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Short Communication

Studying the effect of nature of glass surface on immobilization of glucose isomerase

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ABSTRACT

Immobilization of glucose isomerase (GI) (EC 5.3.1.5) has been carried out on non-porous glass surfaces using γ-aminopropyltriethoxysilane and polyethyleneimine as an activator and gluteraldehyde as cross linking agent for its potential use. Polyethyleneimine was found to be a superior immobilization activator than γ-aminopropyltriethoxysilane. Further, the effects of rough and smooth surfaces of non-porous glass beads on immobilization of glucose isomerase have been investigated. Polyethyleneimine on rough glass beads was found to be better activator material than that of γ-aminopropyltriethoxysilane. The prime objective of study was to immobilize glucose isomerase on an improved surface of non-porous glass beads which will give a significantly higher immobilization yield. After optimizing the parameters the immobilization yield increased from an initial value 29.43% to 79.6%.

1. Introduction

The use of immobilized enzymes in the food, pharmaceutical and chemical industries has increased steadily during the past decade. Engineering of these enzymes from biological entities to industrial reactors is a very exciting goal. The largest market for any enzyme is in its immobilized form as it allows the possibility of enzyme reuse and also improves the enzyme stability and overall process economy. Glucose isomerase (GI) (EC. 5.3.1.5) catalyzes the reversible isomerization of D-glucose to D-fructose was investigated in this study. It is widely used in immobilized form to produce high-fructose corn syrup (HFCS), one of the very valued products in food and healthcare segment. The high cost of GI principally governs the overall economics of the manufacture of HFCS. The GI is expensive because it is an intracellular enzyme and large quantities are needed to compensate for its low activity towards glucose (high Km for glucose when correlated using Michaelis Menton kinetics). The Michaelis constant (Km) of an enzyme is a measure of the affinity of the enzyme for its substrate. The value of Km for a particular enzyme is defined as the substrate concentration at which half of the enzyme molecules are complexed with the substrate. Under these conditions, at any instant, only half of the total enzyme molecules are capable of catalyzing the targeted reactions (Bhosale et al., 1996; Demirel et al., 2006). From industrial perspective, GI with the increased thermo stability, higher affinity for glucose, and lower pH optimum can contribute significantly in the development of an economically feasible commercial process for enzymatic isomerization of glucose to fructose (Levenspiel, 1999). Further, one of the effective ways to reduce cost of production of GI is to recover it efficiently from fermentation broth and reuse it several times. Immobilization of GI offers an excellent opportunity for its effective reuse and all other essential requirements which makes process economically viable. Henceforth, development of immobilized GI has been a subject of great interest (Taqieddin and Amiji, 2003).

A rich literature exits on GI immobilization on various supports. We are referring some of the recent major breakthroughs in this area as follows: As an intracellular enzyme GI immobilization efforts can be classified in two major categories as cell free immobilization and whole cell immobilization (Bhosale et al., 1996). Seyhan Tükel and Alagöz (2008) carried out the immobilization of GI on macroporous beads of Eupergit *C* 250 L showing improved catalytic efficiency after immobilization and reusability of GI without affecting its thermal and storage stabilities. Demirel et al. (2006) compared GI immobilization on different hydrogels based on polymer matrices. Dolia and Gaikar (2006) has purified GI obtained from microbial source and immobilized the same on chitosan beads. They found that immobilized GI was stable over a wide range of pH conditions (Dolia and Gaikar, 2006). Song et al. (2011) have successfully investigated improvement in the activity of the immobilized GI by employing pretreatment strategy with D-glucose or D-xylose prior to immobilization. Recently, DiCosimo et al. (2013) have reviewed economics of immobilized enzymes exclusively citing GI as an example. They have stressed on the fact

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that current industry trends should be combined with the rapid evolution of immobilized enzyme technology to develop sustainable and economically viable processes. Thus, knowing GI's commercial potential many researchers are still engaged in finding out economically viable immobilization techniques which could be globally applicable (DiCosimo et al., 2013).

The objective of the present study was to immobilize glucose isomerase with an aim to improve quantitative immobilization yield. GI was therefore, covalently immobilized onto glass beads, and the effect of various parameters including activation chemical, roughness of bead surface, method of immobilization, enzyme concentration required for immobilization was studied.

2. Materials and methods

2.1. Materials

Non-porous glass micro-beads (class IV A) of particle sizes ranging from 60 to 100 μm were procured from Indo Glass beads, Thane, India. Partially purified GI used for immobilization was produced in our laboratory by fermentation. Polyethylenimine (PEI) with a reported molecular weight range of 50–100 kD was obtained as a 50% (w/v) aqueous solution from Sigma Aldrich, Bangalore, India. Glutaraldehyde (25% aqueous solution) used in the bead derivatization and immobilization procedure was a product of S. D. Fine Chemicals, Mumbai, India. All other chemicals used were of AR grade.

2.2. Screening of activators for derivatization of glass surface

For immobilizing GI onto glass surface, two activator materials *viz*. PEI and APTES were screened for its binding efficiency as well as for the activity of the immobilized enzyme. The nature of the glass surface also plays a major role in binding of enzyme. Therefore, rough and smooth glass beads were used with above mentioned two parameters for preliminary screening. Two different methods were used for two activator materials as explained below. The smooth glass beads could be roughened in two ways, one by treating activated glass beads by a mixture of sulfuric acid and sodium bifluoride solution for a short period; other by simply lyophilisation (which is use here) by freeze drying. Here, we have used lyophilisation to roughen the glass beads.

2.3. Derivatization of glass beads

2.3.1. Using PEI as an activator

The derivatization procedure performed was according to (Wasserman et al., 2004). The procedure is briefly illustrated in following text. Cleaning protocol: 100 g of beads were weighed into a 1-liter beaker, suspended in distilled water, allowed to settle, and the supernatant fraction decanted. After repeating this operation five times, the supernatant became clear. The beads and the water used for suspension were then heated at 90 °C for 15 min with occasional agitation, placed in a sonicator (Heat Systems) at 50–55 kHz of sonication frequency for 2 min for additional cleansing. These beads were then allowed to settle out. This procedure was repeated four times.

Activation protocol: The cleansed beads were then incubated for 1 h at 70 °C in 1 l saturated sodium aluminate solution. At the end of 1 h the cloudy white supernatant fraction was centrifuged using a laboratory centrifuge at 20,000*g* (14,000 rpm) for 5 min. After washing the beads five times with water, 150 ml aqueous solution containing 100 mg PEI/ml (with initial pH 12–13), adjusted to pH 10.0 with 6 N HCl, was added to the cleansed sodium aluminate treated beads. The beads were thoroughly suspended by manual stirring for 5 min, after which they were centrifuged, re-suspended in 150 ml PEI solution, and transferred to a beaker. At this point the beads were lyophilized by freeze drying for four days or washed with buffer depending on the use R-PEIG or S-PEIG, respectively. Smooth PEI glass (S-PEIG) beads and rough PEI glass (R-PEIG) beads were prepared by the same general procedure as given below with one difference; R-PEIG beads were lyophilized before the removal of excess PEI while S-PEIG beads were simply washed with buffer before immobilization. After lyophilisation, the beads were stored in a desiccator at −20 °C (Wasserman et al., 2004).

Conditioning protocol: Before immobilization the R-PEIG and S-PEIG were conditioned to activate the charges on the surface of glass beads by the following procedure (Dishe and Borenfreund, 1951). The beads were then washed three times using a total of 400 ml l0 mM phosphate buffer, pH of 7.6. This was followed by the addition of 200 ml 50 mM phosphate buffer having pH 7.6. The beads were suspended with the help of a mechanical stirring bar, and then 12.5 ml 1 M glutaraldehyde solution was added. The slurry was stirred with mechanical stirrer for 1.5 h and then 1.2 g sodium cyanoborohydride ($NaBH_3CN$) was added. Since the fumes of the same are highly toxic, this and the remaining portion of the procedure were performed under a fume hood. The beads were then rinsed three times with phosphate buffer (50 mM; pH 7.6), and 125 ml of a solution containing 50 mg PEI/ml, adjusted to pH 7.0 with 6 N HCI, were added to react with any remaining aldehyde moieties. An additional 0.2 g $NaBH_2CN$ was then added as a precaution. The solution was stirred overnight, and beads were washed four times with phosphate buffer and four times with distilled water. These beads were used without further treatment for the immobilization studies.

2.3.2. Using APTES as an activator

The derivatization procedure was performed according to Wojtaś-Wasilewska et al. (1988).

2.4. Enzyme immobilization procedure using derivatized beads

Unless otherwise specified, all immobilizations were performed at 25 °C in 50 mM citrate-phosphate, pH 7.5. Enzyme solution (30–180 units) was added to 5 g of conditioned beads (smooth or rough) after pre-processing the same as described earlier to 250 ml flask. Fifteen microliters of 10% glutaraldehyde solution was then added after 30–60 min. Corresponding volume of buffer was added in its place when glutaraldehyde was not used and this was used as blank. The samples were incubated for an additional 30–60 min at 25 °C, after which the supernatant fraction was decanted and discarded. The beads were then re-suspended in 50 mM Citrate-Phosphate, pH 7.5 and agitated on a Vortex mixer. After the beads were settled, the supernatant fraction was removed. Washing of these beads was repeated five times using distilled water.

3. Glucose isomerase activity assay

The reaction mixture contained 2.25 ml of maleate buffer (pH 6.8), 2.5 ml of substrate solution containing 2 M D-glucose, 0.1 M Magnesium sulfate ($MgSO_4 \cdot 7H_2O$), 0.01 M cobalt chloride ($CoCl_2 \cdot 6H_2O$), and 0.25 ml of enzyme immobilized glass micro-beads suspension. The mixture was incubated at 70 °C for 1 h and the reaction was terminated by adding 0.5 ml of 0.2 M perchloric acid. The reaction was stopped by rapid cooling and then 100 fold dilution with distilled water was carried out. Fructose content was determined by the cysteine-carbazole method (Dishe and Borenfreund, 1951). One unit of glucose isomerase activity is