O6-3

Production of isomaltulose by *Serratia plymuthica* cells immobilized.

Carvalho PH. [1#], Kawaguti HY. [1] and Sato HH. [1 *]
[1] Food Science Department, Faculty of Food Engineering, State University of Campinas (UNICAMP), P.O. Box 6121, Campina, SP, Brazil
* Supervisor # prihoff@fea.unicamp.br

INTRODUCTION AND OBJECTIVES

Isomaltulose is a disaccharide industrially obtained from sucrose using immobilized cells of glucosyltransferase-producing microorganisms (Wu et al., 2005). Isomaltulose is found in small concentrations in sugarcane and shows low cariogenic potential. The present study aimed at determining the effect of adding transglutaminase and gelatine in increasing the retention of *Serratia plymuthica* cells immobilized in calcium alginate. The effects of temperature and substrate flow on the conversion of sucrose into isomaltulose and trehalulose were tested. The immobilization of enzymes or microbial cells offers advantages such as the possibility of continuous processing and greater facility in reusing the enzyme.

Many polymerization agents are available but the use of enzymes to form cross-links between the proteins and increase reticulation of the immobilization matrix could be more adequate than the chemical process. The application of hydrocolloids using the interaction between polysaccharides and proteins has been studied for use in capsules by the pharmaceutical industry and in applications for dairy products with and without the use of the enzyme transglutaminase (MTGase) (MATIA-MERINO et al., 2004). MTGase forms cross-links between the lysine and glutamine residues, and is indicated as a substitute for reticulating agents not permitted in food processing (SYNOWIEKI et al., 2006; PARTANEN et al., 2009). It was shown in the present study that the addition of MTGase and gelatine in the immobilization of *S. plymuthica* cells in calcium alginate increased the stability of the immobilized cells. Using a 35% solution of granulated sugar, the best results were obtained at a temperature of 25ºC and flow rate of 0.2 mL/min, obtaining a mean conversion of 63% isomaltulose and 9% trehalulose during six days.

MATERIALS AND METHODS

Production of cell mass by the bacterium Serratia plymuthica

The strain *S. plymuthica* was cultivated in a 6.6L Bioflow IIC fermenter (New Brunswick Scientific, Edison, NJ, USA) as described by KAWAGUTI et al. (2010b). The culture medium was centrifuged at 9,600 x g for 15 minutes at 5ºC. The cell mass was washed twice with distilled water before use in the immobilization process.

Immobilization of Serratia plymuthica cells

The application of Activa TG® transglutaminase (Ajinomoto) in the immobilization of *S. plymuthica* cells in 2% sodium alginate (m:v) was studied. Three immobilization matrixes were prepared in duplicate: Matrix 1: 0.5% gelatine and 1% MTGase; Matrix 2: 0.5% gelatine and 0% MTGase; Matrix 3: 0% gelatine and 0% MTGase. The 34% (m:m) moist *S. plymuthica* cell mass was added to the alginate-gelatine solution in the proportion of 1:2 (v:v), and 3.5% (m:v) of the enzyme MTGase then added with mild stirring until completely homogenised. The cell suspension was dripped into a 0.25 mol/L $CaCl_2$ solution and immobilized as described by KAWAGUTI et al. (2010b). Seven gram samples of the immobilized cell granules were incubated with 35 mL of 35% sucrose solution in 250 mL conical flasks at 27ºC and 50 rpm. The samples of 35 mL of 35% sucrose solution were renewed every 24 hours and the reducing sugar content determined as described by ORSI (2008). From the results obtained in the first test a new matrix composed of a mixture of 1.7% (m:v) alginate, 0.5% (m:v) gelatine and 1% MTGase was used for cell immobilization in the study of the continuous conversion of sucrose into isomaltulose. Ninety-six gram samples of granules containing the immobilized cells were transferred to jacketed columns (30 x 150 mm) and a 35% (m:v) sucrose solution (granulated sugar) circulated in an ascending direction through the packed bed columns at 0.2 and 0.3 mL/min. Four columns were prepared: Column 1: flow of 0.3 mL/min at 25ºC; Column 2: flow of 0.2 mL/min at 25ºC; Column 3: flow of 0.3 mL/min at 27ºC; Column 4: flow of 0.2 mL/min at 27ºC. One milliliter samples were collected every 12 hours and frozen for subsequent analysis of the carbohydrates.

Carbohydrate analysis

The carbohydrates were analyzed in a DIONEX DX-600 chromatograph (Dionex Corporation, 1228 Titan Way Sunnyvale, CA, USA) as described by Kawaguti et al. (2010a).

RESULTS AND DISCUSSION

It was shown that the addition of 1% MTGase and 0.5% gelatine increased the stability of the *S. plymuthica* cells immobilized in calcium alginate (Figure 1).

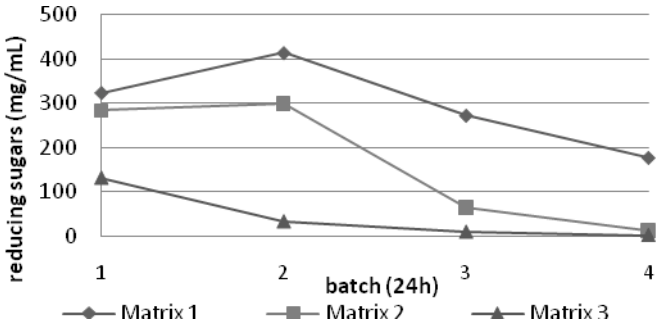

Figure 1: Conversion of sucrose into reducing sugars by *S. plymuthica* cells immobilized in matrixes with different compositions

In the continuous conversion of sucrose into isomaltulose at 25ºC using substrate flows of 0.3mL/min (column 1) and 0.2mL/min (column 2) the *S. plymuthica* cells immobilized in calcium alginate-gelatine- transglutaminase presented mean conversion rates of between 63.17 and 64.03% of isomaltulose (Figure 2 and Table 1). In the conversion of a 35% sucrose solution at 25ºC with a flow rate of 0.2 mL/min substrate, the mean composition obtained was 64.03% isomaltulose, 7.43% trehalulose, 4.0% glucose, 3.58% fructose and a residual sucrose content of 16.71%. The use of a greater substrate flow rate (0.3 mL/min) (column 1) resulted in a greater decrease in the conversion of sucrose into isomaltulose as compared with the column using a flow rate of 0.2 mL/min (column 2), obtaining, respectively, 36.22% and 50.63% (Figure 2) isomaltulose after 200 hours.

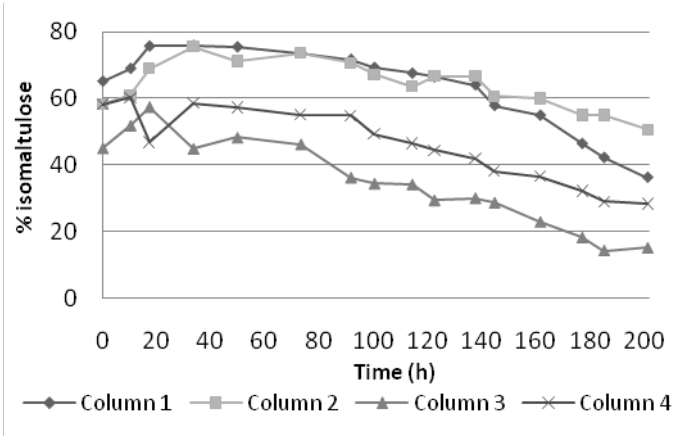

Figure 2: Conversion of sucrose into isomaltulose by *S. plymuthica* cells immobilized in alginate-gelatine-MTGase

The *Serratia plymuthica* cells immobilized on columns 3 and 4 and incubated at 27ºC presented mean conversions of 34.83% and 46.12% isomaltulose, respectively (Figure 2 and Table 1). A greater conversion of sucrose into isomaltulose was observed when a temperature of 25ºC was used, indicating that the glucosyltransferase of *S. plymuthica* was unstable at 27ºC. In a batch process, KAWAGUTI et al. (2010a) showed that the optimum temperature for the conversion of sucrose into isomaltulose by free *S. plymuthica* cells was around 25ºC.

Table 1: Mean conversion of sucrose into isomaltulose, trehalulose and other sugars during ten days of conversion in a continuous process

% Sugars	Average			
	Column 1	Column 2	Column 3	Column 4
Isomaltulose	63.17	64.03	34.82	46.12
Trehalulose	7.31	7.43	3.44	4.63
Sucrose	17.54	16.71	41.67	29.12
Glucose	3.89	4.00	2.77	3.83
Fructose	3.53	3.58	2.08	2.96

CONCLUSIONS

In the study of the conversion of a 35% sucrose solution into isomaltulose in columns containing *S. plymuthica* cells immobilized in calcium alginate-gelatine-MTGase, the best results were obtained using a temperature of 25ºC and flow rate of 0.2 mL/minute, obtaining a mean conversion of 64.03% isomaltulose, 7.43% trehalulose, 4.0% glucose, 3.58% fructose and 16.71% of residual sucrose during 200 hours.

REFERENCES

- KAWAGUTI H.Y. *et al*. (2010a) *Isomaltulose production by free cells of Serratia plymuthica in a bach process*. Food Chemistry, 120, 789-793.
- KAWAGUTI H.Y. *et al*. (2010b) *Effect of concentration and substrate flow rate on isomaltulose production from sucrose by Erwinia sp. cells immobilized in calcium-alginate using packed bed reactor.* Appl. Biochem Biotechnol. 162, 89-102.
- MATIA-MERINO L. *et al.* (2004) *Effects of low metoxyl amidated pectin and ionic calcium rheology and microstructure of acid-induced sodium caseinate gels*. Food Hidrocolloids. 18, 271-281.
- ORSI D.C.(2008) *Produção de isomaltulose a partir de sacarose utilizando a bactéria Serratia plymuthica*. Doutorado. Universidade Estadual de Campinas, Campinas, S.P.
- PARTANEN R. *et al*. (2009) *Effect of transglutaminase-induced cross-linking of sodium caseinate on the properties of equilibrated interfaces and foams*. Colloids and surfaces A: physicochemical and engineering aspects. 334, 79-85.
- SYNOWIECKI J. *et al*. (2006) *Immobilization of thermostable β-glucosidase from Sulfolobus shibatae by cross-linking with transglutaminase.* Enzyme and Microbial Technology. 39, 1417-1422.
- WU L. *et al*. (2005) *Characterization of the highly efficient sucrose isomerase from Pantoea dispersa UQ68J and cloning of the sucrose isomerase gene.* Applied and Environmental Microbiology. 71(13), 1581-1590.