Bioresource Technology 116 (2012) 140–146

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Preparation of a pH-sensitive polyacrylate amphiphilic copolymer and its application in cellulase immobilization

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ARTICLE INFO

Article history:
Received 13 November 2011
Received in revised form 24 February 2012
Accepted 23 March 2012
Available online 10 April 2012

Keywords:
pH-sensitive copolymer
Immobilization
Cellulase
Cellulose

ABSTRACT

P_{MDB}, a pH-sensitive and reversible water-soluble copolymer, was synthesized with methacrylic acid (MAA), 2-(dimethylamino) ethyl methacrylate (DMAEMA), and butyl methacrylate (BMA) and used as carrier for cellulase. The copolymer is insoluble between pH 2.5 and 4.1, and soluble below pH 2.5 or above 4.1. Its recovery in aqueous solution was 97.2% by adjusting its isoelectric point (pI) to 3.1. Cellulase was covalently immobilized on P_{MDB} with 1-ethyl-3-(3-dimethyllaminopropyl) carbodiimide. Under optimized conditions, the activity yield of immobilized cellulase was 63.24% and its recovery was 96.8% by adjusting the pI to 3.5. Maximum activity of the immobilized cellulase was achieved at 60 °C (pH 5.0), while free cellulase exhibited maximum activity at 55 °C (pH 5.0). The immobilized cellulase retained 83.1% of its initial activity after repeated five cycles of hydrolysis reaction. P_{MDB} is a promising carrier for immobilizing enzymes with high and low optimum pH due to its dissolving characteristics.

1. Introduction

In order to significantly reduce the production cost of soluble sugars from cellulosic materials by cellulase, cellulase immobilization techniques have been investigated (Li et al., 2007; Mateo et al., 2007; Sheldon, 2007). Enzymes immobilized on soluble carriers are able to hydrolyze insoluble cellulosic substrates more effectively, but the enzyme cannot be re-used. Whereas enzymes immobilized on insoluble carriers are easy to recover, but bioconversion efficiency is low due to diffusion-controlled mass transfer and steric hindrance in biphasic systems with water-insoluble substrates, and low geometrical congruence with protein surfaces (Goldstein, 1976). Therefore, to overcome these problems, immobilization of enzyme on reversibly soluble-insoluble polymeric carriers has been studied (Fujii and Taniguchi, 1991; Hoshino et al., 1991; Taniguchi et al., 1989). These polymers can easily be dissolved and recovered by changing the physical conditions, such as pH (Arasaratnam et al., 2000; Sardar et al., 1997, 2000; Taniguchi et al., 1992, 1989), temperature (Chen, 1998; Hoshino et al., 1997), light (Wang et al., 2008) and addition of certain ions (Gupta et al., 1993). The soluble–insoluble immobilized enzyme system shows excellent performance for hydrolysis of insoluble substrates in soluble state, and can be recovered in insoluble form from a reaction mixture by centrifugation. Until now, only a few of recyclable polymers have been used in the laboratory since they are often expensive and can only be used as carriers for enzymes immobilization with a high optimal pH due to their dissolving characteristics. In the present study, a new pH-sensitive and reversible water-soluble copolymer P_{MDB} was synthesized by using methacrylic acid (MAA), 2-(dimethylamino) ethyl methacrylate (DMAEMA), butyl methacrylate (BMA) as monomers, and ammonium persulfate and sodium hydrogen sulfite as initiators. Its solubility can be controlled by altering the pH. It is insoluble in aqueous solutions between pH 2.5 and 4.1 and soluble below pH 2.5 or above pH 4.1. To investigate the potential application of this reversible soluble polymer P_{MDB} in enzyme immobilization, a commercial cellulase (Novozymes) from *Trichoderma reesei* was selected as a model enzyme. Cellulase is an enzymatic complex composed of hydrolytic and oxidative enzymes, which synergically promote the degradation of cellulose to glucose. The properties of cellulases immobilized on P_{MDB}, such as optimum operational pH, temperature, thermostability and reusability are reported.

2. Methods

2.1. Materials

Methacrylic acid (MAA), butyl methacrylate (BMA), ammonium persulfate (APS), sodium hydrogen sulfite ($NaHSO_3$) were purchased from Lingfen Chemical Co., Ltd., (Shanghai, China). 2-(dimethylamino) Ethyl methacrylate (DMAEMA) was synthesized according to Zhu (2002). 1-Ethyl-3-(3-dimethyllaminopropyl)

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http://dx.doi.org/10.1016/j.biortech.2012.03.082