

# Enhanced Hydrocarbon Bioremediation Through the Addition of Humic Substances

L. E. Liem and R. M. Facey (Hood Environmental, Sherwood Park, Alberta)

I. Carrigan, J. Blyth, M. Fischer, and A. Henderson (Luscar Specialty Products Division, Edmonton, Alberta)

## **Abstract**

Laboratory experiments to simulate land treatment were conducted to investigate practical aspects of utilising humic substances on the biodegradation of hydrocarbons in soil. A high percentage clay soil was contaminated with diesel to a maximum of 1% by weight. Soluble, powder, and granular humic materials were added between dosages of 0 to 5% by weight and evaluated for its impact on the quality of soil and hydrocarbon biodegradation. The experiments were conducted at room temperature. Aeration was provided through tilling once every week. Soil moisture contents were adjusted weekly to maintain around 50% by weight. The experiments were conducted over 12 weeks period.

Soil samples were taken periodically and analysed for moisture content, particle size distribution, pH, total nutrients, heterotrophic plate count (HPC) bacteria, and total extractable hydrocarbons (TEH). Results of the study revealed that the addition of humic substances increased soil moisture contents up to 21% and improved the soil particle size distribution by reducing percentages of 4 mm or larger soil aggregates by up to 28%. pH was maintained to near neutral to represent a more optimal condition. Carbon to nitrogen (C:N) values were improved to 17:1, or 2.4 times higher than the control. HPC bacteria growth trends were found to follow available nutrients in soil, i.e. humic substances and TEH.

Most importantly, humic substances enhanced TEH biodegradation significantly. Following 12 weeks of treatment, 4,700 and 8,800 mg/kg initial TEH values in soil were reduced to Tier I level at 800 mg/kg by applying humic material between 1 to 3% dosages. These represented up to 128% enhancement over the controls. Tier I level could also be achieved at shorter periods of 9 weeks or less by utilising 5% humic material.

## **Introduction**

The benefits of humic substances are well documented on improving the quality of soil and water ecosystems (Christman and Gjessing, 1983). While widely used in the agricultural industry, humic substances have also been applied in other sectors. These include drilling fluid (Wrightsmann, 1951), waste treatment (Manahan, 1989), and hydrocarbon bioremediation (Lesage et al., 1995 and Roy F. Watson, Inc., 2002). As well, the application of humic substances in conjunction with other soil additives in hydrocarbon bioremediation has an excellent potential market value.

Laboratory experiments of bioremediation were conducted to simulate land treatment at ambient temperature, in which diesel (0.5 to 1.0% concentrations by weight) was used as the hydrocarbon contaminant. Humic materials at 0 to 5% concentrations by weight were added. Once every week, the soil was tilled and the

moisture contents were adjusted to its optimum condition at around 50% by weight. Hydrocarbon and other supporting parameters including moisture content, particle size distribution, pH, heterotrophic plate count bacteria, and nutrients were monitored regularly during 12 weeks of treatment.

The principle objective of this work was to investigate practical aspects of utilising humic substances for enhancing hydrocarbon biodegradation in soil. Results obtained from these experiments could be used as a basis for the field application.

## **Hydrocarbon Guidelines and Treatment**

### ***Hydrocarbon Guidelines***

PHC are defined as mixtures of organic compounds found in or derived from geological substances. The compounds comprise of C<sub>6</sub> to C<sub>10</sub>, C<sub>10</sub> to C<sub>16</sub>, C<sub>16</sub> to C<sub>34</sub>, and > C<sub>34</sub> fractions, excluding benzene, toluene, ethyl-benzene, and

xylene (BTEX) components (CCME, 2001).

CCME (2001) has set guidelines for PHC in soil. Under Tier I levels, i.e. generic numerical levels without considering site-specific conditions, the guideline for diesel (which falls closely under C<sub>16</sub> to C<sub>34</sub> 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***

In bioremediation, hydrocarbons undergo microbial degradation to produce a stable end product of water and carbon dioxide. Compared to others, land treatment is one of the most reliable and economical alternatives with the least impact on the environment. This is an aerobic process performed in the upper soil zone, preferably at depths less than 300 mm (Eweis et al., 1998).

For optimum performance, the soil layer is tilled periodically to provide air circulation within the soil matrix and to allow better contact of microorganisms, nutrients, and contaminants (Fogel, 1994). Over tilling, however, will destroy soil aggregates. It is therefore conducted weekly or once every other week (U.S. EPA, 1993).

The soil is wetted to solubilise the contaminant to water phase that can be reached by microorganisms. Still, excessive moisture may result in the decrease of oxygen transfer. Most aerobic microorganisms operate optimally at moisture contents of 50 to 75% of soil's field capacity (US. EPA, 1985).

The growth of most microorganisms is usually at its best near pH of 7 (Cookson Jr., 1995). Most soil bacteria are mesophiles, which grow best from 15 to 45 °C (US. EPA, 1985). The optimum nutrients balance in terms of carbon to nitrogen (C:N) value has been reported at 50:1 (Genes and Cosentini, 1993). U.S. EPA (1995) suggests a smaller ratio of 20:1.

### ***Humic Substances***

Land treatment operations can be enhanced by improving the quality of soil through the

addition of humic substances. Brief characteristics of humic substances and how they can improve the quality of soil are described below.

Humic substances are complex organic materials that originate from the decomposition of plant and animal residues, but that do not fall into discrete compounds such as proteins, polynucleotides, and polysaccharides (McCarthy and Suffet, 1989). They are made primarily of carbon and oxygen (> 90% by weight), as well as hydrogen, nitrogen, and sulphur (Choudhry, 1984). They are yellow to black in colour, and have molecular weights from hundreds to hundred thousands (Malcolm, 1990).

The benefits of humic substances for bioremediation include providing available carbon to soil and maintaining neutral pH (Jackson, 1993). They also bind nonpolar hydrophobic compounds, such as hydrocarbons, increasing its solubility in water that can be reached by soil microorganisms (Guetzloff and Rice, 1994). Through the hydrogen bonding, these substances retain water on their surfaces and therefore help maintain the soil moisture content (Chen and Shnitzer, 1976). Humic substances have abilities to chelate various cations, resulting in the dissolution of clay structures (Kodama and Schnitzer, 1973). Circulation of air and moisture within the soil matrix will be improved, and better exposure of contaminant and nutrients to the microorganisms will be achieved. Humic substances also allow micronutrients to be retained near the surface of soil particles, benefiting the growth of soil microorganisms (McBride, 1981).

### **Materials**

#### ***Experimental Soil***

The experimental soil was loam type of soil with more than 60% clay content. Its moisture content, field capacity, and porosity were 23, 85, and 50%, respectively. pH was about neutral at 7.3 and the C:N value was 7:1. Detailed properties of the soil can be found in Table 1.

**Table 1: Soil Properties**

Parameter	Unit	Value
Moisture content	%	23
Field capacity	%	85
Porosity	%	50
Particle density	kg/m <sup>3</sup>	1,600
Bulk density	kg/m <sup>3</sup>	1,000
Texture	-	loam
Particle size		
Sand	%	15
Silt	%	22
Clay	%	63
Metals		
Aluminum	mg/kg	18,300
Barium	mg/kg	206
Calcium	mg/kg	10,700
Iron	mg/kg	24,800
Potassium	mg/kg	2,580
Magnesium	mg/kg	5,900
Manganese	mg/kg	530
Sodium	mg/kg	300
Phosphorous	mg/kg	630
Zinc	mg/kg	80
Other metals	mg/kg	< 90
Available Nutrients		
Nitrate	mg/kg	7
Phosphate	mg/kg	6
Potassium	mg/kg	331
Sulfate	mg/kg	126
Total Nutrients		
Carbon	%	2.4
Nitrogen	%	0.34
Phosphorous	mg/kg	710
Sulphur	mg/kg	100
Basic Salinity		
pH	-	7.3
Conductivity	ds/m	1.7
SAR	-	1
HPC bacteria	CFU/g	1,100

### ***Humic Substances***

Humic substances used in these experiments originated from the oxidation of parent materials adjacent to sub-bituminous coal. Soluble, powder, and granular humic materials were tested to investigate their effects on the quality of soil and biodegradation of hydrocarbons, as well as their economy when applied on the field. Important properties of these materials are summarised in Table 2 and described as follows (Luscar, 2002).

The soluble humic material contained 55% organic matter and had pH values in the range of 7.0 and 7.4. More than 80% of this material had particle sizes of 75 microns or less, while the rest sized between 75 and 180 microns. This soluble humic material had a bulk density around 800 to 900 kg/m<sup>3</sup>.

The powder humic material contained more than 80% organic matter, higher than the soluble material. This material had acidic pH values between 3.5 to 4.1. More than 70% of this material had particle sizes of 75 to 180 microns, while the rest sized 75 micron and less.

The granular humic material had the same chemical compositions as those of the powder material. The difference was that more than 70% of this material had particle sizes between 850 and 1,400 microns. About 20% of the particles sized between 180 to 850 microns. Smaller particles (180 microns or less) comprised at less than 10%.

### ***Contaminant***

Diesel was used as the hydrocarbon contaminant due to its low evaporation rate at room temperature. A boiling point analysis from 36.1 to 503.0 °C (C<sub>5</sub> to C<sub>36</sub>) showed that 97% carbon components were in the forms of C<sub>7</sub> to C<sub>22</sub>. It was also found that at temperatures 36 to 69 °C, only 0.1% diesel would be lost. At room temperature (20 ± 0.5°C), the evaporation was expected to be smaller.

### ***Experimental Water***

Distilled water was used to wet the soil matrix. In order to ensure minimum chlorine residuals, the water was aerated for a day.

### ***Testing Apparatus***

The land treatment was 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.

## **Methods**

### ***Experimental Methods***

The experimental soil was thoroughly mixed with diesel at 0.5 and 1% concentrations by dry weight. The mixing was conducted in a cement mixer to achieve homogeneous mixture. The soil mixture was dosed with soluble, powder, and granular humic materials at different concentrations, from 0 to 5% by soil dry weight. The larger dosages were designed to cover a wider range of treatment performances.

Moisture contents of the soil were adjusted to between 50 to 60% by weight, to achieve the optimum moisture conditions at 50 to 75% field capacity. The soil was then placed inside the steel column at 300 mm depth.

The experiments were conducted inside a closed room all the time. Exposure to light was minimal and room temperature was kept constant at  $20 \pm 0.5$  °C. The soil was tilled and wetted manually every week. Moisture contents were adjusted to maintain around 50% at the end of the week. Moisture loss due to a flow through within the soil matrix was negligible. The soil temperature was recorded at  $17 \pm 0.5$  °C.

The design of experiments is summarised in Table 3. Experiments nos. 1 to 4 represented controls. Experiments nos. 5 to 12 were designed to investigate the effect of humic substances on the biodegradation of hydrocarbons in soil. Experiments nos. 13 to 16 were conducted to compare the performance of three different humic materials on the biodegradation of hydrocarbons in soil.

**Table 3: Design of Experiments**

Test No.	Diesel (%)	Humic Material (%)		
		Soluble	Powder	Granular
1	0	1	0	0
2	0	5	0	0
3	0.5	0	0	0
4	1.0	0	0	0
5	0.5	1	0	0
6	0.5	2	0	0
7	0.5	3	0	0
8	0.5	5	0	0
9	1.0	1	0	0
10	1.0	2	0	0
11	1.0	3	0	0
12	1.0	5	0	0
13	1.0	0	1	0
14	1.0	0	5	0
15	1.0	0	0	1
16	1.0	0	0	5

### ***Monitoring and Sampling***

Moisture contents before and after the wetting were measured. Three readings were taken and each average value was recorded. After moisture adjustments, a small volume of soil was sampled, dried, and tested for particle size distribution.

Once every three weeks, soil samples were taken and analysed for pH, conductivity, total nutrients (carbon, nitrogen, sulphur, and phosphorous), HPC bacteria, and total extractable hydrocarbons (TEH). TEH values represented carbon fractions of C<sub>11</sub> to C<sub>30</sub>, which fall closely under C<sub>16</sub> to C<sub>34</sub> fractions as defined by CCME (2001).

### ***Measurement Methods***

Soil moisture content was measured using a portable moisture probe. The results were verified using the gravimetric method. The errors were small at 5% maximum. Particle size distribution analysis was conducted using a standard Tyler sieving apparatus (sieve openings 45 to 4,000 microns) for 15 minutes. All of these were conducted on-site.

Other parameters were measured off-site in a commercial laboratory. These include pH, conductivity, total nutrients (carbon, nitrogen, sulphur, and phosphorous), HPC bacteria, and TEH. It is important to note that TEH was

analysed using EPA 3550/8000-GC-FID method (EPA, 1996), in which hexane was used as solvent. Interferences from other organic compounds were minimised (Roy F. Weston, Inc., 2002). If any, they were eliminated by referring to the control's TEH values. Table 4 summarises measurement methods for parameters of interest.

### **Results and Analysis**

Presented results include soil moisture content, particle size distribution, pH, and C:N values. HPC bacteria and TEH profiles are also presented. Finally, performances of three different humic materials in terms of hydrocarbon biodegradation are compared.

Table 5 summarises soil moisture contents before and after weekly adjustments in 12 monitoring weeks. For each treatment, no significant differences were found from one week to another. Diesel dosages of 1 and 5% were not found to affect the moisture content. Average values for all weeks and their standard deviations (in brackets) are presented.

The table shows that soil moisture contents were adjusted to 60 to 70% and decreased at the end of the week to 48 to 56% as the targeted moisture content. The moisture retaining ability was also presented, which was the moisture ratio before and after adjustment. The table indicates that the addition of humic substances improved the soil moisture retaining ability up to 87%, or 21% higher than the control.

This table also indicates that the soluble humic material performed the best by increasing the moisture retaining ability to 82 and 87% at 1 and 5% dosages, respectively. The powder humic material performed well at a higher dosage of 5%, increasing the moisture retaining ability to 85%. At 1% dosage, the performance was not significantly different from that of the control. The granular humic material at any dosages did not show any significant improvement over the control.

**Table 5: Moisture Contents**

Soil Mixture	Before Adjust-ment (%)	After Adjust-ment (%)	Moisture Re-taining (%)
Soil	66(8.4)	48(11.4)	72(11.7)
Soil+soluble (1%)	63(9.3)	51(11.6)	82(11.5)
Soil+soluble (5%)	60(6.7)	53(8.4)	87(9.2)
Soil+powder (1%)	67(4.0)	50(4.7)	74(6.8)
Soil+powder (5%)	66(6.1)	56(5.8)	85(4.5)
Soil+granular (1%)	67(8.5)	48(4.1)	73(7.5)
Soil+granular (5%)	70(9.0)	53(6.7)	77(8.1)

The experiments found that the addition of humic substances reduced the percentage of larger soil aggregates, allowing better air and moisture circulation within the soil matrix, as well as improving the exposure of contaminant and nutrients to the microorganisms. Soil tilling also became easier with the addition of humic substances.

Percentages for particle sizes 1.4, 2.4, and 4 mm are presented in Table 6. For each treatment, no significant differences were found from one week to another. Average values during all monitoring weeks and their standard deviations are presented.

Table 6 indicates that the soluble humic material performed the best. Percentages of 4 mm soil aggregates or larger were reduced from 87 to 80 and 68% (9 and 28% reductions) with the addition of 1 and 5% of materials, respectively. At 1% dosage, both powder and granular humic materials did not show any improvements over the control. At 5% dosage, the powder humic material reduced the percentage of 4 mm soil aggregates or larger from 87 to 79%, representing 10% reduction. No significant improvement was observed when the granular humic material was utilised.

**Table 6: Particle Size Distribution**

Soil Mixture	Particle Size Distribution (%)		
	4 mm	2.4 mm	1.4 mm
Soil	87(3.7)	4(1.7)	2(0.7)
Soil+soluble (1%)	80(2.8)	9(2.5)	3(0.8)
Soil+soluble (5%)	68(2.7)	15(4.7)	8(1.6)
Soil+powder (1%)	88(2.6)	5(0.8)	2(0.3)
Soil+powder (5%)	79(7.6)	10(4.9)	4(1.5)
Soil+granular (1%)	87(10.8)	5(5.4)	3(2.3)
Soil+granular (5%)	85(5.2)	6(2.1)	3(1.0)

The addition of humic substances also decreased the soil pH to neutral and increased the C:N values. Table 7 shows pH and C:N values for the initial (0<sup>th</sup>) week. The table shows that significant reductions of pH were observed especially when granular and powder humic materials were added. Humic substances increased C:N values up to 140%, in which all humic materials performed almost similarly.

**Table 7: pH and C:N Ratio (Initial Week)**

Soil Mixture	Initial Week Values	
	pH	C:N
Soil	7.6	7
Soil+soluble (1%)	7.4	9
Soil+soluble (5%)	7.2	14
Soil+powder (1%)	7.2	10
Soil+powder (5%)	7.0	17
Soil+granular (1%)	7.0	10
Soil+granular (5%)	7.0	16

The experiments found that the growth of heterotrophic bacteria was a function of the available nutrients in soil, i.e. humic substances and TEH concentrations. Table 8 shows HPC bacteria data for the 3<sup>rd</sup> monitoring week when the soluble humic material was added. Initial TEH values were average readings for each set of experiments. Lower growths were observed for subsequent weeks due to reduced TEH in soil. Similar patterns were also found when granular and powder humic materials were utilised.

**Table 8: HPC Data (3<sup>rd</sup> Week)**

Humic Dosage (%)	Initial TEH (mg/kg)	Millions HPC (# / g)
0	4,700	10.9
	8,800	17.0
1	4,700	45.5
	8,800	101.5
3	4,700	110.5
	8,800	290.0
5	4,700	160.0
	8,800	325.0

Most importantly, the experiments found that the addition of humic substances enhanced the biodegradation of hydrocarbons in soil. Table 9 shows that Tier I level of 800 mg/kg could be achieved following 6 to 9 weeks of treatment from the initial TEH of 4,700 mg/kg by adding

the soluble humic material at 5% dosage. At 1% dosage, Tier I level could be achieved following 9 and 12 weeks of treatment. At the higher initial TEH level of 8,800 mg/kg, 5% humic dosage would result in TEH levels satisfying Tier I level following 9 to 12 weeks of treatment.

Table 9 also indicates that the soluble humic material at 1 to 3% dosages would achieve Tier I level for both initial TEH values of 4,700 and 8,800 mg/kg following 12 weeks of treatment. These represented up to 94% TEH reduction and 128% enhancement over the controls.

**Table 9: TEH Biodegradation**

Humic Dosage (%)	TEH (mg/kg)				
	Initial Week	3 <sup>rd</sup> Week	6 <sup>th</sup> Week	9 <sup>th</sup> Week	12 <sup>th</sup> Week
0	4,700	4,500	3,900	3,500	3,300
	8,800	8,200	7,200	5,900	4,100
1	4,700	4,000	2,700	2,500	1,200
	8,800	8,000	4,700	2,800	2,000
3	4,700	4,200	2,900	1,000	600
	8,800	7,200	5,300	1,800	600
5	4,700	3,300	1,300	700	400
	8,800	5,800	3,500	1,100	700

Figure 1 shows the performances of granular and powder humic materials in terms of hydrocarbon biodegradation relative to that of the soluble humic material. It was found during each monitoring week that the ratio was almost constant. The figure presents average values for all monitoring weeks. As indicated in the figure, at 5% dosage, all humic materials performed almost similarly. At 1% dosage, powder and soluble humic materials performed similarly and better than granular humic material.

This finding suggests that as long as adequate tilling was provided and the optimum moisture content was maintained, powder and soluble humic materials would perform similarly.

### **Conclusions and Recommendations**

Humic substances enhanced soil water retention up to 21% higher than the control, resulting in less moisture adjustments. Humic substances also improved the soil particle size distribution.

Percentages of 4 mm soil aggregates or larger were reduced by up to 28%. Smaller soil aggregates allowed better circulation of air and moisture within the soil matrix. The exposure of contaminant and nutrients to the microorganisms was improved. Soil tilling would also become easier.

Humic substances maintained soil pH values close to neutral. Humic substances also increased carbon to nitrogen (C:N) values up to 17:1, or 2.4 times higher than the control. These factors also benefited the growth of the microorganisms. Heterothropic plate count (HPC) data showed that the microorganisms growth followed available nutrients in soil, i.e. humic substances and total extractable hydrocarbons (TEH).

Initial TEH values of 4,700 and 8,800 mg/kg could be lowered to Tier I level of 800 mg/kg or less following 6 to 9 weeks and 9 to 12 weeks of treatment by adding humic material at 5% dosage by weight, respectively.

After 12 weeks of treatment, initial TEH values of 4,700 and 8,800 mg/kg were reduced to Tier I level or less by utilising humic material between 1 to 3% dosages. These represented up to 128% enhancement over the controls.

Before applying results obtained from these experiments to the field, a specific site investigation is recommended. Parameters to be investigated include type of soil and topography, local climate, type and concentration of contaminant, available tilling and wetting equipment, and usage of the land.

A field pilot scale test is also recommended to confirm findings obtained from these experiments. In the field, humic material and water can be mixed before being applied on the field. Tilling can be done using front-end loaders, which has been identified as the most economical tilling equipment (Cookson Jr., 1995). The pilot test will also include the determination of the total cost required to complete the bioremediation work.

## References

- \_\_\_\_\_. 2001. Canada Wide Standards for Petroleum Hydrocarbons (PHCs) in Soil. Canadian Council of ministries of the Environment. Winnipeg, MB.
- \_\_\_\_\_. 1993. Bioremediation using the Land Treatment Concept, EPA 600/R-93/164. U.S. EPA.
- \_\_\_\_\_. 1995. Remediation Case Studies: Bioremediation, EPA 542/R-95/002. U.S. EPA.
- \_\_\_\_\_. 1996. EPA 3550/8000-GC-FID.
- \_\_\_\_\_. 1996. Health Protection Branch-Method MFHPB18.
- \_\_\_\_\_. 1996. Methods of Soil Analysis: Part 3 Chemical Methods. 3<sup>rd</sup> edition. J. M. Bartels et al, editors. ASA and SSSA, Madison, WI.
- \_\_\_\_\_. 1998. Standard Methods for the Examination of Water and Wastewater. 20<sup>th</sup> edition. L. S. Cleserci et al., editors. APHA, AWWA, WEF, Washington, DC.
- \_\_\_\_\_. 2002. Black Earth Technical Data Sheet. Luscar Ltd., Edmonton, AB.
- \_\_\_\_\_. 2002. Remediation of Diesel and Fuel Hydrocarbons in High Clay Content Soil: A Field Comparison of Amendment Performance Conducted at the Mare Island Naval Shipyard. Technical Report. Roy F. Weston, Inc., Vallejo, CA.
- Chodhry, G. G. 1994. Humic Substances, Structural, Photophysical, Photochemical, and Free Radical Aspects and Interactions with Environmental Chemicals. Gordon and Beach Science, New York, NY.
- Cookson Jr, J. T. 1995. Bioremediation Engineering Design and Application. McGraw-Hill, Inc., New York, NY.
- Christman, R. F. and Gjessing, E. T. 1983. Aquatic and Terrestrial Humic Materials. The Buterworth Grove, Kent, England.
- Eweis, J. B., Ergas, S. J., Chang, D. P. Y., and Schroeder, E. D. 1998. Bioremediation Principles. McGraw-Hill, New York, NY.
- Fogel, S. 1994. Full-Scale Bioremediation of No. 6 Fuel Oil-Contaminated Soil: 6 Months of Active and 3 Years of Passive Treatment. (In) Bioremediation Field Experience. P. E. Flathman et al., editors. Lewis Publishers, Chelsea, MI, pp. 161 – 175.
- Genes, B. R. and Cosentini, C. C. 1993.

Bioremediation of Polynuclear aromatic Hydrocarbon Contaminated Soils at Three Sites. (In) Hydrocarbon Contaminated soils, Vol. III. E. J. Calabrese and P. T. Kostecki, editors. Lewis Publishers, Chelsea, MI, pp. 323 – 331.

Guetzloff, T. F. and Rice J. A. 1994. Does Humic Acid Form a Micelle? The Science of the Total Environment. 152: 31 – 35.

Jackson, W. R. 1993. Humic, Fulvic, and Microbial Balance: Organic Soil Conditioning. Jackson Research Center. Evergreen, CO.

Kodama, H. and Schnitzer, M. 1973. Dissolution of chlorite minerals by fulvic acid. Canadian Journal of Soil Science. 53: 240 – 243.

Lesage, S., Li, W-C., Millar, K, Brown, S., and Liu, Dickson. Effect of Humic Acids on the Bioremediation of Polycyclic Aromatic Hydrocarbons from Aquifers Contaminated with Petroleum. Applied Environmental Microbiology. 54, 1864-1867.

MacCarthy, P. and Suffet, I. H. 1989. Aquatic Humic Substances and Their Influence on the Fate and Treatment of Pollutants. (In)

Aquatic Humic Substances: Influence on Fate and Treatment of Pollutants. I. H. Suffet and P. MacCarthy, editors. American Chemical Society, Washington, DC, pp. xvii – xxx.

Malcolm, R. L. 1990. The Uniqueness of Humic Substance in Each soil, Stream, and Marine Envrionments. Analytica Chimia Acta. 232, 19 – 30.

Manahan, S. E. 1989. Interactions of Hazardous-Waste Chemicals with Humic Substances. (In) Aquatic Humic Substances: Influence on Fate and Treatment of Pollutants. I. H. Suffet and P. MacCarthy, editors. American Chemical Society, Washington, DC, pp. 83 – 92.

McBride, M. B. 1981. Forms and Distribution of Copper in Solid and solution Phase of Soil. (In) Copper in Soils and Plants, J. F. Loneragan, A. D. Robson, and R. D. Graham, editors. Academica, New York, NY.

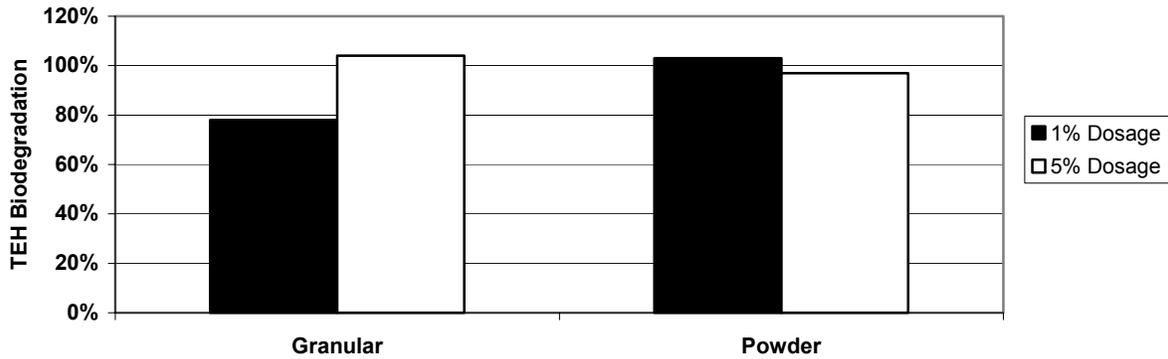
Wrightsmann, G.G. 1951. Improved Drilling Mud Composition. U. S. Patent No. 2,560,380.

**Table 2: Properties of Humic Materials**

Parameter	Unit	Soluble	Powder	Granular
Organic matter	%	80	> 80	> 80
Moisture	%	5 to 10	30 to 35	30 to 35
pH	-	7.0 to 7.4	3.5 to 4.1	3.5 to 4.1
Colour	-	Brown to black	Brown to black	Brown to black
Bulk density	kg/m <sup>3</sup>	866 to 965	790 to 840	790 to 840
Particle size	micron	850 to 1,400 (0%) 180 to 850 (0%) 75 to 180 (17%) < 75 (83%)	850 to 1,400 (0%) 180 to 850 (0%) 75 to 180 (72%) < 75 (28%)	850 to 1,400 (71%) 180 to 850 (22%) 75 to 180 (3%) < 75 (4%)

**Table 4: Measurement Methods**

Parameter	Methods	References
Moisture content	1) Direct measurement 2) Oven dry @105 °C gravimetric	- APHA, AWWA, WEF (1998)
Particle size	Sieve analysis	-
pH	Soil water extraction	APHA, AWWA, WEF (1998)
Conductivity	Soil water extraction	APHA, AWWA, WEF (1998)
Total carbon	Total combustion	ASA and SSSA (1996)
Total nitrogen	Total combustion	ASA and SSSA (1996)
Total sulphur	ICP - HNO <sub>3</sub> / HClO <sub>4</sub> digestion	ASA and SSSA (1996)
Total phosphorous	ICP - HNO <sub>3</sub> / HClO <sub>4</sub> digestion	ASA and SSSA (1996)
TEH	Chromatogram - hexane solvent	EPA 3550/8000-GC-FID (1996)
HPC bacteria	Plate count	Health Protection Branch-Method MFHPB18 (1998)



**Figure 1: TEH Biodegradation Performance Relative to Soluble Humic Material**