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LACTOSE HYDROLYSIS BY IMMOBILIZED WHOLE CELLS OF *K. LACTIS* CBS 2357

A thesis presented in partial fulfilment of the requirements
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ABSTRACT

The application of immobilized yeast for lactose hydrolysis was investigated. The enzyme stability was tested as a function of pretreatment. The stability of *K. lactis* CBS 2357 cells after treatment with glutaraldehyde (GA) and the β-galactosidase activity of whole cells after immobilization in alginate bead and corn particles were studied.

Permeabilization using ethanol and chloroform (10% and 2%, respectively) at 37 °C and 120 rpm for 5 min, followed by stabilization with 10 mM glutaraldehyde at 30 °C for 1 hour with gently shaking deactivated 2.5% of the initial whole cells β-galactosidase activity, tested with the ONPG method. The glutaraldehyde treatment could significantly maintain β-galactosidase activity in phosphate buffer pH 6.5 containing 0.1 mM $MnCl_2$. Manganese and potassium ions in the Mn-Buffer were found to be essential to enhance the activity. The biomass activity of GA stabilized cells in Mn-Buffer can be maintained above 70% during 72 hours of incubation at 30 °C. An increase of incubation temperature from 30 to 37 °C deactivated 10% of biomass activity after 72 hours.

Direct stabilization of alginate biocatalyst with glutaraldehyde caused a significant reduction of β-galactosidase activity with the resulting deactivation depending on glutaraldehyde and alginate concentrations. When 40 g of biocatalyst containing $2x10^9$ cells/g alginate was stabilized in 100 ml of 0 to 4 mM glutaraldehyde, the optimum range of glutaraldehyde concentration was between 0.5 to 1.0 mM. When this concentration range was applied to stabilize 2%- to 3%-alginate biocatalyst, the average biocatalyst activity remained within 56-74% of the initial activity.

It was shown that the adsorption of *K. lactis* on corn particles through a "double liquid cultivation stage" followed by permeabilization of biocatalyst gave a higher activity. The activity obtained was 0.84 μmol lactose hydrolyzed /min/g biocatalyst under the conditions tested. This activity was about 5 times higher than the case without permeabilization and about 2 times higher than that of the permeabilized biocatalyst prepared with a "single liquid cultivation stage". When tested in the packed-bed reactor, during the initial stages the degree of hydrolysis (d.h.) was 45% within the operational conditions tested. Free enzyme was detected during the first 5 hours of operation, especially when non-stabilized corn biocatalyst was used. After 5 hours, free enzyme was no longer detected in the reactor outlet, suggesting that direct adsorption might have rendered good cell confinement inside the corn particles.

Bismillaahirrahmaanirrahiim

(In the name of Allah, Most Gracious, Most Merciful)

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INTRODUCTION

β-Galactosidase is one of the most studied enzymes from a scientific and technological point of view (Richmond, et al., 1981; Gekas and Lopez-Leiva, 1985; Berger, et al., 1995). This enzyme is one of the best examples for studying enzyme kinetics and modeling. This enzyme has been applied to the hydrolysis of lactose into its isomolecular mixtures comprising glucose and galactose (Carrara and Rubiolo, 1994). More recently, it has gained much importance for its application in the synthesis of some promising oligosaccharide compounds via a galactosyl transfer reaction (Berger, et al., 1995; Sheu, et al., 1998).

Lactose is found abundantly in milk and whey. There are two main reasons for the intensive research on β-Galactosidase and its substrate lactose (Santos, 1988; Carrara and Rubiolo, 1996; Siso, 1996). Firstly, lactose is a sugar fastidiously digestible by non-Caucasian people. Secondly, because whey and other dairy wastes are by-products of the dairy industry with high BOD's, they create a major problem for their disposal. In addition, the physical characteristic of lactose to be easily crystallized is troublesome in some processes in the dairy industry. To overcome this, lactose must be hydrolyzed into its more soluble constituents that offers technological advantages, such as improvement of the sweetness and solubility thereby increasing its usability.

There are two hydrolysis methods used so far. The first is the use of acid at a high process temperature (150 ^{o}C), and the second is the use of enzyme operated at a temperature between 4-70 ^{o}C. The use of enzymes has several advantages over acid hydrolysis; i.e. no brown color formation, the protein present in the substrate is not denatured, and formation of undesirable by-products can be prevented. For these reasons, enzyme use is becoming the more popular method in the food industry (Santos, et al., 1988; Siso, 1996).

β-Galactosidase (EC 3.2.1.32) is an intracellular enzyme that exists in many sources. Animal and plant sources are not taken into account for commercial use due to their high cost and low productivity. β-Galactosidases from microorganisms are the first choice because they can be produced in a large quantity. However, not all β-

Galactosidases from microorganisms can be used in the food industry, but only those which are already considered safe (GRAS). The most acceptable β-Galactosidase enzymes are derived from the yeasts *Kluyveromyces* sp. (*K. lactis, K. marxianus* and *K. fragilis*) that can be applied in neutral pH (between pH 6.5–7.0). These enzymes are suitable for the hydrolysis of milk and sweet whey.

Recently, the use of whole cell to accomplish lactose hydrolysis has gained much interest in developing the process technology for lactose hydrolysis; therefore the cost of extracting the intracellular enzyme from yeast is reduced (Siso, 1996). However, the reported results so far have shown that the stability of whole cell biocatalyst is not comparable yet to that conferred by immobilized bulk β-Galactosidase, which can maintain the degree of hydrolysis required in the process range for long periods of time.

The objectives of this research are:

- To study the activity of whole cell β-galactosidase from yeast *Kluyveromyces* sp.;
- To test the stability of treated whole cell biocatalyst on storage at operational temperatures (30 and 37 °C);
- To find an effective immobilized whole cell β-Galactosidase for lactose hydrolysis in a packed-bed reactor.