Technical Discussion #1

BIOREMEDIATION OF SOIL

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In our experience, bioremediation is best accomplished with bio-augmentation, the addition of large numbers of selected microorganisms grown in the production laboratory. In addition to bio-augmentation, several physical and chemical parameters must be controlled in order to obtain optimal growth and maximum degradation of soil contaminants.

The major factors that Custom Biologicals, Inc. (CBI) controls during bioremediation are:

1) Microbial population
2) Nutrient concentrations
3) Oxygen supply
4) Temperature and moisture content
5) pH

1. Microbial Population

CBI has developed a synergistic group of microorganisms named Custom HC. The microorganisms in Custom HC digest short and long chain hydrocarbons, heavy tarry types of oil and grease, coal tars, phenolic compounds, chlorinated organic solvents and many other toxic chemicals.

The organisms in Custom HC are non-pathogenic and use the petroleum products or other chemicals in the soil for both a carbon source and an energy source. They convert the toxic chemicals to cell mass and carbon dioxide and water.

With Bio-augmentation, a sufficient amount of Custom HC is applied to the soil. Our studies have shown that this concentration insures that a sufficient population will be provided. It is not the intent to provide a ready-made population to immediately digest the contaminants, but rather to provide an inoculum that will insure rapid bacterial growth. As the project progresses the population of petrophilic organisms will show up to a one thousand fold increase as they digest the contaminants.

Tightly compacted soil may need additives to increase their permeability and the soil should be treated with either a shredding device or a vibrating screen to produce the smallest size particle possible.

In comparing Bio-augmentation with natural degradation, the former greatly increases the rate of degradation. The microorganisms naturally present that degrade petroleum products are usually present only in the upper few inches of soil and in low concentrations. These organisms are greatly diluted when mixed with a large amount of excavated soil. The dilution factor can be so large that it takes an excessively long time, if ever, to establish a working population of correct organisms.

2. Nutrient Concentrations

Nitrogen is needed for amino acid, purine and pyrimidine biosynthesis, and can be obtained by microorganisms from either inorganic or organic sources. The most commonly used nitrogen sources in Bioremediation are ammonia and nitrate. Many of the bacteria in Custom HC have two pathways for ammonia assimilation and which one functions depends on the ammonia concentration. We purposely keep the ammonia concentration high in order to allow the bacteria to utilize the most simple process.

When nitrate is utilized as the nitrogen source it is reduced to ammonia by a stepwise process. Nitrate is first reduced to nitrite by a molybdenum containing enzyme and then to ammonia. The average nitrogen level recommended is 50-100 mg/L, and should be frequently monitored.

Phosphorus (in the form of phosphate) is utilized by microorganisms primarily to synthesize phospholipids and nucleic acids (DNA and RNA). A minimum of 50-100 mg/L of phosphorous is recommended.

In addition to nitrogen and phosphorus, a variety of minerals is universally required, such as potassium, calcium, magnesium and iron. Many other elements are required only in trace amounts. These include zinc, copper, cobalt, manganese and molybdenum. These metals function in enzymes or coenzymes.

3. Oxygen Supply

Utilization of aliphatic hydrocarbons by microorganisms is strictly an aerobic process. The initial oxidation step of aliphatic hydrocarbons involves molecular oxygen as a reactant and one of the oxygen molecules is actually incorporated into the oxidized product.

The aromatic group of hydrocarbons can be viewed as derivatives of benzene. The breakdown of aromatic hydrocarbons involves the action of either oxygenases or mixed function oxygenases. These two reaction sequences both form catechol which can be degraded in a number of ways leading to either acetyl CoA or TCA cycle intermediates. If the benzene ring contains added methyl groups or other constituents (as in toluene or xylene), these substituents may be attacked before or after the ring is oxidized.

Very importantly, the oxygen concentration is the rate limiting factor in the biodegradation of petroleum based products. Microbial activity is most frequently limited by insufficient oxygen due to slow rates of diffusion into the interior of the soil layers or piles and into the center of soil aggregates. Generally, the greater the mass of oxygen that can be distributed, the more rapid and complete the cleanup. Effective application can result in greatly accelerated cleanups.

4. Temperature and Moisture Control

The bacteria in Custom HC were all isolated from the soil and laboratory studies have shown that their optimum temperature for growth and contaminant degradation is 28C. The temperature of the soil will be maintained as close to 28C as possible. Similar studies have shown that soil moisture should be maintained at 15-20%.

5. pH

The bacteria in Custom HC will achieve optimum results if the pH range is maintained between 6.5 and 8.0. Soils being treated using bioremediation should be monitored frequently and the pH should be manipulated to maintain the soil within the optimum range.

SUMMARY

Bioremediation of contaminated soil is best achieved by bio-augmentation with Custom HC. For the most successful and rapid bioremediation, the oxygen and nutrient concentrations, as well as temperature must be carefully controlled.