

# Soil & Groundwater Cleanup

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Enhancing Bio  
Annual Bioremediation Issue





# Enzyme helps remediate at lightning speed

By Bret Braden and Mark Ryckman

**H**istorically, bioremediation of petroleum-based compound incidents has been limited by the uncertainty of time and completeness of the reactions. Bacterial-based enhancement products have helped in limited applications, but have not been completely successful in all cases. Recently, non-pathogen enzyme agents have come onto the commercial market which, combined with air injection technology, enable companies to look at bioremediation (both in situ and ex situ) as a fast-track alternative.

Soil and groundwater remediations may be accelerated using a blend of bioremediation enhancing agents to stimulate native microbes to reduce biological reaction kinetics to less than eight weeks, and sometimes less than four weeks.

More than 1,814,000 metric tons of contaminated soil have been treated with the enzyme based agent HCZyme on multiple field projects for hydrocarbon and chlorinated hydrocarbon cleanups without generating toxic byproducts. HCZyme is non-toxic, contains no bacteria or genetically engineered microbes, and has been approved (not endorsed) by the Environmental Protection Agency for use in surface water, groundwater and soil remediations.

Most soil, groundwater, and free product contaminated sites have native microorganisms present that are acclimated to destroy hydrocarbons. To accelerate the breakdown process, addition of a blend of enzymes, amino acids, glucose, oxygen, and moisture control is all that is required to compress bioremediation times from years to weeks.

## Case History: Diesel Pipeline Break

A major pipeline experienced a break in a limestone Karstic landscape typified by sinkholes and caves formed from the dissolution of rock by streams, springs, and solution valleys. An estimated 227,000 liters of diesel fuel migrated over soil and entered the groundwater system through sinkholes. Approximately one half

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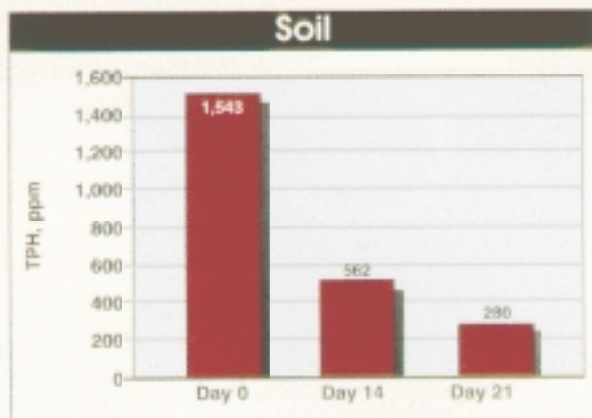


Figure 1

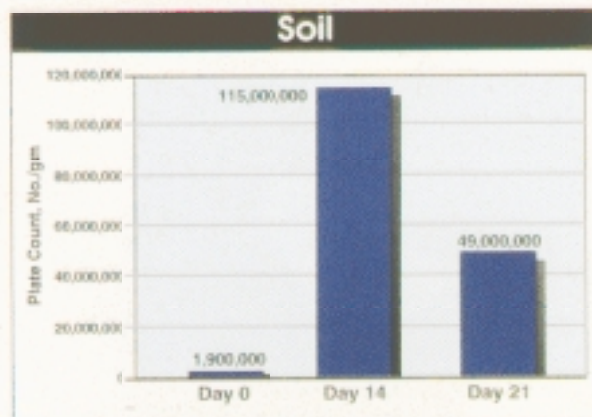


Figure 2

hectare of soil characterized as sandy clay loam was saturated with fuel from 1 to 2.5 meter depths.

Remtech Engineers, Marietta, Ga., was engaged to manage the soil remediation at this site. Alternate technologies, including on-site incineration, off-site incineration, off-site biodegradation, and on-site biodegradation were evaluated. On-site bioremediation using HCZyme was selected as the most cost-effective alternative.

Site sandy clays require soils be conditioned to ensure thorough mass transfer of the enzymes and moisture to the soil matrix. A variety of soil conditioning methods/agents were investigated, including gypsum treatment (9 metric tons/acre), compost, sawdust, wood

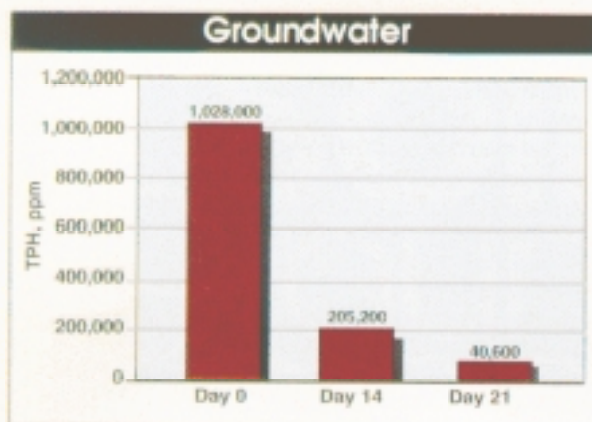


Figure 3

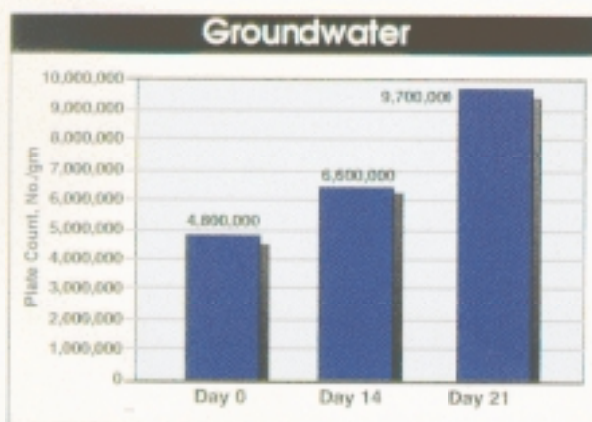


Figure 4

chips, drying, and deep tilling.

The key to handling clay soils is to control moisture content to less than 50 percent of the field holding capacity to prevent clay destabilization and balling which minimizes air and water transfer rates. Several contact configurations were considered for bioremediation, including windrowing, bioheaping, in situ/deep tilling, and in situ non-disturbed.

Field test pitting revealed that soil aeration with a track excavator (deep tilling) would provide adequate soil conditioning. In situ soils would be maintained at 40 percent field moisture holding capacity.

A multi-layered horizontal aeration/injection/extraction manifold was placed in the soil with an integrated water/enzyme application system to treat the material in place. The site cover consisted of black plastic to absorb solar heat using the earth as an insulator. Heated injection air was obtained from the heat of compression from regenerative blowers alternating between positive and negative pressure modes.

Integration of the soil remediation system with groundwater treatment/free product recovery is presently being evaluated. The preliminary functional design calls for using the soil treatment system as an infiltration gallery for enzymes and water to treat vadose

zone soils and attack free product trapped in rock caverns, fractures, and other geological anomalies. Air and enzyme injection through an existing free product monitoring well network (over 100 wells) will accelerate bioremediation of the saturated zone.

#### Soil Degradation Data

A three week pilot test was conducted on soil and groundwater samples extracted from the site.

One week following treatment of the soil, heterotrophic populations increased from 1,500,000 to 150,000,000 colony-forming units (CFU)/gram. Two weeks into the treatment cycle, total petroleum hydrocarbons (TPH - DRO) soil concentrations were degraded from 1,543 ppm to 562 ppm. Three weeks into the treatment cycle, TPH degraded from 562 ppm to 280 ppm. (See figures 1 & 2, page 34)

#### Groundwater/Free Product Degradation Data

One week following treatment, microbial groundwater plate counts jumped to 4,900,000 CFU/ml and free product plate counts increased to 1.2 million CFU/ml. Two weeks into the treatment cycle, free product thickness decreased from 72 mm to 9 mm and free product TPH concentrations fell to 205,200 mg/kg from 1,028,000 mg/kg, constituting an 80 percent reduction. Three weeks into the treatment cycle, TPH concentration dropped to 40,600 mg/kg. (see figures 3&4, this page)

Full-scale remediation will commence following the completion of dye tracer tests to determine aquifer retention times which will influence enzyme and oxygen injection rates.

#### How HCZyme works

HCZyme, like many enzyme groups, has been produced through a biofermentation process using the 26 bacteria and fungus known to digest hydrocarbon compounds. The critical part of the process is the autolysis stage, where the cell wall and the cytoplasmic membrane are dissolved.

The resulting group of enzymes are in a class known as oxygenases, which activate oxygen and convert it to a form in which the oxygen atom can be incorporated directly into the hydrocarbon compound, thus breaking the long-chain bonds. As this process repeats, the hydrocarbon compound is quickly broken down to the corresponding alcohol, aldehyde and finally to the monobasic fatty acids. These simple "easy hydrocarbons" are then readily assimilated by the indigenous bacterial population.

In simple terms, the enzymes tend to attach themselves to the hydrocarbon materials — soils, free-floating or miscible. The enzymes then attract the bacteria surrounding the cell surfaces to catalyze the breakdown reactions.

Once the reaction is completed the enzyme breaks free to attach to another hydrocarbon source in order to repeat the same reaction. This is often referred to as the "lock and key" nature of enzymes, since the enzyme



"key" is used many times. Injection of enzymes in the matrix of petroleum compounds, along with a continuous aerobic environment, ensures completion of the biodegradation process.

In water, hydrocarbon compounds tend to separate into three phases: the floating or free-product phase, the soluble or miscible phase, and the sludge phase. When HCZyme is applied topically, it tends to attach to the top of the floating hydrocarbon layer first. Some of the HCZyme will pass through once saturation of the top layer is completed and will solubilize in the miscible and sludge layer. Generally, there are native microorganisms present in all three phases.

If an accelerated inoculation of the miscible and/or sludge layer is desired, the enzyme should be injected into these phases using injection well or probe techniques. Another inoculation approach, commonly used in industrial wastewater treatment, is to pump the sludge/water to surface, adding the enzymes (and oxygen) to the materials as they are pumped to the surface of a treatment area. Agitation can accelerate the process further.

If sufficient HCZyme is applied to the three phases of petroleum product in a subsurface situation, the enzyme

complex will stay with the hydrocarbon materials until they are broken down into simple molecules.

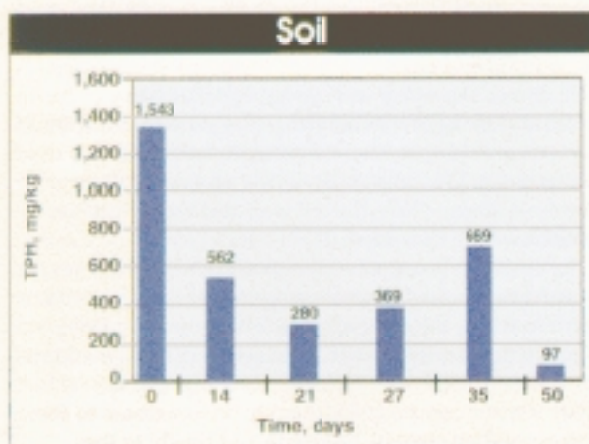
The recommended weekly doses — three to five typically — will ensure a continual reaction process without interruption of the reaction kinetics. If the groundwater body proves to be very dynamic or free-flowing, it is strongly suggested to directly inject the HCZyme into the aquifer zone so it can penetrate and attach to the petroleum molecules as they mobilize in the miscible zones.

One liter of concentrate generally treats 6 cubic meters of soil and is applied once a week for five weeks. Dosages may vary with contaminant concentration and site specific geological conditions. Chemical costs are approximately \$7/m<sup>3</sup> with total treatment costs ranging from \$15 to \$50/m<sup>3</sup> depending on the matrix contact configuration selected.

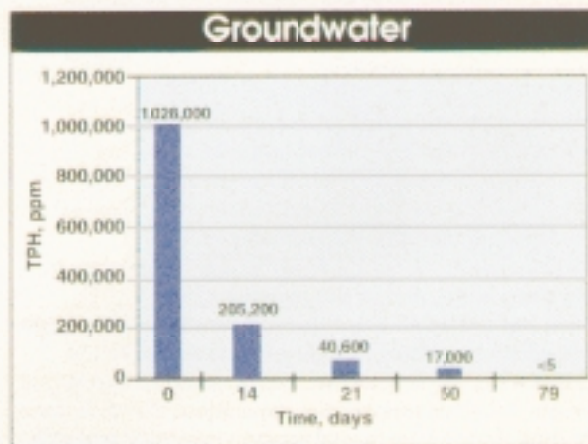
As with any catalyst, this enzyme only performs as well as the mass transfer systems which deliver the enzymes, moisture, and air. Each site presents unique challenges that require the interpretation and application of these delivery systems by an experienced site remediation engineer. ■

#### ***Paper Addendum - Extended Pilot Test Data (2/28/97)***

TPH soil concentrations dropped below 100 mg/kg for a 93.7% reduction in 50 days. The groundwater/free product was allowed to degrade for a total of 79 days (almost completely oxidizing the diesel).



**Figure 1: Extended Pilot Test**



**Figure 3: Extended Pilot Test**

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