

ENHANCED HYDROCARBON BIOREMEDIATION THROUGH THE ADDITION OF HUMIC SUBSTANCES PART II: IMPROVED LAND TREATMENT AND STATIC- PILE COMPOSTING

by

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ABSTRACT

Laboratory experiments were conducted to improve the biodegradation of hydrocarbons in soil through the addition of humic substances. A simulated land treatment operation was conducted, in which a clay type soil was spiked with diesel to achieve initial concentrations of 0.25 to 1.0 %. Bulking agents and soil additives (extra-cellular enzymes and biostimulants) were added in combination with humic substances in a simulated land treatment operation. Parameters investigated included soil moisture retaining ability, soil aggregate size distribution, soil pH, total carbon to nitrogen ratio (C:N), heterothropic plate count (HPC) bacteria, and total extractable hydrocarbons (TEH). The land treatment operation was modified to simulate static-pile composting by increasing the ambient temperature from 20 to 50 °C. The method of aeration was also improved from manual tilling to forced aeration. Bulking agents and humic substances were added and monitored for the growth of HPC bacteria and reduction of TEH.

Bulking agents improved soil aggregate size distribution, reduced soil pH closer to 7, increased C:N value, promoted the growth of HPC bacteria, and enhanced the biodegradation of diesel. Soil additives increased C:N value, promoted the growth of HPC bacteria, and enhanced the biodegradation of diesel. By adding bulking agents or soil additives in conjunction with humic substances, better soil quality was observed and the biodegradation of diesel in soil was improved. However, when large dosages of these materials were added, the improvement became insignificant. The best performance was observed through the addition of 5% humic substances together with 0.1% extra-cellular enzymes and biostimulants containing soil additives. Tier I at 800 mg/kg was achieved between 3 to 6 weeks of treatment, representing 1.3 times improvement over humic substances alone, and 5.5 times more reduction over the control.

Static-pile composting improved the biodegradation of diesel in soil over the land treatment operation. Forced aeration performed better than manual tilling. The best performance was observed when humic substances at 5% dosage and bulking agents at 50% dosage were added. Tier I was achieved between 3 to 6 weeks, representing 1.2 times improvement over humic substances alone, and 3.9 times more reduction over the control. When compared to the land treatment, this represented 2.3 times improvement over humic substances alone, or 17 times more reduction over the control.

INTRODUCTION

Bioremediation is considered to be reliable, cost-effective, and an environmentally sound method to treat hydrocarbon spills in soil (Cookson, 1995). This method utilizes soil microorganisms to degrade the hydrocarbon contaminant into stable end products, ideally water and carbon dioxide. Since the growth of microorganisms depend on the soil condition, improvement on soil quality will also enhance treatment performance. Efforts in improving soil quality to enhance the biodegradation of hydrocarbons in soil were conducted by Liem et al. (2003). In laboratory experiments conducted to simulate land treatment, it was found that humic substances improved soil quality, benefiting the growth of soil microorganisms. The biodegradation was enhanced, achieving in some situations over 90% reductions of up to 1.0% initial diesel concentrations in 12 weeks of treatment or less.

As a follow-up, additional laboratory experiments were conducted to improve the utilization of humic substances in enhancing diesel biodegradation in soil. The first group of experiments was completed to improve the performance of land treatment by incorporating bulking agents and soil additives in addition to humic substances. The second group of experiments was conducted to modify the treatment method, from land treatment to static-pile composting. In this case, the ambient temperature was increased from 20 to 50°C, and aeration was improved from manual tilling to forced aeration. All experiments were monitored for 12 weeks. Parameters monitored included soil pH, moisture content, aggregate size distribution, total carbon and nitrogen ratio (C:N), heterotrophic plate count (HPC) bacteria, and total extractable hydrocarbon (TEH).

HYDROCARBONS AND TREATMENT METHODS

Petroleum hydrocarbons (PHC) are organics found in or derived from geological substances. They comprise of C₆ to C₁₀, C₁₀ to C₁₆, C₁₆ to C₃₄, and >C₃₄ fractions, excluding BTEX components (CCME, 2001). According to Alberta Energy Utilities Board (2001), 42,700 m³ of oil spills occurred in Alberta from 1996 to 2001. CCME (2001) has identified several concerns related to the presence of PHC in soil: 1) most of them are toxic and may pose a fire hazard, 2) lighter hydrocarbons are mobile in soil, while larger and branched-chain hydrocarbons are persistent in the environment, and 3) they degrade soil quality and may create aesthetic problems. CCME (2001) has therefore established guidelines for PHC in soil. Under Tier I levels, or generic numerical levels without considering site conditions, the guideline for diesel (which falls closely under C₁₆ to C₃₄ fractions of hydrocarbons) has been set at 800 mg/kg. This is a guideline for surface fine-grained soil suitable for agricultural applications.

Land treatment is an aerobic process performed in the upper soil zone. This treatment is designed for minimal cost and operation. Process optimization becomes a challenge and the treatment requires a long time. The contaminated soil is spread over the land in thin layers of 300 mm or less (Eweis et al., 1998). The soil is wetted to dissolve the contaminant in water for accessibility by the microorganisms. Still, excessive moisture may result in poor oxygen transfer within the soil medium. Oxygen requirement for the

microorganisms is provided through tilling, which also allows better contact between the contaminants, microorganisms, and nutrients (Fogel, 1994). Tilling is conducted once every week or other week to avoid the destruction of soil aggregates (U.S.EPA, 1993). Mesophilic bacteria growth is best between 15 to 45°C (U.S.EPA, 1985).

Composting, although a higher operating cost and a more complex operation, provides process flexibility and requires a shorter period of treatment. In this operation, contaminated soil is piled, in which the generated heat is trapped. High temperatures are desirable to increase the solubility of contaminants in water and improve mass transfer, enhancing the biodegradation rate. Thermophilic bacteria also have the ability to breakdown a wider range of chemical structures (Eweis et al., 1998). In static-pile composting, the contaminated soil is piled at 2 to 3 m high and spread out in an area as large as 30 x 50 m² (Sellers et al., 1993). The oxygen requirement is provided through forced aeration. Temperature within the pile to achieve thermophilic range of 50 to 60°C is controlled by aeration and by an external heat source.

For both land treatment and static-pile composting, optimum moisture contents range between 50 to 75% of the soil's field capacity (U.S.EPA, 1985). Most microorganisms grow best at a pH around neutral (Cookson Jr., 1995). The optimum C:N value has been reported at 20:1 to 25:1 (U.S.EPA, 1985). Bulking agents and soil additives can be added to improve the quality of soil and the growth of soil microorganisms. Satisfactory microbial inoculum is usually present either in soil, soil additives, or bulking agents.

MATERIALS

Properties of interest of the experimental soil are listed in Table 1.

Table 1: Experimental Soil

| Parameter | Unit | Value |
|------------------|-------------------|-------|
| Bulk density | kg/m ³ | 1,000 |
| Porosity | % | 50 |
| Moisture content | % | 23 |
| Field capacity | % | 85 |
| Particle size | | |
| Clay | % | 63 |
| Silt | % | 22 |
| Sand | % | 15 |
| pH | - | 7.3 |
| Total Nutrients | | |
| Carbon | % | 2.4 |
| Nitrogen | % | 0.34 |
| HPC bacteria | CFU/g | 1,100 |

Diesel was used as the hydrocarbon contaminant due to its low evaporation at tested temperatures. A boiling point analysis was conducted from 36.1 to 503.0°C (C₅ to C₃₆ fractions) and showed that 97% of the carbon components were in the forms of C₇ to C₂₂. At temperatures of 36 to 69°C, it was observed that only 0.1% of diesel would be lost. At lower temperatures, the evaporation loss was expected to be smaller.

According to McCarthy and Suffet (1989), humic substances are complex organic materials from the decomposition of plant and animal residues, excluding discrete compounds such as polynucleotides, proteins, and polysaccharides. Humic substances consist mainly of carbon and oxygen, as well as hydrogen, nitrogen, and sulphur (Choudhry, 1984). The benefits of humic substances for bioremediation include providing available carbon to soil and maintaining neutral pH (Jackson, 1993). They bind non-polar hydrophobic compounds, such as hydrocarbons, increasing its solubility in water to be reached by soil microorganisms (Guetzloff and Rice, 1994). Humic substances, through the hydrogen bonding, retain water on their surfaces and maintain the soil moisture content (Chen and Shnitzer, 1976). They have abilities to chelate various cations, resulting in the dissolution of clay aggregates (Kodama and Schnitzer, 1973). This improves circulation of moisture and air within the soil and exposure of the contaminant and nutrients to the microorganisms. Humic substances retain micronutrients near the surface of soil particles, benefiting the growth of soil microorganisms (McBride, 1981). Humic substances used in these experiments originated from the oxidation of parent materials adjacent to sub-bituminous coal. Humic substances were tested to investigate their effects on the quality of soil and biodegradation of hydrocarbons. Table 2 summarizes properties of interest of the humic material (Luscar, 2002).

Table 2: Humic Substances

| Parameter | Unit | Value |
|---------------|-------------------|-------------------------------|
| Organics | % | 55 |
| pH | - | 7.0 to 7.4 |
| Bulk density | kg/m ³ | 866 to 965 |
| Particle size | micron micron | 75 to 150 (25%) < 75 (75%) |

Bulking agents were added to increase soil porosity and to provide a medium for soil microorganisms to grow. They will also increase available nutrients and the initial number of microorganisms (Eweis et al., 1998). Compost and wood-mulch based of bulking agents were used at 1:1 mixture by weight. Table 3 lists properties of interest of the bulking agents.

Table 3: Bulking Agents

| Parameter | Unit | Compost | Wood-Mulch |
|-----------------|-------------------|--------------------|--------------------|
| Moisture | % | 29 | 62 |
| Porosity | % | 50 | 50 |
| Bulk density | kg/m ³ | 585 | 250 |
| Particle Size | % | 18 (≥ 4.0 mm) | 39 (≥ 4.0 mm) |
| | % | 57 (0.5 to 4.0 mm) | 53 (0.5 to 4.0 mm) |
| | % | 25 (< 0.5 mm) | 8 (< 0.5 mm) |
| Total Nutrients | Carbon | 18.2 | 38.7 |
| | Nitrogen | 0.94 | 0.88 |
| pH | - | 7.2 | 7.1 |
| HPC | CFU/g | 250 millions | 77 millions |

Biostimulants and extra-cellular enzymes were added to the experiment soil to promote

the growth of microorganisms and to catalyze the degradation of hydrocarbons. Biostimulants consisted of nutrients and bacterial culture. Extra-cellular enzymes were designed to break long-chain hydrocarbon rings resulting in more water-soluble products. Six different soil additives were tested during the experiments. Three soil additives contained only extra-cellular enzymes, while the other three contained both extra-cellular enzymes and biostimulants (Bio Huma Netics, 2002, The Moorhead Group, 2002, and Pendragon Technologies, 2002). Table 4 shows the breakdown of the extra-cellular enzymes and biostimulants (Pendragon Technologies, 2002).

Table 4: Soil Additives

| Component (by weight) | Breakdown |
|------------------------------|---|
| Extra-cellular enzymes (10%) | Lipase, protease, amylase, cellulase, hemi-cellulase, fructose, pectinase |
| Bacteria culture (60%) | Bacillus subtilis, bacillus licheniformis, bacillus megaterium, bacillus thuringiensis, bacillus brevis, lactobacillus acidophilus, saccharomyces cerevisiae, pseudomonas putida, pseudomonas alcaligenes, pseudomonas stutzeri, nitrobacter winogradskyi |
| Nutrients (30%) | Inorganic and organic additives |

Distilled water was used to wet the soil medium. One-day aeration prior to the experiment was required to minimize any chlorine residuals in the experimental water.

Land treatment and static-pile composting were simulated in 28 gauge galvanised steel columns. The columns had dimensions of 150 mm diameter and 450 mm tall. A coarse screen and 4 micron filter paper was placed at the bottom of each column. The column was supported by a pan and covered by a coarse screen on the top. For the static-pile composting operation, temperature adjusted ovens were used to control the ambient temperature. An air pump provided air to each testing column.

METHODS

The design of experiments is summarized in Table 5. The soil was placed inside the steel column at 300 mm depth. Soil moisture contents were adjusted to 50 to 60% by weight at the end of each week. Moisture contents before and after the wetting were measured using a portable moisture probe and verified using gravimetric method. The errors were small at 5% maximum. Three readings were taken and the average value recorded. After moisture adjustments, a small volume of soil was sampled, dried, and tested for soil aggregate size distribution. Soil aggregate size distribution was analyzed using a standard Tyler sieving apparatus (sieve openings 45 to 4,000 microns) for 15 minutes.

The land treatment experiments were conducted inside a closed room. Exposure to light was minimal and room temperature was kept constant at 20 ± 0.5 °C. The soil was tilled and wetted manually every week. The soil temperature was recorded at 17 ± 0.5 °C. The static-pile composting experiments were conducted in temperature adjustable ovens at 50 ± 1 °C. Aeration was provided either by weekly tilling or continuous forced aeration at 1 L/min rate. The soil temperature was recorded at 46 ± 1 °C.

Once every three weeks, soil samples were taken and analyzed for different parameters including pH, total carbon and nitrogen, HPC bacteria, and TEH. TEH values represented carbon fractions of C₁₁ to C₃₀, which fall closely under C₁₆ to C₃₄ fractions as defined by CCME (2001). Table 6 summarizes measurement methods for parameters of interest.

Table 5: Experimental Design

| Run No. | Diesel (%) | Treatment (%) | | | | Temperature (°C) | Aeration |
|-----------------|------------|---------------|----------------|----------------------|--------------------------------------|------------------|----------|
| | | Humic | Bulking Agents | Enzymes ² | Enzymes + Biostimulants ³ | | |
| 1 ¹ | 0.50 | 0 | 0 | 0 | 0 | 20 | Tilling |
| 2 ¹ | 1.00 | 0 | 0 | 0 | 0 | 20 | Tilling |
| 3 ¹ | 0.00 | 1 | 0 | 0 | 0 | 20 | Tilling |
| 4 ¹ | 0.00 | 5 | 0 | 0 | 0 | 20 | Tilling |
| 5 | 0.00 | 0 | 50 | 0 | 0 | 20 | Tilling |
| 6 ¹ | 0.50 | 1 | 0 | 0 | 0 | 20 | Tilling |
| 7 ¹ | 0.50 | 2 | 0 | 0 | 0 | 20 | Tilling |
| 8 ¹ | 0.50 | 3 | 0 | 0 | 0 | 20 | Tilling |
| 9 ¹ | 0.50 | 5 | 0 | 0 | 0 | 20 | Tilling |
| 10 ¹ | 1.00 | 1 | 0 | 0 | 0 | 20 | Tilling |
| 11 ¹ | 1.00 | 2 | 0 | 0 | 0 | 20 | Tilling |
| 12 ¹ | 1.00 | 3 | 0 | 0 | 0 | 20 | Tilling |
| 13 ¹ | 1.00 | 5 | 0 | 0 | 0 | 20 | Tilling |
| 14 | 0.25 | 1 | 50 | 0 | 0 | 20 | Tilling |
| 15 | 0.25 | 2 | 50 | 0 | 0 | 20 | Tilling |
| 16 | 0.25 | 3 | 50 | 0 | 0 | 20 | Tilling |
| 17 | 0.25 | 5 | 50 | 0 | 0 | 20 | Tilling |
| 18 | 0.50 | 1 | 50 | 0 | 0 | 20 | Tilling |
| 19 | 0.50 | 2 | 50 | 0 | 0 | 20 | Tilling |
| 20 | 0.50 | 3 | 50 | 0 | 0 | 20 | Tilling |
| 21 | 0.50 | 5 | 50 | 0 | 0 | 20 | Tilling |
| 22 | 0.25 | 0 | 50 | 0 | 0 | 20 | Tilling |
| 23 | 0.25 | 0 | 50 | 0 | 0 | 50 | Tilling |
| 24 | 0.25 | 0 | 50 | 0 | 0 | 50 | Forced |
| 25 | 0.25 | 5 | 50 | 0 | 0 | 20 | Tilling |
| 26 | 0.25 | 5 | 50 | 0 | 0 | 50 | Tilling |
| 27 | 0.25 | 5 | 50 | 0 | 0 | 50 | Forced |
| 28 | 0.50 | 0 | 50 | 0 | 0 | 20 | Tilling |
| 29 | 0.50 | 0 | 50 | 0 | 0 | 50 | Tilling |
| 30 | 0.50 | 0 | 50 | 0 | 0 | 50 | Forced |
| 31 | 0.50 | 5 | 50 | 0 | 0 | 20 | Tilling |
| 32 | 0.50 | 5 | 50 | 0 | 0 | 50 | Tilling |
| 33 | 0.50 | 5 | 50 | 0 | 0 | 50 | Forced |
| 33 | 0.50 | 0 | 0 | 0.1 | 0 | 20 | Tilling |
| 35 | 0.50 | 0 | 0 | 0 | 0.1 | 20 | Tilling |
| 36 | 0.50 | 5 | 0 | 0.1 | 0 | 20 | Tilling |
| 37 | 0.50 | 5 | 0 | 0 | 0.1 | 20 | Tilling |

Note:¹ conducted by Liem et al. (2003).

² three extra-cellular soil additives were tested separately.

³ three extra-cellular + biostimulants soil additives were tested separately.

Table 6: Measurement Methods

| Testing | Methods | References |
|------------------|--|-----------------------------|
| Moisture content | Direct measurement Oven dry @105 °C gravimetric | - APHA, AWWA, WEF (1998) |
| Aggregate size | Tyler sieve analysis | - |
| pH | Soil water extraction | APHA, AWWA, WEF (1998) |
| Total carbon | Total combustion | ASA and SSSA (1996) |
| Total nitrogen | Total combustion | ASA and SSSA (1996) |
| HPC bacteria | Plate count | HPB-Method MFHPB18 (1998) |
| TEH | Chromatogram-hexane solvent | EPA-3550/8000-GC-FID(1996) |

RESULTS AND ANALYSIS

Table 7 summarizes soil moisture content before and after weekly adjustments during 12 monitoring weeks. Average values for the whole weeks and their standard deviations (in brackets) are presented. For each treatment, no significant differences were found from one week to another. Diesel and soil additives did not affect the moisture content.

Table 7: Moisture Content

| Soil Mixture | Before Adjustment (%) | After Adjustment (%) | Moisture Retaining (%) |
|------------------------------------|-----------------------|----------------------|------------------------|
| Soil | 66(8.4) | 48(11.4) | 72(11.7) |
| Soil + bulking agents | 71 (10.0) | 49(12.0) | 68(11.5) |
| Soil + humic (1%) | 64 (7.5) | 50(9.8) | 78(10.0) |
| Soil + humic (5%) | 60(6.7) | 53(8.4) | 87(9.2) |
| Soil + humic (1%) + bulking agents | 66(9.5) | 50(10.0) | 75(10.5) |
| Soil + humic (5%) + bulking agents | 62(7.0) | 54(8.1) | 86(7.2) |

The table shows that soil moisture content was adjusted to 60 to 71% and decreased at the end of each week to 48 to 54% as the targeted value. The moisture retaining ability was also presented, which was the ratio of moisture content before and after adjustment. The table shows that the addition of bulking agents decreased the moisture retaining ability due to larger soil porosity. When humic substances were added, the soil moisture retaining ability increased from 72 to 87% (or 21% higher) for soil without bulking agents, and from 68 to 86% (or 26% higher) for soil with bulking agents.

Percentages for aggregate sizes 1.4 to 2.4, 2.4 to 4.0, and ≥ 4.0 mm (average values for the whole weeks and their standard deviations) are presented in Table 8. For each treatment, no significant differences were found from one week to another. Diesel concentrations and soil additives did not affect the aggregate size distribution.

The experiments found that the addition of bulking agents or humic substances reduced the percentage of larger soil aggregates, allowing better air and moisture circulation within the soil medium. This also improved exposure of contaminant and nutrients to the microorganisms. However, the addition of bulking agents in combination with humic substances did not show any significant improvement.

Table 8: Aggregate Size Distribution

| Soil Mixture | Aggregate Size Distribution (%) | | |
|------------------------------------|---------------------------------|---------------|---------------|
| | ≥ 4.0 mm | 2.4 to 4.0 mm | 1.4 to 2.4 mm |
| Soil | 88(4.9) | 5(1.7) | 2(3.20) |
| Soil + bulking agents | 76(3.9) | 11(8.8) | 4(3.4) |
| Soil + humic (1%) | 84 (3.4) | 6 (1.9) | 3 (1.2) |
| Soil + humic (5%) | 70 (11.2) | 13 (4.5) | 6 (3.8) |
| Soil + humic (1%) + bulking agents | 73(9.7) | 14(6.9) | 6(3.1) |
| Soil + humic (5%) + bulking agents | 75(8.7) | 13(4.4) | 4(3.0) |

Table 9 shows pH and C:N values for the initial week. The addition of bulking agents or humic substances decreased the soil pH closer to neutral and increased the C:N value. The combination of both ingredients increased the C:N value up to three times but did not seem to affect the soil pH significantly.

Table 9: pH and C:N Ratio (Initial Week)

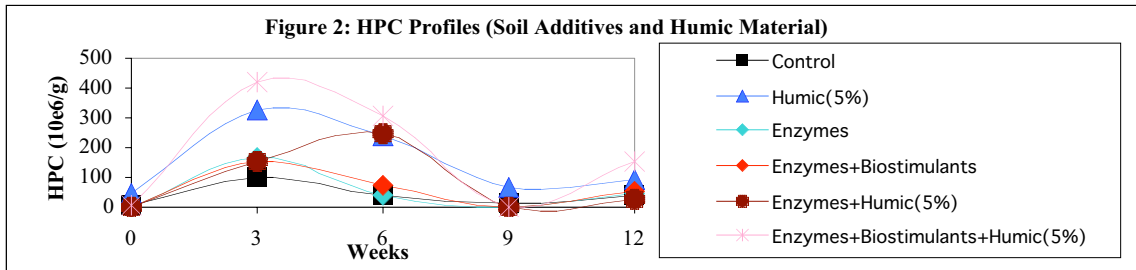
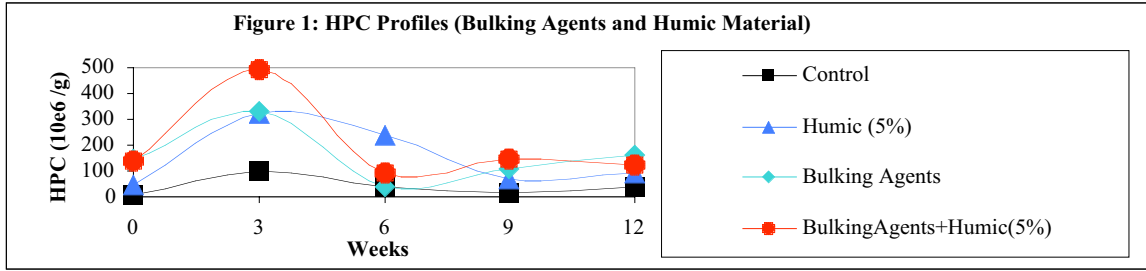
| Soil Mixture | Initial Week Values | |
|---|---------------------|----------|
| | pH | C:N |
| Soil ¹ | 7.6 | 7 |
| Soil + bulking agents | 7.4 | 14 |
| Soil + humic (1%) ¹ | 7.4 | 9 |
| Soil + humic (5%) ¹ | 7.2 | 14 |
| Soil + humic (1%) + bulking agents | 7.4 | 17 |
| Soil + humic (5%) + bulking agents | 7.2 | 21 |
| Soil + additives (enzymes) | 7.4 to 7.5 | 13 to 14 |
| Soil + additives (enzymes + biostimulants) | 7.4 to 7.5 | 13 to 14 |
| Soil + humic (1%) + additives (enzymes) | 7.1 to 7.4 | 17 to 19 |
| Soil + humic (5%) + additives (enzymes + biostimulants) | 7.0 to 7.2 | 16 to 24 |

Note: ¹ Liem et al. (2003).

This table shows that all soil additives did not affect the soil pH significantly but increased the C:N value. The combination of soil additives and humic substances decreased the soil pH to around 7 and increased the C:N value to three times. All soil additives performed similarly.

Figure 1 shows HPC profiles for the land treatment operation at 0.5% initial diesel concentration when bulking agents and humic substances were utilized. The figure shows that the addition of bulking agents increased the initial HPC and promoted their growth to almost the same level as that of humic substances. The combination of them provided the best growth. The growth of HPC bacteria peaked at the 3rd week of treatment and leveled off following the degradation of available nutrients (diesel) in soil.

Figure 2 shows HPC profiles for the land treatment operation at 0.5% initial diesel concentration when soil additives and humic substances were utilized. Only one profile from extra-cellular enzymes containing soil additives is shown in the figure. The other two profiles showed similar trends and are not presented here. The same arrangement was also made for soil additives with extra-cellular enzymes and biostimulants.



The figure shows that the addition of soil additives improved the growth of HPC bacteria, although humic substances performed better than all of them. Significant improvement was observed when soil additives with extra-cellular enzymes and biostimulants were added in combination with humic substances.

Figure 3 shows HPC profiles for the static-pile composting operation at 0.5% initial diesel concentration when bulking agents and humic substances were utilized. This figure shows that continuous forced aeration resulted in better HPC growth than weekly manual tilling. The HPC growth was slow for controls with either method of aeration. With the exception of humic substances with at manual tilling, bulking agents and humic substances improved the HPC growth. The combination of both performed the best.

This figure shows that modifying the treatment method from land treatment to static-pile composting did not increase the population of HPC bacteria. This suggests that different types of bacteria grew at different ranges of temperature as expected. At their optimum condition, the HPC growth peaked at the 3rd week of treatment.

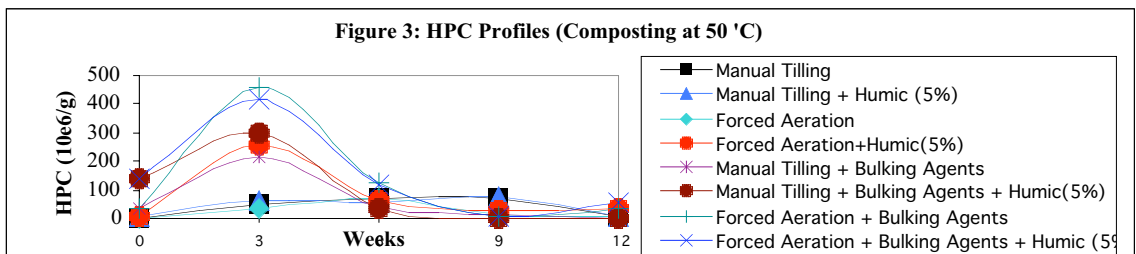


Figure 4 shows TEH profiles for the land treatment operation at 0.5% initial diesel concentration when bulking agents and humic substances were utilized. Independently, humic substances and bulking agents enhanced the biodegradation of diesel in soil. At

large dosages of humic substances and bulking agents, the improvement became smaller. Tier I (800 mg/kg) was achieved between weeks 6 to 9 at 5% of humic substances, with or without bulking agents. This represented 83% diesel reduction, which was 4 times higher compared to that of the control at 21% reduction.

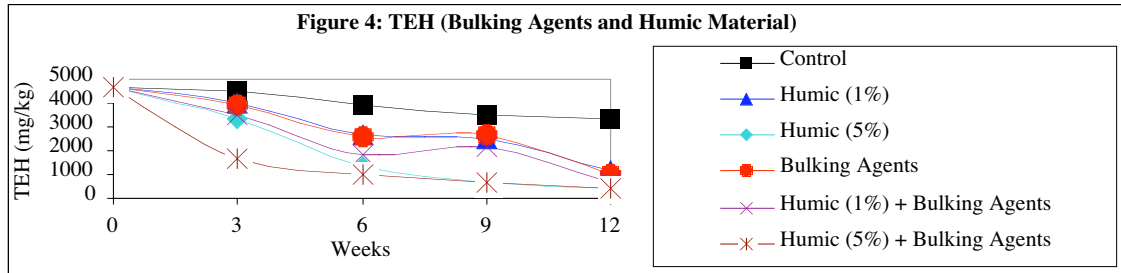
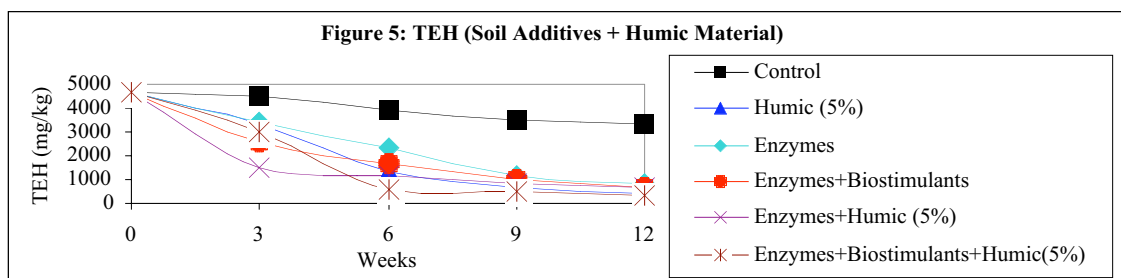


Figure 5 shows TEH profiles for the land treatment operation at 0.5% initial diesel concentration when soil additives and humic substances were utilized. Only one profile from extra-cellular enzymes containing soil additives and one profile from soil additives with extra-cellular enzymes and biostimulants are shown in the figure. The other profiles showed similar trends.

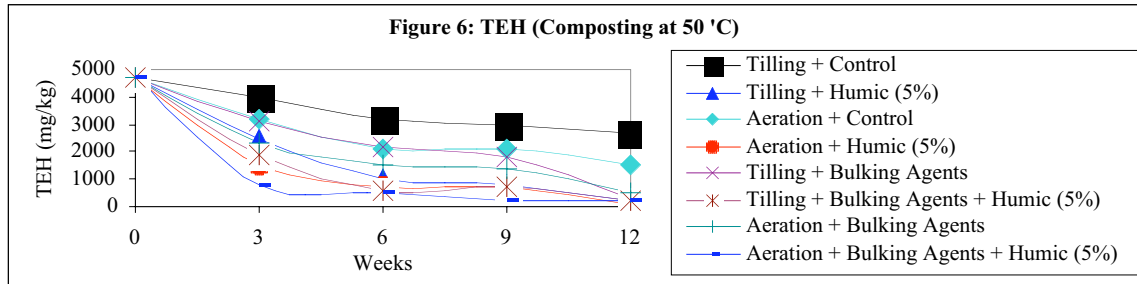


The figure shows that extra-cellular enzymes and biostimulants containing soil additives performed similarly to that of humic substances. Soil additives with extra-cellular enzymes only did not perform as well as that of humic substances. The combination of humic substances and soil additives with extra-cellular enzymes and biostimulants performed the best. Tier I was achieved at the 6th week of treatment. This represented 83% diesel reduction, or 5 times enhancement over the control at 17% reduction.

Figure 6 shows TEH profiles for the static-pile composting operation at 0.5% initial diesel concentration when bulking agents and humic substances were utilized. This figure shows that weekly manual tilling without any treatment was able to reduce the diesel down to 2,700 mg/kg, or representing 43% reduction after 12 weeks of treatment. Using land treatment, the reduction of TEH was smaller at 28%. With the help of forced aeration, the reduction was increased to 66%. These findings revealed that high temperatures played an important role in the reduction of diesel in soil. While biodegradation by soil microorganisms was still the main process, diesel loss due to forced aeration should also be taken into consideration.

Continuous forced aeration outperformed the weekly manual tilling. Independently,

bulking agents and humic substances enhanced the biodegradation of diesel in soil. The combination of both improved the performance slightly. Using forced aeration, humic substances with bulking agents achieved Tier I between 3 to 6 weeks of treatment, representing 83% diesel reduction, or 3 times enhancement over the control at 29% reduction.



CONCLUSIONS AND RECOMMENDATIONS

The utilization of humic substances to enhance the biodegradation of hydrocarbons in soil through land treatment could be improved through the addition of bulking agents and soil additives. At large dosages of materials, the improvement became smaller. Table 10 summarizes the performance of land treatment in achieving Tier I at 800 mg/kg from 4,700 mg/kg initial diesel concentrations.

Table 10: Land Treatment Performance (Initial TEH = 4,700 mg/kg)

| No. | Treatment | Tier I Objective (800 mg/kg) |
|-----|---|------------------------------|
| 1 | Control | 3,300 mg/kg @ 12 weeks |
| 2 | Humic @ 1% | 1,200 mg/kg @ 12 weeks |
| 3 | Humic @ 5% | Tier I @ 9 to 12 weeks |
| 4 | Bulking Agents @ 50% | 1,000 mg/kg @ 12 weeks |
| 5 | 2 + 4 | Tier I @ 12 weeks |
| 6 | 3 + 4 | Tier I @ 6 to 9 weeks |
| 7 | Soil additives (enzymes) @ 0.1% | 1,000 mg/kg @ 12 weeks |
| 8 | Soil additives (enzymes + biostimulants) @ 0.1% | Tier I @ 12 weeks |
| 9 | 3 + 7 | Tier I @ 6 to 9 weeks |
| 10 | 3 + 8 | Tier I @ 3 to 6 weeks |

The biodegradation of hydrocarbons in soil through the addition of humic substances could be improved by modifying the treatment method from land treatment to static-pile composting. The addition of bulking agents together with humic substances improved the biodegradation. At large dosages of materials, the improvement was minimal. Table 11 summarizes the performance of static-pile composting in achieving Tier I at 800 mg/kg from 4,700 mg/kg initial diesel concentrations.

Recommendations for future work include more detailed laboratory experiments to investigate optimum dosages of humic substances, bulking agents, and soil additives to achieve the desired performance. The experiments should also include higher and

different types of initial hydrocarbon concentrations. Most importantly, cost factors should be considered when determining the optimum treatment.

Table 11: Static-Pile Composting (Initial TEH = 4,700 mg/kg)

| No. | Treatment | Aeration | Tier I Objective (800 mg/kg) |
|-----|----------------------|----------|------------------------------|
| 1 | Control | Tilling | 2,700 mg/kg @ 12 weeks |
| 2 | Control | Aeration | 1,600 mg/kg @ 12 weeks |
| 3 | Humic @ 5% | Tilling | Tier I @ 6 to 9 weeks |
| 4 | Humic @ 5% | Aeration | Tier I @ 6 to 9 weeks |
| 5 | Bulking Agents @ 50% | Tilling | Tier I @ 9 to 12 weeks |
| 6 | Bulking Agents @ 50% | Aeration | Tier I @ 9 to 12 weeks |
| 7 | 3 + 5 | Tilling | Tier I @ 6 to 9 weeks |
| 8 | 4 + 6 | Aeration | Tier I @ 3 to 6 weeks |

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Table 1. Physical and Chemical Properties of Pristine, Control and Biotreated Soils used in Study

| | Soil A | Soil B | Soil C | Soil D | Control |
|----------------------------|---------------|---------------|---------------|---------------|----------------|
| Texture^a | Fine | Fine | Fine | Fine | Fine |
| Sand (%) | 40.7 ± 1.3 | 34.7 ± 1.3 | 23.7 ± 0.7 | 47.1 ± 0.7 | 44.0 |
| Silt (%) | 32.8 ± 1.7 | 38.3 ± 0.9 | 41.2 ± 0.5 | 30.2 ± 0.4 | 37.6 |
| Clay (%) | 26.5 ± 0.9 | 27.0 ± 0.2 | 35.1 ± 0.1 | 22.7 ± 0.3 | 18.4 |
| Organic Matter (%) | 4.0 ± 0.1 | 5.8 ± 0.3 | 3.8 ± 0.1 | 2.1 ± 0.2 | 2.7 |
| pH (Sat. Paste) | 7.5 ± 0.0 | 7.5 ± 0.1 | 7.2 ± 0.0 | 7.2 ± 0.1 | 7.1 |
| EC (dS/m) | 1.47 ± 0.03 | 2.19 ± 0.08 | 0.96 ± 0.04 | 2.72 ± 0.09 | 0.53 |
| SAR^b | 1.70 ± 0.0 | 3.00 ± 0.11 | 0.80 ± 0.00 | 1.10 ± 0.00 | 0.10 |
| Bulk Density (g/cc) | 1.20 ± 0.01 | 1.14 ± 0.01 | 1.18 ± 0.02 | 1.26 ± 0.01 | 1.26 |

^aFine textured soils are defined as having greater 50% by mass particles less than 75 μm ($D_{50} < 75 \mu\text{m}$).

^bSodium Adsorption Ratio.

Note physical and chemical properties are reported as the average of triplicate analyses \pm 95 % confidence interval; the control values are the result of a single analysis.

Table 2. Petroleum Hydrocarbons (PHC) and Polycyclic Aromatic Hydrocarbons (PAHs) within Biotreated Soils Compared to AENV/CCME PHC Standards for Various Land Uses Assuming EcoSoil Contact as Exposure Pathway

| | Agriculture | Residential/ Parkland | Commercial | Industrial | Soil A (mg/kg) | Soil B (mg/kg) | Soil C (mg/kg) | Soil D (mg/kg) |
|---|---------------------------------------|---------------------------------------|---------------------------------------|--------------------------------------|-------------------|-------------------|-------------------|-------------------|
| Petroleum Hydrocarbons | | | | | | | | |
| Fraction 2 (>C ₁₀ -C ₁₆) | 900 ^a /2200 ^b | 900 ^a /2200 ^b | 1500 ^a /3000 ^b | 1500 ^a /3000 ^b | 289 ± 9.9 | 117 ± 28.0 | 153 ± 12.8 | 279 ± 39.1 |
| Fraction 3 (>C ₁₆ -C ₃₄) | 800 ^a /3500 ^b | 800 ^a /3500 ^b | 2500 ^a /5000 ^b | 2500 ^a /5000 ^b | 3693 ± 32.7 | 1127 ± 6.5 | 1113 ± 18.2 | 1993 ± 45.7 |
| Fraction 4 (>C ₃₄ -C ₅₀) | 5600 ^a /10000 ^b | 5600 ^a /10000 ^b | 6600 ^a /10000 ^b | 6600 ^a /1000 ^b | 2180 ± 49.3 | 726 ± 4.3 | 921 ± 27.2 | 894 ± 44.2 |
| PAHs^c | | | | | | | | |
| Naphthalene | 0.1 | 0.6 | 22 | 22 | 0.09 ± 0.01 | < 0.05 | 0.07 ± 0.00 | 0.10 ± 0.01 |
| Phenanthrene | 0.1 | 5 | 50 | 50 | 0.22 ± 0.01 | < 0.05 | 0.05 ± 0.01 | 0.13 ± 0.01 |
| Chrysene | - | - | - | - | 0.12 ± 0.01 | < 0.05 | < 0.05 | 0.09 ± 0.00 |
| Fluorene | - | - | - | - | 0.10 ± 0.00 | < 0.05 | < 0.05 | 0.05 ± 0.00 |

All results reported on a dry weight basis.

CCME PHC F1 not determined as results (not shown) from biotreatment indicated that C₅-C₁₀ hydrocarbons were below detection.

^aCCME PHC Standards for surface soil (< 1.5 m below ground surface).

^bCCME PHC Standards for subsurface soil (> 1.5 m below ground surface).

^cOnly those PAHs that were detected are provided in Table.

Note: Detectable PHCs and PAHs are reported as the average of triplicate analyses ± 95 % confidence interval.

Table 3. Results of Microtox™, Seed Germination and Acute and Subchronic Earthworm Bioassays Following Exposure to Biotreated PHC Contaminated Soils

| | Soil A | Soil B | Soil C | Soil D | Control |
|---|--------|--------|--------|--------|---------|
| Earthworm (% Survival)^a | | | | | |
| 14 day | 100 | 100 | 100 | 100 | 100 |
| 10 week | 100 | 100 | 100 | 100 | 100 |
| Plant (% Germinated)[*] | | | | | |
| Radish (day 4) | 100 | 100 | 100 | 100 | 100 |
| Oats (day 4) | 97 | 93 | 90 | 97 | 100 |
| Radish (day 7) | 100 | 100 | 100 | 100 | 100 |
| Oats (day 7) | 100 | 100 | 93 | 100 | 100 |
| Radish (day 11) | 100 | 100 | 100 | 100 | 100 |
| Oats (day 11) | 100 | 100 | 93 | 100 | 100 |
| Microtox (% Sample)[*] | | | | | |
| EC ₅₀ (5 minute) | > 100 | > 100 | > 100 | > 100 | ND |
| EC ₂₀ (5 minute) | > 100 | > 100 | > 100 | > 100 | ND |
| EC ₅₀ (15 minute) | > 100 | > 100 | > 100 | > 100 | ND |
| EC ₂₀ (15 minute) | > 100 | > 100 | > 100 | > 100 | ND |

^a 50% diluted soil data not shown, data are the same as in the 100 % contaminated soil.

ND = not determined.