

Performance of the fixed-bed process for the treatment of oil well effluents using autoclaved cellular concrete as a novel biological support

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ABSTRACT

This study aims to evaluate the performance of a fixed-bed process for treating oily wastewater from oil fields in southern Algeria using autoclaved cellular concrete (ACC) as a novel bacterial support. The experimental setup consists of a column filled with ACC, forming a fixed bed. The bioreactor is inoculated with mixed sludge and operates continuously with upward flow. The inhibitory effect of iron on COD removal was evaluated using iron concentrations of 5, 15, and 85 mg/L. Removal efficiencies of key parameters were measured to assess bioreactor performance. Chemical oxygen demand (COD) removal rates reached 90%, 91%, and 92% at Fe²⁺ concentrations of 5, 15, and 85 mg/L, respectively. Corresponding Fe²⁺ elimination rates were 98%, 95%, and 97%. A high Fe²⁺ concentration of 85 mg/L did not inhibit hydrocarbon biodegradation. Autoclaved cellular concrete was characterized using scanning electron microscopy (SEM), which revealed a porous structure and a rough surface conducive to bacterial adhesion and growth. Energy-dispersive X-ray spectroscopy (EDX) analysis showed that the support is primarily composed of silica (Si), oxygen (O), and calcium (Ca), with minor amounts of iron (Fe), magnesium (Mg), and potassium (K). The absence of toxic materials in the support confirms its suitability as a bacterial support.

Keywords: fixed-bed process, autoclaved cellular concrete, oil well effluents, biological support.

INTRODUCTION

As the oil industry relentlessly expands, it has become a significant source of pollution, attracting intense scrutiny worldwide. This industry encounters major challenges in managing and treating oil drilling wastewater. Petroleum industry operations

use large amounts of water and generate significant effluent containing salts, carbon compounds (dispersed and dissolved), heavy metals, drilling muds, oil, additives, acids, and solvents (Perez et al., 2016; Sanghamitra et al., 2021). All these toxic compounds disrupt the equilibrium of both terrestrial and marine ecosystems (Vieira dos Santos

et al., 2014; Adetunji and Olaniran, 2021). The infiltration of oil effluents contaminates groundwater resources essential to sustaining life in arid regions (Álvarez et al., 2017; Mokif et al., 2022), depletes soil fertility, and causes significant harm to local flora and fauna (Mansour et al., 2024; Tumurbaatar et al., 2024). Beyond their environmental impact, these pollutants take a serious toll on human health. Organic pollutants, such as polycyclic aromatic hydrocarbons, can disrupt the endocrine system, damage the nervous system, and increase the risk of cancer (Turner et al., 2017). Their presence is also linked to lung diseases and persistent skin inflammation (Kadri et al., 2017; Lawan et al., 2023).

Each application of water necessitates compliance with specific water quality standards. Remove dispersed phases before reinjecting water, and eliminate dissolved substances before discharge or reuse for irrigation. In the oil industry, discharge standards govern liquid effluents, atmospheric emissions, and solid waste. Drilling muds, particularly oil-based muds, must be contained and treated. Their direct discharge into the environment is strictly prohibited.

Numerous treatment techniques have been developed for petroleum industry wastewater and are classified into distinct methodological categories. Ozonation (Christodoulis et al., 2024), membrane bioreactors (Di Fabio et al., 2013), coagulation, adsorption, flocculation (Varjani et al., 2019), and hybrid methods that combine adsorption processes with advanced oxidation (Alomar et al., 2022). Recent studies have applied electrochemical oxidation (Mirshafiee et al., 2024), and photocatalysis (Zhang et al., 2024). However, these processes are not fully effective in removing dissolved organic matter and produce harmful byproducts (Islam, 2021; Ferreira et al., 2024).

In contrast, biological treatment is evaluated as an environmentally sustainable and cost-effective solution. This method offers significant advantages, including reduced operational costs, elimination of chemical usage, and enhanced removal efficiencies for a broad range of dissolved compounds compared with physicochemical treatments (Alaidaroos et al., 2023).

Aerobic processes employ either free-living bacterial cultures suspended in the water to be treated, known as activated sludge treatment, or bacterial cultures fixed on a support, referred to as fixed-bed treatment (Lusinier et al., 2021). Fixed-bed biological processes have demonstrated

effectiveness in removing organic matter and are applicable across a broad spectrum of organic load concentrations, ranging from low to very high. Aerobic biodegradation rates are significantly influenced by factors such as nutrient availability, oxygen concentration, pH, humidity, temperature, and the specific nature of the pollutant (Kulkarni, 2016; Zhao et al., 2019).

Heterotrophic bacteria play a crucial role in the final decomposition of organic matter during these processes. Their catabolic activity converts complex organic compounds into simpler substances during decomposition (Marchand et al., 2017; Eregie et al., 2023). Hydrocarbon biodegradation involves oxidation reactions that yield lower-molecular-weight hydrocarbons, carbon dioxide, water, and bacterial biomass upon completion (Atlas and Raymond, 2008; Liu et al., 2023).

The chemical reactions of hydrocarbon biodegradation, catalyzed by bacterial enzymes, are complex and depend on environmental physicochemical conditions, the nature of the hydrocarbon substrate, and the bacterial strain used (Kuyukina et al., 2020). Aerobic biological processes are widely used in oilfield wastewater treatment due to their faster reaction kinetics and higher purification efficiency compared to anaerobic processes. Several aerobic bacterial species, such as *Pseudomonas*, *Acinetobacter*, *Flavobacterium*, *Mycobacterium*, *Brevibacterium*, and *Arthrobacter*, can degrade hydrocarbons (Atlas and Raymond, 2008; Zhao et al., 2025).

During the growth phase, most microorganisms can colonize support surfaces. Attachment is facilitated by exopolymers produced by bacteria. Initial colonization of solid surfaces occurs at specific preferred sites, from which biofilm formation progresses until the substrate is fully covered. Concurrently, cell generation contributes to increased biofilm thickness (Lusinier et al., 2021; Walker et al., 2025). Biofilms formed by microorganisms are subject to diverse hydrodynamic conditions. Fluctuations in nutrient and oxygen availability, along with applied shear stress, significantly influence cell adhesion to support surfaces and may impact treatment efficiency (Bhattacharyya et al., 2022; Afify et al., 2025).

The selection of appropriate support materials is critical for achieving effective treatment in fixed-bed bioreactors (Zhao et al., 2019; Nabaterega et al., 2025). Previous studies have demonstrated that supports in fixed-bed bioreactors

promote biofilm formation on their surfaces, thereby increasing resistance to environmental stresses and improving nutrient transfer (Zhou et al., 2020; Lago et al., 2024).

Numerous types of supports have been investigated in biological processes to address oil wastewater, such as ceramic carriers (Dong et al. 2011), bamboo and coconut husk chips (Antwi-Akomea et al., 2018), biochar (Chen et al., 2019), *Luffa cylindrica*-polystyrene hybrid (Okan et al., 2022), modified biochar (Geng et al., 2024), Anox Kaldnes K5 media (Ferreira et al., 2024). These media are costly and require significant time to synthesize.

So far, no research has explored the potential of fixed biological beds with autoclaved cellular concrete as a bacterial support for treating oily wastewater. This work aims to examine the treatment of oily wastewater from oil fields in southern Algeria in a fixed-bed system using autoclaved cellular concrete (ACC) as an innovative inert bacterial support. ACC is a water-resistant material characterized by an 80% air structure, which confers natural hydrophobicity. It resists moisture and retains structural integrity when exposed to water. The selection of materials is based on properties that facilitate bacterial biofilm adhesion and growth.

MATERIALS AND METHODS

Analytical method

COD measures the optimal amount of oxygen required to oxidize organic and inorganic substances in an effluent. COD was measured at high temperature using Hanna Instruments equipment. Ammoniacal nitrogen (NH_4^+), Phosphate (PO_4^{3-}), and ferrous ion (Fe^{2+}) concentrations were determined with a Carl Zeiss Jena Spekol 11 spectrophotometer, based on a previously established calibration curve. pH was measured using a pH 539 meter. An analysis of the ACC material was carried out using scanning electron microscopy, supplemented by energy-dispersive spectroscopy using a Thermo Scientific Axiom ChemSEM.

Wastewater composition

Wastewater is collected from several discharge points. The characteristics of wastewater are presented in Table 1.

Bioreactor characteristics

The laboratory-scale experimental setup (Figure 1) comprises a tank (a) containing wastewater, a peristaltic pump (b), and a column (c) measuring 155 cm in height and 3.1 cm in diameter. The column is packed with a support material (d) that provides a solid substrate for microbial growth. The effective height of the bioreactor is 125 cm. Treated water is collected in a separate tank (e). An air pump (f) supplies oxygen to the bioreactor. Samples for analysis are collected at the outlet of the column's effective bed. The bioreactor operates in dynamic mode with a flow rate of 0.3 m/h. However, operating a control column devoid of biomass would serve as a complementary study to quantify the abiotic phenomena involved in pollutant removal.

Experimental procedure

Autoclaved cellular concrete (ACC) was selected as the support for the fixed-bed study. ACC is a silico-calcareous material produced by autoclaving a mixture of silica sand, lime, ferrosulphate, alumina powder, and water. The material has a density of 500 kg/m³ and a tensile strength of 3.3 MPa.

The ACC was crushed into small spherical pebbles, each measuring 2 cm in diameter, and placed in the column. The support was submerged in activated sludge containing a diverse microbial community. Before treatment, water was percolated through the bed to remove excess activated sludge, ensuring only the sludge adhered to the material remained. Wastewater was pumped from tank 1 and introduced into the column in an upward flow. The system operated continuously. Air supplied by a compressor provided oxygen to the

Table 1. The characteristics of effluent from oil drilling operations

Parameter	Concentration range (mg/L)
COD	540–710
Fe^{2+}	5–85
NH_4^+	25–35
PO_4^{3-}	17.5–22
Na^+	5.9–11
HCO_3^-	20–27
pH	6.8–8.30
Cl ⁻	9.10–15

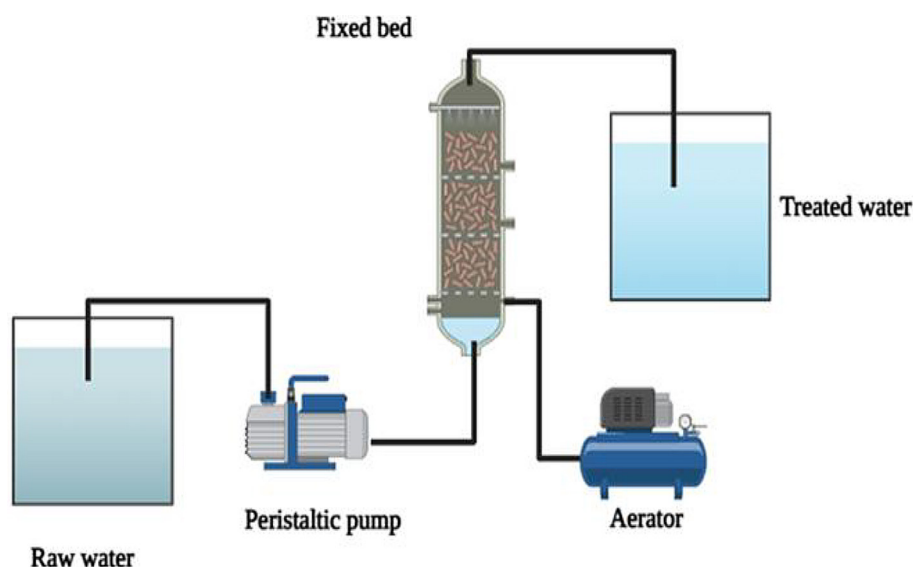


Figure 1. Experimental setup

microorganisms. Samples collected during the column's operation represent successive measurements from a single, continuously operating experimental system and therefore cannot be considered independent replicates. Daily sampling at consistent times was performed to monitor operating parameters and removal performance by analyzing both influent and effluent over time. The inlet and outlet water of the bioreactor were monitored.

RESULTS AND DISCUSSION

The feasibility of the recommended biological treatment in a fixed-bed column was assessed by collecting daily samples at the column inlet and outlet. Analyses included characteristic pollution parameters such as COD, pH, ammoniacal nitrogen, and phosphates. Iron (Fe^{2+}) removal was also monitored. This protocol was repeated at iron concentrations of 5, 15, and 85 mg/L.

Study of the inhibitory effect of iron on COD removal

Chemical oxygen demand is a primary indicator of pollution in industrial effluents, measuring the oxygen required to oxidize organic and inorganic matter in a water sample. Elevated COD values indicate higher concentrations of organic material or pollutants in water, which can reduce dissolved oxygen levels and adversely affect aquatic ecosystems.

The results (Figure 2) indicate the presence of organic matter at the column inlet, as measured by COD, with values ranging from 550 to 590 mg O_2 /L. In this biodegradation test, the evolution of COD was assessed in the presence of a high concentration of Fe^{2+} . To evaluate the inhibitory effect of iron on hydrocarbon biodegradation, the iron concentration in the bioreactor was varied. Chemical oxygen demand was monitored for iron loads of 5, 15, and 85 mg/L.

Figure 2a reveals the decrease in COD over time for an initial Fe^{2+} concentration of 5 mg/L. A decline in effluent COD concentration observed during the initial days of bioreactor operation indicates that a portion of the organic matter is used to synthesize microbial biomass, while the remainder meets the energy requirements for microbial growth and maintenance. Following a brief adaptation phase, the COD concentration at the bioreactor outlet increased to 80 mg/L and subsequently stabilized at 40 mg/L by the 25th day of operation. Bearing in mind that the average COD concentration was around 550 mg/L. The presence of iron at this concentration did not impede hydrocarbon biodegradation. The COD removal efficiency reached 90% after 25 days of system operation.

Figure 2b shows the decrease in COD over time for an initial Fe^{2+} concentration of 15 mg/L. This test was conducted under previously described conditions, with an iron concentration of 15 mg/L. From the 4th day onward, COD at the bioreactor outlet decreased significantly and stabilized at 40 mg O_2 /L by the 15th day. After a

biomass acclimation phase to the new iron load, COD removal efficiency reached high levels, which can be attributed to sufficient contact time between the biomass and the effluent. Indeed, after a few days, COD removal efficiencies stabilized at 91%. It is important to note that stabilization did not require additional time with each increase in iron concentration. The microorganisms successfully adapted to the altered conditions resulting from the increased Fe^{2+} load. In comparison, Zueva et al. (2020) report that the COD removal efficiency in oil industry wastewater treatment is limited to 70%.

The evolution of chemical oxygen demand was monitored over time at an iron concentration of 85 mg/L (Figure 2c). The results indicate a substantial reduction during the initial 10 days of operation, with a 92% decrease and a final concentration below 50 mg/L at the bioreactor outlet.

In the fixed culture technique, microorganisms within biofilms secrete an extracellular matrix that facilitates their attachment to the bedding material. This extracellular matrix increases the diffusion barrier and slows the transfer of toxic compounds within the biofilm. A high concentration of Fe^{2+} did not inhibit hydrocarbon biodegradation. Instead, it promoted the development of a selective microbial community capable of degrading organic matter. Similarly, Lusinier et al. (2021) report that at the lowest hydraulic retention time (HRT) of 12 hours and an organic loading rate of $3.2 \text{ kg COD m}^{-3} \text{ d}^{-1}$, only the fixed-bed hybrid bioreactor (FBHBR) maintained higher COD removal efficiency than the conventional system. The FBHBR's ability to operate effectively under such low HRT conditions is critical to the compactness of treatment processes, particularly for offshore applications.

Variation of ferrous ion Fe^{2+} concentration versus time

At low concentrations, metals such as magnesium, zinc, iron, and copper serve as components of biological catalytic systems and are required as trace elements by most microorganisms. Several factors influence the toxicity of heavy metals, including their concentration, environmental pH, and temperature.

Figures 3a, 3b, and 3c show iron removal rates of 98%, 95%, and 97% at concentrations of 5, 15, and 85 mg/L, respectively. Elevated iron concentrations promote the proliferation of resistant iron bacteria, including stalked species such as *Gallionella ferruginea* and filamentous species such as *Sphaerotilus* (Mansour et al., 2024; Müller et al., 2024). These bacteria oxidize iron and utilize it in anabolic and catabolic reactions essential for their maintenance and growth. Oxidized iron may also be deposited on various components of the bacterial cytoplasmic membrane. Similarly, Zhao et al. (2025) developed an Fe^{3+} enriched bioreactor to improve pollutant removal and enhance the activity of associated microbial communities. The Fe^{3+} -rich system enabled efficient removal of hydrocarbons, chemical oxygen demand (COD), and polymers. High-throughput sequencing demonstrated that Fe^{3+} supplementation strengthened interactions between iron-reducing bacteria and methanogenic archaea, thereby facilitating the biodegradation of long-chain hydrocarbons.

The literature indicates that biological iron removal offers several advantages over conventional processes. i) After fixation on bacterial exudates, oxidized iron forms sludge that is more compact than the amorphous floc produced by physicochemical processes. ii) Rapid oxidation

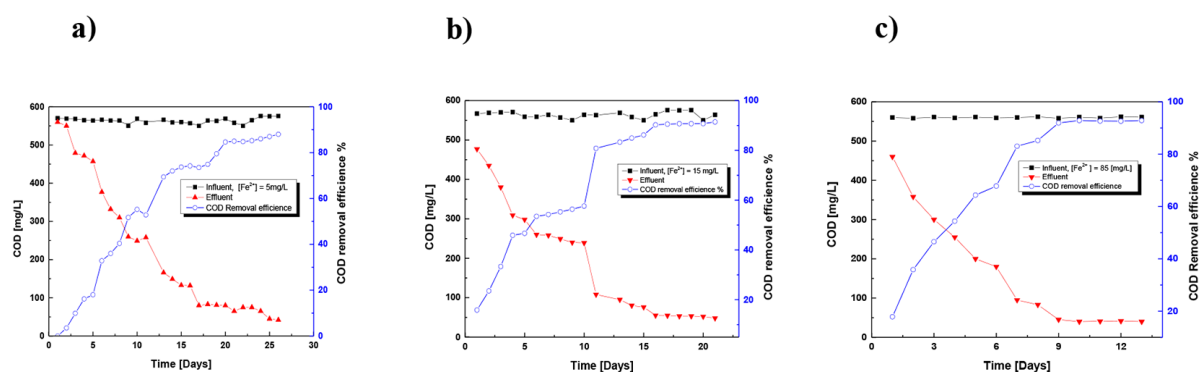


Figure 2. Evolution of chemical oxygen demand (COD) versus time at: (a) $\text{Fe}^{2+} = 5 \text{ [mg/L]}$, (b) $\text{Fe}^{2+} = 15 \text{ [mg/L]}$, (c) $\text{Fe}^{2+} = 85 \text{ [mg/L]}$

of Fe^{2+} enables flow rates of 10 to 15 m/h, contingent on the iron concentration in the raw water. iii) pH adjustment is typically not required, except when correcting the calcium-carbonate balance in treated water. Biological iron treatment resolves common challenges, including iron sequestration by silica and the flocculation of precipitated iron. iv) In contrast to the conventional process, which required decanting when iron concentrations exceeded 50 mg/L, biological iron removal enables direct feeding of high-iron-content water into the system (Müller et al., 2024; Bahuguna et al., 2025).

Evolution of ammoniacal nitrogen (NH_4^+) concentration versus time

High rates of ammonia nitrogen consumption 50, 72, and 75% were observed from the 10th day of bioreactor operation at concentrations of 5, 15, and 85 mg/L, respectively (Figure 4).

The assimilation of ammoniacal nitrogen into living matter results in the synthesis of proteins through the polymerization of amino acids, as well as nucleic acids and coenzymes (Wang et al., 2016). Nitrogen, in its various oxidized and reduced forms, plays an important role in the cellular development of purifying bacteria. It represents the most reduced form of nitrogen and is therefore incorporated at a negligible energy cost. As a result, ammonium ions are the preferred nitrogen source for microorganisms. Biological nitrogen treatment generally involves nitrification and denitrification steps. In the nitrification process, ammonia is oxidized by *Nitrosomonas* bacteria to produce nitrites, which are then, in a second step, oxidized by *Nitrobacter* bacteria to produce nitrates. Denitrification requires ideal conditions, such as temperature, pH range, respiration mode (aerobic or anaerobic), and nutrition mode (autotrophic or heterotrophic), to stimulate the appropriate bacteria in biological treatment.

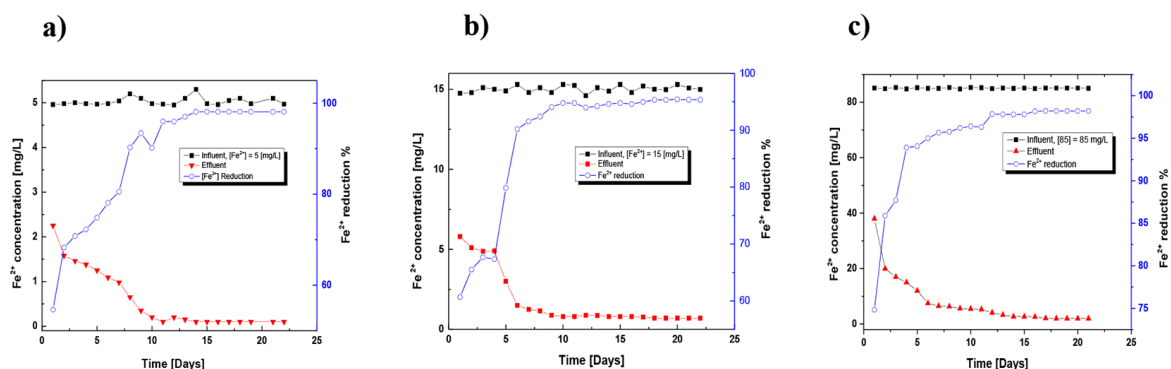


Figure 3. Evolution of Fe^{2+} concentration versus time, (a) $\text{Fe}^{2+} = 5$ mg/L, (b) $\text{Fe}^{2+} = 15$ mg/L, (c) $\text{Fe}^{2+} = 85$ mg/L

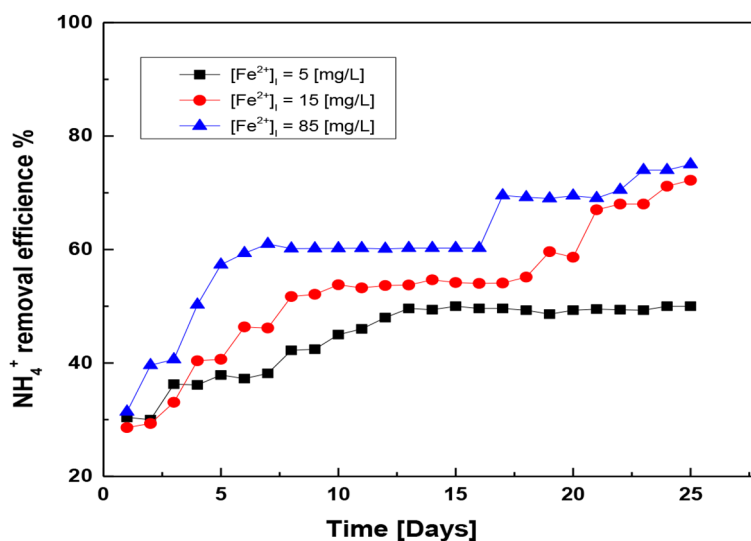


Figure 4. Evolution of ammonia nitrogen concentration versus time

Evolution of phosphate concentration (PO_4^{3-}) versus time

Effective biodegradation requires water to contain sufficient concentrations of mineral elements in bioavailable forms, which are essential for microbial activity. These elements include nitrogen, sulfur, calcium, potassium, and phosphorus. Phosphorus is an essential element for microbial biomass, as it is crucial in the synthesis of adenosine triphosphate (ATP), nucleic acids, and cell membrane components (Bhattacharyya et al., 2022). Figures 5a, 5b, and 5c present the evolution of PO_4^{3-} concentration over time.

At an Fe^{2+} concentration of 5 mg/L, removal efficiency increased from 50% to 72% after the adaptation phase. The significant reduction in PO_4^{3-} concentration, from 26 mg/L to less than 10 mg/L, is attributed to the progressive development of bacterial flora that utilize phosphate as a nutrient for growth and cellular maintenance. Orthophosphate consumption rates of 84% and 93% were observed at iron concentrations of 15 mg/L and 85 mg/L, respectively. Higher Fe^{2+} concentrations in the same fixed bed promoted substantial development of the bacterial flora. The formation of calcium precipitates with PO_4^{3-} ions was ruled out, as the release of calcium into the water would occur only in an acidic medium, following the leaching of lime. However, throughout all the experiments in this study, the pH of the raw water remained between 7.2 and 7.3. Complexation of Fe with PO_4^{3-} was also considered negligible, as the formation of such precipitates would have fouled the column, obstructed water and nutrient flow to the biofilm, and caused biofilm detachment, resulting in highly turbid effluent. In contrast, the column effluent remained clear throughout the experiments.

Evolution of pH versus time

pH is a critical parameter in industrial wastewater treatment, as it directly affects the efficiency of biological processes. The optimal pH for polysaccharide secretion varies among species, but for most bacterial strains, it is approximately pH 7. Microorganisms responsible for degrading organic matter, such as *Pseudomonas*, exhibit optimal activity within specific pH ranges. The production of exopolysaccharides within biofilms confers protection against adverse environmental conditions, particularly pH fluctuations. If pH levels are inadequate, microbial metabolic activity decreases, reducing the efficiency of organic contaminant degradation. Maintaining pH within appropriate ranges is essential for achieving optimal biological treatment efficiency and complying with discharge standards. Therefore, precise pH adjustments to influent water are implemented throughout the wastewater treatment process to meet these objectives (Bhattacharyya et al., 2022).

Figures 6a, 6b, and 6c present the variation of pH over time. The pH values range from 7.3 to 8.3. These results indicate that the pH remains neutral or slightly basic throughout the treatment process, consistently falling within the range recommended for biological treatment.

Scanning electron microscopy analysis of autoclaved cellular concrete

An effective bacterial support medium is characterized by a large specific surface area to facilitate high biomass attachment, high porosity to minimize biofilm fouling, and a microcavity structure that enables the circulation of water, nutrients, and oxygen into the core of the support.

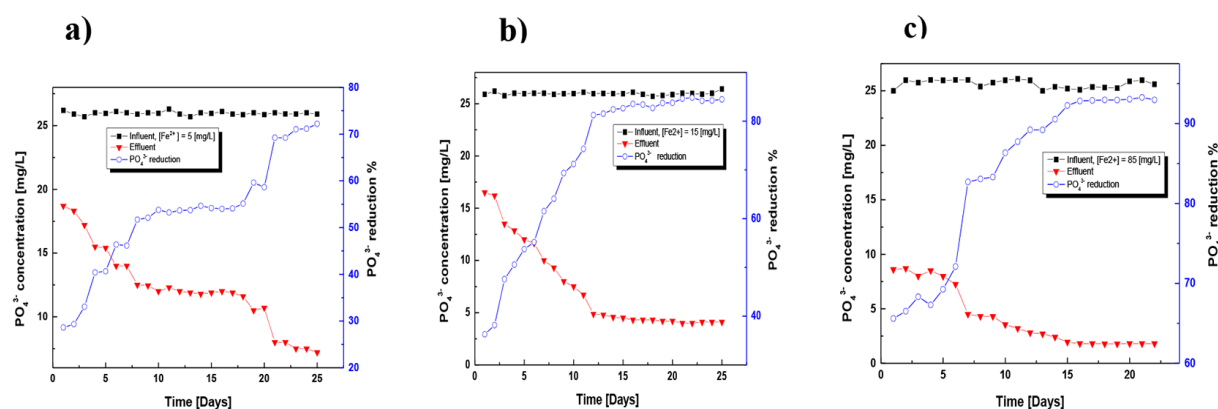


Figure 5. Evolution of phosphate concentration versus time: (a) Fe^{2+} = 5 mg/L, (b) Fe^{2+} = 15 mg/L, (c) Fe^{2+} = 85 mg/L

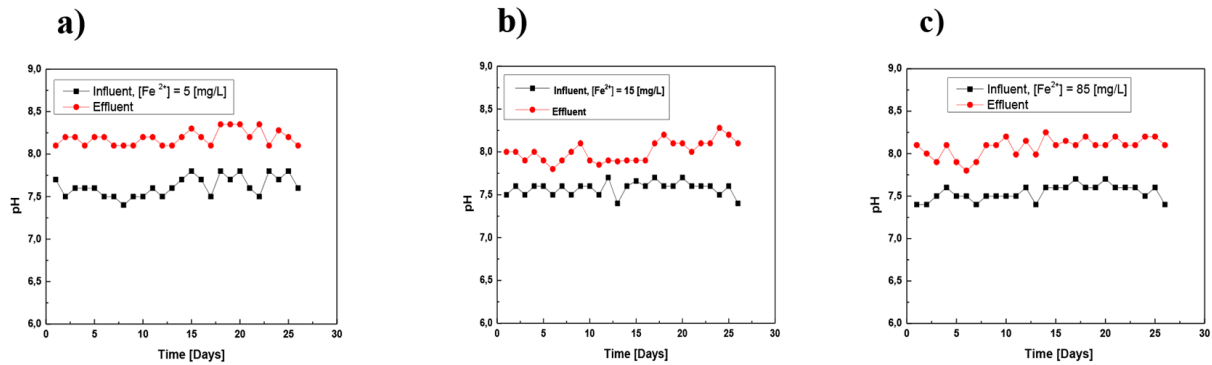


Figure 6. Evolution of pH versus time, (a) $\text{Fe}^{2+} = 5 \text{ mg/L}$, (b) $\text{Fe}^{2+} = 15 \text{ mg/L}$, (c) $\text{Fe}^{2+} = 85 \text{ mg/L}$

Scanning electron microscopy (SEM) analysis of the ACC sample (Figure 7) reveals the alveolar structure, characterized by closed cells, which imparts lightness and insulating properties to autoclaved cellular concrete. SEM is used to assess the chemical and physical bonding between silica grains (ground sand) and the hardened binder paste (cement and lime). During autoclaving or high-pressure curing, SEM reveals the formation of specific hydrated calcium silicate crystals that confer mechanical strength to the material (Figure 7a, b). Additionally, SEM enables visualization of micro-cracking or

heterogeneities within the raw material mixture. The structure of the ACC material enables effective circulation of water and air, both of which are essential for microbial growth. Additionally, the material's relatively granular surface promotes bacterial attachment and supports subsequent growth (Figure 7c, d). SEM indicates that the material possesses a porous matrix, with micropores situated between the macropores. Additionally, the ACC exhibits a high porosity (80%), in contrast to conventional ceramic or plastic supports, which typically have porosity values below 50%.

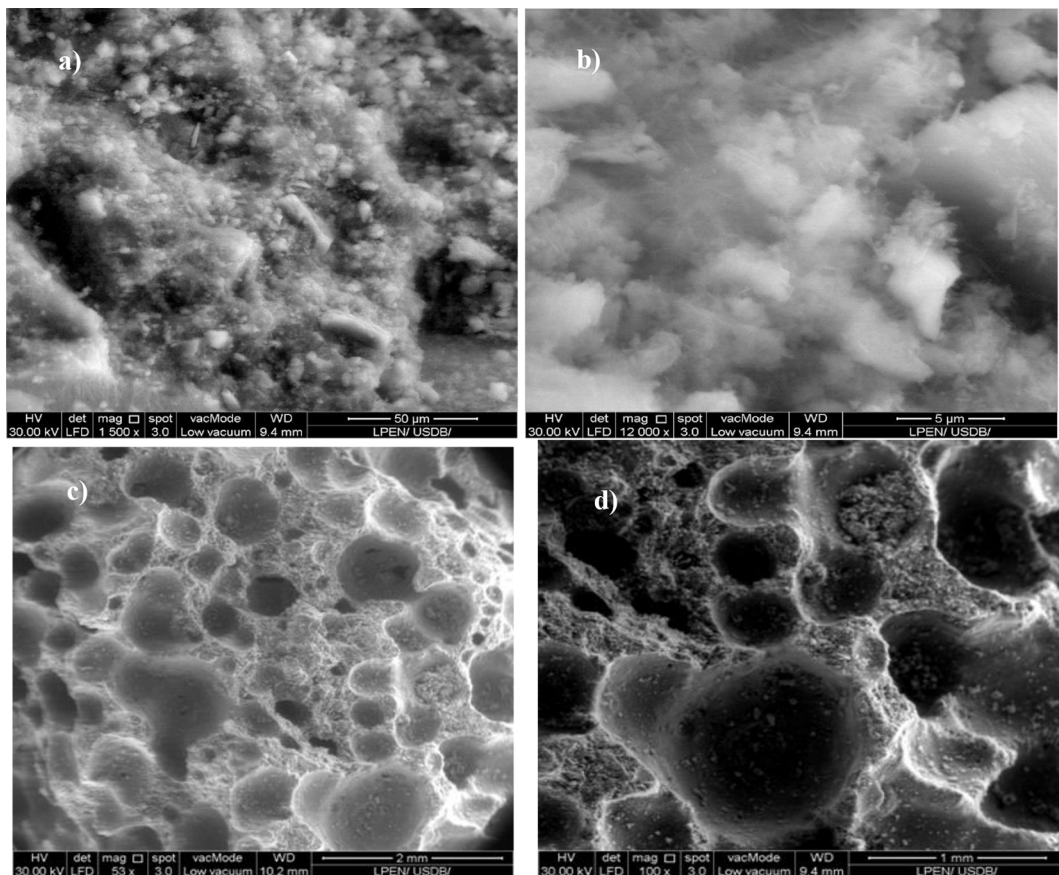


Figure 7. Support ACC analysis by scanning electron microscopy

Energy dispersive X-ray spectroscopy of ACC sample

Energy-dispersive X-ray spectroscopy (EDX) analysis of autoclaved cellular concrete (Figure 8) shows that the material primarily consists of silica (Si) and oxygen (O), forming silicon dioxide (SiO_2). The presence of calcium ions (Ca) is attributed to the use of lime in ACC production. Aluminum (Al) is detected in minor quantities due to the addition of aluminum powder as an expansive agent, which facilitates pore formation by causing the mixture to rise. Trace amounts of magnesium (Mg), iron (Fe), sulfur (S), and potassium (K) are also present. These elements are widely available in nature. Furthermore, ACC does not contain toxic elements and can function as an effective support material.

Effect of the effective bed height on iron removal efficiency

The variation in iron removal efficiency along the column was investigated using four different biological filter heights. The operating conditions included an ambient temperature of $28 \pm 2^\circ\text{C}$, a flow velocity of 0.35 m/h, and an initial Fe^{2+} concentration of 85 mg/L. The results are presented

in Table 2. The results demonstrate that Fe^{2+} concentration at the column outlet decreases as bed height increases. These findings indicate that a substantial biological filter height is necessary for effective treatment.

However, excessive bed height may increase sludge production and reduce water flow through the column. Therefore, optimizing bed height is critical. A bed height of 1 to 2 meters is recommended for biological treatment to minimize pressure drop.

Microbial population

Ongoing research focuses on bacteria-support-substrate mechanisms that enable significant bacterial attachment and optimal removal of organic pollutants. Among the active bacterial species, those that develop most prominently are those capable of degrading the greatest amount of organic matter within the shortest time frame. The composition of the microflora depends on various complex factors that are difficult to define precisely; while estimating bacterial density is relatively straightforward, it is impossible to specify the exact role played by bacterial interactions, particularly competition, in population growth. However, microorganisms utilizing the

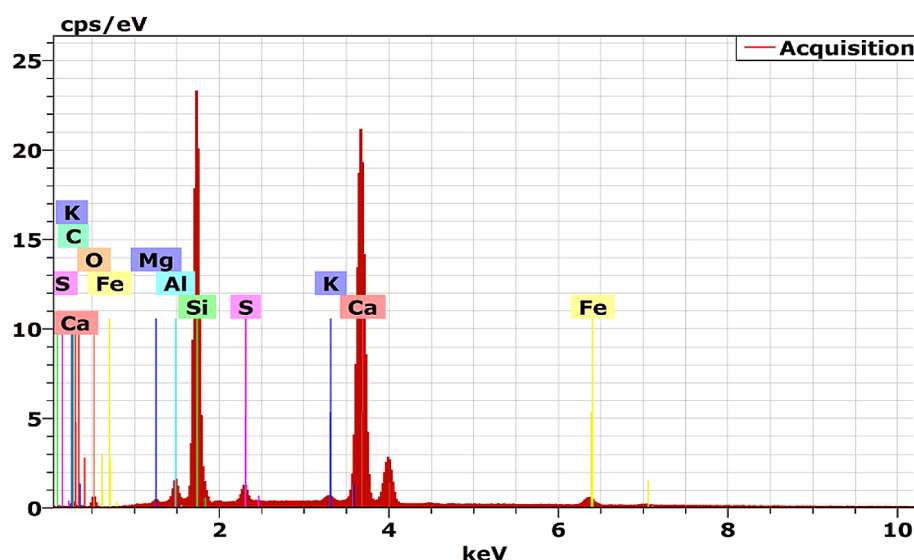


Figure 8. Support AAC analysis by energy dispersive X-ray spectroscopy

Table 2. Variation of iron concentration versus column height

Bed height (m)	0.4	0.75	1	1.36
Fe^{2+} concentration (mg/L)	0.95	0.67	0.63	0.23

Table 3. Microbial population in the seed sludge and in the bioreactor

Microbial Community	Total microbial count (Cells/ml) At 22 °C	Total microbial count (Cells/ml) At 37 °C	Coliforms <i>Escherichia coli</i> (Cells/100 ml)	<i>Pseudomonas aeruginosa</i> (Cells/ml)	Iron bacteria (Cells/ml)
Seed sludge	$1.5 \cdot 10^4$	$3 \cdot 10^5$	$11 \cdot 10^3$	$3 \cdot 10^4$	70
Bioreactor	$5 \cdot 10^4$	$8 \cdot 10^3$	$3 \cdot 10^3$	$5 \cdot 10^4$	10^3

same nutrients are not necessarily in competition; in some instances, certain substrates can only be utilized through the complementary action of multiple species (Atlas et al., 2008; Marchand et al., 2017).

Preliminary Microbiological analyses have identified the bacterial strains collected in the bioreactor. However, the overall stability of the microbial community does not necessarily reflect the stability of its individual populations. When examined separately, each species, subject to the dynamics of growth, dominance, inhibition, and symbiosis, plays a role strictly limited by intense, complex competition for survival within the microbial ecosystem. The results (Table 3) highlight the presence of diverse bacterial species, with *Pseudomonas aeruginosa* predominating. The configuration of the fixed-bed bioreactor significantly influenced the composition of bacterial populations. Notably, bacterial population density differed between the seeding sludge and the biofilm formed within the bioreactor. Substantial shifts in population structure occurred during the biodegradation process. Analyses identified two dominant species within the bioreactor: *Pseudomonas aeruginosa* and iron bacteria. Species richness was greater in the bioreactor compared to the seeding sludge, resulting in increased microbial diversity within the fixed-bed system. The biofilm was predominantly colonized by *Pseudomonas*, which is recognized for its hydrocarbon-degrading capabilities (Wang et al., 2016). These results suggest that the biomass adapted effectively to influent containing hydrocarbons and iron. The bioreactor exhibits high bacterial growth. During the biodegradation process, the removal of chemical oxygen demand (COD) in the fixed-bed bioreactor consistently exceeded 90%, a rate significantly higher than that observed by Lusnier et al. (2021) in a conventional activated sludge bioreactor. This result demonstrates the feasibility of achieving high removal rates in an industrial-scale process and highlights the potential for on-site application. Microbial analysis further revealed a highly

diverse community within the fixed-bed bioreactor, conferring robustness and a strong capacity for degrading complex pollutants. According to a study on the qualitative distribution of saprophytic heterotrophic bacteria in watercourses, Margesin et al. (2003) estimated that 93% of these bacteria are Gram-negative bacilli, with the *Pseudomonas* group comprising more than one third. This group is regarded as a key component of the bacterial community. The predominance of *Pseudomonas* is likely attributable to its high resistance to a broad spectrum of toxins; this microorganism can degrade carbon and utilize it as a carbon source (Varjani et al., 2020).

CONCLUSIONS

This study assessed the performance of a fixed-bed biological process for treating oil-industry wastewater using an innovative bacterial support. COD removal efficiencies reached 90%, 91%, and 92% for Fe^{2+} concentrations of 5, 15, and 85 mg/L, respectively. The proliferation of iron-oxidizing bacteria resulted in iron elimination rates of 98%, 95%, and 97% at Fe^{2+} concentrations of 5, 15, and 85 mg/L, respectively. An Fe^{2+} concentration of 85 mg/L did not inhibit the biodegradation process, demonstrating the effectiveness of biological treatment for oil industry wastewater. Ammonia nitrogen and phosphate removal rates of 50, 72, and 75% and 72%, 82%, and 93% were achieved in the bioreactor at Fe^{2+} concentrations of 5, 15, and 85 mg/L, respectively. The pH values ranged from 7.3 to 8.3. scanning electron microscopy characterization revealed that ACC has a porous structure and exhibits a rough surface that promotes bacterial adhesion. Energy-dispersive X-ray Spectroscopy analysis indicated that ACC does not contain toxic components that inhibit bacterial growth. It acts as an excellent biological filter. The proposed process applies to the present case due to its cost-effectiveness and environmentally sustainable characteristics

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REFERENCES

- Adetunji, A. I., Olaniran, A.O. (2021). Treatment of industrial oily wastewater by advanced technologies: a review. *Applied Water Science*, 11(6), 98. <https://doi.org/10.1007/s13201-021-01430-4>
- Afify, M.G.E., Gomaa, O.M., Abd El Kareem, H., Abou Zeid, M. A. (2025). Promoting bacterial colonization and biofilm formation for enhanced biodegradation of low-density polyethylene microplastics. *Bioresources and Bioprocessing*, 12, 59. <https://doi.org/10.1186/s40643-025-00902-8>
- Alaidaroos, B.A. (2023). Advancing eco-sustainable bioremediation for hydrocarbon contaminants: Challenges and solutions. *Processes*, 11(10), 3036. <https://doi.org/10.3390/pr11103036>
- Alomar, S., Hameed, B.H., Usman, M., Almomani, F.A., Ba-Abbad, M.M., Khraisheh, M. (2022). Recent advances on the treatment of oil fields produced water by adsorption and advanced oxidation processes. *Journal of Water Process Engineering*, 49, 103034. <https://doi.org/10.1016/j.jwpe.2022.103034>
- Álvarez, L.M.M., Ruberto, L.A.M., Balbo, A.L., Mac, W.P. (2017). Bioremediation of hydrocarbon-contaminated soils in cold regions: Development of a pre-optimized biostimulation biopile-scale field assay in Antarctica. *Science of the Total Environment*, 590, 194–203. <https://doi.org/10.1016/j.scitotenv.2017.02.204>
- Antwi-Akomeah, S., Fei-Baffoe, B., Belford, E. J.D., Borigu, M. (2018). Hydrocarbon contaminated water remediation using a locally constructed multi-stage bioreactor incorporated with media filtration. *Global Journal of Environmental Science and Management*, 4(4). <https://doi.org/10.22034/gjesm.2018.04.00>
- Atlas, R.M., Raymond, R.L. (2008). Stimulated petroleum biodegradation, *CRC Critical Reviews in Microbiology*, 5(4), 371–386. <https://doi.org/10.3109/10408417709102810>
- Bahuguna, M., Joshi, N., Bhandari, G., Singh, P., Gupta, S., Rafatullah, M., Anggayasti, W.L., Huda, N. (2025). Biological removal of iron content from water sources using iron-oxidizing bacteria: a review. *Environmental Pollutants and Bioavailability*, 37(1), 2556182. <https://doi.org/10.1080/26395940.2025.2556182>
- Bhattacharyya, A., Liu, L., Lee, K., Miao, J. (2022). Review of biological processes in a membrane bioreactor (MBR): Effects of wastewater characteristics and operational parameters on biodegradation efficiency when treating industrial oily wastewater. *Journal of Marine Science and Engineering*, 10(9), 1229. <https://doi.org/10.3390/jmse10091229>
- Chen, C., Yan, X., Xu, Y.Y., Yoza, B.A., Wang, X., Kou, Y., Ye, H., Wang, Q., Li, Q.X. (2019). Activated petroleum waste sludge biochar for efficient catalytic ozonation of refinery wastewater. *Science of The Total Environment*, 651(2), 2631–2640. <https://doi.org/10.1016/j.scitotenv.2018.10.131>
- Christodoulis, K., Theodoropoulou, M., Tsakiroglou, C.D. (2024). Remediation of oil-drilling cuttings by ozonation in a bubble flow reactor, *Journal of Environmental Chemical Engineering*, 12(2), 112436. <https://doi.org/10.1016/j.jece.2024.112436>
- Di Fabio, S., Malamis, S., Katsou, E., Vecchiato, G., Cecchi, F., Fatone, F. (2013). Optimization of membrane bioreactors for the treatment of petrochemical wastewater under transient conditions. *Chemical Engineering Transactions*, 32, 7–12. <https://doi.org/10.3303/CET1332002>
- Dong, Z., Lu, M., Huang, W., Xu, X. (2011). Treatment of oilfield wastewater in moving bed biofilm reactors using a novel suspended ceramic biocarrier. *Journal of Hazardous Materials*, 196, 123–130. <https://doi.org/10.1016/j.jhazmat.2011.09.001>
- Eregie, S.B., Jamal-Ally, S.F. (2023). Comparison of biodegradative efficiency of wildtype versus mutagenised *Scenedesmus vacuolatus* of spent coolant waste: dehydrogenase activity and total petroleum degradation studies. *International Journal of Environmental Analytical Chemistry*, 103(18), 6996–7022. <https://doi.org/10.1080/03067319.2021.1965593>
- Ferreira, A.R., Skjolding, L.M., Sanchez, D.F., Ntyne, A.G.B., Ivanova, Y.D., Feilberg, K.L., Chhetri, R.K., Andersen, H.R. (2024). Offshore produced water treatment by a biofilm reactor on the sea bed: The effect of temperature and matrix characteristics. *Journal of Environmental Management*, 365, 121391. <https://doi.org/10.1016/j.jenvman.2024.121391>
- Geng, S., Mao, S., Xu, G., Ding, A., Chen, F., Dou, J., Fan, F. (2024). Performance evaluation of modified biochar as a polycyclic aromatic hydrocarbon adsorbent and microbial-immobilized carrier. *Processes*, 12(12), 2939; <https://doi.org/10.3390/pr12122939>
- Islam, B. (2015). Petroleum sludge, its treatment and disposal: A review. *International Journal of Chemistry Science*, 13(4), 1584–1602.
- Kadri, T., Rouissi, T., Brar, S.K., Cledon, M., Sarma, S., Verma, M. (2017). Biodegradation of polycyclic aromatic hydrocarbons (PAHs) by 3 fungal enzymes:

- A review. *Journal of Environmental Sciences*, 51, 52–74. <https://doi.org/10.1016/j.jes.2016.08.023>
19. Kulkarni, S.J. (2016). Biological Treatment of Petroleum Wastewater: A Review on Research and Studies. *International Journal of Petroleum and Petrochemical Engineering*, 2(2), 17–21. <http://dx.doi.org/10.20431/2454-7980.0202004>
 20. Kuyukina, M.S., Krivoruchko, A.V., Ivshina, I.B. (2020). Advanced bioreactor treatments of hydrocarbon-containing wastewater. *Applied Sciences*, 10(3), 831. <https://doi.org/10.3390/app10030831>
 21. Lago, A., Rocha, V., Barros, O., Silva, B., Tavares, T. (2024). Bacterial biofilm attachment to sustainable carriers as a clean-up strategy for wastewater treatment: A review. *Journal of Water Process Engineering*, 63, 105368. <https://doi.org/10.1016/j.jwpe.2024.105368>
 22. Lawan, M.S., Kumar, R., Rashid, J., Barakat, M.A.E.F. (2023). Recent advancements in the treatment of petroleum refinery wastewater. *Water*, 15(20), 3676. <https://doi.org/10.3390/w15203676>
 23. Liu, Y., Zhang, J., Yang, S. (2023). Biodegradation and removal of heavy oil using *Pseudomonas* sp. and *Bacillus* spp. isolated from oily sludge and wastewater in Xinjiang Oilfield, China. *Journal of Environmental Chemical Engineering*, 11(3), 110123. <https://doi.org/10.1016/j.jece.2023.110123>
 24. Lusnier, N., Seyssieq, I., Sambusiti, C., Jacob, M., Lesage, N. (2021). A comparative study of conventional activated sludge and fixed bed hybrid biological reactor for oilfield produced water treatment: Influence of hydraulic retention time. *Chemical Engineering Journal*, 420(2), 127611. <https://doi.org/10.1016/j.cej.2020.127611>
 25. Marchand, C., St-Arnaud, M., Hogland, W., Bell, T.H., Hijri, M. (2017). Petroleum biodegradation capacity of bacteria and fungi isolated from petroleum-contaminated soil. *International Biodegradation*, 116, 48–57. <https://doi.org/10.1016/j.ibiod.2016.09.030>
 26. Mansour, M.S.M., Abdel-shafy, H.I., Ibrahim, A.M. (2024). Petroleum wastewater: Environmental protection, treatment, and safe reuse: An overview. *Journal of Environmental Management*, 351, 119827. <https://doi.org/10.1016/j.jenvman.2023.119827>
 27. Margesin, R., Labbé, D., Schinner, F., Greer, C.W., Whyte, L.G. (2003). Characterization of hydrocarbon-degrading microbial populations in contaminated and pristine alpine soils. *Applied and Environmental Microbiology*, 69(6), 3085–3092. <https://doi.org/10.1128/AEM.69.6.3085-3092.2003>
 28. Mokif, L.A., Jasim, H.K., Abdulhusain, N.A. (2022). Petroleum and oily wastewater treatment methods: A mini review. *Materials Today: Proceedings*, 49(7), 2671–2674. <https://doi.org/10.1016/j.matpr.2021.08.340>
 29. Mirshafiee, A., Nourollahi, M., Shahriary, A. (2024). Application of electrooxidation process for treating wastewater from petrochemical with mixed metal oxide electrode. *Nature*, 14, 1760. <https://doi.org/10.1038/s41598-024-52201-5>
 30. Müller, S., Corbera-Rubio, F., Kegel, F.S., Lauren, M., van Loosdrecht, M.C.M., van Halem, D. (2024). Shifting to biology promotes highly efficient iron removal in groundwater filters. *Water Research*, 262, 122135. <https://doi.org/10.1016/j.watres.2024.122135>
 31. Nabaterega, R., Vesuwe, R.N., Iorhemen, O.T. (2025). Statistical evaluation of different filter media and application of multiple criteria analysis to select the best media for pollutants removal in wastewater biofiltration: A review. *Science of The Total Environment*, 996, 180104. <https://doi.org/10.1016/j.scitotenv.2025.180104>
 32. Okan, O.L., Ugonabor, V.I., Onu, C.E., Chinedu, J.U. (2022). Application of trickling filter with hybrid biofilm support media in the treatment of petroleum effluent. *LAUTECH Journal of Engineering and Technology*, 16(2), 94–105.
 33. Pérez, L.S., Rodriguez, O.M., Reyna, S., Sánchez-Salas, J.L., Lozada, J.D., Quiroz, M.A., Bandala, E.R. (2016). Oil refinery wastewater treatment using coupled electrocoagulation and fixed film biological process. *Physics and Chemistry of the Earth, Parts A/B/C*, 91, 53–60.
 34. Sanghamitra, P., Mazumder, D., Mukherjee, S. (2021). Treatment of wastewater containing oil and grease by biological method- a review. *Journal of Environmental Science and Health*, 56(4), 394–412. <https://doi.org/10.1080/10934529.2021.1884468>
 35. Turner, N.R., Abigail Renegar, D. (2017). Petroleum hydrocarbon toxicity to corals: A review. *Marine Pollution Bulletin*, 119(2), 1–16. <https://doi.org/10.1016/j.marpolbul.2017.04.050>
 36. Tumurbaatar, S., Kováts, N., Hubai, K. (2024). Phytotoxicity testing of atmospheric polycyclic aromatic hydrocarbons. *Atmosphere*, 15(9), 1143–1156. <https://doi.org/10.3390/atmos15091143>
 37. Varjani, S., Kumar, G., Rene, E.R. (2019). Developments in biochar application for pesticide remediation: current knowledge and future research directions. *Journal of Environmental Management*, 232, 505–513. <https://doi.org/10.1016/j.jenvman.2018.11.043>
 38. Varjani, S., Upasani, V. N., Pandey, A. (2020). Bioremediation of oily sludge polluted soil employing a novel strain of *Pseudomonas aeruginosa* and phytotoxicity of petroleum hydrocarbons for seed germination. *Science of the Total Environment*, 737, 139766. <https://doi.org/10.1016/j.scitotenv.2020.139766>
 39. Vieira dos Santos, E., Bezerra Rocha, J.H., Medeiros

- de Araújo, D., Chianca de Moura, D., Martínez-Huitle, C.A. (2014). Decontamination of produced water containing petroleum hydrocarbons by electrochemical methods: a mini review. *Environmental Science and Pollution Research*, 21, 8432. <https://doi.org/10.1007/s11356-014-2780-4>
40. Walker, S., Kolb, T. (2025). Operation of a differential fixed bed reactor for investigations on micro-kinetics of heterogeneous gasification reactions. *Fuel*, 381, 133561. <https://doi.org/10.1016/j.fuel.2024.133561>
41. Wang, X., Wang, X., Liu, M., Zhou, L., Gu, Z., Zhao, J. (2016). Bioremediation of marine oil pollution by *Brevundimonas diminuta*: Effect of salinity and nutrients. *Desalination Water Treatment*, 57(42), 19768–19775. <https://doi.org/10.1080/19443994.2015.1106984>
42. Zhang, W., Xiong, Y., Zhang, D., Guan, Y., Wang, Z., Bao, M., Li, Y. (2024). High-efficiency treatment of oily wastewater by photocatalytic in-situ Fenton oxidation under visible light. *Chemical Engineering Journal*, 500, 156848. <https://doi.org/10.1016/j.cej.2024.156848>
43. Zhao, Q., Chen, C., Chen, Z., Shan, H., Liang, J. (2025). Enhanced oilfield-produced-water treatment using Fe^{3+} -augmented composite bioreactor: Performance and microbial community dynamics. *Bioengineering (Basel)*, 12(7). <https://doi.org/10.3390/bioengineering12070784>
44. Zhao, Y., Liu, D., Huang, W., Yang, Y., Ji, M., Nghiem Duc, L., Trinh, Q.T., Tran, N.H. (2019). Insights into biofilm carriers for biological wastewater treatment processes: Current state-of-the-art, challenges, and opportunities. *Bioresource Technology*, 288, 121619. <https://doi.org/10.1016/j.biortech.2019.121619>
45. Zhou, L., Zhang, Y., Ge, Y., Zhu, X., Pan, J. (2020). Regulatory mechanisms and promising applications of quorum sensing-inhibiting agents in control of bacterial biofilm formation. *Frontiers in Microbiology*, 11, 589640. <https://doi.org/10.3389/fmicb.2020.589640>
46. Zueva, S., Corradini, V., Elena Ruduka, E., Veglio, F. (2020). Treatment of petroleum refinery wastewater by physicochemical methods. *International Conference on Efficient Production and Processing*, 161, 01042, 5. <https://doi.org/10.1051/e3sconf/202016101042>