

ENHANCED BIOREMEDIATION

Delivery of electron donors has been one of the most serious challenges for in-situ enhanced bioremediation. With the introduction of the iSOC gas infusion equipment line in January 2001, the delivery challenge of dissolved oxygen and other gases has been met. This technology will work for aerobic (petroleum hydrocarbons, BTEX, MTBE, PCBs, PCPs,) and anaerobic (chlorinated solvents, nitrates, perchlorate, solvent stabilizers). In addition to treating source areas and migrating off-site plumes, long-lasting pump and treat systems can now be enhanced with a bioremediation component to significantly reduce treatment time and costs.

ELECTRON DONORS AND ACCEPTORS:

Microorganisms use a wide variety of electron donors, including both organic and inorganic compounds, which can be the chemical contaminant or a treatment chemical, frequently rich in carbon, injected into the subsurface to serve as the electron donor. Electron acceptors are much more limited. The most common electron acceptors include O_2 , NO_3^- , NO_2^- , SO_4^{2-} , CO_2 , Fe(III), and Mn(IV). Microbial use of oxygen as the electron acceptor is called aerobic metabolism, whereas microbial use of electron acceptors other than oxygen is called anaerobic metabolism. The complex intrinsic biodegradation processes show a variety of electron donors present in the vicinity of a gasoline plume ([Intrinsic Biodegradation Processes Diagram](#) - courtesy of Richard Woodward, Sierra Environmental)

AEROBIC: Using oxygen as an electron acceptor, microbes reduce contaminant mass. The reactions involve a transfer of electrons from the contaminant mass on a molecular basis and some electrons are transferred to the electron acceptor (oxygen) and some electrons and carbon is transferred to the microbes that use the energy, electrons and carbon to grow. In this biological process, water and carbon dioxide are given off as by-products. Complete conversion of the contaminant to the end product carbon dioxide is called mineralization. Some of the target chemicals that can be broken down aerobically include the petroleum hydrocarbons (gasoline, diesel, jet fuel, kerosene, mineral spirits, Stoddard solvent, oils and others), benzene, toluene, ethylbenzene and total xylenes (BTEX compounds), MTBE, PCPs, PCBs.

NUTRIENTS: Nutrients (ortho-phosphate and ammonium nitrate) are also needed in appropriate concentrations to optimize microbial degradation of the target chemicals.

ACCESSIBILITY OF CONTAMINANTS: Generally, toxic, complex molecular structures of non-aqueous (free-phase), sorbed and gaseous (bubbles) contaminants are not readily degradable by microbes. When the contaminant is non-toxic, water soluble and similar to naturally occurring structures, bioremediation will occur more rapidly. Bioremediation requires a certain level of indigenous heterotrophic degrader microbes, the proper nutrients. High concentrations of metals can make the environment too toxic for microbial growth. Some groundwaters EBS has researched have proven to be sterile to microbial growth due to low water temperatures, high metals concentrations, lack of nutrients or other factors. Contaminant concentrations must be within a range: if the levels are too high, the environment will be toxic to microbial growth, whereas if the

levels are too low, the food source will be sparse and the microbes will starve. A carefully monitored program including bench tests and sampling can help to optimize conditions by adding electron acceptors, additional carbon sources (food), and nutrients. Optimization of the biological processes can serve to significantly lower the time of the remediation process.

Information on bacteria site suitability for enhanced natural attenuation:

“A site’s microbial activity is directly related to the available energy and electron acceptors as long as excessive toxicity isn’t present. A measurement of the natural microbial activity is one procedure for evaluating potential toxic conditions at a site. Low bacteria counts can indicate a potential toxicity problem. Groundwater has typical bacterial counts ranging from 10^3 to 10^8 counts per liter. Typical microbial counts for soil vary between 10^3 and 10^7 counts per gram of soil. Counts below 10^3 organisms per gram of soil in contaminated zones may indicate toxic conditions. Microbial activity is normally greater near the surface and decreases to low levels at depths of over 200 ft.”

From: *Bioremediation Engineering... Design and Application* by John T. Cookson, Jr. Site Characterization for Bioremediation chapter, page 215; McGraw-Hill, Inc., New York, NY.

OXYGEN SOURCES:

SOLID OXYGEN SOURCES: Solid peroxygens such as magnesium peroxide or calcium peroxide have been used over the past several years with varying degrees of success. When injected, solid peroxygens are emplaced with a grout pump and solidify in the subsurface as cement, lowering effective porosity in the area of the plug. The amount of oxygen liberated into solution is somewhat limited and the oxygen-generating reactions generally last no longer than several months. Some manufacturers of the solid peroxygen add a soluble salt, such as potassium phosphate into their product. The salt acts as a biological nutrient as well as creating microfractures as the water leaches the salts out. These microfractures theoretically allow for more of the peroxygen to become wet and to produce more oxygen in the process. Although magnesium peroxide is 10% oxygen by weight, it is doubtful whether a significant portion of the oxygen will be liberated. Microbes in the vicinity of the grout plugs are killed by the exposure to the peroxygen.

LIQUID SOURCES OF OXYGEN: Liquid sources of oxygen include hydrogen peroxide. Mixed with water, the permanganates (potassium, sodium, calcium) are sources of oxygen. These liquids can add free oxygen into the aquifer, however, the reactions are relatively short: seconds to minutes for hydrogen peroxide to minutes to hours for the permanganates. These chemicals will destroy the microbes in the vicinity of the injection locations.

GAS SOURCES OF OXYGEN: Gas sources include air sparging techniques. The best method is delivering oxygen as a soluble gas into the aquifer. Using tanks of 100% oxygen or an oxygen gas generator, several methods have been used to deliver oxygen into the groundwater. By far the most successful method on a mass-transfer of gas into liquid is the iSOC process.

GAS INFUSION: Gas infusion by the iSOC process is described in detail at the website: www.isocinfo.com. The iSOC technology is being used at over 100 sites in 26 states and a few foreign countries. Installation of the iSOC is simple ([Installation FAQs](#)).

[GAS INFUSION OVERVIEW](#)

[GAS INFUSION USER'S GUIDE](#)

[GAS INFUSION OXYGEN REQUIREMENTS](#)

[GAS INFUSION MTBE ABSTRACT](#)

GAS INFUSION (iSOC) CASE STUDIES:

[Englewood, Colorado](#)

[Brush, Colorado](#)

[Chiefland, Florida](#)

[Flying J Truck Station, Indiana](#)

[Glastonbury, Connecticut](#)

[Kessel, Belgium](#)

[Mapleshade, New Jersey](#)

The iSOC gas infusion technology has only been available since January 2001.

PAPERS AT NATIONAL GROUND WATER ASSOCIATION (NGWA) MTBE Focus Conference: June 5-6, 2003, Baltimore, Maryland:

In-Situ remediation of an MTBE and BTEX Groundwater Plume Using a Full-Scale Oxygen Infusion System; presented by Sunoco, Inc. and Handex Environmental

Pilot Study to Evaluate the Effectiveness of Oxygen Infusion Technology to Bioremediate MTBE and TBA in Ground Water; presented by BP Amoco, Inc. and Handex Environmental.

Enhanced Natural Attenuation of MTBE and TBA in Ground Water at a High Dissolved Iron Site in the Northeast US Using iSOC Technology; presented by Global Technologies and inVentures Technologies, Inc.

In addition, there will be iSOC case studies/papers given at the following US national environmental conferences during 2003:

June 2-5, 2003: Battelle Bioremediation Conference, Orlando, FL

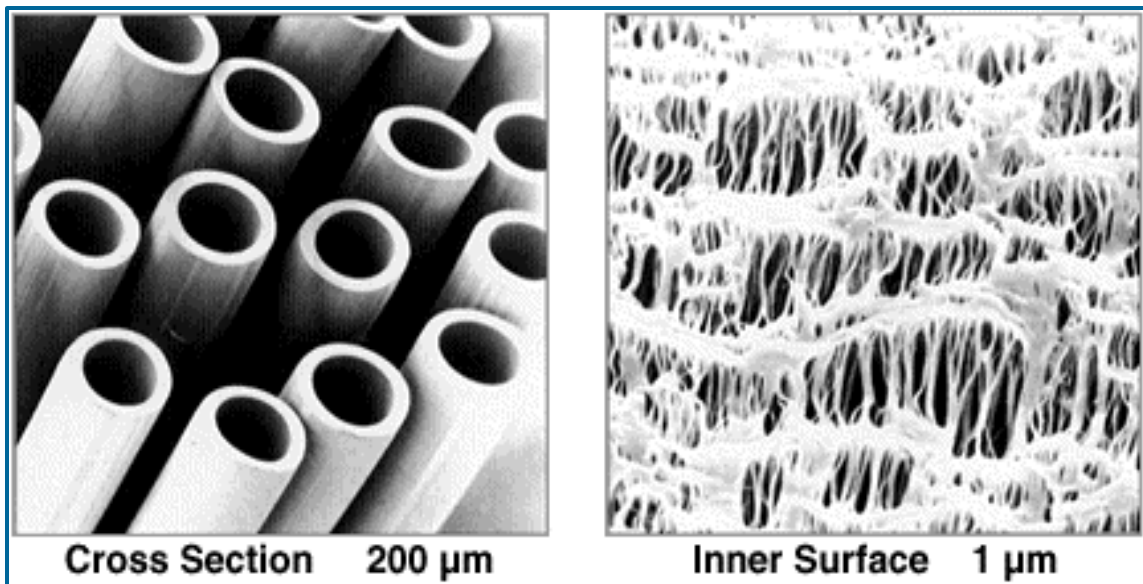
August 20-22, 2003: NGWA Petroleum Hydrocarbons Conference, Costa Mesa, CA

October 20-23, 2003: University of Massachusetts Contaminated Soils and Water Conference, Amherst, MA

November 13-14, 2003: NGWA Remediation Conference: Site Closure and the Cost of Cleanup, New Orleans, LA

Enhanced Bio-Remediation Products: The iSOC Gas Infusion System

EBS represents inVentures Technologies, Inc., an innovative Canadian technology company that has designed equipment for the high efficiency mass transfer of gases into liquids. After spending several million dollars on research and development, the iSOC was developed for gas infusion for enhanced bioremediation. This patented material microporous hollow fiber is designed to effect rapid, no bubble gas transfer (infusion) into the aquifer. This method of gas infusion allows for long-term retention of extremely high levels of dissolved oxygen or other gas. The method has been tested to be about 94% efficient in gas transfer into liquid, achieving gas transfer efficiencies up to ten times better than more conventional methods such as air sparging. For more information please see www.isocinfo.com.



MICROPOROUS HOLLOW FIBER MAGNIFIED FOR VIEWING

The iSOC system achieves enormous mass transfer surface area in a small volume (7,000 square meters of surface area in each cubic meter of material).

FOR NORTHERN/CENTRAL CALIFORNIA*:

EBS-100; iSOC System: (submit purchase order through EBS):

EBS-110; iSOC controller (1 well)

EBS-120: iSOC 3 well controller

***For other service locations, please refer to www.isocinfo.com**

PLEASE CALL FOR PRICING

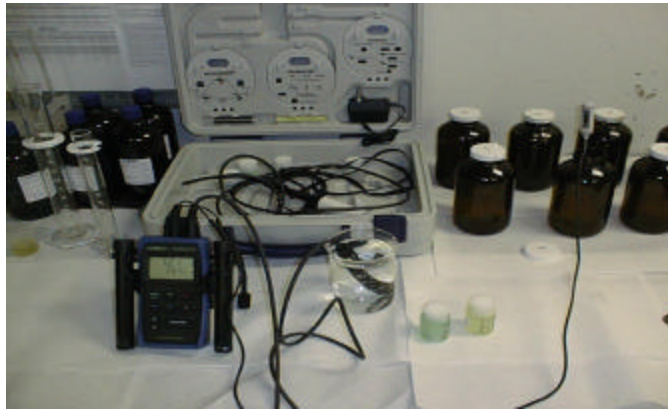
LABORATORY MONITORING RECOMMENDATIONS:

EBS recommends performing remediations in three steps: bench test, pilot test and final remediation. During the writing of the Corrective Action Plan or an Interim Remediation Plan, various remediation options are examined. Once bioremediation is recommended, a microbial parameter study is recommended. For aerobic remediation, EBS performs the following tests:

EBS SERVICES: ALL LOCATIONS:

EBS provides Laboratory Monitoring of iSOC Bioremediation Enhancement

EBS offers laboratory monitoring of microbiological parameters to validate iSOC bioremediation enhancement in the field. Both aerobic and anaerobic biodegradation processes can be characterized and followed over time to document changes in dissolved gas concentrations, macronutrients, and total bacterial populations as well as contaminant-degrading subpopulations of naturally occurring bacteria.



Bench tests allow for optimization of chemical and biological parameters before going into the field.

EBS-300; Aerobic Microbial Parameter Profiling

Bacterial enumeration for total aerobic heterotrophic bacteria, contaminant-degrading bacteria (e.g., gasoline/BTEX, diesel, oil, MTBE), macronutrients (ammonia nitrogen, o-phosphate), Total Inorganic Carbon (TIC) or alkalinity, pH and dissolved oxygen with report regarding in-situ placement of injection ports/wells.

1 week.

EBS-301; Anaerobic Microbial Parameter Profiling

Bacterial enumeration for total anaerobic heterotrophic bacteria, contaminant-degrading bacteria (e.g., chlorinated solvents, nitrate), alternate terminal electron acceptors (nitrate, sulfate, ferric iron), Total Inorganic Carbon (TIC) or alkalinity, pH and REDOX will be performed. 2 weeks.

EBS-302; Aerobic Microcosm Studies for Contaminant Biodegradation

Bench test to document the aerobic biodegradation of MTBE/TBA, gasoline, diesel and motor oil fractions of petroleum hydrocarbons by naturally occurring bacteria in groundwater collected from enhanced bioremediation site wells. Microcosms consist of sample groundwater enhanced with essential macronutrients, pH buffer and iSOC gas infusion oxygen per field protocol in sealed glass serum bottles. Microcosms are uniformly spiked with target contaminants and then sacrificed at time zero and an endpoint (e.g., 1 week) for GC-MS analysis (Total Extractable Hydrocarbons by EPA 8015, modified by CA-LUFT and EPA 8260 for gas oxygenates) of the contaminants before vs. after enhanced bioremediation. Includes concurrent negative (abiotic) and positive controls.

EBS-303; Anaerobic Microcosm Studies for Contaminant Biodegradation

Bench test to document the anaerobic biodegradation of PCE, TCE, cis1-2 DCE, nitrates and perchlorate by naturally occurring bacteria in groundwater collected from iSOC enhanced bioremediation site wells. Microcosms consist of sample groundwater enhanced with essential macronutrients, pH buffer and specific gas infusion gases per field protocol in sealed glass serum bottles. Microcosms are uniformly spiked with target contaminants and then sacrificed at time zero and an endpoint (e.g., 2 months) for GC-MS analysis of the chlorinated solvents and their breakdown products before vs. after enhanced bioremediation. Includes concurrent negative (abiotic) control.

EBS-304; EBS Custom Services: Custom in-situ delivery and microcosm studies and other specialized research projects can be designed. EBS offers technical consulting services for well placement and delivery evaluation by Jim Jacobs, CHG.

Complimentary treatments (chemical oxidation), can be evaluated with the iSOC technology. Laboratory research and field bioremediation protocols by PhD biochemist are available.