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Article

Novel catalytic and effluent decolorization functionalities of sol-gel immobilized *Pleurotus ostreatus* IBL-02 manganese peroxidase produced from bio-processing of wheat straw

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ABSTRACT

Solid state bio-processing of wheat straw was carried out through an indigenous fungal strain *Pleurotus ostreatus* IBL-02 under pre-optimized fermentation conditions. The maximum activity, 692±12 U/mL, of the industrially important manganese peroxidase (MnP) enzyme was recorded after five days of still culture incubation. The crude MnP was 2.1-fold purified with a specific activity of 860 U/mg after purification on a Sephadex-G-100 gel column. On native and SDS-PAGE electrophoresis gels, the purified MnP fraction was a single homogenous band of 45 kDa. An active fraction of MnP was immobilized using hydrophobic sol-gel entrapment comprising tetramethoxysilane (T) and propyltrimethoxysilane (P) at different T:P molar ratios. Characterization revealed that after 24 h incubation at varying pH and temperatures, the MnP fraction immobilized at a T:P ratio of 1:2 in the sol-gel retained 82% and 75% of its original activity at pH 4 and 70 °C, respectively. The optimally active fraction at a 1:2 T:P ratio was tested against $MnSO_4$ as a substrate to determine the kinetic catalytic constants K_M and V_{max}. To explore the industrial applicability of *P. ostreatus* IBL-02 MnP, both the free and immobilized MnP were used for the decolorization of four different textile industrial effluents. A maximum of 100% decolorization was achieved for the different textile effluents within the shortest time period. A lower K_M, higher V_{max}, hyper-activation, and enhanced acidic and thermal resistance up to 70 °C were the novel catalytic features of the sol-gel immobilized MnP, suggesting that it may be a potential candidate for biotechnological applications particularly for textile bioremediation purposes.

1. Introduction

Lignocellulosic biomass is a major renewable resource of potentially fermentable carbohydrates but is mostly discarded in the form of pre-harvest and post-harvest agricultural losses, and waste from food processing industries. The lignocellulosic wastes are present in abundance and therefore, are of great value as raw materials for the production of industrially important enzymes. It has been established that white rot fungi (WRF), a group of generally robust microbes, can produce extracellular ligninolytic enzymes comprising lignin peroxidase (LiP), manganese peroxidase (MnP), and laccase that are involved in the degradation of lignin as well as in bio-remediation processes [1–3]. During recent years, there has been a great interest in the potential applications of MnP in bio-pulping and bio-bleaching as well as in bio-remediation processes [1–4].

MnPs are extracellular glycoproteins with an iron protoporphyrin IX (heme) prosthetic group mostly secreted in multiple forms during secondary metabolism [5]. Mn^{2+} functions as a mediator for MnP where MnP catalyzes the H_2O_2 dependent

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oxidation of Mn^{2+} to Mn^{3+} that subsequently forms complexes with different chelators like pyrophosphate malonate, oxalate, L-tartrate, oxaloacetate, L-malate, and methylmalonate, low-molecular-mass diffusible mediators, which non-specifically oxidize a variety of phenolic and non-phenolic substances including lignin and toxic pollutants [2].

Textile effluents contain several types of chemicals including real dye residues that are toxic, carcinogenic, mutagenic, and pose serious health hazards to the entire living ecosystem. An eco-friendly treatment method of textile waste effluents is still a major environmental concern of the modern world [6]. In spite of the existing physical and chemical technologies, which are usually expensive and commercially unattractive, biological processes provide an alternative cost effective and eco-friendly approach that can be applied to wide range of dye containing industrial effluents [4,6]. Industrial applications of enzymes in most cases involve the use of continuous reactors that require immobilized bio-catalysts in order to improve process efficiency through improvements in enzyme stability, reusability and controlled product release. A variety of immobilization techniques have been investigated to develop immobilized bio-catalysts [5]. However, among the different immobilization methods, gel entrapment is preferred because gel polymers are non-toxic and do not swell in aqueous or organic solvents, thus preventing the leaching of the entrapped enzyme and denaturation of its native conformation [3,7,8].

WRF has the ability to degrade contaminants by virtue of its extracellular ligninolytic enzymes [3–6,9]. During the last few years, we have demonstrated considerable improvement in many processes related to lignocellulose biotechnology and triggered in-depth studies of lignocellulosic materials, ligninolytic microorganisms, and their enzymes. Therefore, in this context, the capability of the indigenously isolated MnP to degrade/decolorize different textile dyes containing industrial waste effluents was investigated for its use in biotechnological applications particularly for textile bioremediation purposes.

2. Experimental

2.1. Chemicals and lignocellulosic substrate

Propyltrimethoxysilane (P), tetramethoxysilane (T), polyvinyl alcohol, Sephadex-G-100, and standard protein markers were purchased from Sigma-Aldrich (USA). Agricultural waste wheat straw was collected from the Student Research Farm, University of Agriculture Faisalabad, Pakistan. The substrate was chopped, dried (60 °C), ground to a fine particle size and stored in polyethylene plastic bags to avoid free moisture until further use. For decolorization studies, four different dyes containing textile industry effluents, i.e., Nishat textile (NIT), K&N textile (KIT), Sitara textile (SIT), and Crescent textile (CRT) were collected on-site from local textile industries in Faisalabad, Pakistan.

2.2. Microorganism and preparation of inoculum

A pure culture of the indigenous fungal strain *P. ostreatus* IBL-02 was raised on potato dextrose agar (PDA) slants at pH 4.5 and 28 °C. Homogenous spore inoculum was prepared by growing the fungus for 5 d in the Kirk's basal nutrient medium in the presence of an additional 1% (w/v) Millipore filtered sterile glucose solution to develop inoculums with 1×10^6–1×10^8 spore/mL. The main constituents of the medium were ammonium tartrate (0.22 g/L), KH_2PO_4 (0.21 g/L), $MgSO_4\cdot7H_2O$ (0.05 g/L), $CaCl_2\cdot H_2O$ (0.01 g/L), thiamine (0.001 g/L), and Tween-80 (10 mL/L).

2.3. Production and extraction of MnP

P. ostreatus IBL-02 was grown in solid state fermentation (SSF) using a wheat straw based fermentation medium under pre-optimized growth conditions. The culture flasks (500 mL) contained 5 g wheat straw, moistened (66% w/w moisture) with Kirk's salt medium that was supplemented with glycerol and urea as an inexpensive carbon and nitrogen source in a ratio of 25:1 (C:N) and 1 mmol/L $MnSO_4$ (1 mL) as optimal nutritional factors. The SSF medium was autoclaved, inoculated (5 mL fresh fungal spores) and allowed to ferment at 30 °C in a still culture incubator (Sanyo, Japan). After 7 d of fermentation, 100 mL of distilled water was added to all of the fermented cultures and the flasks were shaken at 120 r/min for 30 min [10]. The contents were filtered and the filtrates were centrifuged at 3000 g for 10 min (Sanyo, Japan). The supernatants were pooled and used as a crude extract for the MnP activity assay, purification, and immobilization purposes.

2.4. Determination of MnP activity and protein content

MnP activity was measured by adopting the methodology previously described [2,6]. The MnP activity was measured against a reagent blank at 270 nm (ϵ_{270} =11590 mol^{-1} L cm^{-1}, UV 2000, Hitachi, Germany). The recorded activities were expressed as U/mL, where one unit of activity was defined as the amount of enzyme required to produce a unit increase in the absorbance at a specific wavelength (nm) per mL of the reaction mixture. To determine the protein content of the sample, the Bradford microassay was used with bovine serum albumin as the standard [11].

2.5. Purification of MnP

Purification of MnP was carried out by ammonium sulfate fractionation (85% saturation), followed by dialysis and gel filtration chromatography using a Sephadex-G-100 column (2×25 cm) as described previously [2]. Phosphate buffer (100 mmol/L) with 0.15 mol/L NaCl was used as the elution buffer and the flow rate was maintained at 0.5 mL/min. Up to 20 pooled fractions were collected, concentrated by ultra-filtration and used to determine molecular mass, enzyme activity as well as the protein content as described in section 2.4.

2.6. Native and SDS-PAGE

The purified and lyophilized sample was dissolved in a