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Evaluation of the diagnostic ratios for the identification of spilled oils after biodegradation

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Abstract Biodegradation, one of the most important weathering processes, alters the composition of spilled oil, making it difficult to identify the source of the release and to monitor its fate in the environment. A laboratory experiment was conducted to simulate oil spill weathering process of microbial degradation to investigate compositional changes in a range of source- and weathering-dependent molecular parameters in oil residues, and the conventional diagnostic ratios for oil spill identification were also evaluated. The conventional diagnostic ratios of n-alkane displayed obvious changes after biodegradation, especially for Pr/n-C$_{17}$ and Ph/n-C$_{18}$ with relative standard deviation more than 118.84 %, which suggests they are invalid for oil source identification of the middle-serious spill. Many polycyclic aromatic hydrocarbons (PAHs) are more resistant to biodegradation process than their saturated hydrocarbon counterparts, thus making PAHs to be one of the most valuable fingerprinting classes of hydrocarbons for oil identification. Biomarker ratios of hopanes and steranes were also useful for source identification even after moderate biodegradation, and the diagnostic ratios from them could be used in tracking origin and sources of hydrocarbon pollution. Finally, the carbon isotopic type curve may provide another diagnostic means for correlation and differentiation of spilled oils, and be particularly valuable for lighter refined products or severely biodegraded oils, the source of which may be difficult to identify by routine biomarker techniques.

Introduction

With the rapid development of ocean oil exploration and oil transportation, more and more oil spill accidents have taken place. Oil spills cause extensive damage to marine and terrestrial ecosystems, human health, and natural resources (Wang and Fingas 2003). Therefore, characterization of chemical compositions and identification of oil spill sources are, in many cases, critical for providing forensic evidence in the investigation of oil spill accidents and settling disputes related to liability (Wang et al. 2001, 2003). After oil spills into the marine environment, it is subjected to a variety of weathering processes (Fig. 1). These weathering processes mainly include: evaporation, dissolution, microbial degradation, photooxidation, dispersion, water–oil emulsification, hydrolysis, adsorption onto suspended particulate materials, and oil–mineral aggregation (Boehm et al. 1982; Wang et al. 1997; Garrett et al. 1998; Garcia de Oteyza and Grimalt 2006; D'Auria et al. 2009). Biodegradation of hydrocarbon by natural pollutions of microorganisms is general a long-term weathering process and represents the primary mechanisms

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Fig. 1 Processes and pathways by which spilled oil may enter marine ecosystem. Modified after Kingston (2002)
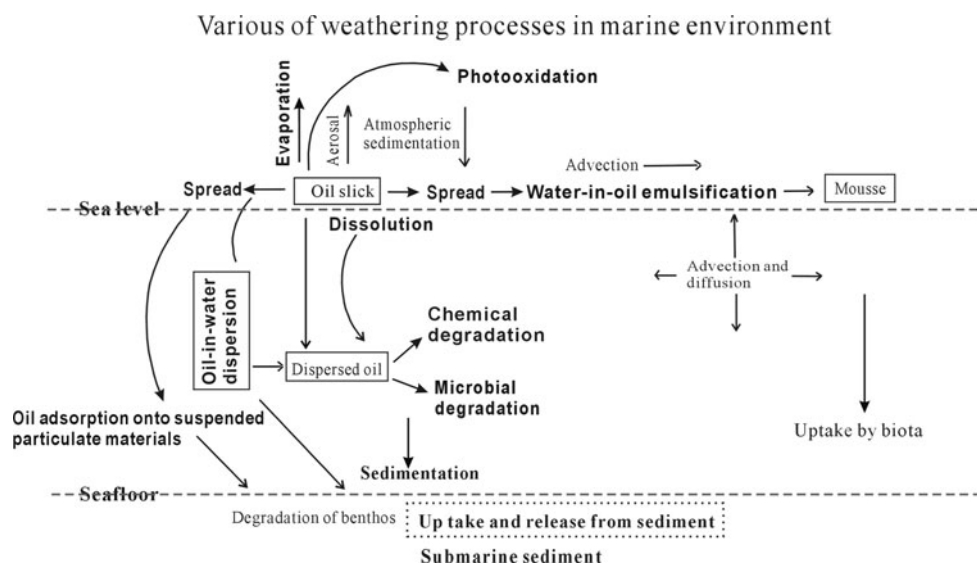
by which petroleum is eliminated from the marine environment. Thus, biodegradation can be one of the most important weathering processes in the sea environment (D'Auria et al. 2009). The biodegradation of petroleum and other hydrocarbons in the marine environment is a complex process, whose quantitative and qualitative aspects depend on the type, nature and amount of the oil or hydrocarbon present, the ambient and seasonal environmental conditions (such as temperature, oxygen, nutrients, water activity, salinity, and pH), and the composition of the autochthonous microbial community (Wang and Fingas 2003).

The molecular fingerprints and parameters used to correlate oil with its source based on the gas chromatography (GC) and gas chromatography mass spectrometry (GC–MS) analysis are strongly modify by biodegradation (Prince et al. 2002, 2003; Wang and Fingas 2003). The effects on the molecular composition of petroleum are reasonably well established (see a review by Peters et al. 2005 and references therein). Several in vitro and in situ studies have shown that hopane and sterane compounds are very resistant to biodegradation. However, it should be noted that for severely weathered or long-term weathered oil, degradation of some biomarkers was observed. Munoz et al. (1997) found that isoprenoids were severely degraded and biomarkers were more or less altered 8 years after an oil spill in a peaty mangrove in a tropical ecosystem. In addition, it has been demonstrated that in some cases even the efficient diagnostic ratios for oil spills identification, such as bulk carbon isotopic ratios of spilled oil fractions, can be affected by biodegradation (Mansuy et al. 1997; Xu et al. 2001). Therefore, it is very important to understand the relationship between the biodegradation processes and the composition changes of oils. Up to now, the effects of biodegradation on oils have been thoroughly documented (Bost et al. 2001; Prince et al. 2002, 2003; Béhar et al.

2006; Bao and Zhu 2008). However, little work has been done about the evaluation of conventional diagnostic ratio for such representative biomarkers in the biodegraded oils for oil spill identification. Although carbon isotopic type curve was used in petroleum exploration in recent years (Duan et al. 2003; Li and Guo 2010), there have been few reports of use it for forensic oil spill investigations.

In this paper, a laboratory experiment was conducted to simulate oil spill weathering process of microbial degradation. In order to evaluate the conventional diagnostic ratio for the biodegraded oils, the distribution of n-alkanes, polycyclic aromatic hydrocarbons (PAHs) and biomarkers of terpane and sterane in the initial crude oil and residual oils are quantitatively determined during the course of the microbial degradation process to reveal the effect of the biodegradation on the chemical composition of spilled oils at the molecular level. The study focuses on two main aspects of the problem: (1) evaluation of possibilities and limitations of distribution of n-alkanes, PAHs and biomarkers of terpane and sterane as a correlation tool in environmental problems; (2) determination of the effects of biodegradation processes on the carbon isotopic type curve, and whether such curve could compromise the correlation of a weathered oil to its non-degraded counterpart.

Experimental

Sample

Different samples from various origins were analyzed in this study (Table 1). Two crude oils (SL-1, SL-2) collected from Shengli oilfield, the second-largest oilfield in China, were used for artificial biodegradation experiment. In addition, other crude oil samples from Liaohe basin (LH-1,

Table 1 Origin of oil samples used in this study and their respective API degree

Sample no.	Location	Well name	API[a] (°)	Type
LH-1	Liaohe basin	Ou-26	22.3	Normal oil
LH-2		Ou-27	15.6	Heavy oil
TH	Tahe oilfield	TK230	31.3	Normal oil
SLB	Songlin basin	S23	36.8	Normal oil
SL-1	Gudong oilfield	S12-13	27.3	Normal oil
SL-2		S12-5	35.3	Normal oil

[a] API = 141.5/specific gravity − 131.5

LH-2), Tahe oilfield in Tarim basin (TH) and Songliao basin (SLB) were examined for comparisons of carbon isotopic characteristic.

Culture enrichment and growth on crude oil under aerobic conditions

The enrichment culture technique was used for culture isolation (Mukherji et al. 2004), using crude oil as the sole source of carbon and energy. Some water samples obtained near an oil well from Shengli oilfield was used for culture isolation. The oilfield waters from Shengli oilfield was added in enrichment medium, and the enrichment medium was put in the incubator at 50 °C for 30 min. Refer to the natural living conditions of bacteria, aerobic bacteria and low inorganic salt concentration of aqueous medium were chosen in this study. The mineral media composition was as specified (concentration in mg/l): $(NH_4)_2PO_4$ (1,000), KH_2PO_4 (500), Na_2HPO_4 (75), $MgSO_4·7H_2O$ (200) and $CaCl_2$ (20). Culture growth and oil biodegradation was studied in 500-ml screw cap flasks each containing 120 ml mineral media, 1 ml crude oil, and 6 ml inoculum, incubated at 120 rpm and 37 °C. In trial experiment, the rate and extent of oil biodegradation was studied under different conditions. We found 14 days is proper time for such simulation experiment. After determining the growth profile, the overall loss of oil after 14 days of incubation was quantified by extracting the cultures. According to our trial experiment, the acclimatized cultures could tolerate salinity up to 3.5 % NaCl. Since the typical salinity in marine environments is 3–3.5 %, the cultures with 3–3.5 % NaCl can be used for in situ oil bioremediation in marine environments. In their study, culture growth and utilization of hydrocarbons from crude oil could occur at salinities of 3.2 %.

Extraction and fractionation

The crude oil and biodegraded oil were deasphalted by precipitation with n-hexane followed by filtration. The deasphalted oil was fractionated by column chromatography on alumina over silica gel. Saturated hydrocarbons, aromatic hydrocarbons and non-hydrocarbons were obtained by successively eluting with n-hexane, toluene and chloroform/methanol (v/v, 98:2), respectively.

GC–MS analyses and isotopic composition

Saturated hydrocarbons and aromatic hydrocarbon were analyzed with a 6890N GC/5973N mass spectrometer equipped with a HP-5 capillary column (50 m × 0.32 mm × 0.25 μm, Agilent Technologies, USA). The GC oven temperature was programmed from 80 to 300 °C at 4 °C min^{-1}, held during 30 min at this temperature. Helium was used as a carrier gas. Mass spectrometer conditions were electron ionization at 70 eV with an ion source temperature at 250 °C. The deuterated surrogate standards that were initially spiked into each sample were treated as internal standards, where each surrogate compound was associated with a group of calibrated alkanes. Individual n-alkanes were identified based on the retention time of the standards (nC_{10-40}, Sigma), and concentrations of each n-alkane were calculated based on the standard calibration curve of each corresponding standard compound. Biomarker ratios were calculated using peak areas from the m/z 191 and m/z 217 chromatograms. For quantitative analysis, the response factor for the surrogate standard was calculated by dividing the surrogate concentration by the respective peak area. On the other hand, individual PAHs were quantified based on the retention time and m/z ratio of an authentic PAHs mixed standard (Sigma), and concentrations of each PAHs were calibrated based on the standard calibration curve.

Stable carbon isotope compositions of the crude oil samples (LH-1, LH-2, TH, SLB, SL-1, SL-1-B) were determined after combustion of the samples in quartz tubes. The tubes were opened in a vacuum line ($2 × 10^{-3}$ torr) and the water was removed by trapping at −70 °C. The produced CO_2 was collected in a sampling ampoule at −196 °C and transferred directly to the inlet system of a Finnigan MAT 252 mass spectrometer for carbon isotope ratio determination. Carbon isotope values are expressed

relative to the international standard 'Vienna Pee Dee Belemnite' (VPDB; $^{13}C/^{12}C = 0.01118$).

Results and discussion

Crude oils are typically composed of many thousands of individual compounds. Nevertheless, the compounds we will discuss in this paper include only saturated hydrocarbon, which are the most abundant and most biodegradable.

Distributions of alkanes

The initial samples in simulation experiment have pristane/phytane (Pr/Ph) ratios of less than 1.0, and contain abundant C_{27} steranes relative to C_{29}, reflecting that they are derived from mixed terrigenous and algal–bacterial organic matter with dominant algal organic matter formed under a weakly reducing environment (Duan et al. 2008; George 2008). The high sterane and hopane isomer ratios, slightly higher than 1.0 of n-alkane carbon preference index (CPI) values (Table 2), indicate that the oil samples are mature (Duan et al. 2008; George 2008).

Although alkanes are not particularly useful for determining the sources of the spill, they can give some information on the degree of weathering or freshness of the samples, which is indicated by the distributions of n-alkanes, isoprenoid alkanes and by the total concentrations of the resolved peaks. The m/z 85 chromatograms of the saturated hydrocarbon fractions of the two crude oil samples, as well as those residues from the corresponding crude oils via biodegradation, are illustrated in Fig. 2. It is apparent that there are significant differences in the distribution of these fractions, with respect to the n-alkanes, pristane and phytane, in the initial oils and biodegraded

oils. The distribution of alkane for the initial oils (SL-1, SL-2) contains n-alkane ranging from n-C_{13} to n-C_{34}, with unimodal distribution and n-C_{20} as the main peak (Fig. 2a, c). Low molecular weight n-alkanes ($<n$-C_{13}) are still seen clearly within original oil sample SL-1 and SL-2 (Fig. 2) during the oil course within the petroleum system, indicating that this sample has only reached the "slight" level of biodegradation. Nevertheless, the residue (SL-1-BD, SL-2-BD) are characterized by predominance of n-C_{19}–n-C_{34} with phytane as the main peak (Fig. 2b, d). According to the scale reported by Peters and Moldowan (1993), almost complete loss of low molecular weight n-alkanes ($<n$-C_{17}) and slight degradation of steranes for the studied oils suggest the two samples were moderately degraded.

As shown in Table 2, the initial oils exhibit a substantially higher value of the L/H-alkanes ratio (0.96, 1.56) compared to that of the biodegraded oils (0.63, 1.01). Based on this observation, the low molecular weight alkanes (LMW alkanes) (n-C_{12}–n-C_{21}) may have undergone microbial degradation compared to the high molecular weight alkanes (HMW alkanes) (n-C_{22}–n-C_{34}). Similar distributions were also reported for the tar-balls collected from the East Coast of Peninsular Malaysia (Chandru et al. 2008). It is probably because that the biodegradation usually favors the short chained or LMW alkanes compared to longer chained ones. Moreover, the CPI does not vary significantly and increase from 1.12 to 1.18 and decrease from 1.28 to 1.15, respectively (Table 2). Normally, the n-alkanes would be degraded prior to significant alteration of other compound classes, resulting in higher Pr/n-C_{17} and Ph/n-C_{18} ratios for slightly or moderately biodegraded oils than their non-biodegraded counterparts. Although the Pr/n-C_{17} and Ph/n-C_{18} ratios increase with biodegradation (Table 2), pristane and phytane appear to be partially degraded in residue oils. Furthermore, the Pr/Ph ratio,

Table 2 Variability of some common indices in alkane and isoprenoid among biodegraded oil and its source oil

Sample no.	Range	Main peak	CPI[a]	Pr/Ph	Pr/n-C_{17}[b]	Ph/n-C_{18}[c]	L/H[d]
SL-1	n-C_{12}–n-C_{37}	n-C_{20}	1.12	0.71	0.89	0.97	0.96
SL-1-BD	n-C_{17}–n-C_{38}	Ph	1.18	0.66	61.0	46.0	0.63
%RSD[e]	–	–	3.80	5.26	137.34	135.57	29.41
SL-2	n-C_{12}–n-C_{38}	n-C_{20}	1.28	0.92	0.40	0.44	1.56
SL-2-BD	n-C_{12}–n-C_{38}	Ph	1.15	0.89	7.38	5.08	1.01
%RSD	–	–	7.57	2.34	126.88	118.84	30.27

[a] $1/2[(C_{25} + C_{27} + C_{29} + C_{31} + C_{33})/(C_{24} + C_{26} + C_{28} + C_{30} + C_{32}) + (C_{25} + C_{27} + C_{29} + C_{31} + C_{33})/(C_{26} + C_{28} + C_{30} + C_{32} + C_{34})]$

[b] The ratios of pristane/n-C_{17}

[c] The ratios of pristane/n-C_{18}

[d] n-C_{14} to n-C_{21} alkanes relative to the sum of n-C_{22} to n-C_{34} alkanes

[e] Relative standard deviation

Fig. 2 GC–MS chromatograms (*m/z* 85) of crude oil after biodegradation and the initial oil. SL-1-BD: the oil after biodegradation for SL-1; SL-2-BD: the oil after biodegradation for SL-2
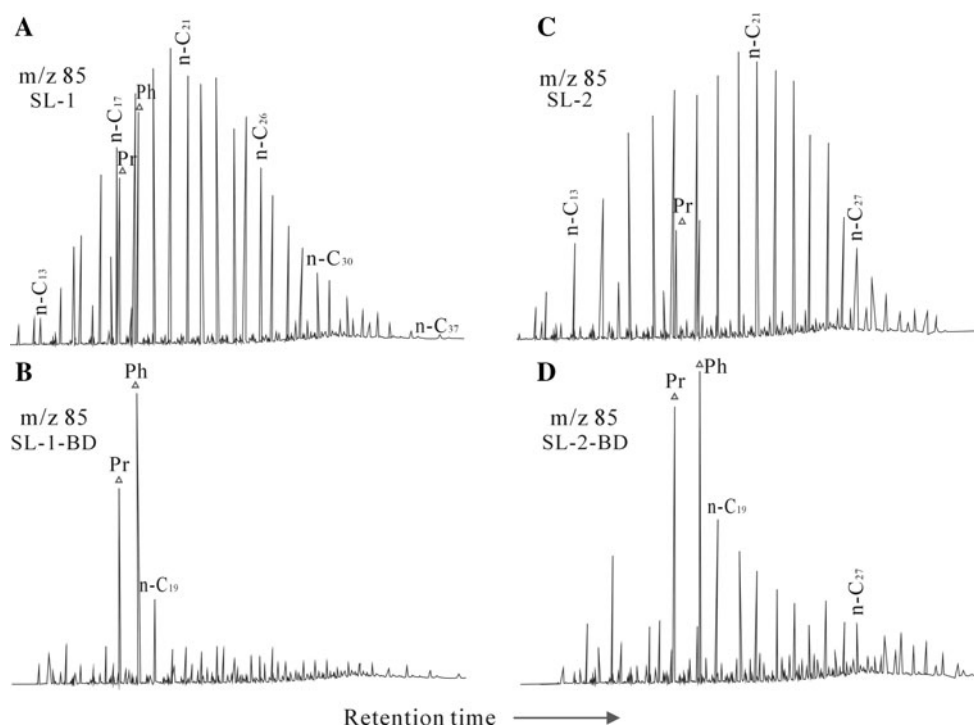
widely invoked as an indicator of the redox condition in the depositional environment, also decreases from 0.71 to 0.66 and 0.92 to 0.89, respectively. In agreement with the interpretation of Bao et al. (2002), we believe that such change in Pr/Ph is likely due to the more readily biodegradation of pristane than phytane. On the basis of these results, it is also concluded that the branched alkanes and acyclic isoprenoids all showed a low susceptibility to degradation while the most sensible fractions were those of the linear alkanes.

Compositions of alkanes and PAHs have been previously used to evaluate the weathering of tar-balls and oil spill samples (Barakat et al. 2001; Hegazi et al. 2004). Parameters generated from *n*-alkane and isoprenoid distributions have a tendency to change under the influence of biodegradation. Based on the evaluation method of indices suggested by Stout et al. (2001) and Li et al. (2009), relative standard deviation (%RSD) is considered as an indicator to evaluate the variability of diagnostic indices in this experiment. The indices with %RSD <5 % are probably not affected by weathering, while a %RSD more than 5 % suggests that weathering has a remarkable effect on the index. The data in Table 2 and Fig. 3a demonstrate that the ratios of CPI, Pr/Ph, and L/H alkanes all display obvious changes over weathering time, especially for Pr/n-C$_{17}$, Ph/n-C$_{18}$ with a %RSD of more than 118.84, indicating that these ratios are not valid for oil source identification of the spill moderately degraded.

Terpanes and steranes

Terpanes and steranes is another group of biomarker compounds that are commonly used in oil correlation, source identification, and maturity determination. The distributions of steranes and hopanes in the biodegraded oil did not change significantly and were similar to the initial crude oil. For example, the distribution profiles of terpanes in such oils are all dominated by hopanes comprised of C$_{27}$–C$_{35}$ members, with the 17α, 21β(H)-hopane isomers being the major compounds (Fig. 4a). In addition, the initial oils contain C$_{27}$–C$_{30}$ steranes dominated by 5α(H),14α(H),17α(H) components, with the 20R isomers more abundant than the 20S isomers (Fig. 4b).

In this paper, five traditional maturity indicators for the biomarkers, widely used in petroleum exploration, are considered (Table 3). As expected, all indices of terpane and sterane biomarkers have lower standard deviations (SDs) and %RSD (<5 %) (Table 3; Fig. 3b), displaying little change after biodegradation. The results are also similar to those of previous study, which showed that C$_{30}$ 17α, 21β(H)-hopane and its extended homologs (homohopanes) are biodegraded in the environment and laboratory (Peters and Moldowan 1991; Moldowan et al. 1995). In general, terpane and sterane biomarkers are more resistant to degradation than *n*-alkanes, isoprenoids (Barakat et al. 2002). This phenomenon was also observed for biomarker maturation of the crude oils from Liaohe basin (Bao and

Fig. 3 %RSD of parameters of saturated hydrocarbon in biodegraded crude oils. C_{31}-A: $C_{31}22S(22S + 22R)$; C_{29}-B: $C_{29}20S/(20S + 20R)$; C_{29}-C: $C_{29}\beta\beta/(\beta\beta + \alpha\alpha)$; *Dotted line* refer to the %RSD <5 %
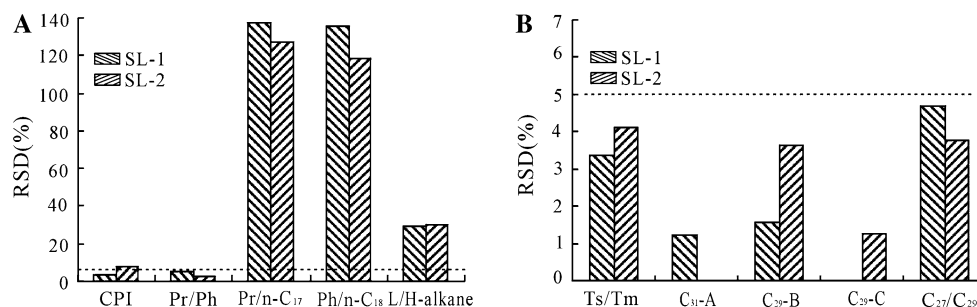

Fig. 4 Mass chromatograms showing the distribution of pentacyclic triterpane (*m/z* 191) and steranes (*m/z* 217) of the representative sample
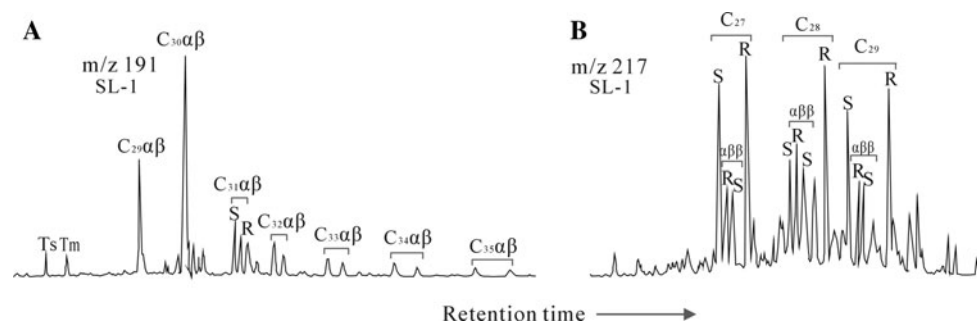

Table 3 Variability of parameters in pentacyclic terpanes (*m/z* 191) and sterane (*m/z* 217) among biodegraded oils and their source oils

Sample no.	Ts/Tm[a]	$C_{31}22S(22S + 22R)$[b]	$C_{29}20S/(20S + 20R)$[c]	$C_{29}\beta\beta/(\beta\beta + \alpha\alpha)$[d]	C_{27}/C_{29}[e]
SL-1	1.08	0.57	0.46	0.37	0.78
SL-1-BD	1.03	0.58	0.45	0.37	0.73
%RSD	3.35	1.23	1.55	0.00	4.68
SL-2	0.94	0.59	0.40	0.57	0.96
SL-2-BD	0.88	0.59	0.38	0.57	0.91
%RSD	4.12	0.00	3.63	1.25	3.78

[a] Ratio of 18α-22,29,30-trisnorhopane relative to 17α-22,29,30-trisnorhopane

[b] 22S(22S + 22R) for C_{31}-17α,21β(H)-homohopane

[c] Ratios of 20S/(20S + 20R) for C_{29}-5α(H),14α(H),17α(H)-steranes

[d] 5α(H),14β(H),17β(H)/[5α(H),14β(H),17β(H) + 5α(H),14α(H),17α(H)] for C_{29} steranes

[e] C_{27}/C_{29} 5α(H),14α(H),17α(H)-20R-steranes

Zhu 2008). Based on the data, it is suggested that the fingerprint patterns of triterpanes and steranes showed no change after biodegradation, despite extensive saturate hydrocarbon losses, and the ratios of selected paired biomarkers also remained constant.

Although both 18a(H)-22,29,30-trisnorneohopane (Ts) and 17a(H)-22,29,30-trisnorhopane (Tm) were degraded in oil sample, a slight increase in the Ts/Tm ratio was observed (Table 2), implying that Tm is more readily biodegraded than Ts. These findings were consistent with the studies conducted by Barakat et al. (2001) and Bost et al. (2001). As shown in Table 3, selective biodegradation of the steranes has occurred. Preferential removal of

the C_{27} homologue and ααα 20R isomer results in a slightly decrease in the C_{27}/C_{29}. In general, the reservoir oil samples show C_{31}–C_{35} to be more susceptible to biodegradation than C_{30}, with preferential degradation of the 22R isomer over 22S isomer in the C_{31}–C_{34} 17α(H),21β(H) series (Bost et al. 2001). Moreover, the biodegradation can also alter regular sterane distributions according in the order: 5α(H),14α(H),17α(H) 20R > 5α(H),14β(H),17β(H) 20R > 5α(H),14α(H),17α(H)20S > 5α(H),14β(H),17β(H)   20S, with $C_{27} > C_{28} > C_{29}$ (Pieri et al. 1996). However, the preferential degradation of such isomer in this study is very weak, which may be probably due to the shorter experimental time.

Table 4 Variability of parameters in PAHs among biodegraded oils and their source oils

Sample no.	C_2D/C_2P^a	C_3D/C_3P^b	$\sum P/\sum D^c$	4-MD/1-MD[d]	C_2C/C_2P^e
SL-1	0.16	0.21	0.92	3.45	0.16
SL-1-BD	0.17	0.20	0.89	3.50	0.18
%RSD	4.29	3.45	2.34	1.02	5.24
SL-2	0.24	0.32	0.58	2.90	0.23
SL-2-BD	0.23	0.35	0.57	2.92	0.22
%RSD	3.01	6.33	1.23	0.49	3.14

[a] C_2-dibenzothiophenes/C_2-phenanthrenes

[b] C_3-dibenzothiophenes/C_3-phenanthrenes

[c] Σphenanthrenes/Σdibenzothiophenes

[d] 4-methyldibenzothiophene/1-methyldibenzothiophene

[e] C_2-chrysenes/C_2-phenanthrenes

As mentioned above, terpanes and steranes retained their molecular compositions during the weathering process of moderate biodegradation and could be used in tracking the origin and sources of hydrocarbon pollution in the marine environment. However, it should be noted that in severely weathered or long-term weathered oil, biodegradation of some sterane and terpane biomarkers was also observed (Wang et al. 2001, 2003).

Distributions of PAHs

PAHs are relatively stable and diagnostic constituents of petroleum and provide more detailed compound-specific data that can be used to identify source (Douglas et al. 1996). The PAHs consist primarily of five families of compounds: naphthalenes, fluorenes, phenanthrenes, dibenzothiophenes, and chrysenes, each consisting of an un-substituted or parent compound and a series of alkylated compounds. Generally, the degree of polycyclic aromatic hydrocarbon degradation decreases with increasing ring size; and within a homologous series, decreases with increasing alkylation (Barakat et al. 2002; Wang and Fingas 2003). Major compositional changes of PAHs observed in this paper are consistent with previous studies (Wang and Fingas 2003; Asif et al. 2009). Biodegradation susceptibility of PAHs is dependent on the number of aromatic rings and the location of the alkyl substituents. Generally, the susceptibility to biodegradation decreases with an increase in the number of aromatic rings and in the number of alkyl substituents on the aromatic moieties. For alkyl naphthalenes and alkyl phenanthrenes the thermodynamically more stable isomers are generally more susceptible.

In previous works (e.g. Barakat et al. 2002; Wang and Fingas 2003), some PAHs ratios have been used as weathering indicators of varying sensitivity for different stages in the weathering process. Table 4 shows that the 4-methyldibenzothiophene/1-methyldibenzothiophene (4-/1-m-DBT), C_2-chrysenes/C_2-phenanthrenes (C_2-C/C_2-P),

C_2-dibenzothiophenes/C_2-phenanthrenes (C_2-D/C_2-P), C_3-dibenzothiophenes/C_3-phenanthrenes (C_3-D/C_3-P), and $\sum$phenanthrenes/$\sum$dibenzothiophenes ($\sum$P/$\sum$D) ratios in the spilled oil were essentially the same as that of the reference oil. In agreement with the interpretation of previous studies (Wang and Fingas 2003), we believe that all the isomers of, for example, the methyl dibenzothiophenes, phenanthrenes, chrysenes, are lost at more or less the same rate for lightly or moderately biodegraded oils in such little experimental time of this study neglecting the simulation experimental conditions.
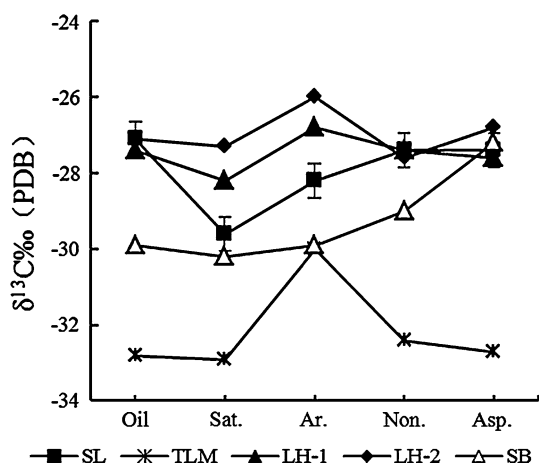
Carbon isotopic type curve

The δ^{13}C values of petroleum components, including whole crude oils, their fractions, and individual compounds, were usually ascribed to differences in organic matter sources, maturity levels, depositional environments, and the input of mixed sources (Murray et al. 1994; Xu et al. 2001; Duan et al. 2003). There are significant differences in the bulk isotopic composition of crude oils from different locations (Table 5). The averaged RSD calculated for oils are higher than 2.50 %, which is far out of the range of the analytical error. Furthermore, the SDs and %RSD between the isotopic values of the biodegraded and unbiodegraded oils are 0.42 and 1.55 ‰, respectively, remaining within the range of reproducibility.

The bulk carbon isotopic ratios of spilled oil samples can also be affected by weathering processes in certain situations. Moreover, the gas chromatography–isotope ratio mass spectrometry (GC–IRMS) technique still suffers from a number of problems and limitations related to the correlation of moderately to severely biodegraded oils, despite the increased specificity. The approach is most effective when applied in the presence of *n*-alkanes and a reduced background. However, the lack of *n*-alkanes in the severely biodegraded oil prevented the GC–IRMS analysis.

Table 5 Carbon isotopic compositions of studied oils

Sample no.	Type	Crude oil	$\delta^{13}C$ (‰)			
			Sat.	Aro.	Non.	Asp.
LH-1	Normal oil	−27.4	−28.2	−26.8	−27.4	−27.6
LH-2	Heavy oil	−27.1	−27.3	−26	−27.6	−26.8
TH	Normal oil	−32.8	−32.9	−30	−32.4	−32.7
SLB	Normal oil	−29.9	−30.2	−29.9	−29.0	−27.2
SL-1	Normal oil	−27.1	−29.6	−28.2	−27.4	−27.4
SL-1-B	Normal oil	−27.7	−30.4	−28.3	−27.3	−27.2
%RSD		1.548409	1.885618	0.250303	0.25854	0.5180269

Fig. 5 Comparison of carbon isotopic type curves of oils. *Bars* indicate standard errors

With the increase of polarity, the type curve drawn according to the $\delta^{13}C$ values of saturated hydrocarbons, aromatics, non-hydrocarbons and asphaltenes, is often employed in the oil–oil and oil–source correlations (Shen 1995). Crude oils of the same source usually possess the similar type curves because of the isotopic inherited effect of parent materials (Xu et al. 2001). When $\delta^{13}C$ was plotted against fraction number, the slope of the $\delta^{13}C$-fraction line varied in oils from different source environments. An alternative method based on the carbon isotopic characteristics of group components should be effective for the characterization of such extensive biodegraded oil samples. As can be seen in Fig. 5, each oil sample has its own characteristic isotopic fingerprint. The crude oil from Songliao basin has a normal type curve of $\delta^{13}C$ values. In contrast, the type curve of $\delta^{13}C$ values for the sample from Shengli oilfield has a reversed trend. Moreover, the samples from Liaohe basin, whose carbon isotopic compositions of saturated hydrocarbon are heavier than aromatics and similar to those of the non-hydrocarbons and asphaltenes, do not show a normal type curve of $\delta^{13}C$ values. Furthermore, it is clear that the crude oils from Liaohe

basin and Shengli oilfield have ^{13}C-enriched values compared to the oils from Tarim basin, in which the algal/bacterial matter input is predominant. The heavier isotope composition of these samples is due to a major input of terrigenous organic matter. In summary, the shape of the isotopic profile for LH-1 and LH-2 sharing the same organic matter inputs appears to be very similar, which is different from that of TH and SLB samples.

The second step was to determine if biodegradation processes can compromise correlations between pollutants and suspected sources. The artificially biodegraded samples were analyzed by Finnigan MAT 252 mass spectrometer, and the results are compared to those of the initial oil. The biggest fractionation of carbon isotopes generally exists between saturated hydrocarbons and asphaltenes for a normal crude oil; if the carbon isotopic compositions are relatively lighter, this fractionation is often greater than 2 ‰ (Xu et al. 2001). The SL-1 sample follows the similar trends after biodegradation, with SDs of 0.56 ‰ for saturated hydrocarbons and 0.14 ‰ for asphaltenes. The SDs the isotopic values of the biodegraded and initial oils remain within the range of reproducibility. In conclusion, no significant carbon isotopic fractionation is observed during the course of this bacterial biodegradation experiment. In certain cases, particularly for the lighter refined products or severely biodegraded oils, the carbon isotopic type curve may provide valuable correlation data in the absence of biomarker data.

Conclusions

Biodegradation can be one of the most important processes in the environment, which can strongly modify the fingerprints and parameters for spilled oil identification. In summary, under aerobic conditions the *n*-alkanes are the most readily degraded hydrocarbons, and the biodegradation of polycyclic aromatic hydrocarbons decreases with increasing size and alkylation. The above discussion also reveals that: the ratios of CPI, Pr/Ph, and L/H alkanes are

not valid for oil source identification of the middle-serious spill after biodegradation. In contrast, terpanes, steranes and PAHs retained their molecular compositions after moderate biodegradation and the diagnostic ratios from them could be efficiently used in oil spill identification. Moreover, the SDs between the isotopic values of the biodegraded and initial oils remain within the range of reproducibility, which means that the carbon isotopic type curve may provide a valuable way for oil spill identification in the absence of biomarker data particularly with lighter refined products or severely biodegraded oils.

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