Biodegradation and Dewatering of an Industrial Waste Oil

By

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(ABSTRACT)

Waste oil generated from industrial operations at a diesel locomotive maintenance facility was investigated to establish its treatability and potential volume reduction. The waste oil and water mixture separated into four distinct layers; free oil, emulsified oil, weathered oil, and wastewater. The research was conducted in a series of three batch experiments and focused on the emulsified and weathered oils. The waste oil was aerobically treated in nutrient amended, 55 gallon (208 L) drums for 38 to 42 days in 10 and 20 % mixtures with sufficient air for mixing and oxygen. Biodegradation, and the role of a synthetic surfactant in promoting biodegradation, was measured using chemical oxygen demand (COD), fluorescein diacetate (FDA), and gas chromatography (GC) analyses with extractable material. Dewatering of biodegraded oil was measured using capillary suction test (CST), time to filter (TTF), and percent cake solids.

Batch 1 examined the role of bioaugmentation by comparing a 10% waste oil mixture that was augmented with a mixture of hydrocarbon degraders to a 10 % mixture of waste oil with no bioaugmentation. Final COD reductions were 59 (± 9) and 38 (±3) % for the bioaugmented and non-bioaugmented reactors, respectively. Chromatographs showed significant reduction in the abundance of peaks by the end of the experiment for both reactors. Overall results suggested that there was no significant difference in biodegradation capabilities between the amended and native microorganisms.

Batch 2 was conducted to determine if a synthetic surfactant (Tween-80) could enhance biodegradation of a 10 % waste oil mixture. The surfactant-amended reactor showed COD

reduction 3 days before the non-surfactant-amended reactor. Chromatographs showed similar results for both reactors with the non-surfactant-amended reactor showing slightly better degradation by the end of the experiment. The total COD reduction by the end of the experiment was the same in both (R1: 85 ± 20%, R2: 84 ± 16 %), suggesting that exogenous surfactant addition did not have a long-term impact in the biodegradation of the waste oil.

Batch 3 examined the effect of different oil phases and concentrations on biodegradation and the dewatering characteristics of post-biodegraded waste oil. The 20 % weathered and emulsified waste oil mixture showed a clear delay in COD reduction (no notable reduction until day 24) compared to the 10 % weathered waste oil mixture. The final COD reductions were the same (R1: 48 ± 13%, R2: 49, ± 23 %). Chromatographs showed similar results for both reactors and indicated that degradation of the waste oil occurred in both reactors. The data suggest that the 20 % waste oil mixture can be degraded to the same extent as the 10 % mixture in 38 days.

Dewatering characteristics, as measured by CST, were poor for the 20 % post-biodegraded combined waste oil mixture without conditioning. Conditioning with alum or ferric chloride substantially improved dewatering of the waste oil for the 20 % mixture but was of limited benefit for the 10 % mixture. Percent cake solids for conditioned 10 % post-biodegraded waste oil mixture was 44 (± 0.3) to 50 (± 1.7) % and 34 (± 0.3) to 50 (± 1.8) % for the 20 % mixture. The cake solids for the unconditioned 10 % mixture was 50 to 65 % and 54 to 68 % for the 20 % mixture. The higher percent cake solids for the unconditioned 20 % mixture was countered by the very high TTF (up to 30 min. to filter 50 mL) and the inability to dewater the sludge during the last five sampling events. Conditioning appeared to have a limited effect on the dewatering properties of the 10 % mixture.

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1. INTRODUCTION

The extensive use of petroleum products in modern society inevitably results in spills, leaks, and the generation of waste products. Managing and remediating these environmental contaminants requires a multifaceted approach that must consider the magnitude, health and environmental risk, and cost. A wide range of physical, chemical, and biological treatment technologies are available for application with hazardous materials and contaminated land (Mohammed et al., 1996). Bioremediation has been shown to be a viable treatment technology for the remediation of petroleum hydrocarbons (Cookson, 1995; Huesemann and Moore, 1994; Salanitro et al., 1997).

2. LITERATURE REVIEW

A. Biodegradability of Petroleum Products

Petroleum products encompass a wide array of hydrocarbons from short-chain aliphatic and simple aromatic hydrocarbons in gasoline to kerosene, diesel, and lubricating oils. Each gradation contains both an increasing number of carbon atoms in the chain and increasing degree of complexity (Fan and Krishnamurhty, 1995). Many components that make up oils have been shown to be biodegradable (Britton 1984; Gibson and Subramanian 1984; Bosset and Bartha, 1984). The time required to degrade petroleum hydrocarbons substantially increases with chain length and degree of branching. Usually, the order of persistence to biodegradation is Bunker C crude oil, diesel, No. 2 heating oil, jet fuel, and gasoline (Song et al., 1980) due to the higher

boiling point and lower solubility of hydrocarbon constituents comprised in heavier products. Additionally, refractory or "higher end" components include more complex structures such as multi-substituted *iso*-alkanes and cyclohexane, *o*-dialkylbenzene derivatives, and ß-branched and quarternary-branched hydrocarbons of *o*-xylene and cyclohexane. These complex structures are highly resistant to biodegradation (Solano-Serena et al., 1999; Britton 1984; Gibson and Subramanian 1984). Biodegradability of oils can be enhanced by introducing sufficient nutrients (Venosa et al., 1995), modifying the bacterial growth environment to enhance biodegradation (Robertiello et al., 1994), designing reactors that enhance mass transfer of the oil fractions into solution (Zappi et al., 1996; Wilson et al., 1994), and/or introducing solublized agents such as surfactants (Strong-Gunderson and Palumbo, 1995; Pinto and Moore, 2000; Zheng and Obbard, 2000).

B. Nutrients

Typical domestic wastewater has ample amounts of macronutrients such as nitrogen and phosphorus for microbial growth. These nutrients are often limiting in soils and must be provided to ensure biological degradation of hydrocarbons (Mohammed et al., 1996). This is also the case for many types of industrial wastewaters, including those that contain petroleum products. Venosa et al. (1995) experimented with crude oil released onto plots on the shoreline of Delaware Bay and concluded that bioremediation by application of fertilizer enhanced the natural removal of spilled oil on cobble and mixed sand and gravel beaches. Venosa et al. (1994) also reported that the minimum nitrate-N concentration needed by oil degraders to grow on hydrocarbons at an accelerated rate under continuous flow conditions was approximately 1.5

mg/L. Often, the lack of sufficient availability of nutrients is the limiting factor in microbial degradation of hydrocarbons (Pritchard 1991). A biostimulation study on the Alaskan beaches contaminated by the *Valdez* oil spill showed that the application of fertilizer alone stimulated indigenous hydrocarbon-degrading species five- to ten-fold (ADEC et al., 1990).

C. Modifying Bacterial Growth Environment

The bacterial growth environment can be modified to promote biodegradation. Environmental modifications can include the adjustment of pH and temperature and the addition of nutrients, oxygen, or surfactants. One common approach to implementing these modifications is the traditional pump and treat technology in remediating contaminated soils and groundwater. In the pump-and-treat systems, wells are installed at the contaminated site for removal of groundwater. Groundwater is pumped to the surface and the contaminants are removed using the appropriate physical, chemical, or biological treatment system. The treated groundwater is then discharged to surface waters, a publicly owned treatment works, or back into the aquifer (Haley et al., 1989). Robertiello et al. (1994) established two different well-point series to a 120 m^2 sandy soil contaminated with gasoline and diesel fuel. The contaminated groundwater was extracted from the subsoil, treated in a series of above ground tanks that encouraged the growth of microorganisms, and reinjected it into the subsoil. The average pollutant content of the treated soil decreased notably in the first 25 days of operation. The pump and treat method continues to be utilized where practical as new technologies are introduced to enhance pump and treat or replace it with more cost-effective measures.

D. Reactor Design

The design of reactors can have a significant impact on the success of biodegrading hydrocarbon contaminated soils, slurries, and wastewater. Reactor size and configuration should be appropriate for the type and volume of waste being treated and thought should be given to how the design could be easily implemented to a full-scale system.

Slurry reactors mix water and contaminated soils via air and/or mechanical means in an above ground vessel to promote biodegradation of the targeted contaminants. These systems can be designed and equipped with various process-control instruments to create ideal conditions for biodegradation and offer maximum control, flexibility, and usually the highest rates of biodegradation among above-ground biotreatment systems for contaminated soils (Zappi et al., 1996). Wilson et al. (1994) developed a mobile system that could be reused for a variety of slurry applications. The system consisted of a watertight 20 yd^3 steel drop box with air introduced at the base and a water recirculation system to aid in the distribution of nutrients, which allowed the monitoring of operational parameters in a flow-through system, in place of discrete points within the reactor vessel. The field-scale reactors were designed based on results obtained from a feasibility study, taking into account the limitations of the laboratory scale, and incorporating future use requirements. The authors noted that the use of a laboratory-scale model and emulating actual conditions of treatment provided sufficient data for the construction of the field units and a model for interpreting operational parameters during the field trials.

E. Surfactants

Contaminants must be in the liquid phase for biodegradation to occur. Contaminants present as nonaqueous-phase liquids (NAPLs) are not available for microbial degradation (Strong-Gunderson and Palumbo, 1995). Surface-active agents (i.e., surfactants) have been used to increase the bioavailability of contaminant compounds by increasing solubilization rates and thus make a greater fraction of the contaminants favorable to biodegradation (Strong-Gunderson and Palumbo, 1995). Surfactants have a hydrophobic component that aligns with the hydrocarbon, while the hydrophilic portion aligns with water to form a micelle. This micelle increases the oil-water interface and provides greater availability of the oil to the microorganisms, which increases the rate of biodegradation. Volkering et al. (1995) reported that PAH in micellar phase were not available to bacteria; therefore, they act as a reservoir that replenishes aqueous phase PAH when consumed by biodegradation.

Microorganisms will produce biosurfactants to aid in their assimilation of both semi-soluble and insoluble hydrocarbons. Falatko and Novak (1992) reported that biosurfactants produced from growth on gasoline compounds acted similarly to commercial surfactants by increasing solubility of the compounds without inhibiting biodegradation; however, biosurfactants produced by growth on glucose and vegetable oil had an inhibitory affect on degradation of gasoline compounds. Mercade et al. (1996) noted four Rhodococcital strains and one Bacillital strain produced biosurfactants growing on waste lubricating oils. The biosurfactants reduced surface tensions from 55 mN/m to less than or equal to 40 mN/m. The surface-active compounds responsible were glycolipids and lipopetide. Page et al. (1999)

compared the effectiveness of a biosurfactant to Tween-80 in enhancing the aqueous concentrations of PAHs from crude oil. The enhanced PAH concentrations ranged from 2.2 times to more than 35 times for the biosurfactant treatment compared to the synthetic surfactant treatment. The biological surfactant was, therefore, more effective than its synthetic counterpart in solubilizing these compounds from a complex mixture to an aqueous solution.

The relationship between the hydrophilic and hydrophobic moieties of a surfactant molecule is referred to as the hydrophile-lipophile balance (HLB) number. The HLB number is a function of the ratio of the formula weights of the hydrophilic half to the entire molecule; i.e., the larger the HLB number, the more hydrophilic the molecule (Rouse et al., 1994). The HLB number indicates a surfactant's preference for oil (HLB 3-6) or water (HLB 10-18).

Bruheim et al. (1997) tested a *Rhodococcus* species for its ability to oxidize alkanes in crude oil emulsified by nonionic chemical and biological surfactants. They reported that bacteria in the exponential and stationary growth phases were negatively affected by surfactant amendment; however, oxidation rates in the stationary growth phase were in some cases stimulated by surfactants. The stimulatory effect depended on both the chemical structure and the physicochemical properties (HLB) of the surfactants. Those surfactants with intermediate HLB values (8-12) gave the best results.

The amount of surfactant used to enhance the solubility of contaminants has varied in the literature. Researchers generally use concentrations of surfactants near the critical micelle concentration (CMC) value because of the documented inhibition of bacterial metabolism by

supra-CMC levels of some surfactants (Laha and Luthy, 1991). Pinto and Moore (2000) reported that the addition of the synthetic surfactant Tween-80 at 10^4 x CMC released an average of 75% of bound ^{14}C-PAH and 64% of aged PAH from a weathered PAH contaminated soil without causing inhibition. Zheng and Obbard (2000) noted that a 2.5% solution of the surfactant Tween-80 solubilized 85% of a total of nine PAHs in an aged (1 month) contaminated soil at a soil/water ratio of 1:10. Other researchers have reported the surfactants Tween-80 and polyoxyethylene 10 lauryl ether (PLE) increased anthracene, pyrene, and benzo (a) pyrene oxidation rate by 2 to 5 fold in a PAH contaminated soil matrix (Kotterman, et al., 1997).

Polycyclic aromatic hydrocarbons (PAH) are low soluble, organic compounds that are toxic, mutagenic, and priority pollutants. The biodegradation of PAH are limited due to their low solubility. Surfactants, both synthetic and microbially produced, have been used to successfully increase biodegradation through solubilization. Tiehm (1994) showed that nontoxic surfactants enhanced the degradation of fluorene, phenanthrene, anthracene, fluoranthrene, and pyrene and that increased PAH degradation occurred in a mixture to which nontoxic surfactants that were not preferred as growth substrates were added.

Microorganisms may prefer to use synthetic surfactants as a substrate in place of hydrocarbons. Volkering et al. (1995) reported that microorganisms utilizing crystalline naphthalene and phenanthrene as target substrates had growth and oxygen uptake rates that were not affected by surfactants and that the increase in growth was not caused by use of surfactants as the sole source of carbon and energy. They further showed that the maximum dissolution rate

of the PAH increased when surfactant was present, indicating facilitated transport of the PAH into the aqueous phase.

The nonionic synthetic surfactant Tween-80 has been shown to promote the solubilization of hydrocarbons in the aqueous phase without producing inhibitory affects. Janiyani et al. (1993) investigated the treatment of oil sludge with the synthetic surfactant Tween-80. Results showed the release of hydrocarbons to the aqueous phase, pH variation did not affect the solubilization process, and mixing with proper contact time improved the release of hydrocarbons into the aqueous phase. The authors observed that hydrocarbon release from oil sludge into the aqueous phase was necessary for the hydrophilic hydrocarbon-utilizing bacteria, which could not access the oil sludge unless it was in water suspension. Strong-Gunderson and Palumbo (1995) reported the addition of Tween-80 increased the bioavailability of toluene, naphthalene, and recalcitrant natural organic matter (NOM) in aqueous and soil-based systems. They further showed that neither the Tween-80 surfactant nor the NOM supported microbial growth as the sole carbon and energy source.

F. Bioaugmentation

Biodegradation can also be enhanced by bioaugmentation where bacterial cultures are grown in sufficient quantities on selected hydrocarbons and then introduced to the waste oil. This approach may be preferred if the waste oil is fresh or if it is believed that native microorganisms are not present in sufficient quantities. However, native microorganisms can prove equally capable of degradation given the proper nutrients. Venosa et al. (1992) reported

that indigenous microorganisms were primarily responsible for the biodegradation of weathered Alaska North Slope crude oil and outperformed exogenous oil degraders with excess nutrients. Huesemann et al. (1993) reported that napthalene, anthracene, phenanthrene, chrysene and benzo (a) pyrene were completely degraded in four weeks using aerobic reactors, inoculated with acclimated cultures. Venkateswaran and Harayama (1995) concluded the initial fraction of microbial species and the catabolic activities in a community are important factors in determining its efficacy for petroleum degradation. Robertiello (1994) found that the quantity and quality of hydrocarbon-degrading microorganisms present in a sandy soil contaminated with gasoline and diesel fuel were closely linked to (and proved to be in equilibrium with) the quantity of contaminants to be broken down. Inocula consisting of strains that are different from those which naturally tend to exist in hydrocarbon-polluted soils would be of little use. In both the field and laboratory, after a few weeks of treatment, a naturally selected microbial flora tended to prevail over those initially introduced.

There are a variety of commercially available bacterial additives designed for specific contaminants. These products are used periodically in industrial wastewater treatment plants to prevent plant upsets due to overload of difficult-to-degrade, toxic, or inhibitory compounds in the wastestreams from food processors, paper mills, chemical plants, and oil refineries (Forsyth et al., 1995). Nerella et al. (1995) reported that eight microbial inoculant products were added to microcosms containing soil from a salt marsh and amended with fertilizer. None of the products increased activity above that of the fertilized control with oil. Addition of oil to microcosms increased populations of hydrocarbon-dgrading microorganisms, but bioagmentation products did not increase populations over time. The authors noted that the natural populations

of hydrocarbon-degrading microorganisms were adequate in the salt marsh soil for bioremediation.

Pearce et al. (1995) performed a pilot-scale study to evaluate the application of landfarming techniques in bioremediating a soil highly contaminated with petroleum products. Differences in total petroleum hydrocarbon concentration (TPHC) reduction from use of a commercial versus an in-house indigenous biosupplement were insignificant. The commercial biosupplement showed an initial lag phase, which was thought to signify an acclimaton period. It was therefore concluded that a biosupplement of indigenous microorganisms was efficient and more cost-effective, compared to the commercial biosupplement, for application at full-scale.

G. Fluorescein Diacetate

Fluorescein diacetate (FDA) has been successfully used as an indicator of intracellular microbial activity. FDA, an esterified dye, has no color until it is hydrolyzed by the cell, which results in a light absorption change that can be measured and correlated to activity. Since fluorescein accumulation depends upon an intact membrane and active metabolism, only active cells should fluoresce (Chrzanowski et al., 1984). Bishop and Safferman (1996) noted that combining the analysis for phospholipid level and FDA activity (i.e., microbial activity) proved to be valuable in approximating the optimum biofilm quantity in a fluidized bed reactor utilizing a synthetic wastewater. Riis et al. (1998) studied the extraction of microorganisms from soil. They evaluated the extraction efficiency by combining biochemical activities (FDA hydrolysis, dehydrogenase activity and dimethylsulfoxide reduction) and cell counting. The highest residual

activities (in samples after sonication) were always measured for the hydrolysis of FDA. De Rosa et al. (1998) reported a method to measure biofilm bacteria activity using FDA noting that experimental tests have shown a good correlation between the absorbance of the solution at 490 nm and bacterial concentrations.

H. Sludge Dewatering

Sludge dewatering is the removal of water from the sludge to reduce its volume, thus lowering the cost of handling and disposal. A variety of chemicals, polymers and inert agents are used to assist in the dewatering process. The extent of reduction of sludge volume or dewatering is a function of the characteristics of sludge, type of dewatering device and treatment process. Sludge characteristics such as specific resistance, capillary suction time, filter yield, and solid content can be determined and combined with varying doses of sludge conditioners to aid in the effective dewatering and sludge handling operations.

Hwa and Jeyaseelan (1997) compared the effectiveness of alum and lime in conditioning oily sludge with oil contents varying from 1.8 to 8.0 % by weight. They reported the degree of reduction of sludge volume or dewatering is a function of the characteristics of sludge and the type of dewatering device. They noted that CST values without alum dosage were much higher than the CST values after addition of alum. Moreover, alum produces fewer solids with lesser optimum dosage than lime and is therefore a better sludge conditioner compared to lime.

How the dewatering agents are introduced to the sludge is also important in the success and economics of sludge dewatering. Novak (1991) studied the effect of mixing on the

performance of sludge conditioning chemicals. A variety of water (alum and lime softening) and wastewater sludges (anaerobically digested, lime softening, waste activated) were used. He reported that sludges comprised of flocculent particles are sensitive to shear, undergoing disaggregation which creates an increased demand for conditioning chemicals. He also noted that the benefits of sludge conditioning to shear resistance are dependent on both the type of conditioning chemical and the conditioning dose.

I. Petroleum Waste Problem and Objectives

Much of the research associated with the biodegradation of petroleum hydrocarbons has focused on contaminated soil and groundwater. The biodegradation of waste oils generated as byproducts from industrial activity has received limited attention. This study was conducted at the request of an industry to assess the potential for biodegradability of waste oil generated at their maintenance facility to reduce the volume of waste oil and disposable sludge that was generated during the servicing of diesel locomotives. Current practice in handling and disposing of the waste oils include collection of the waste from various sources throughout the maintenance complex and storing the combined waste in an outdoor open concrete tank. The waste oil and water mixture in the tank separates into four layers. The top three layers are removed via a commercial contractor twice per year at a considerable expense to the industry. The final layer consists of wastewater that is pumped out, treated with polymers to promote separation, and discharged to sand drying beds. The filter cake is then removed for final disposal to a landfill. This study focused on the middle two layers of waste oil. The objectives of this study were to determine (i) the biodegradability of the waste oil, (ii) the role of bioaugmentation

and surfactants in facilitating biodegradation, (iii) the effect of oil composition on biodegradation, and (iv) the dewatering characteristics of biodegraded oil.

3. MATERIALS AND METHODS

A. Pilot Plant Configuration and Operation

The pilot plant system used in this study is represented in Figure A. The pilot plant consisted of two reactors (R1 and R2) constructed of steel 208-L (55 gal.) drums with clamped steel lids. A working volume of 185 L was maintained per reactor. Three sample ports were installed at 6.35 cm, 36.35 cm and 61.60 cm from the bottom inside of each drum. The sample ports consisted of 2.54 cm, 1.58 cm and 1.58 cm diameter ball valves, respectively, and allowed for collection of representative samples throughout the reactor. Compressed air, filtered, regulated and metered, entered the top of the drum at 0.99 to 0.113 m^3/min, at 11.0 to 13.8 kPa of pressure. Compressed air lines were constructed of 1.27 cm (1/2 in.) rubber tubing and 1.27 cm polyvinyl chloride (PVC) pipe. The PVC pipe inside the drums was configured in the shape of an H and had a series of 0.158 cm (1/16 in.) diameter holes. The airflow rate was set to provide sufficient mixing and oxygen. The exhaust air was vented to the atmosphere by way of a 1.27 cm PVC pipe.

Each reactor was amended with nutrients that varied for each experimental run (see definition of each experimental run or "batch" below). The nutrient amendments for batch 1 were: $CaCl_2 \bullet 2H_2O$, 0.92 g/L; $MgCl_2 \bullet 6H_2O$, 1.46 g/L; $FeCl_3$, 0.14 g/L; $(NH_4)_2SO_4$, 9.32 g/L; and K-Gro All Purpose Plant Food (N:P:K,15-30-15) (Alljack, United Industries Corp., St.

Louis, MO), 1.44 g/L. Batch 2 was identical except that iron was added as $FeSO_4 \bullet 7H_2O$ at a concentration of 0.24 g/L. During batch 3 experiments, R2 was fed nutrients at concentrations two times those fed to R1. Nutrient concentrations

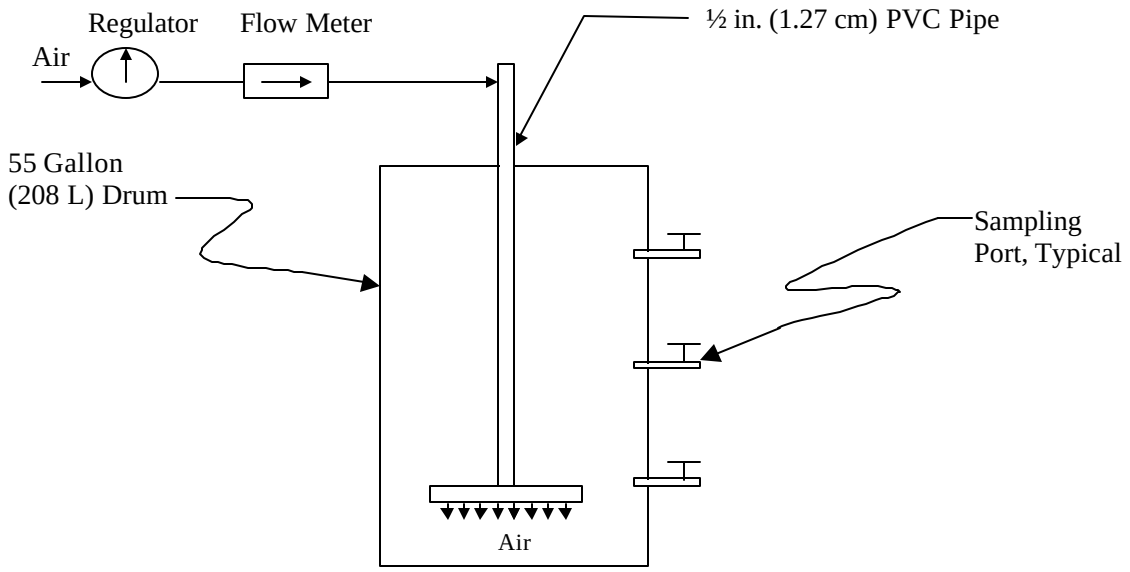

Figure 1. Block Diagram of Pilot Plant Reactor

fed to R1 were the same as those fed to both reactors during batch 2, except that ammonium was fed in two forms, as $(NH_4)_2SO_4$ at 0.63 g/L, and as $(NH_4)_2CO_3 \bullet H_2O$ at 7.43 g/L.

The reactor volume content was maintained at 185 L and tap water was added to make up for any loss due to evaporation. The pH and temperature were routinely recorded and lime was added to the reactors as necessary during batch 2 and 3 to maintain a pH between 6 and 8.

B. Waste Oil Characterization, Experimental Runs, and Sampling

The waste oil and water mixture used for the experiments consisted of four distinct layers. Layer 1 (~5 %) consisted of free oil that accumulated on the surface; Layer 2 (~50 %) was made up of emulsified oil and was the largest volume; Layer 3 (~20 %) was weathered oil with little emulsification; and Layer 4 (~25 %) was wastewater. The bottom of the tank consisted of heavy sludges and inerts. The waste oil and water mixture contained a wide variety of petroleum products including engine-lubricating oil, air compressor oils, diesel fuel and heavy greases with lesser amounts of caustic cleaners, polymers, and flocculents.

Three experimental runs were conducted and included four experimental conditions, as listed in Table 1. Batch 1 compared the effect of bioaugmentation versus relying upon indigenous microorganisms; batch 2 evaluated the effect of adding a synthetic surfactant; and batch 3 compared biodegradability of oil at different strengths and from different sources (i.e., layers).

C. Batch 1

R1 consisted of a 10 percent waste oil mixture of layer 2 (emulsified oil) bioaugmented with a mixture of hydrocarbon degraders. R2 served as the control reactor with a 10 percent waste oil mixture of layer 2 and no bioaugmentation (indigenous microorganisms). Novozymes, Inc. (Salem, VA) supplied the microorganisms used for

Table 1. Pilot Plant Experimental Conditions

Experimental Conditions	Batch 1		Batch 2		Batch 3	
	R1	R2	R1	R2	R1	R2
Bioaugmented	Yes	None	B1-R2	B1-R2	B2-R2	B2-R2
Surfactant	NA	NA	Yes	NA	NA	NA
Percent Oil	10	10	10	10	10	20
Oil Layer	L2	L2	L2	L2	L3	L2 + L3

B1=Batch 1, B2=Batch 2, L2=Layer 2, L3=Layer 3, R1=Reactor 1, R2=Reactor 2, NA=Not Applicable.

bioaugmentation. A total of seven strains of hydrocarbon degraders were used: SSC 79, SSC 82, SSC 84, SSC 86, SSC 87, SSC 90, and H3. The strains were grown on a variety of hydrocarbons, including diesel fuel, naphthalene, and anthracene. Two liters of each strain were added to R1 (14 L inoculum in 185 L of reactor volume) at the beginning of batch 1.

The reactors were filled to approximately 75 L with tap water, nutrients were added, and the contents were mixed with a high-speed mixer for 5 minutes. Waste oil was then added (18.5 L) along with the remainder of the tap water (R1 & R2), bioaugmented (R1, 14 L of inoculum), and the entire contents mixed for 5 minutes with a high-speed mixer at 1,200 revolutions per minute (rpm). The total liquid content in each reactor was 185 L. Airflow was then introduced at 5.9 m^3/hour to provide sufficient mixing.

D. Batch 2

Both R1 and R2 were filled with water, nutrients, and waste oil and mixed as described at the start of batch 1. Next, both reactors were bioaugmented (15 L each) with sludge from batch 1, R2 (indigenous microorganisms). R1 also received 4 L of a stock solution of the nonionic synthetic surfactant Tween-80 to a final concentration of 50 mg/L. This surfactant was chosen because of its ability to liberate hydrocarbons into the aqueous phase without exhibiting toxicity, even at levels above the critical micelle concentration (Rouse et al., 1994). The reactors were then filled to their operating volume (185 L) with tap water and the entire contents mixed for 5 minutes with a high-speed mixer at 1,200 rpm. Airflow was then introduced at 5.9 m^3/hour to provide sufficient mixing.

E. Batch 3

Both R1 and R2 were filled with water and nutrients and mixed as described at the start of batch 1. Waste oil was added as a 10 % mixture to R1 (18.5 L of L3) and as a 20 % mixture to R2 (18.5 L of L2 & 18.5 L of L3). Next, both reactors were bioaugmented (15 L each) with sludge from batch 2, R2 (indigenous microorganisms) and then filled to their operating volume (185 L) with tap water. The entire contents were mixed for 5 minutes with a high-speed mixer at 1,200 rpm. Airflow was then introduced at 5.9 m^3/hour to provide sufficient mixing.

Samples were taken at the start of each batch and subsequently on days 3, 6, 10, 15, 24, 31, 38 and 42 for batch 1. For batches 2 and 3 the samples were taken at the start and on days 3,

6, 9, 14, 24, 31 and 38. All samples (approximately 150 mL) were directly collected in glass amber containers with Teflon lined caps, and were placed on ice for transport to the laboratory. Samples were stored at 4 °C until additional analyses could be initiated (within 24 hours).

F. Analytical Methods

Reactor samples were routinely monitored for chemical oxygen demand (COD), total suspended solids (TSS), volatile suspended solids (VSS), fluorescein diacetate (FDA) bioactivity, hydrocarbon composition via solvent extraction and gas chromatography (GC), capillary suction test (CST), and sludge conditioning with vacuum filtration for cake solids. Due to the oily nature of the samples, multiple phases developed in the sample containers. Special processing methods were developed in order to generate results that were as representative as possible of the entire sample volume. These methods are discussed below.

i. Chemical Oxygen Demand (COD)

Each sample was vigorously mixed in the original sample container using a Teflon-coated magnetic stir bar on a stir plate. A 10-mL aliquot was transferred from each sample into 90 mL of tap water (1:10 dilution). Two mL from the well mixed 1:10 dilution was transferred into the appropriate amount of tap water to obtain the desired final dilution: (1:200, 1:250, or 1:500). From this well mixed dilution, 5 mL was transferred into a 25 mm x 150 mm (50 mL) test tube. Volumetric pipets were used for each transfer during the dilution procedure. Each sample was analyzed in triplicate. Duplicate samples from each reactor were diluted twice to derive two independent dilution samples per original reactor sample. One of the triplicate

samples was spiked with 100 µL of potassium hydrogen phthalate (KHP) to yield 100 mg/L of COD. This allowed the COD recovery to be estimated in the presence of the sample matrix to discern the reliability of the COD data. Spiked recoveries for the samples were the following: Batch 1, 95 (± 12) mg/L, n=40; Batch 2, 94 (± 6) mg/L, n=31; and Batch 3, 101 (± 15) mg/L, n=30. Standards were prepared using a 10 or 20% mixture (depending on percent oil in reactor) of mineral salts diluted with tap water in a manner that was identical to how the unknown samples were diluted. The standards were spiked with KHP to determine COD recoverability within the salt matrix used. Spiked recoveries for the standards were the following: Batch 1, 101 (± 10) mg/L, n=20; Batch 2, 96 (± 6) mg/L, n=14; and Batch 3, 102 (±5) mg/L, n=26. Two cold and two hot blanks were prepared with each set of samples. CODs were analyzed according to Method 5220C of *Standard Methods* (1995).

ii. Total Suspended Solids and Volatile Suspended Solids (TSS and VSS)

TSS and VSS were measured using Method 2540D and E, respectively, of *Standard Methods* (1995). A known volume of sample was filtered through a previously weighed 1.5 µm glass fiber filter (Whatman 934-AH) in a filter apparatus and then dried overnight at 103 °C and weighed again. The mass of solids on the filter was the mass of the TSS in the filtered volume. The solids remaining from the TSS test were dried at 550 °C for 20 minutes and weighed again. The weight lost between the two drying temperatures was the VSS in the filtered volume.

iii. Methylene Chloride Extraction

Approximately 5 mL of a well-mixed, undiluted, reactor sample was placed in a clean, dry, preweighed 40-mL glass centrifuge tube. The tube was capped and weighed and the sample weight determined. Methylene chloride (MeCl) was added in a w/w MeCl: sample ratio of 2.5:1 for the 10 percent waste oil mixtures and 5:1 for the 20 percent waste oil mixture. Samples were prepared in duplicate. An internal standard (stock solution of 1,4-dichlorobenzene, 800 mg/L for batch 1, 400 mg/L for batch's 2 & 3) was added to each tube (0.5 mL for the 2.5:1 and 1.0 mL for the 5:1 ratio of MeCl to sample). A standard consisting of 10 or 20 mL (for the 10 or 20 % waste oil mixtures) of mineral salts, MeCl and the internal standard was developed to evaluate any effects they may have on the extracted sample. The tubes were weighed, tumble mixed for 24 hours, then centrifuged (Beckman Model J-21C) for 20 minutes at 750 x g to separate the water and methylene chloride layers. Tubes were weighed and the top layer of the centrate (nonextractable) was removed and placed into a clean, dry preweighed ceramic crucible and weighed. The water phase of the nonextractable portion was allowed to evaporate and the crucible was weighed. The crucible was then placed in a muffle furnace at 550 °C for 1 hour. The crucible was removed from the furnace, allowed to cool to room temperature, and weighed (nonextractable portion). The nonextractable fraction data is not presented in this document.

After the nonextractable fraction was removed from the centrifuge tube, the centrifuge tube cap was immediately replaced and the tube was weighed. The remaining portion of the sample (extractable) was collected into preweighed 4 mL vials with Teflon lined caps. The tube and sample vials were weighed to determine the mass of the extractable portion of the

sample. The sample to MeCl weight ratio collected in the 4 mL vials was further diluted to 1:10, 1:5, 1:2, or 1:1 (w/w) as necessary to obtain appropriate gas chromatograph output.

Standards were developed to qualitatively compare degradation of the waste oil to known amounts of clean oils that make up the three largest components of the waste oil: diesel fuel, engine lubricating oil, and air compressor oil. Clean, unused batches of each standard were obtained from the industry source and were extracted in a manner identical to the waste oil samples. Standards of 1,000 mg/L, 10,000 mg/L, and 100,000 mg/L were developed. The standard to MeCl weight ratio collected in the 4 mL vials were further diluted to 1:100, 1: 20, 1:10, or 1:5 (w/w) as necessary to obtain appropriate gas chromatograph output. Representative chromatograms showing the fingerprints for the standard oils are shown in Figure 5. Peaks in the diesel fuel, compressor oil and engine oil appear from 4 to 28 minutes, 16 to 43 minutes, and 24 to 44 minutes, respectively.

iv. Gas Chromatography

GC analysis was performed to observe changes in hydrocarbon abundance over time. The methylene chloride extracted oil was analyzed using a Shimadzu GC-14A Gas Chromatograph (GC) equipped with a flame ionization detector (FID). A J & W Scientific DB5-MS fused silica capillary column with 0.25-µm film thickness, 0.25-mm internal diameter, and 30-m length was used. The temperature program started at 70°C for 1 minute, and then was increased to 300°C at a rate of 7°C/min where it was held for 5 minutes. The temperature was further increased to 310°C at a rate of 10°C/min for 5 minutes for a total analysis time of 48 minutes. Injector and detector temperatures were maintained at 295°C and 310°C, respectively.

Samples were analyzed in a splitless/split fashion using a Shimadzu AOC-20I autoinjector unit. A splitless injection sample volume of 2 µL and helium carrier gas flow of 1 mL/min was used. Air, hydrogen, helium (column), and helium (make-up) gauge pressures were maintained at 45, 55, 72, and 78 Pa, respectively. Purge and split flow rates were 10 and 50 mL/min, respectively. Shimadzu CR501 integrator settings were as follows: slope = 5000; peak width = 1.5; drift = 0; time to double = 0, attenuation = 4 or 5; stop time = 48 min.; chart speed = 10 mm/min; minimum peak area = 2000; method = 0041; and format = 2040.

v. Fluorescein Diacetate (FDA) Test

The FDA test was performed on freshly collected samples using a modified version of the method described by Schnurer and Rosswell (1982). Tests were carried out in duplicate on samples using a 60 mM (pH 7.6) sodium phosphate buffer (PB) stock solution, diluted from a 1 M stock. In a 10 mL test tube, 1 mL of undiluted sample was added to 4 mL of PB and 100 µL of FDA solution dissolved in acetone (2 mg/mL). The test tubes were vortexed and then shaken for 1 hour in a water bath set at the temperature corresponding to the temperature in the reactor for the sample day (21 ± 3 °C). Test tube contents were removed, vortexed, and filtered using 0.2 µm Supor®-200 membrane filters (Gelman Sciences, P/N 60300). The filtrate was diluted 1:5 with distilled water and absorbance was read at 490 nm using a Beckman Model DU 640 spectrophotometer. When microorganisms enzymatically hydrolyze FDA, a color change occurs producing a subsequent change in light absorption that can be measured and associated with activity. Blanks were prepared using 4 mL of PB, 1 mL of deionized water and 100 µL of FDA and analyzed in a manner that was identical to the unknown samples.

vi. Surface Tension

Surface tension measurements were performed to determine the appropriate dosage of surfactant to be used in batch 2. Measurements were performed in triplicate with a Fisher Surface Tensionmeter Model 20. The tensionmeter was calibrated with distilled water. The measurements were performed in a 45 mm diameter flat bottom container with approximately 40 mL of sample. The platinum-iridium ring used for the measurements was cleaned with benzene and chromic acid followed by a distilled water rinse after each sample. Samples were taken from a 100 mL mixture of 10 percent waste oil (v/v) to which various doses of the nonionic, synthetic surfactant Tween-80 (Atlas Chemical Industries, Wilmington, DE) were added (See Table 2). The sample was vigorously mixed with a high-speed mixer for 3 minutes and then placed on a stir plate and mixed with a Teflon-coated magnetic stir bar at a moderate rate for 15 minutes prior to surface tension measurements.

vii. Percent Solids

The percent solids in the reactors were determined during batch 3 using two different methods. The first method was applied for sampling events on days 1, 3, and 6. A well-mixed sample (20 to 40 mL measured by beaker) from each reactor was placed into a clean, dry, preweighed aluminum dish. The dish was reweighed, then placed into a drying oven at 103 ^{o}C for approximately 2 days. The dish was removed from the oven, allowed to cool in a desiccator, and weighed again to determine percent solids. After day 6, the second method was used. A known amount of a well-mixed sample (20 mL) was collected with a wide-mouth pipet and placed into a clean, dry, preweighed aluminum dish. The dish was reweighed, then placed into a drying oven at 103 ^{o}C for approximately 2 days. The dish was removed from the oven,

allowed to cool in a desiccator, and then weighed to determine percent solids. Next, the dish was placed in a muffle furnace at 550 ^{o}C for 20 minutes, removed from the oven, allowed to cool in a desiccator, and then weighed to determine percent volatile solids fraction.

viii. Sludge Conditioning

A known volume (50 or 100 mL) of a well-mixed reactor sample was poured in a 250 mL beaker. A variable speed jar tester was used to mix the sample and various coagulant (Alum or $FeCl_3$) doses were added. Applied doses were 0.75, 0.10, 0.15, and 0.125 lb/lb (pound of coagulant per pound of sludge solids). The percent solids of the sample, converted to grams/liter, combined with the sample size, (50 or 100 mL) and the concentration of coagulant (100 g/L) were used to determine the dose volume of coagulant. Coagulant was dosed during a 1 minute mixing period (100 rpm) and the sample was then immediately transferred to the capillary suction test apparatus. Varying doses were applied to samples collected at the beginning, middle, and end of batch 3. The 0.10 lb/lb dose proved to be optimum at each stage tested with the exception of day 0 (no conditioning was optimum) and day 38, where the 0.125 lb/lb dose was optimum for ferric chloride and the 0.75 lb/lb was optimum for alum.

ix. Capillary Suction Test (CST)

CST was performed using Method 2710G of *Standard Methods* (1995). Whatman No. 17 chromatography grade paper was cut into 7 x 9 cm sections, with the grain placed parallel to the long side. A piece of paper was placed into a CST apparatus, and a representative reactor sample (100 mL), mixed for 1 minute at 100 rpm with a jar tester, was poured into the test cell until it was full. The time for the liquid to be drawn from the inner pair

of electrical contacts to the outer contact was recorded. The test was repeated a minimum of twice per sample. The remaining sample was discarded and new CST paper was installed for each measurement.

x. Percent Cake Solids

A well-mixed reactor (100 mL) sample was placed in a 250 mL beaker and mixed with a variable speed jar tester for 1 minute at 100 rpm. Conditioned samples were dosed during the 1 minute mixing period. Immediately after mixing, the sludge samples were dewatered using a 9 cm diameter Buchner funnel with a Whatman No. 40 ashless filter paper for sludge cake retention. Samples were dewatered at an applied vacuum differential of 38 cm Hg, and filtrate volume was quantified as a function of filtrate time. Cake solids were collected from a 5 cm x 5 cm square on top of the filter and placed in a clean, dry, preweighed aluminum dish. The sample was weighed, then placed into a drying oven at 103 ^{o}C for 24 hours. The pan was removed from the oven and allowed to cool in a desiccator and then weighed to determine percent cake solids. Unconditioned reactor samples (no coagulant added) were treated in a manner identical to conditioned reactor samples.

xi. Time-To-Filter (TTF)

TTF test was performed using Method 2710H of *Standard Methods* (1995). A 100 mL well-mixed representative reactor sample was poured into a Buchner funnel containing a pre-wetted Whatman No. 40 ashless filter paper under a constant vacuum of 51 kPa. A stopwatch was used to determine the time required for 50 and 100 mL of sample to collect in a graduated cylinder.

xiii. Statistical Analysis

A statistical analysis was applied where replicates were performed. Analyses applied were pooled standard deviation for COD and FDA data and standard deviation for sludge dewatering, where applicable. For COD and FDA data, the averages provided on the graphs are duplicate measurements of duplicate samples per original reactor sample. Error bars around each COD and FDA data point equal the pooled standard deviation of the two sets of samples. Statements of significance regarding COD and FDA data are based on whether or not the pooled standard deviations overlap. For the dewaterability studies, the averages provided on the CST, TTF, and percent cake solids graphs are measurements of duplicate samples per original reactor sample. Error bars around the data points represent the standard deviation of the samples. Data points without error bars indicate single measurements. Statements of significance regarding CST, TTF and percent cake solids data are based on whether or not the standard deviations overlap.

4. RESULTS AND DISCUSSION

A. Batch 1 – Effect of Bioaugmentation

The total COD percent removed and bioactivity changes measured during batch 1 indicate that bioaugmentation with nonindigenous bacterial strains did not improve the overall biomass activity relative to indigenous microorganisms. The initial measured COD values were 28,320 (± 980) mg/L and 32,140 (± 1,143) mg/L for R1 and R2, respectively (data not shown). Percent COD reduction (Figure 2) was based on COD values measured at day 3 for both reactors, as the initial values were not representative of the actual COD of the waste oil. By day

3, the oil was visibly dispersed and it was possible to obtain a more representative sample, which resulted in a higher COD value than initially measured. By day 3, measured COD values had peaked to 46,530 (± 1,060) mg/L and 41,550 (± 1,080) mg/L for R1 and R2, respectively. Between day 3 and 10, the COD in both reactors decreased by 50 percent. Final reductions (day 42) were 59 (± 9) and 38 (± 3) percent for R1 and R2, respectively.

The variability in the percent COD removal was most likely due to sample representation and oil dispersion. Obtaining a representative sample proved to be difficult due to the hydrophobic nature of the oil. The emulsified oil was especially resistant to dispersion in the early stages of the study but was visibly dispersed after 3 to 6 days of operation, which coincided with a reduction in COD. Berwick and Stafford (1985) also reported difficulties in initial oil dispersal in their efforts to biodegrade waste oil. They found that initially the oil was never evenly dispersed in the liquor. However, they found that a second phase was invariably marked by a homogeneous oil-in-liquor suspension which could be determined by analyzing samples withdrawn from different depths with the aeration system in operation. The explanation for this transition was that the increase in bacterial biomass would have resulted in the release of variable amounts of soluble protein, phospholipids, and glycolipids from the lysed cells. These compounds are believed to have contributed to the emulsification and dispersion of oil.

The drop in COD reduction (increase in COD) from day 15 to day 24 may have been the result of further dispersion of emulsified oil or break down of more complex hydrocarbons. This dispersion of oil is most likely due to the production of biosurfactants. Microorganisms produce surfactants to disperse the oil into the aqueous phase where the contaminants are amenable to

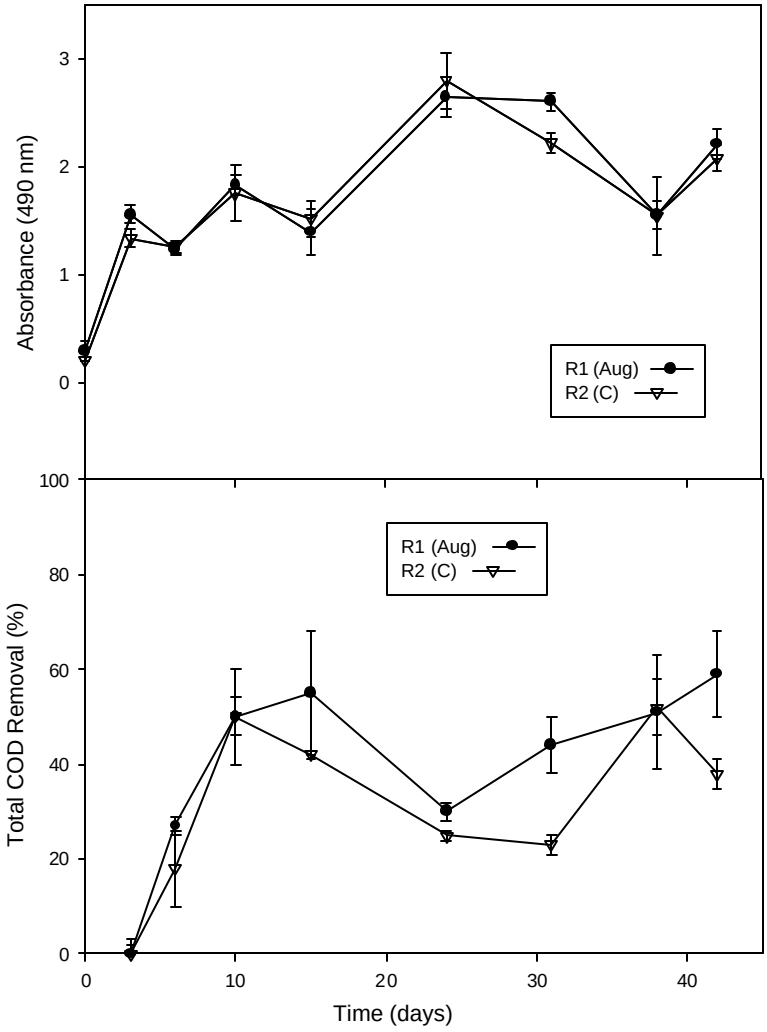

Figure 2. Total Percent COD Removal and Bioactivity Changes: Batch 1, R1-Bioaugmented 10% Waste Oil Mixture, R2-Nonaugmented 10% Waste Oil Mixture. Layer 2: Emulsified Oil.

biodegradation (Strong-Gunderson and Palumbo, 1995). Additional surfactant production is necessary for continued solubilization of the remaining nonaqueous-phase oil (Bruheim, et al., 1997). This cycling of surfactant production and carbon utilization appears to have occurred throughout the study and is the most likely reason for the decrease and subsequent increase in

total COD removal values shown in Figure 2. It is interesting to note that R2 showed a decrease in COD reduction between day 38 and 42 while R1 showed and increase in COD reduction during the same period. The data suggests continued reactor operation may have resulted in another cycle of COD increase (as seen in days 10 to 24) followed by COD reduction (observed during days 24 to 38).

The bioactivity of the microorganisms as measured by FDA (See Figure 2) was similar in both R1 and R2. FDA activity initially increased in both reactors from start to day 3 while there was no measured reduction in COD. Bioactivity showed corresponding growth in both reactors with decreasing COD removal (increase in COD) from day 15 to day 24 followed by comparative reduction with increasing COD removal from day 24 to day 38. These trends suggest that with minimal induction of biosurfactants the microorganisms can degrade the oil when the oil is dispersed and in a usable form. When the oil is emulsified or consists of complexed hydrocarbon structures, biosurfactants are necessary for hydrocarbon utilization and are represented by an increase in bioactivity.

The pH of each reactor dropped from an initial value of 7 to 6.2 by day 24 and to 5.2 by the end of the experiment (data not shown). The reactors were not buffered and this drop in pH most likely had a negative influence on the bioactivity of the microorganisms by the end of the experiment. Most studies indicate that pH 7 to 8 is optimum for degradation of petroleum hydrocarbons (Mohammed, et al., 1996).

Comparing the chromatographs from day one for both R1 and R2 (See Figures 3 and 4) to the standards (Figure 5), it can be seen that diesel fuel was a significant component of the samples. The diesel fuel steadily degraded in both reactors during the batch study and by day 38 was effectively degraded. The most persistent peaks observed in chromatographs from both reactors occurred from 21 to 23 minutes and 42 to 44 minutes. The peaks between 21 and 23 minutes appear to be related to the diesel fuel while the peaks occurring between 42 and 44 minutes occur in both the compressor and engine oils but seem to be more closely related to the compressor oil. These later peaks were still present by day 38, indicating that it was difficult for the microorganisms to degrade these higher molecular weight compounds.

Biodegradation of the waste oil appeared to be similar for both reactors based on total COD removed, bioactivity, and GC analyses. Near the end of the study (day 38), the measured COD reduction was the same in both reactors at 51 (± 12) and 52 (± 6) percent in R1 and R2, respectively. R1 showed a small increase in bioactivity over R2 on day 31 but was otherwise the same as R2, suggesting that introducing known hydrocarbon degraders was not advantageous in terms of COD reduction or increased bioactivity. The chromatographs show very similar results for both R1 and R2 throughout the study (Figures 3 and 4). These results suggest that there were minimal differences in biodegradation capabilities between the amended and native microorganisms, and that amendment with non-native microorganisms is not required.

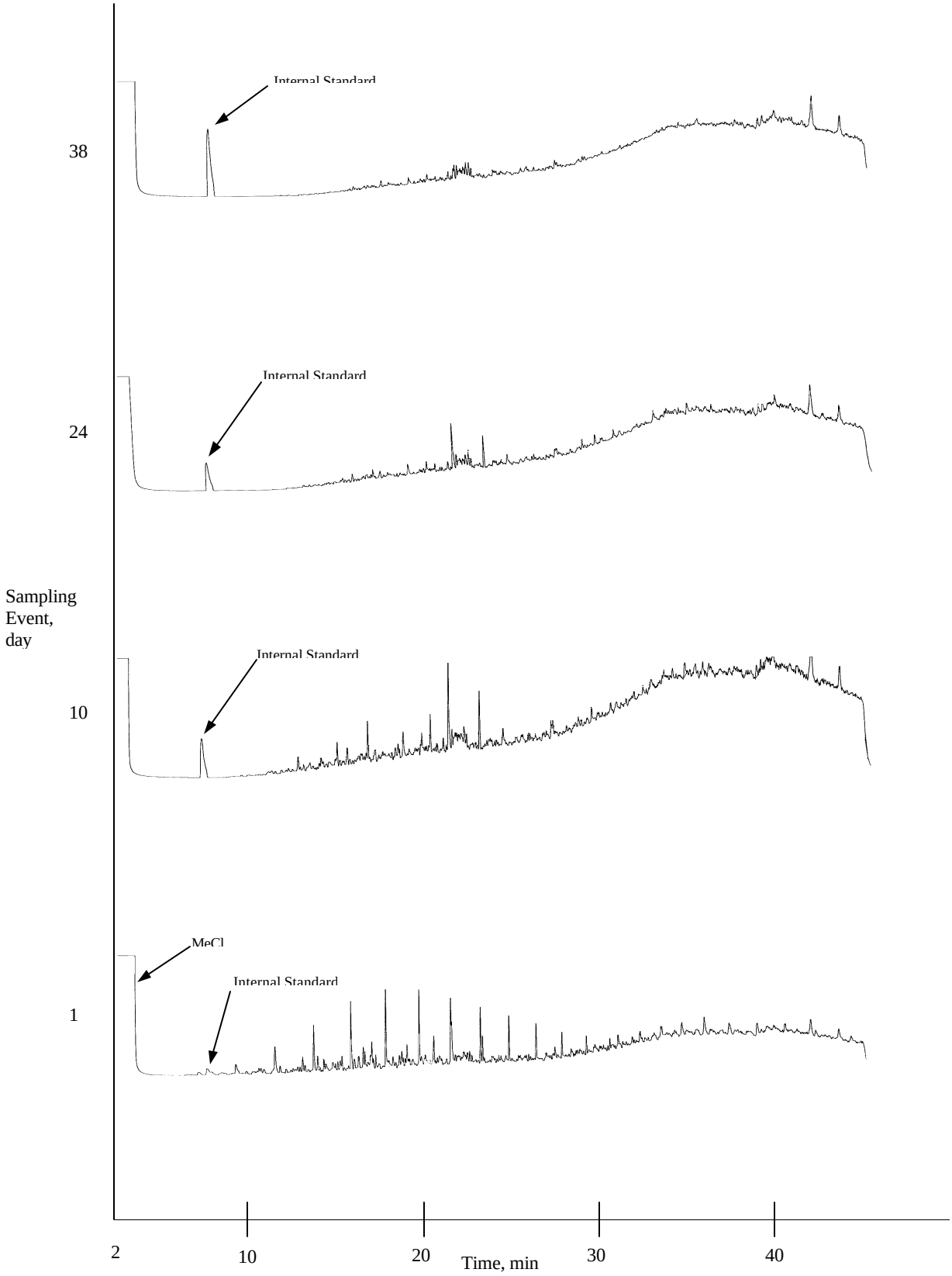

FIG. 3. Selected chromatographs of waste oil from Batch 1, R1 (Layer 2). Dilutions: 1:5 for day 1, 1:2 for days 10 and 24, and 1:1 for day 38.

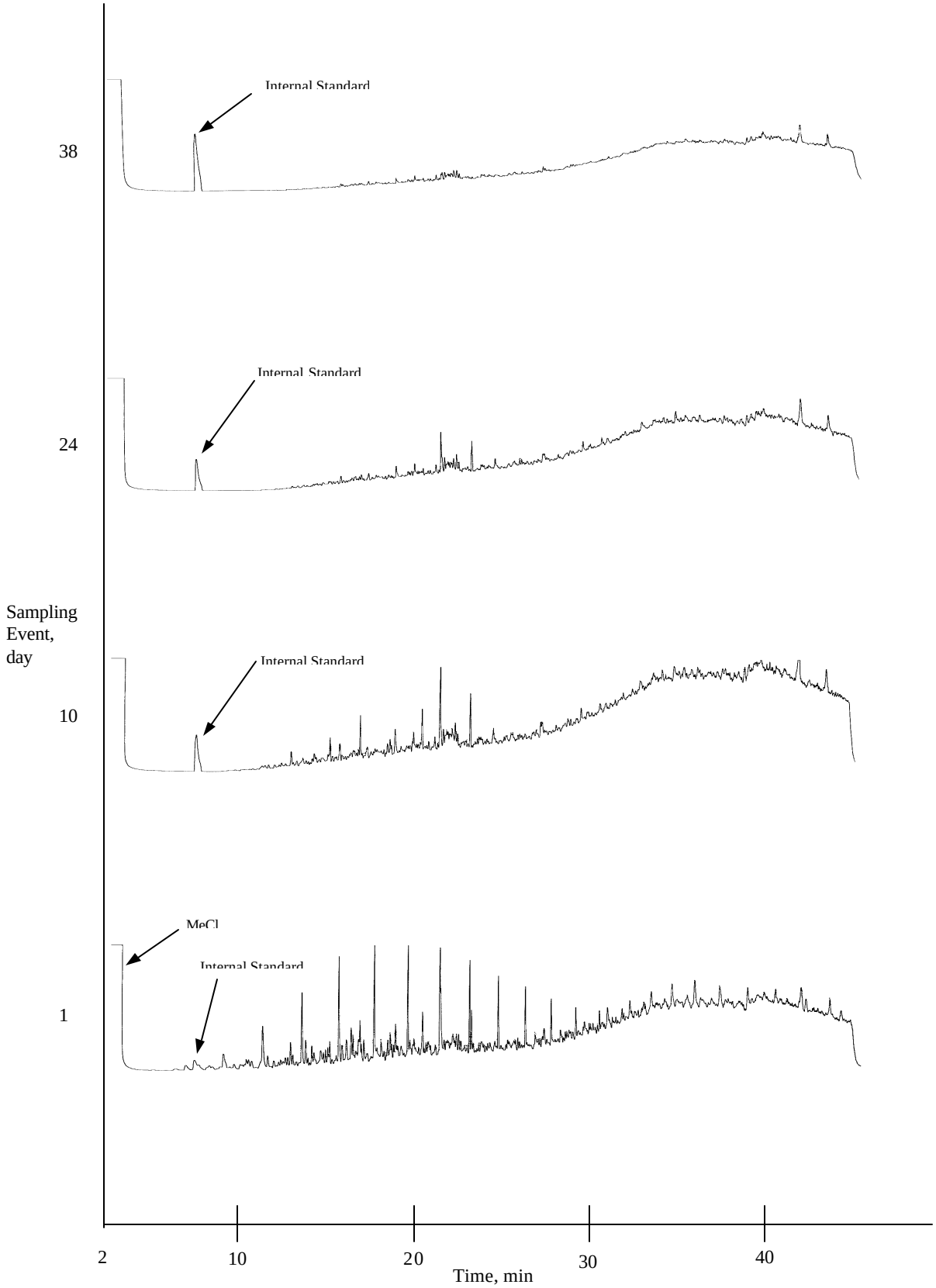

FIG. 4. Selected chromatographs of waste oil from Batch 1, R2 (Layer 2). Dilutions: 1:10 for day 1, 1:2 for days 10 and 24, and 1:1 for day 38.

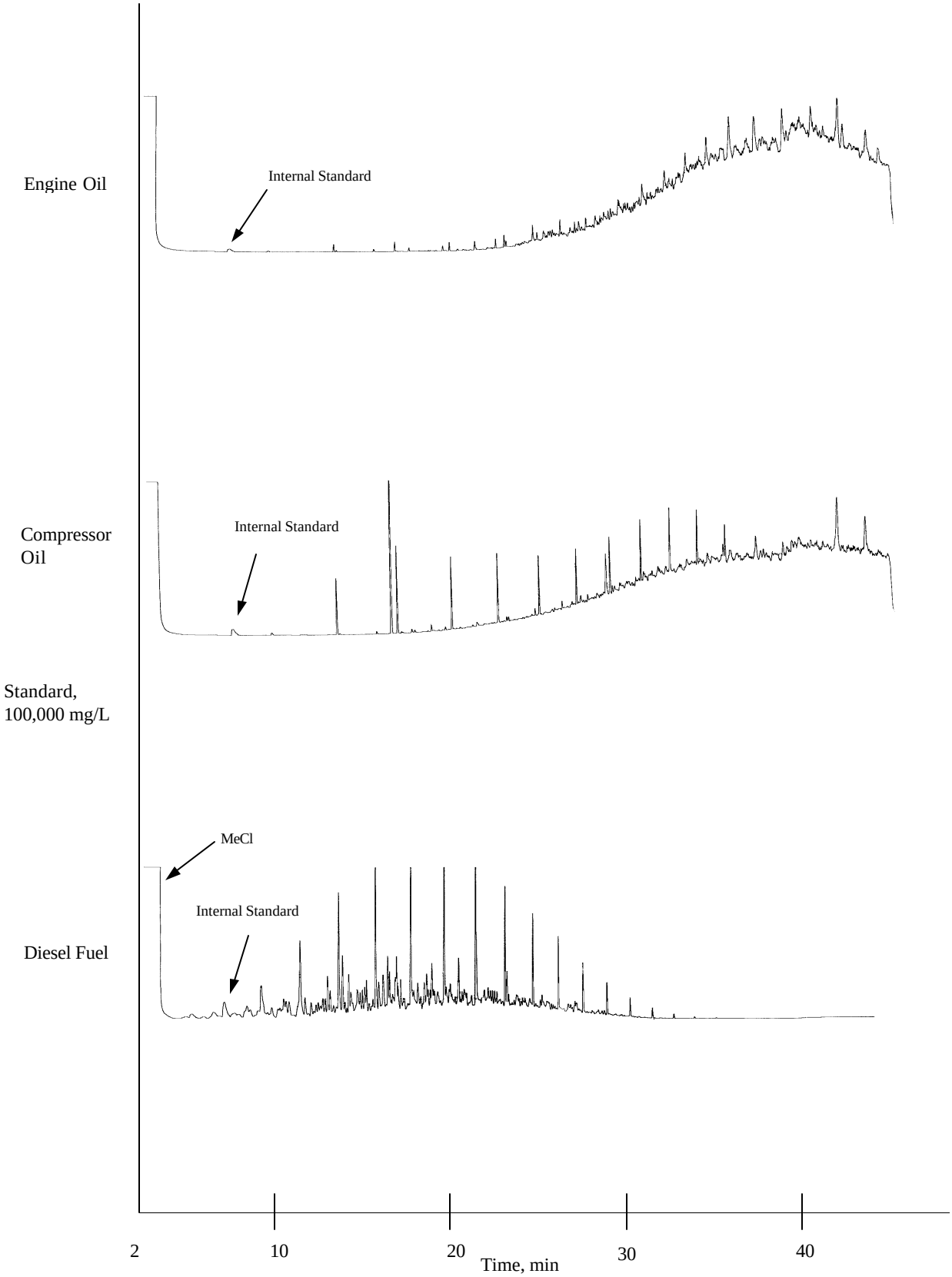

FIG. 5. Selected chromatographs of Standards (clean oil). Dilutions: 1:100 for Diesel Fuel, 1:10 for Compressor Oil, and 1:20 for Engine Oil.

B. Batch 2 – Effect of an Exogenous Synthetic Surfactant

Surface tension measurements were conducted with 10 % solutions of layer 2 waste oil and varying amounts of the surfactant Tween-80. The dose of 5.0 mg of surfactant for a 10 percent waste oil sample (See Table 2) had the lowest apparent surface tension and was selected as the amount to be used in batch 2. The average pH for both reactors was 6.3 and ranged from 5.6 to 7.0. Lime was added on days 6, 9, 14, and 31 to both reactors to adjust pH at or near 7.0.

Figure 6 shows the total COD percent removed and bioactivity changes during batch 2. Percent reduction was based on measured COD values at day 3 for R1 and day 6 for R2, as the initial values were not representative of the actual COD of the waste oil. By day 3, the oil in R1 was dispersed and it was possible to obtain a more representative sample, which resulted in a higher COD value than initially measured. This was also true by day 6 for R2. The initial COD values were 29,200 (± 1,410) mg/L and 23,000 (± 570) mg/L for R1 and R2, respectively (data not shown). By day 3, the COD for R1 had peaked to 50,670 (± 190) mg/L and by day 6, R2 peaked at 54,140 (± 380) mg/L.

The surfactant-amended reactor R1 showed that COD removal was initiated 3 days before the nonsurfactant-amended reactor R2. This coincided with visible observations that showed dispersion of waste oil in R1 by day 3 while R2 was still significantly emulsified. The rapid dispersion of oil in R1 was attributed to the presence of Tween-80. By day 6, the oil was dispersed in R2 and COD removal was increasing. This delay in dispersion of oil in R2 was most likely due to the time required for production of sufficient biosurfactants. This was also seen in the first three days and again between days 15 and 24 of batch 1. Unlike batch 1, there

did not appear to be any cycling of surfactant production and carbon utilization based on the COD removal and bioactivity changes shown in Figure 6. By day 38 both reactors had essentially the same COD removal, 84 (± 20) and 85 (± 16) percent in R1 and R2, respectively.

Table 2. Apparent Surface Tension

Waste Oil Layer 2 (%)	Surfactant Tween-80 (mg)*	Average Apparent Surface Tension (dynes/cm)
10	0	35.8
10	1.0	33.0
10	3.0	31.6
10	5.0	30.8
10	7.0	31.6
10	10.0	31.1
0 (DI Water)	0	75.3

DI = Deionized, *Mass applied to 100 mL of a 10 % waste oil mixture

The synthetic surfactant appeared to help in the early dispersal of waste oil, which coincided with the decrease in measured COD by day 3 for R1; however, by day 6 the oil in R2 had visibly dispersed and COD reduction followed. The total reduction was the same for both reactors, suggesting the exogenous surfactant did not have a long-term impact in the biodegradation of the waste oil. This suggests that there is no significant difference in COD removal, 84 (± 20) and 85 (± 16) percent in R1 and R2, respectively by day 38, between the

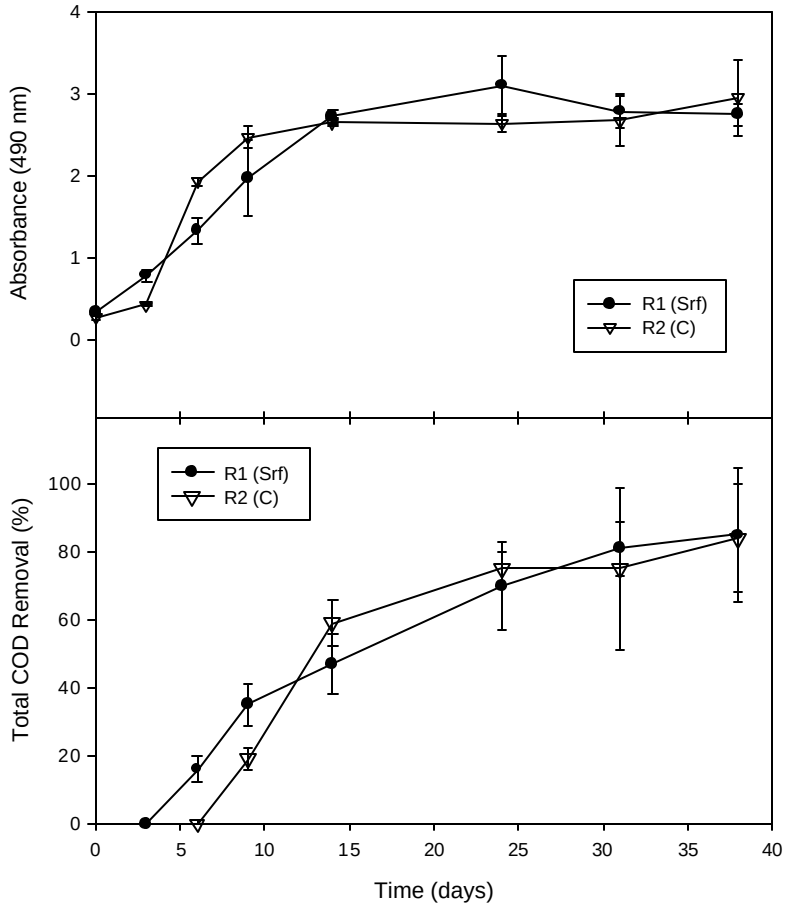

Figure 6. Total Percent COD Removal and Bioactivity Changes: Batch 2, R1-Surfactant Amended, Indigenous10% Waste Oil Mixture, R2-Indigenous 10 % Waste Oil Mixture. Layer 2: Emulsified Oil.

surfactant amended and the control reactor beyond 6 days of operation, and that surfactant addition is not required.

Bioactivity changes during batch 2 coincided with the reduction in COD (See Figure 6). Bioactivity in both reactors steadily increased in the first 14 days, mirroring the reduction in COD. Bioactivity in R1 was slightly higher than R2 at day 3, which may be attributable to the oil being dispersed and thus more accessible to the microorganisms. R2 showed higher activity by day 6, which correlated with significant dispersion of the waste oil. R2 continued to show the same or higher bioactivity than R1 through the remainder of the study. In summary, synthetic surfactant Tween-80 addition appears to have had minimal impact on the bioactivity of the microorganisms and did not enhance biodegradation beyond the first 6 days of operation.

Figures 7 and 8 show selected chromatographs for R1 and R2, respectively. The data show a significant reduction in the peaks appearing from 10 to 30 minutes by day 6, with the majority of the peaks diminished by day 38 for both reactors. The peak reductions for R1were slightly less than those of R2 in the 20 to 22 minute and 40 to 44 minute ranges.

Comparing the chromatographs from day one for both R1 and R2 (See Figures 7 and 8) to the standards (Figure 5) it can be seen that diesel fuel is a significant component of the samples. The diesel fuel steadily degraded in both reactors during the batch study and by day 38 was effectively degraded. The most persistent peaks observed in chromatographs from both reactors occurred from 21 to 22 minutes and 40 to 44 minutes. The peaks between 21 and 22 minutes appear to be related to the diesel fuel while the peaks occurring between 42 and 44 minutes occur in both the compressor and engine oils, but seem to be more closely related to the compressor oil. These later peaks are still present in both chromatographs by day 38, indicating the difficulty the microorganisms had in degrading these higher molecular weight, more complex

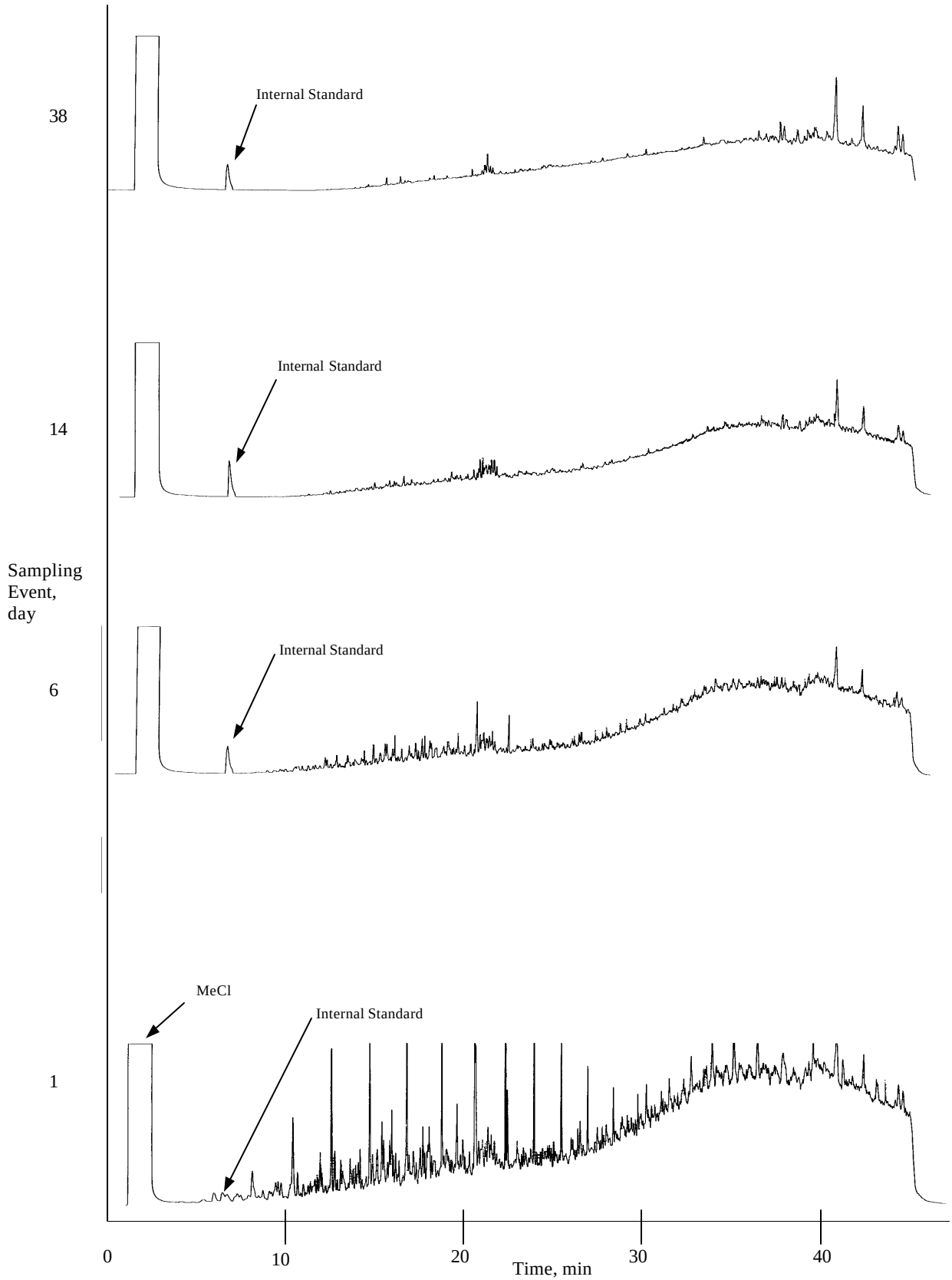

FIG. 7. Selected chromatographs of waste oil from Batch 2, R1 (Layer 2). Dilutions: 1:5 for day 1, 1:2 for days 6 and 14, and 1:1 for day 38.

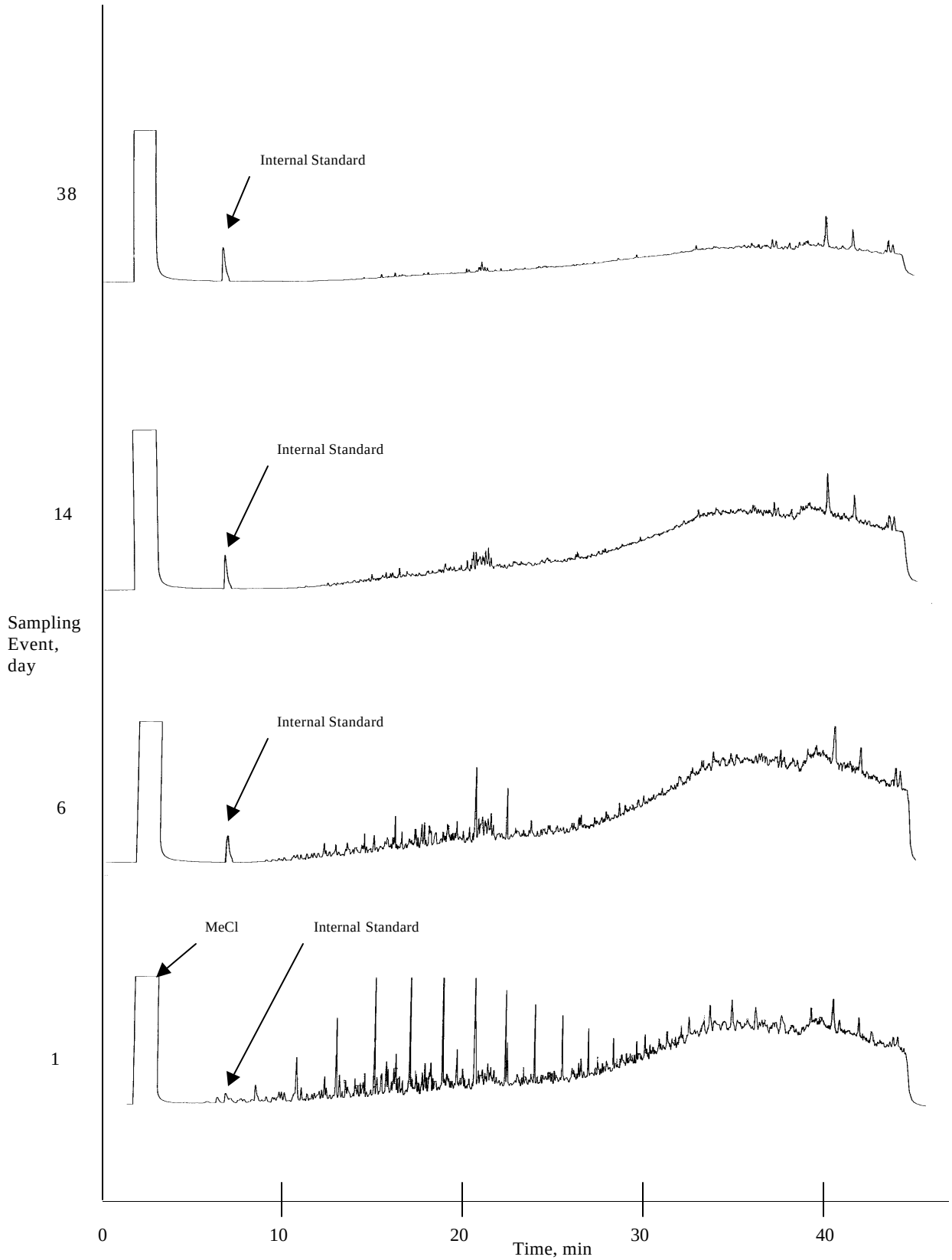

FIG. 8. Selected chromatographs of waste oil from Batch 2, R2 (Layer 2) with dilutions of 1:5, 1:2, 1:2, and 1:1 for days 1, 6, 14, and 38, respectively.

compounds. These data suggest that extensive degradation of the waste oil occurred in both reactors.

The chromatographs show very similar results for both R1 and R2 throughout the batch 2 experiment, with R2 showing slightly better degradation by day 38. These data suggest that there were minimal differences in biodegradation between the surfactant amended and control reactors and that amendment with an exogenous surfactant is not required.

Comparing data from the control reactors in batch 2 with batch 1, the reduction in COD in batch 2 steadily continued until reaching a final reduction of 85 (± 16) percent while batch 1 (38 days) achieved a reduction of only 52 (± 6) percent. There was no cycling of COD reduction in batch 2 as was observed in batch 1 and the bioactivity followed the same trend as the COD reduction for batch 2 while bioactivity in batch 1 increased when COD increased and decreased when COD reduction occurred. Additionally, the chromatographs are very similar for both reactors at the end of each batch study (day 38). There are two possible reasons for the differences between batch 1 and 2. First, lime was added during batch 2 to maintain pH at or near 7.0 while no additional buffering agents were added to batch 1. Near the end of batch 1 (day 38), the pH had dropped to 5.2 while the end of batch 2 the pH was at 5.9. Second, both reactors were inoculated with indigenous microorganisms from R2 of batch 1. The microorganisms in batch 2 were pre-exposed to the emulsified oil during batch 1 (42 days) and between batches (21 days), thus providing a longer time for the microorganisms to adapt to the waste oil. It appears that these two factors account for the higher final degradation, as measured by COD, in batch 2 as compared to batch 1.

C. Batch 3 – Effect of Waste Oil Concentration and Composition

Figure 9 shows the total percent COD removed and bioactivity changes during batch 3 for R1 (10 % mixture of layer 3) and R2 (20 % mixture of layers 2 and 3). Percent reduction was based on initial COD values for R1 and day 6 for R2, as the initial value for R2 was not representative of the actual COD of the waste oil. By day 6, the oil in R2 dispersed and it was possible to obtain a more representative sample, which resulted in a higher COD value than initially measured. The initial COD values were 26,300 (± 610) mg/L and 44,930 (± 13,250) mg/L for R1 and R2, respectively (data not shown). By day 3, the COD for R2 had increased to 53,010 (± 4,250) mg/L and by day 6 had peaked at 63,550 (± 8,460) mg/L respectively (data not shown). This increase in COD in R2 paralleled the observation of oil dispersion, which was proceeded by a decrease in COD with a final reduction of 49 (± 23) percent. R1 showed a steady decrease in COD from the beginning of batch 3, ending with a final reduction of 48 (± 13) percent.

There was a clear delay in COD removal in R2 compared to R1. The emulsified component of layer 3 appeared to be minimal, coinciding with COD reduction in R1 from the start and continuing throughout the experiment. R2 did not appear to show notable COD reduction until day 24. This delay in COD reduction was observed in the two previous experiments; however, the delay was more pronounced in batch 3, which may be attributable to competition between layers 2 (emulsified oil) and 3 (weathered oil). Foam appeared in both reactors, in small to moderate amounts, until day 35. It is interesting to note that by day 28, small, sticky, ball-like clumps of sludge were observed floating on the surface of both reactors.

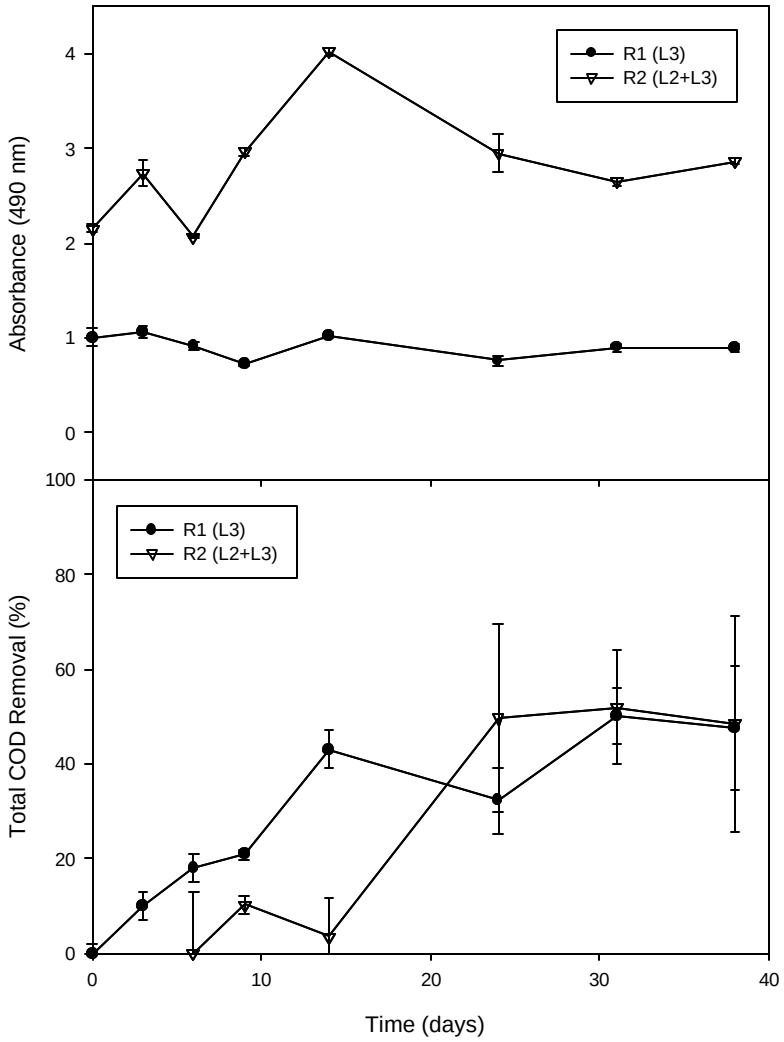

Figure 9. Total Percent COD Removal and Bioactivity Changes: Batch 3, R1-Indigenous 10% Waste Oil Mixture, R2-Indigenous 20% (10% Layer 2,10% Layer3) Waste Oil Mixture. Layer 2: Emulsified Oil, Layer 3: Weathered Oil.

The sludge balls remained until the end of the experiment, though reduced in abundance. This sludge ball formation was not observed in the first two experiments and was attributed to layer 3. The average pH for R1 was 7.4 and ranged from 5.8 to 8.8. The average pH for R2 was 7.6 and

ranged from 6.0 to 8.9. Lime was added on days 24, 31, 35, and 38 to both reactors to adjust pH at or near 7.0.

The bioactivity changes as measured by the FDA test show significant differences between the two reactors (Figure 9). The bioactivity in R1 remained mostly steady around 1 while the bioactivity of R2 varied from 2 to 4 and inversely correlated with the total COD removed (when COD removal was low, bioactivity increased). These trends for R2 are similar to what was observed for R2 in batch 1 and again suggest that with minimal induction of biosurfactants the microorganisms can degrade the oil when the oil is dispersed and in a usable form. When the oil is emulsified (Layer 2 in R2) or consists of complexed hydrocarbon structures, biosurfactants are necessary for hydrocarbon utilization and are represented by an increase in bioactivity.

Figure 10 shows selected chromatographs from R1. The data show minor reduction in peaks from 10 to 25 minutes by day 14 with the majority of the peaks diminished by day 38. The two large peaks occurring at 42 and 44 minutes show minimal reduction throughout the study and are attributable to more complex hydrocarbon structures, which are more difficult to degrade.

Comparing the chromatographs from day one for R1 to the standards (Figure 5) shows that the amount of diesel fuel in the sample is significantly less than that observed in batches 1 and 2, which consisted of waste oil from layer 2. Layer 3 consisted of less emulsified and more weathered waste oil than layer 2. Layer 3 was located beneath layer 2 and therefore did not

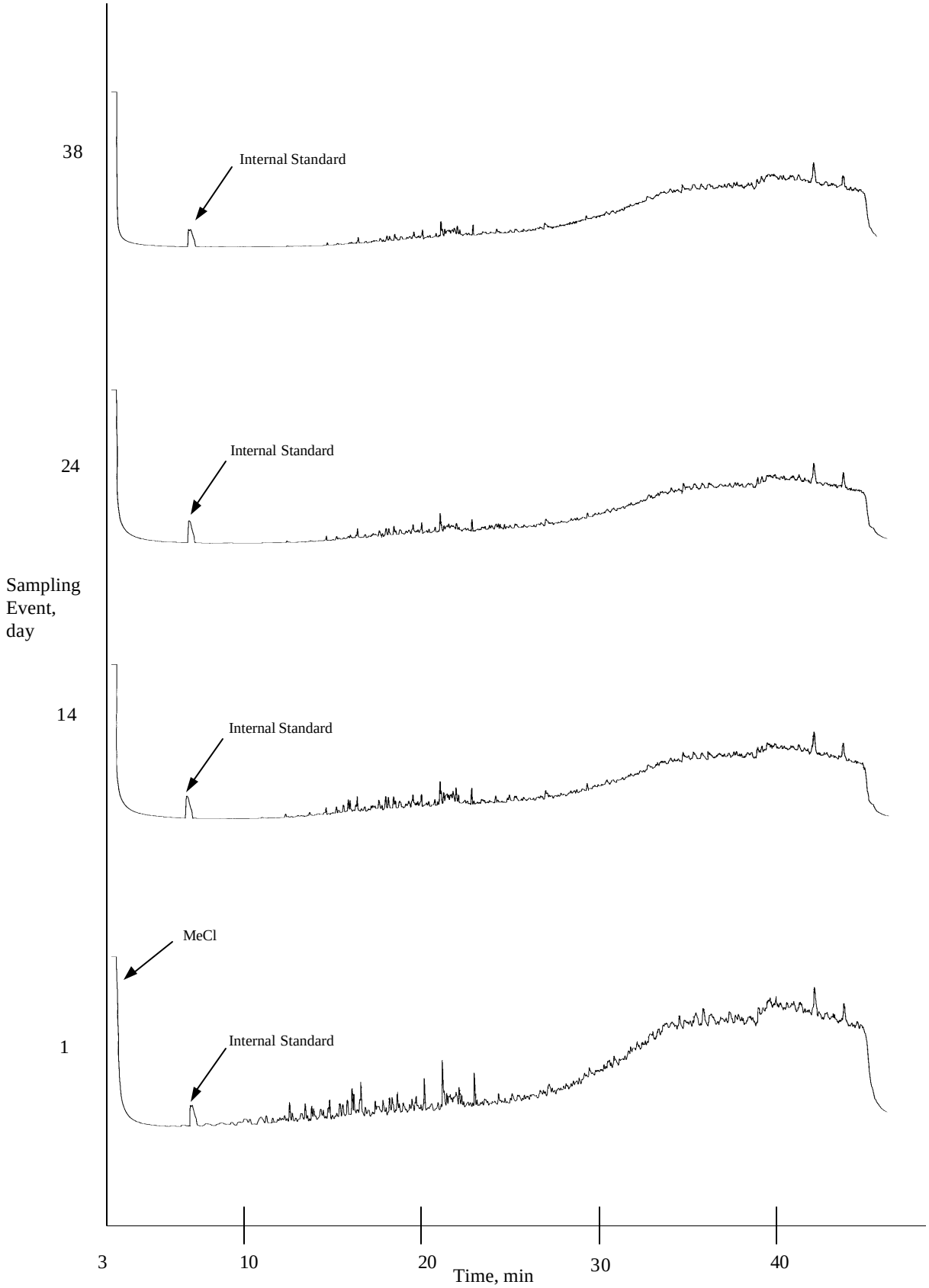

FIG. 10. Selected chromatographs of waste oil from Batch 3, R1 (Layer 3). Dilutions: 1:2 for days 1, 14, and 24 and 1:5 for day 38.

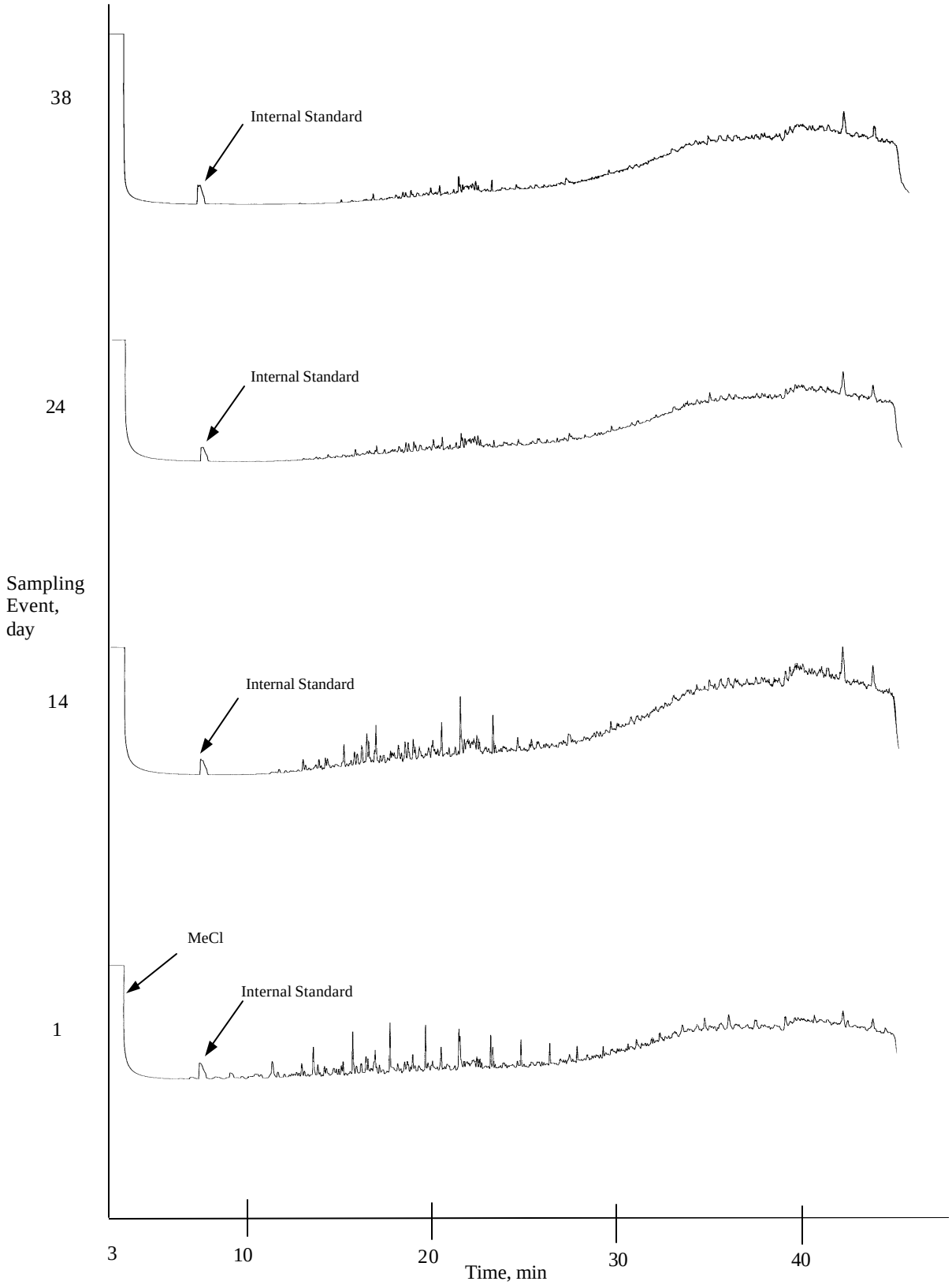

FIG. 11. Selected chromatographs of waste oil from Batch 3, R2 (Layers 2 & 3). Dilutions: 1:2 for days 1, 14, and 24 and 1:1 for day 38.

contain significant amounts of the less dense, lighter fraction diesel fuel that was a principal component of layer 2. The compressor and engine oil components of layer 3 also appear to be less than observed in layer 2 (batch's 1 and 2). Therefore, layer 3 contained hydrocarbons and other compounds that were not represented by the chosen standards and may have been poorly extractable.

Selected chromatographs from R2 are presented in Figure 11. The data show a minor reduction in a few peaks from 12 to 25 minutes by day 14; however, some of the peaks within this range and the peaks at 42 and 44 minutes appear larger by day 14 than they were at day one. This is most likely due to the inability to obtain a representative sample at day one. Chromatographs for day 6 for R2 show a greater abundance of the entire range of peaks, especially in the diesel fuel range (data not shown). This also correlates with the higher COD at day 6 than at days 1 and 3.

The most persistent peaks observed in chromatographs from both reactors occurred from 21 to 23 minutes and 42 to 44 minutes. The peaks between 21 and 23 minutes appear to be related to the diesel fuel while the peaks occurring between 42 and 44 minutes occur in both the compressor and engine oils but seem to be more closely related to the compressor oil. These later peaks were still present in both chromatographs by day 38, indicating the difficulty the microorganisms had in degrading these higher molecular weight, more complex compounds.

The chromatographs show very similar results for both R1 and R2 throughout the study and suggest that degradation of the waste oil occurred in both reactors. Based on the

chromatographs, there appears to be minimal differences in biodegradation between the 10 percent waste oil mixture of Layer 3 and the 20 percent waste oil mixture from layers 2 and 3 by the end of the study. This suggests that a 20 percent waste oil mixture can be degraded to the same extent as a 10 percent mixture in 38 days.

The COD data for batch 3 did not show the steady COD reduction as observed in batch 2, even though the reactors were inoculated with indigenous microorganisms from the control reactor in batch 2. By the time batch 3 started, the indigenous microorganisms had been exposed to layer 2 for two batch studies (42 and 38 days, respectively). Additionally, the indigenous microorganisms were sustained between studies on a portion of the contents of layer 2 from each control reactor, for a total exposure time of 146 days. This longer exposure time to layer 2 would likely result in a more efficient and complete oxidation of the waste oil than what was observed in the previous two batch studies. However, the overall decrease in COD for R2 (49 ± 23 percent) did not match the final level of reduction observed in R2 for batch 2 (85 ± 16 percent) despite the longer exposure time. The difference in the batch 3 study was the presence of layer 3. The microorganisms had not been previously exposed to this layer, which is the likely reason why the final percent removal of COD was less than what was measured in batch 2. Comparing the chromatographs and COD data from R1 (layer 3) to R2 (layers 2 and 3) and to similar data from batch's 1 and 2, it appears that the layer 3 COD fraction is more reflective of the non-extractable component than in the other batch's.

D. Batch 3 – Dewaterability Studies

Dewatering studies were conducted during the batch 3 experiment. Dewatering characteristics were poor for R2 without conditioning and showed minimal improvement for R1 (data for R1 conditioned not shown), as characterized by CST and time to filter test. The emulsified waste oil combined with the weathered oil (R2) proved to be the most difficult to dewater. At the start of batch 3 (through day 6), R2 readily dewatered; however, as the oil became dispersed the CST increased significantly for the unconditioned sludge (Figure 12). The poor dewaterability of the unconditioned sludge in R2 correlated with the increase in bioactivity after day 6 and the dispersion of oil, suggesting that biosurfactants were being produced and contributing to the difficulty in dewatering. Conditioning with alum or ferric chloride substantially improved dewatering of the waste oil. The most effective dose of coagulant proved to be 0.10 (lbs. of coagulant to lbs. of dry sludge) at each point of the study for both reactors with the exception of day 0 (data for R1conditioned sludge not shown) and day 38. The initial sampling at the start for both waste oils showed the unconditioned waste oil more readily dewatered than the conditioned waste oil. Both alum and ferric chloride were effective conditioners after day 0, as measured by CST, for the sludge from R2; however, they were of limited benefit for R1 (data not shown). It is interesting to note the drop in the CST of the unconditioned sludge for R2 from day 31 to 38. These data indicate continued operation of the reactor beyond 38 days may result in more readily dewaterable sludge.

The relationship between conditioned and unconditioned sludge was also clearly seen with the time to filter (TTF) test using 50 mL of sludge (Figure 13). The unconditioned waste oil

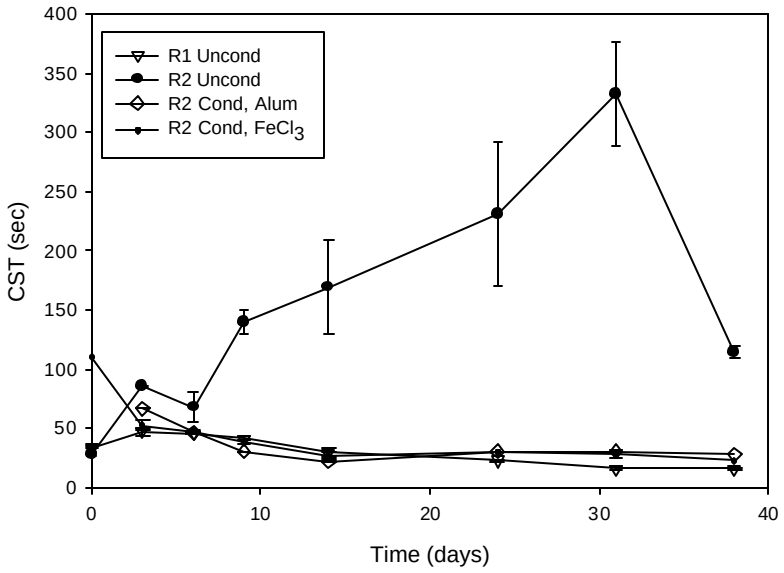

Figure 12. Capillary Suction Time Test: Batch 3, R1-10%, R2-20% Waste Oil Mixtures, Indigenous Inoculated. Layer 2: Emulsified Oil, Layer 3: Weathered Oil. Conditioned with coagulant at 0.10 lb/lb, except for ferric chloride on day 38, which was 0.125 lb/lb. Data points without error bars indicate single measurements.

in R2 (L2+L3) showed poor filterability, taking up to 30 minutes to filter 50 mL. Once more there was a correlation between an increase in bioactivity and a decrease in dewaterability after day 6 for the waste oil in R2 (TTF of 30 min. by day 14), suggesting that biosurfactants may have played a role in the poor dewaterability.

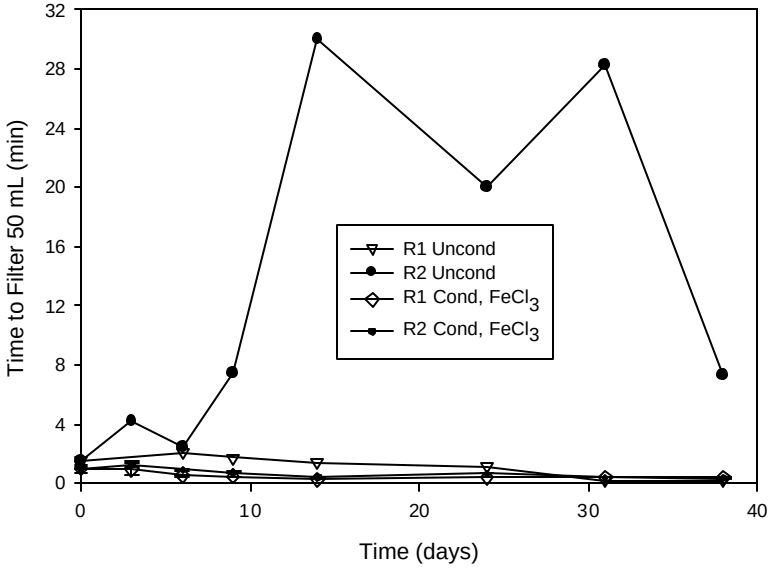

Fig. 13. Time to Filter 50 mL of Sludge: Batch 3, R1-10%, R2-20% Waste Oil Mixtures, Indigenous Inoculated. Layer 2: Emulsified Oil, Layer 3: Weathered Oil. Conditioned with coagulant at 0.10 lb/lb except day 38, which was 0.125 lb/lb. Data points without error bars indicate single measurements.

The unconditioned waste oil in R1 (L3) filtered in 2 min. or less with minimal improvement by conditioning. The dewaterability of the waste oil in R2 dramatically improved with conditioning (up to 98 % reduction in TTF for 50 mL). Again, a substantial improvement in dewaterability

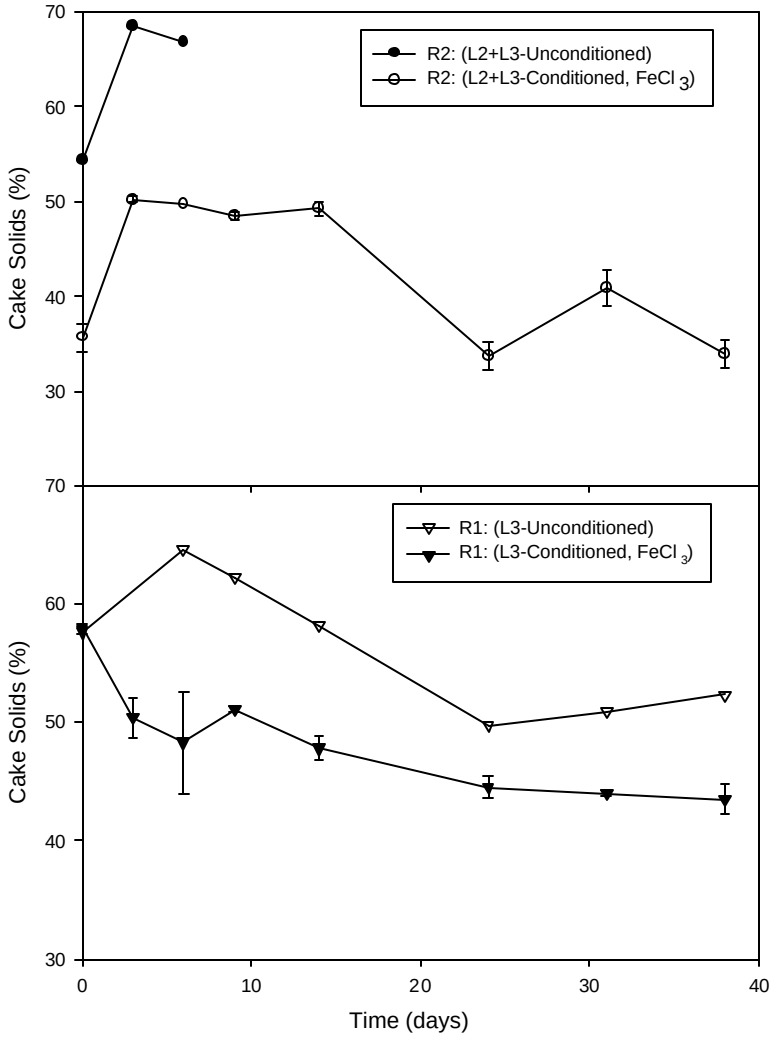

Figure 14. Percent Cake Solids of Sludge: Batch 3, R1-10%, R2-20% Waste Oil Mixtures, Indigenous Inoculated. Layer 2: Emulsified Oil, Layer 3: Weathered Oil. Conditioned with coagulant at 0.10 lb/lb except day 38, which was 0.125 lb/lb. Data points without error bars indicate single measurements.

was observed from day 31 to 38 for the unconditioned waste oil in R2, providing a second independent test that yielded similar results for the same time frame, suggesting that extended operational time might improve dewatering characteristics.

Percent cake solids for conditioned sludge throughout batch 3 was 44 (± 0.3) to 50 (± 1.7) percent for R1 and 34 (± 0.3) to 50 (± 1.8) percent for R2 (Figure 14). Percent cake solids for unconditioned sludge throughout batch 3 was 50 to 65 percent for R1 and 54 to 68 percent for R2 (Figure 14). The values for R2 represent 3 out of 8 sampling events due to the inability to dewater the samples for sampling days 14, 24, 31 and 38.

The higher percent cake solids for the unconditioned sludge from R2 was countered by the very high TTF and the inability to dewater the sludge during the last 5 sampling events. The filter appeared to be blinded by the sludge shortly after applying a vacuum, which prevented the bulk of the water in the sample from being filtered. Once again, there was a correlation between the poor dewaterability and the increase in bioactivity after day 6, suggesting biosurfactants may have contributed to the poor dewaterability of the unconditioned sludge. These data suggest conditioning of the sludge from R2 improves the dewatering properties considerably while lowering the percent cake solids minimally.

The data for R1 sludge conditioning revealed that it lowered the percent cake solids by as much as 32 percent while improving TTF and CST (data not shown) minimally indicating that conditioning did not substantially improve dewatering properties.

5. CONCLUSIONS

Biodegradation and the role of bioaugmentation and surfactants in promoting biodegradation, the effect of oil composition on biodegradation, and the dewatering characteristics of biodegraded oil were studied. Biodegradation, and the role of a synthetic

surfactant in promoting biodegradation, was measured using COD, FDA, and GC analysis of extractable material. Dewatering of biodegraded oil was measured using CST, TTF, and percent cake solids. Based on the results obtained from this study, the following conclusions can be made:

1. The addition of non-native microorganisms did not facilitate significant degradation of the waste oil.
2. The presence of the synthetic surfactant Tween-80 dispersed the waste oil initially, but did not enhance biodegradation relative to a nonamended control beyond the first six days of reactor operation.
3. The longer exposure time for the indigenous microorganisms (63 days from batch 1 to batch 2) to layer 2, combined with lime addition for pH adjustment, resulted in a greater final COD reduction of the waste oil for the control reactor in batch 2 (85 ± 16 percent) as compared to batch 1 (52 ± 6 percent).
4. The extent of waste oil biodegradation was not dependent on the initial concentration or composition of the waste oil; however, the rate of degradation was affected by the contents and concentration of waste oil.
5. The unconditioned, post-biodegraded combined waste oil mixture of layers 2 and 3 exhibited poor dewatering properties. Conditioning with either ferric chloride or alum improved dewatering characteristics as measured by CST. Conditioning with ferric chloride improved dewatering characteristics as measured by the TTF test while minimally reducing percent cake solids.
6. The unconditioned, post-biodegraded waste oil dewatering properties of layer 3 showed limited improvement by conditioning with either ferric chloride or alum, as

measured by CST. Conditioning with ferric chloride did not substantially improve dewatering characteristics as measured by the TTF test and reduced percent cake solids by as much as 32 percent.

6. ENGINEERING SIGNIFICANCE AND RECOMMENDATIONS

Treatment of the waste oil could be accomplished by biological oxidation via an aerobic, completely mixed batch operation. The reactor vessel would be sized based on estimated quantities of waste oil generated, accounting for existing storage capacity. The waste oil would need to be diluted to at least a 20 percent mixture and appropriate nutrient amendment and oxygen would be required. Either diffused air or mechanical means would accomplish sufficient mixing. Mechanical mixing was not utilized in this study; however, it may provide more complete mixing than diffused air, and therefore be a more cost-effective treatment. Some form of pH adjustment (e.g., lime or NaOH) would be required, combined with continuous pH monitoring for proper treatment control.

Bioaugmentation or the addition of a synthetic surfactant would not be required; however, depending on desired treatment time, either or both may be necessary to minimize treatment time and thus, cost. These additional operational expenditures would need to be balanced with the initial cost of proprietary bioaugmentation inoculations and synthetic surfactants. Additionally, careful selection of either exogenous microorganisms or synthetic surfactants would be necessary for effective treatment. Bioaugmentation would only be recommended with the initial batch treatment. Each succeeding batch treatment would be

inoculated with indigenous microorganisms from the previous batch, thus eliminating the need for exogenous bioaugmentation.

It is assumed that layer 1 (free oil, mostly diesel fuel and lightweight components of lubrication oils) would continue to be removed via commercial contractor due to cost effectiveness. A completely mixed regime of combined waste oil (layers 2-emulsified oil, 3-weathered oil, and 4-wastewater containing dissolved petroleum products) would be treated in batches, for a specified period of time, dependent on periodic reactor sampling for dewaterability and ultimate disposal. The goal would be to minimize treatment time while maximizing volume reduction. The dewatered waste could be handled as either a solid waste or a special waste, depending on treatment goals and disposal regulations and costs.

Dewatering costs (operational and disposal) could dictate whether or not biological treatment is fundamentally viable. As seen in this study, chemical conditioning was required for effective dewatering of post-biodegraded waste oil. Additional chemicals/polymers could be applied that may reduce operational costs, therefore presenting a more cost-effective means of sludge handling and disposal.

A waste oil survey was performed in the fall of 1996 that revealed approximately 86,000 gallons of waste oil mixture, from 24 different sources, was disposed of in the outdoor waste oil concrete tank on a monthly basis. A new waste oil survey would need to be conducted to identify current sources, quantities, and make up of waste oil and petroleum impacted water being collected. Certain high volume waste oil sources have since been removed, and a new treatment plant has been added, since the initial waste oil survey was conducted. Additionally,

current disposal methods would need to be balanced against upfront design and capital investment in treatment processes along with long-term operational and maintenance costs such as labor and consumables (i.e., chemicals and utilities). Additional consideration must be given to costs associated with initial and periodic operator training, necessary for effective treatment control.

A strong emphasis on waste reduction at the source would prove to be the most cost-effective measure the industry could implement at this facility. It is typically cheaper to manage a small amount of waste oil at the point of origin than to attempt to treat large volumes of combined waste products at a convenient, end of line, collection point (i.e., outdoor, catch all, concrete tank). Establishing an effective pollution prevention plan at the shop level would most likely prove to be a much more cost-effective measure than the expensive capital and operational and maintenance costs associated with end of pipe treatment.

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APPENDIX A

TABLE A1: COD Concentration and Percent Reduction Values for Batch 1

COD Batch 1

Date	Day	COD Avg. (mg/L) R1 (Aug)	COD Avg. (mg/L) R2 (C)	s_p (mg/L) R1 (Aug)	s_p (mg/L) R2 (C)
1/16/98	1	28322	32144	980	1143
1/19/98	3	46534	41552	1060	1081
1/22/98	6	33864	34068	645	2660
1/26/98	10	23400	20696	1040	2172
1/31/98	15	20972	24206	2772	196
2/9/98	24	32693	31282	565	351
2/16/98	31	26186	32066	1614	784
2/23/98	38	22658	19835	2792	1225
2/27/98	42	19123	25958	1717	869

COD Batch 1 Percent Reduction

Date	Day	COD Avg. (mg/L) R1 (Aug)	COD Avg. (mg/L) R2 (C)	s_p (mg/L) R1 (Aug)	s_p (mg/L) R2 (C)
1/16/98	1				
1/19/98	3	0.00	0.00	0.02	0.03
1/22/98	6	0.27	0.18	0.02	0.08
1/26/98	10	0.50	0.50	0.04	0.10
1/31/98	15	0.55	0.42	0.13	0.01
2/9/98	24	0.30	0.25	0.02	0.01
2/16/98	31	0.44	0.23	0.06	0.02
2/23/98	38	0.51	0.52	0.12	0.06
2/27/98	42	0.59	0.38	0.09	0.03

TABLE A2: COD Concentration and Percent Reduction Values for Batch 2

COD Batch 2

Date	Day	COD Avg. (mg/L) R1 (Srf)	COD Avg. (mg/L) R2 (C)	s_p (mg/L) R1 (Srf)	s_p (mg/L) R2 (C)
3/20/98	1	29200	23000	1414	566
3/23/98	3	50666	23226	188	707
3/26/98	6	42770	54144	1733	376
3/29/98	9	32775	43605	2055	1330
4/3/98	14	26656	22246	2368	1580
4/13/98	24	15206	13440	2003	633
4/20/98	31	9600	13594	768	3240
4/27/98	38	7757	8832	1513	1382

COD Batch 2 Percent Reduction

Date	Day	COD Avg. (mg/L) R1 (Srf)	COD Avg. (mg/L) R2 (C)	s_p (mg/L) R1 (Srf)	s_p (mg/L) R2 (C)
3/20/98	1				
3/23/98	3	0.00	0.57	0.00	0.03
3/26/98	6	0.16	0.00	0.04	0.01
3/29/98	9	0.35	0.19	0.06	0.03
4/3/98	14	0.47	0.59	0.09	0.07
4/13/98	24	0.70	0.75	0.13	0.05
4/20/98	31	0.81	0.75	0.08	0.24
4/27/98	38	0.85	0.84	0.20	0.16

TABLE A3: COD Concentration and Percent Reduction Values for Batch 3

COD Batch 3

Date	Day	COD Avg. (mg/L) R1 (L3)	COD Avg. (mg/L) R2 (L2/L3)	s_p (mg/L) R1 (L3)	s_p (mg/L) R2 (L2/L3)
6/11/98	1	26304	44932	607	13246
6/14/98	3	23655	53010	685	4249
6/17/98	6	21534	63552	613	8457
6/20/98	9	20813	57024	154	1152
6/25/98	14	14976	61248	633	4624
7/5/98	24	17797	31948	1264	6284
7/12/98	31	13133	30528	827	3623
7/19/98	38	13762	32712	1836	7406

COD Batch 3 Percent Reduction

Date	Day	COD Avg. (mg/L) R1 (L3)	COD Avg. (mg/L) R2 (L2/L3)	s_p (mg/L) R1 (L3)	s_p (mg/L) R2 (L2/L3)
6/11/98	1	0.00		0.02	
6/14/98	3	0.10		0.03	
6/17/98	6	0.18	0.00	0.03	0.13
6/20/98	9	0.21	0.10	0.01	0.02
6/25/98	14	0.43	0.04	0.04	0.08
7/5/98	24	0.32	0.50	0.07	0.20
7/12/98	31	0.50	0.52	0.06	0.12
7/19/98	38	0.48	0.49	0.13	0.23

TABLE A3.1: COD Spike Recoveries for Batch 3

Pooled Standard Deviation:

$s_p = [(n_1-1)s_1^2 + (n_2-1)s_2^2/(n_1+n_2-2)]^{1/2}$

s_1 = std. dev. of data set 1 (duplicates from R1A)

s_2 = std. dev. of data set 2 (duplicates from R1B)

n = size of sample

COD Spike Recoveries	COD avg. (mg/L)	Std. Dev. (mg/L)	No. of Samples
Spike R1	102	16	14
Spike R2	99	14	16
Overall	101	15	30
Std.	102	5	26

TABLE A4: FDA Absorbance Values (Bioactivity) at 490 nm for Batch 1

Batch 1		Avg. A_{490} of	Avg. A_{490} of	s_p A_{490}	s_p A_{490}
Date	Day	R1 (Aug)	R2 (C)	R1 (Aug)	R2 (C)
1/16/98	0	0.2893	0.2013	0.094	0.007
1/19/98	3	1.561	1.341	0.082	0.083
1/22/98	6	1.242	1.258	0.063	0.057
1/26/98	9	1.838	1.758	0.096	0.261
1/31/98	15	1.396	1.523	0.215	0.164
2/9/98	24	2.647	2.804	0.182	0.264
2/16/98	31	2.609	2.222	0.083	0.088
2/23/98	38	1.553	1.552	0.359	0.126
2/27/98	42	2.213	2.085	0.136	0.122

TABLE A5: FDA Absorbance Values (Bioactivity) at 490 nm for Batch 2

Batch 2		Avg. A_{490} of	Avg. A_{490} of	s_p A_{490}	s_p A_{490}
Date	Day	R1 (Srf)	R2 (C)	R1 (Srf)	R2 (C)
3/20/98	0	0.3379	0.2712	0.028	0.028
3/23/98	3	0.789	0.429	0.074	0.019
3/26/98	6	1.338	1.934	0.158	0.045
3/29/98	9	1.974	2.469	0.462	0.135
4/3/98	14	2.728	2.653	0.086	0.034
4/13/98	24	3.105	2.638	0.351	0.094
4/20/98	31	2.789	2.675	0.197	0.312
4/27/98	38	2.747	2.948	0.129	0.464

TABLE A6: FDA Absorbance Values (Bioactivity) at 490 nm for Batch 3

Batch 3		Avg. A_{490} of	Avg. A_{490} of	s_p A_{490}	s_p A_{490}
Date	Day	R1 (L3)	R2 (L2/L3)	R1 (L3)	R2 (L2/L3)
6/11/98	0	1.003	2.155	0.090	0.037
6/14/98	3	1.055	2.739	0.067	0.134
6/17/98	6	0.913	2.068	0.039	0.016
6/20/98	9	0.722	2.961	0.017	0.038
6/25/98	14	1.022	4.022	0.031	0.046
7/5/98	24	0.756	2.944	0.061	0.200
7/12/98	31	0.901	2.645	0.042	0.041
7/19/98	38	0.897	2.864	0.036	0.029

Pooled Standard Deviation:

$s_p = [(n_1-1)s_1^2 + (n_2-1)s_2^2/(n_1+n_2-2)]^{1/2}$

s_1 = std. dev. of data set 1 (duplicates from R1A or R2A)

s_2 = std. dev. of data set 2 (duplicates from R1B or R2B)

n = size of sample

TABLE A7: Apparent Surface Tension Values for Layer 2 (Emulsified Oil)

Apparent Surface Tension				Graph	
Waste Oil Layer 2 (%)	Surfactant Tween 80 (g)	Apparent Surface Tension (dynes/cm)	Avg. Apparent Surface Tension (dynes/cm)	Surfactant (g)	Avg. AST (dynes/cm)
10	0	35.9	35.8	0	35.8
		35.9			
		35.7			
10	0.001	33.1	33.0	0.001	33.0
		33.1			
		32.9			
10	0.003	31.7	31.6	0.003	31.6
		31.7			
		31.4			
10	0.005	30.8	30.8	0.005	30.8
		30.8			
		30.7			
10	0.007	31.6	31.6	0.007	31.6
		31.6			
		31.7			
10	0.010	31.1	31.1	0.01	31.1
		31.1			
		31.1			
0 (DI Water)	0	76.6	75.6	0	75.3
		75.8			
		75.4			
		75.3			
		75.1			

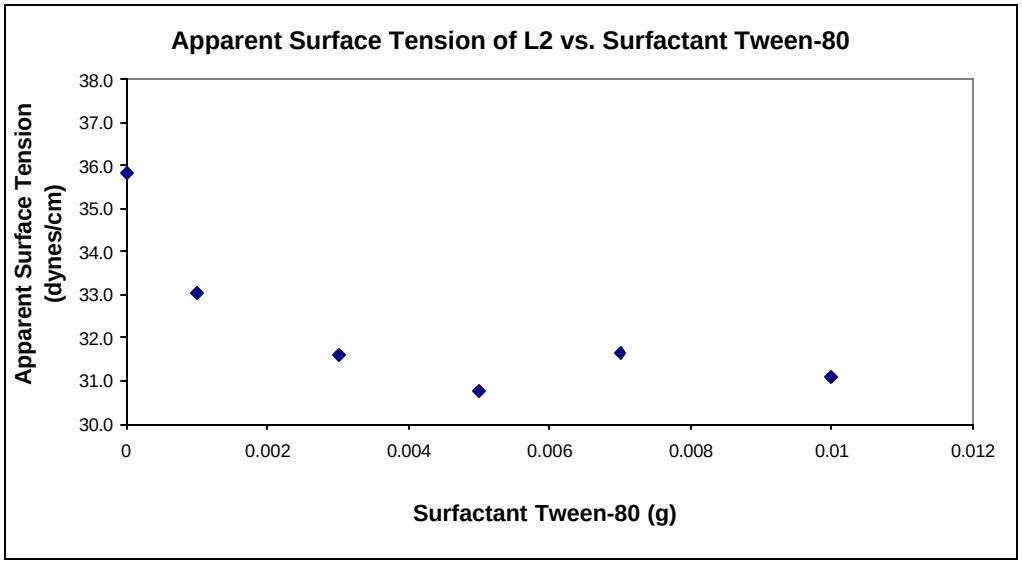

Figure A1: Apparent Surface Tension of Layer 2 Using Surfactant Tween-80

TABLE A8: Capillary Suction Time Test for R1: Raw and Conditioned Sludge

Batch 3 Day	Avg. CST of Raw Sludge (sec) R1:Raw	Std. Dev. CST Raw Sludge (sec) R1:Raw	Avg. CST of Cond. Sludge Alum (sec) R1:Alum	Std. Dev. CST Cond. Sludge Alum (sec) R1:Alum	Avg. CST of Cond. Sludge $FeCl_3$ (sec) R1:$FeCl_3$	Std. Dev. CST Cond. Sludge $FeCl_3$ (sec) R1:$FeCl_3$
1	34.1	0.8	47.0	2.4	38.3	
3	46.8	3.7	39.2	1.4	29.7	0.5
6	45.0	0.6	33.5	0.6	26.5	2.0
9	41.3	1.6	23.7	0.3	23.5	1.1
14	29.3	3.2	18.9	0.8	18.9	0.6
24	22.9	0.7	20.5		26.7	
31	15.9	1.6	18.1	0.4	26.9	1.1
38	15.6	1.1	17.7		24.7	

TABLE A9: Capillary Suction Time Test for R2: Raw and Conditioned Sludge

Batch 3 Day	Avg. CST of Raw Sludge (sec) R2:Raw	Std. Dev. CST Raw Sludge (sec) R2:Raw	Avg. CST of Cond. Sludge Alum (sec) R2:Alum	Std. Dev. CST Cond. Sludge Alum (sec) R2:Alum	Avg. CST of Cond. Sludge FeCl3 (sec) R2:$FeCl_3$	Std. Dev. CST Cond. Sludge FeCl3 (sec) R2:$FeCl_3$
1	28.1	1.7			110.1	
3	85.1	1.2	66.8	0.1	52.6	4.7
6	67.8	12.2	46.1	0.4	46.9	0.6
9	139.9	10.7	30.2	0.5	38.5	1.3
14	169.3	40.0	22.3	1.2	27.3	2.2
24	230.9	61.4	30.6		29.9	
31	332.4	43.5	30.2	0.9	27.8	3.4
38	114.1	5.1	27.5		27.9	

TABLE A10: Optimum Dose of Coagulant: Based on CST Test

Optimum Dose

Batch 3 Day	Sample	Alum Dose lb/lb	CST (sec)	$FeCl_3$ Dose lb/lb	CST (sec)
1	R1A-to (L3)	0.10	47.3	0.10	38.3
	R2A-to (L2/L3)			0.10	110.1
24	R1A (L3)	0.075	20.2	0.10	26.7
	R1A (L3)	0.10	20.5	0.125	25.4
	R2A (L2/L3)	0.075	29.9	0.10	29.9
	R2A (L2/L3)	0.10	30.6		
38	R1A (L3)	0.075	15.8	0.10	24.7
	R1A (L3)	0.10	17.7		
	R2A (L2/L3)	0.075	21.8	0.10	27.9
	R2A (L2/L3)	0.10	27.5	0.125	22.6

TABLE A11: Time to Filter Conditioned Sludge

Time To Filter Conditioned Sludge ($FeCl_3$)

Batch 3 Day	Average Time to 50 mL (min)	Average Time to 50 mL (min)	Std. Dev. Time to 50 mL (min)	Std. Dev. Time to 50 mL (min)
Day	R1 (L3)	R2 (L2/L3)	R1 (L3)	R2 (L2/L3)
1	1.03	0.95	0.07	0.24
3	0.93	1.29	0.42	0.23
6	0.53	0.90	0.04	0.04
9	0.45	0.71	0.04	0.06
14	0.23	0.40	0.00	0.03
24	0.41	0.66	0.01	0.01
31	0.39	0.38	0.01	0.01
38	0.41	0.34	0.06	0.05

TABLE A12: Time to Filter Unconditioned Sludge

Time To Filter Unconditioned Sludge ($FeCl_3$)

Batch 3	Average Time to 50 mL (min)	Average Time to 50 mL (min)
Day	R1 (L3)	R2 (L2/L3)
0	1.53	1.52
3		4.17
6	2.03	2.42
9	1.73	7.50
14	1.42	30.00
24	1.13	20.00
31	0.18	28.25
38	0.20	7.33

0.10 lb/lb = mL $FeCl_3$ x 100 g/L $FeCl_3$/(100 mL sample x % solids (converted to g/L))

TABLE A13: Percent Cake Solids of Conditioned Sludge

Percent Cake Solids for Conditioned ($FeCl_3$) Sludge

Batch 3 Day	Average Cake Solids (%)	Average Cake Solids (%)	Std. Dev. Cake Solids (%)	Std. Dev. Cake Solids (%)
Day	R1 (L3)	R2 (L2/L3)	R1 (L3)	R2 (L2/L3)
1	57.9	35.7	0.4	1.5
3	50.4	50.2	1.7	0.3
6	48.3	49.7	4.3	0.0
9	51.1	48.5	0.3	0.5
14	47.8	49.3	1.0	0.7
24	44.5	33.8	0.9	1.5
31	44.0	40.9	0.3	1.8
38	43.5	33.9	1.3	1.5

TABLE A14: Percent Cake Solids of Unconditioned Sludge

Percent Cake Solids for Unconditioned Sludge

Batch 3	Cake Solids, %	Cake Solids, %
Day	R1 (L3)	R2 (L2/L3)
0	57.6	54.4
3		
6	64.6	68.5
9	62.2	66.8
14	58.2	33.3
24	49.7	
31	50.9	
38	52.3	43.5

0.10 lb/lb = mL $FeCl_3$ x 100 g/L $FeCl_3$/(100 mL sample x % solids (converted to g/L))

APPENDIX B

TABLE B1: Volatile Solids: Batch 3

Batch 3	Volatile Solids, %	Volatile Solids, %	Volatile Fraction, %	Volatile Fraction, %
Day	R1 (L3)	R2 (L2/L3)	R1 (L3)	R2 (L2/L3)
0	36.1	37.2	62.8	68.3
3				
6	32.5	49.9	50.2	72.8
9	32.6	48.9	52.4	73.1
14	29.9	25.6	51.5	76.8
24	31.1		62.7	
31	29.2		57.3	
38	31.2	31.7	59.7	72.9

TABLE B2: Density of Waste Oil

Sample	Density (g/mL)
L2-Source Drum	0.98
L3-Source Drum	0.99
L3-Goop*-Source Drum	1.0
B2-R2-d0-unconditioned	0.91
B3-R1-d0-unconditioned	1.1
B3-R2-d0-unconditioned	1.1
B3-R1-d31-unconditioned	1.0
B3-R2-d31-conditioned	1.1
B3-R1-d38-unconditioned	1.1
B3-R1-d38-conditioned	1.1
B3-R2-d38-unconditioned	1.0
B3-R2-d38-conditioned	0.98

*Goop: Heavy foam on top of waste oil

TABLE B3.1: CST for Batch 2

Sample	**Batch 2** Day	**R1** Avg. CST (sec) R1 (Srf)	**R2** Avg. CST (sec) R2 (C)	**R1** CST Std. Dev. (sec) R1 (Srf)	**R2** CST Std. Dev. (sec) R2 (C)
t0	0	39.9	24.6	4.8	1.2
t2hr	0.2	41.5	37.2	0.9	2.3
d3	3	151.2	59.1	7.1	6.1
d6	6	305.4	432.7	23.5	60.2
d9	9	329.7	511.3	20.0	54.5
d14	14	276.7		22.6	
d24	24	336.4	799.9	69.3	168.2
d31	31	345.1	593.6	13.5	84.0
d38	38	252.6	419.7	2.8	73.4

TABLE B3: CST for Batch 1

Sample	**Batch 1** Day	**R1** Avg. CST (sec) R1 (Aug)	**R2** Avg. CST (sec) R2 (C)	**R1** CST Std. Dev. (sec) R1 (Aug)	**R2** CST Std. Dev. (sec) R2 (C)
t0	0	39.9	24.6	4.8	1.2
d15	15	382.4	529.0	3.0	4.5
d33	33	498.0	524.7		7.9
d36	36	729.6	412.9	168.5	77.0
d38	38	779.8	547.8	184.5	7.9
d42-mix	42	448.4	295	119.1	35.1
d42-op	42	266.9	85.1	72.0	26.4
d42-wp	42	17.7	15.2	0.4	0.1
d42-sp	42	1839.0	1081.9	342.6	87.4

TABLE B4: Percent Solids and Time to Filter for Batch 1, Day 42 and Batch 2

Sample (100 mL)	Day	Solids (%)	Alum added (mL)	Time to 50 mL (min)	Time to filter (min)	Vol. of filtrate (mL)	FeCl3 Added (mL)	Dosage lb/lb
B1-R1-d42	42	4.73	9.70	3.50	*	12	-	0.10
B2-R1-d0	0	4.61	4.73	0.88	5.7	98	-	0.05
B2-R1-d3	3	3.38	6.93	3.37	18.0	99	-	0.10
B2-R1-d6	6	3.86	7.91	16.0	*	-	-	0.10
B2-R1-d9	9	3.74	7.66	10.0	*	36	-	0.10
B2-R1-d14	14	3.33	6.82	6.00	*	35	-	0.10
B2-R1-d24	24	2.61	5.35	6.00	*	37	-	0.10
B2-R1-d31	31	2.13	4.36	1.37	6.0	98	-	0.10
B2-R1-d38	38	1.55	1.60	2.30	10.0	93	-	0.05
B2-R1-d38	38	1.55	**	1.00	6.0	93	0.78	0.10
B1-R2-d42	42	5.35	10.96	1.97	*	12	-	0.10
B2-R2-d0	0	3.79	3.89	0.95	6.0	97	-	0.05
B2-R2-d3	3	3.25	6.66	0.72	6.0	98.5	-	0.10
B2-R2-d6	6	3.73	7.64	4.90	25.5	94	-	0.10
B2-R2-d9	9	3.83	7.85	2.63	14.0	98	-	0.10
B2-R2-d24	24	2.64	5.41	6.00	*	33	-	0.10
B2-R2-d31	31	2.20	4.51	2.00	10.0	98	-	0.10
B2-R2-d38	38	1.54	1.60	1.20	6.0	91	-	0.05
B2-R2-d38	38	1.54	**	1.28	6.0	92.5	0.78	0.10

Notes:

*Sample took too long to filter 50 mL; stopped after noted time to filter 50 mL.

**Used $FeCl_3$ as a coagulent

Sludge was conditioned with a dosage of 0.10 lb/lb of Alum or $FeCl_3$.

Cake solids were collected from a 5 cm x 5 cm square on top of filter.

TABLE B5: Percent Solids Batch 3

Sample R1 (L3)	Avg. Solids, %	Std. Dev. Solids, %	Sample R2 (L2+L3)	Avg. Solids, %	Std. Dev. Solids, %
B3-R1-d0	2.11	0.13	B3-R2-d0	10.2	1.28
B3-R1-d3	1.97	0.02	B3-R2-d3	4.79	0.15
B3-R1-d6	1.72	0.02	B3-R2-d6	4.69	0.02
B3-R1-d9	1.76	0.08	B3-R2-d9	4.19	0.03
B3-R1-d14	1.65	0.01	B3-R2-d14	4.37	0.13
B3-R1-d24	1.53	0.03	B3-R2-d24	3.86	0.05
B3-R1-d31	1.36	0.01	B3-R2-d31	3.33	0.01
B3-R1-d38	1.51	0.12	B3-R2-d38	3.25	0.20

TABLE B6: Total Suspended Solids and Volatile Suspended Solids for Batch 1

Batch 1		TSS Avg. (g/L)	TSS Avg. (g/L)	Std. Dev. (g/L)	Std. Dev. (g/L)	VSS Avg. (g/L)	VSS Avg. (g/L)	Std. Dev. (g/L)	Std. Dev. (g/L)
Date	Day	R1 (Aug)	R2 (C)	R1 (Aug)	R2 (C)	R1 (Aug)	R2 (C)	R1 (Aug)	R2 (C)
1/16/98	0	15.1	27.7	5.4	12.3	13.9	25.8	5.1	11.6
1/19/98	3	17.3	16.0	2.0	2.1	15.9	14.7	1.9	2.0
1/22/98	6	20.5	16.7	3.4	1.3	18.8	15.2	3.3	1.1
1/26/98	10	15.4	18.0	4.0	1.7	14.1	16.6	3.8	1.6
1/31/98	15	16.4	17.1	2.4	0.4	15.2	15.8	2.1	0.4
2/9/98	24	15.4	18.1	4.5	0.5	14.4	17.0	4.2	0.5
2/16/98	31	17.8	17.7	0.6	0.8	16.6	16.4	0.6	0.8
2/23/98	38	17.9	17.2	2.5	9.2	17.0	16.2	2.1	9.3
2/27/98	42	13.6	13.3	0.1	1.2	12.5	12.2	0.1	1.0

TABLE B7: Total Suspended Solids and Volatile Suspended Solids for Batch 2

Batch 2		TSS Avg. (g/L)	TSS Avg. (g/L)	Std. Dev. (g/L)	Std. Dev. (g/L)	VSS Avg. (g/L)	VSS Avg. (g/L)	Std. Dev. (g/L)	Std. Dev. (g/L)
Date	Day	R1 (Srf)	R2 (C)	R1 (Srf)	R2 (C)	R1 (Srf)	R2 (C)	R1 (Srf)	R2 (C)
3/20/98	0	22.0	13.4	2.5	2.6	21.2	12.3	2.3	2.3
3/23/98	3	20.0	14.1	0.2	0.6	18.7	12.8	0.2	0.6
3/26/98	6	19.0	22.9	4.7	1.1	17.3	21.0	4.5	1.0
3/29/98	9	21.2	23.5	1.0	1.9	19.2	21.4	1.0	1.7
4/3/98	14	16.6	15.9	0.5	1.1	14.8	14.2	0.3	1.0
4/13/98	24	11.2	13.3	1.7	1.3	9.5	11.6	1.6	1.3
4/20/98	31	8.1	9.3	0.8	2.5	6.4	7.4	0.7	2.4
4/27/98	38	7.5	9.4	1.4	4.2	5.5	7.6	1.7	4.0

TABLE B8: Total Suspended Solids and Volatile Suspended Solids for Batch 3

Batch 3		TSS Avg. (g/L)	TSS Avg. (g/L)	Std. Dev. (g/L)	Std. Dev. (g/L)	VSS Avg. (g/L)	VSS Avg. (g/L)	Std. Dev. (g/L)	Std. Dev. (g/L)
Date	Day	R1 (L3)	R2 (L2/L3)	R1 (L3)	R2 (L2/L3)	R1 (L3)	R2 (L2/L3)	R1 (L3)	R2 (L2/L3)
6/11/98	0	14.6	19.6	0.4	2.5	9.0	14.2	0.2	2.3
6/14/98	3	15.1	31.0	0.1	1.6	9.2	22.2	0.1	1.5
6/17/98	6	14.8	33.0	0.3	0.6	9.0	24.2	0.2	0.6
6/20/98	9	15.6	35.4	0.5	0.7	9.6	27.0	0.3	0.8
6/25/98	14	13.2	34.9	0.1	1.4	8.0	26.5	0.1	1.3
7/5/98	24	11.7	18.0	0.2	1.9	7.5	12.8	0.1	1.7
7/12/98	31	11.5	23.4	0.2	1.8	7.0	17.2	0.2	1.7
7/19/98	38	11.7	22.1	1.2	0.5	7.1	16.0	0.8	0.4

TABLE B9: Metal Concentrations in Sludge from Control Reactors on Last Day of Each Batch Study

SAMPLE RESULTS

Description	Arsenic (mg/L)	Barium (mg/L)	Cadmium (mg/L)	Chromium (mg/L)	Lead (mg/L)	Mercury (mg/L)	Selenium (mg/L)	Silver (mg/L)
R1C-d38-B3	0.065	ND	0.090	0.66	1.8	ND	0.006	0.04
R1C-d38-B3*	0.064	ND	0.082	0.64	1.8	ND	0.004	0.04
R2-d42-B1	0.027	ND	0.070	0.34	0.8	ND	ND	ND
R2-d42-B1*	0.027	ND	0.064	0.26	0.8	ND	ND	ND
R2-d38-B2	0.030	ND	0.152	0.28	1.0	ND	ND	ND
R2-d38-B2*	0.030	ND	0.154	0.28	1.0	ND	ND	ND
R2C-d38-B3	0.095	ND	0.178	1.32	3.0	ND	0.005	0.08
R2C-d38-B3*	0.097	ND	0.178	1.22	3.0	ND	0.008	0.08
Digestion Blank	ND	ND	ND	ND	ND	ND	ND	ND
TCLP LIMIT	**5.0**	**100.0**	**1.0**	**5.0**	**5.0**	**0.200**	**1.0**	**5.0**
DETECTION LIMIT	**0.002**	**1.0**	**0.005**	**0.05**	**0.1**	**0.0005**	**0.002**	**0.02**

QA/QC DATA

Description	Arsenic % RECOVERY	Barium % RECOVERY	Cadmium % RECOVERY	Chromium % RECOVERY	Lead % RECOVERY	Mercury % RECOVERY	Selenium % RECOVERY	Silver % RECOVERY
Cal. Check Standard	105	103	98	95	98	100	83	103
Matrix Spike	110	113	97	99	100	100	108	101
SPEX Certified Std.	94	Did not analyze	99.5	101	100	100	96	107

NOTES:

*= Duplicate sample

ND=Result was below the detection limit.

1) Samples were digested in duplicate using EPA SW 846: Method 3050, "Digestion of Sediments, Sludges and Soils".

2) Mercury analysis was performed as per EPA SW 846: Method 7470, "Mercury in Liquid Waste".

TABLE B10: Pilot Plant Operational Settings for Batch 1

Batch 1		pH (s.u.)		Temp. (°C)		Air Flow (Working/Set) (SCFM)		Tap Water Added (L)		Lime Added (g)
Day	Date	R1	R2	R1	R2	R1	R2	R1	R2	R1&R2
1	1/16/98	7.0	7.0	20	20	3.5	3.5	-	-	N/A
3	1/19/98	6.2	6.2	19	19	3.5/3.5	3.7/3.5	3.5	1.75	N/A
6	1/22/98	6.2	6.2	19	20	3.5/3.5	3.0/3.5	3.5	1.75	N/A
10	1/26/98	6.0	6.0	17	17	3.5/3.5	3.5/3.5	0.875	0.875	N/A
15	1/31/98	6.2	6.2	17	17	3.3/3.5	3.4/3.5	2.2	2.2	N/A
24	2/9/98	6.2	6.2	17	16.3	3.3/3.5	3.5/3.5	0	1.75	N/A
31	2/16/98	6.0	6.0	17	17	3.65/3.5	3.65/3.5	1.75	0.875	N/A
38	2/23/98	5.3	5.3	17.5	17.5	3.3/3.5	3.2/3.5	4.375	4.375	N/A
42	2/27/98	5.2	5.2	18	17.5	3.6	3.7	7.875	7.875	N/A

TABLE B11: Pilot Plant Operational Settings for Batch 2

Batch 2		pH (s.u.)		Temp. (°C)		Air Flow (Working/Set) (SCFM)		Tap Water Added (L)		Lime Added (g)
Day	Date	R1	R2	R1	R2	R1	R2	R1	R2	R1&R2
1	3/20/98	7.0	7.0	19	19	3.5	3.5	-	-	-
3	3/23/98	7.0	7.0	19	17.2	3.0/3.5	3.2/3.5	5.25	6.125	-
6	3/26/98	6.3	6.3	18	17.8	3.8/3.5	4.1/3.5	6.125	7.875	20
9	3/29/98	6.0	6.0	21.5	21	3.3/3.8	3.6/3.8	8.75	8.75	40
14	4/3/98	6.0	6.5	20	20	3.4/3.5	3.7/3.5	8.75	7.875	20
24	4/13/98	6.0	6.0	20	18.5	2.55/4.0	3.5/4.0	5.25	5.25	-
31	4/20/98	5.6	5.8	19.5	20	2.7/3.7	2.6/3.7	6.125	6.125	60
38	4/27/98	6.4	5.9	20	20	3.2	3.75	7.75	7.75	-

TABLE B12: Pilot Plant Operational Settings for Batch 3

Batch 3		pH (s.u.)		Temp. (°C)		Air Flow (Working/Set) (SCFM)		Tap Water Added (L)		Lime Added (g)
Day	Date	R1	R2	R1	R2	R1	R2	R1	R2	R1&R2
1	6/11/98	8.2	8.4	22	21	3.5	4.0	-	-	-
3	6/14/98	8.8	8.9	20.5	19.8	3.2/3.5	4.3/4.0	7.0	3.5	-
6	6/17/98	8.6	8.7	21	21	4.3/3.5	4.1/4.0	8.75	10.5	-
9	6/20/98	8.4	8.6	21	21	3.7/3.7	4.0/4.0	8.75	10.5	-
14	6/25/98	7.2	8.2	22	21	3.5/3.5	4.1/4.0	5.25	5.25	-
24	7/5/98	5.8	6.2	21.2	21.8	3.3/3.5	3.1/3.8	7.0	5.5	60/40
31	7/12/98	6.0	6.0	21.6	21.5	3.5/3.5	3.5/4.0	8.75	7.0	60
38	7/19/98	6.0	6.0	22	21.5	3.2	3.9	9.625	9.625	60

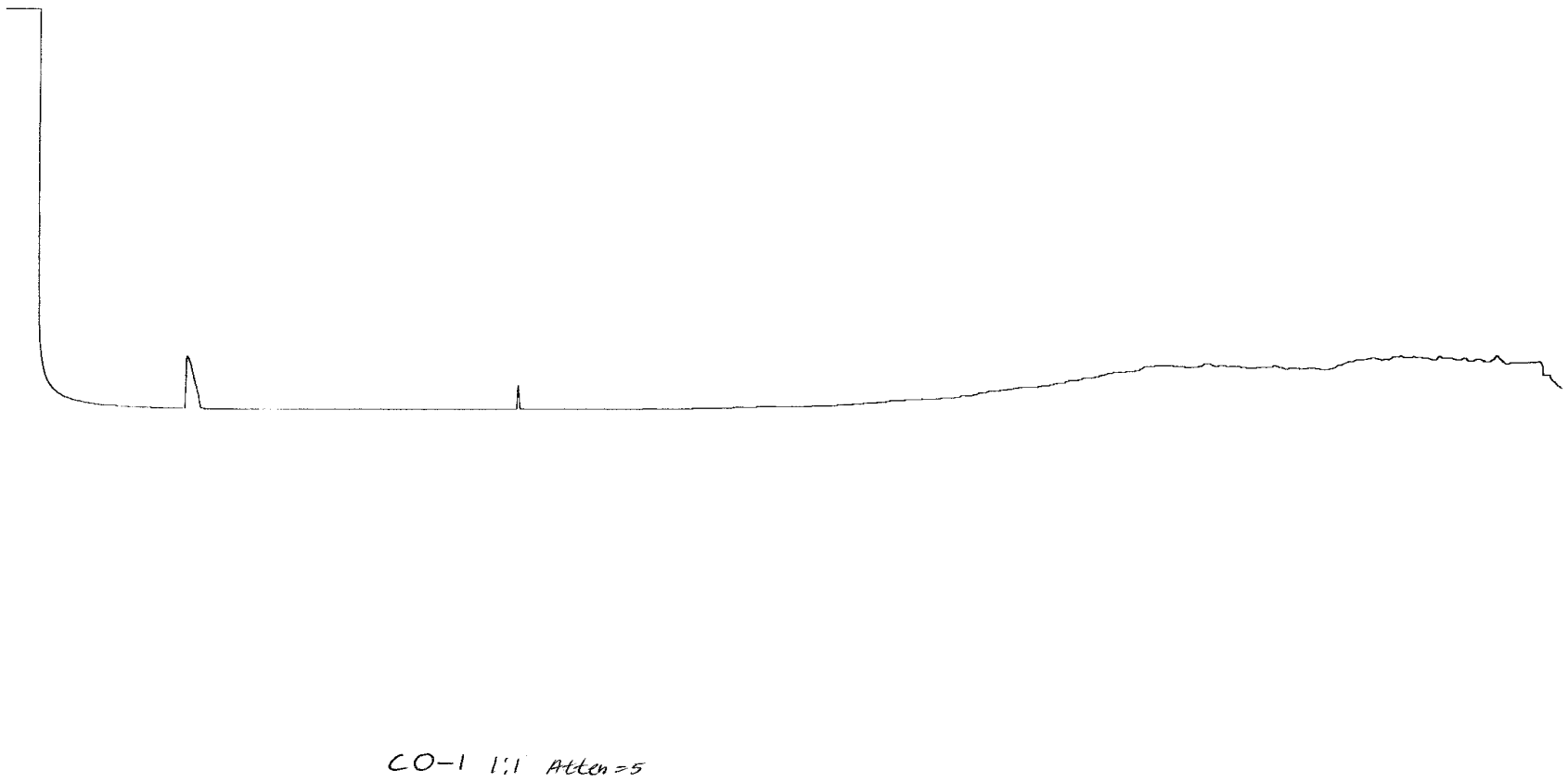

Figure B1: Standard Chromatograph: Compressor Oil (Clean Oil), 1,000 mg/L.

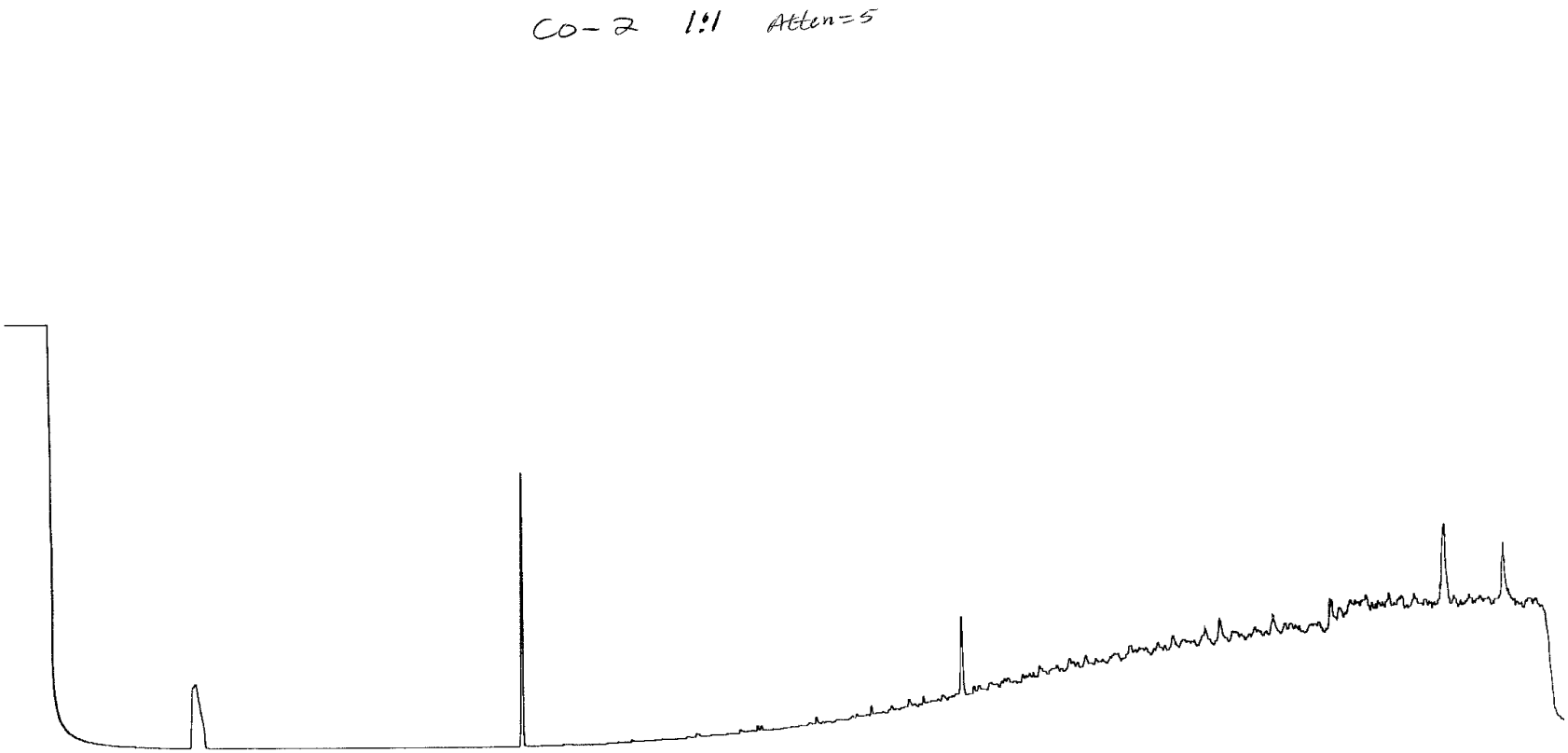

Figure B2. Standard Chromatograph: Compressor Oil (Clean Oil), 10,000 mg/L.

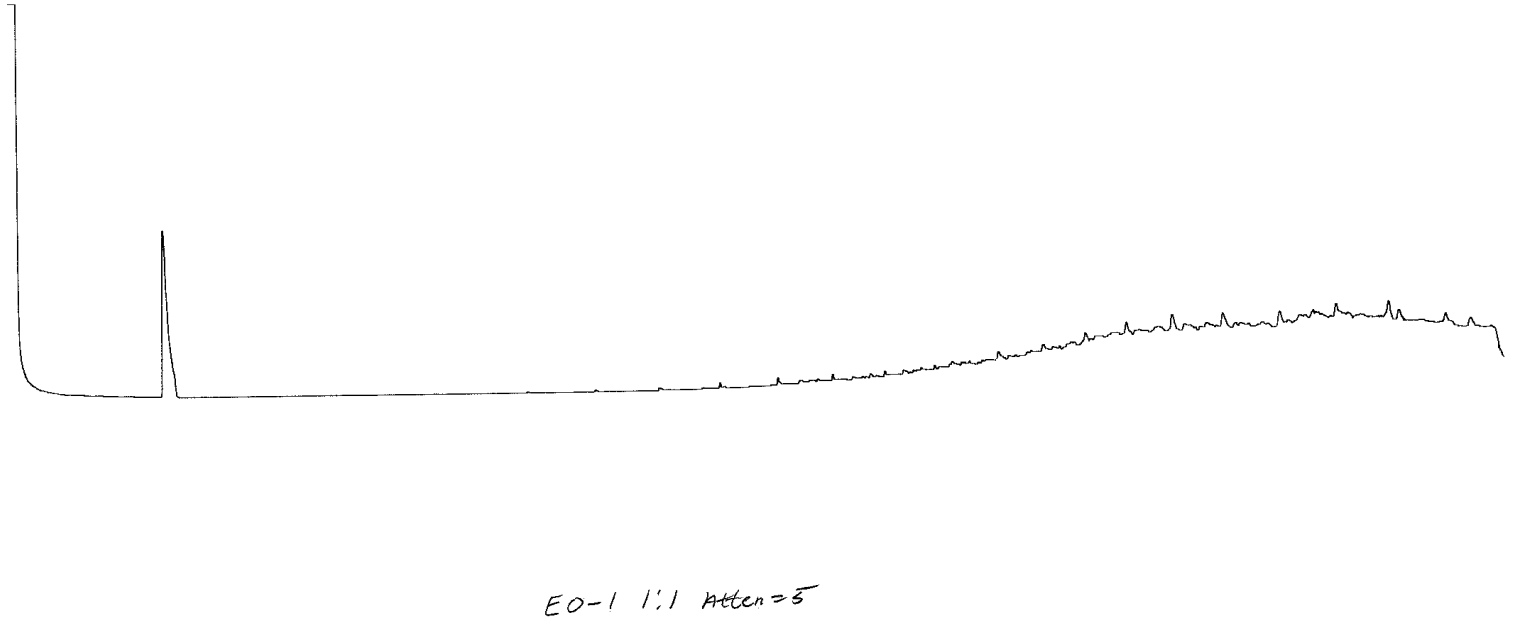

Figure B3. Standard Chromatograph: Engine Oil (Clean Oil), 1,000 mg/L.

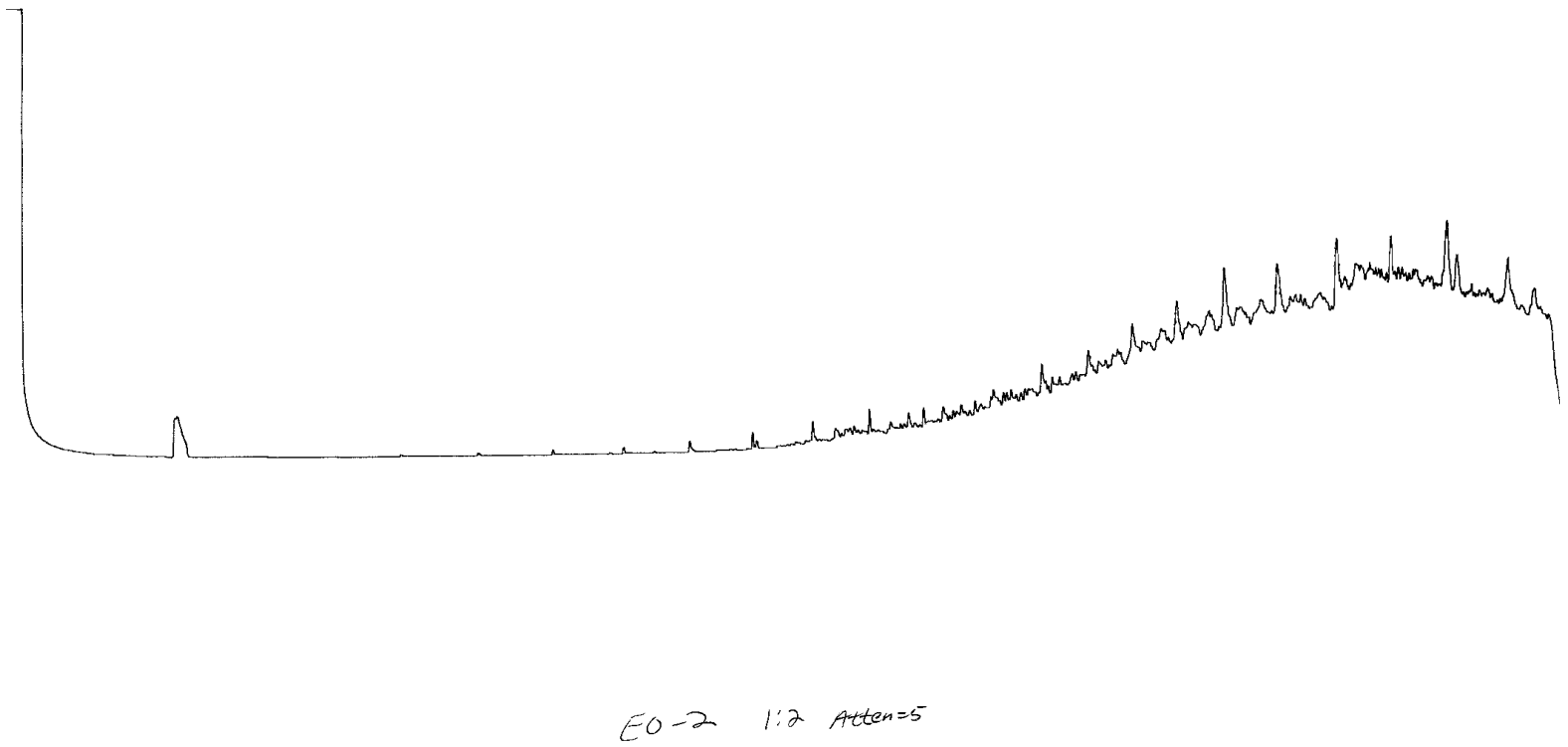

Figure B4. Standard Chromatograph: Engine Oil (Clean Oil), 10,000 mg/L.

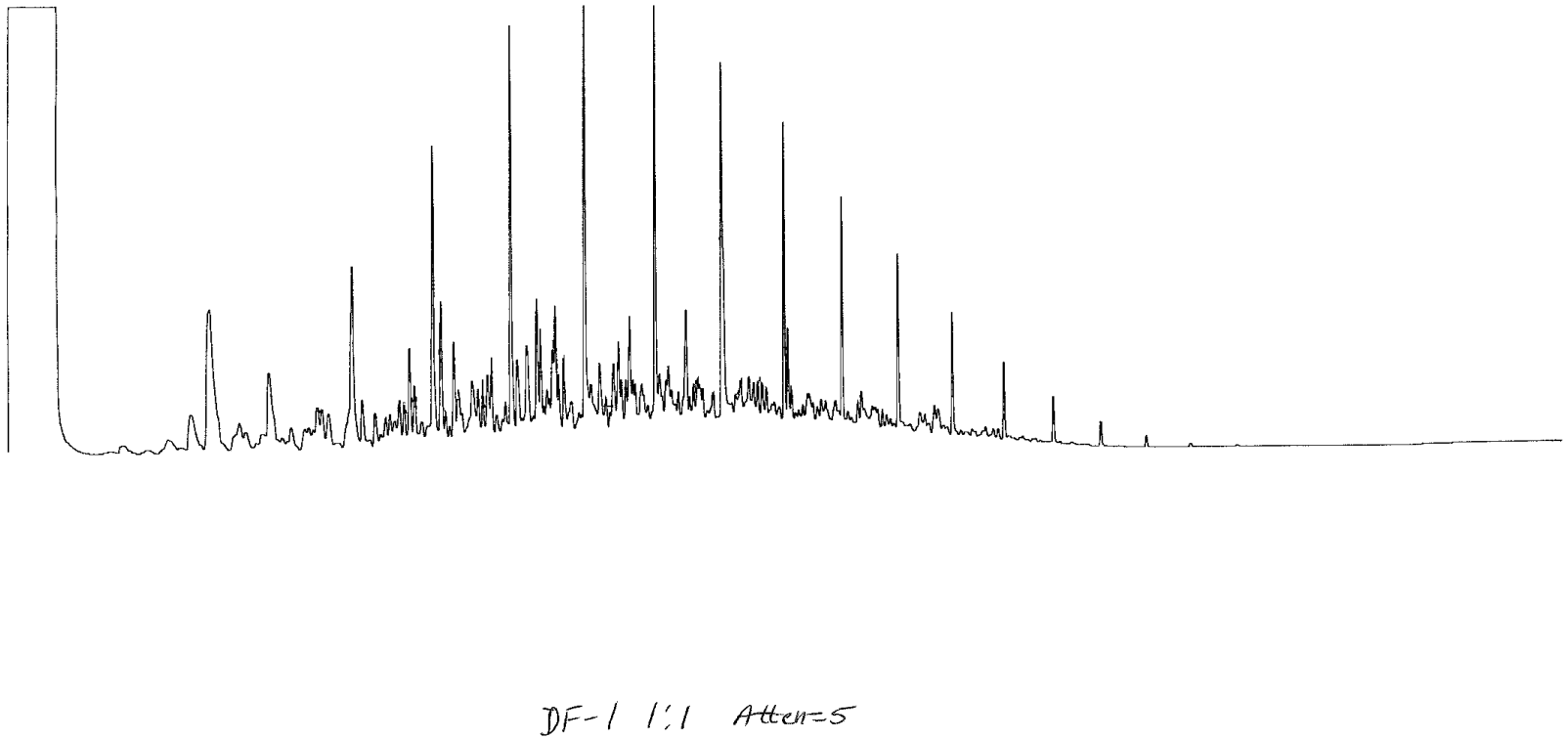

Figure B5. Standard Chromatograph: Diesel Fuel (Clean), 1,000 mg/L.

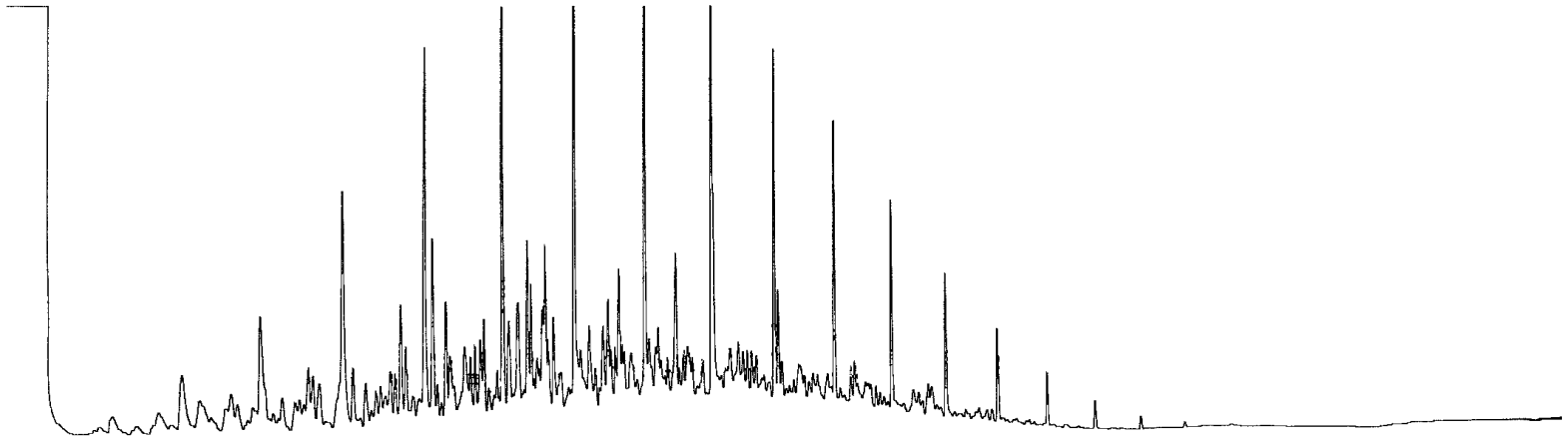

Figure B6. Standard Chromatograph: Diesel Fuel (Clean), 10,000 mg/L.

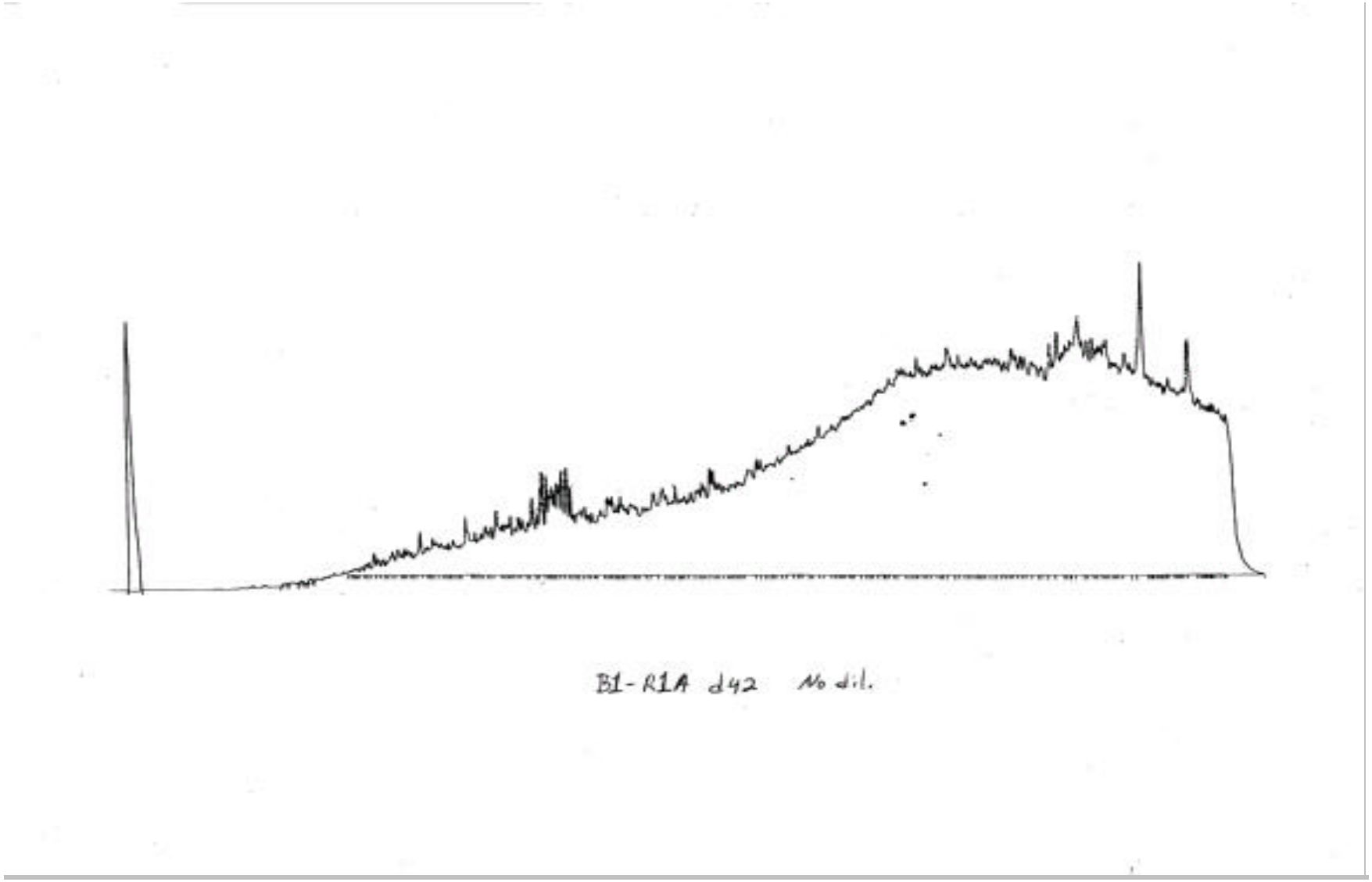

Figure B7. Chromatograph: Batch 1, R1, Day 42 with no dilution

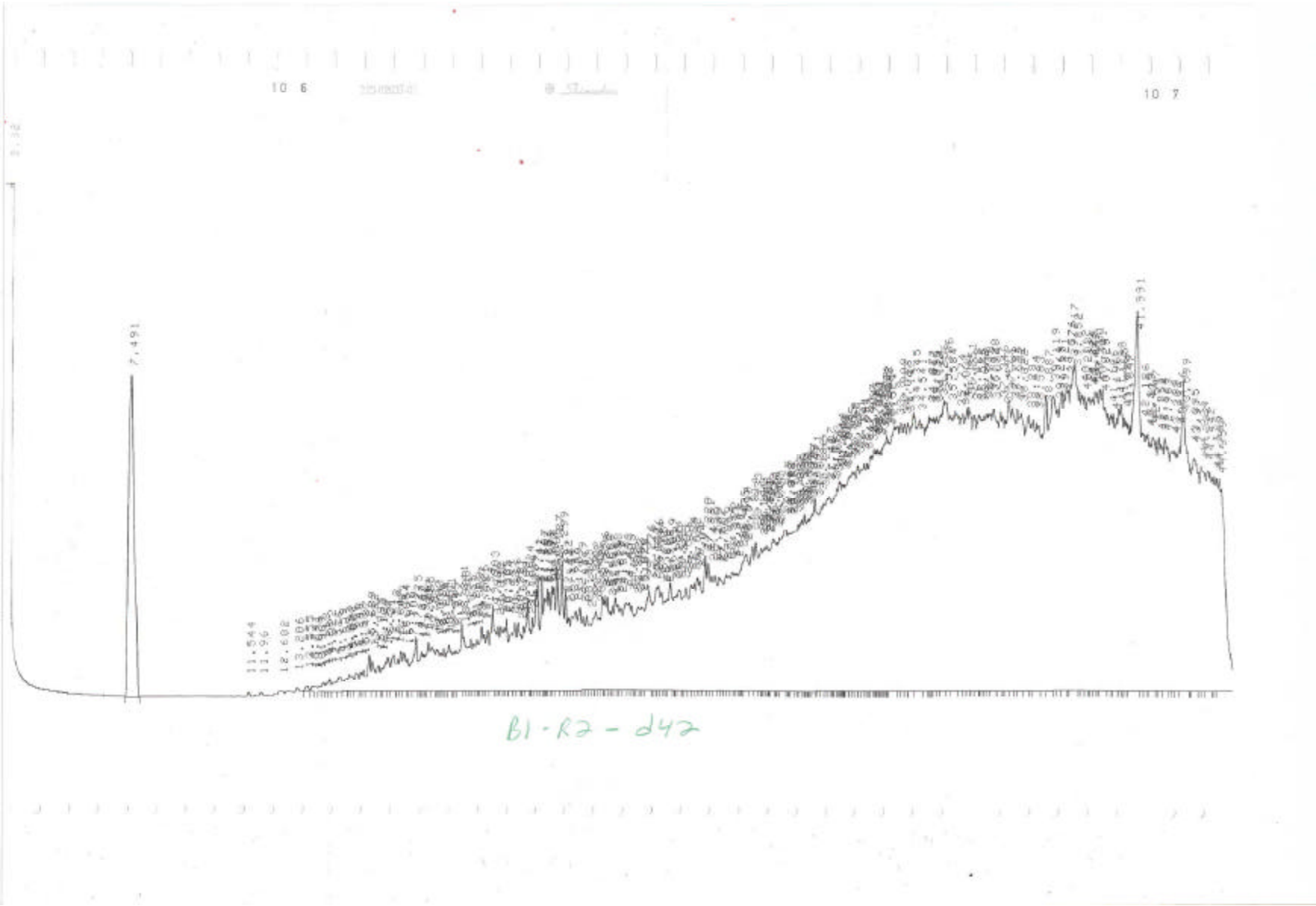

Figure B8. Chromatograph: Batch 1, R2, Day 42 with no dilution

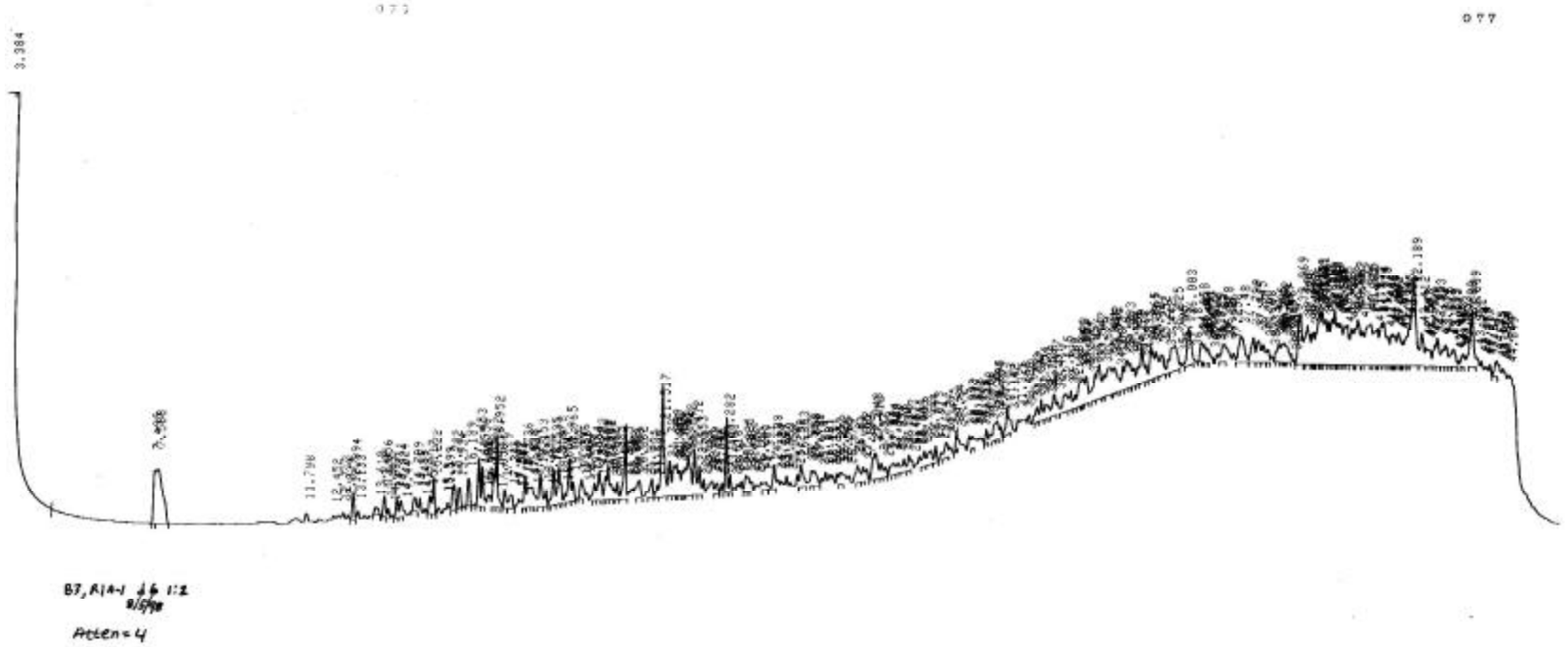

Figure B9. Chromatograph: Batch 3, R1, Day 6, with a 1:2 dilution

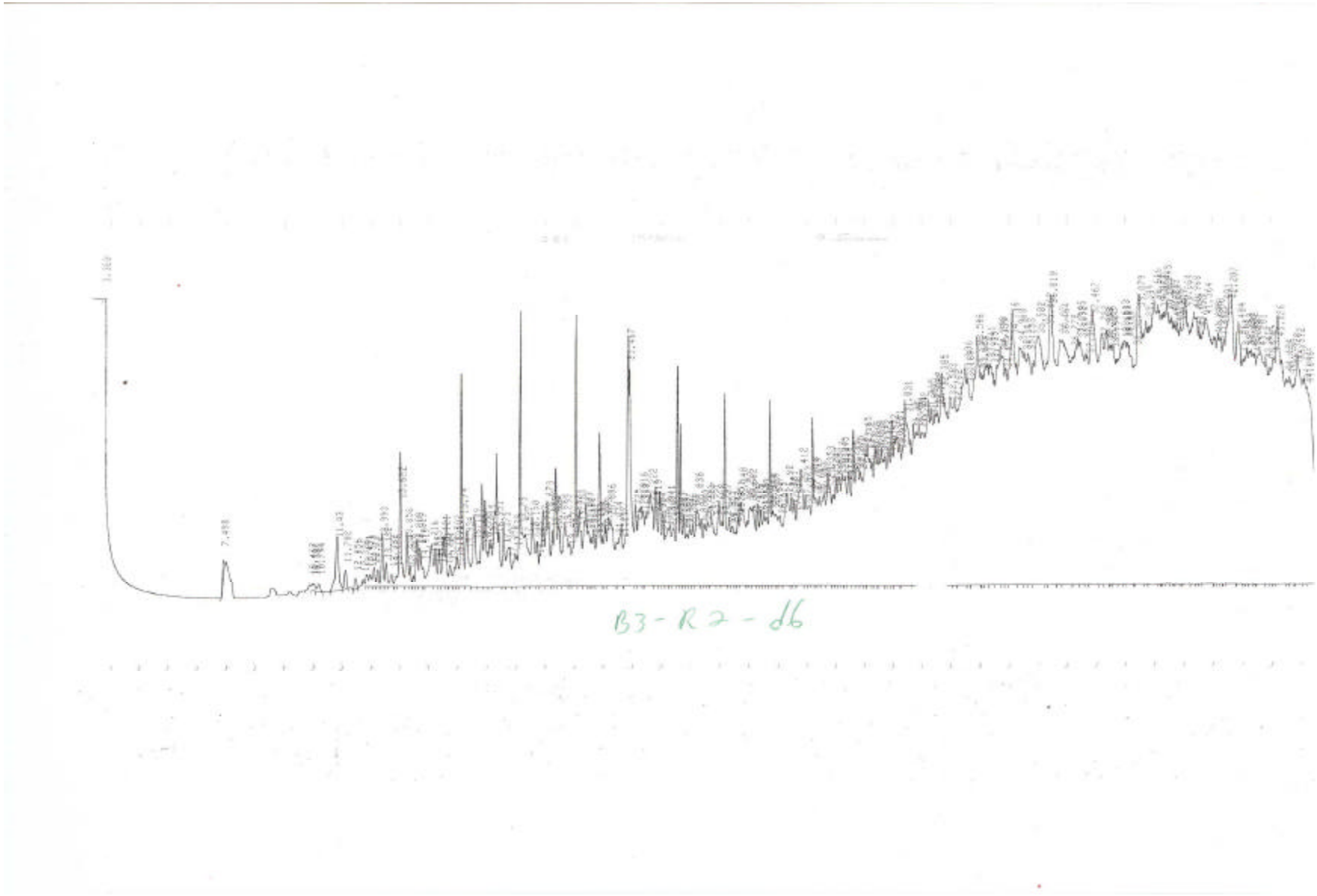

Figure B10. Chromatograph: Batch 3, R2, Day 6 with a 1:2 dilution

VITA

Jeffrey A. McInnis was born in Bath, Maine and graduated from Morse High School in 1981. He served in the United States Air Force from 1984 to 1991 and served in Operation Desert Storm. He graduated from Virginia Tech in 1996 with a B.S. in civil engineering and continued on with his M.S. in environmental engineering at Virginia Tech starting in the fall of 1996. He began work at Hayes, Seay, Mattern and Mattern (HSMM) in Roanoke, Virginia in the fall of 1998 and completed (finally) his thesis in March 2003. He is currently a staff engineer at HSMM.