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Immobilization of Horseradish Peroxidase onto Bio-Linked Magnetic Particles with Allium Cepa Peel Water Extracts

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Abstract : Enzyme peroxidases are biological catalysts and play a major role in phenolic wastewater treatments and other environmental applications. The most studied species from the peroxidases family is horseradish peroxidase (HRP). In environmental processes, HRP could be used in its free or immobilized form. Enzyme immobilization onto solid support is performed to improve the enzyme properties, prolong its lifespan and operational stability and allow its reuse in industrial applications. One of the enzyme supports of a newer generation is magnetic particles (MPs). Fe_3O_4 MPs are the most widely pursued immobilization of enzymes owing to their remarkable advantages of biocompatibility and non-toxicity. Also, MPs can be easily separated and recovered from the water by applying an external magnetic field. On the other hand, metals and metal oxides are not suitable for the covalent binding of enzymes, so it is necessary to perform their surface modification. Fe_3O_4 MPs functionalization could be performed during the process of their synthesis if it takes place in the presence of plant extracts. Extracts of plant material, such as wild plants, herbs, even waste materials of the food and agricultural industry (bark, shell, leaves, peel), are rich in various bioactive components such as polyphenols, flavonoids, sugars, etc. When the synthesis of magnetite is performed in the presence of plant extracts, bioactive components are incorporated into the surface of the magnetite, thereby affecting its functionalization. In this paper, the suitability of bio-magnetite as solid support for covalent immobilization of HRP across glutaraldehyde was examined. The activity of immobilized HRP at different pH values (4-9) and temperatures (20-80°C) and reusability were examined. Bio-MP was synthesized by co-precipitation method from Fe(II) and Fe(III) sulfate salts in the presence of water extract of the Allium cepa peel. The water extract showed 81% of antiradical potential (according to DPPH assay), which is connected with the high content of polyphenols. According to the FTIR analysis, the bio-magnetite contains oxygen functional groups (-OH, -COOH, C=O) suitable for binding to glutaraldehyde, after which the enzyme is covalently immobilized. The immobilized enzyme showed high activity at ambient temperature and pH 7 (30 U/g) and retained ≥ 80% of its activity at a wide range of pH (5-8) and temperature (20-50°C). The HRP immobilized onto bio-MPs showed remarkable stability towards temperature and pH variations compared to the free enzyme form. On the other hand, immobilized HRP showed low reusability after the first washing cycle enzyme retains 50% of its activity, while after the third washing cycle retains only 22%.

Keywords : bio-magnetite, enzyme immobilization, water extracts, environmental protection
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