Anekwe and Isa
Biotechnology for the Environment (2024) 1:12
https://doi.org/10.1186/s44314-024-00012-3

Biotechnology for the Environment

RESEARCH

Open Access

Sustainable remediation of acid mine drainage (AMD)- and crude oil-contaminated sites through wastewater-enhanced *Pseudomonas aeruginosa* ATCC 15442 treatment

Ifeanyi Michael Smarte Anekwe[1*] and Yusuf Makarfi Isa[1]

Abstract

The increasing rate of soil contamination poses a major challenge to the sustainability of the ecosystem. Acid mine drainage (AMD) and crude oil are among the major soil contaminants contributing to the degradation of soil organic matter. This study aims to evaluate the application of *Pseudomonas aeruginosa* ATCC 15442 (*PA*-15442) and wastewater for the treatment of AMD- and crude oil-contaminated soils. A microcosm containing 1 kg each of AMD- and crude oil-contaminated soils was inoculated with *PA*-15442, and brewery and domestic wastewater were added for the bioaugmentation study. The result of the 28-day study conducted under mesophilic conditions showed an average TPH and metal removal efficiency of 58.84% and 52.75%, corresponding to 51.07, 47.29, 59.32, 58.98, and 47.1% for the individual metals (Fe, Al, Cu, Zn, and Mn), respectively, and 49% for the average sulfate removal after the treatment period. This study has shown that bioaugmentation of contaminated soils with the strain of *PA*-15442, and the addition of wastewater could be an environmentally friendly and sustainable approach for the remediation of AMD- and petroleum-contaminated soils.

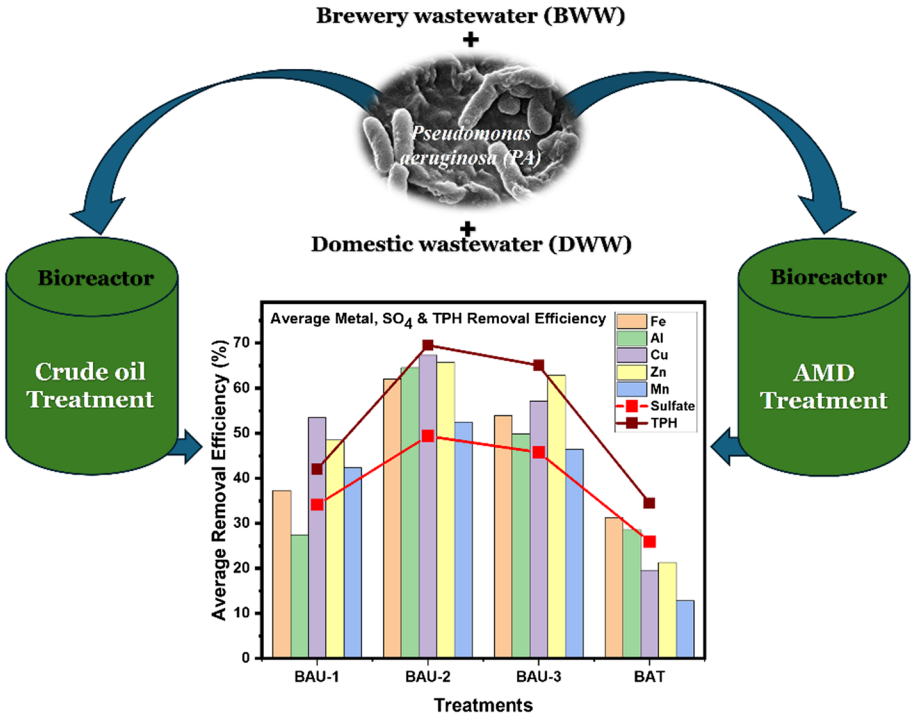
Keywords Acid mine drainage (AMD), Brewery wastewater, Crude oil, Contaminated soil, Domestic wastewater, *Pseudomonas aeruginosa*

Introduction

Environmental pollution is a global issue that threatens the existence of the ecosystem. This problem has contributed immensely to water, soil, and air contamination, climate change, poor standard of living, and reduction in economic activities. The high rate of soil pollution has created a serious issue of urgent concern to prevent both the aquatic and terrestrial habitats from total annihilation. Among these contaminants, acid mine drainage (AMD) and crude oil (also known as petroleum) are the major forms of land/soil and water pollutants that deteriorate and degrade soil structure, reduce soil fertility, and pollute proximate water bodies, thereby decreasing the availability of arable land for agricultural purposes and accessibility of potable water. Oil spillage from petroleum and petrochemical industries attributed to exploration activities, instrument malfunction, and recklessness are the major source of this form of soil pollution which has degraded the environment and negatively impacted the lives of host communities [33, 56]. AMD is a mine waste that emerges from old, abandoned, or active mine sites containing sulfate and heavy metals that contaminate the soil and proximate water bodies. AMD is generated when sulfide-bearing minerals, pyrites (iron disulfide, FeS_2), are exposed to oxygen and water. Pyrite oxidation is triggered

*Correspondence:
Ifeanyi Michael Smarte Anekwe
anekwesmarte@gmail.com; Ifeanyi.anekwe@wits.ac.za
Full list of author information is available at the end of the article

Graphical Abstract



which facilitates the formation of AMD (ferric hydroxide) and sulfuric acid as shown in Eq. 1. AMD formation is characterized by pyrite oxidation, low pH (< 3.0), high sulfate contents, heavy metals such as Fe, Cu, Al, Mg, Zn, Cd, Pb, Ni, and Co, and the presence of radioactive elements (Ra, Ur) depending on the type of mine site [12].

$$4FeS_2 + 15O_2 + 14H_20 \rightarrow 4Fe(OH)_3 + 8H_2SO_4 - \text{(Pyrite Oxidation)} \quad (1)$$

Bioremediation is a biological method that utilizes microorganisms to degrade biodegradable contaminants [36, 37]. This can be achieved through biostimulation, bioaugmentation, or bioventing, which involves the application of organic nutrients, genetically modified microbes, or air injection, respectively, for the degradation of contaminants [36]. The bacteria strain, *Pseudomonas aeruginosa*, is classified as a gram-negative bacterium, gammaproteobacterial, aerobic, rod, and belonging to the family Pseudomonadaceae, which can utilize hydrocarbon as an energy source [11, 70] and can tolerate a variety of heavy metals like copper, cadmium, chromium, nickel, among other metals [12, 17, 69].

The biodegradation of pollutants involves various microbial processes that utilize pollutants as energy sources or convert them into less harmful substances. These processes can occur under different environmental conditions and utilize different mechanisms to convert harmful pollutants into benign substances [6, 50, 67]. One of the primary methods is aerobic biodegradation, in which microorganisms break down complex hydrocarbons, such as those found in petroleum products, in the presence of oxygen. This process leads to the conversion of these hydrocarbons into simpler compounds such as carbon dioxide and water. Anaerobic biodegradation, on the other hand, takes place in an environment where oxygen is not present. Under such conditions, microorganisms use alternative electron acceptors, such as nitrate, to break down organic compounds. This enables the degradation of pollutants in environments where oxygen is not available [66]. Reductive dechlorination is another important process in bioremediation, especially in the treatment of chlorinated organic compounds. Certain bacteria can remove chlorine atoms from these toxic substances under anaerobic conditions and convert them into less harmful forms [71]. In methanogenesis,

organic material is broken down by methanogenic bacteria, producing methane and carbon dioxide. This is often utilized in anaerobic digestion for waste treatment [42]. In addition, cometabolism occurs when microorganisms incidentally degrade pollutants during their regular metabolic activities, even if the pollutants are not their primary energy source [67]. These diverse biological processes emphasize the versatility and effectiveness of bioremediation in treating a wide range of environmental pollutants. To this end, the objective of this study is to evaluate the application of *Pseudomonas aeruginosa* ATCC 15442 strain and wastewater amendment as a carbon source for the treatment of crude oil- and AMD-contaminated soils.

Materials and method

Materials

The soil samples for this study were collected in Durban, South Africa. The crude oil was sourced from a local oil refinery in South Africa, while the wastewaters (WW): brewery wastewater (BWW) and domestic wastewater (DWW) were obtained from the South African Brewery (SAB) and a wastewater treatment plant, respectively. A pure culture of *Pseudomonas aeruginosa* ATCC 15442 (*PA*-15442) used in this study was provided by Anatech Microbial Laboratories, South Africa. The soil samples were air dried for 24 h and then sieved using a 2-mm standard sieve. The soil composition was 79.32% sand (2–0.02 mm), 14.71% silt (0.02–0.002 mm), and 5.97% clay (< 0.002 mm).

Methodological approach

Preparation of crude oil-contaminated soil

The soil sample (1 kg) was spiked with 50 g of crude oil and stirred to achieve a homogeneous mixture of the two components in a mechanical shaker, according to our previous studies [14]. To obtain extreme soil sample contamination, the concentration of 5% w/w was adopted as a concentration > 3% has been reported to affect soil structure [59]. The bioremediation methods were applied after 4 days of aging to simulate a real polluted soil scenario.

Simulation of mine water and preparation of AMD-contaminated soil

Acid mine drainage (AMD) was simulated with a metal composition similar to that in the central Witwatersrand [45], as described in previous studies [10, 13]. The composition comprised 25.70 mg/kg $ZnSO_4{\cdot}H_2O$, 10.70 mg/kg $CuSO_4{\cdot}5H_2O$, 200.01 mg/kg $FeSO_4{\cdot}7H_2O$, 20.00 mg/kg $MnSO_4{\cdot}H_2O$, and 50.50 mg/kg $Al_2(SO_4)_3$-$18H_2O$, with a sulfate concentration of 798 mg/kg. These components were dissolved in deionized water, and the pH was adjusted to 2.7 with H_2SO_4 to obtain an acidic AMD medium. The solution was stirred at 200 rpm for 60 min to ensure homogeneity. To prepare AMD-contaminated soil, the simulated mine water was mixed with the soil sample and stirred at 180 rpm. The contaminated samples were then dried and left undisturbed for 48 h before undergoing bioremediation treatment.

Microbial culture and inoculum preparation

The strain of *PA*-15442 used in the present study was stored at 2–8 °C prior to cultivation and application in the bioremediation process. The starter culture was first grown in a solid medium prepared by dissolving 10 g of McConkey agar medium in 200 mL deionized water using a 300-mL flask. *PA*-15442 was gently transferred to the slant media and incubated for 48 h at 37 °C. For the crude oil (BAUc) treatment, bacteria cells were activated according to Varjani and Upasani [83] by transferring culture from the nutrient agar slant to Bushnell-Hass (BH) broth ($MgSO_4$, 0.200 g/L; $CaCl_2$, 0.020 g/L; KH_2PO_4, 1.000 g/L; K_2HPO_4, 1.000 g/L; NH_4NO_3, 1.000 g/L; $FeCl_2$, 0.050 g/L; pH at 25 °C, 7.1) amended with 1% v/v crude oil and incubated at 37 ± 1 °C, in continuous agitation at 180 rpm for 24 h. The inoculum was prepared in 200 ml BH medium by inoculating activated culture broth at 4% v/v of optical density (OD) of 1.0 at AU_{600}, and incubated at 37 ± 1 °C, 180 rpm for 24 h. For the AMD (BAUa) treatment, the culture was transferred to Luria–Bertani (LB) broth in an Erlenmeyer flask prepared by dissolving peptone (10 g/L), NaCl (5 g/L), and yeast (2 g/L) in distilled water. The LB solution was autoclaved at 120 °C for 90 min and cooled for 30 min.

The medium was incubated at 37 ± 1 °C, with constant shaking at 180 rpm for 48 h. Bacteria cells used for this study were immobilized according to Philip et al. [63] after 48 h of incubation (optical density 1.0 at 600 nm), and cells were harvested and separated from media using centrifugation at 4000 rpm for 30 min at 4 °C. The supernatants were decanted, and immobilized cells recovered as sediment. The isolated biomass was rinsed with distilled water. The inoculum was prepared in a 200-mL LB medium using the immobilized cells.

Bioaugmentation study of crude oil- and AMD-contaminated soils

The bioaugmentation treatment of crude oil and AMD was carried out in four bioreactors, each designated as BAUc and BAUa, containing 1 kg of crude oil- and AMD-contaminated soils, respectively. One hundred milliliters of BH and LB inoculum each was added to three BAUc or

BAUa bioreactors, respectively, followed by inoculation of enriched culture (OD: 1.0 at AU_{600}) at 5% (w/w), while some bioreactors were amended with WW effluents as shown in Table 1.

Sample preparation and instrumentation

The total nitrogen (TN) was obtained by the semi-micro Kjeldahl technique [54], and the available phosphorus (P) was determined by Brays No. 1 procedure [58], while the total degrading bacteria (TDB) were obtained by the vapor phase transfer technique according to Amanchukwu et al. [8]. Soil pH was determined according to the following [60]: 20 g of homogenized soil sample was mixed with 20 ml of distilled water (1:1 *w/v*) in a 50-ml beaker, and the suspension was agitated for 60 min. Bacteria media were autoclaved using a vertical-type steam sterilizer HL-341. Optical cell density was determined with Thermo Scientific GENESYS™ 150 UV–visible spectrophotometer, and Eppendorf 5810R refrigerated centrifuge w/A-4–81 rotor was used for cell immobilization. The soil samples from the BAUc and BAUa treatments were dried in an oven at low temperature (35 °C), pulverized using mortar and pestle and sieved using a standard sieve size of 63 µm to ensure grain size homogeneity.

For the mechanical extraction of the remaining crude oil from the sample, 5 g of homogenized soil was mixed with dichloromethane (DCM) and acetone in a ratio of 2:1 in a 250-mL glass vessel. The jar was covered with aluminum foil and shaken vigorously for 90 min at 200 rpm on a mechanical shaker to ensure effective extraction of total petroleum hydrocarbons (TPH). The resulting solution was filtered through Whatman filter paper followed by a syringe filter. The filtrate (extract) was transferred to a 50-ml volumetric flask and diluted to a known volume. TPH was determined by gas chromatography-mass spectrometry (GCMS-QP2010 SE). The carbon–oxygen demand (COD) was measured according to Standard Methods 5220D (APHA 1995) [16] using a Hach COD reactor (DRB200, Hach, USA) and a spectrophotometer (DR3900, Hach, Germany) [78].

Table 1 Bioaugmentation treatment of contaminated soil

Bioreactors	BWW (mg/kg^{-1} soil)	MWW (mg/kg^{-1} soil)	*PA*-15442 (5% w/w)	Loading ratio (BWW:MWW)
BAUc-1	0	0	√	4:0
BAUc-2	100	0	√	3:1
BAUc-3	0	100	√	2:2
BATc (control)	0	0	-	0:0
BAUa-1	0	0	√	0:0
BAUa-2	100	0	√	4:0
BAUa-3	0	100	√	0:4
BATa (control)	0	0	-	0:0

Samples from AMD treatments were analyzed using scanning electron microscopy (SEM) (Tescan Mira3, Tescan, Czech Republic), energy dispersive X-ray spectroscopy (EDS) (Thermo Fisher Nova NanoSEM, FEI, USA), and X-ray fluorescence analysis using Wirsam XRF-500. For the sulfate analysis, aqueous extraction was used, and color was detected at 420 nm using a Gallery plus discrete analyzer.

Results and discussion

Results

Characterization results

Table 2 shows that brewery wastewater has a higher nitrogen content, a higher bacterial count, and a higher COD content compared to domestic wastewater (DWW), indicating that DWW has a nutrient deficiency compared to brewery wastewater. These results suggest that brewery wastewater can increase microbial density and activity, making it an effective stimulant for the biodegradation of hydrocarbon-contaminated soils. Furthermore, the organic matter in brewery wastewater, represented by COD, is generally readily biodegradable and consists mainly of sugars, soluble starch, ethanol, and volatile fatty acids [19].

Bioaugmentation of crude oil (BAUc)—results

Bioaugmentation treatment of crude oil-contaminated soil with *Pseudomonas aeruginosa* ATCC 15442 showed significant changes in crude oil accumulation and degradation. In all treatments inoculated with *PA*-15442, an accumulation of crude oil was observed within the first 2 weeks (Fig. 1a), which then appeared to decrease by week 4 (Fig. 1b). This contrasts with the control treatment where no visible changes were observed (Fig. 1c). Figure 2 shows a visible reduction in TPH from week 1, with BAUc-2 showing the highest weekly removal efficiency throughout the treatment period, except at week 2 where it showed a decrease compared to the other treatments. BAUc-2 reduced the initial TPH concentration from 50,000 to 15,260 mg/kg by week 4, corresponding to a removal efficiency of 69.48% at an average removal rate of 1318.75 mg/day. BAUc-3 showed the highest removal efficiency at week 2 with an increase in efficiency of 20.14 compared to 10.88% and 9.43% for BAUc-1 and BAUc-2, respectively. Overall, BAUc-3 removed 32,315 mg/kg of hydrocarbons at an average removal rate of 1161.25 mg/day, corresponding to a TPH removal efficiency of 65.03%. In comparison, BAUc-1 had a removal efficiency of 42.02% at an average rate of 750 mg/day during the 28-day remediation period. The control treatment (BATc) had the lowest and slowest TPH reduction, removing 17,250 mg/kg at an average rate of 616 mg/day. These results indicate that the application of *PA*-15442 in

Table 2 Physicochemical and microbiological characterization of soil, brewery, and domestic wastewaters

Wastewater/ composition	pH	COD (mg/L)	Total nitrogen	Available phosphorus	Microbial count
BWW	8.2	750	52.7 ± 0.07 mg/L	9.6 ± 0.04 mg/L	1.7×10^6 CFU/mL
DWW	7.9	704	45.5 ± 8.7 mg/L	15.5 ± 0.5 mg/L	1.1×10^6 CFU/mL
Soil	7.2	-	2.8 ± 0.1 g kg soil^{-1}	2.1 ± 0.01 g kg soil^{-1}	3.0×10^6 CFU/g

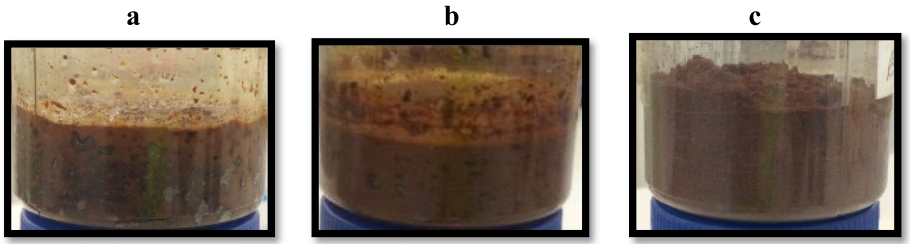


Fig. 1 **a** The accumulation and immobilization of crude oil in treatment amended with *PA*-15442 and BWW due to the effect of biosurfactant which reduced in week 4 (**b**) as a result of biodegradation, while no visible changes or effect was observed in **c**, the control treatment

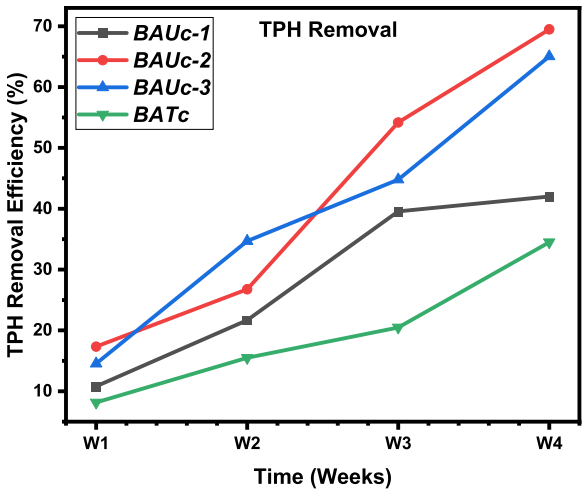


Fig. 2 TPH removal efficiency from BAUa treatment for a 28-day treatment with and without microbial and wastewater amendment, including *PA*-15442 only (BAUa-1), *PA*-15442 + BWW (BAUa-2), *PA*-15442 + DWW (BAUa-3), and no amendment–control (BATa)

combination with wastewater increases the efficiency of TPH removal, suggesting that the TPH reduction in the supplemented treatments is due to the microbial activities stimulated by these biostimulants.

Bioaugmentation of AMD (BAUa)—results

The treatment started in the first week with a remarkable metal reduction in the different bioreactors, especially in those inoculated with PA and enriched with wastewater. A black precipitate was only observed on the surface of the *PA*-15442 + WW-enriched treatments. The BAUa-1 treatment inoculated with *PA*-15442 showed an average metal removal efficiency of over 50 for Fe, Cu, Zn, and Mn, while Al was removed at less than 40% in week 4 (Fig. 3a). The average sulfate removal efficiency for this treatment was 40.22%. BAUa-2 (*PA*-15442 + BWW) showed the highest weekly removal efficiency in week 1 with over 80% for Fe, Al, Cu, and Zn, except for Mn, with a subsequent decrease from week 2 to week 4, reaching an average metal removal efficiency of 62.40% after 28 days (Fig. 3b). BAUa-3 treatment amended with *PA*-15442 + DWW showed more than 40% removal for all metals except Al (< 40%) in week 1. The removal effects improved in week 2, but then decreased by more than 15% in the following 2 weeks (Fig. 3c). This treatment finally reduced all metals (Fe, Al, Cu, Zn, and Mn) by more than 50% on average and achieved an overall removal efficiency of 54.04%.

The control treatment (BATa) had a lower metal removal efficiency compared to the other treatments, with an average metal reduction of less than 30% and an average sulfate removal efficiency of 31.55% (Fig. 3d). For sulfate removal, a reduction of 4.52 and 2.13 at week 3 and week 2 was observed in treatments BAUa-2 and BAUa-3, where *PA*-15442 was supplemented with BWW and DWW, respectively, resulting in a cumulative sulfate removal of 447 mg/kg and 410 mg/kg, corresponding to an average removal efficiency of 56.01% and 51.37% with average removal efficiency of 15.96 mg/day and 14.64 mg/day, respectively (Fig. 4). An increase in pH from 6.9 to 7.3 was observed in all BAUa treatments. The metal removal efficiency ranged from 24–84% for Fe, 21–81% for Al, 43–88% for Cu, 36–89%

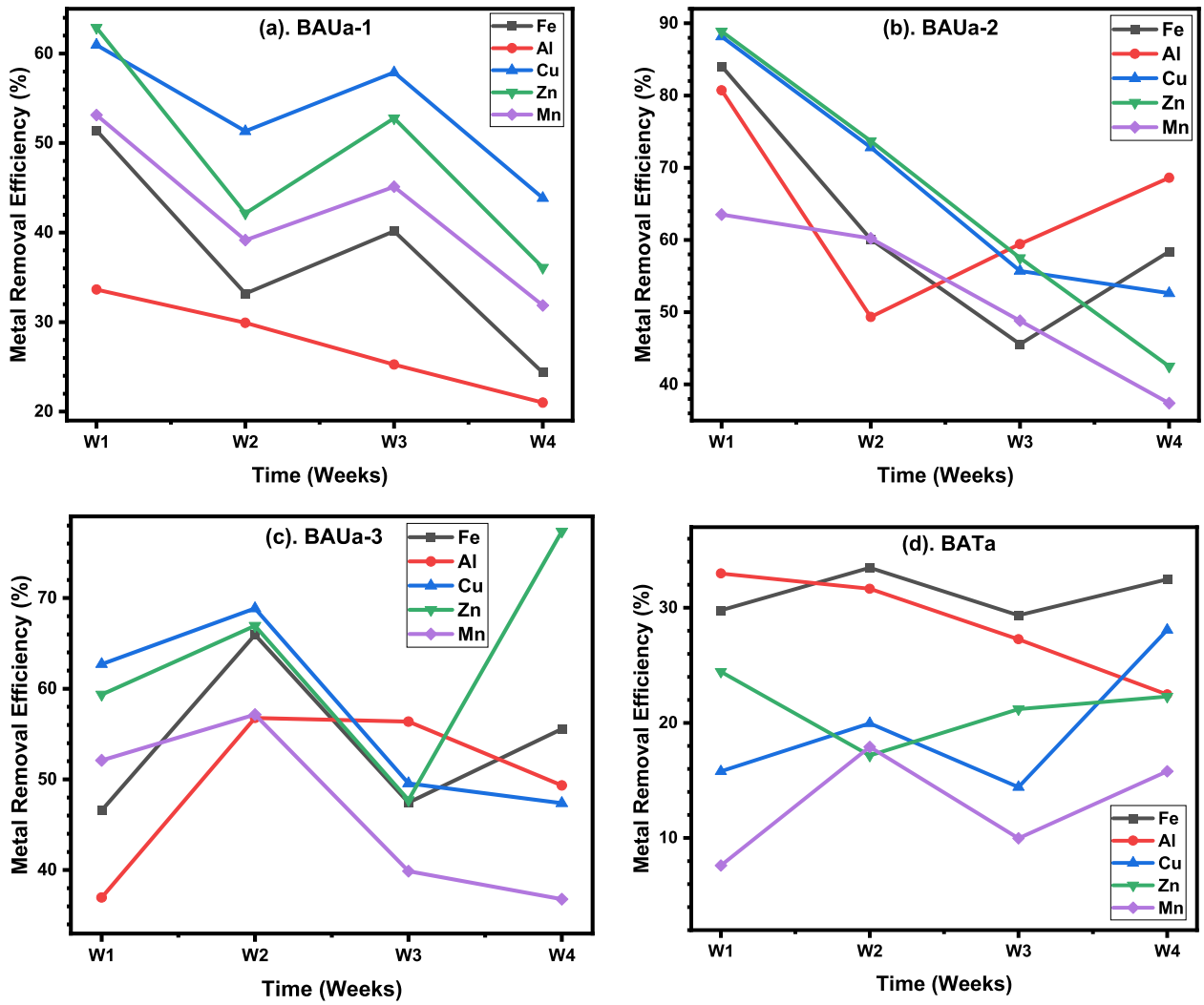


Fig. 3 Heavy metal removal efficiency for different BAUa methods for 28-day treatment period **a** *PA* only (BAUa-1), **b** *PA* + BWW (BAUa-2), **c** *PA* + DWW (BAUa-3), **d** no amendment–control (BATa)

for Zn, and 31–64% for Mn. These results show that the combined application of *PA*-15442 and WW effectively removes metals and sulfates. Figure 5 shows the average metal and sulfate removal efficiency of the different treatments, which emphasizes the feasibility of the wastewater-enhanced *PA*-15442 treatment method for the remediation of AMD-contaminated soils.

Scanning electron microscopy (SEM)–energy dispersive X-ray spectroscopy (EDS)

SEM analysis of the samples of BAUa-1 and BAUa-2 treatment systems revealed that their surfaces were covered with extensive layers (Fig. 6a and b). These layers consisted of Fe, Al, Zn, and O, together with trace elements and small amounts of other metals, as shown by the peaks in the EDS spectrum (Fig. 7a and b). These peaks corresponded to the main components of the metals present in the AMD treatment system and suggest that Fe, Al, and other metals were precipitated as metal sulfides, resulting in some metals being undetectable in the EDS spectrum of the samples after treatment. The degree of metal reduction or removal was determined by XRF analysis as described above. However, the sample from the wastewater-enriched treatment (BAUa-2) showed that more microelements were added by the addition of wastewater.

Discussion

Bioaugmentation of crude oil-contaminated soils (BAUc)

The bioaugmentation treatment of crude oil-contaminated soil using *PA*-15442 observed the accumulation of crude oil evident in all treatments inoculated with *PA*-15442 as shown in Fig. 1a within the first 2 weeks of the treatment and seems to have reduced in week 4 (Fig. 1b) which is contrary to the control treatment (BATc) with

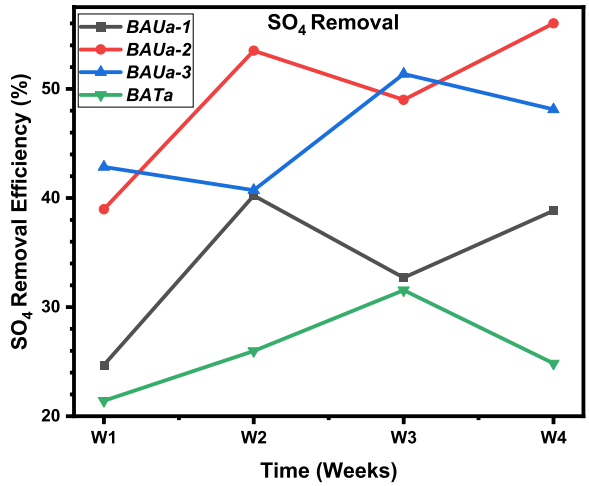


Fig. 4 Sulfate removal efficiency from different BAUa treatment methods

no visible changes in treatment (Fig. 1c). The accumulation of crude oil as shown in Fig. 1 evident in treatments amended with *PA*-15442 after the first week of treatment can be attributed to the result of solubilization and immobilization of crude oil to the degrading bacteria by the produced rhamnolipids [68], and the subsequent degradation of the accumulated crude oil [43] results in the decrease in the concentration of the accumulated crude oil (Fig. 2) in week 4. Figure 2 shows that the reduction in TPH was visible from week 1, with BAUc-2 recording the highest weekly removal efficiency throughout the treatment except in week 2, which observed a decline when compared with other treatments and reduced the initial concentration of 50,000 mg/kg to 15,260 mg/kg which represents 69.48% TPH removal efficiency in week 4 at 1318.75 mg/day average removal rate. However, BAUc-3 was able to remove 32,315 mg/kg of hydrocarbon at 1161.25 mg/day average removal rate from the treatment, corresponding to 65.03% TPH removal efficiency, while BAUc-1 recorded 42.02% after the remediation period at 750 mg/day average removal rate (28 days). Biodegradation of crude by *PA*-15442 was feasible through the production of rhamnolipid biosurfactant, which is a low molecular weight glycolipid [81] that explains the reason for no visible changes in the control treatment (Fig. 3). Rhamnolipids by *Pseudomonas aeruginosa* is a well-known biosurfactant, which allows the biological absorption of crude oil by accumulated biomass [20] which can have an impact on the actual removal of crude oil [20, 43] as evident in treatments inoculated with *PA*-15442 [25].

The amendment of the organic substrates like BWW and DWW as additional carbon sources introduced alkalinity that triggered increases in pH of the system, which facilitates dehydrogenase to enhance biodegradation of hydrocarbons evident in treatment BAUc-2 and BAUc-3, amended with BWW and DWW respectively which recorded 25% average increase TPH removal efficiency more than the treatment without wastewater amendment (BAUc-1). This agrees with the results of several studies [3, 9, 15]. The control treatment (BATc) recorded the lowest and slowest TPH reduction from week 1 till the end of the treatment removing 17,250 mg/kg at 616 mg/day. It is evident from the BAUc treatment that the application of *PA*-15442 + WW enhances TPH removal

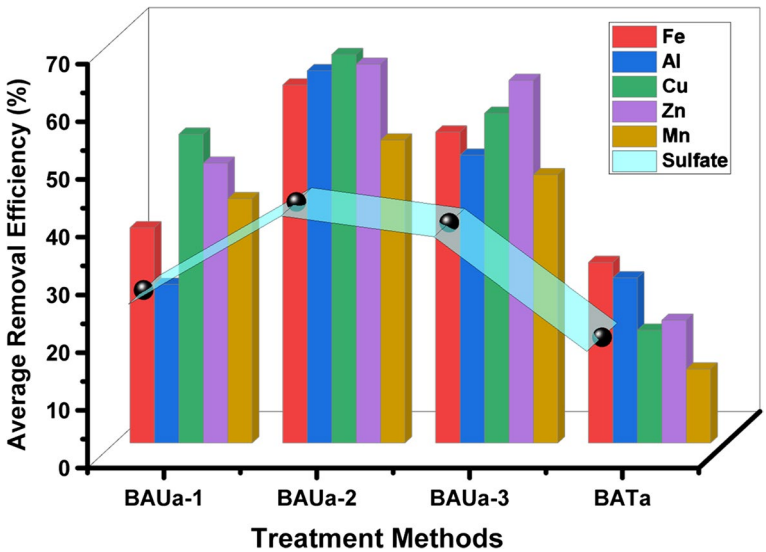


Fig. 5 Average metal and sulfate removal efficiency from different BAUa treatments

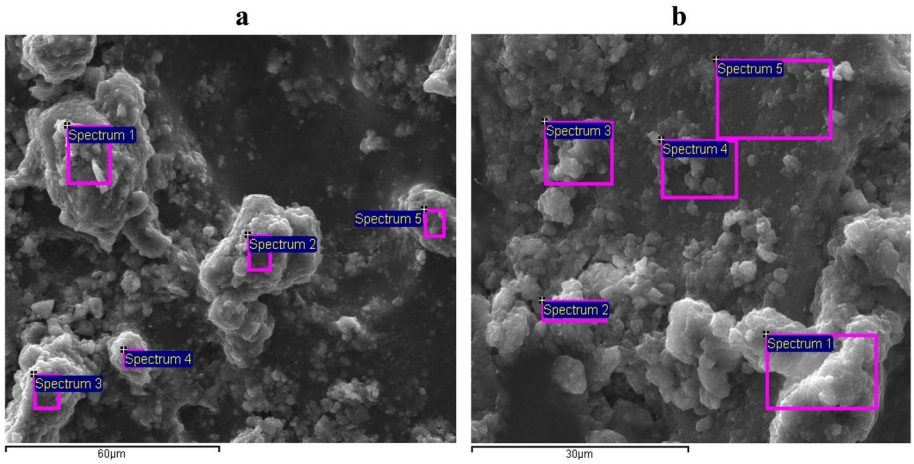


Fig. 6 SEM images from **a** BAUa-1 and **b** BAUa-2 treatment systems showing the cryptocrystalline coating layer of spherulitic aggregates

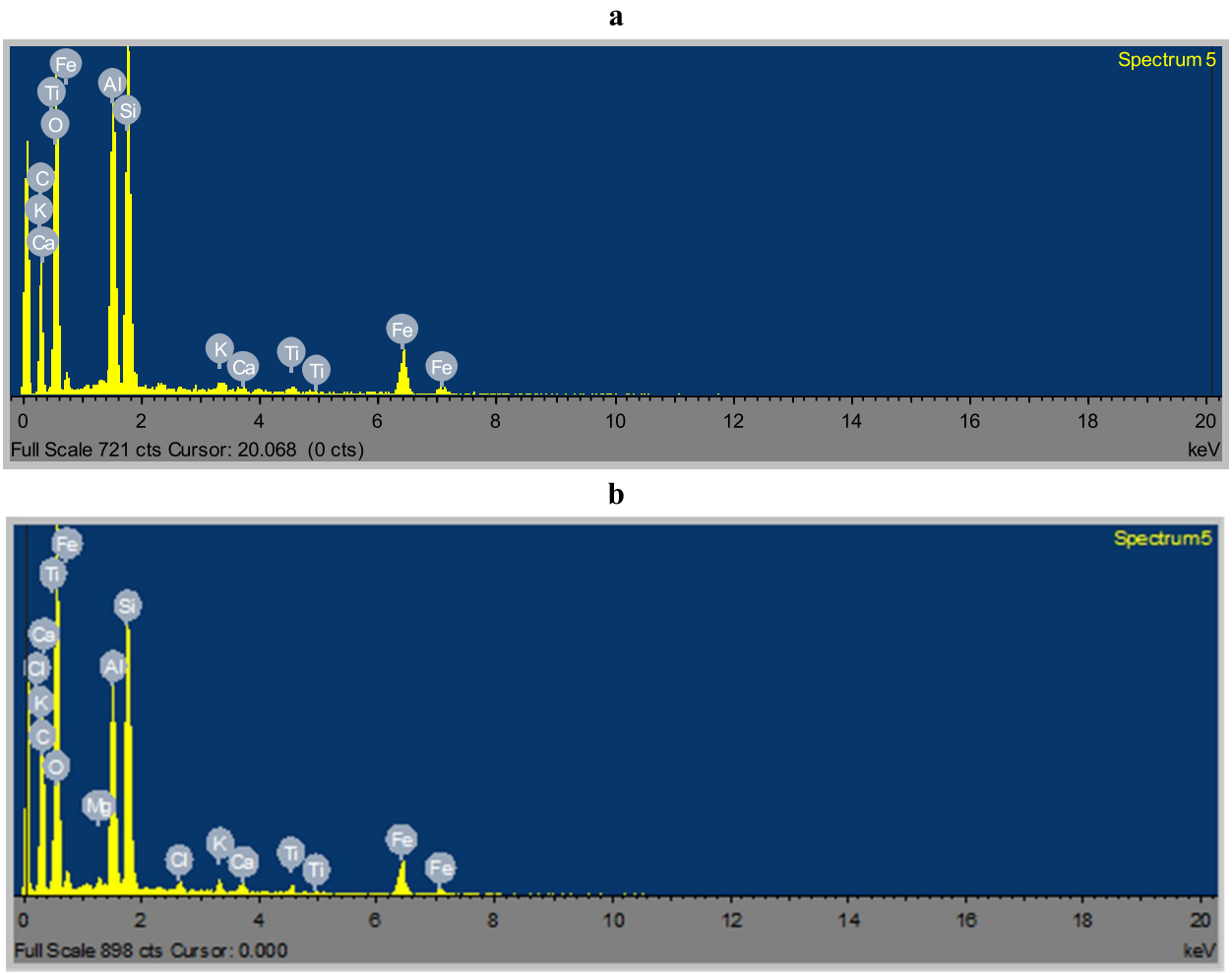


Fig. 7 EDS micrographs of samples from **a** BAUa-1 and **b** BAUa-2 treatment systems, consisting of Fe, Al, O, microelements, and minor amounts of other metals

efficiency. This showed that TPH reduction from treatments supplemented wastewaters is due to the microbial activities induced by these biostimulants. The results of this study, which recorded 67.47% average TPH removal efficiency with *PA* + WW after 28 days, are in agreement with studies by Abdulsalam et al. [2], Qiao et al. [65], and Benyahia and Embaby [21] which reported removal efficiencies of 66%, 46–64%, and 56–77% after 10 week-, and 90- and 156-day treatment period, respectively. Moreover, Mohajeri et al. [53] observed that the amendment of organic nutrient to the microbial population increases the biodegradation efficiency with removal efficiencies of 73.89, 73.76, and 58.31% reported for initial oil concentrations of 3, 30, and 60 g/kg soil, respectively, after 90-day study period which corresponds to the present study which recorded average removal efficiency of 67.47% for 50 g/kg soil with *P. aeruginosa* and WW amendment after 28-day study period.

The accumulation of crude oil, as shown in Fig. 1, evident in treatments amended with *PA* after the first week of treatment, can be attributed to the result of solubilization and immobilization of crude oil to the degrading bacteria by the produced rhamnolipids [68], and the subsequent degradation of the accumulated crude oil [43] results to the decrease in the concentration of the accumulated crude oil (Fig. 2) in week 4. Biodegradation of crude by *Pseudomonas aeruginosa* was feasible through the production of rhamnolipid biosurfactant which is a low molecular weight glycolipid [81] that explains the reason for no visible changes in the control treatment (Fig. 3). Rhamnolipids by *Pseudomonas aeruginosa* is a well-known biosurfactant, which allows the biological absorption of crude oil by accumulated biomass [20] which can have an impact on the actual removal of crude oil [20, 43] as evident in treatments inoculated with *Pseudomonas aeruginosa* [25]. Rhamnolipid biosurfactants are characterised by special properties, including non-toxicity, biodegradability, biocompatibility and high efficiency at low concentrations. Their ability to be produced from natural substrates under mild environmental conditions makes them particularly suitable for bioremediation applications. Guo-liang et al. [43] and Uzoigwe et al. [81] reported that crude oil adsorption to dead *Pseudomonas aeruginosa* may be negligible in hydrocarbon degradation since *P. aeruginosa* utilize crude oil as a sole energy source for biological activities through the synthesis of rhamnolipids. The amphiphilic nature of the rhamnolipids enables them to dissolve and immobilize hydrophobic solvents like crude oil to enhance bioavailability and degradation [62, 72, 76]. This process is accomplished by reducing the surface and interface stress between the relatively high liquid–liquid phase and the formation of stable emulsion in order to enhance degradation.

In agreement with the present BAUc study which recorded 42–69% with an average of 58.84% TPH removal efficiency after 28 days, the study by Kumari et al. [48] reported 67.1% hydrocarbon removal efficiency with *Pseudomonas aeruginosa* after 45 days of treatment, while Tavassoli et al. [79] recorded 46% removal efficiency with an appreciable growth of *Pseudomonas* spp. observed during the treatment. Bezza and Chirwa [22] recorded 62% after 8 days of treatment using biosurfactant produced by *P. aeruginosa* for the treatment of PAH-polluted soil as the substantial reduction in hydrocarbon was due to a combined solubilization and biodegradation process as reported by Shin et al. [73]. The study by Das and Mukherjee [32] showed that *Pseudomonas aeruginosa* M and MN strains were more effective than *B. subtilis* strain in the TPH degradation process which accounts for an average removal efficiency of 75% as against 46% recorded by the latter after 120 days of treatment attributed to the possession of energy-dependent system by *P. aeruginosa* strain which mediates the rapid absorption (in the presence of rhamnolipid) of hydrophobic components as reported by Noordman and Janssen [55], while the microbial biodegradation of resins fractionated from Arabian light crude oil using *Pseudomonas aeruginosa* isolated from emulsified mixed population recorded 50% removal efficiency from 5000 ppm TPH concentration of crude oil after 7 days of treatment as reported by Venkateswaran et al. [84].

However, the study by Song et al. [77] showed variation in TPH removal efficiencies of *P. aeruginosa* S and *P. aeruginosa* Y strains with 69% and 52% biodegradation efficiencies respectively which can be attributed to cell surface hydrophobicity (CSH) as reported by Bouchez Naïtali et al. [26] where *P. aeruginosa* S showed low cell hydrophobicity and decrease in broth's surface tension as the strain was able to grow on alkanes, while *P. aeruginosa Y*, on the contrary, observed significant cell surface hydrophobicity with no substantial variation visible in the surface tension. This showed that *P. aeruginosa* Y relates directly to oil droplets, while *P. aeruginosa* S engaged hydrocarbon droplets by means of biosurfactant-medium mode which correlates to a higher removal efficiency. However, the results noted that the biosurfactant-producing mode is still effective to the direct mode degradation which suggests that biosurfactant could achieve an optimal reduction of hydrocarbon concentration [40]. This may explain and contribute to the variation in biodegradation efficiencies with *Pseudomonas aeruginosa* strains as the synthesized biosurfactant plays a vital role in petroleum biodegradation.

The amendment of the organic substrates like BWW and DWW as additional carbon sources introduced alkalinity that triggered increases in the pH of the

system, which facilitates dehydrogenase to enhance biodegradation of hydrocarbons [3, 9, 15] evident in treatment BAUc-2 and BAUc-3, amended with BWW and DWW respectively which recorded 25% average increase TPH removal efficiency more than the treatment without wastewater amendment (BAUc-1). Also, the synergy between the microbial load present in wastewater and *P. aeruginosa* attributes to the increase in removal efficiencies as reported by Agarry and Latinwo [4]. In accordance with the present study which increased the TPH removal efficiency from 40 to 65% and 69% with BWW and DWW amendment respectively after 28 days, the investigation by Al-Hadhrami et al. [7] observed an increase in TPH removal efficiency from 20 to 50% using the organic substrate as an energy source for *Pseudomonas aeruginosa*, while mineral fertilizer recorded from 14 to 20% after the 24-h treatment period. According to the findings by Al-Hadhrami et al. [7], the appreciable removal efficiency recorded with the organic substrate (cane sugar molasses) more than mineral fertilizer can be attributed to the increased respiration, which enhanced the oxidation rate associated with significant hydrocarbon breakdown. This showed the effect and selectivity of energy source by bacteria (*PA*) [7].

Similar to the result of this investigation which recorded 67.47% average TPH removal efficiency with *Pseudomonas aeruginosa* and wastewater after 28 days, Abdulsalam et al. [2], Qiao et al. [65], and Benyahia and Embaby [21] reported removal efficiencies of 66%, 46–64%, and 56–77% after 10 week- and 90- and 156-day treatment period respectively, while Mohajeri et al. [53] observed that amendment of organic nutrient to the microbial population increases the biodegradation efficiency with removal efficiencies of 73.89, 73.76, and 58.31% reported for initial oil concentrations of 3, 30, and 60 g/kg soil respectively after 90-day study period which corresponds to present study which recorded average removal efficiency of 67.47% for 50 g/kg soil with *P. aeruginosa* and wastewater amendment after 28-day study period. The findings of Mohajeri et al. [53] noted that a high concentration of crude oil affects the rate of microbial biodegradation. The high degradation efficiency with combined BAU and BST can be attributed to a low concentration of initial TPH (3 g/kg or 700 mg/kg soil) when compared to the present study as reported by Mohajeri et al. [53]. This validates the efficacy of bioaugmentation and biostimulation (*Pseudomonas aeruginosa* inoculation and WW amendment) in the treatment of crude oil-contaminated sites, which recorded appreciable removal efficiency when compared with control treatment which received neither inoculation nor wastewater amendment.

Mechanism of metal removal by P. aeruginosa ATCC 15442

The treatment started on the first week with appreciable metal reduction observed in different bioreactors, especially treatments inoculated and amended with *PA*-15442 + WW, respectively, with black precipitate observed on the surface of treatments amended with P.A + wastewater only. BAUa-1 treatment inoculated with *PA*-15442 recorded an average metal removal efficiency of > 50% for Fe, Cu, Zn, and Mn, except for Al with < 40% in week 4 (Fig. 3a) with an average sulfate removal efficiency of 40.22%. BAUa-2 (*PA*-15442 + BWW) recorded the highest weekly removal efficiency in week 1 (> 80% for Fe, Al, Cu, Zn except for Mn) and a decline from week 2 to week 4 with an average metal removal efficiency of 62.40% after 28 days (Fig. 3b). However, the BAUa-3 inoculated and amended with *PA*-15442 + DWW showed > 40% removal for all metals except for Al (< 40%) in week 1 and the removal efficiencies for the treatment appreciated in week 2 with a decline of > 15% in the remaining 14 days (Fig. 3c). The BAUa-3 treatment reduced all metals (Fe, Al, Cu, Zn, and Mn) by > 50% (average) to attain an average removal efficiency of 54.04%. The control treatment (BATa) observed a slow metal removal efficiency when compared to other treatments with the average metal reduction below 30% efficiency with 31.55% average sulfate removal efficiency (Fig. 3d). However, for sulfate removal, BAUa-2 and BAUa-3 treatment where *PA*-15442 was amended with BWW and DWW respectively observed a 4.52% and 2.13 decline in week 3 and week 2 respectively which attribute to a cumulative sulfate removal of 447 mg/kg and 410 mg/kg to represent 56.01% and 51.37% average removal efficiency at 15.96 mg/day and 14.64 mg/day average removal rate, respectively (Fig. 4). An increase in pH was observed in all BAUa treatment to 7.3 from 6.9. Metal removal efficiencies were 24–84%, 21–81%, 43–88%, 36–89%, and 31–64% for Fe, Al, Cu, Zn, and Mn, respectively. Hence, it can be deduced that the combined application of bacteria strain, *PA*-15442, and wastewaters was effective for metal and sulfate removal.

The reduction of metal concentration in treatment amended with *PA* showed the bacteria's potential to remove varying metals from contaminated soils [1, 85]. According to the findings by Fomina and Gadd [39], through surface complexation on cells and other external layers, biosorption can be performed as a passive absorption by cell and tissue fragments or through dead biomass or live cells [41]. Also, as a metal removal mechanism, *P. aeruginosa* synthesizes siderophore known as pyoverdine (*iron carriers*)—a high-affinity iron-chelating compound as reported by Peek et al. [61] which can bind metal during bioremediation treatment. Peek et al. [61] noted that *siderophores are essential for the acquisition*

of iron by certain pathogenic bacteria like P. aeruginosa sp. The unavailability of Fe which is a significant nutrient for the *P. aeruginosa* bacteria prompted the secretion of siderophore which mobilize and transport Fe across cell membranes. These siderophores are released by *P. aeruginosa* to scavenge available Fe from the mineral phase in the treatment system by the gradual formation of soluble Fe^{3+} complexes (under acidic condition) for active transport and uptake mechanism [44, 52] which contributes to the effective reduction of Fe and other metals. Siderophore can chelate and detoxify other metal ions (aside Fe), such as Cu, Al, Pb, Mn, Zn, Cd, Cr, and Ur among others [28, 34] to enhance metal reduction as evident in the current study.

However, the black precipitate visible on the surface of the treatment suggests sulfidogenic process is feasible due to the introduction of wastewater into the system which also contributes to metal reduction through metal precipitation as sulfides, hydroxides, or carbonates, and sorption into organic materials [18, 35, 38, 49, 51], van den [82]. Silva et al. [74] reported that metal removal mechanism using *P. aeruginosa* can be facilitated by the introduction of organic energy source for the bacteria for the maintenance of active, stable, and sensitive microbial community needed for effective bioaccumulation or biosorption process for enhanced metal removal [27, 74]. This vividly elucidates the increase in average metal removal efficiencies in treatments amended with *P. aeruginosa* and wastewater (BAUa-3 and BAUa-4) where the presence of an organic substrate in the form of wastewater (BWW and DWW) increased the average metal removal efficiency by 20.59% and 12.23% respectively when compared to the treatment without nutrient amendment (BAUa-1) which recorded 41.81% average removal efficiency. According to Sinha and Mukherjee [75], the increase in *P. aeruginosa* KUCd1 survival rate can be linked to the amendment and availability of extra energy source during the treatment for rapid metabolism which facilitated metal removal efficiency to 89%, while media treatment recorded 75% and nutrient-deficient media removes less than 20% Cd from the wastewater after 96-h treatment period which corresponds to 41.81% (media nutrient only) as against 58.22% (avg. removal with wastewater nutrient amendment) recorded in the present study which corresponds to 16.41% increase in removal efficiency.

The metal removal efficiencies of 24–84%, 21–81%, 43–88%, 36–89%, and 31–64% were recorded for Fe, Al, Cu, Zn, and Mn (with 51.07, 47.29, 59.32, 58.98, and 47.10% average) respectively after 28 days which correspond to the study by Juwarkar et al. [47] which reported heavy metal removal efficiencies of 86–96% with *P. aeruginosa* BS2 isolated from oil sludge after 96 h, while Awasthi et al. [17] reported 79.5, 52.4%, and 61% removal efficiencies for Cu, Zn, and Fe after a 24–72-h treatment period. Also, Chen et al. [31] reported 87.2% and 99.8% removal efficiencies for Cu and Zn, respectively, with *Pseudomonas putida* (CZ1) strain during the active growth cycle. The appreciable metal removal efficiency of these metals, also evident in the present study, can be due to their (Fe, Cu, Zn) indispensable nature as essential nutrients required for bacterial growth and cell survival, which enhance their tolerance in metal concentration [17] where excess of these metals may be detrimental to microbial growth [57, 80]. However, in accordance with this investigation, with average Al removal efficiency of 47%, Purwanti et al. [64] and Boeris et al. [23] recorded 46% and 44% using *P. aeruginosa* and *P. putida*, respectively, as Al exposure is detrimental to the growth of the bacteria [64].

Silva et al. [74] reported that Zn removal was effective at pH 7.0 with 87.7% removal efficiency after 72 h of treatment, while Mn removal was low (21.69%), which is also consistent with the present study where Mn had the lowest average removal efficiency of <50% compared to the other metals. This could be due to the possession of high atomic mass, which interferes with the adsorption process [29]. Also, the findings from Silva et al. [74] showed that *P. aeruginosa*'s adsorption from metals from individual solutions is higher than in a multi-metal mixture as the growth of *P. aeruginosa* was more visible in Zn, followed by Cu and Mn, which buttresses Mn's low removal efficiency. Hence, the discrepancy and fluctuations in metal removal efficiencies observed during the treatment may be due to the complexation of metal in the multi-metal treatment systems, which lowered the bioavailability of metals and hence increased toxicity [75], depletion of organic substrates and/or lack of bioavailability of metals in the soil to degrading microbes. Also, the low metal removal recorded in the control treatment (BATa) in the present study is due to a lack of inoculum or organic substrate amendment [24]. The study showed that the application of *PA*-15442 with wastewater as an extra carbon source was effective for the reduction of multi-metal concentration in the soil and can serve as a potential remediation alternative. Figure 8 shows the overall performance of different methods for the remediation of TPH- and AMD-contaminated soils. It is evident that the addition of wastewater to PA microbes (BAU-2 and BAU-3) improved the remediation efficiency.

Sulfate removal

The BAUa treatment with the bacteria strain *PA*-15442 showed sulfate reduction as evident in BAUa-1 because the bacteria species has been found to grow in organic or inorganic sulfur sources [46] while utilizing the same

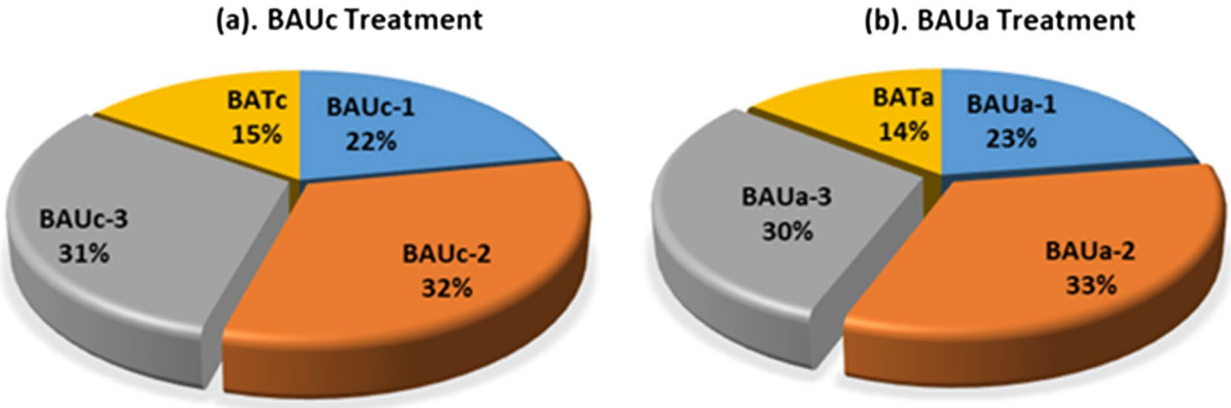


Fig. 8 Performance efficiency of all bioaugmentation methods for the treatment of TPH (BAUc)- and AMD (BAUa)-contaminated soils

as the sole sulfur source in the presence of glucose as an energy source. However, *Pseudomonas* sp. produced varying amounts of biosurfactants depending on the available sulfur sources. In agreement with the present BAUa study which recorded 40–56% average sulfate removal efficiency after 28 days, Ajao et al. [5] reported 62.8% removal efficiency for sulfate with *P. aeruginosa* and *B. subtilis* after 15-day treatment period. The introduction of wastewater (BWW and DWW) into the system as an extra energy source for *PA*-15442 facilitated the sulfate removal efficiency as evident in BAUa-2 and BAUa-3 treatment which increased the average removal efficiencies by 15.80% and 11.16% respectively (when compared to BAUa-1 inoculated with the bacteria strain only—40.22%) which is attributed to the possible synergy between *P. aeruginosa* and other microbial community present in wastewater [4]. This was further buttressed in the study by Chen et al. [30] which reported the ability of *P. aeruginosa*, (heterotrophic denitrifiers) to effectively reduce sulfate as a result of positive interaction with SRB present in wastewater to attain > 70% sulfate removal efficiency after 10 days of treatment.

Conclusion

Bioaugmentation treatment of contaminated soils with *PA*-15442 adopts the biosorption process for hydrocarbon, metal, and sulfate reduction with 42.02, 41.81, and 40.23% average removal efficiencies, respectively. However, the amendment of organic nutrients (wastewaters) introduced alkalinity, which triggers pH increase and facilitates microbial activities to account for an increase in the removal efficiencies of these pollutants by 25.24 and 16.23 and 13.47% for TPH, metals, and sulfate, respectively, as brewery wastewater amendment recorded slightly appreciable efficiency than domestic wastewater. By using the wastewater as an extra carbon source, the bacteria can enhance their metabolic activity and potentially increase their efficiency in degrading or immobilizing metals in the soil. This method leverages biological processes to transform or sequester metals and offers a sustainable and potentially cost-effective solution compared to traditional remediation methods such as chemical treatments or physical removal. This study showed that the wastewater-enhanced *PA*-15442 method can serve as a potential bioremediation tool for the treatment of acid mine drainage- and crude oil-contaminated soils.

Authors' contributions
Conceptualization: Yusuf Makarfi Isa; methodology: Yusuf Makarfi Isa and Ifeanyi Michael Smarte Anekwe; formal analysis and investigation: Ifeanyi Michael Smarte Anekwe; writing—original draft preparation: Ifeanyi Michael Smarte Anekwe; writing—review and editing: Yusuf Makarfi Isa; resources: Yusufi Makarfi Isa; supervision: Yusufi Makarfi Isa.

Funding
No funding was received to assist with the preparation of this manuscript.

Availability of data and materials
Data is available on request from authors.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Authors consent to the publication of this manuscript.

Competing interests
The authors declare no competing interests.

Author details
[1]School of Chemical and Metallurgical Engineering, University of the Witwatersrand, Johannesburg 2050, South Africa.

Received: 1 July 2024 Accepted: 27 July 2024
Published online: 26 September 2024

References

1. Abdi O, Kazemi M. A review study of biosorption of heavy metals and comparison between different biosorbents. J Mater Environ Sci. 2015;6:1386–99.
2. Abdulsalam S, Bugaje IM, Adefila SS, Ibrahim S. Comparison of biostimulation and bioaugmentation for remediation of soil contaminated with spent motor oil. Int J Environ Sci Technol. 2011;8:187–94.
3. Adekunle AA, Adekunle IM, Badejo AA, Alayaki FM, Olusola AO. Laboratory scale bioremediation of crude oil impacted soil using animal waste compost. Tehnički glasnik. 2017;11:45–9.
4. Agarry S, Latinwo G. Biodegradation of diesel oil in soil and its enhancement by application of bioventing and amendment with brewery waste effluents as biostimulation-bioaugmentation agents. J Ecol Eng. 2015;16:82–91.
5. Ajao A, Adebayo G, Yakubu S. Bioremediation of textile industrial effluent using mixed culture of Pseudomonas aeruginosa and Bacillus subtilis immobilized on agar-agar in a bioreactor. J Microbiol Biotech Res. 2011;1:50–6.
6. Akpasi SO, Anekwe IMS, Tetteh EK, Amune UO, Shoyiga HO, Mahlangu TP, Kiambi SL. Mycoremediation as a potentially promising technology: current status and prospects—a review. Appl Sci. 2023;13:4978.
7. Al-Hadhrami M, Lappin-Scott H, Fisher P. Studies on the biodegradation of three groups of pure n-alkanes in the presence of molasses and mineral fertilizer by Pseudomonas aeruginosa. Mar Pollut Bull. 1997;34:969–74.
8. Amanchukwu S, Obafemi A, Okpokwasili G. Hydrocarbon degradation and utilization by a palm-wine yeast isolate. FEMS Microbiol Lett. 1989;57:151–4.
9. Amhakhian SO, Faleke BA. Bioremediation of soils contaminated with hydro carbon (oil spillage) in Nigeria. Int J of Pharm Life Sci. 2014;5(12):4026–30.
10. Anekwe IMS, Isa YM. Comparative evaluation of wastewater and bioventing system for the treatment of acid mine drainage contaminated soils. Water-Energy Nexus. 2021;4:134–40.
11. Anekwe IMS, Isa YM. Bioremediation of Crude oil-contaminated Soils-A Review. Petroleum & Coal. 2022;64(3):632–64.
12. Anekwe IMS, Isa YM. Bioremediation of acid mine drainage–Review. Alex Eng J. 2023;65:1047–75.
13. Anekwe IMS, Isa YM. Bioremediation of acid mine drainage contaminated soils using bioattenuation, wastewater and air-injection system. Bioremediat J. 2023;27:363–81.
14. Anekwe IMS, Isa YM. Application of biostimulation and bioventing system as bioremediation strategy for the treatment of crude oil contaminated soils. Soil and Water Research. 2024;19:100–10.
15. Ani KA., Chukelu C, Government RM, Ochin E. Analysis and optimization processes of goat dung as a potential co-substrate in bioremediation. 2018.
16. APHA, American Water Works Association. Standard methods for the examination of water and wastewater. In Standard Methods for the Examination of Water and Wastewater. American Public Health Association. 1995;1000–1000.
17. Awasthi G, Chester A, Chaturvedi R, Prakash J. Study on role of Pseudomonas aeruginosa on heavy metal bioremediation. Int J Pure App Biosci. 2015;3:92–100.
18. Bai H, Kang Y, Quan H, Han Y, Sun J, Feng Y. Treatment of acid mine drainage by sulfate reducing bacteria with iron in bench scale runs. Biores Technol. 2013;128:818–22.
19. Bassey E, Inyang J. Characterization of brewery effluent fluid. J Eng Appl Sci. 2012;4:67–77.
20. Beal R, Betts W. Role of rhamnolipid biosurfactants in the uptake and mineralization of hexadecane in Pseudomonas aeruginosa. J Appl Microbiol. 2000;89:158–68.
21. Benyahia F, Embaby AS. Bioremediation of crude oil contaminated desert soil: effect of biostimulation, bioaugmentation and bioavailability in biopile treatment systems. Int J Environ Res Public Health. 2016;13:219.
22. Bezza FA, Chirwa EMN. Biosurfactant from Paenibacillus dendritiformis and its application in assisting polycyclic aromatic hydrocarbon (PAH) and motor oil sludge removal from contaminated soil and sand media. Process Saf Environ Prot. 2015;98:354–64.
23. Boeris PS, Liffourrena AS, Lucchesi GI. Aluminum biosorption using non-viable biomass of Pseudomonas putida immobilized in agar–agar: performance in batch and in fixed-bed column. Environ Technol Innov. 2018;11:105–15.
24. Boopathy R. Factors limiting bioremediation technologies. Biores Technol. 2000;74:63–7.
25. Bouchez-Naïtali M, Vandecasteele J-P. Biosurfactants, an help in the biodegradation of hexadecane? The case of Rhodococcus and Pseudomonas strains. World J Microbiol Biotechnol. 2008;24:1901–7.
26. Bouchez Naïtali M, Rakatozafy H, Marchal R, Leveau J, Vandecasteele J. Diversity of bacterial strains degrading hexadecane in relation to the mode of substrate uptake. J Appl Microbiol. 1999;86:421–8.
27. Brown MJ, Lester J. Role of bacterial extracellular polymers in metal uptake in pure bacterial culture and activated sludge—I. Effects of metal concentration. Water Res. 1982;16:1539–48.
28. Carrillo-Castañeda G, Muños JJ, Peralta-Videa J, Gomez E, Tiemannb K, Duarte-Gardea M, Gardea-Torresdey J. Alfalfa growth promotion by bacteria grown under iron limiting conditions. Adv Environ Res. 2002;6:391–9.
29. Chen B-Y, Utgikar VP, Harmon SM, Tabak HH, Bishop DF, Govind R. Studies on biosorption of zinc (II) and copper (II) on Desulfovibrio desulfuricans. Int Biodeterior Biodegradation. 2000;46:11–8.
30. Chen C, Ren N, Wang A, Yu Z, Lee DJ. Microbial community of granules in expanded granular sludge bed reactor for simultaneous biological removal of sulfate, nitrate and lactate. Appl Microbiol Biotechnol. 2008;79:1071.
31. Chen XC, Shi JY, Chen YX, Xu XH, Xu SY, Wang YP. Tolerance and biosorption of copper and zinc by Pseudomonas putida CZ1 isolated from metal-polluted soil. Can J Microbiol. 2006;52:308–16.
32. Das K, Mukherjee AK. Crude petroleum-oil biodegradation efficiency of Bacillus subtilis and Pseudomonas aeruginosa strains isolated from a petroleum-oil contaminated soil from North-East India. Biores Technol. 2007;98:1339–45.
33. Datta K, Chakraborty S, Roychoudhury A. Management of soil, waste and water in the context of global climate change. Environmental Nexus for Resource Management: CRC Press; 2025.
34. del Olmo A, Caramelo C, Sanjose C. Fluorescent complex of pyoverdin with aluminum. J Inorg Biochem. 2003;97:384–7.
35. Deng D, Lin LS. Two-stage combined treatment of acid mine drainage and municipal wastewater. Water Sci Technol. 2013;67:1000–7.
36. Dey S, Parida SN, Rout AK, Dei J, Maharana J, Pradhan SP, Pradhan SK, Behera BK. Chapter 5 - Nanobioremediation: a sustainable approach for environmental monitoring with special reference to the restoration of heavy metal contaminated soil and wastewater treatment. In: Srivastav AL, Grewal, AS Markandeya, Pham TD (eds.) Role of green chemistry in ecosystem restoration to achieve environmental sustainability. Amsterdam: Elsevier; 2024.
37. Dhar S, Devnath S, Kumar V, Roy S, Rout AK, Mistri A, Parida SN, Bisai K, Jana AK, Behera BK. Bioremediation and its application in aquaculture. In: Behera BK, editor. Biotechnological tools in fisheries and aquatic health management. Singapore: Springer Nature Singapore; 2023.
38. Dvorak DH, Hedin RS, Edenborn HM, McIntire PE. Treatment of metal-contaminated water using bacterial sulfate reduction: results from pilot-scale reactors. Biotechnol Bioeng. 1992;40:609–16.
39. Fomina M, Gadd GM. Biosorption: current perspectives on concept, definition and application. Biores Technol. 2014;160:3–14.
40. Gautam K, Tyagi V. Microbial surfactants: a review. J Oleo Sci. 2006;55:155–66.
41. Gavrilescu M. Removal of heavy metals from the environment by biosorption. Eng Life Sci. 2004;4:219–32.
42. Getabalew M, Alemneh T, Bzuneh E. Review on methanogenesis and its role. World J Agri Soil Sci. 2020;6:1–7.
43. Guo-Liang Z, Yue-Ting W, Xin-Ping Q, Qin M. Biodegradation of crude oil byPseudomonas aeruginosa in the presence of rhamnolipids. J Zhejiang Univ Sci B. 2005;6:725–30.
44. Hider RC, Kong X. Chemistry and biology of siderophores. Nat Prod Rep. 2010;27:637–57.
45. Humphries MS, McCarthy TS, Pillay L. Attenuation of pollution arising from acid mine drainage by a natural wetland on the Witwatersrand. S Afr J Sci. 2017;113:1–9.
46. Ismail W, El Nayal AM, Ramadan AR, Abotalib N. Sulfur source-mediated transcriptional regulation of the rhlABC genes involved in biosurfactants production by Pseudomonas sp. strain AK6U. Front Microbiol. 2014;5:423.

47. Juwarkar AA, Dubey KV, Nair A, Singh SK. Bioremediation of multi-metal contaminated soil using biosurfactant—a novel approach. Indian J Microbiol. 2008;48:142–6.
48. Kumari S, Regar RK, Manickam N. Improved polycyclic aromatic hydrocarbon degradation in a crude oil by individual and a consortium of bacteria. Biores Technol. 2018;254:174–9.
49. Lyew D, Sheppard JD. Effects of physical parameters of a gravel bed on the activity of sulphate-reducing bacteria in the presence of acid mine drainage. J Chem Technol Biotechnol Int Res Process Environ Clean Technol. 1997;70:223–30.
50. Malik S, Kishore S, Kumar SA, Dhasmana A. Role of bacteria in biological removal of environmental pollutants. Emerging Technologies in Applied and Environmental Microbiology. Amsterdam: Elsevier; 2023.
51. Martins M, Faleiro ML, Silva G, Chaves S, Tenreiro R, Costa MC. Dynamics of bacterial community in up-flow anaerobic packed bed system for acid mine drainage treatment using wine wastes as carbon source. Int Biodeterior Biodegradation. 2011;65:78–84.
52. Miethke M, Marahiel MA. Siderophore-based iron acquisition and pathogen control. Microbiol Mol Biol Rev. 2007;71:413–51.
53. Mohajeri L, Zahed MA, ABDUL AZIZ, H. & HASNAIN ISA, M. Assessment of bioaugmentation and biostimulation efficiencies for petroleum contaminated sediments. Environ Energy Econ Res. 2017;1:89–98.
54. Mulvaney BJ, Page A. Nitrogen-total. Methods Soil Anal Part. 1982;2:595–624.
55. Noordman WH, Janssen DB. Rhamnolipid stimulates uptake of hydrophobic compounds by Pseudomonas aeruginosa. Appl Environ Microbiol. 2002;68:4502–8.
56. Numbere AO, Gbarakoro TN, Babatunde BB. Environmental degradation in the Niger Delta ecosystem: the role of anthropogenic pollution. Sustainable utilization and conservation of Africa's biological resources and environment: Springer; 2023.
57. O'Brien S, Hodgson DJ, Buckling A. Social evolution of toxic metal bioremediation in Pseudomonas aeruginosa. Proc Royal Soc B Biol Sci. 2014;281:20140858.
58. Olsen SR, Sommers LE. Phosphorus. In "Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties" ed. By A. L. Page. Soil Sci Soc Amer Inc. (USA): 1982:403–30.
59. Osuji L, Egbuson E, Ojinnaka C. Chemical reclamation of crude-oil-inundated soils from Niger Delta, Nigeria. Chem Ecol. 2005;21:1–10.
60. Peech M, Alexander L, Dean L, Reed JF. Methods of soil analyses for soil fertility investigations. US Department of Agriculture, Circ. N757i, 23. 1947.
61. Peek ME, Bhatnagar A, Mccarty NA, Zughaier SM. Pyoverdine, the major siderophore in Pseudomonas aeruginosa, evades NGAL recognition. Interdisciplinary perspectives on infectious diseases. 2012.
62. Perfumo A, Banat IM, Canganella F, Marchant R. Rhamnolipid production by a novel thermophilic hydrocarbon-degrading Pseudomonas aeruginosa AP02-1. Appl Microbiol Biotechnol. 2006;72:132.
63. Philip L, Iyengar L, Venkobachar C. ORIGINAL PAPERS Biosorption of U, La, Pr, Nd, Eu and Dy by Pseudomonas aeruginosa. J Ind Microbiol Biotechnol. 2000;25:1–7.
64. Purwanti IF, Kurniawan SB, Imron MF. Potential of Pseudomonas aeruginosa isolated from aluminium-contaminated site in aluminium removal and recovery from wastewater. Environ Technol Innov. 2019;15:100422.
65. Qiao J, Zhang C, Luo S, Chen W. Bioremediation of highly contaminated oilfield soil: bioaugmentation for enhancing aromatic compounds removal. Front Environ Sci Eng. 2013;8:293–304.
66. Reineke W. Aerobic and anaerobic biodegradation potentials of microorganisms. In: Beek B, editor. Biodegradation and persistence. Berlin, Heidelberg: Springer Berlin Heidelberg; 2001.
67. Reineke W, Schlömann M. Microbial degradation of pollutants. Environmental microbiology: Springer; 2023.
68. Ron EZ, Rosenberg E. Natural roles of biosurfactants: minireview. Environ Microbiol. 2001;3:229–36.
69. Roy R, Samanta S, Pandit S, Naaz T, Banerjee S, Rawat JM, Chaubey KK, Saha RP. An overview of bacteria-mediated heavy metal bioremediation strategies. Appl Biochem Biotechnol. 2024;196:1712–51.
70. Rudra B, Gupta RS. Phylogenomics studies and molecular markers reliably demarcate genus Pseudomonas sensu stricto and twelve other Pseudomonadaceae species clades representing novel and emended genera. Front Microbiol. 2024;14:1273665.
71. Sassetto G, Lorini L, Lai A, Petrangeli Papini M, Zeppilli M. Groundwater bioremediation through reductive dechlorination in a permeable bioelectrochemical reactor. Catalysts. 2024;14:208.
72. Satpute SK, Banpurkar AG, Dhakephalkar PK, Banat IM, Chopade BA. Methods for investigating biosurfactants and bioemulsifiers: a review. Crit Rev Biotechnol. 2010;30:127–44.
73. Shin K-H, Kim K-W, Ahn Y. Use of biosurfactant to remediate phenanthrene-contaminated soil by the combined solubilization–biodegradation process. J Hazard Mater. 2006;137:1831–7.
74. Silva RMP, Rodríguez AÁ, de Oca JMGM, Moreno DC. Biosorption of chromium, copper, manganese and zinc by Pseudomonas aeruginosa AT18 isolated from a site contaminated with petroleum. Biores Technol. 2009;100:1533–8.
75. Sinha S, Mukherjee SK. Pseudomonas aeruginosa KUCd1, a possible candidate for cadmium bioremediation. Braz J Microbiol. 2009;40:655–62.
76. Smyth T, Perfumo A, Marchant R, Banat I. Isolation and analysis of low molecular weight microbial glycolipids. Handbook of Hydrocarbon and Lipid Microbiology.: Springer; 2010.
77. Song R, Hua Z, Li H, Chen J. Biodegradation of petroleum hydrocarbons by two Pseudomonas aeruginosa strains with different uptake modes. J Environ Sci Health Part A. 2006;41:733–48.
78. Talinli I, Anderson G. Interference of hydrogen peroxide on the standard COD test. Water Res. 1992;26:107–10.
79. Tavassoli T, Mousavi S, Shojaosadati S, Salehizadeh H. Asphaltene biodegradation using microorganisms isolated from oil samples. Fuel. 2012;93:142–8.
80. Teitzel GM, Geddie A, de Long SK, Kirisits MJ, Whiteley M, Parsek MR. Survival and growth in the presence of elevated copper: transcriptional profiling of copper-stressed Pseudomonas aeruginosa. J Bacteriol. 2006;188:7242–56.
81. Uzoigwe C, Burgess JG, Ennis CJ, Rahman PK. Bioemulsifiers are not biosurfactants and require different screening approaches. Front Microbiol. 2015;6:245.
82. van den Berg M, Botes M, Slabbert E, Cloete T. Evaluating sulphate removal and identifying the bacterial community present in acid mine drainage treated with synthetic domestic wastewater sludge. Water SA. 2016;42:475–82.
83. Varjani SJ, Upasani VN. Biodegradation of petroleum hydrocarbons by oleophilic strain of Pseudomonas aeruginosa NCIM 5514. Biores Technol. 2016;222:195–201.
84. Venkateswaran K, Hoaki T, Kato M, Maruyama T. Microbial degradation of resins fractionated from Arabian light crude oil. Can J Microbiol. 1995;41:418–24.
85. Vijayaraghavan K, Yun Y-S. Bacterial biosorbents and biosorption. Biotechnol Adv. 2008;26:266–91.

Publisher's Note