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Fate of Hydrocarbons During Oily Sludge Disposal in Soil†

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A 1,280-day laboratory simulation of the "landfarming" process explored the fate in soil of polynuclear aromatics (PNAs) and total extractable hydrocarbon residues originating from the disposal of an oily sludge. In addition to the measurement of CO_2 evolution, periodic analyses of PNAs and hydrocarbons monitored biodegradation activity. The estimation of carbon balance and of soil organic matter assessed the fate of residual hydrocarbons. Seven sludge applications during a 920-day active disposal period were followed by a 360-day inactive "closure" period with no further sludge applications. A burst of CO_2 evolution followed each sludge addition, but substantial amounts of undegraded hydrocarbons remained at the end of the study. Hydrocarbon accumulation did not inhibit biodegradation performance. Conversion of hydrocarbons to CO_2 predominated during active disposal; incorporation into soil organic matter predominated during the closure period. In this sludge, the predominant PNAs were degraded more completely (85%) than total hydrocarbons. Both biodegradation and abiotic losses of three- and four-ring PNAs contributed to this result. Some PNAs with five and six rings were more persistent, but these constituted only a small portion of the PNAs in the sludge. The study confirmed that the microbially mediated processes of mineralization and humification remove sludge hydrocarbons from soils of landfarms with reasonable efficiency.

Throughout the industrial world, petroleum is the principal source of fuel and chemical feedstocks. Like other large-scale industrial processes, petroleum refining is a potential source of environmental pollution, necessitating elaborate and costly effluent treatment systems (3). These treatment systems are highly effective in improving effluent quality but also produce oily sludges that constitute a disposal problem. Among the available disposal alternatives, the "landfarming" process has gained increasing acceptance (R. Bartha and I. Bossert, *in* R. Atlas, ed., *Petroleum Microbiology*, in press). In this process, the oily sludge is spread on land, and the decomposition of its hydrocarbon components by soil microorganisms is encouraged.

The landfarming of oily sludges and waste oils has been studied in the field (6–11) and also in the laboratory (5, 15). From these studies, optimal conditions for landfarming emerged and were condensed to practical landfarming guidelines by the American Petroleum Institute (1). Some polynuclear aromatics (PNAs), e.g., benzo[*a*]pyrene, contained in the oily sludge, are considered to be potential carcinogens, and the fate of these and other recalcitrant hydrocarbon components of oily sludges was not clarified in earlier studies. Additional questions to be answered by this study were the dynamics of hydrocarbon biodegradation during periods of intensive use (frequent reapplication schedule) and during prolonged inactivity or "closure" of the landfarm. The need for precise sludge applications, controlled incubation conditions, and measurement of carbon balance dictated a laboratory incubation approach.

MATERIALS AND METHODS

Sludge and soil. A batch of oily sludge originating from the dissolved air flotation unit of a petrochemical plant effluent treatment system was used throughout this study. When received, the sludge contained ca. 80% water. To permit reproducible sludge reapplications over a 3-year period, the water was allowed to evaporate outdoors in a plastic-lined drying frame over a period of several weeks. The drying process was finished by a 24-h period in a 50°C drying room. The drying process probably resulted in partial loss of some of the more volatile sludge hydrocarbons, but this was unavoidable. The dried sludge was homogenized in a Waring blender, and samples were placed in individual tin cans. These were flushed with nitrogen gas, sealed, and stored at −20°C until used. The dry sludge contained (per gram): 299 mg of methylene chloride ($MeCl_2$)-extractable material, including 3.6 mg of PNAs; 360 mg of $MeCl_2$-inextractable organic matter (humus), and 131 mg of total nitrogen (N). A sandy loam from the untreated periphery of an active landfarm was sieved and kept in a semimoist state at 4°C. The soil, containing 17 mg of humus per g and 1.5 mg of total N per g and having an original pH of 6.4, was limed with 15 mg of $CaCO_3$ per g of soil to a pH of 7.5 10 days before the biodegradation experiments (5).

Incubation. Incubations were carried out in three types of containers. For measurement of CO_2 evolution, 10-g (dry weight) soil samples were incubated in Biometer flasks (2). For extraction and analysis of residual hydrocarbons, 100-g soil samples were incubated in 1-liter beakers covered with polyethylene film to reduce evaporation. A 7-kg batch of soil was incubated in a stainless steel pan for leaching and plant growth studies (to be reported separately). All three incubation systems were identically treated in terms of liming, fertilization (60 μmol of N as NH_4NO_3 per g of soil and 5 μmol of P as K_2HPO_4 per g of soil), sludge application rates (140 mg of dry sludge containing 42 mg of hydrocarbons per g of soil at each application), sludge application frequency (seven times during a 25-month period), and soil moisture (50% of holding capacity). All samples were incubated at 20°C in the dark with periodic aeration and water addition to compensate for oxygen consumption and evaporative water losses. Poisoned controls (2% $HgCl_2$) were included to monitor abiotic hydrocarbon losses.

At sludge reapplications, the new material was thoroughly mixed with the soil sample, and sufficient water was added to hydrate the sludge soil mixture to 50% of its holding

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capacity. No new fertilizer or lime was added, since the fertilizer was assumed to be recycled within the samples, and the pH decreased only moderately to 6.6 after 1,280 days of incubation.

The simulated landfarming operation had two phases. During the first 751 days of the active phase, sludge was applied in 90 to 150-day intervals. A 360-day closure study simulating a cessation of sludge disposal followed, 169 days after the last sludge application. Incubation conditions were identical, and the total length of the two phases was 1,280 days.

Analytical. Carbon dioxide evolution in the Biometer flasks was measured as described earlier (2, 5). Before reloading, replicate sludge-soil samples were extracted in a Soxhlet apparatus by using methylene chloride ($MeCl_2$). Extraction was continued until the solvent in the Soxhlet tube showed no more fluorescence under UV light. The extract was dried over Na_2SO_4, concentrated by evaporation under a nitrogen gas stream, and adjusted to a volume of 100 ml. Samples of this extract were used for UV scans, hydrocarbon determinations, and analysis of PNAs. By using a Carey model 17 spectrophotometer, the spectra of the diluted extracts were recorded between 240 and 400 nm.

$MeCl_2$-extractable material was determined gravimetrically after evaporation of the solvent under a nitrogen stream at room temperature. The last traces of solvent were removed in a desiccator containing paraffin chips. It was recognized that, in addition to residual hydrocarbons, the described procedure also determined solvent-soluble hydrocarbon biodegradation products, e.g., long-chain fatty acids. However, for the purpose of this discussion, all solvent-extractable substances are being referred to as total residual hydrocarbons.

Before gas chromatographic-UV analysis, PNAs were separated from other hydrocarbons. A sample of the $MeCl_2$ extract was spiked with [^{14}C]phenanthrene, [^{14}C]benzo[*a*]anthracene, and [^{14}C]benzo[*a*]pyrene (Amersham Corp., Burlington Heights, Ill.), each contributing approximately one-third to a total count of 1.2×10^5 dpm. The radiolabeled PNAs served as internal standards to correct for PNA recovery losses in sample clean-up procedures. The PNA fraction was cleaned up by partitioning into dimethyl sulfoxide equilibrated with phosphoric acid. This was followed by counter extraction of PNAs into Isooctane after dilution of the dimethyl sulfoxide extract with water (21 CFR 178-3620C). After cleanup, the PNAs were displaced into toluene, concentrated under nitrogen gas to a 20- to 30-μl volume, and analyzed by the gas chromatographic-UV procedure described by Pancirov et al. (12).

Analysis of soil and sludge for water content, water-holding capacity, nonhydrocarbon organic matter, and total nitrogen was performed as described by Pramer and Schmidt (13).

Replication and statistical control. The unusual length of the study and the high expense of PNA analysis necessitated some compromises in terms of replication and statistical analysis. Biometer flasks were set up in quadruplicate, and the CO_2 data represent averages of four flasks during the first 920 days and of two flasks during the last 360 days of the study. The untreated soil control was measured only for 920 days; the dashed line (see Fig. 1) represents a projection. For clarity, the standard deviations are not indicated on individual data points; the coefficients of variation were below 3.5% for both curves.

Incubations for hydrocarbon and PNA analyses were set up in triplicate beakers. At sampling times, two beakers were extracted, and the third was frozen to serve as a reserve sample to be used only in case of analytical mishaps or questionable results. The two extracts were compared by UV scans, which were typically superimposable. Only one of the extracts was routinely subjected to complete hydrocarbon and PNA analyses. To evaluate the accuracy of these measurements, hydrocarbons were determined in triplicate at time 0 and at 920 days when the coefficients of variation were 2.4 and 2.1% respectively. PNA analyses of triplicate samples were performed at time 0 and at 419 days. For total PNAs, the coefficients of variation were 8.8 and 4.9%, respectively. For individual PNAs, the coefficients of variation ranged between 4 and 22%, with the exception of fluorene and fluoranthene which exhibited relatively high coefficients of variation (38 and 31%, respectively).

RESULTS AND DISCUSSION

CO_2 evolution. As compared with CO_2 evolution in untreated soil, applications of oily sludge increased the rate of CO_2 evolution ca. 20-fold (Fig. 1). This increase was not linear. After each treatment, a strong acceleration of CO_2 evolution was evident. As the more available hydrocarbons of the sludge became exhausted, CO_2 evolution rates tapered off and approached the rates exhibited by untreated soil. At these points, new sludge was applied, yielding the step pattern of CO_2 evolution evident in Fig. 1. This step pattern was fairly consistent in all seven applications, indicating that the biodegradation performance was not decreased by accumulation of residues or by another physiochemical change brought about by the treatment. A borderline overload condition may have developed after the first three closely spaced applications, as evidenced by the relative flatness of the third step (days 156 to 280). This condition was corrected by slightly decreasing the frequency of treatments.

During the closure period (days 920 to 1,280), CO_2 evolution was essentially linear at a rate only slightly higher than that of untreated soil. Subtracting the CO_2 production by untreated soil, only 5.3 mmol of CO_2 (15 μmol per day) was attributable to sludge hydrocarbons during the 360-day closure period. This contrasted with 97.7 mmol of CO_2 (106 μmol, daily average) produced due to hydrocarbons during

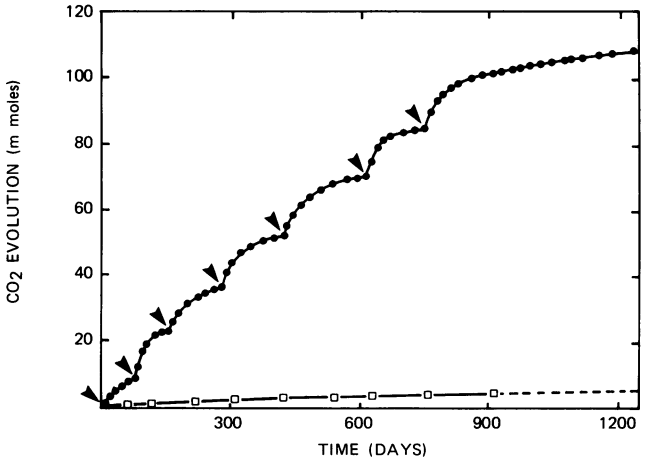

FIG. 1. Cumulative CO_2 evolution from 10-g samples of untreated soil (□) and soil treated with oily sludge (●). The arrows indicate the time of sludge additions, 1.4 g of sludge containing 420 mg of hydrocarbon per application. Curves represent the average of quadruplicates; the coefficient of variation for each point is less than 3.5%. The dashed portion of the soil curve represents a projection.

the 920-day active period of the experiment. It appears that hydrocarbons not mineralized during the first 3 to 4 months after their application do not serve as primary substrates for microbial growth. During a closure period, mineralization of residual hydrocarbons appears to be a slow and relatively minor process of elimination.

Fate of total hydrocarbons. Of 293 mg of hydrocarbon applied per g of soil in seven increments (Fig. 2), 137 mg (47%) was eliminated during 1,280 days of the study. Evaporative losses during drying of some low-molecular-weight and readily biodegradable hydrocarbons may be a reason for this relatively low figure. Of the above, 102 mg (35%) was eliminated during the active period and 35 mg (12%) was eliminated during the closure period. It is interesting that the average rate of hydrocarbon decrease was only slightly slower (97 μg per day) during the closure period than during the active phase (111 μg per day). This was in marked contrast to the greatly decreased CO_2 evolution during the closure period. Therefore, it is clear that during the closure period the major route of hydrocarbon disappearance was not mineralization but humification, i.e., the conversion of solvent-extractable hydrocarbons and hydrocarbon metabolites to solvent-insoluble soil organic matter. The material presented below is also relevant to the overall fate of hydrocarbons.

Attempts to stimulate hydrocarbon elimination during the closure period by supplying an alternate substrate (ground wheat straw [1% by soil weight], applied in combination with proportional amounts of NH_4NO_3 and K_2HPO_4 fertilizer) increased CO_2 evolution but inhibited, rather than stimulated, hydrocarbon elimination (data not shown). In soil poisoned with 2% $HgCl_2$, there was no measurable decrease in hydrocarbons (data not shown), indicating that virtually all hydrocarbon loss was biologically mediated.

Fate of PNAs. With the oily sludge, a total of 3.49 mg of PNA per g of soil was applied (Fig. 3). At 920 days, only 0.45 mg (12.9%) of PNA remained. By the end of the closure period (1,280 days), a further decrease to 417 μg (11.9%) occurred. Viewed as a class, the PNAs of this sludge were eliminated more rapidly than total hydrocarbons. It should be noted, however, that PNAs in this sludge constituted only 1.2% of the total hydrocarbons. In contrast to the decrease

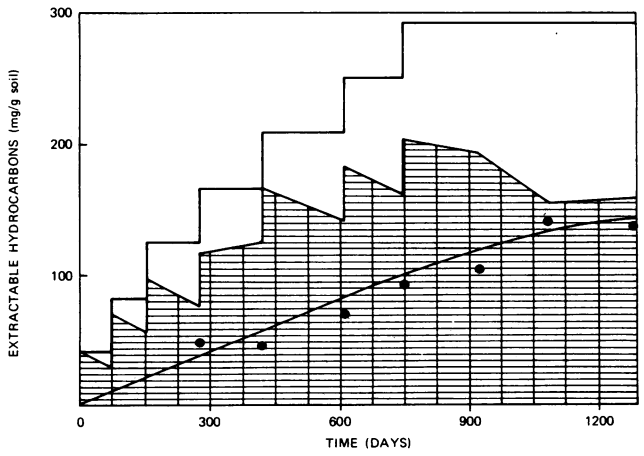

FIG. 2. Extractable hydrocarbons in sludge-treated soil. The unshaded area indicates the amount of hydrocarbon added; the cross-hatched area indicates the amount of hydrocarbon remaining after incubation. Cumulative hydrocarbon biodegradation (●) was calculated from the difference.

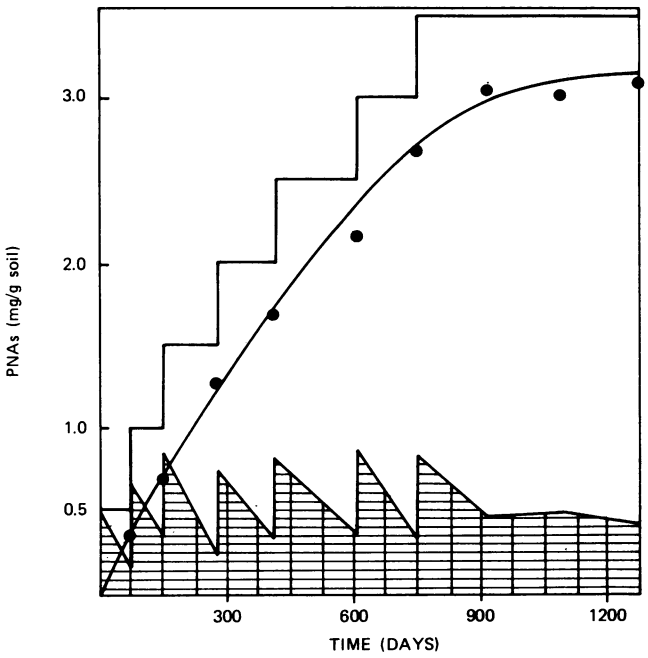

FIG. 3. PNAs in sludge-treated soil. The unshaded area indicates the amount of PNAs added; the cross-hatched area indicates the amount of PNAs remaining after incubation. Cumulative PNA biodegradation (●) was calculated from the difference.

of hydrocarbons, the average rate of PNA decrease was much slower (0.1 μg/g of soil per day) during the closure period than during the active period (3.3 μg/g of soil per day).

The fates of individual PNAs and PNA subclasses according to numbers of condensed aromatic rings are summarized in Table 1. The degradation of three-ring PNAs was very extensive. Anthracene was degraded somewhat more slowly than other three-ring PNAs. The degradation of some four-ring PNAs was also extensive, but pyrene and triphenylene were relatively recalcitrant, reducing the average elimination of this subclass to about half of the previous one. The five- and six-ring PNAs were all quite resistant to degradation.

The results (Table 1) may be summarized by the statement that PNA persistence increases with the number of condensed rings. Within a subclass, e.g., the four-ring PNAs, recalcitrance showed some positive correlation with increasing degree of ring condensation. Highly condensed PNAs such as pyrene and triphenylene were more stable than the less-condensed benz[*a*]anthracene or chrysene. Most PNA degradation occurred during the active period of the experiment, with no clear trend of PNA loss during the closure period. The addition of ground straw during the closure period failed to increase PNA disappearance (data not shown).

In contrast to hydrocarbons, PNA loss from soil samples poisoned by 2% $HgCl_2$ was substantial, amounting to 48% of the total PNA loss from biologically active soil. Loss of three- and four-ring PNAs was relatively high from poisoned soil (data not shown). The frequent aeration periods necessary to replenish oxygen provided some opportunity for volatility losses, but, because of the high molecular weights of the PNAs, such losses are unlikely to be substantial. At 2%, $HgCl_2$ effectively suppresses biological activity in soil (14), but autoxidation followed by incorporation into humus provides a possible mechanism for PNA loss from the

TABLE 1. PNA content of sludge-treated soil samples

PNA	PNA content of sludge-treated soil samples (μg/g of soil)										
	Elapsed time (days)										Total remaining (%)[a]
	0	75	156	280	419	612	751	920	1,092	1,280	
Three-ring											
Fluorene	77.4	<5.0	<34.0	<0.1		<8.0	<6.0	22.0	22.0	8.2	1.5
Phenanthrene	265.0	60.3	115.0	2.2		2.0	5.4	4.1	4.0	3.4	0.2
Anthracene	55.6	11.1	20.1	11.2	20.2	19.3	19.0	23.0	27.0	27.0	6.9
Four-ring											
Fluoranthene	19.9	14.6	48.5	17.6	18.9	12.0	10.9	11.0	19.0	6.6	4.7
Pyrene	40.9	47.0	96.9	115.0	182.0	213.0	200.0	279.0	280.0	245.0	85.6
Benz[*a*]anthracene	9.4	7.2	15.9	9.7	7.4	5.2	4.0	6.8	5.5	1.0	1.5
Chrysene	7.9	7.0	19.1	12.7	11.6	6.8	<5.0	5.8	5.6	1.7	3.1
Triphenylene	3.0	3.2	7.3	10.7	11.2	12.4	12.0	8.5	21.0	15.0	71.4
Five-ring											
Benzo[*ghi*]fluoranthene	<4.0	<4.0	<5.0	<4.0	<12.0	<8.0	<20.0	7.1	9.5	14.0	~100.0
Benzo[*b*]fluoranthene	1.8	2.1	3.8	5.0	7.9	8.5	7.6	9.5	9.7	10.0	79.4
Benzo[*j*]fluoranthene	0.9	0.9	1.8	2.3	4.3	2.8	2.4	5.0	4.6	5.0	79.4
Benzo[*k*]fluoranthene	1.1	1.3	2.4	3.6	4.5	5.0	5.4	3.0	2.5	2.3	29.9
Perylene	1.2	1.1	1.8	3.1	3.7	4.9	2.8	4.8	4.7	5.0	59.5
Benzo[*a*]pyrene	7.2	5.1	12.0	15.8	23.1	25.4	28.6	29.0	25.0	28.0	55.6
Benzo[*e*]Pyrene	4.6	4.0	8.4	10.9	16.5	18.3	19.6	28.0	26.0	28.0	87.0
Six-ring											
(Benzo[*ghi*]perylene)	3.1	2.7	3.5	7.9	10.4	13.6	15.5	6.5	17.0	17.0	

[a] Based on the total amount applied during the experiment. The total remaining of three-ring, four-ring, five-ring, and six-ring PNAs was 1.4, 47.4, 78.5, and 78.3%, respectively.

poisoned samples. Additional studies will be necessary to clarify all mechanisms of PNA loss. Meanwhile, the PNA losses documented in Table 1 should be viewed as resulting from a combination of biodegradation and undefined abiotic mechanisms.

Materials balance. One of the aims of this study was to account quantitatively for the fate of the organic carbon in the oily sludge. This has not been attempted in previous studies and is nearly impossible to achieve under field conditions. It was assumed that sludge hydrocarbons contain an average of 90% carbon (Table 2). For humus material, both in the sludge and the soil, an average carbon content of 55% was assumed (16).

In the form of hydrocarbons and humus, the landfarm soil received a total amount of 466 mg of C per g of soil. Of this, 97% was accounted for in terms of residual hydrocarbon, residual humus, and evolved CO_2. Total humus changed only very slightly from a 203-mg C input to a recovery of 190 mg of C per g of soil. Of course, this does not mean that humus was not mineralized during the experiment, but, as in most soil systems, humus was in a dynamic equilibrium (16). Humus mineralized was almost quantitatively replaced by humic materials generated from hydrocarbon biodegradation products. Total CO_2 C evolved from sludge was 123 mg/g of soil (corrected for CO_2 evolution from untreated soil). If the 13 mg of CO_2 C contributed by humus mineralization is subtracted from the total, then 110 mg of CO_2 C can be attributed to mineralization of 123 mg of hydrocarbon C (89% recovery).

TABLE 2. Carbon balance of the landfarm soil at the start and conclusion of the 1,280-day experiment

Carbon	mg of C per g of soil		Balance (%)[a]
	Input	Residual	
Hydrocarbon	263	140	53.2
Humus	203	190	93.6
CO_2		122	

[a] The total carbon balance at the conclusion of the experiment was 96.8%.

In terms of nitrogen balance, the soil contained 1.5 mg of N per g. The sludge additions contributed 18.3 mg of N per g of soil and the NH_4NO_3 fertilizer contributed 0.8 mg of N per g of soil, providing a total of 20.6 mg of N per g of soil. At the end of the experiment, 17.9 mg of N per g was recovered (87% of the input). Limitation of hydrocarbon biodegradation by mineral nutrients (5) was not evident during the experiment, justifying the initial assumption that fertilizer will be recycled within the system. The observed slight N loss was probably by denitrification. In landfarm operations, it may be possible to use only moderate fertilizer supplements after an initial heavy fertilization. However, such projections should be first verified in actual field studies, since both leaching and denitrification losses in an open system are likely to be much greater than in the contained laboratory model.

Conclusions. From the above study, the following conclusions are applicable to the landfarming disposal of oily sludges. (i) Application of oily sludge is followed by rapid hydrocarbon biodegradation activity. The percentage of hydrocarbon mineralized (converted to CO_2) will vary with composition, but some hydrocarbon accumulation can be expected in the soils of intensively used landfarms. These hydrocarbon residues appear to be relatively inert and do not interfere with the biodegradation process. (ii) Mineralization is the predominant hydrocarbon removal mechanism during the active phase of the landfarming process. During a closure period, mineralization declines strongly, but hydrocarbon removal continues at relatively high rates by the humification process. (iii) Polynuclear aromatics as a class were degraded-transformed in the sludge tested more extensively than were total hydrocarbons. The number of PNA rings and the degree of ring condensation correlate negative-

ly with the loss of individual PNA compounds. Next to biodegradation, abiotic loss plays a major role in PNA removal. A sludge with a different PNA content or composition may not behave in the same fashion.

ACKNOWLEDGMENTS

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LITERATURE CITED

1. **American Petroleum Institute.** 1980. Manual on disposal of refinery wastes, 1st ed. American Petroleum Institute, Washington, D.C.
2. **Bartha, R., and D. Pramer.** 1965. Features of a flask and method for measuring the persistence and biological effects of pesticides in soil. Soil Sci. **100**:68–70.
3. **Davis, J. B.** 1967. Petroleum microbiology. Elsevier/North-Holland Publishing Co., New York.
4. **DeBorger, R., T. Jacobs, J. Pijck, R. Vanloocke, A. Verlinde, W. Verstraete, and J. P. Voets.** 1974. Belgian national R and D program on the environment—groundwater pollution—annual report 1974. Offices of the Prime Minister, Ministry of Science Policy Programming, Brussels.
5. **Dibble, J. T., and R. Bartha.** 1979. Effect of environmental parameters on the biodegradation of oil sludge. Appl. Environ. Microbiol. **37**:729–739.
6. **Francke, H. C., and F. E. Clark.** 1974. Disposal of oily wastes by microbial assimilation. U.S. Atomic Energy Commission report Y-1934. U.S. Atomic Energy Commission, Washington, D.C.
7. **Gudin, C., and W. J. Syratt.** 1975. Biological aspects of land rehabilitation following hydrocarbon contamination. Environ. Pollut. **8**:107–112.
8. **Jensen, V.** 1975. Decomposition of oily wastes in soil, p. 278–287. G. Kilbertus, O. Reissinger, A. Mourey, and J. Cancela da Fonseca (ed.), Proceedings of the First International Conference on Biodegradation and Humification, 1974, Nancy, France.
9. **Kincannon, C. B.** 1972. Oily waste disposal by soil cultivation process. Environmental Protection Agency publication no. R2-72-110. Environmental Protection Agency, Washington, D.C.
10. **Lehtomake, M., and S. Niemela.** 1975. Improving microbial degradation of oil in soil. Ambio **4**:126–129.
11. **McGill, W. B., and M. J. Rowell.** 1977. The reclamation of agricultural soils after oil spills. *In* J. A. Toogood (ed.), Part I: research. Alberta Institute of Pedology publication no. M-77-11. University of Alberta, Edmonton, Canada.
12. **Pancirov, R. J., T. D. Searl, and R. A. Brown.** 1980. Methods of analysis for polynuclear aromatic hydrocarbons in environmental samples, p. 123–142. *In* L. Petrakis and F. T. Weiss (ed.), Advances in chemistry series, no. 185: petroleum in the marine environment. American Chemical Society, Washington, D.C.
13. **Pramer, D., and E. L. Schmidt.** 1964. Experimental soil microbiology. Burgess Publishing Co., Minneapolis.
14. **Rozycki, M., and R. Bartha.** 1981. Problems associated with the use of azide as an inhibitor of microbial activity in soil. Appl. Environ. Microbiol. **41**:833–836.
15. **Schwendinger, R. B.** 1968. Reclamation of soil contaminated with oil. J. Inst. Petrol. **54**:182–197.
16. **Stevenson, F. J.** 1982. Humus chemistry: genesis, composition, reactions. John Wiley & Sons, Inc., New York.