Research Article
Hittite Journal of Science and Engineering, 2023, 10 (3) 219–228
ISSN NUMBER: 2148–4171
DOI: 10.17350/HJSE19030000310

Use of Heat-killed *Aspergillus ochraceus* NRRL 3174 Discs as Biosorbent for Petroleum Removal

Nermin Hande Avcioglu **Sezen Bilen Ozyurek** **Isil Seyis Bilkay**
Hacettepe University, Department of Biology, Ankara, Türkiye

Article History:
Received: 2023/03/13
Accepted: 2023/07/11
Online: 2023/09/30

***Correspondence to**: Nermin Hande Avcioglu,*
E-mail: hurkmez@hacettepe.edu.tr, Phone: +90 312 297 8024;
Fax: +90 312 299 2028

ABSTRACT

The purpose of this work was to evaluate the petroleum sorption capacity of heat-killed fungal discs obtained from *Aspergillus ochraceus* strain. Effect of various parameters such as biosorbent dose (0.5g-2.5g/100mL), petroleum concentration (0.5-5%), pH (4.0-8.0), contact time (1-12h) and re-usability of biosorbent (1-6) were investigated. Accordingly, the highest biosorption capacity was obtained with 1% petroleum concentration, 1.5 g/100mL heat-killed fungal discs, 10h contact time at pH: 5.0 and at room temperature. Additionally, each disc was able to actively use for at least 6 more cycles in biosorption experiments. The specific removal rate was calculated as 0.114 day^{-1}, the rate constant and half-life period were also 1.609 day^{-1}, $t_{1/2}$ = 0.431, respectively. The kinetic study was described by the pseudo-second order model and the equilibrium modeling was found to be well fitted with Langmuir isotherm. The biosorbent(s) were characterized by Focused Ion Beam Scanning Electron Microscopy (FIB-SEM). Over 80% removal of long-chain *n*-alkanes by the heat-killed fungal discs was confirmed by GC-MS analysis. Since there has been no similar study investigating the sorption of petroleum with heat-killed *Aspergillus ochraceus* discs, this novel bio-based sorbent with its low cost, environmentally friendly and easy-to-apply properties can be used in advanced biosorption studies.

Keywords:
Petroleum; Biosorption; Heat-killed *Aspergillus ochraceus*; Biosorption Isotherm and Kinetics

INTRODUCTION

In recent years, vast amounts of petroleum pollutants have been released into the environment due to petrochemical activities. Physical, chemical, physicochemical, and biological methods are used in the treatment of polluted water (1-5). Although, these conventional methods are efficient, most of them are not cost-effective and have limitations (6). The biosorption and bioaccumulation processes which completely remove pollutants, have become remarkable with their low-cost, efficiency potential, high selectivity and easy designed (7, 8). Biosorption is known as physicochemical adsorption and ion exchange that occurs on the surfaces of organisms or biomaterials. Recent studies show that low-cost adsorbents are effective in the removal of organic pollutants and heavy metals from the aquatic ecosystem (9-15). Low-cost adsorbents are also used in the removal of petroleum and polycyclic aromatic hydrocarbons (PAHs) from contaminated areas (16, 17). Adsorbents like corn stack (18), orange peel (19) and pomegranate peel (20) have been used in the biosorption of petroleum hydrocarbons.

The biomass of bacteria, fungi and algae can be used as biosorbent in removing of pesticides, dyes, heavy metals, and organic pollutants from the environment (21, 22). Among them, indigenous microorganisms due to prolonged exposure to these pollutants have higher tolerance with increased adaptive capacity. Fungi, which constitute a large part of biodiversity, play an important role in ecological cycle. Therefore, it is crucial to determine the fungal tolerance and diversity in heavily polluted environments (23). Several filamentous fungi such as *Penicillium* sp., *Aspergillus* sp., *Trichoderma* sp., *Fusarium* sp., *Alternaria* sp., *Geotrichum* sp., *Rhizopus* sp., and *Monilia* sp. have high remediation potential (24). Filamentous fungi, which are ubiquitous in the environment, are among the most economical and bio-friendly biosorbents due to their vegetative properties. They also have advantages such as large-scale biomass production, low cost, and high production capacity when compared to other microorganisms (25, 26). Previous studies have been reported that different *Aspergillus* species as *A. niger*, *A. flavus*, *A. versicolor*, and *A. tamarii* NRC3 were potently used in biosorption processes (25, 27). Among

Cite as:
B. BICAK and S. KECEL GUNDUZ, "Interaction of Ghk Tripeptide with Receptors Targeted in Some Cancer Studies: A Theoretical Approach with Molecular Docking" Hittite Journal of Science and Engineering, vol. 10, no. 3, pp. x–x, 2023. doi:10.17350/hjse19030000xxx

them, *Aspergillus ochraceus* is a fungus that can be frequently encountered in decaying vegetation, soils, various agricultural products, and grains. In addition to mycotoxin, antimicrobial agent, and enzyme production that can be used in industrial processes, it has a great capacity to biosynthesize various metabolites (28, 29).

Fungi are also frequently used in the fermentation industry as they can adapt to changes in pH, oxygen levels and temperature, as well as nutrient concentration. So, they are particularly abundant in industrial waste products after fermentation processes (25). The use of high amount of fungal biomass as a biosorbent in the removal of organic pollutants from an aquatic environment is an effective, reliable, and economical method. Since the biosorption process does not involve metabolic activity, it can occur in any living or dead cell. Dead cell is more advantageous to be used as a biosorbent in biosorption processes when compared to livings cell as it does not need any other nutrients and can be used in many cycles (30). Similar studies reported that dead fungal biomass has higher sorption efficiency in the removal of toxic organic pollutants (31-33). Raghukumar et al. (34) also reported that a dead fungal biomass of marine fungi NIOCC#312 could rapidly absorb phenanthrene from contaminated aquatic environment. So, organic pollutants can be adsorbed by dead cells by surface absorption, carbon sequestration and chemical reactions (30). Accordingly, the fungal biosorption is based on three basic mechanisms as (1) Extracellular accumulation (2) Intracellular accumulation and (3) Cell surface sorption/precipitation (25). Adsorption capacity of biosorbent vary depending on their porosity, specific surface area and functional groups. An ideal biosorbent should be non-toxic, easily available, reusable, has high affinity, and be used in large scale areas (30).

This study aims to investigate the biosorption capacity of heat-killed *Aspergillus ochraceus* disc with its intriguing structural features. In this context, heat-killed *Aspergillus ochraceus* discs were used as a novel and eco-friendly biosorbent for petroleum biosorption. Although a few studies have been reported on biosorption of organic pollutions using low-cost sorbents, it has not been reported a similar study that investigates the sorption of petroleum with heat-killed *Aspergillus ochraceous* discs. Accordingly, the main objectives of this study are (i) to investigate the efficiency of a new biosorbent in terms of petroleum biosorption; (ii) to optimize the biosorption parameters such as biosorbent dose, petroleum concentration, pH, contact time, and reusability; (iii) to clarify the biosorption process by studying its kinetic and equilibrium modeling (iv) to characterize the surface morphology of biosorbent, and (v) to approve the biosorption of short-, medium- and long-chain *n*-alkanes in the petroleum with using heat-killed fungal discs.

N. H Avcioglu at al / Hittite J Sci Eng, 2023, 10 (3) 219–228

MATERIALS AND METHODS

Fungal strain and cultivation conditions

Aspergillus ochraceus NRRL 3174 strain was obtained from Hacettepe University Culture Collection Laboratory, Beytepe, Ankara, Turkey. To enhance fungal spores, *A. ochraceus* was inoculated into Potato Dextrose Broth (PDB, Merck, Millipore, Germany) and incubation was carried out at 30 °C and 150 rpm for 7 days (Miprolab, Turkey). The fungal cultures were stored at 4 °C in the refrigerator for further use.

Biosorbent preparation

To prepare fungal discs, *A. ochraceus* strain was inoculated on Potato Dextrose Agar (PDA, Merck, Millipore, Germany) and incubation was carried out at 30 °C for 7 days in a static incubator (Miprolab, Turkey). Following this, the fungal culture was cut with a perforator (a diameter of 7 mm) and discs were autoclaved three times to obtain heat-killed fungal discs to be used as biosorbent for further experiments (35).

Optimization of biosorption capacity

Distilled water (pH=7.0) containing 1% (v/v) Triton X: 100 was sterilized at 121 °C for 15 min (Prior Clave, UK) for petroleum biosorption. Following the cooling, 1% (v/v) of petroleum sterilized with 0.22µm pore size cellulose acetate syringe filter (Millipore, Sartorius, Germany) was added. To determine the optimal parameters for petroleum biosorption, biosorbent dose (0.5g-2.5g/100mL), petroleum concentration (0.5-5%), pH (4.0-8.0), contact time (1-12h) and re-usability of heat-killed discs (1-6) were investigated. The biosorption capacity of heat-killed *A. ochraceus* discs were determined as follows:

$$Q_e = \frac{C_i - C_f}{m} xV \quad (1)$$

(Qe: adsorption capacity (g/g), Ci: initial concentration of petroleum (g/L), Cf: concentration of petroleum in equilibrium (g/L), m: mass of biosorbent (g), V: volume (L)) (36). Experiments were carried out in 3 parallels with abiotic control group.

Petroleum extraction and gravimetric analysis

Following the sorption of petroleum by heat-killed fungal discs under optimized conditions, the remaining petroleum was extracted with dichloromethane (DCM) (CH_2Cl_2) (1:2) (Sigma- Aldrich, USA). The flasks were left at 90 °C for 1 hour to evaporate the solvent (Memmert, Schwabach, Germany). The biosorption of petroleum was also calculated using the following equation:

$$Removal(\%) = \frac{(p0 - p1 - p2)}{p0} \times 100 \quad (2)$$

p_0 and p_1 indicate the initial and remaining concentrations of petroleum at different incubation periods, p_2 is the abiotic loss of petroleum (35, 37).

The kinetics of petroleum removal

The specific removal rate was calculated as described before Bilen Ozyurek and Seyis Bilkay (38). The specific rate was expressed by the following formula:

$$d_x \,/\, x_0 \times d_t \quad (3)$$

d_x shows the change in petroleum concentration (mg/L), x_0 indicates the initial concentration of petroleum (mg/L), and dt is the time interval ($hour^{-1}$).

The rate constant and half-life period of petroleum was calculated using the following equations:

$$ln_{ct} = ln_{co} - K_t \quad (4)$$

$$t_{1/2} = ln2 / k \quad (5)$$

c_o and c_t denote the initial and remaining concentrations of petroleum (mg/L), K is rate constant of the change in the petroleum content ($hour^{-1}$), and *t* is contact time (hour) (24, 39).

Re-usability of heat-killed fungal discs

Following the petroleum adsorption, fungal discs were removed from the biosorption medium and dried at 30˚C for 24h. Dried fungal discs were autoclaved three times to be used as biosorbent for re-usability experiments. Biosorption was carried out with re-used fungal discs under optimized conditions (1-6 times).

Characterization of heat-killed *A. ochraceus* discs with FIB-SEM (Focused Ion Beam Scanning Electron Microscopy)

A. ochraceus discs were coated with gold (Leica ACE 600) and FIB-SEM (FIB-SEM GAIA3, Tescan, Czech Republic) was used to observe petroleum biosorption by heat-killed *A. ochraceus* discs operating at 2 kV and 4 kV with magnifications of 150x, 100x and 1kx.

GC-MS (Gas Chromatography-Mass Spectrometry)

The GC-MS analysis was carried out to determine the change in the petroleum content according to Bilen Ozyurek et al. (2021) by using TRB-1 GCMS-QP-2020 (Shimadzu, Tokyo, Japan), fitted with a capillary column, 100% Dimethyl polysiloxane, (TRB-1 Teknokroma, Spain) (TR-110132) (30m × 0.25mm × 0.1mm) (length: column ID: film thickness) by the Petroleum Research Center at Middle East Technical University (METU) in Ankara, Turkey. The extraction of residual petroleum was carried out with DCM (1:2). The carrier gas was helium at 1.5 mL/min and the injection temperature was set to 250 °C; the temperature program was adjusted to 40 °C (5 min) to 180 °C at 8 °C/min and then to 320 °C (16 min) at 10 °C/min.

RESULTS AND DISCUSSION

Optimization of biosorption capacity

In recent years, researchers have focused on the surface and cell absorption studies with different groups of microorganisms in the removal of petroleum hydrocarbons. So, as the petroleum removal efficiency of live *Aspergillus ochraceus* discs was found as 94% in a previous study, the biosorption capacity of heat-killed *A. ochraceus* discs has also been investigated (35). Due to the ubiquity of this fungi in aquatic ecosystem, there has been a tendency to use heat-killed cells of fungi as biosorbent (30, 40, 41). The biosorption activities of *A. ochraceus* were also reported in a few studies (29, 42). It has been reported that low-cost, environmentally friendly, and easy to scale fungal biomasses are used in biosorption processes (33, 43). However, the environmental factors such as pH, temperature, and salinity should be optimized for the maximum petroleum biosorption (30).

In this study, the biosorption of petroleum with 0.5-2.5 g/100mL (1–5 pieces of disc) heat-killed fungal discs was investigated. Thus, it was determined that the highest biosorption obtained with 1.5 g/100mL heat-killed fungal discs (Figure 1a). Due to the competition and overlap between the heat-killed fungal discs, the increase in the number of discs causes a decrease in petroleum biosorption. Similarly, Kumar and Mukherji (44) reported that 0.1% algae biomass in the range of 0.1-2% (v/v) was used in the sorption of diesel and motor oil.

Biosorption of 0.5% - 5% (v/v) petroleum with heat-killed fungal discs was also investigated. While a maximum biosorption was found with 1% (v/v) (96 mg/L) petroleum, a decrease was observed at values <1% and >1% (Figure 1b). Due to the complex structure of petroleum, there was a decrease in biosorption with the increasing of petroleum concentration. The certain compounds in petroleum are easily adsorbed by heat-killed cells. However, since reversible adsorption occurs in these polar compounds, the adsorbed components are released by dead cells over time (30). Similarly, Al-Hawash et al. (24) reported that petroleum hydrocarbons were adsorbed effectively by heat-killed *Aspergillus* sp. strain. The fungi with high biosorption capacity can adsorb not only petroleum hydrocarbons but also heavy metals. Accordingly, several filamentous fungi have been reported to be effective in the sorption of copper and cobalt (25). Simonescu and Ferdes (45) reported that an increasing copper ion concentration caused an increase in copper uptake, but a decrease in uptake efficiency. They also reported that the specific copper sorption of *Aspergillus oryzae* ATCC 11489, *Aspergillus oryzae* ATCC 20423, *Fusarium oxysporum* MUCL 791, and for *Polyporus squamosus*, and *Aspergillus niger* ATCC 15475 varied between 1.66 mg/g and 7.52 mg/g. Moreover, *Fusarium oxysporum* MUCL 791 showed a maximum specific copper uptake in the presence of 100 mg/L of copper ion concentration. The success of fungi in metal biosorption can be explained by their distinctive features such as solid cell wall composed of mineral ions and nitrogen, and filamentous branching growth habits (25, 46).

It is known that pH affects the biosorption by changing the chemistry of the solution, the competition of sorbate ions and the activity of the functional groups of the biosorbent (47). In this study, the biosorption of petroleum with heat-killed fungal disc was investigated in the range of pH: 4.0-8.0. Thus, a decrease was observed at values pH <5 and >5 (Figure 1c). This can be explained by the increased hydrogen ions preventing the adsorption of petroleum to the cation binding sites (48). Hence adsorption capacity decreases with increasing H^+ ions. Low biosorption capacities of heat-killed fungi were obtained at pH: 4.0 due to the protonation of functional groups on the cell wall (49). Devi et al. (50) reported that pH: 4.0 was found to be optimum for the removal of organic pollutants. Similarly, Mwandira et al. (51) showed that lead removal capacity of *Oceanobacillus profundus* increased with up to pH 5 and then decreased. And another study reported that the maximum petroleum sorption with live fungal pellets of *Aspergillus* RFC-1 was observed at pH: 6.0-7.0, a decreased was detected at pH: 8.0 (24). Additionally, the optimal pH value for copper uptake of *Trametes versicolor* was reported as pH: 5.0 (52) and pH: 6.5 for *Aspergillus niger* (53). While it is known that changes in zeta potentials of petroleum at different pH levels affect biosorption, especially alkaline pH levels cause a decrease (30, 54). Also, the low amount of adsorption at acidic pH is due to the competition between hydrogen (H^+) and hydronium (H_3O^+) ions for cation binding sites in the solution, low solubility of petroleum hydrocarbons, and/or precipitation of ions (55).

The biosorption amount and concentration of petrochemical waste varies depending on the contact time, the structure of chemical and the type of microorganism (56). In this study, the biosorption of petroleum with heat-killed fungal discs was investigated at contact time between 1-12 hours. So, it was found that the petroleum biosorption gradually increased over time and reached a maximum value of Qe = 2.45. Since various *n*-alkanes, aromatics, resins and asphaltenes in the structure of petroleum were adsorbed more slowly on the surface of heat-killed fungal cells, the amount of adsorption reached equilibrium after 10^{th} hour (Figure 1d and Figure 2). In accordance, Xu et al. (57) reported that the adsorption of petroleum on dead biomasses gradually decreases due to the sorption of only certain compounds in the petroleum at a given contact time. Al-Hawash et al. (24) showed that the maximum adsorption of naphthalene, phenanthrene and pyrene by the dead fungal biomass reached maximum level at 10^{th} minute, but the maximum adsorption of petroleum by the dead fungal biomass was achieved at 40^{th} minute. In addition, *Aspergillus oryzae* ATCC 20423 reached its maximum removal efficiency (88.21%) for copper on the 5^{th} day of incubation (45).

To sum up, the optimum conditions were found to be as pH:5.0, 1% for petroleum concentration, 1.5 g/100mL for heat-killed fungal discs, 10h for contact time at room temperature (Qe= 2.45 g petroleum/g fungal disc) (Figures 1a, 1b, 1c and 1d). Besides, the specific removal rate was 0.114 with 0.004 h^{-1} of abiotic loss for petroleum. The rate constant and half-life period were calculated as 1.609 h^{-1}, $t_{1/2}$ = 0.431 according to the first-order kinetics model equation. Despite its low half-life, high biosorption efficiency of the fungal strain with high-rate constant has been emphasized in the literature (37, 58). The change in the petroleum concentration with heat-killed fungal disc between 1-12 hours was shown in Figure 2.

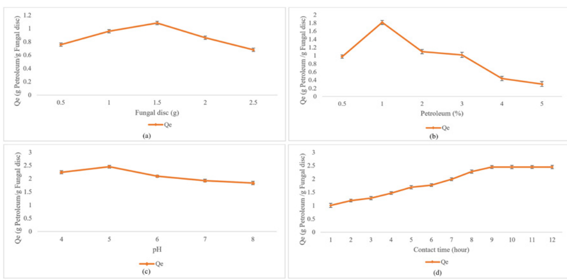

Figure 1. Optimization parameters for petroleum biosorption on heat-killed *A. ochraceus* discs, (a) Fungal disc (b) Petroleum concentration, (c) pH and (d) Contact time. *Each experiment is the mean of three data and the error bars represent the standard deviation

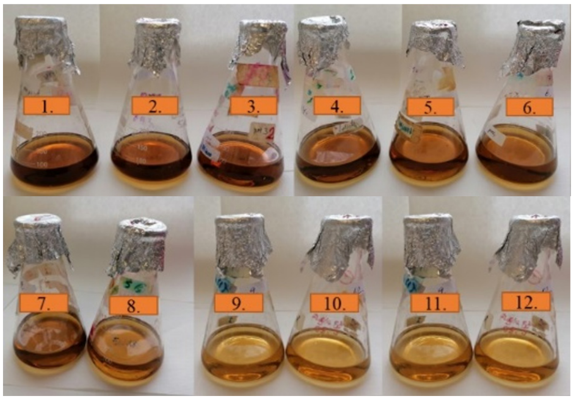

Figure 2. The biosorption of petroleum with heat-killed fungal disc at contact time between 1-12 hours. *The experiments were carried out under the optimum conditions for petroleum biosorption as 1% petroleum concentration, 1.5 g/100mL heat-killed fungal discs, at pH:5.0 and room temperature.

Kinetic study

To evaluate the rate and the mechanism of petroleum biosorption, kinetic studies were performed in terms of pseudo-first order and pseudo-second-order models with following equations (47);

The pseudo-first-order kinetic model was expressed as;

$$ln\left(q_e - q_t\right) = ln\left(q_e\right) - k_1 t \quad (6)\ (59)$$

The second-order kinetic model was expressed as;

$$\frac{t}{q_t} = \left(\frac{1}{k_2 q_e^{\,2}} + \frac{t}{q_e}\right) \quad (7)\ (60)$$

q_e is the amount of adsorbed petroleum at equilibrium in g/g, q_t is the amount of adsorbed petroleum at time t, k_1 and k_2 are the first and second order constants, respectively. Additionally, k_1 and k_2 values can be determined from the slopes of the linear plot of log (q_e-q_t) versus t and the linear plot of t/q_t versus t, respectively (61).

When the coefficients of R^2 values are examined, it is observed that the biosorption kinetic of petroleum on heat-killed *A. ochreceus* discs was well fitted with the pseudo-second order model (Figure 3). Pseudo-second kinetic model explains the kinetics of this reaction as the chemical interaction between the biosorbent and the sorbent surface in the rate-limiting step (47). Similar to our research, pseudo-second kinetic was found as the most suitable kinetic model to explain heavy metal removal with citric acid functionalized *Bougainvillea spectabilis* (7), Cd (II) removal with *B. spectabilis* (11), Alizarin Red S and Bromophenol blue dyes removal with phthalate-functionalized *Sorghum bicolor* (8), crude oil with chitosan (62), Cr (III) removal with chemically modified *Trifolium alexandrinum* (14) and heavy metal removal with magnetic adsorbent (15). Kinetic model parameters are shown in Table 1.

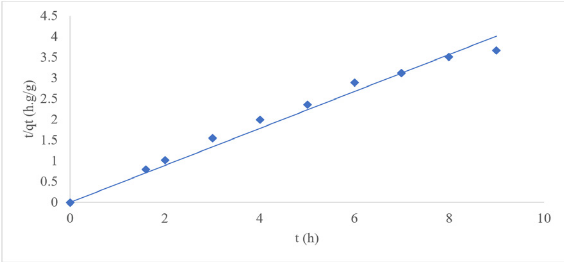

Figure 3. Pseudo-second order kinetic model of petroleum biosorption on heat-killed *A. ochraceus* discs. *The experiments were carried out under the optimum conditions for petroleum biosorption as 1% petroleum concentration, 1.5 g/100mL heat-killed fungal discs, at pH:5.0 and room temperature. Each experiment is the mean of three data.

Table 1. Kinetic model parameters for petroleum biosorption by heat-killed *A. ochraceus* discs (pH=5.0, 1.5 g/100mL heat-killed fungal discs, 1% petroleum concentration, contact time: 10h at room temperature, Qe= 2.45 g petroleum/g fungal disc).

Kinetic model	Parameter
Pseudo-first order	K_1- 0.0034 R^2- 0.7066
Pseudo-second order	K_2- 0.22 R^2- 0.8848

Biosorption isotherms

Langmuir and Freundlich isotherm models were applied to examine the biosorption mechanism of the petroleum by heat-killed *A. ochraceus* discs. Langmuir isotherm expresses a mono-layer system by means of the interaction between the specific homogeneous sites of the adsorbent surface and the adsorbate (61, 63). The linear form of the Langmuir isotherm is expressed as follows;

$$q_e = \frac{K_L q_m C_e}{1 + K_L C_e} \quad (8)\ (63)$$

R_L (separation factor) is used as the fundamental features of the Langmuir isotherm and expressed as;

$$R_L = \frac{1}{1 + K_L C_o} \quad (9)\ (64)$$

The adsorption process is defined as unfavorable if R_L >1, linear if R_L=1, favorable if 0<R_L<1 and irreversible if R_L=0 (64).

Freundlich isotherm model expresses a multilayer sorption by means of the interaction between the heterogenous surface of the adsorbent and the adsorbate (61). The linear form of the Freundlich isotherm is expressed as follows;

$$\ln q_e = \ln K_f + \frac{1}{n}\ln C_e \qquad (10)\ (61)$$

q_e is the amount of adsorbed petroleum at equilibrium in (g/g), K_L is the Langmuir constant (Lg^{-1}), q_m is the maximum adsorption capacity of the adsorbent (g/g) and C_e is the concentration of the adsorbent under equilibrium (g/L) (63). The slope of the linear plot of the ($1/q_e$) versus ($1/C_e$) gives ($1/q_mK_L$) and the intercept gives ($1/q_m$) in Langmuir isotherm (20) and the slope of the linear plots of ln q_e versus ln C_e gives 1/n and K_f in Freundlich isotherm (61).

According to the results obtained from isotherm analysis of heat-killed *A. ochraceus* discs it was observed that the isotherm of heat-killed fungal discs is well fitted with the Langmuir model (Figure 4) which describes the reaction as the biosorbent adsorb on the surface of sorbent as a monolayer. Moreover, it also explains the surface of the discs is homogeneous and all sides on its surface have the same adsorption energy (62, 64). Additionally, separation factor (R_L) is proved the favorability of this biosorption process (Table 2). Similarly, Langmuir isotherm is well fitted for the explanation of heavy metal, dye, and crude oil removal by using different sorbents in literature (7, 8, 11, 61-63, 65).

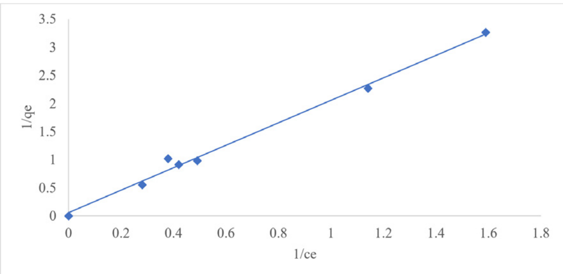

Figure 4. Langmuir isotherm model of petroleum biosorption on *A. ochraceus* discs. *The experiments were carried out under the optimum conditions for petroleum biosorption as 1% petroleum concentration, 1.5 g/100mL heat-killed fungal discs, 10h contact time, at pH:5.0 and room temperature. Each experiment is the mean of three data.

Table 2. Isotherm model parameters for petroleum biosorption by heat-killed *A. ochraceus* discs (pH=5.0, biosorbent dose: 1.5g/100mL, 1% petroleum concentration, contact time:10h at room temperature).

Isotherm	Parameter
Langmuir	q_{max} (mg/g)- 6.67 K_L(1/mg)-0.076 R_L-0.354-0.845 R^2- 0.9922
Freundlich	K_F (mg/g)-2.71 1/n- 1.2069 R^2- 0.766

Re-usability of heat-killed fungal discs

Investigating the re-usability of heat-killed fungal discs at laboratory scale is important in terms of determining the suitability of these biomaterials for industrial use. El-Gheriany et al. (19) reported that petroleum sorption decreases with each cycle of the re-usability experiment. Accordingly, although the petroleum sorption of heat-killed fungal discs decreased with each cycle, it was clearly observed that the discs could be used actively in petroleum biosorption for at least 6 more cycles (Figure 5). Similarly, Sasidharan and Kumar (15) reported that MMPSS (magnetically modified pretreated biogas slurry solid) adsorbent can be preserved with less material loss in the continuous adsorption process. In this context, it has been clearly emphasized that heat-killed fungal discs are low-cost biomaterials that can be applied on an industrial scale with their re-usability feature.

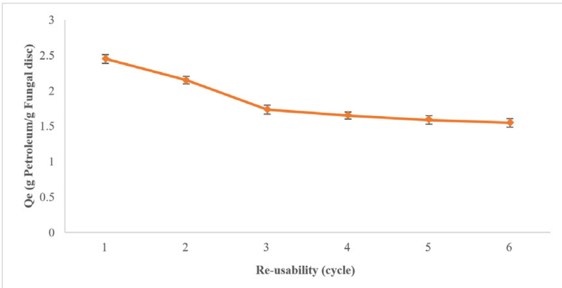

Figure 5. Re-usability of fungal discs for petroleum biosorption. *The experiments were carried out under the optimum conditions for petroleum biosorption as 1% petroleum concentration, 1.5 g/100mL heat-killed fungal discs, 10h contact time, at pH:5.0 and room temperature. Each experiment is the mean of three data and the error bars represent the standard deviation.

Focused Ion Beam Scanning Electron Microscopy (FIB-SEM)

To better understand the mechanisms involved in the binding of contaminants to the biomass surface, it is important to characterize the structure and chemical properties of the cell surface. Various environmental factors such as temperature, pH, ionic strength, availability of nutrients and natural lights affect the chemical composition of biomass (30). Accordingly, Figure 6 shows the un-treated and treated heat-killed *Aspergillus ochraceus* NRRL 3174 discs with petroleum. Moreover, the petroleum sorption on surface of heat-killed fungal disc was characterized by FIB-SEM images (Figures 6). The hyphae structure of the heat-killed fungal disc was clearly shown in Figure 7a. SEM images of heat-killed fungal discs treated with petroleum showed different morphology due to the attachment of petroleum on the

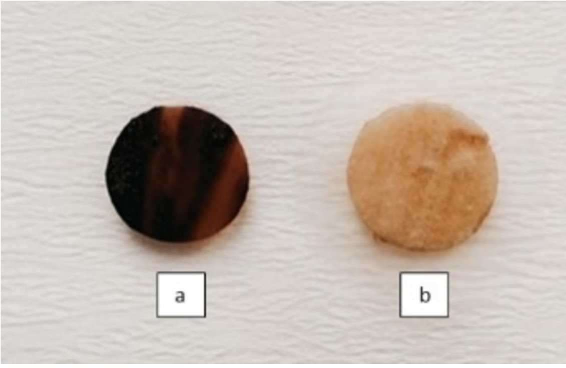

Figure 6. Heat-killed *Aspergillus ochraceus* NRRL 3174 discs a. treated b. un-treated form with petroleum

surface of biosorbent (Figures 7a and 7b). The heat-killed fungal discs have various functional groups (-OH, –NH, –COOH) on their surface, which supplied various sites for binding of toxic compounds (33). The adsorption efficiency of the fungal discs was positively affected by increase in the active sites due to heat denaturation operation on the cell wall proteins. It is also suggested that heat increases the hydrophilicity of the fungal biomass by removing $-CH_3$ groups responsible for the hydrophobic nature of the cell wall. Also, autoclaving disrupts the fungal cell integrity, resulting in greater porosity. As a result, more petroleum molecules can be fixed on the enlarged pores of the heat-killed fungal biomass (43).

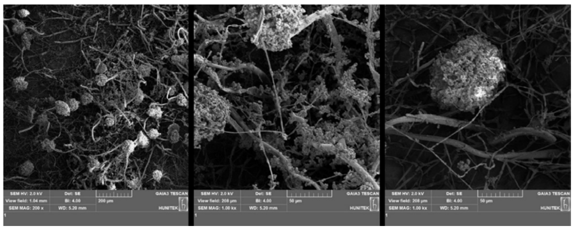

Figure 7a. FIB-SEM images of fungal discs before the biosorption of petroleum.

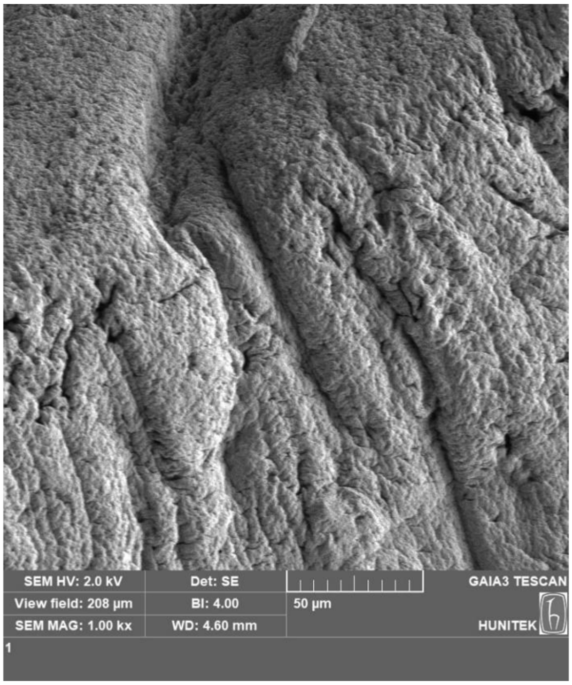

Figure 7b. FIB-SEM images of fungal discs after the biosorption of petroleum

GC-MS

Table 3 represents the *n*-alkane concentrations (ppm) of petroleum content before and after biosorption. According to Table 3, the removal of *n*-alkane fractions of petroleum by heat-killed *A. ochraceus* discs was also calculated in Figure 8. Figure 8. clearly shows that the *n*-alkane fractions in the range of C_{10} - C_{14} were removed above 90%; C_{15} - C_{21}, C_{28} and C_{30} - C_{36} were removed around and above 80%; C_{22} - C_{27}, C_{29} were also removed approximately 65% - 75%. It was detected that short-chain- and some of medium-chain *n*-alkanes of petroleum were biosorbed by heat-killed fungal discs over 90% and 80%, respectively. The most striking result is that the sorption of long-chain *n*-alkanes by heat-killed fungal disc was over 80%. In similar studies, the bio removal of petroleum was approved by GC-MS analysis (66-68). Short and long-chain *n*-alkanes are the most common components of the petroleum. Short-chain *n*-alkanes are highly toxic, while long-chain *n*-alkanes are solid, low soluble, and less bioavailable, and persist in the environment for a long time. Therefore, the biosorption of long-chain *n*-alkanes is of great importance for environmental remediation (69, 70).

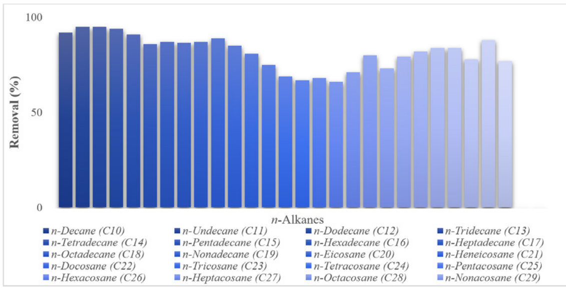

Figure 8. The removal of *n*-alkane fractions of petroleum by heat-killed fungal disc. *The experiment was carried out under the optimum conditions for petroleum biosorption as 1% petroleum concentration, 1.5 g/100mL heat-killed fungal discs, 10h contact time, at pH:5.0 and room temperature.

CONCLUSION

Fungal biomass has great advantageous to be used as biosorbent due to their high growth and reproduction rates, capability to use various carbon sources, and ability to adapt to adverse environmental conditions. In this context, the heat-killed fungal discs obtained from *Aspergillus ochraceus* were used as biosorbent in the biosorption process of petroleum. For the maximum biosorption, various parameters were optimized as pH:5.0, 1% petroleum concentration, 1.5 g/100mL heat-killed fungal discs, and

Table 3. Gas chromatography-mass spectrometry analysis of petroleum

***n*-Alkane fractions**	**Initial**	***Remaining**
n-Decane (C_{10}), ppm	151.3	12.2
n-Undecane (C_{11})	219.4	11.4
n-Dodecane (C_{12})	239.0	12.5
n-Tridecane (C_{13})	246.7	14.5
n-Tetradecane (C_{14})	227.3	20.9
n-Pentadecane (C_{15})	258.7	37.2
n-Hexadecane (C_{16})	225.1	29.4
n-Heptadecane (C_{17})	249.6	33.7
n-Octadecane (C_{18})	195.6	24.9
n-Nonadecane (C_{19})	213.1	22.8
n-Eicosane (C_{20})	187.0	28.5
n-Heneicosane (C_{21})	193.6	36.8
n-Docosane (C_{22})	198.4	50.0
n-Tricosane (C_{23})	222.5	69.1
n-Tetracosane (C_{24})	235.8	78.5
n-Pentacosane (C_{25})	239.2	77.5
n-Hexacosane (C_{26})	181.7	61.5
n-Heptacosane (C_{27})	149.6	43.7
n-Octacosane (C_{28})	111.4	30.1
n-Nonacosane (C_{29})	93.8	22.3
n-Triacontane (C_{30})	70.1	15.1
n-Hentriacontane (C_{31})	67.8	12.3
n-Dotriacontane (C_{32})	56.7	9.28
n-Tritriacontane (C_{33})	49.2	7.70
n-Tetratriacontane (C_{34})	40.8	8.95
n-Pentatriacontane (C_{35})	30.7	<3.78
n-Hexatriacontane (C_{36})	12.3	<2.81
n-Octatriacontane (C_{38})	<5.73	<5.73
n- Tetracontane (C_{40})	<6.93	<6.93

* The results show the remaining *n*-alkane fractions of the petroleum
*The values are given in ppm (parts-per million)

10h contact time at room temperature. The interaction of the adsorbent with the petroleum in the aqueous phase followed pseudo-second order kinetics. The Langmuir isotherm, which is the best fit for petroleum biosorption, indicates that physisorption plays a major role in biosorption process. In addition, the reuse of heat-killed fungal disc, which is effective, low-cost, and environmentally friendly, in a wide variety of industrial areas was also emphasized in this study. It should be considered that no similar study has been found investigating the sorption of petroleum with heat-killed *Aspergillus ochraceous* discs. This study pointed out the use of *Aspergillus ochraceous* discs as an efficient biosorbent in the biosorption process of petroleum. The results should be supported by further studies to increase its biosorption capacity and to fill the existing gaps.

ACKNOWLEDGEMENT

This work is funded by Hacettepe University Scientific Research Projects Coordination Unit (Project number: FHD-2019-17719).

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTION

All authors contributed to the study conception and design. Project administration, Conceptualization, Investigation, Methodology, Writing-original draft, and editing were performed by [Nermin Hande Avcioglu] and [Sezen Bilen Ozyurek]; Resources, Data curation and Software were carried out by [Nermin Hande Avcioglu] and Funding acquisition by [Sezen Bilen Ozyurek]. The first draft of the manuscript was written by [Nermin Hande Avcioglu], [Sezen Bilen Ozyurek], [Isil Seyis Bilkay] and all authors read and approved the final manuscript.

REFERENCES

1. Pi Y, Chen B, Bao M, Zhang B. Microbial degradation of four dispersed crude oils by Rhodococcus sp. evaluated using carbon stable isotope analysis. J Chem Technol Biotechnol 2019; 94 (6): 1800–7.
2. Chen ZK, An C, Boufadel M, Owens E, Chen Z, Lee K, Cao Y, Cai M. Use of surface-washing agents for the treatment of oiled shorelines: Research advancements, technical applications and future challenges. Chem Eng J 2020; 391: 123565.
3. Liu B, Chen B, Zhang B, Song X, Zen G, Lee K. Photocatalytic ozonation of offshore produced water by TiO2 nanotube arrays coupled with UV-LED Irradiation. J Hazard Mater 2020; 40: 123456.
4. Tian Y, Li J, Whitcombe TW, McGill WB, Thring R. Application of oily sludge-derived char for lead and cadmium removal from aqueous solution. Chem Eng J 2020; 384: 123386.
5. Zhu Z, Zhang B, Cai Q, Ling J, Lee K, Chen B. Fish waste based lipopeptide production and the potential application as a bio-dispersant for oil spill control. Front Bioeng Biotechnol 2020; 8: 734.
6. Kapahi M, Sachdeva S. Bioremediation options for heavy metal pollution. J Heal Pollut 2019; 9 (24): 191203.
7. Nazir H, Salman M, Athar M, Farooq U, Wahab A, Akram M. Citric acid functionalized Bougainvillea spectabilis: a novel, sustainable, and cost-effective biosorbent for removal of heavy metal (Pb2+) from wastewater. Water Air Soil Pollut 2019; 230: 1-16.
8. Akram M, Salmana M, Farooq U, Saleem U, Tahir S, Nazira H, Arsalan HM. Phthalate-functionalized Sorghum bicolor L.; an effective biosorbent for the removal of alizarin red S and bromophenol blue dyes from simulated wastewater. Desalin Water Treat 2020; 190: 383-92.
9. Shen J, Huang G, An C, Zha S, Rosendahl S. Immobilization of tetrabromobisphenol a by pinecone-derived biochars at solid-liquid interface: synchrotron-assisted analysis and role of inorganic

fertilizer ions. Chem Eng J 2017; 321: 346–57.
10. Cai L, Zhang Y, Zhou Y, Zhang X, Ji L, Song W, Zhang H, Liu J. Effective adsorption of diesel oil by crab-shell-derived biochar nanomaterials. Materials 2019;12 (2): 236.
11. Nazir H, Salman M, Athara M, Farooq U, Akrama M, Saleem N. A novel biosorbent B. spectabilisis stalks leaves for removal of Cd (II) and Cu (II) from wastewater. Desalin Water Treat 2019; 148: 222-28.
12. Hess BJ, Kolar P, Classen JJ, Knappe D, Cheng JJ. Effects of co occurring species present in swine lagoons on adsorption of copper on eggshell. Int J Environ Res 2019; 13: 613–22.
13. Joseph EE, Azorji, JN, Iheanacho JC, Nwachukwu CU, Iheagwam KS, Okoli CJ. Adsorption of petroleum hydrocarbons from crude oil polluted soil using agro-waste. J Environ Treat Tech 2022; 10 (4): 284-89.
14. Saleem U, Akram M, Salman M, Farooq U, Dar A, Tahir S. Biosorption activity of chemically modified Trifolium alexandrinum for the detoxification of Cr (III) contaminated water. Desalin water Treat 2022; 246: 212–25.
15. Sasidharan R, Kumar A. Magnetic adsorbent developed with alkali thermal pretreated biogas slurry solids for the removal of heavy metals: optimization, kinetic, and equilibrium study. Environ Sci Pollut Res 2022; 29 (20): 30217–32.
16. Olivella MÀ, Jové P, Bianchi A, Bazzicalupi C, Cano L. An integrated approach to understanding the sorption mechanism of phenanthrene by cork. Chemosphere 2013; 90 (6): 1939–44.
17. Christensen KM, Rorrer GL. Equilibrium partitioning behavior of naphthalene and phenanthrene with axenic microplantlets of the temperate green seaweed Acrosiphonia coalita. Chemosphere 2009; 76 (8): 1135–42.
18. Hussein M, Amer AA, Zahran HF, Ali SM, Elgohary M, Nasr M. Agricultural waste as a biosorbent for oil spills. Int J Dev 2013; 2 (1): 127-35.
19. El Gheriany IA, El Saqa FA, Amer AAER, Hussein M. Oil spill sorption capacity of raw and thermally modified orange peel waste. Alex Eng J 2020; 59(2): 925-32.
20. Al Zubaidi I, Al Zubaidi M, Tajik M, Al Zubaidi M, Al Mutair M, Sheikh M, Chen Y, Al-Yasiri M, Alsudays A. Pomegranate peels powder for the remediation of oil polluted water from waste lubricating oil. Proceedings of the 5th International Conference of Fluid Flow, Heat and Mass Transfer, Niagara Falls, Canada, 2018.
21. Wu J, Yu HQ. Biosorption of 2,4-dichlorophenol from aqueous solution by Phanerochaete chrysosporium biomass: isotherms, kinetics, and thermodynamics. J Hazard Mater 2006; 137: 498–508.
22. Chung M, Tsui MT, Cheung K, Tam NF, Wong MH. Removal of aqueous phenanthrene by brown seaweed Sargassum hemiphyllum: Sorptionkinetic and equilibrium studies. Sep Purif Technol 2007; 54 (3): 355–62.
23. Crognale S, Annibale AD, Pesciaroli L, Stazi SR. Fungal community structure and as-resistant fungi in a decommissioned gold mine site. Front Microbiol 2017; 8: 2202.
24. Al-Hawash AB, Zhang X, Ma F. Removal and biodegradation of different petroleum hydrocarbons using the filamentous fungus Aspergillus sp. RFC-1. Microbiol Open 2019; 8 (1): e00619.
25. Dusengemungu L, Kasali G, Gwanama C, Ouma KO. Recent Advances in Biosorption of Copper and Cobalt by Filamentous Fungi. Front Microbiol 2020; 11: 582016.
26. Shah SS, Palmieri MC, Sponchiado SRP, Bevilaqua D. Enhanced bio-recovery of aluminum from low-grade bauxite using adapted fungal strains. Braz J Microbiol 2020; 51: 1909-18.
27. Oladipo OG, Awotoye OO, Olayinka A, Bezuidenhout CC, Maboeta MS. Heavy metal tolerance traits of filamentous fungi isolated from gold and gemstone mining sites. Braz J Microbiol 2018; 49: 29–37.
28. Chen L, Li E, Wu W, Wang G, Zhang J, Guo X, Xing F. The secondary metabolites and biosynthetic diversity from Aspergillus ochraceus. Front in Chem 2022; 10: 938626.
29. Hareeri RH, Aldurdunji MM, Abdallah HM, Alqarni AA, Mohamed SGA, Mohamed GA, Ibrahim SRM. Aspergillus ochraceus: metabolites, bioactivities, biosynthesis, and biotechnological potential. Molecules 2022; 27 (19): 6759.
30. Vahabisani A, An C. Use of biomass derived adsorbents for the removal of petroleum pollutants from water: a mini review. Environ Syst Res 2021; 10 (1): 1-10.
31. Rao J, Viraraghavan T. Biosorption of phenol from an aqueous solution by Aspergillus niger biomass. Bioresour Technol 2002; 85 (2): 165–71.
32. Kumar R, Bishnoi NR, Bishnoi K. Biosorption of chromium (VI) from aqueous solution and electroplating wastewater using fungal biomass. Chem Eng J 2008; 135 (3): 202–8.
33. Lu N, Hua T, Zhai Y, Qin H, Aliyeva J, Zhang H. Fungal cell with artificial metal container for heavy metals biosorption: Equilibrium, kinetics study and mechanisms analysis. Environ Res 2020; 182: 109061.
34. Raghukumar C, Shailaja M, Parameswaran P, Singh S. Removal of polycyclic aromatic hydrocarbons from aqueous media by the marine fungus NIOCC 312: Involvement of lignin-degrading enzymes and exopolysaccharides. Indian J Mar Sci 2006; 35 (4): 373-79.
35. Bilen Ozyurek S, Avcioglu NH, Seyis Bilkay I. Mycoremediation potential of Aspergillus ochraceus NRRL 3174. Arch Microbiol 2021; 203: 5937-50.
36. Mohammadi M, Sedighi M, Hemati M. Removal of petroleum asphaltenes by improved activity of NiO nanaoparticles supported on green AlPO-5 zeolite: process optimization and adsorption isotherm. Petroleum 2020; 6 :182-88.
37. Benguenab A, Chibani A. Biodegradation of petroleum hydrocarbons by filamentous fungi (Aspergillus ustus and Purpureocillium lilacinum) isolated from used engine oil contaminated soil. Acta Ecologica Sinica 2021; 41 (5): 416-23.
38. Bilen Ozyurek S, Seyis Bilkay I. Comparison of petroleum biodegradation efficiencies of three different bacterial consortia determined in petroleum contaminated waste mud pit. SN Appl Sci 2020; 2: 1-12.
39. Kachieng'a L, Momba MNB. Kinetics of petroleum oil biodegradation by consortium of three protozoan isolates (Aspidisca sp., Trachelophyllum sp. and Peranema sp.). Biotechnol Rep 2017; 15: 125–31.
40. Cheng Z, Feng K, Su Y, Ye J, Chen D, Zhang S, Zhang X, Dionysiou DD. Novel biosorbents synthesized from fungal and bacterial biomass and their applications in the adsorption of volatile organic compounds. Bioresour Technol 2020; 300: 122705.
41. Kim N, Seo JH, Yun YS, Park D. New insight into continuous recirculation process for treating arsenate using bacterial biosorbent. Bioresour Technol 2020; 316: 123961.
42. Saratale GD, Kalme SD, Govindwar SP. Decolorisation of textile dyes by Aspergillus ochraceus (NCIM-1146). Ind J Biotechnol 2006; 5: 407-10.
43. Legorreta-Castañeda AJ, Lucho-Constantino CA, Beltrán-Hernández RI, Coronel-Olivares C, Vázquez-Rodríguez GA. Biosorption of water pollutants by fungal pellets. Water 2020; 12 (4): 1155.
44. Kumar Mishra P, Mukherji S. Biosorption of diesel and lubricating oil on algal biomass. 3 Biotech 2012; 2: 301–10.
45. Simonescu CM, Ferdes M. Fungal biomass for Cu (II) uptake from aqueous systems. Pol J Environ Stud 2012; 21 (6): 1831-39.

46. Latha JNL, Babu PN, Rakesh P, Kumar KA, Anupama M, Susheela L. Fungal cell walls as protective barriers for toxic metals. Adv Med Biol 2012; 53: 181-98
47. Boleydei H, Mirghaffari N, Farhadian O. Comparative study on adsorption of crude oil and spent engine oil from seawater and freshwater using algal biomass. Environ Sci Pollut Res 2018; 25: 21024-35.
48. Jalali R, Ghafourian H, Asef Y, Davarpanah S, Sepehr S. Removal and recovery of lead using nonliving biomass of marine algae. J Hazard Mater 2002; 92 (3): 253–62.
49. Aracagok YD. Biosorption of lead by a soil isolate Aspergillus neoalliaceus. Arch Microbiology 2022; 204 (9): 547.
50. Devi MG, Al-Hashmi ZS, Sekhar GC. Treatment of vegetable oil mill effluent using crab shell chitosan as adsorbent. Int J Environ Sci Technol 2012; 9: 713–18.
51. Mwandira W, Nakashima K, Kawasaki S, Arabelo A, Banda K, Nyambe I, Chirwa M, Ito M, Sato T, Igarashi T, Nakata H, Nakayama S, Ishizuka M. Biosorption of Pb (II) and Zn (II) from aqueous solution by Oceanobacillus profundus isolated from an abandoned mine. Sci Rep 2020; 10 (1): 21189.
52. Tsekova T, Todorova D, Dencheva V, Ganeva S. Biosorption of copper (II) and cadmium (II) from aqueous solutions by free and immobilized biomass of Aspergillus niger. Bioresour Technol 2010; 101 (6): 1727-31.
53. Tsekova T, Todorova D, Ganeva S. Removal of heavy metals from industrial wastewater by free and immobilized cells of Aspergillus niger. Int Biodeterior Biodegrad 2010; 64 (6): 447-51.
54. Lim MW, Von Lau E, Poh PE A. A comprehensive guide of remediation technologies for oil contaminated soil—present works and future directions. Mar Pollut Bull 2016; 109 (1): 14–45.
55. Zhou JL, Kiff RJ. The uptake of copper from aqueous solution by immobilized fungal biomass. J Chem Technol Biot 1991; 52 (3): 317–30.
56. Hasan IF. Role of filamentous fungi to remove petroleum hydrocarbons from the environment, In Microbial Action on Hydrocarbons, Springer, Singapore; 2019.
57. Xu N, Bao M, Sun P, Li Y. Study on bioadsorption and biodegradation of petroleum hydrocarbons by a microbial consortium. Bioresour Technol 2013; 149: 22–30.
58. Barnes NM, Khodse VB, Lotlikar NP, Meena RM, Damare SR. Bioremediation potential of hydrocarbon-utilizing fungi from select marine niches of India. 3 Biotech 2018; 8: 1-10.
59. Sokker HH, El-Sawy NM, Hassan MA, El-Anadouli BE. Adsorption of crude oil from aqueous solution by hydrogel of chitosan-based polyacrylamide prepared by radiation induced graft polymerization. J Hazard Mater 2011; 190 (1-3): 359-65.
60. Singh S, Parveen N, Gupta H. Adsorptive decontamination of rhodamine-B from water using banana peel powder: a biosorbent. Environ Technol Innov 2018; 12: 189-95.
61. Peng D, Ouyang F, Liang X, Guo X, Dang Z, Zheng L. Sorption of crude oil by enzyme-modified corn stalk vs. chemically treated corn stalk. J Mol Liq 2018; 255: 324-32.
62. Mottaghi H, Mohammadi Z, Abbasi M, Tahouni N, Panjeshahi MH. Experimental investigation of crude oil removal from water using polymer adsorbent. Journal of Water Process Engineering 2021; 40: 101959.
63. Mallampati R, Xuanjun L, Adin A, Valiyaveettil S. Fruit peels as efficient renewable adsorbents for removal of dissolved heavy metals and dyes from water. ACS Sustain Chem Eng 2015; 3 (6): 1117-1124.
64. Zhang B, Yu S, Zhu Y, Shen Y, Gao X, Shi W, Tay JH. Adsorption mechanisms of crude oil onto polytetrafluoroethylene membrane: kinetics and isotherm, and strategies for adsorption fouling control. Sep Purifi Technol 2020; 235: 116212.
65. Yu X, He Y. Optimal ranges of variables for an effective adsorption of lead (II) by the agricultural waste pomelo (Citrus grandis) peels using Doehlert designs. Sci Rep 2018; 8 (1): 729.
66. Liang J, Cheng T, Huang Y, Liu J. Petroleum degradation by Pseudomonas sp. ZS1 is impeded in the presence of antagonist Alcaligenes sp. CT10. AMB Expr 2018; 8 (1): 1-13.
67. Al-Dhabi NA, Esmail GA, Arasu MV. Enhanced production of biosurfactant from Bacillus subtilis strain Al-dhabi-130 under solid-state fermentation using date molasses from Saudi Srabia for bioremediation of crude-oil-contaminated soils. Int Env Res Pub He 2020; 17 (22): 8446.
68. Luo X, Chen L, Zhao S, Lei Z, Xia M, Zhang C. Study of the characteristics of two immobilized microbial materials in degradation and evolution of petroleum hydrocarbon. ACS Omega 2020; 5 (31): 19402–8.
69. Guo H, Tang S, Xie S, Wang P, Huang C, Geng X, Jia X, Huo H, Li X, Zhang J, Zhang Z, Fang J. The oil removal and the characteristics of changes in the composition of bacteria based on the oily sludge bio electrochemical system. Sci Rep 2020; 10 (1): 15474.
70. Liu S, Yuan W, Yang X, Li C, Ji Y, Feng X, Li M, Xie Y. Microbial degradation of petroleum characteristic pollutants in hypersaline environment, emphasizing n-hexadecane and 2,4 di-tert-butylphenol. Environ Monit Assess 2023; 195 (6): 771.