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Comparison of oil composition changes due to biodegradation and physical weathering in different oils

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Abstract

The well-characterized Alberta Sweet Mixed Blend oil and several other oils which are commonly transported in Canada were physically weathered and then incubated with a defined microbial inoculum. The purpose was to produce quantitative data on oil components and component groups which are more susceptible or resistant to biodegradation, and to determine how oils rank in relation to each other in terms of biodegradation potential. The biodegraded oils were characterized by quantitative determination of changes in important hydrocarbon groups including the total petroleum hydrocarbons, total saturates and aromatics, and also by quantitation of more than 100 individual target aliphatic, aromatic and biomarker components. The study reveals a pattern of distinct oil composition changes due to biodegradation, which is significantly different from the pattern due to physical or short-term weathering. It is important to be able to distinguish between these two forms of loss, so that loss due to weathering is not interpreted as loss due to biodegradation in the laboratory or in the field. Based on these findings, the oil composition changes due to biodegradation can be readily differentiated from those due to physical weathering. To rank the tested oils with respect to biodegradability, losses in total petroleum hydrocarbons and aromatics were used to calculate biodegradation potential indices, employing equations proposed by Environment Canada and the US National Oceanic and Atmospheric Administration. The different methods produced very similar biodegradation trends, confirming that patterns of oil biodegradability do exist.

Keywords: Oils; Polynuclear aromatic hydrocarbons

1. Introduction

Bioremediation is of interest world-wide as a potential oil spill clean-up option [1–9], especially for inaccessible or sensitive environments. However, before bioremediation is considered as a clean-up option by spill response decision-makers, it is essential to know whether the spilled oil is amenable to biodegradation. Other than simple observations, such as lighter oils degrade more quickly than heavier oils or *n*-alkanes degrade more rapidly than branched alkanes, little quantitative data have been available for evaluating the biodegradation of the wide range of crude oils and petroleum products commonly transported in Canada.

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The Emergencies Science Division (ESD) of Environment Canada and the US National Oceanographic and Atmospheric Administration (NOAA) independently conceived of testing oils and petroleum products to determine their relative biodegradability. It was felt that standardized laboratory testing could produce quantitative data on oil components and component groups which are more susceptible or resistant to biodegradation, and additionally, on how oils rank in relation to each other in terms of biodegradation potential. Standardized methods have been developed by ESD [10,11] and the National Environmental Technology Applications Centre (NETAC) [12] for laboratory-based testing of crude oil biodegradation. The Environment Canada test currently uses a defined freshwater microbial inoculum while the NOAA approach generally followed the NETAC protocols by using a natural seawater inoculum. Both methods are based on a laboratory shaker flask test, with and without the addition of nutrients [13]. Information from laboratory tests will not necessarily predict the actual behaviour of an oil in the environment, since it does not account for the numerous environmental variables that determine the actual rate of biodegradation in a given environment. However, the laboratory tests could provide a screening tool to determine which oils are likely to be degradable, by how much, and to what degree, so as to facilitate more informed judgements about the potential for bioremediation of a particular spill.

Environment Canada has developed detailed analytical methods for characterizing and quantifying oil composition [14–18]. Since 1993, hundreds of samples generated during controlled laboratory shaker flask studies for evaluating oil biodegradability by defined inocula [19] have been analyzed for quantitative determination of oil composition changes. These data have been analyzed to permit general statements to be made about oil biodegradation. The purpose of this paper is to provide an overview of the oil composition changes that commonly occur during biodegradation, and how to distinguish biodegradation from weathering based on distinct patterns of oil composition changes and on changes of a number of characteristic oil biodegradation ''diagnostic ratios''. At present, little information is available regarding similarities and differences in oil composition due to biodegradation versus physical weathering. This knowledge is particularly valuable when evaluating oil samples from field studies or spill sites to determine whether the composition changes which have occurred are due to biodegradation or weathering. Finally, calculated biodegradation potential indices are briefly described in order to rank the tested oils with respect to biodegradability by the defined bacterial consortium.

2. Experimental

2.1. Weathering of oils

Eight oils which are commonly transported in Canada were selected for freshwater biodegradation tests. They are Alberta Sweet Mixed Blend (ASMB, the reference oil), Cold Lake Bitumen (CLB), Bent Horn (BH), Norman Wells (NW), Oseberg (OS), Prudhoe Bay (PB), Terra Nova (TN) and Bunker C (BC). The main property description and hydrocarbon group distribution of the eight oils are presented in Table 1. Prior to biodegradation, all of the oils were weathered by evaporation in a fumehood for 24 h in ESD's Oil Laboratory to reduce volatile hydrocarbon losses during incubation. The evaporative mass losses for the eight oils were determined to be 17.3% (ASMB), 9.0% (CLB), 12.7% (BH), 15.3% (NW), 10.7% (OS), 5.6% (PB), 4.7% (TN) and 0.1% (BC), respectively.

2.2. Biodegradation method

Six microbial stains (three aliphatic degraders and three aromatic degraders) comprise the standard freshwater inoculum used in the freshwater oil biodegradation tests. The bacteria used in these experiments were not selected on the basis of extraordinary biodegradation performance and they were obtained from environmental samples using a wide variety of oils and media; there was no selection of isolates specifically for degradation of these oils [19]. In other words, a defined reproducible consortium was created rather than a ''super-inoculum''. The individual isolates were prepared in bulk at the start of the project and cryo-preserved in

Table 1
Main property description and hydrocarbon group distribution of tested oils[a]

Oil	Origin	API gravity	Boiling range (°C)	Hydrocarbon group			
				Saturates	Aromatics	Polars	Asphaltenes
ASMB	Alberta	37.0	40–650	84	13	1	2
CLB[b]	Alberta	9.8	200–740	(Sat.+Aro.=56)		29	16
BH	Northwest Territories	41.3	60–700	94	5	0	1
NW	Northwest Territories	38.4	40–700	85	11	2	1
OS	North Sea, Norway	34.4	100–700	65	25	8	2
PB	Alaska	24.8	40–700	78	18	3	2
TN	Newfoundland	37.1	40–700	79	15	4	3
BC	(Heavy Fuel oil)	14.1	160–750	32	32	17	19

[a] The hydrocarbon group data are cited from Ref. [45].
[b] The hydrocarbon group data for CLB are cited from Ref. [46].

suitable replicate aliquots for use throughout the entire project.

Briefly, the tests were conducted as follows: 200 ml of standard freshwater medium [19] was added to each 500-ml Erlenmeyer flask. The equivalent of 2 g/l (0.2%, v/v) of the oil of interest was then added to the cool, sterile medium aseptically, except for CLB and BC, which were added gravimetrically. Replicate aliquots of the standard freshwater inoculum were thawed and aseptically transferred to appropriate flasks, to a final concentration of 106 viable cells per ml of medium for each strain. A sterile standard nutrient solution containing nitrate, ammonium and phosphate was added to the flasks as indicated in Table 2. All flasks were incubated at 10°C in the dark at 200 rpm on a gyrotory shaker for 28 days. The PB oil was incubated additionally at 4, 10, 15 and 22°C for 7, 14 and 28 days to examine the changes in composition of the PB oil with time and temperature. All treatments except the sterile control (SC) were performed in duplicate or triplicate. At the end of the incubation period, cultures were acidified to pH≤2 with 2 *M* H_2SO_4 and 1.0 ml of surrogate standard solution (*o*-terphenyl, [$^2H_{10}$]phenanthrene and squalane) was added to the cultures to monitor extraction recovery. Residual oil was exhaustively extracted with multiple aliquots of HPLC-grade dichloromethane. The extract was dried over anhydrous sodium sulfate, concentrated by rotary evaporation and under N_2 to less than 1 ml, then quantitatively transferred to 2-ml glass vials with PTFE liners for chemical analysis.

2.3. Analysis of oil composition changes

For quantitative assessment of oil degradation, 16 to 20 mg of extracted oil was fractionated into saturate and aromatic fractions by silica gel liquid column chromatography and analyzed by gas chromatography–flame ionization detection (GC–FID) and GC–mass spectrometry (MS) [14–17]. Half of the saturate fraction (F1) was used for analysis of saturates and biomarker compounds; half of the aromatic fraction (F2) was used for analysis of target polycyclic aromatic hydrocarbons (PAHs) and

Table 2
Summary of treatments for evaluating oil composition changes due to biodegradation by a defined freshwater microbial inoculum (+=present, −=absent)

	Oil and nutrients (positive control) ($n=3$)	Oil without nutrients (negative control) ($n=2$)	Sterile control
Standard freshwater medium	+	+	+
Standard freshwater inoculum	+	+	−
Standard nutrients	+	−	+
Oil of interest	+	+	+

alkylated PAH homologues. The remaining half of F1 and F2 were combined (F3) and used for determination of total GC-detectable TPH and the unresolved complex mixture (UCM) of hydrocarbons. The following groups will be referred to when describing and discussing oil composition changes during biodegradation:

TPH or total gas chromatographic detectable petroleum hydrocarbons (GC-TPH): the sum of all GC-resolved and unresolved hydrocarbons. The resolvable hydrocarbons appear as peaks and the unresolvable hydrocarbons appear as the area between the lower baseline and the curve defining the base of resolvable peaks;

Total saturates: the sum of all resolved and unresolved saturate hydrocarbons including the total *n*-alkanes, branched alkanes and cyclic saturates;

Total aromatics: GC-TPH minus the total saturates;

Total *n*-alkanes: the sum of all resolved *n*-alkanes (from C_8 to C_{40} plus pristane and phytane);

Total of five alkylated PAH homologues: the sum of five target alkylated homologues of naphthalene, phenanthrene, dibenzothiophene, fluorene and chrysene determined by GC–MS;

Total biomarkers: the sum of target terpane (m/z 191) and sterane (m/z 217 and 218) compounds determined by GC–MS.

Note that the measured values of hydrocarbons lost (such as % TPH lost, % saturates lost, and so on) were obtained using the SC as the reference. Hence, the reported losses of hydrocarbon groups in this work are definitely due to biodegradation rather than to abiotic losses.

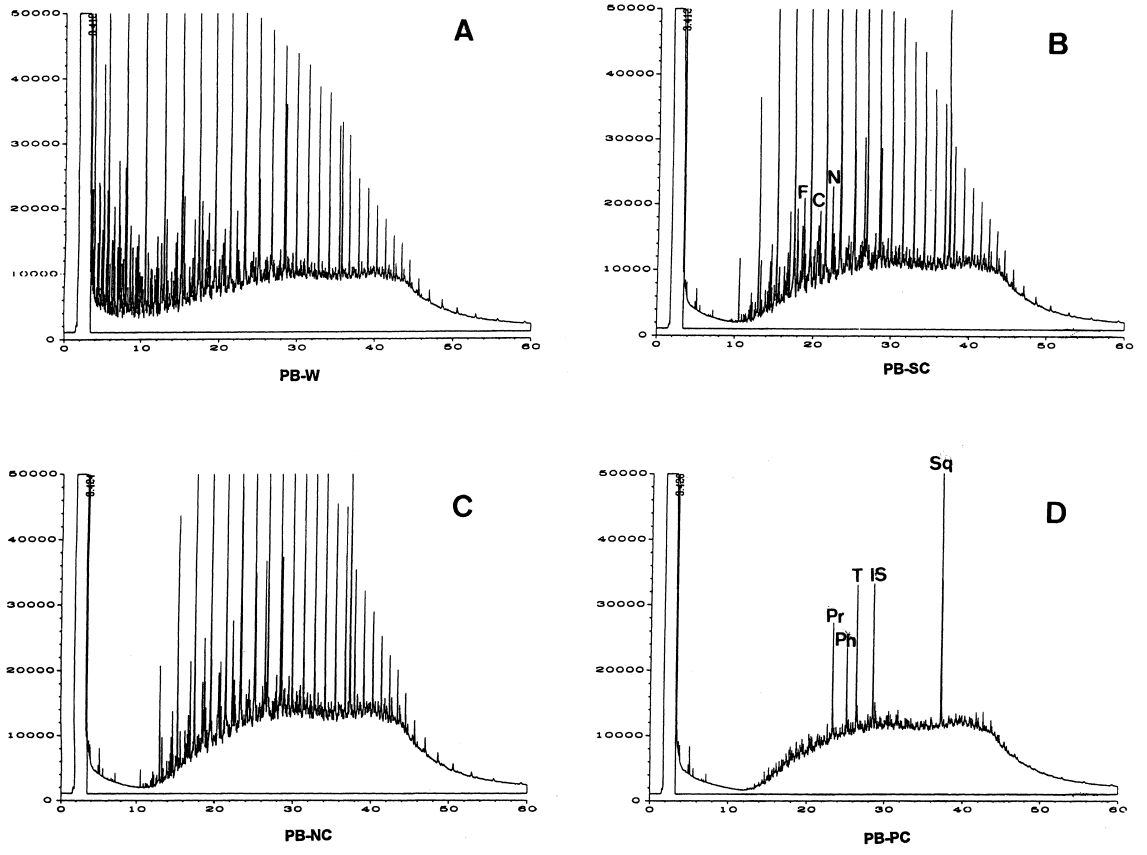

Fig. 1. Representative GC chromatograms for TPH analysis of PB oil incubated under the freshwater conditions. (A) Weathered PB source oil; (B) SC; (C) NC; (D) PC. F, C, T, Pr, Ph, T, Sq and I.S. represent farnasane, trimethyl-C_{13}, norpristane, pristane, phytane, surrogates *o*-terphenyl and squalane, and internal standard 5-α-androstane, respectively.

3. Results and discussion

3.1. Composition changes of TPH and saturate hydrocarbons

As a representative example, Fig. 1 shows GC chromatograms for TPH analysis of PB oil incubated under freshwater conditions using the standard inoculum. Fig. 2 presents GC–MS chromatograms of the saturate fraction at m/z 85 fragment for PB oil biodegradation series samples, clearly illustrating the changes in *n*-alkane content. Fig. 3 depicts quantitatively the *n*-alkane distributions of ASMB and PB oil biodegradation series. A number of hydrocarbon composition changes under various laboratory and field inoculum conditions have been reported [20–25]. The detailed quantitative composition changes of saturate hydrocarbons in the eight oils after exposure to the defined inoculum are summarized as follows (Table 3). Some of the observations on saturate composition changes are in agreement to the previous findings by other researchers [20–25], as discussed below.

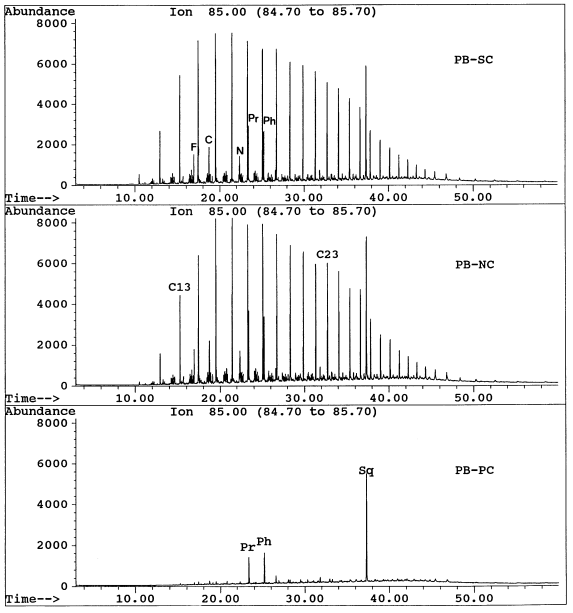

Fig. 2. Representative GC–MS (m/z 85) chromatograms of saturate fractions for the SC (top), NC and PC (bottom) of PB oil biodegradation series under the standard freshwater inoculum conditions. C_{13} and C_{23} represent normal alkanes with the carbon number of 13 and 23. Refer to Fig. 1 for identification of peaks F, C, T, Pr, Ph, T and Sq.

(1) For all crude oils tested, TPH, total saturates (including straight-chain, branched, GC-resolved and unresolved hydrocarbons), and total aromatics showed degradation to various degrees. In general, TPH, total saturate and total *n*-alkane degradation were greatly enhanced (Figs. 1–3) when nutrients were present (that is, positive controls, PCs). When nutrients were present, TPH, total saturate and total *n*-alkane losses were determined to be 9–41%, 5–47% and greater than 90%, respectively, for the eight oils. In contrast, for the corresponding negative controls (NCs, in the absence of nutrients), TPH, total saturate and total *n*-alkane losses were determined to be only 3–15%, 0–13% and 16–28%, respectively (Table 3).

(2) Alkane susceptibility was clearly correlated with chain length, i.e., the shorter the chain length, the more susceptible [1,2]. In the presence of nutrients, *n*-alkanes were nearly completely degraded and only a fraction of the isoprenoids remained.

(3) GC-resolved saturates degraded much faster than the unresolved complex saturate hydrocarbon mixtures, indicated by the significant decrease of the ratios of the total GC-resolved peaks to UCM. Table 3 clearly demonstrated significant decrease in the ratios of GC-resolved peaks to UCM for all PC samples of the eight test oils except the CLB, which demonstrated little change in the ratio (GC analysis demonstrates that the chemical composition of CLB is specific and very different from most crude oils. The chromatograms of either fresh or weathered CLB are dominated by UCM with very limited GC-resolved hydrocarbons). In contrast, the corresponding NCs only showed a small decrease in the ratios, illustrating the effect of nutrients on biodegradation of saturate hydrocarbons. For example, the ratios of the total GC-resolved peaks to UCM were determined to be 0.24, 0.08 and 0.24 for the SC, PC and NC of the ASMB oil, respectively. This parameter is a useful indicator of biodegradation degree for most conventional oils. The ratios show good consistency from run to run and are subject to little interference from instrument fluctuation.

(4) Branched alkanes were less susceptible to biodegradation than straight-chain *n*-alkanes. Figs. 1–3 clearly show that *n*-alkanes were nearly com-

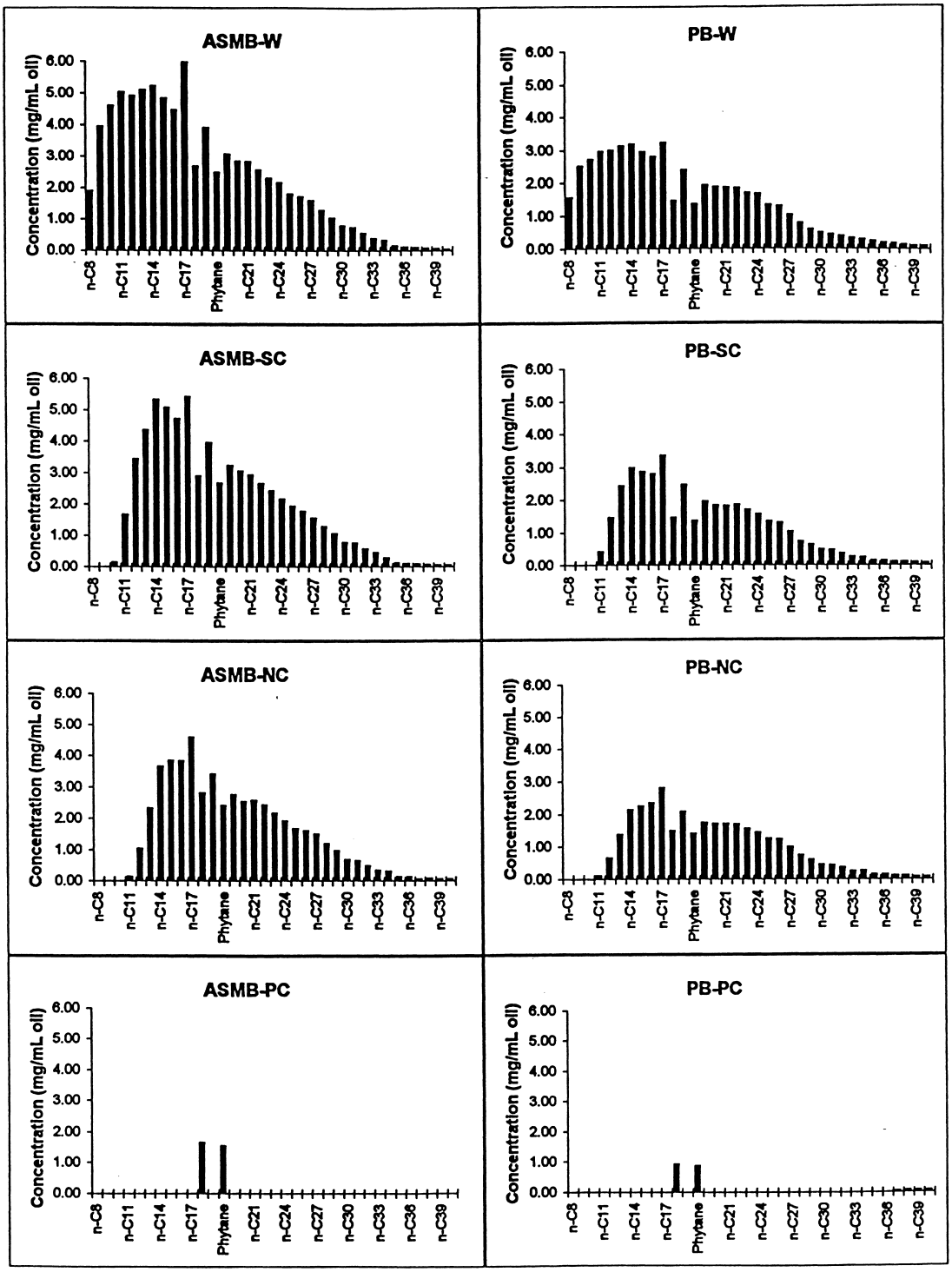

Fig. 3. Changes of *n*-alkane distributions (mg/ml oil) of ASMB and PB oil biodegradation series under the standard freshwater inoculum conditions. W, SC, NC and PC represent the weathered source oil, SC, NC and PC oil biodegradation samples.

Table 3
Composition changes of eight oils during biodegradation under freshwater conditions (28 days at 10°C)

	Concentration of hydrocarbon groups[b]					% Hydrocarbons lost[c]					Ratios of target hydrocarbons				
Eight oils[a]	TPH (mg/ml)	Total saturates (mg/ml)	Total aromatics (mg/ml)	Total *n*-alkanes (mg/ml)	Total 5-PAH homologues (μg/ml)	TPH lost (%)	Saturates lost (%)	Aromatics lost (%)	*n*-Alkanes lost (%)	5-PAH homologues lost (%)	n-C_{17}/ pristane	n-C_{18}/ phytane	Pristane/ phytane	GC-resolved/ UCM	*n*-Alkanes/ (pristane+phytane)
PB-W	567	436	131	64.2	11 548						2.25	1.78	1.07	0.25	18.45
PB-SC	460	324	136	39.1	10 291						2.31	1.81	1.07	0.15	14.02
PB-PC	315	205	111	1.9	3710	32	37	19	95	64	0.00	0.00	1.10	0.09	1.07
PB-NC	393	281	112	32.1	3978	15	13	18	18	33	1.92	1.52	1.07	0.14	11.75
ASMB-W	570	449	121	80.6	8501						2.00	1.58	1.07	0.35	15.77
ASMB-SC	516	398	118	66.5	7696						1.88	1.48	1.08	0.24	12.01
ASMB-PC	365	275	90	4.3	4492	29	31	24	94	42	0.00	0.00	1.19	0.08	1.00
ASMB-NC	452	363	90	53.6	3790	13	9	24	19	51	1.65	1.41	1.15	0.24	10.18
CLB-W	446	296	150	9.1	5554						1.53	0.91	0.79	0.09	26.57
CLB-SC	418	276	142	3.6	5608						1.46	0.92	0.80	0.07	10.44
CLB-PC	382	261	120	0.4	3411	9	5	15	90	39	0.00	0.00	0.84	0.07	3.53
CLB-NC	406	279	127	2.6	3554	3	2	11	27	37	0.92	0.61	0.81	0.07	8.47
BH-W	568	484	84	80.1	1307						3.41	1.90	0.73	0.37	22.78
BH-SC	473	390	83	60.6	1269						3.23	1.81	0.74	0.27	16.62
BH-PC	277	207	70	0.6	490	41	47	16	99	61	0.00	0.00	0.76	0.05	1.00
BH-NC	408	339	70	43.9	412	14	13	16	28	68	2.58	1.60	0.80	0.23	13.55
NW-W	549	405	144	43.4	7716						1.53	1.13	0.96	0.26	12.58
NW-SC	503	353	150	32.8	7465						1.54	1.14	0.99	0.17	8.80
NW-PC	373	270	103	3.1	3465	26	23	32	91	54	0.00	0.00	0.99	0.09	1.00
NW-NC	460	325	135	27.4	3917	9	8	11	17	48	1.33	0.97	0.98	0.15	7.85
BC-W	310	218	92	33.9	15 943						2.89	2.00	0.71	0.27	36.93
BC-SC	303	213	91	33.8	18 263						2.64	2.03	0.81	0.24	34.68
BC-PC	234	151	83	1.3	9963	23	29	9	96	45	0.00	0.00	0.52	0.10	22.87
BC-NC	274	188	86	25.4	13 357	10	12	5	25	27	1.80	1.41	0.84	0.20	27.43
TN-W	497	391	106	81.8	5208						3.02	1.37	0.62	0.36	18.63
TN-SC	430	320	110	56.0	4303						2.83	1.35	0.65	0.25	16.25
TN-PC	276	180	96	1.1	2118	36	44	13	98	51	0.00	0.00	0.45	0.07	1.00
TN-NC	420	326	94	50.0	2200	3	0	15	16	49	2.50	1.19	0.65	0.25	13.55
OS-W	612	475	137	76.1	5766						2.30	1.72	1.07	0.30	18.53
OS-SC	503	380	123	54.2	5165						2.30	1.72	1.07	0.20	13.40
OS-PC	372	264	107	2.6	3067	26	31	13	95	41	0.00	0.00	1.19	0.07	1.00
OS-NC	475	375	94	45.7	1944	6	1	24	16	62	2.02	1.58	1.10	0.19	8.69

[a] PB: Prudhoe Bay; CLB: Cold Lake Bitumen; BH: Bent Horn; NW: Norman Wells; BC: Bunker C; TN; Terra Nova; OS: Oseberg. [b] The values of TPH, total saturates, total aromatics and total *n*-alkanes were determined by GC methods and expressed in mg/ml for all samples except CLB and BC samples which are expressed in mg/g or μg/g oil. [c] The values of hydrocarbons lost were obtained using the SC as the reference; W, SC, PC and NC represent the weathered source oil, sterile control, positive and negative control, respectively. The values are mean values for PC ($n=3$) and NC ($n=2$).

pletely degraded and the isoprenoids pristane and phytane were the only two branched alkanes detected in the PCs after incubation but that even these had been reduced in abundance, resulting in a decrease in the ratios of n-C_{17}/pristane and n-C_{18}/phytane from 0.6–1.1 to 0.00 for the eight tested oils (Table 2). Changes in ratios of n-C_{17}/pristane and n-C_{18}/phytane have been widely used for years as indicators of biodegradation. However, one must be cautious in using the ratios to estimate the degree of oil biodegradation, because the use of the ratios may underestimate the magnitude of oil biodegradation due to the fact that the isoprenoids are also biodegradable, even though at slower rates under most environmental conditions [7,16,17,26–28]. A new method using the highly biodegradation-resistant hopanes as conserved internal standards to estimate the depletion of residual oil have been proposed [26,29] and successfully used for determining the weathered percentages of oiled sediment samples from an Arctic beach [17]. It has been demonstrated that this method can provide a more accurate representation of the degree of degradation than do the traditional n-alkane/isoprenoid ratios.

(5) Among isoprenoids, the preferential degradation of shorter chain length compounds was also observed. Fig. 1D clearly shows that smaller isoprenoids (such as farnesane, trimethyl-C_{13} and norpristane) were completely gone in the PCs, and only pristane and phytane with reduced abundances were detected.

3.2. Composition changes of aromatic hydrocarbons

Fig. 4 shows the total ion GC–MS chromatograms of the aromatic fraction of PB oil biodegradation series. Note that removal of peaks from the chromatogram does not necessarily mean that full biodegradation of aromatics to CO_2 has occurred. Incomplete oxidation of the aromatic ring by cometabolism would be sufficient to reduce the size of the corresponding peak in the chromatogram. Fig. 5 graphically depicts the distribution changes of five target alkylated PAH homologues in ASMB and PB oil series. Table 4 summarizes changes in the relative distribution of PAHs in each alkylated PAH homologous family and within isomeric group of ASMB and

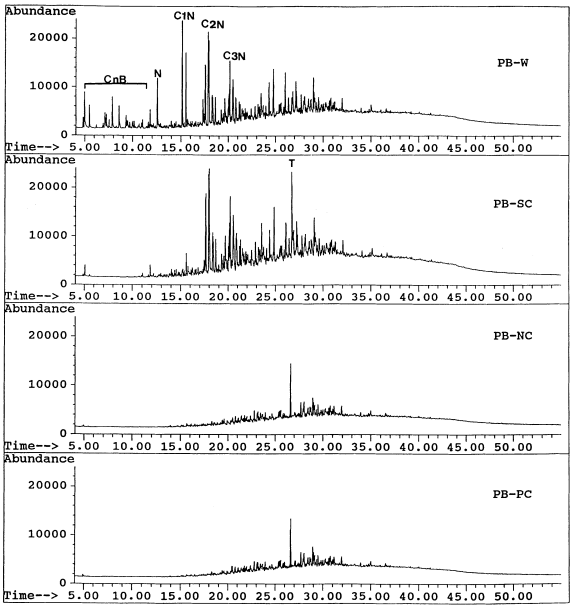

Fig. 4. Total ion chromatograms of the aromatic fraction of PB oil biodegradation series under the standard freshwater inoculum conditions, illustrating chemical composition changes of aromatics caused by biodegradation. W, SC, NC, and PC have the same meaning as indicated in Fig. 3. T, CnB, N, C1N, C2N and C3N represent surrogate o-terphenyl, alkylbenzenes, naphthalene and alkylated (C_1-, C_2- and C_3-substituted) naphthalenes, respectively.

PB oil due to biodegradation. Key points from analyses of aromatic fractions can be summarized as follows. Some of the observations on aromatic composition changes are in agreement to the previous findings by other researchers [7,8,27–35], as discussed below.

(1) Among the five target alkylated PAH homologues, the alkyl homologues of naphthalene (the most abundant PAH, 2-rings) were the most easily degradable, followed by alkyl homologues of dibenzothiophene (sulphur-containing 3-rings, 12 carbons), fluorene (3-rings, 13 carbons), and then phenanthrene (3-rings, 14 carbons). This is consistent with previous reports on that the rate of degradation of PAHs is inversely proportional to the number of rings in the PAH molecule [16,17,32–35]. Alkylated chrysenes (4-rings) are the most biodegradation-resistant homologues monitored. No degradation of chrysene or alkylated homologues was observed by using this inoculum. However, this should not be taken to imply that these compounds cannot be

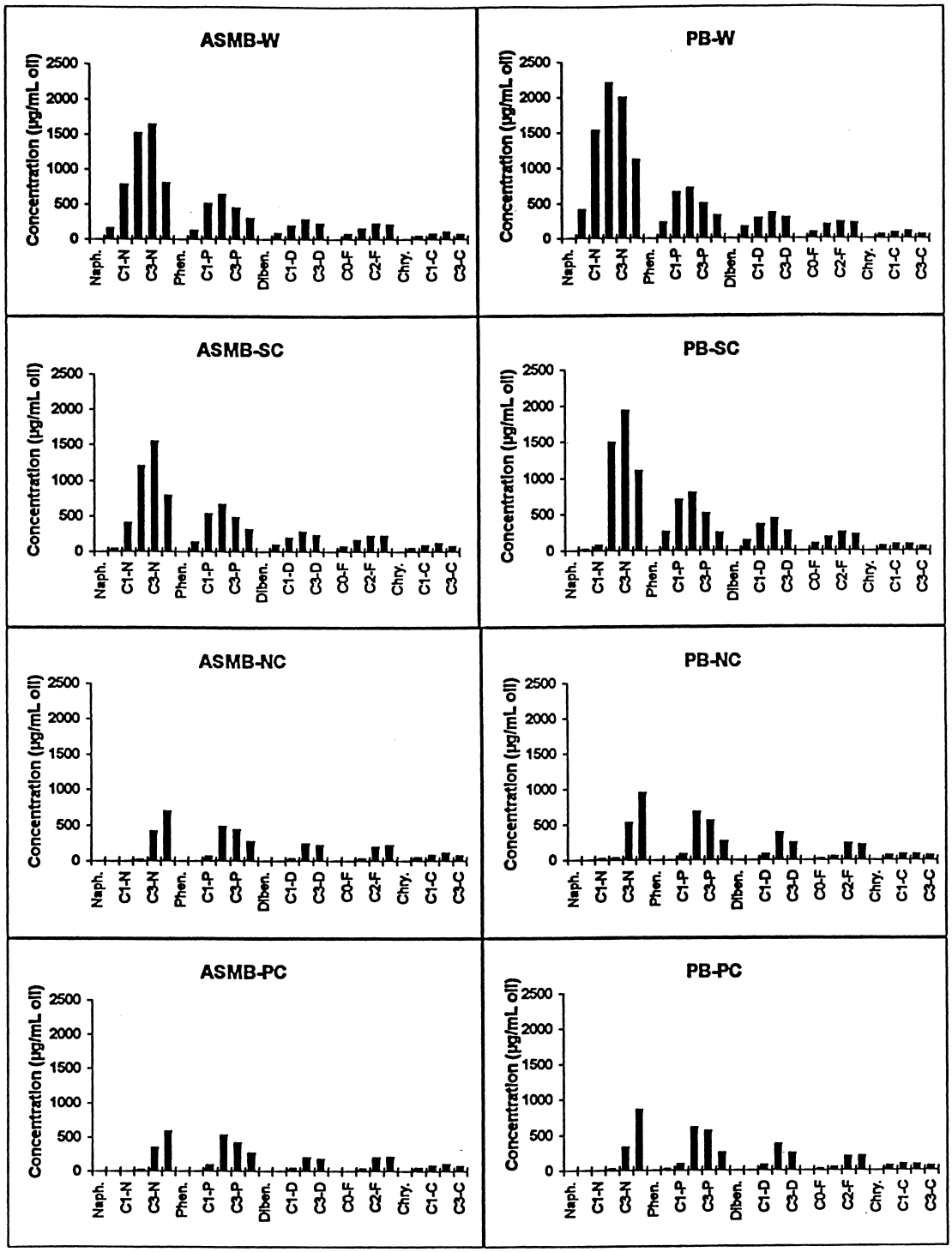

Fig. 5. Distribution changes (in µg/ml) of five target alkylated homologous PAHs in ASMB and PB oil biodegradation series under the standard freshwater inoculum conditions. W, SC, NC and PC have the same meaning as indicated in Fig. 3. N, P, D, F and C represent naphthalene, phenanthrene, dibenzothiophene, fluorene and chrysene, respectively; 0–4 represent carbon number of alkyl groups in alkylated PAH homologues.

Table 4
Changes in the relative distribution of PAHs in each alkylated PAH homologous family and within isomeric group of ASMB and PB oil due to biodegradation and due to weathering.

	Relative distribution within alkylated homologous families													
	PB (biodegradation)				ASMB (biodegradation)				ASMB[b] (after physical weathering)					
	W[a]	SC[a]	PC[a]	NC[a]	W	SC	PC	NC	0%	9.8%	19.5%	29.8%	34.5%	44.5%
Naphthalenes														
C_0-N	0.06	0.00	0.00	0.00	0.06	0.03	0.00	0.00	0.08	0.08	0.08	0.07	0.04	0.00
C_1-N	0.18	0.05	0.00	0.00	0.21	0.17	0.00	0.00	0.19	0.19	0.19	0.19	0.17	0.02
C_2-N	0.30	0.36	0.01	0.01	0.33	0.35	0.03	0.07	0.32	0.31	0.31	0.32	0.34	0.25
C_3-N	0.29	0.40	0.26	0.24	0.28	0.31	0.61	0.56	0.29	0.29	0.29	0.29	0.31	0.47
C_4-N	0.17	0.19	0.73	0.74	0.12	0.14	0.35	0.37	0.13	0.13	0.13	0.14	0.15	0.26
Sum	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Phenanthrenes														
C_0-P	0.09	0.10	0.00	0.00	0.09	0.08	0.00	0.00	0.08	0.08	0.08	0.08	0.08	0.08
C_1-P	0.27	0.27	0.05	0.03	0.31	0.31	0.26	0.19	0.28	0.28	0.28	0.28	0.28	0.28
C_2-P	0.30	0.31	0.40	0.40	0.27	0.27	0.34	0.35	0.34	0.34	0.34	0.34	0.34	0.34
C_3-P	0.21	0.20	0.37	0.40	0.21	0.22	0.25	0.29	0.19	0.20	0.20	0.20	0.19	0.20
C_4-P	0.13	0.12	0.17	0.17	0.12	0.12	0.15	0.17	0.11	0.11	0.11	0.11	0.11	0.11
Sum	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Dibenzothiophenes														
C_0-D	0.15	0.11	0.00	0.00	0.15	0.15	0.00	0.00	0.15	0.15	0.15	0.15	0.15	0.14
C_1-D	0.26	0.29	0.09	0.05	0.27	0.27	0.24	0.18	0.28	0.28	0.28	0.28	0.28	0.28
C_2-D	0.33	0.36	0.54	0.57	0.34	0.34	0.44	0.48	0.34	0.34	0.34	0.35	0.34	0.35
C_3-D	0.26	0.24	0.36	0.37	0.24	0.25	0.31	0.34	0.23	0.23	0.23	0.23	0.23	0.23

Sum	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Fluorenes														
C_0-F	0.11	0.11	0.01	0.01	0.10	0.10	0.00	0.00	0.09	0.09	0.09	0.09	0.09	0.08
C_1-F	0.26	0.24	0.08	0.06	0.22	0.22	0.18	0.14	0.22	0.22	0.22	0.22	0.22	0.21
C_2-F	0.33	0.34	0.44	0.45	0.34	0.34	0.41	0.41	0.36	0.36	0.36	0.36	0.36	0.36
C_3-F	0.30	0.30	0.46	0.48	0.34	0.35	0.41	0.44	0.33	0.33	0.34	0.34	0.34	0.36
Sum	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Chrysenes														
C_0-C	0.19	0.21	0.20	0.20	0.16	0.16	0.16	0.16	0.14	0.15	0.15	0.15	0.15	0.15
C_1-C	0.29	0.30	0.30	0.29	0.24	0.24	0.24	0.25	0.22	0.22	0.22	0.22	0.22	0.22
C_2-C	0.31	0.31	0.32	0.31	0.34	0.33	0.33	0.34	0.32	0.32	0.33	0.32	0.33	0.33
C_3-C	0.20	0.19	0.18	0.20	0.27	0.27	0.27	0.26	0.32	0.31	0.31	0.31	0.30	0.30
Sum	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	Relative distribution within given alkylation isomeric series													
(1,3+1,6-dimethyl-*N*)/ total of C_2-N	0.50	0.51	0.26	0.21	0.50	0.50	0.03	0.08	0.49	0.49	0.49	0.49	0.49	0.52
(3+2-methyl-P)/ (4-/9+1-methyl-P)	0.72	0.70	0.04	0.24	0.98	0.98	0.68	0.13	0.96	0.95	0.96	0.95	0.96	0.96
4-:2-/3-:1-methyl-DBT	1.00:	1.00:	1.00:	1.00:	1.00:	1.00:	1.00:	1.00:	1.00:	1.00:	1.00:	1.00:	1.00:	1.00:
	0.63:	0.62:	0.00:	0.00:	0.73:	0.73:	0.44:	0.00:	0.76:	0.75:	0.76:	0.75:	0.75:	0.76:
	0.29	0.29	0.45	0.69	0.27	0.26	0.35	0.26	0.28	0.29	0.28	0.28	0.28	0.29
Methyl-F/total of C_1-F	0.31	0.33	0.02	0.00	0.35	0.35	0.29	0.06	0.36	0.35	0.35	0.36	0.35	0.36

[a] W: Weathered oil; SC: sterile control; PC: positive control and NC: negative control. The values given in this Table are mean values ($n=2$ for SC and NC, $n=3$ for PC).

[b] See Ref. [42] for detailed weathering results.

biodegraded. Mineralization of chrysene by other microorganisms in soil has been reported by Carmichael and Pfaender [36], and also in 22-year-old Arrow oil samples [16].

Among other target PAHs, low-molecular-mass (M_r) biphenyl, acenaphthalene and acenaphthene were nearly completely lost, while other higher ring-number PAHs were shown to be only minimally degraded if at all.

(2) An increase in alkylation level decreases susceptibility to microbial attack, as previously described by Fedorak and Westlake [30]. The degradation order of alkylated PAHs was $C_0>C_1>C_2>C_3>C_4$ in each alkylated PAH family, It was especially obvious within the naphthalene family that, as the size of the molecule increased, the amount of degradation decreased. Table 4 lists the relative distribution of PAHs in each alkylated PAH homologous family of ASMB and PB oils. Within homologous series the relative ratios of parent and C_1-substituted PAHs (and C_2-naphthalenes too) strikingly decreased, while ratios of C_2, C_3 and C_4 alkylated homologues significantly increased, in comparison to the weathered and SCs. For example, compared to the SC of ASMB oil, the relative percentages of C_0-N, C_1-N, C_2-N, C_3-N and C_4-N within the alkylnaphthalene series of the PCs were decreased or increased from 0.03 to 0.00, 0.17 to 0.00, 0.35 to 0.03, and from 0.31 to 0.61 and 0.14 to 0.35, respectively. This degradation trend and pattern was observed for all oils studied.

(3) GC-resolved aromatics degraded, in general, faster than unresolved aromatic hydrocarbons, indicated by a much higher percentage of the five target alkylated PAH homologues lost than that of the total aromatics lost (see Table 3, 27–64% of five target PAH homologues lost versus 5–32% of total aromatics lost). For example, the positive and negative controls of PB oil showed 64% and 33% of five target alkylated PAH homologues lost, while only 19% and 18% of the aromatics degraded. The parameter of five target alkylated PAH homologue loss can be used as an indicator of the degree of biodegradation of petroleum aromatics;

(4) Microbial degradation was demonstrated to be isomer specific for alkylated PAHs [28,37–39]. The details will be discussed in Section 3.4. The use of the ratios of three isomers of methyldibenzothiophenes (4-methyl-DBT:2-/3-methyl-DBT:1-methyl-DBT) for source identification and indicating the occurrence of biodegradation has been reported [37]. For example, the ratios of three isomers of methyldibenzothiophenes were determined to be 1.0:0.75:0.29 and 1.0:0.62:0.29 for the weathered ASMB oil and the corresponding SCs, and weathered PB oil and the corresponding SCs, respectively (Table 4). However, as biodegradation proceeded, not only did the absolute concentrations of C_1-dibenzothiophenes pronouncedly decrease, but the three isomers of methyldibenzothiophenes showed great alteration in their relative ratios (1.0:0.44:0.35 and 1.0:0.00:0.26 for the positive and negative controls of ASMB oil, and 1.0:0.00:0.45 and 1.0:0.00:0.69 for the positive and negative controls of PB oil, respectively). Obviously, 2-/3-methyldibenzothiophene biodegraded at the fastest rate within the isomeric series, indicated by the striking decrease of its relative ratio to 4-methyldibenzothiophene; while 1-methyldibenzothiophene is slightly more resistant to biodegradation than 4-methyldibenzothiophene, indicated by the increase of its relative ratio to 4-methyldibenzothiophene. This effect is mirrored in the significant decrease in the relative isomeric distribution of (3+2-methylphenanthrenes) to (4-/9-+1-methylphenanthrenes), (1,3+1,6-dimethylnaphthalenes) to the total of C_2-naphthalenes, and methylfluorene to the total of C_1-fluorenes (see Table 4 for detailed ratio values).

(5) The weathered crude oils still contained significant amounts of BTEX (the collective name of benzene, toluene, ethylbenzene and the xylene isomers) and other alkylbezenes despite the physical weathering treatment, but the corresponding SCs only contained very small amounts. This indicates that evaporative loss of BTEX and other alkylbenezenes occurred during the incubation period. The concentrations of BTEX and C_3-benzenes were determined to be well under 20 μg/g oil for the SCs. Therefore, the effect of loss of BTEX and alkylbenzenes due to biodegradation on the loss of the total aromatics can be considered negligible for the oils tested. However, BTEX would be present, at least initially, in a spill and might be important, depending on the contaminant composition and other environmental factors such as temperature.

(6) The ease of microbial attack is very dependent on oil composition and biodegradation conditions. In general, degradation of TPH, saturates and *n*-alkanes was significantly enhanced in the presence of nutrients (Table 3) [1,2]. However, for many oils, the degradation of total aromatics and five alkylated PAH homologous series was not enhanced by addition of nutrients. In some cases, greater losses occurred in the absence of nutrients, such as the degradation of Oseberg oil showed. This finding is not completely understood yet, but it has also been reported by Fedorak and Westlake [40] from a study of PB oil in saltwater collected off the coast of the Washington State, by Fayad and Overton [28] from a study of oil mousse collected during the 1991 Gulf Spill, and by Wrenn et al. [41] from a study of effects of nitrogen source on crude oil biodegradation. One possible hypothesis is that aromatic oxidation occurs in the absence of growth through co-metabolism; that is, through existing enzyme activity even in the absence of nutrients. More experiments are underway by Environment Canada to investigate this phenomenon.

3.3. Composition changes of biomarker compounds

The experiments clearly demonstrated that there was no observable sign of alteration of biomarker compounds, regardless of oil type, incubation conditions and nutrient presence (Table 5). The concentrations of all monitored terpane and sterane compounds in each oil tested were found to be quite constant, indicating that these compounds are resistant to biodegradation by the consortium used in these experiments. The ratios of selected paired terpanes and steranes also remained constant. To illustrate that, the average of the sum of ratios for the seven paired target terpanes C_{23}/C_{24}, Tm/Ts [Tm: 17α(H),21β(H)-22,29,30-trisnorhopane, Ts: 18α(H),21β(H)-22,29,30-trisnorhopane], C_{29}/C_{30}, $C_{32}(S)/C_{32}(R)$, $C_{33}(S)/C_{33}(R)$, C_{23}/C_{30}, C_{24}/C_{30} and 1 paired steranes C27-αββ/C29-αββ from 70 biodegradation samples of ASMB oil inoculated under various inoculum conditions during 1994 were determined to be 8.2 ± 0.2 with the relative standard deviation under 4%. Table 5 presents, as an example, relative ratios of eight paired target biomarker compounds of four tested oils. In contrast, pristane and phytane showed reduction in their concentrations, and the ratios of C_{17}/pristane, C_{18}/phytane and pristane/phytane were significantly altered as well (Fig. 3 Table 3), indicating that degradation of pristane and phytane had occurred.

3.4. Characteristic oil biodegradation patterns and diagnostic ratios for distinguishing biodegradation from physical weathering

Physical weathering and biodegradation are both important factors to consider after an oil spill. Differentiation of the characteristic oil composition changes due to biodegradation and/or due to physical weathering is very important for planning an effective clean-up strategy, assessing the biodegradability of spilled oil and evaluating the efficacy of any bioremediation products and other oil-spill treating products employed at the spill site.

Comprehensive quantitative results from the study of effects of weathering on chemical composition changes of ASMB oil at various degrees of weathering (0–45% loss by mass) have been reported [42]. The main findings are briefly summarized as follows (Table 4):

(1) Simultaneous loss of low-M_r and a corresponding increase in the proportion of high-M_r oil hydrocarbons was obvious.

(2) Ratios of *n*-C_{17}/pristane, *n*-C_{18}/phytane, pristane/phytane were virtually unaltered because these compounds have about the same volatility and solubility.

(3) Six ASMB oil samples weathered 0% to 45% showed only small changes in total *n*-alkanes (in the range of 70–76 mg/g oil) as the weathering percentages increased. However, the five target alkylated PAH homologues at all alkylation levels (except naphthalene for 45% weathered ASMB) showed concentration increases as the weathering percentages increased. This has been demonstrated to be due to loss of low-M_r components by evaporation, with the corresponding effective concentration of high-M_r components due to oil volume reduction.

(4) Naphthalene and C_1-naphthalenes showed significant decreases in distribution ratios within the alkylnaphthalene family only at the highest weathering percentage, 45%.

(5) No significantly noticeable loss due to evapo-

Table 5
Comparison of diagnostic ratios of target biomarkers under freshwater inoculum conditions (28 days at 10°C)

	ASMB oil							CLB oil							NW oil							TN oil						
	W	SC	PC1	PC2	PC3	NC1	NC2	W	SC	PC1	PC2	PC3	NC1	NC2	W	SC	PC1	PC2	PC3	NC1	NC2	W	SC	PC1	PC2	PC3	NC1	NC2
C_{23}/C_{24}	1.73	1.67	1.71	1.75	1.70	1.71	1.77	1.85	1.82	1.87	1.84	1.80	1.83	1.89	1.27	1.29	1.31	1.33	1.32	1.34	1.29	0.86	0.94	0.91	0.84	0.81	0.82	0.84
Tm/Ts	0.98	1.23	1.21	1.22	1.06	0.93	0.99	3.70	3.72	4.19	4.20	3.82	4.16	3.84	2.68	2.96	2.65	2.77	2.73	2.56	2.58	1.41	1.17	1.19	1.18	1.20	1.30	1.34
C_{29}/C_{30}	0.53	0.51	0.53	0.50	0.52	0.53	0.52	0.75	0.77	0.76	0.77	0.74	0.77	0.76	0.65	0.65	0.62	0.62	0.61	0.64	0.65	0.32	0.34	0.34	0.33	0.35	0.33	0.35
$C_{32}(S)/C_{32}(R)$	1.58	1.60	1.61	1.59	1.52	1.59	1.56	1.44	1.50	1.59	1.47	1.51	1.44	1.38	1.49	1.48	1.52	1.59	1.63	1.58	1.48	1.44	1.48	1.42	1.53	1.49	1.45	1.43
$C_{33}(S)/C_{33}(R)$	1.52	1.47	1.48	1.37	1.43	1.53	1.53	1.49	1.43	1.43	1.48	1.48	1.49	1.52	1.58	1.46	1.42	1.57	1.58	1.49	1.46	1.46	1.48	1.51	1.55	1.53	1.47	1.57
C_{23}/C_{30}	0.44	0.47	0.47	0.48	0.47	0.45	0.46	0.53	0.46	0.45	0.46	0.46	0.46	0.47	0.63	0.59	0.55	0.56	0.65	0.51	0.65	0.05	0.05	0.05	0.05	0.05	0.05	0.05
C_{24}/C_{30}	0.25	0.28	0.28	0.27	0.28	0.26	0.26	0.29	0.25	0.24	0.25	0.25	0.25	0.25	0.47	0.46	0.42	0.42	0.49	0.38	0.50	0.06	0.06	0.05	0.06	0.06	0.06	0.06
$C_{27}\alpha\beta\beta/C_{29}\alpha\beta\beta$ steranes	0.97	0.93	1.01	1.02	1.02	1.01	0.92	0.94	0.76	0.74	0.83	0.92	0.85	0.88	0.68	0.71	0.70	0.78	0.71	0.70	0.72	1.54	1.64	1.51	1.54	1.65	1.48	1.54
Total	8.0	8.2	8.3	8.2	8.0	8.0	8.0	11.0	10.7	11.3	11.3	11.0	11.3	11.0	9.5	9.6	9.2	9.6	9.7	9.2	9.3	7.1	7.2	7.0	7.1	7.1	7.0	7.2

C_{23}, C_{24}, Tm, Ts, C_{29}, C_{30}, C_{32} and C_{33} represent biomarker triterpanes; $C_{27}\alpha\beta\beta$ and $C_{29}\alpha\beta\beta$ represent biomarker steranes (Ref. [14,15]).

ration was observed for chrysenes, phenanthrene, dibenzothiophenes, fluorenes and their alkylated homologues, especially for the alkylated chrysene and phenanthrene series. More importantly, PAHs within each alkylated homologous family did not show alterations of their relative distributions.

(6) Isomeric distributions within C_1-dibenzothiophenes, C_1-, C_2- and C_3-naphthalenes, C_1-phenanthrene, C_1-fluorenes exhibited great consistency in their relative ratios for ASMB weathered from 0 to 45%. Excellent consistency of the relative distribution within the C_1-dibenzothiophene series has been demonstrated not only for artificially weathered oils, but also for many short-term field weathering and burned oil samples, and has been used for source identification and differentiation of crude and weathered oils [37].

(7) No evaporative losses were observed for biomarker terpanes and steranes and their relative ratios within isomers and between isomeric groups were virtually constant.

The data from the laboratory shaker flask biodegradation studies of many different oils and oil products demonstrate that biodegradation causes a number of characteristic and distinct oil composition changes to occur, which are significantly different from composition changes caused by physical weathering.

Changes in n-C_{17}/pristane and n-C_{18}/phytane ratios have been long recognized and used as indicators of biodegradation. As for alkylated PAH isomers, however, only some very limited results on their unique composition changes due to biodegradation have been reported [27,28,37,38]. Table 4 summarizes the relative distribution changes of alkylated PAHs in each alkylated homologous family and within isomeric groups of ASMB and PB oils due to biodegradation.

Table 4 clearly demonstrates pronounced differences in both absolute and relative distribution changes caused by biodegradation and weathering. First, as described above, all target parent PAHs (except chrysene), 2- and 1-methylnaphthalene, plus biphenyl, acenaphthalene and acenaphthene in the tested oils were significantly biodegraded, and their concentrations decreased to around 5 μg/g oil from 100–400 μg/g oil (Table 3). Second, the relative distributions of PAH groups with different levels of alkylation within their alkylated homologous families was greatly altered (Table 4). This alteration is significantly different from the change in the distribution patterns caused by weathering [42]. For example, the relative distributions of the parent and alkylated naphthalenes (C_0-N:C_1-N:C_2-N:C_3-N:C_4-N) were determined to be 0.00:0.02:0.25:0.47:0.26 for the most highly weathered (44.5% mass loss) ASMB oil sample, and 0.00:0.00:0.03:0.61:0.35 for the biodegraded ASMB PC sample. Thirdly, a number of isomeric groups demonstrated unique degradation pattern changes, in both concentrations and relative distributions (see Fig. 5 and Table 4 for details). Fig. 6A–E show representative GC–MS fragmentograms of C_2-naphthalenes (m/z 156, after 14 days), C_3-naphthalenes (m/z 170, after 28 days), C_1-phenanthrene (m/z 192, after 14 or 28 days), C_1-dibenzothiophenes (m/z 198, after 14 or 28 days) and C_1-fluorenes (m/z 180, after 28 days) in the SC PB oil and the corresponding PCs. The circled regions in Fig. 6 indicate the characteristic changes in distribution within isomers that were preferentially altered by biodegradation and/or co-metabolic oxidation. Fig. 6 clearly shows that among six identified C_2-naphthalenes (Fig. 6A: ethyl-, 2,6-, 1,3-, 1,6-, 2,3- and 1,2-dimethylnaphthalene), 10 identified C_3-naphthalenes (Fig. 6B: C3-, ββ-ethylmethyl-, αβ-ethylmethyl-, 1,3,7-, 1,3,6-, 1,3,5-, 2,3,6-, 2,3,5-, 1,2,7- and 1,2,5-trimethylnaphthalene), three identified C_1-fluorenes (Fig. 6C: methyl-, 2- and 1-methylfluorene), four identified C_1-phenanthrenes (Fig. 6D: 3-, 2-, 4-/9- and 1-methylphenanthrene) and three identified C_1-dibenzothiophenes (Fig. 6E: 4-, 2-/3- and 1-methyldibenzothiophene), bacteria preferentially degraded 1,3- and 1,6-dimethylnaphthalene; C_3-, ββ-ethylmethyl-, 1,3,7-, 1,3,6-, 1,3,5- and 1,2,5-trimethylnaphthalene; methylfluorene (completely gone after 28 days); 2-methylphenanthrene (after 14 days) and then 3- and 1-methylphenanthrene (after 28 days); and 2-/3-methyldibenzothiophene (significantly decreased in abundance after 14 days and completely removed after 28 days), resulting in great changes in the relative ratios of (1,3-methylnaphthalene+1,6-dimethylnaphthalene)/total of C_2-N, methylfluorene/total of C_1-F, (3-methylphenanthrene+2-methylphenanthrene)/(9-methylphenanthrene + 1-methylphenanthrene) and 2-/3-methyl-dibenzothiophene/4-

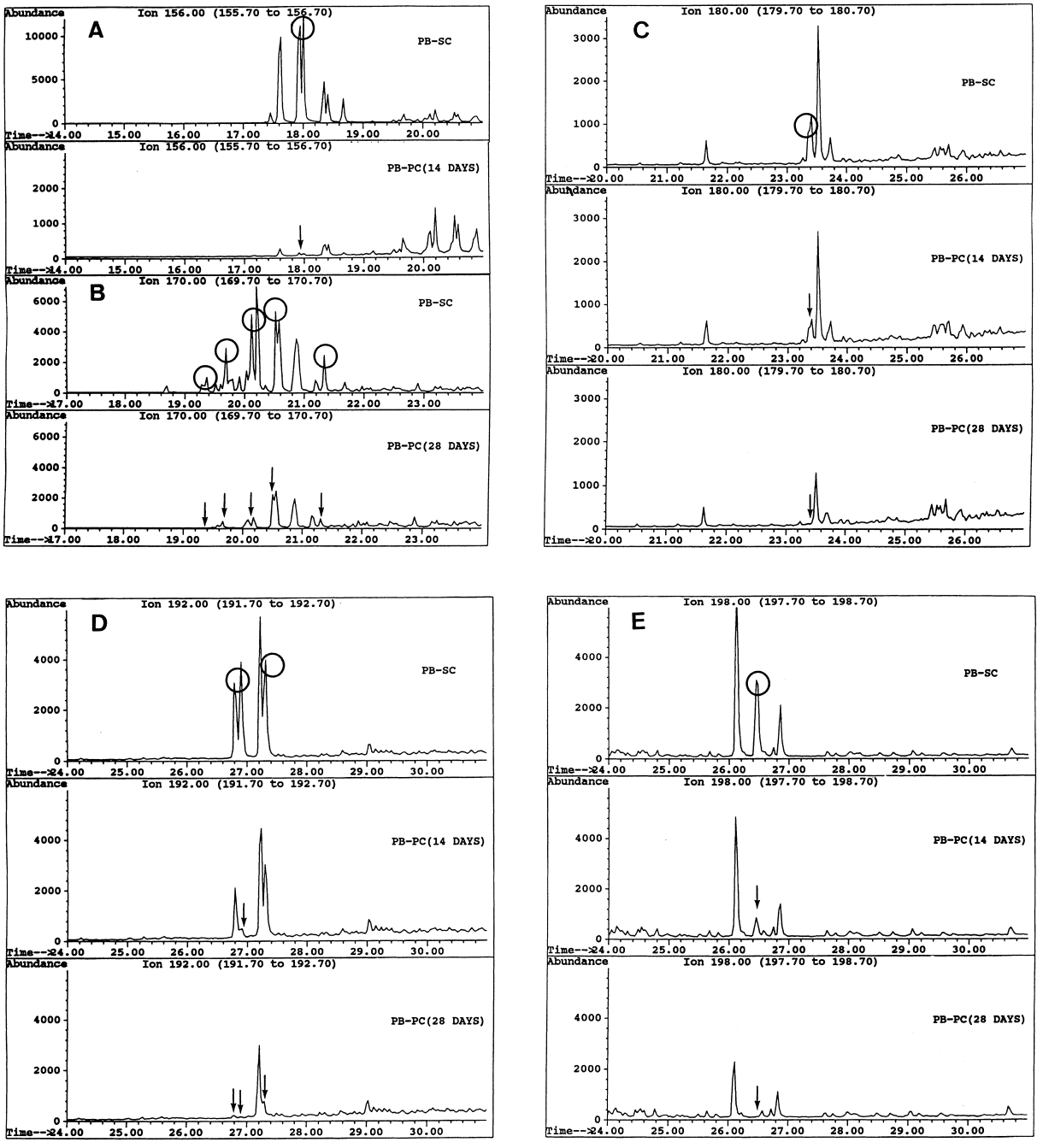

Fig. 6. Extracted ion chromatograms for C_2-naphthalenes [ion 156, after 14 days, (A)], C_3-naphthalenes [ion 170, after 28 days, (B)], C_1-fluorenes [ion 180, after 14 and 28 days, (C)], C_1-phenanthrenes [ion 192, after 14 and 28 days, (D)] and C_1-dibenzothiophenes [ion 198, after 14 and 28 days, (E)] in the source sterile control PB oil and the corresponding positive controls, illustrating distinct and characteristic composition changes of PAHs due to biodegradation. The circled regions indicate the characteristic changes in distribution within isomers that were preferentially altered by biodegradation and/or co-metabolic oxidation.

methyldibenzothiophene. In sharp contrast, no such characteristic distribution pattern and diagnostic ratio changes were observed for physically and/or short-term weathered oils [37,42]. This kind of preferential biodegradation of certain isomers over other isomers within isomeric groups were observed not only for PB oil incubated for different periods (7, 14 and 28 days) at different temperatures (4, 10, 15 and 22°C), but also observed for other tested oils under varying incubation conditions and for nine Alaskan oils [43]. Therefore, these ratios derived using the defined consortium, may be useful when extrapolated to the natural environment for indicating the occurrence of biodegradation. These data, when used in conjunction with quantitative data on the changes in distribution of the entire PAH homologous groups, can more accurately assess the extent and progress of oil biodegradation.

The concentrations and relative distribution changes among and between isomers of alkyl PAH homologous groups determined from hundreds of different oil biodegradation samples in the past four years have demonstrated the usefulness of these biodegradation-characteristic parameters for indication of occurrence of biodegradation and in evaluating biodegradation degree and biodegradability of oils. The preference for the biodegradation of different isomers of the alkylated PAHs was also seen for the 25-year-old Nipisi oil spill samples in the natural environment [44].

3.5. Biodegradation potential index

All of the oil tested had measurable losses of hydrocarbons as a result of incubation with the defined inoculum under the stated conditions. In terms of ranking oils with respect to biodegradation potential, Environment Canada and NOAA put forward two equations which combine losses in TPH and aromatics to produce a biodegradation index on a scale with a maximum value of ten [13].

Equal weighting of TPH and aromatic fraction losses:

$$\text{Index value} = [0.5(\text{mean \% GC} - \text{TPH loss}) + 0.5(\text{mean \% total aromatics loss})]/10 \quad (1)$$

A 30:70 weighting of TPH and aromatic fraction losses:

$$\text{Index value} = [0.3(\text{mean \% GC} - \text{TPH loss}) + 0.7(\text{mean \% total aromatics loss})]/10 \quad (2)$$

Obviously, the ranking index is relatively simple, so that it can be understood and used by a wide audience. Generally speaking, an overall indicator of oil biodegradation is desired, and that is provided by the ''GC-TPH loss'' grouping. However, since the GC-TPH grouping is largely composed of readily degradable saturates and *n*-alkanes, some means of emphasizing the aromatics degradation is desired, since aromatics including five target alkylated PAH homologues are considered to be more recalcitrant and some of their bio-oxidation products are potentially more toxic and carcinogenic than those derived from saturate metabolism. Therefore, the total aromatics is also chosen as a factor in the index. As for total saturates, since they are generally quite degradable, and are already accounted for in the TPH grouping, this class is not considered a relevant addition to the biodegradation index calculation.

Eqs. (1) and (2) were used to generate index values for each oil tested in this study (Fig. 7A,B). For example, given that the PB oil had mean TPH loss of 32% and a mean total aromatics loss of 19% in the PC (Table 3), the biodegradation index values were readily determined to be 2.55 and 2.29 using Eqs. (1) and (2), respectively. It can be seen from Fig. 7 that the light oils NW, BH and ASMB appear to be more biodegradable, while CLB and BC are consistently less biodegradable, as would be predicted by their high asphaltenic composition (Table 1). Within the light oils (e.g., NW and BH), there is a considerable difference in the aromatic content (11% and 5%, respectively); this parameter is more heavily weighted in Eq. (2), resulting in a differentiation between NW and BH degradability that is not evident when using Eq. (1) to calculate the biodegradability index (Fig. 7). The utility of these two proposed equations in predicting in situ biodegradability, and whether one equation is more suitable than the other remains to be seen as more studies of actual oil spill bioremediation data are reported.

The most biodegradable oil tested ranked only 3.02 on a scale of 10, using the defined inoculum. It is important to note that this value would be expected to vary, depending upon the inoculum and on the prevailing environmental conditions, so it cannot

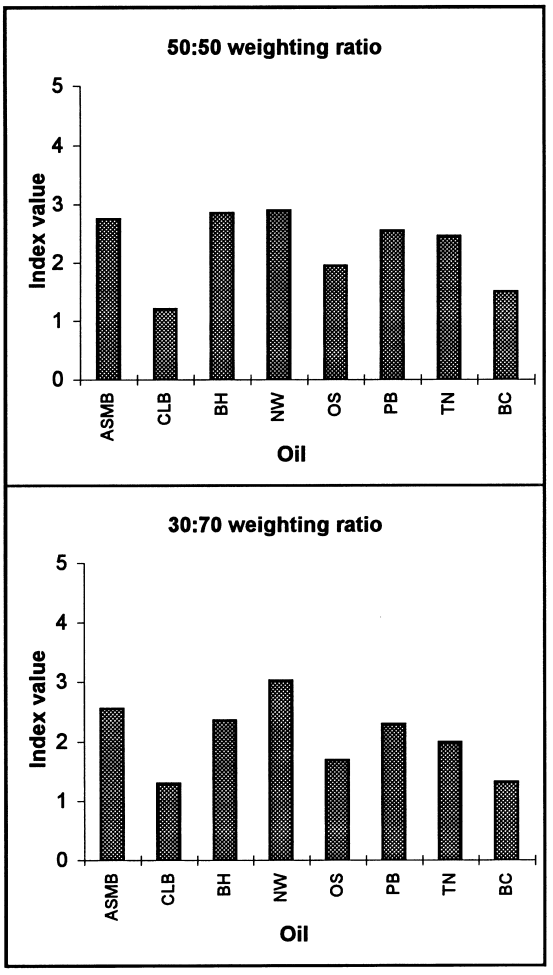

Fig. 7. Biodegradation indices for evaluating the biodegradation potential of eight oils tested under the freshwater conditions with nutrients. The index values were calculated from Eqs. (1) and (2), respectively.

be considered an absolute. In the context used here, the relative ranking of the oils is valid because all parameters are held constant, with only the oil composition varying. Similar results were also obtained from the NOAA warm marine biodegradation study [13]. These patterns of oil biodegradability, when used in conjunction with data on the efficacy of various oil spill bioremediation agents [10], would be valuable to spill clean-up decision makers when trying to determine if bioremediation is an appropriate clean-up tool.

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