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Short communication

Hydrolysis of milk/whey lactose by β galactosidase: A comparative study of stirred batch process and packed bed reactor prepared with calcium alginate entrapped enzyme

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ABSTRACT

Calcium alginate entrapped β galactosidase preparations were used for the hydrolysis of lactose from solution, milk and whey in batch processes as well as in continuous packed bed columns. The efficiency of columns, containing calcium alginate entrapped soluble and crosslinked concanavalin A complex of β galactosidase was examined at various flow rates at room temperature 32 °C, for the continuous hydrolysis of 0.1 M lactose for over 2 months. From the kinetic study the Michaelis constant (K_m) were found to be 2.51 mM and 5.18 mM for the free and immobilized β galactosidase, respectively. The V_{max} for the soluble enzyme and immobilized preparation was 4.8×10^{-4} mol/min and 4.2×10^{-4} mol/min, respectively. Furthermore, we found that entrapped crosslinked Con A–β galactosidase was more efficient in the hydrolysis of lactose present in milk (77%) and whey (86%) in batch processes as compared to the entrapped soluble β galactosidase.

1. Introduction

Enzymatic hydrolysis of lactose is an important biotechnological process because the hydrolyzed products can be consumed by lactose maldigesters [1]. The enzyme β-D-galactosidase galactohydrolase (β galactosidase, E.C.3.2.1.23 lactase) hydrolyzes lactose into glucose and galactose [2]. There are basically two different ways to use β galactosidases. The soluble enzyme is normally used for batch processes while the immobilized form lends itself to continuous operation [3]. Despite the high cost of enzyme attachment, immobilized β galactosidase systems remain more economically feasible than free enzyme systems, as these processes may be performed continuously and offer the possibility of reutilizing the enzyme [4]. Various types of supports and techniques have been used for β galactosidase immobilizations and their applications [5]. Reactors such as fluidized fixed bed, hollow fibre, plug flow, capillary bed and rolled membrane all containing immobilized β galactosidases have been reported for lactose hydrolysis [6,7]. Various β galactosidases has also been immobilized on supports such as cotton, alumina, silanized porous glass, phenol formaldehyde resin, corn grits, Sepharose 4B and fructogel derivatives [8–10]. Membrane reactors often lower enzymatic activity in comparison to the use of soluble enzyme in batch reactors [7]. Other researchers have studied the behavior of soluble enzymes in hollow fibre reactors and have proposed kinetic models considering an equilibrium constant of adsorption [11]. It has also been seen that the enzyme adsorbed reversibly onto the hollow fibre membrane suffered conformational changes [12]. In many of these cases the result has been considerable reduction in enzyme activity as well as binding capacity. In addition many of these supports are very expensive, mechanically poor in terms of rigidity, hardness and flexibility and also subject to microbial attack [13]. The process for immobilization should be mild enough so that during immobilization enzyme would not get denatured. Immobilization has great potential in food bioprocessing and has been widely used for hydrolysis of lactose in milk, milk products and whey. The use of packed bed reactors in biological processes would allow the application of new methodologies to transform an environmental problem, such as permeate whey elimination of dairy industries, in a commercial affair [14]. The choice of lactose hydrolysis in batch and continuous mode depends primarily on the enzymatic characteristics and the economics encompassing the production, storage and reusability [15].

Abbreviations: Con A, concanavalin A; ONPG, *o*-nitrophenyl β-D-galactopyranoside; K_m, Michaelis constant; V_{max}, maximum velocity; ε_m, molar extinction coefficient.

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In the present paper reactor efficiency and operational stability both in terms of continuous and batch processes have been studied by taking an inexpensive support of Con A and calcium alginate. The purpose of our present work is to set up a large-scale model for the industrial application of hydrolyzing milk and whey.

2. Materials and methods

2.1. Materials

Aspergillus oryzae β galactosidase (3.2.1.23) and lactose were obtained from Sigma Chem. Co. (St. Louis, MO, USA). Sodium alginate was the product of Merck, India. Jack bean meal was purchased from DIFCO (Detroit, USA). Whole milk was purchased from local dairy industry. All other chemicals and reagents used were of analytical grade and were used without further purification.

2.2. Preparation of alginate beads

The soluble and crosslinked Con A–β galactosidase complex were mixed independently with 5.0% aqueous sodium alginate solution and added drop wise to a stirred solution of 0.2 M $CaCl_2$ prepared in distilled water. A 5.0-mL syringe with attached needle number 20 was used for the preparation of calcium alginate beads [15].

2.3. Hydrolysis of lactose using packed bed columns

Calcium alginate entrapped crosslinked Con A–β galactosidase (1525 U) was packed in a (2.0 cm × 10 cm) column. A column of similar dimensions containing alginate entrapped soluble β galactosidase (1500 U) was also prepared. The packed volume of the column was 8 mL. Lactose (0.1 M) dissolved in 0.1 M sodium acetate buffer; pH 4.6 containing 0.001 M sodium azide was passed through both the columns at different flow rates at room temperature (32 °C).

2.4. Assay of β galactosidase

β galactosidase activity of the enzyme was determined by measuring the release of *o*-nitrophenol from ONPG (*o*-nitrophenyl β-D-galactopyranoside) at 405 nm. The reaction was carried out in a total volume of 2.0 mL containing 1.7 mL of 0.1 M sodium acetate buffer, pH 4.6, 0.1 mL suitably diluted enzyme and 0.2 mL of 20 mM ONPG at 32 °C for 15 min. The reaction was stopped by adding 2.0 mL 2.0N sodium carbonate solution [15].

One unit of β galactosidase activity is defined as the amount of enzyme that liberates 1.0 μmol of *o*-nitrophenol (ε_m = 4500 L mol^{-1} cm^{-1}) per min under standard assay conditions.

2.5. Glucose estimation

An appropriate amount of β galactosidase treated lactose, suitably diluted with 0.5 M phosphate buffer at pH 7.0 was taken. The hydrolysis of lactose was estimated by using solution C. Solution C was prepared by taking 5 mg of glucose oxidase, 1 mg of peroxidase dissolved in 5.0 mL of 0.1 M potassium phosphate buffer, pH 6.1, 15 mg *o*-dianisidine HCl prepared in 2.5 mL of distilled water and 40 mL glycerol (20%). Solution C, 1.5 mL was added in all assay tubes. The test tubes were again incubated at 37 °C for 15 min and the reaction was stopped by adding 1.0 mL of 6.0N HCl and developed color was measured at 540 nm [15].

2.6. Lactose hydrolysis in batch process

Lactose solution (500 mL, 0.1 M) was independently incubated with (400 U) of soluble β galactosidase, entrapped Con A–β galactosidase complex and crosslinked Con A–β galactosidase. Each mixture was stirred continuously in water bath at 50 °C and 60 °C. The aliquots from the reacting mixture were taken at different time intervals up to 10 h and assayed for the analysis of glucose by using glucose oxidase–peroxidase assay procedure described in Section 2.5 [15].

2.7. Hydrolysis of milk by β galactosidase in batch process

The milk was skimmed by centrifuging the cold milk at 8000 × *g* for 20 min. The fat layer was removed from milk and stored at 4 °C for further use. Skimmed milk (500 mL) was treated with β galactosidase (1000 U) in a batch process at 32 °C. The aliquots of 250 μL were taken out at indicated time intervals for 12 h. The hydrolysis of lactose was estimated by assay procedure described in Section 2.5 [15].

2.8. Hydrolysis of whey by β galactosidase in batch process

The milk was skimmed by centrifuging the cold milk at 8000 × *g* for 20 min. The fat layer was removed and whey was prepared from the skimmed milk by acidifying with HCl until the pH reached 4.8. The casein was removed by centrifugation. Prepared whey was stored at 4 °C for further use [17]. Whey (500 mL) was treated with β galactosidase (400 U) in batch process at 32 °C. The aliquots of 250 μL were taken out at indicated time intervals for 12 h. The hydrolysis of lactose was estimated by assay procedure described in Section 2.5 [15].

2.9. Protein estimation

Protein concentration was determined by the dye binding method [18]. Coomassie brilliant blue G-250 (100 mg) dye was dissolved in 50 mL of absolute alcohol by shaking till all dye went into the solution. To this 100 mL of 85% orthophosphoric acid (v/v) was added and the final volume was made up to 1.0 L with distilled water. The dye was filtered through Whatman no. 1 paper before use.

To 1 mL of the protein solution 5 mL dye solution was added and the color intensity was recorded at 595 nm after 5 min against an appropriate blank prepared similarly without protein. BSA was taken as standard protein.

2.10. Statistical analysis

The data expressed in various studies was plotted using Sigma Plot-9 and expressed as S.E. (±). Each value represents the mean for three independent experiments performed in duplicate, with average standard deviation <5%.

3. Results and discussion

3.1. Determination of kinetic constants

The Michaelis constant (K_m) and maximum velocity (V_{max}) were calculated from Lineweaver Burk plots (Table 1). It is interesting that V_{max} is affected less than K_m upon immobilization. The change in the affinity of the enzyme for its substrate is caused by lower affinity of the substrate to the active site of the immobilized enzyme [19]. Due to the entrapment of crosslinked Con A–β galactosidase K_m