Oklahoma Water Research Institute
Annual Technical Report
FY 1998

Introduction

Research Program

Basic Project Information

Basic Project Information	
Category	**Data**
Title	Geochemical and Microbiological Influences on Terminal Electron Accepting Processes and Its Relation to the Biodegradation of Pollutants in the Subsurface: A Study of an Aquifer Contaminated by Landfill Leachate
Project Number	C-02
Start Date	09/01/1997
End Date	08/31/2000
Research Category	Water Quality
Focus Category #1	Groundwater
Focus Category #2	Hydrogeochemistry
Focus Category #3	Toxic Substances
Lead Institution	Oklahoma Water Research Institute

Principal Investigators

Principal Investigators			
Name	**Title During Project Period**	**Affiliated Organization**	**Order**
Joseph M. Suflita	Professor	University of Oklahoma, Dpt. of Botany and Microbiology	01
Ethan		Texas A&M University, Dpt. of Geology and	

Grossman	Professor	Geophysics	02
Luis Cifuentes	Professor	Texas A&M University, Dpt. of Oceanography	03

Problem and Research Objectives

Accurate prediction concerning the fate of pollutants in the environment depends upon precise observations of a number of ecological parameters. Subsurface microorganisms can play a central role in the destruction of contaminants within aquifers. The geochemical makeup of an aquifer can influence the biological activity of these organisms which can in turn, have an effect on geochemistry. The work performed by implementation of this proposal has been to determine the presence and distribution of microbial processes that are occurring in the aquifer and to assess the impact of these processes on the quality of the groundwater. This project has been addressed in a variety of ways which include on-site field measurements and observations as well as laboratory experimentation and analyses. The findings are certainly not restricted to the Norman landfill. Many of our results will be useful to other investigators who are working in related areas at other field sites.

Methodology

TASK 1: GEOCHEMICAL ANALYSIS OF HYDROGEN IN GROUNDWATER This task involves the measurement of dissolved hydrogen gas in the groundwater that underlies the landfill. We are investigating the usefulness of hydrogen as an indicator of the dominant microbial processes which could be occurring at the site. Information concerning the three-dimensional distribution of microbial processes is required in order to accurately describe a subsurface ecosystem and predict the rate of biodegradation of pollutant chemicals. If hydrogen can be used to make this description, then the task of characterizing the environment is greatly simplified. Traditionally, this characterization involves obtaining sediment cores which are used in laboratory experiments that can incur a significant expense while often requiring long incubation times and tedious sampling. Groundwater sampling for hydrogen is much simpler and cost effective in that no sediment is required. Water can be pumped from wells which have been installed at the site of interest and can then be removed after hydrogen monitoring is complete, resulting in minimal disturbance to the environment. To directly determine the microbial processes occurring in the aquifer below the landfill, and as a way to test the validity of hydrogen measurements, we have obtained sediment cores of landfill material and used them in a variety of laboratory experiments. These samples have been used to determine the types of bacteria that are present in the leachate-impacted zone and how they are distributed. Sediment from the aquifer has also been used in direct rate measurements of microbial processes. These rate experiments were used to elucidate the dominant microbially catalyzed redox reactions occurring in the aquifer. The results were compared with the predictions made based on dissolved hydrogen measurements. My laboratory has used sediment from the landfill to develop a procedure for extracting and quantifying reduced sulfur compounds from water and sediment. The procedure is an adaptation of a previous method which provides comparable results while requiring significantly less time, effort, and expense. TASK 2: ORIGIN AND CYCLING OF METHANE Methanogenesis is an important terminal electron accepting process in landfills and aquifers impacted by landfill leachate (Beeman and Suflita, 1990; Adrian et al., 1994). Methane produced in landfills like the Norman landfill can be an explosion hazard, a source of atmospheric greenhouse gases, and a substrate for microbial activity. This methane may be produced by aceticlasis or CO2 reduction. These pathways impart distinctive isotopic signatures that can be used to identify the origin of the gas (Whiticar et al., 1986; Hackney et al., 1996; Grossman, 1996). Previous studies suggest that methane in landfills forms predominantly from aceticlasis. Geochemical modeling of data for methane d13C and dD, leachate dD, and DIC d13C should provide a test of this hypothesis. Another important issue is the fate of methane in aquifers containing landfill leachate. How does this methane impact the geochemistry and microbiology of the aquifer, and influence groundwater quality? Methane may be oxidized aerobically by methylotrophs (Hanson and Wattenberg, 1991; Kightly et al., 1995) or anaerobically by methanogens (Hoehler et al., 1994) and perhaps sulfate reducers (Reeburgh, 1980; Kelly et al., 1985). These groups have varied capabilities to metabolize organic contaminants, thus the factors controlling their activity and distribution have important implications for contaminant degradation. Because methane oxidation leads to large carbon and hydrogen isotopic fractionations, the process can be traced with stable isotopic measurements. To study the origin and fate of methane at the Norman Landfill site, carbon and hydrogen isotopic analyses of methane, DIC, and water will be performed on water samples collected seasonally from wells located along a flow path. Additional hydrochemical data will be collected to assess the availability of electron acceptors (oxygen, nitrate, sulfate, DIC), presence of degradation products (CH4 , DIC, H2S, Fe2+ , etc.), and changes in the carbon cycle (d13CDIC, alkalinity, pH, DIC). Such chemical and isotopic data will be collected in collaboration with researchers from the University of Oklahoma and the U.S. Geological Survey. We plan to focus on waters along a southwest transect starting at site 35 in the west cell of the Norman landfill site (Scholl and Christenson, 1998). Waters upgradient of the landfill will also be sampled to provide a baseline for study. Waters will be sampled at least quarterly to provide seasonal coverage.

Principal Findings and Significance

TASK 1: GEOCHEMICAL ANALYSIS OF HYDROGEN IN GROUNDWATER Hydrogen measurements indicate that different bacterial processes are occurring at various locations within the aquifer. In fact, significant differences in hydrogen values have been measured at different depths and at different times of the year within the same well. A recent finding has been that of elevated hydrogen concentrations after an isolated rainfall event. During the particular sampling period, the soil was saturated with rainwater, which may have prevented the diffusion of oxygen, fostering a more reduced condition in the aquifer resulting elevated hydrogen levels. Alternatively, rain can deliver a short-lived pulse of oxygen to the surface of the water table that can cause the partial oxidation of otherwise recalcitrant organic molecules. Once the oxygen is depleted, the partially oxidized compounds left behind can serve as substrates for the anaerobic communities whose activity would also raise hydrogen values. We are currently monitoring dissolved hydrogen in several wells at monthly intervals in an effort to observe temporal changes in the dominant microbial processes. Our work has shown that the concentration of hydrogen in groundwater can indeed be a useful indicator. However, under some environmental conditions its usefulness is limited and needs to be considered in concert with other groundwater geochemical measurements. Future work in this area will further define the usefulness of dissolved hydrogen measurements and provide an additional tool for site characterization to workers in several disciplines including microbiology, geochemistry and various engineering sciences. Our findings also indicate that the distribution of microorganisms and microbial activities is not uniform but that 'hot spots' exist where a particular type of microorganism is numerically dominant over all others. Some locations in the aquifer contain several different types of bacteria, which are all in relatively high numbers. Furthermore, we have shown that even though some types of bacteria may be greatly outnumbered in a particular area, they are still active and are able to survive in the midst of fierce competition. This phenomenon has long been assumed by researchers in this field but seldom shown in laboratory experiments. These types of studies are critical in advancing our understanding of how microorganisms survive in the subsurface and how they compete against one another. Not only does this information describe the aquifer that underlies the Norman landfill but can also be used to help predict the status of other contaminated sites and aid in their characterization. Direct rate measurements made in the laboratory suggest that sulfate reduction and methanogenesis are the most important microbial reactions at the aquifer locations. At one location, the hydrogen values measured in the field agree with the laboratory experiments in predicting sulfate reduction as a dominant process. In another area of the aquifer, the hydrogen suggests that iron reduction is the dominant reaction but laboratory rate measurements could not confirm this prediction. This finding implies that caution is necessary in the interpretation of dissolved hydrogen data and that other methods should be employed, in addition to hydrogen measurements, in efforts to accurately map the distribution of microbially catalyzed redox reactions. The quantitation of inorganic sulfur compounds in sediment can indicate the activity of sulfate-reducing bacteria which can be responsible for a variety of metabolic processes including the souring of oil formations, corrosion of steel equipment and biodegradation of pollutant chemicals. The improved method for quantitating reduced sulfides, which we developed, has now been published and is of interest to researchers not only in microbiology but in the engineering and geosciences as well. In addition to determining the dominant microbial processes that are the factors that limit the activity of the microorganisms also need to be investigated. We have found that sulfate reduction is an important process at the landfill and our experimental evidence has suggested that the sulfate concentration in the aquifer is maintained at a level which limits the rate of sulfate reduction. Sulfate-reducing bacteria are widely distributed in many environments and have the capacity to degrade compounds that are metabolized slowly or not at all under other conditions. At some sites it may be necessary to know what limits the activity of these organisms in order to maximize their biodegradative capacity. We have examined possible sources of sulfate to the aquifer. In addition to recharge from upgradient water and the refuse itself, the cycling of reduced iron-sulfur compounds near the water table is an important regeneration of sulfate. The water table at the landfill responds almost immediately to rainfall events, suggesting that oxygen dissolved in rain reaches the water table and can oxidize the reduced iron and sulfur species that are abundant at that location. This cycle produces sulfate, which is soluble and becomes available for reduction by the resident microflora. At locations further below the water table, barite dissolution is likely responsible for the maintenance of a low, background concentration of sulfate. Barite (barium sulfate), which is typically a stable mineral, will dissolve at very low sulfate concentrations, releasing soluble sulfate to the aquifer. Since this dissolution happens only when sulfate is very low, this process does not occur at the water table where sulfate is found in the mM range. It is more likely that barite dissolution is an important sulfate source at other locations as a mechanism for maintaining a low but constant supply of sulfate to the resident microorganisms. The spatial distribution of sulfate reduction activity can be visualized based on a direct imaging procedure that we have developed using cores obtained from the leachate impacted area downgradient from the landfill. Radioactive sulfate is applied to the face of a core sample, the sulfate is reduced by the resident microflora yielding radioactive sulfide which precipitates on the soil particles. The unreacted sulfate is washed away leaving only sulfide, which can be examined qualitatively, and quantitatively using a specialized autoradiography instrument. This procedure allows for direct viewing of the distribution of microbial activity in a minimally disturbed sample giving the researcher an accurate representation of how the processes occur in the field. TASK 2: ORIGIN AND CYCLING OF METHANE Groundwater samples for isotopic analyses have been collected from Norman landfill during 6/98, 4/99, and 6/99 field excursions. Analyses of 6/98 samples are complete and those of 4/99 samples are in progress. Carbon isotope analyses of methane clearly show evidence for methane oxidation on the margins of the plume. In the plume center, methane d13C values are about -56‰, whereas these values increase above and below the plume to values at least as high as -22‰. This increase in methane d13C results from the preferential oxidation of 12CH4 by bacteria. Preliminary data suggest that methane is oxidized along the flow path as well. It is unclear whether methane is oxidized aerobically or anaerobically. Future modeling studies in conjunction with U.S. G. S. hydrogeochemical studies will address this issue. The carbon isotopic compositions of dissolved inorganic carbon (DIC) show the influence of methanogenesis on the DIC in the plume center, with d13C values as high as 13‰. As expected, DIC contents are also high with values of ~50mmol/L within the plume and even higher values (>60mmol/l) in the transition zone below the plume. The carbon isotopic compositions of DIC decrease away from the plume due mostly to mixing with pristine groundwater. Methane oxidation also contributes 13C-depleted DIC to the groundwater, but mass balance modeling suggests that the amount of carbon added by methane oxidation is small compared with the total amount of DIC in the groundwater. Future efforts will examine the mechanism of methanogenesis, CO2 reduction versus aceticlasis, through hydrogen isotopic analyses of methane. REFERENCES Adrian, N.R., Robinson, J.A., and Suflita, J.M., 1994. Spatial variability in biodegradation rates as evidenced by methane production from an aquifer. Appl. Environ. 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Descriptors

Aquifer Characteristics, Anaerobic Treatment, Bacteria, Biodegradation, Biogeochemistry, Geochemistry, Groundwater Quality, Isotopes, Landfill, Microbiology, Pollutants, Toxic Substances

Articles in Refereed Scientific Journals

Cozzarelli, I. M., J. M. Suflita and G. A. Ulrich. Heterogeneity of Anaerobic Microbial Processes in a Shallow Aquifer at the Norman Landfill. In Preparation. 1434HQ-96-GR-02692-02 Ulrich, G. A., G. N. Breit, I. M. Cozzarelli, and J. M. Suflita. Biogeochemistry of Barium and the Ecology of Sulfate-Reduction under Electron Acceptor Limitations in a Landfill Leachate-Contaminated Aquifer. In Preparation. 1434HQ-96-GR-02692-02 Krumholz, L. R., J. Li, W. W. Clarkson, G. G. Wilber, and J. M. Suflita. 1997. Tranformation of TNT and Related Aminotoluenes in Groundwater Aquifer Slurries under Different Electron Accepting Conditions. J. Indus. Microbiol. Biotechnol. 18:161-169. 1434HQ-96-GR-02692-02 Krumholz, L. R., and J. M. Suflita, 1997, Anaerobic Aquifer Transformations of 2,4,-Dinitropnenol under Different Terminal Electron Accepting Conditions. Anaerobe 3:399-403. 1434HQ-96-GR-02692-02 Ulrich, G. A., L. R. Krumholz, and J. M. Suflita, 1997. A Rapid and Simple Method for Estimating Sulfate Reduction Activity and Quantifying Inorganic Sulfides. Appl. Environ. Microbiol. 63:1627-1630. 1434HQ-96-GR-02692-02

Book Chapters

Dissertations

Water Resources Research Institute Reports

Conference Proceedings

Cozzarelli, I. M. , J. M. Suflita, G. A. Ulrich, S. H. Harris, M. A. Scholl, J. L. Schlottmann, and J. B. Jaeschke, 1999, Processes in a Contaminant Plume Downgradient from a Landfill, Norman, Oklahoma, in Morganwalp D.W. and T. H. Buxton eds., U.S. Geological Survey Toxic Substances Hydrology Program - Proceedings of the Technical Meeting, Charleston, South Carolina, Volume 3 in Subsurface Contamination from Point Sources: U.S. Geological Survey Water Resources Investigations Report 99-4018C 1434HQ-96-GR-02692-02 Harris, S. H. , G. A. Ulrich , and J. M. Suflita, 1999, Dominant Terminal Electron Accepting Processes Occurring at a Landfill Leachate-Impacted Site as Indicated by Field and Laboratory Measures, in Morganwalp D.W. and T. H. Buxton eds., U.S. Geological Survey Toxic Substances Hydrology Program - Proceedings of the Technical Meeting, Charleston, South Carolina, Volume 3 in Subsurface Contamination from Point Sources: U.S. Geological Survey Water Resources Investigations Report 99-4018C 1434HQ-96-GR-02692-02

Other Publications

Information Transfer Program

Basic Project Information

Basic Project Information	
Category	**Data**
Title	Prism
Description	Bimonthly newsletter of the Environmental Institute, including the OWRRI
Start Date	
End Date	
Type	Newsletter
Lead Institution	Oklahoma Water Research Institute

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Category	**Data**
Title	Technical Reports and General Publications
Description	Listing of technical reports and other publications generated by the OWRRI and Environmental Institute. Available in print or on Institute web site.
Start Date	
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Type	Publications
Lead Institution	Oklahoma Water Research Institute

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USGS Internship Program

Student Support

Student Support					
Category	**Section 104 Base Grant**	**Section 104 RCGP Award**	**NIWR-USGS Internship**	**Supplemental Awards**	**Total**
Undergraduate	N/A	N/A	N/A	N/A	N/A
Masters	1	N/A	N/A	N/A	1
Ph.D.	N/A	5	N/A	N/A	5
Post-Doc.	N/A	N/A	N/A	N/A	N/A
Total	1	5	N/A	N/A	6

Awards & Achievements

Publications from Prior Projects

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