Bio-Remediation

An in-situ solution to petroleum contaminated soils.

A natural solution that saves time AND money!

From This

To This

In 60-180 days!

Presented by:

The ARK Enterprises, Inc.
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INTRODUCTION

Bio-remediation is the act of using natural life (bio) to break down and remediate a contaminated site, surface or object. Restoring a surface or object to a condition which is no longer hazardous to the environment, is the prime objective of this process.

Using microorganisms and special enhancement solutions to break down the hydrocarbon pollutant is the newest and most successful technology being utilized in this process. There are many different methods accepted in these processes. However, biodegration is the preferred system in most cases. The related system can be categorized as follows:

BIODEGRATION: The degrading or chemical breakdown of a substance or compound that's using living microorganisms.

BIORESTORATION: The restoring of an object or site to a biologically suitable condition similar to it's original state by the utilization of natural microorganisms. Nature in itself acts as a built in check and balance system. Within time (years), nature will usually create a defensive mechanism (microbial) to re-establish a natural balance.

BIOSTIMULATION: The act of physically manipulating a contaminated site to enhance the growth of existing or added microorganisms. Nature in time will populate an area with microorganisms to start a natural remediation process. This process is drastically enhanced during properly engineered biostimulation.

BIOAUGMENTATION: This is the utilization of specific living forms to enhance or augment the natural processes taking place.

The major benefit of bio-remediation is transformation and not transportation. The cost of transformation is lower and requires little or no specialized equipment. Bio-remediation is the newest technology in remediating hydrocarbon contamination in an environmentally conscious world.

The bioremediation services offered by The ARK Enterprises, Inc. Technologies,Inc. are a combination of biorestoration, biostimulation and bioaugmentation. The results are faster and more efficient remediation than other methods. This enhanced approach can achieve dramatic results in relatively short periods of time.

THE DIFFERENCE

**Save 30-50%
in actual costs**

Complete remediation in as few as 60 days**
Depending on level of contamination and weather

Requires less: labor, heavy equipment, machinery

Does not deplete the soil of it's natural enzymes and bacterias which allows for for faster and more complete remediation

HOW DOES IT WORK?

Throughout the past twenty-five years, many technologies have been introduced to help with the remediation of petroleum contaminated soils.

The major benefit of bio-remediation is transformation and not transportation. The cost of transformation is lower and requires little or no specialized equipment. Bio-remediation is the newest technology in treatment of hydrocarbon contamination in an environmentally conscious world.

The bioremediation services offered by The ARK Enterprises, Inc. are a combination of biorestoration, biostimulation and bioaugmentation. The results are faster and more efficient remediation than other methods. This enhanced approach can achieve dramatic results in relatively short periods of time.

The first stage of this technology includes the introduction of a proper mix of "activated" peat moss and nitrates to target the contaminates. After initial application and tilling, the process now requires monitoring and repeated tilling and/or watering, depending on the weather conditions. The "activated" peat, has been dried to create voides in the capillaries which will only absorb hydrocarbons. Once encapsulated, acceleration of the breakdown of the hydrocarbon is increased in part due to the added surface area and exposure to oxygen of the hydrocarbons.

The activated peat technology was researched by the Federal Government (FORSCOM) using a bio-cell (landfarming) approach with the omission of repeated watering and tilling. The study showed that the activated peat technology reduced TPH-GRO contaminations from 615 ppm to 10 ppm (81%), within 84 days. Similarly, the "no frills" treatment reduced TPH-DRO contaminations on a second site from 494 ppm to 56 ppm in the same amount of time. The purpose of this study was to research and find bioremediation methods that would dramatically reduce their costs. The comparative costs, including the construction of two biocells have been determined to result in an annual savings of 60% over soil safe or chem waste disposalalternatives (from $209,900 or $242,000 to an annual cost of $66,800). Permanent implementation of this technology has been approved for this Federal Facility to begin in 2002.

The The ARK Enterprises, Inc. services would include the P.O.L. Sorb peat moss technolgy with the option of utilizing the addition of specific enzymes enhance the performance of the activated peat. Acting as a "host" to the cultured enzymes, P.O.L. Sorb provides optimum absorption of the hydrocarbons in its capillaries, while preserving the natural occuring bacteria in the soil. The enzymes have the added protection of the peat, which allows them ample access to oxygen so that they do not die off. In addition, while the peat moss is providing accelerated oxygenation to the hydrocarbons for faster breakdown, the enzymes are assisting by the conditions that are created when using activated peat. These technologies are available as separate services or as a combined service.

The combination of the two technologies results in a very efficient and effective "team" that preserves the natural bacterias in the soil, and result in faster turnaround time to restore the contaminated soil into useful land.

can be used to absorb:
All Petroleum, Oils & Lubricants

P.O.L. Sorb® is all natural, 100% organic, non-toxic, lab-tested, field proven, industrial absorbent that is economical, efficient, non-abrasive, non-leaching and in its natural state is already biodegraded.

With P.O.L. Sorb You Will:
Use Less Product
Absorb More Liquids
Dispose Of Less Product
Save Time
Save Money
Save Labor
and
PROTECT THE ENVIRONMENT because you are using **ENVIRONMENTALLY RESPONSIBLE PRODUCTS!**

P.O.L. Sorb® provides superb absorbent solutions for land and off shore applications. The inherent capillary action of the activated peat provides a powerful wicking action that absorbs and encapsulates oils, solvents, heavy metals, pesticides, herbicides and all other organic chemicals on contact.

P.O.L. Sorb® absorbents provide cost effective and environmentally intelligent clean up and restoration solutions.

P.O.L. Sorb:

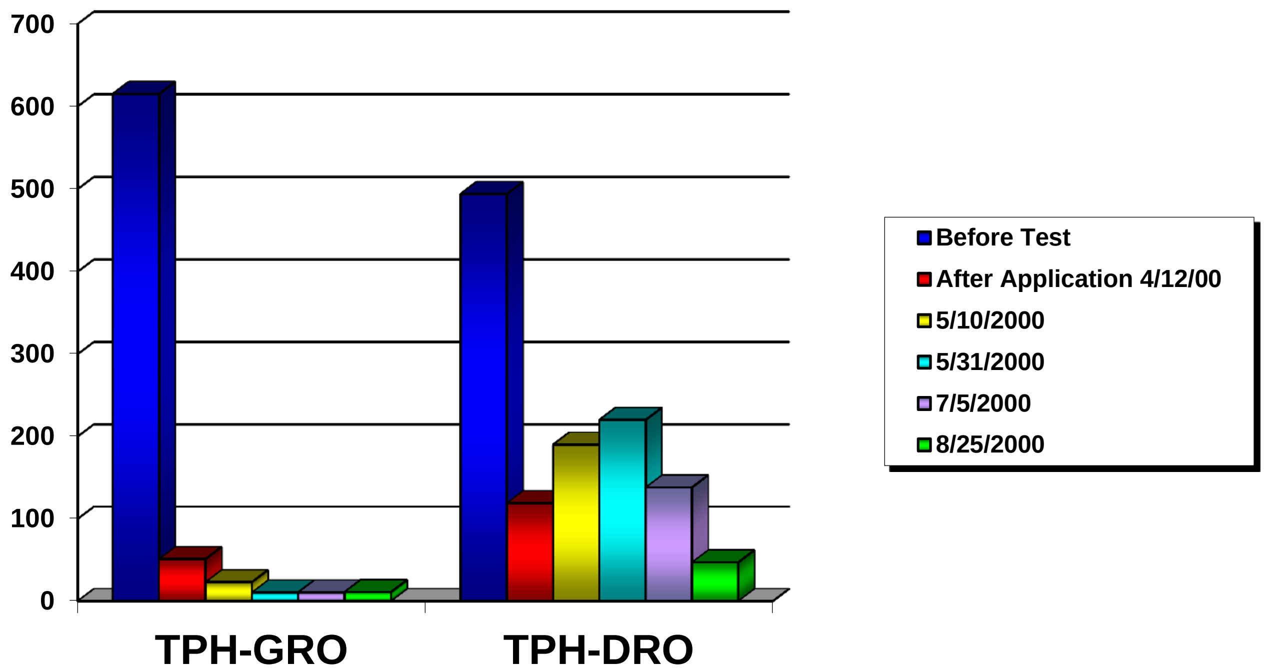
Meets EPA & OSHA & ANSI standards
Is excellent for spill response and bioremediation
Will not leach: passes TCLP test
Offers High BTU value for incineration
Safely cleans oil from any surface
Leaves no oily sheen or residue
Saves time and money
Reduces waste
Requires only One Pound per Gallon!
Saves time, money and labor before, during and after spills!

P.O.L. Sorb® is ideal for:

- Maintenance areas
- Parking Areas
- Refueling Areas
- HM/HW Storage & Collection Points
- HazMat Pharmacies
- Contingency or Deployment Operations
- Bioremediation
- Water Filtration
- Waterways
- Peat Over Sand Sewage Filtration
- Industrial Wastewater Treatment Plants
- Vehicle Repair Shops
- Ports/Marinas
- Open Sea Oil Spill/Shoreline Cleanup
- UST Removal Sites

Test Results from Hunter Army Airfield Pilot Study

Biocell #1 is silty sand clay with gasoline contamination
Biocell #2 is silty sand with diesel contamination



Cell 1 Summary:

TPH-GRO from 615 mg/kg to 10 mg/kg (98% reduction) by day 84
Benzene from .458 to .001 ug/kg (99% reduction) by day 84
Toluene from .15 to .003
Ethylbenzene from 2.675 to .009
Xylenes from 5.133 to .014

Cell 2 Summary:

TPH-DRO from 494 mg/kg to 47 mg/kg (90% reduction) by day 84
Benzene from .458 to .001 ug/kg (99% reduction) by day 84
Toluene from .15 to .003
Ethylbenzene from 2.675 to .009
Xylenes from 5.133 to .014

Test Results from Venezuela Test

BIOREMEDIATION WITH THE ACTIVATED PEAT MOSS PRODUCT IN A SAVANNA SOIL AFFECTED BY SPILLED PETROLEUM

Cermeno Jose (•); Ortiz, Elba (•); Martinez, Pedro (• •); Bastardo, Mugel (• •)

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ABSTRACT

The preservation of environment is fundamental in order to get a self sustainable development, by keeping a harmonic relationship between oil exploration and environmental equilibrium. This is a necessary condition in order to share the oil production with other economic activities like agriculture and cattle production. In reality, the oil production is the most important factor in the national income of Venezuela; and, the oil is a strategic energy source in our modern industrial process. Therefore, it is particularly significant that all the attention be paid in protecting the environment in the course of the oil production process. The objective of this study was to evaluate the bioremediation of a soil affected by an oil spill, using direct applications of Activated Peat Moss in the soil. During the investigation, cow manure was used as a bioindicator. The results of this study demonstrated that the germination level was the highest using 1875 kg/ha of Activated Peat Moss and 14 days of incubation. Further the contamination of oil greatly reduces the native microbiological activity of soils; and the Activated Peat Moss product improves this activity and provides a clear contribution in the soil's biological reactivation, reducing the pollution levels in 88% (TPH) in terms of 8.45% 14 days after application of Activated Peat Moss versus 85.2% after the initial spill. The practices of mechanization, application of the fertilizer and Activated Peat Moss improves the physical, chemical, and biological conditions of the soils affected by the oil spill.

The bioremediation technique used in this study involves tilling a layer of the soil between 4 to 6 inches, and turning of the soil using farming equipment (raking, plowing, tilling) to promote the oxidation and partial mechanical rupture of the compound. Fertilizers and water were added later to stimulate the biodegradation process. Some native microorganisms (located in the native soil) with the capacity to metabolize contaminated substances and its growth were stimulated in place in order to increase the biodegradation activity.

The biodegradation of hydrocarbons by native or introduced populations of microorganisms is an effective mechanism in the removal of contaminants from the environment which has been recognized as a recent viable technology (Scott and Baker, 1995).

The goal of this study was to determine effective treatment of contaminated soil from the spill of crude oil by adjusting the critical environmental factors such as: type and pH of the soil, humidity of the soil, oxygenation, administration of necessary nutrients, temperature, etc. with the purpose of increasing the microbial activity and the biodegradation of hydrocarbons.

Methods and Materials

1. Agro-Ecological description of the area in which the investigation was done in a soil with the following characteristics:
   a) Predominant Vegetation - opened field
   b) Life Zone - dried tropical forest
   c) Precipitation - 31 inches/year
   d) Temperature - 79 degrees Fahrenheit
   e) Vocational Use - agricultural
   f) Type of Soil - ultisol (clay, sand, and silt)

2. Description of the test
   a) The planting of bean (vigna unguiculata (L) walp) was used as a bioindicator with the purpose of evaluating the bioremediation capacity of the product ACTIVATED PEAT MOSS which was used in the soil affected by the crude oil spill.
   b) The statistical design of random blocks, with 10 treatments and 3 repetitions was used, and for the differences of averages the Bayes test at 95% probability was used.

3. Fertilization of the test
   a) The equivalent of 500 kg of the commercial formula of 12-24-12 and the agricultural classifier was applied at the time of the planting (based on soil analysis) which were incorporated on two different stages of tilling.

4. Planting and application of the product
   a) The planting of the bean was done in 3 stages and in the following order: at the time of application of the product, at 7 (seven) days later, and at 14 (fourteen) days later; after the application of the bioremediator ACTIVATED PEAT MOSS/HA.

5. Parameters evaluated - The following parameters were evaluated:
   a) % of germination
   b) Contents of fungi and bacteria present in the soil
   c) Contents of fungi and bacteria present in the ACTIVATED PEAT MOSS
   d) Types of fungi and bacteria present in the soil
   e) Types of fungi and bacteria present in the ACTIVATED PEAT MOSS
   f) Microbial activity of the soil before and after application of ACTIVATED PEAT MOSS
   g) Total content of hydrocarbons present (TPH) before and after the application of ACTIVATED PEAT MOSS

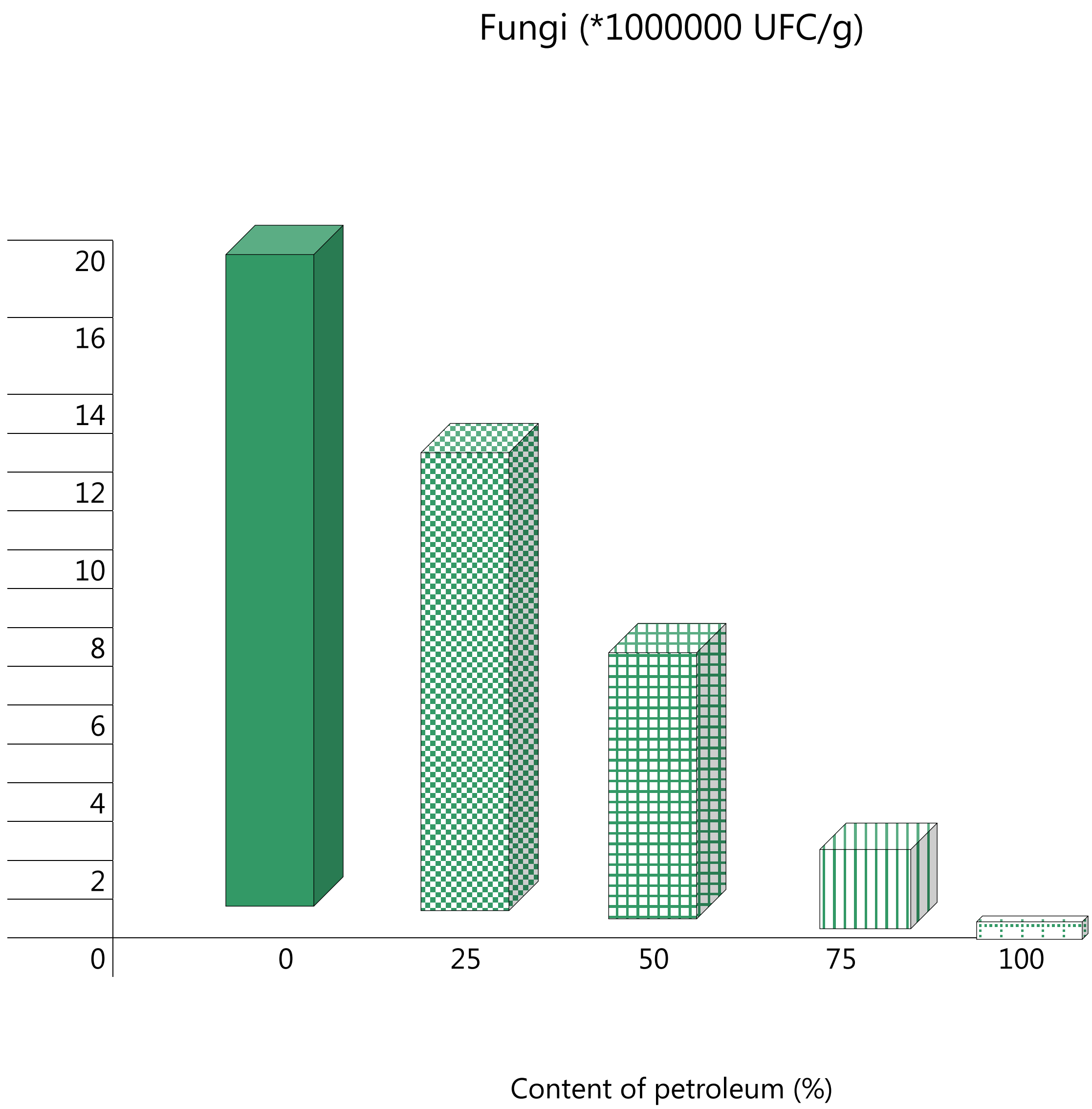

Figure:
Content of fungi in the soil contaminated by the spill of crude oil (petroleum)

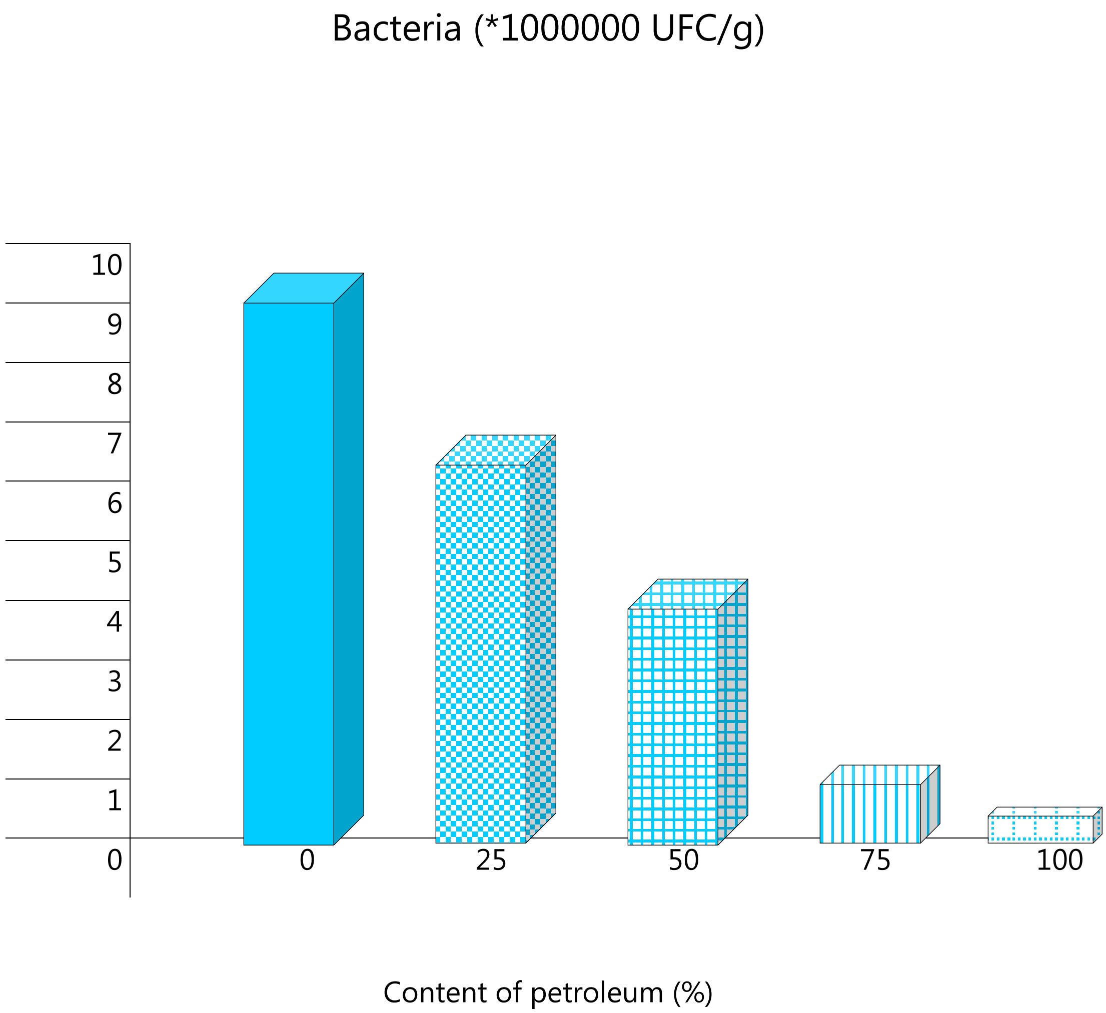

Figure:
Content of bacteria in the soil contaminated by the spill of crude oil (petroleum)

Results and Conclusions

1. The time of application of 14 days and the amount of 1875 and 2500 kg of ACTIVATED PEAT MOSS/HA showed the highest percentages of germination in the planting of the bean.

2. The high content of TPH present in the soil significantly reduces the contents and the growth of microorganisms present in the soil.

3. ACTIVATED PEAT MOSS contains a great variety and quantity of fungi and bacteria with a high capacity for the biodegradation of hydrocarbons.

4. The time of application of 7 and 14 days with the amount of 1875 kg of ACTIVATED PEAT MOSS/HA showed the highest content of fungi and bacteria present in the soil.

5. The time of application of 14 days and the amounts from 625 to 2500 kg of ACTIVATED PEAT MOSS/HA showed less content of TPH present in the soil with a significant reduction (of 88%) in comparison with the initial TPH present in the soil.

6. The product ACTIVATED PEAT MOSS is a good bioremediator when using 1875 kg/ha, and the ACTIVATED PEAT MOSS substantially increases the physical, chemical, and biological conditions of the soils affected by the spill of crude oil.

Test Results from South Africa Test

Picture 1. Old engine oil soaked into the ground at an Industrial site. The depth of oil was 6" /125mm

Picture 2. circa 3 hours later. Activated Peat Moss blended into soil at a ratio of 3 parts product to 1 part oil. Oil is immediately encapsulated locking up the spill & the odor.

Picture 3. After 3 months. Complete bio-remediation, Soil and Activated Peat Moss was tilled and nutrients added.

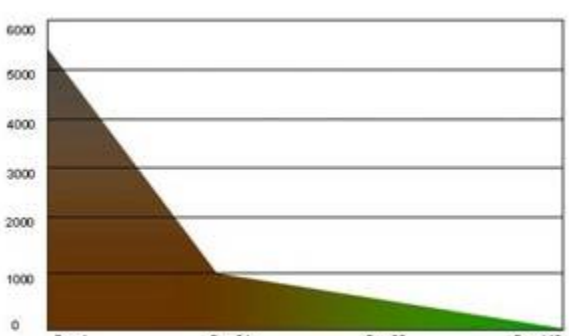

Chart showing average bio-remediation of hydrocarbons milligrams per kg over a 110 day period.

book on HUMIC ACIDS - Message (Plain Text)

File Edit View Insert Format PGP Tools Actions Help

Type a question for help

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From: Dr. Kim H. Tan [khtan@negia.net]

Sent: Wed 10/29/2003 4:45 PM

To: info@arkent.com

Cc:

Subject: book on HUMIC ACIDS

I read with interest in the web site your advertisement about your product POL-SORB, and was amazed about your wonderful explanations and illustrations. I couldn't agree more that Sphagnum peat has the adsorption capacity as described and is ENVIRONMENTALLY FRIENDLY. Its salient action is now traced not only to its enormous pore spaces, but also to its HUMIC ACID content, forming the bulk in Peat.

A book is now available on HUMIC and FULVIC ACIDs, providing complete coverage. Read all about the more Modern Concepts and Genesis of Humic Substances, their distribution worldwide in Terrestrial and Aquatic Ecosystems, and their isolation from SOIL, PEAT, BLACK WATER and LEONARDITE. Read about Differences in Composition, Chemistry and effect on BIODETOXIFICATION, REDOX or ELECTRON TRANSFER and PHOTOCHEMICAL REDUCTION of toxic metals and pollutants in soils. Examine the influence of Humic Substances on SOIL CONDITIONS,SOIL AMELIORATION, PLANT GROWTH, MICROBIAL ACTIVITY and HUMAN HEALTH. Study the Important role of Humic Matter in the Environment, Agriculture, Industry, Medicine and Pharmacy, AND MORE in:

Kim H.Tan. 2003. HUMIC MATTER IN SOIL AND THE ENVIRONMENT, PRINCIPLES AND CONTROVERSIES. Marcel Dekker Inc, 270 Madison Ave, New York, NY 10016, USA.

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Thank you,
Dr. Kim Howard Tan
Professor Emeritus

As environmental awareness is at an all time high, now, at a time when it is possible for our comparatively young industries to improve operations and reverse the trend of polluting our environment. This can be done at a reasonable cost, and without jeopardizing the financial stability or the health of our present and future generations.

P.O.L. Sorb effluent filtration:

Industrial wastes, untreated effluent from textile dye houses, metal plating, or battery manufacturing plants are high in colourants and heavy metal pollutants. Although colourants are generally biodegradable, special treatments are necessary to restore significant amounts of oxygen that are used up. Nature can also eliminate the heavy metals that are dumped into the environment during manufacturing processes, but it takes a long time for these to break down, and even the traces that are left can be highly toxic.

Chemicals treating chemicals is not the answer! They are costly, dangerous to handle, both before and after the treatment process, and are very difficult to safely dispose of.
Tests show that after just two passes of effluent through a mat of peat and water, the concentration of common transition metals was lowered to well below acceptable environmental limits for these toxic substances. Even though this peat would now be considered “polluted” it is completely safe to handle or store, and disposal presents no problem.

P.O.L. Sorb can be used as a cleansing agent. It can absorb 8–12 times its own weight and is able to remove or neutralize 95% to 100% of contaminants present in water without any specialized training, and won’t complicate the problem further by being hazardous to handle or difficult to dispose of.
Its unique cellular structure allows P.O.L. Sorb to absorb dyes and other colour compounds. Because of its chemical composition, P.O.L. Sorb can stabilize or neutralize these elements. With its ability to absorb through its porous exterior it can encapsulate, surround, and lock liquids and soluble solids into its gelatinous interior; thus virtually eliminating any chance of leaching when disposed of in landfill sites. Accepted current processes of purifying water using P.O.L. Sorb as the filter satisfies the stringent pollution control requirements of many well known governmental agencies including the United States Environmental Protection Agency. The federal government of Canada and affected provincial governments have also approved landfill as an acceptable disposal method for used peat.

This spent peat can also be burnt without any danger to the atmosphere. Companies doing research into water filtration using peat have found that the spent peat can continue to be used for horticultural purposes with excellent results. There is no danger of anything leaching out of the peat and contaminating ground waters.

Costs involved would depend on the degree of pollution, daily capacity, and other factors unique to each individual situation. The cost of P.O.L. Sorb as a natural resource is minimal. The technology is priced much lower than filtration processes now in place. P.O.L. Sorb has secured raw peat resources available for all current and anticipated uses to carry this company and its customers for well over a hundred years.

Test Results of Removal of Heavy Metal Pollutants using the Husson/Couplan Water Treatment System

METAL	EFFLUENT LIMIT	BEFORE	AFTER
Cyanide	0.03	36.00	0.03
Fluoride	18.00		
Aluminum	0.20	40.00	0.30
Barium	1.00		
Cadmium	0.10	25.00	0.10
Chromium +6	0.05	300.00	0.04
Chromium +3	0.25	300.00	0.25
Copper	0.20	250.00	0.20
Iron	0.50	31.50	0.25
Lead	0.05	8.40	0.03
Manganese	1.00		
Nickel	1.00	67.50	0.05
Silver	0.05		0.05
Zinc	0.05	7.50	0.08
Antimony		30.00	0.05
Mercury		15.00	0.01

Test Results of Removal of Pollutants (in addition to heavy metals) from a Sample of a Typical Dyehouse Effluent using the Husson/Couplan Water Treatment System

CHARACTERISTIC	BEFORE TREATMENT	AFTER TREATMENT
Colour Sample "A"	1250 APHA	65 APHA
Colour Sample "B"	2700 PT/CO	10 PT/CO
Turbidity Sample "A"	21.5 APHA	3 APHA
Turbidity Sample "B"	530 PPM SIO2	1.1 PPM SIO2
Turbidity Sample "C"	660 JTU	0 JTU
C.O.D.	1200 PPM	85 PPM
B.O.D.	150 PPM	8 PPM
T.O.D.	1200 PPM	156 PPM
Phosphates	33.6 PPM	0.76 PPM
Suspended Solids	216 PPM	4 PPM

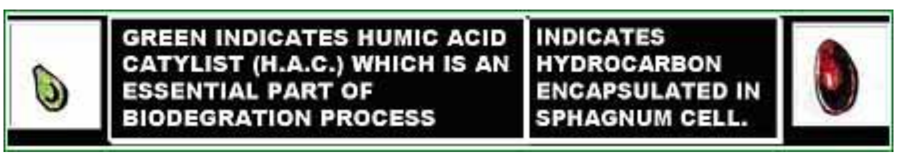
How Does P.O.L. Sorb Break Down Hydrocarbons?

HYDROPHILIC

During the heat activation process, the sphagnum moss changes from hydrophilic (absorbs water) to hydrophobic (repels water) and only encapsulates hydrocarbons.

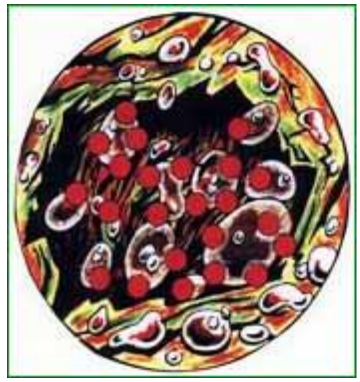
Exploded View of one cell in hydrophilic state able to encapsulate water up to 20 times it's own weight.

Exploded View of one cell now in hydrophobic state Will now repel water and encapsulate hydrocarbons.



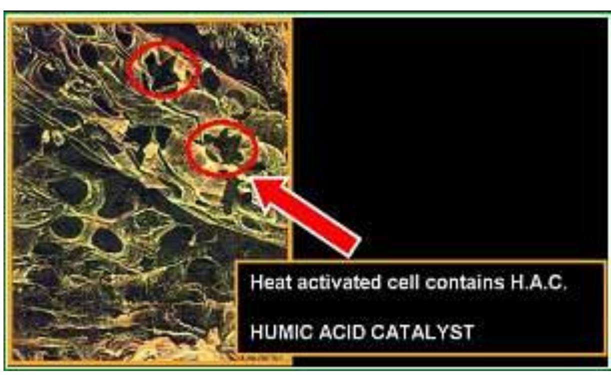
Hydrocarbons encapsulated in the cell

Magnified view of humic acid and naturally occurring microbes breaking down hydrocarbons.



View of Cell after biodegration. The cell has returned to a HYDROPHILIC state and will encapsulate water.

Exploded view of one cell in HYDROPHILIC state (holding water),completing the P.O.L. Sorb evolution cycle.



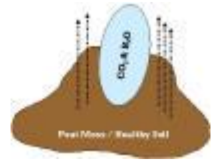
This piece of heat treated Sphagnum moss magnified thousands of times, the dark areas are the empty cells that were full of water prior to dehydration, these cells encapsulate and lock in the hydrocarbon.

MICROBIAL ACTIVITY WITH THE PEAT CELL

Activated Peat Moss near oil spill

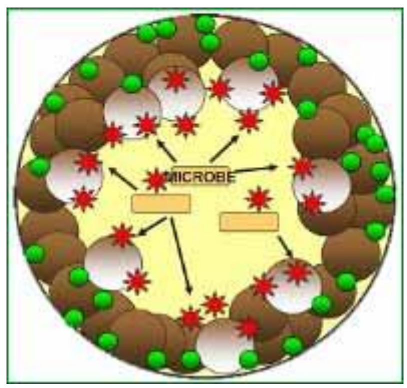
Peat and microorganisms eat oil and other organics (hydrocarbons)

Peat and microorganisms digest hydrocarbons and convert them into Carbon Dioxide (CO2) and Water (H2O)

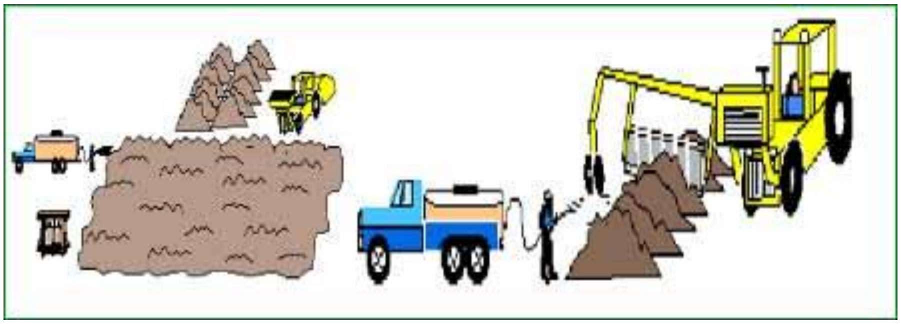


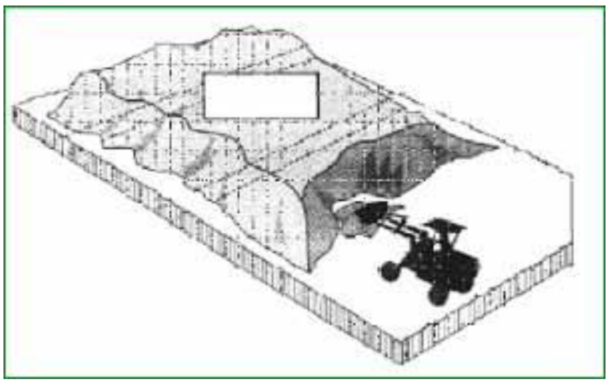
Peat and microorganisms give off CO2 and H2O

Humic Acid,
Oil,
Microbes,
Enzymes,
BioDegraded Oil



LAND FARMING or IN SITU ?
Bio-remediation ~ Accelerated Natural Attenuation





Soil

- Enzymes (Microbes / Bacteria)
- Oxygen (air)
- Water / Heat
- Nitrogen (Urea / Fertilizer)
- P.O.L. Sorb (The Host)
- Aeration

This plant is growing in peat that was used to clean up an oil spill

...Why soil bacteria that clean up metal pollution find it so hard to let go.

31 May 2000 by: **DAVID ADAM**

Bacteria wearing sticky protein coats could soon become front-line soldiers in the battle to clean up polluted soil. Bugs covered with metal-binding proteins can mop up pollution and boost plant growth, new research shows.

The cell surfaces of many enzymes latch onto heavy metals, but this metal 'sequestering' is too feeble to significantly reduce metal pollution in soil. Bugs can be made stickier by modifying them so that they produce cadmium-binding mouse proteins, Victor de Lorenzo of the Centro Nacional de Biotecnología in Madrid, Spain and his colleagues now report. Although the toxic metal remains in the soil, once bound to the modified bacteria it is less likely to be taken up by plants and animals.

The problems of heavy metal pollution are well known in Spain. In April 1998, about five million cubic metres of polluted water spilled from a mine residue pool near Seville, ruining crops and leaving surrounding farmland useless. Although the floodwater was successfully diverted away from a nearby national park, the ecological effects are expected to linger for years, as the zinc, lead and other metals left in local groundwater work their way through the food chain.

Microorganisms offer a cheap way to clean up pollution. Bugs that convert toxic substances, including oils and solvents, to harmless gas are becoming increasingly popular, not least because they are robust, easy to manage and don't stop working to read the newspaper. But they are not alchemists -- metal pollutants stay metal, whatever bacteria do.

But 'treating' metal pollution does not necessarily mean removing it from soil, de Lorenzo's team shows. They can make cadmium in soil less harmful by attaching it to the bacteria Ralstonia eutropha, a strain naturally resistant to heavy metal pollution.

They do this using 'metallothioneins': proteins produced by animals including mice that have an affinity for heavy metals. Few microrganisms produce metallothioneins, but de Lorenzo and his colleagues find that R. eutropha can be persuaded to do so -- by giving it the mouse gene.

These genetically modified bacteria escape many of the strict conditions governing other GM experiments. "There are regulations, but they are far more simple than those applied to GM plants," de Lorenzo says. "None of the engineered genes is this case can pose any imaginable risk, even in the very unlikely case that other enzymes capture them," he adds. The researchers also give the soil bacteria part of another gene that encourages the cadmium-luring proteins to sit on the bacterial outer membrane, so that they are fully accesible to the pollutant.

In tests using cadmium-contaminated soil and tobacco plants, the modified bacteria bind three times as much cadmium as unaltered bugs, reducing the cadmium's toxic affects on the plants. Over four times as many plants grow in 'treated' as in 'untreated' soil, but plant growth is still almost 90 per cent lower than in uncontaminated ground, the researchers report in Nature Biotechnology1.

The results are "promising, but not spectacular," says Derek Lovley, a microbiologist at the University of Massachusetts who specializes in bacterial pollution treatment. "Further improvements will be required before inoculation of soil with these organisms can be considered an effective remediation strategy."

But the study does prove that the technique could work, making further work in this area crucial. As Lovley says: "there are currently so few other practical options for effective, yet inexpensive remediation of metal-contaminated environments."

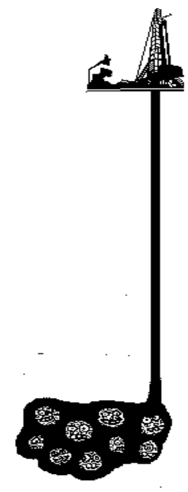
References

Valls, M., Atrian, S., de Lorenzo, V. & Fernández, L. A. Engineering a mouse metallothionein on the cell surface of Ralstonia eutropha CH34 for immobilization of heavy metals in soil. Nature Biotechnology 18, 661 - 665 (2000).

http://www.nature.com/nsu/000601/000601-6.html

Digging deeply
Subsurface science unearths previously unknown organisms that could be a gold mine for environmental remediation.

Fascinating creatures are at work deep in the earthþand they do interesting things. Environmental Sciences Division researcher Tommy Phelps has encountered them during the past several years in his work with the DOE Subsurface Science program, which has investigated the techniques for drilling holes deep in the earth and the problems that arise when you dig way down.



What Phelps and fellow researchers have found in the deep subsurface sediments are thermophilic bacteria—that means they like it hot—that have the capability to alter metals to produce magnetite, or magnetic iron. Some researchers are interested in them from an evolutionary standpoint. However, because these bacteria reduce metal compounds in an electron-swapping process similar to our breathing, they could be useful in waste remediation technologies as well as other applications.

Phelps touts these subterraneous microorganisms because of their comparative efficiency in producing their magnetic leavings."We haven't found any microorganism that doesn't have a cousin on the surface that can do the same thing, but the thermophilic bacteria can do it better, faster, and under more extreme conditions. We believe this capability can be applied to a variety of problems, the most obvious being mixed waste treatment."

Environmental remediation was a major reason DOE continued to study deep drilling when other agencies' incentive to dig ended at around 100 meters. Because, for instance, plumes of the solvent trichloroethyleneþa notorious pollutantþcan sink more than 100 meters deep and expand for several kilometers, shallow holes don't address the DOE researchers' questions. "When you dig deeper than 100 meters, the costs go up and so do the problems," Phelps said."Deep holes take an entirely different set of technologies and expertise. It is also expensive—you have to allocate your resources to where you dig one hole every two or three years."

The thermophilic bacteria were first encountered at an oil and gas deposit exploration site near Fredericksburg, Virginia. Researchers discovered the magnetite-producing bacteria at a depth of about 1000 meters. The site lay in the Taylorsville Triassic Rift Basin, a region with an underground layer of sediment that was formed 200 million years ago during the Triassic Period, then covered by tectonic activity. The ancient layer is effectively sealed off from the surface."It is geologically and hydrologically isolated from surface effects," Phelps said."It has groundwater that is probably 100 to 150 million years old."

That insulating layer has eroded over time enough to make it accessible, although not *easily* accessible. To support the 76-meter drilling rig and be able to truck in supplies in rainy weather, the oil and drilling companies literally floored a 1.5-acre tract of land with two to three layers of oak planking. It took 67 semi trucks to haul in the equipment. "The DOE assistant secretary at the time came out to visit. He was intrigued by the scope of the project—the hole went to a depth of 2.3 kilometers—and the industry-laboratory-DOE collaboration," Phelps said.

A similar hole in the loftier terrain of the Piceance Basin near Grand Junction, Colorado, produced similar bacteria, although the two regional rock formations were formed at different times and are far apart."We started looking for new organisms, and found some," the ESD researcher said."We found organisms that reduced every metal that we threw at them—iron, manganese, chromium, cobalt, and uranium.

These scanning electron micrographs show two bacteria cultures with externally deposited magnetic oxides.

These microorganisms use metals as electron acceptors, like we use oxygen. Just like we make carbon dioxide from oxygen, they take iron and make magnetite and maghemite—magnetic minerals." Phelps is currently working with two postdocs, Chuanlun Zhang, a geoscientist, and Shi Liu, a microbiologist specializing in anaerobic organisms. They are working with the Chemical and Analytical Sciences Division to explore the potential of the enzymes."CASD has been immensely helpful in the basic sciences avenue, and Zhang's background in geoscience has helped us in collaborating with CASD's Dave Cole."

Phelps notes a good deal of interest in these netherworld enzymes in a number of venues."An oil industry rep said that where he finds magnetite, he finds natural gas," he said. Other scientists want to study the isolated bacteria's respiratory systems on an evolutionary basis—thermophilic iron reduction as a possible precursor to oxygen respiration.

One speculative application could be rheomagnetic fluids, in which a fluid like oil with magnetic particles added can be rendered solid by applying an electric current. Other uses could lie in the production of high-speed lubricants, paints, photographic films, and even magnetic tracers for diagnostics.

The real interest is in treating mixed waste, including metals and solvents."All waste, really," Phelps said. Phelps theorizes that these bacteria could be introduced into water polluted with heavy metals. Once the organisms have reduced the pollutants into insoluble metal compounds, retrieving it could involve a simple separation process.

And, if their environment can be heated enough, thermophilic bacteria could be very efficient at cleaning up groundwater polluted with solvents, Phelps said.

A patented Westinghouse technology that arose from the early stages of the DOE Subsurface Science program, PHOSter, received an R&D 100 award. The phosphate- removing technology is now used in seven states. The subsurface program, however has ended, and its biotechnological applications have been rolled into the Natural and Accelerated Bioremediation program. The thermophilic bacteria studies are currently in funding limbo.

That hasn't kept news from getting out about the weird bugs underground. Recently, a Web page about unidentified flying objects made mention of the Subsurface Science Program and its work with "bacillus infernus,"—bacteria from hell—and offered a link to an article on the subject.

It's fairly safe to say that drilling a hole to these depths represents a journey into the unknown. Any suggestion, however, that these underground bacteria might be long-sequestered alien life forms leaves Phelps in a merry state.

http://www.ornl.gov/publications/labnotes/aug96/phelps.htm

Bioremediation: Bacteria Do Our Dirty Work

By **Douglas Page**

In August of 1992, 133,000 liters (35,000 gallons) of Jet A aircraft fuel were discovered to have somehow seeped out of the pipeline and underground storage tanks into two areas of soil beneath Van Nuys Airport in Van Nuys, California.

By toxic spill standards, this wasn't headline news, but it was big enough to draw the attention of environmental agencies that feared the fuel could reach ground water. One gallon of contaminant can pollute 300 million gallons of ground water. The two soil areas contained elevated petroleum hydrocarbon concentrations of 24,000 parts per million (measured in total petroleum hydrocarbon, or TPH, units), three times the level that requires remediation. The combined plumes soaked an area some 150 by 90 feet, to a depth of 90 feet. At discovery, the plume had reached one-third of the way to ground water.

Traditional cleaning methods for the site (located adjacent to a major runway) could have disrupted airport activity for three to five years. These traditional methods involve applying caustic, solvent-based cleaners or excavating and hauling. Caustic chemicals often create even more environmental problems, and excavation essentially just sweeps the contamination under a different rug.

Instead, the airport tenant chose a proposal from Biotreatment, Inc., San Diego, which used a technology called bioremediation that cost less, took less time, and did not disrupt airport activities to any extensive degree. Within 90 days, in situ bioremediation reduced TPH levels by an average of 80 percent. Monitoring at that time showed the degradation rate had leveled off at 2,000 to 2,500 ppm--75 percent below action levels.

How Bioremediation Works

Basically, bioremediation harnesses the ability of microorganisms (such as bacteria or fungi) to remove pollutants from the environment. In its natural state, this process is called biodegradation. Biodegradation is as old as life itself. ***When humans enter the picture by manipulating conditions, the process is called bioremediation.***

Bioremediation is the natural way of reducing toxic organic materials to harmless carbon dioxide, water, and various forms of salt. It is, in fact, the same process that takes place when grass clippings and garden waste are composted for later use as a soil nutrient for future plantings. Enzymes existing naturally in all soil and water produce enzymes that break down hydrocarbons into smaller, less toxic materials.

Using modern methods, science has found ways to accelerate and improve the effectiveness of biodegradation. Naturally occurring enzymes identified and isolated for their ability to degrade specific hydrocarbon contaminants such as oil and gasoline are now being cloned and applied in industrial strength to hazardous waste sites. The enzymes, when combined with nutrients, pH stabilizers, oxygen, and surfactants (detergents), yield a product that, when introduced into contaminated soil or water, optimizes the environment so bioremediation can take place.

Bioremediation is currently being used to manage municipal sewage, clean up oil spills, remediate ground water contaminated by underground storage leaks, treat industrial waste water, and reclaim a variety of hazardous waste sites.

Biotechnology firms such as Advanced BioTech, Visalia, California, market naturally occurring microorganisms packaged in a dry, dormant state. BioTech's hydrocarbon-digesting enzymes, for instance, are sold in one-half pound (227 gram) or 2.5 pound (1.1 kilogram) containers, including specially formulated biochemical nutrients--a concoction well suited to remediate benzene, amines, phenols, cresols, naphthalene, alcohols, petroleum hydrocarbons, and pesticides from refinery and petrochemical waste sites.

Once bioremediation treatment commences, the hazard is swiftly and dramatically reduced, neutralized, or eliminated through mineralization--a botanical term for the decomposition of organic matter in soils by microorganisms, which releases mineral elements (nitrogen, phosphorus, potassium, sulphur) as inorganic ions. Altogether, more than 70 different enzymes are known to be capable of degrading petroleum components. The technology is approved by the U.S. Environmental Protection Agency and the EPA's Canadian compliment, Environment Canada, as well as other regulatory bodies worldwide.

The bugs have appetites for more than petroleum byproducts. Researchers are using genetically engineered fungi, bacteria, and algae as "biosorption" systems to capture polluting metals and radionuclides, including mercury, copper, cadmium, and cobalt. One company, The Institute for Genomic Research (TIGR), has successfully sequenced a microbe that can absorb large amounts of radioactivity. TIGR scientists hope to use the genes that code for "uranium-gobbling" to fashion new biological means of cleaning up radioactive dump sites.

Some of the early work in the microbial technology behind bioremediation was conducted in the 1950s by a scientist named Howard Worne, who was commissioned by the U.S. Army during the Korean conflict to develop a new generation of military fatigues that would not rot in the moist, humid Asian climate. In the course of his work, Worne uncovered a microorganism that broke down fabrics that were previously thought to be non-biodegradable.

From this discovery, Worne speculated other microorganisms might exist that could degrade other materials. Eventually, Worne isolated an organism that was capable of degrading phenol (carbolic acid), a common organic pollutant. The bioremediation industry took root in Worne's work.

Bioremediation Today

Biotechnology has emerged has an industry with one of the highest growth potentials for the twenty-first century. After just 10 years in the biotechnology revolution, there are already more than 1,300 biotechnology companies in the U.S., with a total of nearly $13 billion in annual revenue and more than 100,000 employees. With more than 200 million tons of hazardous materials being generated annually in the U.S. alone, the cost of cleaning up toxic waste sites is now estimated to be in excess of $1.7 trillion. Bioremediation, a practical and cost-effective method of removing hydrocarbons from contaminated areas, could do a lot of the dirty work for us.

Bioremediation, however, is not a panacea for soil and ground water contamination. There are limits to bioremediation's effectiveness. Microbe growth is inhibited by heavy metal concentrations, making bioremediation unsuitable for some cleanup efforts. Plus, not every chemical has an appropriate bacterium, and when genetically engineered enzymes are used, this constitutes release of a new organism with unknown consequences.

Employing bioremediation is not as simple as just pouring a microbe soup over a spill site. Many smaller environmental firms have stopped doing bioremediation because they have realized there is much more to bioremediation than mixing a few nutrients and water with soil and then seeding with a few grams of the common soil bacteria Pseudomonas aeruginosa. Bioremediation, like the rest of the environmental industry, is maturing. Regulators and clients have gotten smarter. Bioremediation is now a technically viable remedial option based on good science and engineering.

Successful, cost-effective bioremediation programs are dependent on hydrogeologic conditions, contaminant signature, microbial ecology, and other factors. Biotreatability studies are necessary to evaluate site conditions, including such analyses as:

Screening studies to obtain biodegradation parameters such as electron acceptors/donors, oxidation-reduction potential, and pH (a logarithmic index for hydrogen ion concentration)

Microbiological assays to determine microbial growth conditions, degrader population densities, and presence of enzymes capable of destroying contaminants of concern

Microcosm studies to evaluate bioremediation potential under controlled conditions

The preliminary analysis is performed by experts in the field--environmental engineers, soil scientists, hydrogeologists, and chemical engineers. Indeed, this may be the one place where chemical engineers can apply their skills to the remediation, instead of the creation, of toxins.

After the biotreatability assessments are complete, those involved can decide whether to apply intrinsic (passive) bioremediation or enhanced (engineered) bioremediation.

Intrinsic bioremediation of toxic organic compounds is accomplished by using indigenous microorganisms, principally heterotrophic (carbon-requiring) bacteria, which transform the contaminant into an innocuous by-product. In this simplest form of bioremediation, contaminated soil is "bio-stimulated" with nutrients and an oxygen source, like peroxide, to encourage the proliferation of existing enzymes, which in turn degrade the contaminant more rapidly. When the nutrients and contaminants are depleted, the organisms are left to return to their original levels or are removed via a filtration process.

In other cases where risk to the water table is more immediate, enhanced bioremediation may be necessary. This engineered form of bioremediation can be performed in situ through biosparging (spraying), bioventing, or hydrogen peroxide/inorganic nutrient supplementation. In these cases, the contaminated soil is often tilled into uncontaminated surface soil along with the nutrients that will stimulate indigenous bacteria to degrade the contaminants. In some cases the soil may be aerated or flushed with liquids to assist in the removal of pollutants. The duration of the process varies from a few weeks to several months.