Available online at www.sciencedirect.com
SCIENCE DIRECT®
ELSEVIER
Catalysis Communications 6 (2005) 525–530
CATALYSIS COMMUNICATIONS
www.elsevier.com/locate/catcom

Glucoamylase immobilized on montmorillonite: Synthesis, characterization and starch hydrolysis activity in a fixed bed reactor

G. Sanjay, S. Sugunan *

Department of Applied Chemistry, Cochin University of Science and Technology, Kochi 682 022, Kerala, India

Received 20 December 2004; revised 18 April 2005; accepted 19 April 2005
Available online 17 June 2005

Abstract

Glucoamylase from *Aspergillus Niger* was immobilized on montmorillonite clay (K-10) by two procedures, adsorption and covalent binding. The immobilized enzymes were characterized using XRD, surface area measurements and ^{27}Al MAS NMR and the activity of the immobilized enzymes for starch hydrolysis was tested in a fixed bed reactor (FBR). XRD shows that enzyme intercalates into the inter-lamellar space of the clay matrix with a layer expansion up to 2.25 nm. Covalently bound glucoamylase demonstrates a sharp decrease in surface area and pore volume that suggests binding of the enzyme at the pore entrance. NMR studies reveal the involvement of octahedral and tetrahedral Al during immobilization. The performance characteristics in FBR were evaluated. Effectiveness factor (η) for FBR is greater than unity demonstrating that activity of enzyme is more than that of the free enzyme. The Michaelis constant (K_m) for covalently bound glucoamylase was lower than that for free enzyme, i.e., the affinity for substrate improves upon immobilization. This shows that diffusional effects are completely eliminated in the FBR. Both immobilized systems showed almost 100% initial activity after 96 h of continuous operation. Covalent binding demonstrated better operational stability.
© 2005 Elsevier B.V. All rights reserved.

Keywords: Glucoamylase; Immobilization; Montmorillonite; Adsorption; Covalent binding; Fixed bed reactor; Michaelis constant

1. Introduction

Immobilized enzymes have been used in food technology, biotechnology, medicine and also analytical chemistry. They provide various advantages over free enzymes including easy separation of the reactants and products from reaction media, easy recovery of the enzyme and repeated or continuous use [1]. Glucoamylase (α-1,4-D-glucan glucohydrolase, EC 3.2.1.3) an exo enzyme acts on the 1,4-glucosidic linkages from the non-reducing ends of amylose, amylopectin and glycogen in a consecutive manner liberating D-glucose. It also hydrolyses α-1,6- and α-1,3-glucosidic linkages but at a much slower rate compared to its action on α-1,4-linkages. Glucoamylase is an industrially important enzyme and is used for large-scale saccharification of malto-oligosaccharides into glucose and various syrups required in the food and beverages industry [2]. Studies on immobilization of glucoamylase are in rapid progress and many supports have been utilized. These include ceramic membranes [3], polymer microspheres [4,5], magnetic supports [6], etc. Inorganic supports for enzyme immobilization are of great interest because of their durability and high mechanical strength for usage in packed or fluidized bed reactors and relatively low cost. In addition, immobilization by adsorption is economically feasible and attractive.

In the present study, we have immobilized glucoamylase on montmorillonite clay via two methods, i.e.,

* Corresponding author. Tel.: +91 484 2575804; fax: +91 484 577595.
E-mail address: ssg@cusat.ac.in (S. Sugunan).

1566-7367/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.catcom.2005.04.016

adsorption and covalent binding. Montmorillonite, which is a 2:1 dioctahedral smectite, has been successfully used as a support as well as a catalyst for the past few decades [7]. The swelling ability of these naturally occurring phyllosilicate minerals provide unusual properties and appreciable surface area for adsorption of organic molecules [8]. A large number of modifications on clay that provide a variety of textural and catalytic properties are possible which include acid treatment, cation exchange, pillaring with robust metal ions, intercalation of polymeric organic moieties, etc. Depending on the type of modification, the properties of clay can be tuned in such a way as to suit specific applications. Here we have employed montmorillonite K-10, which is the acid activated form. Since clays are aluminosilicates, they possess acidic sites that are capable of interaction with amino groups of enzymes leading to ionic binding. This linkage is much stronger than mere physical binding and hence the enzyme will be retained on the support for a larger duration. The immobilized glucoamylase was characterized by XRD, surface area measurements and NMR spectroscopy and the activity for starch hydrolysis was tested in a packed bed reactor. K_m and V_{max} were calculated from the Hanes–Woolf plot. The effectiveness factor η was also determined from a study of kinetics of the reaction. Operational stability in continuous mode was tested for 100 h.

2. Experimental

2.1. Materials used

Glucoamylase from *Aspergillus Niger*, Montmorillonite K-10, 3-amino propyl triethoxy silane, glutaraldehyde and bovine serum albumin were purchased from Sigma Aldrich Chemicals Pvt. Ltd., Bangalore. All other chemicals were of high purity available commercially.

2.2. Immobilization of glucoamylase

In case of adsorption, montmorillonite K-10 was first mixed with deionized water and vigorously stirred for 6 h. It was filtered, dried at 120 °C for 12 h and calcined at 350 °C for 12 h. This calcined clay was mixed with equal volumes of 0.1 M buffer solution and enzyme solution of required concentration and shaken for 1 h in a thermostated water bath shaker at room temperature. It was then centrifuged in a cooling centrifuge for 1 h. The centrifugate was tested for presence of protein by developing colour using Folin Phenol Ciocaltaue's reagent [9] and measuring the absorbance at 640 nm in a Shimadzu 160A UV–Vis spectrophotometer. A standard calibration curve was plotted using bovine serum albumin and the amount of un-adsorbed protein was calculated. The residue was washed several times with deionized water; each time the amount of protein in solution was measured. The immobilized enzyme was stored in 0.1 M buffer at 5 °C for further use. In order to covalently bind the enzyme, calcined montmorillonite K-10 was functionalized by mixing with a 10% (v/v) solution of 3-amino propyl triethoxy silane in acetone and vigorously stirred for 3 h at room temperature [10]. It was filtered, washed several times with acetone until the washings became colourless and later dried at 80 °C for 12 h. This silanized clay was treated with 10% aqueous solution (v/v) of glutaraldehyde and stirred vigorously for 3 h. It was filtered, washed free of excess glutaraldehyde and dried at 60 °C for 12 h. This functionalized clay was used for immobilization as per the procedure described above. The covalently bound enzyme was stored in 0.1 M buffer at 5 °C for further use.

2.3. Characterization

A *Micromeritics* model Gemini 2360 surface area analyzer was used to measure the nitrogen adsorption isotherms of the samples at liquid nitrogen temperature. The specific surface area and pore volume were determined from the BET plot ($p/p_0 = 0.05$–0.95). Prior to the measurement, the samples were degassed at room temperature for 12–16 h in nitrogen flow. Powder XRD of the immobilized enzyme systems and the support were taken on a *Rigaku* D/Max-C system with Ni filtered Cu Kα radiation ($\lambda = 1.5406$ Å) within the 2θ range 2–15° at a scanning rate of 0.5°/min at room temperature. Solid-state ^{27}Al MAS-NMR experiments were carried out over a *Bruker* DSX–300 spectrometer at a resonance frequency of 78.19 MHz for ^{27}Al. For all experiments a standard 4 mm double-bearing Bruker MAS probe was used. The sample spinning frequency was 8 kHz with a single pulse excitation corresponding to $\pi/2$ flip angle. The pulse length for the experiments was 10 µs whereas the pulse delay was 2 s. The spectra were externally referenced with respect to a dilute solution of $AlCl_3$. XWINNMR software operating in a UNIX environment on a silicon graphics computer was employed to acquire and retrieve data.

2.4. Catalytic activity measurements

A silica glass tube of 1.2 cm id and 25 cm length was used as the reactor. Provision for water circulation was also made available. The immobilized enzyme (0.5 g) was packed into a bed at the middle of the reactor, which was filled with glass beads. The substrate was fed from the top of the reactor using a *Cole Palmer 74900 series* syringe pump and the products were collected at the bottom. The reactor was operated at a space velocity of 3.26 h^{-1}. After the reaction time, an aliquot (1 mL) of the product was removed from the

Download English Version:

https://daneshyari.com/en/article/10243316

Download Persian Version:

https://daneshyari.com/article/10243316

Daneshyari.com