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Ketoprofen and aspirin removal by laccase immobilized on date stones

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HIGHLIGHTS

- Functionalized date stone surface area increased about thirty-six-fold.
- Immobilized laccase retained 54% of its initial activity after 6 cycles of recycling.
- Ketoprofen and Aspirin were nearly completely degraded.

GRAPHICAL ABSTRACT
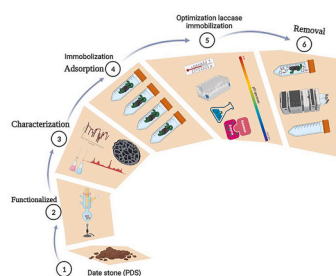
ARTICLE INFO

ABSTRACT

In recent years, enzymatic remediation/biocatalysis has gained prominence for the bioremediation of recalcitrant chemicals. Laccase is one of the commonly investigated enzymes for bioremediation applications. There is a growing interest in immobilizing this enzyme onto adsorbents for achieving high pollutant removal through simultaneous adsorption and biodegradation. Due to the influence of the biomolecule-support interface on laccase activity and stability, it is crucial to functionalize the solid carrier prior to immobilization. Date stone (PDS), as an eco-friendly, low-cost, and effective natural adsorbent, was utilized as a carrier for laccase (fungus *Trametes versicolor*). After activating PDS through chemical treatments, the surface area increased by thirty-six-fold, and carbonyl groups became more prominent. Batch experiments were carried out for ketoprofen and aspirin biodegradation in aqueous solutions. After six cycles, the laccase maintained 54% of its original activity confirmed by oxidation tests of 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS). In addition, the storage, pH, and thermal stability of immobilized laccase on functionalized date stone (LFPDS) were found to be superior to that of free laccase, demonstrating its potential for ongoing applications. In the aqueous batch mode, this immobilized laccase system was used to degrade 25 mg L^{-1} of ketoprofen and aspirin, resulting in almost complete removal within 4 h of treatment. This study reveals that agricultural wastes such as date stone can successfully be valorized through simple activation techniques, and the final product can be used as an adsorbent

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and substrate for immobilization enzyme. The high efficiency of the LFPDS in removing ketoprofen and aspirin highlights the potential of this technology for removing pharmaceuticals and merits its continued development.

1. Introduction

The rate at which pharmaceutical production grows has exceeded those of the most concerning global challenges such as carbon dioxide emission and nutrient pollution (OECD, 2019). Third of the prescribed pharmaceutical is reported to become waste in the United States of America (Product Stewardship Institute, 2018). The wasted pharmaceuticals are likely to end up in water systems. These compounds are designed to interact with living systems under certain circumstances, making them stable and hard to remove in water treatment systems. Common techniques for the treatment of pharmaceuticals include adsorption (Yin et al., 2020; Mahmoodi et al., 2011, 2014, 2019), membrane separation (Zhang et al., 2015, 2019, 2021; Meng et al., 2020), advanced oxidation (Du et al., 2020), biodegradation (Zhang et al., 2022), and enzymatic degradation (Zhou et al., 2022; Mahmoodi and Saffar-Dastgerdi, 2020). Among these, enzymatic degradation has received considerable attention endorsing its strong oxidative ability under mild reaction conditions with minimal energy consumption, making it much safer and energy-effective (Zhou et al., 2022; Moha-jershojaei et al., 2015).

According to several studies, laccase, as an oxidative enzyme, can easily biodegrade a wide range of organic pollutants, such as pharmaceuticals, into less harmful molecules (Alharbi et al., 2019; Bilal et al., 2019). Laccase employs a radical-based process to oxidize a range of non-aromatic and aromatic hydrogen donors by utilizing molecular oxygen (Majeau et al., 2010). While laccase has the potential for pollutant biodegradation, its commercial usage may be economically unfeasible owing to the difficulties in collecting and reusing the enzyme from the reaction medium. Laccase can be immobilized on a solid carrier in order to circumvent these constraints. Advantages such as simple enzyme separation from the reaction mixtures, increased enzymatic activity and stability, as well as reusability promote the continuing development of enzyme immobilization techniques (Wahab et al., 2020). The immobilization of enzymes has been accomplished using various substances as solid carriers (Wahab et al., 2020).

Researchers have mainly focused on exploring biodegradable and cost-effective materials as carriers for enzymes. An example of these materials would be agro-industrial waste. The sustainable conversion of agro-industrial wastes into valuable products has economic and environmental benefits (Girelli et al., 2020). One of the agro-industrial wastes widely found in the Middle East and North Africa regions (MENA) is the date stone. Based on the date production figures reported by the Food and Agriculture Organization (Faostat, 2020) and considering the weight percentage of the date stone (11–18%), the annual production of date stones is about 1.5 tons. This presents an attractive opportunity for utilizing this waste for industrial applications in the MENA region. This study proposes using this waste as an adsorbent and enzyme carrier for pharmaceuticals removal from water.

The activated date stone is categorized as having pores on different size scales; micro, meso and macro combination. Because of its high lignocellulosic composition, carbon content and density, and low ash content (Ahmed, 2016), the date stone is suitable for conversion into adsorbents. Thus, turning date stone waste into activated carbon is a highly promising and useful method for decomposition while also providing a financial benefit to the date fruit industries, especially in the MENA region. Chemical or thermal modifications are often applied to improve the absorbability of carbonous materials (Adewuyi, 2020). Chemical modification such as pretreatment with alkaline (e.g., Ca(OH)$_2$, NaOH, and Na$_2$CO$_3$), inorganic acids (e.g., HNO$_3$, H$_2$SO$_4$, HCl, and C$_6$H$_8$O$_7$), hydrolysis of carboxylate groups, delignification, ester-ification of phosphate and carboxyl groups are commonly used (Abegunde et al., 2020). Chemical techniques require less energy compared to thermal modification, and hence they are desirable.

Among pharmaceutical compounds, ketoprofen and aspirin are of particular interest due to their wide use as over-the-counter medicines. Ketoprofen (2-(3-benzoylphenyl) propionic acid) is a nonsteroidal anti-inflammatory drug used to treat muscular and joint pain, as well as conditions such as arthritis, gout and rheumatoid osteoarthritis (ALOthman et al., 2021). Aspirin (acetylsalicylic acid, ASA) is a common drug used to relieve fever and pain. Aspirin is an anti-inflammatory, analgesic, and antipyretic medication (Li et al., 2015). Aspirin prevents platelet aggregation (Jia et al., 2019), myocardial infarction (Stolarek et al., 2015), and cardiovascular disease (Stuntz and Bernstein, 2017). Every year, around thirty-five thousand tons of aspirin are used globally, and it is on the WHO's List of Essential Medicines (WHO, 2021). This could result in a significant amount of aspirin being disposed into the environment. Ketoprofen and aspirin characteristics are illustrated in Table S1. Unfortunately, conventional water treatment processes are ineffective in removing aspirin and ketoprofen from water (Moghiseh et al., 2019; Zhao et al., 2019; Kermia et al., 2016).

Enzyme immobilization has proven to strengthen the adsorbent reusability and the pollutant removal rate while decreasing the operating cost (Zhou et al., 2021). For instance, dye removal has been reported utilizing a biomimetic dynamic membrane (BDM) with adsorption materials and enzymes in the biomimetic layer. Chen and his colleagues developed BDM via physical adsorption and combined filtration of CNTs and laccase for the advanced treatment of dye wastewater (Chen et al., 2019). Due to the synergistic effect of adsorption, enzymatic degradation, and membrane separation, BDM demonstrated effective dye removal and antifouling ability. Shao and co-workers suggested that after laccase immobilization, the maximum removal efficiency of hollow mesoporous carbon spheres for tetracycline hydrochloride increased from 55% to 98% (Shao et al., 2019). It was also reported that the immobilization of enzymes on magnetic chitosan–clay composite beads (Aydemir and Güler, 2015) and magnetic bimodal mesoporous carbon (Liu et al., 2012) improved the removal efficiency of phenol by 62% and 58%, respectively. Naghdi and his co-workers examined the bioremediation of carbamazepine (CPZ) by laccase enzyme immobilized on acid-treated pine wood nanobiochar. After three cycles of reusability, the resulting immobilized biocatalytic system retained 70% of its initial activity, removing 83% of CPZ from contaminated water (Naghdi et al., 2017). In another investigation, Nguyen and his colleagues observed more than 90% removal effectiveness for bisphenol A, diclofenac, carbamazepine, and sulfamethoxazole from wastewaters utilizing laccase immobilized on granular activated carbon with biocatalytic conversion and adsorption. The proportion of adsorption in overall removal efficiency varied across compounds; nevertheless, adsorption was more prominent (over 50%) for carbamazepine and diclofenac, which are resistant to enzymatic treatment (Nguyen et al., 2016).

Although enzyme immobilization helps overcome the limitations of applying free enzymatic techniques for water treatment, such as high solubility and low stability, the immobilization techniques still have limitations. The common laccase immobilization techniques reported in the literature include enmeshment, covalent binding, self-immobilization and adsorption. Among these techniques, the latter captured the attention of many research investigations, including this work. Despite its limitation represented by enzyme leakage and low immobilization efficiency compared to covalent binding, it still offers good enzyme protection with low fabrication cost and ease of operation compared to the other methods (Zhou et al., 2021). Hence, this study focuses on the adsorption technique for immobilization using recycled

agricultural waste.

Several studies have examined the ability of PDS to remove different pollutants such as dyes, heavy metals, pesticides, and phenols (Ahmed, 2016). However, to the author's best knowledge, there are no studies that assessed PDS as a carrier for enzyme immobilization for the ultimate goal of pharmaceutical removal. Thus, this work has been dedicated to evaluating the capacity of a chemically functionalized PDS (FPDS) for dual functionality as a laccase carrier and as an adsorbent for the simultaneous removal and degradation of ketoprofen and aspirin. The study investigates the effect of operating parameters such as pH, shaking speed and temperature on the absorbability of laccase onto date stone. The study also evaluates the stability of adsorbed laccase on FPDS and its activity.

2. Materials and methods

2.1. Materials

Analytical-grade chemicals and reagents were used in this work without further purification. Sulfuric acid (H_2SO_4), sodium bicarbonate $NaHCO_3$, 2,2-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt (ABTS) (98%) and laccase from fungus *Trametes versicolor* ($\geq$0.5 U mg^{-1}) were purchased from Sigma-Aldrich. Ultrapure water was used for washing and solution preparation throughout the experiments. Whatman® glass microfibre filters, Grade GF/F (47 mm circles) were employed to separate PDS activated carbon from the aqueous phase medium.

2.2. Biosorbent preparation

The date stones were obtained from pitting the *Phoenix dactylifera* L. fruit (PDS) and cleaned manually to remove any loosely attached fiber from the surface. Thereafter, PDS was washed with a copious amount of distilled water to remove dust and impurities from its surface. After that, the cleaned PDS were dried in an oven at 105 °C for one day to remove the excess amount of moisture. The dried biomass was crushed using a grinder and sieved to obtain a particle size range of 500 μm–750 μm. About 5 g of PDS was added to 500 mL of 5 M H_2SO_4 while being subjected to heat under reflux for 6 h at 80 °C. Then, after cooling, PDS was washed several times to remove the excess concentration of sulfuric acid. The resulting PDS was mixed with sodium bicarbonate $NaHCO_3$ (1%) and placed in an incubator shaker (Compact SteadyShake 757) for 6 h at 150 rpm at 25 °C to neutralize the PDS. The functionalized PDS (henceforth termed FPDS) was heated to 105 °C for a day. Afterwards, FPDS were sieved through 150 μm–200 μm to obtain a uniform-size particle with an increased surface area. FPDS stored in air separate tight bottles for subsequent uses.

2.3. Laccase immobilization

The laccase immobilization process was performed according to the procedure reported by (Pandey et al., 2022; Imam et al., 2021) with some modifications. Four Spinwin™ tube conical bottom with 15 mL total volume were used in the laccase immobilization. Briefly, each tube contained a cocktail of 250 mg FPDS with 10 mL buffer solution (pH 4) that had 40 units of laccase (4 U/0.025 g). The mixture was incubated in VorTemp 1550 shaking incubator (provided by Labnet) for 1 day at 35 °C and constant shaking of 150 rpm. Afterwards, samples were centrifuged for 5 min at 10,000 rpm using Eppendorf™ Centrifuge 5810. The laccase immobilization was determined by subtracting the initial activity from the immobilized one. The cocktail was washed twice with 5 mL of buffer solution (pH 4) for each rinse to remove unbonded laccase with measuring the enzyme activity. The final immobilized sample (LFPDS) was stored to determine the optimum parameters such as pH, stability, temperature, laccase concentrations, as well as pollutant removal.

2.4. Laccase activity assay

Laccase activity was measured by monitoring the ABTS oxidation rate to ABTS$^+$ radical and followed the previously published literature (Suman et al., 2018). This was carried out at room temperature by monitoring the increase in the absorbance at 420 nm (ε = 36,000 M^{-1}cm^{-1}). A UV–visible spectrophotometer-double beam (Shimadzu UV-1601) was utilized in the experiments. Under the standard assay conditions, one unit of laccase activity corresponded to the amount of required laccase to oxidize one μmol of ABTS per min. A mixture of 375 μL (0.5 mM ABTS) and 125 μL (1 mg mL^{-1}) of laccase in a buffer solution of pH 4 was incubated for 2 min at room temperature. It can be noticed that after the mixing process, the cocktail color changed to green color because of the enzymatic oxidation of ABTS to ABTS$^+$. The activity of LFPDS was investigated by placing 50 mg in 2 mL buffer (pH 4) containing 0.5 mM ABTS and shaking it at 150 rpm at room temperature for 3 min. After that, the samples were centrifuged at 10,000 rpm, and color development was determined spectrophotometrically at 420 nm.

Free and immobilized laccase activities were determined by Equations (1) and (2) (Zhang et al., 2020; da Silva et al., 2022; Pandey et al., 2022):

$$\text{Free laccase activity } (\text{U.mL}^{-1}) = \frac{\Delta abs \times D_f \times R_v}{\varepsilon \times t \times E_v} \quad (1)$$

$$\text{Immobilized laccase activity } (\text{U.g}^{-1}) = \frac{\Delta abs \times D_f \times R_v}{\varepsilon \times t \times M_{\text{carrier}}} \quad (2)$$

Where Δabs is the absorbance of ABTS$^+$, D_f is the dilution factor, and R_v is the reaction volume (mL). ε is the molecular extinction coefficient of ABTS$^+$, t is reaction time (min). E_v is the volume of laccase (mL), and M_{carrier} is the mass of the carrier on which the laccase was immobilized (g).

2.5. Optimization of laccase immobilization and its reusability

The immobilization of laccase onto FPDS was optimized with regard to pH, temperature, and shaking speed. A pH range of 2–7 was assessed, along with temperature and shaking speed ranges of 15–55 °C and 100–250 rpm, respectively. To calculate the immobilization yield, the initial and residual activity after immobilization were measured. The remaining activity was subtracted from the initial activity to obtain the amount of immobilized laccase. The immobilization yield is the value obtained as a percentage of the quotient of the immobilized activity and the initial activity. The free and immobilized laccase were tested for a pH range of 2–7 for an hour at room temperature. The stabilities of free and immobilized laccase were also investigated within different temperatures (15–55 °C) for an hour. Free and immobilized laccase storage was evaluated for one month by testing their activity each day. The laccase immobilized date stone reusability was examined up to six sequential cycles with the assistance of ABTS. Additionally, an ABTS range of 0.1–0.6 mM at the optimized conditions was examined for the activity of both immobilized and free laccase.

The reusability of the LFPDS experiment was conducted to determine its stability for consecutive cycles. At room temperature, about 50 mg of LFPDS was added to 10 mL of 0.5 mM ABTS. In each cycle, the amount of formed product per mM of ABTS was determined. Then, the cocktail was centrifuged at 10,000 rpm for 3 min, and enzyme activity was determined in the obtained supernatant. The oxidation of ABTS to ABTS$^+$ with the immobilized laccase with respect to the time was monitored in the laccase activity assay. Once the measurement of each cycle had finished, the LFPDS was washed twice with MilliQ water to remove ABTS and then re-equilibrated with a mixture containing new ABTS at pH 4. The process's operational stability was assessed over six cycles.

2.6. Physical and chemical characterization of PDS

2.6.1. Surface area and porosity

The Brunner – Emmett – Teller (BET) specific surface area and pore size distribution of produced materials were determined using the adsorption-desorption isotherm of nitrogen at a liquid temperature of 77 K by using Micromeritics (3Flex). The relative pressure of high-performance adsorption analyzers was $P/P_0 = 0.95$. Both surface area and micropore volume were determined by using the t-plot method.

2.6.2. Morphology and elemental composition

The morphology of PDS, FPDS, LFPDS and LFPDS (after removal) samples was examined using a scanning electron microscope (SEM) and energy dispersive x-ray (EDS) analyzer. For this analysis, the FEI/ThermoFisher Apreo S LoVac SEM and the EDAX AMETEK Octane Elect Plus were utilized at an accelerating voltage of 10 kV. The examined sample powder was fixed onto a conductive carbon tape and coated with an ultrathin gold film for 10 min.

2.6.3. Fourier transform infrared spectrometer (FTIR)

The FTIR spectra can provide vital details about a material's chemical structure and functional groups. Infrared spectra were obtained using FTIR (Nicolet™ iS™ 5 FTIR Spectrometer, Thermo Fisher, USA) equipped with an iD7 ATR accessory. With 200 scans and a resolution of 4 cm^{-1}, the IR spectra were measured in the wavenumber range of 400–4000 cm^{-1}. The ATR head was adjusted with one click to deliver the optimal pressure on the sample through the tip. Before each analysis, a blank background spectrum was obtained to provide a relative scale for absorption strength and blank subtraction.

2.6.4. Boehm titration

The amount of surface functional groups is one of the essential features of a produced date stone activated carbon that was determined by the Boehm titration method (Boehm, 1994; Bohli et al., 2015). In a series of Erlenmeyer flasks, about 500 mg of activated date stones were added to 50 mL of 0.05 N hydrochloric acid HCl, sodium hydroxide NaOH, sodium bicarbonate NaHCO$_3$, sodium carbonate Na$_2$CO$_3$, and Sodium ethoxide C$_2$H$_5$ONa solutions, respectively. The suspensions were filtered through a 0.45 μm membrane filter to remove the tested materials after 24 h of continuous agitation (150 rpm and 25 °C). Then, about 10 mL of aliquots base or acid filtrate was titrated with 0.05 N HCl or NaOH solutions, depending on the original solution that was utilized.

Blank samples (without PDS) were also run with each reaction at the same experimental conditions. During titration, the pH of the solution was monitored using a pH meter until the endpoint was reached at a neutral pH. The number of acidic groups on the activated date stones was calculated as follows: C$_2$H$_5$ONa neutralized carbonyl, carboxylic, lactones, and phenolic groups, whereas NaOH neutralized total acidic functional groups (carbonyls, phenols, carboxyl, lactones, and any other acidic components); Na$_2$CO$_3$ neutralized carboxylic and lactones and NaHCO$_3$ neutralized only strong acidic functional groups (carboxyl groups). The amount of HCl interacting with activated date stones determines the number of basic surface sites. The reaction between the acidic oxygenated functional groups and the reagents on the surface is based on the difference in acid/base strength.

2.6.5. Proximate analysis and ultimate analysis of adsorbents

The approximate analysis of PDS biomass was accomplished according to ASTM specifications. The production of ash was calculated at 550 °C for 4 h in a furnace. Carbon, hydrogen, nitrogen, and oxygen elemental compositions of PDS were determined using an elemental analyzer Model EA 1108 (Carlo Erba Instruments) and ASTM D3176 standard techniques (Hai et al., 2021).

2.7. Testing and measurements for pharmaceuticals removal

Ketoprofen (C$_{16}$H$_{14}$O$_3$, molar mass: 254.281 g mol^{-1}, purity ≥98%, Sigma -Aldrich) and aspirin (C$_9$H$_8$O$_4$, molar mass: 180.158 g mol^{-1}, Acetylsalicylic acid, purity >99%, Sigma -Aldrich) were selected as model pharmaceuticals in this study. Pharmaceutical degradations were determined by high-performance liquid chromatography (LC-20AD Prominence, Shimadzu) coupled with a diode array detector. The full scan spectrum was between 190 and 800 nm. Ketoprofen and aspirin were detected at 254 and 229 nm (Andraws and Trefi, 2020; Massimi et al., 2015), respectively. The LC-20AD mobile phase flow was carried out at a ratio of 20:80 (acetonitrile/water) and 0.5% CH$_2$O$_2$. The flow rate was kept at 1 mL min^{-1}. A column (C18 Inertsil ODS-3, Its pH range 2–7.5, the temperature was 25 °C) with a dimension of Length 250 mm × 4.6 mm Inner Diameter, 5 μm particle size was utilized for the measurements. The injected sample volume was 10 μL. Triplicate measurements were made, and the findings represent the average of the repeated measurements. The detections limits (0.015 mg L^{-1} for ketoprofen and 0.017 mg L^{-1} for aspirin) were determined by (3 × standard noise deviation) over the angular coefficient of the straight, while the quantifications limits (0.05 mg L^{-1} for ketoprofen and 0.055 mg L^{-1} for aspirin) were determined (10 × standard noise deviation) over the angular coefficient of the straight. The retention times and the standard deviations (SD) (min) were 22.1 min, and 0.026 min for ketoprofen and for aspirin were 14.6 min and 0.014 min.

Ketoprofen and aspirin degradation was carried out by adding 0.25 g of LFPDS in 75 mL of a solution containing 25 mg L^{-1} concentration of these compounds in a 150 mL Erlenmeyer flask at pH 4 and 150 rpm. After 6 h of stirring, 1 mL of the supernatant liquid was withdrawn from the flask and centrifuged at 10,000 rpm for 10 min. Separate samples of the supernatant were collected and examined using a UV–Vis spectrophotometer. As the laccase catalytic process is selective, the adsorption process of pharmaceutical compounds could occur (Zdarta et al., 2019). Therefore, other sets of experiments for each compound have also been conducted to evaluate the degradation via manipulation in the activated PDS status (i.e. one has activated date stone without immobilization process (FPDS), the other one with the presence of the ABTS (LFPDS-ABTS) and finally with just laccase). The degradations of the pharmaceutical compounds in these experiments were monitored for 6 h at 35 °C. Each hour, 1 mL of the supernatant was collected and centrifuged at 10,000 rpm for 10 min and then measured by HPLC for quantification.

2.8. LFPDS and FPDS reusability for pharmaceuticals removal

The reusability of both LFPDS and FPDS for removing ketoprofen and aspirin was investigated. Degradation and adsorption experiments for ketoprofen and aspirin were carried out in batches mode (see section 2.7). After each 4 h cycle, the samples were rinsed with buffer solution, centrifuged, and the recovered date stone was dried with compressed air and reused. Nevertheless, washing did not result in a complete recovery of date stones. In order to maintain the same solids/liquids ratio as the first batch, the quantity of date stone was supplemented after each cycle.

2.9. Kinetic of free and immobilized laccase

The Michaelis–Menten constant (K_m) and the maximum rate of reaction (v_{max}) were determined based on the oxidation reaction of ABTS at varying substrate concentrations under optimal conditions. The apparent kinetic parameters (K_m and v_{max}) of free and immobilized laccase were calculated. The following equation was applied (da Silva et al., 2012):

$$v = \frac{v_m \, [S]}{K_m + [S]} \tag{3}$$

Where the reaction velocity is indicated as v (mM min^{-1}), the maximum velocity of the reaction is represented as v_m (mM min^{-1}), the concentration of substrate is defined as [S] (mM), and the Michaelis–Menten constant is denoted by K_m (mM).

2.10. Pollutant adsorption isotherm, kinetics and thermodynamics

2.10.1. Kinetic modelling

The solute adsorption rate onto the adsorbent is referred to as an adsorption dynamic study. The residence period of adsorption at the solid-solution interface is controlled by this rate. Pseudo-first-order and pseudo-second-order are the most widely employed kinetic models. The pseudo-first-order model suggests a direct correlation between adsorption capacity and time depending on the saturation concentration and adsorbent uptake. Equation (4) describes the linear form of the pseudo-first-order (Lagergren, 1898). The pseudo-second-order is based on the assumption of chemisorption of the adsorbate onto the adsorbent (Srivastava et al., 2008; Soroush et al., 2022; Hayati and Mahmoodi, 2012) and it is described in the linear form in Equation (5).

$$\ln(q_e - q_t) = \ln q_e - K_1 t \tag{4}$$

Where q_e is the amount of pharmaceutical compound adsorbed at equilibrium (mg g^{-1}), q_t is the amount of pharmaceutical compound adsorbed at time t in minute (mg g^{-1}), K_1 is the equilibrium rate constant of the pseudo-first-order equation (min^{-1}). The rate constants are obtained from the straight-line plots of ln (q_e– q_t) versus t.

$$\frac{t}{q_t} = \frac{1}{K_2 q_e^2} + \frac{t}{q_e} \tag{5}$$

Where K_2 (g mg^{-1} min^{-1}) is the equilibrium rate constant of the pseudo-second-order equation. The straight-line plot of t/qt against t allows for obtaining the rate constants.

Equations (6) and (7) were applied to calculate the adsorption capacity and the removal efficiency at equilibrium as follows:

$$Q_e = \frac{(C_0 - C_e)V}{W} \tag{6}$$

$$Removal\ efficiency\ (\%) = \frac{(C_0 - C_e)}{C_0} \times 100 \tag{7}$$

Where C_o and C_e (mg L^{-1}) are the pharmaceutical compound concentrations at the initial and equilibrium conditions, respectively. The volume of the solution is V (L), and the mass of the activated date stone is W (g).

2.10.2. Langmuir isotherm

The isotherms that describe ketoprofen and aspirin adsorption onto date stone was examined by applying two commonly used models, namely Langmuir and Freundlich. Langmuir isotherm model assumes that the adsorption resulted in a homogenous surface forming a monolayer of adsorbate without interaction between adsorbed molecules (Langmuir, 1916). The model is presented in equation (8), and its linearized form is presented in equation (9).

$$q_e = \frac{q_m \times K_L \times C_e}{1 + K_L \times C_e} \tag{8}$$

$$\frac{1}{q_e} = \frac{1}{q_m} + \frac{1}{K_L \times q_m} \times \frac{1}{C_e} \tag{9}$$

Where q_m is maximum adsorption with complete monolayer coverage on the adsorbent surface (mg g^{-1}), and K_L is the Langmuir constant related to the energy of adsorption (L mg^{-1}). Both q_m and K_L can be calculated from the linear plot of $1/C_e$ versus $1/q_e$. The separation factor (R_L) was determined by using Equation (10).

$$R_L = \frac{1}{1 + K_L \times C_0} \tag{10}$$

Where R_L is the dimensionless Langmuir constant which indicates that the adsorption possibility is either favorable ($0 < R_L > 1$), unfavorable ($R_L > 1$), linear ($R_L = 1$) or irreversible ($R_L = 0$).

2.10.3. Freundlich isotherm

The Freundlich model is an empirical formula that assumes adsorption takes places on heterogeneous surface with varying levels of energies (Freundlich, 1906). The mathematical expressions of the model and its linearized form are presented in Equations (11) and (12).

$$q_e = K_f \times C_e^{1/n} \tag{11}$$

$$\text{Log } q_e = \text{Log } K_f + \frac{1}{n}\text{Log } C_e \tag{12}$$

Where q_e is the amount of absorbed target compounds per unit adsorbent at the equilibrium (mg g^{-1}). Ce is the concentration of adsorbate in the solution at the equilibrium (mg L^{-1}). Both n and K_f are the Freundlich adsorption isotherm constants, and they are determined from the intercept and slope of the linear plot between log C_e and log q_e.

2.10.4. Adsorption thermodynamics

The thermodynamic behaviour of ketoprofen and aspirin adsorption onto date stone was studied by determining standard Gibbs free energy ($\Delta G°$), standard enthalpy ($\Delta H°$), and standard entropy ($\Delta S°$) as presented in the Equations below (Lonappan et al., 2018a):

$$\Delta G° = -R \times T \times \ln(K_L) \tag{13}$$

$$K_L = \frac{q_e}{C_e} \tag{14}$$

$$\ln(K_L) = \frac{-\Delta H°}{RT} + \frac{\Delta S°}{R} \tag{15}$$

$$\Delta G° = \Delta H° - T \times \Delta S° \tag{16}$$

Where R is the universal gas constant (8.314 J mol^{-1} K^{-1}), and T is the temperature (K). $\Delta H°$ and $\Delta S°$ can be calculated using the slope and intercept of the In(K_L) against 1/T plots (Fig. 9). $\Delta G°$ can be computed using Equation (13).

2.11. Data quality check

All experiments were performed in triplicate, and the results were represented in mean values with and standard deviation. Origin software version 2019 was employed to visualize Figures and data checked by the Kolmogorov-Smirnov test with a significant level of 0.05.

3. Results and discussions

3.1. PDS and FPDS characterization

The BET surface area of the raw PDS sample was measured to be 25 m^2 g^{-1}. The FPDS exhibited a vast increase in the surface area, reaching 903 m^2 g^{-1}. A larger surface area indicates that the FPDS has a more porous structure (Irbe et al., 2006). The total pore volume, micropore volume and mesopore volume of FPDS were 0.932 cm^3 g^{-1}, 0.386 cm^3 g^{-1}, and 0.191 cm^3 g^{-1}, respectively.

The SEM micrographs illustrated in Fig. 1 show the morphology of PDS (A), FPDS (B), LFPDS (C), and LFPDS after removal (D). As can be seen, there was a clear difference between FPDS and LFPDS. Upon activation of PDS, the porosity further developed. The surface of functionalized date stone exhibits a homogenous and porous structure. The
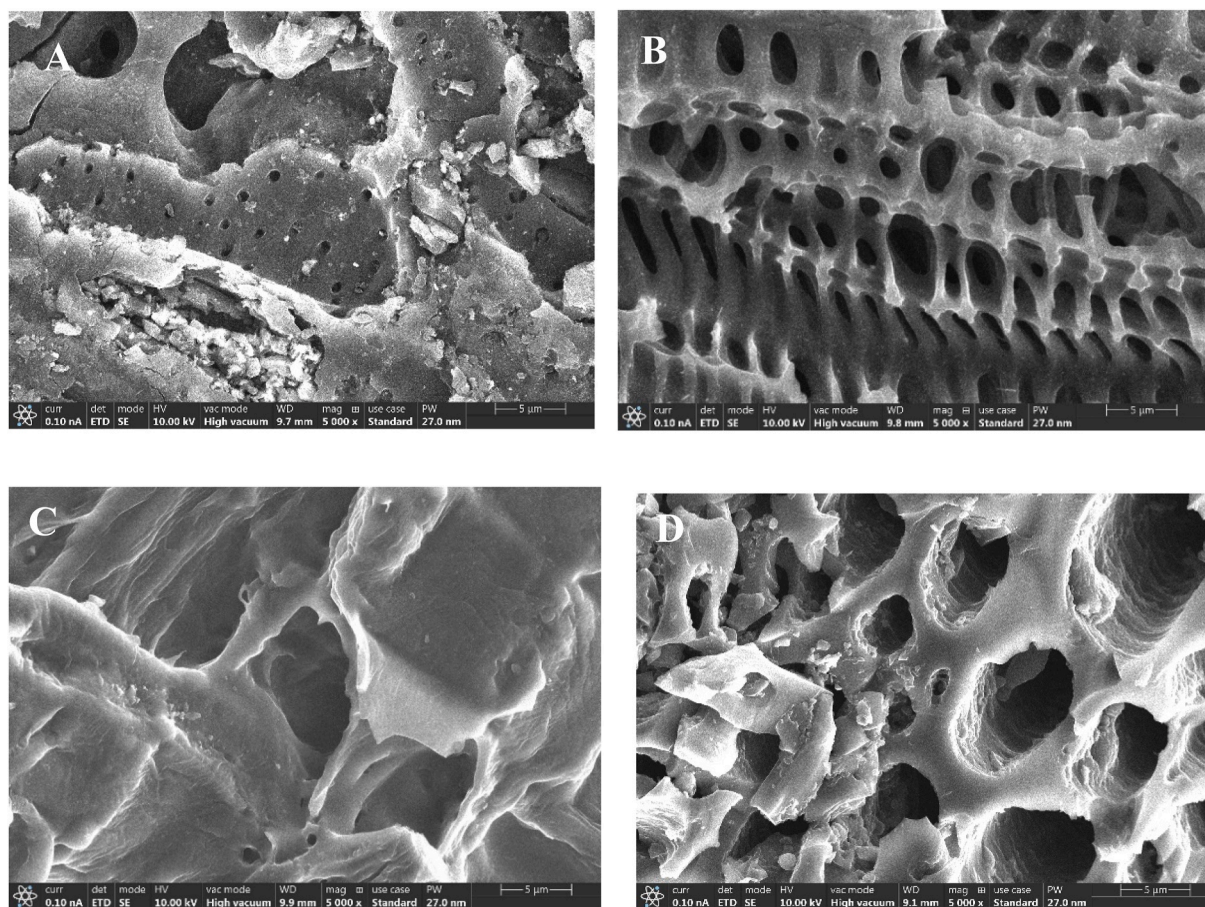
Fig. 1. SEM for PDS (A), FPDS (B), LFPDS (C), and LFPDS (after removal) (D).

presence of macroporous, mesoporous, and microporous FPDS increase surface accessibility for adsorption with some heterogeneity. In terms of elemental analysis, the EDX spectrum (Figure S1) revealed that the principal elements found on PDS were C and O. After activation, minor amounts of Ca, K, Mg, H, Na, S, Si, and N appeared on the surface. Following the adsorption of laccase, the C and N percentages increased. The increase of N content indicates the presence of laccase on the FPDS surface (Uygun, 2013; Pandey et al., 2022). After the removal, a number of minerals that were likely obscured by pharmaceuticals adsorbed during degradation processes and dissolved in the water disappeared.

Fig. 2 shows the FTIR for PDS, FPDS and LFPDS. The three infrared spectra demonstrate the existence of a wide band at 3436 cm^{-1}, which corresponds to the O–H bond vibration of elongation for PDS (Belala et al., 2011). The vibration of asymmetric and symmetric stretching, C–H bands of cellulose, is represented by the bands at 2922 cm^{-1} (Danish et al., 2014). The peak at around 1711 cm^{-1} is indicative of the stretching vibration of C=O of the carboxylic acids of xylan found in hemicelluloses (Sun et al., 2005; Pavan et al., 2008). This peak is also indicative of the amide bond in the laccase protein, which confirms its immobilization on the surface of date stones (Pandey et al., 2022; Zhang et al., 2020). The vibration at 1529 cm^{-1} is ascribed to the aromatic deformation (C=C) of lignin (Bouchelta et al., 2008). The band found at 1260 cm^{-1} corresponds to the vibration of lignin's C–O methoxy groups. The band at 870 cm^{-1} is a result of the C–H deformation in cellulose (Al-Ghouti et al., 2013).

The findings of surface functional group analysis by the Boehm titration method are shown in Table S2. This table demonstrates that acidic functional groups predominate the PDS surface. Table S2 also shows that FPDS acidic character (2.246 meq g^{-1}) is greater than the basic one (0.429 meq g^{-1}). This increase is probably due to the use of
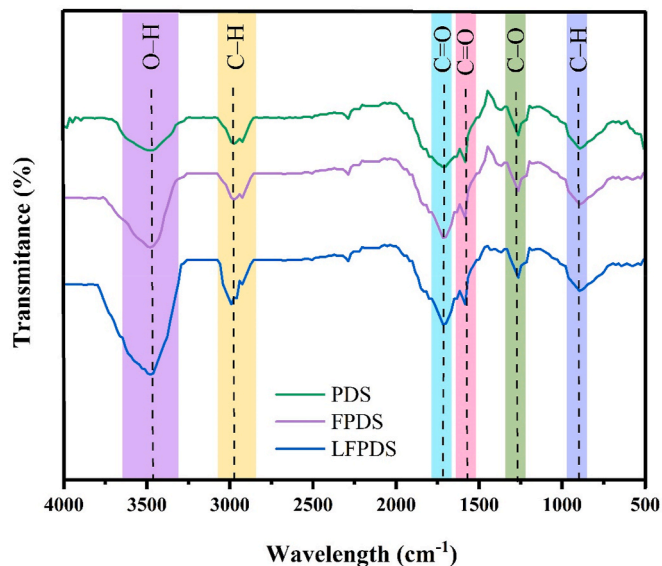

Fig. 2. FTIR spectrum of PDS, FPDS and LFPDS.

strong acid as an activating agent with a weak base afterwards which also appeared to have an increase in the activated date stones. The increase was also confirmed by the FTIR spectrum. Similar findings were reported by (Claoston et al., 2014; Han et al., 2013), who speculated that the carboxylic groups were entrapped in the large structure and hence were very surface inactive whereas the lactone and phenolic groups

were substantially surface exposed.

The fixed carbon content of PDS was 23.6% which is lower than the carbon content in the ultimate analysis 30.42% (Table 1). This can be explained as some of the carbon content might have been lost in the form of hydrocarbons as volatile compounds (Sellaperumal, 2012). Furthermore, both O and H contents in the PDS biomass were high (44.71% and 6.51%, respectively), and nitrogen content was low (0.98%). Other minerals such as sulfur, phosphorus, Ca, Si, Mg, Fe, Na, K and Al were presenting around 17%. These findings are in line with Mahdi et al. (2017) findings, who reported that O and H contents of date stone biomass were about 46% and 7.5%, respectively.

3.2. Laccase immobilization

The immobilization of laccase on activated stone was produced by employing the adsorption technique. Immobilization conditions were optimized using a wide range of pH, temperature, shaking speed and laccase dose in order to maximize laccase immobilization. The pH of the matrix has an impact on the enzyme's stability; thus, it is an important factor to consider throughout the immobilization procedure. Fig. 3 depicts the effect of varying pH (A), temperatures (B), shacking speed (C), laccase concentrations on date stone (D), and immobilization yield (E). The influence was always evaluated by measuring ABTS activity. As shown in Fig. 3 (A), laccase immobilization was very low at pH 2, possibly because of a loss of laccase activity, but increased gradually when the pH was raised to 3, and the maximal enzyme immobilization was attained at pH 4 with 45 U g^{-1}. Afterwards, the laccase immobilization decreased with a pH increase. Another close range of pH (from 3.8 to 4.2) for laccase immobilization was also investigated to identify more precisely the optimal point for immobilization, which also confirmed that pH 4 is important for optimum laccase function.

Temperature is another crucial aspect of laccase (fungus *Trametes versicolor*) immobilization since it is heat sensitive and only works at its ideal temperature range. Laccase immobilization improved from 33 U g^{-1} to 52 U g^{-1} when the temperature was raised from 15 °C to 35 °C, as illustrated in Fig. 3 (B). This is attributable to an increase in the rate of laccase adsorption on the surface of FPDS. At 55 °C, the immobilization rate of laccase decreased to 15 U g^{-1}, perhaps as a result of a reduction in laccase viability as the temperature rose (Wang et al., 2021).

Increasing the laccase units from 50 to 250 U g^{-1} of date stone contributed to raising the laccase immobilization from 17 to 72 U g^{-1}. Nevertheless, increasing enzyme units over 250 U g^{-1} showed no effect on laccase immobilization, which is due to the exhaustion of accessible locations on the date stone surface. With regard to the shaking speed, 150 rpm seems to be the optimal shaking speed for immobilization.

Overall, the optimal immobilization conditions to obtain functional laccase on the surface of FPDS were determined to be pH 4, 35 °C, 150 rpm, and 250 laccase units per gram of date stone. About 71.2% of laccase immobilization yield under these circumstances was achieved. The optimum conditions and acid-functionalized PDS with numerous carbonyl groups contributed to the high immobilization yield (Lonappan et al., 2018b). A slightly lower immobilization yield was reported by other studies. For example, a 64.23% immobilization yield for

Table 1
Physicochemical properties of date stone biomass.

Proximate analysis	(wt%) ±SD
Moisture content	7.54 ± 0.21
Volatile matter	66.75 ± 2.94
Ash	2.11 ± 0.06
Fixed carbon	23.6 ± 2.65
Ultimate analysis	**(wt%) ± SD**
Carbon	30.42 ± 1.2
Hydrogen	6.51 ± 1.1
Nitrogen	0.98 ± 0.02
Oxygen	44.71 ± 2.3

lignocellulosic biochar (Li et al., 2018). Imam and his colleagues achieved a 66% immobilization yield for immobilized laccase on functionalized rice husk biochar (Imam et al., 2021). This indicates that a simple modification of PDS made it a promising substrate for hosting laccase.

3.3. Stability and reusability of LFPDS

Fig. 4 shows the stability of free and immobilized laccase with respect to pH (A), temperature (B), ABTS (C), and storage time (D). It was observed that LFPDS is more stable than free laccase possibly due to its decreased conformational flexibility and improved rigidity which enhances its stability under varied pH circumstances (Chong-Cerda et al., 2020). At neutral pH, the LFDPS stability was still showing an increase activity of 11.5% over the free laccase. Additionally, in all pH ranges, the stability of LFDPS was higher than the free laccase, with an optimum activity recorded for both cases at pH 4. In temperature experiments, both free and immobilized laccase exhibited an optimum temperature of 35 °C, after which the activity decreased with raising the temperature. The change in activity with temperature was sharper with free laccase compared to immobilized laccase. The activity of laccase as a function of increasing ABTS concentration reaching an equilibrium at 0.5 mM is probably related to the K_m of the protein towards ABTS (Latif et al., 2022).

The stability of both free and immobilized laccase was evaluated by storing them at two different temperatures of 25 °C and 35 °C and measuring their daily activity for up to one month, as shown in Fig. 4 (D). The high storage stability of the immobilized enzyme is a key criterion for evaluating the effectiveness of an enzyme, which makes the solid biocatalyst preferable to the free enzyme.

The major causes of activity loss during storage are oxidation and reduction losses, both of which are decreased following immobilization. Because of the constrained conformation changes, the immobilized laccase displayed higher storage stability than the free laccase (Rouhani et al., 2020). After one month, the relative activity of free laccase decreased to 60.94% at 25 °C and 52% at 35 °C, but the relative activity of immobilized laccase exhibited a light decrease to 82.4% and 75% at the same temperatures.

The stability of immobilized laccase is a crucial assessment criterion. Through the batch experiment study, the operational stability of the LFPDS system was assessed over several sequential cycles. Throughout this operation, the LFDPS activity was measured, and the findings are shown in Fig. 5.

During the first cycle, no activity loss was noticed; however, losses of 3%, 6%, and 17% were confirmed during the second, third, and fourth cycles, respectively. The laccase activity dropped significantly in the last two cycles 43% and 54%, respectively.

The reduction in laccase activity could be explained in three ways: 1) laccase leaching from the adsorbent, 2) denaturation of the laccase and 3) a reduction of the adsorption ability of the pharmaceuticals into the surface of LFPDS. In light of this, the activity drop observed in this study was consistent with previous researchers' outcomes (Wang et al., 2021; Imam et al., 2021; Zhang et al., 2014). Wang and his colleagues used adsorption to immobilize laccase onto biochar treated with cetyl-trimethylammonium bromide and discovered that after six cycles, the immobilized laccase had lost roughly 55% of its activity (Wang et al., 2021). In a different study, six cycles of immobilization of laccase on rice straw charcoal maintained around 40% of its initial activity (Imam et al., 2021). Zhang et al. (2014) observed a 70% decrease in laccase activity immobilized on a nanofiber membrane after ten ABTS oxidation cycles for decolorization (Zhang et al., 2014).

Overall, the immobilized laccase demonstrated good stability and reusability. Strong interactions between the date stone surface and laccase, which promote its successful immobilization, are primarily responsible for the immobilized enzyme's excellent operational stability.
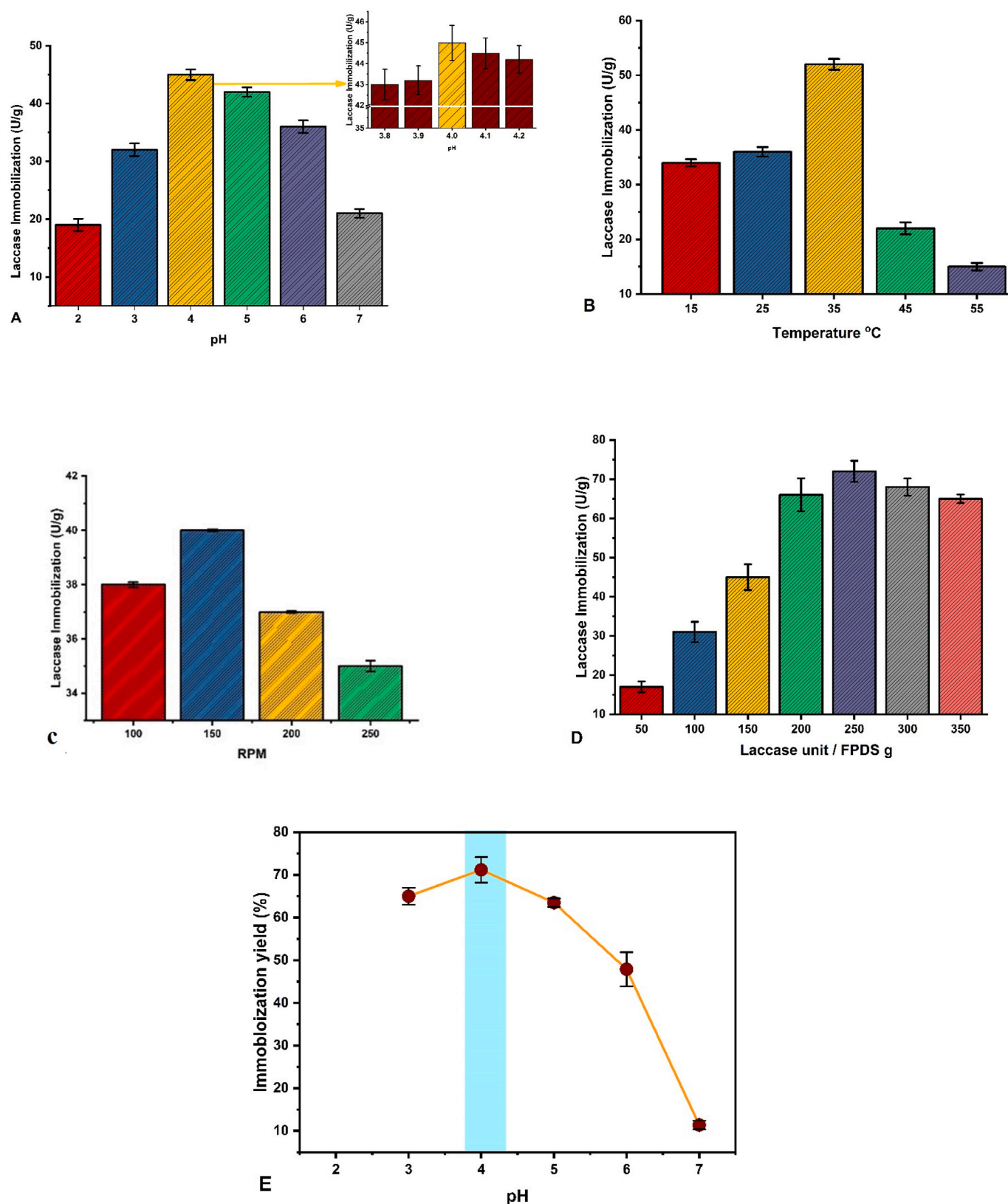
Fig. 3. Influence of altering pH (A), temperatures (B), rpm (C), laccase concentrations on date stone (D), and immobilization yield (E). The influence of the parameters was always assessed by measuring activity towards ABTS.
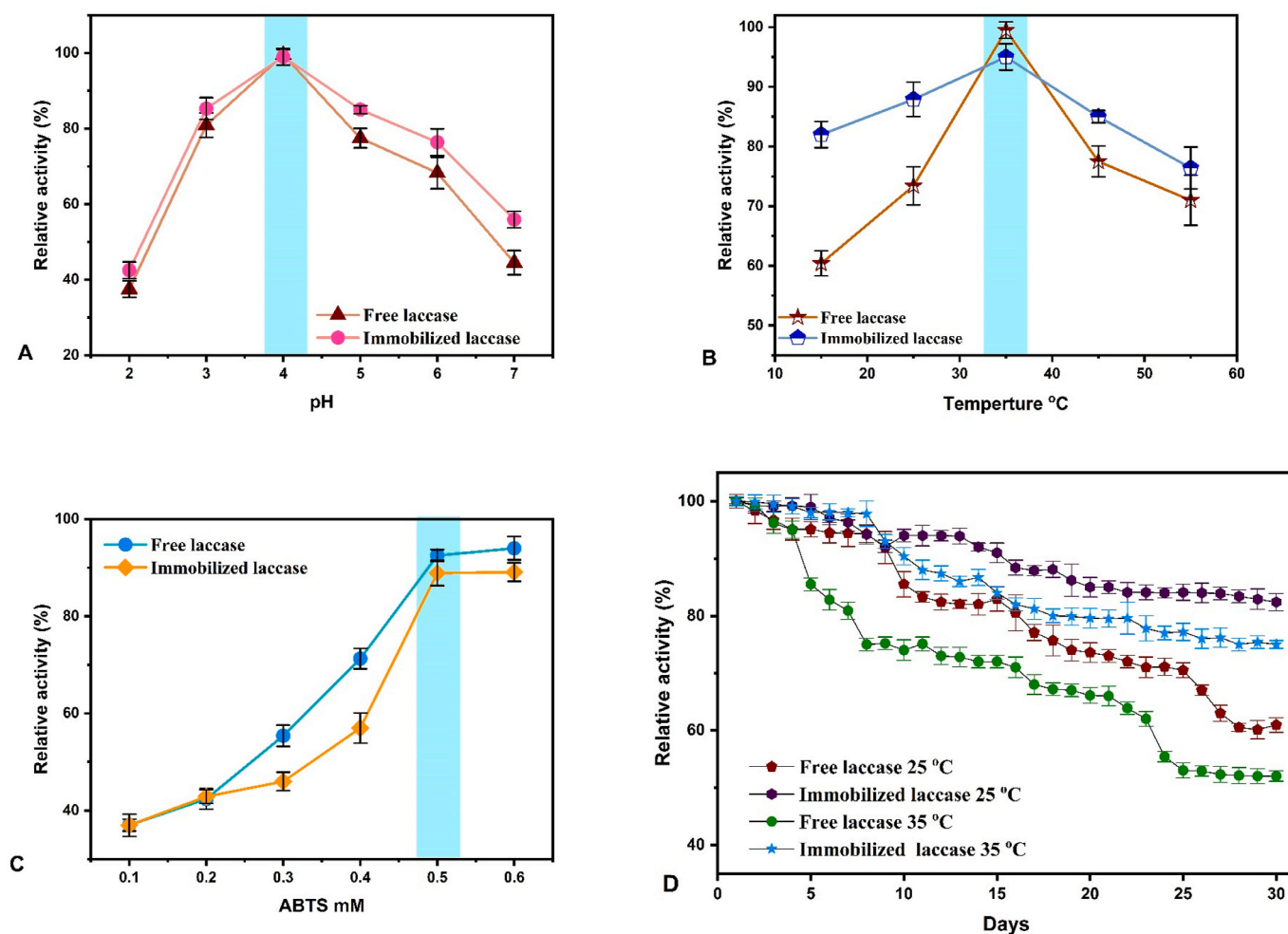
Fig. 4. Stability of free and immobilized laccase with respect to several parameters pH (A), temperature (B), ABTS (C), and storage (D).
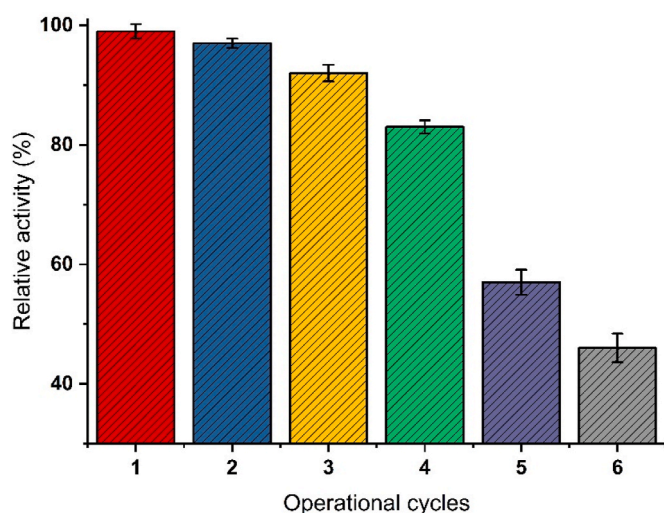

Fig. 5. LFPDS operational stability.

Laccase, both free and immobilized demonstrated the normal behaviour of an enzyme reaction with ABTS as a substrate. Accordingly, the kinetic parameters were determined using the Michaelis– Menten equation. Using Origin software, the Michaelis–Menten parameters K_m and v_{max} were determined by nonlinear fitting of reaction rate against substrate concentration. Fig. 6 shows Michaelis–Menten fitting model to free and immobilized laccase. It is widely known that the parameter K_m provides an indicator of the affinity of the enzyme to the substrate. More specifically, a higher K_m reported for a certain substrate indicates a lower affinity for that substrate. With a K_m value increase from 0.46 to 0.65 mM, it can be concluded that immobilization reduces laccase's
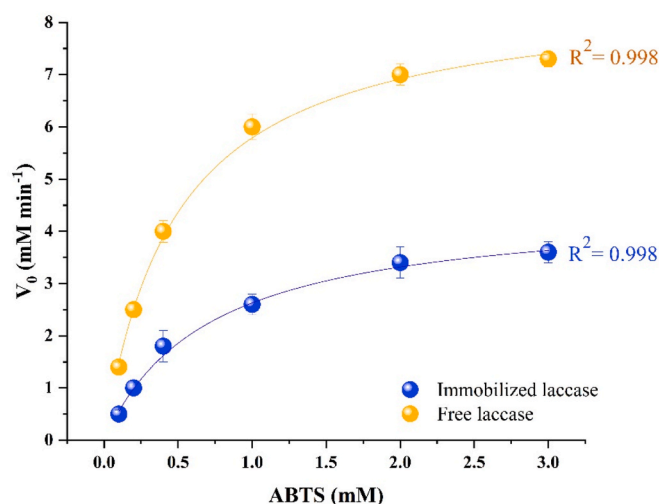

Fig. 6. Michaelis–Menten fitting model to free and immobilized laccase.

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affinity for ABTS in LFPDS. This could be owing to restrictions on the mass transfer of the substrate to the surface of LFPDS. The slight changes in the three-dimensional conformation of the laccase upon adsorption onto the support directly or indirectly impact the active site of the laccase (bib_citation_to_be_resolvedCristóvão et al., 2012). Other studies have drawn the same conclusion (Wang et al., 2008; Cabana et al., 2009), showing a reduced affinity for the substrate due to diffusional limitations and decreased enzyme flexibility after immobilization. The v_{max} value of the immobilized enzyme (4.42 mM min^{-1}) was lower than that of the free enzyme (8.57 mM min^{-1}). The lower maximum reaction rate could be explained by the formation of substrate diffusional limitations after enzyme binding.

3.4. Pollutants adsorption isotherm, kinetics and thermodynamics

Fig. 7 shows the results of the fitting experimental data against Langmuir and Freundlich isotherm models. It can be seen that both Langmuir and Freundlich isotherm models fit well with the aspirin adsorption data, but Langmuir exhibits a slightly better fit. In comparison, the Freundlich isotherm model produces a slightly better fit for ketoprofen adsorption data compared to Langmuir isotherm model. This may indicate the formation of a uniform monolayer of both aspirin and ketoprofen onto activated date stone. Similar findings have been observed in other studies (e.g. Mahmoodi et al., 2010). The calculated maximum sorption capacity (q_m) was 568.18 and 458.71 mg g^{-1} for ketoprofen and aspirin respectively.

The dimensionless separation factor (R_L) identifies the kind of isotherm as irreversible ($R_L = 0$), favorable ($0 < R_L > 1$), linear ($R_L = 1$), or unfavorable ($R_L > 1$) (Ho et al., 2002). Table S3 shows R_L determined value for both pharmaceutical compounds, and it was found to be between 0 and 1, indicating favorable adsorption. As for Freundlich isotherms, K_f, n, and R^2 are 2.100, 1.858, and 0.954, respectively (Table S3). The value of n > 1 implies favorable adsorption (Ghaedi et al., 2013). The value of 1/n, which varies between 0 and 1, is known as the heterogeneity factor. For the case of this study, the 1/n values were close to 1 for both pharmaceuticals indicating lower degree of surface heterogeneity.

Fig. 8 shows the pseudo-first-order model (A) and pseudo-second-order (B) kinetic models fitting for aspirin and ketoprofen adsorption. The correlation coefficient for the pseudo-second-order kinetic model was 0.999 for both compounds, compared to 0.984 and 0.983 for ketoprofen and aspirin respectively for the pseudo-first-order model. This suggests that chemisorption was the rate-regulating step in the adsorption process. The fitting parameters for kinetic models are shown in Table S3.

Fig. 9 shows a plot of lnK_1 versus 1/T for the estimation of the

thermodynamic parameters for the adsorption of ketoprofen and aspirin on FPDS. The thermodynamic parameters of ketoprofen and aspirin adsorption such as free energy change ($\Delta G°$), enthalpy change ($\Delta H°$) and entropy change ($\Delta S°$) at various temperatures (i.e. 288, 298, 308 and 318 K) are shown in Table S4. $\Delta G°$ values reduced as temperature increased, demonstrating the positive impact of temperature on the adsorption's effectiveness. Negative values of $\Delta G°$ obtained at four different temperatures show that the adsorption of ketoprofen and aspirin onto FPDS occurs spontaneously. As the values of Positive $\Delta H°$ values were observed, indicating the endothermic nature of the adsorption process. Positive values of $\Delta S°$ suggest that the adsorption process is random and stable (Yao et al., 2020).

3.5. Pharmaceutical compounds removal and reusability

The present study investigated the biodegradation of ketoprofen and aspirin using laccase immobilization on functionalized date stones. The adsorption of these pharmaceuticals onto date stones was also assessed through control experiments. Fig. 10 depicts the decrease in ketoprofen (A) and aspirin (B) concentrations observed over a period of 6 h under different experimental circumstances, i.e., with the laccase, FPDS, LFPDS, and LFPDS-ABTS. It is inferred that in the case of LFPDS and LFPDS-ABTS, the reduction in ketoprofen and aspirin concentrations was the synergistic effects of adsorption and the enzymatic degradation. Ketoprofen and aspirin concentrations were measured by their respective retention times (22.1 and 14.6 min) and the area under the curve.

In all scenarios shown in Fig. 10, the concentration of ketoprofen declined precipitously within the first hour, dropping by 49%, 55%, 75%, and 72% from its initial concentration of 25 mg L^{-1} in FPDS, laccase, LFPDS, and LFPDS-ABTS, respectively. During the same time frame, the concentration of aspirin in FPDS, laccase, LFPDS, and LFPDS-ABTS decreased by 44%, 50%, 63%, and 66%, respectively.

The highest decrease in the ketoprofen concentration after 4 h was 95% and 98% in the case of LFPDS-ABTS and LFPDS, respectively but the measurements were continued till 6 h in order to confirm the highest removal percentage. A similar profile was also obtained with aspirin, where around 97% and 92% decrease was recorded in the case of LFPDS and LFPDS-ABTS, respectively, after 4 h. Nonetheless, FPDS and laccase exhibited a delay in removing both contaminants. Ketoprofen removal efficiencies were 67% for FPDS and 70% for laccase, while aspirin removal efficiencies were 65% for FPDS and 72% for laccase. The adsorption of FPDS was due to the large surface area of the date stone. Both sets of experiments with LFPDS and LFPDS-ABTS for both pollutants removal had almost the same results. This shows that laccase has improved the removal percentages with both pollutants by around 20%.

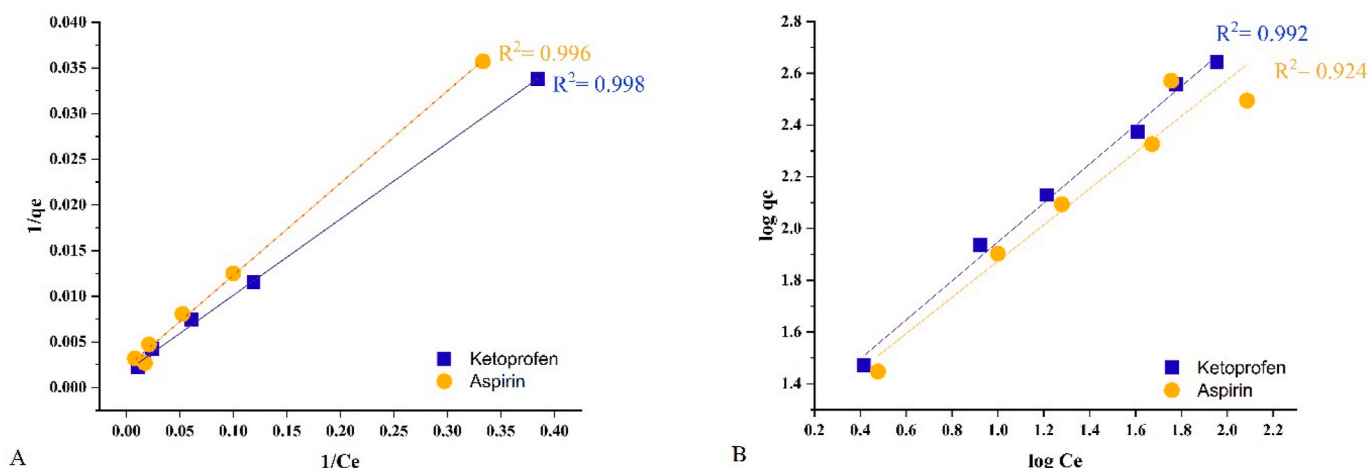After 6 h of treatment, laccase activity decrease was also assessed. In

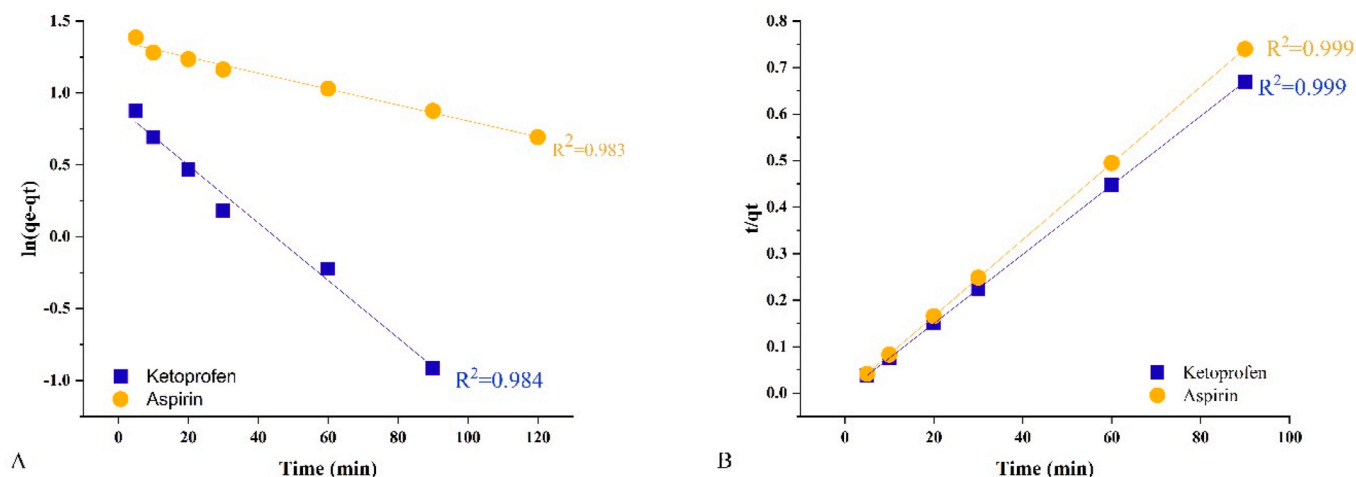**Fig. 7.** Langmuir (A) and Freundlich (B) isotherms for ketoprofen and aspirin.

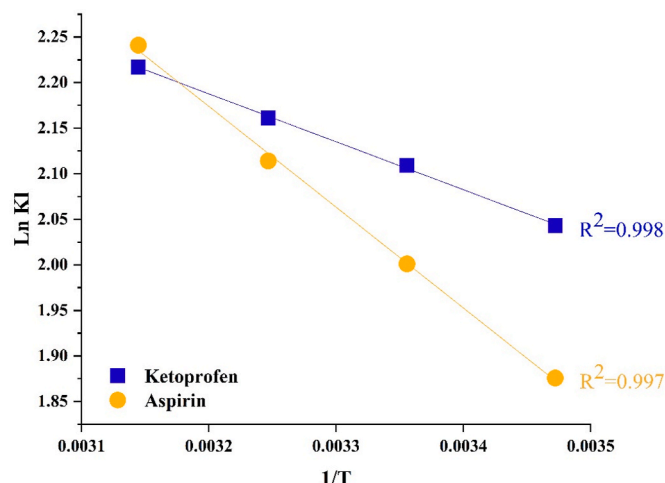**Fig. 8.** Pseudo-first-order model (A) and pseudo-second-order (B) kinetic models for FPDS.

Fig. 9. Plot of lnK$_l$ vs. 1/T for the estimation of the thermodynamic parameters for the adsorption of ketoprofen and aspirin on FPDS.

the instance of the free laccase, there was a 63% decrease in laccase activity relative to the original enzyme activity. In contrast, 50% of LFPDS's activity was lost, which presents a decent improvement in laccase stability.

Due to the presence of biodegradation and adsorption sites on LFPDS, two removal strategies for both pharmaceutical compounds could be postulated. In the first mechanism, it is assumed that the main removal process is adsorption on free sites of LFPDS and that there is no degradation. In the second mechanism, chemicals were adsorbed onto free sites of biocatalyst, and after the initiation of biodegradation by laccase, the occupied sites were freed, allowing the sorption-biodegradation cycle to begin again. In order to evaluate both mechanisms, the contributions of degradation and adsorption were evaluated for each cycle.

The reusability of the immobilized laccase for the removal of the pharmaceutical compounds was examined, and the findings are shown in Fig. 11. As presented in Fig. 11, after five removal cycles, the removal efficiency of LFPDS for both compounds was still maintained at over 75%. However, the removal efficiency of FPDS was maintained at over 51%. In cycles 6 and 7, the aspirin removal efficiency decreased from 76% (fifth cycle) to 51% (seventh cycle) when LFPDS was used while it decreased from 52% (fifth cycle) to 30% (seventh cycle) in the case of FPDS. Ketoprofen removal efficiency by LFPDS also dropped in the sixth

and seventh cycles from 75% to 50% whereas it decreased from 51% to only 29% by applying FPDS. Comparing LFPDS and FPDS removal efficiencies for both pharmaceutical compounds, it can be stated that there was around a 20% increase in the case of LFPDS in each cycle. This could be explained by the improvement in laccase addition introduced.

The declining trend in removal effectiveness (Fig. 11) could be due to the leaching and denaturation of the enzyme, as was found for ABTS oxidation (Spinelli et al., 2013). This degrading system has the potential to be deployed as part of the water treatment scheme to eliminate pharmaceutically active compounds. However, it is necessary to study the economics of the whole process and optimize the operating parameters for a wide variety of pharmaceutical chemicals.

Fig. 12 shows the conceptual mechanism of LFPDS in absorbing and degrading ketoprofen and aspirin. In general, the incorporation of enzymes introduces degradation as an additional removal mechanism besides the known adsorption ones such as covalent bonding (chemical interaction) and hydrogen and π-π interactions (physical interactions). Functional groups, especially carboxylic and amine, can play a significant role in the interaction between laccase and date stone surface. The attached laccase, in turn, may interact with aromatic functional groups on aspirin and ketoprofen, converting them to free radicals that start domino reactions (Arregui et al., 2019).

4. Future prospects

The results obtained in this study show that immobilizing laccase onto bio-waste materials such as activated date stone achieves promising removal of pharmaceuticals or at least the tested ones in this work. However, this was obtained in a controlled environment using synthetic water samples. Testing the removal capacity of this technique with natural samples and with a wider range of pharmaceuticals are important prior to planning the scale-up of the process. In the authors' opinion, the scale-up of immobilized laccase on activated date stone can be envisaged in two configurations: packed column design and fluidized bed reactor. In both cases, monitoring laccase activity and adsorption capacity of the loaded adsorbents are needed to determine the exhaustion time for the system. The challenge in such systems is the difference in the rate of the two removal mechanisms namely adsorption and degradation. In order to overcome this challenge, a series of treatment units should be implemented to ensure the maximum utilization of laccase activity and adsorbent capacity. Given the sensitive stability of laccase, it is likely to lose activity before the adsorbent reaches exhaustion. In this case, laccase can be fed with the water being treated so it can adsorb onto the active sites of the adsorbent in the column and the degradation can be resumed through free and adsorbed laccase. To
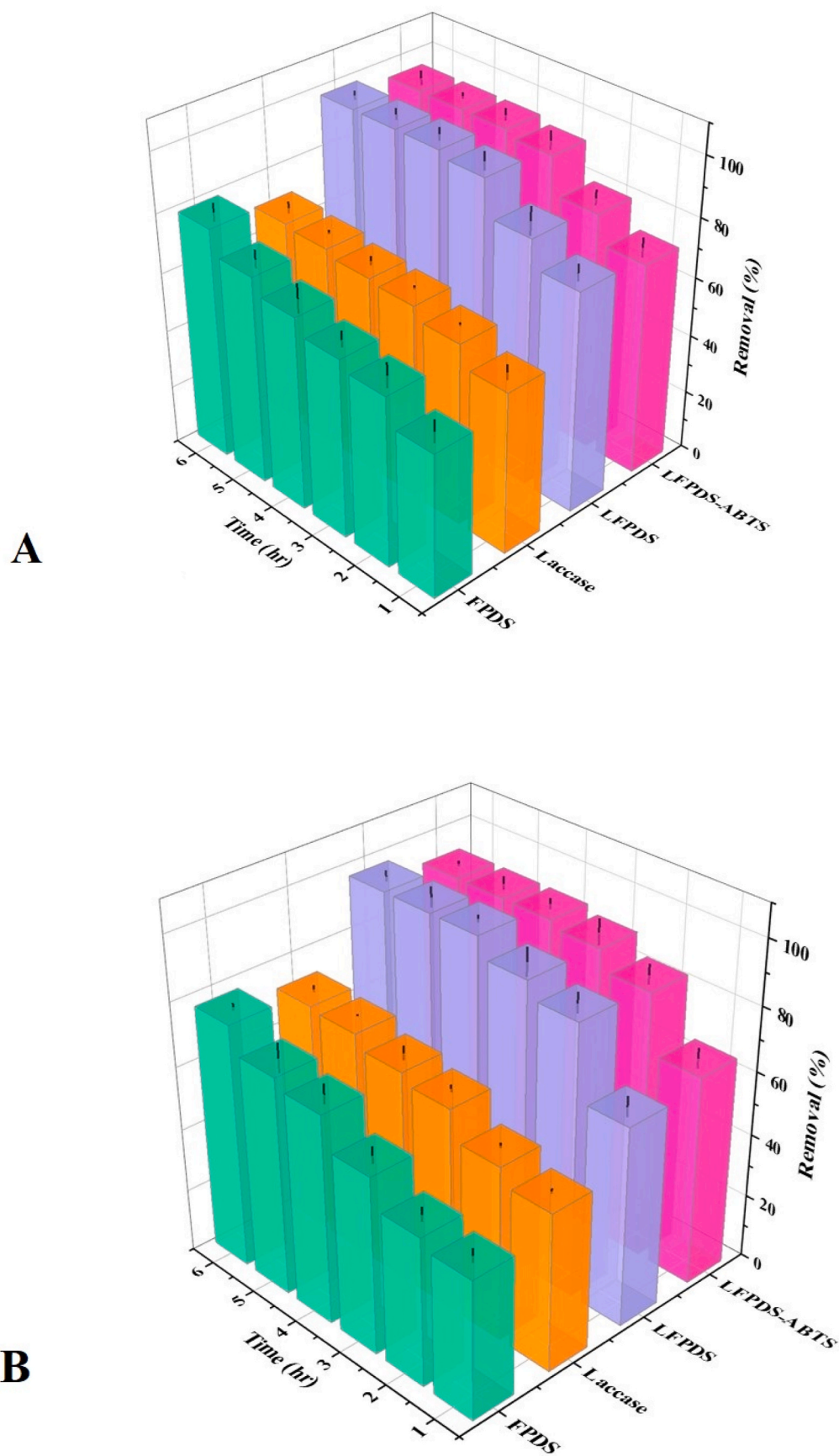
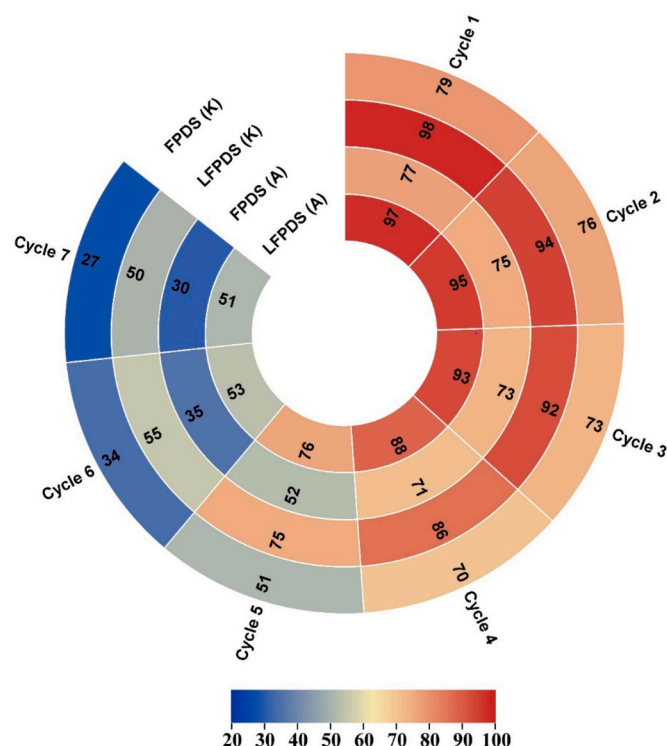**Fig. 10.** Ketoprofen (A) and aspirin (B) removal (%).

Fig. 11. Comparison of the removal efficiency of ketoprofen (K) and aspirin (A) over several sequential recovery runs using the LFPDS and FPDS. Standard deviations were ≤1.4 in LFPDS and FPDS within all cycles. All numbers in percentage.
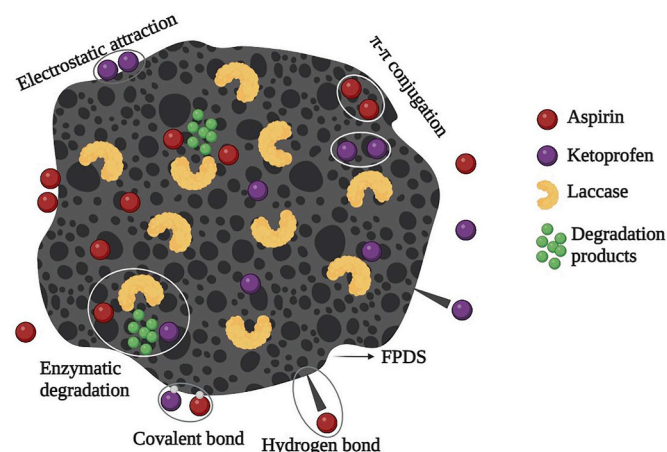

Fig. 12. An illustration of degradation and removal of pharmaceuticals with LFPDS.

avoid the accumulation of laccase on the column bed close to the feed entrance, multiple feeding ports for laccase should be implemented. In the case of a fluidized bed reactor, the loaded adsorbent with laccase can be fed as needed with the water being treated. The authors would like to emphasize that their opinion about the future development of the laccase-enhanced adsorption system is by no mean intended to limit the scale-up configuration options to the above-mentioned ones. Rather, it is only meant to provide researchers with food for thoughts for the future development of such systems.

5. Conclusion

This research emphasizes the significance of agricultural waste (date stone) recovery in the establishment of a cost-effective and sustainable immobilized laccase system. The ABTS oxidation reusability assays revealed that the immobilized laccase retained 54% of its original activity after six cycles. Laccase degradation and FPDS adsorption as separate processes achieved about 60% removal of ketoprofen and aspirin in 4 h. However, the combined adsorption and degradation using immobilized laccase nearly reached a complete removal of these pharmaceuticals for the same treatment time. These findings confirm the significant potential for the utilization of agricultural wastes and enzymatic treatment for environmental reclamation applications. The next step is to evaluate the efficiency of this process with real wastewater samples on a large scale to confirm its feasibility for industrial applications.

Author Contributions Statement

Osamah J. Al-sareji: Formal analysis, Writing – original draft, Data curation, Visualization, Writing – review & editing, Methodology, Investigation. **Mónika Meiczinger**: Conceptualization, Supervision, Validation, Writing – review & editing. **Jasim M. Salman**: Investigation, Methodology. **Raed A. Al-Juboori**: Writing- Reviewing and Editing. **Khalid S. Hashim**: Writing- Reviewing and Editing, Methodology, Resources, Visualization. **Viola Somogyi**: Conceptualization, Supervision. **Miklós Jakab**: Data curation, Methodology.

Funds

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.chemosphere.2022.137133.

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