



Research paper

Immobilization of laccases from *Pycnoporus sanguineus* on magnetized carbon nanofibers for the degradation of psychiatric drugs: Venlafaxine and carbamazepine

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ARTICLE INFO

Keywords:

Biocatalysis
Enzyme immobilization
Laccases
Nanobiocatalysts
Nanofibers

ABSTRACT

The COVID-19 pandemic has significantly increased the consumption of psychiatric medications, such as venlafaxine (VFX) and carbamazepine (CBZ), leading to their accumulation in wastewater and subsequent environmental concerns. These compounds are classified as emerging pollutants, presenting challenges for conventional wastewater treatment systems. This study, faced the immobilization of laccases derived from a native strain of *Pycnoporus sanguineus* CS43, isolated from northeastern Mexico, onto magnetically modified carbon nanofibers (mCNF) to enhance degradation efficiency, enzyme stability, and reusability. The enzyme immobilization on mCNF resulted on high loading value of 73.24%. The performing of the degradation assays revealed that this innovative system achieved 51.51% and 57.14% removal of VFX and CBZ respectively within just 4 h at pH 5 and 25°C. A remarkable stability of the system was demonstrated retaining between 70% and 100% the enzyme activity on the nanomaterial. After 28 days of storage, the nanobiocatalysts system retained 74.50% of their initial activity. These results highlight the potential of mCNF as an effective support for laccase immobilization, providing a sustainable and efficient strategy for the bioremediation of psychiatric drug pollutants in aquatic environments.

1. Introduction

The COVID-19 pandemic has led to a significant increase in the consumption of psychiatric medications, such as venlafaxine (VFX) and carbamazepine (CBZ), resulting in their accumulation in wastewater and raising environmental concerns [1,2]. These compounds, which are classified as emerging pollutants (EPs), persist in aquatic environments due to the low efficiency of conventional wastewater treatment plants (WWTPs) in degrading/removing them effectively. Studies have shown that pharmaceutical residues released from WWTPs can disrupt gene expression, mobility, and neurophysiological development in aquatic organisms, plants and humans, highlighting the urgent need for alternative strategies that enhance the removal of these EPs [3,4].

Several strategies have been explored for the degradation of VFX and CBZ, including advanced oxidation processes (AOPs), electrochemical oxidation (EO), and biological degradation (BD). AOPs, such as photocatalytic degradation using TiO₂ catalysts, have demonstrated complete removal of VFX [5,6]. Similarly, CBZ degradation through Electro-Fenton process catalyzed by Fe@Fe₂O₃ nanowire from aqueous solution has shown promising results achieving a removal efficiency of 91.2 % [7]. However, these methods often produce toxic byproducts or demand high energy inputs to achieve high removal yields, which limits their efficiency and sustainability [8]. In contrast, biological based treatments, particularly enzyme mediated processes, have emerged as greener, environmentally-friendlier alternatives. For example, Laccases from *Trametes versicolor* and *Ganoderma lucidum* have demonstrated

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<https://doi.org/10.1016/j.rineng.2025.106956>

Received 19 July 2025; Received in revised form 22 August 2025; Accepted 26 August 2025

Available online 26 August 2025

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efficient degradation of VFX and CBZ and other EPs [3,9], but challenges such as prolonged reaction times, enzyme instability, and non-reusability hinder large-scale applications [10].

To address these challenges, this study investigates the application of a carbonaceous magnetic nanostructured material for the immobilization of laccases from *Pycnoporus sanguineus*. Enzyme immobilization has been widely recognized for its advantages over free enzyme applications, particularly exhibiting improved stability, easier enzyme recovery, and enhanced catalytic efficiency [11–13]. Among the various nanomaterials available, carbon-based materials have gained significant attention as enzyme carriers due to their unique physicochemical properties, including high surface area, biocompatibility, and chemical stability [14]. Additionally, the integration of magnetic nanoparticles into the nanobiocatalytic system enables efficient recovery of the immobilized biocatalyst, minimizing enzyme loss, prolonging its functionality in better conditions compared with other materials [15]. Moreover, these materials have been proven to be efficient for biological and industrial applications of immobilized enzymes, since the magnetic separation minimizes negative impacts on biocatalysts [16]. However, since pure magnetite cannot effectively combine with biomolecules, literature has reported its utilization in combination with other materials, such as nanoflowers, metal organic frameworks, carbonaceous materials, polymeric composites, or even core-shell structures [16–18].

Therefore, this study presents an innovative strategy for addressing emerging pollutant contamination in aquatic systems through the immobilization of native *P. sanguineus* laccases onto magnetized carbon nanofibers. This approach not only enhances enzyme stability but also enables reusability providing a more sustainable alternative to conventional degradation methods. Moreover, the immobilization of this specific laccase species onto magnetic nanomaterials has not been explored previously, positioning this research as a novel contribution to the field. By demonstrating the effectiveness of this nanobiocatalytic system in removing VFX and CBZ, this work advances eco-friendly solutions for mitigating pharmaceutical pollutants in water treatment applications.

2. Experimental section

2.1. Chemicals and materials

2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS, $\geq 98\%$ purity), venlafaxine hydrochloride ($\geq 99\%$), carbamazepine ($\geq 99\%$), sodium phosphate monobasic monohydrate ($\geq 98\%$), sodium phosphate dibasic heptahydrate ($\geq 98\%$), citric acid anhydrous ($\geq 99\%$), iron (II) sulfate heptahydrate ($\geq 99\%$), nitric acid ($\geq 99\%$), iron (III) chloride hexahydrate ($\geq 99\%$), ammonia hydroxide ($\geq 99\%$), and carbon nanofibers ($\geq 98\%$, $D \times L$ 100 nm $\times$ 20–200 μ m) were purchased from Sigma-Aldrich (Monterrey, Mexico). HPLC-grade water was prepared in-house using a Milli-Q/Milli-RO system (Millipore, USA), and HPLC-grade methanol was obtained from J.T. Baker (Monterrey, Mexico).

2.2. Enzymes production

Laccase was obtained from an indigenous strain of *P. sanguineus* CS43, provided by the Universidad Autónoma de Nuevo León, Mexico. Fungal cultures and enzyme production were performed following the conditions reported by Ramírez-Cavazos et al. with some modifications [19,20]. Laccase production was carried out at 28 °C for 14 days using 14 L bioreactors (New Brunswick Scientific Co., Germany) with a working volume of 10 L. After fermentation, the samples were collected, filtered, and centrifuged at 4000 $\times$ g for 10 min to obtain a clear supernatant, which was used as the crude enzyme extract for subsequent biotransformation reactions [21]. Two laccase isoforms, Lac I and Lac II, derived from this strain, have been previously reported and characterized [20,22].

2.3. Synthesis of the magnetic carbon nanofibers (mCNF)

The functionalization of iron oxide nanoparticles was performed by modifying a previously reported method [23]. This method involved the acid treatment of CNF followed by a chemical co-precipitation process. Initially, 1 g of CNF was mixed with 100 mL of nitric acid and treated at 80 °C for 6 h under vigorous magnetic stirring. After the acid treatment, the mixture was washed several times with deionized water until the pH was adjusted to approximately 7. The sample was then centrifuged and dried in a vacuum oven at 60 °C under 700 mbar. The magnetic functionalization of CNF was carried out using a co-precipitation method [24]. First, 0.450 g of oxidized CNF (CNFox) was dispersed in 200 mL of distilled water using ultrasonic probe sonication for 15 min. Then, 0.450 g of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ was added, and the sonication continued for another 15 min before heating the mixture was heated to 55 °C under a nitrogen (N_2) atmosphere. Next, 0.231 g of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ was added, and the sonication was maintained for 30 min at 55 °C under nitrogen. The pH value was adjusted to approximately 10 by adding 15 mL of 3.5 M NH_4OH dropwise to precipitate ferric and ferrous salts. Finally, the magnetic carbon nanofibers (mCNF) were recovered via magnetic separation, washed with distilled water and ethanol until a neutral pH was reached, and then dried under vacuum at 60 °C under 550 mbar.

2.4. Immobilization of laccases

Crude laccases from the CS43 strain were immobilized onto the mCNF via physical adsorption, following the method described by González-Coronel et al. (2017) with some modifications [25]. First, 10 mg of mCNF was suspended in 3 mL of PBS (pH 7) and sonicated for 15 min using an ultrasonic probe. Then, a 23 U/mL laccase solution was added to the suspension, and the mixture was mechanically stirred at 180 rpm for 2 h at 25 °C. Afterward, the immobilized enzyme (mCNFs@Lcc) was magnetically separated and washed with PBS buffer to remove any unbound enzyme. The immobilized enzymes were stored at 4 °C until further use.

2.5. Characterization of nanobiocatalyst

The modified carbon nanofibers were characterized using multiple analytical techniques. Transmission Electron Microscopy (TEM; Hitachi H-7500, Japan) was employed to examine the morphology of the nanoparticles, operating at an acceleration voltage of 80 kV with spot size of 5 for high-resolution imaging. Samples were deposited onto 75-mesh copper grids coated with formvar/carbon support films (TED Pella, prod. # 01,802-F). Energy-dispersive X-ray spectroscopy (EDS; XFlash6110, Bruker, Germany) was used to analyze the chemical composition of CNF, oxidized CNF (CNFox), and mCNF, with all samples sputtered with a thin layer of gold to prevent charging during analysis. Fourier Transform Infrared Spectroscopy (FTIR; Frontier, Perkin Elmer, Germany) equipped with an ATR accessory was utilized to assess structural changes in the chemical composition of the CNF, CNFox, mCNF, and mCNF@Lcc. Zeta potential (ζ) was measured to monitor surface charge changes after CNF magnetization (Zetasizer Pro Blue, Malvern Panalytical). Finally, the crystalline phases of the magnetic nanoparticles in CNF and mCNF were examined using X-ray diffraction (XRD; Rigaku MiniFlex600, USA), with all samples dried under vacuum before analysis. No additional treatment was necessary for the XRD analysis.

2.6. Enzymatic activity and stability assessments

Enzymatic activity was determined spectrophotometrically by monitoring the oxidation of 0.5 mM 2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS). The oxidation reaction was performed in a 0.1 M citrate/0.2 phosphate buffer (pH 3) at 25 °C, and the increase in absorbance at 420 nm ($\epsilon = 36 \text{ mM}^{-1} \text{ cm}^{-1}$)

was measured using a BMG LabTech plate spectrophotometer (Durham, USA). One unit (U) of laccase activity was defined as the amount of enzyme required to oxidize 1 μmol of ABTS per minute, it was calculated using Eq. (1):

$$\text{Laccase activity} \left(\frac{U}{L} \right) = \frac{\Delta A * V_t * 10^6}{\Delta t * d * \epsilon * V_s} \quad (1)$$

Where ΔA is the difference in absorbance, Δt is the period of incubation (s), ϵ is the extinction coefficient, d is the cuvette diameter (1 cm), V_t is the total assay volume, and V_s is the enzyme sample volume.

The percentage of enzyme immobilization was determined by measuring laccase activity and calculating the difference between the initial activity and the residual activity in the supernatant and wash solutions, using Eq. (2):

$$\% \text{ of immobilization} = \frac{(A_i - A_r)}{A_i} * 100 \quad (2)$$

where A_i and A_r were the activity of the laccases and the recovered activity from supernatant of immobilization solution.

The pH stability of the nanobiocatalyst was evaluated by measuring the activity of the immobilized system at pH values ranging from 3 to 7. Since the enzymatic activity of the nanobiocatalyst cannot be measured directly, a relative activity assessment was performed by mixing the immobilized system with ABTS under mechanical stirring in the absence of light for 10 min. The nanobiocatalyst was then magnetically separated, and the reaction was halted by adding an equal volume of acetonitrile. The absorbance at 420 nm was measured, and the highest recorded absorbance was set as 100 % relative activity. The reusability of the immobilized enzyme system was evaluated through repeated activity assays under identical conditions. Immobilized laccase activity was measured at pH 3 and 25 °C using ABTS as the substrate. Each reaction cycle lasted 10 min, after which the nanobiocatalyst was magnetically separated from the reaction mixture. Acetonitrile was added to inactivate any desorbed enzymes, and absorbance was measured. The recovered material was washed with PBS (pH 7) and resuspended in fresh ABTS solution for the next cycle, with the laccase activity in the first cycle set as 100 %

2.7. Removal assays

Carbamazepine and venlafaxine removal tests were conducted in batch systems using a 2 mL reaction volume containing 30 mg mL⁻¹ of the respective compound, PBS buffer (pH 7), and 10 mg of immobilized enzyme (16.79 U) in carbon nanofibers. The systems were incubated at 25 °C and 60 °C with shaking at 180 rpm for 4 h, with temperatures selected based on previous reports [21]. To determine concentration of contaminants, 200 μL of the reaction solution was collected and mixed with acetonitrile (1:1) to halt the reaction. The mixtures were subsequently filtered through a 0.22 μm Nylon filter (Thermo Scientific) and stored at -20 °C for further analysis. Controls were included to distinguish between enzymatic degradation and adsorption effects. The first control consisted of only free laccases to assess biodegradation capability. The second consisted of only mCNF to evaluate adsorption. Both controls were conducted in the presence of the respective drugs. All experiments were conducted in duplicate.

Quantitative analysis was carried out using High-Performance Liquid Chromatography (HPLC, Agilent, UltiMate 3000) equipped with a reverse-phase Zorbax Eclipse C18 column (4.6 $\times$ 150 mm). The analysis was carried out in isocratic mode with two mobile phases: phase A (methanol with 0.1 % formic acid) and B (water with 0.1 % formic acid). The mobile phase composition was set at 60:40 % for CBZ and 50:50 % for VFX. The flow rate was maintained at 1 mL/min, with a 20 μL sample injection per run. The column temperature was set at 40 °C, and equilibration was performed for 10 min before each analysis. Each drug was monitored for 15 min to ensure complete separation and accurate

quantification, with detection wavelengths set at 285 nm for CBZ and 226 nm for VFX. Concentrations were normalized to the initial value (time 0 h, set as 100 %). Any fluctuations above 100 % in control samples are attributed to small variations in sampling, extraction efficiency, or analytical sensitivity.

3. Results and discussion

3.1. Characterization

3.1.1. Microstructural and chemical characterization

Various carbon-based nanomaterials combined with iron oxide nanoparticles have been previously reported, encompassing multiple phases, including Fe₃O₄, α -Fe₂O₃, γ -Fe₂O₃, ϵ -Fe₂O₃, and β -Fe₂O₃. Among these, Fe₃O₄ is considered the most favorable due to its superior biocompatibility, high chemical stability, and non-toxic nature [26]. Following the visual confirmation of CNF magnetization, as evidenced by material's separation upon exposure to a magnetic field (Fig. S1), XRD characterization was performed to determine the phase of the synthesized iron oxide. Although Fe₃O₄ (magnetite) was the expected phase, it has been reported that side reactions and uncontrolled synthesis conditions can lead to the formation of undesired phases like hematite (Fe₂O₃) or maghemite (γ -Fe₂O₃), which can arise when reaction parameters such as temperature, pH, or precursor concentration deviate from optimal conditions, affecting the control over the iron oxidation states [27]. Fig. 1 presents the characterization of synthesized magnetic nanoparticles (MNPs), alongside a comparison between the non-magnetized carbon nanofibers (CNF) and the magnetized nanofibers (mCNF). The XRD pattern of magnetite (Fe₃O₄), referenced from the JCPDS database (JCPDS: 19-0629), was compared with the experimental data. The characteristic diffraction peaks of Fe₃O₄ were observed at 2θ values of 30.2°, 35.5°, 43.2°, and 53.5°, corresponding to the (220), (311), (400), (422), (511), and (440) crystalline planes, respectively. These diffraction peaks confirm the successful formation of Fe₃O₄ via the co-precipitation method assisted by an ultrasonic bath [28]. The structural integrity of CNF before and after magnetization was further examined through comparative XRD analysis. The diffractogram of the CNF exhibited two prominent peaks: one at 2θ = 25.9°, corresponding to the (002) plane of graphite, indicating a well-aligned graphitic structure, and another at 2θ = 44°, corresponding to the (100) plane, which is associated with in-plane diffraction of carbon atoms within graphitic sheets [24]. The sharp intensity of the (002) reflection suggests a high degree of crystallinity, indicative of well-ordered graphitic layers, while the relatively weaker signal at 44° suggests the presence of both graphitic and amorphous carbon regions [29]. Following the magnetization process, the XRD pattern of mCNF exhibited strong diffraction signals at 30.2° (220), 35.5° (311), and 53.5° (511), characteristic of Fe₃O₄. Meanwhile, the CNF signal at 25.9° (002) remained visible, confirming the structural retention of the carbon nanofiber. Additionally, a reduction in the intensity of the 44° (100) peak was observed, which is likely due to overlapping diffraction signals from the magnetite phase. This result contrasts with previous reports; for example, in a similar material -magnetic nanofibers synthesized at high temperatures via pyrolysis- an amorphous carbon phase was observed, which could directly impact the thermal and chemical stability of the fiber for further applications [30]. This difference is likely attributed to the pre-acidic treatment applied to CNF before magnetization and the use of ultrasound during co-precipitation, which has been reported to remove structural defects and disordered carbon regions [31]. Additionally, the cavitation effect of sonication allows the system to reach high energy levels in solution, thereby eliminating the need for high-temperature pyrolysis to achieve the desired structural properties [32].

Moreover, Fig. 1b presents the FTIR characterization of CNF, magnetite, and mCNF, providing qualitative confirmation of the formation of magnetic carbon nanofibers. As observed, the FTIR spectrum

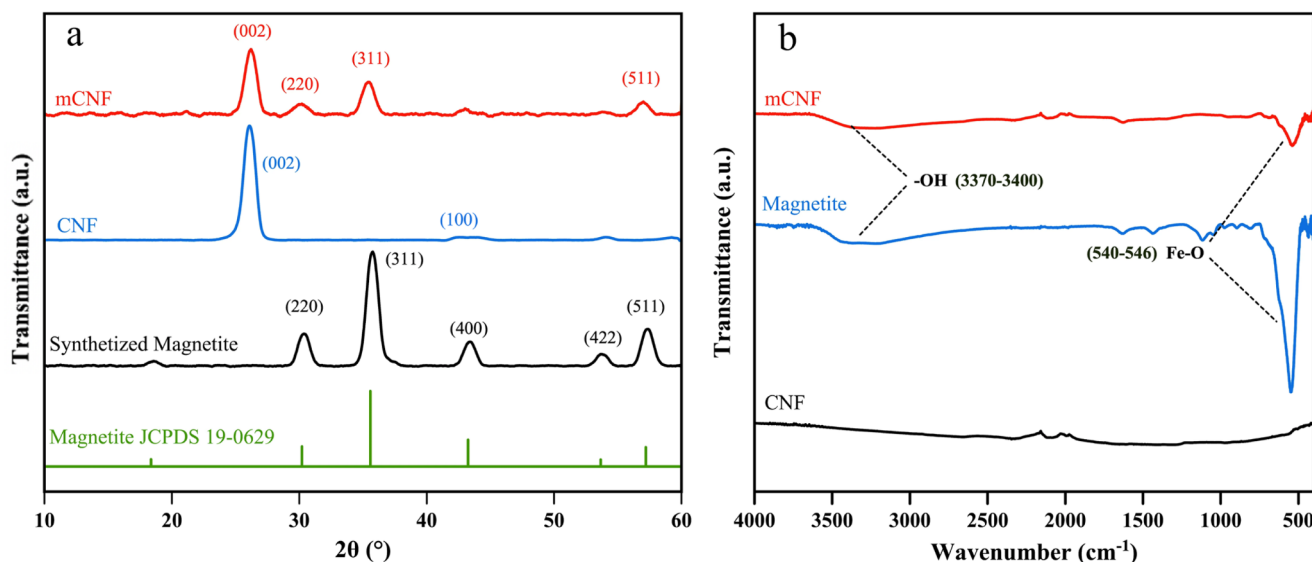


Fig. 1. Characterization of the materials by a) X-ray diffraction, and b) FTIR spectroscopy. Carbon nanofibers (CNF), magnetized carbon nanofibers (mCNF).

of CNF does not exhibit any significant functional groups. Although the acid treatment of CNF led to an increase in elemental oxygen content within the fibers (Table S1), the material's high crystallinity—attributed to the strong sp^2 bonds in the carbon lattice—limits its ability to effectively absorb infrared light. As a result, although the presence of certain functional groups might be expected, the strong and highly ordered bonding within the material reduces the vibrational modes necessary for FTIR detection [33,34]. In contrast, both magnetite and modified mCNF exhibit two distinct characteristic bands. The first band, observed at approximately 540 cm^{-1} , corresponds to the stretching vibrations associated with Fe–O interactions, confirming the presence of magnetite. The second band, appearing near 3400 cm^{-1} , is attributed to O–H stretching vibrations, likely arising from adsorbed water or hydroxyl groups on the surfaces of either the carbon nanofibers or the magnetite [26]. These findings are further supported by the ζ potential measurements in water (Fig. S2). The free CNF exhibits a strong negative ζ -potential (-33.79 mV), indicating a surface charge, which leads to electrostatic repulsion between particles and, consequently, high stability in suspension [35]. In contrast, magnetite shows a low positive ζ -potential ($+9.33\text{ mV}$), suggesting a tendency for particle aggregation,

likely due to the presence of hydroxyl groups, as indicated by FTIR analysis. A similar trend is observed for mCNF, which exhibits a lower negative ζ -potential (-18.58 mV) compared to CNF, suggesting that modifications to the surface chemistry during the functionalization reduced electrostatic repulsion [36,37]. The moderate ζ -potential of mCNF falls within the range observed for other carbonaceous and magnetic materials used in aqueous applications, as explored in previous reports [38,39]. These materials have demonstrated good dispersion stability and applicability in areas such as adsorption, catalysis, and biomedical applications, supporting the potential of mCNF for enzyme assisted catalysis.

3.1.2. Morphological characterization

The morphologies and size distributions of magnetite particles, CNF and mCNF were examined using transmission electron microscopy (TEM). Fig. 2a–b depicts the spherical morphology of the synthesized magnetite, with an average diameter of $107.97 \pm 8.57\text{ nm}$. The CNF exhibited an average diameter of $10.62 \pm 0.59\text{ nm}$. Fig. 2c–d illustrate the formation of a network of bare carbon nanofibers (CNFs), exhibiting their characteristic cylindrical channels. The commercial and untreated

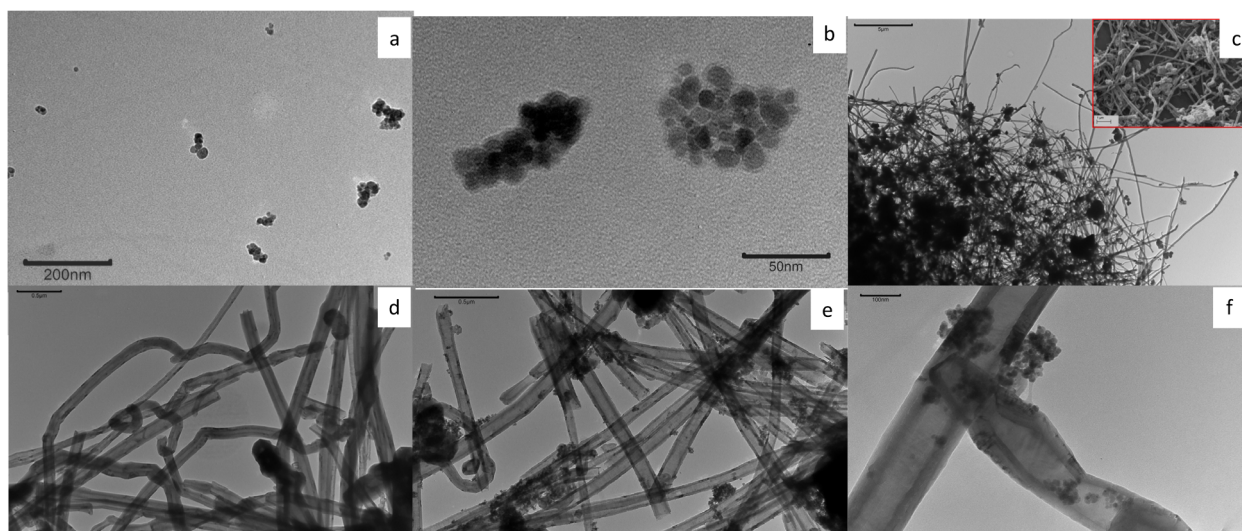


Fig. 2. TEM images of the materials, (a–b) magnetite, (c–d) CNF and (e–f) mCNF magnification of 25k, 120k, 2k, 15k, 20k and 70k, respectively from (a–f). The insert of Fig. 2c shows the SEM image of commercial and untreated CNFs.

CNFs used in this work showed fuzz-like agglomerates and deformations (Fig. 2c). In the insert of Fig. 2c is shown the Scanning Electron Microscopy (SEM) image, in which these formations are evidenced. A similar distribution has been reported by several authors [40,41]. Additional images are provided in (Fig. S3). The coating of magnetite on carbon nanofibers (Fig. 2e–f) appears randomly distributed across the CNF surface. A similar distribution of magnetic nanoparticles on CNF was reported in a previous study, which found that magnetic NPs tend to form random clusters that remain anchored even after prolonged ultrasonic treatment [23]. It is important to highlight that the use of ultrasound in the functionalization of CNF not only preserves crystallinity but also enables the synthesis of well-distributed magnetic nanoparticles in a short time and under safe conditions. This contrasts with previous reports that employed high-energy-consuming techniques, which resulted in broader size distributions [42]. Furthermore, ultrasound prevents agglomeration during the precipitation process, facilitating the formation of uniformly sized nanoparticles [43,44].

3.2. Enzyme immobilization, stability and reusability

Enzyme immobilization addresses the key limitations of using free enzymes in biotransformation applications by enhancing stability, enabling easy recycling, and preserving catalytic activity. In this context, laccase immobilization onto mCNF demonstrated high efficiency, overcoming the challenges associated with free enzymes systems and making easy and practical the large-scale application by the use of magnetic field in its separation [14]. The adsorptive interaction between laccases and mCNF was confirmed through FTIR analysis (Fig. S4), which revealed the presence of characteristic functional groups from laccase proteins without direct chemical interactions with mCNF. This suggests that non-covalent interactions played a dominant role in enzyme attachment.

The adsorption-based immobilization approach leveraged the synergistic properties of magnetite and carbon nanofibers, achieving an immobilization yield of 73.24 ± 1.28 %, significantly higher than previously reported magnetic supports (Table 1). For instance, Fe_3O_4 @ SiO_2 -based entrapment resulted in a 43.3 % immobilization yield [45], while Fe_3O_4 @Chitosan covalent attachment achieved 51.8 % [46]. Even advanced Fe_3O_4 @ SiO_2 @PDA systems and CuFe_2O_4 cross-linking methods demonstrated lower yields (≤ 56.4 %) compared to mCNF [47,48]. The enhanced immobilization efficiency of mCNF can be attributed not only to its large surface area, porous structure, and favorable surface chemistry, but also to the multiple intermolecular forces involved in enzyme binding. These include hydrogen bonding, electrostatic interactions between charged groups on the enzyme and the support, hydrophobic interactions, and π - π stacking between aromatic residues of the laccase and the graphitic domains of the carbon nanofibers. This combination of forces promotes strong, non-covalent enzyme adsorption without the need for additional chemical modification [46]. In contrast, entrapment-based techniques, such as Fe_3O_4 @ SiO_2 and Fe_3O_4 @ SiO_2 @PDA, often lead to low enzyme loading due to steric hindrance within polymer matrices. Even though covalent attachment (e.g., Fe_3O_4 @Chitosan) and cross-linking (e.g., CuFe_2O_4), can provide stable immobilization, it may cause partial enzyme deactivation due to strong chemical interactions that alter the enzyme's native conformation [49]. These findings highlight mCNF as a highly efficient

immobilization platform, offering higher yields, structural integrity, and ease of enzyme recovery, making it a promising alternative for biotransformation applications.

The pH activity profile of both free and immobilized laccases was evaluated based on the oxidation of ABTS (Fig. 3). The results demonstrate that free laccase exhibited maximum activity at acidic pH, but enzymatic activity gradually decreased as pH increased, becoming completely inactive at pH 7. This behavior is consistent with previous studies, which have reported that laccases are susceptible to inactivation at higher pH values due to multiple factors, including hydroxyl ions interaction with copper atoms in the active site, self-denaturation, and changes in the ionization states of key amino acid residues, which disrupt substrate binding and catalytic activity [50,51]. In contrast, immobilized laccases exhibited a broader pH stability range, maintaining higher activity between pH 3 and 5 and retaining approximately 64 % of activity at pH 7, a significant improvement over free enzymes. This enhanced stability is attributed to enzyme immobilization, which restricts conformational flexibility, reducing denaturation and promoting long-term catalytic efficiency [52]. These findings are particularly relevant for wastewater treatment, where operational pH conditions typically range between 5 and 7, making immobilized laccases more suitable for practical applications [53]. However, the poor activity presented at pH <4 might hinder applications of immobilized laccases when acidic conditions are given in wastewaters, which might force the use of free enzymes or make some modification to the immobilized matrix. This trend is consistent with previous studies magnetic modified nanomaterials for enzyme stabilization. For example, Fe_3O_4 @Chitosan laccases demonstrated improved pH tolerance but exhibit lower immobilization yields compared to mCNF [46]. Similarly, Fe_3O_4 @ SiO_2 @Kit-6 showed improved retention of activity at higher pH levels but required additional chemical modifications to optimize stability [54]. Moreover, studies on single magnetic nanoparticles such as Fe_2O_3 and Fe_3O_4 also revealed increase in pH resistance, though enzyme leaching was a limitation in some cases [55]. The improved pH tolerance observed in mCNF-immobilized laccases suggests that adsorption-based immobilization on carbon nanofibers plays crucial role in maintaining enzymatic activity in less favorable pH conditions.

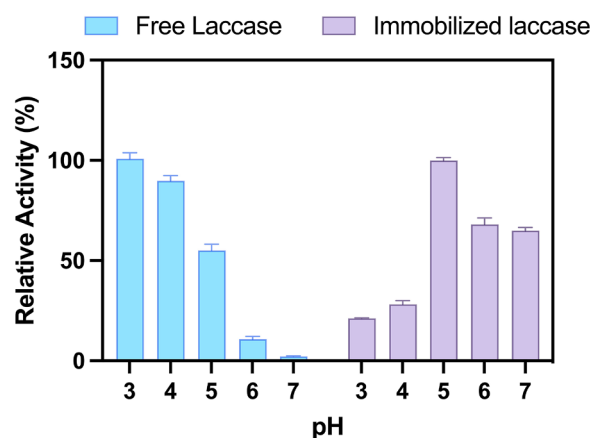


Fig. 3. pH-dependent activity of free and immobilized laccases.

Table 1

Comparison of magnetic materials in the immobilization of laccases.

Magnetic material	Enzyme	Immobilization technique	% immobilization	References
Fe_3O_4 @ SiO_2	Laccase	Entrapment	43.3	[45]
Fe_3O_4 @Chitosan	Laccase	Covalent	51.8	[46]
Fe_3O_4 @ SiO_2 @PDA	Lignin peroxidase	Entrapment	56.4	[47]
CuFe_2O_4	Laccase	Cross-link	50.0	[48]
mCNF	Laccase	Adsorption	73.24	This work

The long-term stability of immobilized laccases was evaluated by storing the mCNF/Lcc system 4 °C for 28 days, measuring residual enzymatic activity every 7 days (Fig. 4a). The results show that mCNF system retained approximately 75 % of its initial activity after four weeks, demonstrating high stability under storage conditions. One of the main limitations of physical adsorption. For example, previous studies reported significantly lower retention (23.06 % - 46.24 %) for laccase adsorbed on polyvinyl alcohol (PVA), sodium alginate (SA), and chitosan over the same period [21]. In contrast, covalent immobilization using poly (methyl methacrylate) and magnetic nanoparticles coated with chitosan retained 75 % and 70 % of enzymatic activity, respectively, after one month [56,57]. These findings highlight the effectiveness of mCNF as an immobilization platform, where the high surface area and porosity of CNF and MNP contribute to enhanced enzyme retention and long-term stability [12].

Fig. 4b presents the reusability profile of immobilized laccases, evaluated over five consecutive cycles of ABTS oxidation. After the fifth biotransformation cycle, the system retained 23.49 % of its initial activity. The gradual activity loss is likely associated with enzyme leaching, mechanical stress during handling, or product adsorption at the enzyme's active sites, thereby limiting catalytic efficiency [58]. Nevertheless, this decrease does not necessarily indicate that the support material is unsuitable for further use, as it can be reloaded with fresh enzyme for subsequent immobilization cycles. Moreover, a significant improvement in recyclability is evident when compared with other adsorptive immobilization systems. For instance, laccase immobilized on PVA and PVA/SA nanofibers lost approximately 80 % of its initial activity after just three cycles, demonstrating the inferior stability of these carriers [21]. The enhanced retention of enzymatic activity in mCNF suggests that its structural and chemical properties facilitate stronger enzyme-substrate interactions, reducing the rapid loss of catalytic efficiency observed in other adsorbents. These results reinforce the potential of mCNF as an effective nano-adsorbent for enzyme immobilization, offering both long-term stability and reusability. This approach aligns with circular economy principles, promoting the sustainable application of immobilized enzymes for the removal of emerging pollutants [4].

3.3. Removal of venlafaxine and carbamazepine

The degradation of venlafaxine and carbamazepine was evaluated using free and immobilized laccases, along with controls containing free mCNF, at two temperatures (25 °C and 60 °C). These experiments were conducted to assess the influence of temperature on drug removal efficiency and to apply a first-order degradation kinetics model (Table S2). At 25 °C, the mCNF@Lcc system exhibited the highest VFX removal

efficiency (51.50 %), with a sharp concentration decline observed within the first 4 h (Fig. 5a). This enhanced degradation is attributed to the synergistic effect of mCNF adsorption and enzymatic biotransformation. The role of substrate adsorption by mCNF was evident, as mCNF alone adsorbed approximately 20 % of VFX within the first hour, after which the concentration plateaued. In contrast, free laccases achieved 44.60 % degradation after 4 h, slightly lower than immobilized laccases, suggesting that the bionanocatalyst's performance is driven by both adsorption and enzymatic transformation. Since VFX adsorption onto mCNF occurred primarily at the beginning of the reaction, this indicates that biodegradation dominated the later phases, particularly in the immobilized enzyme system [59].

At 60 °C, mCNF alone exhibited improved performance (40.88 %), likely due to enhanced molecular interactions at elevated temperatures (Fig. 5c). However, immobilized enzymes showed slightly lower removal efficiency (37.83 %) compared with mCNF alone, suggesting increased substrate adsorption at higher temperatures may hinder enzymatic degradation [59,60]. For CBZ degradation, a similar trend was observed at 25 °C, where mCNF demonstrated strong adsorption capacity, removing 47.63 % of the CBZ within the first hour, after which the drug remained adsorbed with no significant degradation by the bionanocatalyst (Fig. 5b). This indicates that CBZ removal primarily occurred via adsorption, consistent with previous findings on carbonaceous materials, where weak interactions between CBZ and the matrix can lead to desorption over time [61]. Additionally, mCNF@Lcc showed slightly higher CBZ transformation compared to mCNF alone, maintaining a sustained removal trend, suggesting that adsorption facilitated enzymatic degradation [62]. However, at 60 °C, no CBZ removal was observed for any system (Fig. 5d), which aligns with previous reports indicating that temperatures above 35 °C weaken CBZ interactions with carbon materials, reducing adsorption stability and catalytic efficiency [63].

Comparing these results with previous enzyme-based degradation systems, a notable improvement in removal efficiency and reaction time was observed. The use of *Trametes versicolor* fungi for VFX degradation resulted in only 25 % removal in 3 days, reaching a maximum of 60 % after 15 days [9]. Llorca et al., 2019. Similarly, laccase enzyme from *Pleurotus ostreatus* removed only 22 % of VFX within the same timeframe [64], likely due to diffusion limitations, where the substrate struggles access active sites embedded in the fungal matrix. Further evidence of VFX recalcitrance was reported when soybean peroxidase (SBP) immobilized on semiconductors failed to degrade over 95 % of the drug, even when redox mediators were introduced [65]. In contrast, the present study demonstrated significantly higher and faster VFX degradation using immobilized laccases, highlighting the effectiveness of enzyme immobilization in enhancing catalytic efficiency and overcoming mass

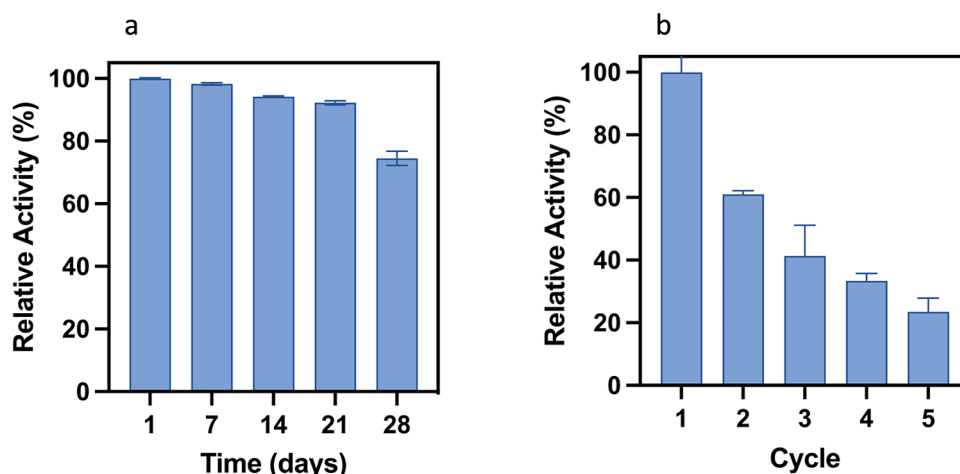


Fig. 4. Storage stability (a) and reusability (b) of the system mCNF@Lcc.

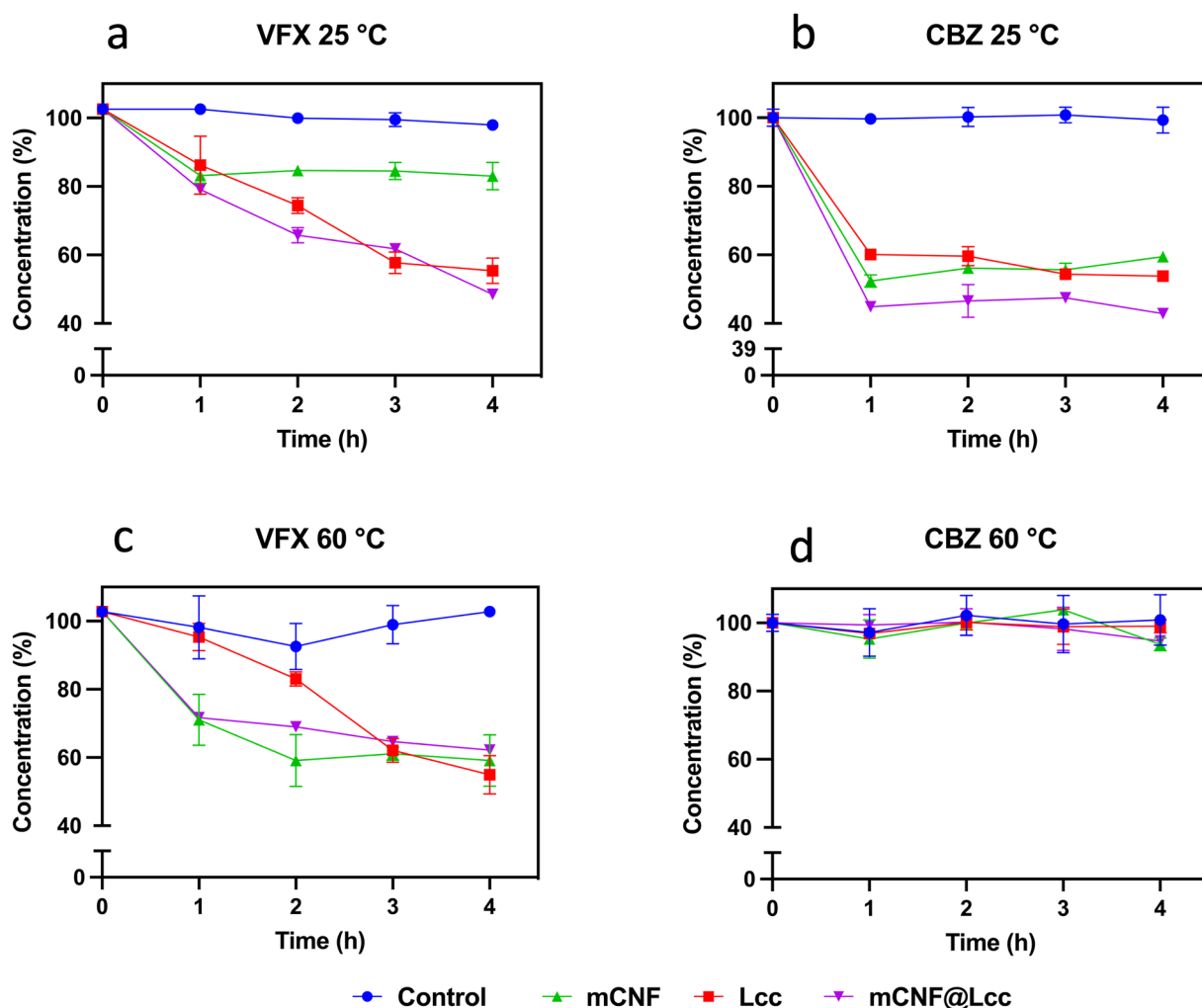


Fig. 5. Removal trials of (a, c) venlafaxine and (b, d) carbamazepine at 25 °C and 60 °C.

transfer limitations [66]. These findings suggest that mCNF-immobilized laccases represent a promising alternative for wastewater treatment applications, particularly for rapid and efficient removal of persistent pharmaceuticals. For CBZ degradation, immobilization of commercial laccases from *Trametes versicolor* onto MWCNT/PVDF supports via covalent bonding showed limited effectiveness, degrading <10 % of CBZ within the first 5 h and achieving only 23.18 % removal after 72 h [67]. In the present study, high CBZ adsorption onto mCNF prevented clear biotransformation detection, reinforcing the need to optimize enzyme-support interactions to balance adsorption and catalysis. Interestingly, laccases from *Pycnoporus sanguineus* have exhibited higher catalytic efficiency toward polycyclic hydrocarbons, likely due to their higher redox potential compared to *T. versicolor* laccases [62,68]. The superior performance of *P. sanguineus* laccases in VFX degradation further supports their potential for environmental applications, particularly in the removal of emerging contaminants.

4. Future scope

This study highlights the potential of mCNF-immobilized laccases for the efficient degradation of pharmaceuticals in aqueous environments. However, further research is needed to explore alternative immobilization techniques, such as covalent bonding, cross-linking, or encapsulation, which may enhance enzyme stability, reusability, and catalytic efficiency under different environmental conditions. Investigating the

long-term performance of immobilized enzymes in continuous flow systems will also be crucial for real-world wastewater treatment applications. Additionally, scaling up the immobilization process and assessing the economic feasibility of mCNF-based biocatalysts will provide valuable insights for industrial implementation expanding this approach to the degradation of other emerging contaminants, including pesticides and personal care compounds.

5. Conclusions

A magnetic nanobiocatalyst (mCNF@Lcc) was successfully developed by immobilizing native laccases from *P. sanguineus* onto magnetic carbon-based nanomaterials (mCNF@Lcc). The system was evaluated for the biotransformation of two psychiatric drugs, VFX and CBZ. Within four hours, 51.5 % of VFX was transformed, while CBZ primarily adsorbed onto the magnetic support, following adsorption kinetics at 25 °C. The dual functionality of the nanobiocatalyst, enabling both catalytic transformation and pollutant adsorption, highlights its potential for wastewater treatment and environmental remediation. The stability and reusability of mCNF@Lcc are crucial for real-world applications. In this study, variables such as pH and temperature influenced the notable action of mCNF@Lcc, remaining stable for 28 days post-synthesis and retaining activity over five reuse cycles, despite a gradual decline. Given the increasing presence of pharmaceuticals in aquatic environments and their potential risks to public health, hybrid nanobiocatalytic systems that integrate the adsorptive properties of nanomaterials with the

catalytic efficiency of enzymes represent a sustainable and scalable solution for removing recalcitrant pharmaceutical contaminants.

Funding information

This research was partially supported by Secretaria de Ciencia, Humanidades, Tecnología e Innovación (SECIHTI) and Tecnológico de Monterrey. Prof. Parra-Saldivar is funding by Research England, UK.

CRediT authorship contribution statement

Saúl Antonio Hernández Martínez: Writing – original draft, Validation, Methodology, Investigation, Data curation. **Alberto Balderas-Saucedo:** Writing – original draft, Methodology, Investigation. **Reyna Berenice González-González:** Writing – review & editing, Conceptualization. **Roberto Parra-Saldivar:** Conceptualization, Resources. **Rafael Vazquez-Duhalt:** Conceptualization, Writing – review & editing, Resources, Supervision. **Elda M Melchor-Martínez:** Conceptualization, Formal analysis, Writing – review & editing, Project administration, Resources, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The PhD scholarship awarded by Consejo Nacional de Humanidades, Ciencias y Tecnologías (CONAHCYT), Mexico to Saúl Antonio Hernández Martínez (1077066) is thankfully acknowledged. The authors would like to gratefully acknowledge the Latin American and Caribbean Water Center from Tecnológico de Monterrey for the laboratory facilities. All authors are also grateful to their representative universities for providing literature access.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.rineng.2025.106956](https://doi.org/10.1016/j.rineng.2025.106956).

Data availability

Data will be made available on request.

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Immobilization of laccases from *Pycnoporus sanguineus* on magnetized carbon nanofibers for the degradation of psychiatric drugs: venlafaxine and carbamazepine

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2025-09-01

Elsevier

<https://doi.org/10.1016/j.rineng.2025.106956>

Martínez SAH, Balderas-Saucedo A, González-González RB, et al., (2025) Immobilization of laccases from *Pycnoporus sanguineus* on magnetized carbon nanofibers for the degradation of psychiatric drugs: venlafaxine and carbamazepine. *Results in Engineering*, Volume 27, September 2025, Article number 106956