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Immobilization studies of cellulase on three engineered polymer surfaces

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ABSTRACT

Enzymes are valuable micro-machines that catalyze a variety of making and breaking reactions. It is well known that immobilization confer stability to enzymes, however, it came at a cost of calculative optimization, support selection and choice of linking method. Desirable properties like hydrophilicity, biocompatibility, resistance to microbial attacks and easy access at low cost has enabled agarose, sepharose, silica, celite, glass and certain plastics as frequently used supports for enzyme immobilization. Herein, we have compared three common plastic polymers- polystyrene, polypropylene and polyethylene for chemical immobilization and subsequent biotransformation using the cellulase enzyme. The results show that the immobilized cellulase on polystyrene, polypropylene and polyethylene beads retain almost two-fold residual activity compared to free enzyme after three-hour incubation at 65 °C. Also, the immobilized cellulase retained significant activity after multiple uses and upon ambient storage for 35 days. Overall, improved catalysis was observed for cellulase upon immobilization, especially to polystyrene surface.

1. Introduction

Cellulases, the multicomponent enzymes (endocellulases, cellobiohydrolase, and β-glucosidase) produced primarily by fungi, bacteria, and protozoans catalyze the hydrolysis of the β-(1-4) linkages in cellulose to convert it to soluble sugars (Zhang and Zhang, 2013). Cellulases covers a large sphere of industrial applications including textile, detergent, brewery and wine, food, animal feed, leather, pulp, and paper, as well as in research and development (Pazarlioğlu et al., 2005; Filos et al., 2006; Juturu and Wu, 2014). The cellulosic materials serve as a substrate for the production of chemicals, and fuel ethanol by enzymatic conversion (Wayman et al., 1992; Philippidis et al., 1993; Fujita et al., 2002). However, industrial setups relying on the use of free cellulases can suffer an unfavorable economic because of the high requirement, low recycling, and susceptibility to inactivation of the enzyme. The immobilization, on the other hand, can overcome some of these shortcomings for applications such as the production of sugar from cellulosic materials (Tébéka et al., 2009). The quest to reduce the cost while enhancing activity and stability of enzyme to a wide range of pH and temperature has resulted in various innovative and effective physical and chemical methods for immobilization of the cellulases (Hirsh et al., 2010). Usually, covalent methods are preferred for immobilization as it restricts the loss (leaching) of enzyme by making a stable covalent bond with the support. Available information suggests the use of various materials such as liposome, PIII-treated polystyrene, Si wafers, and polyvinyl alcohol membrane for covalent immobilization of cellulases (Wu et al., 2005; Li et al., 2007; Tébéka et al., 2009; Hirsh et al., 2010). The choice of chemical linkers for immobilization varies depending on the linking chemistry. Several studies have used photolabile linkers for covalent immobilization of a variety of biomolecules (Liu and Yan, 2010). Photolabile linkers allow easy and efficient cleavage reactions that can be performed directly into solvents suitable for many biological analyses. Particularly, 1-fluoro-2-nitro-4-azidobenzene (FNAB) is used efficaciously for surface engineering of various polymers for immobilization and bioconjugation of proteins and enzymes (Kumar et al., 2016). For example, biomolecular surfaces constructed using FNAB were used in applications like diagnostic assays (e.g. ELISA), aptamer screening (SELEX), microarray fabrication, and to construct conducting polymers and biosensors (Bora et al., 2002; Deepa et al., 2009; Ahirwar et al., 2014, 2016). Considering the diverse applications of FNAB, the present communication has tried to explore the utility of FNAB constructs in biocatalysis of cellulose. The immobilized cellulase on polystyrene, polypropylene and polyethylene polymer beads was analysed for its relative and residual activity, stability and reusability.

2. Materials and methods

The plastic polymer beads of polystyrene (spherical; diameter =

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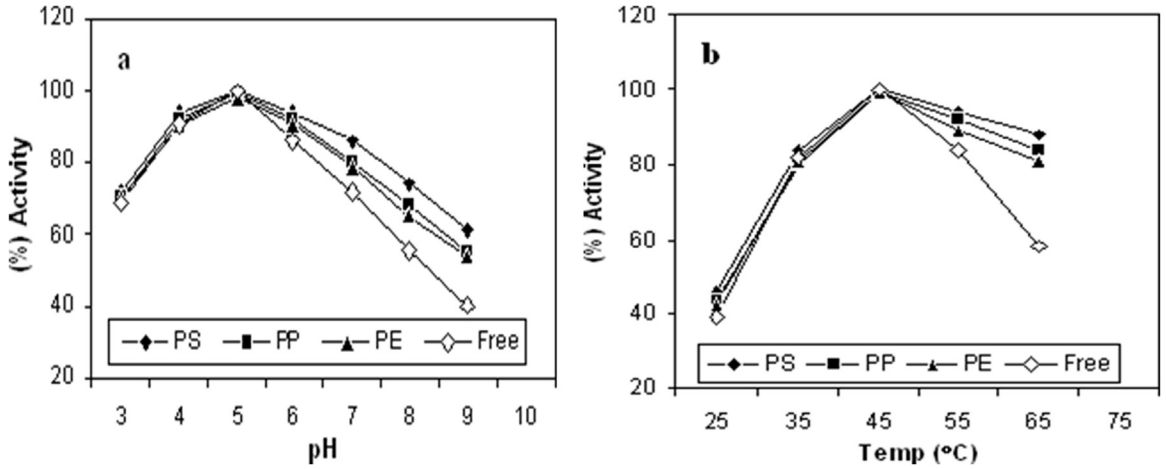

Fig. 1. The effect of pH (a) and temperature (b) on the catalytic activity of immobilized and free cellulase.

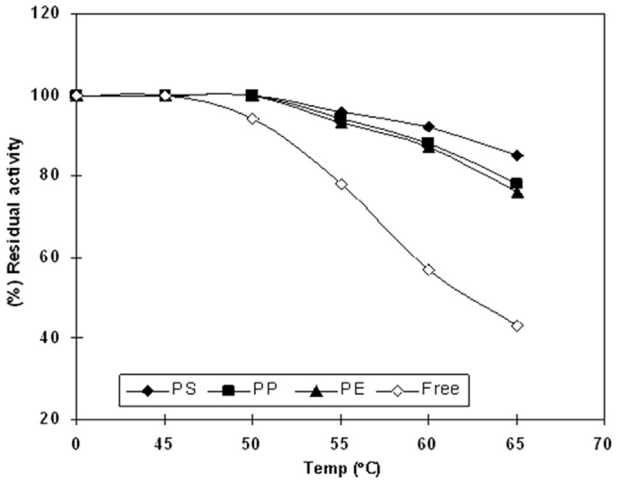

Fig. 2. Thermal stability of immobilized and free enzyme.

3.30 ± 0.36 mm), polypropylene (diameter = 3.40 ± 0.30 mm) and polyethylene (diameter = 3.43 ± 0.55 mm) were prepared for enzyme immobilization by spreading the ethanolic solution of FNAB (300 mg FNAB in 7.5 ml of ethanol) onto respective category of beads, followed by the evaporation of solvent under dark conditions. After complete evaporation of methanol, the beads were irradiated with UV radiations (365 nm) for 15 min in a UV Stratalinker. Afterwards, cellulase was immobilized to the activated polymer beads by incubating 1 g of each polymer beads with cellulase solution (1 mg Cellulase from *Aspergillus niger* in 1 ml of 0.1 M phosphate buffer saline, pH 7.2) for 45 min at 50 °C. The efficacy of FNAB for activation of polymer beads and subsequent immobilization of cellulase was checked by enzymatic assay of cellulase (Ghose, 1987).

The effect of pH on the activity of immobilized enzyme was studied by incubating cellulase-immobilized polymer beads with 1% carboxymethyl cellulose (CMC) solution having different pH (sodium citrate buffer, pH 3–6; PBS, pH 7–9) at 45 °C for 60 min. The resulting reducing sugars were estimated on spectrophotometer at 546 nm using Dinitrosalicylic Acid (DNS) method (Ghose, 1987). Control experiments for comparative analysis were carried out with free cellulase.

Similarly, the effect of temperature on the activity of immobilized and free cellulase was studied by incubating the 1% CMC solution with respective enzyme preparations at 25, 35, 45, 55 and 65 °C respectively, for 60 min. Percentage activity was calculated with respect to the maximum activity of the enzyme. In another experiment, the thermal stability of immobilized and free cellulase was checked by incubating enzyme preparations at 45, 50, 55, 60 and 65 °C for 180 min, followed by assessment of the residual activity by analysing the degradation of 1% CMC solution. Initial activity (before incubation) of the untreated enzyme was taken as control. In subsequent experiments, the reusability of PS, PP and PE- immobilized cellulase was checked for 8

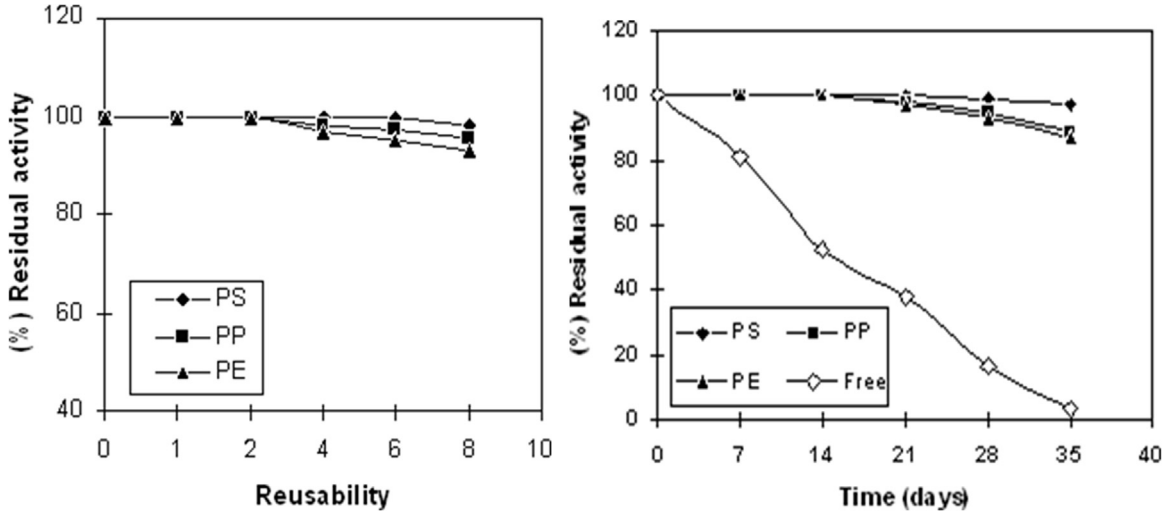

Fig. 3. (a) Reusability of immobilized enzyme. (b) Storage stability of immobilized and free enzyme.