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Role of Microbial Activity in Fe and S Cycling in Sub-Oxic to Anoxic Sulfide-Rich Mine Tailings: A Mini-Review

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Acid-mine drainage is an important environmental problem associated with base-metal mining and numerous studies have looked at the chemical and microbial processes involved in the oxidation of metal sulfides under oxic conditions. However, little is known about the activity of bacteria living in the same sulfide-rich tailings, but under sub-oxic to anoxic conditions. Recent work on Cu-Zn mine tailings have shown that sulfate-reducing bacteria (SRB) are present and active in these tailings (under a wide range of pH conditions) and that their activity is season dependent. In fact, acidic conditions and low organic carbon availability in the spring tend to greatly limit the activity of SRB in the tailings. On the other hand, IRB populations tend to increase in number in the spring, maybe as a result of the lack of activity of SRB. These findings are in agreement with studies on acidic coal mining lakes in Germany, which showed that IRB were mainly active in acidic and oxic sediments whereas SRB dominated in the more anoxic and pH neutral sediments. The presence and activity of SRB and IRB also represents a potential bioremediation tool, because both microbial pathways generate alkalinity. Recent work on acidic mining lakes indicates that IRB and SRB activity could be enhanced in a control manner and be used as an acidity neutralizing process to treat environments impacted by AMD.

Introduction

Base-metal mines generate large quantities of mining residues, which are commonly called mine tailings. These mine tailings often contain large concentrations iron sulfide minerals (pyrite, pyrrhotite), along with small amounts of the ore itself and they are generally disposed of in open-air impoundments. Once exposed to air and humidity (from rain or snow), the sulfide-rich minerals can undergo oxidation as a result of various microbial and chemical processes.[1] The oxidative dissolution of iron sulfides generate acidity and releases large quantities of sulfate and soluble heavy metals (Fe, Cu, Zn, Ni, Pb, Hg, As, etc.), producing acid-mine drainage (AMD). A large body of literature is available on the generation of AMD,[2] the microbial populations involved in the oxidation of metal sulfides[3] and on potential remediation technologies[4–7] because AMD represents a serious environmental problem facing the mining sector. Despite all of the above research, the fact remains that little is known about microbial processes occurring in the deeper sub-oxic to anoxic portion of the tailings, where metal sulfide oxidation is no longer occurring. Until recently, it was thought that anaerobic microbial processes could not be sustained in the anoxic portion of tailings because of the lack of organic electron donors essential to several heterotrophic bacteria. However, studies performed on Cu-Zn and Au tailings and acidic mining lakes (from coal mining) have shown that sulfate-reducing bacteria are indeed present in these environments and that they actively participate in Fe and S cycling.[8–13] Moreover, iron-reducing bacteria are also thought to play an important role in iron cycling and alkalinity generation.[14] The present paper is intended as a mini-review on the occurrence, role and activity of sulfate- and iron-reducing bacteria in mine tailings. However, given the limited number of studies on sulfide-rich mine tailings, this mini-review also includes recent work on acidic mining lakes associated with coal mining and other environments impacted by mining activity. These environments possess chemical and mineralogical conditions similar to those of sulfide-rich mine tailings, i.e., presence of large quantities or Fe-rich minerals, acidic and oxic conditions and low organic carbon content.

Microbial Sulfate Reduction

In aquatic systems (freshwater and marine environments), microbial sulfate reduction represents an important carbon mineralization process, where sulfate serves as the final electron acceptor in the oxidation of simple organic carbon substrates (eq 1).

$$2CH_2O + SO_4^{-2} \rightarrow 2HCO_3^- + H_2S \qquad (1)$$

Soluble sulfide is produced during the reduction, along with alkalinity. Sulfide can either accumulate in the sediment porewaters or react with soluble reduced iron (Fe(II) to form iron monosulfides, commonly known as acid volatile sulfides (AVS). These iron monosulfides can however transform over time into pyrite, a common mineral of sedimentary environments.[15] Microbial sulfate reduction is in fact the driving process behind the formation of metal sulfides in low temperature environments.[15] The reduction of sulfate to sulfide is carried out by sulfate-reducing bacteria (SRB). SRB are considered strictly anaerobic bacteria and tend to prefer neutral pH conditions,[16] even though they have been recovered in acidic environments.[17] SRB use several low molecular weight compounds, such as acetate, formate, propionate, butyrate, pyruvate, lactate, but also hydrogen.[16]

SRB Occurrence and Activity in Mine Tailings and Mining Impacted Environments

A priori, sulfide-rich mine tailings do not represent a suitable habitat for sulfate-reducing bacteria, because they are generally acidic and oxic and contain very little organic carbon (Figure 1). However, large SRB populations have been recovered in several acidic and oxic Cu-Zn mine tailings,[9–13] as

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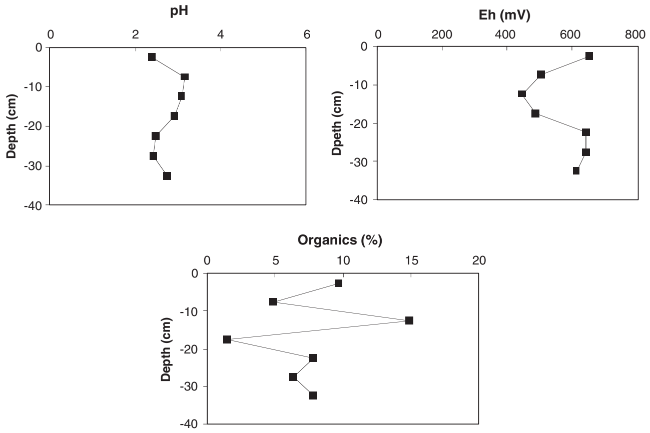

Figure 1. pH, redox potential and organic carbon content of acidic and oxic Cu-Zn tailings.

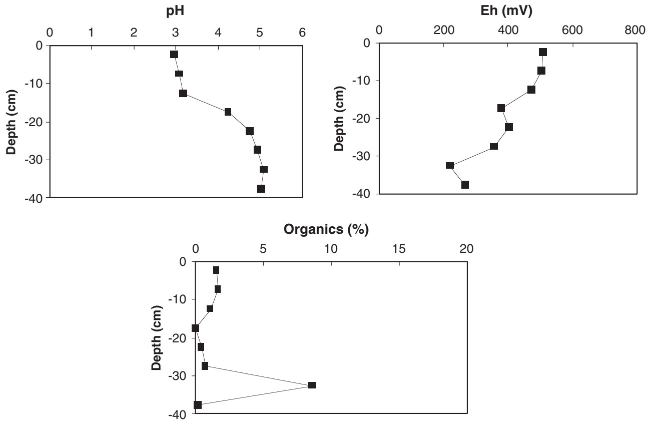

Figure 3. pH, redox potential and organic carbon content of Cu-Zn tailings showing the presence of oxic conditions at the surface and anoxic conditions in the sub-surface.

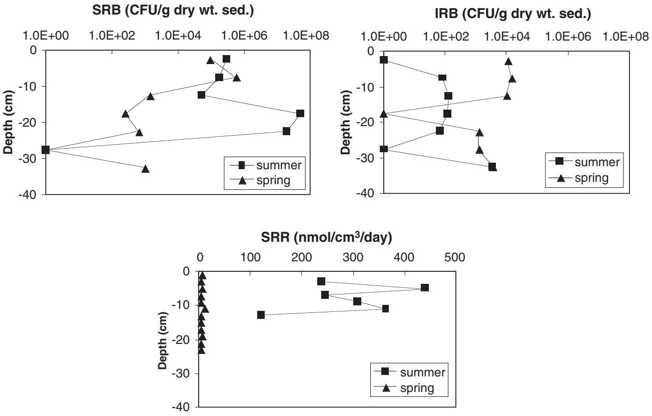

Figure 2. Seasonal variations in SRB and IRB populations and sulfate-reduction rates in acidic and oxic Cu-Zn tailings.

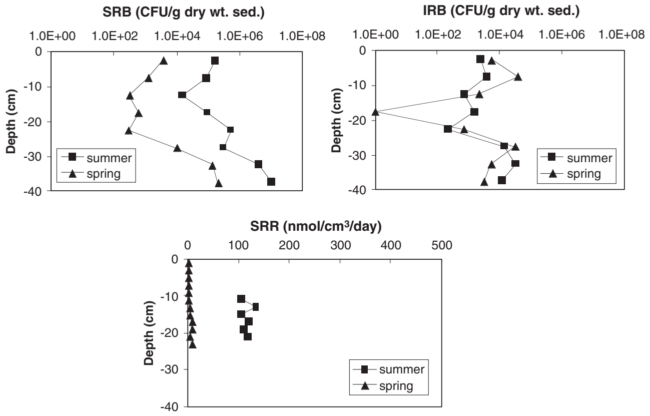

Figure 4. Seasonal variations in SRB and IRB populations and sulfate-reduction rates in Cu-Zn tailings showing the presence of oxic conditions at the surface and anoxic conditions in the sub-surface.

shown in Figure 2. Large SRB populations have also been recovered from the anoxic portion of some Cu-Zn tailings (Figures 3 and 4), where anoxic and slightly acidic pH condition prevail.[9–12] Studies of mining impacted environments, such as acidic mining lakes and river sediments have also been shown to host large SRB populations.[18–19] Past research on Cu-Zn mine tailings showed that there was a close relationship between the presence of SRB and the decline of sulfate in the porewaters and pyrite enrichment zones within the tailings.[9–11] However, the activity of SRB within these tailings was always based on circumstantial evidence because their microbial activity was never established. In addition, it was thought the SRB were not active under oxic and acidic conditions, but merely present as dormant cells,[9–13] because Gram-positive SRB (belonging to the *Desulfotomaculum* genius) are known to sporulate under stressful conditions.[16] However, recent work by Praharaj and Fortin,[8] has shown that SRB are indeed active in Cu-Zn tailings and their activity appears to be season dependent (Figures 2 and 4). The study showed that SRB populations were particularly active during the summer months when the tailings contained more organic carbon, as compared to the spring, when the tailings were depleted of organic carbon and were more acidic, following snowmelt. In Cu-Zn mine tailings sites where anoxic and pH neutral conditions prevailed in the subsurface, sulfate reduction rates were as high as 1000 nmol/cm^3/day during the summer.[8] Interestingly, SRB activity was not limited to anoxic tailings, high sulfate reduction rates (ranging from 100–450 nmol/cm^3/day) were measured in very acidic and oxic tailings (pH 2–3) in the summer.[8] These high rates (~ 1000 nmol/cm^3/day) are in fact comparable to rates measured in salt marsh environments, where large sulfate concentrations exist.[20] The rates measured in mine tailings are also in the same order of magnitude as the ones reported for natural acidic lakes,[17] but slightly higher than the ones measured in acidic lakes impacted by coal mining.[18, 21] In these acidic lakes, Blodau et al.[18] reported that sulfate reduction rates were generally very low in the surficial oxic and acidic sediments (pH 3) but rapidly increased with depth and pH.

The microbial diversity of SRB living in mine tailings is still under investigation, but preliminary results have indicated that they can use simple organic acids, such as lactate, acetate and formate, which have been identified in the porewaters of acidic Cu-Zn tailings.[12] In addition, SRB can completely oxidize these electron donors, but results from batch experiments have also shown that some SRB are incomplete oxidizers, i.e., they oxidize lactate to acetate (unpublished data).

The activity of SRB in sulfide-rich mine tailings suggests that new iron sulfides are being formed *in situ*. Sequential chemical extractions performed on Cu-Au tailings have indeed shown that there exists zones of pyrite enrichment (extracted with concentrated HNO_3) at various depths, which coincide with large SRB populations.[9–11] Large chromium-reducible sulfide fractions (CRS) also appeared to coincide with zones of high sulfate reduction rates in Cu-Zn tailings, as shown by Praharaj and Fortin.[8] The presence of high SRR also corresponded to slightly negative δ^{34}S values of the CRS fractions around the same depth, which is generally indicative of the presence of biogenic sulfides.[8] However, the δ^{34}S values of the CRS fractions in the sulfide-rich tailings were less negative than the values generally reported for biogenic pyrite in cold marine sediments.[8] It is suspected that the isotopic composition of the CRS fractions in sulfide-rich tailings corresponds to a mixture of abiotic pyrite (present during the ore formation) and biogenic pyrite formed following FeS formation and microbial sulfate reduction.[22] It is important to mention here that the AVS fraction of sulfide-rich mine tailings, known to include biogenic iron mono-sulfides,[23] does not provide useful information on the activity of SRB, because pyrrhotite, a common component of sulfide-rich tailings (associated with the ore), is also extracted, along with biogenic Fe-monosulfides, thus biasing the results.[22] Sulfur isotopic composition of sediments from acidic lakes impacted by coal mining activity has however been used to infer microbial sulfate reduction and sulfur cycling.[24–25] In summary, the best indicator of microbial sulfate reduction in mining environments is the *in situ* measurement of sulfate reduction rate,[20] however when it is not possible to do so, the combination of porewater and solid phase geochemistry, along with sulfur isotopic analyses can be used to assess the role of SRB in Fe and S cycling in these environments.

Microbial Iron-Reduction

Fe(III) reduction in sedimentary environments is driven by both abiotic and biotic reactions.[26–27] However, microbial iron reduction represents an important carbon mineralization process in non-sulfidogenic sediments, because abiotic iron reduction is limited. Various Fe(III)-oxides[27] can serve as the final electron acceptor in the oxidation of organic carbon (eq 2)

$$\mathrm{Fe(OH)_3 + 0.25\ CH_2O + 2H^+ \rightarrow Fe^{2+} + 0.25\ CO_2 + 2.75\ H_2O} \qquad (2)$$

The microbial reduction of ferric iron oxides releases soluble reduced iron in solution and generates alkalinity. Various dissimilatory iron-reducing bacteria are involved in the reduction of Fe(III) in sedimentary environments.[28] Several of them can gain energy from the direct oxidation of simple electron donors, such as acetate and formate, but other more complex electron donors can be used.[26] Unlike SRB, iron-reducing bacteria (IRB) can carry Fe(III) reduction under both oxic and anoxic conditions and under a wide range of pH conditions. Acidophilic iron-reducing bacteria tend to be active under oxic conditions, whereas neutrophilic iron reducing bacteria are generally active under anoxic conditions.[29]

IRB Occurrence and Activity in Mine Tailings and Mining Impacted Environments

The presence and activity of iron reducing bacteria have been investigated in acidic lakes impacted by coal and base metal mining activity[14, 18, 21, 30–32] and fluvial sediments.[19] In these environments, large populations of IRB were recovered in Fe-rich oxic and anoxic sediments showing a wide range of pH conditions. In acidic lake sediments, the availability of Fe(III) is not the limiting factor in the *in situ* activity of IRB, it is rather the availability of suitable electron donors.[21] In addition, Fe is readily recycled in these acidic lakes, especially near the sediment-water interface. Depending on the season and on the concentration of dissolved oxygen, Fe(II) oxidation rates can surpass Fe(III) reduction rates.[21] The rapid cycling of Fe in the upper portion of the sediments is partially caused by the absence of soluble sulfide in the porewaters, which prevents FeS precipitation and immobilization. Iron reduction rates in these sediments can be as high as 250 nmol/cm^3/day in the upper acidic and oxic sediments, but decline with depth.[14] The low Fe reduction rates in the deeper sediments is linked to the increased *in situ* activity of SRB and to the potential competition for electron donors between IRB and SRB.[14] Iron reducers present in these acidic and oxic sediments are either autotrophic or hetorotrophic acidophilic bacteria.[21] Autotrophic IRB include *Acidithiobacillus ferrooxidans*, whereas acidophilic heterotrophic bacteria belonging to the genus *Acidiphilium* have been identified as key players in the microbial reduction of Fe(III) in acidic mining lakes.[33] In particular, *A. cryptum* JF-5 has been shown to oxidize several sugars, alcohols, H_2, and most organic acids of the tricarboxylic acid cycle.[21, 33]

In mine tailings, little is known about the *in situ* activity of IRB, even though they can be present in large numbers. They have been recovered under acidic and oxic tailings (Figure 2), but also in anoxic and slightly acidic tailings (Figure 4). Interestingly, populations numbers do not appear to be affected by seasonal variations, in fact, results indicate that they might be more abundant in the spring shortly after snowmelt, than in the summer (Figures 2 and 4). Their *in situ* activity has not been measured as a function of sampling season, but the results tend to indicate that IRB populations might be sustained and even thrive under more acidic conditions in the spring, when the *in situ* pH drops.[8] In addition, in the spring, SRB activity has been shown to decline, as a result of various physico-chemical factors, including low pH, thus lowering any potential competition for suitable electron donors between IRB and SRB. If it is the case, the behaviour of IRB in mine tailings would be in agreement with the findings about acidic lakes impacted by mining activity, where IRB are more active in the upper acidic sediments, due to low pH conditions and less active SRB populations. Preliminary batch experiment results have shown that IRB isolated from mine tailings can completely oxidize formate, lactate and acetate, but cannot compete with SRB under anoxic and more neutral pH conditions (unpublished data). It is clear that more work is needed on the ecology of IRB in sulfide-rich mine tailings since they can affect Fe cycling and the generation of alkalinity, as discussed in the last section of this paper. Such work in currently underway in the author's laboratory.

Stimulation of Microbial Iron and Sulfate Reduction as a Potential Bioremediation Technology

Equations 1 and 2 indicate that alkalinity is being generated during the microbial reduction of sulfate and iron. As a result, these two anaerobic processes could be used to increase alkalinity in sulfide-rich tailings and sediments of mining lakes and combat acidity production or existing acidity originating from acid-mine drainage. Some studies have been performed on acidic coal mining lakes in Germany,[34–38] where the sediments are characterized by low pH (pH ~2) and low organic carbon content.[21] Given the fact that microbial sulfate and iron reduction are carried out by heterotrophic bacteria (with the exception of *A. ferrooxidans*), it is essential to have a large supply of suitable organic carbon in these sediments in order to stimulate microbial growth and alkalinity generation. Laboratory experiments performed with sediments from acidic mining lakes have shown that the addition of simple organic substrates (lactate, pyruvate, etc.) and cheap industrial wastes can stimulate microbial sulfate reduction, which had for effect to increase the pH of the sediment slurry by several pH units.[36] Small microcosms filled up with lake sediments and water from acidic mining lakes have also been tested to determine the effect of organic carbon substrate addition on alkalinity production.[34] Results showed that the addition of cheap industrial wastes (a combination of Carbokalk (a mixture of organic carbon and lime from the sugar industry), ethanol and whey) stimulated microbial sulfate reduction, neutralized the acidity and increased the pH of the microcosm to near neutral-pH conditions.[34] Large enclosures (amended with Karbokalk) were also setup in these acidic mining lakes in order to assess the importance of Fe(III) reduction in alkalinity generation.[37] Results showed that microbial iron reduction was the initial microbial process to take place, but the presence of suitable anions for the precipitation of Fe(II) minerals (either carbonate or sulfides) is critical in maintaining the gain of alkalinity because Fe(II) has the potential to be re-oxidized and generate acidity.[37] Overall, these studies indicate that controlled microbial iron and sulfate reduction processes might represent an important remediation tool to maintain alkalinity generation in environments impacted by acid-mine drainage. In sulfide-mine tailings, several studies have looked at chemical and physical processes designed to eliminate acidity generation within the mining waste or prevent it, prior to open-air disposal.[39–40] Controlled *in situ* iron and sulfate reduction experiments have however never been investigated in such environments, but based on the information that *in situ* SRB and IRB populations are present and active in Cu-Zn tailings, these alkalinity generation processes should be further investigated.

Conclusion

Base-metal mines and their associated tailings can generate acidity and release large concentrations of toxic heavy metals in the surrounding ecosystems. If left unattended and untreated, these sulfide-rich tailings can oxidize for hundreds of years. It is then important to find practical and cost-effective treatment processes to reduce or stop acidity generation. Based on the research that has been carried out in Cu-Zn mine tailings and acidic coal mining lakes in Germany, it appears that *in situ* microbial processes, namely iron and sulfate reduction, might represent an interesting approach in controlling and neutralizing acidity within these mining-impacted sites. However, several factors must be kept in account, such as the type of organic substrates used to stimulate microbial activity, the *in situ* conditions of the sites (temperature, pH, Eh, concentration of electron acceptors, etc.) and the potential competition between variable microbial communities for the available electron donors. All of the above have the potential to severely impact the activity of SRB and IRB and the long term effectiveness of the bioremediation processes.

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References

(1) P. C. W. Singer and W. Stumm, Science **176**, 1121 (1970).
(2) I. Suzuki, Can. J. Microbiol. **45**, 97 (1999).
(3) B. J. Baker and J. F. Banfield, FEMS Microbiol. Ecol. **44**, 139 (2003).
(4) S. G. Benner, D. W. Blowes, W. D. Gould, R. B. Herbert Jr, and C. J. Ptacek, Environ. Sci. Technol. **33**, 2793 (1999).
(5) S. G. Benner, D. W. Blowes, W. D., C. J. Ptacek, and K. U. Mayer, Appl. Geochem. **17**, 301 (2002).
(6) A. H. M. Hulshof, D. W. Blowes, C. J. Ptacek, and W. D. Gould, Environ. Sci. Technol. **37**, 5027 (2003).
(7) T. K. Tsukamoto, H. A. Killion, and G. C. Miller, Water Res. **38**, 1405 (2004).
(8) T. Praharaj and D. Fortin, Geomicrobiology J. **21**, 457 (2004).
(9) D. Fortin, J. P. Rioux, and M. Roy, Water Air Soil Poll. Focus **2**, 37 (2002).
(10) D. Fortin, M. Roy, J. P. Rioux, and P-J Thibeault, FEMS Microbiol. Ecol. **33**, 197 (2000).
(11) D. Fortin and T. J. Beveridge, Geomicrobiology J. **14**, 1 (1997).
(12) D. Fortin, B. Davis, and T. J. Beveridge, FEMS Microbiol. Ecol. **21**, 11 (1996).
(13) D. Fortin, B. Davis, G. Southam, and T. J. Beveridge, J. Ind. Microbiol. **14**, 178 (1995).
(14) A. Peine, A. Tritschler, K. Kusel, and S. Peiffer, S. Limnol. Oceanogr. **45**, 1077 (2000).
(15) R. A. Berner, Geochim. Cosmochim. Acta **48**, 605 (1984).
(16) J. R. Postgate, *The sulfate-reducing bacteria, 2nd Ed.* Cambridge University Press. Cambridge (1984).
(17) M. Koschorreck, K. Wendt-Potthoff, and W. Geller, Environ. Sci. Technol. **37**, 1159 (2003).
(18) C. Blodau, S. Hoffman, A. Peine, and S. Peiffer, S. Water Air Soil Poll. **108**, 249 (1998).
(19) B. Wielinga, J. K. Lucy, J. N. Moore, O. F. Seastone, and J. E. Gannon, Appl. Environ. Microbiol. **65**, 1548 (1999).
(20) J. E. Kostka, B. Gribsholt, E. Petrie, D. Dalton, H. Skelton, and E. Kristensen, 2002. Limnol. Oceanogr. **47**, 230 (2002).
(21) K. Kusel, Water Air Soil Poll. Focus **3**, 67 (2003).
(22) T. Praharaj and D. Fortin, D. Water Air Soil Poll. **155**, 35 (2004).
(23) J. W. Morse, F. J. Millero, J. C. Cornwell, and D. Rickard, Earth Sci. Rev. **24**, 1 (1987).
(24) K. Friese, K. Wendt-Potthoff, D. W. Zachmann, A. Fauville, B. Mayer, and J. Veizer, Water Air Soil Poll. **108**, 231 (1998).
(25) K. Knoller, A. Fauville, B. Mayer, G. Strauch, K. Friese, and J. Veizer, Chem. Geol. **204**, 303 (2004).
(26) D. R. Lovley and E. Phillips, Appl. Environ. Microbiol. **51**, 683 (1986).
(27) E. E. Roden, Environ. Sci. Technol. **37**, 1319 (2003).
(28) D. R. Lovley, *Fe(III)- and Mn(IV)-reducing prokaryotes*, eds. M. Dworkin, S. Falkow, E. Rosenberg, K-H. Schleifer, and E. Stackebrandt, The Prokaryotes, Springer-Verlag, New-York (2001), p. 325.
(29) D. B. Johnson and T. A. M. Bridge, J. Appl. Microbiol. **92**, 315 (2002).
(30) K. Kusel, U. Roth, T. Trinkwalter, and S. Peiffer, Environ. Exp. Bot. **46**, 213 (2001).
(31) K. Kusel, U. Roth, and H. L. Drake, Environ. Microbiol. **4**, 414 (2002).
(32) D. E. Cummings, A. W. March, B. Bostick, S. Spring, F. Caccavo, S. Fendorf, and R. F. Rosenzweig, Appl. Environ. Microbiol. **66**, 154 (2000).
(33) K. Kusel, T. Dorsch, G. Acker, and E. Stackebrandt, Appl. Environ. Microbiol. **65**, 3633 (1999).
(34) R. Frömmichen, K. Wendt-Potthoff, K. Friese, and R. Fischer, Environ. Sci. Technol. **38**, 1877 (2004).
(35) R. Frömmichen, S. Kellner, and K. Friese, Environ. Sci. Technol. **37**, 1414 (2003).
(36) A. Fauville, B. Mayer, R. Frömmichen, K. Friese, and J. Veizer, Chem. Geol. **204**, 325 (2004).
(37) K. Wendt-Potthoff, R. Frömmichen, P. Herzsprung, and M. Koschorreck, Water Air Soil Poll. Focus **2**, 81 (2002).
(38) K. Kusel and T. Dorsch, Microbiol. Ecol. **40**, 238 (2000).
(39) M. Ueshima, D. Fortin, and M. Kalin, Geomicrobiol. J. **21**, 313 (2004).
(40) J. Jurjovec, C. J. Ptacek, and D.W. Blowes, Geochim. Cosmochim. Acta **66**, 1511 (2002).