DOE/ER/61636--T/

FINAL PROJECT REPORT

The Microbial Methane Cycle in Subsurface Sediments

Award no.: DE-FG03-93ER61636

July 1, 1993 to August 31, 1997

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MASTER

STUDIES OF A TEXAS AQUIFER SYSTEM

The objectives of this study were to determine the factors controlling microbial activity and survival in the subsurface and, specifically, to determine whether microbial communities in aquitards and in aquifer microenvironments provide electron donors and/or acceptors that enhance microbial survival in aquifers. Although our original objectives were to focus on methane cycling, we pursued an opportunity to study sulfur cycling in aquifer systems, a process of much greater importance in microbial activity and survival, and in the mobility of metals in the subsurface. Furthermore, sulfur cycling is pertinent to the Subsurface Science Program's study at Cerro Negro, New Mexico.

Our study combined field and laboratory approaches and microbiological, molecular, geochemical, and hydrogeological techniques. During drilling operations, sediments were collected aseptically and assayed for a variety of microorganisms and metabolic capabilities including total counts, viable aerobic heterotrophs, total anaerobic heterotrophs, sulfate reducing bacteria (SRB) and sulfate reduction activity (in situ and in slurries), methanogens, methanotrophs, and Fe- and S- oxidizers, among others. Geochemical analyses of sediments included organic carbon content and $^{13}\text{C}/^{12}\text{C}$ ratio, sulfur chemistry (reduced sulfur, sulfate), $^{34}\text{S}/^{32}\text{S}$ ratios, and "bioavailable" Fe(III). Finally, to characterize chemical environments, flow patterns, and groundwater ages, groundwater levels were monitored and groundwater samples were regularly collected and analyzed for major ions, pH, dissolved oxygen, Fe, $^{34}\text{S}/^{32}\text{S}$, $^{13}\text{C}/^{12}\text{C}$, ^{14}C , tritium, etc.

We drilled eight boreholes in the Eocene Yegua formation at four localities on the Texas A&M University campus using a hollow-stem auger drilling rig. The drilling pattern forms a "T", with three well clusters along the dip direction and two along strike. Four boreholes were sampled for sediments and screened at the deepest sand interval encountered, and four boreholes were drilled to install wells in shallower sands. Boreholes range in depth from 8 to 31 m, with screened intervals ranging from 6 to 31 m. Below are the results of these field studies.

Microbial Abundance and Activity in a Low Conductivity Aquifer System in East-Central Texas (Martino et al., in press)

The influence of sediment properties and groundwater geochemistry on microbial abundance and activity was examined in a Gulf Coast aquifer system. Three boreholes were drilled into the sands, silts, clays, and lignite of the Eocene Yegua formation and wells were installed in all water-bearing sands. Total numbers of microorganisms ranged from 10^6 to 10^8 cells per gram dry weight (gdw^{-1}), and viable counts ranged from 0 to 10^6 cells gdw^{-1} . The highest densities of anaerobic heterotrophs and SRB (10^5 and 10^6 cells gdw^{-1} , respectively) were measured in the deepest aquifer sands (28-31 m) where the total organic carbon content was lowest. Rates of anaerobic H_2 , lactate, and formate consumption were also high in aquifer sands relative to the other strata. The higher microorganism numbers and activities in the aquifer sediments likely reflect the importance of increased electron donor and acceptor transport in higher hydraulic conductivity sands relative to other strata. The presence of sulfate and near absence of other electron acceptors (O_2 , NO_3^- , and Fe^{3+}), absence of methanogens, high numbers of SRB, and relatively high sulfate reduction activity suggest that sulfate reduction is the dominant terminal electron accepting process in the deep aquifer. In contrast to aerobic Atlantic Coastal Plain sediments, aquifer sediments at 30 m in this east-central Texas aquifer contain greater numbers of anaerobes than aerobes.

Low pH and high sulfate contents argue for oxidation of pyrite in or near the shallow aquifer. Pyrite from the deep aquifer sediments with the greatest abundance and activity of SRB is too enriched in ^{34}S (up to 58‰) to have formed from modern or typical groundwater sulfate. Overall, our data suggest that the resident microflora are capable of cycling carbon and sulfur in the aquifer, but probably without substantial remineralization of pyrite in the formation.

28-01-81

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Microbial Interactions in the Terrestrial Subsurface: Sulfur Cycling, Heterogeneity, and Survival (Ulrich et al., in prep.)

Microbiological, geochemical, and isotopic analyses of sediment and water samples from the unconsolidated Yegua formation in east-central Texas were used to assess microbial interactions in the terrestrial subsurface. Sediment of varied lithology (sands, silts, clays, lignite) was collected from two boreholes to depths of 30 m. Our findings suggest that pyrite oxidation strongly influenced the geochemical environment in the shallow sediments (~5 m) and produced acidic waters (pH 3.8) that were rich in sulfate (28 mM) and ferrous iron (0.3 mM). S- and Fe-oxidizing bacteria were readily detected in shallow sediments and likely played an indirect role in pyrite oxidation. In consistent fashion, there was a relative paucity of pyrite in shallow sediments and a low $^{34}\text{S}/^{32}\text{S}$ -sulfate ratio (0.2 ‰), reflecting contributions from ^{34}S -depleted sulfides, in shallow sediments. Pyrite oxidation likely provides a sulfate source for both oxic and anoxic aquifers in the region. A variety of assays and direct imaging techniques of ^{35}S -sulfide production in sediment cores indicated that sulfate reduction occurred in both the oxidizing and reducing portions of the sediment profile with a high degree of spatial variability. Narrow zones of activity were detected in sands that were juxtaposed to clay or lignite-rich sediments. The fermentation of organic matter in the latter laminae provided low molecular weight organic acids to support sulfate reduction in neighboring sands. Consequently, sulfur cycling in shallow sediments and the transport of sulfate represents an important mechanism for the survival of microorganisms in the subsurface by providing electron donors for chemoautotrophic microorganisms and electron acceptors for sulfate-reducing bacteria. The activity of the latter group of microorganisms was linked to the provision of suitable electron donors from spatially distinct zones.

Community Analysis of Iron- and Sulfur-oxidizing Bacteria in the Subsurface using Molecular Methods

The community structure of bacteria associated with the terrestrial subsurface sediments of a Gulf Coast aquifer system was examined by polymerase chain reaction (PCR) amplification of the 16S rRNA gene from microbial communities recovered from various depths. Sediment and groundwater were collected to depths of 30 m and enriched for iron and sulfur oxidizing microorganisms. DNA was extracted from frozen sediment and water samples along with enrichment cultures and isolates. Two different primer sets were used for the PCR, one for amplification of DNA from the domain bacteria and another specific for organisms of the genera *Thiobacillus*. Amplified DNA fragments of the V3 region of 16S rRNA were resolved by denaturing gradient gel electrophoresis (DGGE), and the resulting profiles were reproducible and specific for the communities from different depths. Bacterial diversity estimated from the number and intensity of specific fragments in DGGE profiles decreased with depth. The subsurface DNA was also amplified with primers specific for *T. ferrooxidans* and *T. thiooxidans*. This amplification showed the presence of sulfur oxidizers to be most abundant above the oxidizing-to-reducing transition zone. Several dominant fragments in the DGGE profiles of sulfur-oxidizing enrichments were excised and sequenced. Among the dominant populations were representatives related to *T. ferrooxidans* and *T. intermedius*.

A Rapid and Simple Technique for Measuring Sulfate Reduction Activity and Quantifying Sulfides (Ulrich et al., 1997)

A simplified procedure for the extraction of reduced sulfur compounds (S^0 , FeS , FeS_2 , and S^{2-}) is presented. This technique may be used for determination of either sulfate reduction rates or the levels of reduced sulfur in sediments or water. Complete extraction and recoveries of FeS , FeS_2 , and S^{2-} were observed. The extraction efficiency for S^0 depended on the form that was tested. This technique can be used with samples containing up to 20 mg reducible sulfur. We demonstrate the utility of this technique in a determination of both sulfate reduction rates and

reduced sulfur pools in marine and freshwater sediments. A side by side comparison with the established single step technique has yielded comparable results with a fraction of the effort.

OTHER STUDIES

Fine-scale Chemical and Isotopic Heterogeneity in Deep Groundwaters near Cerro Negro, New Mexico--Relation to Lithology and Microbial Activity (Grossman et al., 1997)

As part of the DOE Subsurface Science Program's study of microbial origins in the deep subsurface, cores and groundwaters were collected from the Cretaceous Dakota sandstone and Mancos shale near Cerro Negro, NM. To examine the impact of lithology and microbial activity on groundwater chemistry, we deployed a multi-layer sampler between the depths of 183 to 204 m and performed chemical and isotopic analyses at a resolution of 30 cm.

Groundwaters are Na-HCO₃ waters with pH ranging from 7.7 to 8.6 and total dissolved solids ranging from 1050 to 1470 mg/L. Water chemistry correlates with lithology as indicated by electric logs and core samples. Shale aquitards above and below the Cubero sandstone member contain waters with high Na and dissolved inorganic carbon (DIC) contents. Additionally, DIC from shale aquitards have homogeneously low ¹³C compositions (mostly -7 to -6‰). Conversely, near the upper and lower shale-Cubero sandstone contacts, ¹³C contents of waters in the sandstone systematically increase to maxima of > -4.5‰ in the middle of the Cubero. Thus, lithology exerts local control on groundwater chemistry.

Microbiological evidence from Cerro Negro suggests that fermentation products diffusing from shales enhance sulfate reduction at sand-shale contacts (Krumholz et al., 1997, Nature 386:64). A small ¹³C minimum at the upper shale-Cubero contact, presumably caused by respired carbon, coincides with an interval of enhanced sulfate reduction activity. This interval also exhibits unusually high dissolved sulfide contents and downward-decreasing sulfate contents. Thus, fine-scale sampling of groundwaters reveals chemical and isotopic gradients associated with lithologic and microbiologic variation.

Origin of Gaseous Hydrocarbons from Upper Cretaceous and Tertiary Strata in the Piceance Basin, Western Colorado (Katz and Grossman, 1996; Katz, 1997)

Natural gas samples were collected for geochemical analyses from Upper Cretaceous and Tertiary strata of the Piceance basin in western Colorado to: 1) determine the origin of gases (i.e., microbial versus thermogenic), 2) determine the thermogenic source rock(s) for the gas-rich Williams Fork Formation, and 3) assess the nature of gas migration.

Mud logging gases were sampled approximately every 60 m between 350 and 2800 m and analyzed for ¹³C compositions and C₁/C_{1,3} ratios. Samples collected from low gas content intervals above 1950 m define two parallel trends of increasing ¹³C_{C1} content with depth. Data from the first trend are based on eighteen analyses and range from -69.9 to -38.3‰ (R² = 0.92). These data suggest a microbial and mixed microbial/thermogenic origin for methane. Only one sample from above 1950 m contained sufficient amounts of C₂ for isotopic analysis (δ¹³C_{C2} = -27.0‰ at 1718 m). Data from the second trend are based on seven analyses and are offset by approximately +20‰ compared with the primary trend at comparable depths. These data range from -65.0 to -38.5‰ (R² = 0.84). δ¹³C_{C1} and C₁/C_{1,3} data from both trends are similar when viewed on a crossplot, thus suggesting that large-scale, vertical gas migration has occurred. Migration was probably aided by fractures that formed during maximum burial and peak gas generation. Except for one sample collected at 1718 m, ¹³C_{C2} compositions above 1950 m were not determined due to insufficient sample sizes. Below 1950 m, gas contents abruptly increase. These gases have ¹³C compositions indicative of thermogenic origin. Gases between 1950 and 2450 m have relatively

uniform geochemistries ($\delta^{13}\text{C}_{\text{C}_1} = -39.9 \pm 0.3\text{‰}$, $\delta^{13}\text{C}_{\text{C}_2} = -27.4 \pm 1.1\text{‰}$, $\text{C}_1/\text{C}_{1+2} = 0.91 \pm 0.03$), and are chemically distinct and therefore genetically different from gases between 2450 and 2791 m ($\delta^{13}\text{C}_{\text{C}_1} = -37.9 \pm 0.2\text{‰}$, $\delta^{13}\text{C}_{\text{C}_2} = -26.4 \pm 0.5\text{‰}$, $\text{C}_1/\text{C}_{1+2} = 0.88 \pm 0.01$). Gases of the latter group were probably derived from coalbeds that comprise the Cameo Group, as abundant coals are found between 2450 and 2630 m. Only three thin coalbeds occur within the Coal Ridge Group between 1950 and 2450 m, so gases from this interval were probably derived from interbedded shales.

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RESEARCH

28 JUN 1997