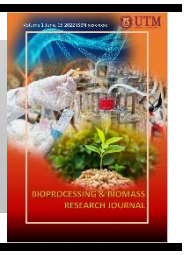
Bioprocessing and Biomass Technology

Journal homepage: https://bioprocessing.utm.my



Research Article

Biodegradation of Oxytetracycline by Using Laccase Entrapped in Mesoporous Silica Microparticles

Nur Hazimah Abdul Halim, Azmi Fadziyana Mansor*, Noorashikin Md Salleh, Nur Farahah Mohd Khairuddin, Nur Atiqah Mohamad Aziz,

Department of Chemical and Process Engineering, Faculty of Engineering and Built Environment, Universiti Kebangsaan Malaysia, Selangor, Malaysia

ARTICLE INFO

Article History:
Received 06 November 2025
Received in revised form 24 November 2025
Accepted 24 November 2025
Available online 30 December 2025

Keywords:
Immobilized laccase,
Oxytetracycline,
Degradation

ABSTRACT

This study evaluates the catalytic efficiency of free laccase and laccase entrapped in mesoporous silica microparticles (LSM) for the degradation of oxytetracycline (OTC) in aqueous media. The LSM system demonstrated a degradation efficiency of up to 82% within 24 hours, outperforming the free enzyme in terms of pH tolerance and operational stability. Temperature-dependent experiments revealed that degradation at elevated temperatures was primarily due to OTC thermal instability rather than enzymatic activity. Furthermore, LSM retained approximately 90% of its degradation capacity after seven reuse cycles, indicating strong potential for repeated application. These findings underscore the applicability of LSM as a robust biocatalyst for antibiotic remediation in water treatment systems.

INTRODUCTION

The pervasive application of antibiotics across healthcare, veterinary, and agricultural domains has resulted in their continuous release into aquatic ecosystems, raising serious environmental and public health concerns. Even at minimal concentrations, residual antibiotics can disturb microbial equilibrium, accelerate the emergence of antimicrobial resistance, and potentially endanger human health (Kumar et al., 2022; Sarmah et al., 2025). The detection of these compounds in potable water supplies highlights the critical need for efficient and sustainable removal technologies.

Oxytetracycline (OTC), a widely utilized tetracycline-class antibiotic, is frequently identified in terrestrial and aquatic matrices due to its partial metabolic breakdown and excretion in biologically active forms (Solanki et al., 2022). Its strong affinity for soil particles, coupled with the possibility of desorption under variable environmental conditions, contributes to its environmental persistence and mobility (Kim et al., 2023; Yang et al., 2024). Notably, OTC concentrations reaching up to 50 mg/L have been documented in effluents from pharmaceutical manufacturing, significantly surpassing levels typically observed in domestic wastewater streams (Leichtweis et al., 2022; Zhong et al., 2022).

Traditional treatment methods such as ozonation and photolytic irradiation have demonstrated high efficacy in degrading OTC; however, they often result in the formation of hazardous secondary products (Nguyen et al., 2021). In contrast, enzymatic degradation particularly via laccase presents a more environmentally favourable approach (Younus et al., 2023). Laccase, a multicopper oxidase, is capable of oxidizing a broad spectrum of organic pollutants. Its catalytic performance can be significantly enhanced through immobilization techniques, which improve enzyme stability, activity retention, and operational reusability (Datta et al., 2021; Chen et al., 2022; Ren et al., 2023).

Building on this, laccase immobilization has been widely investigated using diverse supports such as carbonaceous materials, polymers, and metal–organic frameworks (MOFs), each offering distinct advantages in terms of enzyme stability and reusability (Datta et al., 2021). Carbon-based supports, including activated carbon and graphene

*Corresponding Author
E-mail address: afadziyana@ukm.edu.my
DOI address: 10.11113/bioprocessing.v4n2.82

derivatives, provide high surface area and strong adsorption capacity, while polymeric matrices offer flexibility and ease of functionalization for tailored enzyme–support interactions (Ren et al., 2023). MOFs have recently attracted attention due to their tunable porosity and ability to enhance enzyme stability, though limitations in mass transfer and pore blockage remain challenges (Ren et al., 2023). In comparison, silica-based supports are particularly advantageous because of their chemical inertness, mechanical robustness, and highly porous structure, which facilitate efficient enzyme entrapment and minimize leaching. Recent studies have demonstrated that silica-bound laccase exhibits enhanced catalytic activity, stability, and operational reusability under environmental conditions, making it especially suitable for applications such as wastewater treatment (Alokpa et al., 2025; Rodríguez-Couto, 2023). Thus, the choice of mesoporous silica microparticles in this study is well justified, as they combine structural stability with biocompatibility, offering a reliable platform for sustainable enzymatic degradation of antibiotics.

This research therefore focuses on the enzymatic breakdown of OTC using laccase entrapped in mesoporous silica microparticles (LSM). The study compares the degradation efficiency of immobilized versus free laccase under varying physicochemical conditions, including pH, temperature, and reaction duration. Furthermore, the recyclability of the LSM system is evaluated to determine its feasibility for real-world wastewater treatment applications.

MATERIALS AND METHOD

Chemicals and Reagents

All reagents utilized in this study were of analytical grade to ensure experimental accuracy and reproducibility. Oxytetracycline hydrochloride (OTC), acetonitrile, sodium acetate, orthophosphoric acid, and n-hexane were procured from Sigma-Aldrich (USA). Additional chemicals including 2,6-dimethoxyphenol, ammonia solution, triethylamine (TEA), isopropanol (IPA), and tetraethyl orthosilicate (TEOS) were obtained from Merck (Germany). Laccase enzyme from *Trametes versicolor* was supplied by Daiwa Kasei Co. Ltd. (Japan). This enzyme preparation contained 30% protein by weight, with a molecular mass of approximately 62 kDa, activity 500 U/g and an isoelectric point of 3.

Preparation of LSM

Immobilization of laccase within silica microparticles was achieved through a sol-gel synthesis approach using TEOS as the silica precursor. The initial sol was prepared by mixing TEOS, IPA, deionized water, (1:0.8:0.6 by molar ratio) and $2.5x10^{-6}$ mol hydrochloric acid (0.05 M), followed by continuous stirring at 500–600 rpm for 2 hours to facilitate hydrolysis and condensation. Subsequently, 5 mg/ml laccase solution and $0.4x10^{-3}$ mol TEA were added to initiate gelation. The gel was aged at 30 °C for 1 hour to enhance structural integrity. This method follows the procedure described by Mansor et al. (2015), in which the physical properties of LSM and the performance of immobilized laccase were characterized. The present study extends that work by investigating the degradation of oxytetracycline (OTC) using LSM, building upon the established immobilization technique and previously reported material properties.

Post-gelation, a solvent exchange process was conducted using a ternary mixture of silane, IPA, and n-hexane in a volumetric ratio of 1:1:2 for 2 hours to remove residual reactants and stabilize the matrix. The resulting microparticles were thoroughly rinsed with IPA, air-dried at ambient temperature, and stored at 5 °C to preserve enzymatic activity. To evaluate enzyme retention, leaching studies were performed by incubating the LSM in phosphate buffer (pH 7), and the amount of released protein was quantified using the Biuret assay (Noor, 2025). The amount of protein released confirms the effectiveness of the immobilization process and the structural stability of the silica microparticles.

OTC Degradation Procedure

A stock solution of OTC (1000 mg/L) was prepared in methanol and stored at −20 °C to maintain stability. Working solutions at 100 mg/L were freshly diluted with deionized water prior to each experiment. For degradation studies, 50 mg of LSM was added to 25 mL of OTC solution and incubated at 30 °C with agitation at 200 rpm. All reactions were conducted under light-protected conditions to prevent photodegradation. Samples were filtered through 0.22 µm membranes before analysis. The degradation percentage was determined using the **Equation (1)**.

$$C(\%) = \frac{(C_0 - C_t)}{C_0} \times 100 \qquad \textbf{Equation (1)}$$

Where, C_o and C_t represent the initial and residual concentrations of OTC, respectively. In a typical experiment, free laccase (FL) was used as the comparison to LSM. Control experiments which is OTC in methanol without laccase were conducted to account for non-enzymatic degradation. All the reaction were performed in triplicate and covered with aluminium foil to eliminate the influence of light on OTC degradation.

OTC Concentration Assay

Quantification of OTC was performed using a Varian 5000 HPLC system equipped with a UV detector set at 355 nm. Separation was achieved using a LiChroSpher 100 RP-18E column (125 × 4.6 mm I.D., Merck) fitted with a 5-µm LiChroSpher 100 RP-18E guard column (4.6 × 34 mm I.D.). The mobile phase consisted of acetonitrile and 0.02 M orthophosphoric acid in a 24:76 (v/v) ratio, adjusted to pH 2.3. The flow rate was maintained at 1.2 mL/min, with the column temperature set at 30 °C. Each sample injection volume was 100 µL, ensuring consistent chromatographic performance throughout the analysis.

Evaluation of Stability and Reusability

The effect of reaction time on oxytetracycline (OTC) degradation was initially investigated at pH 7 and 30 °C, with reactions monitored for up to 92 hours. For pH variation studies (pH 4–8), the reaction time was fixed for 24 hours, while the temperature was maintained at 30 °C. Conversely, for temperature variation studies (30–70 °C), the reaction time 24 hours and pH 4 were set based on the results obtained from the preceding pH experiment. This sequential one-factor-at-a-time (OFAT) approach ensured that each parameter was systematically evaluated while controlling for the influence of other variables.

To evaluate the reusability performance, the LSM were recovered after each reaction cycle, thoroughly washed, and

reintroduced into fresh OTC solution. This process was repeated for seven consecutive cycles to determine the retention of catalytic activity over time.

RESULTS AND DISCUSSION

Reaction Time Effects on OTC Degradation

The successful entrapment of laccase within the silica matrix was confirmed through the sol–gel immobilization procedure, during which all reaction mixtures consistently transitioned into stable gels, indicating effective incorporation of the enzyme into the silica network (Mansor et al., 2015). Following immobilization, no protein leaching was detected after drying, as verified by the Biuret assay, thereby confirming that the enzyme was securely entrapped within the matrix. The absence of leaching not only validates the robustness of the immobilization process but also highlights the protective role of the silica framework, which shields laccase from direct environmental stress and minimizes conformational changes that could compromise activity.

The temporal profile of oxytetracycline (OTC) degradation revealed a progressive increase in removal efficiency over the 24-hour incubation period. Initially, both free laccase and laccase immobilized in mesoporous silica microparticles (LSM) exhibited modest degradation rates of 24% and 35%, respectively within the first 3 hours (**Figure 1**). This lag phase can be attributed to the structural complexity of OTC, which may hinder direct enzymatic oxidation due to steric hindrance and limited accessibility to reactive sites. The superior performance of LSM in the early phase suggests that immobilization may enhance enzyme-substrate interactions by stabilizing the enzyme conformation and facilitating substrate diffusion through the porous matrix (Wang et al., 2021; Xu et al., 2023).

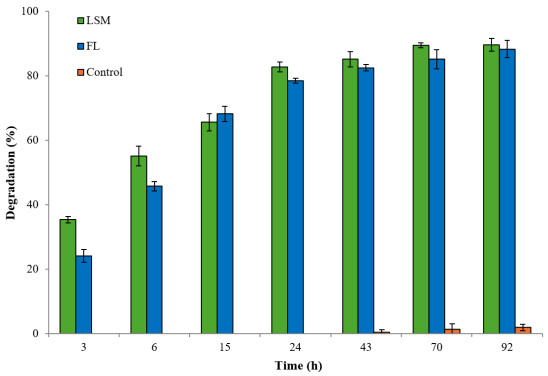


Figure 1 Effect of reaction time on degradation of OTC

As the reaction progressed, degradation efficiencies increased significantly, reaching 78% for free laccase and 82% for LSM after 24 hours. These results demonstrate that laccase, even without the aid of redox mediators such as 1-hydroxybenzotriazole (HBT), possesses intrinsic catalytic capability to transform OTC. The gradual escalation in degradation may reflect cumulative enzymatic activity and partial breakdown of OTC into intermediates more amenable to further oxidation. However, beyond 24 hours, the degradation plateaued, and control experiments indicated non-enzymatic degradation, likely due to OTC's inherent instability under prolonged exposure to aqueous conditions (Bashory et al., 2025; Ulucan-Altuntas et al., 2021). This underscores the importance of distinguishing enzymatic activity from antibiotic degradation when evaluating treatment efficacy. The findings align with previous studies that employed laccase-mediated systems for antibiotic removal, although many relied on mediators to enhance redox potential (Gu et al., 2021). The ability of LSM to achieve comparable degradation without additives highlights its potential for simplified and cost-effective deployment in environmental remediation (Ezra et al., 2024).

pH Effects on OTC Degradation

pH is a critical determinant of enzymatic activity and substrate stability, particularly in environmental applications where pH fluctuations are common. The study examined OTC degradation across a pH range of 4 to 8, revealing distinct performance differences between free and immobilized laccase. **Figure 2** shows LSM maintained high degradation efficiency (>80%) throughout the tested pH spectrum, with peak removal observed at pH 4 (92.6%). However, control experiments indicated that acidic conditions alone contributed to 24% OTC degradation, suggesting that hydrolytic breakdown may complement enzymatic activity at low pH (Solanki et al., 2022). Importantly, LSM demonstrated consistent performance even as pH increased, indicating enhanced structural resilience and catalytic stability conferred by immobilization. This broad pH tolerance is advantageous for real-world applications, where wastewater pH can vary significantly (Chen et al., 2022).

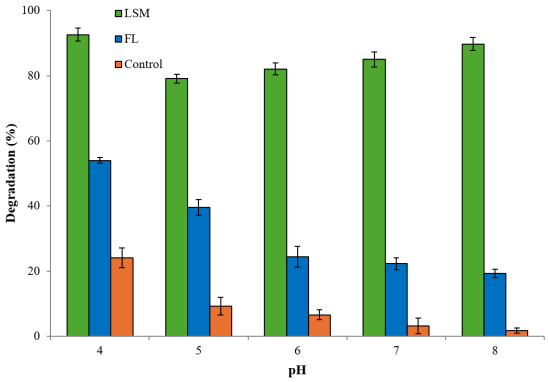


Figure 2 Effect of pH on degradation of OTC

In contrast, free laccase exhibited declining activity with increasing pH, dropping from 54% at pH 4 to just 19% at pH 8. This trend is consistent with the known behavior of fungal laccases, which typically operate optimally under acidic conditions. Alkaline pH can disrupt the enzyme's internal electron transfer by promoting hydroxide ion binding to the copper centers in the active site, leading to conformational changes and reduced catalytic efficiency (Sharma, 2025). The enhanced pH stability of LSM supports findings from other immobilization strategies, such as cross-linking with glutaraldehyde or encapsulation in alginate beads, which have similarly improved enzyme robustness (Lassouane et al., 2022). These results affirm that immobilization not only preserves enzymatic function but also extends its operational range, making LSM a versatile biocatalyst for diverse environmental conditions.

Temperature Effects on OTC Degradation

Temperature influences both enzymatic kinetics and substrate stability. In this study, OTC degradation was

assessed across temperatures ranging from 30 °C to 70 °C. Both free laccase and LSM showed increased degradation with rising temperature, with maximum removal observed at 50 °C and above (**Figure 3**). This result however does not completely represent the OTC removal either by free laccase or LSM, respectively. Similar result was also observed in relative degradation percentage through the negative control experiment despite the absence of enzymatic activity. This is because higher temperatures favoured lower drug stability, resulting in a very short half-life and speeded the OTC degradation (Zouanti et al., 2020). This observation complicates the interpretation of enzymatic activity, as the degradation did not solely reflect the biocatalytic transformation.

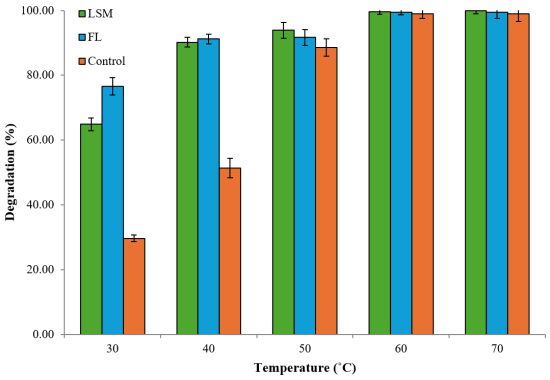


Figure 3 Effect of temperature on degradation of OTC

OTC is known to exhibit reduced stability at higher temperatures, with accelerated hydrolysis and photodegradation pathways contributing to its breakdown (Olumee-Shabon and Knutson, 2023). Furthermore, Wang et al. (2021) reported that the specific activity of free laccase decreased sharply at elevated temperatures, while immobilized laccase on CTAB-KOH modified biochar exhibited remarkable catalytic stability and a broader temperature profile. Immobilization often enhances thermal stability by restricting enzyme mobility and protecting against denaturation (Xu et al., 2023). However, the remarkable relative removal observed at ≥50 °C in the present study is largely attributed to OTC instability rather than enhanced catalytic activity.

Reusability of Immobilized Laccase

Operational stability and reusability are key metrics for evaluating the economic feasibility of immobilized enzymes in large-scale applications. The study demonstrated that LSM retained approximately 90% of its initial degradation capacity after seven consecutive cycles, indicating minimal loss of activity and structural integrity (**Figure 4**). This high level of reusability is consistent with recent reports on immobilized laccase systems, where activity retention over multiple cycles was attributed to reduced enzyme leaching and enhanced mechanical stability of the support matrix. For instance, Zainal et al. (2024) demonstrated efficient immobilization of laccase on magnetic spent tea carriers, achieving high activity yields and stability over repeated cycles. Similarly, Bera et al. (2025) highlighted that interconnected carrier systems significantly reduce enzyme inactivation and physical loss during handling, thereby sustaining catalytic performance. Tadesse and Liu (2025) further emphasized that novel nanomaterials and dynamic carrier systems enhance enzyme reusability by minimizing leaching and improving mechanical robustness. The robust performance of LSM in repeated use scenarios underscores its potential for continuous flow systems and batch reactors in wastewater treatment. By minimizing enzyme consumption and replacement costs, LSM offers a sustainable and scalable solution for antibiotic remediation.

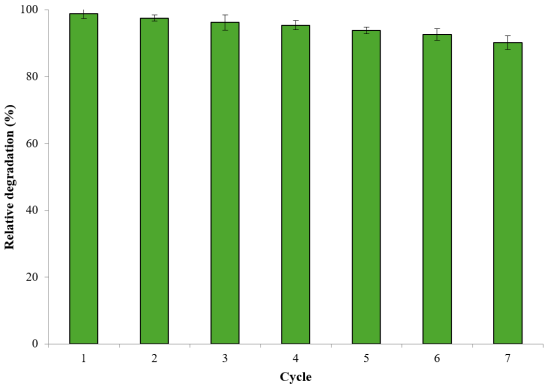


Figure 4 Relative degradation of OTC by LSM in subsequent processes

CONCLUSION

This study presents a comprehensive evaluation of laccase immobilized in mesoporous silica microparticles (LSM) for the degradation of oxytetracycline (OTC) in aqueous environments. The findings demonstrate that LSM not only enhances degradation efficiency compared to free laccase but also significantly improves enzyme stability across a wide range of pH values and operational conditions. While temperature-induced degradation was largely attributed to OTC instability, LSM maintained structural integrity and catalytic potential under thermal stress. The reusability of LSM further reinforces its suitability for industrial and environmental applications, achieving nearly 90% degradation efficiency after multiple cycles. These attributes high catalytic performance, broad pH tolerance, thermal resilience, and operational reusability which position LSM as a promising biocatalyst for sustainable antibiotic removal from contaminated water sources. Future work may focus on integrating LSM into pilot-scale treatment systems, exploring its performance in complex wastewater matrices, and assessing its compatibility with other bioremediation strategies. Overall, the immobilization of laccase in mesoporous silica represents a significant advancement in enzymatic water treatment technologies.

Acknowledgement

The financial supports from the Universiti Kebangsaan Malaysia for funding this research via Geran Galakan Penyelidik Muda (Grant number: GGPM-2024-020) are gratefully acknowledged.

Conflicts of Interest

The author declares that there is no conflict of interest regarding the publication of this paper.

References

Alokpa, K., El-Yagoubi, Y. and Cabana, H. (2025). Development and optimization of a silica-bound laccase biocatalyst and its application in hospital wastewater treatment. *Environmental Science and Pollution Research*, 32, 5399–5418.

Bashory, F. N., ALshibeh ALwattar, N. H. and AL Masalma, M. R. (2025). Stability of oxytetracycline in different types of solutions and stored at different temperatures. *ACS Omega*, 10(18), 12037–12042.

Bera, K., Bhattacharya, D., & Mukhopadhyay, M. (2025). Breakthroughs into laccase immobilization: a glance of techniques and interconnected carriers. *Brazilian Journal of Chemical Engineering*, 1-37.

Chen, Z., Oh, W.D. and Yap, P.S. (2022). Recent advances in the utilization of immobilized laccase for the degradation of phenolic compounds in aqueous solutions: A review. *Chemosphere*, 307, 135824.

Datta, S., Veena, R., Samuel, M. and Ethiraj, S. (2021). Immobilization of laccases and applications for the detection and remediation of pollutants: A review. *Environmental Chemistry Letters*, 19(1), 521–538.

Ezra, R., Vanti, G., & Masaphy, S. (2024). Sustainable, targeted, and cost-effective laccase-based bioremediation technologies for antibiotic residues in the ecosystem: A comprehensive review. *Biomolecules*, 15(8), 1138.

Gu, Y., Yuan, L., Jia, L., Xue, P. and Yao, H. (2021). Recent developments of a co-immobilized laccase–mediator system: A review. *RSC Advances*, 11, 29498–29506.

Kim, S. H., Park, S. Y., Kim, G. E. and Jho, E. H. (2023). Effect of pH and temperature on the biodegradation of oxytetracycline, streptomycin, and validamycin A in soil. *Applied Biological Chemistry*, 66(63).

Kumar, A., Pal, D. and Singh, R. (2022). Antibiotics in aquatic environments: a review on occurrence, fate, and ecological risks. *Environmental Science and Pollution Research*, 29(45), 67812–67830.

Lassouane, F., Aït-Amar, H. and Rodriguez-Couto, S. (2022). High BPA removal by immobilized crude laccase in a batch fluidized bed bioreactor. *Biochemical Engineering Journal*, 184, 108489.

Leichtweis, J., Vieira, Y., Welter, N., Silvestri, S., Dotto, G.L. and Carissimi, E. (2022). A review of the occurrence, disposal, determination, toxicity and remediation technologies of the tetracycline antibiotic. *Process Safety and Environmental Protection*, 160, 25-40.

Mansor, A. F., Atikah, N., Nurul, W., Wan, I., Zawawi, M., Othman, N. S. and Endud, S. (2015). Preparation and characterization of in-situ entrapment of laccase in silica microparticles via an ambient drying procedure. *Journal of Sol-Gel Science and Technology*, 75(2), 323–335.

Nguyen, C.H., Tran, M.L., Van Tran, T.T. and Juang, R.S. (2021). Efficient removal of antibiotic oxytetracycline from water by Fenton-like reactions using reduced graphene oxide-supported bimetallic Pd/nZVI nanocomposites. *Journal of the Taiwan Institute of Chemical Engineers*, 119, 80-89.

Noor, R. (2025). Biuret Reagent: Principle, Procedure, Protein Detection. Cliff Scientific World. Retrieved from https://cliffscientificworld.com/biuret-reagent/.

Olumee-Shabon, Z. and Knutson, S. (2023). Impact of temperature and water hardness on the stability of mixed tetracyclines (chlortetracycline, oxytetracycline, and tetracycline) used in veterinary medicine. *American Journal of Veterinary Research*, 85(4), 1–10.

Ren, S., Wang, F., Gao, H., Han, X., Zhang, T., Yuan, Y., and Zhou, Z. (2023). Recent progress and future prospects of laccase immobilization on MOF supports for industrial applications. *Applied Biochemistry and Biotechnology*, 196, 1669–1684.

Rodríguez-Couto, S. (2023). Immobilized-laccase bioreactors for wastewater treatment. *Biotechnology Journal*, 18(1), e202300354.

Sarmah, K., Saikia, K. and Datta Roy, J. (2025). Laccase enzyme as a sustainable tool for improving environmental health. *Biodegradation*, 36, 124.

Sharma, N.D. (2025). Reaction mechanism and redox potential of laccase. In Laccase and Polyphenol Oxidase. *Academic Press*, 111-132.

Solanki, R., Patel, D., Patel, H. and Patel, M. (2022). Occurrence and fate of oxytetracycline residues in the environment: a review. *Environmental Science and Pollution Research*, 29(12), 17568–17582.

Tadesse, M. and Liu, Y. (2025). Recent advances in enzyme immobilization: the role of artificial intelligence, novel nanomaterials, and dynamic carrier systems. *Catalysts*, 15(6), 571.

Ulucan-Altuntas, S., Yazici Guvenc, S., Can-Güven, E., Ilhan, F. and Varank, G. (2021). Degradation of oxytetracycline in aqueous solution by heat-activated peroxydisulfate and peroxymonosulfate oxidation. *Environmental Science and Pollution Research*, 28, 48238–48250.

Wang, Z., Ren, D., Jiang, S., Yu, H., Cheng, Y., Zhang, S., Zhang, X. and Chen, W. (2021). The study of laccase immobilization optimization and stability improvement on CTAB-KOH modified biochar. *BMC Biotechnology*, 21, 47.

Xu, R., Zhang, X., Zelekew, O. A., Schott, E. and Wu, Y. (2023). Improved stability and activity of laccase through de novo and post-synthesis immobilization on a hierarchically porous metal–organic framework (ZIF-8). *RSC Advances*, 13, 17194-17201.

Yang, Z., Zhang, Q., Jiang, Y., Kong, W., Wang, W. and Wu, Y. (2024). Study on the adsorption process and mechanism of oxytetracycline in different loess: roles of soil characteristics and influencing factors. *Water, Air, and Soil Pollution*, 235(419).

Younus, H., Khan, M. A., Khan, A. and Alhumaydhi, F. A. (2023). Eco-friendly biocatalysts: Laccase applications, innovations, and future directions in environmental remediation. *Catalysts*, 15(10), 921.

Zainal, D. A. A., and Mohd Syukri, M. S. (2024). Effects of incubation time and laccase concentration on immobilization of laccase on magnetic spent tea: adsorption and cross-linking method. *Bioprocessing and Biomass Technology*, 3:2, 123 – 127.

Zhong, S.F., Yang, B., Lei, H.J., Xiong, Q., Zhang, Q.Q., Liu, F. and Ying, G.G. (2022). Transformation products of tetracyclines in three typical municipal wastewater treatment plants. *Science of The Total Environment*, 830, 154647.

Zouanti, M., Bezzina, M. and Dhib, R. (2020). Experimental study of degradation and biodegradability of oxytetracycline antibiotic in aqueous solution using Fenton process. *Environmental Engineering Research*, 25(3), 316-323.