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Mobility and enhanced biodegradation of a dilute waste oil emulsion during land treatment[1]

Virginia G.M. Calabrese, Robert E. Elsavage, Gary K. Bissonnette and Alan J. Sexstone

Division of Plant and Soil Sciences, West Virginia University, Morgantown, WV, USA

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SUMMARY

The mobility and biodegradability in soil of a dilute waste oil emulsion generated by an aluminium rolling industry was investigated. Laboratory simulations and field evaluation of waste disposal suggested that the majority of the oil emulsion was retained in surface soil following application. However, potential leaching of waste to the subsurface was demonstrated, particularly at higher loading rates in soils of sandy texture. Strategies to enhance rates of biodegradation in surface soils were investigated, including fertilization and microbial inoculation. A single strain inoculum was obtained from a group of 81 isolates selected for their ability to partially mineralize the waste oil emulsion, and was tentatively characterized as a hydrocarbonoclastic *Corynebacterium* sp. Inoculation did not effectively stimulate waste removal in soil compared with fertilization, which significantly increased respiration and biodegradation. The maximum loss of the applied oil emulsion from soil was 30% during a 56-day in vitro incubation. Fertilized, aerated liquid waste emulsion was more rapidly degraded, resulting in loss of 65% of the waste emulsion within 18 days.

INTRODUCTION

Previous reports of land treatment studies deal primarily with disposal of concentrated wastes [2,20]. Land application and soil retention of dilute waste coolant emulsions have received comparatively little attention [9,16]. Absorptive capacity for oil depends on soil properties, viscosity of the waste and the presence of chemical and/or microbial emulsifiers and surfactants [4,6]. In the present study, we investigated land treatment of a dilute (0.5%) waste oil emulsion (OE) generated from a rolling oil emulsion (ROE) utilized by an aluminium metal-working mill. The ROE was comprised primarily of mineral oils and triethanolamine oleate soaps used as an oil-in-water emulsion (Fig. 1). The spent emulsion was retained in holding ponds, where oil was separated from the aqueous phase and reclaimed. The remaining aqueous OE was periodically pumped from the holding ponds and irrigated over two disposal sprayfields. The disposal site used in this study appeared well suited for land treatment of oily wastes, since the soil has a loamy texture in the upper horizon with a higher underlying clay content as recommended by the American Petroleum Institute. However, potential mobility of OE to lower soil horizons was a concern, since the waste oil originated as a biodeteriorated dilute emulsion.

Previous work by Neal et al. [16] established the presence of hydrocarbon-degrading bacteria in these sprayfield soils. Elsavage and Sexstone [9] observed retention and biodegradation of OE in the upper 48 cm of this soil during a 104-day period following application. However, data from this field study did not account for small amounts of OE leached below 48 cm. Accumulation of oil residues suggested that strategies to increase degradation efficiency also were needed. Therefore, the objectives of this study were to investigate retention of OE in surface sprayfield soils and to evaluate whether fertilization and bacterial inoculation stimulated biodegradation.

MATERIALS AND METHODS

Site description and sampling regime

The OE land treatment site consisted of two 5.7-ha waste irrigation sprayfields located within the Ravenswood Works (Ravenswood, WV, USA; 81°46′ W, 39°25′ N) of Kaiser Aluminium and Chemical Corporation, Inc. (KACC), and designated the east and west sprayfields (ESF and WSF). Details of sprayfield operation, OE components, and soil properties have been described previously [9].

All sampling was performed in July 1988. Bulked samples were collected by removing six soil cores (2 × 24 cm) at each of four points 34 m (the point of heaviest OE application) from two designated irrigation hydrants in both the ESF and WSF. Samples were returned to the laboratory on ice, sieved through 5-mm wire mesh, combined in plastic ziplok bags, and stored at 4 °C prior to in vitro biodegradation

Correspondence to: A.J. Sexstone, Division of Plant and Soil Science, West Virginia University, Morgantown, WV 26506-6057, USA.

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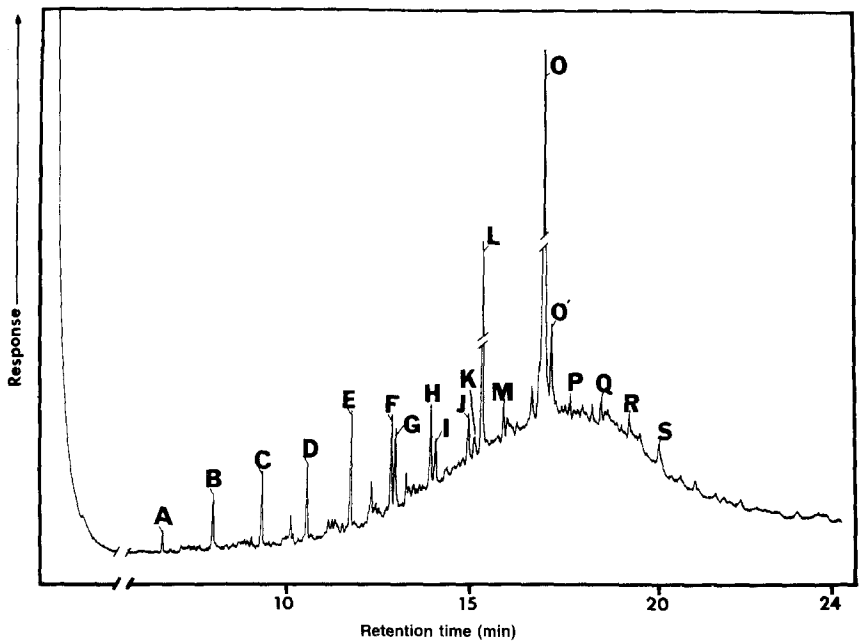

Fig. 1. A representative gas chromatogram illustrating resolvable components extracted from the waste oil emulsion: A, *n*-dodecane; B, *n*-tridecane; C, *n*-tetradecane; D, *n*-pentadecane; E, *n*-hexadecane; F, *n*-heptadecane; G, pristane; H, *n*-octadecane; I, phytane; J, *n*-nonadecane; K, 1,2-benzene-dicarboxylic acid-dibutylester; L, hexadecanoic acid; M, *n*-eicosane; O, oleic acid; O', octadecanoic acid; P, *n*-docosane; Q, *n*-tricosane; R, *n*-tetracosane; S, *n*-pentacosane.

experiments. Shelby tube and split spoon cores were sampled by the firm of Dames and Moore (Cincinnati, OH, USA). Twenty-four soil cores were collected in stainless steel Shelby tubes (8 cm × 76 cm) in a cluster approximately 22 m from a selected spraybird in each sprayfield. Twelve samples were obtained from the upper 60 cm of soil. Twelve additional cores from the same location extended from 60 to 120 cm. Shelby tubes were closed at both ends with plastic caps and transported to the laboratory where they received simulated loadings of OE. Two deep cores, which extended from the soil surface to a depth of 4.6 m, were obtained from each sprayfield using split spoon samplers (13 cm × 61 cm). Each boring consisted of soil in 30-cm increments, which were transferred to ziplok bags and returned on ice to the laboratory. Liquid waste samples were collected in 20-l plastic containers from a valve in the pipe connecting the holding ponds to the sprayfields and returned to the laboratory where they were stored at 4 °C.

Soil column loading experiment

The Shelby tubes were placed in wooden racks with each core resting in a funnel with a 250-ml beaker underneath it. In the original experimental design, triplicate cores were to be loaded at one, three and six times the normally permitted sprayfield application rate (2.5 cm week^{-1}). Infiltration of OE into many of the soil cores was so restricted, however, that higher amounts could not be applied without pooling of the waste above the soil surface. The actual amount of OE loaded onto individual replicates ranged from 1.3 to 6.1 cm week^{-1} core^{-1} and contained an average concentration of 3.06 mg of solvent-extractable material (SEM) ml^{-1} of OE. Individual cores received average OE applications between 1.3 and 6.1 cm core^{-1} week^{-1} for an 8-week period. Three control cores from each sampled depth at each site received equivalent applications of distilled water during this period. Leachate collected in the beakers under each core was extracted for total recoverable oil and grease (EPA method 413.1) at the end of the experiment. Cores were cut into three equal sections and the soil extruded, air dried, sieved (4.75 mm), and thoroughly mixed. One 10-g subsample from each section was extracted twice with trichlorotrifluoroethane (TCTFE) as detailed by Elsavage and Sexstone [19]. Evaporated soil and leachate extracts were weighed and diluted to 500 μg ml^{-1} in TCTFE prior to analysis by gas chromatography (GC).

Analysis of deep cores

Each 30-cm section from the 4.6-m deep cores was air dried, sieved (4.75 mm), and thoroughly mixed. Two 10-g subsamples were then extracted with TCTFE. Dried extracts were weighed and dissolved in solvent to between 500 and 1000 μg ml^{-1} for GC analysis.

Biodegradation experiments

Soil samples (ESF, 10 g wet weight) were distributed into 250-ml Erlenmeyer flasks and adjusted to approximately 25% gravimetric moisture content. Four samples were extracted with TCTFE to measure background OE in the soil (6.7 ± 0.4 mg g^{-1}). Each of the remaining samples was amended with 70 mg of OE substrate delivered in 1 ml of pentane, after which flasks were left open for 1.5 h to allow evaporation of the solvent. This OE amendment was obtained

by concentration SEM from a TCTFE extract of the waste oil emulsion as previously described [9]. Samples then were treated in a factorial design with a bacterial inoculum ($I = 10^7$ cells strain GM22 g^{-1}, described below) and 0.5 ml fertilizer solution ($F = 20$ mg NH_4NO_3 and 3 mg KH_2PO_4 per flask). Sterile controls were autoclaved for 20 min at 121 °C and 103 kPa (15 psi) on 2 consecutive days. Flasks were capped with butyl rubber stoppers equipped with rubber septa and incubated in the dark at 25 °C for 56 days. Headspace gas of all flasks was sampled weekly and analysed for CO_2 by GC. Flasks were uncapped for 1 h after each sampling to allow reaeration of the soil, and ambient CO_2 was subtracted from subsequent CO_2 measurements. Four flasks from each treatment were sacrificed for TCTFE extraction of OE on days 0, 7, 14, 28, 42 and 56. Sterile control flasks were sacrificed on days 14, 28 and 56. Microbial activity was stopped by the addition of 2 ml of chloroform for 2 h, followed by drying in air for 24 h. Solvent extracts were treated as described in the previous section.

A similar factorial experiment was performed using the liquid OE. Homogeneous liquid waste (100 ml) averaging 0.58 mg SEM ml^{-1} was distributed into 250-ml Erlenmeyer flasks. Samples were treated with bacterial inoculum (10^5 cells ml^{-1}) and fertilizer, which was added as 0.325 g $flask^{-1}$ Bushnell-Haas (BH) salts (Difco, Detroit, MI, USA). This mineral medium, designed to provide necessary nutrients for growth of hydrocarbon-degrading bacteria, provided 0.35 mg N, 0.33 mg P and 0.74 mg K ml^{-1} OE. Controls were sterilized by autoclaving. Flasks were capped with rubber stoppers and connected in series as a gas train by vinyl and glass tubing attached to a brass aquarium gang valve (Penn-Plax Inc., Garden City, NY, USA) through which filtered air was pulled at a rate of 8 l min^{-1} by a vacuum pump. Flasks were incubated at 25 °C for 18 days. Sterile water was periodically added to flasks to offset evaporation. Four flasks from each treatment group and two sterile control flasks were sacrificed on each of days 4, 8, 12 and 18 for TCTFE extraction and GC analyses. Samples were first acidified to pH 1.0 with 12 N HCl in order to protonate fatty acid metabolites and allow their extraction in the organic phase. Acidified samples were heated to 90 °C for 7.5 h to break the emulsion, then cooled and extracted three times in a separatory funnel with 50 ml TCTFE. Methanol was added to inoculated samples to minimize formation of a stable emulsion during extraction. Organic layers were pooled, washed with H_2O to remove residual methanol, dried by filtration through 5 g Na_2SO_4 in a Whatman no. 1 filter, and collected for gravimetric and GC analyses.

Isolation of the inoculum

Microorganisms capable of growth on ROE as sole carbon source were isolated from sprayfield soil and from OE by serial enrichment in a liquid medium, designated BHROE, consisting of BH mineral salts broth (Difco) and 0.5% ROE (v/v). Microorganisms were isolated from the final serial enrichment by spread plating on solidified BHROE (bacteria), modified Czapek agar in which sucrose was replaced with ROE (fungi), and modified glycerol-arginine agar [1] in which glycerol was replaced with ROE (actinomycetes). Ability to degrade ROE was confirmed in liquid media by inoculating single-colony isolates into Hungate tubes containing BHROE or BH alone. Tubes were incubated for 2 weeks, followed by CO_2 analysis. Isolates which produced significantly more CO_2 in BHROE compared with BH were maintained as presumptive ROE degraders. The ability of each presumptive isolate to degrade hydrocarbons was examined in BH supplemented with 1% (v/v) each of ROE and hexadecane (>99% purity; Aldrich Chemical Co., Milwaukee, WI, USA). Following incubations of 7 and 14 days, flasks were extracted with TCTFE and analysed by GC to determine hexadecane degradation.

One isolate, designated GM22, was used as an inoculum in subsequent biodegradation experiments because of its ability to grow extensively both on fatty acid emulsifiers and mineral oil hydrocarbons. The isolate was characterized by conventional microbiological testing as well as by whole cell fatty acid analysis (Microbial I.D. Inc., Newark, DE, USA). Prior to biodegradation experiments, GM22 was grown at room temperature in 100 ml of shaken (200 rpm) Tryptic Soy broth (TSB; Difco), examined to ascertain purity, transferred to fresh media (100 CFU ml^{-1}), grown to late log phase, harvested by centrifugation, washed twice in sterile 0.85% saline to remove nutrients, and diluted to produce an inoculum of suitable density.

Analytical measurements

Headspace CO_2 samples were measured by GC using a Carle 100 gas chromatograph (Hach Company, Loveland, CO, USA) equipped with a thermal conductivity detector. Separations were achieved using a 2 m × 3 mm Poropak Q column (Waters Associates, Milford, MA, USA). Solvent extracts of OE or culture fields were analysed by GC on a Varian 3400 gas chromatograph (Varian Associates, Inc., Sunnyvale, CA, USA) equipped with an autosampler, on-column injector, and flame ionization detector. Separations were achieved using a 20-m × 0.53-mm i.d. DB-1 megabore column (J & W Scientific, Inc., Folsom, CA, USA). Some solvent extracts were also analysed by GC-MS using a Finnigan 9600 gas chromatograph and 4500 mass spectrometer (Finnigan Corp., Cincinnati, OH, USA) connected to an INCOS data system equipped with an NBS internal library. Separations were achieved using a 30-m × 0.25-mm i.d. DB-1 capillary column. Detailed operating conditions and data quantitation procedures for the GC and GC-MS analyses have been described previously [9].

Statistical analyses

Differences among treatments in the biodegradation experiments were determined by analysis of variance (SAS Institute Inc., Cary, NC, USA). Data were analysed using a factorial arrangement of treatments with $\alpha = 0.05$ or 0.01. Cumulative carbon dioxide evolution was compared on individual days due to the decreasing number of replicates with time.

RESULTS AND DISCUSSION

Soil column loading experiment

OE was loaded onto intact, relatively undisturbed soil columns to simulate waste applications to the 0–60-cm and 60–120-cm depths of the sprayfield. All leachate was collected, and the distribution of OE in the soil column was determined after 56 days (Fig. 2). Background concentrations of SEM in the soil cores prior to simulated waste applications were higher in 0–60-cm cores than in 60–120-cm cores from both ESF and WSF. Higher background concentrations of SEM were observed in WSF cores, which was expected because of active waste applications on this site prior to sampling.

Gravimetric analyses subsequent to 56 days of OE loading confirmed that much of the freshly applied SEM was retained in the upper 20 cm of all samples regardless of sampling location. However, extractable material was detected in leachate from several cores, including controls. In some cases it accounted for 2–20% of the SEM applied to treated cores. Greater leaching of OE was observed in the 0–60-cm than in the 60–120-cm cores of the ESF, a result consistent with the higher clay content of deeper soils. The opposite was true at the WSF site, but the soils were sandier at the lower depth of this field [V.G. Matheson, 1989, MS thesis, West Virginia University]. The higher leachate amounts from individual replicates were not considered an artifact resulting from soil sampling because all core sections were visibly unfragmented when the cylinders were disassembled for SEM analyses. Concentrated SEM in column leachates did not always correspond with the higher OE loading rates, and probably resulted from saturated flow through soil samples of greater macroporosity and sandier soil texture, or through soil macropores [23].

Analysis of deep cores

One of the 4.6-m cores from each field showed evidence of subsurface OE accumulation, while the second core indicated retention of OE in surface soil (Fig. 3). Deep core no. 1 from the ESF and WSF exhibited SEM accumulations of 4 and 8 mg g^{-1}, respectively, at the 3–4.3-m depth. Deep core no. 2 from the ESF retained 4 mg g^{-1} SEM in the

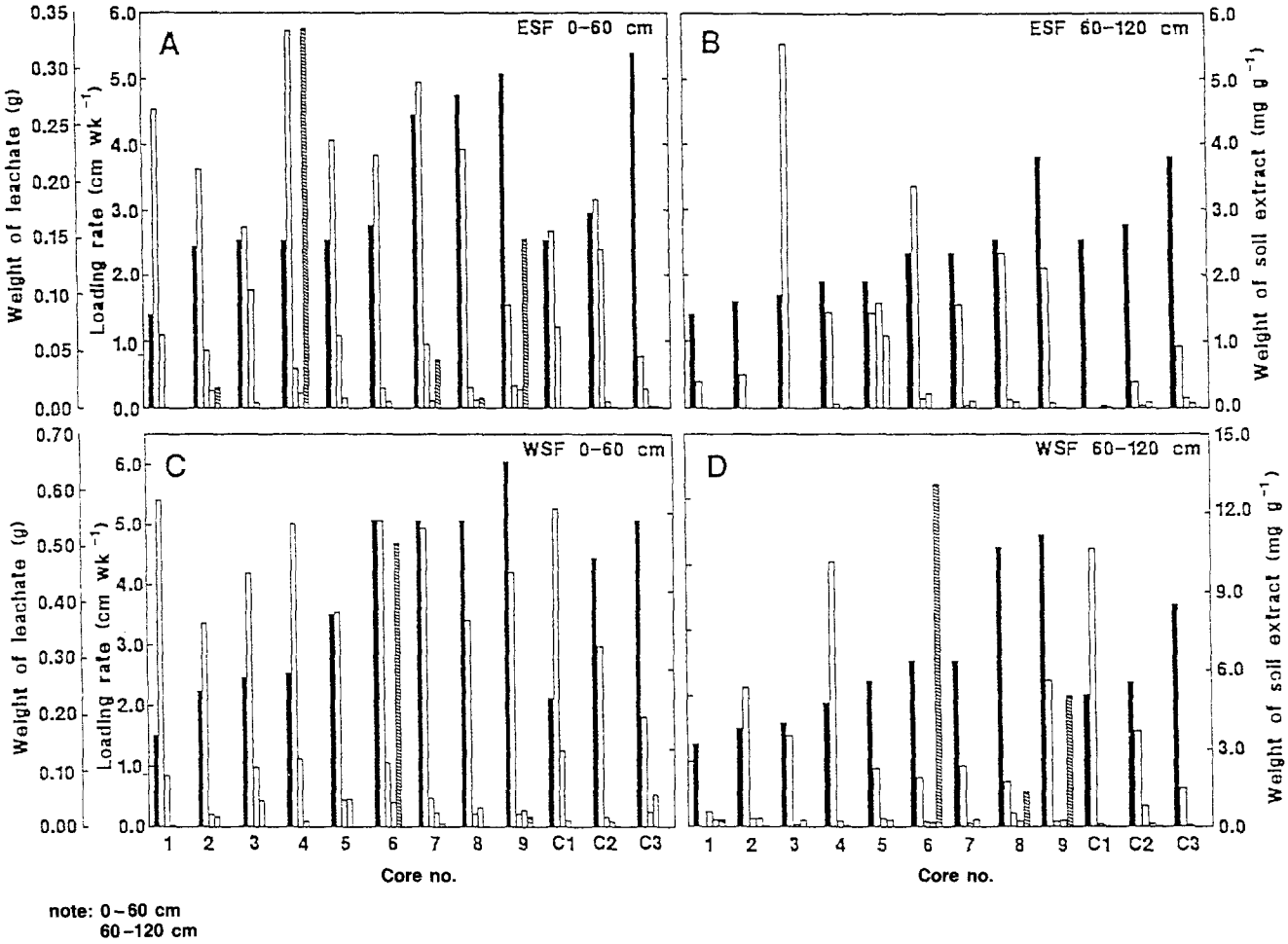

Fig. 2. Simulated applications of oil emulsion (OE) to sprayfield soils. Average OE loading rate (■); weight of solvent (TCTFE)-extractable material from soil core sections (0–20, 20–40 and 40–60 cm) following 56 days of OE application (□); and weight of TCTFE extractable material from soil leachate (▨). Soils: A, east spray field (ESF) 0–60 cm; B, ESF 60–120 cm; C, west spray field (WSF) 0–60 cm; D, WSF 60–120 cm.

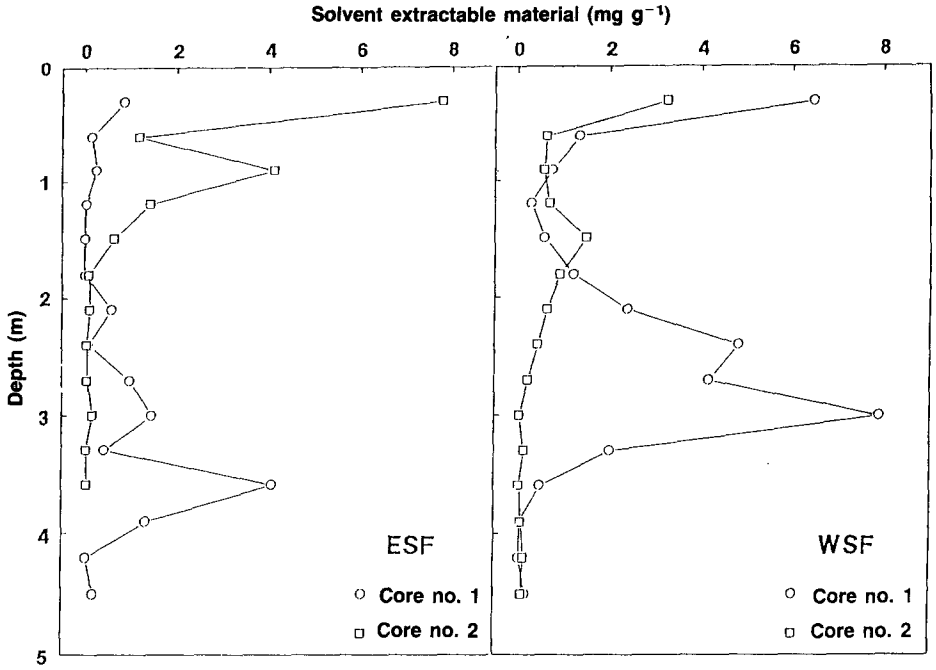

Fig. 3. Weight of solvent (TCTFE)-extractable materials from deep soil cores obtained from the east (ESF) and west (WSF) sprayfield during summer, 1988. Points are means of two replicate samples.

upper 1.0 m, while core no. 2 from the WSF retained 1.5 mg g^{-1} SEM between the 1.5–3-m depth. The SEM from core no. 1 of the ESF contained few hydrocarbons resolvable by GC. It appeared to be an accumulation of non-degraded residues such as those noted in previous studies [6,7]. The SEM derived from core no. 1 from the WSF contained significant amounts of resolvable hydrocarbons which appeared to have undergone less biodegradation. We noted similar GC chromatograms of SEM derived from the previously described soil column leachates. These chromatograms suggested that OE accumulations in the WFS were of recent origin and were derived from a waste of high hydrocarbon concentration. This was consistent with: (i) infrequent use of the ESF since June 1987, with subsequent increased dependence on the WSF; (ii) temporary closure of one waste holding pond during the study period, necessitating more frequent irrigation of the WSF with an emulsion that had undergone less extensive physical separation and thus contained a higher concentration of oil; and (iii) the sandy texture of the WSF subsurface, which may have allowed infiltration of OE once the sorptive capacity of surface soils had been saturated (Fig.2).

Results of these analyses, together with production of OE-containing leachate during waste application to soil columns, demonstrate the potential mobility of this waste to the subsurface. Soils high in clay, such as those in the lower horizons of the ESF, would hinder downward flow of much of the emulsion. If these areas are adjacent to sandy areas, then small amounts of waste could continue leaching to the subsurface under conditions of saturated flow. Clearly, strict waste application limits and correct field management must be exercised to prevent unwanted mobility of undegraded hydrocarbons to the subsurface. The presence of sandy areas in these fields calls into question the suitability of this site for waste application. A more extensive sampling regime will be necessary to determine the true current extent of OE distribution throughout the soil profile of the entire sprayfield.

Isolation of the inoculum

Enrichment procedures resulted in isolation of 133 bacteria, actinomycetes and fungi, 81 of which produced significantly greater CO_2 in mineral salts broth containing 0.5% ROE than in mineral salts broth alone. These results indicated active microbial growth on constituents of the emulsion.

Growth of isolates in BHROE with 1% hexadecane (BHROEH) revealed that most isolates were not active hydrocarbon degraders in axenic culture. Solvent extraction of culture fluids followed by gas chromatographic analysis confirmed that isolates in each group biodegraded isomers of octadecanoic acid and other fatty acids contained in the triethanolamine oleate soap emulsifying pack, but biodegradation of the added hexadecane and resolvable hydrocarbons contained in the mineral oil fraction did not occur. During the original enrichments, the ROE apparently exerted a stronger selective pressure for fatty acid degraders than for hydrocarbon degraders, in part because alkanes were present in low initial concentrations relative to the fatty acid-containing emulsifiers. A similar phenomenon might occur in the sprayfield soils, where plentiful fatty acids could act as competitive carbon substrates and retard hydrocarbon biodegradation [11,15].

Two isolates, nos 22 and 126, significantly reduced hexadecane concentrations in BHROEH medium. Fungal isolate no. 126 was originally obtained from a waste oil

pond and was identified as *Penicillium frequentans* by Dr Dale Hindal and Dr Horace Barnett, West Virginia University. Alkane-degrading *Penicillium* species, including *P. frequentans*, have been reported [17]. They could contribute to hydrocarbon degradation in soil [24]. Isolate no. 22 was obtained from sprayfield soil and was characterized as a slow-growing (5-h doubling at 25 °C on TSB), obligately aerobic, gram-positive, acid-fast, pleomorphic to rod-shaped bacterium. Although the organism was not conclusively identified, whole cell fatty acid analysis placed this isolate within the broad grouping of the coryneform bacteria (Dr Myron Sasser, Microbial ID, Inc., Newark, DE, USA, personal communication). When grown on mineral salts plus 1% hexadecane (BHH) in the absence of ROE, isolate no. 22 emulsified the insoluble hydrocarbon to droplets ranging from 0.5 to 3 μm in size, as viewed by Nomarski and phase contrast microscopy. Growth of cells adhering directly to hexadecane droplets was abundant. Many cells contained intracellular refractile bodies, which were absent when grown on TSB. These bodies resembled hydrocarbon inclusions accumulated by alkane-growth *Corynebacterium* sp. [21], or other intracellular storage polymers produced by hydrocarbonoclastic bacteria [13]. Production of emulsifiers has been reported when *C. hydrocarbonoclastis* was grown on a mixture of linear alkanes or kerosene [25] and when *C. lepus* was grown in kerosene [8].

Biodegradation experiments

Efficient degradation of OE retained in surface soils would help to minimize the potential for subsurface contamination. A previous study suggested that application rates in excess of 460 mg SEM m^{-2} wk^{-1} were potentially detrimental to rates of biodegradation, whereas continuous low level exposure was necessary to optimally maintain a microbial population capable of metabolizing OE [9]. The KACC sprayfields were not routinely fertilized, a treatment found to enhance biodegradation of oily wastes in other studies [3]. We were thus able to investigate the effect of supplemental N, P and hydrocarbonoclastic bacterial inoculum on in vitro rates of OE biodegradation.

Despite ready utilization of hydrocarbons and fatty acids by isolate GM22 in culture, inoculation of sprayfield soil did not stimulate biodegradation of OE. The inoculum was harvested during exponential growth on BHROE, was viable by plating procedures, and was added to soil at a rate sufficient to double the viable heterotrophic plate count (10^7 g^{-1}). However, soil respiration was not significantly increased by inoculation ($P < 0.05$), indicating lack of active metabolism by isolate GM22 in soil (Fig. 4). Percentage recovery with time of oil components by gas chromatography was significantly higher in the inoculated treatment ($P < 0.01$), suggesting actual inhibition of biodegradation (Fig. 5). However, interpretation of these data is complicated by unequal gravimetric recovery of OE. In this study it averaged 71% from non-inoculated samples compared with 83% from inoculated samples. The greater recovery of OE from inoculated samples is at present unexplained. Emulsifiers produced by isolate no. 22 may have helped to desorb

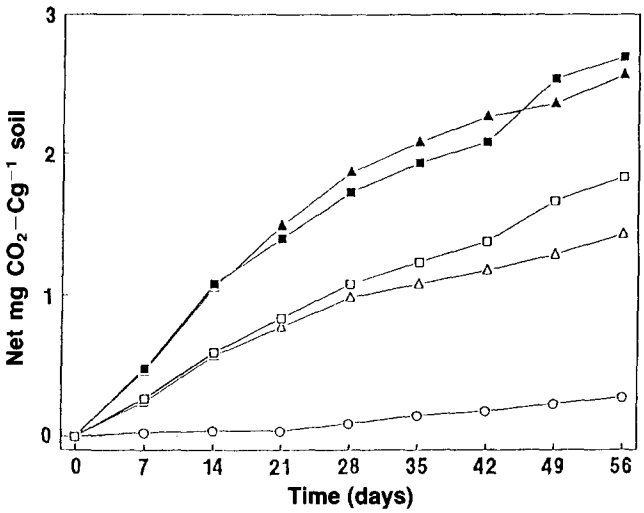

Fig. 4. Effects of inoculation (I) and fertilization (F) on cumulative in vitro CO_2 evolution from oil emulsion amended sprayfield soil during a 56-day laboratory incubation. Symbols: ○, autoclaved; ▲, +I +F; △, +I −F; ■, −I +F; □, −I −F. Individual determinations are means of replicates which decreased from 24 to 4 in number as described in the 'Materials and methods'.

greater amounts of OE from the soil matrix during solvent extraction. Such an effect could account for greater recovery (1.25×) of non-resolvable components from inoculated samples at day 7 compared with those added at day 0.

Successful bioremediation by inoculation of exogenous microorganisms into soil appears to be rare [3,14]. Numerous reports have shown that microorganisms capable of degrading light oil fractions can be readily enriched in soil. An inoculum would therefore have to compete with adapted natural populations [12,22].

In contrast to inoculation, addition of N and P enhanced biodegradation compared to corresponding unfertilized samples. Hydrocarbon wastes have wide ratios of carbon to mineral nutrients, an imbalance which can be corrected by appropriate fertilization [5]. Soil respiration was stimulated significantly ($P < 0.01$) at all sampling times in fertilized compared with unfertilized samples. Twice as much CO_2 accumulated in these samples over the 28-day incubation (Fig. 4). Biodegradation of oil components also was enhanced in fertilized samples ($P < 0.01$), particularly during the first 14 days (Fig. 5).

Stimulation of OE biodegradation was studied by fertilization and/or inoculation of continually aerated liquid obtained from the waste holding ponds. Biodegradation of components resolvable by GC was stimulated significantly by fertilizer ($P < 0.01$) but it was unaffected by inoculation (Figs 6, 7). Clearly, naturally occurring microbial populations present in the waste oil ponds were capable of degrading hydrocarbons and fatty acids. Percentage recovery of components non-resolvable by GC also showed a positive fertilization effect ($P < 0.01$), but suggested that inoculum hindered biodegradation of OE. Unlike soil, OE was extracted with equal efficiency (> 98%) from both inoculated and non-

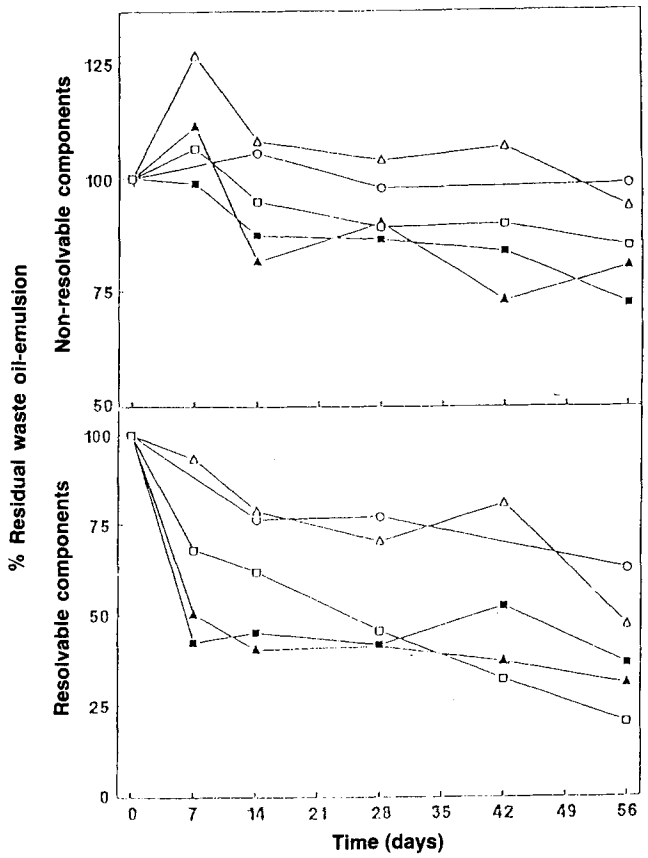

Fig. 5. Effects of inoculation and fertilization on in vitro disappearance of components resolvable and non-resolvable by gas chromatography contained in solvent (TCTFE) extracts of oil emulsion amended sprayfield soil. Points are the means of four replicate determinations. Symbols as in Fig. 4.

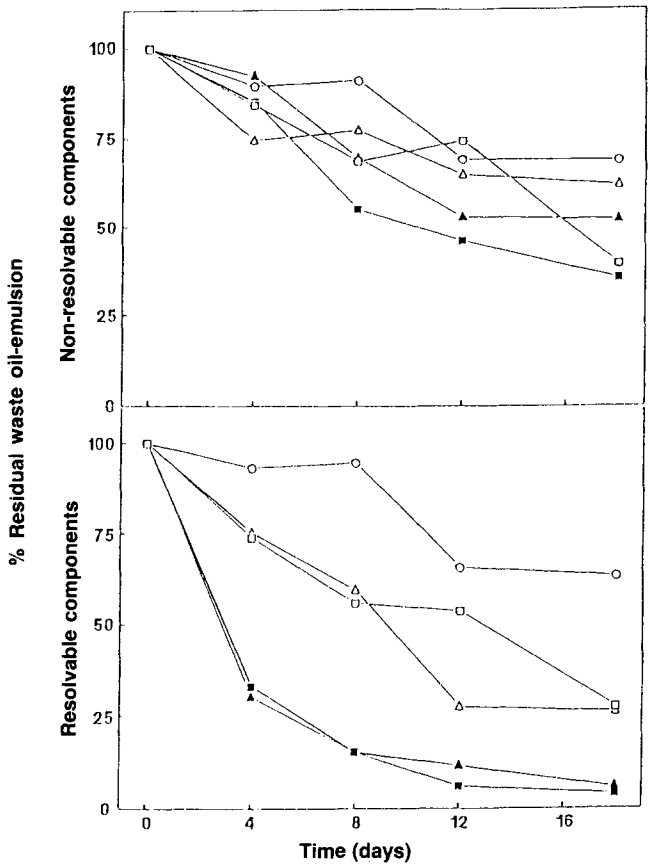

Fig. 6. Effects of inoculation and fertilization on in vitro disappearance of components resolvable and non-resolvable by gas chromatography contained in solvent (TCTFE) extracts of aerated liquid oil emulsion collected from the waste holding ponds. Points are the means of four replicate determinations. Symbols as in Fig. 4.

inoculated samples. Bacterial emulsifiers, such as those released by isolate GM22, can coat hydrocarbon droplets, preventing adherence to droplets by competing bacteria [19] and/or pseudosolubilization by other bacterial surfactants [10,18]. Bacterial metabolites, emulsifiers and dead biomass also may have served as alternate carbon sources for autochthonous organisms, thus retarding OE degradation. Up to 25% of the OE substrata was degraded in autoclaved controls in both the soil and liquid systems. This may have been due to contamination, since these systems were not entirely contained. However, degradation was significantly greater in non-sterile treatments.

Our data indicate the need for disposal strategies which minimize aqueous inputs to the holding ponds, maximize emulsion separation time in the holding ponds, and decrease the OE load prior to waste application. Spray regimes which maximize areal coverage and minimize saturated flow of the waste into the soil should be adopted. Results with liquid waste suggest that preincubation of OE in fertilized, aerated ponds or in bioreactors could effectively decrease the hydrocarbon load prior to application to soil where biodegradation appears to be slower and less extensive. Fertilization and tillage of sprayfield soil should be carried out routinely to remove nutrient limitations and promote aeration. Soil inoculation is not necessary to improve bioremediation at this site. The OE can be readily immobilized and biodegraded in surface sprayfield soils. However, careful management of this land treatment facility is necessary to prevent mobility of the waste to subsurface soils, where conditions for waste oil biodegradation are less favourable.

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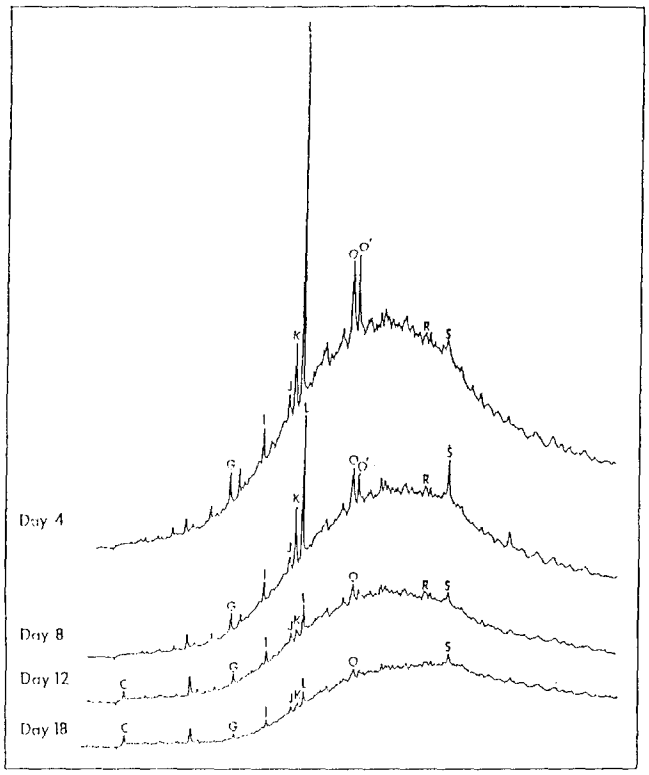

Fig. 7. Representative gas chromatograms illustrating in vitro biodegradation of components resolvable and non-resolvable by gas chromatography in aerated and fertilized liquid oil emulsion during an 18-day incubation. See Figure 1 for designation of resolvable components.

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