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Immobilization of glucosyltransferase from *Erwinia* sp. using two different techniques

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ABSTRACT

Two different techniques of glucosyltransferase immobilization were studied for the conversion of sucrose into isomaltulose. The optimum conditions for immobilization of *Erwinia* sp. glucosyltransferase onto Celite 545, determined using response surface methodology, was pH 4.0 and 170 U of glucosyltransferase/g of Celite 545. Using this conditions more than 60% conversion of sucrose into isomaltulose can be obtained. The immobilization of glucosyltransferase was also studied by its entrapment in microcapsules of low-methoxyl pectin and fat (butter and oleic acid). The non-lyophilized microcapsules of pectin, containing the enzyme and fat, showed higher glucosyltransferase activity, compared with lyophilized microcapsules containing enzyme plus fat, and also lyophilized microcapsules containing enzyme without fat addition. The non-lyophilized microcapsules of pectin containing the glucosyltransferase and fat, converted 30% of sucrose into isomaltulose in the first batch. However the conversion decreased to 5% at the 10th batch, indicating inactivation of the enzyme.

1. Introduction

Isomaltulose, also called Palatinose® and Lylose® is a reducing sugar and structural isomer of sucrose. This sugar is an interesting substitute for sucrose, in the food industry, considering that it is 50% as sweet as sucrose (Takazoe, 1989), non-carcinogenic (Sasaki et al., 1985) and has low glycemic index (Achten et al., 2007). The insulin release after isomaltulose ingestion is correspondingly reduced as compared to other sugars, creating the possibility of its application in diabetic and sports food and drinks. Considering that isomaltulose has no toxicity, this sugar is commercially used as a sucrose substitute in foods, soft drinks and medicines (Lina et al., 2002). Isomaltulose can be converted into sugar alcohol, Isomalt®, which has other useful properties for foods.

Isomaltulose is naturally present in very small quantities in honey, sugarcane extract and other sugar-rich fluids (Huang et al., 1998). A few bacterial species are known to have the ability to produce glucosyltransferase, which converts sucrose into isomaltulose. These microorganisms include *Erwinia* sp. D12 (Kawaguti et al., 2010), *Serratia plymuthica* (Kawaguti and Sato, 2010), *Klebsiella* sp. k18 (Orsi et al., 2009). For industrial purposes, the immobilization of the biocatalyst (enzyme and/or cells) offers several advantages, including reusability, easy product separation, enhancement of enzyme stability and continuous operation (Oliveira et al., 1997). Adsorption and entrapment are generally simple and cheap methods previously well-studied for other biocatalysts. Moreover, the adsorption technique using Celite as the support is reported as being very interesting for other enzymes such as lipases (Khare and Nakajimab, 2000; Silva et al., 2008).

There are several works in the literature reporting bacterial cell immobilization in isomaltulose production (Kawaguti et al., 2006; Oliva-Neto and Menão, 2009). However, few studies are focused on the immobilization of extracted glucosyltransferase, which converts sucrose into isomaltulose. The immobilization of the enzyme presents some advantages compared to cell immobilization, such as lower risk of microbial contamination of the product, the former prevents the risk of unwanted catalytic activity; whole cells bring along further resistance to mass transfer due to the presence of the cell wall, which drastically reduces reaction rates (Chen, 2007). Thus, this work aimed to immobilize the glucosyltransferase from *Erwinia* sp. D12, in two different supports by adsorption (Celite) and entrapment (low-methoxyl pectin

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microcapsules), considering they are simple and cheap techniques. Afterward, the conversion of sucrose into isomaltulose catalyzed by the immobilized preparations was evaluated. This *Erwinia* sp. strain has been studied by our research group and presented interesting applications in the production of isomaltulose (Kawaguti et al., 2006, 2007; Kawaguti and Sato, 2010). Initially, response surface methodology (RSM) was used to evaluate the influence of pH value in the adsorption of glucosyltransferase onto Celite 545, the concentration of enzyme adsorbed onto the support and the addition of glutaraldehyde to retain the enzyme. RSM has never been used for the study of the immobilization of glucosyltransferase. This technique is used to identify the relationships between the variables and the response, generally resulting in the optimization of the process and simultaneously limiting the number of experiments. In the second part of this study, the glucosyltransferase was immobilized in microcapsules of low-methoxyl pectin with the addition of fat material (butter and oleic acid). The morphology of the microcapsules was examined using optical microscopy and scanning electron microscopy. The immobilized glucosyltransferase in low methoxyl pectin was used in different batches for the conversion of sucrose into isomaltulose. It must be highlighted that those two supports have never been studied for the immobilization of glucosyltransferase that converts sucrose into isomaltulose.

2. Material and methods

2.1. Microorganism

The *Erwinia* sp. sp D12 strain that produces glucosyltransferase, that converts sucrose into isomaltulose, was previously isolated and obtained from the Food Biochemistry Laboratory, College of Food Engineering – UNICAMP and maintained on nutrient agar, at 4 °C.

2.2. Glucosyltransferase production

For the preparation of the pre-inoculum, a 15 h culture of the *Erwinia* sp. strain was inoculated in 250 mL Erlenmeyer flasks containing 50 mL of the fermentation medium previously optimized by Kawaguti et al. (2007). The medium was composed of sugar cane molasses (Companhia Energética Santa Eliza, BR) (150 g/L) Milhocina® (corn steep liquor) (Corn Products do Brasil, BR) (20 g/L), Yeast Extract Prodex Lac SD® (Produtos Especiais para Alimentos S/A, BR) (15 g/L), and the pH adjusted to 7.5. The flasks were incubated at 200 rpm, 30 °C, for 15 h. A 300 mL aliquot of this pre-inoculum and 3 mL of Dow Corning® FG-10 antifoam (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, USA) containing 2700 mL of culture environment with the same composition. Agitation and aeration were kept at 200 rpm and 1 vvm, respectively. After 8 h of fermentation at 27 °C, cell mass was recovered by centrifugation at 9600 × *g* for 15 min at 5 °C and washed twice with distilled water. A cell suspension of 10% (m/v) containing wet cells, in distilled water, was prepared. Cells were disrupted by sonicator treatment (Labline Instruments, Inc., IL, USA), for 20 s at 180–200 W, and this step was repeated 15 times. The suspension was then centrifuged at 9600 × *g* for 15 min at 5 °C and the supernatant was used as crude glucosyltransferase. The glucosyltransferase activity was assayed by measuring the reducing sugars formed from the sucrose using the Somogyi method (1945) with glucose as the standard. A mixture of 450 μL of a 10% (m/v) sucrose solution in 0.05 M citrate-phosphate buffer, pH 6.3, and 50 μL of enzyme solution (23 U/mL) was incubated for 25 min at 33 °C. One unit of glucosyltransferase activity (U) was defined as the amount of enzyme that releases 1 μmol of reducing sugars from sucrose per minute under the above assay conditions.

2.3. Optimization of the immobilization conditions of glucosyltransferase onto Celite 545

For the immobilization of glucosyltransferase onto Celite 545 (Merck, Darmstadt, Germany with particle size of 0.02–0.1 mm), Erlenmeyers flasks (250 mL) containing 40 mL of 0.1 M buffer (citrate-phosphate pH 4.0–5.6, phosphate pH 6.5–8.0), 10 g of Celite 545 and different concentrations of the enzyme (14.1–246.2 U/g of Celite 545) were incubated for 12 h, 130 rpm, at 5 °C. The samples of immobilized glucosyltransferase onto Celite 545 were filtered using qualitative paper, washed with distilled water and transferred to Erlenmeyer flasks (250 mL) containing 40 mL of different concentrations of glutaraldehyde solutions. The flasks were incubated for 1 h, at 130 rpm, at 5 °C, and then the samples of immobilized enzyme were filtered using qualitative paper and washed with distilled water. The Erlenmeyers flasks containing 10 g of immobilized glucosyltransferase preparation and 50 mL of 10% (m/v) of sucrose solution in distilled water were incubated for 2.5 h, at 33 °C and 130 rpm. The sucrose solution was changed three times and the carbohydrates were analyzed as described in Section 2.5.

Two sequential central composite designs were employed to optimize the immobilization of glucosyltransferase onto Celite 545. The first design was a 2^3 five level central composition design (2^3-CCD) consisted of 2 axial points (+/−) at a distance of $\alpha = 1.68$, with four replicates at the center point. A total of 17 runs were performed in each experiment to study the independent variables. The independent variables were pH value for the adsorption of glucosyltransferase onto Celite 545, concentration of enzyme and glutaraldehyde, while the conversion of sucrose into isomaltulose was the dependent variable.

The second design (2^2-CCD) was carried out, fixing glutaraldehyde concentration for all the assays as 0.1%; pH value and enzyme concentration were varied at different levels. The immobilization process and the system reaction were carried out as described earlier and the conversion of sucrose into isomaltulose (dependent variable) was determined.

The variables were coded according to Eq. (1):

$$X_i = \frac{X_i - X_0}{\Delta X_i} \quad (1)$$

where X_i is the dimensionless coded value of an independent variable, X_i is the real value of an independent variable, X_0 is the real value of an independent variable (X_i) at the center point and ΔX_i is the step change value. The variables and levels are shown in Tables 1 and 2. The system behavior was determined by a second-order polynomial equation, based on the equation below

$$Y = \beta_0 + \Sigma\beta_i x_i + \Sigma\beta_{ii} x_i^2 + \Sigma\beta_{ij} x_i x_j \quad (2)$$

where Y is the predicted value for the response, β_0 is the offset term, β_i is the linear effect coefficient, β_{ii} is the squared effect coefficient and β_{ij} is the interaction effect. $x_i x_j$ represents the interaction between different coded values, where i is one parameter and j is other.

The experimental results were considered only for the first batch and they were fitted to the second-order polynomial function. Student's *t*-tests were used to determine the statistical significance of the regression coefficients and analysis of variance (ANOVA) was performed to evaluate the statistical significance of the model. The response model was expressed in terms of coded variables, ignoring the statistically non-significant terms. Variables with a confidence level greater than 90% were considered to have a significant influence on the response (%). All data were treated with the aid of the Statistica® software 7.0 (Statsoft, Inc., Tulsa, USA).

Table 1
First central composite design 2^2 coded for the study of the effect of pH, enzyme concentration and glutaraldehyde concentration on the immobilization process of glucosyltransferase onto Celite, for conversion of sucrose into isomaltulose; the statistical analyses were carried out only in the first batch of 2.5 h, at 33 °C and 130 rpm.

Assay	Variables			Conversion of sucrose into isomaltulose (%)		
	pH	Enzyme (U/g of Celite)	Glutaraldehyde (%)	1° batch	2° batch	3° batch
1	−1 (5.6)	−1 (32.6)	−1 (0.10)	7.38	7.38	9.03
2	+1 (7.4)	−1 (32.6)	−1 (0.10)	0.00	0.00	0.00
3	−1 (5.6)	+1 (87.0)	−1 (0.10)	21.92	21.92	23.63
4	+1 (7.4)	+1 (87.0)	−1 (0.10)	1.34	1.34	1.59
5	−1 (5.6)	−1 (32.6)	+1 (0.40)	1.51	0.00	1.59
6	+1 (7.4)	−1 (32.6)	+1 (0.40)	0.00	0.00	0.00
7	−1 (5.6)	+1 (87.0)	+1 (0.40)	12.75	8.73	10.64
8	+1 (7.4)	+1 (87.0)	+1 (0.40)	0.00	1.52	1.15
9	−1.68 (5.0)	0 (59.8)	0 (0.25)	19.81	18.09	20.32
10	+1.68 (8.0)	0 (59.8)	0 (0.25)	0.00	0.00	0.09
11	0 (6.5)	−1.68 (14.1)	0 (0.25)	0.00	0.00	0.00
12	0 (6.5)	+1.68 (105.5)	0 (0.25)	7.23	8.00	7.19
13	0 (6.5)	0 (59.8)	−1.68 (0.00)	16.94	14.12	11.54
14	0 (6.5)	0 (59.8)	+1.68 (0.50)	3.25	2.87	3.77
15	0 (6.5)	0 (59.8)	0 (0.25)	4.31	6.33	4.62
16	0 (6.5)	0 (59.8)	0 (0.25)	6.18	5.96	4.29

2.4. Immobilization of glucosyltransferase in microcapsules of low-methoxyl pectin

The immobilization of glucosyltransferase was carried out as described by Correa et al. (2004). The mixture of 95 mL of 2% (m/m) of amidated low methoxyl pectin (Cp Kelco) in water, and 5 mL of glucosyltransferase (23.39 U/mL), was homogenized using Ultra Turrax T-50 (IKA, Works do Brasil, RJ), at 10,000 rpm for 1 min and it was pulverized in a $CaCl_2$ solution (2%, m/v) with an air pressure of 0.15 kgf/cm^2. The distance between the pulverizer and the ionic solution was set at 12 cm. The microcapsules formed were kept in the $CaCl_2$ solution for 30 min and washed with cold distilled water. The effect of the addition of fat (2%, m/m), composed of butter and oleic acid (1:1, m/m), on immobilization of glucosyltransferase in microcapsules of low-methoxyl pectin was also studied. Samples of microcapsules with and without fat addition were lyophilized. The morphology and the glucosyltransferase activity of microcapsules was studied as described below.

2.4.1. Morphology and microstructure of the microcapsules

The particle size and distribution of the microcapsules containing glucosyltransferase were analyzed with a NIKON optical microscope (Eclipse E800 – Japan). Image Pro Plus 4.0 software was used for image capture. The morphology of the lyophilized microcapsules was observed by scanning electron microscopy. The encapsulated samples were fixed in stubs on a double-faced metallic tape and covered with a layer of gold using a Balzer's evaporator (model SCD050, Baltec, Liechtenstein, Austria) for 120 s, while applying a current of 40 mA. Observations were made using the scanning electron microscope (JEOL, JSM-T300, Tokyo, Japan) at an accelerating voltage of 10 kV (Rosenberg and Young, 1993).

2.4.2. Determination of glucosyltransferase activity in microcapsules of low-methoxyl pectin

Erlenmeyers flasks containing 20 g of glucosyltransferase immobilized in microcapsules of low-methoxyl pectin and 100 mL of 10% (m/v) sucrose in distilled water were incubated at 33 °C for 30 min. The glucosyltransferase activity was determined as described by Park et al. (1996). The reducing sugars formed from sucrose were measured by the Somogyi method (Somogyi, 1945). Glucosyltransferase immobilized in microcapsules with and without fat addition, both lyophilized and non-lyophilized, were used in six consecutive batches.

2.4.3. Conversion of sucrose into isomaltulose using microcapsules of low-methoxyl pectin containing glucosyltransferase

The mixture of 50 mL of 2% (m/m) of amidated low methoxyl pectin solution, 2 g of fat (butter and oleic acid, 1:1, m/m) and 48 mL of glucosyltransferase (23.39 U/mL) was homogenized and the microcapsules were prepared as described in Section 2.4. Erlenmeyers flasks (500 mL) containing 20 g of immobilized glucosyltransferase and 160 mL of 10% sucrose solution in distilled water was incubated at 22 °C and 130 rpm. The microcapsules were reused in twelve batches of 8 h and the sugars were determined as described in Section 2.5.

Table 2
Second central composite design 2^2 coded to study the effect of pH and enzyme concentration on the immobilization process of glucosyltransferase onto Celite treated with 0.1% (m/v) of glutaraldehyde solution for the conversion of sucrose into isomaltulose (one batch of 2.5 h, at 33 °C and 130 rpm).

Assay	Variables		Glucose (%)	Fructose (%)	Trehalulose (%)	Sucrose (%)	Isomaltulose (%)
	pH	Enzyme (U/g of Celite)					
1	−1 (4.2)	−1 (65.9)	1.95	0.802	23.74	18.75	54.76
2	+1 (5.3)	−1 (65.9)	1.25	ND	17.28	48.81	32.76
3	−1 (4.2)	1 (215.5)	3.15	3.00	27.52	1.88	64.46
4	+1 (5.3)	1 (215.5)	2.10	0.91	25.61	18.35	53.02
5	−1.41 (4.0)	0 (104.7)	3.43	1.56	21.69	10.43	62.89
6	+1.41 (5.5)	0 (104.7)	1.68	ND	20.96	35.27	42.09
7	0 (4.7)	−1.41 (35.2)	1.82	ND	20.39	34.87	42.91
8	0 (4.7)	+1.41 (246.2)	2.41	0.95	28.31	6.57	61.78
9	0 (4.7)	0 (104.7)	2.22	1.78	25.31	14.72	55.97
10	0 (4.7)	0 (104.7)	2.21	0.96	23.25	17.33	56.25
11	0 (4.7)	0 (104.7)	2.12	ND	17.33	17.18	56.05

Table 3
Analysis of variance of the effect of pH, enzyme concentration and glutaraldehyde concentration on the immobilization process of glucosyltransferase onto Celite, for conversion of sucrose into isomaltulose. Analysis of the first CCD.

Source of variation	Sum of squares	Degrees of freedom	Mean squares	*F* test
Regression	789.94	7	112.85	18.10
Residues	56.10	9	6.23	
Lack of fit	53.93	7		
Pure error	2.17	2		
Total	846.04	16		

$R^2 = 0.9337$; $F_{0.90;7;9} = 2.51$.

2.5. Carbohydrate analyses by Dionex liquid chromatograph

Carbohydrate analyses were performed in a Dionex-DX600 chromatograph (Dionex Corporation, Sunnyvale, CA) equipped with an IP25 isocratic pump and ED50 gold electrochemical detector. The sugars were separated using a CarboPac™ PA 1 (4 mm × 250 mm) column, CarboPac™ PA 1 (4 mm × 50 mm) guard column, and 250 mM sodium hydroxide solution as the mobile phase, with a 1 mL/min flow, at 20 °C. The carbohydrates were analyzed by retention time, as compared with fructose, glucose, sucrose and isomaltulose standards (Sigma Ultra®, Sigma Chemical Co., St. Louis, MO).

3. Results and discussion

3.1. Immobilization of glucosyltransferase onto Celite 545

A considerable variation in the results was observed in the conversion of sucrose into isomaltulose using different samples of immobilized glucosyltransferase onto Celite 545, in the first CCD (Table 1). This indicates the significant influence of variables on the immobilization of glucosyltransferase onto Celite 545. Statistical analyses were carried out in the first batch, and all independent variables were statistically significant with a 90% confidence level ($p < 0.1$). Isomaltulose formation was analyzed in the other two batches, for all the assays, to evaluate the importance of glutaraldehyde in the retention of glucosyltransferase onto Celite 545. In assay number 13, with no glutaraldehyde treatment, the conversion of sucrose into isomaltulose decreased throughout the other two batches, indicating that the presence of glutaraldehyde was important for enzyme retention. In assay number 3 the conversion of sucrose into isomaltulose had the highest value (21.92%) of all three batches, compared to the other assays. In this assay the concentration of the glucosyltransferase was of 87.0 U/g of Celite 545, the pH value equal to 5.5 and the treatment with 0.1% of glutaraldehyde. The mathematical model can be obtained according to the analysis of variance analysis (ANOVA) and *F* test (Table 3). The R^2 was equal to 0.93; the model is illustrated in Eq. (3).

$$\begin{aligned}\text{Isomaltulose}(\%) = {} & 3.83 - 5.53 \cdot \text{pH} + 1.50 \cdot \text{pH}^2 + 2.88 \cdot \text{enzyme} \\ & -2.88 \cdot \text{glutaraldehyde} + 1.57 \cdot \text{glutaraldehyde}^2 \\ & -3.05 \cdot \text{pH} \cdot \text{enzyme} + 1.71 \cdot \text{pH} \cdot \text{glutaraldehyde}\end{aligned} \quad (3)$$

As observed in Fig. 1, the highest conversion of sucrose into isomaltulose values were achieved when the concentration of enzyme was higher, and pH and glutaraldehyde concentrations were lower. However, the treatment of the immobilized enzyme with glutaraldehyde was considered important to maintain isomaltulose production. Thus for the second CCD (CCD 2^2) the samples of immobilized enzyme onto Celite 545 were treated with 0.1% of glutaraldehyde. Only one batch of 2.5 h was carried. The highest conversion value (64.46%) was achieved in assay number 3, when the pH value was equal to 4.2 and the enzyme concentration was 215.5 U/g of Celite 545. Conversely, the lowest conversion value (32.76%) was observed in the assay number 2, where the pH value was 5.3 and the concentration of enzyme 65.9 U/g of Celite

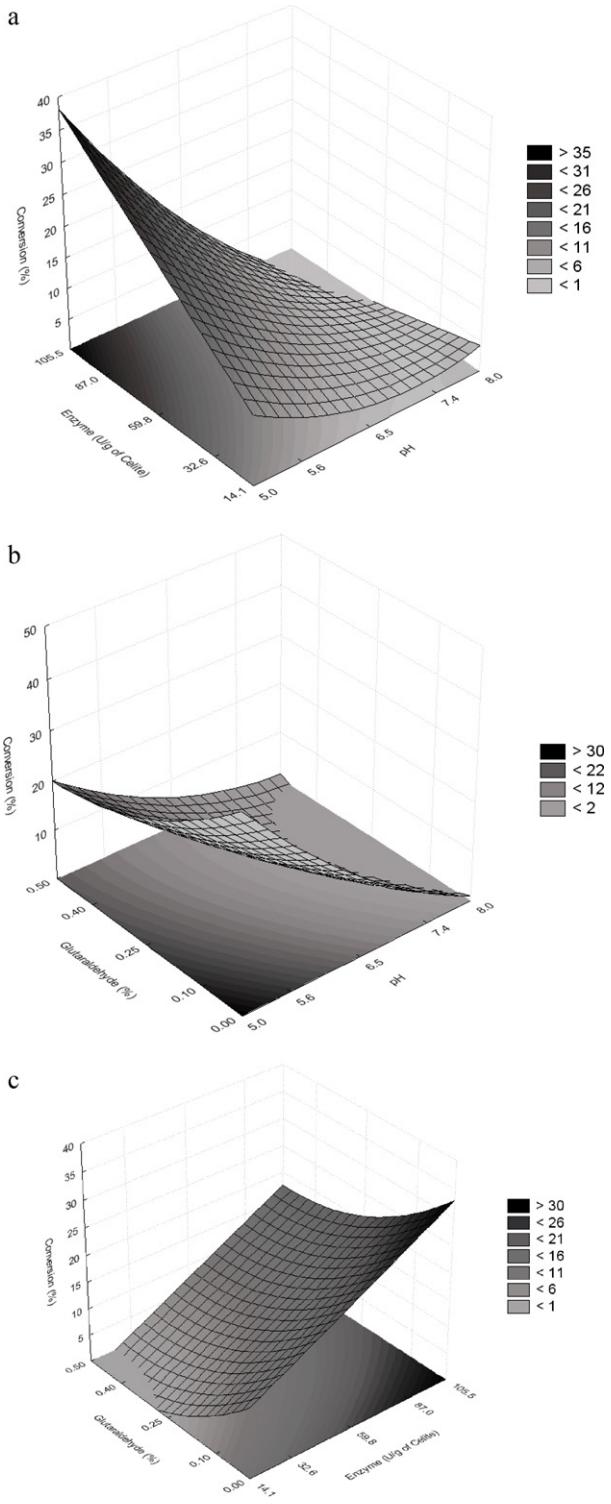

Fig. 1. Response surfaces and contour plots of pH, enzyme concentration and glutaraldehyde effect on the immobilization process of glucosyltransferase onto Celite 545, for conversion of sucrose into isomaltulose. The statistical analyses were carried out only in the first batch. (a) pH × enzyme, (b) glutaraldehyde × pH, and (c) glutaraldehyde × enzyme.

Table 4
Analysis of variance of the effect of pH and enzyme concentration on the immobilization of glucosyltransferase onto Celite, treated with 0.1% (m/v) glutaraldehyde on the conversion of sucrose into isomaltulose. Analysis of the second CCD.

Source of variation	Sum of squares	Degrees of freedom	Mean squares	*F* test
Regression	962.65	5	192.53	164.47
Residues	5.85	5	1.17	
Lack of fit	5.81	3		
Pure error	0.04	2		
Total	968.51	10		

$R^2 = 0.9939$; $F_{0.95;5;5} = 5.05$.

545 (Table 2). In an attempt to retain the enzyme onto the support, glutaraldehyde was added in immobilization process. Several manuscripts report the use of glutaraldehyde for immobilization of different enzymes through the covalent bond formed between the amine groups of the enzymes (NH2-Enz) and carriers (NH2-R). (Chae et al., 1998; Palomo et al., 2007.) In this work, considering that the support was not activated the covalent bond between enzyme and support was not expected. On the other hand it was expected that the protein molecules formed cross-links with other protein molecules and then cross-linked proteins would be adsorbed onto Celite 545. In addition, it was expected that the glutaraldehyde might decrease enzymatic activity, as observed in other experiments with lipase immobilized onto Celite by adsorption followed by the use of glutaraldehyde (Khare and Nakajimab, 2000).

The mathematical model (Eq. (4)) is obtained with ANOVA with 95% confidence level ($p < 0.05$). The *F*-test indicated that the model was statistically significant and the R^2 was equal to 99.39% (Table 4). It can be observed in Fig. 2 that when the glucosyltransferase was immobilized onto Celite 545 using a buffer with the lowest pH value (4.0) and almost the highest glucosyltransferase concentration (170.0–226.3 U/g of Celite 545), the sucrose conversion into isomaltulose would be higher than 60%.

$$\text{Isomaltulose}\,(\%) = 56.09 - 7.87 \cdot \text{pH} - 2.1 \cdot \text{pH}^2 + 7.09 \cdot \text{enzyme} - 2.17 \cdot \text{enzyme} + 2.64 \cdot \text{pH} \cdot \text{enzyme} \quad (4)$$

Kawaguti et al. (2006) reported the influence of the agents polyethyleneimine and glutaraldehyde in the immobilization of *Erwinia* sp. D12 cells on calcium alginate for the conversion of sucrose into isomaltulose. The authors observed that only glutaraldehyde was efficient in the immobilization process. The conversion of sucrose into isomaltulose showed a minimum of 38.99% in the second assay (0.65%-polyethylenimine and 0.02% of glutaraldehyde) and a maximum of 72.27% in the fifth assay (0%-polyethylenimine and 0.06% of glutaraldehyde).

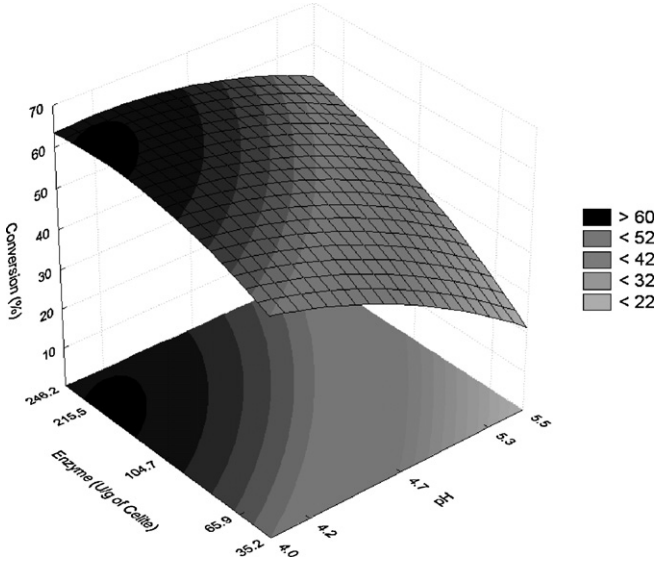

Fig. 2. Response surface and contour plot of pH and enzyme concentration on immobilization process of glucosyltransferase onto Celite 545, for conversion of sucrose into isomaltulose.

Mundra et al. (2007) described the immobilization of cells of *Erwinia rhapontici* NCPPB 1578 on calcium alginate in conversion of sucrose into isomaltulose. The authors observed that the maximum yield, corresponding to 140 mg/mL of isomaltulose, was achieved in a batch of 1 h, at alginate concentration of 5% (w/v), cell loading of 5 g/L and bead diameter of 2.25 mm.

3.2. Immobilization of glucosyltransferase on microcapsules of low-methoxyl pectin

3.2.1. Morphology and microstructure of the microcapsules

The microcapsules of low methoxyl pectin containing immobilized glucosyltransferase presented a spherical shape. The microcapsules without fat (Fig. 3a) showed average size of 173 ± 88 μm, while the microcapsules with fat (Fig. 3b) showed average size of 196 ± 88 μm. As can be seen in the Fig. 3b, the fat material was internalized in the microcapsules, therefore the microcapsules were expected to be larger in comparison to the microcapsules without fat addition. Fig. 4 shows the electronic scan microscope analysis of lyophilized microcapsules with and without fat before they were applied on the batches for conversion of sucrose into isomaltulose. The microcapsules with fat addition (Fig. 4b) presented a more preserved structure compared to the

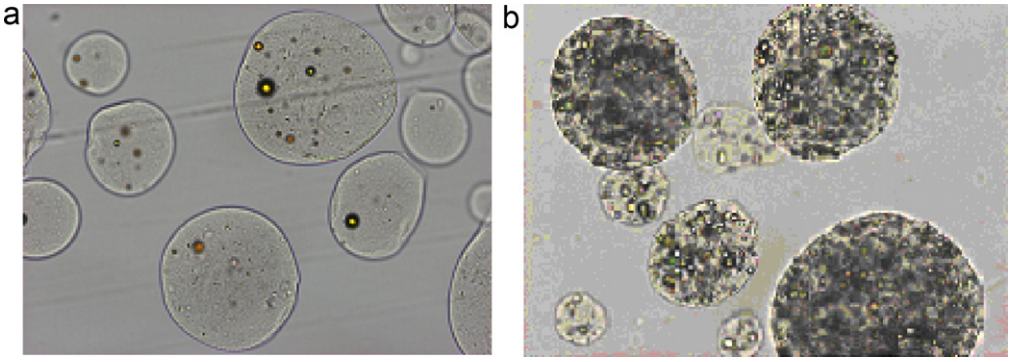

Fig. 3. Optical microscopy analyses of non-lyophilized microcapsules of low methoxyl pectin containing crude glucosyltransferase before six batches of conversion of sucrose into isomaltulose (magnification 10×). (a) Microcapsules without fat addition; and (b) microcapsules with fat addition.

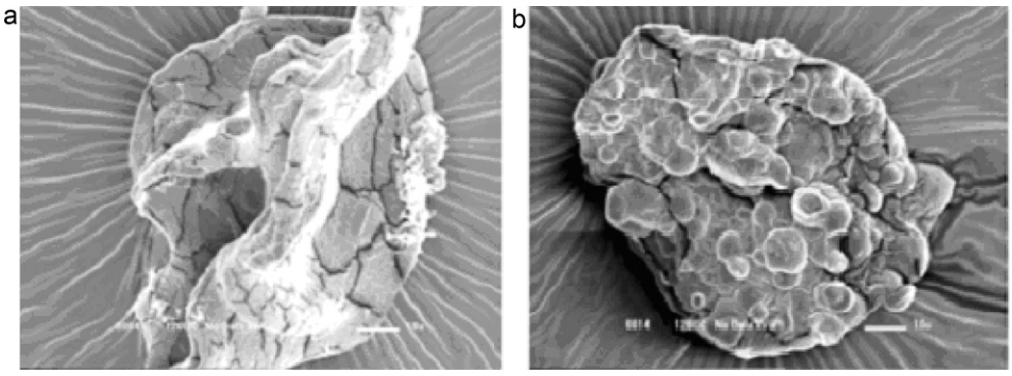

Fig. 4. Micrograph of lyophilized microcapsules of low methoxyl pectin containing glucosyltransferase (magnification 1200×). (a) Microcapsules without fat addition; and (b) microcapsules with fat addition.

microcapsules without fat (Fig. 4a), indicating the advantage of the addition of the lipophilic material in retaining glucosyltransferase.

3.2.2. Retention of enzymatic activity of immobilized glucosyltransferase

The microcapsules of immobilized glucosyltransferase prepared with low-methoxyl pectin and 2% fat (50% butter and 50% oleic acid) showed higher enzyme activity than lyophilized and non-lyophilized microcapsules of immobilized glucosyltransferase prepared with low-methoxyl pectin without fat addition (Fig. 5). According to the literature, lyophilization can cause pronounced structural perturbations in the enzyme molecule, often leading to deactivation (Sheldon, 2007). The addition of fat in the preparation of microcapsules of immobilized glucosyltransferase with low-methoxyl pectin increased the retention of the enzyme, however the activity of the enzyme decreased after the third batch.

3.2.3. Conversion of sucrose into isomaltulose using microcapsules of low-methoxyl pectin containing glucosyltransferase

Fig. 6 shows the results of conversion of sucrose into isomaltulose using glucosyltransferase immobilized in microcapsules of low-methoxyl pectin with fat addition. In the first batch the conversion value was almost 30%. However, the conversion capacity in further batches decreased. In the second batch a conversion value of 25% was observed. After nine batches the conversion values were lower than 5%. The decrease in the conversion rate of sucrose into isomaltulose was probably due to loss of the enzyme from the microcapsules or the inactivity of the enzyme during the tests.

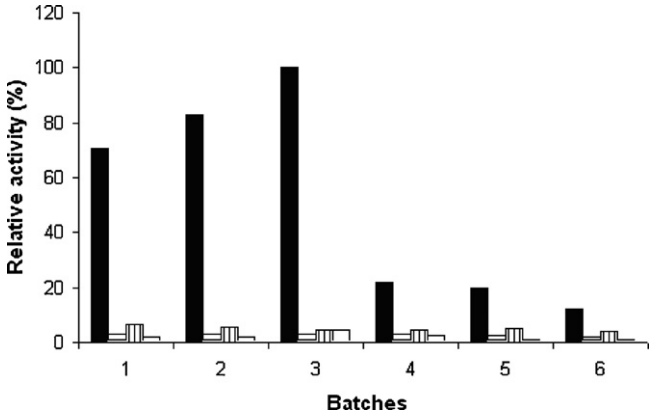

Fig. 5. Relative glucosyltransferase activity of the different low methoxyl pectin microcapsules containing glucosyltransferase after six batches of 30 min each of conversion of sucrose into isomaltulose. (■) Microcapsules with fat; (▤) microcapsules without fat; (▣) lyophilized microcapsules with fat; and (□) lyophilized microcapsules without fat.

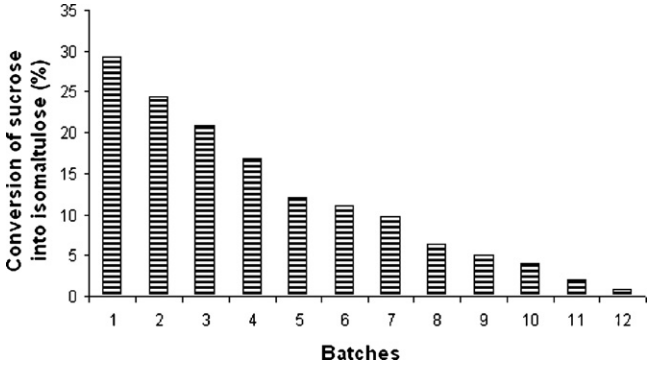

Fig. 6. Conversion of sucrose into isomaltulose using microcapsules with fat containing glucosyltransferase in the batch system (30 min each) at 33 °C.

There are few works published on the immobilization of glucosyltransferase, which converts sucrose into isomaltulose, extracted from the cell. Kawaguti and Sato (2007) studied the immobilization of *Erwinia* sp. D12 whole cells, an extract containing disrupted cells and enzyme, and also an enzymatic extract, on 2% calcium alginate. All the three immobilized preparations were then treated with 0.06% of glutaraldehyde and applied for conversion of 35% sucrose solution into isomaltulose using 24 h batches. Immobilized whole cells converted 64.6% of sucrose into isomaltulose in the first batch, which was almost identical to the results using immobilized disrupted cells (62.6%). Immobilized glucosyltransferase in calcium alginate showed a conversion value of 43.1% in the first batch. All preparations showed a decrease in their conversion of sucrose into isomaltulose in the further batches.

4. Conclusion

Celite 545 presented the most interesting results of conversion of sucrose into isomaltulose when compared with the enzyme immobilized in pectin microcapsules plus fat. The optimized conditions in immobilization of the glucosyltransferase onto Celite 545 were pH value of 4.0 and 170.0 U of glucosyltransferase/g of Celite 545, resulting in more than 60% of conversion of sucrose into isomaltulose. In the immobilization of the glucosyltransferase by its entrapment in microcapsules of low methoxyl pectin, the presence of lipophilic material was important in retaining glucosyltransferase activity. The lyophilization of microcapsules decreased the activity of glucosyltransferase. The non-lyophilized microcapsules containing glucosyltransferase entrapped in low methoxyl pectin and fat showed 30% conversion of sucrose into isomaltulose. The

conversion values decreased, indicating that the immobilized glucosyltransferase shows low stability.

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