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Microbial Degradation of Oil Spills Enhanced by a Slow-Release Fertilizer

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The improved cleanup of marine oil spills by stimulating biodegradation through the use of a slow-release fertilizer is reported. A paraffin-supported fertilizer containing $MgNH_4PO_4$ as active ingredient was developed and evaluated in laboratory and field experiments using quantitative infrared spectrometry and chromatographic techniques. The biodegradation of Sarir crude oil in the sea was considerably enhanced by paraffin-supported fertilizer. After 21 days 63% had disappeared as compared to 40% in the control area.

The estimated yearly influx of petroleum pollutants in the sea has been estimated to be as high as 10 million tons (3). Large amounts of crude oil disappear by weathering and microbial degradation (18). Weathering includes evaporation of volatile constituents, emulsification in water, and sinking or beaching. Degradation occurs by spontaneous and microbial oxidation. Several approaches for solving the problem of oil pollution have been described (5, 10, 17), but each has its limitations. Numerous investigators have studied the possibility of using microorganisms (1, 4, 9, 12, 13, 15) to increase the natural biodegradation of petroleum, but it is too slow to have practical importance for oil cleanup. Inoculation of oil-polluted areas with oil-decomposing microorganisms seems to be ineffective because of growth-limiting nitrogen and phosphorus concentration in seawater (9, 19).

The effectiveness of nitrogen and phosphorus compounds as fertilizers for petroleum biodegradation has been established in laboratory experiments (1), but because of their rapid dissolution these salts have little or no effect when applied in the sea. In an attempt to develop a slow-release fertilizer, which also sticks to the oil, paraffinized urea and octylphosphate were tested in laboratory experiments and under simulated marine conditions (2). This study describes a similar approach to the problem of crude oil biodegradation using a new lipophilic slow-release fertilizer formulation with a single nitrogen- and phosphorus-containing compound.

MATERIALS AND METHODS

Crude oil. The type, origin, and physicochemical properties of the crude oil used in these experiments are reported in Table 1.

Organisms. Counts of viable microorganisms were performed by plating on marine agar 2216 (Difco) after incubation for 2 days at 25 C. Hydrocarbon-oxidizing bacteria were determined by the most-probable-number technique as described by Gunkel (6), using a liquid medium containing crude oil as the sole carbon source. The pH of the fresh seawater from the port of Ortona, (Pescara, Italy) was 7.9 to 8.2.

PSF. Paraffin-supported fertilizer (PSF) was prepared in the following way. A 40-g portion of $MgNH_4PO_4 \cdot 6H_2O$ (Riedel de Haën) was added to 100 ml of a 10% solution of paraffin (melting point, 58 to 60 C; Schuchardt) in $CHCl_3$, and the solvent was evaporated to dryness in a Rotavapor. The dried preparation was treated in a Waring blender, and the powder was passed through a sieve of 0.630-mm mesh.

Biodegradation. (i) Batch experiments were carried out in cotton-plugged 500-ml Erlenmeyer flasks containing 100 ml of seawater (from Ortona), 40 mg of crude oil, and 20 mg of PSF. The above amount of PSF was chosen to supply 2 g of nitrogen per 100 g of crude oil. This concentration was determined as the optimum in preliminary experiments carried out in our and other laboratories (1). Two controls were included: flasks without PSF, to evaluate the biodegradation that occurs in seawater naturally; and flasks with 0.2% (vol/vol) formaldehyde, to evaluate the loss of crude oil components by evaporation and other nonbiological causes.

The flasks were incubated for 45 days at 15 and 25 C on a rotary shaker at 100 rpm. (These temperatures correspond to the average winter and summer surface temperatures of the Mediterranean Sea.)

(ii) Field experiments, in duplicate, were made in the bay of Ortona, about 300 m off the coast. A sea surface of 25 m^2 was delimited, using a system of floats (Fig. 1) consisting of 20 aluminum elements (70 by 50 cm) connected (liquid tight) to each other by a rubber element. The submersed part was about 30 cm; the emergent part was 20 cm.

A 250-g amount of Sarir crude oil was poured inside each experimental area. After spreading the

TABLE 1. *Physicochemical properties of Sarir crude oil from Libya*

Property	Amt
Specific weight (15 C)	0.8395
Pour point[a]	+19 C
Paraffin (%, wt/wt)[b]	18.2
Conradson carbon (%, wt/wt)[c]	4.10
Total sulfur (%, wt/wt)[d]	0.16
Paraffin, aromatic hydrocarbon ratio[e]	4.15

[a] Determined by American Standard Testing Material (A.S.T.M.) method D36-66T.
[b] Determined by British Petroleum method 237-39.
[c] Determined by A.S.T.M. method D 189-65.
[d] Determined by A.S.T.M. method D-129-64.
[e] Determined by infrared spectroscopy.

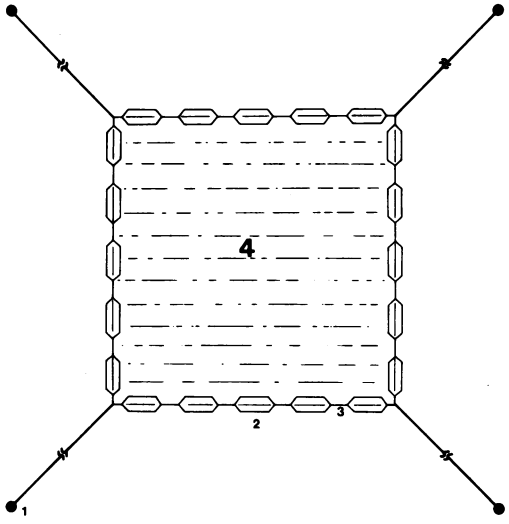

FIG. 1. *System of floats used in the field experiments. Mooring buoy (1); aluminum float element (2); connecting rubber panel (3); experimental area (4).*

oil, 125 g of PSF was distributed homogeneously on the surface of the oil film using a small powder bellows and was exposed for 21 days. To avoid drifting of the crude oil by the sea breeze and clotting and sticking along the inside walls of the floats, a net of 5-cm mesh was laid on the water surface.

As controls, identical areas were used: without PSF, to evaluate the loss of crude oil components due to chemical-physical causes and to the natural biodegradation; and adding to the crude oil the same amount of paraffin as contained in 125 g of PSF, to evaluate and differentiate their contribution to the total paraffinic residue.

Analytical methods. In laboratory experiments, the oil constituents were extracted by adding 20 ml of CCl_4 to each flask, after acidification to pH 4 with 1 N HCl. The CCl_4 phase was removed, after centrifugation, and dehydrated by anhydrous Na_2SO_4. The amount of total hydrocarbons was determined by infrared spectroscopy (14), using a calibration curve and measuring the area of the bands between 2,980 cm^{-1} and 2,820 cm^{-1}. The analysis of *n*-saturated (alkanes) was made by gas chromatography using a Hewlett-Packard model 7620 gas chromatograph equipped with a flame ionization detector and automatic integrator.

In the case of field experiments, extractions were carried out by washing the net and the inside area of the floats three times with CCl_4. The solvent fractions were pooled, evaporated to dryness in a rotary evaporator, and weighed. The crude oil residues were then analyzed as follows: the asphaltenes on a Hyflo Supercel column; the saturates (alkanes) and aromatic and NSO (polar N-, S-, and O-containing materials) fractions on a silica gel-alumina column, using *n*-pentane-benzene-methanol (7). Determination of *n*-alkanes was made by gas-liquid chromatography.

RESULTS

Counts of heterotrophic bacteria in seawater samples ranged between 2×10^4 to 5×10^4 viable cells/ml; the hydrocarbon-oxidizing bacteria ranged between 90 to 100/100 ml.

Microbial degradation in laboratory experiments. The effect of temperature on the rate of crude oil biodegradation is shown in Fig. 2.

The utilization of alkanes, evaluated by gas-liquid chromatography (Fig. 3), is reported in Table 2.

The losses of the lower paraffins due to evaporation and to "spontaneous" utilization (attributable to nitrogen and phosphorus naturally present in seawater) were about 32.5 and 2.5%

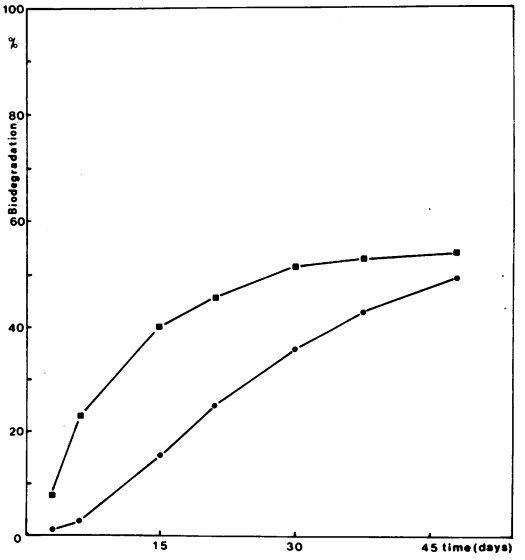

FIG. 2. *Effect of temperature on the biodegradation (batch) of Sarir crude oil in seawater fertilized with $MgNH_4PO_4$ supported by paraffin. Symbols: 25 C incubation temperature (■); 15 C incubation temperature (●).*

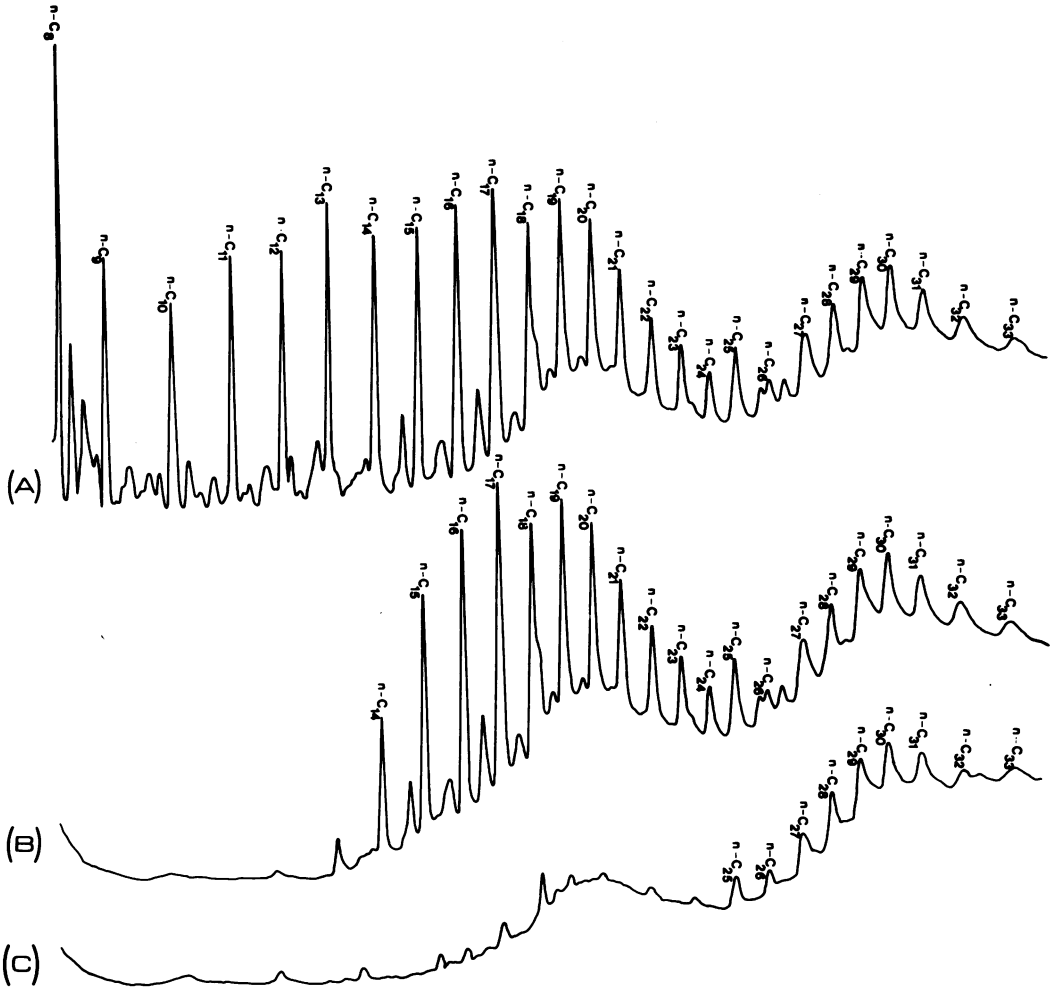

FIG. 3. *Gas chromatograms of Sarir crude oil. (A) Intact sample. (B) Control (losses by weathering and naturally occurring biodegradation) after 21 days. (C) In the presence of the PSF. The analysis was made using a Hewlett-Packard model 7620 gas chromatograph equipped with a flame ionization detector and automatic integrator. The operating conditions were: a stainless-steel column 1.83 m long, 2.2-mm ID, packed with 10% UCC-982 (methyl vinyl silicone) on Chromosorb W, 80 to 100 mesh. The column temperature was programmed at 60 C for 4 min; from 60 to 140 C at 6 C/min, held isothermally for 1 min; from 140 to 220 C at 6 C/min, held isothermally for 14 min; from 220 to 300 C at 6 C/min, held isothermally for 30 min. Injector block temperature was 400 C, detector temperature was 370 C, and helium flow rate was 60 ml/min.*

of the total paraffins up to C_{33}, respectively. In the presence of the fertilizer, 75% of the paraffins up to C_{33} (including the paraffins added with PSF) were depleted.

Biodegradation in field experiments. The fate of Sarir crude oil from field and laboratory experiments is summarized in Fig. 4. The recovery from the control area (no PSF added) was about 60% (wt/wt) after 21 days. The loss is attributable to the weathering processes and to spontaneous microbial metabolism. The recovery of petroleum products from the fertilized area, after 21 days, was about 46% (wt/wt). Up to 20% of the recovered oil may be attributable to the paraffin of PSF. In other words, the net microbial degradation of crude oil ascribed to PSF is about 38% (wt/wt). Using the isoprenoid hydrocarbon phytane as biological marker (8, 11), the net crude oil depletion in the fertilized area corresponded to about 34%. Some authors suggest that the use of phytane as a biological marker has to be interpreted with care (7). In the present work the crude oil recovery data, calculated by using phytane as internal standard, are in good agreement with the value obtained from gravimetric analysis.

TABLE 2. *Gas chromatographic analysis of n-paraffin losses from Sarir crude oil at 15 C after 45 days*

Paraffin	Evaporation (%, wt/wt)	Spontaneous utilization (%, wt/wt)	Total loss in presence of PSF (%, wt/wt)
n-Paraffin	32.5[a]	2.5[a]	75[b]
C_8-C_{15}	77.5[c]	7.5[c]	100[c]
C_{16}-C_{24}	0	0	81[c]
C_{25}-C_{33}	0	0	27[c]

[a] Calculated on the basis of the *n*-paraffins up to C_{33}.
[b] Calculated on the amount of *n*-paraffins up to C_{33} plus paraffins added with the PSF.
[c] Calculated on the basis of the indicated paraffin range.

The results of liquid chromatographic analysis of Sarir crude oil are reported in Table 3. The loss of hydrocarbon classes in the field experiments, as compared to unexposed but topped (250 C) Sarir crude oil, is reported in Fig. 5. The saturated fraction shows a decrease of about 57% as compared to the topped crude oil. A part of the loss (30%) is to be attributed to natural processes such as evaporation, solubilization, and spontaneous biodegradation; the rest (27%) is the result of the fertilizer treatment. It should be noted that the paraffin added to the nutrient salts of the PSF contribute to the total 45% paraffin residue. The total loss of aromatics is about 44%, 30% of which is attributed to PSF-stimulated biodegradation.

DISCUSSION

The PSF fertilizer was chosen out of the following considerations. (i) $MgNH_4PO_4$ has a low solubility, which is characteristic of a slow-release fertilizer (it is used for this reason also in agriculture). It does not need complex and expensive coating procedures based on a controlled porosity matrix as some soluble fertilizers do. (ii) $MgNH_4PO_4$ contains N and P in the same molecule and this avoids unbalanced release of the two elements, as in the case of mixtures of different salts. (iii) It is not toxic. (iv) Paraffinization of the salt results in a floating system consisting of spongelike aggregates able to absorb oil and water to form microenvironments suitable for microbial growth.

$MgNH_4PO_4$ has a low nitrogen content (9.0% in the monohydrate form, 5.7% in the hexaydrate), and this requires the use of 30 to 50 parts of PSF per 100 parts of oil to reach the optimal salt/oil ratio as determined from batch experiments. In the sea, the natural turnover of nitrogen seems to be high enough (12) to allow for the reduction of the fertilizer-oil ratio. To evaluate the use of PSF in practical and economical terms, further field experiments are necessary with the aim of determining the minimum amount of PSF still stimulatory to the microbial degradation of oil.

In conclusion, we believe, on the basis of our work, that the use of PSF, alone or combined with microbial inocula depending on environmental conditions, can be a way to solve the problem of oil clean-up.

We suggest that PSF could be used as a complement to the mechanical recovery, acting on the portion of the oil left behind by this process, and as a valid alternative to dispersion or sinking, which are, up to now, the most frequently used treatments. The latter two measures are effective only from the cosmetic point of view, but they do not eliminate the toxic components of the oil. In fact, the sinking process extends the toxicity from the surface to the seafloor (13),

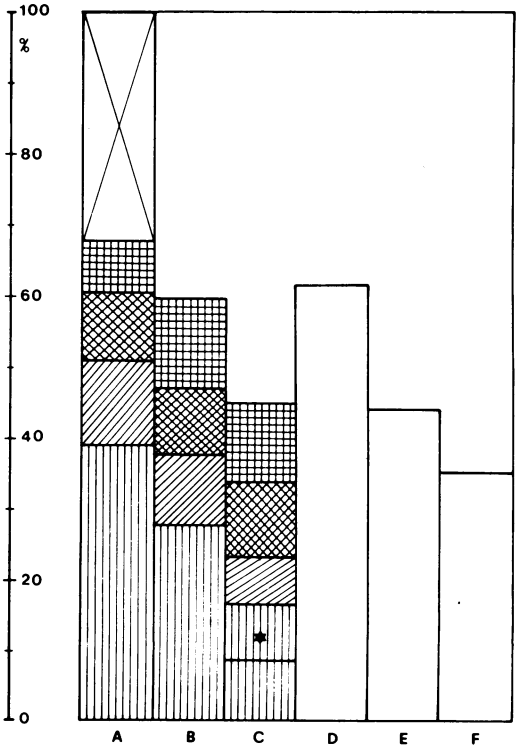

FIG. 4. *Fate of Sarir crude oil after 21 days of field and laboratory experiments. (A) Intact sample. (B, D) Controls (losses by weathering and naturally occurring biodegradation) in field and batch experiments, respectively. (C) Field experiments with fertilizer. (E, F) Batch experiments at 15 and 25 C, respectively. Symbols:* ▥, *Saturates;* ▩, *saturates from PSF;* ▨, *aromatics;* ■, *NSO;* ■, *asphaltenes;* ⊠, *fraction boiling point < 250 C.*

TABLE 3. *Liquid chromatographic analysis of crude oil*

Oil fractions	% (wt/wt) of crude oil					
	Topped (250 C)		Control after 21 days		Residue after 21 days	
Saturates	57.60	38.88[a]	45.90	27.54[b]	36.78	16.73[c]
Benzene-soluble asphaltene	7.70	5.19	7.24	4.34	11.24	5.11
Benzene-insoluble asphaltene	2.41	1.60	14.80	8.84	15.39	7.00
Aromatics	17.28	11.67	17.34	10.50	14.31	6.50
Soluble NSO	10.90	7.35	9.60	5.72	5.26	2.39
Insoluble NSO	4.16	2.81	5.11	3.06	16.96	7.70

[a] The data in this column were normalized on the basis of topped (250 C) weight of oil (67.50%).
[b] The data in this column were normalized on the basis of the recovery value (60.00%).
[c] The data in this column were normalized on the basis of the recovery value (45.43%).

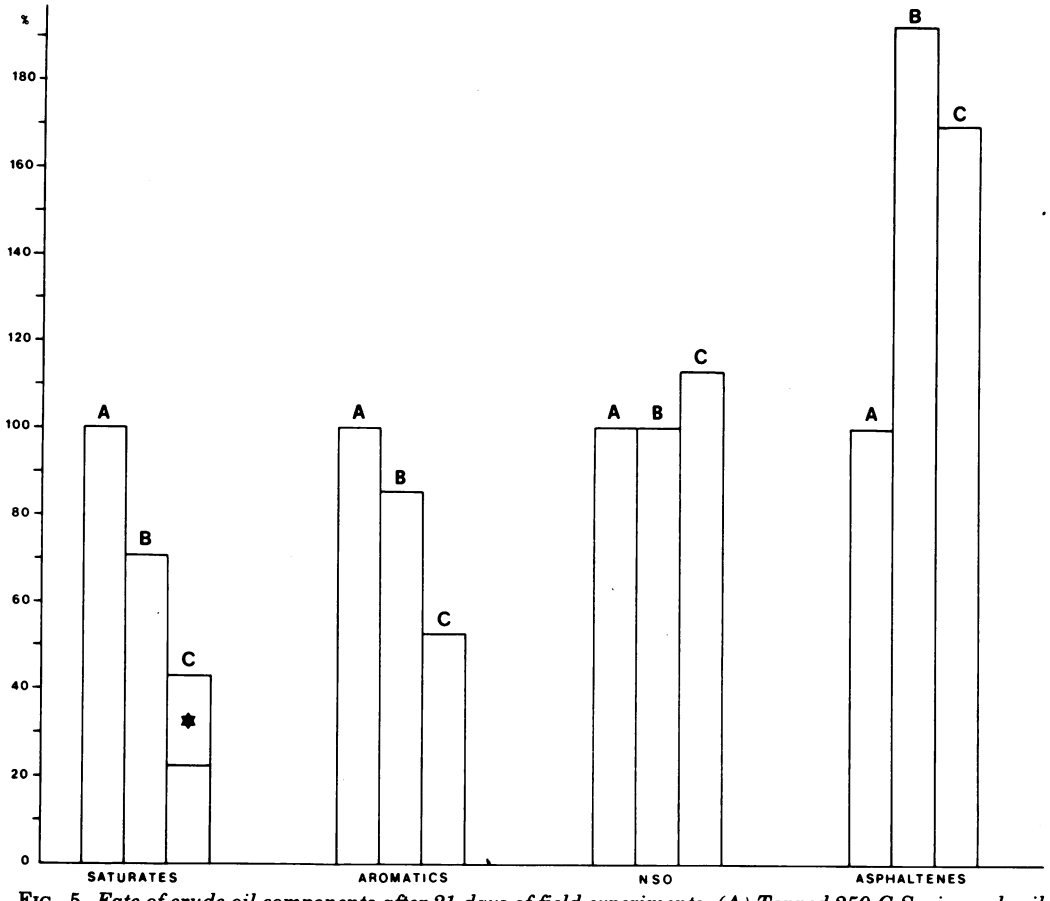

FIG. 5. *Fate of crude oil components after 21 days of field experiments. (A) Topped 250 C Sarir crude oil. (B) Controls (losses by weathering and naturally occurring biodegradation). (C) Residuals after fertilization treatment. Symbol: (★) contribution from the paraffins added to coat the nutrient salts.*

and dispersants often produce oil emulsions that are more toxic than either the crude oil or the dispersant alone (16).

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