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Catalytic activity of soluble versus immobilized cauliflower (*Brassica oleracea*) bud peroxidase-concanavalin A complex and its application in dye color removal

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ABSTRACT

Biocatalytic activity of soluble against immobilized cauliflower (*Brassica oleracea*) bud peroxidase (CBP) on calcium alginate gel beads have been assessed; internal conditions including temperature, pH and stability with denaturants optimized for application in removing color of textile industrial effluent. CBP–concanavalin A complex expressed ~84% of original peroxidase whereas in entrapped state on calcium alginate pectin gel the activity reduced to ~64%. Immobilized CBP–Con A complex retained ~56.6% activity even at 70 °C which was achieved at pH 4.0. Prolonged urea treatment resulted in activity loss of entrapped CBP-Con A complex by ~19.8%. With dioxane at 50% (v/v), entrapped CBP-Con A complex exhibited an activity of over 58.7%; whereas an increasing concentration of dimethylformide caused reduction in peroxidase activity to 35.8% at 80% (v/v). With immobilized proteins there was considerable color removal upto ~90.6% and ~81.1% from disperse red 19 and dye mixture (disperse red 19+disperse black 9) respectively, in continuous two reactor system and total organic carbon analysis was quite comparable to color loss. Entrapped CBP-Con A complex were reusable upto ten cycles in dye color removal. This study suggests that immobilized and entrapped CBP catalytic system can be efficiently exploited for dye color removal from industrial effluent due to its sustainability, durability and reusability over its soluble counterpart.

1. Introduction

Synthetic dyes are difficult to remove from effluents by conventional biological processes as they are highly stable and resistant to microbial attack (Pala and Toket, 2002). Although physico-chemical methods are available for dye color removal but being cost effective finds limited application (Moreira et al., 2000). Another concern of such approaches are the generation of intermediates and end products that are carcinogenic and mostly more toxic than the dyes *per se*. Approaches utilizing biodegradative abilities of some white rot fungi and peroxidases from vegetable sources seems promising (Liu et al., 2004; Jamal et al., 2012). Owing to their extracellular nonspecific free radical-based enzymatic system, they can completely eliminate a variety of xenobiotics as well as synthetic dyes, giving rise to nontoxic compounds (Bezalel et al., 1997; Pointing, 2001).

Peroxidases are monomeric heme-containing and hydrogen peroxide dependent oxidoreductase glycoproteins. The scope and applications of such enzymes in detoxification, dye decolorization and removal of various toxic organic pollutants especially phenolic compounds which contaminate water and industrial effluents is gaining prominence (Azevedo et al., 2003; Husain, 2006; Lopez et al., 2002). As compared to microbial treatment enzymatic treatment are potentially better due to shorter treatment period; operation of high and low concentrations of substrates; absence of delays associated with the lag phase of biomass, reduction in sludge volume and ease of controlling the process. Further on immobilization such enzymes are advantageous over their soluble counterparts due to enhanced stability, easier product recovery and purification, protection against denaturants, proteolysis, and reduced susceptibility to contamination over prolonged usage (Zille et al., 2003). The strategy adopted for immobilization of

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peroxidases is limited primarily due to the use of commercially available enzyme/expensive supports (Norouzian, 2003). Among the known techniques employed for immobilization bioaffinity based physical adsorption using crude homogenate is useful and cost effective, with additional benefit, such as proper orientation of enzyme on the support (Mislovicova et al., 2000).

Novel research in the area of enzyme technology has provided significant clues and approaches that facilitate large scale optimum utilization of enzymes by entrapping and immobilizing (Gomez et al., 2006; Husain and Husain, 2008). Although, such enzyme system has its own limitations of undergoing leakage and loss, such leakages can be minimized by controlling the pore dimensions. Alternatively, entrapping cross-linked or pre-immobilized enzyme preparations could be better and sustainable approaches (Betancor et al., 2005).

The present study demonstrates the biocatalytic activity of soluble and entrapped cauliflower bud peroxidase (CBP)–Con A complex among various internal conditions and the performance of immobilized system in dye color removal of glycosylated ***Brassica oleracea*** bud peroxidase. ***B.oleracea*** popularly known as cauliflower is widely planted in tropical areas and consumed as vegetables and has earlier been shown by our group to be significantly effective in decolorizing synthetic recalcitrant dye. Salt fractionated immobilized CBP with lectin Con A was entrapped into calcium alginate-pectin beads. A comparative study on the biocatalytic activity of immobilized form of CBP (CBP–Con A complex and CBP–Con A–calcium alginate–pectin complex) under different experimental conditions has been presented for using such enzymes effectively in waste water treatment. Its ability to decolorize dyes in the batch processes and continuous two reactor system has also been examined. This is the first report on the effectivity and suitability of immobilized CBP in dye color removal.

2. Material and methods

2.1. Materials

Sodium alginate, bovine serum albumin, Concanavalin A, O-dianisidine HCl, Disperse Red 19 (DR19), Disperse Black 9 (DB9) was procured from Sigma Chemical Co. (St. Louis, MO, USA). Dioxane, dimethylformide (DMF), sodium dodecyl sulfate (SDS) and pectin were obtained from SRL Chemicals, Mumbai, India. All other chemicals were of analytical grade. The cauliflower buds were procured from Narendra Dev University of Agriculture and Technology, Faizabad, U.P., India. The samples were aseptically transferred into sterilized plastic bags.

2.2. Partial purification, protein estimation and cauliflower bud peroxidase (CBP) activity assay

Cauliflower buds (~350 g) was homogenized in 700 ml of 100 mM sodium acetate buffer, pH 5.6; salt purification and measurement of peroxidase activity was done as described by Jamal et al. (2012) (Jamal et al., 2010). This preparation of protein was aliquoted and stored for further use. Protein was estimated using BSA as a standard protein and following the procedure of Lowry et al. (1951).

2.3. Preparation of insoluble CBP–Con A complex and entrapment in calcium alginate–pectin beads

The peroxidase proteins (1200 U) were mixed with an increasing concentration of Con A (0.1–1.0 ml) in a series of tubes and the precipitate (CBP–Con A complex) exhibiting maximum activity was obtained following the procedure of Jamal et al. (2012) (Jamal et al., 2010). Calcium alginate capsules were prepared by extrusion using a simple one-step process as described by Nigma et al. (1988). CBP–Con A complex (1230 U) was mixed with sodium alginate (2.5%) and pectin (2.5%) in 10 ml of 100 mM sodium acetate buffer (pH 5.6) to obtain beads following the method of Jamal et al. (2012) (Jamal et al., 2010).

2.4. Peroxidase activity assay and effect of enzyme loading

Peroxidase activity was determined by the method of Jamal et al. (2012) (Jamal et al., 2010). One unit (1.0 U) of enzyme activity is the amount of enzyme protein that catalyzes oxidation of 1.0 μmole of *o*-dianisidine HCl per min at 37 °C into colored product. An increasing concentration of enzyme (110–1000 U) was mixed to calcium alginate-pectin gel in a series of tubes. Expression of loaded enzyme was monitored by assaying the peroxidase activity.

2.5. Measurement of encapsulation efficiency and leakage of CBP

CBP concentration was measured both in calcium chloride solution and capsule to assess the encapsulation efficiency. The encapsulated enzyme concentration was estimated by cutting the capsules in half and placing it in 5 mL phosphate buffer (pH = 7.4) solution. In order to obtain encapsulated protein concentration of protein in buffer was measured according to the Lowry's assay after 2 h (Nigma et al., 1988). The percentage of encapsulated enzyme was obtained from the difference between initial protein introduced to the calcium chloride hexahydrate solution and encapsulated protein measured as mentioned above.

Enzyme leakage measurement was carried out by placing capsules in a test tube filled with Tris buffer (pH = 8.0) for 18 h. The capsules were cut in half and placed in phosphate buffer (pH = 7.4) solution. The protein concentration was measured according to Lowry's assay and the leakage percentage was calculated from the differences between encapsulated protein at the beginning of time interval and the value found according to the above procedure (Nigma et al., 1988).

2.6. Optimization of internal conditions in the presence of contaminants

2.6.1. Effect of time, temperature and pH on immobilized CBP

CBP–Con A complex and calcium alginate–pectin entrapped CBP–Con A complex (1.20 U) were incubated at 60 °C in 100 mM sodium acetate buffer (pH 5.6) for varying time interval to obtain time activity plot following the method of Jamal et al. (2012) (Jamal et al., 2010). The temperature activity (20–90 °C) and pH activity plots (pH 2–9) were obtained following similar methods.

2.6.2. Effect of urea, organic solvents, detergents, sodium azide and mercuric chloride on immobilized CBP

CBP–Con A complex and calcium alginate–pectin entrapped CBP–Con A preparations (1.20 U) were incubated separately in 4.0 M urea; 10–80% (v/v) of water-miscible organic solvents; dioxane and DMF and increasing concentrations of sodium dodecyl sulfate (SDS) and non-ionic detergents; Triton X-100 and Tween-20 (0.5–6.0%, v/v); with sodium azide, mercuric chloride ($HgCl_2$) (0.05–6.0 mM) prepared in 100 mM sodium acetate buffer (pH 5.6) at 37 °C for 1 h. Catalytic activity was monitored at all the indicated detergent concentrations (Jamal et al., 2010). Activity of enzyme without exposures to contaminants was taken as control (100%).