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A novel and sustainable composite of L@PSAC for superior removal of pharmaceuticals from different water matrices: Production, characterization, and application

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ABSTRACT

This study endeavors to develop cost-effective environmentally friendly technology for removing harmful residual pharmaceuticals from water and wastewater by utilizing the effective adsorption of pistachio shell (PS) biochar and the degradation potency of laccase immobilized on the biochar (L@PSAC). The carbonization and activation of the shells were optimized regarding temperature, time, and NH3/PS ratio. This step yielded an optimum PS biochar (PSAC) with the highest porosity and surface area treated at 700 °C for 3 h using an NH3NO3/PS ratio of 3% wt. The immobilization of laccase onto PSAC (L@PSAC) was at its best level at pH 5, 60°C - C. The optimum L@PSAC maintained a high level of enzyme activity over two months. Almost a complete removal (>99%) of diclofenac, carbamazepine, and ciprofloxacin in Milli-Q (MQ) water and waste-water was achieved. Adsorption was responsible for >80% of the removal and the rest was facilitated by laccase degradation. L@PSAC maintained effective removal of pharmaceuticals of ≥60% for up to six treatment cycles underscoring the promising application of this material for wastewater treatment. These results indicate that activated carbon derived from the pistachio shell could potentially be utilized as a carrier and adsorbent to efficiently remove pharmaceutical compounds. This enzymatic physical elimination approach has the potential to be used on a large-scale.

1. Introduction

The escalating consequences of rapid industrialization and urbanization have led to the accumulation of a substantial volume of persistent pollutants in the environment, comprising of a wide range of hazardous materials most importantly are pharmaceuticals and endocrine-disrupting chemicals (Shakerian et al., 2020). Emerging pollutants, a categorization encapsulating substances not immediately considered in routine monitoring programs, are identified through meticulous works on their toxicity, hazardous health impacts, and public perception, with data scrutinized across diverse environmental domains (Yadav et al., 2021). The effects of these contaminants present major negative impacts...
on human health and the environment. Common strategies for pharmaceuticals removal approaches involve but are not limited to adsorption (Al-sareji et al., 2024; Li et al., 2012), advanced oxidation (Yu et al., 2024), biodegradation (Zheng et al., 2020a; Zhou et al., 2021), membrane separation (Zhang and Jiang, 2019; 2021; 2022; Chen et al., 2019), and enzymatic degradation (Harguindeguy et al., 2024). The potential application of bioremediation methods seems very feasible considering its cost-effectiveness and sustainable characteristics (Serbent et al., 2024). In this field, organisms or their enzymatic components are utilized to eliminate contaminants by degrading them into forms that are less toxic or less toxic to the ecosystem. The unique biocatalytic properties of this method have attracted the attention of the scientific community for their potential applications in bioremediation. Thus, bioremediation is particularly suitable for water treatment because of its low activation energy and mild conditions (Mousavi et al., 2021).

Lactase, oxidoreductases enzyme, could work on a wide range of substrates and catalyse oxidation in a variety of complexes. Despite its benefits, there are limitations accompanied by the utilization of this enzyme. These include challenges regarding its storage, sensitivity to pH and temperature changes, cost, reusability, and difficulty in separating the enzyme from the reaction medium (Liu et al., 2018). Immobilization on solid support brings the best solution to these issues (DiCosimo et al., 2013). The utilization of waste materials by-products such as biochar is a viable option for enzyme industries, especially with emphasizing on using environmentally friendly materials. This solution not only brings a cost reduction for enzyme production, but also offers to tackle waste management issues. Improperly disposed of these wastes could negatively impact the environment by gases like ammonia (NH3) and methane (CH4). These gases contribute to climate change on a global scale (Bilal and Iqbal, 2019). Agricultural and food wastes have several favorable properties such as high porosity, large surface area, and the presence of various chemical groups such as amino, hydroxyl, carboxyl, thiol, and phosphate groups. These chemical properties can be associated with various complex processes such as surface adsorption, ion exchange, complexation, and microdeposition (Saravanan et al., 2023).

One notable source of biochar that warrants attention is the shells of the Pistacia vera tree. According to data released by FAOSTAT in 2018, the primary regions responsible for the worldwide production of pistachios are the United States, Iran, Turkey, and China (FAostat, 2018). In the year 2016, the combined output of these countries exceeded one million tons. The pistachio’s shell accounts for 51–69% of its total fruit weight (Tekin et al., 2021). Consequently, a substantial quantity of pistachio shells is generated after nut consumption, and due to their lack of economic value, the majority of shells are ultimately discarded in landfills or incinerated. Thus, there is an immediate need for the handling of this waste by-product.

The existing research on pistachio shells has primarily centered around the generation of biochar from this waste material, as extensively explored in the review conducted by Igwegbe et al. (2023). Scientists have also used an adsorption-based methodology in developing biochar. Zinc oxide (ZnO) nanoparticles were incorporated into pistachio shells. The resulting nanoparticles of pistachio shell covered with ZnO were developed. This system was used to eliminate tetracycline (TEC) from synthetic wastewater samples with 84.87% removal percentage of the selected contaminant (Mohammed and Kareem, 2019). However, the study did not further investigate the long-term performance of the system. The same group has further conducted a study to remove a mixture of contaminants including tetracycline (TEC), amoxicillin (AMO), and ciprofloxacin (CIP) from water (Mohammed et al., 2020). Regrettably, the highest removal percentages could not be identified (Mohammed et al., 2020). Another study by Hassan et al. (2013) has examined the deltamethrin pesticide removal by using pistachio shells as an activated carbon. The finding indicated that maximum adsorption capacity of the system was 162.60 mg/g at 35 °C.

Based on the above and the studies mentioned, it is clear that the use of pistachio shell waste as a carrier of new pollutants, in particular lactase immobilizers, has been underutilized. To date, there is a significant gap in the literature, as no study has investigated the viability of using pistachio shell waste as a carrier material, particularly for the removal of pharmaceuticals. Hence, this research work attempts to fill this knowledge gap by assessing the efficacy of chemically functionalized pistachio shells as a support for lactase, specifically targeting the removal of emerging contaminants such as diclofenac (DCF), carbamazepine (CBZ), and ciprofloxacin (CIP) in Milli-Q (MQ) water and wastewater samples. The pollutants selection is determined by their widespread distribution in aquatic ecosystems and the potential environmental and health effects they cause (Fekadu et al., 2019; Chaves et al., 2022; Al-Juboori and Hilal, 2023). Studies on the effects of operational variables such as temperature, pH, and concentration of enzyme on immobilization in pistachio shells are also tested. Additionally, the system is evaluated through multiple runs along with its performance through multiple operational cycles.

2. Materials and method

2.1. Materials

The diclofenac (CAS number: 15307-86-5), carbamazepine (CAS number: 298-46-4), and ciprofloxacin (CAS number: 85721-33-1) were acquired from Merck KGaA. Ammonium nitrate (NH4NO3) (>99.0%, CAS No.: 6484-52-2), Hydrochloric acid (HCl, 37%), nitric acid (HNO3, 99.8%), sulfuric acid (H2SO4, 99.6%), laccase (Trametes versicolor, ≥0.5 U/mg, EC 1.10.3.2) and 2,2’-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) (98%) were provided by Merck Chemicals. The necessary ingredients for the formation of the McIlvaine citrate-phosphate solution, which included citric acid (C6H8O7) and disodium phosphate (Na2HPO4) were purchased from AVANTOR. Sodium hydroxide (NaOH) 98%, sodium bicarbonate (NaHCO3) 99.5–100.5%, sodium carbonate (Na2CO3) 99.5%, and sodium ethoxide 95%, were supplied from Sigma-Aldrich. Pistachio (Pistacia vera L.) shell waste (PS) was acquired from a local supermarket free of charge. Milli-Q (MQ) water, produced by the Millipore Direct-Qt® 5 UV purification system with a resistivity of 18.2 MΩ·cm−1 was used for cleaning and preparing solutions. Whatman® glass microfibre 47 mm circles) filters were utilized in filtration. All chemicals and reagents utilized were of analytical grade and did not require further purification.

2.2. Adsorbent and activation

The PS was washed with tap water to remove contaminants that might appear on the material and thereafter dried at 105 °C for 48 h. Subsequently, the PS material reduced in size to reach a range of 0.5–2 mm. Next, the method outlined by Sajjadi et al. (2019) was employed to develop an activated carbon by using ammonium nitrate (NH4NO3). The applied conditions were different NH4NO3/PS ratio wt. (%), (2,4,6, and 8), temperatures ranging from 500 °C to 800 °C and 2–3.5 h as activation time. An 80 mg of PS is combined with a polluted solution (50 mg/L) in a 250 mL Erlenmeyer flask. The study was conducted for 3.5 h under controlled conditions of 25 ± 1 °C, pH 5, and 150 rpm. Table S1 shows a summary of the findings of this procedure and emphasizes the optimal parameters for producing Pistachio Shell Activated Carbon (PSAC). This parameter revealed that an NH4NO3/PS ratio of 6% and a pyrolysis temperature of 700 °C for 3 h. A vertical stainless-steel reactor placed within an electric furnace was used to carry out the pyrolysis process. Throughout the process, nitrogen gas flows continuously at a rate of 150 cm3/min. The PSAC washed with water and then 0.1 M HCl to remove inorganic salts and any remaining ash. Then, the material was washed further to neutralize and kept overnight for 105 °C. An amount of 2 g of PSAC was mixed with 5 M H2SO4 and 5 M HNO3 (1:1) for 6 h and 90 °C (Al-sareji et al., 2023a). The final product was cooled and washed with MQ water to set the pH to 6–7. Then, it was dried for one night at 105 °C and stored for further utilization.
2.3. Analysis and equipment

Analytical techniques were employed to describe and understand the properties of produced material (PSAC). FTIR model Nicolet™ iS™ 5 from Thermo Fisher, USA was used to analyze the functional groups. The device was equipped with attenuated total reflection (ATR), within the range of 400–4000 cm⁻¹ and a resolution of 2 cm⁻¹. Boehm titration was carried out to determine functional groups on the surfaces of PS and PSAC, as described in our earlier study (Al-sareji et al., 2023a). SEM (JEOL JIB-4700F) - EDS (GENTLEBEAM™ (GB)) with an accelerating voltage of 3–5 kV and a current of 10 pA was used to understand the materials surface morphology and chemistry. The samples were coated with a 4 nm layer of Au-Pd to enhance their conductivity. XRD (Rigaku D/Max 2500 VB + X) provided with Cu radiation source was employed for evaluating PSAC crystallinity at 40 kV and 35 mA and 2θ scan of 10–80°. The Micromeritics 3Flex was used to measure the pore size and specific surface area (S BET) employing Brunauer-Emmett-Teller (BET) method. The adsorbents’ point of zero-charge (pH ZPC) was determined using the drift process as outlined by Kosmulski in 2009. The proximate analysis of PS and PSAC was conducted according to the guidelines set by the American Society for Testing Materials (ASTM). The ash content, moisture, and volatile matter were measured following ASTM D3174-04, D3173-03, and D3175-0 standards, respectively. The fixed carbon was determined via deducting the sum of moisture, ash, and volatile matter from 100%. The elemental analysis of PS and PSAC was carried out using a Carl Erba Instruments Model EA 1108 following the ASTM D3176 standard.

2.4. Enzyme immobilization

The laccase was immobilized onto PSAC following the procedure reported in (Costa et al., 2019; Al-sareji et al., 2023b) by mixing 2 mg of a PSAC with 1.20 mL containing 4 mg/mL laccase at pH 5. The solution was stirred using orbital agitation for 4 h at 30 °C. The selection of pH for immobilization was determined based on initial evaluations of its influence on the efficacy of free-state laccase as shown in Fig. S1. After immobilization, the resultant product, known as L@PSAC, was carefully washed with a buffer solution. After washing, the laccase activity was evaluated in the buffer as a control measure to check for enzyme detachment (section 2.5). No enzymatic activity was found in any of the materials used. The immobilization yield was quantified by subtracting the activity of the free-state laccase in the solution from the activity of the laccase that remained in the supernatant after immobilization and expressing the result as a percentage. Ultimately, the L@PSAC samples were preserved at 4 °C for further examination.

2.5. Free and immobilized laccase activity measurement

The activity of enzyme is gauged through its ability to oxidize a 0.50 mM ABTS substrate. The analysis was conducted in a buffer solution consisting of 0.05 mM citrate and 0.10 mM phosphate, at a pH of 5. The procedure included mixing 0.10 mL of the laccase solution with 1.9 mL of the ABTS solution, resulting in a total volume of 2.0 mL (Costa et al., 2019; Imam et al., 2021). The solution was placed in an incubator at controlled temperature of 25 ± 2 °C for 3 min (Pandey et al., 2022). The spectrophotometric measurement of ABTS oxidation was conducted at a wavelength of 420 nm using a Shimadzu UV-1601 double-beam UV–visible spectrophotometer in kinetic mode. The laccase activity was assessed by examining the first linear portion of the kinetic curve, which represents the relationship between absorbance and time. The measurement of laccase activity is based on the oxidation of 1 μmol of ABTS per minute, which is equivalent to one unit (U) of laccase activity. The activity of the unbound enzyme was quantified in units per liter (U/L). The immobilized laccase activity was measured by adding 105 mL of citrate/phosphate buffer at concentrations of 0.05 M and 0.10 M, respectively, with a pH of 5. The experiments were conducted at 25 ± 2 °C, with the addition of 37.50 mL of a 0.5 mM ABTS solution, and a stirring speed of 150 rpm. The samples were filtered through 0.45 μm polypropylene filters. Laccase activity was quantified using the following equation (Imam et al., 2021):

\[
\frac{U}{g} = \frac{\Delta ab \times F_{\text{dil}} \times R_v \times 10^6}{\varepsilon \times M_{\text{carrier}}}
\]

\[
U/g \text{ denotes the quantity of laccase that can oxidize } 1 \mu\text{mol of ABTS during a minute and per unit mass of } M_{\text{carrier}}. \text{ The absorbance per minute } (\Delta ab) \text{ is obtained using linear regression. } F_{\text{dil}} \text{ represents the dilution factor, } R_v \text{ represents the reaction volume in mL, } \varepsilon \text{ is the molar extinction coefficient, and } M_{\text{carrier}} \text{ is PSAC mass (g). The conversion factor from molarity to micro molarity is } 10^6.
\]

2.6. Enzyme kinetic

The Michaelis-Menten kinetics of both free and L@PSAC were investigated by exposing them to different doses of ABTS in buffer solutions that were adjusted for this purpose. The kinetic parameters, namely the Michaelis-Menten constant (Km) and the maximal reaction velocity (v max), were obtained using the following equation (Briggs and Haldane, 1925; Cornish-Bowden, 2013).

\[
v = \frac{v_m [S]}{K_m + [S]}
\]

where, ‘v’ represents the reaction rate measured in mM per minute, ‘v m’ indicates the highest achievable reaction velocity, ‘[S]’ represents the concentration of the substrate in mM, and ‘K m’ represents the Michaelis–Menten constant, likewise measured in mM.

2.7. Immobilization influencing factors

The effect of pH, temperature, concentration, and storage time on laccase activity in free and immobilized forms was investigated. For testing pH effect, separate tubes were set up with each tube containing 100 μL of 4 mg/mL of free state laccase or 20 mg of L@PSAC. These tubes were filled with 4 mL of buffer solutions with various pH in the range of 3–8. Afterward, the tubes were stirred at a speed of 150 rpm at 30 °C for 6.0 h. The enzyme activity was measured after that. The impact of temperature and concentration was evaluated in a similar fashion by varying the temperature from 15 to 65 °C at pH 5 and maintaining the concentration as above or varying the concentration from 0.5 to 5 mg/mL for free laccase and to 20 mg for L@PSAC maintaining a pH 5 at 30 °C. Regarding storage stability, both free laccase and L@PSAC samples were stored for a maximum duration of 2 months at 4 °C, 25 °C, and 30 °C. Throughout this storage period, the enzyme activity was measured weekly.

2.8. Reusability tests

A 100 mg of L@PSAC was put with 2.0 mL of a buffer solution containing 0.50 mM ABTS at a pH of 5. The mixture was then placed in an incubator at 30 °C for 10 min while being stirred at 150 rpm. After the incubation period, the mixture was centrifuged at 7000 rpm for 3 min. The solids were extracted, washed with MQ water, and then reused in several cycles.

2.9. Assessment of removal and recyclability effectiveness

The efficacy of L@PSAC in eliminating emerging contaminants from a liquid solution was evaluated via a sequence of controlled experiments utilizing MQ water and wastewater. Prior to conducting an analysis of the wastewater samples, a filtration process was used. In the investigations, 100.0 mg of L@PSAC was introduced into a solution consisting of 40 mL of a mixture of pollutants, where each pollutant had a
concentration of 50 mg/L. The solution was then agitated at a speed of 150 rpm at a temperature of 25 °C. At regular intervals of 15 min, 2 mL samples were taken throughout the experiment duration. The purpose of this step was to identify the duration needed for the system to achieve equilibrium, which was found to be 60 min. The removal effectiveness was calculated by comparing the starting and final concentrations in the aqueous phase. Afterward, the L@PSAC was cleaned with MQ water. The adsorption of pollutants onto PSAC (without laccase) was also studied to determine the extent to which physical removal and degradation contribute to the process. To this end, 100 mg of PSAC was added to a 40.0 mL mixture of pollutants with a concentration of 50.0 mg/L, and the same experimental procedure was applied. The pharmaceuticals concentration was measured using ultra-performance liquid chromatography-mass spectrometry (UPLC-MS) equipped with an electron spray ionization (ESI) interface. The Exactive Focus mass spectrometer conducted a thorough scan over the mass-to-charge ratio range of 50–1000, exhibiting adequate accuracy with a low error rate of less than 5 parts per million (ppm). The ESI was conducted with a

Fig. 1. SEM-EDS for PS (A), L@PSAC (B) and PSAC (C).
negative polarity, with a voltage of -4.5 kV. The ion transfer capillary was operated at a temperature of 550 °C and was linked to a ground potential established at 0 V.

2.10. Quality check

In order to confirm the accuracy of the experimental results, they were run in triplicate and were shown as the mean value and standard deviation (in case of the Figures). The data was tested using the Kolmogorov-Smirnov with significance level of 0.05 (p < 0.05). Blanks were run in parallel with each run to enhance the precision of the results. The grayscale was subjected to a thermal cleaning process at a temperature of 100 °C with washing by methanol to minimize the risk of cross-contamination.

3. Results and discussion

3.1. Characterization

3.1.1. Surface morphology and pores structure

The textural characteristics of PSAC, denoted by a BET surface area (SBET) of 1056.38 m²/g and a total pore volume (Vt) of 0.648 cm³/g, surpassed those of PS (SBET: 127.56 m²/g, Vt: 0.207 cm³/g) and L@PSAC (SBET: 218.49 m²/g, Vt: 0.326 cm³/g). The heightened porosity observed in PSAC can be ascribed to the infiltration of gases into the lignocellulose matrix during the activation process, induced by the detonation of ammonium-nitrate and the execution of activation at an elevated pyrolysis temperature of 700 °C. Upon comparing SBET values, it is evident that the modification led to an approximately 8.28-fold increase in SBET for PS. In contrast, the SBET for L@PSAC experienced a substantial decrease of 79.31% following the immobilization step. This pronounced reduction in surface area serves as quantitative evidence for the successful immobilization process through PSAC surface adsorption. The reduction in SBET following enzyme immobilization echoes findings from various prior studies. For example, HE and co-authors (2006) investigated the immobilization of lipase on mesoporous silica, revealing an 89.1% reduction in surface area (He et al., 2006).

The morphology as illustrated by the SEM-EDS micrographs of PS, PSAC and L@PSAC is shown in Fig. 1. PSAC possessed highly regular-shaped and well-developed pores in the form of long and parallel channels, while the PS surface was relatively smooth, and its amorphous porous structure consisted of uneven distributions of holes. Regarding the L@PSAC, there were no noticeable changes in surface texture after the immobilization of laccase. Because laccases have a small size range between 60 and 90 kDa, which is approximately equal to a particle size of less than 5 nm. Micrographs at a magnification of 1 µm struggle to capture this specific dimension (Lonappan et al., 2018a, b). Following the immobilization process, the surface of L@PSAC exhibited enhanced smoothness, presumably due to the application of a laccase coating on the activated carbon surface (Zhang et al., 2020a, b). The well-defined, regularly shaped pores observed in PSAC can be ascribed to the synergetic interplay of two principal processes: the explosive decomposition of NH₄NO₃ at elevated temperatures and pyrolysis carried out at 700 °C. The mechanistic sequence unfolds as follows: NH₄NO₃, experiencing melting at 169 °C, permeates the lignocellulose structure of wood particles. With a temperature rise, the quantity of NH₄NO₃ infiltrating the wood increases, concurrently generating micro-cracks because of heightened internal stresses at high temperatures and the degradation of cellulose content within wood at temperatures ranging from 210 to 220 °C (Taghiyari, 2013; Hill, 2007). Above this temperature range (260 °C), NH₄NO₃ undergoes an explosive reaction, with the liberated gases presumed to play a pivotal role in creating parallel channels and fostering the development of pores with diverse sizes. The activation process further progresses, culminating with the elevation of the temperature to 700 °C (Chaturvedi and Dave, 2013). Fig. 1 presents the EDS results derived from elemental analysis of PS, PSAC, and L@PSAC. PSAC exhibits elevated carbon content and a larger surface area compared to PS, rendering it a promising carbonaceous porous material with potential applications in adsorption technology, and various other applications. The high carbon content also contributes to the augmentation of graphic sheets within the structure of PSAC. The presence of enzyme on the surface of the adsorbent is evidenced by the increased nitrogen in L@PSAC compared to PSAC as seen in Al-sareji et al. (2023c). It was also observed that sulfur contents in the material decreased after carbonization at 700 °C due to the loss to the gas phase (Zhao et al., 2018). It was stated that S vaporization temperatures on thin films vary between 150 °C and 400 °C (Shin et al., 2013). Similar observations were reported by Al-Wabel et al. who found that increasing pyrolysis temperature reduced sulfur content due to its volatility at 200-800 °C (Al-Wabel et al., 2013).

3.1.2. Functional groups analysis

It was essential to conduct Fourier Transform Infrared (FTIR) spectroscopy on the adsorbent to acquire a more comprehensive understanding of the functional groups on the surfaces of PS, PSAC, and L@PSAC. As depicted in Fig. 2 A, all samples demonstrated asymmetrical stretching of CH₂ groups at 2914 cm⁻¹. Absorption bands associated with the carbonyl group (1589 cm⁻¹) and the phenolic group (1135 cm⁻¹) were observed in the FTIR spectra of PS, PSAC, and L@PSAC. PSAC exhibited a C=O stretching band at 1368 cm⁻¹, attributed to the chemical treatment involving HNO₃ and H₂SO₄, with the COOH absorption band indicated via peak at 1368 cm⁻¹. The L@PSAC manifested a slight increase in intensities and broadening of the peaks at 3439 cm⁻¹. The peak at 3439 cm⁻¹ was correlated with the overlapping O-H and N-H bonds present in laccase (Al-sareji et al., 2023c). It is hypothesized that the wavelength at 1589 cm⁻¹ is regarded as N-H stretching vibration, indicative of the presence of laccase protein (Hu et al., 2015). Within the range of 1135 cm⁻¹, it was observed that laccase protein exhibited characteristic properties of proteins (Al-sareji et al., 2023b).

It has been documented that incorporating carboxyl groups onto a carrier’s surface can enhance the immobilization of enzymes (Cho and Bailey, 1979). Consequently, the oxygen-containing functional groups on the surfaces of PS and PSAC were analyzed via Boehm titration. The outcomes revealed a prevalence of acidic over basic groups, affirming the acidic nature of PS and PSAC (Fig. 2 B). Specifically, the total oxygenated acidic functional groups in PS measured 2.12 meq/g, while the total basic groups amounted to 0.94 meq/g. Notably, there was an increase in surface functional groups when PS was Pyrolyzed and activated, with a rise of approximately 4.92 meq/g for the acidic groups and 0.21 meq/g for the overall surface basicity. The results align consistently with the estimations made, as the activation process facilitates the generation of additional acidic substances through the interaction between inorganic acids and the carbon precursor. The assessment of functional groups through Boehm titration corresponds harmoniously with the qualitative FTIR findings, providing further confirmation of the observed trends and reinforcing the effectiveness of the analytical approach employed in this investigation. The results also align with the observations reported by Zhang et al. (2020b), who also reported a similar pattern of elevated acidic groups and reduced basic groups after activation. A different research investigation used sonication in a blend of sulfuric acid and nitric acid to alter single-walled carbon nanotubes. The findings demonstrated that after subjecting the nanotubes to 14 h of sonication, they were cut and the quantity of carboxyl groups increased from 0.91 to 6.4 mmol/g (Marshall et al., 2006).

3.1.3. Crystallinity and surface charge

To assess the graphitization of PSAC, and L@PSAC, the powder XRD of these carbonaceous porous adsorbents were scrutinized. As depicted in Fig. 3, the patterns reveal distinct peaks at 2θ = 25.72⁰ and 42.22⁰, corresponding to the (002) and (100) diffraction peaks of the graphite plane and disordered graphite plane, respectively (Guo et al., 2017). Notably, the XRD pattern of PSAC and L@ PSAC exhibits more
pronounced and well-defined peaks which is indicative of possessing higher crystallinity and graphitic pore walls. The findings substantiate that the chemical activation by NH$_4$NO$_3$ engenders highly ordered pores with enhanced graphite crystallinity. This phenomenon is attributed to the synergistic influence of the explosive properties of NH$_4$NO$_3$ and the pyrolysis of the carbon sample at elevated temperatures, leading to the alignment of graphite along the pore walls. The immobilization of lac-case did not seem to affect the crystallinity of PSAC.

The surface charges of materials are notably influenced by the solution pH (Grmasha et al., 2024). One important characteristic that is utilized to study such changes is pH$_{pzc}$. The surface carries a negative charge in solutions with a pH greater than pH$_{pzc}$ and a positive charge in solutions with a pH lower than pH$_{pzc}$. Adsorption, being a surface-centric separation process, hinges on the charge distribution across the adsorbent surface, thereby dictating the electrostatic interactions between the adsorbate and the adsorbent. Under conditions where the pH is less than pH$_{pzc}$, anionic species are attracted to the positively charged adsorbent, while at pH levels exceeding pH$_{pzc}$, cationic species are adsorbed by the negatively charged adsorbent material (Al-sareji et al., 2024). As depicted in Fig. 3, it is evident that the surface charge of the PSAC exhibits a pronounced dependence on the initial pH of the solution, with the determined pH$_{pzc}$ value being 6.44. This result agrees with the values reported elsewhere. For example, MH Al-Awadhi and colleagues utilized PS for Lead (II) and Aluminum (III) removal from real water samples and the study found that PS has pH$_{pzc}$ value of 6.37 (MH Al-Awadhi et al., 2023). Another study reported a pH$_{pzc}$ of PS biochar to be 6.1 (Saghir et al., 2022).

3.1.4. Proximate and ultimate analysis

Table S2 shows the results for the approximate analysis of PS and PSAC. The reduction in volatile matter within the PSAC is notable, decreasing from 81.61% to 7.05%. The production of a highly stable and compact carbon structure under elevated pyrolysis temperatures is responsible for a substantial increase in fixed carbon percentage, elevating it from 12.72% to 90.17%. This might be explained by the high level of polymerization and the evaporation of volatile substances (Tomczyk et al., 2020). Simultaneously, the pyrolysis process resulted in a slight rise in the ash content, suggesting the existence of non-volatile and non-combustible components. The primary cause for this phenomenon is most likely associated with the general decrease in mass of the material during the pyrolysis process, resulting in the evaporation of volatile substances. Nevertheless, it is important to emphasize that inorganic substances that are not easily evaporated or burned also remain, leading to a perceived increase in the amount of ash present.

![FTIR for PS, PSAC, and L@PSAC as well as titration for PS and PSAC (meq/g).](image1)

![XRD for PSAC, and L@PSAC and pH$_{pzc}$ for PSAC.](image2)
(Hanif et al., 2023). The examination of PS and PSAC through ultimate analysis reveals that the PSAC manifests a heightened carbon content, witnessing a 39.73% increase, accompanied by a decrease in hydrogen by 4.29% and in oxygen by 40.57% compared to PS. The observed reduction in hydrogen and oxygen during the pyrolysis process is plausibly attributed to the removal of volatile components (Das et al., 2021). The findings derived from this investigation align with the structural attributes of PS in prior research. İstıtan and co-researchers delineated the properties of PS as follows: moisture (6.8%), volatile matter (83.8%), ash (2%), fixed carbon (14.2%), carbon (47.9%), oxygen (45.2%), hydrogen (6.4%), and nitrogen (0.5%) (İstıtan et al., 2016).

3.2. L@PSAC optimization

To optimize laccase immobilization, adjustments were made to pH, temperature, and laccase concentrations, as illustrated in Fig. 4. The assessment of its impact was conducted by evaluating ABTS activity. Elevating the enzyme concentrations from 0.50 to 4.0 mg/mL revealed in a corresponding increase in immobilization from 11.28 to 67.38 U/g, as depicted in Fig. 4a. However, exceeding an enzyme concentration of 4 mg/mL did not exert any influence on laccase immobilization likely attributed to the reduction in available active sites on PSAC surface. The pH of the matrix plays a pivotal role in influencing enzyme stability; hence, it constitutes a crucial parameter in the immobilization process. As shown in Fig. 4b, laccase immobilization is notably reduced at pH 3, potentially attributable to a decline in laccase activity (see Fig. 5B). The optimum immobilization rate for L@PSAC, amounting to 55.80 U/g, was observed at pH 5. Subsequently, at pH 6, laccase immobilization declined to 40.59 U/g and continued this decreasing trend with the elevating the solution pH. Concerning temperature, the immobilization of laccase increased from 35.29 to 63.89 U/g as the temperature rose from 10 °C to 30 °C, as illustrated in Fig. 4B. This enhancement is attributed to the heightened rate of enzyme adsorption onto PSAC. However, at 55 °C and 60 °C, the rate of laccase immobilization deteriorated to 27.09 U/g and 20.43 U/g, respectively, potentially owing to a reduction in enzyme viability with elevated temperatures (Wang et al., 2021). The laccase immobilization yield under optimal conditions reached 68.72%. The substantial immobilization yield achieved in this system under the specified optimal conditions may be attributed to the presence of carbonyl groups on PSAC (Lonappan et al., 2019). In a prior study, Imam et al. (2021) investigated laccase immobilization on acid-treated rice husk biochar for anthracene biodegradation (İmam et al., 2021). Their findings indicated a noteworthy immobilization yield of 66% and demonstrated high operational stability in the immobilized system. In our earlier research, the optimal conditions for laccase immobilization on date stone resulted in a 71.2% yield, (Al-sareji et al., 2023a) slightly surpassing the present work.

Free laccase and L@PSAC manifested typical enzymatic behavior in response to the substrate ABTS, prompting the application of the Michaelis–Menten equation for the assessment of kinetic parameters. The values of $K_m$ and $v_{max}$ were determined through the nonlinear fitting of the reaction rate against substrate concentration (Fig. 4 D). It is widely acknowledged that the $K_m$ value reflects the enzyme’s affinity for the substrate. Notably, the $K_m$ for free laccase reduced from 0.488 mM to 0.377 mM when immobilized on PCAS. This suggests that the process of immobilization diminishes the affinity of laccase for ABTS, possibly

![Fig. 4. L@PSAC optimization concerning laccase dosage (A), pH (B), temperature (C) and kinetics (D).](image-url)
attributable to mass transfer constraints between ABTS and the adsorbent surface. Subtle alterations in the three-dimensional conformation of the enzyme during adsorption onto the support exert indirect or direct effects on the active site of enzyme (Cristovão et al., 2012). Consistent with prior research, analogous outcomes have been observed, elucidating a reduced substrate affinity because of diffusional restrictions and diminished enzyme flexibility after immobilization (Al-sareji et al., 2023c). L@PSAC demonstrated a lower $v_m$ value of 4.679 mM/min in comparison to the free enzyme’s $v_m$ of 7.919 mM/min. This decline in the maximal reaction rate could be ascribed to the imposition of diffusional constraints on the substrate after enzyme binding.

### 3.3. Free-state and L@PSAC stabilities

![Fig. 5](#) Free laccase and L@PSAC activities in different temperatures (A) and pH levels (B). SD were less than 3.81% in both temperatures and pH experiments.

Fig. 5 illustrates the stability of L@PSAC and free laccase with respect to temperature (A) and pH (B). L@PSAC exhibited superior stability compared to free-state laccase across the selected temperatures and pH ranges. The enhanced robustness and adaptability are because of its decreased structural flexibility and increased strength, rendering it remarkably resistant to different settings (Imam et al., 2021). The L@PSAC and free-state laccase performed well at temperatures of 30 °C and 25 °C, respectively. As the temperature rises, their activity was noticed to decline. The free-state laccase was more temperature sensitive compared to L@PSAC. This induted that L@PSAC has successfully encountered the increase in temperature. Regarding pH, at pH 5, L@PSAC stability resulted in a significant 9.68% enhancement, resulting in greater activity compared to free-state laccase. On the other side, the free-state laccase exhibited the maximum activity at pH 4 with a 4.57% increase in activity compared to L@PSAC. When comparing the stability of L@PSAC with free-state laccase at various pH levels, L@PSAC demonstrates superior stability.

The decrease in laccase activity seen at higher pH values is most likely due to laccase denaturation. The broader active pH range observed in immobilized enzyme is presumed to stem from the functional groups present on the adsorbent’s surface, capable of scavenging H+ from the solution and sustaining laccase activities (Rouhani et al., 2020). Comparable results were highlighted in the literature but with some fluctuation in the ideal temperature. The laccase from Trametes versicolor, whether free or immobilized on a metal-organic framework, exhibited the maximum levels of activity at a temperature of 30 °C (Rouhani et al., 2020). The most favorable temperature for laccase, derived from Aspergillus and present in both free and immobilized forms on silica nanoparticles, was found to be 40 °C and 50 °C, respectively (Hu et al., 2015). The disparity in the ideal operational temperature of unbound and bound laccase could be attributed to the variety in laccase sources and the materials used for immobilization.

### 3.4. Operational stability L@PSAC and storage stability

When assessing the viability of a process, particularly in an industrial context, operational stability emerges as a pivotal factor. To gauge the operational stability of the L@PSAC system, a series of consecutive cycles were carried out as part of batch tests. ABTS was employed as the standard substrate for evaluating the recyclability of L@PSAC over ten cycles. Throughout this procedure, scrutiny was applied to the activity of the immobilized laccase, and the findings are presented in Fig. 6. No discernible decline in activity during the initial three cycles; however, reductions of 3.50%, 4.57%, and 5.38% in laccase activity were observed in subsequent cycles (4th to 6th). Notably, the 7th and 8th cycles witnessed substantial declines of 11.26% and 23.72% in laccase activity. The 9th and 10th cycles exhibited a pronounced reduction of 48.46% and 64.71%, respectively. The diminishing laccase activity may be attributed to either the enzyme’s detachment from the adsorbent during the washing process or enzyme denaturation. In a comparable investigation, Zang and co-authors (2014) observed a significant decrease of 70% in enzyme activity when immobilized on a nanofiber membrane and subjected to 10 ABTS oxidation cycles, highlighting parallels with the current study. The plausible operational stability of immobilized laccase is largely credited to robust ionic interactions between the biochar surface and the immobilized protein.

In general, the inherent instability of enzymes in their free state during storage, leading to a gradual loss of activity, has hindered their widespread application. The rapid decline in catalytic activity during storage and challenges in recovery post-reactions have constrained the utility of free-form enzymes. Consequently, research has focused on investigating versatile solid supports for enzyme immobilization to surmount these challenges. The enhanced storage stability exhibited by immobilized enzymes stands as a crucial criterion in evaluating enzyme utility of free-form enzymes. Consequently, research has focused on investigating versatile solid supports for enzyme immobilization to surmount these challenges. The enhanced storage stability exhibited by immobilized enzymes stands as a crucial criterion in evaluating enzyme utility of free-form enzymes. Consequently, research has focused on investigating versatile solid supports for enzyme immobilization to surmount these challenges. The enhanced storage stability exhibited by immobilized enzymes stands as a crucial criterion in evaluating enzyme utility of free-form enzymes. Consequently, research has focused on investigating versatile solid supports for enzyme immobilization to surmount these challenges. The enhanced storage stability exhibited by immobilized enzymes stands as a crucial criterion in evaluating enzyme utility of free-form enzymes. Consequently, research has focused on investigating versatile solid supports for enzyme immobilization to surmount these challenges. The enhanced storage stability exhibited by immobilized enzymes stands as a crucial criterion in evaluating enzyme utility of free-form enzymes. Consequently, research has focused on investigating versatile solid supports for enzyme immobilization to surmount these challenges.
In the initial month of storage, L@PSAC demonstrated activity reductions of 32.70%, 25.68%, and 39.58% from its initial activity at 4°C, 25°C, and 30°C, respectively. On the other hand, the free laccase displayed higher activity reductions of 29.57%, 39.57%, and 39.58% from its initial activity at 4°C, 25°C, and 30°C, respectively, during the same period. More than 50% of L@PSAC retained its activity under all three conditions, while the free laccase experienced a substantial decline with approximately a 90% loss. Previous studies have reported a comparable enhancement in stability, with the percentage of improvement varying between 30% and 70% across different investigations (Tavares et al., 2013; Pezzella et al., 2014). The extent of improvement is influenced by factors such as the carrier’s nature and the mechanisms involved in binding. The observed augmentation in storage stability is attributed to the reinforcement of enzyme stability on the support, imparting structural rigidity, and shielding the enzyme from unfolding and denaturation (Rhushan et al., 2015).

3.5. System preferences and recyclability

To assess the operational robustness of L@PCAS for potential industrial applications, an investigation into the removal of a mixture of emerging contaminants from both MQ water and wastewater was conducted, and the outcomes are demonstrated in Fig. 7. Notably, L@PCAS presents both biodegradations, facilitated by the presence of laccase, and adsorption sites, thereby proposing two distinct mechanisms for pollutant removal. In the first mechanism, the exclusive removal process is presumed to be adsorption onto available sites of PCAS, without any concurrent degradation. Conversely, the second mechanism envisions pollutants adhering to unoccupied sites of the biocatalyst, and, upon initiation of laccase-mediated biodegradation, the previously occupied sites are liberated, initiating a sorption-biodegradation cycle anew. To differentiate between the adsorption contribution to pharmaceuticals removal by PSAC from that of combined adsorption and degradation of L@PSAC in Fig. 7, the former is denoted as A and the latter is denoted as L. The data presented in Fig. 7 are for 10 cycles. The removal of DCF, CBZ, and CIP is more or less on the same level for both water matrices. The enzymatic degradation of the tested pharmaceuticals constitutes approximately 20% of the total reduction while this percentage is a little lower for MQ water (~11.5–13%). This is related to the decrease in adsorption efficiency in wastewater compared to MQ water which led to an increase in the enzymatic contribution to the degradation. The enhanced pollutant degradation efficiency of biocatalysts in wastewater could be associated with the facilitation of electron transfer in electro-chemical processes due to the presence of ions in the effluent (Naghdi et al., 2017). The decrement in catalytic performance observed over cycles in both water matrices may be ascribed to the occupation of adsorption sites by non-degradable compounds, particularly discernible in wastewater. The diminishing trend in removal efficiency (Fig. 7) could also be attributed to enzyme leaching and denaturation, consistent with observed behavior in ABTS oxidation (Spinelli et al., 2013). This degradation system holds promise for integration into the wastewater treatment plants to mitigate the release of emerging contaminants into the environment. However, a comprehensive economic analysis of the entire process and fine-tuning of operational parameters for a diverse range of pollutants are imperative before progressing to a scale-up phase. Table 1 shows the pollutants removal efficiencies by immobilized enzyme from selected studies. Comparing the current findings with those provided in Table 1, it can be concluded that the developed method successfully eliminated a combination of emerging pollutants from both MQ water and wastewater.

4. Pros, cons and future prospective

The carrier represents about 47% of the overall cost of an immobilized enzyme system (Bilal and Iqbal, 2019). Activated carbon derived from pistachio shells, which are carbon-rich precursor materials, could be used to reduce system expenses and provide matrices for immobilizing enzymes. This agro-industrial by-product is abundantly available and economically advantageous. Furthermore, using it as a solid carrier in immobilization matrices would enhance waste management and show to be economically feasible (Al-sareji et al., 2023b). In addition, L@PSAC achieved a high rate of removing contaminant mixtures when employed in both MQ water and wastewater. The study revealed that L@PSAC could be used in the water treatment, as opposed to using free-state laccase. One of the enzyme immobilization cons is the challenge of finding the ideal support to immobilize the enzymes for different uses. Given this obstacle, it is essential to carry out a more thorough analysis to eliminate these limitations. Identifying the best immobilization approach for agro-industrial by-product properties is challenging due to the wide range of available technologies and specific operational requirements. Despite the extensive studies on agro-industrial waste effectiveness in enzyme immobilization, there is still a lack of a molecular structural model feasible (Al-sareji et al., 2023b). Thus, developing a molecular structure model for biochar from agro-industrial waste is essential as it could offer valuable insights into the reactivity of biochar. This might also enhance the process of adding functionality to the material for a wide range of applications. Furthermore, the replacement of crude extract with purified enzymes increases the cost of biocatalysis, an issue that requires further investigation. Furthermore, for an immobilized system to be cost-effective, it must not only use agricultural waste as support materials but also sustain its activity across several cycles.
5. Conclusion

The pervasive presence of emerging contaminants such as pharmaceuticals poses a formidable threat to the ecological health and sustainability of ecosystems. The removal of some of the most prominent pharmaceuticals was explored in this study utilizing a cost-effective, reusable, and thermally stable waste material (PSAC) as a carrier for laccase enzyme. The activation and pyrolysis of PS along with laccase immobilization were optimized to devise a robust system that harnesses adsorption and enzymatic degradation mechanisms. The best activation conditions were found to be 700 °C for 3 h using NH₄NO₃/PS ratio of 3% wt. Laccase was successfully immobilized onto PSAC at 60 U/g, pH 5, and 30 °C. The resultant product L@PSAC was capable of achieving almost complete removal of DCF, CBZ, and CIP in MQ water and wastewater in the first cycle. About 70–80% of the removal was due to adsorption in wastewater and this percentage was higher (85–88%) in MQ water. The stability of laccase was proven to be enhanced upon immobilization even for long periods of 60 days. Nevertheless, further research is necessary to determine the approach’s reliability when applied to a more complicated sample matrix. In addition, testing this material in a fixed bed column system is important for future research to affirm its potency in this application. It is vital to also investigate the system design parameters for a continuous large-scale process. Factors such as the competition between pharmaceuticals and other pollutants for adsorption sites and their interaction with enzymes are particularly important.

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Table 1
Pollutants removal efficiencies by immobilized enzyme from selected studies.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Matrix</th>
<th>Method</th>
<th>Removal efficiency (%)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anthracene</td>
<td>Spiked water</td>
<td>Immobilized on rice straw biochar</td>
<td>100</td>
<td>Imam et al. (2021)</td>
</tr>
<tr>
<td>Acetaminophen</td>
<td>Wastewater</td>
<td>Cross-linked enzyme aggregates</td>
<td>97</td>
<td>(Kumar and Cabana, 2016)</td>
</tr>
<tr>
<td>Atenolol</td>
<td>Wastewater</td>
<td>Immobilized on Pineewood Biochar</td>
<td>68</td>
<td>(Naghdi et al., 2017)</td>
</tr>
<tr>
<td>Diazepam</td>
<td>Spiked water</td>
<td>Immobilized on Pineewood Biochar</td>
<td>83</td>
<td></td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>Wastewater</td>
<td>Immobilized on Pineewood Biochar</td>
<td>86</td>
<td></td>
</tr>
<tr>
<td>Sulfathiazole</td>
<td>Spiked water</td>
<td>Immobilized on CPC silica beads</td>
<td>71.7</td>
<td>(Rahmani et al., 2015)</td>
</tr>
<tr>
<td>Malachite green dye</td>
<td>Spiked water</td>
<td>Immobilized on pine needle biochar</td>
<td>85%</td>
<td>Pandey et al. (2022)</td>
</tr>
<tr>
<td>Bisphenol A</td>
<td>Spiked water</td>
<td>Immobilized on magnetic bagasse</td>
<td>100</td>
<td>Zhang et al. (2020b)</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>Mill-Q water</td>
<td>Immobilized on pistachio shell</td>
<td>&gt;99%</td>
<td>Current study</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>Wastewater</td>
<td>Immobilized on pistachio shell</td>
<td></td>
<td></td>
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<tr>
<td>Ciprofl oxacin</td>
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References

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.2024.118565.


Spinelli, D., Fararella, E., Di Michele, A., Pogni, R., 2013. Immobilization of Fungal (Trametes versicolor) Lacase onto Amberlite IR-120 H B.


