



Fungal proliferation and hydrocarbon removal during biostimulation of oily sludge with high total petroleum hydrocarbon

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Abstract

A laboratory-scale study was conducted to investigate the effect of bioaugmentation (BA) and biostimulation (BS) on the remediation of oily sludge with high total petroleum hydrocarbon (TPH) content (269,000 mg/kg d.w. sludge). TPH concentration significantly decreased by 30.4% ($P < 0.05$) in the BS treatment after 13-week incubation, and 17.0 and 9.1% of TPH was removed in the BA and control treatments (amended with sterile water only), respectively. Aliphatic and other fractions (i.e., saturated *n*-alkanes and cyclic saturated alkanes) were reduced in the BS treatment, whereas no decrease in aromatic hydrocarbons occurred in any treatment. Gas chromatography–mass spectrometry analysis of aliphatic fractions showed that low-chain-length alkanes (C8–C20) were the most biodegradable fractions. The BS treatment supported fungal proliferation, with Sordariomycetes and Eurotiomycetes as the dominant classes. BS increased fungal diversity and decreased fungal abundance, and changed bacterial community structure. The findings show the potential of using BS to treat oily sludge with high TPH content.

Keywords Oily sludge · Biostimulation · Bioaugmentation · Total petroleum hydrocarbon · Fungal proliferation

Introduction

Oily sludge is a complex mixture of petroleum hydrocarbons, mineral substances, solid particles, and water (Lazar et al. 1999). Generally, it is formed from the processes of petroleum production, transportation, storage, and refining (Hu et al. 2013; Mishra et al. 2001). Carcinogens and immunotoxins, e.g., polycyclic aromatic hydrocarbons, in oily sludge threaten

human health (Asgari et al. 2017; Shuai et al. 2018). Oily sludge contamination, e.g., through oil spills, has caused concern globally due to its increasing accumulation and threat to the environment, with potential detrimental impacts on human health (Roy et al. 2018).

Bioremediation, including bioaugmentation (BA) and biostimulation (BS), has been recognized as an effective treatment for oily sludge and petroleum-contaminated soil (Adetutu et al. 2015; Mansur et al. 2016; Wu et al. 2016). BA is defined as adding exogenous microorganisms to sludge to enhance pollutant degradation. This approach has been widely studied and practiced (Poi et al. 2017; Ying et al. 2016). According to reported literatures, various classes of contaminants such as polychlorinated biphenyls and polycyclic aromatic hydrocarbons could be effectively bioremediated using BA approach under different kinds of contaminated matrices including soils, wastewater, and sediments (Castiglione et al. 2016; Di Gregorio et al. 2015; Payne et al. 2011; Siracusa et al. 2017). BS is designed to improve the metabolic activity of indigenous microorganisms by regularly replenishing contaminated soil or sludge with nutrients to meet the needs of functional bacteria or fungi (Bento et al. 2005; Tyagi et al. 2011). This method has also proven to be effective. For instance, Simpanen et al. (2016) reported that BS was the most effective method for remediating freshly

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contaminated soil after a fuel spill accident. Similarly, Al-Saleh and Hassan (2016) showed that the amendment of organic materials in soil increased microbial activity and enhanced petroleum degradation. Due to varied physical and biological characteristics of different contaminated sites, laboratory studies should be carried out to determine the most suitable treatment prior to the in situ bioremediation of oil contamination.

Generally, the optimal total petroleum hydrocarbon (TPH) concentration of bioremediated soil ranges from 0.2 to 5.5% (w/w) (Huesemann et al. 2002). However, oily sludge with high TPH content can be directly bioremediated, and high TPH removal (> 80%) has been achieved during biological treatment of sludge with a TPH concentration of more than 20% (He et al. 2015; Sarkar et al. 2016).

Fungi and bacteria play key roles in the degradation of contaminants during bioremediation (Bardi et al. 2017; Yuan et al. 2018). Previous studies revealed shifts in microbial community structure during the bioremediation of oily sludge or petroleum-contaminated soil (Liu et al. 2011; Roy et al. 2018; Ye et al. 2016). Most studies have focused only on bacterial community structure and few have paid attention to fungal community structure. Owing to their high biomass and ability to survive in harsh environments, the role of fungi should be considered during bioremediation (Rousk and Frey 2016; Wardle et al. 2004).

Xinjiang Uyghur Autonomous Region is an important petroleum-producing region in China, with over 10 million tons of oil being mined and 200,000 tons of high-TPH oily sludge being produced annually (Chen and Luo 2014; Sa 2009). Oily sludge output is increasing yearly, and its accumulation in the environment has many negative ecological impacts. It is therefore crucial to develop efficient bioremediation methods for oily sludge treatment. Therefore, the objectives of this study were to (1) assess the effects of BA and BS on the hydrocarbon degradation of oily sludge with a high TPH concentration collected from Xinjiang oilfield and (2) investigate the shift of bacterial and fungal community structure during bioremediation.

Material and methods

Petroleum-contaminated sludge for microcosm study

The sludge used in this study was collected from a district adjacent to an oil refinery in Karamay, Xinjiang Uyghur Autonomous Region, China (44.83° N, 88.35° E). It was generated from oil extraction and has a relatively high TPH concentration (269,000 mg/kg d.w. sludge). After sampling, the sludge was immediately refrigerated at 4 °C for further study. The basic properties of sludge are shown in Table 1.

Table 1 Basic properties of sludge used in this study

Initial properties	Value
Total petroleum hydrocarbon (g/kg)	269.5
pH	7.1
Moisture content (%)	71.9
Total carbon (g/kg)	67.1
Total nitrogen (g/kg)	2.8
Total phosphorus (g/kg)	0.5
Soluble salts (g/kg)	11.5
Organic matter (g/kg)	133.5

Experimental bioremediation design

Bioremediation, through BA and BS, was conducted in butyl rubber-capped flasks containing 300 g sludge. BA was performed by adding a mixed culture of petroleum-degrading consortia enriched from oil-contaminated coastal sediment from Zhoushan, Zhejiang Province, China. This mixed culture was dominated by *Alcaligenes* sp. (11.1%), *Shewanella* sp. (22.2%), and *Bacillus* sp. (66.7%), which could completely degrade 2% (v/v) crude oil within 5 days. The culture was inoculated into the sludge at a ratio of 10% (v/v). BS was performed by adding a mixed solution of (NH₄)₂SO₄ and KH₂PO₄ to oily sludge to achieve a C/N/P ratio of 100:10:1 (Wu et al. 2016). A control treatment was carried out by adding sterile water (SW) to oily sludge to keep moisture content at 75%. Three replicates were prepared for each treatment. All treatments were statically incubated at room temperature (25 °C) for 13 weeks. The sludge was stirred weekly to maintain sufficient oxygen content. Sterile water was added regularly to all microcosms to supplement the water evaporated during incubation. During incubation, the pH of sludge samples was analyzed as described by Asgari et al. (2017) using a pH meter (Mettler-Toledo FiveEasy Plus, USA).

Determination of TPH and aliphatic and aromatic hydrocarbons

The TPH content of the oily sludge was first analyzed by gas chromatography–mass spectrometry (GC–MS) and then quantified gravimetrically. TPH extraction was conducted as described by Gurav et al. (2017). Wet sludge (4 g) was sampled from each flask (in triplicate) for TPH degradation analysis at 0, 2, 5, 6, 8, 11, and 13 weeks (sludge was thoroughly mixed before each sampling). Samples were dried at room temperature (25 °C) in a desiccator for 48 h immediately after sampling and placed in separate round flasks.

TPH extraction was performed by using a Soxhlet extractor with dichloromethane as extracting agent. The extraction process was conducted at 65 °C for 12 h, followed by evaporation of dichloromethane using a rotary evaporator. TPH was

designated as the weight of residues remaining in the flasks. Subsequently, TPH obtained from each sample was re-dissolved in hexane and analyzed by GC–MS (QP2020, Shimadzu, Japan) equipped with a SH Rxi-5Sil MS column (30 m × 0.25 μm × 0.25 mm, Shimadzu). GC–MS parameters were set according to a modified protocol of Patowary et al. (2016).

Analyses of aliphatic and aromatic fractions were performed using a modified method of Gurav et al. (2017). Neutral alumina column (10 × 300 mm) chromatography was applied for separation of aliphatic and aromatic fractions. Alumina powder (8 g, analytical grade, Sigma-Aldrich, USA) was loaded in the column with hexane and eluted several times. Extracted TPH was dissolved in hexane and loaded in the column. Aliphatic and aromatic fractions were obtained by successively eluting the column with 30 mL hexane and 70 mL dichloromethane-hexane (1:1, v/v). After evaporation, the aliphatic and aromatic fractions were gravimetrically determined. The residual fraction of TPH consisted of saturated *n*-alkanes and cyclic saturated alkanes were defined as “other fractions.”

GC–MS analysis of aliphatic fractions

Aliphatic hydrocarbons account for the main part of TPH, and different chain-length fractions were further analyzed. The obtained aliphatic fractions were re-dissolved in hexane, and the fractions of C8–C40 were quantified via GC–MS (QP2020, Shimadzu) equipped with an SH Rxi-5Sil MS column (30 m × 0.25 μm × 0.25 mm, Shimadzu). Helium was used as carrier gas with a flow rate of 1.2 mL/min. The injection volume was 1 μL with a split ratio of 10:1. The parameters of the column oven temperature were set as follows: The initial temperature was set at 50 °C with a hold time of 2 min, and the temperature was subsequently increased to 300 °C at 6 °C/min, with a hold time of 25 min. To acquire the mass spectra, the ion source and interface temperatures were set at 230 and 300 °C, respectively. The acquisition mode was set to selected ion monitor mode, with the molecular ion of each aliphatic component corresponding to the retention time of external standards.

Determination of microbial community structure

Microbial community structures of initial and 13-week treated sludge were analyzed via Illumina high-throughput sequencing. Total DNA of bacteria and fungi was extracted using EZNA® Soil DNA Kits (Omega Bio-Tek, USA) according to the manufacturer's instructions. The polymerase chain reaction (PCR) method was used to amplify the bacterial 16S rRNA V3–V4 region by using 338F (ACTCCTACGGGAGGCAGCA) and 806R (GGACTACHVGGGTWTCTAAT) primers and the fungal ITS1 region by using ITS5F (GGAAGTAAAAGTCG

TAACAAGG) and ITS1R (GCTGCGTTCATCGATGC) primers. PCR amplification products were examined by 2% agarose gel electrophoresis, followed by purification using an AxyPrep™ DNA Gel Extraction Kit (Axygen Biotechnology, USA). The QuantiFluor™ system (Promega, Italy) was subsequently employed for DNA library quantification. DNA libraries were then loaded on an Illumina-Miseq device for sequencing. Raw reads were quality checked through Quantitative Insights Into Microbial Ecology pipeline version 1.8.0 (Caporaso et al. 2010). After quality checking, operational taxonomic unit (OTU) clustering was conducted based on a 97% sequence identity threshold using the pick_otus.py command with UCLUST algorithm (Edgar 2010). The Greengenes 13.8 and UNITE 5.0 databases were used as reference databases for OTU classification status identification of bacteria and fungi, respectively.

Statistical analysis

All data were mean values of the three replicates. To test for significant differences in hydrocarbon removal efficiency between different treatments, one-way analysis of variance was employed by using SPSS 19.0 software (IBM Corp., USA).

Results

Hydrocarbon removal

The GC–MS chromatograms of TPH in the initial sludge and sludge subjected to 13-week SW, BA, and BS treatments are shown in Fig. 1. The BA and SW treatments exhibited low TPH removal, whereas a remarkable reduction in TPH was achieved in the BS treatment.

The TPH concentrations and corresponding hydrocarbon fractions in different treatments are shown in Fig. 2. The TPH concentration of initial sludge was 269.5 g/kg, which consisted of 160.1 g/kg aliphatic hydrocarbons, 25.9 g/kg aromatic hydrocarbons, and 83.5 g/kg other fractions (Fig. 2). The removal efficiency and rate of TPH and hydrocarbon fractions were calculated by the following equations: $(C_{\text{Initial concentration}} - C_{\text{Final concentration}})/C_{\text{Initial concentration}}$ and $(C_{\text{Initial concentration}} - C_{\text{Final concentration}})/91$, respectively; the results are listed in Table 2. At the end of the 13-week bioremediation, TPH decreased from 269.5 g/kg to 245.0, 223.8, and 187.5 g/kg in the SW, BA, and BS treatments, corresponding to removal efficiencies of 9.1, 17.0, and 30.4%, respectively (Table 2). Compared with the TPH content of initial sludge, only the BS treatment significantly enhanced TPH removal ($P < 0.05$) (Fig. 2).

As shown in Fig. 2, at the end of bioremediation, the aliphatic fractions were 146.7, 139.6, and 118.5 g/kg, the aromatic fractions were 26.9, 25.0, and 26.3 g/kg, and the other fractions were 71.4, 59.2, and 42.7 g/kg in the SW, BA, and BS

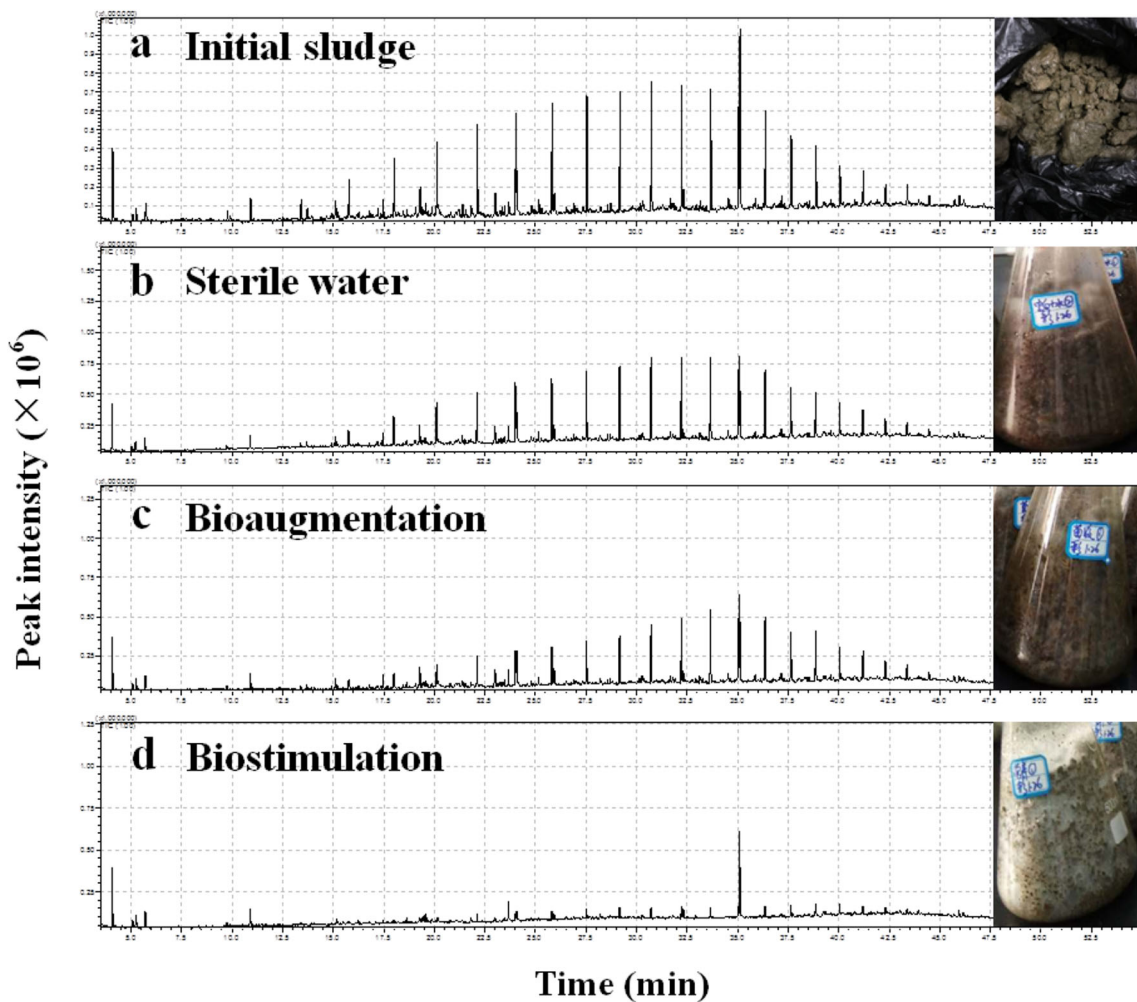


Fig. 1 Gas chromatography–mass spectrometry chromatographs of total petroleum hydrocarbons in initial sludge (a) and sludge subjected to sterile water (b), bioaugmentation (c), and biostimulation (d) treatments (the

abundance on y-axis is the same for all samples). Embedded images are of initial sludge and treated sludge

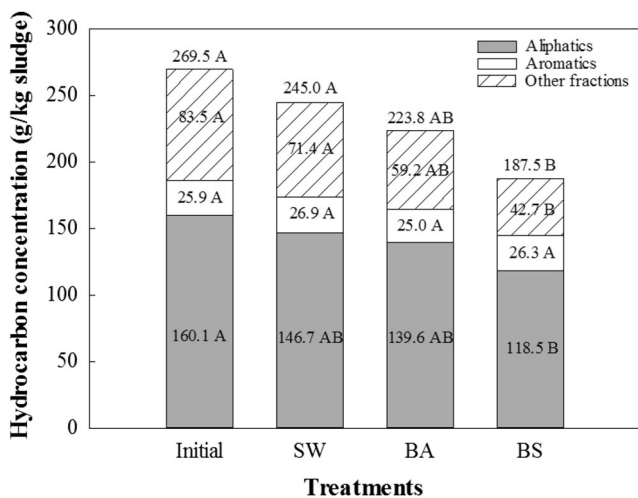


Fig. 2 Total petroleum hydrocarbons and different hydrocarbon concentrations in initial sludge and sludge in sterile water (SW), bioaugmentation (BA), and biostimulation (BS) treatments. Numbers in bars show hydrocarbon concentrations. Different letters in the same hydrocarbon concentration bars indicate significant differences ($P < 0.05$)

treatments, respectively. Further study showed that the degradation of aliphatic and other fractions contributed to the main

Table 2 Removal efficiency and rate of total petroleum hydrocarbons (TPH) and hydrocarbon fractions in sterile water (SW), bioaugmentation (BA), and biostimulation (BS) treatments after 13-week bioremediation

Treatments	SW	BA	BS
Removal efficiency (%)			
TPH	9.1 ± 3.5	17.0 ± 6.2	30.4 ± 0.2
Aliphatics	8.3 ± 8.0	12.8 ± 6.9	26.0 ± 1.9
Aromatics	-3.9 ± 20.8	3.5 ± 26.9	-1.5 ± 6.2
Other fractions	14.5 ± 2.5	29.1 ± 25.0	48.9 ± 2.4
Removal rate (mg/kg/day)			
TPH	300 ± 100	500 ± 200	900 ± 6
Aliphatics	100 ± 100	200 ± 100	400 ± 30
Aromatics	-10 ± 60	10 ± 80	-4 ± 20
Other fractions	100 ± 20	300 ± 200	400 ± 20

TPH removal in all treatments (Table 2). A significant decrease in aliphatic hydrocarbons was observed in the BS treatment ($P < 0.05$) compared to initial sludge (Fig. 2). The aromatic fractions remained unchanged during the bioremediation process in all treatments (Fig. S1). Interestingly, the other fractions showed a remarkable decrease from 83.5 g/kg in initial sludge to 42.7 g/kg in the BS treatment, corresponding to a removal efficiency of 48.9% (Fig. 2).

The concentration of aliphatic hydrocarbon with different chain lengths (i.e., C8–C20, C21–C30, and C31–C40) and their corresponding degradation rates in different treatments are shown in Table 3. Middle-chain-length alkanes constituted the main part of aliphatic fractions (66.6%), and the BS treatment showed the best degradation performance of alkanes with different chain-length ranges. Moreover, low-chain-length fractions (C8–C20) exhibited the highest reduction in all treatments, indicating that low-chain-length alkanes were the most readily degraded fractions.

Determination of pH

Monitoring pH is crucial since it reflects microbial activity and oxidative degradation of petroleum hydrocarbons (Ji et al. 2014). In all treatments, pH decreased rapidly during the first 5 weeks and increased until the eighth week. After that, the pH varied slightly in the SW and BA treatments, but decreased in the BS treatment (8–13 weeks). Generally, the changes in pH, ranging from 6.1 to 7.1, were not significant (Fig. S2).

Illumina high-throughput sequencing analysis

The results of the Illumina high-throughput sequencing and alpha-diversity index are presented in Table 4. After the quality check, a total of 199,481 16S rRNA and 176,406 ITS gene sequences for all treatments were obtained. The bacterial OTU

numbers in initial sludge and the SW, BA, and BS treatments were 947, 651, 345, and 1178, respectively, and fungal OTU numbers were 80, 755, 119, and 67, respectively. Diversity indexes of sludge in different treatments were calculated by mothur software version 1.35.1. The alpha-diversity index depends on both the richness and uniformity of a microbial community. The abundance-based coverage estimator, Chao 1, and Simpson index represent the richness, while the Shannon index demonstrates evenness in a community (Wu et al. 2017). The BS treatment had the highest bacterial community richness and diversity (Table 4). BS increased bacterial community diversity and richness in the oily sludge. Fungal community diversity noticeably increased in the BA treatment and slightly increased in the BS treatment (Table 4).

Microbial community structure

The structures of the fungal and bacterial communities at different taxonomic levels, including dominant phyla, classes, and genera, were determined for all treatments (Figs. 3 and 4). During bioremediation, fungal growth was slightly visible in the SW and BA treatments, and fungi proliferated in the BS treatment (embedded images in Fig. 1). The fungal community structures were similar in different treatments except for the BA treatment, which showed higher diversity. At phylum level, the most abundant fungal phylum was Ascomycota in all treatments (Fig. 3a). At class level, the dominant fungi in initial sludge were Sordariomycetes (98.21%) with Saccharomycetes (0.45%) as a minor class (Table S1). After the 13-week bioremediation, the relative abundance of Eurotiomycetes increased from 0.23 to 14.27% in the BS treatment, and a shift in fungal community structure occurred in the BA treatment (Fig. 3b). There was a negligible difference between the microbial community structures of the SW treatment and initial sludge (Fig. 3b). At genus level, there was a wide distribution of *Scedosporium* in initial sludge

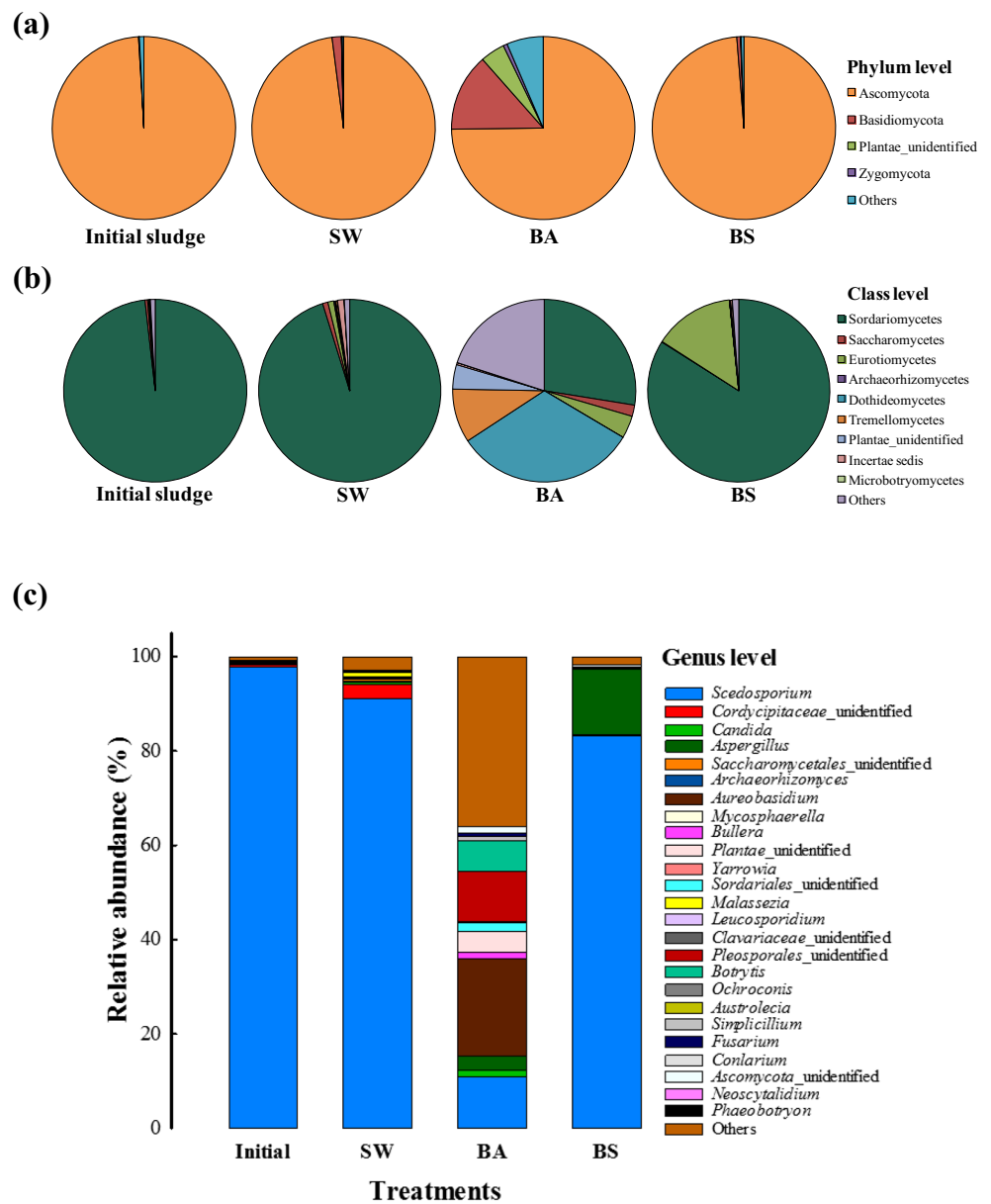
Table 3 Concentration of aliphatic hydrocarbons with different chain lengths and their corresponding degradation rate in sterile water (SW), bioaugmentation (BA), and biostimulation (BS) treatments after 13-week bioremediation

Treatment	Fractions	Hydrocarbon concentration (g/kg)	Degradation efficiency (%)
Initial	C8–C20	35.4	–
	C21–C30	77.0	–
	C31–C40	3.1	–
BA	C8–C20	27.2	23.2
	C21–C30	68.5	11.0
	C31–C40	2.8	9.7
BS	C8–C20	20.5	42.1
	C21–C30	56.9	26.1
	C31–C40	2.3	25.8
SW	C8–C20	30.1	15.0
	C21–C30	70.6	8.3
	C31–C40	2.9	6.4

Table 4 Observed operational taxonomic units (OTUs) and alpha-diversity indexes of the bacterial (bac.) and fungal phylotypes in sterile water (SW), bioaugmentation (BA), and biostimulation (BS) treatments; ACE, abundance-based coverage estimator

	Reads	OTU	Phylum	Class	Order	Family	Genus	Simpson	Chao1	ACE	Shannon
Initial-bac.	45,259	947	17	33	53	90	138	0.795528	947	947	5.27
SW-bac.	46,456	651	14	28	39	70	110	0.566941	657.21	676.74	3.19
BA-bac.	63,079	345	16	28	44	72	110	0.381918	400.15	413.95	1.86
BS-bac.	44,687	1178	8	17	26	55	120	0.933843	1178	1178	6.79
Initial-fungi	44,032	80	3	12	17	15	17	0.630553	86.07	90.11	1.87
SW-fungi	49,618	755	9	21	55	85	126	0.644802	124.08	124.01	2.2
BA-fungi	37,592	119	10	22	59	92	134	0.936602	755	755	5.89
BS-fungi	45,164	67	4	10	15	15	21	0.613572	69.8	72.95	1.96

Fig. 3 Relative distribution of major taxonomic groups at phylum (a), class (b), and genus (c) levels for fungi in initial sludge and sludge in sterile water (SW), bioaugmentation (BA), and biostimulation (BS) treatments. The most abundant operational taxonomic units (OTUs) at different levels for all treatments are listed, and all other OTUs were combined and shown as “Others”



(97.8%) (Fig. 3c). After the 13-week treatment, the relative abundance of *Scedosporium* in the SW, BA, and BS treatments was 91.1, 10.8, and 83.3%, respectively. Compared to that in initial sludge, the relative abundance of *Scedosporium* decreased in the BS treatment. An increase in *Aspergillus* was detected in the BS treatment (Fig. 3c).

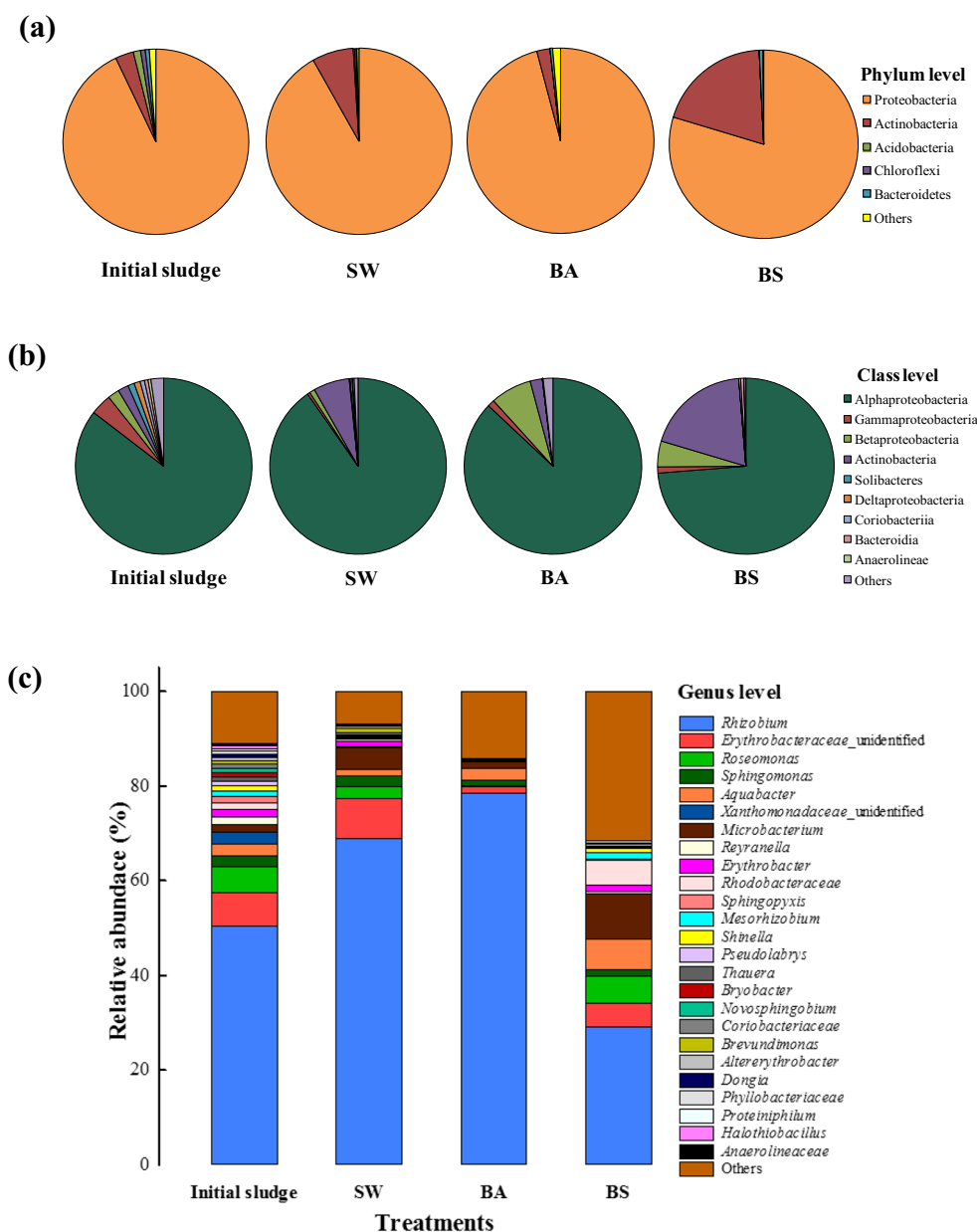
With regard to bacterial community structure, Proteobacteria was the predominant phylum in all the treatments, and a distinct increase of Actinobacteria was observed in the BS treatment (Fig. 4a). At class level, all treatments were dominated by Alphaproteobacteria (Fig. 4b). The relative abundance of Actinobacteria in the BS treatment increased from 1.9 to 19.1% (Table S1), which was noticeably higher than that in other treatments. At genus level, *Rhizobium* was dominant in

the initial sludge, and the relative abundance of this genus increased in the SW and BA treatments and decreased in the BS treatment. Moreover, the BS treatment increased the diversity of bacterial community structure compared to that of other treatments (Fig. 4c).

Discussion

In this study, a laboratory-scale bioremediation was conducted to investigate the effects of BA and BS on oily sludge with a high TPH concentration (~27%) collected from Xinjiang oilfield, China. Enhanced TPH reduction (30.4%) was achieved in the BS treatment with fungal proliferation

Fig. 4 Relative distribution of major taxonomic groups at phylum (a), class (b), and genus (c) levels for bacteria in initial sludge and sludge in sterile water (SW), bioaugmentation (BA), and biostimulation (BS) treatments. The most abundant operational taxonomic units (OTUs) at different levels for all treatments are listed, and all other OTUs were combined and shown as “Others”



(Figs. 1 and 2). The TPH removal showed small variations between the BA and SW treatments (17.0 and 9.1%, respectively), indicating that the addition of exogenous bacteria exhibited slight promotion in TPH degradation. The degradation mostly occurred during the first 5 weeks of bioremediation and was limited during the remaining weeks. This was probably due to the depletion of nutrients that supported the growth of fungi in all three treatments at the initial stage (0–5 weeks). He et al. (2015) reported a similar pattern in the bioremediation of oily sludge. However, this could be easily solved by regularly supplying nutrients to oily sludge. The highest TPH removal and fungal biomass indicated the feasibility of applying BS in remediating local oily sludge in Xinjiang Autonomous Region.

The pH is an important parameter that indicates microbial degradation activity (Joubert et al. 2007). In this study, the pH of oily sludge decreased rapidly during the first 5 weeks, which might have resulted from the degradation of organic matter and production of carbon dioxide. During the next 3 weeks, the pH in all treatments increased, possibly due to the degradation of organic acids. After 8 weeks, the pH in the SW and BA treatments stabilized, whereas that in the BS treatment decreased, probably as a result of fungal biomass decomposition. During the initial (0–5 weeks) and final (8–13 weeks) stages, the pH in the BS treatment decreased rapidly, indicating the highest microbial degradation activity. This was consistent with the superior performance of the BS treatment.

The moisture content of oily sludge is another important factor affecting hydrocarbon degradation efficiency (Ayotamuno et al. 2006; Leahy and Colwell 1990). Insufficient water may inhibit microbial metabolism, but excessive moisture content may hinder microbial access to oxygen (Schjønning et al. 2011). The moisture content in all treatments was kept constant at 75%, which was slightly higher than that of the initial sludge (71.9%). A removal efficiency of 9.1% in the SW treatment was achieved in this study (Table 2). The much higher fungal biomass in this treatment compared to that in the initial sludge (embedded images in Fig. 1) might be attributable to the addition of water, which accelerated fungal proliferation by dissolving nutrients adsorbed onto oily sludge, thereby making the nutrients more bioavailable.

The results of hydrocarbon fraction analysis showed that the concentration of aliphatic hydrocarbons was reduced, whereas the degradation of aromatic hydrocarbons was limited in this high-TPH oily sludge; similar results were found by Wu et al. (2017). The removal of biodegradable hydrocarbon generally causes accumulation of residual fractions such as resin and asphaltenes (Yanto and Tachibana 2014). However, in our study, a noticeable reduction of other fractions including saturated *n*-alkanes and cyclic saturated alkanes was observed in the BS treatment. Since this fraction was also responsible for the toxicity of TPH (Di Gregorio et al. 2016), its removal was of great significance. In this study,

microorganisms such as *Rhizobium* sp., *Microbacterium* sp., and fungi genus belonging to Ascomycetes (e.g., *Aspergillus*) were detected. These microorganisms have previously been reported to be capable of degrading cyclic alkanes and *n*-alkanes (Al-Saleh and Hassan 2016; Becarelli et al. 2019; Vela et al. 2002), suggesting the potential roles in the removal of other fractions in the current study. Apart from that, the BS treatment exhibited the highest degradation efficiency of aliphatic hydrocarbons with different chain-length ranges (Table 3). These results further proved that BS was more effective in treating local oily sludge than BA.

The removal efficiencies of TPH in the SW, BA, and BS treatments were consistent with fungal growth. The BS treatment showed the greatest fungal growth (embedded images in Fig. 1), indicating the important role of fungi in TPH removal. Generally, the degrading capacity of fungi is better than that of bacteria (Schink 2002). In this study, fungal proliferation was observed in the BS treatment, with *Scedosporium* as the dominant genus (Fig. 3c), suggesting that the high TPH removal in this treatment might be attributable to *Scedosporium* spp. The ability of *Scedosporium* to degrade many complex pollutants, such as aromatic hydrocarbons (Reyes-César et al. 2014), aliphatic hydrocarbons (April et al. 1998; Yuan et al. 2018), and mixtures of these contaminants (Morales et al. 2017), has been widely reported. Previous study has also reported that *Scedosporium* spp. could grow well in various nutrient conditions, and easily proliferate under the stimulation of nutrient (Meletiadis et al. 2001). Moreover, high relative abundance of *Scedosporium* in initial sludge indicated its ubiquitous existence in local oily sludge (Fig. 3c) and high resistance to petroleum hydrocarbons. Apart from that, the relative abundance increase of *Aspergillus* sp. (Fig. 3c), which was also known as a petroleum degrader (Al-Hawash et al. 2018; Bovio et al. 2017; Essabri et al. 2019), could be attributable to the nutrients supplement. This further supported the approach of adding nutrients to local sludge to stimulate the growth of widely distributed indigenous fungi, thereby remediating the sludge.

Microbial community analysis showed that the BS treatment exhibited a higher bacterial community diversity (Table 4). This result differed from that of previous studies that reported that the addition of nutrients could reduce bacterial diversity in oil-contaminated soil (Al-Saleh and Hassan 2016; Wu et al. 2016). Generally, there was higher bacterial diversity in low-TPH oily sludge than in high-TPH oily sludge (Ramadass et al. 2015). The decrease in TPH in the BS treatment probably resulted in increased bacterial diversity. Yuan et al. (2018) revealed that the addition of the exogenous fungus *Scedosporium* to an indigenous bacterial consortium provided a suitable environment for the formation of a bacterial biofilm and markedly increased bacterial diversity. In this study, the abundant growth of *Scedosporium* by BS might create beneficial conditions for bacterial growth and lead to increased microbial diversity.

Conclusions

The effect of BA, BS, and SW treatments on the remediation of high-TPH oily sludge and microbial community structure was investigated. The BS treatment significantly enhanced TPH removal (30.4%), reducing aliphatic and other fractions. During bioremediation, fungi proliferated in the BS treatment, with *Scedosporium* as the dominant genus that might play a role in TPH degradation. BS was the most efficient method for treating high-TPH oily sludge collected from Xinjiang Autonomous Region. However, more research is needed to improve BS efficiency, e.g., by optimizing the C/N/P ratio, adding biosurfactants, or combining oil-degrading microorganisms.

E-supplementary data of this work can be found in the online version of the paper.

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Compliance with ethical standards

Conflicts of interest The authors declare that there is no conflict of interest.

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