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Effects of Temperature and Crude Oil Composition on Petroleum Biodegradation

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The biodegradability of seven different crude oils was found to be highly dependent on their composition and on incubation temperature. At 20 C lighter oils had greater abiotic losses and were more susceptible to biodegradation than heavier oils. These light crude oils, however, possessed toxic volatile components which evaporated only slowly and inhibited microbial degradation of these oils at 10 C. No volatile toxic fraction was associated with the heavier oils tested. Rates of oil mineralization for the heavier oils were significantly lower at 20 C than for the lighter ones. Similar relative degradation rates were found with a mixed microbial community, using CO_2 evolution as the measure, and with a *Pseudomonas* isolate from the Arctic, using O_2 consumption as the measure. The paraffinic, aromatic, and asphaltic fractions were subject to biodegradation. Some preference was shown for paraffin degradation, especially at low temperatures. Branched paraffins, such as pristane, were degraded at both 10 and 20 C. At best, a 20% residue still remained after 42 days of incubation. Oil residues generally had a lower relative percentage of paraffins and higher percentage of asphaltics than fresh or weathered oil.

Following the wreck of the Torrey Canyon many studies were undertaken to determine the fate of petroleum contaminants on the world's oceans. The ability of microorganisms to degrade a great number of hydrocarbons of varied structure found in petroleum has now been examined (7, 8, 10, 12, 14, 16). However, the biodegradability of only a limited number of crude oils of varied composition has been similarly studied (4, 6, 13, 17). A recent study by Westlake et al. (15) did compare the relationship between the composition of four crude oils and their biodegradability. Jobson et al. (9) also have reported that both low- and high-grade crude oils are subject to microbial degradation at 4 and 30 C. These studies report that certain hydrocarbons are more readily degraded than others and that environmental factors such as temperature can influence the ability of microorganisms to degrade petroleum hydrocarbons.

One should therefore consider individually the biodegradability of the great variety of crude oils that may contaminate the oceans under the various environmental conditions where contamination may occur. Concern should be given to the overall changes in the composition of each crude oil that occur during the process of microbial degradation and to the differences in the degradability of individual hydrocarbon components within the various crudes. Since petroleum contamination of the seas can occur at the point of production or en route to destination, biodegradation by different microorganisms under various environmental conditions, including different temperatures, should be examined. Special concern should be extended to the cold marine environment since: (i) a large percentage of the world's oceans are below 5 C; (ii) new oil production in polar regions is underway; (iii) low temperatures limit microbial activities; and (iv) oil contamination may upset the especially delicate ecosystem balance of cold environments.

In the study reported in this paper, the biodegradability of seven crude oils of varied composition was examined. Studies were carried out at 10 and 20 C with microbial communities and isolates from the Pacific and Arctic Oceans to model different conditions in which crude oil composition and temperatures could effect petroleum hydrocarbon degradation.

MATERIALS AND METHODS

Biodegradation experiments. Seawater samples were collected off the coasts of Santa Monica, Calif., and Barrow, Alaska. The seawater samples were supplemented with 10 mM nitrate and 0.5 mM phosphate ions, pH 8.1. Crude oils (1.0 g) were added to

100-ml samples of seawater in a gas train apparatus (3) with reciprocal shaking, 10 oscillations/min. Crude oils were sterilized by passage through silver filters (0.2-μm pore size; Flotronics, Spring House, Pa.). For controls, seawater was sterilized by passage through membrane filters (0.2-μm pore size; Millipore Corp., Bedford, Mass.). The crude oils used in these biodegradation experiments were Sweden crude, Solvent mix crude (27.7% Jameson, 8.6% Dori North, 2.6% Bateman Lake, 29.3% W. T. Intermediate, 4.0% Bronte, 19.0% Wilshire, and 8.8% Amacher-Tippett crudes), South Louisiana crude, Lagomedio crude, Mirando Humble crude, Prudhoe Bay crude, and Barrow crude (gifts of Sun Ventures, Exxon, Atlantic Richfield, and Naval Petroleum Reserve 4). These crudes represent a variety of oils that are presently or shortly will be transported by ship for refining. These crudes were chosen for this study because of their varied chemical and physical properties, e.g., specific gravities from 0.771 to 0.896 (Table 1). The names of the crudes are common names and do not necessarily reflect their composition or origin; e.g., Sweden crude is produced from wells in the Duval region of Texas. Descriptions of the general properties of these crude oils are available from the suppliers.

Densities of the seven oils (Table 1) were determined at 25 C by weighing replicate 10-ml volumes with a Cahn electrobalance (Cahn Instruments, Paramount, Calif.). Biodegradation experiments were carried out in replicate at 10 and 20 C. These temperatures were chosen because Arctic and northern Pacific Ocean surface waters were found to be between 10 and 20 C during the summer months. Mineralization, the conversion of organic petroleum components to inorganic CO_2, was monitored as CO_2 release as determined by periodic standard titration procedures (1). The residual oil was recovered from experimental flasks after 42 days of incubation by continuous liquid-liquid extraction for 24 h with diethyl ether. The solvent was then evaporated under nitrogen at 25 C, and the residual oil was analyzed by gravimetric and chromatographic procedures as described below.

In other experiments, oxygen consumption was measured with a Gilson respirometer (Gilson Electronics, Middleton, Wis.) by adding 0.05 ml of fresh crude oil or 0.05 ml of preweathered oil to 5 ml of Alaskan seawater samples which were supplemented with sodium nitrate and sodium phosphate.

TABLE 1. *Densities of tested crude oils*

Crude oil	Sp gravity (g/ml; 25 C)
Solvent mix crude	0.771
Sweden crude	0.813
South Louisiana crude	0.875
Lagomedio crude	0.884
Prudhoe crude	0.887
Barrow crude	0.889
Mirando Humble crude	0.896

The final nitrogen and phosphorus concentrations were 10 mM N and 0.5 mM P. Filter-sterilized water samples with added oil and inoculated seawater without added oil were included as controls. Samples were shaken at 60 oscillations/min. Final pH was 8.1. Preweathered crude oil was prepared by evaporation of the volatile fraction of fresh oil for 24 h at 25 C under a stream of nitrogen. Preweathered crude oil was used to separate possible inhibitory effects of compounds in the volatile fraction from direct temperature effects. The flasks in the Gilson respirometer were also inoculated with a *Pseudomonas* sp. which previously had been isolated from experimental oil slicks floated in Prudhoe Bay (5). A control inoculated with the *Pseudomonas* sp., but without crude oil, and a sterile control were also included. The inoculum was prepared by growing the organism on marine broth 2216 (Difco, Detroit, Mich.) at 20 C for 24 h, harvesting the cells by centrifuging at 5,000 × *g* for 15 min, and washing the cells with and suspending the cells in sodium nitrate (10 mM) and sodium phosphate (0.5 mM) buffer, pH 8.1. The inoculum was diluted with this buffer solution to yield a protein concentration of 1 mg of protein per ml. Protein concentration was determined by the technique of Lowry et al. (11). An inoculum equivalent to 0.1 mg of protein was added to flasks in the Gilson respirometer.

Analytical. Residual oil was separated into class fractions by silica gel chromatography using a glass column (2 by 40 cm outer dimension) packed with Silica Gel G (70 to 230 mesh; bed volume, 60 ml). Separation of the paraffinic, aromatic, and asphaltic fractions was accomplished by successive elution with 120-ml aliquots of hexane, benzene, and methanol, respectively. The solvent was evaporated under nitrogen, and the weight and weight percent of the three hydrocarbon classes was determined gravimetrically. This class separation is based on the method of Davis (6).

A mixture of known hydrocarbons containing 20% (by wt) each *n*-hexadecane, pristane, pentadecylcyclohexane, ethylnaphthalene, and cyclohexane carboxylic acid was used to test the efficiency of the column chromatographic separation procedure. An 0.5-g amount of the test mixture was added to the column and eluted with 120 ml each of hexane, benzene, and methanol. After solvent evaporation at 25 C under nitrogen, the eluted fractions were weighed and dissolved in 1 ml of diethyl ether. The ether solutions were analyzed by gas-liquid chromatography as described below. To facilitate the detection of cyclohexane carboxylic acid, aliquots of each eluted fraction were treated with 10% (wt/vol) BCl_3-CH_3OH (Applied Science Lab., State College, Pa.) to form the methyl ester, prior to gas chromatographic analysis. Compounds in the eluted fractions were identified by comparison with gas chromatographic retention times of the individual components in the test mixture and quantitated by comparison with the peak height response of these individual components.

The gas chromatographic analysis showed that, of the original 0.1 g of each component added to the column: for hexadecane, 99.98% was recovered in

the hexane eluate and 0.02% was recovered in the benzene eluate; for pristane, 99.7% was recovered in the hexane eluate and 0.3% in the benzene; for pentadecylcyclohexane, 100% was recovered in the hexane eluate; for ethylnaphthalene, 0.2% was recovered in the hexane eluate and 99.8% in the benzene eluate; and for cyclohexane carboxylic acid, 99.1% was recovered in the methanol eluate and 0.9% remained on the column. Thus, greater than 99% of each component was separated into its proper class fraction by this method; i.e., the paraffins (hexadecane, pristane, and pentadecylcyclohexane) were eluted in the hexane fraction, the aromatic component (ethylnaphthalene) was eluted in the benzene fraction, and the asphaltic component (cyclohexane carboxylic acid) was eluted in the methanol fraction.

More detailed changes in specific components in the various crude oils were determined by gas-liquid chromatography using a Hewlett-Packard (Palo Alto, Calif.) model 5700 gas chromatograph equipped with a dual flame ionization detector and stainless-steel columns (1.8 by 0.3 m) packed with 10% Apiezon L on 60/80 chromosorb W. Temperature-programmed runs of 5-min isothermal at 130 C, 8 C/min to 250 C, and 30-min isothermal at 250 C were used for all crudes. Retention times of the petroleum components were compared with known components of Sweden crude that had been identified previously by combined gas chromatography-mass spectrometry (3) and with pure C10-C30 *n*-paraffins (Aldrich Chemical Co., Milwaukee, Wis.).

RESULTS

The volatilization of crude oils as determined with sterile controls was found to vary between 25 to 40% during 42 days of incubation with sterile seawater at 10 and 20 C (Table 2). Losses are shown in Table 2 by weight and as percentage of weight loss compared to the weight of the fresh oil. Although the rate of evaporation was not measured, identical abiotic losses occurred at 10 and 20 C during the 42-day incubation period. As expected, the percentage of abiotic loss showed a direct relationship to the specific gravity of the oil—the lighter the oil, the greater the evaporative losses. Gas chromatography of the residual oil showed that compounds above molecular weight 170 were not significantly lost abiotically.

Biological losses from these oils showed that the heavier oils were more resistant to biodegradation. When crude oils were incubated with California seawater, losses at 20 C varied between 25 and 50% as the result of biological activity (Table 2). The combination of abiotic losses and biodegradation resulted in removal of up to 80% of the lighter oils but only 50% of the heavier oils. There was about a 30 to 40% reduction in biodegradative losses for the heavier oils and a 50 to 60% reduction for the lighter oils at 10 C (Table 2). Losses from replicates varied less than 0.05 g.

Mineralization of the oils by the indigenous microorganisms of the California seawater showed the same pattern as the biodegradative losses (Fig. 1). No CO_2 was measureable from the sterile seawater-oil controls or from controls without added oil. At 20 C measureable CO_2 release began at about 1 week for all oils. The rate of CO_2 production was similar for the three lighter oils and was significantly higher than the three heavier oils. CO_2 production

TABLE 2. *Losses from different crude oils incubated with California coastal seawater*

Crude oil	Temp (C)	Losses				Residue	
		Nonbiological		Biological		g	%
		g	%	g	%		
Solvent mix crude	20	0.40	40	0.39	39	0.21	21
	10	0.40	40	0.21	21	0.39	39
Sweden crude	20	0.29	29	0.50	50	0.21	21
	10	0.29	29	0.11	11	0.50	50
South Louisiana crude	20	0.28	28	0.41	41	0.31	31
	10	0.28	28	0.28	28	0.44	44
Lagomedio crude	20	0.24	24	0.30	30	0.46	46
	10	0.24	24	0.18	18	0.58	58
Prudhoe crude	20	0.26	26	0.30	30	0.44	44
	10	0.26	26	0.23	23	0.51	51
Mirando Humble crude	20	0.24	24	0.26	26	0.50	50
	10	0.24	24	0.16	16	0.60	60

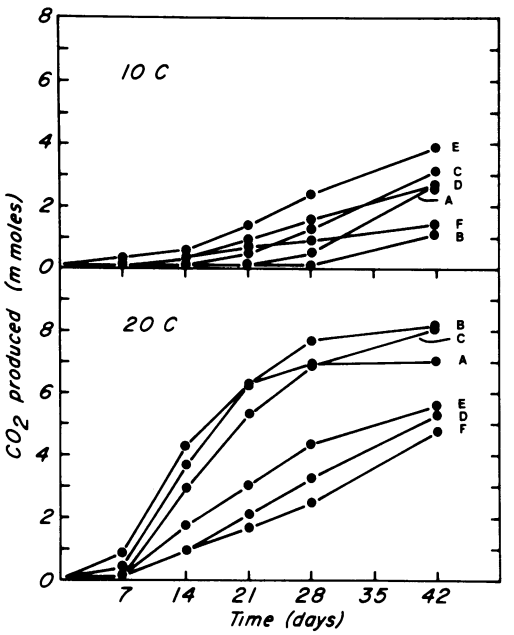

FIG. 1. *Mineralization of different crude oils at 10 and 20 C. (A) Solvent mix crude; (B) Sweden crude; (C) South Louisiana crude; (D) Lagomedio crude; (E) Prudhoe crude; (F) Mirando Humble crude.*

from the lighter oils leveled off after 1 month but was still being produced at a nearly constant rate for the heavier oils at 42 days when the residual oil was recovered. At 10 C mineralization of the three heavier oils began within 14 days, but onset of detectable mineralization of the lighter oils was delayed up to 1 month. The rate of CO_2 release from the heavier oils was about 40% lower at 10 C than at 20 C. In all cases the pH of the seawater after 42 days was within 0.5 pH units of the starting value of 8.1. Total CO_2 produced from replicates varied less than 0.2 mmol.

Similar patterns of oil utilization were found when Alaskan seawater was supplemented with crude oils and inoculated with the *Pseudomonas* sp. (Fig. 2). At 20 C oxygen consumption associated with crude oil utilization was observed for all seven oils within 18 h. The rate of O_2 consumption with the three lighter oils was significantly higher than with the four heavier oils. At 10 C no oxygen consumption occurred with fresh Sweden and Solvent mix crudes. Oxygen consumption with preweathered Sweden and Solvent mix crudes was observed, however, within 24 h. Oxygen consumption with fresh South Louisiana crude at 10 C was observed by 48 h as compared to 24 h with the same oil that had been preweathered. The earlier onset of measureable O_2 consumption indicated that weathering had removed inhibitory components present in these fresh oils. With the heavier crudes no difference was observed in the onset of oxygen consumption between fresh and preweathered oil. Prudhoe crude and Lagomedio crude showed oxygen consumption after 12 h, and Barrow crude and Mirando Humble crude showed oxygen consumption after 36 h. Onset of O_2 consumption was identical in replicate flasks. The rates of O_2 consumption with these four heavier oils were lower than with the preweathered lighter oils.

Analysis of the compositional changes that occurred during 42 days of incubation with the California seawater samples showed that all hydrocarbon classes were subject to biodegradation at both temperatures. The data in Table 3 show the weight and relative percentages of the three hydrocarbon classes for fresh oil, preweather oil, oil biodegraded at 20 C, and oil biodegraded at 10 C. Changes in the relative percentage of a hydrocarbon class indicate whether that hydrocarbon class was degraded at a higher or lower rate than the other component classes. Compared to weathered oil, the percentage of aromatic components was

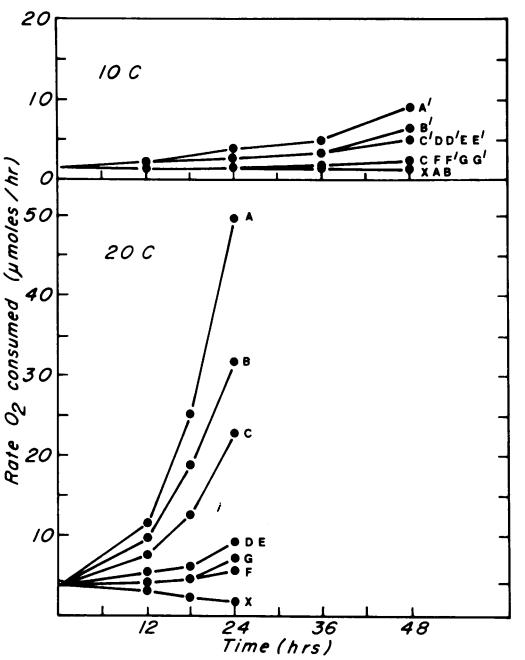

FIG. 2. *Oxygen consumption by a Pseudomonas sp. at 10 and 20 C with different fresh crude oils (A, B, C, D, E, F, G) and preweathered oils (A', B', C', D', E', F', G'). (A) Solvent mix crude; (B) Sweden crude; (C) South Louisiana crude; (D) Lagomedio crude; (E) Prudhoe crude; (F) Mirando Humble crude; (G) Barrow crude; (X) no oil.*

TABLE 3. *Crude oil composition by weight (g) and percent hydrocarbon class of fresh weathered and biodegraded oils*

Crude oil	Paraffin[a]		Aromatic[b]		Asphaltic[c]		Total wt (g)	Recovery (%)
	g	%	g	%	g	%		
Solvent mix crude								
Fresh	0.839	83.9	0.126	12.6	0.032	3.2	1.000	99.8
Weathered	0.482	80.6	0.087	14.5	0.028	4.7	0.598	99.8
Biodegraded at 20 C	0.158	80.6	0.026	13.4	0.010	5.2	0.196	99.2
Biodegraded at 10 C	0.290	74.3	0.072	18.5	0.023	5.9	0.390	98.7
Sweden crude								
Fresh	0.791	79.1	0.179	17.9	0.021	2.1	1.000	99.1
Weathered	0.553	77.3	0.137	19.2	0.019	2.7	0.715	99.2
Biodegraded at 20 C	0.158	74.5	0.034	17.2	0.017	7.9	0.212	99.6
Biodegraded at 10 C	0.351	70.1	0.106	21.1	0.039	7.7	0.501	98.9
South Louisiana crude								
Fresh	0.784	78.6	0.171	17.1	0.043	4.3	1.000	99.8
Weathered	0.540	75.2	0.135	18.5	0.045	6.3	0.720	100.0
Biodegraded at 20 C	0.229	74.3	0.051	16.6	0.026	8.7	0.308	99.6
Biodegraded at 10 C	0.311	71.3	0.086	19.7	0.035	8.1	0.436	99.1
Lagomedio crude								
Fresh	0.712	71.2	0.225	22.5	0.058	5.8	1.000	99.5
Weathered	0.518	68.1	0.185	24.3	0.052	6.8	0.761	99.2
Biodegraded at 20 C	0.311	67.3	0.105	22.7	0.040	8.6	0.462	98.6
Biodegraded at 10 C	0.373	64.5	0.161	27.8	0.040	7.0	0.578	99.3
Prudhoe crude								
Fresh	0.811	81.1	0.169	16.9	0.017	1.7	1.000	99.7
Weathered	0.600	81.0	0.119	16.0	0.018	2.4	0.741	99.4
Biodegraded at 20 C	0.346	78.1	0.077	17.3	0.018	4.2	0.443	99.6
Biodegraded at 10 C	0.387	75.7	0.102	19.9	0.019	3.7	0.511	99.3
Mirando Humble crude								
Fresh	0.680	68.0	0.279	27.9	0.036	3.6	1.000	99.6
Weathered	0.495	65.4	0.223	29.4	0.036	4.8	0.757	99.6
Biodegraded at 20 C	0.310	61.5	0.156	31.0	0.036	7.2	0.504	99.7
Biodegraded at 10 C	0.344	57.8	0.203	34.1	0.045	7.6	0.595	99.5

[a] Hexane eluate.
[b] Benzene eluate.
[c] Methanol eluate.

slightly lower in oil degraded at 20 C, but slightly higher in the same oil degraded at 10 C. Thus, the aromatic fraction appears to be slightly more readily degraded at 20 than at 10 C. Biodegraded oil had a lesser weight of paraffins than either fresh or weathered oil. The percentage of paraffins in the oils degraded at 10 C were lower than in the oils degraded at 20 C, indicating a preferential utilization of paraffins at the lower temperature as compared to the other component classes at the higher temperature. In some biodegraded oil residues there was a greater weight of asphaltic components than in fresh or weathered oil.

More detailed gas-liquid chromatographic analysis showed no preferential degradation of particular chain length paraffins (Fig. 3). Both straight-chain and branched paraffins were degraded. Branched paraffins, however, appeared to be somewhat more slowly degraded than straight-chain paraffins. The numbered components of Sweden crude were previously identified by mass spectrometry (3). The components of the other oils were numbered in an identical manner based solely on retention time. Gas chromatographic tracings of residual oils incubated at 10 C, which are not shown in Fig. 3, confirmed the lesser extent of degradative losses at the lower temperature. Extensive differences in the biodegradability of branched

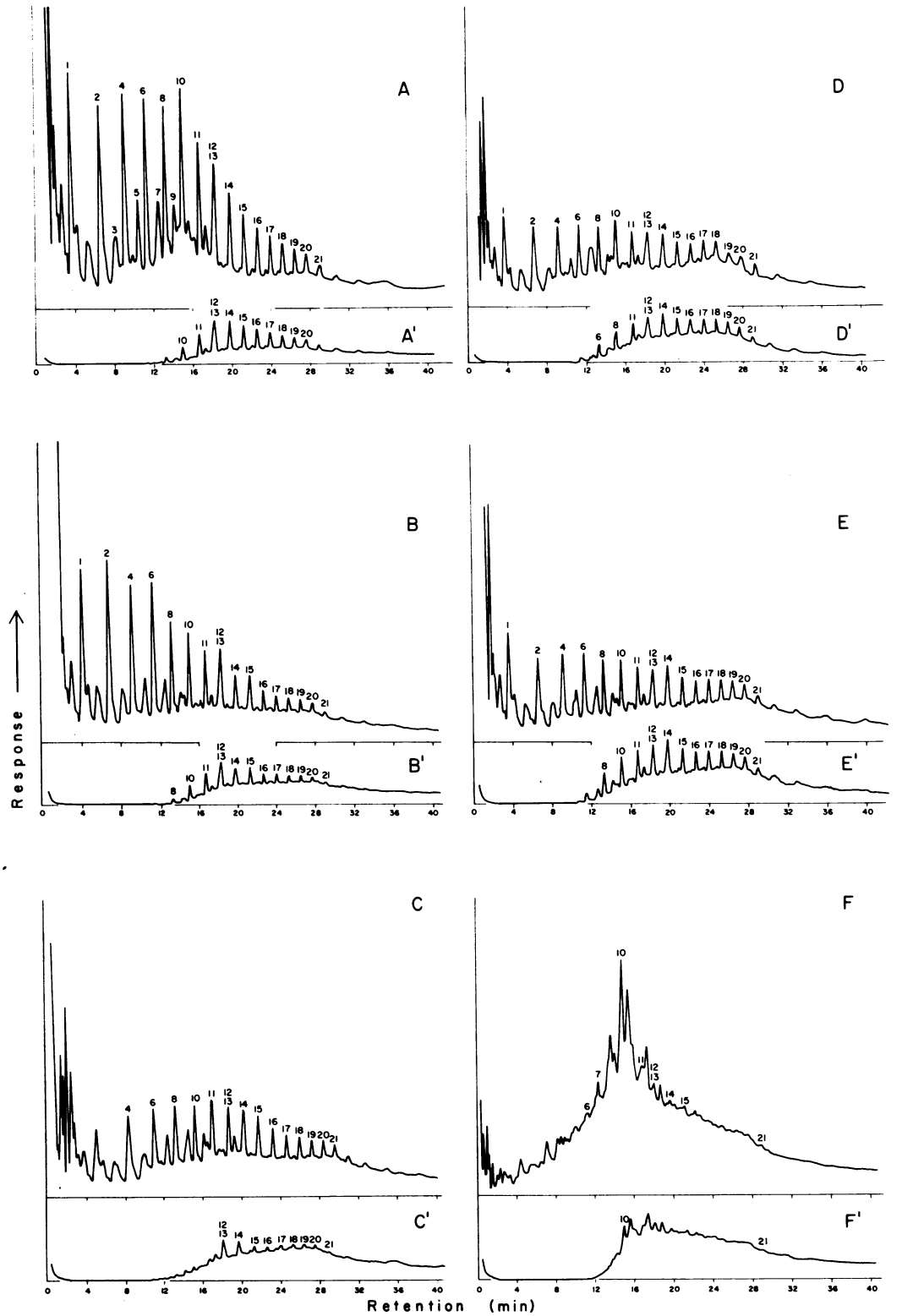

FIG. 3. *Gas chromatographic tracings of various fresh crude oils (A, B, C, D, E, F) and residues after biodegradation (A', B', C', D', E', F') at 20 C. (A) Solvent mix crude; (B) Sweden crude; (C) South Louisiana crude; (D) Lagomedio crude; (E) Prudhoe crude; (F) Mirando Humble crude.*

versus straight-chain paraffins were no more apparent at 10 C than at 20 C. Compounds such as pristane and phytane were degraded at both temperatures. High-molecular-weight compounds above molecular weight 422 and very highly branched or condensed ring components were not resolved by the gas chromatographic analysis, and information on their degradation is speculative.

DISCUSSION

The biodegradability of a variety of crude oils was found to be highly dependent on crude oil composition and temperature. Of special importance was the relative amounts of high- and low-molecular-weight compounds in the various crudes. Heavy oils contain a greater percentage of high-molecular-weight components, whereas light oils contain a greater percentage of low-molecular-weight components. Abiotic losses were highest for the lighter oils because of the volatility of these low-molecular-weight components. The low biological losses from heavy oils probably reflects the resistance to microbial degradation of the complex high-molecular-weight compounds.

As was previously reported (2), Sweden crude oil contains a fraction which apparently fails to evaporate at reduced temperatures and which is inhibitory to the microbial degradation of oil. The present study indicates that this is not a unique phenomenon but one associated with lighter crude oils. Mineralization of the lighter fresh crude oils by mixed microbial communities was delayed at 10 C. Oil degradation at 10 C by a *Pseudomonas* sp. was also inhibited by these fresh light oils, as shown by inhibition of O_2 consumption at 10 C. Preweathering these oils removed this inhibition. Heavier oils, on the other hand, showed no similar evidence for the presence of inhibitory fractions. The heavier oils were degraded at slower rates than lighter oils at 20 C. The lack of an inhibitory fraction, however, makes these heavier oils more readily degraded at reduced temperatures than the lighter oils.

Unlike some predictions based on findings with pure hydrocarbons (4; R. E. Kallio, personal communication), all hydrocarbon classes were found to be subject to biodegradation within the context of the whole crude. It is possible that some components were degraded by co-metabolism. Some preference was shown for degradation of paraffins. Whereas this preference was somewhat heightened at reduced temperatures, both paraffins and aromatics were biodegraded at 10 and 20 C in all oils. Jobson et al. (9) also reported apparent degradation of aromatic petroleum components at 4 and 30 C. Branched compounds, such as pristane, were not found to be recalcitrant to microbial degradation at reduced temperatures as previously reported by Westlake et al. (15). The increase in the weight of asphaltic components in some degraded oils may reflect, in part, a conversion of other hydrocarbons to more polar forms and an actual resistance of these polar components to microbial degradation. Westlake et al. (15) reported a similar increase in polar components during microbial degradation.

Even those crude oils exhibiting greatest susceptibility to biodegradation had a 20% fraction that resisted degradation. This residual fraction, which was not resolvable by gas chromatography, probably is composed of complex, highly branched and condensed molecules. The relative resistance of light oils to degradation at low temperatures should be considered in choosing shipping routes for these oils. Accidental spillages in the Arctic will most likely be of regional heavy type oil, e.g., Prudhoe crude, which will be subject to slow but constant microbial degradation.

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