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Study of weathering effects on the distribution of aromatic steroid hydrocarbons in crude oils and oil residues

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The composition and distribution of triaromatic steroid hydrocarbons in oil residues after biodegradation and photo-oxidation processes were detected, and the diagnostic ratios for oil spill identification were developed and evaluated based on the relative standard deviation (RSD) and the repeatability limit. The preferential loss of C_{27} methyl triaromatic steranes (MTAS) relative to C_{28} MTAS and C_{29} MTAS was shown during the photo-oxidation process. In contrast to the photochemical degradation, the MTAS with the original 20*R* biological configuration was preferentially degraded during the biodegradation process. The RSD of most of the diagnostic ratios of MTAS ranged from 9 to 84% during the photo-oxidation process. However, the RSDs of such ratios derived from MTAS were all <5% even in high biodegradation, and such parameters may also provide new methods on oil spill identification. The parameters of monoaromatic sterane and monoaromatic sterane are not used well for oil spill identification after photo-oxidation. The triaromatic steroid hydrocarbons retained their molecular compositions after biodegradation and photo-oxidation and most of the diagnostic ratios derived from them could be efficiently used in oil spill identification.

Environmental impact

The identification of oil spill sources is, in many cases, critical for providing forensic evidence in the investigation of oil spill accidents and settling disputes related to liability. The "multicriteria approach" for spill source identification is necessary. Besides the biological action, sunlight irradiation is another effect which alters the physicochemical properties of crude oil in the natural environment. In this paper, weathering effects on aromatic steroid hydrocarbon distribution in crude oils and oil residues derived from China were quantitatively studied and a number of diagnostic indices are developed and evaluated for oil correlation and differentiation.

1. Introduction

Oil spills refer to petroleum hydrocarbons naturally or accidentally released to the environment and can usually lead to long-term negative impacts. Annual worldwide estimates of petroleum input to the sea exceed 1 300 000 Mt.[1] During the investigation of oil spill accidents and settling disputes related to liability, characterization of chemical compositions and identification of oil spill sources are, in many cases, critical for providing forensic evidence.[2] The most common approach to characterize spilled oil and identify its potential sources relies on analyses by gas chromatography (GC) and GC-mass spectrometry (GC-MS). Consequently correlations can be quantified on the basis of molecular distribution of aliphatic and aromatic hydrocarbons or through biomarker fingerprints. Biomarkers are geochemical organic compounds that have carbon skeletons, which can be related to their biological precursors.[3] Biomarkers play a growingly important role in characterization, correlation, differentiation, and source identification in environmental forensic investigations of oil spills. The biomarkers most commonly used in forensic investigations are pentacyclic terpanes and steranes.[4–8]

Once entering the environment, oil spilled is subjected to a variety of weathering processes, such as evaporation, dissolution, dispersion, flushing due to wave energy, emulsification, photochemical oxidation, microbial biodegradation, and adsorption to suspended matter and deposition onto the seafloor.[2,8–11] Biological reactions and sunlight irradiation have strong influences on physicochemical properties of crude oil in the environment.[12] It has been reported that analytical results could be ambiguous and inconclusive due to the weathering of oil samples. For example, some previous studies disclosed that severe biodegradation could cause losses of some of the C_{27}–C_{29}

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steranes and demethylation of C_{27}–C_{35} hopanes.[13,14] There is no single one that could be used as a definitive and universal forensic criterion. Therefore, a multicriteria approach by characterizing more than one suite of analysis for spill source identification is necessary.

An important class of biomarkers formed by diagenesis and maturation of sterols are the aromatic steroids. Such biomarkers from sedimentary organic matter can provide valuable information to assess organic input, maturity, correlation of crude oils and the effect of biodegradation in reservoirs, particularly when saturated biomarkers are removed, as in the case of severely biodegraded oils/shales and in condensates or highly mature oils.[15–19] The aromatic steroid hydrocarbons are hardly affected until reaching level 10 in the Peters and Moldowan's scales (PM 10).[19] Based on this, it can be concluded that aromatic steroid hydrocarbons may provide another useful diagnostic means for spill source identification. Thus, more attention should be paid to employ these compounds for forensic oil spill investigations. The combined effects of weathering can strongly modify the fingerprints and parameters used to correlate the oil sample with its source on the basis of GC and GC-MS analysis. It is also important to understand the relationship between the biodegradation and photo-oxidation processes as well as the distribution of aromatic steroid hydrocarbons. However, limited efforts have been made on examining the effect of biodegradation and especially photo-oxidation on such compounds although considerable information is now available about their structures observed in petroleum and sedimentary rocks. To help fill the knowledge gaps, an experimental study aimed at evaluation on the capability and suitability of using triaromatic steroid hydrocarbons as biomarkers for oil spill fingerprinting. To achieve the goal, the following tasks were carried out: (1) environmental biodegradation and photochemical oxidation processes of spilled oil; (2) investigation of weathering effects on distribution of aromatic steroid hydrocarbons in crude oils and oil residues; and (3) based on the selected biomarkers, development of a set of corresponding diagnostic indices for oil correlation and differentiation.

2. Materials and methods

2.1. Biodegradation

The crude oil collected from Shengli oilfield (SL-B-0), the fourth-largest oilfield in China, was used for biodegradation experiments. The enrichment culture technique was used for culture isolation,[20] using crude oil as the sole source of carbon and energy. The procedure used here has been described elsewhere.[21] Briefly, the oilfield water from Shengli oilfield was added in enrichment medium, and the enrichment medium was put in the incubator at 50 °C for 30 min. To mimic the natural living conditions of bacteria, aerobic bacteria and a low inorganic salt concentration of aqueous medium were chosen in this study. The mineral media composition was (mg L^{-1}): $(NH_4)_2HPO_4$ (1000), KH_2PO_4 (500), Na_2HPO_4 (75), $MgSO_4 \cdot 7H_2O$ (200) and $CaCl_2$ (20). The chemical reagents are analytical reagent obtained from Beijing Chemical Reagent Company. Culture growth and oil biodegradation were 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 previous studies,[20,21] culture growth and utilization of hydrocarbons from crude oil could occur at salinities of 3.2%. Each experiment was carried out in triplicate. After determining the growth profile, the overall loss of oil after 21 days of incubation was quantified.

2.2. Photo-oxidation degradation

Photo-oxidation was performed by irradiating a layer of oil with a sunlight simulator for a defined period of time. The crude oil collected from Shengli oilfield was used for the artificial photo-oxidation experiment. The crude oil (SL-P-0) (degassed with nitrogen for 2 days in order to eliminate compounds with a high volatility, 0.1 g) was suspended in water (20 mL) in a sealed vial. An evaporative loss of 25 wt% was estimated by comparison of the chromatograms of the distillation residues. The vial was placed in a ventilating cabinet which was fixed with anti-UV cloth. The mixture was irradiated with a 40 W high-pressure mercury arc for 6, 18, 24 h (SL-P-1, SL-P-2, SL-P-3) respectively in the presence of stirring.

2.3. Extraction, separation and analysis

The procedure used for separation and quantitation of individual alkanes and aromatic steroid hydrocarbons has been described elsewhere.[21,22] The oil and residue were liquid–liquid extracted three times with 50 mL of dichloromethane in a separatory funnel. After drying with anhydrous sodium sulfate, the organic extracts were concentrated by rotary evaporation, and the solvent was exchanged to 1 mL of hexane. The oil samples were deasphalted by precipitation with *n*-hexane followed by filtration. Then, about 5–50 mg of the concentrated extract was fortified with hexane solutions of surrogate standards containing d_{10}-phenanthrene and d_{10}-deuterated chrysene. The fortified extract were further fractioned by column chromatography using a 50 × 1 cm i.d. column packed with 6 g alumina (70–230 mesh, activated for 12 h at 450 °C) and 9 g silica gel (80–120 mesh, activated for 12 h at 150 °C). Saturated hydrocarbons, aromatic hydrocarbons and non-hydrocarbons were obtained by successively eluting with *n*-hexane, toluene and chloroform–methanol (98 : 2), respectively. The extract was eluted with 15 mL hexane and concentrated to 0.5 mL under a gentle N_2 flow for cleanup and then analyzed by GC-MS.

The saturated hydrocarbons and aromatic hydrocarbons were analyzed with a 6890N GC-5973N mass spectrometer (Agilent Technologies, USA). Sample extracts were injected in a splitless mode onto a HP-5 capillary column (50 m × 0.32 mm × 0.25 μm, Agilent Technologies, USA) at an initial temperature of 80 °C. The GC oven temperature was programmed to 300 °C at 4 °C min^{-1} and was held at the final temperature for 30 min. The injector temperature is 300 °C. Helium was used as a carrier gas. Mass spectrometer conditions were electron ionization at 70 eV with an ion source temperature of 250 °C.

Individual *n*-alkanes were identified based on the retention time of the authentic standards (nC_{10-40}, Sigma). On the other hand, aromatic hydrocarbons were quantified based on the retention time and *m*/*z* ratio of an authentic polyaromatic hydrocarbon (PAH) mixed standard (Sigma), and

concentrations of each PAHs were calibrated based on the standard calibration curve. The aromatic steroid hydrocarbons were detected in their key mass chromatograms (*m*/*z* 253, 231 and 245) based on the relative retention times and by comparing their mass spectra with published data.[23,24] A standard reference oil sample was analyzed as part of the internal laboratory QA/QC procedures. Recovery of surrogate standards for aliphatic and aromatic steroid hydrocarbon fractions ranged from 86% to 108% and 92% to 106%, respectively. Instrumental reproducibility assessed by triplicate analysis was around 5%.

3. Results and discussion

3.1. Distribution of *n*-alkane

Although alkanes are not particularly useful for determining the sources of the spill after the biodegradation process, 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 and the profile of the unresolved complex mixture (UCM). Briefly, there were not obvious differences in the distribution of these fractions, with respect to the *n*-alkanes, pristane and phytane, in the initial oil (SL-P-0) and oil residues (SL-P-1, SL-P-2, SL-P-3) after photo-oxidation. Nevertheless, *n*-alkane of oil residues after biodegradation (SL-B-1, SL-B-2) was almost completely biodegraded compared with their initial oil (SL-B-0) (Fig. 1). Both the depletion in *n*-alkanes and the small but UCM are signs of biodegradation.

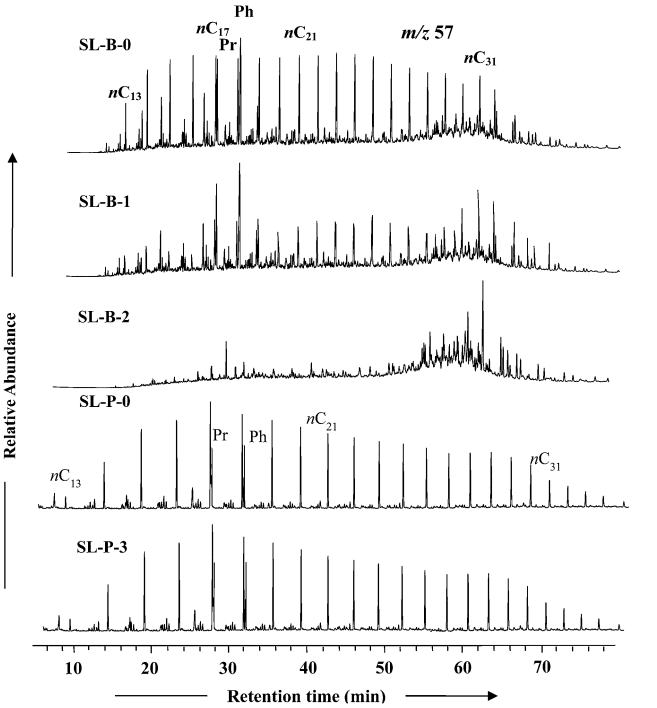

Fig. 1 GC-MS chromatograms (*m*/*z* = 85) of the degraded oils compared to the initial oils. Note: Pr: pristane; Ph phytane; SL-B-0: crude oil for biodegradation; SL-B-1: oil residues after biodegradation for 7 days; SL-B-2: oil residues after biodegradation for 21 days; SL-P-0: crude oil for photo-oxidation; SL-P-3: oil residues after photo-oxidation for 24 h.

3.2. Effect of biodegradation on the distribution of aromatic steroid hydrocarbons

Of the various types of aromatic steroid hydrocarbons, only the distributions of the monoaromatic sterane (MAS, *m*/*z* = 253), triaromatic sterane (TAS, *m*/*z* = 231) and methyl triaromatic sterane hydrocarbon (MTAS, *m*/*z* = 245) were widely presented.[15,18,19,22,24] The distributions of MAS, TAS and MTAS species for samples SL-B-0 are shown in Fig. 2. Peak identification is summarized in Table 1. The sample SL-B-0 had the same distributions of all three aromatic series as that of sample SL-B-2. The ratios of TAS to MTAS for SL-B-1 and SL-B-2 are 0.92 and 0.53, respectively, which showed that the relative abundance of TAS was less than that of MTAS. In addition, the ratios of TAS to MAS for SL-B-1 and SL-B-2 are 0.27 and 1.94, respectively. The initial oils contain C_{21}-MTAS, C_{22}-MTAS, C_{27}–C_{29} MTAS, dominated by C_{27}–C_{29} MTAS components, with C_{29}-MTAS more abundant than C_{27}-MTAS and C_{28}-MTAS. The C_{27}, C_{28} and C_{29} MAS have roughly the same abundance in the initial oil. In addition, the relative abundance of C_{27}–C_{29} MAS of initial oils is also higher than C_{21}-MAS and C_{22}-MAS.

TAS was detected in the *m*/*z* 231 SIM fragmentogram (Fig. 2) which shows no evidence of biodegradation. Connan (1984) reported that biodegradation of aromatic steroids is rare, indicating their bacterial resistance.[25] From examination of the *m*/*z* = 231 and 245 chromatograms, there appear to be no significant differences on the distributions of TAS and MTAS components between the crude oils and their corresponding oil residues after biodegradation.

No obvious change in MTAS distributions was observed after biodegradation (Fig. 3a), which suggested that MTAS was also resistant to biodegradation. The extent of cracking in the side chains of TAS can be used to provide information about petroleum maturity.[3,24] The triaromatic sterane cracking ratios TA(I)/TA(I + II) : $(C_{20} + C_{21})/(C_{20} + C_{21} + C_{26} + C_{27} + C_{28})$ and $C_{20}/C_{20} + C_{28}(20R)$ are commonly used as maturity indicators.[26] The two ratios of SL-B-0 were similar to those of SL-B-1 and SL-B-2. It suggested that such indices will be well used for the correlation of biodegraded crude oils. Another major difference was that the ratio of component G (C_{28} 20*R* TAS) to E (C_{28} 20*S* TAS) in the *m*/*z* = 231 chromatogram showed decreasing trends from 0.90 to 0.83 with the biodegradation level. Based on this, we concluded that the preferential removal of the 20*R* isomer of triaromatic sterane resulted in a slight decrease in the *R*/*S*. These observations were consistent with reports that the 20*R* configuration (the original biological configuration) in regular steranes is preferentially degraded relative to the 20*S* form.[2] In addition, the loss of the lower molecular weight C_{21}-MAS and C_{22}-MAS is weak for the biodegraded oil residue.

3.3. Effect of photo-oxidation on the distribution of aromatic steroid hydrocarbons

The distributions of MAS, TAS and MTAS species for samples SL-P-0 are shown in Fig. 2. Peak identification is also

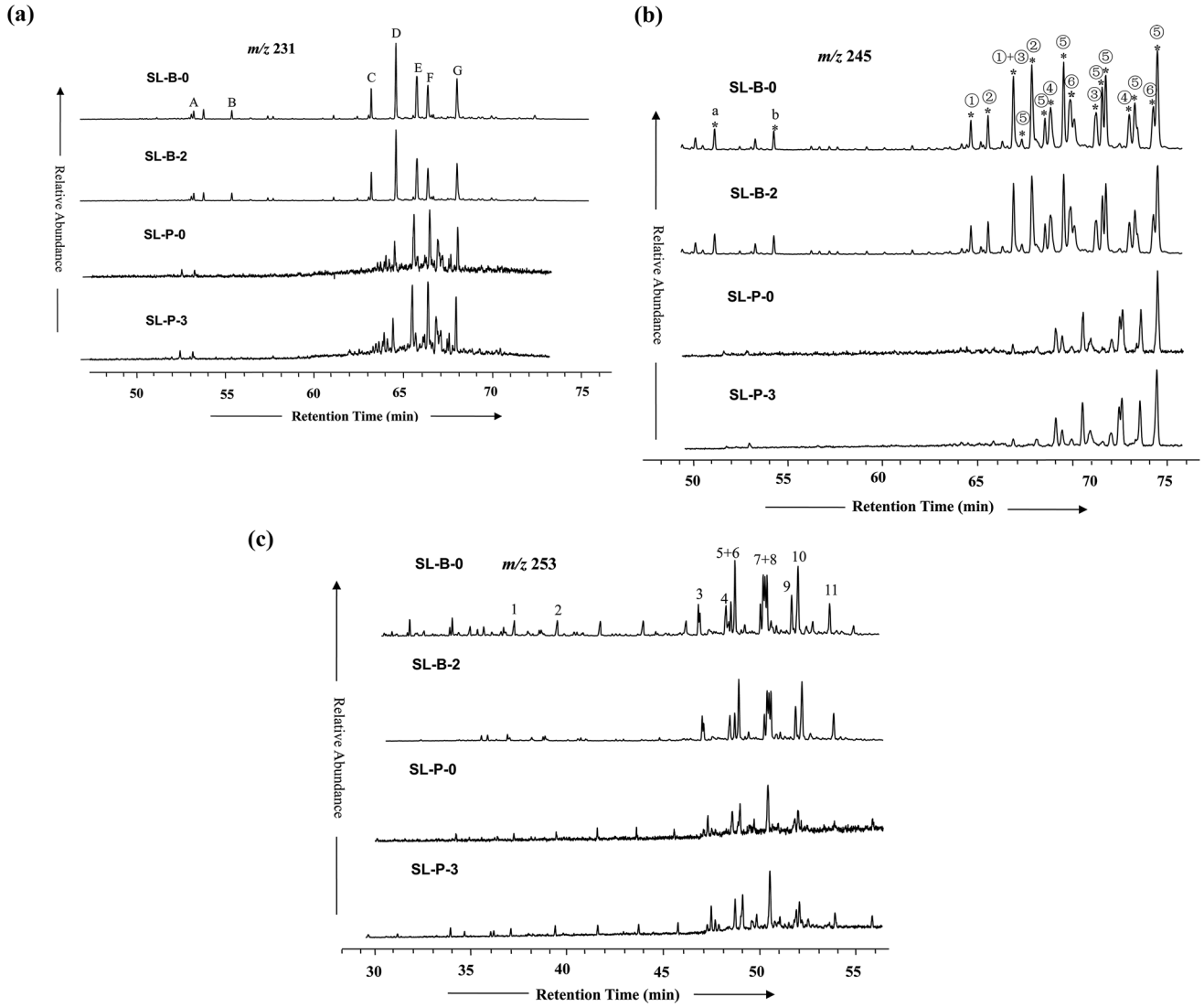

Fig. 2 Mass chromatograms (*m*/*z* 231, 245, 253) showing the distribution of triaromatic steranes (a) and methyl triaromatic steranes (b) and monoaromatic sterane (c) in crude oils and degraded oil residues, respectively. For mass chromatograms, the detail of peak identifications may refer to Table 1.

summarized in Table 1. The sample SL-P-0 had the same distributions as that of SL-P-2. The triaromatic sterane cracking ratios decreased from 0.17 (SL-P-0) to 0.15 (SL-P-3) with photo-oxidation time. The ratios of C_{27}/C_{28}, C_{28}/C_{29}, C_{27}/C_{29} all showed some decreasing trends after photo-oxidation. These results were in accord with the sterane data, which showed that the C_{27} steranes were degraded preferentially to the C_{27}–C_{29} species. The abundance of C_{21}-MAS and C_{22}-MAS relative to C_{27}–C_{29} series in the samples (SL-P-0, SL-P-1, SL-P-2) is roughly the same even in the extensively weathered sample (SL-P-3). The depletion of MAS, TAS and MTAS in the higher molecular weight members in photo-oxidation oil samples (Fig. 3b) was ascribed to some degradation.

Another major difference was that the ratio of component G (C_{28} 20*R*) to E (C_{28} 20*S*) in the $m/z = 231$ chromatogram (G/E) showed increasing trends from 0.74 to 0.84 with the photo-oxidation biodegradation level. Based on this, we concluded that the preferential removal of the 20*S* isomer results in a slightly increase in the *R*/*S*. It was also quite different from that seen in the biodegradation process.

3.4. Evaluation on the diagnostic ratios based on relative standard deviation

Parameters derived from GC and GC-MS data may change under the influence of the weathering process. Based on the evaluation method of indices suggested by Stout *et al.* (2001) and Li *et al.*, (2009), the relative standard deviation (RSD) is considered as an indicator to evaluate the variability of diagnostic indices in this experiment.[27,28] 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 indices.[29]

The parameters of tricyclic terpanes and sterane biomarkers are far different between SL-B-0 and SL-P-0. For example, the

Table 1 Peak identifications in the *m/z* 231, *m/z* 245 and *m/z* 253 mass fragmentograms in oil samples.Modified after (Mackenzie *et al.*, 1981; Zhou and Zhang, 1989; Mi *et al.*, 2007)[15,30,31]

Peak label	Compound classes	Base peak	Compound name	Mass spectra (*m/z*) EI
A	$C_{20}H_{20}$	260	C_{20}-triaromatic sterane	231, 260, 246, 215, 203
B	$C_{21}H_{22}$	274	C_{21}-triaromatic sterane	231, 274, 259, 215, 203
C	$C_{26}H_{32}$	344	C_{26}-triaromatic sterane (20*S*)	231, 344, 329, 215, 203
D	$C_{26}H_{32}$	344	C_{26}(20*R*) + C_{27}(20*S*)-triaromatic sterane	231, 344, 329, 215, 203
E	$C_{28}H_{36}$	372	C_{28}-triaromatic sterane (20*S*)	231, 372, 357, 215, 203
F	$C_{27}H_{34}$	358	C_{26}-triaromatic sterane (20*R*)	231, 368, 343, 215, 203
G	$C_{28}H_{36}$	372	C_{28}-triaromatic sterane (20*R*)	231, 372, 357, 215, 203
a	$C_{21}H_{22}$	274	C_{21}-methyl triaromatic sterane	245, 274, 259, 217, 229
b	$C_{22}H_{24}$	288	C_{22}-methyl triaromatic sterane	245, 285, 273, 217, 229
①	$C_{27}H_{34}$	358	C_{27}-3-methyl triaromatic sterane	245, 358, 343, 217, 229
②	$C_{27}H_{34}$	358	C_{27}-4-methyl triaromatic sterane	245, 358, 343, 217, 229
③	$C_{28}H_{34}$	372	C_{28}-3,24-dimethyl triaromatic sterane	245, 372, 357, 217, 229
④	$C_{29}H_{38}$	386	C_{29}-3-methyl-24-ethyl triaromatic sterane	245, 386, 371, 217, 229
⑤	$C_{29}H_{38}$	386	C_{29}-4,23,24-trimethyl triaromatic sterane	245, 386, 371, 217, 229
⑥	$C_{29}H_{38}$	386	C_{29}-4-methyl-24-ethyl triaromatic sterane	245, 386, 371, 217, 229
1	$C_{21}H_{30}$	282	C_{21}-monoaromatic sterane	253, 282, 267, 143
2	$C_{22}H_{32}$	296	C_{22}-monoaromatic sterane	253, 296, 281, 143
3	$C_{27}H_{42}$	366	C_{27}-5β(*H*)-monoaromatic sterane (20*S*)	253, 366, 351, 143
4	$C_{27}H_{42}$	366	C_{27}-5β(*H*)-monoaromatic sterane (20*R*)	253, 366, 351, 143
5	$C_{27}H_{42}$	366	C_{27}-5α(*H*)-monoaromatic sterane (20*S*)	253, 366, 351, 143
6	$C_{28}H_{44}$	380	C_{28}-5β(*H*)-monoaromatic sterane (20*S*)	253, 380, 365, 143
7	$C_{27}H_{42}$	366	C_{27}-5α(*H*)-monoaromatic sterane (20*R*)+	253, 366, 351, 143
	$C_{28}H_{44}$	380	C_{28}-5α(*H*)-monoaromatic sterane (20*S*)	253, 380, 365, 143
8	$C_{28}H_{44}$	380	C_{28}-5α(*H*)-monoaromatic sterane (20*R*)+	253, 380, 365, 143
	$C_{29}H_{46}$	394	C_{29}-5β(*H*)-monoaromatic sterane (20*S*)	253, 394, 379, 143
9	$C_{29}H_{46}$	394	C_{29}-5α(*H*)-monoaromatic sterane (20*S*)	253, 394, 379, 143
10	$C_{28}H_{44}$	380	C_{28}-5α(*H*)-monoaromatic sterane (20*R*)+	253, 380, 365, 143
	$C_{29}H_{46}$	394	C_{29}-5β(*H*)-monoaromatic sterane (20*R*)	253, 394, 379, 143
11	$C_{29}H_{46}$	394	C_{29}-5α(*H*)-monoaromatic sterane (20*R*)	253, 394, 379, 143

relative deviation for the ratio of 18α-22,29,30-trisnorhopane relative to 17α-22,29,30-trisnorhopane (Ts/Tm), 22*S*(22*S* + 22*R*) for C_{31}-17α,21β(*H*)-homohopane (C_{31} 22*S*/22*S* + 22*R*), the gammacerane index, and 20*S*/(20*S* + 20*R*) for C_{29}-5α(*H*),14α(*H*),17α(*H*)-steranes C_{29} 20*S*/(20*S* + 20*R*) is 160.60, 137.52, 107.20 and 124.08, respectively. It means that the two source oil samples are far different. The variation of the suggested diagnostic ratios between different crude oils (SL-B-0 and SL-P-0) is shown in Tables 2 and 3. In heavily biodegraded oils, the *n*-alkanes, and even the isoprenoids in some cases, may be completely lost. Under such circumstances, GC-FID analysis of *n*-alkanes for the heavily biodegraded oil sample is of little value for suspect source identification. Based on this, the suggested diagnostic ratios of MAS, TAS and MTAS may also be useful to distinct the different oils. For the biodegradation weathering oil residue after 21 days, all the diagnostic ratios of TAS and MTAS displayed little changes over weathering time (Table 2), indicating that these ratios are well used for oil source identification, even after serve biodegradation. The RSDs of ratios derived from TAS were all <5% (Table 3), which showed that such diagnostic ratios were probably not affected by photo-oxidation weathering. Except MTAS-4, the RSDs of other diagnostic ratios from MTAS were all higher than 5%, indicating that these ratios were not valid for oil source identification after photo-oxidation. On the whole, the diagnostic ratios derived from TAS are well used for oil source identification after biodegradation and photo-oxidation.

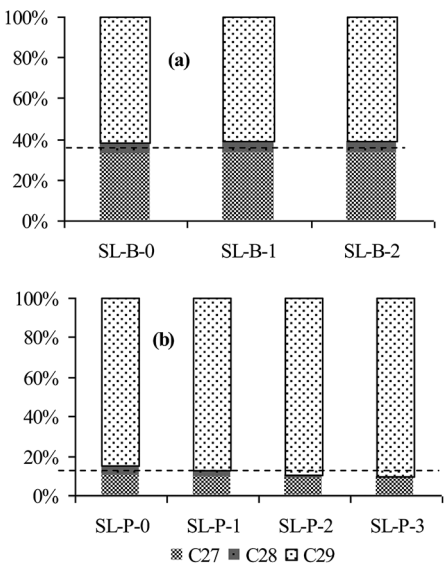

Fig. 3 Relative composition of C_{27}, C_{28}, C_{29} MTAS during the biodegradation (a) and photo-oxidation (b).

3.5. Evaluation on the diagnostic ratios based on the repeatability limit

The repeatability limit, *r*, is the value below which the absolute difference between two single test results obtained under

Table 2 Data on screening of TAS, MTAS and MAS fingerprints for biodegraded oils samples[a]

Parameters	Abbreviations of parameters	Average ($\bar{x}$)	RSD (%)	Range	Repeatability limit	Evaluation
$C_{20}/[C_{20} + C_{28}(20R)]$	P-TAS-1	0.19	2.20	0.01	0.03	Y
TA(I)/TA(I + II)	P-TAS-2	0.06	2.76	0.01	0.01	N
$C_{28}(20R)/C_{28}(20S)$	P-TAS-3	0.87	4.38	0.07	0.12	Y
$C_{27}(20R)/C_{28}(20S)$	P-TAS-4	1.20	0.40	0.01	0.17	Y
$C_{27}(20R)/C_{28}(20R)$	P-TAS-5	0.85	2.96	0.04	0.12	Y
$C_{28}(20S)/[C_{26}(20R) + C_{27}(20S)]$	P-TAS-6	0.61	2.29	0.02	0.08	Y
⑥/④	P-MTAS-1	1.31	1.29	0.03	0.18	Y
③/⑥	P-MTAS-2	0.78	0.23	0.01	0.11	Y
⑥/⑤	P-MTAS-3	0.14	0.34	0.00	0.02	Y
④/⑤	P-MTAS-4	0.11	0.94	0.00	0.02	Y
③/⑤	P-MTAS-5	0.11	0.43	0.00	0.02	Y
③/④	P-MTAS-6	1.03	1.34	0.02	0.14	Y
$a/(a + b)$	P-MTAS-7	0.47	0.30	0.01	0.07	Y
$b/(a + b)$	P-MTAS-8	0.53	0.26	0.01	0.07	Y
$\sum C_{28}/\sum C_{27}$	P-MTAS-9	0.16	1.04	0.00	0.02	Y
$\sum C_{28}/\sum C_{29}$	P-MTAS-10	0.55	1.46	0.01	0.08	Y
3/4	P-MAS-1	0.94	6.34	0.12	0.13	Y
3/5	P-MAS-2	0.41	1.51	0.01	0.06	Y
9/11	P-MAS-3	1.25	1.53	0.03	0.17	Y

[a] P-TAS: abbreviations of parameters from triaromatic sterane; P-MTAS: abbreviations of parameters from methyl triaromatic sterane; P-MAS: abbreviations of parameters from monoaromatic sterane. Average: average value of parameters among the initial oil (SL-B-0) and oil residues (SL-B-1, SL-B-2) after biodegradation; range: the difference value of parameters between maximum value and minimum value; $r_{95\%} = 2.8 \times \bar{x} \times 5\% = 14\%\bar{x}$.

Table 3 Data on screening of TAS, MTAS and MAS fingerprints for photo-oxidation oils samples

Parameters	Abbreviations of parameters	Average[a]($\bar{x}$)	RSD (%)	Range	Repeatability limit	Evaluation
$C_{20}/[C_{20} + C_{28}(20R)]$	P-TAS-1	0.16	3.72	0.01	0.02	Y
TA(I)/TA(I + II)	P-TAS-2	0.06	2.54	0.00	0.01	Y
$C_{28}(20R)/C_{28}(20S)$	P-TAS-3	0.77	2.68	0.05	0.11	Y
$C_{27}(20R)/C_{28}(20S)$	P-TAS-4	0.51	3.59	0.03	0.07	Y
$C_{27}(20R)/C_{28}(20R)$	P-TAS-5	0.66	5.97	0.09	0.09	N
$C_{28}(20S)/[C_{26}(20R) + C_{27}(20S)]$	P-TAS-6	1.08	3.99	0.09	0.15	Y
⑥/④	P-MTAS-1	0.21	20.22	0.10	0.03	N
③/⑥	P-MTAS-2	0.75	61.56	0.92	0.10	N
⑥/⑤	P-MTAS-3	0.04	16.17	0.01	0.01	N
④/⑤	P-MTAS-4	0.21	3.83	0.01	0.03	Y
③/⑤	P-MTAS-5	0.04	80.69	0.07	0.01	N
③/④	P-MTAS-6	0.18	84.35	0.33	0.03	N
$a/(a + b)$	P-MTAS-7	0.47	10.02	0.10	0.07	N
$b/(a + b)$	P-MTAS-8	0.53	8.93	0.10	0.07	N
$\sum C_{28}/\sum C_{27}$	P-MTAS-9	0.26	72.56	0.41	0.04	N
$\sum C_{28}/\sum C_{29}$	P-MTAS-10	0.11	9.63	0.10	0.01	N
3/4	P-MAS-1	0.84	8.74	0.17	0.12	N
3/5	P-MAS-2	0.68	4.57	0.07	0.10	Y
9/11	P-MAS-3	1.32	9.73	0.31	0.19	N

[a] Average: average value of parameters among the initial oil (SL-P-0) and oil residues (SL-P-1, SL-P-2, SL-P-3) after photo-oxidation.

repeatability conditions may be expected to lie with a probability of 95%. This limit is obtained as:

$$r_{95\%} = 2\sqrt{2}S_r = 2.8S_r \quad (1)$$

where S_r is the standard deviation, the relative standard deviation is assumed as ≤5% in oil spill identification; $\bar{x}$ is the mean value. Thus it can been concluded that

$$r_{95\%} = 2.8 \times \bar{x} \times 5\% = 14\%\bar{x}. \quad (2)$$

When the range is smaller than the reproducibility limit, the diagnostic ratios may be well used for oil source identification. The evaluation result is denoted as Y. If the range is larger than the reproducibility limit, the evaluation result is denoted as N. Such diagnostic ratios are of little value for suspect source identification.

Based on the repeatability limit method of oil source identification, a number of diagnostic ratios derived from MAS, TAS and MTAS were used as indicators for oil spill identification. These are briefly summarized in Tables 2 and 3. The result in tables agrees well with the analytical result of relative deviation.

Pentacyclic triterpanes and steranes, the biomarkers most commonly used in forensic investigations, are generally absent or in very low abundances in lighter petroleum products such as jet fuels and mid-range diesels. In comparison with steranes and terpanes, the aromatic steroid hydrocarbons are generally less susceptible to biodegradation. Thus, it may be concluded that aromatic steroid hydrocarbons may also provide some useful diagnostic indices for spill source identification.

4. 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. The preferential removal of the 20*R* isomer of triaromatic sterane results in a slight decrease in the *R*/*S* in oil residues after biodegradation. It is also quite different from that seen in photochemical degradation.

The above discussion also reveals that: despite the decreasing abundance, the distributions of paraffinic hydrocarbons in the crude oil at times 0, 6, 18 and 24 h after photo-oxidation were all similar. All the diagnostic ratios, such as TA(I)/TA(I + II), C_{20}TAS/[C_{20} + C_{28}(20*R*)]TAS, C_{28}(20*R*)/C_{28}(20*S*)TAS, C_{27}(20*R*)/C_{28}(20*S*)TAS, C_{28}(20*S*)TAS/[C_{26}(20*R*) + C_{27}(20*S*)]TAS, (C_{21}MTAS + C_{22} MTAS)/MTAS, C_{27}-5β(*H*)-(20*S*)/C_{27}-5α(*H*) (20*S*), could be efficiently used in oil spill identification after biodegradation and photo-oxidation. Except MTAS-4, the RST of other ratios derived from MTAS ranged from 9% to 84%, suggesting that photo-oxidation has a remarkable effect on these indices. However, all the ratios derived from MTAS display little changes over weathering time with an RSD of less than 5% even after high biodegradation. Triaromatic sterane retained their molecular compositions after biodegradation and photo-oxidation and the diagnostic ratios from them could be efficiently used in oil spill identification.

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