

# Using Electrolytic Respirometry to Monitor Biotreatment

Method could lower cost in determining the feasibility and effectiveness of various soil and groundwater bioremediation methods.

By Thomas G. Zitrides, Richard Bleam and Christopher Hyde

**E**lectrolytic respirometry (ER) has proven a valuable tool in evaluating the biological treatment of contaminated groundwater. The technique was initially used to determine the treatability of compounds contaminating the water, as well as the amounts of additives, such as nutrients and microbial strains, required to optimize biodegradation. Results of ER analyses are used to design and monitor the treatment program.

After biotreatment is initiated, ER analysis of extracted groundwater can determine the progress of biodegradation, duplicating on-site conditions in the laboratory. It can also assess the relative degradation rate over time to determine the program length required to reach desired levels of target compounds.

## What Is ER?

Respirometry is the measurement of the respiration of microorganisms. Aerobic respiration is the energy production mechanism for most organisms. It commonly involves consumption of oxygen and release of CO<sub>2</sub>.

Aerobic microorganisms, primarily bacteria, consume oxygen and give off CO<sub>2</sub> as they convert the available food supply into cell mass. The amount of oxygen consumed, over and above the base rate of respiration (known as the endogenous rate), is

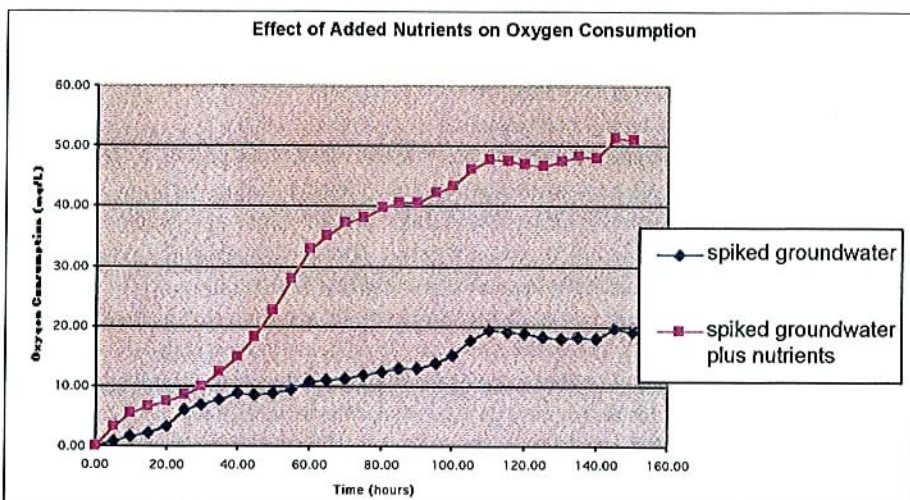
a measure of the activity of the biomass and its metabolism of the food supply.

Other types of microorganisms, such as anaerobic bacteria use nitrate, sulphate, ferric ion, manganic ion, or carbonate in place of oxygen. Aerobic autotrophic microbes use reduced inorganic compounds, such as ammonium or sulfide, in place of organic compounds. For evaluating bioremediation, respirometers usually measure the oxygen consumption and/or production of gases, such as CO<sub>2</sub>, by microorganisms to determine their activity in various environments. The technique can also be used, with

appropriate modifications, to measure the metabolic rate of some anaerobic bacteria.

Short of building a pilot plant, ER provides the closest approximation to what happens in the actual groundwater, or at the site of a chemical or petroleum spill. Base modules of modern ER units, like the BI-2000 made by Bioscience Inc., contain eight reactors and a computer. The systems can be expanded to simultaneously accommodate and analyze data for up to 16 reactor vessels online.

The heart of this process is the reactor vessel, which contains measured amounts of a microbial culture and the compounds – usu-



Graph produced by ER data shows effect of nutrient addition on microorganisms in groundwater contaminated with BTX/MTBE.



Spill site with contaminated groundwater monitored by ER.

ally in an aqueous solution – to be digested. The culture may be a sample of an existing biomass (from a wastewater treatment plant, for example), microorganisms from soil or groundwater, or specific microbial strains selected for activity on certain classes of compounds. Calculated amounts of nutrients may also be added to provide the proper environment for microbial growth or to ascertain the optimum onsite conditions.

When (and if) metabolism begins, the microbes consume existing oxygen inside the reactor and give off CO<sub>2</sub>. The CO<sub>2</sub> is absorbed by a potassium hydroxide solution in the reactor vessel head, resulting in a slight negative pressure. The partial vacuum triggers an electrolytic cell, also in the reactor, which supplies oxygen by means of electrolysis of a dilute acid solution until the original headspace pressure is restored. The coulombs of electrical power consumed are proportional to the oxygen produced. ER can thus provide an accurate measure of the amount of oxygen metabolized by the microbes.

One of the advantages of ER is that the headspace may contain pure oxygen, air or various gas mixtures to simulate an in-situ atmosphere. A minimum oxygen level is required to allow oxygen consumption to occur. Any oxygen concentration in the headspace above this minimum level will be maintained as the electrolytic cell replaces

the oxygen consumed.

Electrical readings are digitized and sent to a computer, which records and displays them. The data may be graphed directly, imported into a spreadsheet to perform calculations, or inserted into a program written to assist in groundwater bioremediation project design. The results of respirometry correlate closely with those of pilot plants and with actual studies in the field, but typically at a lower cost.

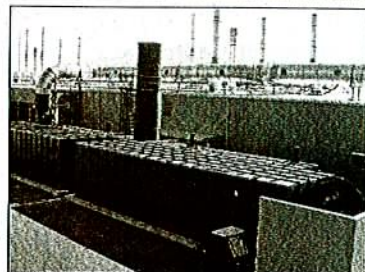
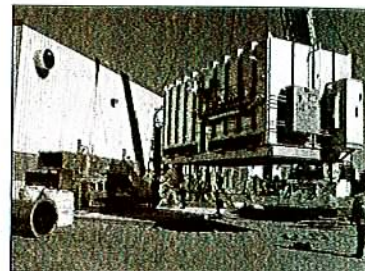
ER is often used to determine the feasibility and effectiveness of various soil and groundwater bioremediation methods. Groundwater samples from wells in the contaminated area can be rapidly evaluated at intervals to ensure that the biotreatment program progresses as planned, and to provide a timeframe for its completion. Regular sampling is necessary, as metabolic processes can reduce levels of required nutrients (in addition to the target compound) or generate inhibitory byproducts. Chemical analyses are used to confirm that oxygen consumption correlates with contaminant degradation.

While standard ER reactor vessels are usually appropriate for groundwater monitoring, special reactors are also available to evaluate samples with very low levels of microbial activity, or to measure respiration in soils rather than aqueous environments.



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# Electrolytic Respirometry

## Case Studies

### Study No. 1:

A site remediation for dichloromethane from a ruptured pipeline at a chemical plant implemented ER to determine biodegradability. There was a possibility that unknown compounds in the soil might inhibit microbial growth, or that methylene chloride-degrading microbes might be present at an insufficient population, even though the compound had been shown to be readily biodegradable in laboratory studies.

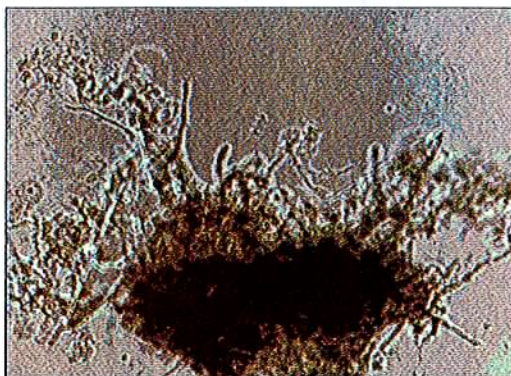
The test parameters – in addition to oxygen consumption – were dichloromethane loss and chloride release from biodegradation. Groundwater from the site was used for the studies. Two of the reactors were inoculated with sludge from a wastewater treatment plant that had been exposed to low levels of the compound over the years, and was thought to contain an acclimated microbial population.

Dichloromethane was added to all reactor vessels at 100 ppm, approximating the average concentration in groundwater. Another two reactors included indigenous microbes, while two served as abiotic controls to quantify non-biological loss of dichloromethane.

After two weeks of observation, no dichloromethane loss nor chlo-

ride release was detected in the abiotic reactors. The same was true of the reactors seeded with indigenous microbes. In the reactors containing microbes from the treatment plant, acclimation and degradation of dichloromethane occurred after just one day.

Based on the treatability studies, biological treatment at the site was begun using a batch reactor seeded with bacteria from the plant's wastewater treatment system. Continuous treatment of the compound was initiated after two weeks. Removal of dichloromethane from the groundwater took a total of 50 days – after a hiatus due to freezing weather – reducing levels to 1 ppb.



Photomicrograph of MTBE-degrading enrichment culture.

### Study No. 2:

A similar ER study was performed on a site contaminated with ethylene glycol. In this case, the indigenous microorganisms were shown to degrade ethylene glycol if proper nutrients were supplied and pH

was regulated. To create a closed-loop system in which biodegradation occurred in the soil, groundwater and reactor, the groundwater was treated in an above-ground reactor with the effluent re-injected into the vadose zone.

Between 85 and 93 percent of the ethylene glycol was removed during the first 26 days of treatment. After a six-month maintenance program, the level was reduced throughout the site to below the analytical limit of detection.

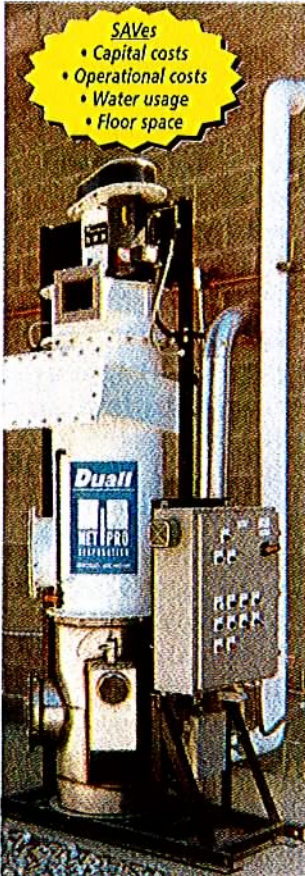
### Study No. 3:

Groundwater from a site contaminated with gasoline was tested to determine the ability of indigenous microbes to biodegrade benzene, toluene, ethylbenzene and xylene (BTEX) and methyl-t-butyl ethyl (MTBE). Duplicate reactors were prepared for each of four treatments: 1.) groundwater as received, 2.) groundwater plus nutrients, 3.) groundwater spiked with BTEX and MTBE, and 4.) groundwater spiked with BTEX and MTBE plus nutrients.

Nutrients had no effect on the groundwater as-received, with MTBE at 6.4 ppm and BTEX at non-detectable levels initially and all compounds at below detection after one month. However, in the spiked groundwater, both oxygen uptake and CO<sub>2</sub> evolution increased when nutrients were added. After one month, about 20 percent of the added benzene and toluene remained in the reactors without nutrients, while the reactors with nutrients were non-detectable for these contaminants. About 5 percent of the MTBE remained in the spiked groundwater with or without nutrient addition.

Using ER in preliminary site studies provides a cost-effective tool to measure bioremediation's potential for cleaning a site. The method determines biodegradation rate, molecular oxygen and nutrient requirements, and allows comparison of treatment methods to enhance the natural biodegradation rate. **PE**

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