IMMOBILIZATION OF CELLS CONTAINING GLUCOSE ISOMERASE USING A MULTIFUNCTIONAL CROSSLINKING REAGENT.

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Summary: This paper describes a simple method for the immobilization of cells containing glucose isomerase enzyme activity, by using an epoxypolyamine crosslinking reagent. One kg of immobilized preparation could convert 5,700 kg of glucose to glucose-fructose syrups; the conversion was 42%.

Introduction

In recent years a great number of techniques for the immobilization of cells and enzymes have been developed. By selecting the proper carrier materials and coupling procedures, it is possible to obtain immobilized derivatives with good activity and stability. Using the epoxypolyamine (EPA), a multifunctional crosslinking reagent, offers some functional groups for crosslinking microbial cells or enzymes and also introduces a certain length of spacer groups. The result of using the crosslinking reagent will be illustrated by immobilized Actinoplanes missouriensis cells containing glucose isomerase.

Materials and methods

Chemicals and microorganism.
Epoxypolyamine reagent was purchased from a local market. The rest of the analytical reagent-grade chemicals were obtained from standard sources. The microorganism used to produce glucose isomerase was Actinoplanes missouriensis (ATCC 14538).

Culture of microorganism.
Actinoplanes missouriensis (ATCC 14538) used in this investigation was cultured in a medium containing beet molasses 1.5%, soy bean powder 2%, K2HPO4 0.2% and MgSO4 0.25%. The initial fermentation pH was 7.2 and temperature was 30°C, with shaking at 110 rpm for 48 h. The cell mass was harvested by centrifuging at 4,000 g for 20 min, followed by washing with distilled water.

Glucose isomerase assays.
For assaying glucose isomerase (EC 5.3.1.5.) activity, the actinomycyes cells were resuspended in 0.2 M maleate buffer (pH 6.8) and were disrupted by sonification. After centrifugation at 10,000 g for 10 min at 4°C, the supernatant was retained for the enzyme activity assays. Glucose isomerase activity was determined by the formation of fructose from glucose. The enzyme reaction mixture contained 4.5 ml of 0.2 M maleate buffer (pH 6.8),
5 ml of 2 M glucose containing 0.1 M MgSO$_4$ and 0.01 M CoCl$_2$, and 0.5 ml of enzyme extract. The mixture was incubated at 70°C for 1 h, and the reaction was stopped by adding 1 ml of 0.2 M perchloric acid. The final volume of the enzyme reaction mixture was made up to 25 ml with distilled water. The fructose concentration was measured by the cysteine-carbazole method (Dische et al.). One unit of glucose isomerase activity was defined as the amount of the enzyme that produce 1 /u mole of D-fructose per min under the assay conditions. Protein was determined by the method of Lowry et al.

Immobiliation.
Wet cells (10 g) were suspended in 100 ml distilled water then the EPA reagent 0.25 ml and 0.5 g MgCO$_3$ were mixed thoroughly with the cells. The mixture was stirred well and allowed to stand for 30-60 min at room temperature, then centrifuged at 4,000 g for 30 min. The precipitate was dried at 60°C and broken or shaped into 2-3 mm particles for immobilized glucose isomerase preparations.

Continuous isomerization of glucose.
A packed column of EPA crosslinked-glucose isomerase was continuously operated at 60°C for 41 days. Glucose was dissolved at 0.2 M, and the resulting substrate solution was then fortified with mineral salts. The substrate solution (60°C) was pumped through the column (2.5 x 12.5 cm) at a flow rate of 167 ml/h at highest conversion. Hot water (60°C) was pumped through a water jacket surrounding the column.

Results
Immobiliation of glucose isomerase.
Various levels of EPA crosslinking reagent were used to immobilize the cells containing glucose isomerase. It was found that levels in the range 0.15-0.25 ml per 10 g wet cells were effective in the crosslinking reaction. The immobilized glucose isomerase retained about 80% of its original activity (Table 1),

Effect of crosslinking pH on the immobilized enzyme activity.
Because the EPA reagent is an acidic solution, pH is very important in the crosslinking reaction. We found that if the pH was in the range 7.0-8.5, good enzyme activity recovery could be obtained, but if it was not in the range, the results were less good (Fig.1).

Continuous isomerization of glucose.
Fig.2 shows the result when using the immobilized glucose isomerase for the continuous isomerization of glucose. The addition of Mg$^{++}$ could enhance the stability of the enzyme. After operating at 60°C for 26 days, the remaining enzyme activity was 50%; after operating for 41 days, 10 g of immobilized enzyme preparations could convert 57,000 g of glucose (dry base) to fructose-glucose syrups and the conversion was 42%.