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Bio-catalytic performance and dye-based industrial pollutants degradation potential of agarose-immobilized MnP using a Packed Bed Reactor System

Muhammad Bilal[a,b,*], Muhammad Asgher[b], Hafiz M.N. Iqbal[c,**], Hongbo Hu[a], Wei Wang[a], Xuehong Zhang[a]

[a] State Key Laboratory of Microbial Metabolism, and School of Life Sciences and Biotechnology, Shanghai Jiao Tong University, Shanghai 200240, China
[b] Industrial Biotechnology Laboratory, Department of Biochemistry, University of Agriculture, Faisalabad 38040, Pakistan
[c] School of Engineering and Science, Tecnologico de Monterrey, Campus Monterrey, Ave. Eugenio Garza Sada 2501, Monterrey, N.L., CP 64849, Mexico

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ABSTRACT

In this study, the matrix-entrapment technique was adopted to immobilize a novel manganese peroxidase (MnP). Agarose beads developed from 3.0% agarose concentration furnished the preeminent immobilization yield (92.76%). The immobilized MnP exhibited better resistance to changes in the pH and temperature as compared to the free counterpart, with optimal conditions being pH 6.0 and 45 °C. Thermal and storage stability characteristics were significantly improved after immobilization, and the immobilized-MnP displayed higher tolerance against different temperatures than free MnP state. After 72 h, the insolubilized MnP retained its activity up to 41.2 ± 1.7% and 33.6 ± 1.4% at 55 °C and 60 °C, respectively, and 34.3 ± 1.9% and 22.0 ± 1.1% activities at 65 °C and 70 °C, respectively, after 48 h of the incubation period. A considerable reusability profile was recorded with ten consecutive cycles. Moreover, to explore the industrial applicability, the agarose-immobilized-MnP was tested for bioremediation of textile industry effluent purposes. After six consecutive cycles, the tested effluents were decolorized to different extents (with a maximum of 98.4% decolorization). In conclusion, the remarkable bioremediation potential along with catalytic, thermo-stability, reusability, as well as storage stability features of the agarose-immobilized-MnP reflect its prospects as a biocatalyst for bioremediation and other industrial applications.

1. Introduction

The development of highly efficient, cost-competitive and environmentally-friendlier industrial processes has led to the search for robust biocatalyst as “green tools” in various applications. Immobilized enzymes (generally referred to enzymes physically restricted and confined within a certain defined region of space) present advantages of improved pH and temperature range adaptability along with excellent catalysis properties and repeated use capability [1–5]. In last few years, various approaches have been envisioned to improve the catalytic functions of manganese peroxidases (MnP). For example, physical (adsorption, entrapment or encapsulation) and chemical meth1ods (covalent bonding and cross-linking) [1,2,6–9]. Entrapment is believed to be an excellent choice for enzyme immobilization since it induces no conformational modification on the native structure of the enzyme [10–12]. So far, a variety of supporting materials has been assessed for immobilizing MnPs by several different methods. Among them, agarose (polysaccharide polymer material) is considered to be an inert support exhibiting excellent biocompatibility as well as biomechanical properties [13]. It is a linear biopolymer derived from various marine algae and seaweeds. Structural porosity provides an efficient microenvironment for enzyme-substrate reaction and eases discharge of end product into the reaction mixture [14]. Several reports revealed that agarose matrix-encapsulation technique offers better enzymatic performance and stability [13].

Textile effluents contain several types of dye residues that are toxic, carcinogenic and mutagenic, in nature. The discharge of such industrial effluents into the water streams by textiles

* Corresponding author at: State Key Laboratory of Microbial Metabolism, and School of Life Sciences and Biotechnology, Shanghai Jiao Tong University, Shanghai 200240, China.
** Corresponding author.
E-mail addresses: bilaluaf@hotmail.com (M. Bilal), hafiz.iqbal@my.westminster.ac.uk, hafiz.iqbal@itesm.mx (H.M.N. Iqbal).

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Table 1
Physiochemical characteristics of textile industrial waste effluents.

Source name	Strength color	λ_{max} (nm)	pH	Dyes
ART	Maroon	515	6.3	Disperse and direct
AST	Light yellow	414	4.7	Disperse, direct and reactive
CRT	Navy blue	579	4.8	Disperse, direct and reactive
ITT	Dark green	629	5.8	Disperse and direct
KAT	Blue	660	10.8	Disperse and direct

along with other industries, with little or no treatments, causes significant contamination issues. Majorly, the treatment of such industrial effluents is still a major environmental concern of the modern world, particularly in some lower developed countries. To date, several treatment technologies have been developed to effectively process such wastewater such as adsorption by activated charcoal, precipitation, chemical reduction, advanced ionizing radiation and ultrafiltration [15]. Therefore, despite the existing physical/chemical technologies for color removal which are usually expensive and commercially unattractive, biological processes provide an alternative to existing physicochemical technologies because they are cost effective, eco-friendly and can be applied to wide range of dye containing industrial effluents. In this context, the ability of white-rot fungi (WRF) to degrade industrial contaminants by their extracellular ligninolytic enzymes, particularly manganese peroxidases, and laccases, has been extensively investigated [2,5,9]. Like other enzyme, issues that seriously restricted the practical utilization of free MnPs are prohibitive cost, non-reusability, the hardness of enzyme retrieval, environment sensitivity, and poor storage and thermal stabilities [16]. Enzyme immobilization technology is an effective way to alleviate these inadequacies. A wider range of substrate specificity makes these enzymes valuable in large industrial use e.g. energy exploitation, environmental protection and bio-detection, food processing, green chemistry, detergents and pharmaceutical processes [17–19]. Fungal MnPs are among the most important biocatalyst with significant biotechnological potential [3,6,7].

Herein, agarose was chosen as support matrix to immobilize MnP through entrapment technique. Varying polymer concentrations were examined to achieve the maximum immobilization yield (IY) of MnP. Thermal and functional stabilities of both MnPs i.e. non-immobilized and agarose-immobilized-MnP were also investigated to analyze its potentiality for commercialization applications. The investigation also involved the practical implementation of immobilized-MnP for decolorization of textile industry effluents using a Packed Bed Reactor System (PBRS).

2. Materials and methods

2.1. Chemicals and textile effluents

All the chemicals/reagents used, in this study, were of analytical laboratory grade and mainly obtained from Sigma-Aldrich (USA) and Scharlau (Spain). Five different dye containing textile effluents were collected from locally available industries i.e. Arzoo Textile (ART), Ayesha Textile (AST), Crescent Textile (CRT), Itmad Textile (ITT), and Kalash Textile (KAT) and used in a Packed Bed Reactor System (PBRS) for decolorization purposes. The physiochemical characteristics of collected effluents are summarized in Table 1.

2.2. MnP source and entrapment within agarose matrix

A 3.43-fold purified MnP fraction with a specific activity of 539.59 U/mg was recovered from the culture filtrate of an indigenous fungal strain *Ganoderma lucidum* IBL-05, as reported earlier [19]. Following that the MnP entrapment was carried out by mixing equal volume (1:1 ratio) of purified MnP form and agarose solution (3.0% w/v), as previously described [13]. The agarose solution was prepared in 50 mM Na-malonate buffer of pH 4.5 by vigorous shaking at 100 °C. After cooling to room temperature (30 °C), the enzyme was incorporated and mixed at 120 rpm for 30 min. The resulting suspension was directly poured on pre-assembled sterile glass plates and placed at 4 °C for 1 h to solidify the gel matrix completely. The agarose microspheres with and with-out MnP were analyzed for enzyme activity using standard assay procedure.

2.3. Influence of varying agarose concentrations on immobilization yield

Varying concentrations of agarose (1.0–7.0%) were tried to find the optimal point for maximum immobilization yield (IY) of MnP. The agarose solution was prepared in Na-malonate buffer by heating in boiling water bath (at 100 °C), and control, as well as enzyme incorporated beads, were developed from clear solution. Enzyme loading efficiency (LE) and immobilization yield (IY) was determined according to the equations indicated below [11]:

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

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.

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

2.4. Effect of reaction pH

Effect of reaction pH on the activities of free MnP and agarose-immobilized-MnP was evaluated by carrying out the enzyme-substrate assay in varying pH ranges (ranging from 2.0 to 10.0) buffers. Different buffer systems such as potassium chloride (pH 2.0), Na-malonate buffer (pH 3.0 and 4.0), citrate phosphate (pH 5.0 and 6.0), Na-phosphate (pH 7.0 and 8.0), and potassium carbonate (pH 9.0 and pH 10.0) were used. The MnP activity was calculated by monitoring H_2O_2-mediated oxidation of manganic-malonate complexes at 270 nm (ε_{270}: 11.59 mM^{-1} cm^{-1}).

2.5. Effect of reaction temperature

The catalytic activities of non-immobilized and agarose- immobilized MnPs were examined by conducting the standard enzyme assay at the temperature ranging from 20 °C to 70 °C (5 °C interval) using above-discussed optimized conditions.

2.6. Effect of reaction time duration

Effect of different reaction time on the catalytic activities of non-immobilized and agarose- immobilized MnPs was investigated by performing the enzyme assay at varying time durations (ranging from 0.0 min to 60.0 min). Standard assay conditions were used for activity determination purposes, as described in the Section 2.10.

2.7. Thermal stability profile

The thermal-stability profile was studied by heating the free and immobilized-MnP in 50 mM Na-malonate buffer of pH 4.5 at different temperature ranges (ranging from 35.0 °C to 70.0 °C) for up to 120 h. Enzyme assay was performed following standard assay conditions from aliquots recovered after each 24 h time, and percent remaining activities were compared with control, i.e., a sample