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MICROBIOLOGICAL CHARACTERISTICS IN A ZERO-VALENT IRON REACTIVE BARRIER

Baohua Gu,[1*] David B. Watson,[1] Liyou Wu,[1] Debra H. Phillips,[1]
David C. White,[2] and Jizhong Zhou[1]

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* Corresponding author. Phone: 865-574-7286; Fax: 865-576-8543; e-mail: b26@ornl.gov
1. Environmental Sciences Division, Oak Ridge National Laboratory, P.O. Box 2008, Oak Ridge, TN 37831.
2. Center for Biomarker analysis~~Institute for Applied Microbiology~~, University of Tennessee, Knoxville, TN 37932-2575.

ABSTRACT

Zero-valent iron (Fe^0)-based permeable reactive barrier treatment has been generating great interest for passive groundwater remediation, yet few studies have paid particular attention to the microbial activity and characteristics within and in the vicinity of the Fe^0-barrier matrix. The present study was undertaken to evaluate the microbial population and community composition in the reducing zone of influence by Fe^0 corrosion in the barrier at the Oak Ridge Y-12 Plant site. Both phospholipid fatty acids and DNA analyses were used to determine the total microbial biomass ~~population~~ and microbial functional groups, including sulfate-reducing bacteria, denitrifying bacteria, and methanogens, in groundwater and soil/iron core samples. A diverse microbial community was identified in the strongly reducing Fe^0 environment despite a relatively high pH condition within the Fe^0 barrier (up to pH ~10). In comparison with those found in the background soil/groundwater samples, the enhanced microbial biomass ~~population~~ ranged from ~1 to 3 orders of magnitude and appeared to increase from upgradient of the barrier to downgradient soil. In addition, microbial community composition appeared to change over time, and the bacterial types of microorganisms increased consistently as the barrier aged. DNA analysis indicated the presence of sulfate-reducing and denitrifying bacteria in the barrier and its surrounding soil. However, the activity of methanogens was found to be relatively low, presumably as a result of the competition by sulfate/metal-reducing bacteria and denitrifying bacteria because of the unlimited availability of sulfate and nitrate in the site groundwater. Results of this study provide evidence of a diverse microbial biomass ~~population~~ within and in the vicinity of the iron barrier, although the important roles of microbial activity, either beneficially or detrimentally, on the longevity and enduring efficiency of the Fe^0 barriers are yet to be evaluated.

Keywords: iron barriers, microorganisms, microbial activity, diversity, and DNA.

INTRODUCTION

Microbiological processes may play an important role in the long-term performance of the zero-valent iron (Fe^0)-based permeable reactive barriers, yet few studies have paid particular attention to the microbial activity and characteristics within and in the vicinity of the Fe^0-barrier matrix (Gu et al., 1999; Scherer et al., 2000). Major uncertainties need to be resolved with respect to the adaptation of indigenous microorganisms to the strongly reducing Fe^0 environment, changes in the microbial community composition, and more importantly, their beneficial or detrimental effects on the longevity and enduring efficiency of the Fe^0 barriers. Depending on the groundwater geochemistry, an enhanced microbial activity may reduce the porosity and the hydraulic performance of the barriers as a result of the accumulation of biomass or biofilm, the production of gas bubbles (e.g., denitrification), and the formation of mineral precipitation (Taylor and Jaffe, 1990; Gu et al., 1999; Phillips et al., 2000; Scherer et al., 2000). A combination of excessive microbial growth and mineral precipitation on Fe^0 surfaces could also result in a decreased reactivity of Fe^0 with contaminants and the subsequent diversion of groundwater flow around the barrier (as corrosion products accumulate) and, thus the reduced long-term treatment efficiency of the barrier (Blowes et al., 1997; Tratnyek et al., 1997; O'Hannesin and Gillham, 1998; Liang et al., 2000).

Conversely, microorganisms could enhance the performance of the reactive barriers by contributing to the degradation of contaminants, by consuming H_2 gas bubbles produced by iron corrosion in groundwater, and by contributing to mineral dissolution (Matheson and Tratnyek, 1994; Allen-King et al., 1997; Weathers et al., 1997; Novak et al., 1998). More specifically, the depletion of dissolved oxygen and the production of cathodic H_2 by Fe^0 corrosion provide a reducing environment favorable to many H_2-consuming anaerobic microorganisms, such as sulfate- and metal-reducing bacteria, methanogens, and denitrifying bacteria, which may stimulate the biotransformation of many redox-sensitive contaminants. As an example, recent

studies have shown that a combination of Fe^0 with methanogenic consortium significantly enhanced both the rate and extent of transformation of chlorinated methanes, such as chloroform and carbon tetrachloride (Weathers et al., 1997; Novak et al., 1998; Till et al., 1998).

Although more than a dozen of in situ Fe^0 reactive barriers have been installed across North America (Liang et al., 2000; Scherer et al., 2000), field evidence for the enhancement of microbial populations and diversity as a result of Fe^0-corrosion is lacking. There were no detailed studies and monitoring of microbial activity and diversity at many of the reactive barrier sites, which have resulted in inconsistent observations to date. For example, no significant microbial activities were observed in several Fe^0-reactive barriers, such as at the Somersworth Sanitary Landfill site in New Hampshire (Duwart, 2000) and an industrial site in New York (Clark, 2000). On the other hand, significant microbial activities were observed in the water effluent and Fe^0 filings both in a laboratory column study (Gu et al., 1999) and a field flow-through experiment at the U.S. Department of Energy (DOE) Portsmouth, Ohio site (Liang et al., 1997). Increased levels of sulfide and sulfide-mineral precipitates were reported in these studies, presumably as a result of microbial reduction of sulfate to sulfide. Similarly, ferrous sulfides have been detected as coatings on mineral surfaces in an Fe^0-reactive barrier at the U.S. Coast Guard air base near Elizabeth City, North Carolina, although no specific analysis of microbial activity was reported (Puls et al., 1999).

The primary objective of this work was to determine and evaluate the microbial population and community composition in the reducing zone of influence by Fe^0 corrosion as part of the major study of an in situ iron reactive barrier designed for the sequestration or removal of uranium and other contaminants, such as technetium (^{99}Tc) and nitrate, in groundwater at the Oak Ridge Y-12 Plant site, Oak Ridge, Tennessee (Gu et al., 2001). Both phospholipid fatty acids (PLFA) and DNA analyses were performed using groundwater and

soil/iron core samples to determine the total microbial abundance and microbial functional groups, including sulfate-reducing bacteria, denitrifying bacteria, and methanogens.

MATERIALS AND METHODS

Site Description. The construction of the iron-reactive barrier trench at the Oak Ridge Y-12 Plant site was completed in late November 1997 as part of the technology demonstration using Fe^0 to retain or remove uranium and other contaminants in groundwater. The trench dimensions are ~225 ft in length, 2 ft in width, and ~30 ft in depth with an Fe^0-filled midsection of ~26 ft in length between two ~100-ft sections of pea gravel (Figure 1). Guar-gum biopolymer slurry was used during soil excavation to prevent the trench walls from collapsing, and the Peerless Fe^0 filings (about –1/2 to 25 mesh size from Peerless Metal Powders and Abrasives, Detroit, Michigan) were used as the reactive medium in the midsection of the barrier. The soil around the barrier is a heterogeneous mixture of fill materials, native soil, saprolite, and rock fragments (Watson et al., 1999). Native soil and saprolite from the Nolichucky Shale formation are present near the bottom of the barrier. The barrier trench is oriented nearly parallel to the direction of groundwater flow and was designed to use both the natural groundwater gradient and the permeability contrast between the iron/gravel in the trench and the native silt/clay outside the trench to direct groundwater flow through the iron treatment zone (Figure 1). Hydraulic monitoring at the site indicated that the hydraulic gradient is ~0.025 ft/ft across the site but flattens to 0.01 ft/ft in the vicinity of the barrier trench. More detailed site information and its hydrogeology were given elsewhere (Watson et al., 1999; Phillips et al., 2000).

Groundwater and Core Sampling. Approximately 48 piezometers including 6 multi-port monitoring wells within the iron barrier were installed at the site, although only several selected monitoring wells were sampled and analyzed for microbiological characteristics

as shown in Figure 1. These sampling wells include (1) DP-20m, screened at ~27.5 to 28 ft below the surface and located in the upgradient portion of the iron; (2) TMW-9, screened at ~15 to 30 ft below the surface and located in the center of the iron; (3) DP-11, screened at ~14.5 to 24 ft belowground and located in soil (downgradient of the iron) where groundwater exits the barrier; and (4) TMW-5, screened at ~14 to 24 ft belowground and located ~50 ft upgradient of the trench (Figure 1). Groundwater from TMW-5 was not influenced by the Fe^0 barrier and was therefore used as a background or reference well for comparison. Similarly, a vertical soil core sample was obtained in the vicinity of TMW-5 and used as a control for the assessment of changes in microbial population and composition as influenced by the Fe^0 barrier. The geochemical properties of groundwater in these monitoring wells are given in Table 1. In general, the site groundwater is aerobic with a near neutral pH and relatively high concentrations of nitrate, bicarbonate, and calcium in comparison with other anions (e.g., sulfate and chloride) or cations (e.g., Mg and Na). Groundwater samples for microbial assay were collected in 1-L polyethylene bottles, unfiltered and unacidified, but were preserved with 10-mL of formaldehyde (36%) prior to the analysis by the phospholipid fatty acids (PLFA) technique. No preservatives were added for those samples used for DNA analysis.

About 15 months after the barrier was installed, core samples were collected in polyurethane tubes (~4 ft length × 1¼ in. diameter) from the Fe^0 barrier and adjacent soil with a Geoprobe by Miller of Huntsville, Alabama. The northern upgradient and southern downgradient interfaces of the trench and soil/iron material were intercepted by coring at a 60-degree angle, as described in detail in Phillips et al (2000) and Gu et al. (2002). The cores were taken from three sections along the Fe^0 barrier at the ~14 to 22 ft and ~21 to 29 ft levels, although only selected core samples from the middle section of the Fe^0 barrier were used for microbial analysis (Figure

1). The soil or Fe^0 core samples were collected in the field in sterilized Whirl-Pak bags, placed in an ice-filled cooler, transported to a laboratory, and immediately frozen until analysis.

Phospholipid Fatty Acids Analysis

Phospholipid fatty acids (PLFA) are important components of all cellular membranes of living organisms, and PLFA analysis is based on the extraction and separation of phospholipid classes, followed by quantitative analysis using gas chromatography mass spectrometry (GC/MS). Briefly, some selected groundwater and core samples were obtained, and the microbial population and community composition were determined in the laboratory of Microbial Insights (Rockford, Tennessee). First, ~1 to 2 L of groundwater were collected and filtered on 47-mm Whatman Anodisc filters (0.22-μ pore diameter). The filter disks were then lyophilized and the lipids extracted with one-phase chloroform-methanol buffer extractant (Guckert et al., 1986). For soil and iron filing samples, an ~60-g subsample was used for the extraction of PLFA by the chloroform-methanol buffer (90 mL). The extracted total lipid was further fractionated into neutral lipids, glycolipids, and polar lipids on disposable silicic acid columns before analysis by GC/MS (Ringelberg et al., 1988). Results of PLFA analysis were expressed in picomoles (*p*mol) per mL of groundwater or in *p*mol per gram soil or iron mass, which were then converted to microbial biomass (cells mL^{-1} or cells g^{-1}) based on an assumption that each *p*mol of PLFA yields approximately 2×10^6 cells (Balkwill et al., 1988).

DNA Analysis

Although PLFA provides a means of measuring microbial biomass and community composition, it provides little information on specific microbial functional groups that are present in the sample. The DNA analysis was therefore performed to determine these functional groups, including sulfate-reducing bacteria, denitrifying bacteria, and methanogens.

Experimentally, about 2-L groundwater samples were taken; microbial cells were collected by centrifuging the groundwater at 12,000 rpm and used for the extraction of DNA. For soil and iron samples, a 5-g subsample was used for DNA extraction. The samples were ground in the presence of liquid N_2 and sterilized sand as described elsewhere (Zhou et al., 1996). The DNA was then extracted by sodium dodecyl sulfate (SDS)-based extraction buffer and purified with Wizard resin columns (Zhou et al., 1996).

In order to obtain semiquantitative information about specific functional groups of microorganisms, a series of tenfold dilutions of the original extracted samples were used and analyzed by polymerase chain reaction (PCR) with group-specific primers for various microbial functional groups, including sulfate-reducing bacteria (Karkhoff-Schweizer et al., 1995), denitrifying bacteria with heme-(nirS) and copper-(nirK) nitrite reductases (Braker et al., 2000), and methanogens (Springer et al., 1995). The PCR amplifications were carried out as described by Zhou et al. (1996). The highest dilution that gives PCR amplification was used as a semi-quantitative method to determine the relative abundance of each microbial functional group.

RESULTS AND DISCUSSION

Microbial Biomass ~~Population~~ and Diversity in Groundwater. Approximately 1 year after the Fe^0 reactive barrier was installed, a substantially enhanced microbial population was observed in the groundwater within and in the vicinity of the Fe^0 barrier from three sampling events between January 1999 and April 2000 (Figure 2). The total microbial abundance (in cells per mL groundwater) was ~1 to 3 orders of magnitude higher than that of the background groundwater (TMW-5) located ~50 ft upgradient of the barrier trench. The enhanced microbial biomass ~~population~~ is particularly evident in groundwater in the downgradient soil (DP-11) and in the center of the iron barrier (TMW-9). A lower microbial biomass ~~population~~, however, was found in the well (DP-20m) located in the upgradient portion of the iron where groundwater

enters the iron barrier. It therefore appears that the microbial biomass ~~population~~ increased from the upgradient to the downgradient soil of the iron barrier (or along the flow path). The microbial population appears to vary slightly in the three sampling events, which took place between January 1999 and April 2000. In the center well within the iron barrier (TMW-9), the microbial biomass ~~population~~ increased threefold to tenfold in April 2000 in comparison with those observed in January and August 1999. A slightly increased microbial population was also observed over time in the upgradient monitoring well in the iron barrier (DP-20m). These observations may suggest a rapid colonization of microbial communities in the first year in a Fe^0 environment with a relatively high pH and a low Eh. These results are consistent with previous observations that the microbial community ~~population~~ was able to adapt to the strongly reducing Fe^0 environment that resulted in an increased sulfate reduction over time in a laboratory column flow-through experiment using simulated groundwater (Gu et al., 1999). However, it is possible ~~pointed out~~ that the increased microbial biomass ~~population~~ in the Fe^0 barrier also may be partially attributed to the use of Guar gum during the trench excavation. Although the trench was flushed with ~3 bed volumes of water with enzyme for the degradation of Guar gum polymers after installation, the residual Guar gum degradation byproducts, such as glucose, may have greatly stimulated the initial growth of microorganisms.

A variety of microbial communities were also detected by the PLFA analysis in groundwater (Figure 3a,b). Despite the fact that many microbial species have overlapping PLFA patterns, ~~although PLFA analysis suffers from its inability to identify the functional groups of microorganisms because many microbial species have similar PLFA patterns. However,~~ PLFA analysis is able to provide insights into microbial community composition, because some specific functional groups of microorganisms contain characteristic fatty acid profiles or lipid biomarkers (Dowling et al., 1986; Guckert et al., 1986; Tunlid and White, 1991). As shown in

Figure 3, six general groups of microbial groups were classified on the basis of the following PLFA structural groups: monoenoic, polyenoics, normal saturated, terminally branched, mid-chain branched, and branched monoenoic PLFAs. The normal saturated PLFAs are found in almost all microorganisms except Archea, whereas ~~the~~ monoenoic ~~and polyenoic~~ PLFAs are found in most Gram-negative bacteria ~~and some types of~~as well as eukaryotic microorganisms. In particular, the monoenoic PLFA may represent ~~fast growing~~ Gram-negative bacteria that can utilize a wide range of carbon sources and adapt quickly to a variety of environments (Guckert et al., 1986; Balkwill et al., 1988). The branched PLFA ~~last three groups~~ are commonly found in Gram-positive and anaerobic microorganisms, such as sulfate- and metal-reducing bacteria, and some Gram-negative facultative anaerobes.

Results (Figure 3a) indicated that ~~these fast growing Gram negative bacteria (~~monoenoic PLFAs~~)~~ were among the most abundant found in all groundwater samples, and ~~their population as well as~~ the proportions ~~contents~~ of the monoenoic and terminally branched PLFAs ~~(or its related microbial community~~ (Figure 3b) appeared to increase slightly over time (between January 1999 and April 2000). On the other hand, the relative proportions ~~abundance~~ of polyenoic PLFAs decreased consistently with time, and no significant amounts of polyenoic PLFAs were found in groundwater samples obtained in April 2000 (or ~2.5 years after the Fe^0 barrier was installed). These observations may suggest that, over time, the polyenoic containing microorganisms or possibly polyenoics released from the Guar gum ~~populations~~ were gradually replaced by ~~other~~ eubacteria ~~bacterial types~~ (e.g., an increased terminally branched PLFA and its associated microbial community ~~population~~, Figure 3b). ~~However, it is pointed out that these observations may be complicated by the initial use and later degradation of residual Guar gum because polyenoics are commonly found in such organisms as fungi, protozoa, algae, higher plants, and animals~~. The Guar itself may contain some residual polyenoics, although a number

of elliptical 50 to 100 um long protozoa ~~and small organisms~~ were ~~indeed~~ observed in groundwater by a ~~colloidal~~ borescope (with a 200× microscope) that was initially intended to observe colloids in groundwater and the flow directions in the Fe^0 barrier in December 1998 (data not shown).

The terminally branched saturated PLFA, representing largely the Gram-positive bacteria, dominated among the branched PLFA group (Figure 3b), although the branched PLFA was much less abundant than the monoenoic PLFA (Figure 3a). In particular, the branched PLFA constituted less than 5% of total PLFA in the center well of the iron barrier (TMW-9). However, the relative abundance of these terminally branched PLFA increased consistently over time within the iron barrier (TMW-9 and DP-20m), which was in contrast to that of polyenoic PLFAs, as discussed earlier. These observations may again indicate the adaptation or growth of ~~these~~ Gram positive, anaerobic microorganisms under the site-specific environmental conditions within the Fe^0 barrier.

To identify specific functional groups of microorganisms in the groundwater, various tenfold dilutions of DNA extracts were analyzed with DNA primers for sulfate-reducing bacteria, denitrifying bacteria, and methanogens because these are the anaerobic microorganisms most likely influenced by the reduction zone of the Fe^0 barrier (Gu et al., 1999; Scherer et al., 2000). A typical image of the DNA gel electrophoresis is shown in Figure 4 (for sulfate-reducing bacteria). As expected, results (Table II) indicated that the sulfate-reducing and denitrifying bacteria were among the most dominant groups of microorganisms identified in these groundwater samples. The relative abundance of these microorganisms also increased along the flow path from upgradient of the Fe^0 barrier (DP-20m) to downgradient soil (DP-11), which was consistent with the results of PLFA analysis (Figure 2). These observations may provide direct evidence of microbial involvement in the reduction of sulfate and nitrate in the Fe^0

barrier under anaerobic conditions. As reported previously, significantly decreased concentrations of sulfate and nitrate were found in the reducing zone of the Fe^0 barrier (Gu et al., 1999; Watson et al., 1999; Phillips et al., 2000). Sulfates were reduced to sulfides, resulting in the formation of FeS precipitates in the Fe^0 barrier. Similarly, elevated sulfide concentration and sulfide mineral precipitation, such as FeS and mackinawite (Fe_9S_8), were also reported in laboratory Fe^0 columns after addition of a broad microbial inoculum (Gu et al., 1999). To date, sulfate reduction is considered primarily as microbiologically mediated reduction processes (Ehrlich, 1990). There is little or no direct evidence showing an abiotic reduction of sulfate by Fe^0 although the reduction of nitrate and sulfonic acid by Fe^0 metal has been reported (Lipczynska-Kochany et al., 1994; Huang et al., 1998).

Microbial Biomass ~~Population~~ and Diversity in Core Samples. As in groundwater samples, a substantially enhanced microbial biomass ~~population~~ and diversity were observed in soil and iron core samples that were taken ~15 months after the iron reactive barrier was installed in the field (Figures 5 and 6). The total microbial abundance in the soil/iron core samples was ~2 to 3 orders of magnitude higher than that in the background soil sample located approximately 50 ft upgradient of the Fe^0 barrier. In addition, the microbial biomass ~~population~~ appeared to increase from the upgradient iron barrier to the downgradient soil as found in groundwater samples in Figure 2, except in the upgradient soil (located approximately 6 in. from the soil/iron interface), which showed the highest microbial biomass ~~abundance~~ (Figure 5). This observation may be explained by the fact that the interface soil also is a strongly reducing zone influenced by the iron corrosion and H_2 and a zone with a relatively high concentration of sulfate and nitrate where groundwater enters into the Fe^0 reactive barrier. The soil could have been a better environment (with nutrients and carbon sources) than Fe^0 filings for the colonization and/or growth of indigenous microorganisms. Additionally, it is noted that the microbial

biomass ~~population~~ was expressed on a unit mass basis of soil or Fe^0 (Figure 5), which may also underestimate the total biomass content in the Fe^0 samples (on a unit volume basis) because of a relatively high particle density of metallic Fe^0.

Nevertheless, diverse microbial communities were present in these soil/iron core samples (Figure 6a,b), and the microbial community composition appears to differ slightly from those in the groundwater samples. In general, the relative abundance of polyenoics decreased from the upgradient portion of the iron to downgradient soil, whereas both terminally branched and monoenoic PLFAs increased, presumably related to the growth of both Gram-positive or Gram-negative anaerobic microorganisms. The relative percentages of polyenoics were particularly high in samples from the iron barrier compared with those in the soil, and these observations again may be complicated by the initial use of Guar gum.

Soil/iron core samples were also analyzed for DNA primers to identify microbial functional groups of sulfate-reducing bacteria, denitrifying bacteria, and methanogens (Table III). Results showed a relatively higher microbial population (or extracted DNA content) in both the upgradient and downgradient soil cores than in the iron samples and are in general agreement with the PLFA analysis (Figure 5). However, a much lower PCR amplification signal was observed in all these solid samples than those in groundwater samples (Table II). This discrepancy may be largely attributed to a poor recovery of DNA using the current methodology for extracting DNA from soil or iron samples because of sorption of these DNA macromolecules on the mineral or iron surfaces (Zhou et al., 1996). Nevertheless, sulfate-reducing bacteria, denitrifying bacteria, and methanogens were identified in most of these soil/iron core samples.

Although methanogens are known to have a great affinity for H_2 (Lovley and Goodwin, 1988), relatively low levels of methanogenic microorganisms were identified in both groundwater and soil/iron samples in comparison with those of sulfate-reducing bacteria and

denitrifying bacteria (Tables II and III). The low levels of methanogens may be related to the relatively high concentrations of nitrate and sulfate present in groundwater, which could have inhibited the proliferation of methanogens. Such observations are consistent with previous studies that showed that sulfate-reducing bacteria have a greater affinity for H_2 than methanogens and may therefore outcompete hydrogenotrophic methanogens because of an unlimited availability of sulfate in groundwater (Lovley and Goodwin, 1988).

Summary and Conclusions. The present study provides evidence of a stimulated microbial population in an in situ Fe^0 reactive barrier used for the sequestration or removal of such contaminants as uranium, ^{99}Tc, and nitrate. An increased biomass and diversified microbial community composition ~~structure~~ was observed by PLFA which provides ~~can provide~~ quantitative measures of in situ community composition and indications of nutritional status~~analysis of characteristic fatty acids or lipid biomarkers, although such analyses were unable to provide information regarding specific microbial species and therefore their functional roles in sequestering or degrading a variety of contaminants~~. On the other hand, DNA analysis provided direct measurements of microbial functional groups, including anaerobic sulfate-reducing bacteria, denitrifying bacteria, and methanogens. Some important implications of the presence of these microorganisms are probably twofold, either beneficial or detrimental, to the performance of the Fe^0 barrier. For example, denitrifying bacteria may have greatly increased the rate and extent of nitrate reduction in the reducing zone of the Fe^0 barrier. As reported previously (Gu et al., 2001), nitrate was found to be completely degraded in groundwater in most of the monitoring wells within and downgradient of the Fe^0 barrier. Similarly, sulfate-reducing bacteria may have played an essential role in reducing sulfate to sulfide (Gu et al., 1999; 2001) because there is no evidence to date showing that sulfate could be chemically reduced to sulfide by Fe^0. These observations illustrate some beneficial contributions of microbial activity for enhanced degradation of contaminants or co-contaminants in groundwater. In addition, previous studies also indicated that these anaerobic microorganisms might potentially benefit the

hydraulic flow of the system by consuming cathodic H_2 gas generated by the corrosion of Fe^0 in groundwater (Gu et al., 1999).

However, increased microbial biomass ~~population~~ also may adversely impact the performance of the Fe^0 barrier because of the potential biofouling and gas production from microbial respiration and denitrification (such as CO_2, N_2, and nitrous oxides). More importantly, perhaps, the reduction of sulfate to sulfide has been reported to contribute to FeS mineral precipitation, which forms coatings on Fe^0 surfaces, decreases the surface reactivity of Fe^0 with contaminants, and causes the cementation of Fe^0 grains or system clogging (Gu et al., 1999; 2001; Phillips et al., 2000). The present study provided no evidence of excessive microbial growth or biofouling in the Fe^0 barrier, probably because of a limited supply of nutrients and a relatively high pH condition. However, the highest microbial biomass ~~population~~ appears to be associated with soils adjacent to the Fe^0 barrier (Figures 2 and 5), where H_2 could diffuse in to stimulate the growth of indigenous anaerobic microorganisms. A close monitoring of microbial activity in the soils in the vicinity of the Fe^0 barrier may thus be important to assess potential biofouling. Future studies are certainly needed for a better understanding of the beneficial or detrimental effects of microbial activity on the long-term performance of the Fe^0 barriers.

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Table 1. General groundwater chemistry at the Oak Ridge Y-12 Plant reactive barrier site (sampled in January 1999).

	TMW-5	DP-20m	TMW-9	DP-11
pH	6.8	7.4	9.0	6.6
Eh	215	-260	-162	-112
Conductivity (μS)	1605	1006	301	845
Nitrate (mg/L)	904	2.4	0.1	0.1
Sulfate (mg/L)	46.8	43.5	67.9	77.5
Chloride (mg/L)	54.9	119.6	21.1	27.6
$HCO_3^-/CO_3^=$ (mg/L)	139	474	97	592
Calcium (mg/L)	361	158	29.8	162
Magnesium (mg/L)	20.5	22.8	12.1	20.8
Sodium (mg/L)	8.9	34.6	18.9	20.6
Potassium (mg/L)	2.9	2.7	2.2	5.0

Table 2. DNA functional group analysis of microorganisms[a] in groundwater at the Y-12 Pathway 2 site.

	TMW-5	DP-20m	TMW-9	DP-11
By DNA Analysis				
Sulfite reductase	nd[b]	10^2	10^2	10^4
Denitrifying bacteria	nd[b]	10	10^3	10^3
Nitrite Reductase	nd[b]	nd[b]	10^2	10^4
Methanogens	nd[b]	1	nd[b]	10

a. Microbial populations are presented as the maximum dilution (tenfold incremental) at which they are detected by the DNA PCR analysis. Sulfate-reducing bacteria, denitrifying bacteria, nitrite reductase, and methanogens were determined by the DSR, Heme, Cu, and MCR primers, respectively.

b. nd = not detected.

Table 3. DNA functional group analysis of specific microbial populations in core samples at the Y-12 Pathway 2 site.

	Background soil	Upgradient soil (~21 ft)	Upgradient iron (~22 ft)	Downgradient iron (~21 ft)	Downgradient soil (~22 ft)
Sulfite reductase	nd[a]	10^2	1	1	nd[a]
Denitrifying bacteria	nd[a]	1	nd[a]	1	1
Nitrite reductase	1	10^2	10	10	10^2
Methanogens	nd[a]	10	nd[a]	1	nd[a]

a. nd = not detected.

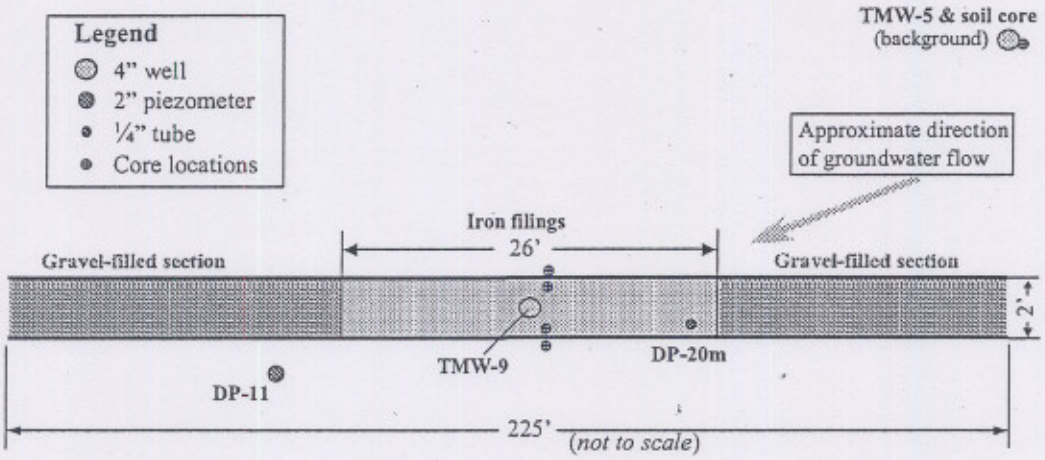

Figure 1. Schematic drawing of the iron/gravel barrier at the Oak Ridge Y-12 Plant site and the locations of groundwater sampling wells and soil/iron cores used for the determination of microbial population and community structure.

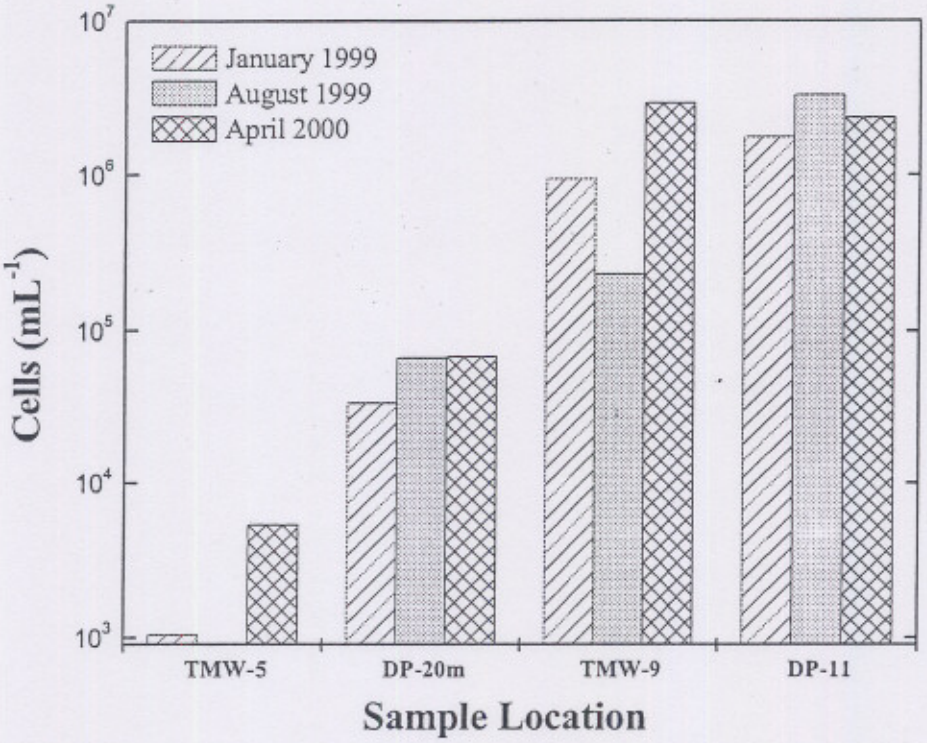

Figure 2. Microbial ~~population or abundance~~biomass by PLFA analysis in groundwater from monitoring wells located at the upgradient soil (TMW-5), within the Fe^0 barrier (DP-20m and TMW-9), and at the downgradient soil (DP-11).

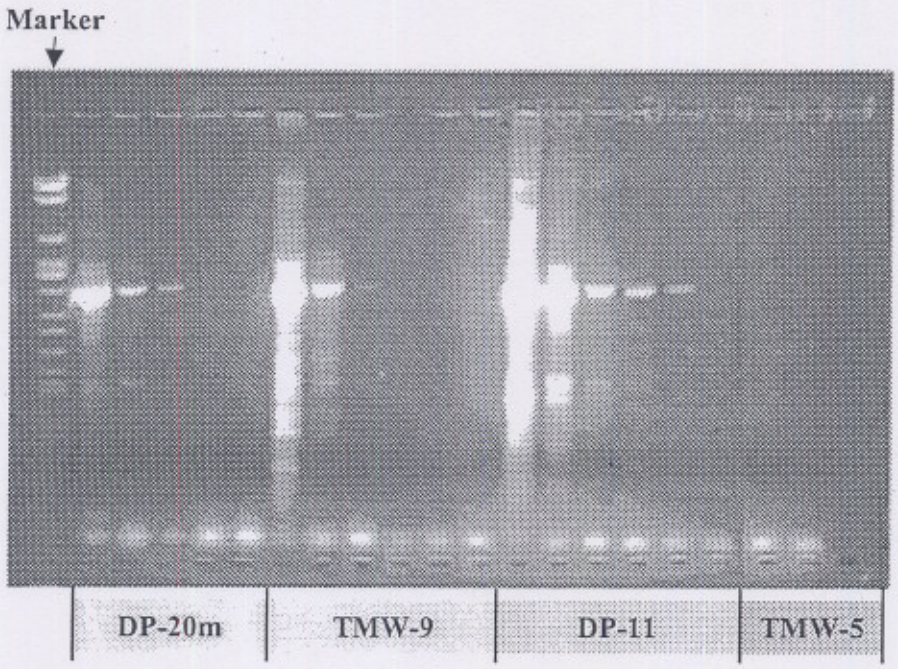

Figure 4. DNA gel electrophoresis image of dissimilatory sulfite reductase for sulfate-reducing bacteria in groundwater samples within and in the vicinity of the Fe^0 barrier. Different bands in each sample represent a series of tenfold dilutions for obtaining semiquantitative information on the abundance of sulfate-reducing bacteria.

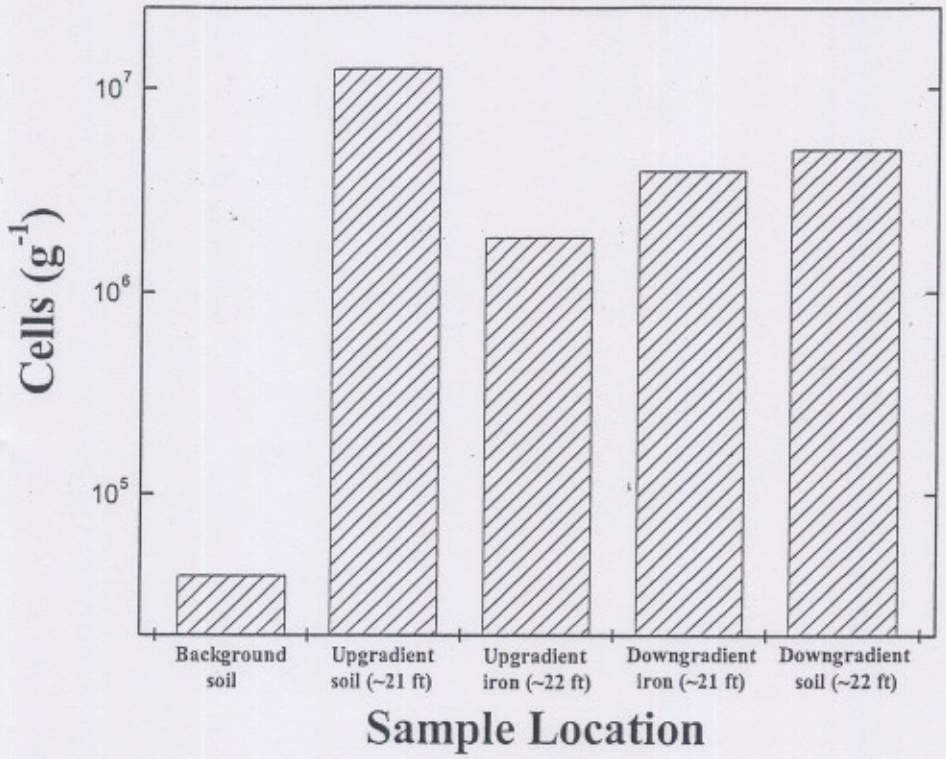

Figure 5. Microbial ~~population or abundance~~biomass by PLFA analysis in soil/iron core samples within and in the vicinity of the Fe^0 barrier.