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Effect of Concentration and Substrate Flow Rate on Isomaltulose Production from Sucrose by *Erwinia* sp. Cells Immobilized in Calcium-Alginate Using Packed Bed Reactor

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Abstract Isomaltulose was obtained from sucrose solution by immobilized cells of *Erwinia* sp. D12 using a batch and a continuous process. Parameters for sucrose conversion into isomaltulose were evaluated using both experimental design and response surface methodology. *Erwinia* sp. D12 cells were immobilized in different alginates, and the influence of substrate flow rate and concentration parameters to produce isomaltulose from sucrose were observed. Response surface methodology demonstrated that packed bed columns containing cells immobilized in low-viscosity sodium alginate (250 cP) presented a mean isomaltulose conversion rate of 47%. In a continuous process, both sucrose substrate concentration and substrate flow rate parameters had a significant effect ($p<0.05$) and influenced the conversion of sucrose into isomaltulose. Higher conversion rates of sucrose into isomaltulose, from 53–75% were obtained using 75 g of immobilized cells at a substrate flow rate of 0.6 mL/min.

Keywords Calcium alginate · Immobilized cells · Isomaltulose · Response surface

Introduction

Isomaltulose and trehalulose are reducing disaccharides, structural isomers of sucrose. Isomaltulose is also known as Palatinose® and Lylose®, naturally present in honey and sugar cane, in very small quantities, and is currently considered very promising as a sugar substitute [1]. Isomaltulose is used in Japan as an ingredient and as a substitute for sucrose in the production of chewing gum, milk-based and bakery products, sweets, pastries, chocolates, desserts and beverages, due to its low cariogenic potential [2]. Isomaltulose is approximately 50% as sweet as sucrose [2–4] and has physical and sensorial properties very similar to those of sucrose [2, 3] and, when used as a food ingredient, as a substitute for sucrose in chocolate and candy products, no differences in sweetness or in residual taste are observed [3].

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Recently production and application of trehalulose have been studied as it is an ingredient of foods with high amounts of sweeteners such as jams and gum drops, as trehalulose is approximately 70% as sweet as sucrose and has a low cariogenic potential [5, 6]. Isomaltulose and trehalulose present low hydrolysis activity and minimum by-production of monosaccharides in the body; consequently they have a low glycemic index. Therefore, insulin release is correspondingly reduced as compared with other sugars, creating the possibility of their application in food and drinks for those who are diabetic and practice sports [1, 7, 8]. Isomaltulose is referred to as a promising sugar substitute as physical and chemical properties of isomaltulose have functional advantages over sucrose [9]. The world production of isomaltulose has been estimated to be 70.000 ton/year [10, 11].

Isomaltulose and trehalulose can be simultaneously formed in the enzymatic conversion of sucrose, by microbial sucrose isomerase. However, the proportion of conversion products depends on the strain [2, 6, 12–14]. *Protaminobacter rubrum* CBS571, *P. rubrum* 574.77, *Erwinia rhapontici* NCPPB1578, *Serratia plymuthica* NCIB8285, *S. plymuthica* ATCC 15928, *Pantoea dispersa* UQ68J produced mainly isomaltulose, approximately 50–90%; *Klebsiella* strains produced approximately 65% of isomaltulose; and *Pseudomonas mesoacidophila* MX45 and *Agrobacterium radiobacter* MX-232 produced mainly trehalulose, approximately 90%.

Several products derived from isomaltulose have potential industrial uses. Intermediate disaccharides, polymers, biodegradable detergents, and surfactants of industrial interest can be obtained. Isomaltulose can also be applied in the obtention of isomaltulose-based oligomers, which have prebiotic potential, stimulating proliferation of bifidobacterium strains from the intestinal microbiota [10, 11].

Isomalt is the main derivative of isomaltulose, a low caloric and non-cariogenic equimolar mixture of sugar-alcohol, obtained by hydrogenation. Isomalt is also known as Isomalt® and Palatinit® and has attracted attention of researchers due to its industrial application as a sucrose substitute in pharmaceutical formulations and in the food industry, used as a dietetic non-cariogenic sweetener [5, 15, 16].

Currently, isomaltulose is produced on an industrial scale in reactors using immobilized cells [14]. Alginate is the most common support employed for whole-cell immobilization [17]. This study aimed to study sucrose conversion into isomaltulose using immobilized *Erwinia* sp. D12 cells in different sodium alginates, and to observe the production of these disaccharides in a continuous process, in a packed bed column.

Material and Methods

Microorganisms and Culture Maintenance

Erwinia sp. D12 cells isolated from fruits were cultivated in agar slants, composed of 6.0% sucrose (*w*/*v*; Merck, Darmstadt, DE), 4.0% bacteriological peptone (*w*/*v*; Oxoid, Cambridge, UK), 0.4% de meat extract (*w*/*v*; Merck, Darmstadt, DE), and 2.0% agar (*w*/*v*; Merck, Darmstadt, DE), for hours, at 30 °C. After incubation sterile vaseline was added to the tubes and the culture was kept at 5 °C and replicated every 2 months.

Producing *Erwinia* sp. D12 Cells in a 6.6-L Fermenter

Erwinia sp. D12 cell biomass was obtained by microorganism fermentation in culture media containing corn steep liquor with 150 g/L sugar cane molasses (Usina Santa Elisa,

Sertaozinho, SP, BR), 20 g/L corn steep liquor (Corn Products Brasil, Mogi Guaçu, SP, BR), 15 g/L commercial yeast extract Prodex Lac SD® (Prodesa Produtos Especiais para Alimentos S/A, Valinhos, SP, BR), and pH adjusted to 7.5 [18].

Pre-inoculum and Fermentation

The 15-h culture of the microorganism described above was inoculated in six 250 mL Erlenmeyer flasks containing 50 mL of the culture medium described in item 2.2. The flasks were incubated at 200 rpm, 30 °C, for 15 h. A 300-mL aliquot of pre-inoculum, prepared as described previously, and 3 mL of Dow Corning® FG-10 anitfoam (D'altomare Química, São Paulo, SP, BR) were aseptically added to a New Brunswick Bioflo IIc 6.6-L fermenter (New Brunswick Scientific, Edison, NJ, EUA) containing 2.700 mL culture medium. Agitation and aeration were kept at 200 rpm and 1 vvm. After 8 h of fermentation at 27 °C, cell mass was recovered by centrifugation at 9.600×*g* for 15 min at 5 °C and washed twice, under aseptic conditions, with previously sterilized distilled water.

Studying the Immobilization of *Erwinia* sp. D12 Cells in Different Alginates and Conversion of Sucrose (Crystal Sugar) into Isomaltulose

Erwinia sp. D12 cell immobilization was carried out using different types of alginate to observe the effect of sucrose conversion into isomaltulose and trehalulose. Trials were performed in shaken Erlenmeyer flasks. Experimental design and response surface methodology were then used to study the influence of the substrate flow rate and concentration in the production of isomaltulose in a packed bed column.

Cell Immobilization Using Calcium Alginate

The wet cell mass of the *Erwinia* sp. D12 strain was obtained as described in item 2.2 For immobilization, a cell suspension of 47.0% (*m*/*v*) containing wet cells, in sterilized distilled water, was mixed with a sterile solution (autoclavated at121 °C for 15 min) of sodium alginate 3.0% (*m*/*v*) in the proportion 1:2 (*v*/*v*). The mixture was then dripped with the aid of a MasterFlex® L/S peristaltic pump (Cole-Parmer Instruments Co., Vernon Hills, IL, USA) and a needle in 0,3 M $CaCl_2$ solution, previously sterilized, to form small beads (3-mm diameter) which were maintained in the same $CaCl_2$, solution, at 5 °C, for 12 h. The beads were then washed with distilled water to remove excess $CaCl_2$. All stages were carried out under aseptic conditions. Sigma high viscosity (14,000 cP), Sigma low viscosity (250 cP; Sigma Chemical Co., St. Louis, MO, USA), Aldrich high viscosity (20,000 cP; Sigma Chemical Co., St. Louis, MO, USA), Fluka (Sigma Chemical Co., St. Louis, MO, USA), and Synth (LabSynth Ltda, Diadema, SP, BR) sodium alginates were evaluated.

Converting Sucrose into Isomaltulose Using Immobilized Cells in a Continuous and Batch Processes

In the batch process, trials were carried out in 250 mL Erlenmeyer flasks, which were incubated in a shaker New Brunswick Scientific Series 25 (New Brunswick Scientific, Edison, NJ, USA) at 100 rpm, at temperatures 25 and 30 °C. Each flask contained 50 mL of 35% sucrose solution (*m*/*v*) and 10 g of beads containing immobilized cells. After each 24-h period the sucrose solution was removed from the flasks and substituted for a new 35% sucrose solution. Beads containing immobilized cells were reused for seven batches.

Carbohydrate analyses were carried out in a DIONEX DX-600 chromatograph. All trials were carried out in duplicate.

Stability of the column containing immobilized cells was tested in a continuous process in order to convert sucrose into isomaltulose. The beads of immobilized cells, obtained as described above, were transferred to a jacketed column (30×150 mm) and a sucrose solution was circulated upwards in the packed bed column, using a MasterFlex® L/S peristaltic pump. Temperature of the column was maintained at 25–30°C using an ultra-thermostate Quimis® Q-14 M2 bath (precision temperature ±0.1°C) and the conversion of sucrose into isomaltulose was analyzed as described in "Carbohydrate Assay Using a Dionex Liquid Chromatography".

Carbohydrate Assay Using a Dionex Liquid Chromatography

Carbohydrate assays were performed in a DIONEX DX-600 chromatograph (Dionex Corporation, 1228 Titan Way Sunnyvale, CA, USA) equipped with an IP25 isocratic pump and an ED50 gold electrochemical detector. Sugar separation was carried out using a CarboPac™ PA 1 column (4 mm×250 mm), CarboPac™ PA 1 guard column (4 mm× 50 mm), and a 250 mM sodium hydroxide solution for the mobile phase, at a flow rate of 1 mL/min, at 20°C. Carbohydrates were analyzed through retention time, by comparing fructose, glucose, sucrose, and isomaltulose standards (Sigma Ultra®, Sigma Chemical Co., St. Louis, MO, USA).

Results and Discussion

Converting Sucrose into Isomaltulose and Trehalulose Using Immobilized Cells in a Batch Process

Figures 1a–e show the conversion of sucrose into isomaltulose and other sugars using immobilized *Erwinia* sp. D12 cells at 25°C in a batch process. Trials A, B, C presented the highest conversion rates of sucrose into isomaltulose, in which approximately 50–60% of isomaltulose was obtained in the first three batches. There was also a little conversion into glucose and fructose. Immobilized cells in alginates A, B, and C presented the best results in stability and conversion of sucrose into isomaltulose, alginates A and B presented slightly superior results compared to alginate C. The highest values of isomaltulose were obtained in trials where cells were immobilized with Sigma (14,000 cP) and Sigma (250 cP) alginates, respectively, during the third batch. In trial A, 65.53% of isomaltulose was obtained and 64.41% during trial B. During trials D and E, the highest isomaltulose values were obtained from the second batch, respectively, 63.19% and 63.32%. The immobilized cells from trials D and E showed the lowest stability, presenting a loss of activity from the third batch. Regarding trehalulose production from sucrose, in a batch process at 25°C, the highest values obtained were 42.94% during trial A, using cells immobilized in Sigma alginate (14,000 cP), and 40.34% during trial C, with cells immobilized in Aldrich alginate (20,000 cP).

Figures 2a–e showed a conversion of sucrose into isomaltulose and other sugars by immobilized *Erwinia* sp. D12 cells using different alginates at 30°C in a batch process. During the first two batches, cells immobilized with alginates A, B, C, and D presented conversion values of 50–59% for isomaltulose. We can observe that cells immobilized with alginate B (Sigma Alginate 250 cP) presented the best results for stability and conversion of

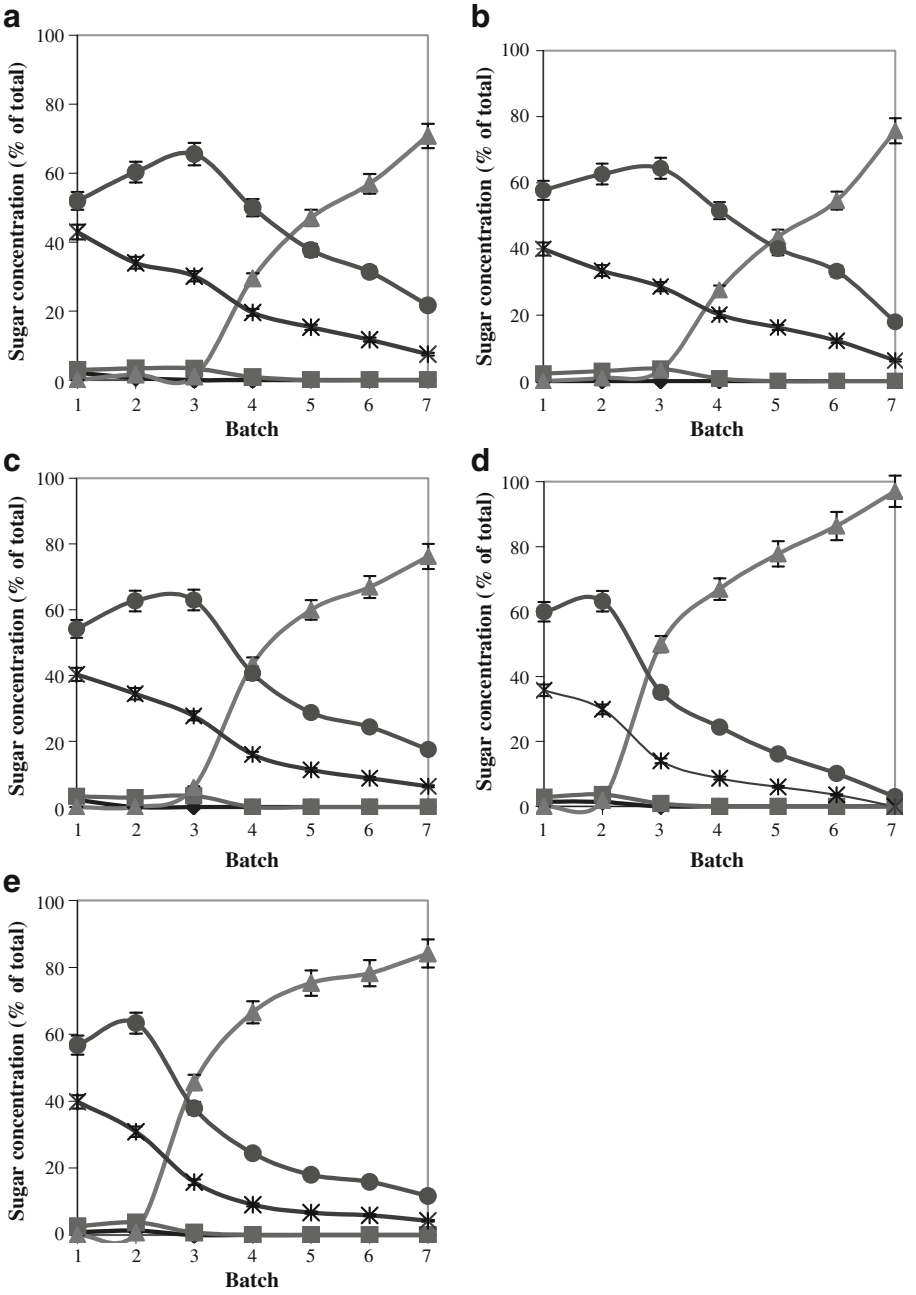

Fig. 1 Conversion of sucrose into isomaltulose and other sugars by immobilized *Erwinia* sp. D12 cells in different alginates in a batch process at 25 °C: **a** Trial A: Alginate Sigma 14000cP; **b** Trial B: Alginate Sigma 250cP; **c** Trial C: Alginate Aldrich 20000cp; **d** Trial D: Alginate Fluka; **e** Trial E: Alginate Synth (*filled diamonds*, glucose; *filled squares*, fructose; *filled triangles*, sucrose; *filled circles*, isomaltulose; *asterisks*, other disaccharide)

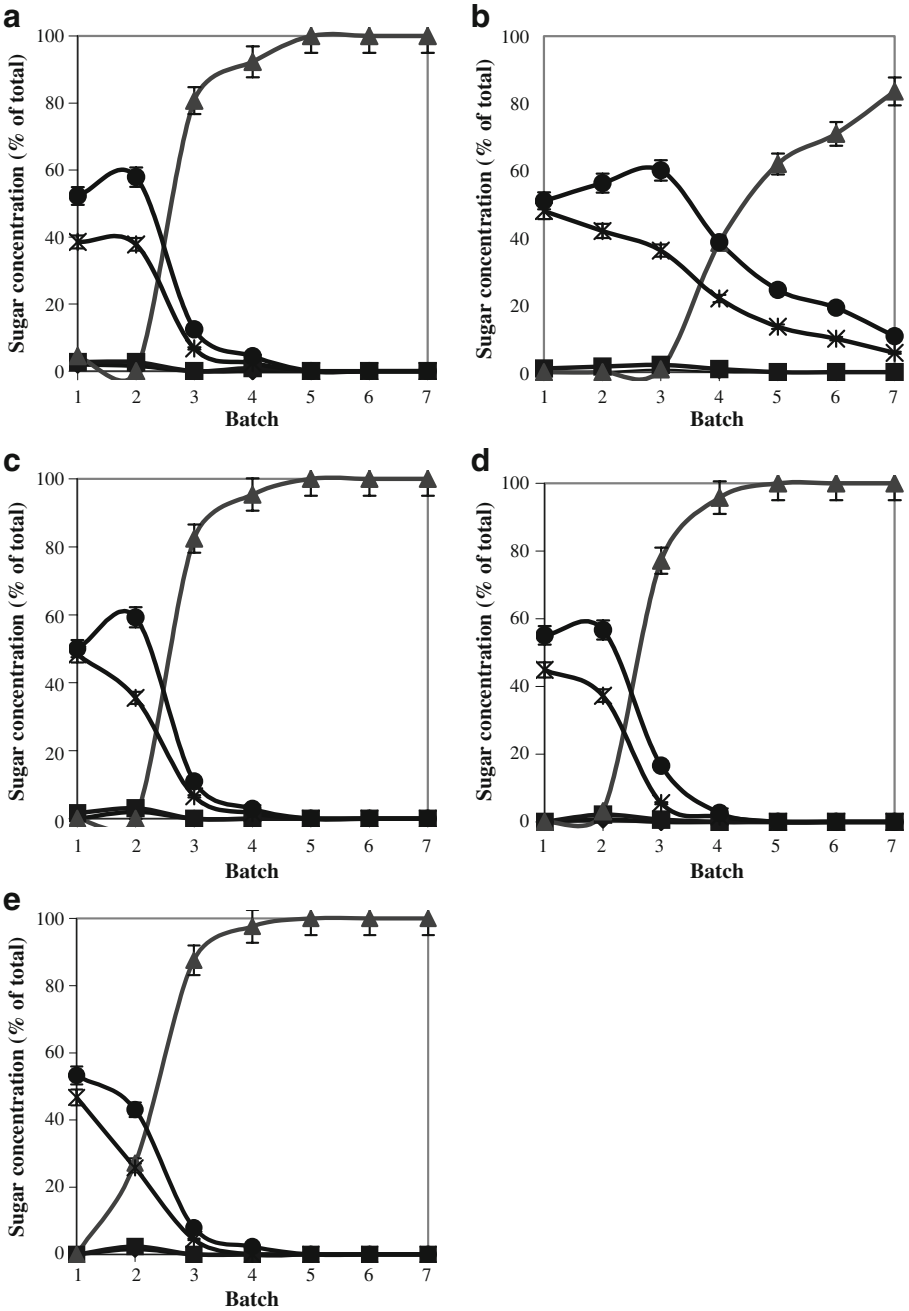

Fig. 2 Conversion of sucrose into isomaltulose and other sugars by immobilized *Erwinia* sp. D12 cells in different alginates in a batch process at 30°C: **a** Trial A: Alginate Sigma 14000cP; **b** Trial B: Alginate Sigma 250cP; **c** Trial C: Alginate Aldrich 20000cp; **d** Trial D: Alginate Fluka; **e** Trial E: Alginate Synth. (*filled diamonds*, glucose; *filled squares*, fructose; *filled triangles*, sucrose; *filled circles*, isomaltulose; *asterisks*, other disaccharide)

sucrose into isomaltulose and trehalulose. The highest yield was obtained for isomaltulose 60.04% with the third batch, with a gradual decrease in the conversion rate, obtaining 10.69% with the last batch. Cells immobilized during trial B were more stable, as cells immobilized for the remaining trials were inactivated from the fifth batch on, at 30 °C.

During the past years there has been significant increase in the research of biocatalysis field involving immobilization of either enzymes or whole-cell systems [19]. Cells immobilized in a matrix can be protected from unfavorable environmental conditions, such as pH, temperature, organic solvent and toxic substances, and can therefore be manipulated and recovered from solutions [20]. Alginate is one of the most used prevailing microbial immobilization supports for whole cells [21] as the methodology is simple, the technique is reproducible and mild conditions are used during the immobilization process [22, 23].

Many microorganisms that produce isomaltulose also produce trehalulose in great quantities. Tsuyuki et al. [17] described the immobilization of *Klebsiella planticola* MX ten cells in calcium alginate to convert sucrose into ismaltulose and trehalulose. The cell mass was mixed in a solution of sodium alginate at 4% (*w*/*v*) in the proportion 1:1 (*v*/*v*) and cell suspension was dripped in a solution of $CaCl_2$ 0.25 while shaking, forming beads with immobilized cells. These beads were maintained in the solution for 1 h and then washed with distilled water and put in a solution of polyethyleneimine. After 5 min, these beads were separated and treated with a 0.5% glutharaldeyde solution at 5 °C for 20 min and washed with distilled water. Immobilized cells treated with polyethyleneimine and glutharaldeyde totally converted the 25% sucrose solution obtaining 65.4% isomaltulose and 29.7% trehalulose.

P. rubrum cells were immobilized in calcium alginate gel [3]. Maximum activity of the immobilized cells, whose pH was 5.5, occurred after 3 h and was proportional to cell mass quantity. Conversion rates for isomaltulose were obtained at 18%, 30% and 44% for 10, 20, and 40 mg cells/mL of solution, respectively.

Mundra et al. [24] studied the effect of alginate concentration, bead diameter and cell mass quantity when immobilizing *E. rhapontici* NCPPB 1,578 cells in calcium alginate in order to convert sucrose into isomaltulose using a batch process. Through both experimental design methodology and response surface analysis a maximum yield of isomaltulose 140 mg/mL was observed from a 30% sucrose solution, using batch process, when the cells were immobilized in sodium alginate in a concentration of 5%, 2.25-mm diameter, and 5-g/L cell suspension.

This study shows that comparing the results obtained to convert sucrose into isomaltulose by immobilized cells, in a batch process at 25 and 30 °C, the Sigma sodium alginate (250cP) was chosen to continue this study, for presenting greater stability as to the remaining alginates.

Converting Sucrose Into Isomaltulose by Immobilized Cells in a Continuous Process

After selecting the alginate for immobilizing *Erwinia* sp. D12 cells, the column stability was tested, containing beads of immobilized cells, for the conversion of sucrose into isomaltulose, in a continuous process. These immobilized cell beads (50 g), prepared as aforementioned, were transferred to a jacketed column (30×150 mm) and a sucrose solution was circulated upwards, as described in "Converting Sucrose into Isomaltulose Using Immobilized Cells in a Continuous and Batch Processes".

Both experimental design and response surface methodology were used to study the effect of an independent sucrose solution substrate and substrate flow rate parameters when

Table 1 Complete factorial design 2^2 codified and uncodified (real values in parentheses) to study the substrate flow and concentration parameters of sucrose conversion into isomaltulose at 25 °C by calcium alginate-immobilized *Erwinia* sp. D12 cells in a continuous process.

Trial	Parameter		
	Substrate (%)	Flow rate (mL/min)	Isomaltulose[a] (%)
1	−1 (35)	−1 (0.5)	49.37
2	+1 (55)	−1 (0.5)	24.29
3	−1 (35)	+1 (1.5)	31.95
4	+1 (55)	+1 (1.5)	18.55
5	0 (45)	0 (1.0)	30.23
6	0 (45)	0 (1.0)	30.08
7	0 (45)	0 (1.0)	33.87

[a] Values of mean conversion rate of sucrose into isomaltulose during a period of 24 to 104 h, where conversion stabilization occurred

converting sucrose into isomaltulose. Trials were carried out in packed bed columns, kept at 25 °C, and followed for a 104-h period.

A complete factorial design 2^2 (CFD-2^2) was used to evaluate the independent parameters and the dependent parameter, given by the conversion of sucrose into isomaltulose (%), illustrated in Table 1. Results were analyzed considering the yield of isomaltulose, the main sugar formed.

Trials referring to CFD-2^2 included seven experiments, four of which referred to the complete factorial 2^2 (±1) and three repetitions of the central point (0) to estimate error and the consequent analysis of variance to obtain the polynomial equation of the first order, generating, after validation, both response surface and contour curve. The conversion of sucrose into isomaltulose, as can be observed, varied a minimum of 18.55% during trial 4 (substrate concentration 55% and substrate flow rate 1.5 mL/min) and reached a maximum conversion rate of 49.37% in trial 1 (substrate concentration 35% and substrate flow rate 0.5 mL/min).

Figures 3, 4, and 5 show the conversion of sucrose into isomaltulose by immobilized *Erwinia* sp D12 cells in calcium alginate, in a continuous process, in packed bed columns, during a period of 104 h. After the first 12 h, stabilization of the isomaltulose conversion

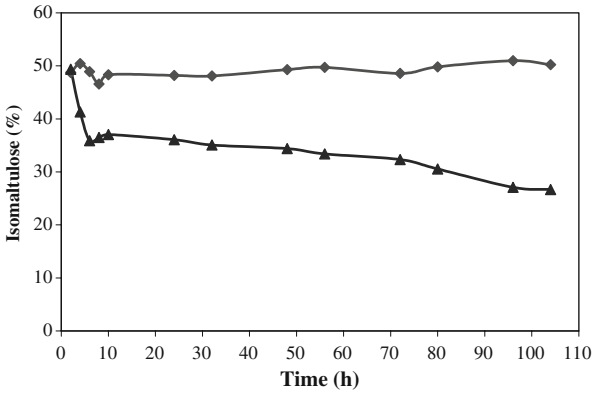

Fig. 3 Conversion of sucrose into isomaltulose: Evaluation of substrate flow and concentration parameters on the conversion of sucrose into isomaltulose at 25 °C by calcium alginate-immobilized *Erwinia* sp. D12 cells in a continuous process: 35% sucrose solution substrate (*filled diamonds*, Trial 1; *filled triangles*, Trial 3)

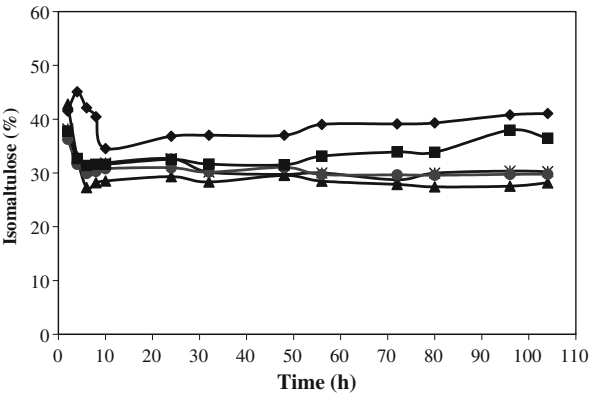

Fig. 4 Conversion of sucrose into isomaltulose: evaluation substrate flow and concentration parameters on the conversion of sucrose into isomaltulose at 25°C by calcium alginate-immobilized *Erwinia* sp. D12 cells in a continuous process: 45% sucrose solution substrate (*filled diamonds*, Trial A; *filledtriangles*, Trial B; *asterisks*, Trial 5; *filled circles*, Trial 6; *filled squares*, Trial 7)

rate was observed. Figure 3 shows that using a 35% sucrose substrate produced a mean conversion of 49.37% at a substrate flow rate of 0.5 mL/min (trial 1), and 31.95% at a substrate flow rate of 1.5 mL/min (trial 3).

Figure 4 shows the results of conversion that refers to the CFD-2^2 central point trials (trials 5, 6, and 7) with a 45% sucrose substrate and a substrate flow rate of 1.0 mL/min. Two other trials were carried out simultaneously, for comparison: trial A, in which 45% sucrose and substrate flow rate of 0.5 mL/min were used; and trial B, in which a sucrose substrate of 45% and a substrate flow rate of 1.5 mL/min were used.

Trials 5, 6, and 7, regarding the three replicates at the central point, presented isomaltulose conversion rates of 30.23%, 30.08%, and 33.87%, respectively. During trial A, where a lower substrate flow rate was used (0.5 mL/min), the isomaltulose conversion rate was slightly higher, 38.77%. However, during trial B, a higher substrate flow rate was used (1.5 mL/min) and a slightly lower isomaltulose conversion rate was obtained, 28.33%.

Using a sucrose substrate concentration of 55%, the lowest conversion rates were obtained (Fig. 5). The mean conversion was 24.29% at a substrate flow rate of 0.5 mL/min (Trial 2), and 18.55% at a substrate flow rate of 1.5 mL/min (trial 4). Figure 3 shows that the two parameters studied, substrate flow rate and concentration were important and influenced the conversion rates of sucrose into isomaltulose, as it becomes evident through the statistical analysis carried out below.

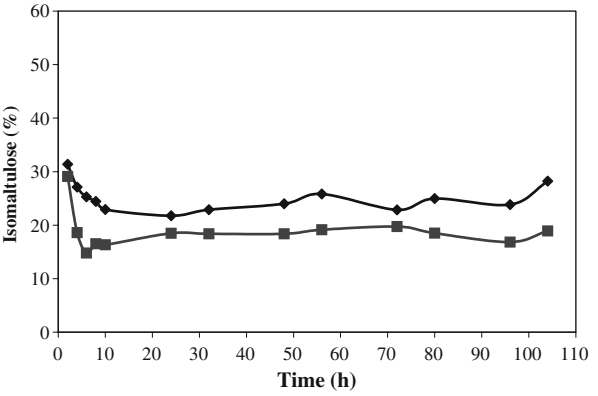

Fig. 5 Conversion of sucrose into isomaltulose: Evaluation of substrate flow and concentration parameters on the conversion of sucrose into isomaltulose at 25°C by calcium alginate-immobilized *Erwinia* sp. D12 cells in a continuous process: 55% sucrose solution substrate (*filled diamonds*, Trial 2; *filled triangle*, Trial 4)

Table 2 Main effects and interactions of substrate flow and concentration parameters of sucrose conversion into isomaltulose at 25 °C by calcium alginate-immobilized *Erwinia* sp. D12 cells in a continuous process.

	Effect	Standard error	*t* (2)	*p*
Substrate (1)[a]	−18.8620	2.1484	−8.7794	0.0127
Flow rate (2)[a]	−11.9610	2.1484	−5.5674	0.0308
1×2	4.8416	2.1484	2.2536	0.1530

[a] Statistically significant parameters with a 95% confidence level

Table 2 presents the effects of the independent parameters when converting sucrose into isomaltulose. As observed the independent parameters substrate flow rate and concentration presented significant and negative effects in the studied range of 95% ($p<0.05$) confidence level. These results indicate that an increase in these parameter values, in the range studied, leads to a lower conversion rate of sucrose into isomaltulose; therefore, a lower sucrose substrate concentration and a lower substrate flow rate would be indicated.

Table 3 presents values for *t*, *p*, and regression coefficient for the conversion of sucrose into isomaltulose used to construct the first order polynomial equation. Using analysis of variance (ANOVA; Table 4) an equation was obtained with a satisfactory R^2 value, a significant *F* value, and a low lack of fit value as to the regression value, characteristics that would generate an equation, which could be efficient when used to predict sucrose into isomaltulose conversion rate responses as a function of the parameters studied.

The 0.94 determination coefficient (R^2) indicates that the statistical model is capable of predicting the conversion of sucrose into isomaltulose, explaining approximately 94% of response variability and only 6% of total variance would remain unexplained by that equation.

To confirm the validity and also the equation to represent the system, an analysis of variance and an *F* test were carried out, in which the $F_{\text{experimental}}$ value obtained (30.50) was approximately 4.40 times greater than the $F_{\text{tabulated}}$ ($F_{0.90,\ 2,\ 4}=6.94$) value, a confidence level of 90% considered excellent, which allow a coded first order polynomial equation to be obtained. This equation describes a response as a function of the analyzed variables.

After the ANOVA and validation of the variables studied, an equation was obtained, which is representative of the behavior of conversion of sucrose into isomaltulose, through beads containing immobilized *Erwinia* sp. D12 cells in calcium alginate, which generated the response surface and the contour curve (Fig. 6). Equation 1 was obtained according to parameters and regression coefficients indicated in Table 3:

$$\text{Isomaltulose}\ (\%) = 31,48 - 9,43.x_1 - 5,98.x_2 \qquad (1)$$

Table 3 Regression coefficients, standard deviation and confidence limits of the substrate flow and concentration parameters of sucrose conversion into isomaltulose at 25 °C by calcium alginate-immobilized *Erwinia* sp. D12 cells in a continuous process.

	Regression coefficient	Standard deviation	*t* (2)	*p*
Mean[a]	31.4767	0.8120	38.7638	0.0007
Substrate (1)[a]	−9.4308	1.0742	−8.7794	0.0127
Flow rate (2)[a]	−5.9804	1.0742	−5.5674	0.0308
1×2	2.4208	1.0742	2.2536	0.1530

[a] Statistically significant parameters with a 95% confidence level

Table 4 Variance analysis of the study of the substrate flow and concentration parameters of sucrose conversion into isomaltulose at 25 °C by calcium alginate-immobilized *Erwinia* sp. D12 cells in a continuous process.

Variation source	Quadratic sum	Freedom level	Quadratic mean	*F* test
Regression	498.8208	2	249.4104	30.5022
Residues	32.7072	4	8.1768	
Lack of fit	23.4761	2		
Pure error	9.2311	2		
Total	531.5280	6		

Correlation Coefficient: $R^2 = 0.94$; $F_{0.95;2;4}=6.94$.

where: x_1 and x_2 represent the coded values corresponding to sucrose concentration and substrate flow rate, respectively.

Figure 6 shows the effects of the parameters of concentration and substrate flow rate in the conversion of sucrose into isomaltulose. As can be observed, in the substrate concentration range studied, there is a tendency towards a higher conversion rate when a lower amount of sucrose is used, below 40%, with optimum concentration at approximately 35%. Regarding substrate flow rate, lower flows have a tendency towards greater conversion rates of isomaltulose, with an optimum concentration at approximately 0.5 mL/min, in the studied range.

After the study of variance of concentration and substrate flow rate and the influence of sucrose conversion into isomaltulose by immobilized *Erwinia* sp. D12 cells in calcium alginate, in packed bed columns, a trial was carried out to check the shelf life of beads

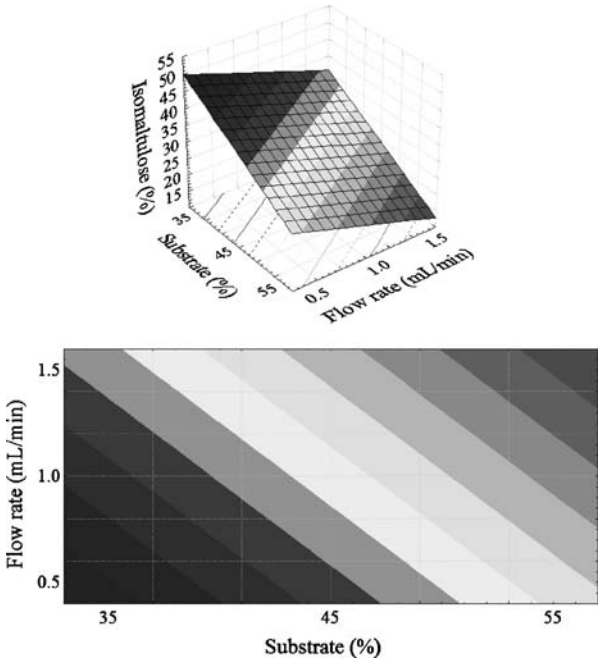

Fig. 6 Response surface and contour curve in the evaluation of the effect of the substrate flow and concentration parameters on the conversion of sucrose into isomaltulose at 25° by calcium alginate-immobilized *Erwinia* sp. D12 cells in a continuous process

containing these immobilized cells. Two columns were used, one containing 50 g of beads with immobilized cells and a 35% sucrose substrate with a flow rate of 0.4 mL/min (column 1), and another column containing 75 g of beads with immobilized cells and a 35% sucrose substrate with a flow rate of 0.6 mL/min (column 2). The conversion rate of sucrose into other sugars was followed for 312 h.

Figure 7 illustrates the conversion of sucrose into isomaltulose. Column 1 presented isomaltulose values of 47–51% during the first 144 h, presenting from then on a gradual decrease in the conversion rates with a consequent increase in residual sucrose. Column 2 presented greater stability and higher values of isomaltulose, 53–75 during the first 192 h, and also had a gradual decrease in conversion rates. The presence of glucose and fructose was observed, though in a low amount. Column 1 presented lower conversion rates of sucrose into isomaltulose than column 2; however, there were similarities in behavior regarding conversion stability.

The results obtained from conversion of sucrose into isomaltulose are according to data of similar studies by other experiments cited in the literature. Moraes et al. [25] studied the production of isomaltulose using immobilized *Erwinia* sp. cells in calcium alginate in a continuous process, using a packed bed column. The authors evaluated the effect of temperature (25–35 °C) and substrate concentration (12.5–60% *p*/*v*) on the conversion of sucrose into isomaltulose, with the aid of response surface methodology. A yield of approximately 50% of isomaltulose was obtained in sucrose solutions from 20–30% at 35 °C. Excess of sucrose affected the immobilized cell activity, decreasing conversion of sucrose into isomaltulose.

Other matrices and methodologies of immobilization have been used to convert sucrose into isomaltulose in continuous process. *S. plymuthica* cells were immobilized in chitosan [26]. Effect of the substrate sucrose concentration (30–70%), temperature at 30–60 °C and residence time of the substrate solution on the specific volumetric productivity of the biocatalyst in a tubular fixed-bed reactor was studied. Residence time of 1.7–3.0 h was necessary to achieve 98–100% conversion of sucrose at substrate concentration of 30–50%. A longer residence time (4–5 h) was needed for reaching the same conversion rate of concentrated sucrose solutions (60–70%). Krastanov et al. [27] used immobilized cells of *S. plymuthica* in hollow fiber biorector to produce isomaltulose from sucrose solution. Specific productivity of the membrane reactor was 16.8 g $m^{-2}h^{-1}$ using a flow rate of 1.3 $cm^3 min^{-1}$ and 40% substrate concentration in continuous mode of action. Biocatalyst activity decreased as the operation time increased.

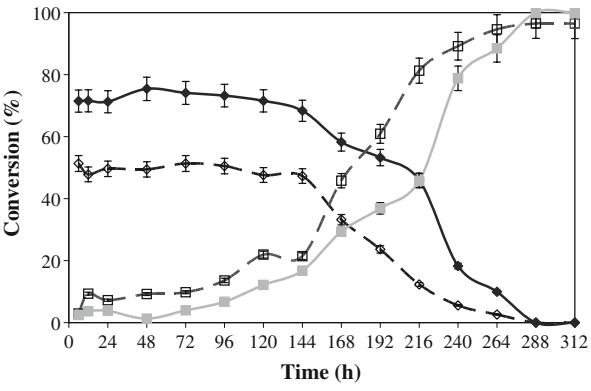

Fig. 7 Conversion of sucrose into isomaltulose by calcium alginate-immobilized *Erwinia* sp. D12 cells in a continuous process, in packed bed columns: *Full lines* represent the results from Column 1, and *Dotted lines* are the results from Column 2 (*filled diamonds* Isomaltulose 1; *empty diamonds* isomaltulose 2; *filled squares* sucrose 1; *filled squares* sucrose 2)

Conclusions

Imobilized cells of *Erwinia* sp. D12 were used to produce isomaltulose from sucrose solution. In preliminary studies, in a batch process, it was verified that the Sigma sodium alginate (250 cP) was the best matrix to immobilize bacteria cells. An experimental design and response surface methodology were used to determine the influence of a sucrose substrate concentration and flow rate in order to enhance stability of *Erwinia* sp. D12 immobilized cells on isomaltulose production using continuous process. It was observed that sucrose substrate concentration and substrate flow rate parameters had a significant effect ($p<0.05$) and influenced the conversion of sucrose into isomlatulose by immobilized cells in calcium alginate in a continuous process. The values obtained for isomaltulose were 53–75% using a 35% sucrose substrate and substrate flow rate at 0.6 mL/min, in a packed bed column containing 75 g of beads of immolbilized *Erwinia* sp. D12 cells. New investigations are necessary to optimize the conversion of sucrose into palatinose.

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