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Mutagenicity and cytotoxicity assessment of biodegraded textile effluent by Ca-alginate encapsulated manganese peroxidase

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ABSTRACT

Present study was aimed to appraise the potential of free and immobilized manganese peroxidase (MnP) for the decolorization and detoxification of textile effluent. MnP was immobilized in Ca-alginate beads at optimized conditions of sodium alginate, calcium chloride and enzyme concentrations. Maximum effluent decolorization of 87.4% was achieved in the presence of H_2O_2 (1 mmol l^{-1}), 1-hydroxybenzotriazole (1 mmol l^{-1}), pH (5.0) and temperature (40 °C) for 5 h of incubation time. The effluent treated at optimized conditions was subjected to toxicity evaluation. The cytotoxicity was evaluated using *Allium cepa*, brine shrimp and heamolytic bioassays; whereas mutagenicity was tested using Ames test of both treated and untreated effluent. The cytotoxicity of treated sample reduced significantly and *A. cepa* showed increase in root length, root count and mitotic index up to 38.46%, 43.47% and 41.83%, respectively, whereas red blood cells (RBCs) lysis reduced 69.84% and brine shrimp nauplii death reduced up to 63.64% in immobilized MnP treated effluent. The mutagenicity reduced up to 73.44% and 75.43% for TA98 and TA100 strains, respectively. The Ca-alginate beads encapsulated MnP revealed promising bio-catalytic efficiency and could be used for the decolorization and detoxification of textile effluents.

1. Introduction

Uncontrolled discharge of untreated wastewater in to the water bodies from textile industries is a serious threat to aquatic living organisms and humans being since some dyes are mutagenic, carcinogenic and genotoxic in nature [1,2]. Being synthetic origin and complicated structures, the dyes are extremely recalcitrant to degradation by conventional physicochemical approaches [3]. The treatment cost, dye removal efficiency, feasibility, generation of residual sludge and secondary pollutants are some of inherent limitations of physicochemical methods [4]. Also, these methods may not effectively degrade or detoxify a wide variety of synthetic dyes under certain environmental conditions in comparison to biological treatment [5]. Elimination of environmental contaminants by biological methods using microorganisms is regarded to be closer to nature since it is eco-friendly and able to degrade the compounds recalcitrant in nature without compromising the eco-system essentials [6–8]. The technology is scalable and offer possibility to mineralize a variety of organic pollutants. Dye degradation at higher reaction rate and operation under milder reaction conditions are the main advantages of microbial-enzymatic system. Several dyes have been decolorized by peroxidases, such as soybean peroxidase (SBP; EC 1.11.1.7), manganese peroxidase (MnP EC 1.11.1.13), lignin peroxidase (LiP EC 1.11.1.14), versatile peroxidase (VP EC 1.11.1.16), and horseradish peroxidase (HRP EC 1.11.1.7). The enzymatic treatments oxidize the dye structures to low molecular weight harmless end products [9–11].

MnP has outstanding prospect as catalysts in the textile, dye and paper industries, wastewater treatment and bioremediation [12,13]. Despite its great potential, the practical utilization of enzymes on industrial scale may be hindered by poor stability, recovery challenges and recyclability [14,15]. Immobilization technique has widened the enzymes applications i.e., recycling and higher stability under wide pH and temperature ranges and toleration toward toxic chemicals [16,17]. These enhanced features have facilitated the implementation of immobilized enzyme in a continuous reactor to remediate large volumes of dye-harboring wastewaters [15] and for this, it is necessary to select appropriate matrices for immobilization. Enzyme entrapment in calcium alginate gel has attracted much attention due to its suitability as support for immobilization of enzymes and is also simple to prepare, provide good mechanical strength, eco-friendly,

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cost effective and very efficient for degradation of organic compounds having different origin and nature [18,19]. Researchers have recently been conducted experiments using Ca-alginate beads as carrier for biocatalyst immobilization, and trials have been conducted successfully for the removal of pollutant from wastewater [5,14,19–21]. Bio-remediation of pollutant to reduce their toxic effects is of great concern and short-term bioassays have been developed and employed successfully for toxicity monitoring of treated and untreated effluents [1]. Therefore, in present investigation, *Ganoderma lucidum* MnP was immobilized in Ca-alginate beads and used for the treatment of textile effluent. The treatment efficiency was evaluated on the basis of degradation, cytotoxicity and mutagenicity reduction. For cytotoxicity, *Allium cepa*, brine shrimp and heamolytic bioassays were used, whereas Ames test was employed for mutagenicity measures.

2. Materials and methods

2.1. Materials

Sodium alginate (viscosity 20,000–40,000 cps), calcium chloride dihydrate ($CaCl_2{\cdot}2H_2O$), bovine serum albumin (BSA), cyclophosphamide monohydrate, methyl methanesulfonate (~99%), potassium dichromate (≥99.0%) and hydroxybenzotriazole from Sigma Chemical Co. (St. Louis, MO, USA) were supplied by local suppliers. Other chemicals and reagents were of analytical grade and used without any purification. Untreated textile industrial effluent was collected from the Sitara Textile industry (STI), located in Faisalabad, Pakistan. At the time of sampling, the industry was using disperse, direct and reactive dyes. The characteristics of the wastewater were pH 10.86 ± 0.37, BOD 243 ± 1.70, COD 826.7 ± 7.93, TSS 392.1 ± 3.30 and TOC 712.3 ± 8.02 mg/l. Wheat bran to be used for MnP production was obtained from Students Research Farms, University of Agriculture; Faisalabad.

2.2. MnP production and purification

MnP enzyme was produced from *G. lucidum* through solid state fermentation using wheat bran at pre-optimized growth conditions of pH 5.5; temperature 30 °C; substrate 5 g; moisture 50%; carbon source, 2% glucose; nitrogen source, 0.02% yeast extract; fungal inoculum, 5 ml; and fermentation time 5 days [22]. The crude enzyme extract produced was subjected to partial purification by ammonium sulphate precipitation, and dialysis as precisely described earlier [23].

2.3. Entrapment of MnP in alginate gel

In order to achieve high immobilization efficiency and retain maximum enzyme activity, the gelling agent (sodium-alginate and calcium chloride solution) and the enzyme quantity to be introduced was optimized in a manner as reported by other authors [14,18,19]. To this end, varying concentrations of sodium alginate (1.0, 2.0, 3.0, 4.0, and 5.0% w/v) and calcium chloride (50, 100, 150, 200, and 250 mmol l^{-1}) were used. Alginate solution was prepared by mixing sodium alginate in deionized water containing a certain amount of MnP enzyme (0.01–1.5 mg ml^{-1}). The gel mixture was extruded drop wise through a syringe into 50 ml of $CaCl_2$ solution under magnetic agitation (250 rpm) for 2 h, forming beads of different sizes (1.7–2.5 mm diameter). The beads were recovered from $CaCl_2$ solution by vacuum filtration, washed twice with distilled water, and kept in 50 mM sodium malonate buffer (pH 4.5) at 4 °C. The beads were dried and percentage immobilization efficiency (IE) was determined using relation shown in Eq. (1).

$$\mathrm{IE}(\%) = \left(\frac{A_0 \times V_0 - A_F \times V_F}{A_0 \times V_0}\right) \times 100 \quad (1)$$

where A_0, and A_F are the activities of MnP solution, and filtrate respectively, V_0, and V_F are the volumes of MnP solution, and filtrate, respectively.

2.4. MnP enzyme assay

MnP activity was assayed as reported by Warrishi et al., [24]. Assay mixture (2.6 ml) containing 1 ml of 1 mM $MnSO_4$, 1 ml of 0.05 M sodium malonate buffer (pH 4.5), 0.5 ml of H_2O_2 and 0.1 ml enzyme solution was incubated at 25 °C for 10 min. Same mixture containing 0.1 ml of distilled water instead of enzyme solution was used as blank. Absorbance's of each sample was recorded at 270 nm (ε_{270} = 11570 M^{-1} cm^{-1}) on UV double beam spectrophotometer (UV 2000, Hitachi, Japan).

2.5. Protein contents determination

Bradford micro-assay [25] was followed for the determination of total protein contents. Enzyme sample (10 μl) was added to 1 ml of Bradford reagent followed by incubation at 37 °C for 15 min. After incubation the absorbance was recorded at 595 nm and protein was estimated from standard curve using Bovine Serum Albumin (BSA) as standard.

2.6. Decolorization of textile industry effluent

For decolorization, triplicate Erlenmeyer flasks containing 10 ml of F-MnP (free MnP) or 5 g of I-MnP (immobilized MnP), 100 ml of textile effluent, 1 ml of 1 mM $MnSO_4$ as MnP mediator, 0.1 mM H_2O_2 and Na-malonate buffer (50 mM; pH 4.5) were incubated in a temperature-controlled shaker (120 rpm) for 5 h reaction time. After stipulated time period, samples were filtered, centrifuged (5000 × *g* for 15 min) and percentage color removal was determined by considering the initial and final absorbance of treated and untreated effluent. Decolorization of effluent was determined by a reduction in optical density at the wavelength of maximum absorbance (546 nm) by UV–vis spectrophotometer.

2.7. Effect of operational variables on decolorization

Effluent was treated with F-MnP and I-MnP in the presence of varying concentrations of H_2O_2 (0.2–1.6 mM), and activities were determined under standard assay procedure [5]. Effect of various redox mediators i.e., veratryl alcohol (VA), vanillin (VN), violuric acid (VLA), 1-hydroxybenzotriazole (HOBT), and syringaldehye (SA) (1.0 mM) on the F-MnP and I-MnP-mediated effluent decolorization was monitored in 50 mM sodium malonate buffer, pH 5.0 in the presence of 1.0 mM H_2O_2 for 1 h at 37 °C. Effluent decolorization by F-MnP was terminated by heating mixture in a boiling water bath for 5 min to stop the enzymatic activity. However, in case of I-MnP, the reaction was terminated by removing enzyme by centrifugation. The remaining effluent decolorization was monitored at 546 nm. The percent decolorization calculated using untreated effluent was considered as control (100%). The decolorization of textile effluent by F-MnP and I-MnP was conducted in the buffers of varying pH (pH 2.0–10.0) to study pH effect, while the influence of temperature was investigated at different temperatures in the range of 20–80 °C. In order to investigate the optimum contact time, required for maximum effluent decolorization, experiments were carried out for 1.0–6.0 h both for F-MnP and I-MnP. Each experiment was performed in triplicate and results were averaged.