



INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI  
SHORT ABSTRACT OF THESIS

Name of the Student : ANAMIKA GHOSE

Roll Number : 196154003

Programme of Study : Ph.D.

Thesis Title: Biodegradation of fluoroquinolone antibiotics by laccase enzyme extracted from spent mushroom waste (SMW) of *Pleurotus florida* and its immobilization on biochar derived from SMW: a sustainable approach

Name of Thesis Supervisor(s) : PROF. SUDIP MITRA

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**SHORT ABSTRACT**

It is crucial to develop urgent and innovative strategies for removing persistent organic micropollutants (OMPs) from soil, groundwater, and surface water. Antibiotics commonly found in contaminated soils and wastewater treatment plant effluents are classified as environmentally persistent pharmaceutical pollutants (EPPPs). These pollutants pose serious ecological threats to non-target species. Since conventional wastewater treatment plants (WWTPs) cannot completely eradicate these pollutants, the excessive use of antibiotics has a negative impact on the environment's ability to regenerate and recover. In recent years, various water bodies such as surface water, tap water, groundwater, common wastewater treatment plants, and rivers persist fluoroquinolone antibiotic residues specifically ciprofloxacin, levofloxacin, and norfloxacin. To address this pressing issue, it is essential to develop alternative, sustainable remediation strategies that can effectively degraded theses pollutants. This study explores an innovative approach to antibiotic bioremediation using spent mushroom waste (SMW), a type of agro-waste. SMW, a byproduct of mushroom cultivation, is rich in ligninolytic enzymes like laccase and peroxidase, which facilitate the biodegradation of complex organic pollutants. The tri-nuclear cluster of copper ions in the laccase enzyme has excellent oxidizing capacity for organic and inorganic compounds. However, its low reduction potential limits its ability to oxidize non- phenolic compounds. To address this limitation, small-sized mediators (both synthetic and natural mediators) are used to bind to the enzyme's active site, reducing steric hindrance. By adjusting the reaction conditions like pH, temperature, and mediator concentration, this study aims to optimize laccase output from *Pleurotus florida* SMW.

The biodegradation of OMPs relies heavily on optimal reaction conditions and the presence of susceptible functional groups. This study focuses on the degradation of fluoroquinolone antibiotics with and without a synthetic mediator. By adjusting the parameters mentioned above, we aim to achieve higher levels of degradation. While free enzymes are prone to denaturation in environmental conditions, immobilization is a technique that involves fixing enzymes in a solid support. In this study, a biochar-based technique for removing antibiotics from wastewater by improving the immobilization of enzymes through adsorption was attempted. The research focuses on identifying parameters that can facilitate the process of adsorptive immobilization. Specifically, we explored the immobilization of laccase on raw biochar surface through an adsorption process, taking into account parameters such as pH (3-6), temperature (30-50 °C), and enzyme dosage (1-5 U mL<sup>-1</sup>). Our findings demonstrate that immobilizing laccase on SMW biochar enhances its productivity and stability. This was achieved at pH 4.5, temperature 30 °C and enzyme

dosage ( $5 \text{ U mL}^{-1}$ ). Additionally, it was observed that the relative activity of laccase immobilized biochar was retained up to 65% after 8 consecutive cycles of 2, 2'-azino-bis 3-ethylbenzothiazoline-6-sulphonic acid (ABTS). This approach provides a promising alternative to conventional adsorption technologies in WWTPs for OMP remediation. This biochar-immobilized enzyme system provides a sustainable, agro-waste based approach with potential real-world applications in WWTPs, where it could help address the challenges of persistent pollutants, ultimately contributing to environmental restoration and ecological health.

