Effect of Environmental Parameters on the Biodegradation of Oil Sludge†

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A laboratory study was conducted with the aim of evaluating and optimizing the environmental parameters of "landfarming", i.e., the disposal by biodegradation in soil of oily sludges generated in the refining of crude oil and related operations. Oil sludge biodegradation was monitored by CO_2 evolution and by periodic analysis of residual hydrocarbons. The parameters studied were soil moisture, pH, mineral nutrients, micronutrients, organic supplements, treatment rate, treatment frequency, and incubation temperature. Oil sludge biodegradation was optimal at a soil water-holding capacity of 30 to 90%, a pH of 7.5 to 7.8, C:N and C:P ratios of 60:1 and 800:1, respectively, and a temperature of 20°C or above. Addition of micronutrients and organic supplements was not beneficial; sewage sludge interfered with hydrocarbon biodegradation. Breakdown of the saturated hydrocarbon (alkane and cycloalkane) fraction was the highest at low application rates, but higher application rates favored the biodegradation of the aromatic and asphaltic fractions. An application rate of 5% (wt/wt) oil sludge hydrocarbon to the soil (100,000 liters/hectare) achieved a good compromise between high biodegradation rates and efficient land use and resulted in the best overall biodegradation rate of all hydrocarbon classes. Frequent small applications resulted in higher biodegradation than single large applications. Two 100,000-liter/hectare (255 barrels per acre) or four 50,000-liter/hectare oil sludge hydrocarbon applications per growing season seem appropriate for most temperate zone disposal sites.

Petroleum refining unavoidably generates considerable volumes of oil sludges. Common sources of these sludges are storage tank bottoms, oil-water separators, flotation and biological wastewater treatment units, cleaning of processing equipment, and soil from occasional minor spills on refinery grounds. The composition of these sludges varies according to their origin, storage, and treatment history. In a typical case hydrocarbons, water, and mineral solids are present in roughly equal proportions. Oily sludges constitute a disposal problem and, among other options, biodegradation in soil or "landfarming" offers a cost-effective yet environmentally acceptable alternative. In contrast to burial in anaerobic landfills, this mode of disposal leads to the relatively rapid biodegradation of the hydrocarbons by soil microorganisms, thus reducing the danger of groundwater contamination.

The biodegradation of accidentally spilled or waste oils in aquatic and terrestrial environments has been the subject of several recent reviews (3, 5, 7). A careful and extensive study of oil biodegradation in soil by Raymond et al. (20), as well as previous work reviewed in this paper, were all conducted in the field under local weather and precipitation conditions. To complement this work, we conducted a laboratory study on the effects of controlled environmental parameters on oil hydrocarbon biodegradation under simulated landfarming conditions. We expected this study to clarify the feasibility and efficiency of the landfarming practice under a variety of climatic and soil conditions. We were also seeking to optimize those parameters of the landfarming practice that can be readily controlled. Using an actual refinery sludge and soil from a prospective disposal site, we evaluated the effects of soil moisture content, soil pH, levels of inorganic fertilizer, micronutrients, and organic supplements, sludge loading rates, application frequencies, and temperature on hydrocarbon degradation in the landfarming process.

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MATERIALS AND METHODS

Soil. The soil for these studies was collected during the summer season from a prospective landfarming site on the grounds of a petroleum refinery in central New Jersey. After removal of surface litter, soil was collected to a 20-cm depth, passed through a 3-mm sieve, and stored at 20°C in plastic bags. The site has a history of industrial use; its soil was highly compacted and had poor drainage and aeration characteristics. The plans for site preparation included the admixture of sand to improve the above characteristics of the soil, and in our laboratory experiments we also mixed this soil with an equal weight of distilled water—washed sharp gardeners' sand. This sand passed through a 0.5-mm sieve but was retained on a 0.16-mm sieve. Out of concern that the poor quality of this soil may have resulted in a low microbial diversity, before use in experiments the soil was inoculated with 0.1% (wt/wt) good-quality agricultural soil preincubated with 0.5% (wt/wt) oil sludge hydrocarbons.

Oil sludge. An oil sludge sample was collected from an open storage pit at the refinery. The sources of the material were oil-water separators, storage tank bottoms, and small spills on the refinery property. To eliminate the water content and to facilitate handling, the sludge was air dried until sufficiently friable to pass through a 3-mm sieve. The air-dried oil sludge was stored at 5°C in sealed glass containers.

Analytical methods. Soil analyses were performed by the New Jersey Soils Testing Laboratory, Cook College, Rutgers University. Soil pH was determined for a soil–distilled water slurry (1:1, wt/vol) with a pH meter. Soil reaction was adjusted by the addition of $CaCO_3$. Water-holding capacity was determined by the Hilgard cup technique. Moisture contents of the soil and oil sludge were measured gravimetrically after drying at 105°C for 12 h. Soil moisture was adjusted by the addition of distilled water.

The ash content of the oil sludge was determined gravimetrically after dry ashing 10-g samples in a muffle furnace at 850°C for 12 h. The hydrocarbon contents of the oil sludge and soil were extracted by the following method. A 25-g sample was mixed with 25 g of anhydrous Na_2SO_4 and extracted with diethyl ether in a Soxhlet extractor for 12 h. The ether extract was concentrated over steam in a Kuderna-Danish concentrator with a Synder column to a volume of less than 10 ml. The extract was dried with an additional 0.1 g of anhydrous Na_2SO_4 and transferred, with rinsings, to a tared vial. After evaporation of the solvent at 25°C, the extractable hydrocarbon was quantified gravimetrically. Extraction efficiency of the hydrocarbons from the sludge–soil–sand mixture was identical to that from the oil sludge alone.

The extracted hydrocarbons were separated further into class fractions by silica gel column chromatography. The silica gel was activated at 105°C for 12 h. The glass column (outer dimensions, 2 by 28 cm; bed volume, 65 ml) was packed with silica gel (Woelm, Eschwege, West Germany) suspended in hexane. Hydrocarbon samples (0.2 to 0.5 g) were absorbed on 3 g of silica gel and placed on the column. A 3-g layer of anhydrous Na_2SO_4 was placed over the sample to prevent mixing of the sample with solvents. The saturated, aromatic, and asphaltic classes were successively eluted by 120-ml volumes of hexane, benzene, and chloroform–methanol (1:1, vol/vol), respectively. The solvents were dried with anhydrous Na_2SO_4 for use. South Louisiana crude oil of known composition (2, 18) was used for calibrating column separation efficiency. The fractions were collected in tared 250-ml Erlenmeyer flasks and weighed after solvent evaporation. The n-alkanes in the saturated hydrocarbon class were analyzed by gas-liquid chromatography as described earlier (8). Carbon dioxide evolution was monitored in Biometer flasks (6) and plotted cumulatively.

Biodegradation experiments. Unless specified otherwise, the Biometer flasks were prepared and incubated in the following manner. Each flask was charged with 10 g of soil, 10 g of sand, and 2.08 g of air-dried oil sludge containing 500 mg of extractable hydrocarbons. Water was added to 60% of the holding capacity, and the soil pH was adjusted to 7.5 by using 7.5 mg of $CaCO_3$ per flask. To avoid any interference with the CO_2 evolution measurements from the oil sludge, $CaCO_3$ treatment of the soil was performed at least 10 days before the oil sludge addition. Nitrogen fertilizer was added as NH_4NO_3 in a C:N ratio of 60:1. Phosphorus and potassium were added as K_2HPO_4 in C:P and C:K ratios of 800:1 and 400:1, respectively. Incubation was at 28°C. The replacement of trapping alkali and a flushing of the Biometer flasks with CO_2-free air were performed as dictated by the CO_2 evolution activity in 1- to 10-day intervals.

Individual environmental parameters were varied as specified below. All parameters not otherwise specified below were maintained in the following experiments at the standard conditions described above.

Soil moisture. Soil moisture was adjusted with distilled water to 30, 60, and 90% of the holding capacity of the soil. Oil sludge was added at 0.5 g/flask (120 mg of extractable hydrocarbons).

Soil pH. Soil pH was adjusted to 5.0, 6.0, 7.0, and 7.8 with varying amounts of $CaCO_3$. The buffering characteristics of this soil prevented its adjustment to a higher pH without the use of unrealistic amounts of $CaCO_3$. Oil sludge was applied at 0.5 g/flask. Control flasks received $CaCO_3$ but no sludge. After 103 days of incubation, the active flasks received a second 0.5 g of oil sludge.

Mineral nutrients. Mineral nutrients were added as NH_4NO_3 and K_2HPO_4 to give a range of initial carbon-mineral nutrient ratios. In respective flasks, these were as follows: C:N ratios, 15:1, 60:1, and 300:1; C:P ratios, 200:1, 800:1, and 4,000:1; and C:K ratios, 100:1, 400:1, and 2,000:1. Control flasks received no mineral nutrient supplementation. On day 131, all flasks were reloaded with the initial amount (2.08 g) of oil sludge.

Micronutrients and organic supplements. Trace elements were added to a series of flasks as 2.6 ml of a 1:1,000 dilution of Hoagland trace element solution (21). The tested organic supplements included 7.5 mg of Difco yeast extract per flask. Controls received yeast extract but no oil sludge. Another series of flasks received dried domestic sewage sludge (31.4%

organic matter) at a 1% (wt/wt) concentration. Controls received sewage sludge but no oil sludge, or oil sludge only.

Treatment rate. Treatment rate was tested at increasing sludge loading rates, corresponding to 0.25, 0.5, 1.0, 2.0, and 3.0 g of extractable hydrocarbons per flask. After 131 days of incubation, flasks received a second charge corresponding to the initial one.

Treatment frequency. Treatment frequency was tested at an overall sludge loading rate corresponding to 2.0 g of extractable hydrocarbons per flask. This amount of sludge was added as a single charge on day 0, as two 1.0-g charges on days 0 and 87, or as four charges of 0.5 g each on days 0, 57, 87, and 124. A proportional amount of fertilizer accompanied each oil sludge application.

Temperature effects. Temperature effects were tested by incubation of oil sludge treated and untreated control flasks at 5, 13, 20, 28, and 30°C. The soil used in these experiments was preincubated for 40 days at corresponding temperatures.

Treatments were made in duplicate. In experiments that involved reloading, initial treatment was in quadruplicate. From the four flasks, two were reloaded and two were sacrificed for analysis. All data points represent the mean of duplicate or quadruplicate samples. Variation between replicates was within ±7%.

RESULTS

Soil analysis. The soil from the prospective landfarming site had a loam texture (42% sand, 34% silt, 24% clay). Its organic matter content was 3.2%, total nitrogen was 0.03%, the cation exchange capacity was 8.1 mg/100 g of soil, and the pH was 3.7. Major mineral nutrient concentrations were found to be as follows (in milligrams per kilogram): Mg, 371; P, 12; K, 13; NO_3^-, 6; NH_4^+, 8. Because of the poor soil quality indicated by the above data, the soil was conditioned before use in our experiments as described above. Similar conditioning was recommended for the disposal site.

Oil sludge analysis. The composition of the air-dried oil sludge used in our experiments was as follows: extractable hydrocarbon, 24%; ash, 72%; water, 4%. By column chromatography, the hydrocarbon composition was found to be: saturated hydrocarbons, 57%; aromatic hydrocarbons, 29%: asphaltic hydrocarbons, 14%. The high ash content of the sludge was consistent with its origin and storage history. The absence of alkanes shorter than C_{14} reflected the effects of weathering during open pit storage. The relatively high proportion of saturated hydrocarbons was expected to favor microbial utilization of the sludge hydrocarbons. As sludges from various sources vary in their oil content, for comparison purposes we found it useful to express our loading rates in terms of extractable hydrocarbons rather than as total sludge weight.

Soil moisture. Carbon dioxide evolution of oil sludge-treated soil was monitored for 60 days at 30, 60, and 90% of the water-holding capacity. Identical CO_2 evolution rates were recorded at all three moisture levels (data not shown).

Soil pH. Raising the soil pH by the addition of $CaCO_3$ had a marked positive effect on oil sludge biodegradation (Fig. 1). The CO_2 evolution of controls not receiving oil sludge showed a similar trend, but the increases were smaller. The CO_2 evolution of untreated controls was subtracted from the CO_2 evolution of the treated samples to show the net effect of pH on oil sludge biodegradation. The highest pH tested (pH 7.8) resulted in the highest rate of CO_2 evolution. Although with this particular soil it was not practical to test a higher pH, we believe this value approaches the optimum.

After 60 days of incubation, CO_2 evolution from the oil sludge stopped for all practical purposes. This could have been due to the exhaustion of metabolically accessible hydrocarbon or to the accumulation of toxic metabolites. The reapplication of oil sludge on day 103 showed that the former was the case. CO_2 evolution was more rapid upon the second application than it was at the beginning of the experiment. The positive effect of increased soil pH was more marked after the second sludge application, with pH 7.0 and pH 7.8 giving the greatest rate and extent of CO_2 evolution.

Information gained from residual hydrocarbon extraction is shown in Fig. 2. In this histogram, percent hydrocarbon degraded gave a higher conversion percentage than did CO_2 evolution. This is to be expected, since some hydrocarbons are converted to cell material and degradation products other than CO_2.
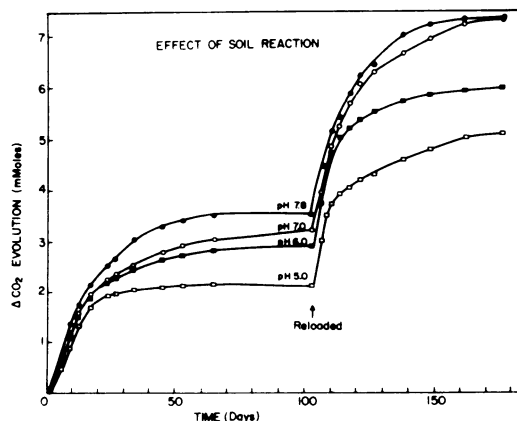

FIG. 1. *Effect of soil reaction on carbon dioxide evolution.*

The greatest extent of hydrocarbon conversion occurred at the highest pH tested (pH 7.8). Subtracting the values for days 0 to 103 from the values for days 0 to 180 shows a greater biodegradation activity during the last 77 days than during the first 103 days. The residual hydrocarbon from the first oil sludge application was not inhibitory to the biodegradation of the second charge. On the contrary, the apparent enrichment of a hydrocarbon-degrading population resulted in a more rapid rate and a greater extent of hydrocarbon biodegradation. Residual recalcitrant hydrocarbons from the first application may have been degraded by cometabolism during the biodegradation of the second charge. The overall decrease in extractable hydrocarbons for the 180-day period was 57%.
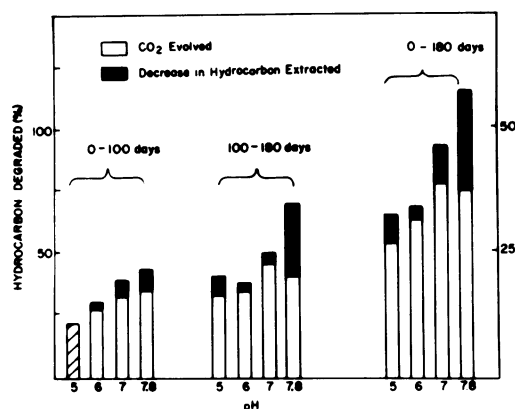

FIG. 2. *Effect of soil reaction on oil sludge biodegradation. The total height of the histograms represents hydrocarbon degradation; the white portion of the histograms represents CO_2 evolution as a percentage of the theoretical total. The CO_2 evolution and hydrocarbon degradation at 100 days and pH 5.0 were identical and are represented by the cross-hatched bar. The group of histograms on the right is on a different scale because of the reloading on day 103.*

Mineral nutrients. The CO_2 evolution data of this 285-day experiment with reloading at day 131 are summarized in Table 1. The kinetics of CO_2 evolution resembled those in Fig. 1. Between days 0 and 131, the two lower levels of fertilization gave almost identical CO_2 evolution values (24.0 and 23.3%, respectively), but for the period from day 131 to day 285 and for the whole duration of the experiment the intermediate treatment level was clearly superior. The highest fertilization level resulted in only a slight CO_2 evolution increase as compared with the unfertilized control.

Determination of residual hydrocarbons confirmed that percent biodegradation was greatest at the intermediate fertilization level. At the highest level of fertilization, biodegradation was only slightly greater than in the control, which received no mineral nutrients. The greater amount of fertilizer itself did not inhibit biodegradation. In other experiments discussed below, flasks were prepared with identical amounts of mineral nutrients, but amounts of hydrocarbon (2 g/20 g of soil) were added to bring the ratios to the following values: C:N, 60:1; C:P, 800:1; and C:K, 400:1. No loss of activity was observed in these treatments. Apparently, conditions of overly low ratios of carbon to mineral nutrients do not favor oil sludge biodegradation.

It should be pointed out that here and in other experiments, unless specifically noted otherwise, no new fertilizer was added upon reloading with oil sludge. Little or no fertilizer loss was expected in the aerobic closed-flask system, and it was anticipated that the bulk of the fertilizer elements, after exhaustion of the readily utilizable hydrocarbons and decline of the responsible microbial population, would be available for use in biodegradation of the subsequent oil sludge load.

Micronutrients and organic supplements. Hoagland trace element solution slightly stimulated CO_2 evolution (Table 2) but not biodegra-

TABLE 1. *Effect of mineral nutrients on oil sludge biodegradation*

Mineral nutrient ratios	% Biodegradation as determined by:[a]					
	CO_2 evolution for days:			Residual hydrocarbon for days:		
	0–131	131–285	0–285	0–131	131–285	0–285
No nutrients added	13.5	22.1	17.8	26.1	35.1	30.0
C:N, 300:1; C:P, 4,000:1; C:K, 2,000:1	24.0	30.0	27.0	28.3	53.7	39.9
C:N, 60:1; C:P, 800: 1; C:K, 400:1	23.3	38.5	30.9	33.2	59.6	45.2
C:N, 15:1; C:P, 200: 1; C:K, 100:1	13.9	28.9	21.4	26.5	48.9	36.7

[a] Loadings were 0.5 g of oil sludge hydrocarbon each on days 0 and 131. The total biodegradation period was 285 days.

dation, as measured by residual hydrocarbon. Yeast extract acted in a similar manner. The latter test was prompted by a report (15) that addition of brewers' waste yeast, at the same concentration as the yeast extract added in this experiment, stimulated the biodegradation of two oils in soil, perhaps by supporting cometabolic hydrocarbon transformations. In our tests, neither trace element nor yeast extract supplementation proved to be of substantial value. An observed slight stimulation, which was nevertheless greater than the excess CO_2 produced by the yeast extract treatment alone, must be ascribed to an enhanced mineralization of hydrocarbon biodegradation intermediates. However, the overall hydrocarbon biodegradation did not increase.

In past years, domestic sewage sludge has received much attention as a soil conditioner (1). Since some organic components of sewage sludge may support cometabolic transformations of the more recalcitrant hydrocarbons, we tested the effects of this readily available and inexpensive additive. Although sewage sludge at 1% (wt/wt) increased CO_2 evolution of soil without oil sludge, it depressed both CO_2 evolution and soil

TABLE 2. *Effect of trace elements, yeast extract, and sewage sludge on oil sludge biodegradation*

Treatment	% Biodegradation as determined by:[a]	
	CO_2 evolution	Residual hydrocarbon
Control	19.0	34.4
Hoagland trace elements	22.1	33.2
Yeast extract	22.6	33.7
Sewage sludge	13.6	26.1

[a] Loading was 0.5 g of oil sludge hydrocarbon. The biodegradation period was 83 days. The control received oil sludge but no other additives. The data are corrected for CO_2 evolution from soil alone, from soil with yeast extract but no oil sludge, and from soil with sewage sludge but no oil sludge, respectively. Consequently, the CO_2 evolution data represent net CO_2 evolution from the oil sludge.

biodegradation in treated samples (Table 2). Consequently, this material does not appear to be a desirable conditioner for landfarming sites.

Oil sludge loading rate. To make the most efficient use of the limited land area available for disposal, a high rate of oil sludge application is desired. However, oil sludge concentration in the soil should not be so high as to depress the rate of biodegradation.

With an increase in the oil sludge loading rate, there was an increase in the absolute amount of CO_2 evolution up to 1 g of hydrocarbons per flask containing 20 g of soil–sand mixture (data not shown). A loading rate of 2 g of hydrocarbon per flask did not result in a further increase in CO_2 evolution, and at 3 g/flask a 12% decrease in CO_2 evolution was observed as compared with the 1- and 2-g/flask loading rates. Table 3 shows the percent biodegradation of the hydrocarbons in the oil sludge as measured by CO_2 evolution and by residual hydrocarbon analysis. As expected, the greatest percentage of degradation occurred at the lowest application level. A reduction in percent biodegradation occurred above the 1-g/flask application rate. Of course, the lowest loading rate would not make optimal use of the biodegradation capacity of the available land area. Table 4 shows the absolute amounts of hydrocarbon degradation and the resulting residue levels. The best compromise between high degradation rates and relatively low residue levels was achieved with two additons of 1.0 g of hydrocarbon per flask, and we consider this the maximum useful loading rate. With the usual conversion factors to the plough layer of field soil, this translates to approximately 100,000 liters of hydrocarbon per hectare (255 barrels per acre). This amount can be applied twice during a 9-month growing season. The above application rate refers to the hydrocarbon content of the sludge rather than to the total bulk of the sludge, since its water and inorganic solid content is not expected to influence greatly the maximum useful loading rate. The above projection should not be considered

TABLE 3. *Effect of loading rate on oil sludge biodegradation*

Loading rate[a] (g)	% Biodegradation as determined by:					
	CO_2 evolution for days:			Residual hydrocarbon for days:		
	0–131	131–285	0–285	0–131	131–285	0–285
3.00	6.3	8.7	7.5	12.0	20.5	14.5
2.00	11.7	14.5	13.1	20.4	26.0	22.4
1.00	23.7	26.3	25.0	34.5	48.8	40.4
0.50	23.3	38.5	30.9	36.0	56.9	45.2
0.25	32.0	48.0	40.0	36.0	60.7	48.7

[a] Grams of extractable hydrocarbon per 20 g of soil–sand mixture. The flasks were reloaded with equal quantities on day 131, and the total biodegradation period was 285 days.

valid for so-called "biosludges" from units for the biological treatment of oily effluents. These sludges usually have a low hydrocarbon content combined with a high amount of nonhydrocarbon organic material, and their maximum useful loading rates remain to be determined.

Analysis of the residual hydrocarbons by component classes revealed that the loading rate had an effect on the types of hydrocarbons degraded. This heretofore unrecognized effect deserves additional consideration in decisions on treatment rates. Of interest is both the absolute mass and also the percentage of each hydrocarbon class degraded in relation to the amount applied. Biodegradation of the saturated hydrocarbon class, expressed as mass degraded, closely followed the pattern established by CO_2 evolution (Fig. 3A). The mass degraded after one and two loadings increased with increasing loading rates up to 1 g of hydrocarbon per flask. The mass degraded at a loading rate of 2 g/flask was only slightly higher, and a loading rate of 3 g of hydrocarbon

per flask resulted in a lesser mass degraded. The percent biodegradation of the saturated hydrocarbon class (Fig. 3B) decreased with increasing charge size, leaving a larger residue of saturated hydrocarbons at higher loading rates.

During the biodegradation of the first load of oil sludge, the mass of aromatic hydrocarbons degraded was highest at a loading rate of 1 g of hydrocarbon per flask; at higher loading rates biodegradation decreased. The mass of aromatic hydrocarbons degraded after two oil sludge loadings increased with increasing loading rates. However, the percentage of aromatics degraded after two loadings decreased at rates above 1 g of hydrocarbon per flask. Enrichment of organisms that can degrade aromatics and/or their enhanced cometabolic activity in the presence of saturated hydrocarbons from the second sludge application are the most plausible explanations for the improved utilization of the aromatics at the higher application rates. Some highly condensed polynuclear aromatic hydrocarbons are considered to be potentially carcinogenic. Loading rates of up to 1 g of hydrocarbon per flask (100,000 liters/hectare) resulted in the highest percentage of biodegradation of the aromatic hydrocarbon residue.

The asphaltic hydrocarbons are considered to be the least susceptible to biodegradation, their complex structure rendering them relatively recalcitrant to microbial attack. Some asphatic material is considered analogous to the humic matter in soil. The asphaltic class also has a nonhydrocarbon component consisting of compounds that contain nitrogen, sulfur, and oxygen. During the metabolism of hydrocarbon compounds, the accumulation of oxygenated intermediates contributes to the asphaltic material in the soil (12).

TABLE 4. *Comparison of hydrocarbon biodegradation and residue at various loading rates*

Loading rate[a] (g)	No. of loadings	Amt of hydrocarbon biodegraded (g)	Amt of residual hydrocarbon (g)
3.00	2	0.88	5.12
2.00	2	0.90	3.10
1.00	2	0.81	1.19
0.50	2	0.45	0.55
0.25	2	0.24	0.26

[a] Grams of extractable hydrocarbon per 20 g of soil-sand mixture. The flasks were reloaded with equal quantities on day 131, and the total biodegradation period was 285 days.
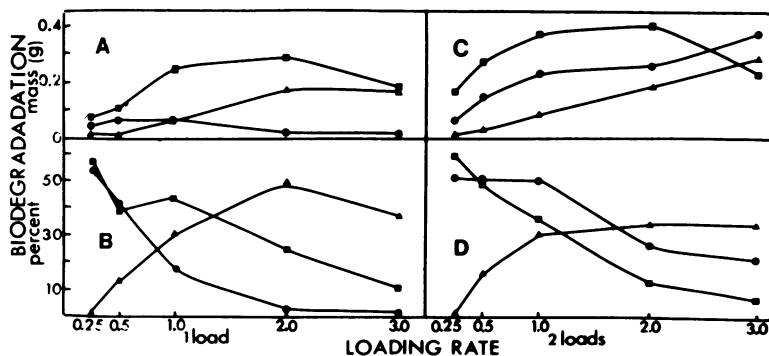

FIG. 3. *Effect of loading rates on the biodegradation of hydrocarbon classes. (A) Absolute amount (mass) of a class removed afer one loading; (B) percentage of a class removed after one loading; (C) absolute amount of a class removed after two loadings; (D) percentage of a class removed after two loadings. Loadings were at 0 and 131 days. The total biodegradation period was 285 days. Symbols:* ■, *saturated hydrocarbons;* ●, *aromatic hydrocarbons;* ▲, *asphaltic hydrocarbons.*

The level of asphaltic residues is the net result of the degradation of hydrocarbon and nonhydrocarbon components of the asphaltic class and the accumulation of oxygenated intermediates from the metabolism of hydrocarbons from all of the hydrocarbon classes. The results of this experiment (Fig. 3A and C) show that the net removal in mass of asphaltic material increases with increasing rates of oil sludge application. The percentage of net asphaltic material removal was at a maximum at a loading rate of 2.0 g of hydrocarbon per flask after the first loading and at a loading rate of between 2.0 and 3.0 g/flask after the second loading. Apparently, the net removal of asphaltic material was greatest under the conditions where saturated hydrocarbons are least biodegraded. It seems possible that the rapid utilization of saturated hydrocarbons at low loading rates results in the accumulation of metabolites that contribute to the asphaltic material.

Considering the above data on hydrocarbon class removal as a function of loading rate, the 1.0-g/flask (100,000 liters/hectare) rate still remains the loading rate of choice since it results in an efficient removal of potentially carcinogenic aromatics and at least strongly limits accumulation of the recalcitrant asphaltic material.

Treatment frequency. An identical load of 2 g of oil sludge hydrocarbons per flask was applied as a single charge or divided into increments of 0.5 or 1 g and applied, with corresponding amounts of fertilizer, over the same time period. The incremental loading of small charges resulted in a higher rate and a higher total of hydrocarbon biodegradation than did the single-charge loading (Fig. 4). The percent biodegradation data (Table 5) confirmed that the extent of biodegradation was greatest in flasks receiving four loadings of 0.5 g of hydrocarbon per flask. Small multiple loadings apparently kept the hydrocarbonoclastic microbial population in an active growth phase with metabolic processes at their peak. After four consecutive loadings, the residual material was not inhibitory to biodegradation.

Separation of the residual hydrocarbons into component classes revealed that the saturated

hydrocarbon class was preferentially utilized at a smaller charge size (Table 5). The percentage of aromatic hydrocarbons biodegraded was also slightly higher at a charge size of 0.5 g. The net removal of asphaltic material, as in the loading rate experiment, was greatest at the higher charge size of 2.0 g.

The results of this and the preceeding experiment were in good agreement and suggested that the pattern of biodegradation of the hydrocarbon classes may be controlled by regulation of the oil sludge loading rate and frequency. Small charge size loadings resulted in a preferential utilization of the saturated hydrocarbon class and in a maximum overall rate and extent of biodegradation. Larger charge size loadings resulted in slightly lower overall biodegradation, but they favored the net removal of asphaltic material.

Temperature. Temperature profoundly influences all biological processes and can be expected to have a significant effect on oil sludge biodegradation. The CO_2 evolution curves (Fig. 5) indicate negligible activity at 5°C, a 2-week
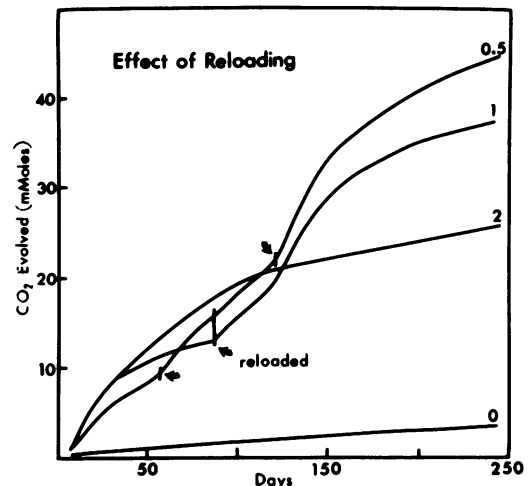

FIG. 4. Effect of reloading on carbon dioxide evolution. Increment charge size, expressed as grams of hydrocarbon per loading, appears at the right. CO_2 evolution was determined at 1- to 10-day intervals as dictated by the rate of evolution. Because of their dense spacing, the data points were omitted.

TABLE 5. Effect of loading frequency on the biodegradation of oil sludge hydrocarbons

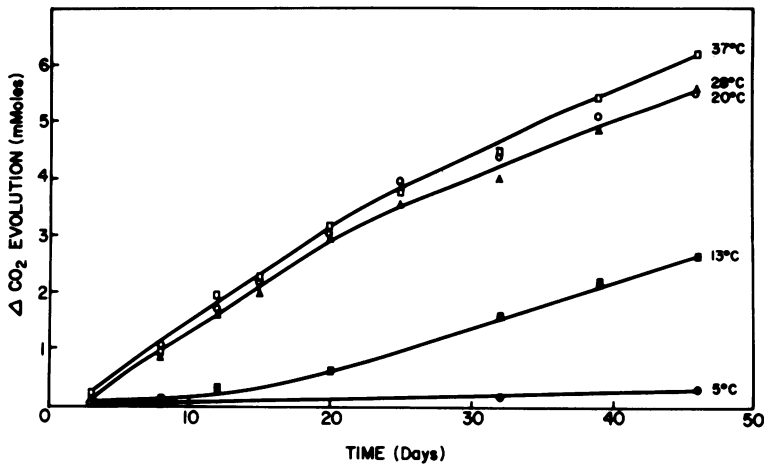| Charge size (g)	No. of loadings	% Biodegradation as determined by:		% of the following hydrocarbon classes biodegraded:		
		CO_2 evolution	Residual hydrocarbon	Saturated	Aromatic	Asphaltic
2.0	1	17.9	24.5	17.7	34.8	31.9
1.0	2	25.8	33.1	35.1	33.8	24.5
0.5	4	30.8	45.5	54.0	40.7	22.0

FIG. 5. *Effect of temperature on CO_2 evolution of oil sludge-treated soil. All curves were corrected for CO_2 evolution of untreated soil at the respective temperature; the curves represent net CO_2 evolution (ΔCO_2) from oil sludge.*

lag phase at 13°C followed by a fair rate of CO_2 release, almost identical rates at 20 and 28°C, and only a slightly higher rate at 37°C.

Hydrocarbon biodegradation, calculated from the residue levels at the conclusion of a 47-day experiment, was plotted as a function of incubation temperature (Fig. 6). It verifies the unexpected pattern indicated by the CO_2 data, i.e., little or no increase of the biodegradation activity above 20°C.

DISCUSSION

Soil moisture. The aerobic biodegradation of simple or complex organic material in soil is commonly greatest at 50 to 70% of the soil water-holding capacity (19). Inhibition at lower values is due to inadequate water activity, and higher values interfere with soil aeration. The thin soil layer (1 cm) in our experimental design minimized the effects of waterlogging on soil aeration, and, therefore, the extrapolation of this result to the field may not be valid. The lack of inhibition at 30% of the water-holding capacity, although unexpected, cannot be regarded as an experimental artifact. It suggests that the moisture requirements for maximum activity on hydrophobic petroleum may be different than the optimal moisture levels for the biodegradation of hydrophilic substrates. It is likely that the hydrocarbons, by rendering some surfaces hydrophobic, reduce the water-holding capacity of the soil and thus increase the availability of the water that is present.

Soil pH. Extremes of pH are inhibitory to the great majority of microbial degradation processes. Most fungi are less adversely affected by
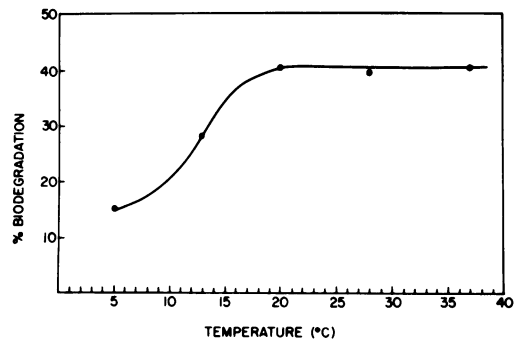

FIG. 6. *Effect of temperature on hydrocarbon biodegradation in oil sludge-treated soil.*

low pH values than are bacteria. Although the relative role of bacteria versus fungi in the biodegradation of hydrocarbons in soil has not been determined, liming in our experiments favored the biodegradation of oil. Our results are in agreement with previous fundings (15, 23). With our test soil, liming to an over-optimal pH was not practical, but considering that Verstraete et al. (23) found optimal activity at a pH of 7.4 and a considerable inhibition at pH 8.5, our highest pH tested (pH 7.8) should be close to the optimum.

Mineral nutrients. The limitation of petroleum biodegradation due to a nutritional imbalance between the substrate carbon supplied by petroleum and the nitrogen and phosphorus required for microbial growth has been extensively reviewed in relation to the marine environment (3, 5). As the nitrogen and phosphorus reserves of most soils are low, a similar situation can be expected to exist in soil. Several previous reports

(13, 14, 16, 20, 23) on the stimulation of oil biodegradation by mineral fertilizers have appeared, but no systematic effort was made to determine the optimal fertilizer levels.

The quantity of nitrogen and phosphorus required to convert 100% of the petroleum carbon to biomass may be calculated from the C:N and C:P ratios found in cellular material. Accepted values for a mixed microbial population in the soil are: C:N, 10:1 (24); and C:P, 100:1 (22). In reality, a complete assimilation of petroleum carbon into biomass is not achievable under natural conditions. Some of the petroleum compounds are recalcitrant or are metabolized slowly over long periods. From petroleum compounds that are readily metabolized, some carbon will be mineralized to CO_2. Thus, efficiency of conversion of substrate (petroleum) carbon to cellular material is less than 100%. Population turnover allows for the recycling of nutrients. The optimal C:N and C:P ratios for petroleum biodegradation were expected to be wider than the theoretical values of 10:1 and 100:1, respectively, but they could not be predicted accurately and required experimental determination.

The mineral nutrient ratios resulting in the greatest biodegradation were similar to the ratios found to be optimal for stimulating the biodegradation of crude oil in seawater (4). Although the seawater ratios were rather broadly bracketed in the soil work, i.e., four times higher and five times lower, the results do suggest a similarity in petroleum biodegradation in the two systems.

Potassium, a standard ingredient in agricultural fertilizer formulations, was present in excess of its actual microbial requirement, and no effort was made to determine the exact requirement level for this element.

Our experiment design eliminated leaching and minimized denitrification losses. These factors are likely to remove at least some of the applied nitrogen fertilizer in a landforming operation, and it is expected that fertilizer in the optimal C:N and C:P ratios will have to be reapplied with every new oil sludge charge. The use of slow-release fertilizers is beneficial in terms of fertilizer savings and minimized leaching. These aspects of mineral fertilization are dealt with in a separate publication (Dibble and Bartha, Soil Sci., in press).

Micronutrients and organic supplements. Trace element deficiency in soils is rarely encountered, and the marginal response to the trace element solution was not unexpected. Our finding that yeast extract failed to stimulate hydrocarbon biodegradation contrasts with the report of Lehtomäke and Niemelä (15) on the

stimulatory effect of brewers' waste yeast. The discrepancy may be due to differences in materials, soils, and test conditions. The fact that Lehtomäke and Niemelä did not use mineral fertilizer in addition to the waste yeast, whereas we did, is particularly relevant. In the former case, waste yeast may have supplied merely nitrogen and phosphorus for hydrocarbon-degrading microorganisms.

The reduced hydrocarbon biodegradation in the presence of sewage sludge, evident from both the CO_2 and the residual hydrocarbon data (Table 2), is explainable by either a diauxic effect (17) or by population selection unfavorable for hydrocarbon degradation. The positive effect of the sewage sludge on CO_2 production of control soil excludes a general antimicrobial effect by the sewage sludge.

Treatment rate. To date, oil sludge application rates in landfarming operations were chosen in an arbitrary manner, the criteria being land availability and the appearance and surface stability of the land after application. Jensen (11) studied the influence of oily waste concentration on biodegradation activity as monitored by oxygen uptake. Oily waste was incorporated into soil at concentrations varying from 0 to 30% (wt/wt). The highest oxygen uptake rate was at a concentration of 5%, but only small positive or negative differences were detected at other concentrations. Total microbial counts increased with oil application to the 5% level but declined thereafter, supporting the O_2 uptake data. No other systematic effort for the optimization of application rates could be located in the literature. On a hydrocarbon basis, the 5% optimal application rate shows a surprisingly good agreement with our own results (1.0 g of hydrocarbon per 20 g of soil–sand mixture).

To date, the effect of the loading rate on the absolute and relative removal of the three hydrocarbon fractions (saturated, aromatic, and asphaltic) has gone unrecognized. Our results show that application rates that are over optimal for a rapid removal of saturated hydrocarbons favor removal of the aromatic and asphaltic classes. Our interpretation of this effect is at this time, by necessity, speculative. We believe that the biodegradation of the higher aromatics and of the asphaltic compounds, few of which can serve as growth substrates by themselves, is dependent upon a continued presence of saturated hydrocarbons to support the cometabolic biodegradation of the former classes. Additional studies will be required for the verification or rejection of this hypothesis.

Treatment frequency. Results showing that smaller and more frequent applications yield

higher overall biodegradation rates than does the infrequent application of large batches are not unexpected, but this parameter has not been evaluated previously specifically with oily wastes. Small, frequent applications minimize the adverse effects of toxic oil sludge components and, at the same time, keep the hydrocarbon-degrading microbial population in a continuous state of high activity. In a practical situation a compromise has to be reached between efficient site use through high biodegradation rates and an avoidance of excessive labor costs for frequent applications. At most temperate zone landfarming sites two 100,000-liter/hectare (255 barrels per acre) or four 50,000-liter/hectare oil sludge hydrocarbon applications per growing season seem appropriate. In the latter case 91,000 liters of oil sludge hydrocarbon per hectare (232 barrels per acre) was degraded per season. This is slightly less than one-half of the applied amount, and longer-term experiments will be necessary to determine whether eventual accumulation of hydrocarbon residues might affect site performance. In a 250-day experiment (Fig. 4) involving four reapplications, we observed a steady increase instead of a decline in hydrocarbon biodegradation, suggesting that a steady state between the described application rates and biodegradation may be reached with no further residue accumulation. Nevertheless, this assumption will need to be verified by experiments and/or long-term field experience.

Temperature effects. A rise in metabolic activity of microbial communities is common throughout the mesophilic range, and the levelling-off of the hydrocarbon biodegradation rate in soil above 20°C was unexpected. Preacclimation of the soil samples at the respective incubation temperatures seems to exclude the possibility of an experimental artifact. As a reasonable but nevertheless speculative explanation we suggest that an increased toxicity of hydrocarbons at higher temperatures may counteract the stimulation of metabolic processes.

Direct temperature regulation of the disposal sites is not technically or economically feasible. Gudin and Syratt (10) suggested the use of black plastic film mulch as a means of stimulating waste oil biodegradation, thus increasing soil temperatures during the winter. The use of film mulch would preclude tilling of the soil and would decrease its aeration. For these reasons, we do not believe this measure would have a positive effect. It is also likely to be unnecessary, since Freedman and Hutchinson (9) reported that the albedo decrease due to oil contamination raised the temperature in the upper 10 to 20 cm of tundra soils by as much as 5°C. Similar effects would be expected in temperate zone soils during the winter.

General remarks. A laboratory approach allowed the testing of a large number of controlled environment parameters within a relatively short time. We are, however, acutely aware of the limitations of such studies in reproducing field situations. Important parameters, such as tillage and soil texture, could not be meaningfully tested in our laboratory system. Poorly drained, waterlogged conditions in the field may drastically reduce biodegradation rates projected from laboratory studies, although this effect was not observed in our laboratory test system. It also remains to be determined whether temperatures that fluctuate around a daily mean during a 24-h cycle can be equated with a steady incubation temperature at the same mean. Consequently, our findings will need validation and possible adjustments in the field. Nevertheless, we feel that our laboratory study will greatly reduce and simplify field experimentation by providing pilot data that can be bracketed in future field tests.

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