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UNDER LABORATORY AND FIELD CODITIONS

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Presentation at:

Ninth International

IGT Symposium On Gas, Oil, and Environmental Technology
and Site Remediation Technologies

December 9-11, 1996

The Broadmoor Hotel

Colorado Springs, Colorado, USA

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ABSTRACT

Bacterial degradation of coal tar from a contaminated soil using a heavy enrichment culture inoculum and vigorous soil mixing was investigated under laboratory (25°C) and pilot scale conditions (10°C avg.). Under laboratory conditions, bacterial degradation of 795 mg/kg Polycyclic Aromatic Hydrocarbon (PAH) within a coal tar contaminated soil was reduced to mg/kg of dry weight soil in twelve weeks. Degradation of the 2,3, and 4 ringed PAHs occurred at similar rates (25-33 mg/kg days⁻¹) while removal of 5 ringed PAHs was much slower (1.4 mg/kg days⁻¹). Overall, 94% of the 16 PAH priority pollutants were removed biologically. A pilot scale project at a MGP contaminated soil site in the Southeast was performed using the laboratory approach. Beginning in December 1995, approximately 90 cubic yards of soil was treated with nutrients and an enrichment of PAH degrading biosolids. The application was accomplished by spraying the inoculum and required nutrients thoroughly into the soil after the soil passed through a hammermill/screen. This approach provided reasonable assurance that most all of the soil received coverage with high concentrations of PAH degrading bacteria and proper nutrient concentrations. Degradation was monitored by an independent laboratory. After 102 winter days, the total PAHs were reduced from 483 mg/kg to 95 mg/kg (80% removal). The degradation rates for the 2, 3, and 4 ring PAHs were once again nearly equal (3.60-3.85 mg/kg days⁻¹) with the 5 ringed PAHs being degraded much more slowly (0.68 mg/kg days⁻¹). Both total heterotrophic and PAH degrading bacterial populations increased during degradation of the coal tar. PAH degrading bacterial population increases correlated with an increase in the PAH degradation rate.

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INTRODUCTION

Bacterial Degradation of Coal Tar

- Degradation of 2, 3, and 4 ring PAHs by bacteria has been well documented [1] while less is known of the fate 5 ring PAHs [2]. Coal tar degradation by bacteria was investigated under laboratory and field conditions by monitoring the degradation of the 16 PAH priority pollutants. The purpose of these studies was to investigate total coal tar degradation in soil that has been treated with high concentrations of a mixed PAH degrading bacterial consortium using previously proven soil handling and inoculation procedures under laboratory and field conditions.

Factors Affecting Biodegradation

- Innovative Remediation Technologies Inc. has bio-remediated over 2.0 million tons of petroleum hydrocarbon contaminated soils throughout the United States and Europe. The process utilized for treatment involves the application of nutrients and a petroleum degrading bio-solids inoculum containing 500-1,000 mg/L of aerobic and facultative bacteria in combination with mechanically intensive soil processing methods. This process has allowed for the rapid bio-degradation of many organic contaminants in a variety of soil types.

Rate limiting factors for the bio-degradation of any compound in soil may be pH, temperature and nutrient or oxygen availability. Recent research has produced data which suggests that contact between the bacteria, nutrients and the compound of interest may play a role in the degradation rate [3]. Other investigations of bacteria and nutrient transport have been addressed and tested experimentally in order to accelerate the frequency of contact [4]. Attempts to enhance contact frequency and therefore, degradation of selected recalcitrant organic chemicals by adding surfactants to overcome solubility problems has also been studied [3]. In addition, certain soil parameters such as soil type, organic content and water and nutrient holding capacity have been taken into consideration in an attempt to understand the effects on transport and degradation rate [4].

Limiting factors may be overcome in excavated soils by; 1) improved soil handling techniques such as screening and aerobic composting and, 2) careful and complete coverage of the soil with nutrients and a microbial inoculum with specific contaminant degrading bacteria. Transport problems are minimized with this approach due to the immediate contact of bacteria, nutrients and the contaminants with one another.

The conventional wisdom of many microbiologists and engineers is that the addition of bacteria

is unnecessary due to the presence of indigenous bacteria which can be stimulated to degrade PAHs within coal tar or petroleum hydrocarbon contaminated soil. While it is certainly true that a host of researches have successfully stimulated the indigenous bacteria population and reduced PAH concentrations in soil, comparatively little work has been done to treat soils using vigorous soil handling procedures in combination with the heavy bacteria augmentation demonstrated in this study.

MATERIALS AND METHODS

Laboratory Study

An enrichment culture of coal tar degrading bacteria was made from known coal tar contaminated soil. Approximately 2 g of soil was placed in a 250 mL flask with 80 mL media. The mineral salts media (MSM) consisted of, in g/L; diammonium phosphate, 1.0; ammonium nitrate, 1.0; magnesium sulfate, 0.05; sodium chloride, 0.002; ferrous sulfate, 0.002; manganese sulfate, 0.002; calcium chloride, 0.002. Coal tar (0.1g) was added as a carbon source and the pH was adjusted to 7.5. Twenty milliliters of supernatant was transferred to a second flask with coal tar and MSM after four weeks of incubation on a lab shaker (150 rpm) at room temperature. Growth of bacteria was noticeable in the culture broth and under microscopic examination after approximately two weeks.

The soil used for the test was a clayey, loam soil obtained from a former MGP site in the southeast. The soil was screened to remove rocks and other debris and analyzed for background nutrients using a portable Hach laboratory. Two plastic pans (12 x 6 x 3in.) with covers were each filled with 500g of soil. One pan served as a control and was brought approximately 30% moisture. The experimental treatment was supplemented with the bacteria enrichment (ten mL of a 3.2×10^8 cfu/mL culture) plus MSM to a 30% moisture content. The soil pH was 7.5 for both treatments. The experiment was maintained at room temperature (approximately 25°C). The soils were monitored for moisture and pH and were well mixed with a spatula weekly. The experimental treatment was supplemented with 1 to 5 mL of a 50 g/L stock solution of ammonium nitrate if the concentration of ammonia or nitrate fell below 100 ppm. One to 5 mL of a 10 g/L stock solution of mono-basic potassium phosphate was added if the phosphate concentration in the soil was below 1 ppm. The concentration of ammonia, nitrate and phosphate was determined as before.

Soil was removed from three locations within the pan and pooled into a single composite at each sampling event to determine the PAH concentration. The soil was extracted and analyzed by EPA methods 3510 and 8100, respectively. Standards for the 16 PAH priority pollutants were obtained from Aldrich (Milwaukee, WI.). The degradation rates for the 2 and 3 ring, 4 ring 5 ring, and total PAHs were determined from the linear portion of the PAH degradation curve.

Pilot Scale-Study

The pilot scale study was initiated in December 1995 at a former MGP site. Approximately 90

cubic yards (c.y.) of soil were screened to remove debris and shredded through a hammermill/screen to pulverize the soil into small particles. The soil was carefully amended with nutrients and bacteria via a pressurized spray system designed to deliver specified volumes of liquid bacteria and nutrients to the soil as it exited the hammermill/screen. The bacterial inoculum used for the pilot scale study was produced over three weeks in a 500 gallon bio-reactor. The inoculum was taken under aeration in a mobile bio-remediation trailer to the field location. A second 500 gallon storage tank was placed on site to contain liquid nutrients.

The treated soil was placed into a 45' x 110' HDPE lined treatment cell with earthen berms for containment. The bottom of the bed was sloped and with a four inch layer of gravel to one corner which contained a sump system to remove excess water. Plastic drain piping was placed within the cell to aid drainage. A layer of geotextile fabric covered the gravel and drain system. A 6 inch lift of clean soil was placed over the drainage system before 6 inches of the contaminated soil was added. The soil was disced with a tractor and sampled every ten days.

Three to four random grab samples were collected every ten days during treatment and tested for total heterotrophic and PAH degrading bacterial populations by standard plate count agar and phenanthrene agar methods, respectively (5,6). The compounds monitored during treatment were the 16 PAH priority pollutants by a modification of EPA methods 3510 and 8100 by an outside laboratory. The data shown represent averages of at least three samples. The degradation rates for the total, 2 and 3 ring, 4 ring and 5 ring PAHs were determined between days 27 and 66. The data reflects the linear portion of the degradation curve.

RESULTS

Laboratory PAH Degradation Study

Table 1 shows the initial and final concentrations of the 16 priority PAHs extracted from the control and treated soil. This soil mostly contains 2 and 3 ringed PAHs (anthracene, phenanthrene and acenaphthylene), the 4 ringed PAHs (pyrene and fluoranthene) and significant amounts of the 5 ringed PAHs (benzo(b)fluoranthene, benzo(a)pyrene, indeno(1,2,3)pyrene and benzo(g,h,i)perylene). The initial total PAH of 795 mg/kg was reduced to 44 mg/kg dry soil. As would be expected the degradation of the 3 ring and less PAHs was the greatest followed by the 4 ring PAHs and, finally, 5 ring PAHs as shown in Table 2.

The laboratory treated soil demonstrated $\geq 95\%$ removal of naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene and anthracene after 12 weeks. The treated soil showed a total of 751 mg/kg PAHs removed while the control lost 281 mg/kg of total PAH after 12 weeks. Losses of PAHs from the soils may have been abiotic as well as biotic. However, the data suggests that considerable biodegradation of PAHs occurred with the bacterial treatment.

Table 1. PAH Concentrations Before and After Bacterial Treatment Compared to a Control.

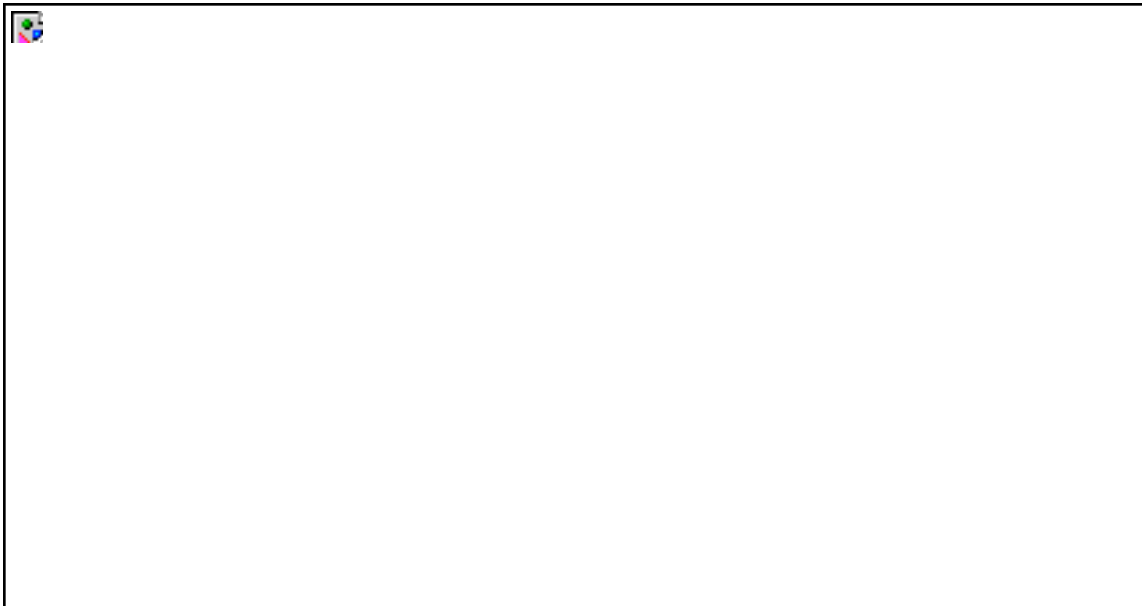
PAH	Initial Concentration (mg/kg)	Biological Treatment Final (mg/kg)	Percent Removal Treated	Control Final (mg/kg)
naphthalene	18.47	0.81	95	13.5
acenaphthylene	65.58	0.89	98	35.2
acenaphthene	52.79	0.83	98	28.1
fluorene	51.23	0.87	98	25.7
phenanthrene	97.9	4.59	95	49.8
anthracene	113.68	1.42	98	82.8
fluoranthene	90.25	5.79	93.5	65.2
pyrene	126.55	8.19	93.5	105.6
benzo(a) anthracene	30.33	2.5	92	16.7
chrysene	33.26	2.8	91.5	19.4
benzo(b) fluoranthene	51.53	4.92	90	39.1
benzo(k) fluoranthene	nd	nd	-	nd
benzo(a) pyrene	23.6	4.23	82	13.7
dibenzo(a,h) anthracene	26.56	3.28	87	14.0
indendo (1,2,3-cd) pyrene	nd	nd	-	nd
benzo(g,h,i) perylene	13.66	3.17	77	5.8
Total	795.39	44.29		514.6

Table 2. Degradation rates for total and PAH priority pollutants based on ring Number.

PAH	Degradation Rate mg/kg/day
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2 and 3 Ring	32.81
4 Ring	24.77
5 Ring	1.36
Total	58.94

Figure 1 shows the degradation of total, 2 and 3 ring, 4 and 5 ring PAHs over time. A dramatic drop is noticeable in the total PAH concentration in the first 2 weeks from 795 to 200 mg/kg which is followed by a slow but constant removal for the remainder of the study. This removal is due almost entirely to the degradation of 2, 3 and 4 ringed PAHs as can be seen in figure 1. The pattern for degradation of the 2, 3 and 4 ringed PAHs are very similar. Degradation of the much larger 5 ringed PAHs occurs at a slow rate from the onset of treatment. This pattern was reproduced in a second experiment. The degradation rates for the total, 2 and 2 ring, 4 ring and 5 ring PAHs are given in Table 2.



As reported consistently in previous literature, the data clearly shows that the degradation of PAHs are inversely proportional to the number of rings. The overall degradation rate is obviously dominated by the loss of 2, 3 and 4 ringed PAHs. As expected, there was a very dramatic difference in the degradation rate between the 4 and 5 ringed PAHs. The observed biodegradation rates correlate directly with the compound solubility.

Pilot Scale PAH Degradation Study

The degradation of the PAHs in 90 cubic yards of soil over time is shown in Figure 2. Temperatures in the range of 0°C dominated the first 30 days of the pilot study. The low temperatures correlate well with the approximate 30 day lag period observed during this time. After 30 days an appreciable amount of PAH degradation occurred as the temperature rose. The PAH contaminated soil was predominantly 2, 3 and 4 ringed PAHs once again. The smaller 2, 3 and 4 ringed PAHs followed similar patterns. The removal of the 5 ringed PAHs occurred much slower. Overall, the total PAH decreased from 483 to 95 mg/kg of dry soil after 100 days (80%).

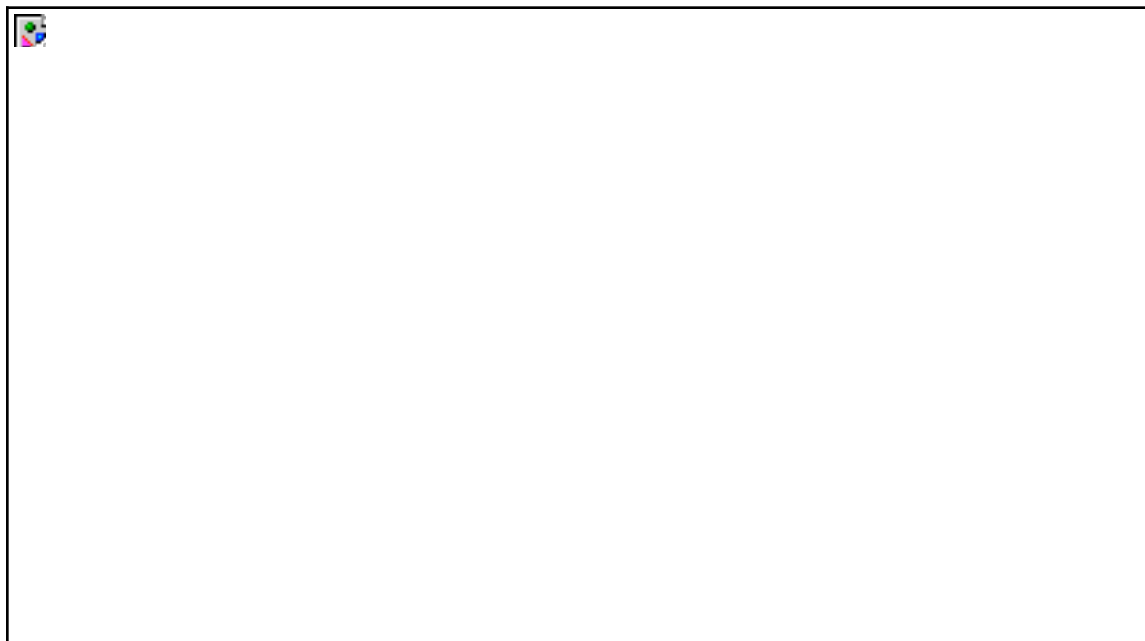


Table 3 shows the degradation rates for the total, 2 + 3 ring, 4 and 5 ring PAHs from the pilot scale study. The degradation rates were determined from the linear portion of the degradation curves between days 27 and 66.

Table 3. Degradation rates for total and PAH priority pollutants based on size for the pilot scale study.

PAH	Degradation Rate mg/kg/day
2 and 3 Ring	3.85
4 Ring	3.60
5 Ring	0.68
Total	8.28

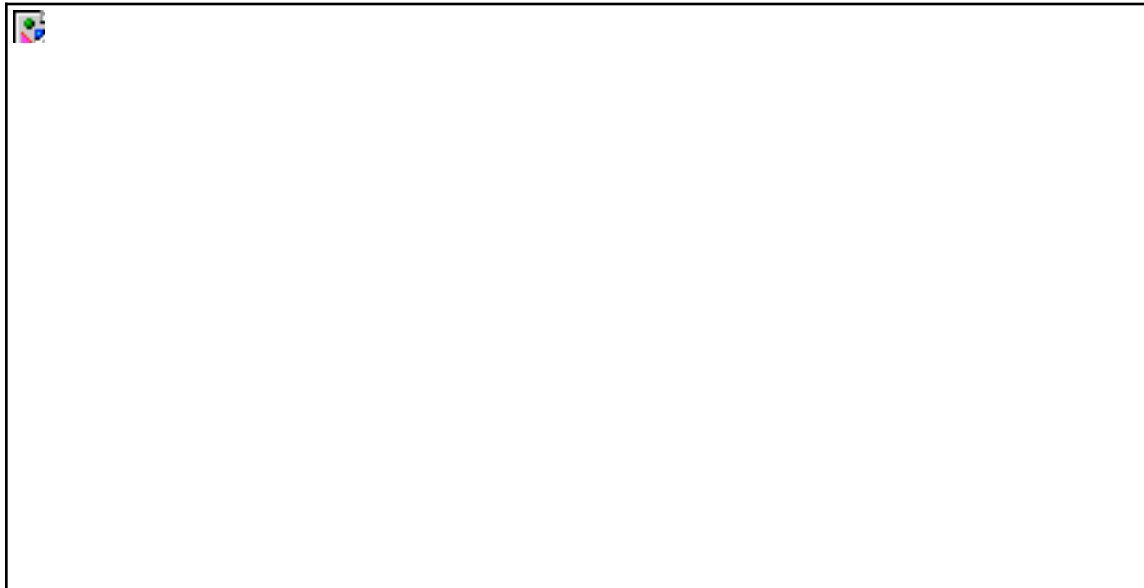
The effects of winter temperatures on PAH degradation are shown in Figure 3. The total PAH began to change as the average daily temperature rose to approximately 13°C, then even after the temperature began to drop to below 4°C, degradation still occurred. Though the temperature fluctuated dramatically, degradation of PAHs occurred as long as the overall temperature remained above 4°C.



Pilot Study Bacterial Populations and PAH degradation

The two bacterial populations of interest are the total heterotrophic bacteria and PAH degrading bacteria which are responsible for the actual degradation of the PAH compounds. Figure 5 shows that both population sizes changed dramatically during the course of treatment.

The heterotrophic bacteria increased in population as the total PAH reduction increased, which correlated with an increase in temperature (Figure 4). The heterotrophic bacteria increased from 6.1×10^6 to a maximum of 5.1×10^7 colony forming units (cfu/g soil) between days 12 and 27. The population stayed at this level until day 40 then slowly decreased to 2.3×10^6 cfu by day 76.



The phenanthrene degrading bacteria were lower in number than the heterotrophic bacteria in the initial sample at 9×10^5 cfu/g soil and never reached the population density as the heterotrophs during treatment. This would be expected due to substrate specificity. Interestingly, this population decreased to 7.5×10^4 cfu/g soil between days 12 and 19 and slowly increased to 5.3×10^5 cfu/g soil between days 19 and 40. This *increase* in population size correlated with the sudden *decrease* in total PAH. The phenanthrene degrading bacterial population decreased at a slow rate to 5.4×10^5 cfu/g soil by day 76. The phenanthrene degrading population followed more closely the degradation of PAH in the soil.

DISCUSSION

The laboratory and field studies demonstrate that the degradation of PAHs in soil is feasible if the proper conditions for bacterial growth are met. It was apparent from these studies that bio-remediation could proceed even in colder temperatures if key conditions for bacterial growth are maintained. It was shown that PAH removal rates were 7.12, 8.52, 6.00 and 2.00 times faster for the laboratory study (25°C) than the field pilot study (10°C) for total, 2 + 3 ring, 4 ring and 5 ring PAHs, respectively.

While no clear evidence was made available in this study that bolsters the argument that augmentation of soil with heavy bacterial inoculum improves performance, the data clearly indicate that the combination of techniques used for the study were effective overall. Previous work has demonstrated with simpler organic contaminants that the re-application of bacteria with mixing to facilitate contact with recalcitrant compounds is effective for further reduction of contaminants. Further studies may be necessary to resolve the augmentation approach as it relates to PAHs.

Certainly the vigorous soil mixing and close maintenance of soil nutrient and moisture levels were critical elements in overcoming transport obstacles within the soil.

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