U.S. Geological Survey
Toxic Substances Hydrology Program—
Proceedings of the Technical Meeting,
Charleston, South Carolina,
March 8-12, 1999

David W. Morganwalp and Herbert T. Buxton, Editors

U.S. GEOLOGICAL SURVEY

Water-Resources Investigations Report 99-4018C

Volume 3 of 3 - Subsurface Contamination From Point Sources

West Trenton, New Jersey

1999

USGS
science for a changing world

In situ Assessment of the Transport and Microbial Consumption of Oxygen in Ground Water, Cape Cod, Massachusetts

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ABSTRACT

Oxygen is a key ground-water constituent, controlling both the geochemistry and microbiology of an aquifer. Accordingly, aerobic respiration, the microbial metabolic process that consumes oxygen, is fundamentally important to the overall functioning of the aquifer. However, despite its significance, few studies have directly examined this process in the subsurface. This study has used several different approaches to investigate oxygen consumption on several different scales in parts of a large (> 5 kilometers) plume of dilute sewage contamination in a sand and gravel aquifer on Cape Cod, Mass. First, oxygen concentration profiles and stable isotope ratios were used to infer the net effect of aerobic respiration on the aquifer scale. Second, natural gradient tracer tests were used at an intermediate scale to measure in situ rates of aerobic respiration within different contours of the ground-water oxygen gradient. Third, two different types of laboratory incubations using aquifer core material, potential electron transport activity (ETS) and oxygen uptake activity, were used for small-scale examination of the process. The latter methods yield estimates of rates and kinetic parameters, which can be compared with the tracer test and isotope results. The sum of these approaches views the aquifer within the context of a subsurface ecosystem, integrating the combined effects of the hydrology, geochemistry and microbiology on the process of oxygen consumption.

INTRODUCTION

While it is actually part of a hydrologic continuum, an aquifer can also be viewed as a discrete ecosystem. Processes that occur in an aquifer involve a complex interaction of biotic and abiotic components. To fully comprehend and predict the effects of this ecosystem on solutes of natural and anthropogenic origin (and vice versa), it is necessary to study the system from a holistic perspective, with the environmental conditions intact. This has been done many times in surface systems. However, in situ assessment of key microbial processes in ground water has been difficult, and thus rarely attempted.

The importance of oxygen as a ground-water constituent is widely recognized. The processes that consume oxygen are of fundamental importance to the overall functioning of an aquifer, both as an ecosystem and as a hydrologic unit. However, there have been relatively few integrated studies of oxygen respiration in ground water, especially from a process-oriented perspective.

This paper is an overview of a project that has examined aerobic respiration in a sand and gravel aquifer at several different scales. The aquifer is characterized by both vertical and horizontal gradients of dissolved oxygen, providing a range of electron acceptor demand from low in the uncontaminated zone of the aquifer, to moderate in the contaminated zone. The study combined recent developments for using ground-water tracer tests to measure biogeochemical processes in situ, improved analytical capabilities both for quantifying low level oxygen concentrations and determining oxygen stable isotope ratios, and laboratory incubations using artificial electron acceptors to assess microbial electron transport system (ETS) activity. These techniques were employed to study oxygen consumption within the aquifer at the kilometer-, meter-, and centimeter-scale. The results provide a better understanding of aerobic respiration as a process in the subsurface, facilitate interpretation of $\delta^{18}O_2$ natural abundance data from this and other field studies, and examine whether the respiration assays that were developed for other environments have utility for ground-water environments.

STUDY SITE

The study was conducted in an unconfined sand and gravel aquifer located on Cape Cod, Mass.

(fig. 1). The aquifer has been contaminated by the disposal of dilute sewage since 1936 (LeBlanc, 1984), which has resulted in a contaminant plume that is more than 5 kilometers long and 25 meters thick, vertically.

A 150-meter-long transect at a site about 2 years travel time from the contaminant source exemplifies ground-water oxygen profiles within the contaminant plume (fig. 2). Sharp vertical gradients of oxygen are evident within the plume. Dissolved oxygen concentrations decrease with depth from levels near atmospheric equilibrium in shallow uncontaminated ground water to anoxia in the core of the plume. There also is a relatively thick vertical interval in which oxygen concentrations persist in the 1-10 micromolar (μM) range. Sampling and analytical procedures for measuring dissolved oxygen in these concentration ranges have been previously described (Kent and others, 1994; Smith, 1997).

The depth of the oxygen gradient sinks relative to the water table as the contaminant plume moves down-gradient, but is evident even after several kilometers of transport. Similar vertical gradients of many other solutes, including dissolved organic and inorganic carbon, as well as microbial populations, coincide with the oxygen gradient, usually increasing in value where the oxygen is decreasing (Smith and others, 1991).

OXYGEN ISOTOPE FRACTIONATION BY AEROBIC RESPIRATION

Microbial processes commonly fractionate low molecular weight molecules because of the tendency of lighter isotopes to react faster than heavier isotopes during enzyme-mediated reactions. The result is an enrichment of the heavier isotopes in the substrate and lighter isotopes in the products. Fractionation of oxygen isotopes by aerobic respiration has been demonstrated in laboratory experiments and open oceans, but has not been examined in detail in ground-water environments. Oxygen isotope fractionation factors for shallow ground-water systems could prove useful to infer relations among mixing, diffusion, and reaction parameters for aerobic respiration, thus yielding aquifer-scale (i.e. up to kilometer-scale) information regarding oxygen consumption along a given flow path. The isotopic ratio of $^{18}O/^{16}O$ in dissolved molecular oxygen changes in the Cape Cod aquifer as a function of the oxygen concentration (fig. 3). As the oxygen concentration decreased, the oxygen became increasingly enriched in ^{18}O; $\delta^{18}O$ values ranged from about +25 per mil (‰) for near air saturation to +45 ‰. These results are consistent with kinetic isotope fractionation by aerobic respiration. However, when fit to the Rayleigh fractionation equation, the apparent

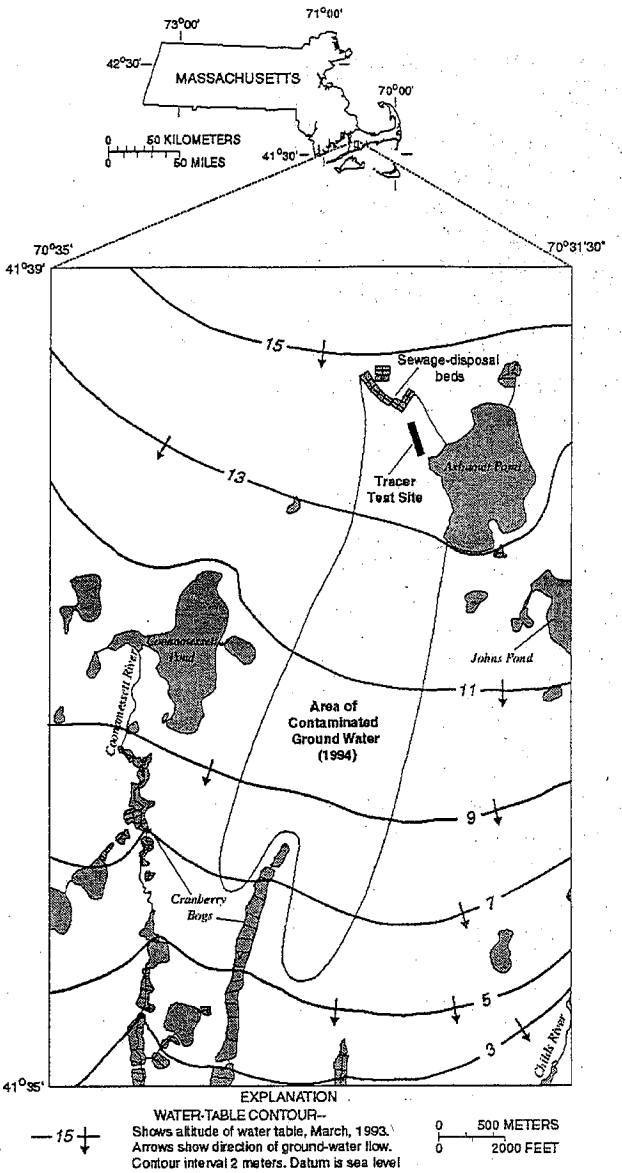

Figure 1. Ground-water study site on Cape Cod, Mass., showing location of the contaminant plume and the large-scale multilevel sampler array used in this study.

fractionation factors (ε = -2 to -10 ‰) are somewhat smaller than many values derived from closed-system experiments or field studies (ε = -20 ‰). This is due, in part, to dilution of oxygen via dispersion and diffusion during the reaction process (Revesz and others, 1999).

NATURAL GRADIENT TRACER TESTS TO ASSESS AEROBIC RESPIRATION IN SITU

Intermediate scale, in situ assessment of aerobic respiration was accomplished using natural gradient tracer tests. These tests were conducted using 15-port multilevel samplers

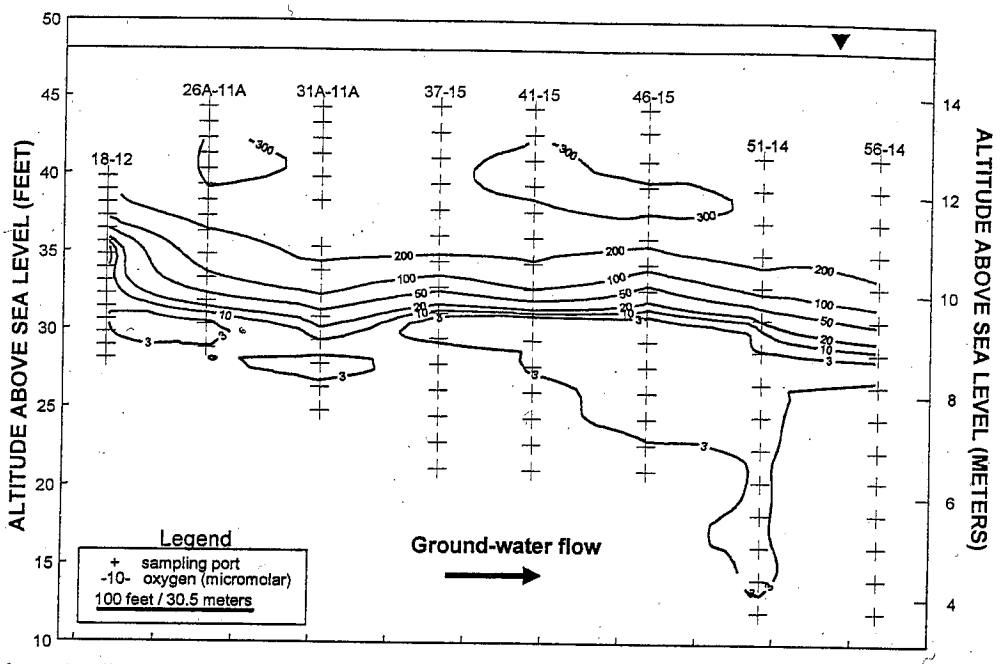

Figure 2. Longitudinal cross section of dissolved oxygen concentrations within the large-scale multilevel sampler array in November 1996. Multilevel sampler identification code is given for each well sampled.

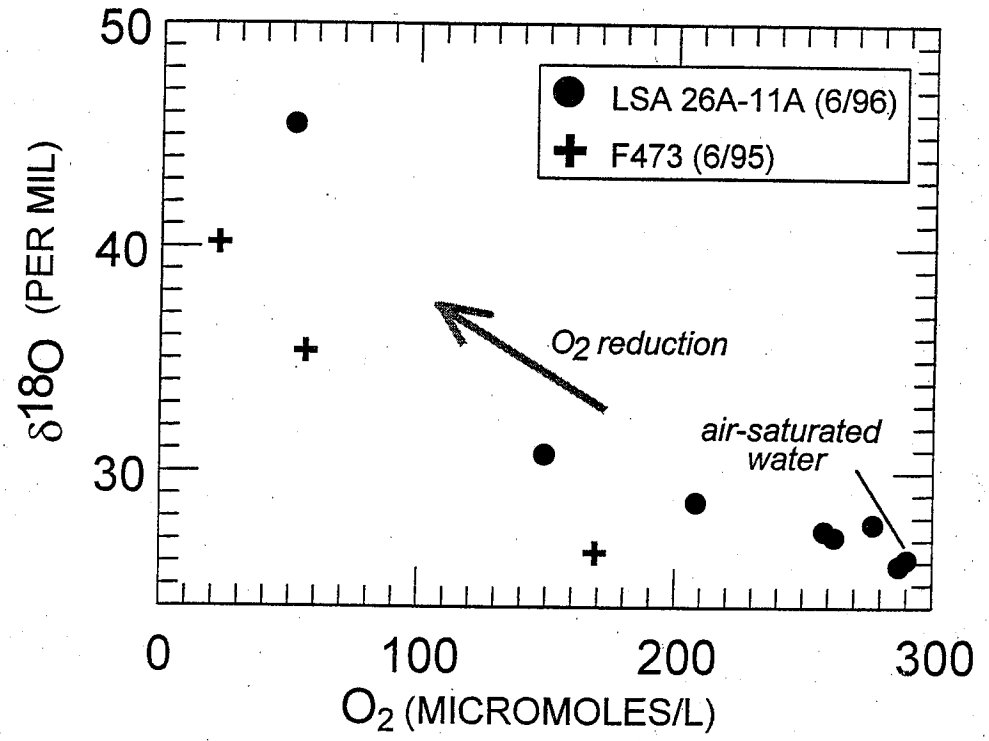

Figure 3. Isotopic content of dissolved oxygen as a function of concentration for ground water from two multilevel samplers located near the large-scale multilevel sampler array.

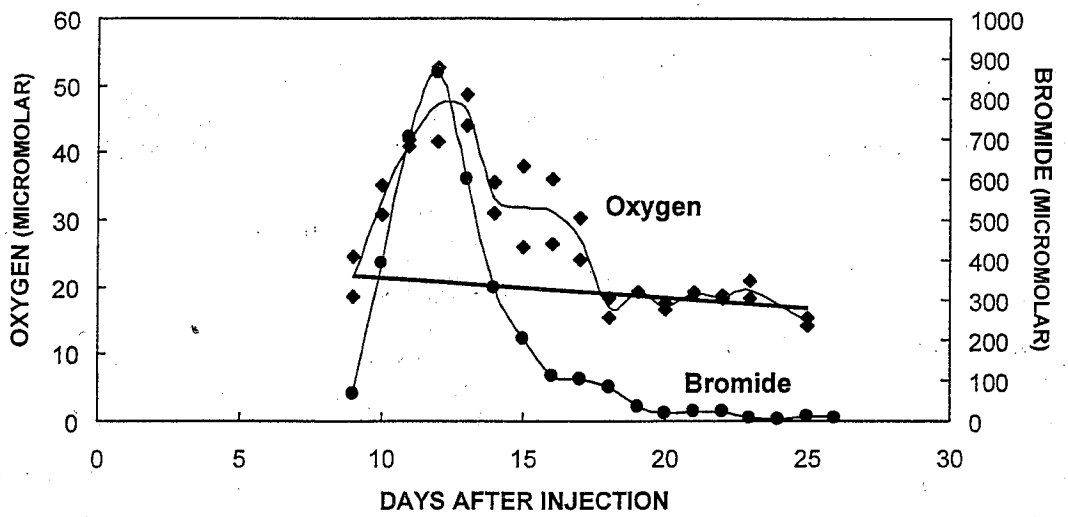

Figure 4. Breakthrough curves of bromide and oxygen at a multilevel sampler located 5.5 meters down-gradient from the injection well during a natural gradient tracer test conducted in the large-scale multilevel sampler array. The solid line is the computed background oxygen concentration. Injectate bromide and oxygen concentrations were 1290 and 111 micromolar, respectively.

(MLS) to introduce a tracer solution (200 liters) into a selected location within the oxygen gradient. The tracer cloud was transported down-gradient by natural ground-water flow and intercepted with rows of MLSs situated perpendicular to the water-table gradient (Smith and others, 1996). The grid of target MLS ports was sampled on a daily basis and analyzed for each of the tracer constituents. Sodium bromide was used as a conservative tracer to determine dispersion and advective transport and to track the path of the tracer cloud.

Aerobic respiration in the Cape Cod aquifer was assessed using two different types of tracers. When oxygen concentrations were relatively high (>30 μM), natural oxygen was replaced by exchange using $^{18}O_2$ (98 atom %) without altering the background concentration. For these tests, samples were collected and analyzed for the product of aerobic respiration, $H_2{}^{18}O$. When the oxygen concentration was low (<20 μM), air was used to increase the dissolved oxygen concentration 20-80 μM above background. Ground-water and injectate samples were analyzed for dissolved oxygen using gas chromatography (Penarrieta, 1998).

A representative breakthrough curve for one of the air tracer tests is shown in figure 4. The amount of oxygen consumed was calculated from the difference between the bromide and oxygen results, after normalizing each to the respective injectate concentration. For the data shown, the rate of oxygen consumption was 1.1 μmoles (liter aquifer x day)$^{-1}$ for 5.5 meters of transport (Penarrieta, 1998). This rate must be considered a potential rate because the in situ oxygen levels were increased above background. However, other in situ conditions were not altered. So the rate is relevant within the context of organic carbon (i.e. available supply of electron donor), microbial populations, and hydrologic flow. The rate can be viewed as an estimate of V_{max} for aerobic respiration within the 20 μM oxygen horizon and subsequently compared with the microcosm oxygen uptake results (see below).

For the $^{18}O_2$ tracer tests, enrichment of ^{18}O in H_2O was ≤ 0.1 ‰ in the breakthrough curves for two separate tests. These results indicate respiration rates ≤ 0.8 μmoles (liter aquifer x day)$^{-1}$. The lower rates of oxygen consumption are consistent with the $^{18}O_2$ test being conducted higher up in the oxygen gradient (i.e. less contaminated). Future work will entail simulating the breakthrough curves for the $^{18}O_2$ and air tracer tests using 1- and 2-dimensional advection-dispersion transport models that include Michaelis Menten-based equations for aerobic respiration.

MICROCOSM DETERMINATION OF AEROBIC RESPIRATION

Assessment of aerobic respiration from the small-scale (centimeter-scale) perspective was accomplished using laboratory incubations (microcosms) with freshly-collected aquifer core material. A variety of incubation techniques have been developed for surface-water systems to measure oxygen consumption and heterotrophic activity for indigenous microbial communities. Two of these were adapted for use in this ground-water study.

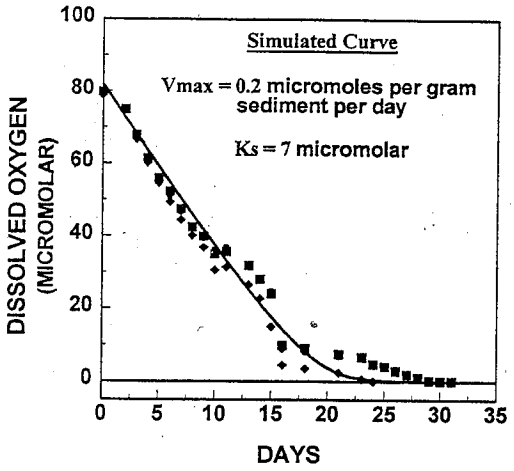

Figure 5. Progress curve of oxygen consumption by slurried aquifer core material collected from the large-scale multilevel sampler array at the 30 micromolar oxygen horizon. Incubations were conducted in triplicate (squares, diamonds and circles) at 15 °C. The line is a best-fit simulation calculated using the kinetic parameters shown in the inset.

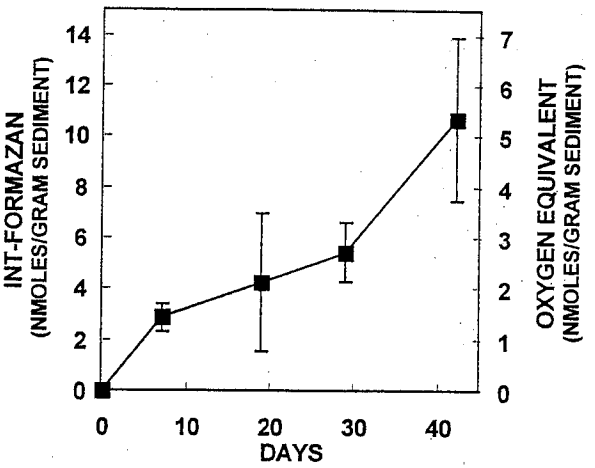

Figure 6. Production of INT-formazan by slurried aquifer core material collected from the large-scale multilevel sampler array at the 6 micromolar oxygen horizon and amended with 1 millimolar INT. Incubations were conducted at 15 °C. Error bars are standard deviation.

The first method is the direct determination of the Michaelis Menten kinetic parameters for aerobic respiration using substrate depletion progress curves. Aquifer core material was slurried with ground water in sealed serum bottles, to which varying amounts of oxygen were added (see Penarrieta, 1998, for details). The bottles were incubated with slow rotational mixing (~6 rpm) at in situ temperatures and oxygen concentrations monitored with time using gas chromatography.

Oxygen uptake in these microcosm incubations was slow, but measurable (fig. 5). Shown are the results for a core sample collected from the 30 μM oxygen horizon. Kinetic parameters were determined using best-fit simulations with a fourth-order Runge Kutta numerical approximation of the Michaelis Menten equation (Smith and others, 1994). The shape of the oxygen uptake curve is dictated by the kinetic parameters for oxygen consumption. Because there was little chemical oxygen demand in these samples, all oxygen uptake was assumed to be due to aerobic respiration. When converted to comparable units, these microcosm rates are >100-fold higher than the tracer test rate estimates. Microcosm replication within a mixed core sample was usually very good, but there was considerable variability between cores. The latter reflects the heterogeneity within the aquifer on this small scale and the disruptive effects that obtaining the core material had upon the attached microbial communities.

The second microcosm incubation method used was the determination of bacterial ETS activity using tetrazolium salts. Tetrazolium salts act as artificial electron acceptors, preferentially intercepting electrons from a cell's electron transport system prior to molecular oxygen, thus functioning as a measure of aerobic respiration. ETS activity assays have been used almost exclusively in surface-water systems; little is known regarding the utility in ground water. In this study the reduction of tetrazolium salt, 2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyltetrazolium chloride (INT) to INT-formazan was measurable in aquifer microcosms, indicating microbial respiratory activity (fig. 6). However, rates of aerobic respiration calculated from INT reduction were 4-10-fold lower than rates obtained from the direct measurement of oxygen consumption in the laboratory microcosms. Additional studies with bacterial cultures isolated from the Cape Cod aquifer revealed that INT is toxic to many ground-water bacteria. Thus, the low rates of aerobic respiration calculated from the INT assay probably reflect the toxicity of INT to some portion of the resident microbial population.

SUMMARY AND CONCLUSIONS

A multi-scale, integrated approach is the best choice for studying a microbial process in the saturated subsurface. This study has demonstrated that aerobic respiration within an aquifer does influence the isotopic signature of molecular oxygen in ground water. That signature is an integrated result of the processes

influencing oxygen concentration along a flow path. More detail about the in situ function of the microbial process relative to transport can be obtained on an intermediate scale using natural gradient tracer tests. These tests provide the in situ quantification of the rates of oxygen consumption and provide a means to compare consumption to oxygen dispersion and diffusion across the oxygen gradient. Finally, mechanistic information, such as the reaction kinetic parameters can be obtained using small-scale laboratory incubations. These incubations also enable the assessment of the short-term responses of the ground-water ecosystem to enviromental perturbations or changes in electron donor supply.

ACKNOWLEDGEMENT

This work was funded in part by a grant (R824787) funded by an Environmental Protection Agency/National Science Foundation Partnership for Environmental Research.

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