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Catalytic Effect of Invertase Loaded Glycidyl Methacrylate Copolymer on Inversion of Sugar by Invertase

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A novel form of invertase covalently 'immobilized' on polymer support is proposed as a reusable form of enzyme. Invertase was immobilized on to a copolymer of glycidyl methacrylate and methyl methacrylate. Polymer particles having glycidyl ether groups were prepared through seeded polymerization in aqeous medium. From the HCl-dioxane back titration method, it was found that about 8–10% of oxirane oxygen remained on the final particles. Invertase 'immobilised' on copolymer exhibited catalytic effect on sucrose inversion. The prepared immobilized invertase could completely hydrolyze sucrose in water at room temperature, at an enzyme concentration of 0.4% based on sucrose weight having invertase activity 30,000BU/mL. The enzyme was stable under stirred conditions and could be reused multiple times without loss of enzyme activity. It was found that the nature of the polymer used for enzyme conjugation had a significant effect on the activity of the invertase used.

Key Words: Invertase, immobilization, Hydrolysis, Sucrose, Polymer support.

INTRODUCTION

Immobilized enzymes may be defined as enzymes whose free movement has been restricted in some manner. One of the major advantages of immobilization is to "fix" the enzyme so as to retain it in a continuous process. Use of soluble enzymes in processing has been limited in part by the cost of the enzymes due to the difficulty and expense of isolation, instability and the fact that in a freely soluble form they usually can be used only once.

The use of immobilized enzymes has several advantages. An immobilized enzyme, in solid or sometimes semisolid form, is readily separable from the product solution and can be reused, thus increasing utility by a large factor. Additionally, the enzymic reaction can be terminated easily simply by removing the enzyme, allowing more precise control of the reaction. Finally, it has turned

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out that enzymes are often much more stable when in fixed form than in solution, allowing an enzymatic conversion to occur over a much longer period without having to replenish the biocatalyst as compared with intact enzymes. Some examples of these effects have been published[1-11].

For the production of the polymer particles having special functional moieties, dispersion homopolymerization and copolymerization have been studied by several research groups. Dispersion copolymerization of methyl methacrylate and glycidyl methacrylate has also been carried out by Lee and Chen[12].

Reactive functional groups assist the formation of a permanent covalent bond between the polymer and the enzyme without affecting its tertiary structure. Oxiranoyl group is perhaps the preferred functional group since covalent binding of enzyme proceeds under ambient, moderate conditions without significant loss of activity[13].

The pore geometry of oxirane polymers, which offer pendent epoxy groups for covalent bonding of enzyme molecule, can be manipulated by strategic interplay of synthesis variables such as crosslink density (CLD), volume of porogen and quantities of protective colloid and polymerization initiator[14].

The significance of these synthesis variables on the physical properties of glycidyl copolymers and immobilization of penicillin G acylase is documented[15].

The sucrose industry is a comparatively minor user of enzymes but provides few historically significant and instructive examples of enzyme technology. The hydrolysis ('inversion') of sucrose, completely or partially, to glucose and fructose provides sweet syrups that are more stable (*i.e.*, less likely to crystallize) than pure sucrose syrups. The most familiar 'Golden Syrup' is produced by acid hydrolysis of one of the less pure streams from the cane sugar refinery but other types of syrup are produced using yeast (*Saccharomyces cerevisiae*) invertase. Enzymatic inversion of sugar requires specific pH and temperature conditions and they were studied[18-20]. Immobilzsed invertase and whole cells of yeast have been investigated for the continuous inversion of sucrose solutions[21-24].

In this work an attempt has been made to achieve complete inversion of sucrose at neutral pH and at 30–37°C temperature. Copolymerization was done in such a way that the polymer should be porous and with sufficient oxirane oxygen for binding the enzyme. This immobilized enzyme showed a catalytic behaviour during the inversion of sucrose with invertase at room temperature and normal pH. Reusability of this copolymer was tested by performing 100 cycles.

EXPERIMENTAL

Glycidyl methacrylate (GMA) and methyl methacrylate were obtained from Lancaster. Polyvinyl alcohol, polyvinyl pyrrolidine, cyclohexanol, methanol and divinyl benzene (DVB) were obtained from Loba Chemie. Invertase was obtained from Biocon India.

Polymer preparation

The porous copolymers were synthesized by suspension polymerization in a jacketed cylindrical polymerization reactor[16, 17]. A volume of water along with

polyvinyl pyrrolidone and sodium chloride was heated in the reaction vessel at 70°C. The reaction mixture components were added rapidly at low stirring speed. The mixture contained initiator, porogen added in the preceding sequence. The addition represented zero time of the co-polymerization. At this time the temperature was about 70°C. Then the stirring speed was slowly increased; temperature remained constant (± 0.5°C) until the end of the reaction. Reaction was allowed to go for completion for 6–7 h. The beads were decanted and washed with water.

The porogen was extracted with methanol in a Soxhlet apparatus and finally dried for 24 h at 60°C. The products result as white opaque spherical beads.

Enzyme preparation and loading

The enzyme solution was prepared by taking 1 mL of enzyme having activity 30,000 BU/mL in 100 mL of water for 1% enzyme solution and 2 mL in 100 mL water for 2% enzyme solution. 10 mL of enzyme solution was used for 10 g of co-polymer for immobilization. 10 mL of 5% sugar solution was used in stirred conditions for all the experiments.

All the experiments were carried out at an ambient temperature and without adjusting the pH of the sucrose solution.

Analysis

The oxirane oxygen group was found by HCl-dioxane back titration method. The purified particles were re-dispersed into dry dioxane, after which 0.2 N HCl solution in absolute dioxane was added and the particles were left to react at room temperature for 15 min. The unreacted fraction of acid was then determined with 0.1 N methanolic sodium hydroxide solution.

Porosity determination: The porosity of the copolymer at various stages was determined by soaking the sample in water and finding out the difference in the dry and wet weight of the sample.

Inversion of sucrose was analysed by copper ion reducing method of Fehling's solution.

Immobilization of invertase on copolymer of glycidyl methacrylate

Enzyme invertase was immobilized on copolymer by keeping the polymer in the solution of enzyme for 24 h in temperature-controlled shaker. This immobilised enzyme in polymer was placed on glass column and washed thoroughly. Enzyme activity was analyzed by measuring the inversion of sugar by copper ion by reducing method of Fehling's solution.

RESULTS AND DISCUSSION

Effect of immobilized invertase on inversion of sucrose

Various concentrations of enzyme were immobilized on polymer and the inversion of 5% sugar solution was studied with and without immobilized invertase.

4 and 2% invertase on sucrose (mL/100 g) were immobilized on copolymer for 24 h.

Inversion of sugar using immobilized invertase: 4% Immobilized enzyme on copolymer showed catalytic effect (Fig. 1). The copolymer without immobilization showed no inversion. 4% enzyme inverted the 5% sugar solution in 60 min which was achieved in less than 5 min with 4% free enzyme and copolymer on which 4% invertase was immobilized.

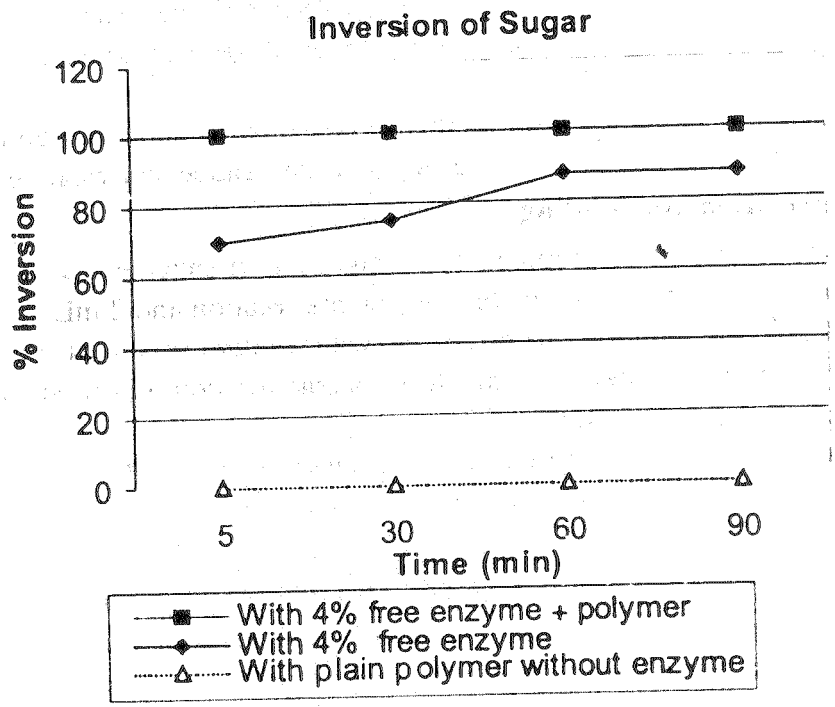

Fig. 1. Sucrose inversion with 4% (based on sucrose) free enzyme using 4% invertase immobilized on copolymer

Similar effect was observed 0.4% free invertase (Fig. 2).

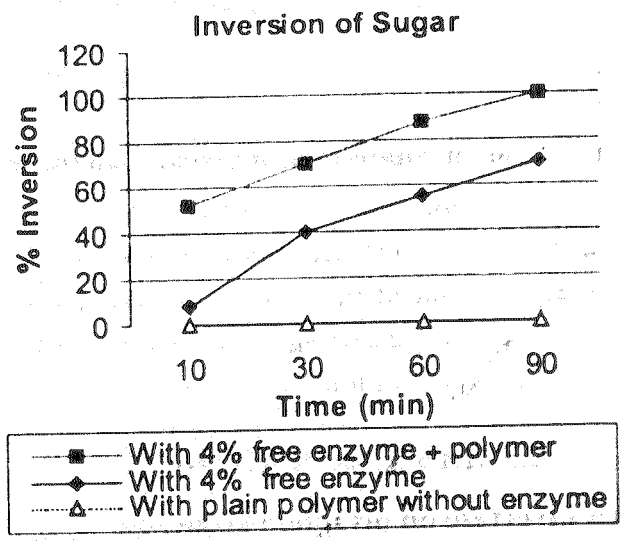

Fig. 2. Sucrose inversion with 4% free enzyme using 4% invertase immobilized on copolymer

For the case of 1% immobilization also results showed a similar pattern. The sucrose inversion is completed in less time if it is passed through the polymer (Figs. 3 and 4).

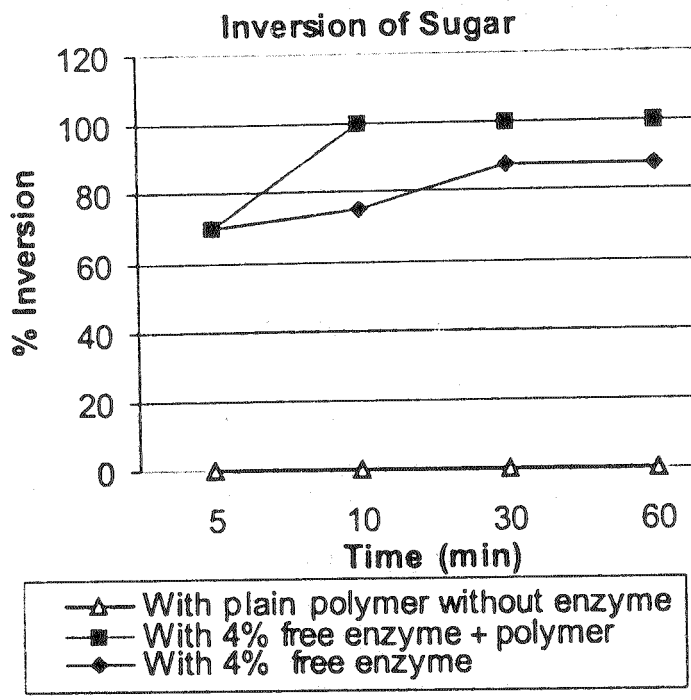

Fig. 3. Sucrose inversion with 4% free enzyme using 2% invertase immobilized on copolymer

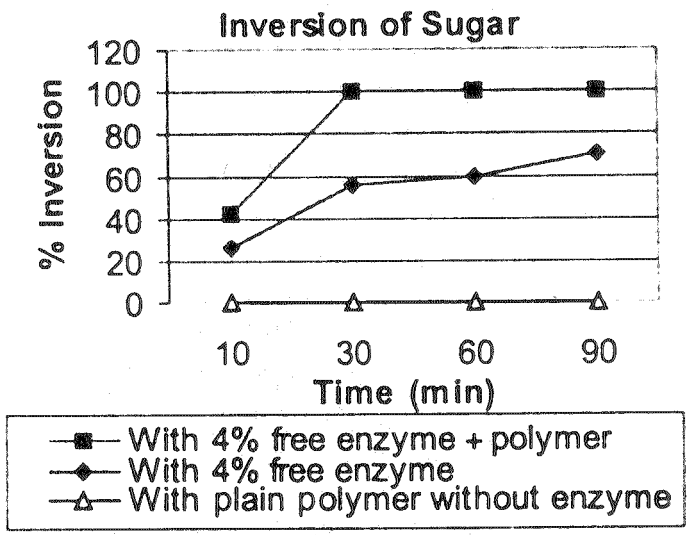

Fig. 4. Sucrose inversion with 10 mL of 4% free enzyme without using 2% invertase immobilized on copolymer

Effect of storage on immobilised invertase stability

The immobilized enzyme was stable for two months. After two months it showed reduction in catalytic activity. The above results clearly indicate that the enzyme has been loaded on the support in a manner in which it is no longer catalyzing the activity, but it appears to prepare the surface for adsorbing fresh enzyme in an orientation that increases the activity of the enzyme towards sugar inversion.

Catalytic activity of polymeric materials is known only for ion exchange resins for acid or base catalyzed reactions. Enzyme supported polymers generally show activity of enzyme itself. In the experiments supported here catalytic activity of

polymer supported enzyme in catalyzing homogenous reaction has been observed, probably for the first time.

Conclusion

Invertase loaded glycidyl methacrylate copolymer facilitates the inversion of sucrose and allows the inversion to take place without adjusting the pH and temperature. The approach has high potential for translation into a commercial process.

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