

## Magnetic Silica Nanoparticles as Viable Support for the Immobilization of Oxidative Enzymes

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**Abstract :** Laccases (benzenediol oxygen oxidoreductases, EC 1.10.3.2) are excellent biocatalysts for biotechnological and environmental applications because of their high activity, selectivity, and specificity. Specifically, these characteristics allow them to perform the oxidation of recalcitrant compounds with simple requirements for the catalysis (presence of molecular oxygen). Nevertheless, the low stability under unfavorable conditions (pH, inactivating agents or temperature) and high production costs still limits their use for practical applications. Immobilization of enzymes has proven particularly valuable to avoid some of the aforementioned drawbacks. Magnetic nanoparticles (MNPs) have received increasing attention as carriers for enzyme immobilization since they can potentially provide an easy recovery of the biocatalyst from the reaction medium under an external magnetic field. In the present work, silica-coated magnetic nanoparticles (Fe<sub>3</sub>O<sub>4</sub>@SiO<sub>2</sub>) were prepared, characterized and used for laccase immobilization by covalent binding. The synthesis of Fe<sub>3</sub>O<sub>4</sub>@SiO<sub>2</sub> was performed in a two-step procedure: co-precipitation and reverse microemulsion. The influence of immobilization conditions: concentrations of the functionalization agent (3-aminopropyl-triethoxy-silane) and the cross-linker (glutaraldehyde) as well as the influence of pH, T or inactivating agents were evaluated. In general, immobilized laccase showed superior stability compared to that of free enzyme. The reusability of the biocatalyst was demonstrated in successive batch reactions, where enzyme activity was maintained above 65% after 8 cycles of oxidation of the substrate 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonate).

**Keywords :** silica-coated magnetic nanoparticles, laccase, immobilization, regeneration

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