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Chitosan beads immobilized manganese peroxidase catalytic potential for detoxification and decolorization of textile effluent

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ABSTRACT

Textile industry has led to severe environmental pollution and is posing a serious threat to the ecosystems. Immobilized biocatalysts have gained importance as potential bio-remediating agent. Manganese peroxidase (MnP) was immobilized onto glutaraldehyde activated chitosan beads by crosslinking and employed for the degradation and detoxification of dyes in textile effluents. The efficiency of chitosan-immobilized MnP (CI-MnP) was evaluated on the basis of decolorization, water quality improvement and toxicity reduction. Maximum color removal of 97.31% was recorded and up to 82.40%, 78.30% and 91.7% reductions in COD, TOC, and BOD were achieved, respectively. The cytotoxicity of bio-treated effluents reduced significantly and 38.46%, 43.47% and 41.83% *Allium cepa* root length, root count and mitotic index were increased, respectively, whereas brine shrimp nauplii death reduced up to 63.64%. Mutagenicity (Ames test) reduced up to 73.44% and 75.43% for TA98 and TA100 strains, respectively. The CI-MnP retained 60% activity after 10 repeated decolorization batches. The CI-MnP showed excellent efficiency for the bioremediation of textile effluents and can be used for the remediation of toxic agents in wastewater. The monitoring of processed wastewater using bioassays is suggested to evaluate bio-efficiency of treatment method for safe disposal of effluents into water bodies.

1. Introduction

Ever-growing chemical pollutants, especially dyes from textile sector are polluting the water reservoirs and in view of toxic nature of dyes, there is need to explore suitable remediation strategies [1]. Annually, 7×10^7 tons dyes are manufactured worldwide and a significant amount is lost during processing and dying, which ultimately mixed with water bodies. The dyes are extremely stable, and some typical dyes are highly resistant to primary treatment methods, including physical or chemical procedures, which are often expensive, and accumulation of concentrated sludge also causes secondary pollution issue [2–4]. Being less expensive, more efficient and environmentally-friendly, the biodegradation has been recognized as an effective tool for treatment of wastewater. Oxido-reductases enzymes (fungi, bacteria) have been performed outstandingly in treating wastes [5,6], especially fungal peroxidases and laccases have shown a great potential for the remediation of dyes [7–13]. Nevertheless, the biological treatment utilizing native enzymes is often unsatisfactory in degrading and detoxifying pollutants because free enzymes encountered operational difficulties (pH, temperature variation and generation of toxic by-products) and complete mineralization of pollutant is not possible due to instability and deactivation of enzymes [13,14]. Enzymes immobilization on suitable matrices is the ultimate solution, which enables enzymes recovery (an essential condition for enzyme reuses). Besides, it provides stability to enzyme against variable conditions in reaction media since enzymes are sensitive to change in reaction conditions and resultantly, denatured under unfavorable conditions [6,15–17]. Therefore, the immobilization offers various advantages over free enzymes under similar reaction conditions which enhanced catalytic efficiency as well as recycling of enzyme [12,18].

Chitosan is an amine polysaccharide, product of deacetylation of chitin and is composed of randomly distributed-(1,4)-linked-glucosamine and *N*-acetyl-D-glucosamine units [19]. At present, chitosan and its derivatives are the focal points as an ideal support in enzyme immobilization [20–23] since it is non-toxic and able to protect enzyme (i.e., toxic by-products, variation in

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reaction conditions and metal ions etc) for efficient activity in view of suitable mechanical stability, biocompatibility and biodegradability. It also enables the enzyme to be reutilized in several successive cycles [23,24–26].

The MnP-mediated degradation is reported by various researchers using single pollutant as model. However, there is lack of comprehensive studies reporting real textile wastewater decolorization (containing mixture of dyes) by CI-MnP. Therefore, the principal objectives of the present study were to immobilize MnP enzyme on chitosan microspheres, characterize and application for the degradation of textile wastewater. The CI-MnP catalytic potential was evaluated by measuring decolorization, water quality and toxicity (cytotoxicity and mutagenicity). For toxicity measurement, standard bioassays were used and finally, several decolorization cycles were run in order to check the stability and reusability of CI-MnP.

2. Materials and methods

2.1. Reagents and chemicals

Chitosan (CTS) (MW 20,000, degree of deacylation = 89.2%), glutaraldehyde (50%, v/v, aqueous solution), calcium chloride dihydrate (≥99%), acetic acid (≥99%), cyclophosphamide monohydrate (≥98%), methyl methanesulfonate (∼99%), potassium dichromate (≥99.0%), and sodium malonate dibasic monohydrate (98%) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Hydrogen peroxide (30% H_2O_2), boric acid (≥99.5%), mono- and dibasic sodium phosphate, and potassium hydroxide (KOH) were procured from Merck (Darmstadt, Germany). *Phanerochaete chrysosporium* manganese peroxidase (MnP) (Sigma-93014; specific activity ≥20 U/g) was obtained from Sigma-Aldrich and Pierce Coomassie Protein Assay Kit was provided by Thermo Fisher Scientific, New York, USA. Other reagents were of analytical grade used as received. Ultra pure water (Millipore, United States) was used for the preparation of solutions.

2.2. Immobilization of MnP

For immobilization, chitosan powder different concentrations (2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0% w/v) were dissolved in acetic acid (1.5% v/v) under heating (50 °C) and continuous agitation [27]. The resultant mixture was filled in syringe and extruded drop wise into alkali coagulating solution (1 *M* KOH) and highly swollen spherical beads were formed. Beads of different sizes were prepared by changing the syringe needle. The beads were separated by filtration, washed with ultra-pure water and stored in Na-malonate buffer (50 m*M*, pH 4.5) at 4 °C. The chitosan beads were activated by treating with glutaraldehyde (0.0–4.0% v/v) at 30 °C for 3 h. The activated beads were incubated with purified MnP enzyme for 24 h at 4 °C for immobilization [26]. The enzyme incorporated beads were washed thrice with Na-malonate buffer to remove uncoupled enzyme and dried. Enzyme loading efficiency (LE) and immobilization efficiency (IE) was determined according to the equations indicated below [28]:

$$\mathrm{LE}(\%) = \left(\frac{C_i V_i - C_F V_F}{C_i V_i}\right) \times 100 \quad (1)$$

where *Ci* is the initial protein concentration, *Vi* the initial volume of enzyme solution, *Cf* the protein concentration in the total filtrate, and *Vf* the total volume of the filtrate.

$$\mathrm{IE}(\%) = \left(\frac{\text{Activity of entrapped enzyme}}{\text{Activity of free enzyme}}\right) \times 100 \quad (2)$$

2.3. Protein contents and enzyme activity measurement

MnP activity was determined by monitoring Mn^{3+}-malonate complexes formation at 270 nm [ε270 = 11.59 L/(mmol.cm)] [29]. Assay mixture comprised of Na-malonate (50 mmol/L, pH 5.0), $MnSO_4$ (0.2 m*M*), H_2O_2 (0.1 mmol/L) and 100 μL free MnP enzyme solution. Activity assay for immobilized enzyme was performed under similar conditions, except stirring and reaction was terminated by separating chitosan beads from the reaction mixture by Buchner funnel before the spectrophotometric analysis. One unit of enzyme activity (U) corresponds to the amount of enzyme needed to oxidize 1 μmole substrate per min. Assay was performed in triplicate; values were averaged and used for enzyme activity calculation. Total protein was determined in comparison to the color response of protein standard assay (bovine serum albumin, BSA) using Pierce Coomassie Protein Assay Kit (Thermo Fisher Scientific, New York, USA).

2.4. Characterization of the free and immobilized MnP

The effect of pH on free and CI-MnP was determined by incubation in buffers of different pH values for 24 h. The buffers used were potassium chloride (200 m*M*, pH 2.0), sodium malonate (pH 3.0 & 4.0), citrate phosphate (pH 5.0 & 6.0), sodium phosphate (pH 7.0 & pH 8.0) and potassium carbonate (pH 9.0 & pH 10.0). Thermal stability was investigated by incubating the free and CI-MnP at 60 °C for 10 h. The residual activity was measured using standard protocol.

2.5. Decolorization of textile effluent

The textile effluent was collected from Crescent Textile industry, Faisalabad, Pakistan following standard sampling method [3]. For decolorization, effluent (100 mL) was mixed with 1 mL of 0.1 m*M* $MnSO_4$ as MnP mediator, 0.1 m*M* H_2O_2 and Na-malonate buffer (50 m*M*; pH 4.5) in Erlenmeyer flasks (250 mL) (triplicate) and flasks were incubated with free MnP and CI-MnP in a temperature-controlled shaker (120 rpm) for 5 h. After stipulated time period, flasks removed, filtered, centrifuged (10,000 rpm, 15 min) and percentage color removal was determined by measuring absorbance at 660 nm (CE Cecil 7200, UK) [13]. The absorbance values for respective supernatants at each time period were corrected by subtracting the values for respective control fraction (containing only reaction medium without enzyme).

2.6. Reusability

Chitosan beads were re-used up to 10 cycles for effluent decolorization to check the recycling capability of CI-MnP. After every cycle, the beads were separated and washed with distilled water followed by 50 m*M* Na-malonate buffer (pH 4.5) washing and subjected to next batch [30].

2.7. Analytical procedures

The pH was monitored by digital pH Meter (WTW pH-meter; InoLab pH 730), whereas BOD and COD meters (Lovibond, water testing systems) were used to measure biological oxygen demand (BOD) and chemical oxygen demand (COD) values, respectively. Un-treated and free and CI-MnP treated effluents were scanned from UV—190–1100 nm (CE Cecil 7200, UK) to measure λ_{max} and degradation pattern. Turbidity was determined by Turbidimeter (TN-100, Eutech). Previously reported methods were adopted for total organic contents (TOC) and total suspended solid (TSS) measurements [3].