dry cell mass).

In order to evaluate the significance of the change of the component content, a statistical test on the equality of two mean values was made. For that purpose, the value of the random variable N was calculated from the following equation

$$N = \frac{\left|\bar{Y}_{ref} - \bar{Y}_c\right|}{\sqrt{s_{ref}^2 \bar{Y}_{ref}/n_{ref} + s_c^2 \bar{Y}_c/n_c}} \qquad (2)$$

where $\bar{Y}$ is the mean value of studied response, s^2 is its variance and n is number of experiments in one set. The indices ref and c represent the reference medium composition and the medium composition with either increased or decreased content of a component. The differences between $\bar{Y}_{ref}$ and $\bar{Y}_c$ were considered significant if the value of N was larger than the critical value of normal distribution, $N_{0.05} = 1.96$.

Activity Assay

The enzyme activity was measured in a reaction mixture consisting of 9.5 cm^3 of 736.8 g dm^{-3} sucrose in 0.1 M-citric acid/K_2HPO_4 buffer, pH 5.5 and 0.5 cm^3 of enzyme solution (final sucrose concentration was 700 g dm^{-3}). The enzyme solution was either the filtrate of culture broth for extracellular activity assay or the suspension of wet cells in 0.1 M-citric acid/K_2HPO_4 buffer, pH 5.5 (0.1 g of wet cells in 1 cm^3 of buffer). The activity assay was carried out in a stirred batch reactor at 55 °C. Samples (1 cm^3) of the reaction mixture were taken off in predetermined time intervals and the reaction was stopped by boiling in a water bath for 2—2.5 min. The samples were diluted and filtered through a membrane filter and frozen before analysis. One unit of fructosylation activity was defined as the amount of enzyme activity which catalyzes the formation of 1 μmol of kestose in 1 min under these conditions.

Experimental Design

In order to find an optimum of initial sucrose concentration and cultivation time for the production of fructosyltransferase, a rotatable central composite design with nine experiments was performed. The levels of coded, x, and true values, z, of the independent variables (factors), which are given in Table 2, are related by the following formula

$$x_i = \frac{z_i - z_i^0}{\Delta z_i} \quad i = 1, 2 \qquad (3)$$

where z_i^0 is a true value of the i-th variable at the centre point and Δz_i is the corresponding step change.

The overall FTase activity Y_1 (U dm^{-3}), the activity in cells Y_2 (U dm^{-3}), the activity in medium Y_3 (U dm^{-3}), the specific cell activity Y_4 (U g^{-1}), and the dry cell mass Y_5 (g dm^{-3}) were dependent variables (responses). The dependence between a response and the factors was described by the second-order polynomial model.

$$Y = b_0 + b_1 x_1 + b_2 x_2 + b_{12} x_1 x_2 + b_{11} x_1^2 + b_{22} x_2^2 \qquad (4)$$

where b_0, $b_1, \ldots, b_{22}$ are the regression coefficients.

RESULTS AND DISCUSSION

Optimization of Medium Composition

The production and the activity of fructosyltransferase by fungus *Aureobasidium pullulans* depends on several parameters such as the storage of microorganism, composition of cultivation medium, and cultivation time. The optimal conditions reported in literature were different in certain degree even at the same strain [1, 3, 5—9]. For that reason, the current publication deals with the optimization of medium composition for the strain of *Aureobasidium pullulans* CCY 27-1-94. The optimization was aimed at the content of the components which were the same as those used in our previous study [4]. It was not dealt here with the effect of chloramphenicol and the effect of sucrose concentration was investigated separately and it is described in the second part of Results and Discussion.

The effect of four medium components on the FTase production and cell growth was investigated in shaken flasks experiments by fluctuating the content of a component in respect to the reference medium composition. The content of all components but sucrose in the reference medium was the same as in our previous study [4]. The sucrose concentration was set to 200 g dm^{-3}. The measured FTase activities and dry cell masses, which are the means of three replicates, are presented in Figs. 1—4. The standard deviations of these quantities, calculated as the square roots of the mean variances (eqn (*1*)), were as follows (in relative values): 10.4 % for the FTase activity, 7.0 % for the dry cell mass, and 12.5 % for the specific cell activity. Figs. 1—4 show that the activity and dry cell mass values in the cultivation media with changed composition did not differ significantly from the values in the reference medium in most cases. The exact comparison of the differences was made using the statistical

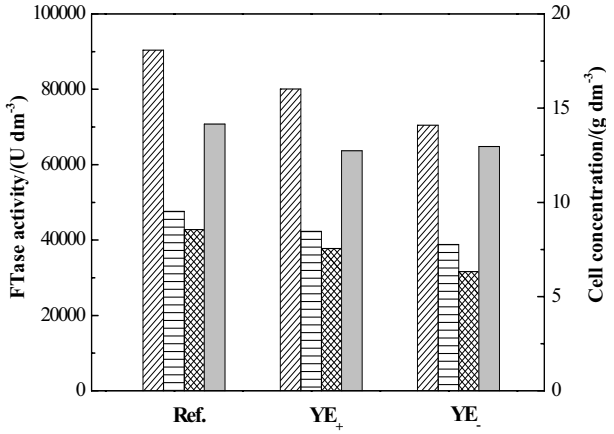


Fig. 1. Effect of the yeast extract on fructosyltransferase activities and cell growth. Overall enzyme activity ▨; activity in cells ▤; activity in medium ▩; dry cell concentration ▭.

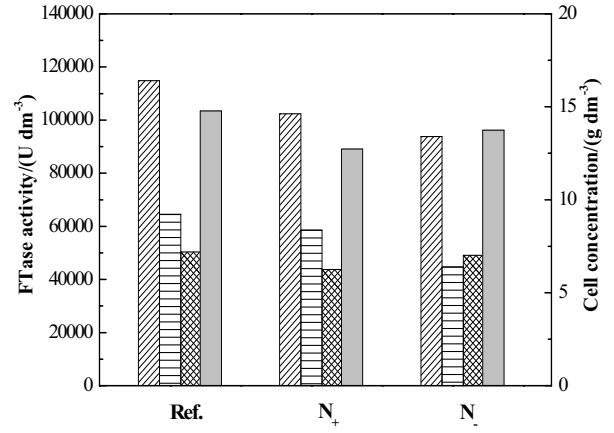


Fig. 2. Effect of $NaNO_3$ on fructosyltransferase activity and cell growth. The symbols are the same as in Fig. 1.

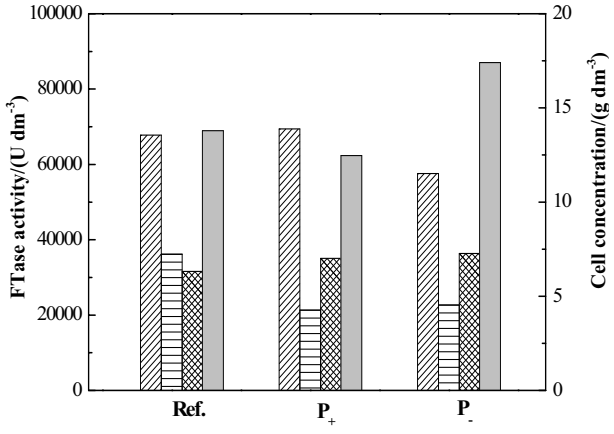


Fig. 3. Effect of K_2HPO_4 on fructosyltransferase activity and cell growth. The symbols are the same as in Fig. 1.

test described in Experimental. The calculated values of random variables N are summarized in Table 3.

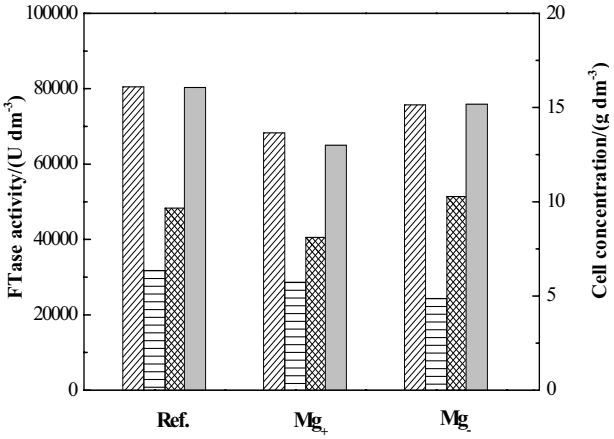


Fig. 4. Effect of $MgSO_4 \cdot 7H_2O$ on fructosyltransferase activity and cell growth. The symbols are the same as in Fig. 1.

Yeast extract is a very suitable source of vitamins and growth factors and also provides some nitrogen needed. The growth of *Aureobasidium pullulans* and FTase production were much larger in the media supplemented with yeast extract than in the media containing only inorganic nitrogen substrates [12]. The effect of yeast extract on the production of FTase and the cell growth is presented in Fig. 1. Table 3 shows that the doubling of yeast extract content did not have any statistically significant effect on the measured variables. On the other hand, the FTase activities decreased both in cells and in medium when the yeast extract content was halved.

$NaNO_3$ has been the most common source of inorganic nitrogen used in previous studies of FTase production by fungi. It was demonstrated to have an influence on the morphology of *A. pullulans* [11]. The results of the investigation of $NaNO_3$ effect on the production of FTase and the cell growth are shown in Fig. 2. It follows from Table 3 that the FTase activities were negatively affected by the decreased supply of inorganic nitrogen. Furthermore, the results in Fig. 2 and Table 3 imply that the cell growth was weakly inhibited by the nitrogen substrate.

K_2HPO_4 served the functions of a source of an important microelement for the cell growth as well as of a buffer component. Fig. 3 and Table 3 give the results of the effect of K_2HPO_4 on the FTase and biomass production. If this salt was omitted from the medium, it had a negative effect on the total FTase produced whereas the cell growth was enhanced. The change of K_2HPO_4 content in comparison to the reference medium resulted in a relatively faster release of the enzyme into the medium. This also explains the decreased FTase cell activities. Very similar observations were made also by *Jung et al.* [1] and *Hayashi et al.* [12].

The addition of magnesium ions is assumed to affect the permeability of cell walls. The influence of

Table 3. The Values of Random Variables N in Individual Experiments

Experiment code	Measured quantity			
	Total FTase activity	FTase in cells	FTase in medium	Dry cell mass
YE_+	1.418	1.384	1.456	1.667
YE_-	2.897	2.372	3.501	1.547
N_+	1.359	1.135	1.653	2.286
N_-	6.327	3.905	0.270	1.039
P_+	0.990	5.562	0.116	1.426
P_-	2.050	5.262	0.221	4.541
Mg_+	1.573	1.081	1.875	2.010
Mg_-	0.587	2.515	0.638	0.631

The critical value of normal distribution was $N_{0.05} = 1.96$. The effect of the change of a variable was significant if $N < N_{0.05}$.

Table 4. The Values of Regression Coefficients and t-Statistics Obtained for the Second-Order Polynomial Function between Responses and Factors (Eqn (*4*))

Term	Total FTase activity, Y_1		Activity in cells, Y_2		Activity in medium, Y_3		Specific activity, Y_4		Dry cell mass, Y_5	
	Coefficient	t-Value	Coefficient	t-Value	Coefficient	t-Value	Coefficient	t-Value	Coefficient	t-Value
b_0	118300*	9.64	72900*	9.64	45370*	9.64	6240*	7.98	11.71*	14.25
b_1	10850*	2.50	5198	1.94	5655*	3.40	−1616*	5.85	2.87*	9.88
b_2	−525	0.12	610	0.23	−1136	0.68	1813*	6.56	−2.90*	9.98
b_{12}	−6141	1.00	−320.8	0.085	−5820	2.47	−531	1.36	−0.37	0.91
b_{11}	−6276	0.87	−4423	1.00	−1853	0.67	770	1.68	−0.95	1.96
b_{22}	−1386	0.19	−2293	0.52	−906	0.33	36	0.80	0.13	0.26

$t_i = |b_i|/s_{\mathrm{bi}}$ where s_{bi} is the standard deviation of b_{i}. The critical values of t-statistics were as follows: $t_{0.05}(38) = 2.02$ for Y_1—Y_4 and $t_{0.05}(17) = 2.11$ for Y_5. The asterisk denotes the statistically significant parameters, $t > t_{0.05}(f)$ where f is the number of the degrees of freedom.

$MgSO_4 \cdot 7H_2O$ is illustrated in Fig. 4. It follows from Table 3 that this factor had the least effect of all the studied ones. As in the case of K_2HPO_4, relatively increased release of FTase was observed in the medium with decreased content of magnesium.

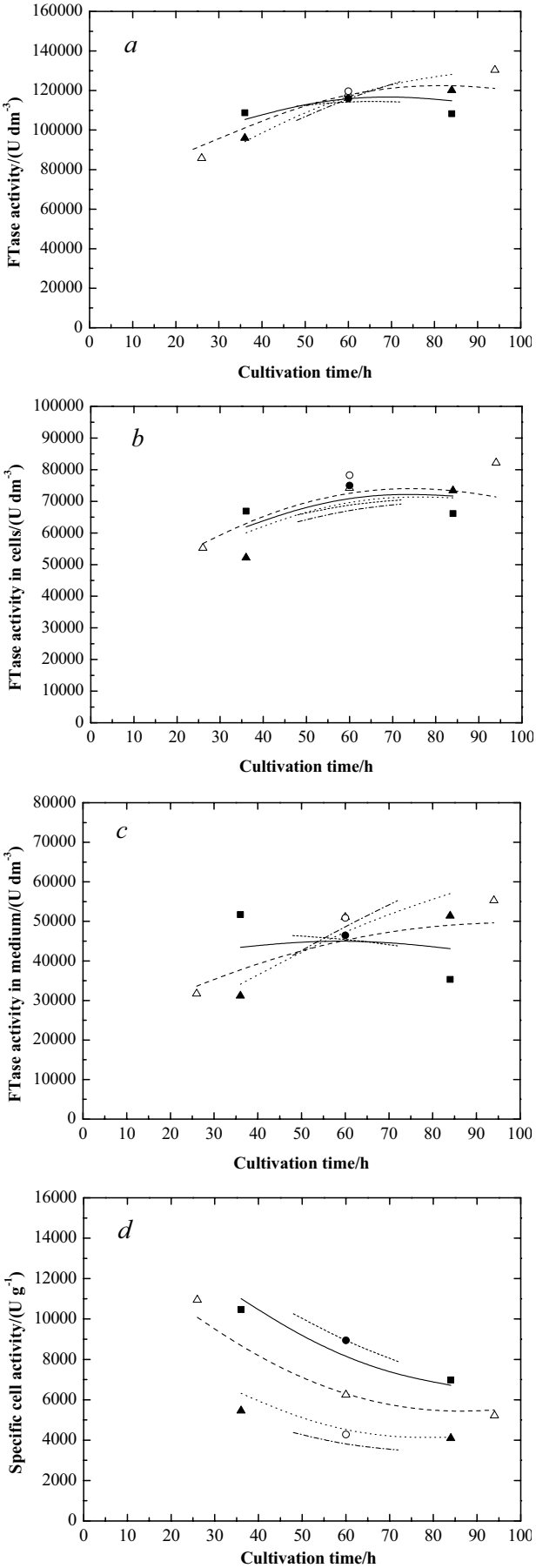
A general conclusion can be made that none of the investigated components of the cultivation medium had a very strong effect either on the cell growth or the FTase production. It was disadvantageous to decrease the content of the components below the values in the reference medium since the cultivation performance was impaired. On the other hand, there was no reason to increase their content either because it would result only in higher costs without improved performance.

Optimization of Sucrose Content and Cultivation Time

A rotatable composite design with two factors was used to optimize the initial sucrose concentration in the cultivation medium and the total cultivation time. The sucrose concentration and the cultivation time in the plan centre were set to 350 g dm^{-3} and 60 h, respectively, which was found favourable in the previous study [4]. The ranges of the investigated factors were 208—491 g dm^{-3} at the initial sucrose concentration and 26—94 h at the cultivation time. The natural and coded variables for the whole plan are included in Table 2.

Five responses were obtained at each point of experimental design. Three of them (cell and medium FTase activities, dry cell mass) were directly measured whereas two remaining ones (total FTase activity, specific cell FTase activity) were calculated from the measured ones. All these results are presented in Figs. 5*a*—5*e*. Figs. 5*b* and *c* indicate that neither the cell FTase activity nor the medium FTase activity depend on the initial sucrose concentration. Naturally, the same conclusion applies to the total FTase activity (Fig. 5*a*), which was obtained as a sum of the cell and medium activities. The same figures also demonstrate that the activities depend slightly on the cultivation time in the range of the experimental design. The activities obviously had to grow from an initial zero value up to a plateau, which was reached at the cultivation time of about 60 h.

Both factors affected the production of the dry cell mass (Fig. 5*d*). The cells grew during the whole cultivation period where the growth was negatively affected by the increasing initial sucrose concentration. On the contrary, the specific cell activity significantly increased with the sucrose content and decreased with the cultivation time (Fig. 5*e*). It is obvious that the FTase production was not directly coupled with the growth of microorganism. The initial increase of the



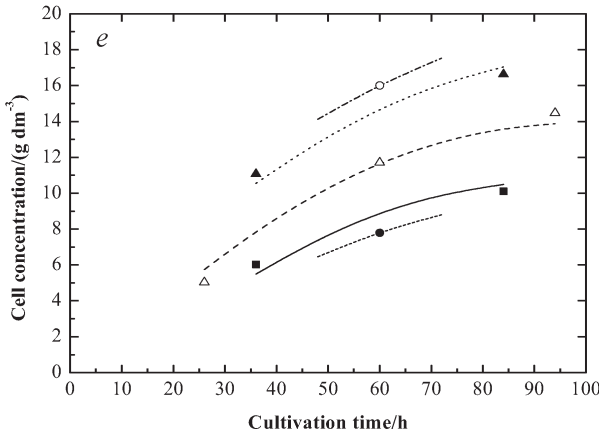


Fig. 5. The results of central composite design. *a*) Total FTase activity, *b*) cell FTase activity, *c*) medium FTase activity, *d*) specific cell FTase activity, *e*) dry cell mass. Symbols represent the measured (calculated) responses at different initial sucrose concentrations ρ/(g dm^{-3}): 208.6 ○ (– · –), 250 ▲ (· · ·), 350 △ (– –), 450 (——), 491.4 (– – –).

Table 5. The Results of the Adequacy Test for the Responses Y_1—Y_5 Described by Eqn (*4*) with the Parameter Values from Table 4

Response	F
Y_1	0.5349
Y_2	2.292
Y_3	1.498
Y_4	1.219
Y_5	0.8709

F-value was calculated from the ratio of residual and experimental variances of the response. The critical values of F-statistics were as follows: $F_{0.05}(3, 38) = 2.852$ for Y_1—Y_4 and $F_{0.05}(3, 17) = 3.197$ for Y_5. The condition of adequacy was $F < F_{0.05}(f_1, f_2)$ where f_1 and f_2 were the degrees of freedom of the residual and experimental variances (eqn (*1*)).

specific activity, which was enhanced at high sucrose concentrations, could be caused by the high osmotic stress. Its decrease in the later phases of cultivations could be a consequence of catabolic repression.

All responses were fitted with the second-order polynomial regression function (eqn (*4*)). The values of estimated regression coefficients and the corresponding values of t-statistics are given in Table 4. The adequacy of the polynomial model was evaluated using the F-test where the experimental variances of individual responses were adopted from the experiments described in the previous subsection. Table 5 shows that all the responses were adequately described by the model. The t-test on the significance of the model regression coefficient suggested that all second-order coefficients were not significant. However, one should not take these results too rigorously since the relative errors of the measured responses were rather

Table 6. The Consumption and Costs of Medium Components per One Ton of FOS's Produced Using Different Biocatalysts

Compound	Immobilized enzyme biocatalyst		Immobilized cell biocatalyst	
	Consumption kg ton^{-1}	Cost* SKK ton^{-1}	Consumption kg ton^{-1}	Cost* SKK ton^{-1}
Sucrose	6.899—17.25**	163.6—408.9	0.6935—1.734**	16.5—41.1
Yeast extract	0.3449	579.5	0.03468	58.3
$MgSO_4 \cdot 7H_2O$	0.01725	0.3	0.001734	0.1
K_2HPO_4	0.1725	13.8	0.01734	1.4
$NaNO_3$	0.3449	26.2	0.03468	2.7

*Costs are in Slovak korunas (SKK). Current exchange rate of SKK to EUR is approximately 42. **The range corresponds to the initial sucrose concentrations 200—500 g dm^{-3}.

large, which consequently made the *t*-values low. According to our opinion, the second-order effect can be neglected only at the effect of sucrose concentrations where the calculated *t*-values were much lower than the critical ones.

The initial sucrose concentration essentially did not affect any of the absolute activity values. It primarily affected the produced dry cell mass, which resulted also in a statistically significant effect on the specific cell FTase activity. The cultivation time had the strongest effect also on the dry cell mass and specific activity. But it had an influence also on the FTase activity, which was stronger on the total and medium activity and weaker on the cell activity. The sole significance of the cultivation time was observed at the total and medium FTase activities.

The results described above do not provide fully unequivocal optimal conditions of fructosyltransferase production. The optimal conditions will depend on the form of the biocatalyst used for the fructooligosaccharides production. If free or immobilized cells were employed, a high specific cell FTase activity would be a great advantage. This would favour shorter cultivation times (not much more than one day) and high sucrose concentrations (over 40 %). On the other hand, if free or immobilized enzyme was used, there would be no need to use very high sucrose concentrations since the total or cell activities did not increase above the sucrose content value of 20 mass %. An optimal cultivation time in this case that would maximize the total amount of produced enzyme appears to be somewhere between 40—60 h. The final recommendations for the optimal conditions of fructosyltransferase production should, however, be made on the cost analysis.

Evaluation of Consumption and Costs of Cultivation Medium Components

The impact of the change of the composition of the cultivation medium on the process economics is strongly related to the form of biocatalyst. We have considered two principle alternatives of the biocatalyst with the FTase activity; immobilized cells and immobilized enzyme. The design criteria for the biocatalyst formulation were taken from literature [13, 14]. The biocatalyst based on immobilized cells was in the form of calcium alginate gel particles with the diameter of 2.2 mm with the entrapped whole cells of *A. pullulans* [14]. A commercial, high porous styrene-derived ion-exchange resin of unspecified size was used for the immobilization of FTase [13]. An estimate of the half-life of immobilized enzyme biocatalyst was 231 days [13]. This period was assumed as the time of one cycle of biocatalyst use. Considering the first-order decay of enzyme activity during the cycle, the average productivity of the bioreactor was calculated to be 72 % of the initial one.

The initial FTase content in the immobilized cell bioreactor (the activity of cells before entrapment per unit volume of fixed-bed bioreactor) was expected to be 50,000 U dm^{-3} [14]. Since the initial FTase concentration in the immobilized enzyme biocatalyst was not given in the original publication [13], we estimated it from the comparison of the bioreactor productivities. The initial volumetric productivities at the initial sucrose concentration of 700 g dm^{-3} and about 60 % sucrose conversion were around 60 g dm^{-3} h^{-1} at the immobilized cell biocatalyst and 600 g dm^{-3} h^{-1} at the immobilized enzyme biocatalyst. *Yun et al.* [14] reported the effectiveness factor of the immobilized cell biocatalyst to be about 0.25—0.3. It is obvious that the effectiveness factor of the immobilized enzyme biocatalyst had to be significantly lower. It was assumed that both biocatalysts operated in the diffusional regime where the effectiveness factor is approximately inversely proportional to the Thiele modulus. Using this assumption and the above given ratio of the volumetric productivities of cell and enzyme biocatalysts, the initial FTase content in the immobilized enzyme bioreactor of 5,000,000 U dm^{-3} was calculated.

The amount of FOS's produced per one biocatalyst cycle was obtained by multiplying the average bioreactor productivity with the biocatalyst half-life time. The division of the initial FTase content with the amount of FOS's produced per cycle gave the amount of enzyme needed per unit mass of FOS's produced. The following values were obtained: 2088 U

kg^{-1} for the immobilized enzyme biocatalyst and 209 U kg^{-1} for the immobilized cell biocatalyst. Our experiments showed that at the optimal conditions the final FTase content in the cultivation bioreactor was approximately 60,000 U dm^{-3}. Using the last two values and the component concentration in the cultivation media, the consumption of the components per unit mass of produced FOS's was obtained (Table 6). The costs of these components were expressed in the prices provided by the distributors of chemicals for large-scale use in Slovakia. Obviously, the consumption and costs were 10 times higher at the immobilized enzyme biocatalyst. We were not able to find the commercial price of FOS's but it is certain that the minimal operating costs can be estimated from about 50,000 to 100,000 SKK per ton of FOS's. If these costs are compared with the values in Table 6, it can be concluded that the contribution of the costs of the cultivation media components to the total operating costs would be either negligible or minimal.

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