

Biodegradation of marine oil spill residues using aboriginal bacterial consortium based on *Penglai 19-3* oil spill accident, China

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ARTICLE INFO

Keywords:

Bioremediation
Indigenous bacteria
Selective depletion
Oil spill
Isomer

ABSTRACT

Bioremediation, mainly by indigenous bacteria, has been regarded as an effective way to deal with the petroleum pollution after an oil spill accident. The biodegradation of crude oil by microorganisms co-incubated from sediments collected from the Penglai 19-3 oil platform, Bohai Sea, China, was examined. The relative susceptibility of the isomers of alkylnaphthalenes, alkylphenanthrenes and alkyldibenzothiophene to biodegradation was also discussed. The results showed that the relative degradation values of total petroleum hydrocarbon (TPH) are 43.56% and 51.29% for sediments with untreated microcosms (S-BR1) and surfactant-treated microcosms (S-BR2), respectively. TPH biodegradation results showed an obvious decrease in saturates (biodegradation rate: 67.85–77.29%) and a slight decrease in aromatics (biodegradation rate: 47.13–57.21%), while no significant difference of resins and asphaltenes was detected. The biodegradation efficiency of alkylnaphthalenes, alkylphenanthrenes and alkyldibenzothiophene for S-BR1 and S-BR2 samples reaches 1.28–84.43% and 42.56–86.67%, respectively. The efficiency of crude oil degradation in sediment with surfactant-treated microcosms cultures added Tween 20, was higher than that in sediment with untreated microcosms. The biodegradation and selective depletion is not only controlled by thermodynamics but also related to the stereochemical structure of individual isomer compounds. Information on the biodegradation of oil spill residues by the bacterial community revealed in this study will be useful in developing strategies for bioremediation of crude oil dispersed in the marine ecosystem.

1. Introduction

Oil spill has been a worldwide challenge in the modern society, which not only causes substantial economic loss, but also poses serious threats to the environmental and human health. Oil spills may be due to releases of crude oil from tankers, offshore platforms, drilling rigs and wells, as well as spills of refined petroleum products and their by-products. On June 4, 2011, the *Penglai 19-3* oilfield caused an oil spill from a sea floor leak that lasted until June 7, with 763 barrels of crude oil flowing into the Bohai Sea, China. It was reported that 840 square kilometers of clean coastal water were polluted due to this oil spill accident By June 17 (Liu et al., 2015). Due to the semi-enclosed characteristics of Bohai Sea, its water exchange with the open sea is limited, which in turn leads to a larger accumulation of pollutants for a long time (Hu et al., 2011). At present, petroleum hydrocarbons are considered as the third most common pollutant followed by nitrogen and phosphorus in this region (SOAC, 2015). Such oil pollutants not only damage the natural environment and ecological resources of Bohai Sea,

but also pose a serious threat to human health (Li et al., 2015; Wang et al., 2018).

Biological treatments (bioremediation) is recognized as the most preferred measures on removal of oil because they are generally cost effective and environmentally friendly (Braddock et al., 1997; Ghazali et al., 2004; Camilli et al., 2010; Zhang et al., 2011; Bacosa et al., 2015; Borah and Yadav, 2017). Although a lot of efficient crude oil degrading bacteria have been isolated, such bacteria may thrive in one environment but may not be able to compete with other microorganisms in another complicated ocean environments. In the past few decades, a variety of crude oil degrading bacteria have been isolated from the oil-polluted locations (Pasumarthi et al., 2013; Wang et al., 2013; Hassanshahian and Boroujeni, 2016; Borah and Yadav, 2017). The bioremediation of the laboratory-cultured functional bacteria is limited to the marine environment (Wang et al., 2013). By contrast, the natural biodegradation processes of indigenous bacteria were believed to play a dominant role on the bioremediation. As we well known, 16 US EPA priority PAHs from oil spill may pose serious threats to coastal habitats

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due to more toxic effects (Pongpiachan et al., 2018). Detailed knowledge of PAHs biodegradation can provide some useful information for further understanding of their environmental fate and potential ecotoxicological effect.

A profound knowledge of the degradation mechanism, efficiency and biodegradation potential of spilled oil in marine ecosystem is thus essential for ecological risk assessment as well as for developing contingency plans for oil spill response. There are many studies about biodegradation of crude oil, but few of them focus on marine oil spill residues using aboriginal bacterial consortium based on a famous large oil spill accident, such as Penglai 19-3 oil spill accident, China. The use of natural microbial remediation on residual oil spill is one of the effective strategies for the restoration ecological environment in Bohai Sea. Overall, the main objectives of this study are (1) investigated the extent of degradation of *n*-alkanes and PAHs; (2) assess the biodegradation effectiveness of crude oil by indigenous bacteria based on Penglai 19-3 oil spill accident; and (3) relative susceptibility to biodegradation of individual isomers (mono, di, and tri) within specific compound classes.

2. Experimental

2.1. Materials and biodegradation experiment setup

Two different microcosm assemblages were set up to conduct the oil bioremediation experiment in this study. The used crude oil was collected from the oil platform in oil-gas mining area of central Bohai Sea. The sediment sample collected from the sea area near Penglai 19-3 oil platform was used for culture isolation. TOC contents of surface sediments in Bohai Sea is 0.23 wt%. The sediment components are mainly composed of sand (53.26%) and silt (38.28%). For biodegradation experiment 1, aliquots (2% w/v) of crude oil and 10 g sediments were co-incubated in 100 mL mineral solution. Refer to the natural living conditions of bacteria in studied area, aerobic bacteria and inorganic salt concentration of aqueous medium were chosen in this study. The mineral media composition was as specified (concentration in g/L): $(\text{NH}_4)_2\text{PO}_4$ (1), KH_2PO_4 (0.5), Na_2HPO_4 (0.075), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (0.2), NaCl_2 (30) and CaCl_2 (0.02). Furthermore, the assemblage (experiment 2) with additional Tween-20 (1%, v/v) was simultaneously performed in order to evaluate the effects of surfactant on bioremediation. Such mixtures in flask were incubated on a constant temperature shaker (120 rpm) and 30 °C for 28 days, and then 15 mL inoculum was transferred to a fresh mineral salt medium and incubated for another cycle. Triplicate treatments were maintained for every sample as well as biotic and abiotic controls. After consecutive transfers, hydrocarbon degraders were isolated by plating on LB solid plates. The effective degradation strains of spilled oil were screened. At the end of the experiment, the original sediments adding crude oil (S0) and samples collected at the end of the incubation for both bioremediation experiments 1 (S-BR1) and experiments 2 (S-BR2) were kept at -80 °C for further analysis. Bacterial populations associated with oil bioremediation were detected by the analysis of 16S rRNA gene based DGGE and clone library analysis. The detail information on the changes of bacterial communities associated with oil degradation will be discussed in other papers.

2.2. Extraction, fractionation

Treatments were freeze-dried, extracted and analyzed after biodegradation experiment. Samples were extracted and analyzed according to the methods that were established at the National Laboratory for Environmental Testing, Environment Canada (1994). Detailed information on sample collection, treatment and analysis were also reported in previous studies (Hu et al., 2011; Wang et al., 2011, 2017). Briefly, extraction was performed using Soxhlet method with dichloromethane and methanol (93:7) as the solvent mixture for 72 h.

The aliphatic hydrocarbons, aromatic hydrocarbons and resins were separated by column chromatography using activated alumina and silica gel, and gradient solvents as eluent: ligarine, ligarine/dichloromethane (3:9, v/v) and MeOH, respectively. Prior to analysis of the fractions by the instrumental analysis, the samples volume were adjusted to 1 mL by evaporation using a stream of filtered N_2 gas. The recoveries of crude oil group varied from 89.54% to 93.75%. In addition, the recovery of crude oil components has increased by 4.16% affected by the addition of Tween-20.

2.3. Instrumental analysis

The instrumental analysis was performed on a Hewlett-Packard 6890 gas chromatograph interfaced with a Hewlett-Packard 5973 mass-selective detector made by Agilent (USA). Helium was used as the carrier gas. *n*-Alkanes and some PAHs were identified by GC-MS in full scan mode. Sample extracts were injected in a splitless mode onto H-5 fused capillary column (30 m × 0.2 mm i.d) coated with 0.25 μm thick. The gas chromatograph oven temperature was programmed at 80 °C for 2 min and ramped at 4 °C/min to a final temperature of 290 °C held for 30 min. The mass spectrometer was operated at an electron energy of 70 eV with an ion source temperature of 250 °C.

Individual *n*-alkanes were identified based on the retention time of the authentic standards ($n\text{C}_{10-40}$, Sigma). On the other hand, individual PAHs were quantified based on the retention time and *m/z* ratio of an authentic PAH mixed standard (Sigma). The minimum method detection limits for an individual aliphatic or aromatic compound is 10 ppb. Relative abundance was calculated from respective mass chromatogram peak areas. Five surrogate standards (naphthalene-d8, phenanthrene-d10, dibenzothiophene-d10, fluoranthene-d10, pyrene-d10) were added to all samples to monitor matrix effects. The calibration curves of PAHs were fitted by peak area of the blank samples spiked with 10, 20, 40, 60, and 80 ng/g standards. The average recoveries of surrogate standards varied from 81.6% to 92%. For detailed analytical procedure, conditions, and quality control refer to previous studies (Hu et al., 2011; Wang et al., 2017).

2.4. Statistical analysis

Repeat values of the sample were presented as the mean ± standard deviation (SD). Statistical analysis was performed with SPSS Version 11.5 analysis. Differences were considered significant at $p < 0.05$. The tests were considered statistically significant if $P < 0.05$. In this paper, we use the average value of triplicate samples in order to better use graph to display the law of data change.

3. Results and discussion

3.1. Bioremediation of crude oil fractions

Crude oil can be divided into four main fractions with a silica gel G column: saturated hydrocarbons (Sat), aromatic hydrocarbons (Aro), resins (Res) and the asphaltenes (Asp) according to their polarizability and polarity. In this study, the relative degradation values of total petroleum hydrocarbon (TPH) are 43.56% and 51.29% for sediments with untreated microcosms (S-BR1) and surfactant-treated microcosms (S-BR2), respectively. The relative abundance of saturated hydrocarbons decreased sharply from 45.30% to 26.28% and 21.60% in S-BR1 and S-BR2 samples, respectively. For aromatic hydrocarbons fraction, the relative abundance decreased slightly from 26.10% to 24.9% and 23.46% in the S-BR1 and S-BR2 sediments, respectively. In contrast, the relative abundance of resins and asphaltene fractions increased dramatically (Fig. 1a). The aromatic fraction (biodegradation rate: 47.13%, 57.21%) is generally more resistant to biodegradation than the fraction of saturated hydrocarbons (biodegradation rate: 67.85%, 77.29%). The saturated hydrocarbons fraction showed the

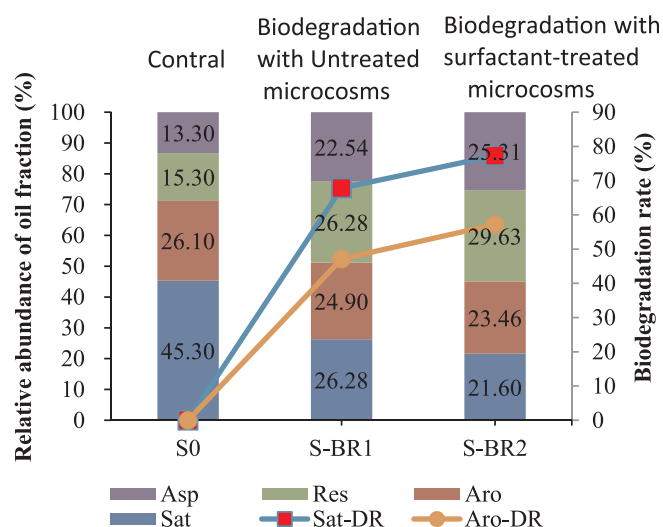


Fig. 1. Variation of four constituent fractions (saturates, aromatics; resins and asphaltenes) and degradation efficiency of saturates and aromatics after remediation. Note: DR: Biodegradation rate.

fastest degradation among all fractions (Fig. 1b). On the whole, an obvious decrease in saturates and a slight decrease in aromatics was observed, and no significant difference of resins and asphaltenes was detected. Tween 20 belongs to non-ionic surfactant. When nonionic surfactant Tween 20 is added, the sorption behavior of oil is affected. Its dispersing effect on crude oil promotes the degradation of crude oil by bacteria (Xie et al., 2004).

3.2. Evolution of *n*-alkane chemical composition during the biodegradation

Efficient degradation of crude oil was shown by TIC of saturated hydrocarbons fractions (Fig. 2). The extent of degradation of the *n*-alkanes was assessed. The oily sediment (S0) shows a typical non-biodegraded profile, having a full suite of *n*-alkanes, while S-BR1 shows significantly lower in abundance of *n*-alkanes. Compared to long-chain *n*-alkanes, the compounds with low- to middle-molecular-weight are generally transformed at higher rates by microbes (Fig. 2b). The oil residue of S-BR2 was highly degraded and completely depleted of *n*-alkanes (Fig. 2c). In the biodegraded oil residue, there is a significant increase in the relative abundance of the unresolved complex mixtures (UCM) (Fig. 2). The Pristane/*n*-C₁₇ (Pr/*n*-C₁₇) and Phytane/*n*-C₁₈ ratios (Ph/*n*-C₁₈ ratios) for the spilled oil were commonly used as early indicators of biodegradation (e.g. Lemkau et al., 2010; Wang et al., 2013). The lower molecular weight *n*-alkanes were preferentially removed before the higher molecular weight compounds, followed by the isoprenoids. According to the already established pattern, higher Pr/*n*-C₁₇ and Ph/*n*-C₁₈ ratios (from 0.68 and 1.18 to 1.02 and 1.56, respectively) for moderately heavy biodegraded oils (S-BR1) than their non-biodegraded counterparts (S0) were observed. The efficiency of *n*-alkane degradation in sediment with surfactant-treated microcosms cultures added Tween 20, was higher than that in sediment with untreated microcosms.

3.3. Evolution of PAHs chemical composition during the biodegradation

The highly degradation-resistant components, such as C₃₀ 17α(H), 21β(H)-hopane, can usually be used as the conserved “internal standards” of weathering rate and extent of weathering for the spilled residual oil (Prince et al., 1994, 2003; Wang and Fingas, 2003). To account for systemic losses not related to biodegradation the PAHs results were normalized to C₃₀ 17α(H), 21β(H)-hopane, which is used as a

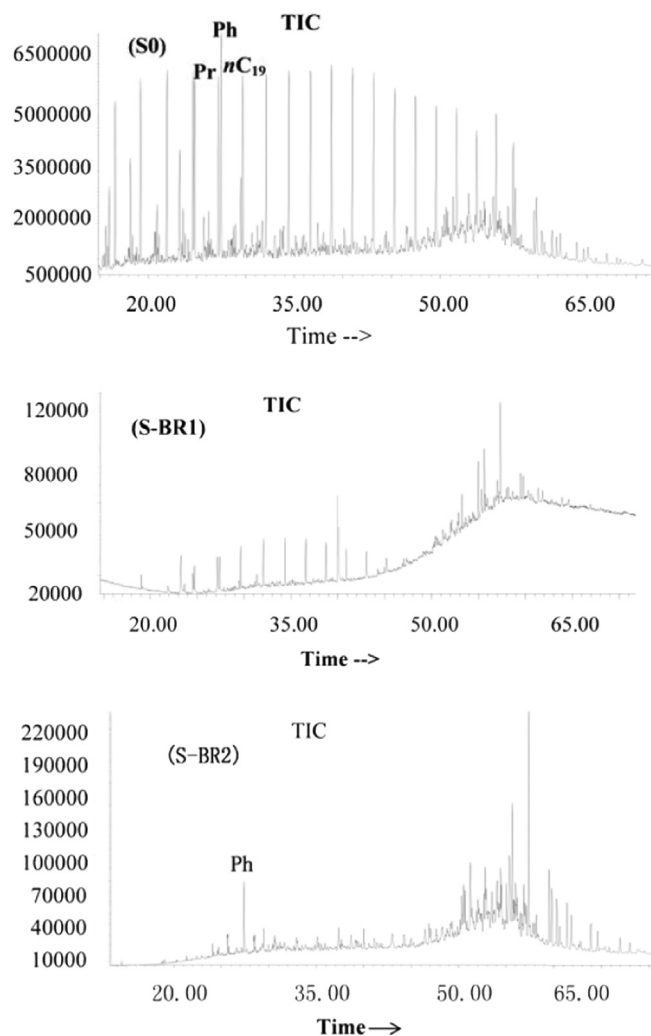


Fig. 2. GC-MS chromatograms of oiled sediment revealing the overall compositional changes before (S0), after biodegradation with untreated microcosms (S-BR1) and with surfactant-treated microcosms (S-BR2).

surrogate for corrections due to non-biodegradable and insoluble (Wang and Stout, 2010; Kristensen et al., 2015; Wang et al., 2016). Fig. 3 clearly shows the effect of degradation on the individual PAHs compositions of the oily sediment with untreated microcosms and with surfactant-treated microcosms. Major compositional changes of PAHs observed in this study are consistent with previous reports (Wang and Fingas, 2003). From Fig. 3, it can be seen that the percentage of crude oil biodegradation of DMP, TMP, CH, MCH, DMCH, DMF, TMDBT, Ret, BaA, Fla, Pyr without surfactant was in the range of 1.28–84.43% (the average value, 39.47) with respect to the abiotic control, which was taken as 100%. In addition, the loss of crude oil caused by the biodegradation of surfactant-treated microcosm's process was in the range of 42.56–86.67% (the average value, 68.57) compared with the control experiment. For some 16PAHs detected in oily sediment, the degradation rates of 2–3 ring PAHs (naphthalene, phenanthrene, fluorene) and 4-ring PAHs (benzaanthracene, fluoranthene, pyrene, chryrene) are 89.06 ± 14.06 and 48.16 ± 6.33 , respectively. For S-BR1 sample with untreated microcosms, some 2–3-ring compounds, such as DMP, TMP, CH, MCH, DMCH, DMF and 4–6 ring PAHs were still largely undegraded (degradation rate: 1.28–16.70%). However, such more recalcitrant compounds are largely degraded for S-BR2 with surfactant-treated microcosms (degradation rate: 42.56–61.36%). The loss of naphthalenes may be caused by evaporation, but the loss of other PAHs is most likely due to biodegradation. The results further supported that

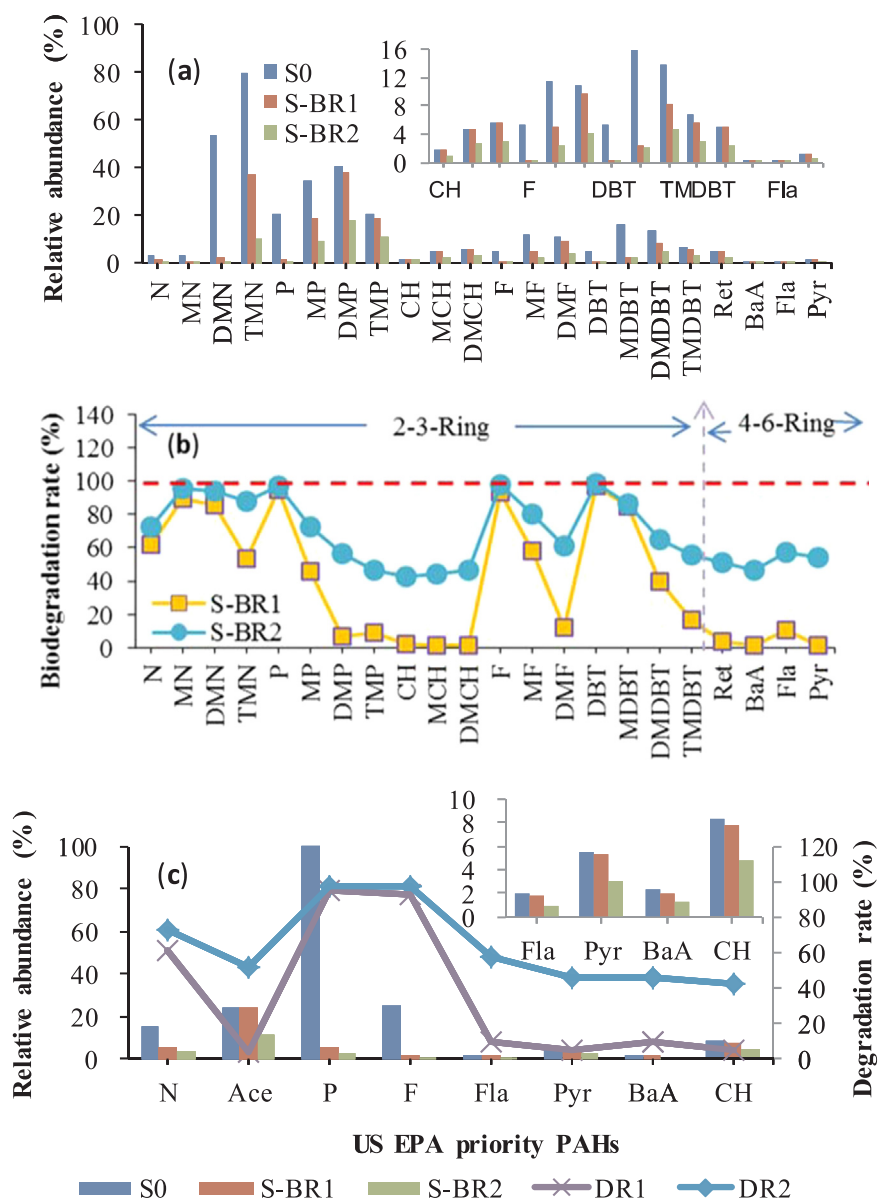


Fig. 3. Distribution and biodegradation on the individual PAHs compositions: distribution of the individual PAHs compounds (a), biodegradation rate on the individual PAHs compositions (b), and distribution and degradation rate of the US EPA priority PAHs (c). Note: The red dotted line shows the 100% normalization line that describes no changes from S0 samples; N: naphthalene; P: phenanthrenes; CH: chrysene; F: fluorine; DBT: dibenzothiophene; Ret: Retene; BaA: Benzanthracene; Fla: fluoranthene; Pyr: pyrene; M: methyl-; DM: dimethyl-; TM: trimethyl-; TeM: tetramethyl-.

the degree of PAHs degradation decreases with increasing in the number of aromatic rings, and decreases with increasing alkylation within a homologous series.

Biodegradation of crude oil is a complex process involving the interaction among bacteria, surface active agents and carbon sources (Tian et al., 2016). Demethylation is a process of leading to the degradation of methyl derivatives of PAHs. The bio-stimulation process may be beneficial to bacteria strains in consortium that decompose methyl isomers (first of all trimethyl-naphthalenes). However, surfactants at high concentrations might inhibit the degradation due to biological toxicity or the micelle formation (Kaczorek et al., 2015). Therefore, the selection of suitable surfactants is of great significance for the degradation of hydrophobic organic pollutants.

3.4. Distribution of isomers in oil after biodegradation

3.4.1. Alkyl-naphthalenes isomers

Naphthalene, phenanthrene compounds are in most of the oil-type

pollutants, and therefore dominant aromatic hydrocarbons in crude oils (Van Aarssen et al., 1999). Moreover, they are also important pollutants in the marine environment and soil environment (Lichtfouse et al., 2005). Combination of mass chromatograms from aromatic fractions for the dimethyl- (DMN), trimethyl- (TMN) of selected least to most biodegraded samples are shown in Fig. 4. The profile in each alkylated PAH family showing the distribution of C₁ (MN) < C₂ (DMN) < C₃ (TMN) is very apparent, similar to the severely weathered oil. The depletion in aromatic hydrocarbons can be seen from the relative intensities of various aromatic compounds by biodegradation identified in the aromatic fractions. Indeed, the percentages of the C₁-, C₂- and C₃-alkyl-naphthalene homologues show considerable variation with increasing degree of biodegradation (Fig. 5a). It shows that increase in alkylation on aromatic rings results in the decrease in susceptibility to biodegradation.

The depletion in aromatic hydrocarbons can be seen from the relative intensities of various aromatic compounds and their isomers by biodegradation identified in the aromatic fractions (Table 1). It is

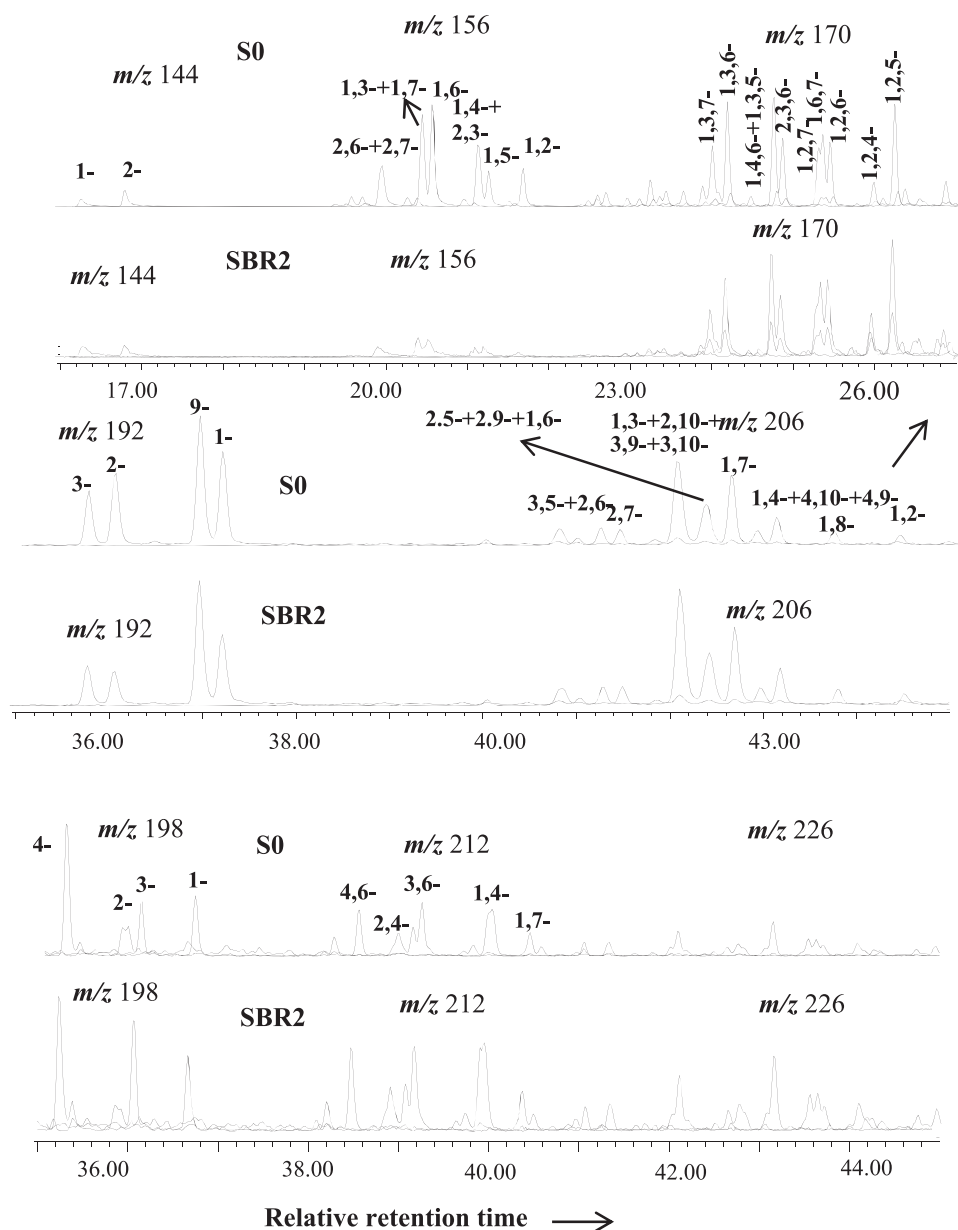


Fig. 4. GC-MS chromatograms for alkyl naphthalene (m/z 144 + 156 + 170; MN, DMN, TMN), alkylphenanthrenes (m/z 192 + 216; MP, DMP) and alkyldibenzothiophene distributions (m/z 198 + 212 + 226; MDBT, DMDBT, TMDBT) in oily sediment before and after biodegradation with surfactant-treated microcosms.

noteworthy to observe that isomers which are structurally similar were applied in these Biodegradation Ratios from every alkylnaphthalenes (Fisher et al., 2008). It contains MN biodegradation ratio (MNBR: 2-MN/1-MN), DMN biodegradation ratio (DMNBR: 1,6-DMN/1,2-DMN), TMN biodegradation ratio (TMNBR: 1,3,7-TMN/1,2,7-TMN). From Fig. 4, we can also see that a significant depletion in the 1-MN isomer relative to the 2-MN isomer is observed with rise in biodegradation levels. Furthermore, the 1,2-DMN, 1,2,7-TMN isomers from DMN, TMNs indicate to be more depleted to biodegradation than the 1,6-DMN and 1,3,7-TMN.

3.4.2. Alkylphenanthrenes isomers

The effect of biodegradation on alkylphenanthrenes was reported using variations in the distribution of methylphenanthrenes (MP), dimethylphenanthrenes (DMP) and trimethylphenanthrenes (TMP). The relative amount of phenanthrene and MP decrease significantly, while the proportion of DMP and TMP increased correspondingly (Fig. 5c), indicating that the DMP and TMP are more difficult to biodegradation

than phenanthrene and MP. It can be concluded that, a decrease in the relative concentration of phenanthrene and lower methyl “homologue” comparing to the higher homologues occurred. This result is largely consistent with field observations by Volkman et al. (1984) and Huang et al. (2004) that higher degree of alkylation leads to strong biodegradability. The difference between S-BR1 and S-BR2 is little.

It is observed that the 2-MP isomer is less resistant to biodegradation while the 9-MP and 1-MP isomers show higher resistance to biodegradation (Fig. 5d). For the MP isomers, the methylphenanthrene ratios on the oil-gas geochemical exploration study, such as (3-MP + 2-MP)/(9-MP + 1-MP), 1-MP/9-MP and 2-MP/MPs show a slight decrease with increasing degree of biodegradation. The reason may be contribute the higher thermally stability of 9-methyl, 1-methyl methylphenanthrenes isomers than 3-methyl, 2-methyl methylphenanthrenes. For the DMP isomers, the susceptibility to the biodegradation of the individual isomers is complicated and less clear due to large number of possible isomers and coelution of different substitution isomers on common stationary phases. In this study, the relative

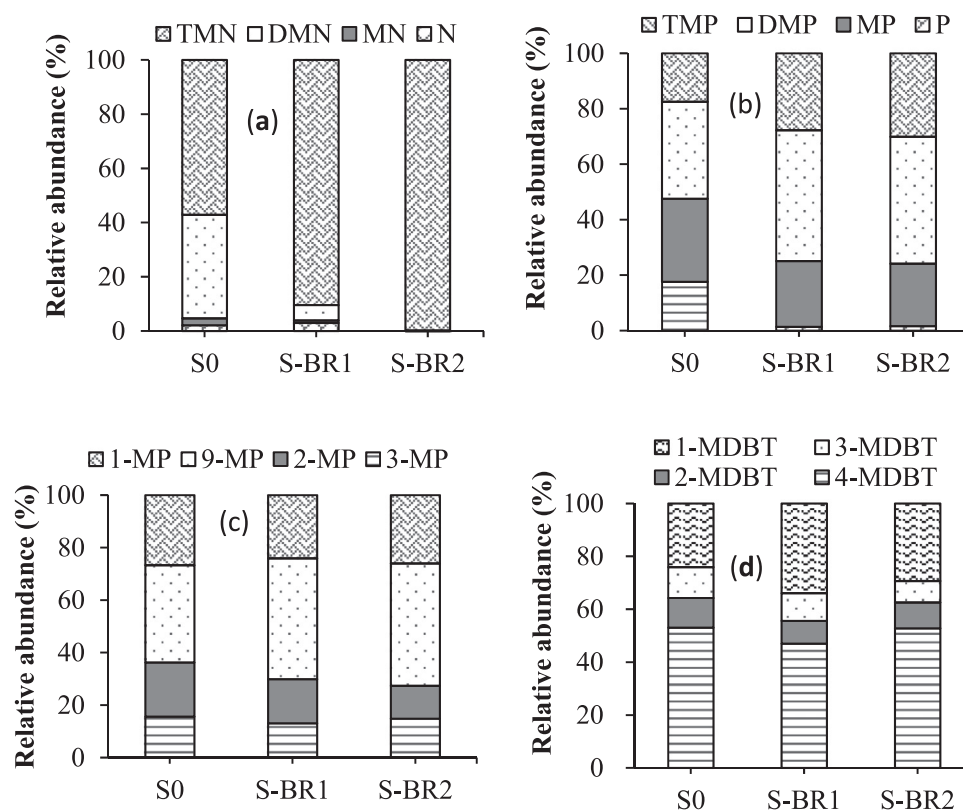


Fig. 5. Biodegradation on the alkylphenanthrenes, alkylphenanthrenes and alkylidibenzothiophene: Biodegradation susceptibility based on the relative abundance of N-MN-DMN-TMN (a), P-MP-DMP-TMP (b), methylphenanthrenes isomers (c) and methylidibenzothiophene isomers (d).

abundance of 1,7-DMP was the highest. On the whole, the order of susceptibility of the methylphenanthrenes to biodegradation in this study is: 2-MP > 3-MP > 1-MP > 9-MP (Figs. 4 and 5d).

The relative abundance of C₂-Ps (DMP) was calculated from peak areas of *m/z* 206 ion chromatogram and is shown in Fig. 4. The 1,7-DMP isomer was regarded as the most resistant isomer among DMP isomers (Chen et al., 2015). However, 1,7-DMP was the most susceptible to biodegradation among the DMP in crude oils from Liaohe oilfield (Huang et al., 2004). Perhaps surprisingly, biodegradation in this does not usually show very much isomer specificity; all the isomers of are lost at more or less the same rate, although this is not necessarily

apparent at first inspection. The degradation rate of DMP isomers reaches 58.21 ± 4.79 for S-BR2. The difference between biodegradation susceptibility of alkylphenanthrenes may contribute to the different bacterial consortium in different laboratory experiments and field observations, which is worth of further investigation.

3.4.3. Alkylidibenzothiophene isomers

The mass fragmentograms of benzothiophenes (BT), dibenzothiophenes (DBT) and their methyl homologues are represented in Figs. 4 and 5e. 4-Methylidibenzothiophene (4-MDBT) is the most abundant compound among the tricyclic sulphur aromatics for various crude oils

Table 1
Biodegradation rate of alkylphenanthrenes, alkylphenanthrenes and alkylidibenzothiophene.

Alkylphenanthrenes			Alkylphenanthrenes			Alkylidibenzothiophene		
Isomers	S-BR1	S-BR2	Isomers	S-BR1	S-BR2	Isomers	S-BR1	S-BR2
2-MN	82.46	92.70	3-MP	55.27	73.84	DBT	96.82	98.47
1-MN	92.00	96.78	2-MP	56.57	83.34	4-MDBT	77.85	79.47
2,7 + 2,6-DMN	93.54	99.22	9-MP	33.81	65.47	2-MDBT	80.77	82.06
1,6-DMN	97.27	99.30	1-MP	52.00	73.25	3-MDBT	77.71	85.60
1,3 + 1,7-DMN	96.54	99.47	EP	23.29	64.62	1-MDBT	64.74	74.90
1,4 + 2,3-DMN	92.87	100.00	2-E + 3,6-DMP	19.65	62.96	4,6-DMDBT	38.39	63.68
1,5-DMN	91.37	100.00	9-EP-DMP	35.15	65.02	2,4-DMDBT	37.95	59.73
1,2-DMN	100.00	100.00	1-EP + 2,6 + 3,5-DMP	17.13	63.25	2,6-DMDBT	40.54	67.00
1,3,7-TMN	63.70	91.72	2,7-DMP	7.61	57.48	3,6-DMDBT	43.72	67.93
1,3,6-TMN	62.71	91.94	2,10 + 1,3 + 3,10 + 3,9-DMP	- 0.93	49.81	3,7-DMDBT	38.58	67.24
1,4,6 + 1,3,5-TMN	54.43	89.85	1,6 + 2,9 + 2,5-DMP	- 4.08	54.33	1,4-DMDBT	35.98	61.46
2,3,6-TMN	63.80	90.33	1,7-DMP	14.18	60.20			
1,2,7-TMN	73.61	100.00	2,3-DMP	12.00	57.48			
1,6,7-TMN	73.20	88.75	4,9 + 4,10 + 1,9-DMP	- 0.54	51.87			
1,2,6-TMN	51.78	87.11	1,8-DMP	8.75	60.25			
1,2,4-TMN	32.89	80.86	1,2-DMP	23.57	57.62			
1,2,5-TMN	69.02	87.63						
1,4,5-TMN	71.50	88.76						

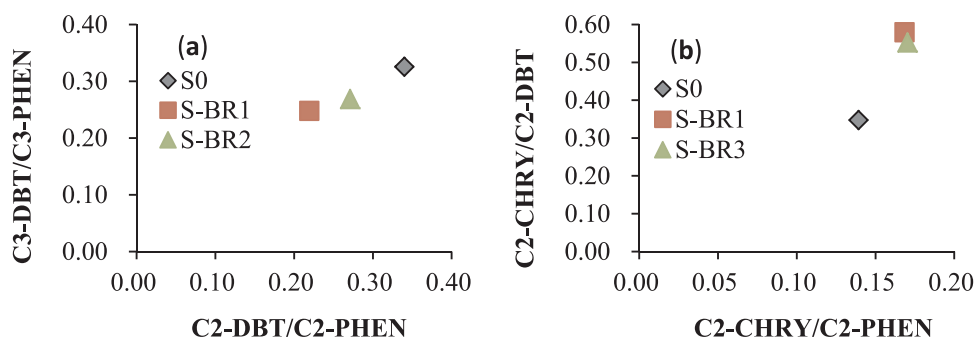


Fig. 6. Cross plot of C₂-DBT/C₂-PHEN, C₃-DBT/C₃-PHEN ratios (a) and C₂-CHRY/C₂-PHEN, C₂-CHRY/C₂-DBT (b).

and sedimentary organic matter (Hegazi et al., 2004). Shielded molecules, 4-alkyl dibenzothiophene and 6-alkyl dibenzothiophene isomers, are relatively difficult to form hydrogen bonds (Xu et al., 2012). The semi-shielded or shielded molecules (4-MDBT and 4,6-DMDBT) increased relatively, leading to an increase in the value of 4-/1-DBT and 4,6-/1,4-DMDBT. The methyl dibenzothiophene ratios (4-MDBT/1-MDBT and 2 + 3-MDBT/1-MDBT), which often used to evaluate the maturity of source rock and crude oils (Alexander et al., 1985; Chakhmakchev et al., 1997), gradually decrease with the increase of the biodegradation degree for biodegraded oils. The reason is that 4-methyl, 2 + 3-methyl dibenzothiophene isomers with higher thermally stability, can be easily destroyed by bacteria than thermally less-stable 1-methyl dibenzothiophene during biodegradation process. For example, the degree of decrease in concentration of 4-methyl, 2 + 3-methyl dibenzothiophene isomers is up to 79% and 86%, respectively, only about 75% for 1-methyl dibenzothiophene isomer. Interestingly, our study shows that the thermally most-stable isomers of the studied PAHs are generally more susceptible to biodegradation than thermally less-stable ones. It suggested that biodegradation and selective depletion of PAHs is not only controlled by thermodynamics but also related to the stereochemical structure of individual compounds. The thermally more-stable isomers are more susceptible to biodegradation than the isomers with lower thermally stability, which suggested that biodegradation processes are different from purely thermodynamic processes.

3.4.4. Other PAHs and their isomers

Some PAHs, such as certain ANTHs and DBTs, FLA and PYR are degraded at about the same rate during weathering process. In several studies, the relative abundance of the PAH alkyl homologue ratio, such as C₂-DBT/C₂-PHEN, C₃-DBT/C₃-PHEN, C₂-CHRY/C₂-PHEN, C₂-CHRY/C₂-DBT, FLA/(FLA + PYR), were used as diagnostic source parameters (Yunker et al., 2002; Wang and Fingas, 2003). The slight shift of such ratios for the extremely degraded samples may be due to the slightly different stability of these compounds. The slight increase of FLA/(FLA + PYR) from 0.75 to 0.73 may contribute to the similar stereochemical structure. In addition, the higher C₂-CHRY/C₂-DBT and C₂-CHRY/C₂-PHEN ratio in the biodegraded oily sediment of this study might be the result of higher environmental stability of chrysene than dibenzothiophenes and higher water solubility of phenanthrene than dibenzothiophene (Bence et al., 1996). The thermally most-stable isomers of PAHs are generally easily biodegraded than thermally less-stable ones. However, C₂-DBT/C₂-PHEN, C₃-DBT/C₃-PHEN ratios display a sharp decrease, followed by a slight increase (Fig. 6). As mentioned above, the results also suggested that the biodegradation is controlled by both thermodynamics and the stereochemical structure of individual compounds.

4. Conclusions

In this study, an efficient bacterial consortium, isolated from petroleum pollution area by *Penglai 19-3* accident, to biodegrade crude oil

spilled in China's Bohai Sea was constructed. The results suggested that crude oil, especially alkanes, driven by indigenous bacteria enriched in sediments from the Bohai Sea, was efficiently biodegraded. The relative degradation values of TPH are 43.56% and 51.29% for sediments with untreated microcosms (S-BR1) and surfactant-treated microcosms (S-BR2), respectively. The relative fractions of saturated hydrocarbons and aromatic hydrocarbons decreased from 26.10% to 23.46% and from 45.30% to 25.60% in the untreated microcosms and surfactant-treated microcosms, respectively. The saturated hydrocarbons fraction showed the fastest degradation among all fractions. There is an obvious decrease in saturates (biodegradation rate: 67.85–77.29%) and a slight decrease in aromatics (biodegradation rate: 47.13–57.21%), while no significant difference of resins and asphaltenes was detected. Furthermore, the relative compositions of various aromatic compounds will change with increasing biodegradation degree. On the whole, the degradation rate of DMP, TMP, CH, MCH, DMCH, DMF, TMDBT, Ret, BaA, Fla, Pyr for S-BR1 and S-BR2 reaches 1.28–84.43% and 42.56–86.67%, respectively. The efficiency of crude oil degradation in sediment with surfactant-treated microcosms cultures added Tween 20, was higher than that in sediment with untreated microcosms. The biodegradation and selective depletion is controlled by both thermodynamics and the stereochemical structure of individual compounds. This study could be helpful in developing strategies for bioremediation of crude oil dispersed in the marine ecosystem.

Acknowledgements

This study was co-supported by International cooperation, CAS, Chinese-foreign cooperation in key projects (133337KYSB20160002), National Natural Science Foundation of China (Grant no. 40806048) and Project of on-site sediment microbial remediation of public area of central Bohai Sea, North China Sea Branch of State Oceanic Administration (QDZC20150420-002).

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