Immobilization and Characterization of Cellulase on Sepabeads and Relizyme

Ramazan Bilgin, M. Serkan Yalcin

Chemistry Department, (Biochemistry Division), Arts & Science Faculty, University of Cukurova, TURKEY, 01330 Balcali, Sarıçam, Adana Province, E-mail: rbilgin@cu.edu.tr

Abstract – In this study, the covalent immobilizations of cellulase from Trichoderma reesei onto Sepabeads and ReliZyme were studied. The optimal immobilization conditions of the cellulase onto the mentioned supports were investigated using response surface methodology. The optimal working conditions and kinetic parameters of free and immobilized cellulase preparations were also determined.

The optimal immobilization pH, immobilization time and cellulase concentration onto Sepabeads were determined as 6.5, 6 h and 1.8 mg/mL, respectively. The corresponding immobilization values onto ReliZyme were 6.5, 2 h and 1.8 mg/mL.

Key words – cellulase, immobilization, sepabeads, relizyme.

I. Introduction

The immobilization of bioactive materials such as proteins (e.g. enzymes and antibodies) and nucleic acids onto inorganic support is almost compulsory for most of their practical applica- tions [1,2]. An appropriately designed immobilization leads to an increased rigidity of the heterogeneous adsorbed materials in bio- logical sphere ranging from antibodies to biocatalysts (enzymes). Subunits dissociation is reduced. Reuse of enzyme is entertained. Thus the immobilization of enzymes and proteins results in an improved stability, simple separation from reaction mixture, possible modulation of the catalytic properties, facilitates prevention of microbial growth and in certain cases higher activity or selectivity [3–6]. For success the enzymes must be appropriately oriented to prevent inefficient performance [7]. Moreover, improved bio- catalytic efficiency can be achieved by manipulating the structure of the support. Enzyme attached on nonporous materials is subject to minimum diffusion limitation though enzyme loading per unit mass of support is usually low. On the other hand, porous materials afford high enzyme loading but suffer a much greater diffusional limitation of substrate [8].

Cellulase is a key biocatalyst enzyme, which hydrolyzes β-1,4 glycosidic bonds of the crystalline complex of cellulosic crystals to produce glucose, which can be fermented to such valuable products as bioethanol [9]. However, the stability and reusability of free cellulas enzyme has been of great concern [10,11]. The multimeric cellulase enzymes may tend to dissociate, resulting in their rapid inactivation. Therefore, the prevention of enzyme dissociation is a first target when trying to stabilize these interesting but complex enzymes [12].There are successful reports on the stabilization of multimeric enzymes by medium engineering, chemical crosslinking, protein engineering or enzyme immo- bilization.

Recently described strategy to prevent the dissociation of multimeric enzymes is to coat their surface with ionic exchangers that may simultaneously interact with several enzyme subunits, preventing enzyme dissociation [13].

II. Material and methods

The immobilization method was described by Alptekin et al. [14].

Cellulase activity was determined according to the method of by Sumner et al. [15].

III. Results

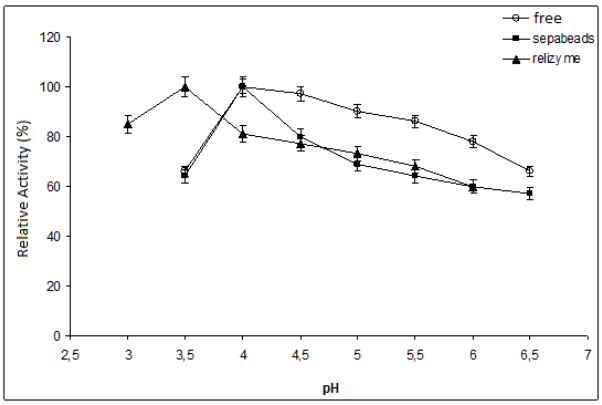

Fig. 1. pH-dependent activity change of free and immobilized cellulase onto Sepabeads and ReliZyme

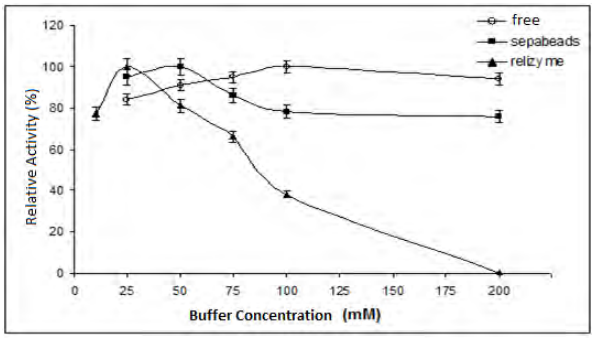

Fig. 2. Buffer concentrations dependent activity change of free and immobilized cellulase onto Sepabeads and ReliZyme

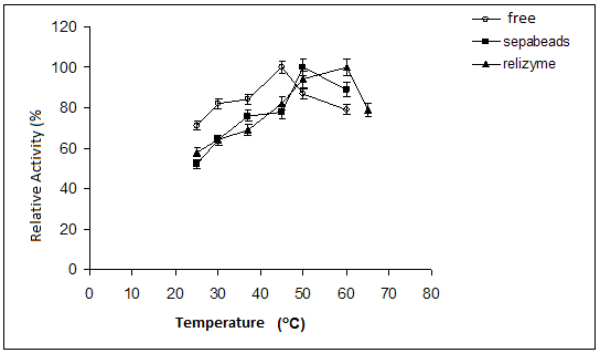

Fig. 3. Temperature-dependent activity changes of free and immobilized cellulase onto Sepabeads and ReliZyme

Conclusions

The optimal immobilization pH, immobilization time and cellulase concentration onto Sepabeads were determined as 6.5, 6 h and 1.8 mg/mL, respectively. The

corresponding immobilization values onto ReliZyme were 6.5, 2 h and 1.8 mg/mL.

The optimal pH, buffer concentration and temperature were determined as 4.0, 100 mM and 45 oC, respectively for the free cellulase; 4.0, 50 mM and 50 oC, respectively for the cellulase immobilized onto Sepabeads and 3.5, 25 mM and 60 oC, respectively for the cellulase immobilized onto ReliZyme. Km and Vmax values were estimated as 0.47 % and 1.05 U/mg prot. for the free cellulase, 1.5 % and 1.1 U/mg prot. for the cellulase immobilized onto Sepabeads and 0.27 % and 3.78 U/mg prot. for the cellulase immobilized onto ReliZyme.

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