Characterization and Bioremediation of a Weathered Oil Sludge

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ABSTRACT ●

The feasibility of bioremediating a weathered petroleum sludge was studied both in the laboratory and in field composting. The sludge consisted of straight-chained alkanes ranging from C_{20} to C_{38}, with a halogen content of 4.1% and a specific gravity of 0.91–0.92 (23–24 API). Twenty indigenous bacteria were isolated from the sludge and identified; all were mesophiles and grew with or without 3.6% sodium chloride. Nine bacteria used sludge as the sole carbon and energy source and were used in the feasibility and composting studies. The sludge-degraders reduced the total petroleum hydrocarbon (TPH) by 97.4% in 10 weeks in shake cultures with 46 μg/g; at TPH values of 4.1×10^4 and 1.0×10^5 μg/g the bacteria reduced the sludge by about 50% in two weeks and at 2.3×10^5 the sludge was toxic. There was no evidence that cometabolism occurred, and a surfactant did not enhance bioremediation. Piles composted with either sawdust or complete compost (composted agricultural wastes) as bulking agents were monitored by measuring the most probable numbers and TPHs through 24 weeks. The initial TPHs ranged from 3.1–3.6×10^4 μg/g, and the bioremediation rate and extent were better using complete compost (72–75% degradation) than with sawdust (56–58%). There was no measurable effect on bioremediation by augmenting with the sludge-degraders, and most probable numbers ranged from 10^5–10^6 cells g^{-1} soil in sawdust-bulked piles and 10^7–10^8 cells g^{-1} in complete compost-bulked piles. After 18 weeks of composting, temperature means were significantly different in the two bulking materials (32°C in complete compost and 41°C in sawdust), but both were 41°C from weeks 19–24, during which time the bioremediation rate did not change in any pile.

Key Words: weathered sludge, oil sludge, bioremediation, composting.

INTRODUCTION ●

To date, research on "sludges" has primarily dealt with those created by spills (Dibble and Bartha, 1979), effluents (Bossert et al., 1984), tank bottom residues (Cansfield and Racz, 1978), or other refinery processes not clearly defined (Marshall and Devinny, 1988; Modi et al., 1980; Prado-Jatar et al., 1993; Shailubhai et al., 1984; Shailubhai, 1986). Of those reported, such as tank bottoms and other refinery residues, none had undergone long-term storage or exposure to climatic conditions, two properties that will alter the chemical composition of the sludge. In addition, there is a lack of complete characterization of the microbes used in these studies, and methods of tracking hydrocarbon degradation have ranged from Soxhlet extraction to CO_2 evolution. These inconsistencies make comparisons difficult and provide little guidance for future studies of other sludges in the laboratory or in the field.

Sludges that are allowed to remain exposed over extended periods to biological and abiological processes, such as limited microbial degradation, volatilization, and auto-oxidation, are known as "weathered sludges" (Floodgate, 1984). Components of weathered sludges become more and more recalcitrant because microbial degradation is limited usually due to oxygen availability and because the hydrocarbon-degrading organisms degrade the most approachable or biodegradable molecules first. In these weathered sludges, the mixture becomes an enrichment of extremely long-chained alkanes, and in some cases may contain high concentrations of aromatic hydrocarbons as well as heavy metals. At high concentrations, toxicity for microbes can result and may even pose health risks to persons handling the sludge (Bartha and Bossert, 1984).

Because individual microbes are limited in the types of hydrocarbons they can metabolize (Britton, 1984), a consortium of hydrocarbon-degrading bacteria is usually employed in an effort to attain degradation of a wider array of hydrocarbons, leading to more complete bioremediation. A process known as cometabolism may also be utilized to increase degradation. In this process, bacteria that partially metabolize some hydrocarbons (e.g., cleaving a ringed compound) provide products that can be degraded by other bacteria. Some non-hydrocarbon-degrading bacteria may then be used in an effort to eliminate a much broader range of hydrocarbons (Perry, 1979). When a particular petroleum, however, contains hydrocarbons that are beyond the degradative abilities of most bacterial consortia, the degradation process cannot be complete.

In the oil fields in East Texas, a process used to remove and dispose of salt water associated with the production of crude oil has led to the creation of weathered sludges that are not conducive to bioremediation due to their anaerobic

Environmental Geosciences, Volume 8, Number 2, 2001 110–122

and toxic nature. After the crude oil is separated by heat from its naturally associated salt water, the residual salt water is placed into large concrete- or plastic-lined pits. Minute droplets of crude oil components, too heavy to be separated in the heating process, accumulate at the surface of the salt water. The salt water is then pumped out of the pits and is disposed of by deep-well injection, leaving a thin layer of heavy hydrocarbons in the pit covered with approximately 10% salt water. After years of repeated separations, the pits can become filled with thick layers of sludge. Allowed to weather without protection from the forces of nature for decades, the sludge becomes primarily composed of the longest alkanes and as viscous as axle grease. The weathered sludge is difficult to handle, has anaerobic microhabitats, and is potentially toxic to microbes used in bioremediation (Bartha and Bossert, 1984; personal observations).

Outdoor storage pits exist in the hundreds in the East Texas oil fields where the sludge they contain is a financial liability to the salt water disposal companies that own them. Most wastes associated with oil and gas exploration, development, and production operations, such as pit sludges, are exempted from federal hazardous waste regulation (Environmental Protection Agency, 1988) and, in Texas, the disposal of such wastes is strictly regulated by the Railroad Commission of Texas (2000). Disposal of the sludge in these pits is of interest from both an environmental standpoint as well as an economic one because bioremediation may be used effectively as a disposal method. Before large-scale bioremediation can be tried, however, studies are needed to investigate the feasibility of bioremediating such wastes.

The purpose of this research was to study the feasibility of bioremediating a weathered petroleum sludge from a salt water collection pit. Studies included are: (1) chemical and physical characterization of the sludge; (2) isolation and identification of the endogenous sludge-degrading microbes; (3) determination of the biodegradability of the sludge; (4) establishment of a sludge concentration nontoxic to bacteria; (5) comparisons of sludge bioremediation rates to establish the efficacy of cometabolism, exogenous surfactant, and crude oil-degrading bacteria; and (6) the feasibility of composting for bioremediation.

MATERIALS AND METHODS

Sludge Preparation and Characterization

Sludge that had weathered three decades in an open collection pit was provided by Ricky Clements of East Texas Salt Water Disposal Co. (Kilgore, Texas). The sludge was solubilized in Freon (1,1,2-trichloro-1,2,2-trifluoroethane) and filtered through glass wool to remove any large particulates of decaying plant and animal materials. After removing the Freon by heating in a water bath (60°C, 1 hr), the resulting material is referred to as filtered sludge (FS) as in Figure 1.

Methods used to characterize the sludge chemically and physically are as follows:

1. general hydrocarbon components: gas chromatography-mass spectrometry (GC-MS) EPA SW 846 method 8015 (Environmental Protection Agency, 1992)
2. total halogen content: Hach method (Environmental Protection Agency, 1992)
3. polychlorinated biphenyl (PCB) content: EPA SW 846 method 8080 (Environmental Protection Agency, 1992)
4. ash content: ASTM-IP method D482-80 (American Society for Testing and Materials, 1980)
5. water content: ASTM method E203 (American Society for Testing and Materials, 1990b)
6. specific gravity and American Petroleum Institute (API) gravity: ASTM method D1429-74 (American Society for Testing and Materials, 1976) and ASTM D1298 (American Society for Testing and Materials, 1990a), respectively
7. BTU content: ASTM method D 3286-17 (American Society for Testing and Materials, 1977)
8. pH determination: EPA method SW846 9045 (Environmental Protection Agency, 1992)
9. ignitability: EPA method SW846 section 2.1.1 (Environmental Protection Agency, 1992)

Bacterial Isolation, Identification, and Culturing Methods

Indigenous bacteria were obtained by spreading sludge on agar dishes and picking clones from isolation streaks made on Tryptic Soy agar (TSA) with and without 3.6% sodium chloride. Multiple slants of each clone were incubated at 25, 35, 40, 45, 50, and 55°C. Conventional microbiological tests were performed on the isolates, and identification was done using Bergey's manuals (Krieg, 1984; Sneath, 1986; Staley, 1989; Holt et al., 1994). Sludge degradation by the isolates was measured by using Bushnell-Haas basal salts medium (B-H BSM) with resazurin as an indicator of oxidation as previously described (Williams et al., 1998). Autoclaved FS (1%) was used as the sole carbon and energy source, and each bacterium was scored as sludge-degrader or nondegrader after four days at 35°C; controls of B-H BSM, those with resazurin but no FS, were inoculated and incubated in the same way to eliminate growth due to organic matter in the inoculum. Inocula were taken from slant cultures.

For all bioremediation experiments, inocula were from Tryptic Soy broth (TSB) cultures shaken overnight (16–18 hr, 70 rpm) on a New Brunswick Scientific Model G-52 gyroshaker at 35°C, and they consisted of 0.1 mL of each isolate of the sludge-degraders. In the cometabolism experiment, inocula included sludge-degraders and nondegraders.

Those experiments comparing sludge-degrading with crude oil-degrading bacteria used the consortia in Table 1 from The University of Texas at Tyler Bacterial Culture Collection, and the bacteria were maintained and cultured as just described. Controls for each experiment received no inoculations, and they were prepared and treated in a manner identical to the experimental flasks.

All laboratory experiments were performed using FS in B-H BSM at a final volume of 150 mL in 500 mL Erlenmeyer flasks, shaken on the gyroshaker at 35°C. Experimental treatments and controls were performed in 3 to 15 replicates, and results of all experiments are reported as means of replicates with standard errors. ANOVAs were used to identify significant changes in total petroleum hydrocarbons.

Feasibility of Bioremediation and Toxicity of Sludge

A concentration of sludge was used that was low enough to eliminate the possibility of toxicity and to maximize the surface area of available carbon source to bacteria, yet high enough to be efficiently extracted using Freon in a separatory funnel and detected by infrared spectroscopy. To facili-

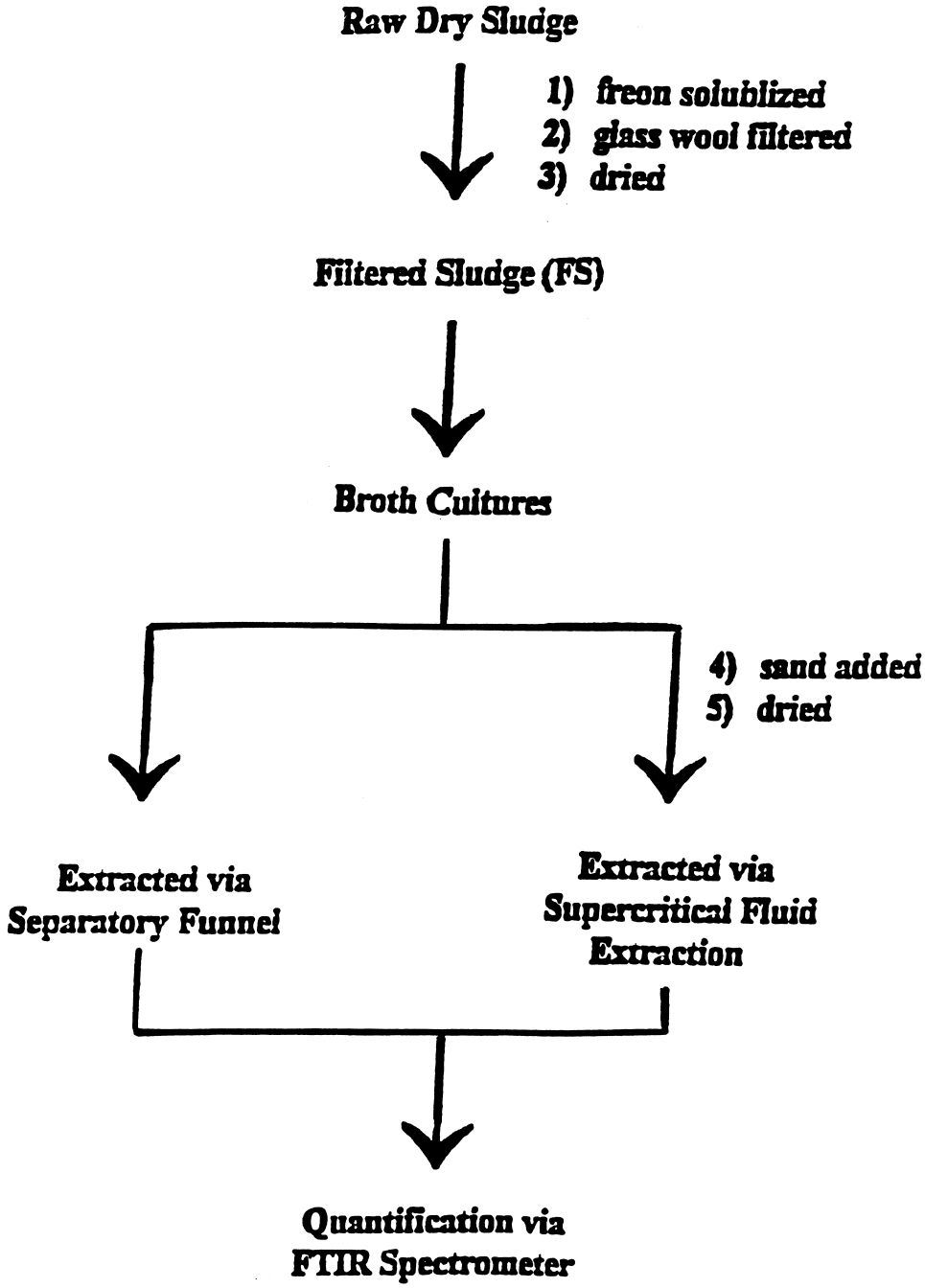

FIGURE 1. Protocols for sludge preparation, extraction, and quantification.

tate such a minute concentration, 15 g of FS were dissolved in 100 mL of Freon; each experimental and control flask received 50 uL of FS, resulting in a TPH of 46 μg/g. Freon extractions of TPHs were performed at the initiation of the study to establish a baseline level; this was followed by extraction of experimentals and controls at one-week intervals for the first four weeks and at the end of 6 and 10 weeks. There were 15 replicates used to establish a baseline with 13 experimental replicates for weeks 1 and 2, and seven for each subsequent interval. Controls consisted of three replicates per interval, and their mean TPH level (auto-oxidation) was subtracted from the baseline mean to eliminate the amount of auto-oxidation; this is referred to as the adjusted baseline level. Biodegradability was reported at each interval as a percentage of TPH degraded in the experimentals as compared to the adjusted baseline TPH.

To determine the qualitative changes at the conclusion of the 10-week period, the seven experimental extractions were combined, volatized to 25 mL, and analyzed for hydrocarbon content by GC-MS. The chromatogram was compared to the chromatogram made on FS before remediation.

To establish the level at which the sludge became toxic to the bacteria, flasks of B-H BSM containing 5, 10, 25, 50, and 75% FS (v/v) were chosen empirically; these percentages resulted in 4.1×10^4, 1.0×10^5, 2.3×10^5, 4.6×10^5, and 6.9×10^5 μg/g, respectively. Baseline, control, and experimental flasks for each interval were in triplicate. Results are presented after two weeks as the percentage of TPH degraded.

Comparative Studies

Cometabolism was investigated after a two-week period by comparing degradation of sludge as the sole carbon and energy source by sludge-degraders to that of sludge-degraders plus nondegraders.

Comparisons were made between experimental cultures containing 1% exogenous, nonionic surfactant (APG 625CS Glycoside; Henkel Corporation, Ambler, PA) and those with no surfactant added over a six-week period, with measurements made at two-week intervals.

A comparison of bioremediation by sludge bacteria to that of crude oil-degrading bacteria was made after two weeks, using the consortia in Table 1.

All comparative studies used triplicate cultures and included baseline extractions and extractions of experimentals and controls at the designated intervals; results are presented as the percentage of TPH degraded. The initial sludge concentration in these three experiments was 9.0×10^4 μg/g.

Extraction and Analytical Methods

In the feasibility experiments, FS was extracted from broth cultures via a separatory funnel (Figure 1). Flasks were ex-

TABLE 1. Bacterial consortia from The University of Texas at Tyler Bacterial Culture Collection.

1	2	3	4	5
Bacillus polymyxa	*Enterobacter intermedius*	*Pseudomonas aeruginosa*	*Acinetobacter calcoaceticus*	*Bacillus megaterium*
Bacillus cereus	*Proteus mirabilis*	*Alcaligenes entrophus*	*Bacillus pasteurii*	*Proteus rettgeri*
Pseudomonas stoutzeii	*Moraxella* sp.	*Alcaligenes paradoxus*	*Propionbacterium acne*	*Corynebacter xerosis*
Arthrobacter globiformis	*Acinetobacter haemolyticus*		*Microbacterium laevaniformans*	*Citrobacter intermedius*
Alcaligenes faecalis	*Microcoleus lylae*		*Cellumonas flavigena*	
Corynebacterium sp.	Sheathed bacterium		*Serratia liquefaciens*	
Proteus sp.	4 group 19 isolates		*Erwinia stewartii*	
Alcaligenes paradoxus	*serratia rabidnea*		*Enterbacter liquefacians*	
	Bacillus coagulans		*Pseudomonas palleroni*	
	Actinomyces viscosus		*Flavobacterium aquatile*	
	Pseudomonas alcaligenes		*Aureobacterium saperdae* or *barkeri*	

The numbers 1–5 are the sources of soil from which the oil-degrading consortia were isolated: 1 = Kenai, AK; 2 = Kilgore, TX; 3 = Marshall, TX; 4 = Rusk, TX; 5 = Median, TX.

TABLE 2. Bacteria isolated and identified from sludge: Degraders and nondegraders

Degraders	Nondegraders
Bacillus firmis	*Bacillus acidocaldarius*
Bacillus fastidiosus	*Bacillus circulans*
Bacillus coagulans	*Capnocytophaga sp.*
Brochothrix campestrus	*Cellulomonas gelida*
Cellulomonas fimi	*Microbacterium imperiale*
Unidentified G+ rod	*Curtobacterium faccumfacions*
Enterobacter cloacae	*Klebsiella oxytoca*
Enterobacter agglomerans	*Escherichia adecarboxylata*
Escherichia blattae	*Aeromonas sp.*
	Pseudomonas solanacearum
	Pseudomonas mendocina

tracted with Freon (25 mL) divided into two equal portions, with the extracts being combined before quantification.

In all other laboratory experiments, hydrocarbons (HCs) were extracted via supercritical fluid extraction using supercritical CO_2 in Freon on a Dionex Series 600 Supercritical Fluid Chromotography/Gas Chromotography. To the aqueous sludge, 200 g of hydrocarbon-free sand was added (Figure 1). After drying the cultures overnight in a drying oven (100°C), the sand-sludge mixture was homogenized using a mortar and pestle and extracted using supercritical CO_2 extraction in Method 3560 proposed by Lopez-Avila et al. (1992) and used by Williams et al. (1998). Extraction time was established by extracting samples of soil containing a known concentration of the sludge used in the laboratory studies. Extractions were performed in duplicate at 30-min intervals until results indicated complete extraction by no increase of TPHs in subsequent extractions. All extracts were treated with 5 g of silica gel before IR analysis (Per-

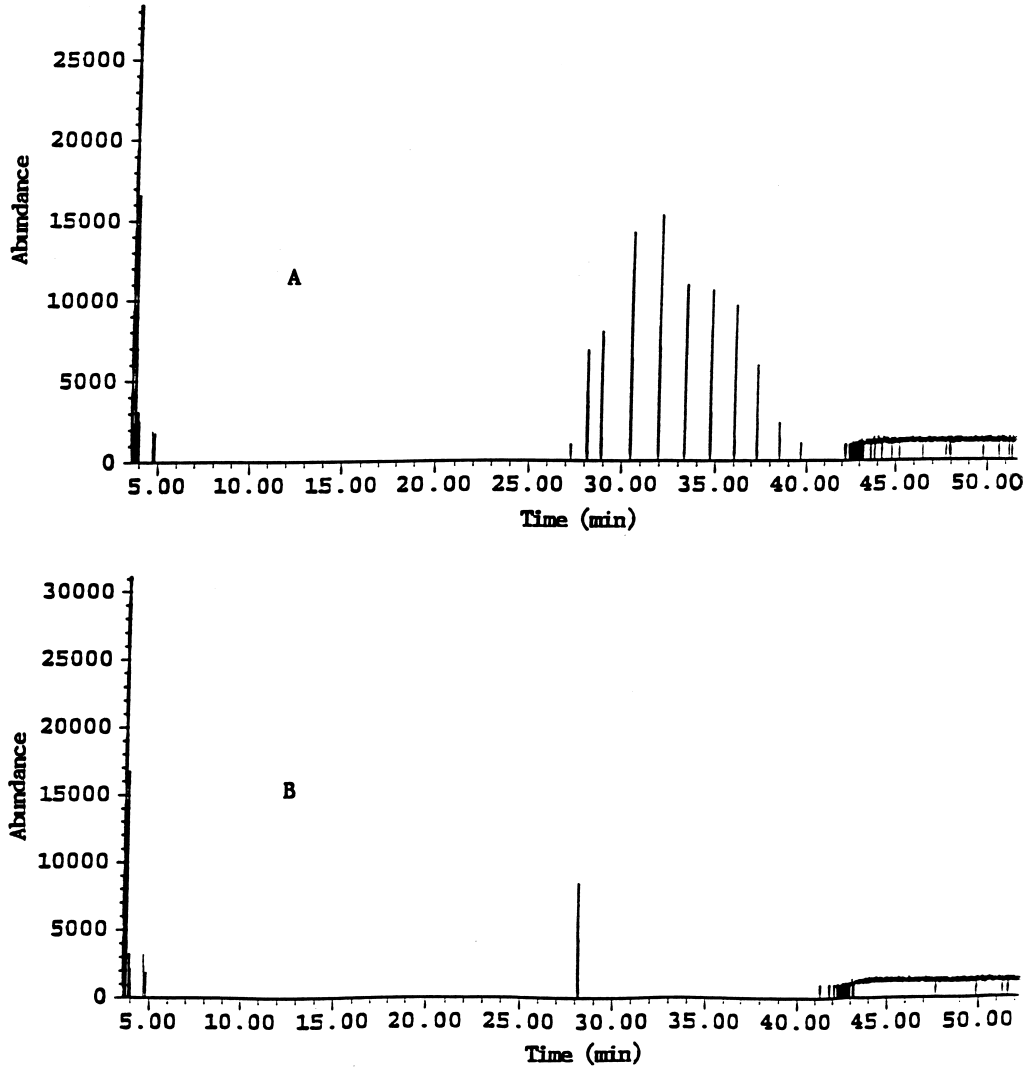

FIGURE 2. Mass spectra of FS before and after 10 weeks of bioremediation: (A) before bioremediation and (B) after bioremediation.

TABLE 3. Physical and chemical properties of filtered sludge.

Specific Gravity	0.91–0.92 (23–24 API)
pH	6.1
BTU	14,388 $BTUlb^{-1}$
Ignitability	Neg.
Water content	2.8%
Total hydrocarbon content	92.1%
Ash content	5.1%
Total halogen content	4.1%
Heavy metals	Negligible
PCB	Negligible

kin-Elmer FTIR spectrometer, Model 1640), using a standard curve according to the EPA Method 418.1 (EPA, 1983).

GC-MS procedures were performed on a Hewlett Packard Gas Chromatogram, Model HPGC 5890, Series II, interfaced with a Hewlett Packard Mass Selective Detector, Model HP 5971, Series II. The column was a Hewlett Packard HP-5MS with dimensions of 30 m × 0.25 um ID. The flow rate was 1 mL min^{-1}, vacuum-compensated with electronic pressure control for constant flow rate. The instrument was calibrated using a mixture of straight-chained alkanes (C^9–C^{18}, C^{20}, C^{22}, C^{24}, C^{26}, C^{28}, C^{30}, C^{32}, C^{34}, C^{36}, C^{38}, C^{40}, C^{42}, and C^{44}).

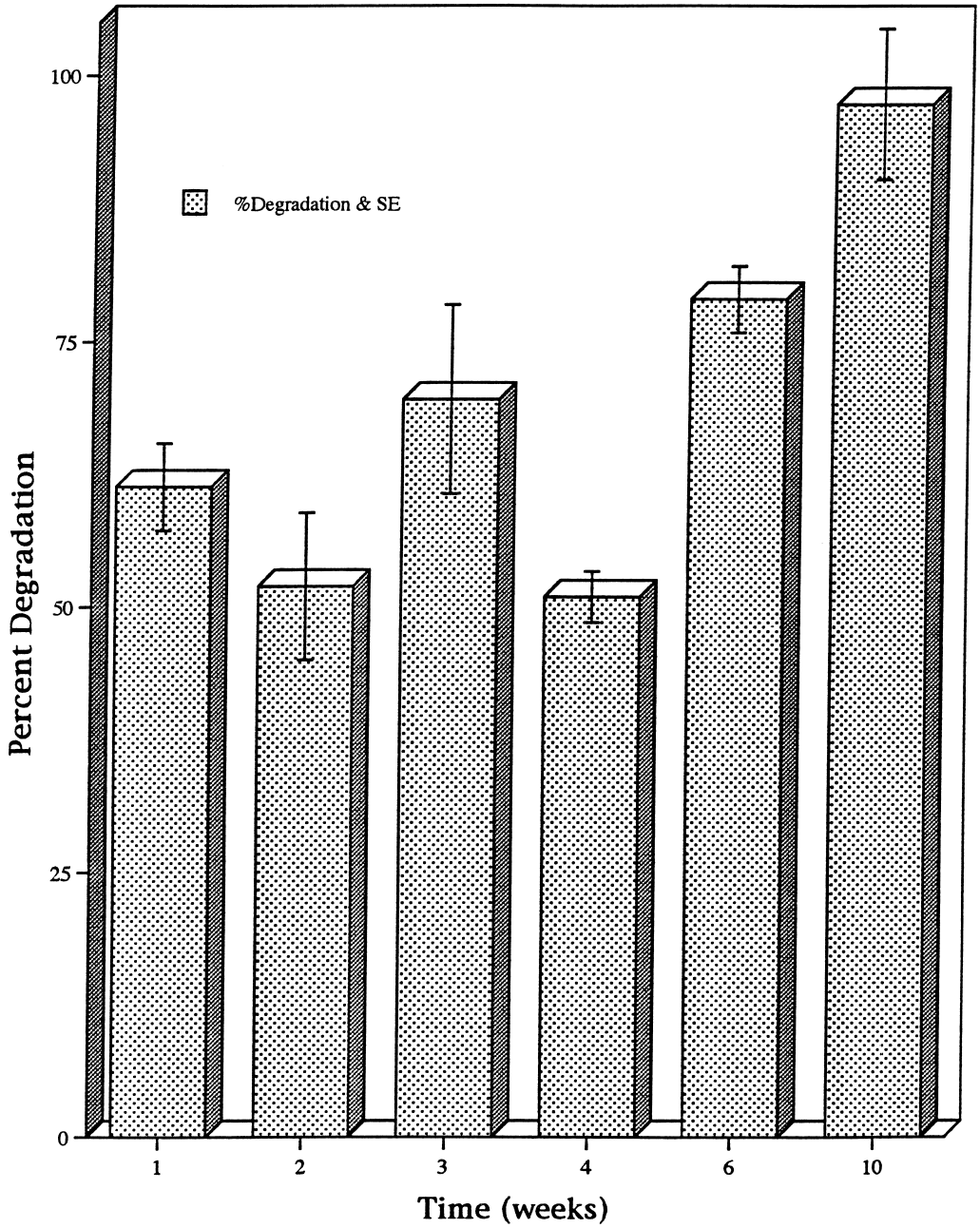

FIGURE 3. Feasibility of sludge degradation in aqueous solution through 10 weeks. Bars represent the means of the percent degradation with standard errors of 13 replicates for weeks 1 and 2 and seven replicates for subsequent weeks at a TPH of 46 μg/g.

Composting Study

Two bulking agents were used: sawdust and a composted material prepared from agricultural waste (complete compost). The complete compost was prepared and supplied by Scott Hammer of Vital Earth Resources, Inc. (Gladewater, TX). Two piles of 14% sludge were prepared by adding 2.74 m^3 of sludge to 16.46 m^3 of each bulking agent and mixing until homogeneous using a front-end loader. The final TPHs of the piles ranged from 3.1–3.6 $\times$ 10^4. To each pile, 37.19 kg of commercial ammonium sulfate (12 N:0 P:0 K, w/w; American Plant Food Corporation (Galena Park, TX) were added. Each of the two piles was divided into two smaller piles for a total of four piles, piles 1 and 2 with sawdust and piles 3 and 4 with complete compost. Piles 2 and 4 were inoculated initially and at 3 and 15 weeks with 4.5 L (0.5 L per isolate) of sludge-degraders, grown in TSB for 24 hr at 35C, while piles 2 and 4 received 4.5 L of water at the same intervals. After mixing, each of the four piles was covered with plastic sheeting to maintain a water-holding capacity (W-HC) of 30% to 90% (Dibble and Bartha, 1979; Sims et al., 1989; Williams et al. 1998) and to prevent cross-

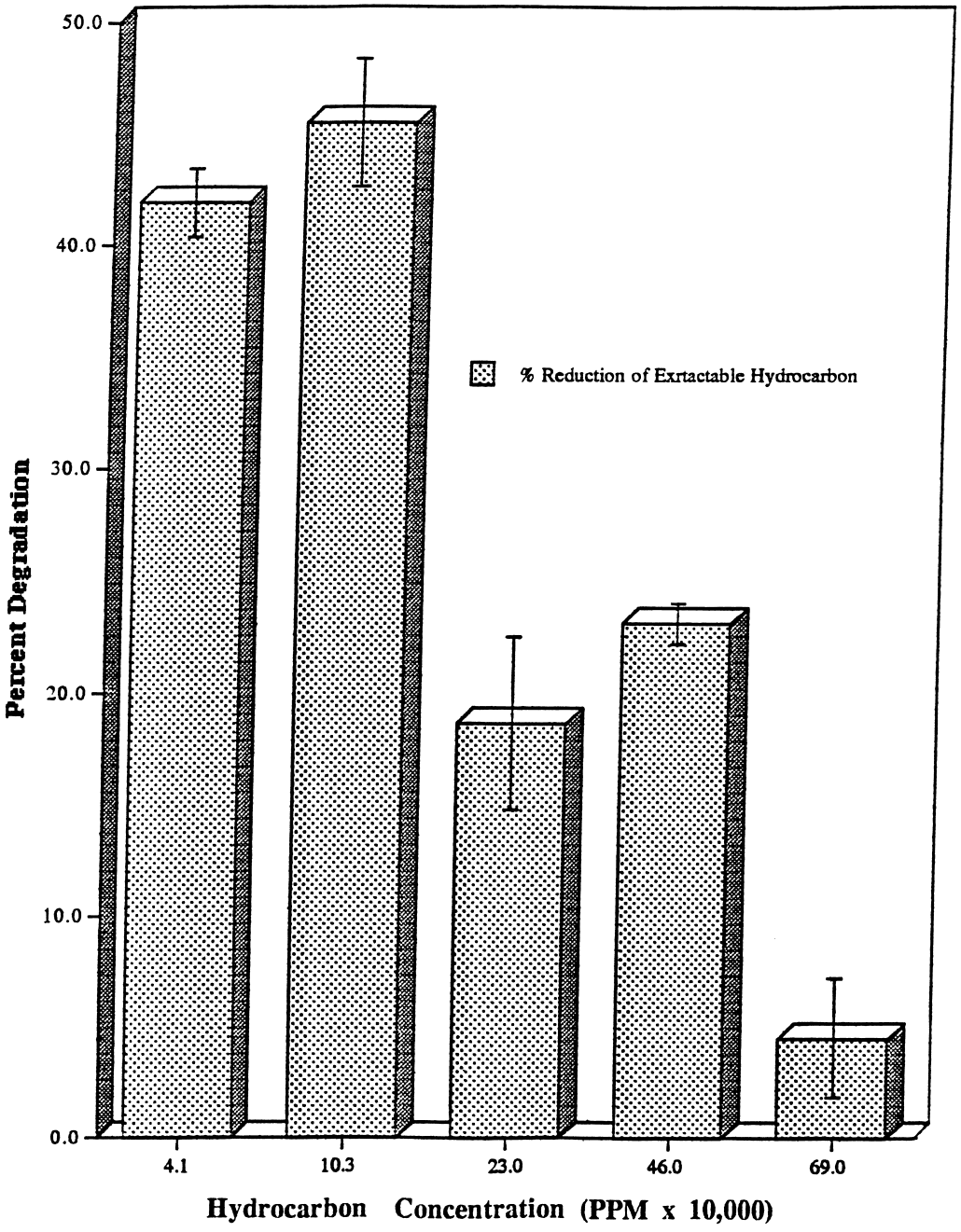

FIGURE 4. Toxicity of sludge at differing TPHs after a two-week period. Bars represent means of percent degradation of three replicates with standard errors.

contamination from runoff of rainwater. Internal temperatures of the piles were monitored by inserting a temperature probe (0.3 m long) into each pile and allowing the temperature gauge to equilibrate before reading. Temperatures were taken twice weekly (first and fourth days of each week) and reported as means with standard errors. ANOVAs were used to determine whether the temperature affected bioremediation significantly.

Bioremediation in the composting piles was monitored for 24 weeks by determining the changes in the most probable number (MPN) of sludge-degraders as described by Williams et al. (1998) and changes in the TPHs at three-week intervals. Four samples were taken from each of the piles after mixing with a front-end loader at each interval, and the samples were homogenized for each MPN and TPH determination. TPHs are presented as the percent of sludge degraded at each interval and MPNs are expressed as cells per gram dry weight of sample. False-positive MPNs were eliminated by using controls identical to the unknowns except without the addition of FS. W-HC of the samples was measured (Klute, 1986) at each interval, and water was added to each pile as needed to maintain 30–90% W-HC as

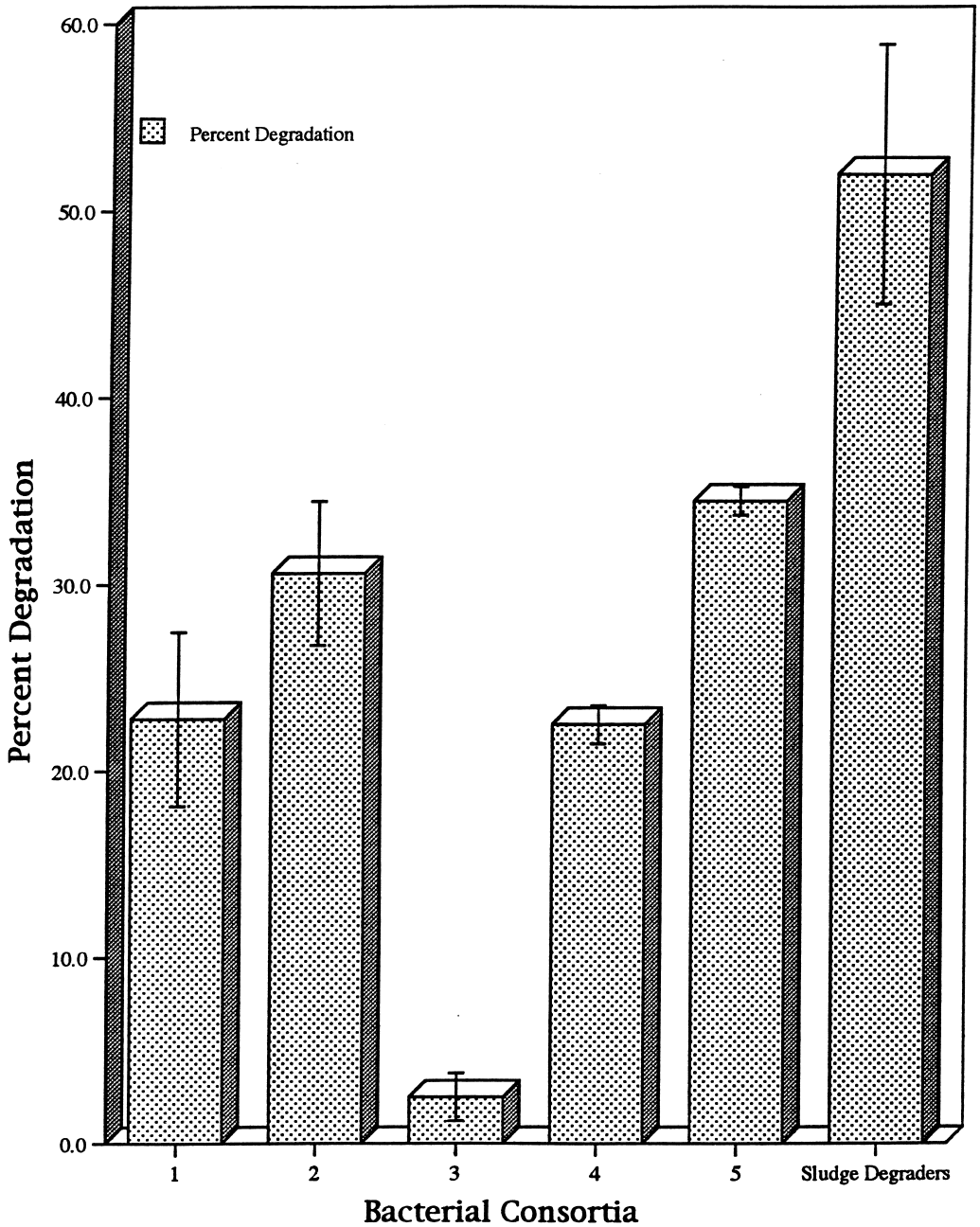

FIGURE 5. Comparative degradation of sludge by six bacterial consortia. Bars represent means with standard errors of three replicates at TPH of 9.0×10^4 μg/g. Consortia 1–5 are listed in Table 1 and the sludge-degraders are identified in Table 2.

recommended by Dibble and Bartha (1979), Sims et al. (1989), and Williams et al. (1998). Initially, the four samples from each pile were extracted separately for TPHs, but after three weeks, when the variations in the four TPHs was minimal as shown by standard deviations of the means, they were homogenized for all of the remaining extractions.

RESULTS ●

There were 20 indigenous bacteria isolated from the sludge and each grew equally well with or without 3.6% sodium chloride. All grew at 25, 35, and 40°C but none grew at 50 or 55°C. The nine sludge-degraders and 11 nondegraders are listed in Table 2. As shown in the GC-MS in Figure 2A, FS had a range of hydrocarbons with approximate retention times of 27 to 40 min, with the most abundant constituent having a retention time of about 32 min. These retention times correspond to straight-chained alkanes ranging in length from C_{20} to $C_{38,}$ with the most abundant component being C_{26} (32min). Other chemical and physical properties of the FS can be seen in Table 3 and include an organic content of 92.1%, a halogen content of 4.1%, and a pH of 6.1. The FS has a specific gravity of 0.91–0.92 (23–24 API), and at room temperature it is a semi-solid.

In all bioremediation experiments, auto-oxidation was less than 1% of the TPH content. As illustrated in Figure 3, the sludge was degraded by an average of 61% after one week and 79% after six weeks, which was the next statistically significant reduction (79%, $p = 0.0128$ with df = 1). At the end of the 10-week period, the TPH had an additional significant reduction ($p = 0.0371$ with df = 1), resulting in 97.4% reduction. The GC-MS performed on the extracted hydrocarbon after bioremediation is seen in Figure 2B. Only one peak existed with a retention time of around 28 min; this corresponds to a straight-chained alkane of C_{21}.

In the toxicity experiment, the sludge was degraded by 40–45% at 4.1×10^4 to 1×10^5 μg/g (5–10% sludge), as shown in Figure 4, but the amount degraded dropped to approximately 20% at concentrations of 2.3×10^5 to 4.6×10^5 μg/g (23–46% sludge). Only 6.7% was degraded at 6.9×10^5 μg/g (69% sludge).

In the cometabolism experiments, 51% of the sludge was degraded by the two consortia and 52% was degraded by the sludge-degraders alone, resulting in no significant difference ($p = 0.4572$ with df = 1).

The difference in bioremediation with surfactant and without surfactant was small, 49–63% with surfactant and 45–54% without surfactant. The difference was not significant ($p = 0.965$ with df = 1).

In the comparative study between the sludge-degrading bacterial consortium and five crude oil-degrading consortia, the consortia of oil-degraders reduced the TPHs by 2.5–34.5% in two weeks, as indicated in Figure 5, while sludge-degraders reduced the TPH by 52% in the same period.

In the composting experiment, shown in Figure 6, the MPNs ranged from 10^5–10^7 cells $g^{-}1$ soil throughout the experiment, except once when the numbers rose to 10^8. The

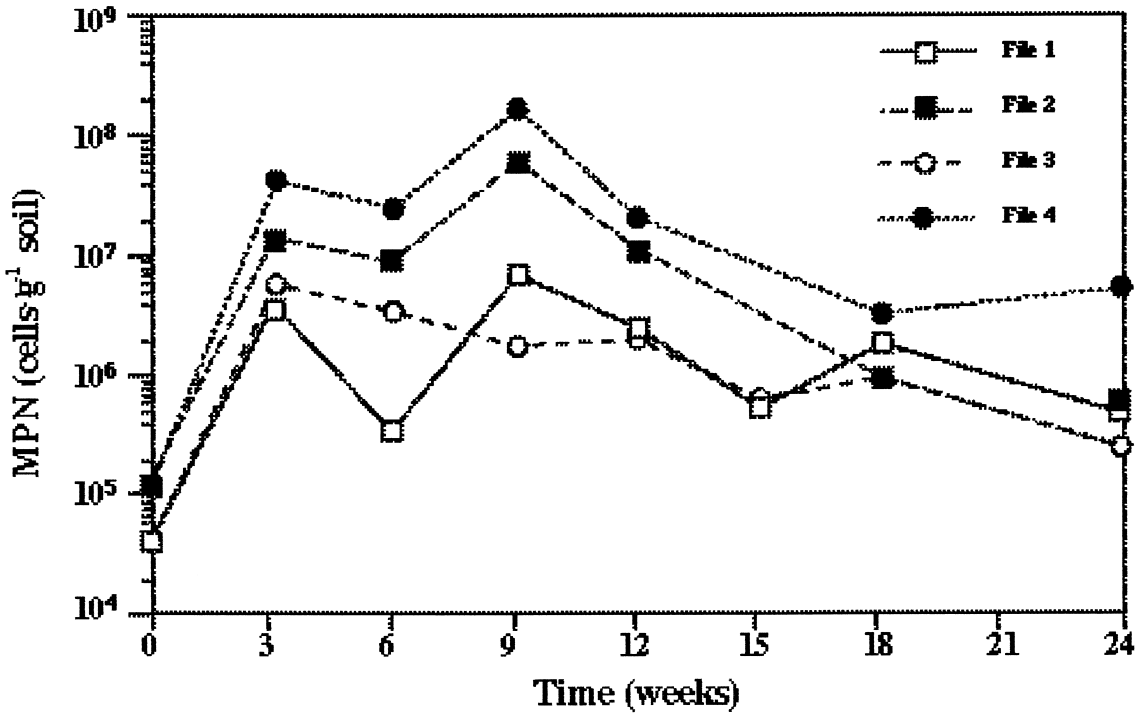

FIGURE 6. Changes in MPNs of sludge-degraders in four composting piles through 24 weeks. Piles 1 and 2 were bulked with sawdust and piles 3 and 4 were bulked with complete compost. Arrows indicate times when bacteria were added (4.5 L) to piles 2 and 4. Samples for MPNs and TPHs were taken at three-week intervals after mixing the piles.

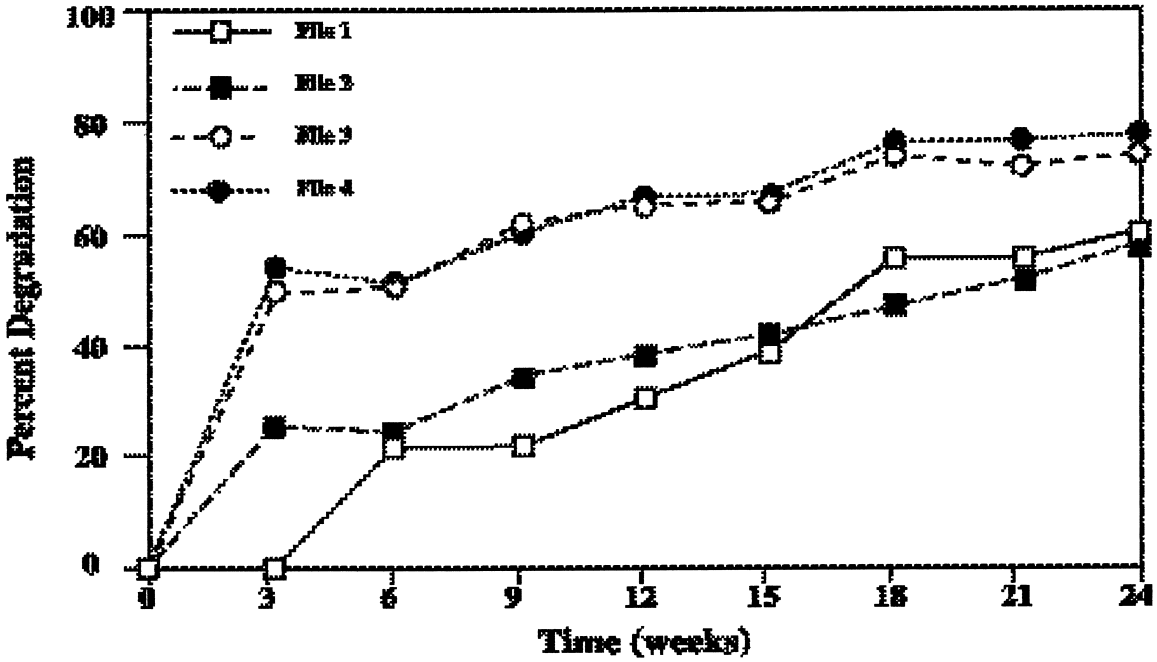

FIGURE 7. Degradation of sludge in the four composting piles through 24 weeks. See Figure 6 for legend.

MPNs were consistently higher in the piles inoculated with sludge-degrading bacteria. The bioremediation of the sludge throughout the experiment, as seen in Figure 7, was almost equal in the two piles bulked with complete compost (72% and 75% at 24 weeks) and essentially equal in the two piles bulked with sawdust (56–58%). W-HCs were 40–60% in the four piles through the 24 weeks.

The temperature means for the composting piles, shown in Table 4, were approximately 41°C for piles 1 and 2 (bulked with sawdust) and 34–35°C for piles 3 and 4 (bulked with complete compost) after 24 weeks; these represent significant differences for the four piles ($p = 0.0$, df = 3) and between the two bulking agents ($p = 0.0$, df = 1) but no significance between the piles with or without bacteria ($p = 0.518$, df = 1; see Table 4). However, during weeks 19–24, the mean temperatures for the four piles ranged from 41–42°C (Table 4), and there were no significant differences between the following: the four piles ($p = 0.583$, df = 3); the bulking agents ($p = 0.213$, df = 1); or the presence and absence of bacteria ($p = 0.480$, df = 1; Table 4). During the last six weeks of composting, the maximum ambient temperatures were 32°C or above for 38 days, with 18 of those being 35°C or higher.

DISCUSSION

Because the nine sludge-degraders grew at temperatures below 45°C but not at temperatures of 50°C and higher, they are mesophiles. Bacteria in this group grow optimally at 20–45°C with tolerances from 15–45°C (Prescott et al., 1996).

The results of the GC-MS on the FS before remediation (Figure 2A) confirmed that the sludge fit the characteristics, both chemically and physically, of a weathered oil sludge. The sludge was a mixture of only straight-chained alkanes of C_{20} to C_{38} with no evidence of short chains, branched chains, or cyclic hydrocarbons. This range is similar to a substance described by Song et al. (1990) as "Bunker C" and also identified as #6 fuel oil by Bruce and Schmidt (1994). Hydrocarbons of the type that would give FS a more

TABLE 4. Temperature means for composting piles and their significance.

	Temperature Means for Weeks[b]			Significance of Temperature Means		
Piles and Treatments[a]	1–24 (n = 41)	1–18 (n = 30)	19–24 (n = 11)	Analysis of Variance for	n = 41	n = 11
1 SD	41.3 ± 4.3	41.0 ± 4.7	41.9 ± 2.8	Piles 1 vs 2 vs 3 vs 4	$p = 0.0$ (df = 3)	$p = 0.583$ (df = 3)
2 SD+	41.1 ± 4.9	41.1 ± 5.6	41.2 ± 2.4	Piles 1 + 2 vs 3 + 4	$p = 0.0$ (df = 1)	$p = 0.213$ (df = 1)
3 CC	35.2 ± 4.8	33.3 ± 3.8	40.5 ± 2.8	Piles 1 + 3 vs 2 + 4	$p = 0.518$ (df = 1)	$p = 0.480$ (df = 1)
4 CC+	34.2 ± 5.7	31.8 ± 4.5	40.6 ± 2.6			

[a] Treatments: SD, sawdust; CC, complete compost; +, bacteria added.
[b] Temperatures were recorded twice weekly except for four weeks when only one was recorded.

fluid character would have been removed during the heating process used in the crude oil production, and there were no hydrocarbons with vapor pressures low enough to be volatile under conditions of open storage. The long-chained hydrocarbons with low volatility account for its semi-solid state at room temperature. The less than 1% level of auto-oxidation in the control flasks is also indicative of weathered oil sludge, as most auto-oxidation would have already occurred in the weathering process.

Some of the properties of the FS were indicative of its origin and provide some interesting questions about the organisms isolated directly from the sludge. The 4.1% halogen-containing sludge was covered by a layer of water with 10% sodium chloride, a level well above ideal for a substrate for freshwater bacteria. This high halogen content, undoubtedly resulting from the associated salt-water that was separated from the crude oil during processing, seemingly had no apparent effect on the bacteria.

It is feasible to bioremediate the sludge since 97% of the TPH was degraded after 10 weeks when starting with 46 μg/g. The sludge was not toxic at 1×10^5 μg/g TPH or 10% (Figure 4) where bioremediation occurred. This is twice the 5% optimum concentration reported by Dibble and Bartha (1979).

The result of the GC-MS after 10 weeks of remediation was qualitative evidence for the removal of hydrocarbons from the sludge (Figure 2B). The flat baseline of the spectrum and the solitary peak with a retention time of 28 min is indicative of only one remaining hydrocarbon type, corresponding to C_{21}. It is curious that a peak with the same retention time was present in the sludge before bioremediation, perhaps indicating that this hydrocarbon was recalcitrant to mineralization.

Cometabolism has been observed in bioremediation of petroleum, and it is generally thought to aid in the biodegradation of cyclic and aromatic hydrocarbons primarily (Perry, 1979). It is not surprising, then, that cometabolism was not observed in this study.

Surfactants are often used to enhance bioremediation rates of hydrocarbons by supplementing the natural production of surfactants. The lack of enhanced degradation by this Henkle surfactant does not mean another type might not yield improved degradation rates. The long-chained alkanes in this sludge may not be miscible with the nonpolar portion of the surfactant molecules to the extent that the surfactant was ineffective.

Those bacteria indigenous to the sludge were far better at degrading the sludge than the consortia of crude oil-degrading bacteria (Figure 5). This illustrates the adaptation of individual bacterial populations to their environment as well as the acclimation of some populations to environments to which they are not indigenous. Those consortia that had the ability to utilize the hydrocarbons in the sludge acclimated quickly and were able to achieve a modest amount of degradation (Figure 5; consortia 1, 2, 4, and 5); consortium 3, however, did not acclimate well to the sludge environment and degraded only 2.5%.

The MPNs in all four composting piles were always within 10^5–10^7 cells g^{-1} soil, except once when the numbers reached 10^8 (Figure 6). The MPNs were consistently higher in piles augmented with sludge-degraders, usually in the 10^7 range. In all instances, where bioremediation occurred, the MPNs were at least in the 10^5–10^6 range, which agrees exactly with reports by Williams et al. (1998) for soils with or without a history of oil pollution and lower than the 10^6–10^8 cells g^{-1} reported by Bossert and Bartha (1984) in oil-polluted soils. The W-HCs of the piles were 40–60%, within the recommended range (Dibble and Bartha, 1979; Sims et al., 1989; Williams et al., 1998).

At the TPHs used in the composting study (3–4%), the MPN method was a valid way to calculate the populations of hydrocarbon-degrading bacteria because the controls (those lacking FS) were negative at the dilutions used to determine cell numbers. This was evidence that the MPNs were due to bacteria utilizing the sludge as sole carbon and energy source and not some other organic material in the samples. Williams et al. (1998) reported similar results in two field studies.

Bioremediation of the sludge (Figure 7) was essentially the same in piles bulked with complete compost (piles 3 and 4) and approximately 16% greater than piles bulked with sawdust (piles 1 and 2, 56 and 58%, respectively). Augmentation of piles 2 and 4 with sludge-degraders had no measurable effect on the extent or rate of bioremediation, contrary to what one might expect. The lower rate of bioremediation using sawdust, augmented or not with sludge-degraders (piles 1 and 2), was a surprise and it suggests that the bulking agent was more important to the process than microbial augmentation. The higher rate using complete compost not augmented with sludge-degraders (pile 3) undoubtedly means there were hydrocarbon-degraders indigenous to the complete compost.

It is obvious from Figure 7 that the extent and rate of bioremediation were greater in the piles bulked with complete compost and, from the temperature means in Table 4, that the complete compost modulated the temperatures better than sawdust, keeping them within the mesophilic range. At the 19- to 24-week period, however, the means of all four piles were in the upper mesophilic range, probably due to the excessive solar heating when ambient temperatures were as high as 35°C. There being no significant differences in temperatures between the four piles during weeks 19–24 (Table 4) perhaps explains why the MPNs declined slightly (Figure 6) and why the degradation rate plateaued (Figure 7).

The conclusions that can be drawn from the combination of the laboratory studies and the field study described are as follows:

1. Weathered oil sludge was primarily long-chained hydrocarbons (C_{20}–C_{38}) with a specific gravity of 0.91–0.92 (23–24 API), forming a semi-solid at room temperature.
2. At a TPH of 46 μg/g, the indigenous microbes degraded 97.4% in 10 weeks in shaken cultures.
3. Bioremediation occurred at 4.1×10^4 and 1.0×10^5 μg/g in two weeks with toxicity resulting at a TPH of 2.3×10^5 μg/g and higher.
4. Neither cometabolism nor exogenous surfactant had any measurable effect on biodegradation of the weathered oil sludge.
5. None of the five bacterial consortia was as effective at degrading the sludge as the bacteria indigenous to the sludge.
6. Composting with complete compost and sawdust as bulking agents were effective means of bioremediating the oil sludge at TPHs of 3–4 $\times 10^4$, and (a) the rate and extent were greater in piles bulked with complete compost than in those with sawdust (73 and 57%, respectively); (b) temperatures in the complete compost-bulked piles through 18 weeks were in the middle of the mesophilic range, probably modulated by the complete compost (complete compost, 32°C; sawdust, 41°C); temperatures in the last 6 weeks, however, approached the thermophilic range in all four piles, during which time the bioremediation rate did not change in any piles; (c) augmentation with the sludge-degraders kept the MPNs higher in piles bulked with complete compost and sawdust, but there were no corresponding increases in sludge degradation.

ACKNOWLEDGMENTS ●

This research was made possible by the generous financial support of the East Texas Salt Water Disposal Company, Kilgore, Texas and the Welch Foundation, Houston, Texas. The cooperation of Vital Earth Resources, Inc., Gladewater, Texas was essential for the composting studies. The use of the Zuckerman Laboratory of Electron Microscopy, Department of Biology, The University of Texas at Tyler, Tyler, Texas in bacterial identification is gratefully acknowledged. This work is in partial fulfillment of the requirements for a Master's of Science (W.R.G.) in the Department of Biology, The University of Texas at Tyler, Tyler, TX 75799.

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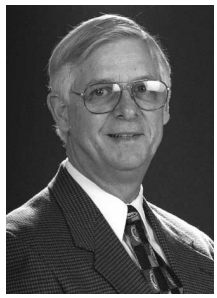

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