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A sustainable nano-hybrid system of laccase@M-MWCNTs for multifunctional PAHs and PhACs removal from water, wastewater, and lake water

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ABSTRACT

This study examined the use of modified multiwall carbon nanotubes (M-MWCNTs) with immobilized laccase (L@M-MWCNTs) for removing ciprofloxacin (Cip), carbamazepine (Cbz), diclofenac (Dcf), benzo[a]pyrene (Bap), and anthracene (Ant) from different water samples. The synthesized materials were characterized using an array of advanced analytical techniques. The physical immobilization of laccase onto M-MWCNTs was confirmed through Scanning electron microscope (SEM)-dispersive X-ray spectroscopy (EDS) analysis and Brunner-Emmet-Teller (BET) surface area measurements. The specific surface area of M-MWCNTs decreased by 65% upon laccase immobilization. There was also an increase in nitrogen content seen by EDS analysis asserting successful immobilization. The results of Boehm titration and Fourier transform infrared (FTIR) exhibited an increase in acidic functional groups after laccase immobilization. L@M-MWCNTs storage for two months maintained 77.8%, 61.6%, and 57.6% of its initial activity for 4 °C, 25 °C, and 35 °C, respectively. In contrast, the free laccase exhibited 55.3%, 37.5%, and 23.5% of its initial activity at 4 °C, 25 °C, and 35 °C, respectively. MWCNTs improved storability and widened the working temperature range of laccase. The optimum removal conditions of studied pollutants were pH 5, 25 °C, and 1.6 g/L of M-MWCNTs. These parameters led to >90% removal of the targeted pollutants for four treatment cycles of both synthetic water and spiked lake water. L@M-MWCNTs demonstrated consistent removal of >90% for up to five cycles even with spiked wastewater. The adsorption was endothermic and followed Langmuir isotherm. Oxidation, dehydrogenation, hydroxylation, and ring cleavage seem to be the dominant degradation mechanisms.

1. Introduction

The release of a diverse range of chemicals into the environment has raised significant concerns over the potential health and environmental risks of these compounds (Deblonde et al., 2011). The population growth and the improper management of municipal sewage have significantly contributed to the contamination of water bodies. It has been reported that around 70–80 % of rivers and streams worldwide are

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burdened with contamination (Husain and Husain, 2007). In addition to the possible negative impacts on human health, the discharge of pollutants into water bodies may have both chronic and acute harmful effects on species within aquatic ecosystems, ultimately resulting in the loss of biodiversity and habitats (Alexander et al., 2012).

PhACs are often found in the effluents of wastewater treatment plants (WWTPs) (Marco-Urrea et al., 2009). PhACs are often used as both prescribed and over-the-counter medications. Following their consumption, these chemicals are excreted in urine and feces, either in their original form or as metabolites, ultimately entering wastewater (Naghdi et al., 2017). The inefficiency of WWTPs in removing persistent pollutants can lead to the escape of these pollutants into surface water bodies (Lienert et al., 2007). Ciprofloxacin, carbamazepine, and diclofenac are related to this group and can be found in water and wastewater (Ashfaq et al., 2017; Zhang et al., 2008; Jos et al., 2003; Parida et al., 2021). In addition to PhACs, PAHs are another class of Persistent Organic Pollutants (POPs), exhibiting an annual global emission estimated at around 504 kt (Fernández et al., 2021). PAHs can travel long distances, and their presence has been consistently observed on a global scale (Yuan et al., 2021; Grmasha et al., 2023a) and caused serval health issues (Neri et al., 2016; Abbas et al., 2013). Considering the harmful effects of PhACs and PAHs, it is of utmost importance to develop technologies capable of removing these contaminants effectively.

The use of bioremediation or biodegradation is a promising field of study that has been shown to be successful in the removal of harmful contaminants from water and wastewater (Saravanan et al., 2021; Al-Sareji et al., 2023). Employing enzymes through immobilization technique in biodegradation offers several advantages over common physiochemical techniques such as greater cost-effectiveness and enhanced safety (Homaei et al., 2013). It is worth noting that carbon materials have typically been favored as carriers for enzyme immobilization due to their ability to provide a high surface area in relation to enzyme loading. The structure and characteristics of carbon nanotubes (CNTs) have been investigated in several fields such as energy storage, biology, and other environmental applications (Xie et al., 2005). The surface of CNTs can simply be modified, allowing for the adjustment of their characteristics to meet specific applications and improve their effectiveness as either support or catalysts. CNTs provide many notable benefits over conventional carriers, such as silica, polymeric materials, etc. These advantages include a larger specific surface area, higher adsorption capacity, and improved enzyme loading capacity, especially when compared to other carbon materials. The literature has documented many methods for immobilizing enzymes on CNTs such as adsorption, covalent bonding, entrapment, or encapsulation. However, adsorption seems to be the most attractive technique due to its cost-effectiveness.

Laccase (EC 1.10.3.2) is an extracellular enzyme that has been proposed to catalyze a wide variety of substrates, including phenols, polyphenols, chlorinated phenols, polyamines, dyes, aromatic substrates, and some inorganic compounds (Dong et al., 2023; Lei et al., 2022). Laccase's broad substrate specificity has made it a valuable tool in wastewater bioremediation, winemaking, textile dyeing, pulp and paper production, finishing, etc. (Widsten and Kandelbauer, 2008; Mahmoodi et al., 2020). The application of laccase enzymatic degradation of complex compounds such as POPs is an attractive technology due to its low cost. Laccase can degrade complex organic compounds without the need for the addition of expensive and harmful chemicals through the electron transfer mechanism facilitated by its four copper atoms that generate strong radicals (Bhardwaj et al., 2022). However, its fragile structure and sensitivity to the surrounding environment (e.g. pH, temperature, hydrodynamic flow forces) make it an inviable option for industrial applications. Hence, recent studies focused on optimizing its immobilization using various carriers. To improve the economics of the treatment process, the carrier should also contribute to or facilitate pollutants removal.

Scientific research has highlighted the ineffectiveness of current wastewater treatment facilities for removing emerging contaminants. When treated wastewater is discharged into receiving water bodies, they may still contain substantial amounts of these pollutants. Therefore, it is crucial to urgently develop innovative technological methods to eliminate these persistent contaminants. The Water Framework Directive (WFD) obligates the member states of the European Union to accomplish a quantitative and qualitative assessment of the status of all bodies of water that is satisfactory. Some Sustainable Development Goals (SDGs) by the United Nations have not been on track to meet the targets by 2030 (or 2025) or no data has been found. In Hungary, SDGs indicated that there is no data or insufficient to meet the milestones set of the following targets (OECD iLibrary): 6.3 "Wastewater treatment", 12.4 "Chemical and hazardous waste", 14.1 "Prevent and significantly reduce marine pollution of all kinds, in particular from land-based activities, including marine debris and nutrient pollution.". This highlights the importance of developing technologies to facilitate achieving these targets.

A combined treatment approach has been proposed in the literature as the best available strategy to mitigate the contamination of emerging pollutants (Osuoha et al., 2023). Hence, we propose the application of combined adsorption using M-MWCNTs and enzymatic degradation employing immobilized laccase as an effective treatment technology. The model pollutants selected in this study are a mix of PhACs and PAHs namely Ciprofloxacin, carbamazepine, diclofenac, benzo[a]pyrene, and anthracene. These compounds were selected based on their frequent occurrence in water bodies and their potential environmental and health risks (Grmasha et al., 2023b; Souza et al., 2022). Some of them are listed on the WFD's updated watch list (Loos et al., 2018) while others need to get more attention as there are no safe limits set for them in Europe yet. Studies that employed MWCNTs as a carrier for laccase immobilization have not fully investigated the effect of both adsorption and degradation. Most of them either focused on the enzyme characteristic improvement (if the immobilization was examined) or adsorption investigation (if adsorption was conducted). Hence, the important question of whether the optimum parameters of immobilization align well with adsorption performance and vice versa remains unaddressed. The present study endeavors to answer this question by examining the simultaneous adsorption and enzymatic degradation for removing PAHs and PhACs from different water matrices which is another unique aspect of this study. The MWCNTs were chemically modified to improve the binding of laccase onto them and enhance their adsorption capacity. The alteration of MWCNTs surface chemistry was tracked using an array of advanced analytical tools. The impact of operational parameters, including pH, temperature, and laccase concentration, on the adsorption capacity of L@M-MWCNTs was investigated. The adsorption behavior of the target contaminants was studied using different isotherm, kinetics, and thermodynamic models. The degradation pathways of the contaminants were followed by identifying the produced metabolites. Finally, the reusability of M-MWCNTs and L@M-MWCNTs was evaluated over 12 treatment cycles.

2. Methodology

2.1. Materials

Ciprofloxacin (Cip, $C_{17}H_{18}FN_3O_3$, CAS No.: 85721-33-1), carbamazepine (Cbz, $C_{15}H_{12}N_2O$, CAS No.: 298-46-4), diclofenac (Dcf, $C_{14}H_{11}Cl_2NO_2$, CAS No.: 15307-86-5), benzo[a]pyrene (Bap, $C_{20}H_{12}$, CAS No.: 50-32-8) and anthracene (Ant, $C_{14}H_{10}$, CAS No.: 120-12-7) were obtained from Merck KGaA. Sulfuric acid (H_2SO_4, 99.6 %), nitric acid (HNO_3, 99.8 %), Hydrochloric acid (HCl, 37%) and laccase (*Trametes versicolor*, ≥0.5 U/mg, EC 1.10.3.2) were provided by Merck Chemicals Company. AVANTOR supplied the citric acid ($C_6H_8O_7$) and disodium phosphate (Na_2HPO_4) necessary to make the McIlvaine citrate-phosphate solution. Sodium hydroxide (≥98%, NaOH), sodium bicarbonate (99.5–100.5%, $NaHCO_3$), sodium carbonate (≥99.5%, Na_2CO_3), sodium ethoxide (≥95%, $NaOC_2H_5$) were purchased from

Sigma-Aldrich. The 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) (98%) for laccase activity measurement was obtained from Roche Diagnostics GmbH. Sigma-Aldrich supplied anthracene ($C_{14}H_{10}$, CAS No.: 120-12-7). MWCNTs (TNNF-6, purity>95 wt%, outer diameter: 10–20 nm, length:5–20 μm, special surface area >120 m^2/g) were obtained from Chengdu Organic Chemicals Co. Ltd, China. Other chemicals were provided by Sigma-Aldrich. Milli-Q water (MQ water) produced by Millipore Direct-Q® 5 UV purification system (resistivity18.2 M cm) was used for washing and solution preparation. Whatman® glass microfiber filters were used for separating MWCNTs from the aqueous media. The analytical-grade chemicals and reagents were utilized without any further purification.

2.2. Acid modification of MWCNTs

Prior to preparation, MWCNTs were rinsed with deionized water, dried at 105 °C for 6 h, and stored in a desiccator. MWCNTs were then modified by incorporating a carboxylic acid group (-COOH). –COOH group facilitates laccase binding with nanotubes as this group creates a stable amide bond (–NH–C=O) with NH_2 group of the laccase. To this end, MWCNTs were immersed in mineral acids, which oxidized them and formed oxygen-containing groups on their surfaces. The pristine MWCNTs (1 g) were activated by refluxing at 80 °C for 5 h in a round-bottom flask (200 mL) containing 100 mL of a 3:1 (v/v) mixture of concentrated HNO_3 and H_2SO_4. Following that, the acid mixture with pristine MWCNTs was exposed to an ultrasonic bath at a temperature of 40 °C for a duration of 3 h to expedite the functionalization. The mixture of MWCNTs-mineral acids was subsequently centrifuged for 10 min at 8000 rpm. MWCNTs were continuously rinsed with deionized water after centrifugation until they were neutralized (pH 6–7) [(Bhardwaj et al., 2022); (Al-sareji et al., 2023a); (Teoh et al., 2018)]. Lastly, the modified MWCNTs (M-MWCNTs) were dried in a vacuum oven at 80 °C for 48 h and then subjected to cooling in a desiccator overnight before sample analysis.

2.3. Physical immobilization

The immobilization process followed the reported procedures in the literature with a slight modification [(Costa et al., 2019); (Al-sareji et al., 2023b)]. Briefly, enzyme immobilization was accomplished by combining 2 mg of M-MWCNTs with 1.2 mL of a 3.75 μL/mL (5 mg/mL) laccase solution in a buffer solution of (pH 5) and agitating orbitally for 3 h at 30 °C. The pH of immobilization was selected based on the initial examinations of the effect of this variable on the activity of free laccase (Fig. S1). Following immobilization, the final product denoted L@M-MWCNTs was rinsed with buffer solution. After washing the L@M-MWCNTs, laccase activity was measured in the buffer as a control test for enzyme leaching. There was no enzyme activity observed in any of the materials employed. Immobilization yield (%) is expressed as the difference between the activity of the free laccase in a solution and the activity of laccase remaining in the supernatant after immobilization, divided by the activity of the free enzyme multiplied by 100. The L@M-MWCNTs were stored at 4 °C for further study.

2.4. Characterization techniques

The drift method was used in identifying the point of zero-charge (pH_{PZC}) of MWCNTs (Kosmulski, 2009). The Brunner-Emmet-Teller (BET) surface area analysis was conducted by evaluating the adsorption-desorption isotherm of nitrogen at a temperature of 77 K using the Micromeritics (3Flex) instrument.The morphology of M-MWCNTs, laccase immobilized on M-MWCNTs (L@M-MWCNTs), and used L@M-MWCNTs were analyzed using FEI/ThermoFisher Apreo S LoVac scanning electron microscope (SEM) equipped with EDAX AMETEK Octane Elect Plus energy-dispersive X-ray spectroscopy (EDS). X-ray powder diffraction (XRD) patterns were acquired with a Max 2500 VB + X X-ray diffractometer at 40 kV, 35 mA, and a 2Θ range of 10-80°. The Fourier Transform Infrared (FTIR) with an iD7 attenuated total reflectance (ATR) measured using Nicolet™ iS™ 5 FTIR Spectrometer (Thermo Fisher, USA). The Boehm titration method was used to measure functional groups (Boehm, 2002). The applied method for the titration and other information for the characterization can be found in the supplementary material (Text 1).

2.5. Adsorption tests, isotherms, kinetics and thermodynamic

The adsorption tests, Langmuir, Freundlich, Dubinin-Radushkevich (D-R), Temkin models [(Langmuir, 1916); (Freundlich, 1906)], kinetics (Lagergren, 1898; Ho and McKay, 1999), Intra-particle diffusion and thermodynamics are explained in detail in the supplementary material (Text 2).

2.6. Free and immobilized laccase activity measurement

The spectrophotometric determination of free laccase activity for the oxidation of 0.5 mM ABTS substrate in 0.05 mM citrate/0.1 mM-phosphate buffer at pH 5 was performed. Laccase solution (0.1 mL) was combined with 1.9 mL of the ABTS solution (total volume of 2.0 mL) and incubated at ambient temperature to measure the activity's concentration. The oxidation of ABTS was recorded by determining the increase in absorbance at 420 nm (ε420nm = 36 000 M^{-1} cm^{-1}). In kinetic mode, the change in absorbance of the solution in the cuvette was measured automatically for 1 min by a UV–visible spectrophotometer-double beam Shimadzu UV-1601. The laccase activity was calculated from the initial linear portion of the kinetic curve (absorbance versus time). The amount of laccase required to oxidize 1 μmol of ABTS per minute was defined as one unit (U) of laccase activity. The activities of the free enzyme were indicated in U/L. The immobilized laccase activity (L@M-MWCNTs) was determined by adding 105 mL of citrate/phosphate buffer 0.05 M/0.1 M, pH 4 at 25 ± 2 °C, and 37.5 mL of ABTS 0.5 mM, with magnetic stirring at 150 rpm. Polypropylene filters (0.45 μm) were utilized to filter the samples. The laccase activity was quantified employing the following equation, [(Costa et al., 2019); (Imam et al., 2021)]:

$$\frac{U}{g} = \frac{\Delta ab \times F_{dil} \times R_v \times 10^6}{\epsilon \times M_{carrier}} \quad (1)$$

where U/g represents the amount of laccase capable of oxidizing 1 μmol of ABTS (per minute and per mass unit of $M_{carrier}$). Δ ab is the absorbance per minute (determined by linear regression), F_{dil} is the dilution factor, R_V is the reaction volume (mL), ϵ (3.6 x10^4 M^{-1} cm^{-1}) is the molar extinction coefficient, $M_{carrier}$ is the mass of the support on which laccase was immobilized (g). 10^6 is the conversion factor for molarity to micromolarity.

2.7. Operational stability and laccase kinetic studies

Both operational stability and laccase kinetic studies methods are explained further in the supplementary material (Text 3).

2.8. L@M-MWCNTs optimum parameters and storage stability

In order to stabilize the pH, separate tubes containing 4 mL of buffer solutions with a pH range of 3–8 were prepared (Naghdi et al., 2017; Al-Sareji et al., 2023). In the tube, 100 μL of free laccase with a concentration of 5 mg/mL was introduced. Then, 20 mg of L@M-MWCNTs was added. The contents of the tubes were shaken at a speed of 150 rpm and 30 °C for 2 h. The residual activity of laccase was assessed in free and immobilized samples. To ensure thermal stability, the experimental procedure used was similar to that used for pH stabilization, with the only difference being that the samples were subjected to different

temperatures (10–60 °C) for 2 h while maintaining a constant pH of 5. The optimal laccase dosage was found by introducing different concentrations of laccase (0.5–5 mg/mL) to 20 mg of L@M-MWCNTs in individual tubes containing 4 mL of buffer solution with a pH of 5. The tubes were then incubated for a duration of 2 h at 30 °C and at 150 rpm. To assess the storability of the enzyme samples, both free and immobilized laccase were kept at 4, 25, and 35 °C for a duration of up to two months. The residual activity of the enzyme samples was then measured weekly.

2.9. L@M-MWCNTs removal test

The efficacy of L@M-MWCNTs for removal of Cip, Cbz, Dcf, Bap, and Ant from various water samples using batch tests (Naghdi et al., 2017; Al-sareji et al., 2023b). Before testing wastewater and lake water (Lake Balaton, Hungary), samples were filtered using Whatman cellulose filter paper with a diameter of 47 mm. Subsequently, the samples were spiked with the aforementioned pollutants. During the experimental procedure, a quantity of 100 mg of L@M-MWCNTs was introduced into a solution containing 50 mL of a pollutant mixture. The concentration of each pollutant in the mixture was 50 mg/L. The resulting solution was then mixed at a speed of 150 rpm at room temperature. Samples of 2 mL were withdrawn at regular intervals of 15 min. The L@M-MWCNTs were cleaned using Milli-Q water, and this process was repeated cyclically.

2.10. Data visualization and check

This section is further explained in Text 4 (supplementary material).

3. Results and discussion

3.1. FTIR, Boehm titration, pHpzc, and XRD

The surface functional groups of MWCNTs, M-MWCNTs and L@M-MWCNTs were qualitatively (FTIR) and quantitatively (Boehm's titration) determined. The FTIR spectra of MWCNTs, M-MWCNTs and L@M-MWCNTs are illustrated in Fig. 1 (A), and Boehm's titration results are presented in Table 1. The existence of functional groups on MWCNTs was confirmed through the FTIR spectra. When comparing the FTIR spectra of pristine MWCNT and M-MWNT, significant peak areas were observed at different wavenumbers as depicted in Fig. 1(A). These include a peak at 1534 cm^{-1}, which indicates the presence of the graphite structure (specifically, the C=C stretching associated with side wall defect) in the MWCNTs. The peak at 2850 cm^{-1} indicates the presence of asymmetric and symmetric C–H stretching bonds in the pristine MWCNT structure. Other notable peaks include the C–O bond stretching at 1082 cm^{-1}, and the hydroxyl group (-OH) at 3444 cm^{-1} [(Bhardwaj et al., 2022); (Khabashesku et al., 2002)]. The peak at 3444 cm^{-1}, which corresponds to the O–H stretching, is observed to have a wide band in both M-MWCNTs and L@M-MWCNTs. The widened peak

Table 1
Functional groups obtained from Boehm titration (mmol/g).

Functional groups	MWCNTs (Pristine)	M-MWCNTs
Carboxylic groups	0.081	0.411
Carbonyl groups	0.041	0.325
Phenolic groups	0.018	0.216
Total surface acidity	0.140	0.952
Total surface basicity	0.157	0.256

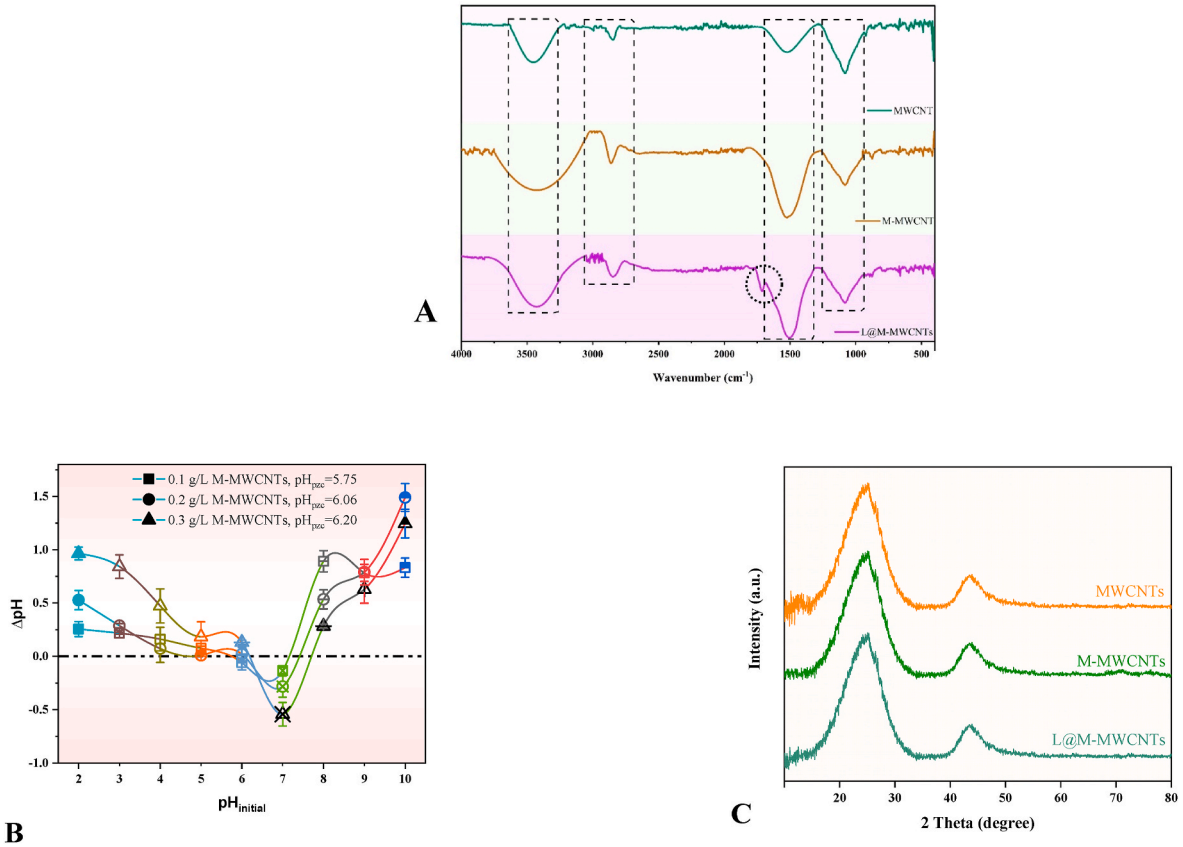

Fig. 1. FTIR spectra for MWCNT, M-MWCNTs, and L@ M-MWCNTs (A); pHpzc of M-MWCNTs with three different concentrations (B); XRD for MWCNT, M-MWCNTs, and L@ M-MWCNTs (C).

at this region could be attributed to the OH in the carboxylic group attached to M-MWCNTs and L@M-MWCNTs after functionalization (Cervantes-Uc et al., 2006; Muhamad et al., 2022). The presence of the peak at 1712 cm^{-1} in L@M-MWCNT indicates the presence of an amide bond inside the laccase protein, which confirms the successful immobilization of laccase on M-MWCNTs surface (Zhang et al., 2020). Table 1 presents the findings obtained from the Boehm titration. The results indicate a noticeable rise in the overall acidity of MWCNTs during the oxidation process. This observed increase could be attributed to the elevated presence of carboxyl, lactones, or phenols on the surface of the MWCNTs. The primary sources of acidity are carboxylic groups, which exhibit the highest acidity, followed by lactonic groups, and finally phenolic groups. This observation suggests that these materials possess acidic properties, which therefore contribute to the enhanced sorption of pollutants from an aqueous solution. The overall basicity of MWCNTs was observed to rise after the oxidation process. This phenomenon may be attributed to the higher number of oxygen functional groups and the presence of pyrone-type structures located at the periphery of the polyaromatic layers (Boehm, 2002). The same trend was also reported in other works (Lu et al., 2008; Yavari and Davarkhah, 2013; Yavari et al., 2010). For instance, Lu and colleagues reported an increase in the total acidity of MWCNTs after oxidation (Lu et al., 2008). Yavari and Davarkhah (2013) investigated the adsorption behavior of zirconium from an aqueous solution using modified multiwall carbon nanotubes treated with a nitric acid solution. The Boehm titration revealed total acidic site on the nanotubes increased from 0.11 to 1.00 mmol/g and a slight increase in terms of basic sites from 0.01 to 0.03 mmol/g.

In accordance with this approach, the samples were dispersed in aqueous media with low ionic strength, upon equilibration; the pH_{pzc} was calculated by measuring the shift in pH values as shown in Fig. 1 (B). The pH_{PZC} measurements are essential for comprehending the behavior of adsorbents in relation to anion or cation exchangers. The pH_{pzc} value of modified MWCNTs was 6. The MWCNTs surface gains a positive charge at pH < pH_{pzc} and a negative charge at pH > pH_{pzc}. This is due to the deprotonation of surface hydroxyl groups increasing the concentration of H^+ ions in aqueous media and O− ions on the surface of M-MWCNTs when pH is greater than pH_{PZC}. The XRD technique is used for quantifying the extent of graphitization, while also offering insights into the degree of alignment of nanotubes. The XRD patterns of MWCNTs samples shown in Fig. 1 (C) demonstrate the presence of a high intense peak at $2\theta = 25.1^{o}$ and low intense peak at $2\theta = 43.4^{o}$, which could be attributed to the (002) and (101) crystallographic planes of graphitic carbon (Yang et al., 2019). These peaks are indicative of the hexagonal graphitic structure of carbon nanotubes (Abdi et al., 2017). XRD patterns obtained for all the examined MWCNTs exhibited a high degree of similarity, suggesting that the carbon nanotubes maintained their graphitic structure (Chen et al., 2016).

3.2. Surface morphology and chemistry

3.2.1. SEM-EDX

Fig. 2 displays SEM images of M-MWCNTs, L@M-MWCNTs, and L@M-MWCNTs after the removal experiments. The characteristic morphology of MWCNTs displays fibrous texture. The modification introduces a range of hydrophilic functional groups, including hydroxyl, carboxyl, and carbonyl groups onto the surface of the MWCNTs (Kang et al., 2015). Consequently, the modified MWCNTs have a strong affinity for binding with laccase. After the immobilization process, it was observed that the surface of L@M-MWCNTs exhibited enhanced smoothness. The correlation between the smoothness of the surface and the immobilization of enzymes in magnetic biochar nanoparticles has been also observed in (He et al., 2020). Furthermore, as it can be observed from Fig. 2 that L@M-MWCNTS has a greater fiber thickness in comparison to pristine MWCNT. This finding suggests that the enzyme has been effectively immobilized onto the M-MWCNTs. Similar findings were reported by Tavares and co-workers (Tavares et al., 2015) about the immobilization of laccase onto MWCNT by adsorption. The study found that the SEM analysis of the laccase (Novozym 51003) over MWCNTs matrix exhibited a discernible layer of a distinct substance. Finally, it also observed that the smooth layer is partially diminished after the removal which indicates that a part of the laccase layer leaches due to repeated uses of MWCNTs.

Regarding elemental analysis, the spectrum obtained from EDS (Fig. 2) indicated that the primary elements detected on the pristine MWCNTs were carbon and oxygen. Following the process of immobilization, there were observable quantities of sulfur and nitrogen present

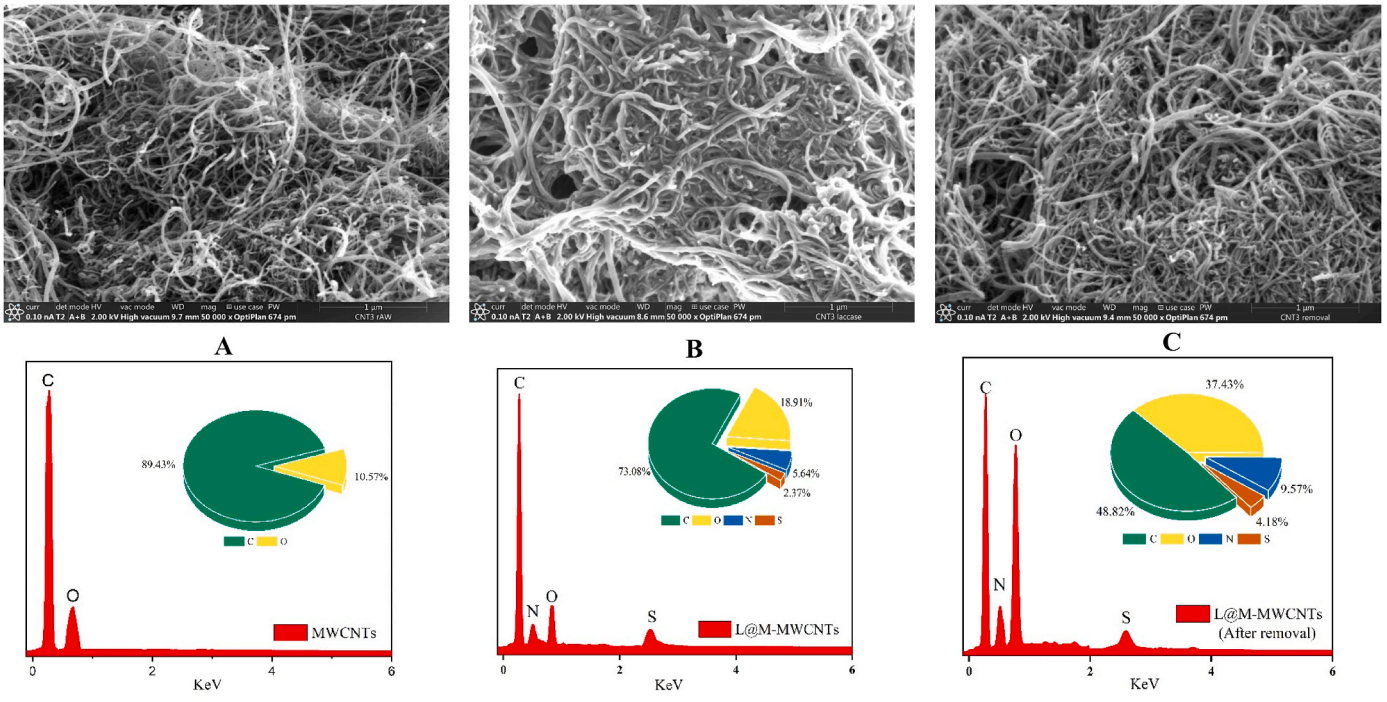

Fig. 2. SEM and EDX for M-MWCNTs (A), L@M-MWCNTs (B), and L@M-MWCNTs after removal (C).

on the surface. The presence of S could be attributed to its oxidation via the use of H_2SO_4. An elevation of nitrogen content signifies the existence of laccase on the surface of MWCNTs (Pandey et al., 2022).

3.2.2. S_{BET}

The experimental results demonstrate that the surface area of MWCNTs showed an increase from 155.1 to 210.3 m^2/g after oxidation with a combination of nitric acid and sulfuric acids (Table 2). Fig. S2 shows the N_2 adsorption–desorption isotherm of MWCNTs, M-MWCNTs, and L@MWCNTs. The observed phenomenon may be attributed to the elimination of impurities, such as carbon block, graphite sheet, amorphous carbon, and nanoparticles from the internal and exterior surfaces of MWCNTs after oxidation. The elimination of these impurities could increase the surface area of MWCNTs. In contrast, the reduction in the overall pore diameter of modified MWCNTs indicates that the process of oxidation facilitates the breakdown of MWCNTs into smaller particles, which have a significant number of surface defects. After that, it may open the tips and probe MWCNT holes (Li et al., 2003). Nevertheless, the S_{BET} for L@M-MWCNTs exhibited a significant reduction of 65% subsequent to the immobilization process, resulting in a value of 73.6 m^2/g. The observed significant decrease in surface area serves as direct evidence supporting the immobilization of laccase by surface adsorption. The decrease of S_{BET} after enzyme immobilization has also been reported in literature. In a study conducted by Badgujar et al. (2013), the authors examined the impact of immobilized lipase on a polymeric composite. The results indicated a significant reduction in the N-accessible surface area, with a fall of 45% from an initial value of 0.804 m^2/g to a final value of 0.437 m^2/g.

3.3. Physical immobilization, laccase kinetic parameters, operational stability, storage

The immobilization by adsorption is regarded as a non-covalent process in which enzymes are physically attracted to the surface of MWCNTs while maintaining their native conformation. To obtain the most effective enzyme immobilization, the experimental conditions were adjusted by altering pH, temperature, and enzyme concentration. The stability of laccase throughout the immobilization technique is influenced by the pH of the solution, which renders it a crucial variable. Fig. 3 (A) displays the effect of pH in the range of 2–8 on the activity of L@M-MWCNTs. The immobilization of laccase was shown to be reduced at pH 2 due to a decline in enzyme activity (8.55 U/g). Conversely, the highest level of enzyme immobilization was achieved at pH 5 (66.43 U/g). It was also noticed that there was a large difference between optimum pH and pH 4 (60.30 U/g) which made the immobilized system better than the free state of laccase. The immobilization of laccase M-MWCNTs exhibited a significant decrease when the pH exceeded 6. At pH below the isoelectric point of 6, the overall charge on M-MWCNTs becomes positive, leading to an enhancement in the electrostatic interaction between the carrier and the negatively charged laccase (Imam et al., 2021; Al-sareji et al., 2023b). The influence of temperature on laccase immobilization is of crucial significance, given that enzymes inherently exhibit sensitivity to heat and exhibit optimal functionality within specified temperature ranges. Fig. 3 (B) depicts the temperature profile of the immobilization process throughout a range of 10–65 °C. The immobilization of laccase exhibited a significant rise in activity from 23.52 U/g to 77.41 U/g when the temperature was elevated from 10 °C to 30 °C. This observed enhancement could be due to the enhanced rate of enzyme adsorption onto M-MWCNTs. The activity of the immobilized laccase at 35 °C is still relatively high (71.32 U/g) suggesting that the system could effectively be operated at two different temperatures. Subsequently, a slow decline became apparent as the temperature ascended. The results shown in Fig. 3 (C) indicate that increasing the concentration of the enzyme from 0.5 mg/mL to 5 mg/mL resulted in a significant improvement in enzyme immobilization, with values increasing from 26.41 U/g to 78.62 U/g. However, the introduction of higher concentrations of laccase did not result in any noticeable impact on the immobilization process of the enzyme. The observed phenomenon could be attributed to the enzyme's capacity to bind to vacant sites onto the surface of M-MWCNTs. Based on a univariate approach, the most favorable conditions for immobilization were identified as a pH of 5, a temperature of 30 °C, and an enzyme concentration of 5 mg/mL, resulting in an immobilization efficiency of 74.56%. The enhanced immobilization efficiency was ascribed to the effective adjustment and modification of M-MWCNTs containing a significant amount of carbonyl groups (Lonappan et al., 2018).

The Michaelis constant (Km) is an essential factor for determining the affinity of laccase with its substrate, whereas the Vmax value indicates the maximal reaction velocity. A lower value of Km indicates a higher rate of binding affinity between the substrate and the enzyme. The impact of substrate concentration (ABTS) on the immobilized activity of laccase at pH 5 is shown in Fig. 3 (D). The substrate concentration varied between 0.05 and 2.50 mM. It is evident that the data aligns well with the nonlinear regression analysis conducted using the Michaelis-Menten kinetic model. The findings demonstrated that the L@M-MWCNTs had a higher Km value (0.229 mM) than the free laccase (0.143 mM). The observed increase in Km values for immobilized enzymes could possibly be attributed to mass-transfer limits arising from internal diffusion constraints. This phenomenon occurs when the substrate is unable to effectively reach certain parts of the immobilized enzyme's active site (Ardao et al., 2011). Furthermore, alterations in the three-dimensional conformation of laccase might potentially impact the active site of the enzyme upon its adsorption onto M-MWCNTs surface. The system's limit rate, Vmax, exhibits a drop of about 2-fold when immobilized laccase is compared to its free enzyme version. The observed decrease in Vmax for immobilized laccase (3.435 mM/min) compared to free laccase (7.004 mM/min) could be attributed to the constrained mobility and decreased availability of active sites in the immobilized enzyme. This reduction in Vmax is consistent with findings published in previous studies. Specifically, it was shown that Vmax decreased by a factor of 5.25 when laccase was immobilized on Amberlite IR-120 H (Spinelli et al., 2013). The immobilization of laccase onto MWCNTs was carried out using adsorption (Tavares et al., 2015), and the Km and Vm values for free and immobilized enzymes were 0.0068 and 1.416 mM/min and 0.1920 and 0.586 mM/min, respectively. A similar trend was identified in the optimization of laccase immobilization on functionalized magnetic nanoparticles, whereby a drop in Vmax and an increase in Km were observed after the immobilization process (Fortes et al., 2017).

The assessment of the reusability of the immobilized laccase includes the measurement of recycling efficiency in each reaction cycle, as seen in Fig. 3 (E). The L@M-MWCNTs demonstrated retention of over 85% of its original activity up to the 8th cycle indicating the sustained preservation of its efficiency. However, after 10 cycles, there was a progressive decline in relative activity reaching 55%. The observed retention of over 50% of the original laccase activity after 10 cycles of catalysis suggests that L@M-MWCNTs exhibit a satisfactory level of stability. The observed decline in laccase activity after the 8th cycle could be attributed to the removal of weak connections by washing. Additionally, the activity of enzymes might decrease over the storage period. Subsequently, the enzyme's catalytic activity experiences a more rapid loss over the subsequent cycles due to frequent use. The literature study revealed that the immobilization approach used played a crucial role in ensuring the

Table 2
S_{BET} for MWCNTs, M-MWCNTs and L@ M-MWCNTs.

	S_{BET}	Total pore volume	Average pore diameter
	m^2/g	cm^3/g	nm
MWCNTs	155.1	0.289	7.58
M-MWCNTs	210.3	0.361	6.77
L@ M-MWCNTs	73.6	0.142	3.82

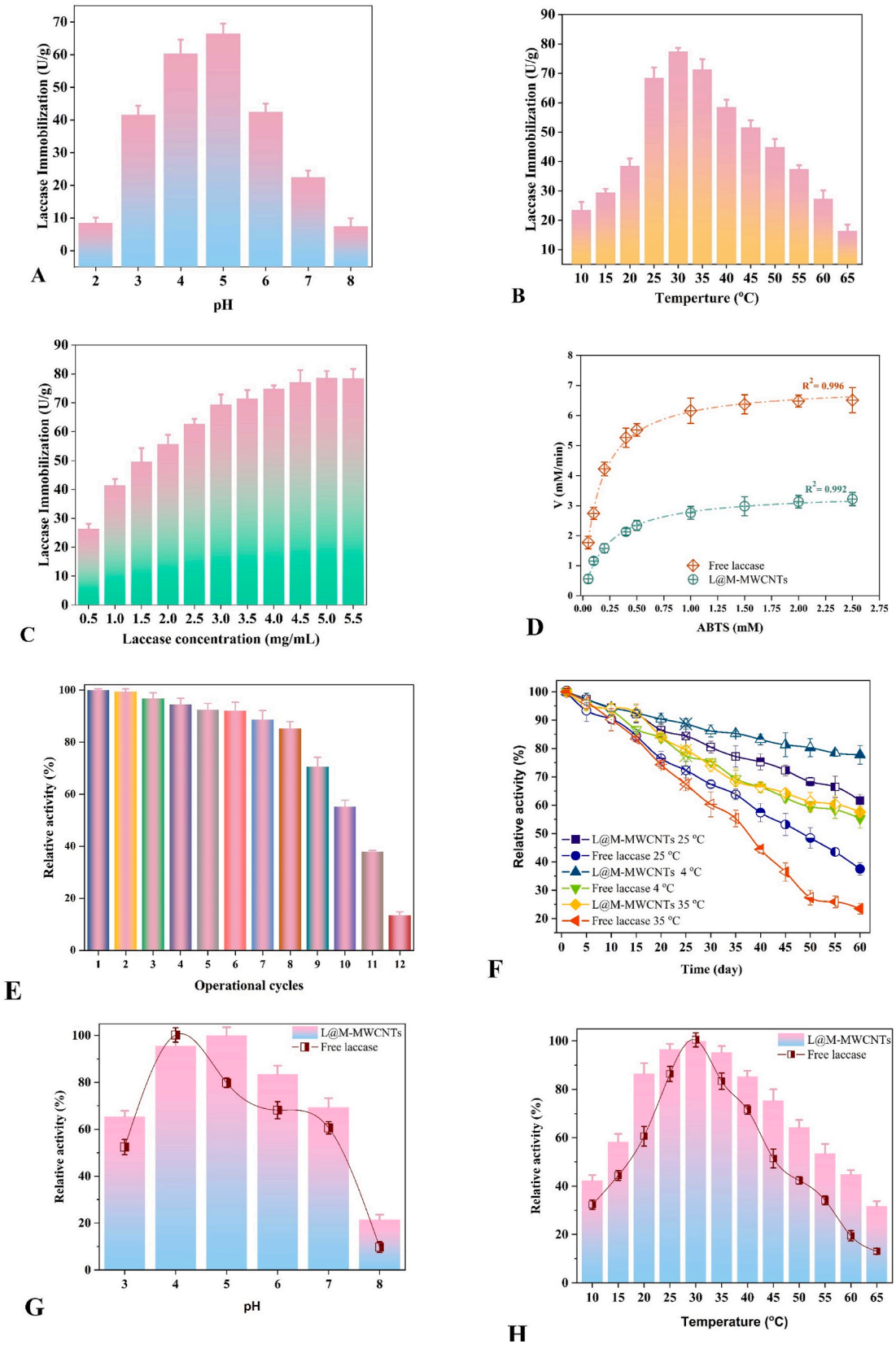

Fig. 3. The impact the pH (A), temperature (B), and laccase concentrations (C) on L@M-MWCNTs; Initial reaction rates for different concentrations of ABTS with free laccase and L@M-MWCNTs. The line in the graph shows the application of the Michaelis-Menten model to the data from experiments (D); Stability results of L@M-MWCNTs over 12 operational cycles (E); Stability of free and L@M-MWCNTs related storage duration with 4 °C, 25 °C and 35 °C (F); Stability of L@M-MWCNTs and free laccase with regard to (G) pH and (H) temperature.

operational stability of laccase (de Oliveira Mota et al., 2023). The stability was shown to be closely associated with the enzyme's high mechanical stability. Mahmoodi and colleagues used a covalent immobilization technique to attach laccase onto nanozeolite-carbon nanotube composites, with the aim of using this system for the biodegradation of Direct Red 23. The reusability assays conducted on the immobilized laccase demonstrated a 69% a retention of original activity after 10 cycles (Mahmoodi et al., 2020). The immobilization of laccase on the M-MWCNTs in the current work, demonstrated consistent and substantial reusability for more than twelve cycles.

The potential for enzyme activity and denaturation can be considerably reduced when the enzyme is immobilized on a carrier (Taheran et al., 2017). Because enzyme activity weakens during prolonged storage, stability is an important criterion to consider when evaluating enzyme activity (Rahmani et al., 2015). The conformational changes of laccase after immobilization are limited due to the reduction of protein structural mobility. Protecting the secondary and tertiary structures of laccase from degradation during long-term storage could substantially reduce the rate of enzyme activity decline and enhance storage stability (Rueda et al., 2016).

The comparative examination of the storage stability of free and immobilized laccase at various temperatures (4, 25, and 35 °C) was conducted for a period of two months and the results are shown in Fig. 3 (F). After a duration of one month, the laccase that was immobilized exhibited a retention of roughly 86.2%, 80.5%, and 73.9% of its initial activity at temperatures of 4 °C, 25 °C, and 35 °C, respectively. For a storage period of two months, a significant proportion of the initial activity of L@M-MWCNTs was preserved. Specifically, 77.8%, 61.6%, and 57.6% of the initial activity were kept at temperatures of 4 °C, 25 °C, and 35 °C, respectively. In comparison, the free laccase enzyme exhibited a lower activity retention of around 75.3%, 67.4%, and 60.3% for its initial activity after one month of storage at temperatures of 4 °C, 25 °C, and 35 °C, respectively. After a two-month incubation period at temperatures of 4 °C, 25 °C, and 35 °C, the free laccase activity exhibited a considerable drop, corresponding to 55.3%, 37.5%, and 23.5% of its initial activity, respectively. These findings highlight the immobilization contribution to the enzyme's stability against conformational denaturation at varied temperatures. The findings exhibit similar results to those previously reported by Xu and co-worker (Xu et al., 2015) who immobilized laccase on a composite nanofibrous membrane consisting of polyvinyl alcohol (PVA), chitosan (CS), and MWNTs. The decline in activity for the free laccases was observed to be much greater than the immobilized laccases when subjected to identical storage conditions over a period of 10 days.

3.4. Effect of pH and temperature on L@M-MWCNTs

The ionization state of amino acids is influenced by pH, hence, changes in pH can influence the functionality of enzymes including laccase (Haki and Rakshit, 2003). Enzymes exhibit excellent stability when immobilized, although remain vulnerable to changes in pH and temperature (Brena et al., 2013). The impact of pH on the activity of both free and immobilized laccase was investigated for a pH range of 3–8 and the findings are shown in Fig. 3 (G). The immobilized form exhibited the maximum laccase activity at a pH of 5, while the free laccase had the highest activity at pH 4. Based on these results, pH 4 will be applied later in the adsorption experiments. It can be noticed that there is a very low variance of <5% between the optimal pH level and pH4 for immobilized laccase. There was a decrease in the activity for both laccase forms at pH levels of 6 and 7, followed by a sharp drop in pH 8. The stability of laccase in its free state decreases when exposed to extremely acidic or basic environments, resulting in notable alterations in laccase activity. It is apparent from Fig. 3 (G) that the process of immobilization has a significant impact on enhancing the stability of laccase.

The impact of temperature on the activity characteristics of L@M-MWCNTs and free laccase was also examined as shown in Fig. 3 (H). It can be noticed that the optimum enzyme activity was recorded at a temperature of 30 °C for both forms of matrix. The findings indicated that the laccase immobilized on M-MWCNTs exhibited superior efficiency compared to the free laccase enzyme, even when subjected to high temperatures. The enhanced enzymatic performance of L@M-MWCNTs at elevated temperatures could be attributed to the formation of multiple bonds between the laccase molecules and the M-MWCNTs, as well as the enhanced capacity of the substrate to diffuse at higher temperatures (Lonappan et al., 2018). The improvement of immobilized laccase activity with temperature could be attributed to the preservation of the conformational structure of the enzyme which in turn could enhance its resistance to different environmental conditions (Cea et al., 2019).

3.5. Adsorption performance

3.5.1. Effect of MWCNTs dosage and contact time

Not only are M-MWCNTs providing an excellent carrier for the laccase, but it is also an effective adsorbent for water pollutants. Fig. 4 (A) illustrates the impact of M-MWCNTs dosages (0.4–2 g/L) on the removal of the target contaminants at 30 °C, 150 rpm, pH 5, and a concentration of 60 mg/L. The rise in the concentration of M-MWCNTs was shown to correspondingly enhance the removal of contaminants to a certain degree, possibly attributable to the enhancement of available adsorption sites. According to Fig. 4 (A), the M-MWCNTs reached saturation at 60 min and exhibited a maximum removal effectiveness range of 67.38% (Dcf) to 72.98% (Bap). However, it was observed that increasing the mass of the adsorbent beyond 1.6 g/L did not provide any further enhancement in the adsorption process. Consequently, a concentration of 1.6 g/L of M-MWCNTs was used in the subsequent batch studies. Different pollutant concentrations were also examined, and the results are presented in Fig. S3. The higher the concentration of pollutants, the lower the removal was.

3.5.2. Effect of pH

Fig. 4 (B) shows the removal percentages of the mixture by the M-MWCNTs at a pH range of 3–7. pH_{PZC} of the M-MWCNTs was determined to be 6. The highest removal for all pollutants was achieved at pH 5. As pH increased, the removal dropped. However, the reduction in the removal at pH 6 is lower compared to pH 4. At a pH < pH_{PZC}, the surface of adsorbent carries a positive charge. As for the pollutants, their charge depends on their ionization which is associated with their dissociation. All the tested contaminants in this study except diclofenac possess a dissociation constant (*pKa*) higher than the pH_{PZC} (Im et al., 2012; NCBI and National Library of Medicine; TMIC; ChemicalBook and CAS DataBase List). This means that they exist in deprotonated form at pH < $pH_{PZC,}$ hence, the electrostatic attraction might have improved the adsorption resulting in the highest removal recorded at pH of 5. Diclofenac has a pKa of 4.15, after which the compound exists in the unionized form which may reduce its solubility which might have led to its high removal at pH 5. Precipitation and electrostatic interaction are not the only removal pathways that might have acted in this work.

3.5.3. Effect of temperature

Fig. 4 (C) illustrates the impact of temperature on the removal of the target contaminants. When the temperature was raised from 15 to 25 °C, there was a noticeable improvement in the efficiency of pollutant removal. Increasing the temperature above 25 °C caused a decrease in the mixture removal efficiency. The process seems to be endothermic since the adsorption efficiency increased with increasing the temperature and this will be discussed in later sections. However, the decline in the removal after 25 °C could be attributed to structural change of the adsorbent upon exposing it to higher temperatures.

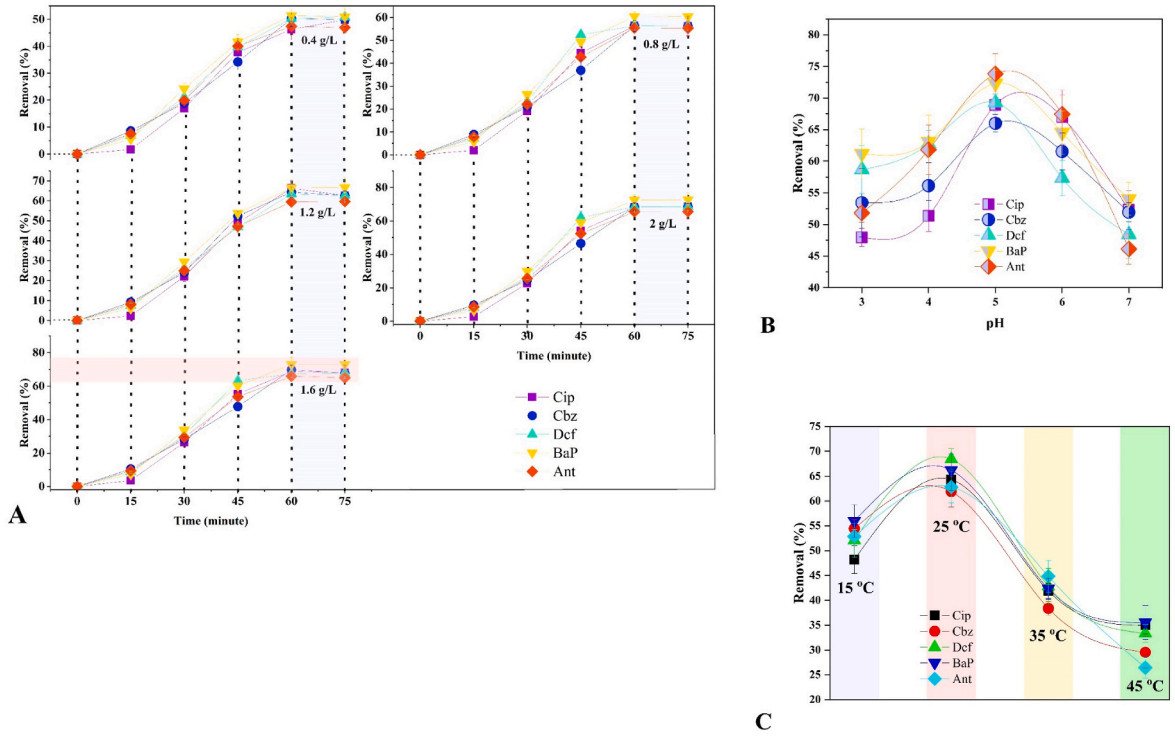

Fig. 4. Effect of 0.4, 0.8, 1.2, 1.6, and 2 g/LMWCNTs dosages on contaminants removal (A); Effect of different pH on pollutants removal (B); and Effect of different temperatures on pollutants removal (C).

3.6. Isotherms, kinetics and thermodynamic

Fig. 5 (A and B) display the results obtained from the fitting of the Langmuir and Freundlich isotherm models to the experimental data. The Langmuir isotherm model demonstrates a better match to the adsorption data of the compounds, as shown by high correlation coefficients ranging from 0.891 (Bap) to 0.998 (Cbz). In contrast, the Freundlich model exhibited correlation values between 0.821 (Ant) to 0.992 (Dcf), suggesting a comparatively weaker fit. This observation suggests the adsorption of the pollutants follows a monolayer development (Azadfar et al., 2021; Eyni et al., 2019; Sharif Nasirian et al., 2021). The maximum adsorption capacity (qm) was determined to be in the range of 507.61 mg/g for CBZ to 892.85 mg/g for Cip. The R_L values of the pollutant compounds, as shown in Table S1, indicate a range between 0 and 1 suggesting a favorable adsorption process which is evident in the obtained results. The heterogeneity factor, denoted as 1/n, represents a numerical number that varies between zero and one. In the case of this study, the reciprocal values (1/n) pertaining to pollutants exhibited a range of 0.5–1, indicating a decreased level of surface heterogeneity (Gholizadeh et al., 2019; Khakpour and Tahermansouri, 2018; Alimohammady et al., 2017). Fig. 5 (C and D) depict the fitting of the adsorption data versus time against the pseudo-first-order and pseudo-second-order models. The data fits better with the second-order model. The fitting parameters for kinetic models are shown in Table S1. The better fit of experimental data to second-order kinetics suggests that the adsorption involves chemical interaction likely to occur between functional groups on the adsorbents' surface and adsorbates (Gholizadeh et al., 2019; Khakpour and Tahermansouri, 2018; Alimohammady et al., 2017). The residual sum of squares (SSE), also known as the error sum of squares, is calculated for kinetic models (Alimohammady et al., 2017). A low SSE indicates that the model, which in this study adhered to pseudo-second-order, effectively matches the data. This is also supported by Adj. R-Square which was also better than pseudo-first-order. Table S2 shows SSE for both models.

Fig. 5 (E) displays the evaluation of the thermodynamic characteristics of the pollutants on M-MWCNTs. The thermodynamic characteristics of pollutants adsorption, namely free energy (G°), enthalpy change (H°), and entropy change (S°), at various temperatures (283 K, 293 K, 303 K, and 313 K), are shown in Table S3. The G° values exhibited a reduction with increasing temperature, indicating that temperature has a favorable impact on the effectiveness of adsorption. The observation of negative values of G° at four distinct temperatures suggests that contaminants exhibit a spontaneous adsorption tendency onto M-MWCNTs. The positive values of the enthalpy suggest that the adsorption processes in endothermic in nature as alluded to earlier (Tahermansouri et al., 2016; Gholizadeh et al., 2020). According to Liu et al. (2012), positive values of S° suggest that the adsorption process exhibits both stability and randomness which is the case in this study.

Fig. S4 shows Dubinin-Radushkevich (D-R) model for the selected pollutants. E value for all chemicals was more than 8 kJ/mol which explained a chemical adsorption has occurred to these contaminants. Fig. S5 shows Temkin isotherm fitting for the PAHs and PhACs. Fig. S6 shows a linear relationship between qt and $t^{1/2}$. The figure indicated that the sorption process is governed by intraparticle diffusion. If the data shows multilinear plots, the sorption process is influenced by two or more stages (Valderrama et al., 2008). Two linear regression lines were derived for PAHs and PhACs. The first line illustrates the process of macropore diffusion, while the subsequent line indicates the process of micropore diffusion till reaching a state of equilibrium.

3.7. Removal, degradation products and recyclability

There are two hypothesized removal mechanisms of pollutants with an immobilized enzymatic system. The first one is the adsorption of these compounds onto the carriers and the second one is the enzymatic degradation of the adsorbed compounds. Based on the isotherm fitting results, it seems that the adsorption that occurred in this work is of a physical nature with the development of a monolayer. The possible mechanisms that could facilitate such adsorption include hydrogen bonding, and π–π interactions, electrostatic interactions, and

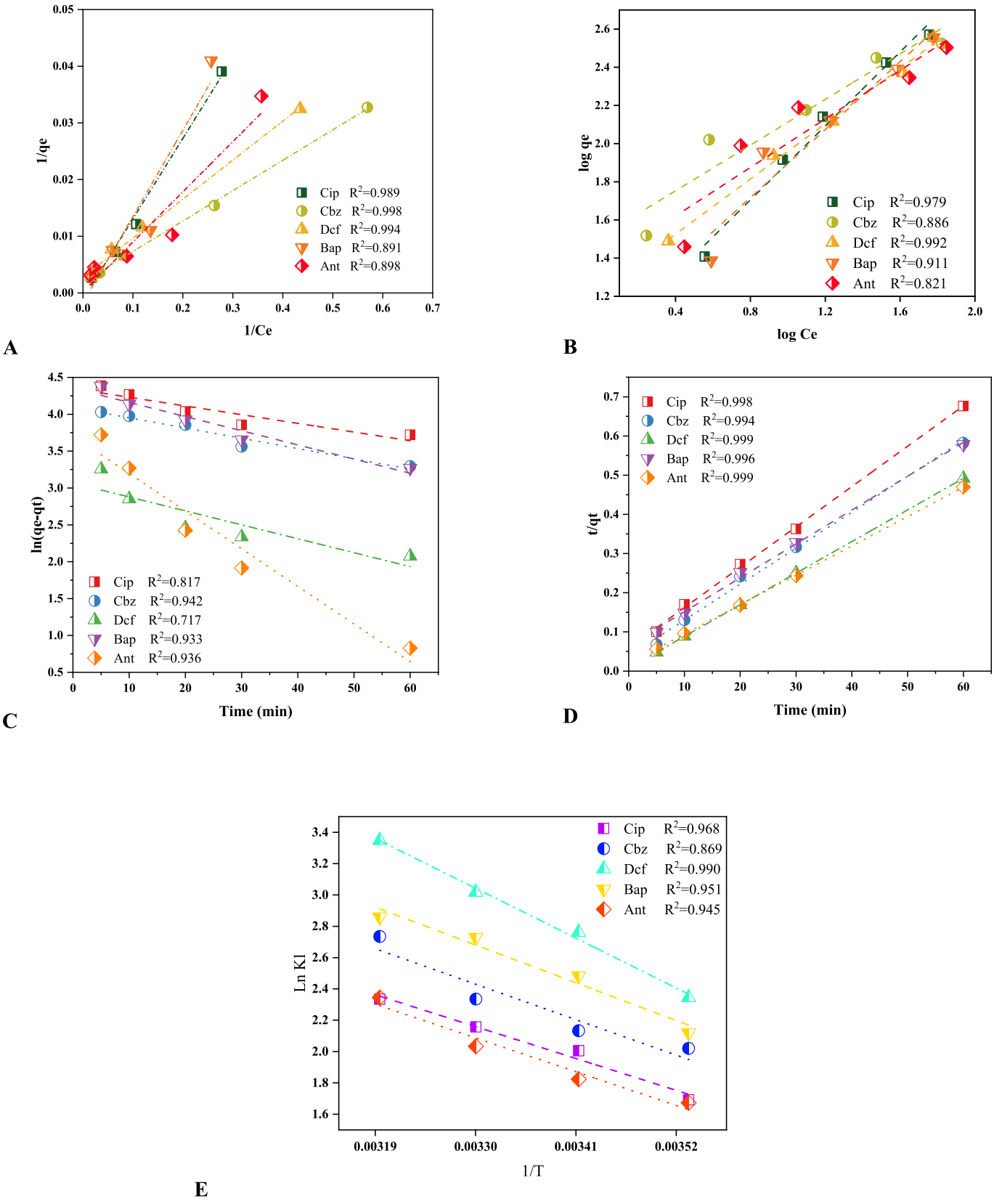

Fig. 5. Langmuir (A), Freundlich (B), the pseudo-first-order model (C), the pseudo-second-order model (D), and thermodynamic characteristics (E) of the pollutants on M-MWCNTs.

hydrophobic-hydrophobic attraction. The laccase enzyme loaded onto M-MWCNTs subsequently degrades the adsorbed pollutants. Functional groups play an important role in the degradation process as they can transform into free radicals with high oxidation potential (Al-sareji et al., 2023b; Al-Sareji et al., 2023c). Fig. 6 (*i-iii*) shows the removal of the selected PAHs and PhACs in synthetic samples and spiked wastewater and lake water samples, respectively for L@M-MWCNTs and M-MWCNTs for 12 successive cycles. In general, L@M-MWCNTs exhibited a better performance compared to M-MWCNTs. As the number of cycles increased, the removal diminished, and this was more pronounced in wastewater samples where only 11 cycles showed measurable removal. PAHs were removed better than PhACs in synthetic samples, but the case is different for spiked surface water and wastewater where the reverse trend was observed especially for Cip. The regenerated adsorbents in some cycles performed slightly better than the proceeding cycles suggesting that the regenerating might have activated some new sites in the adsorbents. L@M-MWCNTs achieved >90% removal of the pollutants for up to 7 cycles in the synthetic samples and

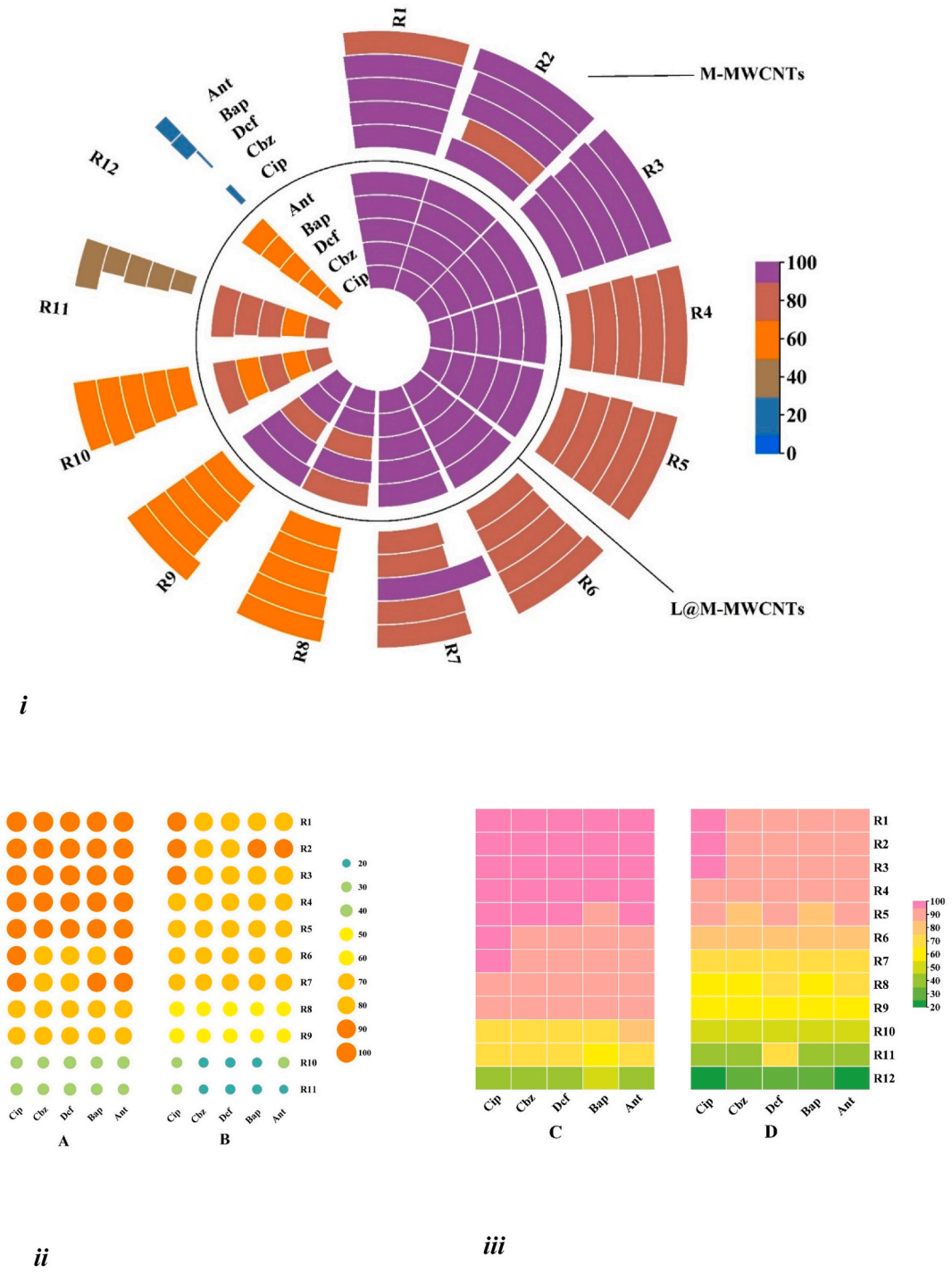

Fig. 6. Pollutant removal from synthetic samples using L@M-MWCNTs and M-MWCNTs. SD was ≤3.56 % for L@M-MWCNTs and ≤2.83% for M-MWCNTs for all cycles (*i*); Pollutants removal from wastewater samples employing (A) L@M-MWCNTs and (B) M-MWCNTs. SD was ≤4.53 % for L@M-MWCNTs and ≤2.70% for M-MWCNTs for all cycles (*ii*); and pollutants removal from the lake (Lake Balaton, Hungary) water samples using (C) L@M-MWCNTs and (D) M-MWCNTs. The SD was ≤4.37 % for L@M-MWCNTs and ≤3.12% for M-MWCNTs for all cycles (*iii*).

5 cycles in wastewater and lake water. In comparison, obtaining >90% removal of most of the tested pollutants was only obtained for the first 4 cycles in the synthetic samples and spiked surface water using M-MWCNTs.

The UPLC-MS technique was used to identify the degradation metabolites of the pollutants. For Ant, the observed products mostly manifested at retention times of 8.5 and 17.4 min, which corresponded to compounds with molecular formulas $C_{14}H_8O_2$ and $C_{16}H_{32}O_2$, respectively (Table S4). The observed products from Bap were $C_{20}H_{10}O_2$, and $C_{22}H_{14}O_2$ at retention times of 7.5 and 9.5 min, respectively (Wang et al., 2023; Hadibarata et al., 2013; Deng et al., 2022) (Table S5). Desethylene ciprofloxacin, $C_{15}H_{17}FN_3O_3$ has been identified as a metabolite of ciprofloxacin degradation (Table S6). This component has been seen not only in biological systems via the activity of the brown-rot fungus *G. striatum*, but also during the process of ciprofloxacin ozonation (De Witte et al., 2009). The compound $C_{13}H_{12}FN_2O_3$ was also detected during the degradation of Cip by the microorganism *G. striatum* (Wetzstein et al., 1999). Furthermore, the compound $C_{17}H_{19}FN_3O_4$ proposed the process of monohydroxylation of Cip. $C_{14}H_{11}Cl_2NO_3$ was identified after diclofenac degradation (Table S7). The 4′,5-dihydroxydiclofenac, $C_{14}H_{11}Cl_2NO_4$ was also detected which was reported to be one of the metabolites that results from diclofenac degradation (Lonappan et al., 2017). Earlier research has documented the process of converting diclofenac into 4′-hydroxydiclofenac using an extracellular peroxigenase derived from the basidiomycete *Agrocybe aegerita* (Kinne et al., 2009). The 10,11-dihydro10,11-epoxycarbamazepine was reported as degradation product of Cbz by whole-cell white-rot fungi, namely *Trametes versicolor*, was described (Hata et al., 2010). In a similar way, it has been shown that some fungus, namely *Umbelopsis ramanniana* and *Cunninghamella elegans*, can produce 10,11-epoxycarbamazepine as their primary metabolite. However, it is worth noting that these fungi also generate secondary metabolites such as 2-hydroxy carbamazepine and 3-hydroxy carbamazepine (Kang et al., 2008). The detected metabolites in this study are similar to those reported in the literature. The primary outcome of Cbz enzymatic degradation seems to be the production of 10, 11-epoxycarbamazepine (Table S8). However, other transformation products have also been detected. Based on the detected metabolites, the likely degradation pathways are oxidation, dehydrogenation, hydroxylation, and ring cleavage. Fig. S7 shows the scheme of reaction for the used materials. Table S9 shows selected studies immobilized laccase on CNT with different matrix. Comparing our work with others, it is clearly shown that better removal performance of a pollutants mixture in addition to better recyclability over 12 successive cycles.

4. Conclusion

The process of immobilizing laccase on MWCNTs by physical adsorption was effectively carried out to produce L@M-MWCNTs. The study compared two systems, namely MWCNTs and L@M-MWCNTs. The MWCNTs had the highest removal efficiency at a pH of 5, temperature of 25 °C, and MWCNTs concentration of 1.6 g/L. On the other hand, L@M-MWCNTs exhibited optimal performance at a pH of 4, temperature of 30 °C, MWCNTs concentration of 1.6 g/L, and enzyme concentration of 5 mg/mL. Overall, L@M-MWCNTs show superior efficacy in removing contaminants compared to M-MWCNTs. L@M-MWCNTs demonstrated a removal efficiency of over 90% for 7 cycles in synthetic samples and 5 cycles in wastewater and Lake Balaton water samples. By contrast, achieving a removal rate of over 90% for the majority of the contaminants examined was only possible during the first 4 cycles in both the synthetic samples and spiked surface water when using M-MWCNTs. The addition of laccase enhanced both the efficiency of removal and the number of cycles. After storing L@ M-MWCNTs for two months, it was observed that the material retained 77.8%, 61.6%, and 57.6% of its initial activity when stored at temperatures of 4 °C, 25 °C, and 35 °C, respectively. During the same period, the free laccase exhibited 55.3%, 37.5%, and 23.5% of its initial activity at temperatures of 4 °C, 25 °C, and 35 °C, respectively. These results emphasize the role of immobilization in enhancing the enzyme's stability against conformational denaturation at different temperatures. Enzymatic degradation metabolites indicate that the primary mechanisms for degradation include oxidation, dehydrogenation, hydroxylation, and ring cleavage.

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CRediT authorship contribution statement

Ruqayah Ali Grmasha: Writing - review & editing, Writing - original draft, Visualization, Validation, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Osamah J. Al-sareji:** Writing - original draft, Visualization, Validation, Resources, Methodology, Investigation, Data curation, Conceptualization. **Mónika Meiczinger:** Writing - review & editing, Supervision, Methodology. **Csilla Stenger-Kovács:** Supervision. **Raed A. Al-Juboori:** Writing - review & editing, Methodology, Investigation. **Miklós Jakab:** Methodology. **Edina Lengyel:** Investigation. **Viola Somogyi:** Writing - review & editing, Validation. **Mohammad Amir Khan:** Writing - review & editing, Data curation. **Khalid S. Hashim:** Writing - review & editing, Methodology.

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.

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at.https://doi.org/10.1016/j.envres.2024.118097.

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