Appl Biochem Biotechnol (2007) 142:95–104
DOI 10.1007/s12010-007-0046-z

Evaluation of Enzymatic Reactors for Large-Scale Panose Production

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Published online: 27 June 2007

Abstract Panose is a trisaccharide constituted by a maltose molecule bonded to a glucose molecule by an α-1,6-glycosidic bond. This trisaccharide has potential to be used in the food industry as a noncariogenic sweetener, as the oral flora does not ferment it. Panose can also be considered prebiotic for stimulating the growth of benefic microorganisms, such as lactobacillus and bidifidobacteria, and for inhibiting the growth of undesired microorganisms such as *E. coli* and *Samonella*. In this paper, the production of panose by enzymatic synthesis in a batch and a fed-batch reactor was optimized using a mathematical model developed to simulate the process. Results show that optimum production is obtained in a fed-batch process with an optimum production of 11.23 g/l h of panose, which is 51.5% higher than production with batch reactor.

Keywords Optimization · Panose · Enzymatic synthesis

Introduction

Panose is a trisaccharide composed by one maltose unit and one glucose unit linked by an α-1,6 glycosidic bond obtained from sucrose and maltose by dextransucrase acceptor reaction [1, 2]. Panose can be widely used in the food industry as an anti-fading agent for food pigments, as food antioxidant and as anticariogenic sweetener [3, 4]. The main application for panose is in orally used products, including food and drinks, as this carbohydrate is non-fermentable by oral microorganisms and imparts thereto a substantial low or anticariogenic property, besides acting as sweetener [4]. Panose can be also used to promote the proliferation

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of microorganisms of the genus difidobacterium in human intestines [5–7]. Despite its potential applications, panose is still not produced in large scale.

Production of panose can be done by enzymatic synthesis using dextransucrase (EC 2.4.1.5) in batch or fed-batch reactors. Dextransucrase (EC 2.4.1.5) is a bacterial extracellular enzyme, which promotes the synthesis of dextran. This enzyme presents high stability under optimum synthesis conditions, allowing for its use in industrial scale [8]. Acceptors can be used in the enzymatic synthesis to deviate some or most of the glucose moieties from dextran production towards the production of acceptor products. Therefore, panose can be produced as the prime acceptor product formed when maltose is used as an acceptor for the synthesis. Figure 1 presents the dextransucrase acceptor reaction with maltose yielding panose. The deviation of the production of dextran towards panose requires a high maltose to sucrose ratio, which will lead to high panose yields [2].

In this paper, a mathematical model for panose production by enzymatic synthesis was used to optimize panose production in batch and fed-batch reactors as to set the optimum operating conditions for panose production in large scale.

Mathematical Model

In this work, a mathematical model for dextransucrase kinetics using maltose as an acceptor was developed based on the path model proposed by Heincke et al. [2]. The kinetic model was used to model and simulate the enzymatic synthesis of panose in batch and fed-batch reactors. Figure 2 presents a scheme of the kinetic model for the dextransucrase acceptor reaction [9].

In this enzymatic synthesis, the acceptor products can also act as acceptors yielding a series of homologous acceptor products having isomaltodextrin residues attached to the *O*-6 nonreducing end glucosyl moiety of maltose [2]. The mathematical model for the pathway presented in Fig. 2, for $j=0$ (panose formation) is given by Eqs. (1, 2, 3, 4). The formation of the enzyme complex (EG_i) is instantaneous and does not have to be considered in the model [2]. The kinetic rate constants of the model are presented in Table 1.

$$\frac{dS}{dt} = \frac{Q \cdot (S_F - S)}{V} - \frac{E \cdot S \cdot (p_1 + p_2 \cdot F + p_3 \cdot M + p_4 \cdot P)}{N} \tag{1}$$

$$\frac{dF}{dt} = \frac{Q \cdot (F_F - F)}{V} + \frac{E \cdot S \cdot (p_1 + p_3 \cdot M + p_4 \cdot P)}{N} \tag{2}$$

$$\frac{dM}{dt} = \frac{Q \cdot (M_F - M)}{V} - \frac{p_3 \cdot E \cdot S \cdot M}{N} \tag{3}$$

$$\frac{dP}{dt} = \frac{Q \cdot (P_F - P)}{V} + \frac{E \cdot S \cdot (p_3 \cdot M - p_4 \cdot P)}{N} \tag{4}$$

$$N = 1 + p_5 \cdot S + p_6 \cdot M + p_7 \cdot F + p_8 \cdot S \cdot F + p_9 \cdot S \cdot M + p_{10} \cdot M^2 + p_{11} \cdot M \cdot P + p_{12} \cdot S \cdot P + p_{13} \cdot S^2 \tag{5}$$

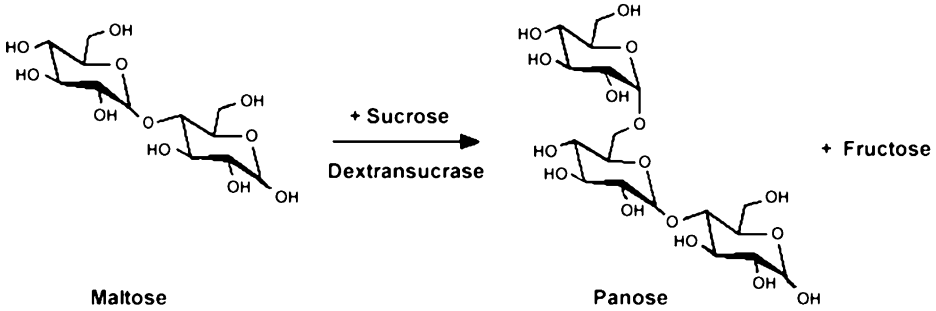

Fig. 1 Dextransucrase acceptor reaction for panose production

where S is the sucrose concentration (mmol/l), F the fructose concentration (mmol/l), M the maltose concentration (mmol/l), P the panose concentration (mmol/l), E the enzyme activity (IU/l), V the reactor volume (l), Q the flow rate (l/h), p_1 to p_{13} are the kinetic rate constants, and the subscript F denotes the concentration of the feed stream.

Five modes of operation (A to E) were studied to find the best operation mode and best operating condition:

A. Batch operation—reactor is filled with 2,000 l of sucrose and maltose solution, enzyme is added and the reaction runs until the conversion of sucrose is at 99%.
B. Fed-batch operation—reactor is filled with 1,000 l of sucrose and maltose solution. Enzyme is added. A solution of sucrose and maltose is fed into the reactor at a fixed flow rate until more 1,000 l of solution is fed into the reactor. The reaction runs until the conversion of sucrose is at 99%. The solution fed into the reactor has the same concentration as the initial solution concentration.

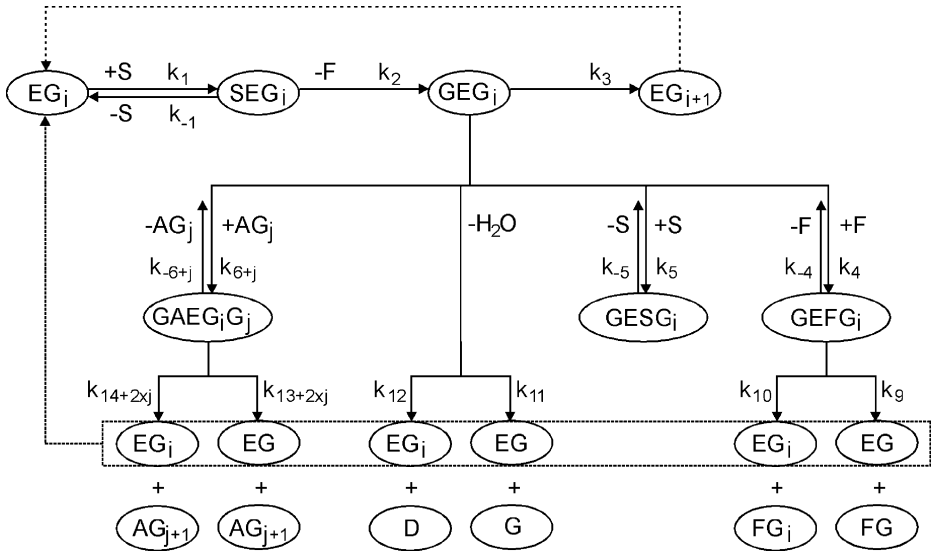

Fig. 2 Kinetic model for the dextransucrase reaction in the presence of acceptors ($i \geq 1, 0 \leq j \leq 2$). Where E is the enzyme, S is sucrose, G is glucose, F is fructose, D is dextran and AG_j is an acceptor such as maltose (AG_1)

Table 1 Kinetic rate constants [2].

Kinetic rate constants	Values
p_1	3.50×10^{-5} (l/mmol s)
p_2	4.76×10^{-8} (l/mmol $s^{0.5}$)^2
P_3	1.81×10^{-6} (l/mmol $s^{0.5}$)^2
p_4	3.52×10^{-6} (l/mmol $s^{0.5}$)^2
p_5	1.45×10^{-2} (l/mmol)
p_6	5.23×10^{-2} (l/mmol)
p_7	2.56×10^{-3} (l/mmol)
p_8	5.81×10^{-5} (l/mmol)^2
p_9	6.66×10^{-4} (l/mmol)^2
p_{10}	2.84×10^{-5} (l/mmol)^2
p_{11}	2.28×10^{-5} (l/mmol)^2
p_{12}	1.06×10^{-4} (l/mmol)^2
p_{13}	3.16×10^{-5} (l/mmol)^2

C. Fed-batch operation—reactor is filled with 1,000 l of sucrose and maltose solution. Enzyme is added. A solution of sucrose and maltose is fed into the reactor at a variable flow rate until more 1,000 l of solution is fed into the reactor. The reaction runs until the conversion of sucrose is at 99%. The solution fed into the reactor has a different concentration as the initial solution concentration.
D. Fed-batch operation—reactor is filled with 1,000 l of sucrose and maltose solution. Enzyme is added. A solution of sucrose is fed into the reactor at a fixed flow rate until more 1,000 l of solution is fed into the reactor. No maltose is fed during the reaction. The reaction runs until the conversion of sucrose is at 99%. The solution fed into the reactor has a different concentration as the initial solution concentration.
E. Fed-batch operation—reactor is filled with 1,000 l of sucrose and maltose solution. Enzyme is added. A solution of sucrose and a solution of maltose are fed into the reactor at a variable flow rate until more 1000 l of solution is fed into the reactor, maintaining a constant concentration of sucrose and maltose during the initial stage. The reaction runs until the conversion of sucrose is at 99%. The solution fed into the reactor has a different concentration as the initial solution concentration.

Three of the five strategies of operation presented in the manuscript represent the main operating strategies for batch and fed-batch reactors (strategies A, B, and C). Other strategies can be implemented but will be special cases of one of these three strategies. Strategies D and E are already special cases of strategies B and C, where no maltose is fed during the operation. Other strategies such as starting the feed at a time different from $t=0$ are also special cases of strategies B and C and were studied as well but with no improvement on panose production.

The possibility of feeding maltose and sucrose during the reaction in the fed-batch process allows keeping the maltose and sucrose concentrations at high levels maintaining high reaction rates for a longer period of time, and may boost production. In the enzymatic synthesis, it also helps to maintain the maltose to sucrose ratio high enough during the reaction increasing panose production and reducing dextran formation and acceptor chain elongation.

In all modes of operation, the enzyme activity was set at 250.0 IU/l. Simulations were carried out for a range of sucrose initial concentration $S_0=10$–100 mmol/l, while maltose to sucrose ratio was $M_0/S_0=0.0$–6.0, and flow rate was $Q=300$–$3{,}000$ l/h. These ranges of the sucrose and the maltose solutions were chosen to keep a low viscosity in the reaction media

because increasing viscosity causes an increase in the diffusion resistance leading to a decrease in the reaction rate.

The concentration of sucrose and maltose were kept at low levels (35% of sugar), if compared to the concentrations used in the sugar industry (> 50% of sugar). As the temperature cannot be raised without compromising the enzyme activity, sugar concentrations of 50 to 60% would lead to a high viscous system. Carbohydrate concentrations of 60% correspond to a viscosity higher than 44 cP (at 25°C), whereas a carbohydrate concentration of 35% of sugar corresponds to a viscosity of 4.8 cP. Furthermore, mixtures of fructose, maltose, sucrose, and glucose cause gelatinization of the sugars leading to even higher viscosities [10]. This higher viscosity causes an increase in the diffusion resistances affecting molecule mobility and a decrease in the reaction rate and selectivity of the process. It also increases substantially the energy costs of the process and the need of special pumps and impellers.

The temperature of the reactor was considered to run at 25°C and to operate isothermically. High temperatures, especially above 30°C, cause the enzyme to denature very quickly. The enzyme system used in the work presents an optimum pH and temperature for its activity and well defined pH and temperatures for the enzyme stability (pH 5.4 and 25°C) [2]. As such, the simulations were carried out at pH and temperatures where the enzyme is active and stable.

The mathematical model [Eqs. (1, 2, 3, 4, 5)] was solved by numerical integration using the method of Runge-Kutta (fifth order RK method). To solve this set of differential equations, a computational program written in FORTRAN was developed and implemented.

Results and Discussion

For the batch process (A), increasing initial sucrose concentrations causes an increase in panose productivity; thus, the highest sucrose concentration (100 mmol/l) should be used in the enzymatic synthesis. Increasing maltose to sucrose ratio helps to enhance productivity up to a maltose to sucrose ratio of 3.5 to 4.0, when after this point, the excess of maltose deviates the reaction equilibrium away from the products, reducing panose productivity (Fig. 3).

Rodrigues [11] has studied the dextran synthesis using maltose as acceptor in a batch reactor and has reported that when the molar ratio of maltose to sucrose is near or higher than 1.0, panose concentration was very high, which is in accordance with the simulations. Higher acceptor products were found in very small concentration (detected by thin layer chromatography). For maltose to sucrose ratios lower than 1.0, decreasing ratios causes a decrease in the panose concentration, and an increase in dextran and higher oligosaccharides concentrations (especially isomaltosyl-1,6-*O*-D-maltose).

Optimization of panose production in the batch process (operating strategy A) was conducted searching for the operating conditions that result in the highest production of panose per hour per liter of reactor (g/l h). A program in Fortran that uses the method of quasi-Newton and a finite-difference gradient was developed to maximize the production of panose and was used to solve the following optimization problem:

$$\text{Find} : S_0 \text{ and } M_0/S_0$$

$$\text{Maximize} : \text{Panose production}\left(\text{g.h}^{-1}.\text{L}^{-1}\right)$$

within ranges of operating conditions :

$$0 \leq S_0 \leq 100\text{mol/L}$$

$$0 \leq M_0/S_0 \leq 6.0$$

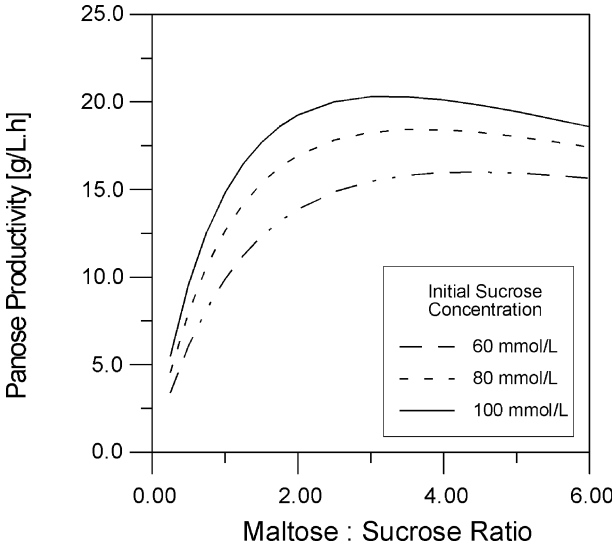

Fig. 3 Panose productivity as function of maltose to sucrose ratio and initial sucrose concentration in the batch process

Where S_0 is the initial sucrose concentration, M_0 the initial maltose concentration, and M_0/S_0 is the maltose to sucrose ratio.

To search for the maximum panose production, first, an initial guess for the values of initial sucrose and maltose concentrations were provided to the optimization algorithm. The mathematical model [Eqs. (1, 2, 3, 4, 5)] is solved by numerical integration (using the fifth order Runge–Kutta method) providing the result (panose productivity) to the optimization algorithm. New values for initial sucrose and maltose concentrations are then calculated increasing the concentrations of sucrose and maltose by a delta (generally, about 1/1,000 to 1/100 of the original concentration). The model is evaluated with these new values, returning the results to the optimization algorithm, which calculates new values for initial sucrose and maltose concentrations based on the gradient (difference) between the panose productivity calculated by the two previous evaluations of the model. The evaluations of the model and calculation of new values for initial sucrose and maltose concentration continues until the value of panose productivity reaches a maximum. These nonlinear optimization problems may find local maxima, instead of the global maxima. To avoid local maxima, the optimization problem was carried out starting from several different initial guesses, and only the global maxima is considered as the true result (optimum operating point).

For the batch process, the higher panose productivity is found at a sucrose initial concentration of 100 mmol/l and a maltose to sucrose ratio of 4.0 (Fig. 3). This operating condition presents high maltose conversion and produces 7.41 g/l h of panose. When lower conversions are allowed, the productivity (g/L h) increases, as the system expends less time during the period where the rate of reaction is low. For conversions of 95 and 90%, the reactor can output 8.98 and 9.86 g/L h of panose, leading to an increase in productivity of 21.2 and 33.0%. The set back of allowing lower conversions is that more sucrose and maltose are left in the reaction mixture to be separated from the final reaction mixture, increasing the separation cost.

As for the batch process (A), in the fed-batch processes (B to E) increasing initial sucrose concentrations causes an increase in panose productivity; thus, the highest sucrose concentration (100 mmol/l) should be used in the enzymatic synthesis (Fig. 4). Panose

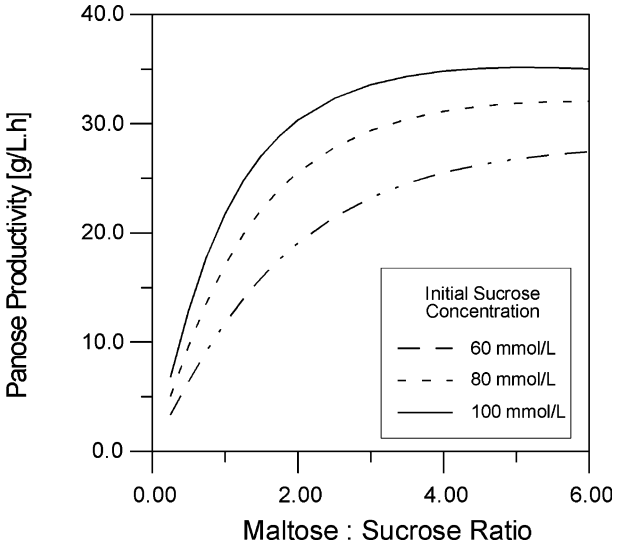

Fig. 4 Panose productivity as function of maltose to sucrose ratio and initial sucrose concentration in the fed-batch process

production increases steeply up to a maltose to sucrose ratio of 5.0, after which, the productivity does not change significantly.

Optimization of panose production in the fed-batch processes (operating strategies B to E) were done following the optimization problem:

$$\begin{aligned}
&\text{Find : } S_0 \text{ and } M_0/S_0\\
&\text{Maximize : Panose production}\left(\text{g.h}^{-1}.\text{L}^{-1}\right)\\
&\text{within ranges of operating conditions :}\\
&0 \le S_0 \le 100\,\text{mmol/l} \quad \text{for all cases}\\
&0 \le M_0/S_0 \le 6.0 \quad \text{for all cases}\\
&0 \le S_\text{F} \le 100\,\text{mmol/l} \quad \text{for cases : B, C, D}\\
&0 \le M_\text{F}/S_\text{F} \le 6.0 \quad \text{for cases : B, C}\\
&300 \le Q \le 3,000\,\text{L/h} \quad \text{for cases : B, C, D}
\end{aligned}$$

Where Q is the maltose and sucrose solution flow rate; S_F is the sucrose concentration in the feed stream; and M_F the maltose concentration in the feed stream.

These optimization problems were also solved using a similar algorithm used for the batch operating strategy (using the method of quasi-Newton and a finite-difference gradient).

The highest productivity of panose obtained for the fed-batch process, at mode of operation B, was found at a sucrose initial concentration of 100 mmol/l, a maltose to sucrose ratio of 3.71 and a flow rate of solution of 79.4 l/min. This operating condition produces 7.35 g/l h of panose, which is 0.8% lower than the productivity found for the batch process.

When the feed flow rate and concentration of feed were allowed to change (mode of operation C), the highest productivity of panose was found at a sucrose initial concentration of 100 mmol/l, initial maltose to sucrose ratio of 4.16 and a solution flow rate of 80.1. The solution fed during the reaction was at a sucrose concentration of 100 mmol/l and a maltose to sucrose ratio of 3.19. This operating condition also produces 7.35 g/l h of panose, equivalent to the one found for mode of operation B. These results indicate that the batch process is better than the fed-batch process operating at modes B and C.

The highest productivity of panose obtained for the fed-batch process, at mode of operation D, was found at a sucrose initial concentration of 100 mmol/l, a maltose to sucrose ratio of 6.0, and a flow rate of solution of 100 l/min. This operating condition yields 7.37 g/l h of panose, which is only 0.3% higher than the productivity found for the batch process.

Although in mode of operation E, the reaction runs at high sucrose concentration during a longer period of time, this operating condition yields 6.42 g/l h of panose, 12.6% lower than the productivity found for the batch process. The highest productivity of panose was found at a sucrose initial concentration of 100 mmol/l, a maltose to sucrose ratio of 6.0 and feeding sucrose at a concentration of 116 mmol/l without feeding maltose during the course of reaction.

If the reaction was allowed to run until a lower sucrose conversion, the panose productivity increases the process and would avoid the low reactions rates that occur when the sucrose concentration is low. When conversions of only 20% of sucrose are allowed, the reactor yields 12.6 g/l h of panose, which is 71.6% higher than the productivity found for the batch process (obtained at a sucrose initial concentration of 100 mmol/l, initial maltose to sucrose ratio of 4.0, and a flow rate of solution of 69.9 l/min. The solution fed during the reaction was at a sucrose concentration of 100 mmol/l and a maltose to sucrose ratio of 5.0). These results indicate that the fed-batch process operating at mode C would be better than the batch process. When more reasonable conversions of 70% of sucrose are allowed, the reactor yields 11.2 g/l h of panose, which is 51.5% higher than the productivity found for the batch process when total conversion of sucrose is achieved. At 90% of sucrose conversion, the reactor yields 9.7 g/l h of panose, which is 32.0% higher than the conversion observed on the batch process.

The major problem with this mode of operation is that a large quantity of substrate has to be purified and recycled. The amount of sucrose and maltose remaining in the substrate is high, and purification may be more difficult to achieve as panose concentration is at 12.7, 38.7, and 47.2 mmol/l, respectively, for conversions of 20, 70, and 90% of sucrose, whereas in the batch process, the concentration of panose in the reaction media is of 48.8 mmol/l with small quantities of maltose and sucrose.

Separations of the constituents of sugar solutions are usually based on chromatographic techniques. The use of high-performance separation units constitutes a key stage in industrial development. Several chromatographic separation processes have been developed in recent years proving techniques applicable at an industrial scale [12]. Although batch chromatography is a relatively simple process offering operating flexibility, it has disadvantages such as the requirement of a large difference in the adsorptive selectivity of the components and ineffectively use of adsorbent bed [13]. To avoid these disadvantages, continuous counter-current methods have been developed where mass transfer is maximized, allowing a more efficient use of the adsorbent bed and requiring only a partial separation of the components [14]. Such processes, however, have the difficulties related to circulating a solid adsorbent. Liquid chromatography in simulated moving bed mode (SMB) allows overcoming these difficulties [15]. The simulated moving bed chromatography (SMB) has the potential to increase productivity and reduce mobile phase consumption in comparison to batch elution chromatography. It allows the continuous counter-current separation of a feed into two streams of products (raffinate and extract) and has been used for many years in the sugar industry for large scale separations. This technology, however, is well established for binary mixtures such glucose and fructose mixtures, but still not well established for ternary mixtures such as panose, maltose, and fructose (studied herein).

Considering total sucrose consumption in the process studied herein, a ternary mixture of fructose, maltose, and panose should be separated to obtain pure panose. Two options should be faced: a simulated moving bed for separating a ternary system, even with low purity in the extract and raffinate, or two simulated moving bed systems in series for binary system separation. The decision is not possible at this point because the operating parameters (equilibrium isotherm of the resin, bed voidage, and adsorbent characteristics) for such systems are not available for the ternary mixture presented in this work.

A second option would be the use of a mixture containing maltose and panose, fructose and panose, or fructose, maltose, and panose. The synthesis of prebiotic carbohydrate as panose is addressed mainly for the food industry to be used as an additive in foods and drinks conferring them a functional characteristic. Although the ingestion of this mixture of carbohydrates is safe for human health, the final product would contain high amounts of non-prebiotic carbohydrates. Fructose and maltose are natural sweeteners, and the use of great amounts of these carbohydrates in food preparations, especially fructose, would lead to a very sweet product. Besides the excessive sweet taste, the product would be also very caloric and its use forbidden to diabetics.

From a productivity point of view the mode of operation C is favored, while analyzing from a purification point of view the mode of operation A (batch process) is favored. The process of choice is conditioned to an economical analysis where the reaction and purification costs have to be calculated and analyzed. If the analysis is based only on the market prices of the products and the reagents, then, the fed-batch process is favored due to the higher panose productivity. Figure 5 shows the relative profit that could be obtained for the batch and the fed-batch process (mode of operation C) as function of the panose market price.

The curves presented in Fig. 5 were calculated taking the market price of food grade sucrose being $1/mol (actual market price: US$ 1.10/kg-July/2005) and the other components being maltose=$2.3/mol and fructose=$10.5/mol. The cost for enzyme production is estimated at $2.0/IU. Relative profit was calculated as $ per mol of sucrose consumed. Currently, no food grade panose is available in the market, and the analytical grade is at $860,000/mol, but as panose begins to be produced in large scale, its market price will tend to diminish.

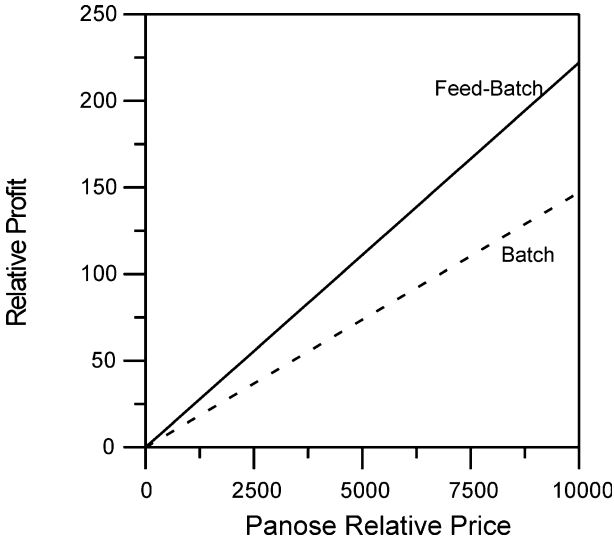

Fig. 5 Relative profit considering only the prices of products and reagents

Conclusions

The optimization of panose has shown that the maximum productivity is obtained in a batch process (mode of operation A) at a sucrose initial concentration of 100 mmol/l and a maltose to sucrose ratio of 4.0, yielding 7.41 g/l h of panose, when the reaction goes to a conversion of 99%. This operating condition presents the highest productivity that can be obtained by enzymatic synthesis using the dextran-saccharase enzyme obtained from *Leuconostoc mesenteroids* NRRL B512F for full conversion. Several other operating strategies were simulated without resulting in a better productivity.

If lower conversions are allowed, the productivity per batch period increases but at the expense of leaving greater quantities of sucrose and maltose to be separated from the desired product. At actual panose prices, lower conversions are acceptable, but as panose becomes a commodity and the separation techniques advances, full conversion will be required.

Taking into account the high market price of panose and the cost of separation, having a higher productivity is, nowadays, an advantage. But as the market prices diminishes, a migration to the batch process is required, as in the batch operation mode, the amount of substrate to be processed is smaller and the sucrose concentration in the substrate is very low, facilitating separation and purification of panose.

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