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Immobilization of α-amylase on zirconia: A heterogeneous biocatalyst for starch hydrolysis

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Abstract

α-Amylase was immobilized on zirconia via adsorption. The support and the immobilized enzymes were characterized using XRD, IR spectra and N_2 adsorption studies. The efficiency of immobilized enzymes for starch hydrolysis was tested in a batch reactor. The effect of calcination temperatures on properties of the support as well as upon immobilization was studied. From XRD, IR and N_2 adsorption studies it was confirmed that the enzyme was adsorbed on the external surface of the support. pH, buffer concentration and substrate concentration had a significant influence on the activity of immobilized enzyme. Immobilization improved the pH stability of the enzyme. The Michaelis–Menten kinetic constants were calculated from Hanes–Woolf plot. K_m for immobilized systems was higher than the free enzyme indicating a decreased affinity by the enzyme for its substrate, which may be due to interparticle diffusional mass transfer restrictions.

Keywords: α-Amylase; Immobilization; Immobilized enzymes; Zirconia; Adsorption; Starch hydrolysis

1. Introduction

Enzymes have been the subject of intense academic interest for many decades and currently poised to become important industrial catalysts. Under the ambit of green chemistry, biocatalysis using renewable resources is very attractive to produce chemicals, which are also safer. Biocatalysis is slowly but steadily gaining importance in various fields of chemical engineering, where chemical synthesis routes are being replaced by enzymatic ones. The major advantage of the enzymatic route is the selectivity with its associated high yield, and exclusivity towards the desired product [1]. The main problems of using the enzymes industrially are the difficulty of their separation from the solution and their inactivation by organic solvent and extreme pH or temperature. Novel designs with immobilized enzymes and without need of separation are of major concern. This also reduces the loss of enzymes and offers the opportunity to use a continuous reactor with a re-use of the enzyme for many reaction cycles and thus lowering the total production cost of enzyme mediated reactions [2]. The immobilized enzyme molecules may also be stabilized against denaturing agents that promote unfolding processes that can destroy the active site [3]. Enhancing both, stabilities can be achieved by immobilization and enzyme engineering (modification of enzyme structure). The traditional acid catalyst methods are now being replaced by enzymic processes [4].

Amylases belong to an enzyme group, which is very commonly used in food and fermentation industry. The hydrolysis of starch to products with low molecular weight, catalyzed by α-amylase (1,4-α-D glucan glucanohydrolase; E.C.3.2.1.1), is one of the most important commercial enzymic processes [5]. Conversion of starch into sugars, syrups and dextrins forms the major part of the starch processing industry. Immobilization of amylase on, mainly, water insoluble carriers, seems to be the most promising way to obtain more stable and reusable forms of enzymes [6]. Immobilization of α-amylase on various particulate

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supports [7], soluble polymers [8] and in ultra filtration membranes [9] has been studied. The disadvantages of polymers as immobilization supports are low pH and thermal stabilities. Porous silica is found to be a good medium for immobilization. Many organic and inorganic supports like clay/modified clays [10], silica [11], zeolite [12] and amorphous aluminium phosphate [13] have been studied for the immobilization of enzymes which are known to be thermally and mechanically stable, non-toxic, and highly resistant against microbial attacks and organic solvents [14]. Solid acid supports can be used to immobilize enzymes since the acidic sites can act as centres of immobilization via the amino groups of enzymes. Immobilization of cellulase, catalase and glucose oxidase on silicate clay minerals has been reported [15–17]. Metal oxides and ceramics were used to immobilize lipase PS (Psuedomonas cepacia) as biocatalyst for the enantioselective acetylation of methyl (±)-mandelate in an ionic liquid solvent system [18]. Increased pH stability and thermal stability was reported for α-amylase immobilized in ordered mesoporous silica (MCM-41, SBA-15 and MCF) [19]. Reports on immobilization of enzymes to metal oxides are limited. As zirconia contains many hydroxyl groups [20] and as the isoelectric point of zirconia (p*I* 4.8) is near that of free α-amylase (p*I* 5.8) maximum immobilization takes place at pH 5. Hence they can be used as a carrier for immobilizing α-amylase.

The main aim of the present work was to produce an immobilized form of α-amylase with advantageous catalytic properties and stability. The present work deals with the synthesis and characterization of zirconia and its use as support for the immobilization of α-amylase. The effect of the pH on activity as well as stability of the immobilized preparations was studied. In the present study, α-amylase was immobilized on zirconia via adsorption. The effect of different calcination temperatures on the properties of support, before and after immobilization was studied. The activity for starch hydrolysis was evaluated in a batch reactor. The support and the immobilized enzymes were characterized using XRD, IR spectra and N_2 adsorption studies. The effect of buffer concentration on the hydrolysis of starch was estimated. The kinetics of the reaction was determined at various substrate concentrations and the kinetic parameters (K_m and V_{max}) were calculated from the Hanes–Woolf plot.

2. Experimental

2.1. Materials

α-Amylase from *Bacillus subtilis* was procured from Sigma–Aldrich Chemicals Pvt Ltd., Bangalore. Zirconium oxychloride was purchased from CDH Chemicals, Mumbai and ammonium hydroxide used was from Qualigens Fine Chemicals, Mumbai. Starch was obtained from SRL Chemicals, Mumbai. All other chemicals were of highest purity commercially available.

2.2. Preparation of the catalyst

Sufficient amount of ammonium hydroxide (1:1 v/v in deionized water) was slowly added to 0.1 M zirconium oxychloride under vigorous stirring at a temperature of 70 °C and the pH was adjusted to 10. The precipitate was kept for stirring at this temperature for 12 h and aged for 24 h and then washed with distilled water until free of chloride, filtered, dried in an air oven at 120 °C for 12 h and calcined at two different temperatures (500 °C and 700 °C) for 12 h.

2.3. Immobilization of α-amylase on oxide carriers

For adsorption, 1 g of zirconia powder was mixed with equal volumes of 0.1 M phosphate buffer and α-amylase solution. It was shaken in a water bath shaker at required temperature for one and a half hour and then filtered. The filtrate was tested for enzyme protein using the spectrophotometric method of Lowry et al. [21] using Folin Ciocaltaue's phenol reagent and measuring the absorption at 640 nm in a Shimadzu 160A UV–Vis spectrophotometer [22]. The influence of pH on immobilization was determined by carrying out the immobilization at various pH (4–8) and determining the enzyme activity under constant conditions. The notations of the catalysts are:

Z-Zirconia, AZ represent α-amylase adsorbed on zirconia, the numbers 1 and 2 represent calcination temperatures of 500 °C and 700 °C.

2.4. Characterization of Immobilized enzymes

Powder XRD of the immobilized enzyme and the supports were taken on a Rigaku D max-C system with Ni filtered Cu Kα radiation ($\lambda - 1.5406$ Å) within the 2θ range 2°–80° at a speed of $2°\text{min}^{-1}$. The samples for XRD were prepared under controlled conditions of humidity. A Micromeritics Gemini 2360 surface area analyzer was used to measure the nitrogen adsorption isotherms of the samples at liquid nitrogen temperature. From this the specific surface area was determined. Prior to the measurement the samples were degassed at room temperature for 12–16 h in nitrogen flow. The IR spectrum of the samples was obtained using an Nicolet Model Magna IR 560 spectrophotometer using KBr disc method. Changes in the absorption bands were investigated in the 500–4000 cm^{-1} region. The resolution and acquisition applied were 4 cm^{-1} and 50 scans respectively.

2.5. Free and immobilized enzyme activity for starch hydrolysis

The activities of the free and immobilized enzymes were tested in a batch reactor. 0.1 g immobilized enzyme (1 ml enzyme solution) was mixed with buffered 5% starch solution and shaken in a water bath shaker. 1 ml of the product was mixed with 5 ml iodine solution and the absorbance was read at 610 nm. One unit of enzyme activity is defined