

## Enhancing Industrial Wastewater Treatment: Efficacy and Optimization of Ultrasound-Assisted Laccase Immobilized on Magnetic Fe<sub>3</sub>O<sub>4</sub> Nanoparticles

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**Abstract :** In developed countries, water pollution caused by industrial discharge has emerged as a significant environmental concern over the past decades. However, despite ongoing efforts, a fully effective and sustainable remediation strategy has yet to be identified. This paper describes how enzymatic and sonochemical treatments have demonstrated great promise in degrading bio-refractory pollutants. Mainly, a compelling area of interest lies in the combined technique of sono-enzymatic treatment, which has exhibited a synergistic enhancement effect surpassing that of the individual techniques. This study employed the covalent attachment method to immobilize Laccase from *Trametes versicolor* onto amino-functionalized magnetic Fe<sub>3</sub>O<sub>4</sub> nanoparticles. To comprehensively characterize the synthesized free nanoparticles and the laccase-immobilized nanoparticles, various techniques such as X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FT-IR), scanning electron microscope (SEM), vibrating sample magnetometer (VSM), and surface area through Brunauer-Emmett-Teller (BET) were employed. The size of immobilized Fe<sub>3</sub>O<sub>4</sub>@Laccase was found to be 60 nm, and the maximum loading of laccase was found to be 24 mg/g of nanoparticle. An investigation was conducted to study the effect of various process parameters, such as immobilized Fe<sub>3</sub>O<sub>4</sub> Laccase dose, temperature, and pH, on the % Chemical oxygen demand (COD) removal as a response. The statistical design pinpointed the optimum conditions (immobilized Fe<sub>3</sub>O<sub>4</sub> Laccase dose = 1.46 g/L, pH = 4.5, and temperature = 66 °C), resulting in a remarkable 65.58% COD removal within 60 minutes. An even more significant improvement (90.31% COD removal) was achieved with ultrasound-assisted enzymatic reaction utilizing a 10% duty cycle. The investigation of various kinetic models for free and immobilized laccase, such as the Haldane, Yano, and Koga, and Michaelis-Menten, showed that ultrasound application impacted the kinetic parameters V<sub>max</sub> and K<sub>m</sub>. Specifically, V<sub>max</sub> values for free and immobilized laccase were found to be 0.021 mg/L min and 0.045 mg/L min, respectively, while K<sub>m</sub> values were 147.2 mg/L for free laccase and 136.46 mg/L for immobilized laccase. The lower K<sub>m</sub> and higher V<sub>max</sub> for immobilized laccase indicate its enhanced affinity towards the substrate, likely due to ultrasound-induced alterations in the enzyme's conformation and increased exposure of active sites, leading to more efficient degradation. Furthermore, the toxicity and Liquid chromatography-mass spectrometry (LC-MS) analysis revealed that after the treatment process, the wastewater exhibited 70% less toxicity than before treatment, with over 25 compounds degrading by more than 75%. At last, the prepared immobilized laccase had excellent recyclability retaining 70% activity up to 6 consecutive cycles. A straightforward manufacturing strategy and outstanding performance make the recyclable magnetic immobilized Laccase (Fe<sub>3</sub>O<sub>4</sub> Laccase) an up-and-coming option for various environmental applications, particularly in water pollution control and treatment.

**Keywords :** kinetic, laccase enzyme, sonoenzymatic, ultrasound irradiation

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