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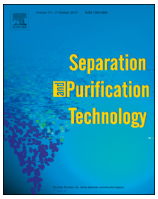

Nano-peroxidase fabrication on cation exchanger nanocomposite: Augmenting catalytic efficiency and stability for the decolorization and detoxification of Methyl Violet 6B dye

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ABSTRACT

In present study polypyrrole-zirconium(IV)selenoiodate cation exchanger nanocomposite (PZrSeI-CENC) was employed to immobilize ginger peroxidase (GP) via strong electrostatic interactions. Fourier transform infrared spectroscopy, transmission electron microscopy and scanning electron microscopy was done to characterize the PZrSeI-CENC before and after immobilization of GP. PZrSeI-CENC bound GP exhibited very high activity yield of around 96%. Immobilized GP was less sensitive to the changes in pH and temperature compared to free GP. In stirred batch processes immobilized GP was able to decolorize around 96% of the Methyl Violet 6B (MV 6B) dye after 3 h of incubation whereas only 81% of the dye was decolorized by free GP under identical experimental conditions. Immobilized GP exhibited excellent reusability in the stirred batch process and decolorized around 60% of the dye even after its 10th repeated use. Furthermore, V_{max} of PZrSeI-CENC bound GP was amplified 10 times which is mainly attributed to polypyrrole aided enhanced electron shuttle system of the enzyme. A degradation pathway was proposed in compliance with gas chromatography-mass spectroscopy analysis that confirms the degradation of MV 6B into low molecular weight products. The genotoxic assessment revealed a significant reduction in toxicity of dye after treatment with nanocomposite bound peroxidase.

1. Introduction

During the past several decades there has been an alarming increase in the colored pollutants in water bodies due to industrialization. Dyes from different groups are released into the water bodies by various industries like textile, tannery, paper and pulp, and paint industries [1,2]. A very low concentration of these dyes in water can add high intensity of color which affects the water transparency and obstructs the path of sunlight [3,4]. Apart from it, dyes discharged into the environment are highly toxic and even carcinogenic in nature, ultimately harming animals and plants both. The group of dyes which is most extensively used in textile, paper, cosmetics, and food industry is triphenylmethane dyes. These dyes are highly toxic to the mammalian cells and mutagenic in nature, and also recalcitrant to the degradation by natural flora and fauna [5]. One of the dyes of this group is Methyl Violet 6B (MV 6B) which is a potential carcinogen, mutagen and mitotic poison with known harmful effects on humans [6,7]. It is widely used in textile industries and to give purple color to paints and printing ink [8]. Therefore, the release of this dye into the environment is a matter of great concern, and its oxidative degradation is highly required.

Many microbial and plant peroxidases have been utilized for the removal of environmental pollutants [9,10]. But the use of free enzymes is circumscribed by several factors like instability under harsh experimental conditions, non-reusability as well as high cost of purification of enzymes [3]. Therefore immobilization of enzyme has been proposed to address these issues [11]. Numerous kinds of supports have been exploited for the immobilization of enzymes such as inorganic carriers, natural polysaccharides, and synthetic polymers by using varying methods of immobilization such as adsorption, covalent attachment, entrapment and microencapsulation [12]. Among them, nano-sized materials gaining much interest due to their several advantages over the bulk supports like large surface area, effective

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Abbreviations: HOBT, 1-hydroxybenzotriazole; GP, ginger peroxidase; MV 6B, Methyl Violet 6B; TEM, transmission electron microscopy; SEM, scanning electron microscopy; FT-IR, Fourier transform infrared; GC-MS, gas chromatography-mass spectroscopy; NPs, nanoparticles; PZrSeI-CE, zirconium(IV)selenoiodate cation exchanger; PZrSeI-CENC, polypyrrole-zirconium(IV)selenoiodate cation exchanger nanocomposite

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enzyme loading and high mechanical strength [13–15]. The nanostructured materials utilized for the immobilization of enzymes are carbon nanomaterials, nanosized polymers beads, metal oxides and metal sulphides nanoparticles (NPs).

The commonly used procedures for the immobilization of enzymes on NPs have been divided into two types; first as reversible ionic bonding or physical adsorption and second as irreversible covalent bonding [16]. Covalent attachments are very strong and stable for enzyme immobilization without any leakage of the enzyme but the enzymes sometimes lose their activities because of blocking or distorting the active sites of enzymes after immobilization. However the physical binding simply involves adsorption which is less expensive, does not require any crosslinking agent and causes no enzyme denaturation. In contrast, the technique is not providing a highly stable form of an enzyme as it engages very weak forces for binding and frequently results into leakage of the enzyme from the support with a change in pH, temperature and ionic strength [17]. In order to strengthen the binding, adsorption requires some strong forces like electrostatic interactions [18]. For such interactions, ion-exchange resins are used for the strong binding of enzymes which involves ionic and electrostatic interactions between charge present on the surface of proteins and opposite charges of the resins [19].

Different conducting polymers like polyaniline, polythiophene, polypyrrole, and polycarbazole have been widely studied for their various useful properties. Among these polymers, polypyrrole is most extensively used for enzyme immobilization due its high electrical conductivity, biocompatibility and low cost of synthesis as compared to other polymers [20]. The activity of enzymes like oxidoreductases which require shuttling of electrons for a catalytic reaction can be augmented by immobilizing them on electron conducting carriers [21]. Toxicity assessment of the dyes and their biologically degraded products is of great concern because sometimes degradation of the chemical pollutants leads to the formation of products which are more toxic than their parent compound [22,23]. Among the toxicity assessments, genotoxicity is of great concern because it may lead to deleterious effects on living organisms [24].

In the present study, polypyrrole-zirconium(IV)selenoiodate cation exchanger nanocomposite (PZrSeI-CENC) was used for the immobilization of GP via adsorption. The immobilized preparation was characterized by Fourier transform infrared (FT-IR) spectroscopy, transmission electron microscopy (TEM) and scanning electron microscopy (SEM). Stability of free and immobilized GP for the degradation of MV 6B dye over a wide range of pH and temperatures were compared. Kinetic parameters for both free and immobilized GP were calculated. Immobilized GP was also evaluated for MV 6B degradation efficiency in batch process and its reusability was also studied. Gas chromatography-mass spectroscopy (GC-MS) analysis was done for the identification of the degraded products and degradation pathway was proposed accordingly. Finally, the genotoxic assessment was carried out to evaluate the reduction in toxicity of the dye after enzymatic treatment.

2. Materials and methods

2.1. Materials

Bovine serum albumin, Histopaque 1077, RPMI 1640 and o-dianisidine-HCl were the products of Sigma Chemical Co. (St. Louis, MO, USA). 1-hydroxybenzotriazole (HOBT) and pyrrole (99%) were obtained from SRL Chemicals (Mumbai, India) and Spectrochem (India Ltd.), respectively. Ginger was purchased from local vegetable market surrounding the AMU Aligarh, India. Other chemicals and reagents were of analytical grades.

2.2. Extraction of peroxidase from ginger

The extraction of GP was done according to the procedure described earlier [25].

2.3. Preparation of zirconium(IV)selenoiodate cation exchanger (ZrSeI-CE)

The preparation of ZrSeI-CE was carried out by taking 0.1 M of zirconium(IV) oxychloride solution prepared in distilled water and gradually mixed with 0.1 M of potassium iodate and 0.1 M of sodium selenite solutions prepared in 1 M HCl with intermittent shaking and by maintaining the pH of the solution as 1.0. Zirconium(IV)oxychloride, potassium iodate and sodium selenite were in the ratio of 1:2:1 (v:v:v) respectively. The resulting white precipitate was left in the mother liquor for 24 h at room temperature. The precipitate was decanted and washed with distilled water, filtered by suction and dried at 50 ± 2 °C [26].

2.4. Preparation of PZrSeI-CENC

For the oxidative polymerization of pyrrole, 33% pyrrole monomer in chloroform and 0.1 M $FeCl_3$ solution in water was added in 1:40 ratio. The mixture was stirred for over 24 h at room temperature. The resultant mixture firstly turned green and then slowly into black slurries. PZrSeI-CENC was prepared by sol-gel mixing of 2:1 (v:v) ratio of polypyrrole into inorganic gels of ZrSeI-CE with constant stirring for 7 h. The obtained mixture was turned slowly into greenish black colored slurries. The slurries were left to stand for 24 h at room temperature. The obtained precipitate was filtered on Buchner funnel, washed with distilled water and finally with methanol. It was kept for drying in an oven at 50 ± 2 °C [26].

2.5. Characterization of PZrSeI-CENC

2.5.1. FT-IR spectroscopy

Characterization of PZrSeI-CENC was done by FT-IR spectroscopy. The spectrum was taken by using FTIR- Perkin Elmer instrument, USA (Spectrum- II, wave no. 4000-400). Calibration was done by using polystyrene film. Phase KBr pellet method was used to run the sample. Background spectrum with the clean atmosphere was obtained before the measurement of sample using identical acquisition parameters.

2.5.2. TEM analysis

To evaluate the size and morphology of PZrSeI-CENC, TEM was performed. Sample of PZrSeI-CENC was prepared by air-drying a drop of diluted solution of PZrSeI-CENC on carbon labelled copper grids at normal atmospheric conditions. The prepared sample was investigated under JEOL-2100 transmission electron microscope at 200 kV.

2.5.3. SEM analysis

Surface morphology of PZrSeI-CENC was examined by SEM. The air dried sample was affixed to carbon tape on a copper stub and 60 nm gold was deposited on it using a sputter coater. JSM-6510 LV scanning electron microscope at 15 kV was employed to examine the prepared sample.

2.6. Immobilization of GP on PZrSeI-CENC

PZrSeI-CENC was prepared according to the procedure described earlier [24]. For the immobilization of GP on PZrSeI-CENC, 36 U of GP was loaded on 200 mg of PZrSeI-CENC in 0.1 M of sodium acetate buffer, pH 5.5 and continuously stirred overnight at 25 °C. The enzyme adsorbed on PZrSeI-CENC was collected by centrifugation at 5000 × *g* for 15 min at 4 °C. GP bound PZrSeI-CENC was washed thrice with assay buffer to remove unbound enzyme and finally suspended in the same buffer at 4 °C for further studies.