ORIGINAL PAPER

Removal of various phenolic compounds from solution using free and entrapped *Lentinus sajor-caju*

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Abstract

White rot fungi have attracted attention for their effectiveness in removing pollutants from aqueous solutions, both in terms of cost and environmental safety. Therefore, the free and entrapped *Lentinus sajor-caju* was used as a model white rot fungus to elucidate its performance in removing different phenolic pollutants from the solution. *L. sajor-caju* was entrapped in Ca-alginate beads and free fungus biomass was used as a control system to remove three different phenolic compounds, namely, phenol (PH), 4-nitrophenol (NP) and pentachlorophenol (PCPH) from aqueous solution. Different characterization techniques such as Fourier Transform Infrared Spectrophotometer, zeta potential and contact angle analysis were performed to characterize the used adsorbents. The performance of fungal preparations for the adsorption of phenolic compounds was optimized under different operational parameters in a batch system. The removal performance of entrapped fungus was also studied in a packed bed reactor. The maximum adsorption capacity of the free and entrapped *L. sajor-caju* were found to be for phenol 170.3 and 125.2 mg/g, for 4-nitrophenol 34.5 and 27.1 mg/g and for pentachlorophenol 398.6 and 516.7 mg/g biomass, respectively. The preferential adsorption of phenolic compounds by both adsorbents was followed in the order of pentachlorophenol > phenol > 4-nitrophenol. All phenolic compounds adsorption on the fungus preparations is well described with the Freundlich isotherm model, and the results are in good agreement with the heterogeneity of free and immobilized *L. sajor-caju* biomass.

Keywords Adsorption · Bioreactor · Ca-alginate · *Lentinus sajor-caju* · Phenolic compounds

Introduction

Increase in the industrial developments and overpopulation attracted the attention of researchers growing detrimental environmental pollution, including air, water, and soil

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pollutants (Bayramoglu et al. 2009; Rubilar et al. 2012). The phenolic pollutants including chlorinated phenols, textile dyes, drugs, pesticides, and others are released into the environment via discharged industrial wastewaters, and the increasing toxicity level, accordingly, can reason various unfavorable effects on human health and living things (Benit et al. 2022; Arica et al. 2022; Ahmaruzzaman and Sharma 2003). Different chemical and physical methods have been reported for the removal of these phenols using various synthetic adsorbents, but these studies are of limited effectiveness. Whereas, the use of cheap microbial biomasses as adsorbents can be an environmentally friendly and economical alternative to remove phenolic pollutants (Arica et al. 2019; Bayramoglu and Arica 2020; Bosso et al. 2015). These organic pollutants are toxic, carcinogenic, and mutagenic and must be carefully treated before being discharged into waterbodies (Bayramoglu et al. 2022; Akinhanmi et al. 2020; Chen et al. 2022). For example, PCPH is one of the most toxic compounds in effluents discharged from diverse industries, they generally bind to cell membranes of living

organisms and block oxidative phosphorylation (Peckova et al. 2021; Said et al. 2021; Sellami et al. 2021). Various microbial biomasses such as yeast, algae, bacteria, and fungi (Bayramoglu et al. 2022, 2009; Yan et al. 2006) have been effectively used to reduce the phenolic compounds contents of the solutions. Among these microbial biomasses, white rot fungi is environmentally friendly and can be grown easily within inexpensive solid/liquid growth medium such as cellulose (Bayramoglu et al. 2009; Rodríguez-Couto 2017). Removal of aromatic phenolic compounds via microbial biomasses can be realized by several functional groups on the fungal biomass surfaces including amine, carboxyl, hydroxyl, sulfhydryl, and phosphonate groups. In previous studies, the removal of phenolic compounds by various white rot fungi species, such as *Pleurotus sajor-caju* (Denizli et al. 2005), *Anthracophyllum discolor* (Rubilar et al. 2012), *Funalia trogii* and *Tramates trogii* (Bayramoglu et al. 2023, 2009) have been studied. Some white rot fungi species have also been used to degrade toxic phenolic compounds. Such as *Funalia trogii* was used for the degradation of Bisphenol A (Bayramoglu et al. 2023), and the decolorization of various dyes solutions have been also realized by various white rot fungi species (Peckova et al. 2021). These have been realized by the release of different extracellular lignocellulosic enzymes (Bayramoglu et al. 2023). The use of free microbial biomass in batch or continuous systems for the adsorption of pollutants causes some problems that complicate the application. These are the difficulty in separating the biomass after adsorption of target pollutant and most importantly the mass loss in the regeneration process of these adsorbents (Qiao et al. 2022). These may be the result of the low strength, low density, and small particle size of the free biomass. Entrapment of microorganisms in a hydrogel system with minimal diffusional restriction can solve these problems. In the present study, the use of free and entrapped *L. sajor-caju* biomass for the efficient adsorption of phenols in batch and packed bed reactors at low cost was investigated. To our knowledge, this is the first report using entrapped *L. sajor-caju* in Ca-alginate beads for the removal of phenols from aqueous solution. The biomass of this fungus can be easily cultivated using solid and liquid growing methods on inexpensive carbon sources (i.e., cellulose). Under different experimental conditions, the performance of this fungus preparation for the adsorption of various phenols was studied. Finally, eco-toxicity of phenolic compounds was performed using different test species, and bio-assays of three different test organisms (i.e., *D. magna, C. reinhardtii,* and *T. aestivum L*) with different phylogenetic were examined.

Materials and methods

Microorganism and cultivation

L. sajor-caju "Reference No: MAFF 430306" is a gift from GenBank (National Institute of Agrobiological Sciences) Japan. The cultivation conditions of the fungus on the solid and liquid medium were realized as described earlier (Bayramoglu and Arica 2016).

Entrapment of fungus in Ca-alginate hydrogel beads

Na-alginate solution (2%) (high viscosity Na-alginate from Sigma Chem. Co., USA) was prepared for the entrapment of *L. sajor-caju*. The cultivated fungus biomass in the pellet form (2.0 g) was homogenized in phosphate buffer (50 mL, 50 mmol/L, pH 6.0) and mixed with Na-alginate solution (50 mL, 4.0%). The mixture was transferred dropwise into a $CaCl_2$ solution (0.5 mol/L) via a nozzle by means of an injection pump (Fisherbrand™ Single Syringe Pump, Model No. 14-831-210). When the sodium alginate-fungus mixture was dropped into $CaCl_2$ solution, sodium ions were exchanged with calcium ions to form an ionically cross-linked insoluble Ca-alginate gel. The fungus entrapped Ca-alginate beads (~1.0 mm size) were further incubated in the $CaCl_2$ for 30 min. Next, the entrapped fungus in Ca-alginate beads were transferred to minimal medium and allowed to grow for 3 days at 30 °C. Then, the fungus biomass-Ca-alginate beads were collected from the $CaCl_2$ solution and used for the removal of phenols, 4-nitrophenol and pentachlorophenol in a batch and packed bed reactor.

Characterization of fungal biomasses

Surface morphology of bare Ca-alginate and fungus entrapped Ca-alginate beads were obtained using SEM, FEI QUANTA 250 FEG, Oregon, USA. The surface functional groups of the fungus preparations were detected using the Nicolet TM ISTM 50 FTIR spectrometer (Thermo Fisher Scientific, USA). The zeta potentials of bare Ca-alginate and free and Ca-alginate-entrapped fungal biomass were obtained at various pH values using a Zeta-sizer (Nano ZS, Malvern Instruments Ltd., Model number: ZEN3600). A digital optical contact angle meter (Phoenix 150, Surface Electro Optics, Korea) was utilized to examine the contact angle values of the samples and the contact angle values were determined with three test liquids namely water, glycerol and diiodomethane at 25 °C as described previously (Van Oss et al. 1988; Bayramoglu and Arica 2005).

Adsorption studies

The adsorption of the phenol, 4-nitrophenol, and pentachlorophenol (obtained from Sigma, St. Louis, USA) with the free and immobilized fungus was realized in a batch system. The effect of experimental parameters such as initial concentrations of phenolic compounds, optimum pH on adsorption rate and capacity was investigated. Stock solutions of phenol, 4-nitrophenol, and pentachlorophenol were prepared in the purified water (1000 mg/L). In each set experiment, solutions including phenolic compounds (50 mL) were added in a glass flask containing 50 mg biomass and incubated at 150 rpm at 25 °C for 6.0 h. The pH of the medium was in the range of 2.0–9.0. The effect of different initial adsorbate concentrations is very critical for the determination of the adsorption performance of the adsorbents and adsorption isotherms investigation. For these reasons, the initial concentration of the phenolic compounds was selected in a broad range between 20 and 800 mg/L. Whereas the concentration of PH, NPH, and PCPH in different oceanic and river waters have been reported between 0.01 and 40 µg/L, 0.1 and 10 µg/L and 5.0×10^{-3} and 2000 µg/L, respectively (Michałowicz and Duda 2007). The amount of adsorbent in the solution was varied in the range of 0.1–1.5 g/L. The initial and residual concentrations of the PH, NPH, and PCPH in the adsorption medium were determined by using HPLC. The equilibrium adsorption capacity for the adsorption of PH, NPH and PCPH for the adsorbents was calculated as described previously (Bayramoglu et al. 2022).

HPLC operating conditions

The phenolic compounds were recognized and quantified with a high-performance liquid chromatography (HPLC). Chromatographic separation of the PH, NPH, and PCPH compounds was carried out with a SUPELCOSIL LC-8 (150 mm × internal diameter 4.6 mm, particle diameter 5 µm) protected by a front column (20 mm, inner diameter 4.6 mm). In order to determine phenolic compounds, as carrier phase; 1.0% acetic acid in methanol was used in line A and 1.0% acetic acid in ultrapure water was used in line B. The solutions were also filtered by a 0.2-µm filter and degassed using an ultrasonic water-bath. The carrier phase of the B line was decreased from 65 to 0% in 20 min compared to the A phase. Then, after 5 min, the A: B carrier phase ratio was changed to 35:65. The flow rate of the mobile phase was chosen as 1.5 mL/min. The column furnace temperature was set to 30 °C and the UV–Vis detector was set to a high absorbance value (280 nm). The injected sample volume was 20 µL. The whole system was automatically controlled under the Windows operating system with the CHROMELLEON data program.

Toxicity evaluation with aquatic organisms

Green alga growth inhibition studies

The Bold Basal Media (BBM) was used for the microalga growth inhibition test as described earlier (OECD 2011). Green microalgae of *C. reinhardtii* were used and grown in BBM as presented earlier (Bayramoglu et al. 2022). The *C. reinhardtii* cells were cultivated in BBM medium containing different amounts of PH, NPH, and PCPH between 5 and 30 mg/L. The control culture was not contained any phenols.

Results and discussion

Properties of the adsorbents

Immobilization of microbial biomass prevents loss of as-prepared adsorbent during the washing and regeneration process. Therefore, the immobilized microbial biomass has greater operational stability compared to its free form. The chosen white rot fungus "*L. cajor-caju*" provides many advantages over other reported algae and bacteria species. These fungi have several degrading extracellular enzymes such as cellulase, laccase, and lignin peroxidase (Bayramoglu et al. 2023). Moreover, compared to other microbial species, white rot fungi have cell walls that contain a high amount of chitin with several functional groups. Therefore, the presence of effective binding sites such as hydroxyl, amine, carboxyl, carbonyl, phosphate, sulfate, lipid, polyuronides, and melanin groups for the adsorption of pollutants on *L. sajor-caju* biomass was observed using the ATR-FTIR spectra. The FTIR spectrum of Ca-alginate exhibited absorption peaks concerning hydroxyl, ether, and carboxylic groups (Fig. 1A). At 3439 cm^{-1}, a stretching vibration peak related to the -OH group was observed. The peak at 1620 cm^{-1} can be ascribed to the stretching vibrations of carboxyl functionality. The bands at 1093 and 1022 cm^{-1} were identified by the C–O stretching of the pyranose ring and C–O stretching vibration with effects from C–O–H deformation. The spectrum of *L. sajor-caju* had a peak at 3260 cm^{-1} in relation to –OH and –NH stretching (Fig. 1B). The peak related to the N–H bending of chitin on the fungus appeared to cause a strong peak at around 1635 cm^{-1}. The peaks at 2927, 1545, 1408, and 1074 cm^{-1}, respectively, could be due to the various useful groups on the fungus structures such as –NH$_2$, –OH, –P$=$O, and –PO groups. These functional groups of fungal biomasses could interact with the phenolic compounds (i.e., phenol, 4-nitrophenol, and pentachlorophenol) and show a significant role in the removal of the phenols from solution. The FTIR spectrum of the alginate–fungus is presented in Fig. 1C. This spectrum offers clear suggestions of the occurrence of fungus biomass in Ca-alginate. After
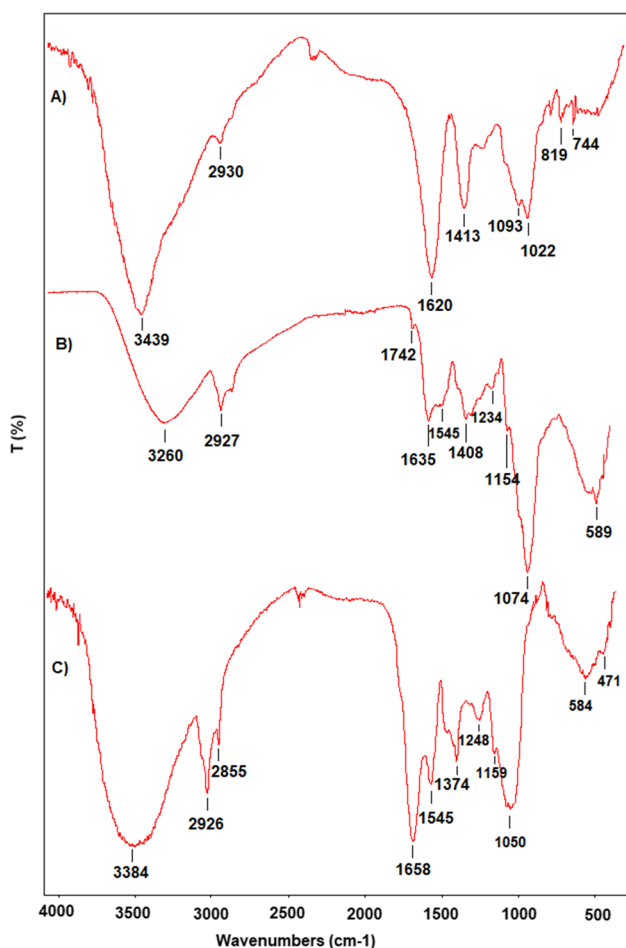
Fig. 1 ATR-FTIR spectra: **A** Ca-alginate, **B** *L. sajor-caju* biomass and **C** Ca-alginate-*L. sajor-caju*

peak of C–O stretching vibrations. The fungus-Ca-alginate bead spectra showed that the density of C=O groups amplified after the entrapment of fungus in Ca-alginate (Fig. 1C).

The BET studies showed that of the bare Ca-alginate, free and entrapped fungus have reasonably high specific surface areas. The specific surface areas of the bare alginate, free, and entrapped *L. sajor-caju* were 6.7, 10.5, and 8.2 g/m^2, respectively. After the entrapment of the fungus, the specific surface area was meaningfully decreased. This could be due to the firm crosslinking of alginate polymer with divalent calcium ions.

The surface micrographs of the fungus preparations were examined by using scanning electron microscopy (SEM). The bare Ca-alginate beads had a rough surface as observed in Fig. 2A, B. The representative SEM image of *L. sajor-caju* entrapped in Ca-alginate beads is presented in Fig. 2C. It was observed that the entrapped fungal mycelia grew uniformly on the surface of the Ca-alginate beads without any restriction. Moreover, the SEM image displayed that the fungal mycelia were uniformly distributed on the Ca-alginate beads. Thus, the fungal biomass entrapped Ca-alginate beads surface was smoother compared to bare Ca-alginate beads (Fig. 2C). The uniform growth of the fungus on Ca-alginate beads can provide an effective adsorption potential over the entire surface area of the fungal biomass entrapped Ca-alginate beads. Thus, the high specific surface area of the fungus could be the result of the mycelial structures and this structure could increase the contact surfaces with phenolic compounds in the aqueous medium.

In general, when a material is contemplated for use as an adsorbent, the surface chemistry of materials is certainly of prime importance. Therefore, the variation of the wetting power of a test liquid is highly sensitive to the surface properties; therefore, the wetting power of the three test liquids was used to determine the surface properties of the fungus samples and investigated by contact angle studies. The hydrophilic and hydrophobic entities on the surface of

the entrapment of fungus in Ca-alginate beads, a peak was detected at 3384 cm^{-1}, and this resulted from the stretching vibration of –NH$_2$ and –OH groups of the Ca-alginate-fungus. A peak was observed at 1658 cm^{-1}, a characteristic

Fig. 2 SEM micrographs: **a** Ca-alginate beads X65; **b** Surface of bare Ca-alginate beads; **c** Surface of the *L. sajor-caju* entrapped Ca-alginate beads
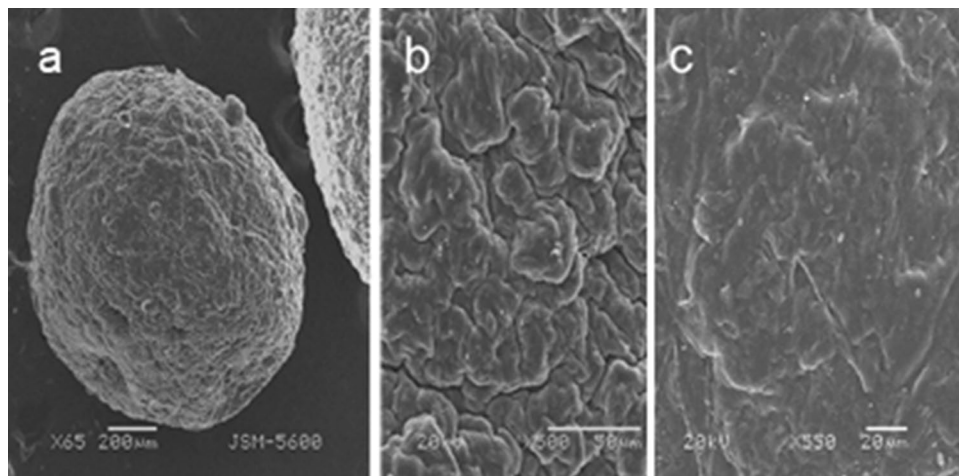
an adsorbent are very important due to the close fit interactions between the adsorbent and the target molecule. Contact angle values of adsorbents for water, glycerol, and diiodomethane are given in Table S1. The surfaces of the free and entrapped fungus were hydrophobic (i.e., $\theta > 90°$). These results suggested that the hydrophobic interaction can play a primary role in the adsorption of phenolic compounds as well as in ionic interactions. This could be accountable for the adsorption performance of both the adsorbents. Different surface properties of samples cause different contact angles with different test liquids (Table S1) and, hence, different surface energies (Table S2). For example, the surface of *L. sajor-caju* has a more hydrophobic character compared to entrapped fungus. Total surface free energy (γ^{Total}), different Lifshitz-van der Waals (γ^{LW}) and acid–base (γ^{AB}) values of both adsorbents were calculated using the van Oss acid–base method. As seen from Table S2, fungus preparations exhibited various acid–base components (γ^{AB}) of surface free energies (γ^{TOT}) due to the presence of various chemical groups on the free and entrapped fungus surfaces. The polar components (γ^{AB}) of the free and entrapped fungus biomass were fragmented into a large base (γ^{-}: 1.5 and 4.6 mN/m^2) and very small acid (γ^{+}: 0.8 and 0.4 mN/m^2) surface energy components, respectively. These data indicated that the surface of free and entrapped fungus had well-defined basic (γ^{-}) properties. As predicted, the highest γ^{AB} value was observed for Ca-alginate-fungus. This could be due to the occurrence of the hydrophilic carbohydrate content of the Ca-alginate.

The efficient groups of the fungus can be dissociated according to the pH of the medium (Fig. 3). The zeta potential plot for free and entrapped fungus has experimentally shown acidic surface properties as experiential for the other microbial biomass studied (Bayramoğlu et al. 2022). The
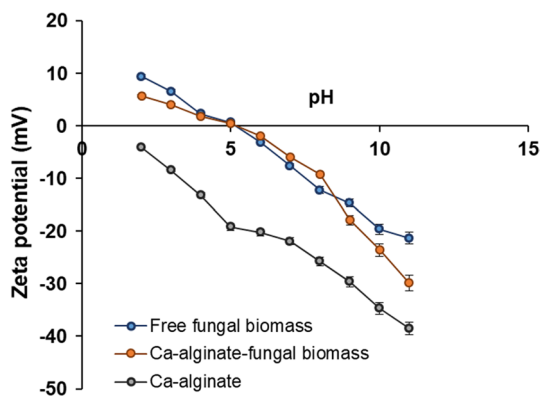
zeta potential values of free and entrapped fungus decreased as the solution pH increased. As observed from Fig. 3, the negative charge density of the Ca-alginate-fungus was higher compared to the free fungus. The surface charge variation in the pH range of 2.0–11 was changed from 11.9 to −21.3 mV for the free fungus and from 5.7 to −29.8 for the entrapped fungus. In the pH range of 2.0–5.0, the surface potentials of free and entrapped fungus were positive and maximum adsorption was detected at pH 5.0. A low negative zeta potential value for the Ca-alginate-fungus may be due to the electrical elimination of negatively charged acidic groups of the acidic polymer in the presence of Ca(II) ions. These statements are in agreement with those obtained by FTIR and contact angle studies. The adsorption of phenolic compounds on adsorbent surfaces can be driven mainly by van der Waals and physical interactions.

Adsorption studies of phenolic compounds in the batch system

Both the free and entrapped fungus were used as natural adsorbents for the adsorption of three different phenolic compounds (i.e., PH, NPH, and PCPH). These compounds are hydrophobic organic pollutants and tend to accumulate in living bodies at a high rate (Xing et al. 2012). The amount of adsorbed phenolic compounds with the free and entrapped fungus was quantified by HPLC. Retention times of PH, NPH, and PCPH compounds from the obtained chromatograms were determined as 3.5, 4.5, and 19.8 min, respectively (Figure S1). The residual phenolic compound concentration in the medium before and after adsorption was calculated from the constructed calibration curves.

Effect of adsorbent dosage

One of the significant parameters in the removal of phenolic compounds in the fungus preparations is the adsorbent dosage. The influence of adsorbent dosage on the removal efficiency was investigated by varying the biomass dosage between 0.1 and 1.5 g biomass/L (Fig. 4A). From this figure, an increase in the amount of adsorbed phenols was observed with the increase in the adsorbent dose. It should be noted that the increase in the percent removal of the tested phenols could be due to the increase in the number of available binding sites with increasing adsorbent dose. On the other hand, the amounts of phenolic compounds adsorbed per unit mass of the tested adsorbents decreased consistently with the increase of the free and entrapped fungus concentrations in the medium. The decrease in the adsorption capacity of the adsorbents used could be due to the fact that the adsorbing sites of the biomass could not be completely filled with phenolic compounds. The percent removal order for phenolic compounds is pentachlorophenol > phenol > 4-nitrophenol,

Fig. 3 The zeta potentials data of the **A** Bare Ca-alginate, **B** *L. sajor-caju* biomass, and **C** Ca-alginate-*L. sajor-caju* biomass as a function of medium pH. Zeta potential measurements were achieved in each condition as three measurement replicates to achieve a reliable data basis for values
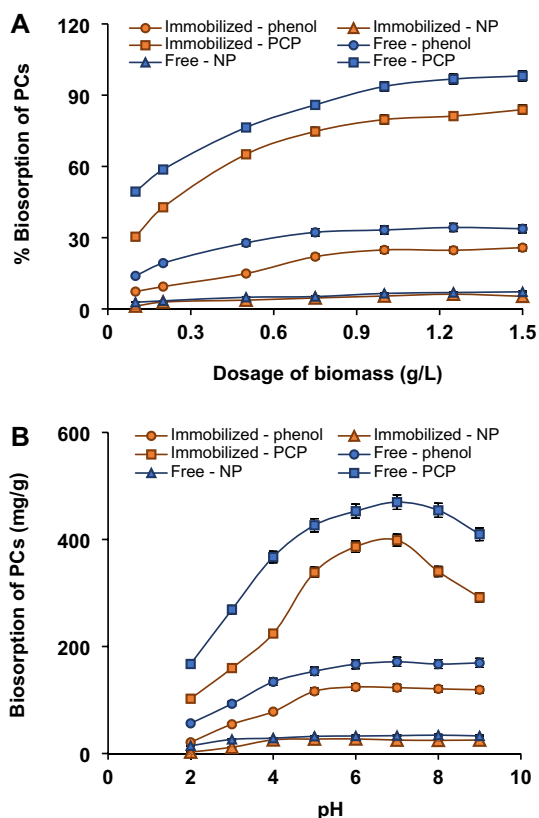
Fig. 4 **A** Sorbent dosage on the adsorption performance of phenol, 4-nitrophenol and pentachlorophenol (initial concentration of the phenolic compounds, 500 mg/L; temperature, 25 °C; medium pH, 5.0 for phenol and 4-nitrophenol and pH 7.0 for pentachlorophenol; contact time, 360 min; stirring rate 150 rpm). **B** Effect of pH on phenol, 4-nitrophenol and pentachlorophenol adsorption on the free and entrapped fungus. (adsorbent dose, 1.0 g/L; initial concentration of the phenolic compounds, 500 mg/L; temperature, 25°C; contact time, 360 min; stirring rate 150 rpm).

and 1.0 g adsorbent dosage was used in the 1.0 L medium in the remaining studies.

Effect of pH on adsorption performance

Solution pHs affect the ionization state of useful groups found on the fungus, such as carboxylate, phosphate, and amino groups, as well as the solubility of phenolic compounds. Therefore, a change in the pH of the solution can lead to an increase or decrease in the removal performance of adsorbents. The effect of pH on the adsorption capacities of the free and entrapped fungus was investigated in aqueous solutions containing phenol, 4-nitrophenol, and pentachlorophenol in the pH range 2.0–9.0 (Fig. 4B). As shown in this figure, the removal efficiencies of PH and NP compounds were observed to be maximum at pH 6.0 for both the free and entrapped fungus, while this value was found to be pH 7.0 for PCPH adsorption. As the pH

values increased from 2.0 to 6.0, the adsorption capacities of both tested adsorbents for phenol and 4-nitrophenol were increased (Fig. 4B), whereas the removal performance of adsorbents was not changed significantly with the increase of medium pH from 6.0 to 9.0. On the other hand, the adsorption capacities of the free and entrapped fungus for pentachlorophenol increased up to pH 7.0 and then declined until the tested pH value of 9.0 (Fig. 4B). At pH 6.0, the maximum amount of adsorbed phenol and 4-nitrophenol on the free and entrapped fungus were found to be 170.3 and 125.2, and 34.5 and 27.1 mg/g, respectively. Whereas the maximum adsorption for PCPH was 516.7 and 398.6 mg/g at pH 7.0 for the free and entrapped fungus, respectively. When the pH value of the solution was increased above 7.0, a reverse trend was detected, and the adsorption capacity of both adsorbents steadily decreased for PCPH. Pentachlorophenol was adsorbed faster than that of phenol and 4-nitrophenol on both adsorbents. This may be due to the higher affinity of the tested adsorbents to PCPH and also to the surface charge distributions of these adsorbents. As reported previously, the amine groups are the major components of the fungus cell surface and can form an important binding site for chlorophenols at neutral pH value via hydrogen bonding interaction. In a reported study, the adsorption of phenol, mono-, di-, tri-, and penta- chlorophenols using granular sludge containing anaerobic bacteria, it has been shown that the removal efficiency of PCPH increases with the increase of the medium pH. Wu and Yu (2006) reported that the amount of adsorbed phenolic compounds is associated with the dissociation constant of phenolic species. The ionic groups of the phenolate ion increase with increasing pH, and the surface charge of the fungus is largely negative in the pH range of 3.0–10.0 (Rao and Viraraghavan 2002). Thus, electrostatic forces between charged fungus surfaces and phenol may play a significant role in adsorption. Radhika and Palanivelu (2006) reported that the pH of the medium will lead to the ionization state of PCP and effects the net charge of the fungus surfaces. Under neutral and basic pH conditions, the biomass of fungi could have a net negative charge distribution and PCPH was totally in anionic form. Therefore, the PCPH was more strongly adsorbed compared to PH and NPH. Kennedy et al. (1992) reported that when the amount of substituted chlorine groups increased on the phenol ring the adsorption performance of the adsorbent increased. Similar optimum pH values were reported in the literature for the adsorption of phenolic compounds on the different white rot fungi. For example, the maximum adsorption of chlorophenols was detected at pH 6.0 for all the tested chlorophenol species (Denizli et al. 2005). The maximum PCPH adsorption *Anthracophyllum discolor* was obtained at pH 5.5 in a fixed-bed column reactor (Rubilar et al. 2012). From these observations, the binding interaction of the phenolic compounds on the free and entrapped fungus

could be considered primarily as non-polar, and physical van der Waals forces.

Adsorption isotherms and kinetic model studies

From Fig. 5A, the phenolic compounds removal efficiency and the adsorption capacities of the free and entrapped fungus preparations improved with the increase of the phenolic compound concentration in the adsorption medium. For both adsorbents, the maximum equilibrium adsorption was observed at 500 mg/L initial adsorbate concentration. It is thought that the increase in the adsorption capacity of the adsorbent with the increase of the phenolic compound concentration in solution is due to the higher probability of collision between the pollutants and the active sites on the

Fig. 5 A Effect of initial concentration of phenol, 4-nitrophenol and pentachlorophenol on adsorption capacity of the free and entrapped fungus biomass. (adsorbent dose, 1.0 g/L; temperature, 25 °C; medium pH, 5.0 for phenol and 4-nitrophenol and pH 7.0 for pentachlorophenol; stirring rate 150 rpm). **B** Effect of contact time on adsorption of phenol, 4-nitrophenol and pentachlorophenol on the free and entrapped fungus biomass, (adsorbent dose, 1.0 g/L; initial concentration of the phenolic compounds, 500 mg/L; temperature, 25°C; medium pH, 5.0 for phenol and 4-nitrophenol and pH 7.0 for pentachlorophenol; stirring rate 150 rpm).

adsorbent (Bayramoglu et al. 2019). The maximum adsorption capacities of the free and entrapped fungus were 170.3 and 125.2 mg/g for PH, 34.5 and 27.1 mg/g for NPH, and 516.7 and 398.6 mg/g for PCPH from the solution, respectively (Fig. 5A). It was observed that in both adsorbents used in the removal of phenolic compounds adsorption capacity, the order was PCPH > PH > NPH.

The adsorption of phenols on the fungus preparations was studied using different adsorption isotherm models (i.e., Langmuir, and Freundlich) to describe experimental data. The equation for the Langmuir adsorption model (Langmuir 1918):

$$q = q_{\mathrm{m}}C / \left(K_{\mathrm{d}} + C\right) \tag{1}$$

The Freundlich adsorption isotherm equation (Freundlich 1907):

$$q = K_{\mathrm{F}}(C)^{1/n} \tag{2}$$

The Langmuir plots were obtained for the adsorption of phenols with the tested adsorbents and the values of R^2 were between 0.999 and 0.895 for the free and entrapped fungus, respectively (Table 1). The values of maximum equilibrium adsorption capacities (q_{m}) of the free and entrapped fungus were determined as 173.9 and 205.9 mg/g for the PH, 37.8 and 39.8 mg/g for NPH, and 522.4 and 895.3 mg/g for PCPH, respectively, and calculated from the slope of the plots (i.e., C vs. C/q). The experimental adsorption capacities (q_{exp}) of the free and entrapped fungus were found to be for phenol 170.3 and 125.2 mg/g, for 4-nitrophenol 34.5, and 27.1 mg/g and for pentachlorophenol 516.7 and 398.6 mg/g, respectively (Table 1). It appears that the determined theoretical values of q_{m} for the free biomass are in agreement with the experimentally achieved adsorption capacities (q_{exp}), but the q_{m} values calculated for immobilized biomass are quite different from the experimental results, especially for PH and PCP. Since the nonlinear Langmuir and semilogarithmic Scatchard curves indicated adsorption heterogeneity of the phenolic compounds. Therefore, the adsorption of phenolic compounds on the entrapped fungus could not be well explained by the Langmuir model. Experimental values obtained in the adsorption of phenolic compounds were plotted according to the Freundlich isotherm model, and the isotherm parameters such as n and K_{F} and the validation coefficients (R^2) were determined (Table 1). Analysis of the correlation regression coefficient R^2 shows that the adsorption process better fits the Freundlich isotherm (Table 1). The main limitation of the Freundlich equation is that the maximum adsorption capacity cannot be evaluated. The magnitude of the K_{F} and n values of the Freundlich model showed that both the used adsorbents had high adsorption capacities and easy uptake of all the tested phenolic compounds from the solution. For both fungus preparations
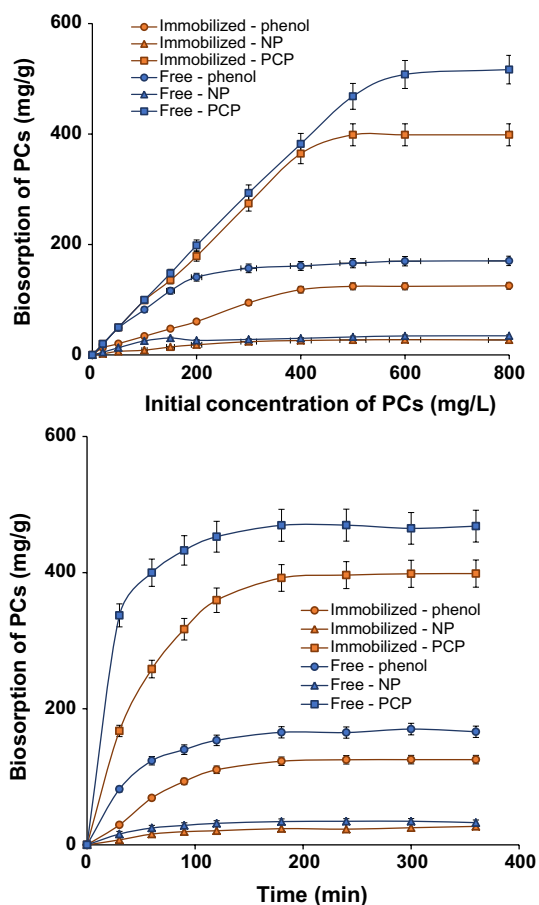
Table 1 Langmuir and Freundlich isotherm model constants for the adsorption of the phenolic compounds using the free and entrapped *L. sajor-caju* in Ca-alginate beads

	q_{exp} (mg/g)	Langmuir constant			Freundlich constant		
		q_m (mg/g)	$K_d \times 10^4$ (M)	R^2	n	K_F	R^2
Free *L. sajor-caju* biomass							
NP	34.5	37.8	4.95	0.988	2.33	2.57	0.879
Phenol	170.3	173.9	1.48	0.999	3.47	33.1	0.906
PCP	516.7	522.4	0.12	0.998	2.89	119.1	0.965
Entrapped *L. sajor-caju* in Ca-alginate beads							
NP	27.1	39.8	19.8	0.970	1.42	0.36	0.978
Phenol	125.2	205.9	43.9	0.946	1.44	1.51	0.987
PCP	398.6	895.3	28.7	0.895	1.16	1.74	0.990

NP 4-nitrophenol, *PCP* pentachlorophenol

examined, the maximum experimental adsorption capacity of phenolic compounds in the order of pentachlorophenol > phenol > 4-nitrophenol. Following the same sequence, the decrease in the K_F values determined by the application of the Freundlich isotherm model, that is, the increase in the K_F value with the increased adsorption capacity, showed that the adsorption process between the phenolic compounds and the tested adsorbent could be explained by the Freundlich model. Thus, this model quite well described the results in agreement with the heterogeneity of the free and entrapped fungus surface (Table 1). In conclusion, the adsorption of phenol, 4-nitrophenol, and pentachlorophenol by free and entrapped fungus appears to be a complex process involving multilayer, interaction, or multisite-type binding.

From Fig. 5B, the adsorption equilibrium time of the PH, NPH, and PCPH at 500 mg/L initial concentration from the aqueous solution with the free and entrapped fungus was reached within 180 min. The observed change in the adsorption equilibrium was very fast in the early stage up to 120 min and gradually reached equilibrium adsorption within 180 min. As reported earlier, the adsorption kinetics of phenols were obtained with different adsorbents and these results exhibited a wide range of adsorption rates. In

this work, the obtained fast adsorption rates were found to be between 120 and 180 min. The presented results are in good agreement with the results of previously reported works (Kennedy et al. 1992, and Calace et al. 2002).

The adsorption kinetics of the free and entrapped fungus were examined using the first-order and second-order kinetic models. The first-order kinetic from the solution is expressed by the following equation (Lagergren 1898; Bayramoglu et al. 2022)

$$\log (q_e - q_t) = \log q_e - (k_1 \cdot t) / 2.303 \tag{3}$$

The following second-order equation (Ho and McKay 1999):

$$t/q_t = 1/(k_2 q_{e2}) t + 1/q_e \tag{4}$$

The initial adsorption rate (h) values of phenol, 4-nitrophenol and pentachlorophenol were 5.11, 1.16 and 44.3 mg/g min and 2.05, 0.67 and 10.6 mg/g min for the free and entrapped fungus, respectively, (Table 2). It is seen that the R^2 values for the second-order equation are greater than 0.998 and 0.994 for the free and entrapped fungus when compared with the first-order equation R^2 values, which are

Table 2 Kinetic model parameters for the adsorption of the phenolic compounds with the free and entrapped *L sajor-caju*

	First-order kinetic model				Second order kinetic model			
	q_{exp} (mg/g)	q_e (mg/g)	$k_1 \times 10^2$ (min)	R^2	q_e (mg/g)	$k_2 \times 10^4$ (g/mg min)	H (mg/g min)	R^2
Free *L. sajor-caju* biomass								
NP	34.5	89.1	3.02	0.961	38.6	7.79	1.16	0.999
Phenol	170.3	489.8	3.17	0.964	191.0	1.40	5.11	0.998
PCP	516.7	933.2	3.99	0.956	536.9	1.05	44.3	0.998
Entrapped *L. sajor-caju* in Ca-alginate beads								
NP	27.1	60.4	0.276	0.976	31.6	6.71	0.67	0.994
Phenol	125.2	478.6	2.43	0.938	156.7	0.84	2.05	0.996
PCP	398.6	756.7	2.83	0.962	454.8	0.51	10.6	0.999

0.956 and 0.938, respectively. These results show that the adsorption process fits the second order equation better than first-order kinetic (Table 2). In addition, the calculated adsorption capacity values (q_e) obtained from the second order kinetic equation for phenolic compounds using fungus preparations were in agreement with the experimental values (q_{exp}). The values of theoretical equilibrium adsorption capacities (q_e) increased for the free and entrapped fungus from 38.6 to 536.9 mg/g and from 31.6 to 454.8 mg/g, respectively. On the other hand, second-order rate constants (k_2) were found to be decreased for the free fungal biomass for adsorption of phenolic compounds from 7.79×10^{-4} to 1.16×10^{-4} and for entrapped counterpart from 6.71×10^{-4} to 0.51×10^{-4}, respectively.

Adsorption mechanisms

Adsorption of PH, NPH, and PCPH by fungus preparations can be identified by hydrogen bonding, π–π interactions, and electrostatic interactions. The adsorption mechanisms of phenolic compounds are given in Fig. 6. As observed from the FTIR spectra, both -OH and –NH$_2$ useful groups were observed between 3400 and 3200 cm^{-1}. These groups are responsible for the hydrogen bond interactions and affected the adsorption capacity of the tested adsorbents to the phenolic compounds. Also, the peak observed around 1900 cm^{-1} was due to the increase due to aromatic ring displacement and may be responsible for π–π interactions. Aromatic rings of PH, NPH and PCPH may have entered into π–π interactions with the presence of π bonds on the adsorbent surface, which increases their adsorption capacity. Phenol-based aromatic structures not only use a strong π–π interaction, but also a high van der Waals interaction with the adsorbent and can also enhance the adsorption capacity of the adsorbent (Xing et al. 2012; De Paula et al. 2022). As the solution pH increases, the negatively charged phenolic compound and positively charged amine groups may have interacted electrostatically, improving the adsorption capacity of fungus preparations. Under the collective result of these interaction mechanisms, entrapped fungus showed good adsorption performance, especially for phenol and pentachlorophenol, and could demonstrate talented performance in wastewater management.

Adsorption studies in the packed bed reactor

Modeling to accurately describe the dynamic behavior of adsorption in a packed bed reactor system is difficult because
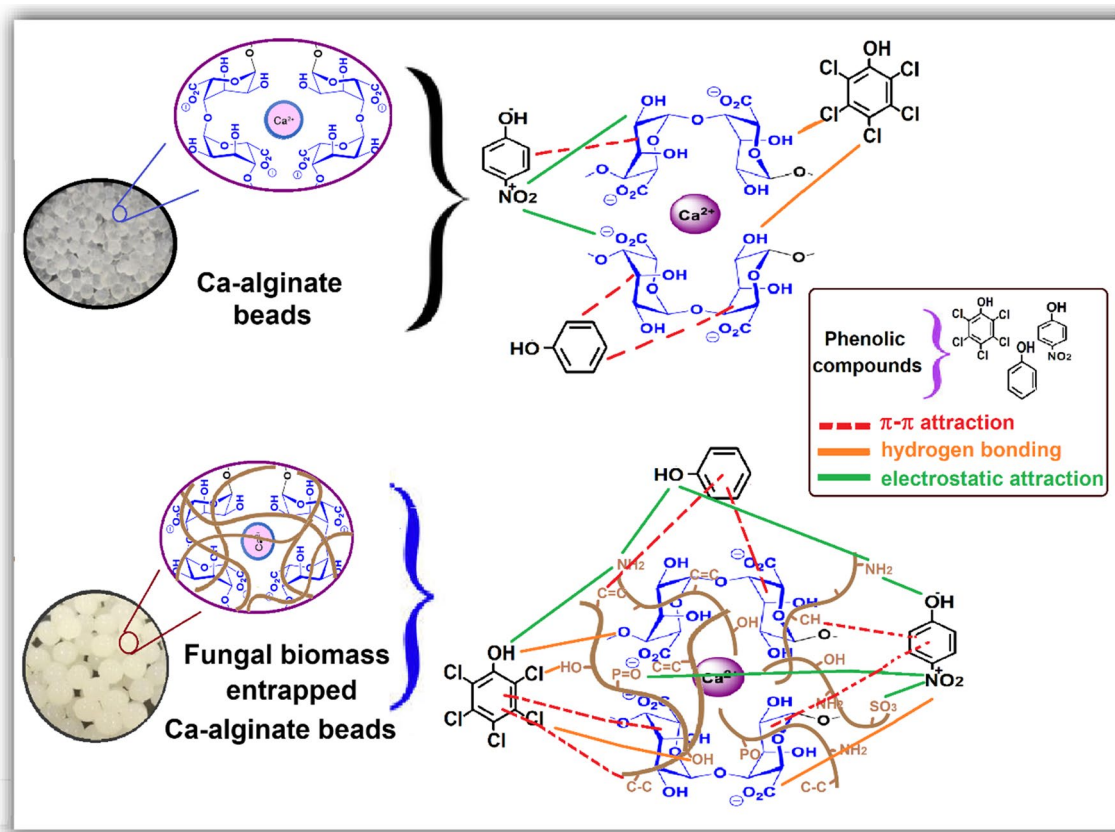

Fig. 6 The description mechanism of the adsorption of phenol 4-nitrophenol and pentachlorophenol

it depends on many parameters. The removal of the phenol, 4-nitrophenol, and pentachlorophenol from the aqueous solution under optimum conditions was investigated in a packed bed reactor system, using the entrapped fungus (Figure S2). The initial concentration of each phenolic compound in the feed solution was selected as 500 mg/L. An individual feed solution containing one of the tested compounds has been introduced to the reactor at a flow rate of 10 mL/h. The adsorption capacity of the entrapped fungus was determined for each phenolic compound. Standard PH, NPH, and PCPH solutions were analyzed by HPLC as described above, and the initial and residual concentrations of phenolic compounds were calculated by integrating the area of the relevant peak from the chromatograms obtained as a result of the HPLC analysis of the samples. The chromatogram peaks for phenol, 4-nitrophenol, and pentachlorophenol are presented in Figure S1. The amounts of adsorbed phenolic compounds by the entrapped fungus in the packed bed reactor are presented in Fig. 7. The adsorption capacity of the entrapped fungus was calculated according to the Thomas model. The reactor was continuously operated by feeding with phenol or 4-nitrophenol at pH 6.0, and pentachlorophenol at pH 7.0 for 180 min. After this period, the amount of the adsorbed PH, NPH, or PCPH on the entrapped fungus was found to be 347.6, 92.8, and 489.5 mg/g, respectively. These values were obtained as 27.1, 125.2, and 398.6 mg/g for PH, NPH, and PCPH in the batch system, respectively. The main reason for achieving higher adsorption capacity in the continuous system should be related that the adsorbent was in contact with the phenolic compounds at around the equilibrium concentration. This value was close to the feed concentration in the continuous system, while in the batch system, the adsorbent contacts the
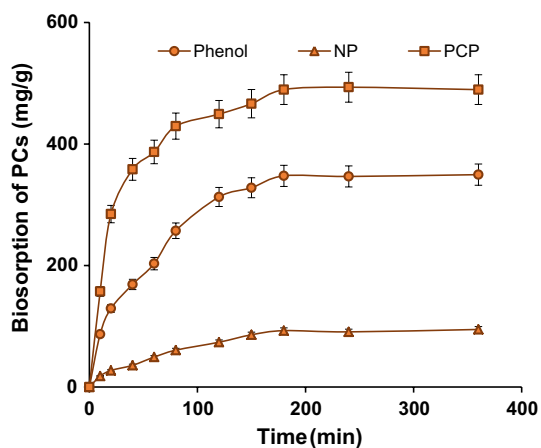

Fig. 7 Effect of initial concentration of phenol, 4-nitrophenol and pentachlorophenol on adsorption capacity of the entrapped fungus biomass in packed bed reactor. (Concentration of the phenolic compounds, 500 mg/L; flow rate, 10 mL/h; contact time, 360 min; pH, 5.0 for phenol and 4-nitrophenol and pH 7.0 for pentachlorophenol)

sorbate at a constantly decreasing equilibrium concentration of the phenolic compounds. It was thought that the high concentration gradient provided in the continuous system caused a significant increase in the adsorption capacity of the entrapped fungus.

Conclusion

The adsorption efficiency of the free and entrapped fungus was studied using PH, NPH, and PCPH as test compounds. The fungal biomass and Ca-alginate are natural materials with reasonable properties and their composite form can act as a good adsorbent system for the removal of toxic phenolic compounds. The entrapped fungus was also operated in a packed bed reactor in a continuous manner and efficiencies in the adsorption of the phenolic compounds from solution were evaluated. This study also showed that an efficient packed-bed reactor could be prepared using composite Ca-alginate-fungus and used as an efficient system for the adsorption of toxic phenolic compounds. Moreover, the packed-bed reactor was operated for a prolonged period without any blockage. The adsorption capacities of both the free and entrapped fungus were affected by the medium pH, and the maximum PCPH adsorption capacities were found to be 516.7 and 398.6, respectively, at pH 7.0. On the other hand, the maximum PH and NPH adsorption capacities were found to be 170.3 and 34.5 mg/g for the free fungus and 125.2 and 27.1 mg/g for the entrapped fungus, respectively, at pH 5.0. The adsorption fitted to the Freundlich isotherm and adsorption kinetics followed pseudo-second order model. The adsorption of the PCPH on both free and entrapped fungus was higher compared to PH and NPH, thus, demonstrating that the adsorption process was majorly influenced by hydrogen binding interaction with functional groups of the adsorbent surfaces. Finally, the potential use of white rot fungi to remove phenol and pentachlorophenol from the solution has been reported in the literature (Bayramoglu et al. 2009; Farkasa et al. 2013; Rubilar et al. 2012). The absorption rates of the free *Pleurotus sajor-caju* (Denizli et al. 2005) and *Funalia trogii* (Bayramoglu et al. 2009) were reported as 89.3 mg/g (or 0.95 mmol/g) and 132.6 mg/g for phenol, respectively. Pentachlorophenol adsorption was studied using a white-rot fungus (i.e., Anthracophyllum discolor) and the amount of adsorbed PCP was reported as 20.5 mg/g (Rubilar et al. 2012). In this work, the adsorption rate of the free *Lentinus sajor-caju* for phenol and pentachlorophenol was found to be 170.3 and 516.7 mg/g and the obtained results were quite comparable with these related studies.

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Availability of data and materials All data and materials are true and valid and can use general repositories saving and also data are provided upon request.

Declarations

Conflict of interests No competing interest.

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication All authors have given consent for publication.

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