Observations on the groundwater chemistry in and below a pyretic tailings pile and microbiology

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ABSTRACT

Despite intense research directed to acid-generating processes a disparity is evident when field conditions are described and explained based on models and laboratory observations. This paper uses the iron oxidation process in groundwater located in and below acid-generating tailings in northern Ontario, Canada as an example of the disparity. A strong control of the iron redox couple is shown in the groundwater regime with iron oxidation processes measured in the laboratory slower than expected from first principals. Microbial activity in 4 year old samples may account for higher than expected pH values. Classical and molecular methodologies to determine presence and activity of microbial consortia reveal differing consortia, but functional information relating it to the acid mine drainage chemistry is lacking.

INTRODUCTION

Research has been underway, for at least two decades, into ecologically-driven treatment alternatives as constructed wetlands, microbial neutralization (Nixdorf & Deneke, 2004). To date, however, this effort has not yielded a practical treatment scenario widely embraced by the mining industry (Kalin 2004a, Kalin 2004b). Although the basic chemical and microbiological processes that create and neutralize AMD/ARD (acid mine drainage/acid rock drainage) are well known, complex homogeneous and heterogeneous processes in the waste streams, e.g. precipitation of solid phases. The status of chemical and physical conditions in a ground water regime associated with a small tailings deposit are presented along with investigations into microbial consortia in the contaminated ground water. The data highlight the need for an integrated, holistic, empirical methodological approach to address the contaminant generation and treatment of acid mine drainage rather than the prevailing chemical physical models based on assumptions derived in the laboratory.

METHOD

Site description

A mining waste management area in northern Ontario has been utilized as a research site, where ecological approaches to mine drainage have been gradually implemented (Kalin, 2001). The site includes a tailings deposit containing about 750,000 t of tailings (41 % pyrite and 4 % pyrrhotite) which accumulated during the life of the mine and mill (1970 to 1981). Hydrological investigations conducted between 1986 and 2002 revealed large variability in ground water contamination. In this paper, only an abbreviated and limited discussion will be given of piezometers screened in and below the tailings area of 25 ha.

Table 1 provides screen characteristics of the piezometers, and the depths and the stratigraphy in which they were screened, all of which were within or below the tailings deposit. For purposes of hydrological modeling, the screen depths of the piezometers were differentiated into four layers the first one, from within the tailings two to four above bedrock.

Sampling procedure

20 piezometers within the tailings basin were sampled September 7 and-8, 1996. Samples were recovered with a 1.5 L bailer, poured into wide-mouth sterilized 500 ml PVC jars which were filled to the brim to exclude oxygen and immediately placed in coolers on ice by a crew of three. to prevent the e precipitation of iron. A second sample was obtained from the same bailer volume, immediately preserved (1% conc. HNO_3, termed "WA") upon recovery of the original water level in the piezometers to determine the elemental concentrations (ICP-OES). E_H readings were performed immediately in a beaker from the same bailed volume. The first reading, after approximately 5 sec, was recorded as an Em value, along with electrical conductivity and pH measurements recorded within the next 5 minutes. The Em readings were converted to Eh according to $E_H = Em + 241 - 0.66 \times ((t - 25)\ ^oC)$.

Table 1 : Information of Piezometers in Tailings Area

Piezo Name	Stratigraphy & (Aquifer)	Hydraulic cond. cm/sec	Total Depth m	Top of Screen m	Width of Screen m
H-1	Tailings (1)	8.90E-05	2.10	1.13	0.91
H-2	Tailings (1)	3.60E-04	2.87	1.89	0.91
H-3	Tailings (1)	6.80E-06	2.90	1.92	0.91
H-4	Tailings (1)	4.80E-04	2.23	1.25	0.92
H-5	Tailings (1)	1.30E-04	2.41	1.43	0.91
H-6	Tailings (1)	3.10E-04	2.50	1.53	0.91
H-7	Tailings (1)	5.40E-03	2.90	1.92	0.92
H-8	Tailings (1)	1.50E-04	2.26	1.28	0.91
M-27S	Tailings (1)	2.10E-05	5.00	4.17	0.76
M-7N	Tailings (1)	3.80E-06	4.85	4.02	0.77
M-24W	Sand (2)	7.70E-05	5.36	4.54	0.76
M-40A	Sand/Bedrock (2)	1.10E-03	6.34	5.52	0.76
M-41	Sand (2)	1.20E-06	4.91	4.08	0.76
M-4	Sand (3)	2.20E-03	5.18	4.36	0.77
M-5W	Coarse Sand (3)	5.20E-05	5.33	4.97	0.31
M-30	Sand (3)	3.70E-04	3.99	3.17	0.76
M-5E	Coarse Sand (4)	4.30E-03	10.82	10.46	0.30
M-7S	Sand (4)	7.70E-04	10.55	10.18	0.31
M-43	Fine Sand (4)	6.00E-04	9.36	8.99	0.30
M-46	Bedrock (4)	1.30E-02	11.25	10.57	0.61

The samples were delivered to the laboratory within 48 hours. When the samples had returned to room temperatures an aliquot of each was used to determine acidity/alkalinity. After 10 to 11 days with concurrent measurements of pH, E_m, acidity and alkalinity, a further aliquot of the samples was filtered through 0.45 µm and preserved with 1% conc. HNO_3 (termed "FAS" for "filtered, acidified supernatant) and sent for elemental analysis. The closed bottles remained at room temperature and stored in the dark until May 1999 when further measurements were taken. At that time further oxidation was observed.

On June 10, 1998, another set of samples was collected, from the piezometers using the same techniques with the exception that in the field, 500 ml aliquot sub-samples were filtered through 0.45 µm filter and then preserved with 1% conc. HNO_3 for elemental analysis. The unpreserved samples were obtained at the same time for repeated measurements of the iron oxidation process, no WA and FAS samples were obtained.

Microbiological isolation and enumeration

In October, 2000 sub-samples of the September, 1996 collection, were prepared in sterile centrifuge tubes after rigorous shaking to suspend iron precipitates, and then shipped to B. Johnson, University of Bangor Wales, U.K. who cultured microbial populations in them. The samples were processed using media for enumerating acidophilic bacteria as described in Johnson (1995). In May 2001 freshly collected water samples were shipped for the same purposes and in May 2002 glass slides suspended for 4 years at the screen depth of piezometers (M7S, M7N, M27C and M27S, cf. Table 1) were shipped immediately after collection in the field in their original water. For each slide an area of 55 mm by 26 mm was cleared using sterile cotton wool swabs. These were placed into 5 ml of sterile basal salts solution (pH 5.8) and vortexed thoroughly to release the trapped bacteria.

A dilution series (10° - 10^{-4}) was prepared from each suspension, and 100 µL aliquots of each used to inoculate solid media selective for extremely and moderately acidophilic iron-oxidizing and heterotrophic bacteria (Johnson, 1995, Hallberg and Johnson, 2001). Plates were incubated for 4-6 weeks at 20^oC, before colonies were counted and preliminary identification made of isolates.

A 25 dm^{-3} ground water sample from the contaminated ground water plume, originating in the tailing was obtained under nitrogen and shipped within 24 h by air to P. Lau at Biotechnology Research Institute of the National Research Council of Canada in Montreal to perform the S16 RNA analysis. Details are given in Lau et al. (2001).

RESULTS

Chemical and biological neutralization processes can be expressed in stoichiometric equations. These reactions provide a basis for predicting the behavior of both biological and chemical treatment systems. The efficiency of both system types, is determined by the rate of iron oxidation. In conventional chemical treatment plants, the dosage of neutralizing chemicals is determined by the pH of in-coming AMD. In an alternative, biological system, iron oxidation serves as the first metal removal step (Lau et al. 2001). It is commonly assumed that in ground water, where reducing conditions prevail, the chemical oxygen demand is high, and precipitation through iron oxidation would occur immediately.

Figure 1 provides E_H and pH values for ground water samples, measured immediately upon capture from the piezometers, correlated with a Pourbaix diagram of water. The four layers were obtained by hydrological modeling using the code MODFLOW. Nearly all piezometers in the first and uppermost layer are located in the tailings.
As elemental concentrations in the ground water varied greatly. It was reasonable to expect that the physicochemical conditions would also differ, particularly as the background groundwater is very dilute with a
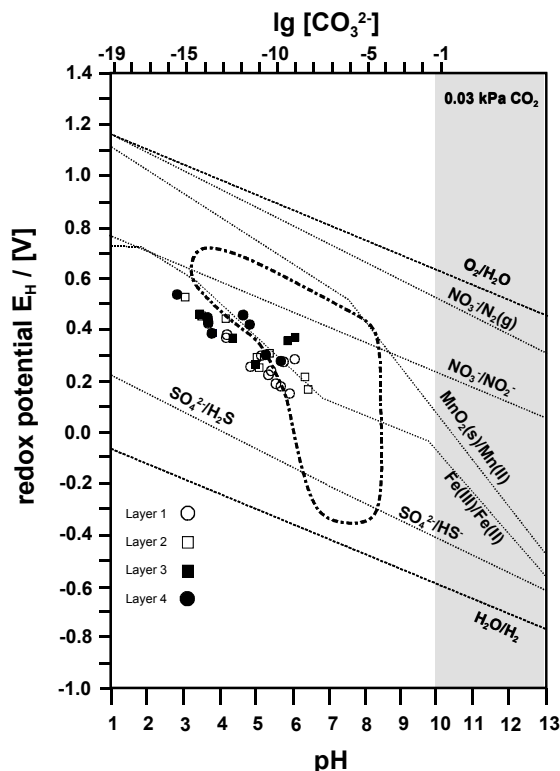
near-neutral pH, E_H of 300 mV to 450 mV and a conductivity of about 50 µS. On inspection of the E_H-pH environment the similarity of the ground water regardless of depth is striking.

Figure 1: E_H-pH data in 20 piezometers sampled in 1996 and 1998 correlated with the boundaries of natural aqueous systems and various redox couples.

The boomerang-shaped field represents the pH-E_H region of natural waters as determined by Baas-Becking et al. (1960). The data follow the lower boundary set for oxidized mine water and correlate closely with the E_H-pH calculated for the Fe(III)/Fe(II) couple (Meinrath et al., 2004). The redox regime is strongly influenced by the shallow layer of tailings, affected by AMD. The ground water just above bedrock display a lower pH range. No effect of the surrounding stratigraphic material can be noted. Comparing the data obtained from different sampling campaigns, a wide variability in the measured pH-E_H values was noted for a given piezometer.
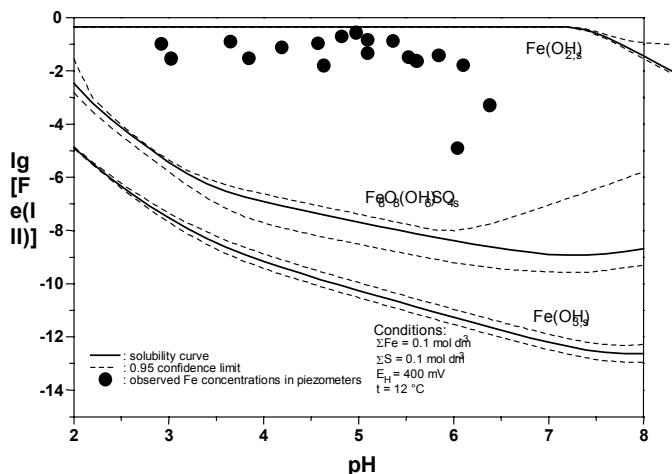

Figure 2: Comparison of observed Fe concentrations in Layer 1 piezometers with theoretical solubility curves of Fe(OH)$_{2,s}$, Fe$_8$O$_8$(OH)$_6$SO$_4$ (schwertmannite) and Fe(OH)$_{3,s}$ (goethite).

Groundwater samples were continued to be measured to observe the iron precipitation and acidification. Between 1996 to 2002, the pH-E_H after 10 days from collection over 4 years show an increase in E_H and decrease in pH values closely the F(III)/Fe(II) redox couple. Several of the samples reach pH 1 or slightly lower, but the precipitation of iron is rather slow, not as expected quite rapid. Geochemical modeling (PhreeqC) and stoichio-metric assessments of the theoretical pH values, using the determined iron concentrations suggested, that indeed, the pH should be lower than that measured (data not shown). This prompted the search for microbial activity in the stored samples, as discussed below.

In Figure 2 a correlation of Fe concentration in piezometers (solid circles) with theoretical solubility curves of $Fe(OH)_{2,s}$, $Fe_8O_8(OH)_6SO_{4,s}$ (schwertmannite) and $Fe(OH)_{3,s}$ (goethite) is given. Solid Lines give mean solubili-ties, while dashed lines represent 95% confidence limits estimated by Ljungskile probabilistic speciation code (Ödegaard-Jensen et al. 2004). The observed concentrations correspond almost with the solubility of Fe^{2+}(aq) in equilibrium with a $Fe(OH)_2$,s phase. The question, whether a Fe(II) sulfate phase should be solubility limiting will not be further discussed. The authors are well aware of the limitations of thermodynamic modelling (Meinrath et al., 2000, Meinrath 2001, Meinrath & May, 2002, Meinrath et al., 2004, Ödegaard-Jensen et al., 2004).
In Figure 3 the concentrations of Fe and S in the WA sample from the l996 sampling campaign are correlated with concentrations which remain after 10 days in solution, after filtration through 0.45 µm filter paper (FAS).
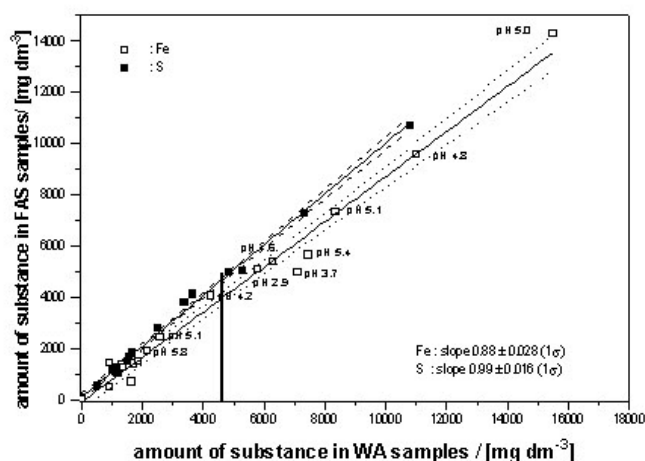

Figure 3: concentrations of Fe and S in acidified samples (WA) from the l996 campaign correlated with concentrations which remain after 10 days in solution, measured after 0.45 µm filtration (FAS).

Figure 3 shows that Fe and sulfate (given as sulfur in Fig. 3) may reach very high concentrations. Under the E_H and pH conditions observed in the piezometers these high concentrations are rather stable. The pH values, given in Fig. 3 for higher Fe concentrations, indicate a low acidity or, in other words, an enormous potential to form highly acidic solutions upon oxidation. In a conventional treatment plant (e.g. by lime neutralization) the associated precipitation process would inhibit neutralization immediately.

Microbial consortia

The contaminant plume leaving the tailings basin has been subjected to 16s rDNA analysis (Lau et al, 2001) to assess applicability of in-situ treatment. The contaminant status, as discussed above, was considered extremely hostile to microbial life. A total of 298 recombinants from five sampled piezometers were determined. From105 16s rDNA sequences, a total of 18 Eubacteria taxa were identified by matching with known taxa. The most frequently found were *Flavobacterium*, *Cytophaga*, *Zooglea* and *Gallionella ferruginea*. The well known Fe(II) oxidizers *Acidithiobacillus ferrooxidans* and *Acidophilum* which are widely found in other studies were both found in only one recombinant. Surpisingly *Leptospirillum* was not present in these low redox groundwaters, in contrast to their abundance in other studies including the highly oxidized Iron Mountain biofilm samples (Bond et al. 2000). *Gallionella ferruginea*, another iron oxidizer, was frequent in the Ontario study. In this study, there was also a notable absence of Archaea and Eucarya. Archaea, which include many sulphate reducers, present in other studies (e.g. Bond et al., 2000; Edwards et al., 1999, 2000) and might be expected in groundwater samples. No known sulphate or iron reducers were found despite moderately high concentrations of TOC (2.9 to 9.4 mg dm^{-3}) needed to support sulphate reduction. However *Desulfitobacterium dehalogenans*, a taxon known to carry out sulphate, sulphite and thiosulphate reduction in culture, was detected.

The general absence of iron and sulphur oxidizers in this study is interesting. Abundance of *Zooglaea* and *Cytophaga*, potential denitrifiers and chemoorganotrophs is also notable. It further indicates the potential for

anaerobic respiratory processes to take place. *Flavobacteria* are generally considered as aerobes and would therefore be unexpected in the groundwater environment. *Gallionella ferruginea* is also considered as an obligate aerobe and important Fe (II) oxidizer. Microbial survey using the 16s rDNA technique supported tentatively, that in-situ treatment adding organic carbon and urea may be successful to generate microbial alkalinity.

The results were far to varied to expand them to the tailings ground water. To determine microorganisms present in situ, microscope slides were suspended within the piezometers for a period of four years from the 10th of June l998 to the 6th of July 2002, at the screen depth of the piezometers. After the first year slides were recovered and microscopic revealed extensive colonization. Some slides were send to B. Johnson for identification with the conventional culturing technique. On all slides of the 4 piezometers investigated, the isolates were *'Acidithiobacillus ferrooxidans*-like', the extremely acidophilic and no moderate acidophiles were isolated. From one of the four slides '*Leptospirillum ferrooxidans*-like' colonies were isolated and from one slides an the *Acidiphilium*-like taxa , a heterotroph. The dominant heterotrophic isolate was a *Frateuria*-like bacterium, previously found in AMD in Norway (Johnson *et al*., 2001)but on one other slide the dominant heterotroph was as *Acidiphilium*-like.

The microbial diversity in 4.6-year stored groundwater from the same piezometers screened in and below the tailings, as discussed for the chemistry was also been determined, since these samples had reached pH values as low as 1.0 during storage at room temperature in the dark . The samples were collected in September 1996 and sent for microbial analysis in May 2001.The same iron-oxidizing bacterial groups were present on the slides as in the stored samples, along with fungi and yeasts. The major iron-oxidizing group was *Acidithiobacillus ferrooxidans*.
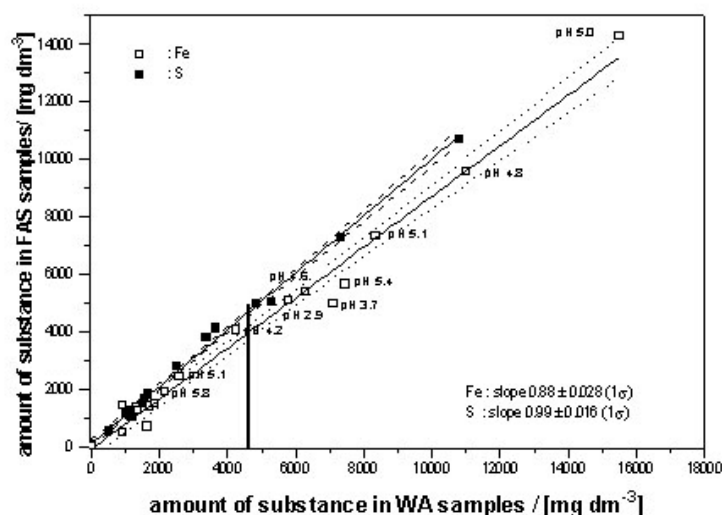
With these observations the important point can be made, that microbes affecting all the geochemical processes are present, alive and active, be it in the ground water, in a reducing environment, as it changes to the oxidizing condition in the previously sterilized bottle, and of course on the precipitated products, the metal sludges or the natural secondary iron precipitates.

CONCLUSION

Measurements performed within this study on piezometers in and below tailings show that geochemical correlations on basis of thermodynamic data are a helpful tool in assessing the physicochemical conditions in the subsurface. Interpretation of geochemical data on the basis of hydro-geological, hydrological and geochemical modeling is affected by a considerable amount of assumptions and approximations (Meinrath & May 2002). More often than not, these assumptions and approximations cannot be validated to the degree common in laboratory experiments and more academic settings. The object of the study is often an underground aquifer (system) accessible only at a few selected points. These points in fact represent disturbances of the object under study because the boreholes provide for the access of oxygen and release of dissolved gases, e.g. CO_2. In some cases, the object of study is interpreted in terms of a numerical hydrological model where the numerical algorithms are known to be approximate and affected by lack of numerical accuracy (e.g. numerical dispersion). The application of thermodynamic equilibrium concepts to the geochemical interpretation of the values for certain parameters obtained by chemical analysis is a basic deficiency of geochemical modeling.

A major criticism towards the application of physicochemical information obtained in a laboratory to the natural environment is the neglect of biological, e.g. bacterial, contributions. In this study, the effect possible of micro-organisms in the oxidation of metal sulphides (e.g. pyrite) has been studied in the subsurface of a former metal mining site. The presence of iron-oxidizing bacteria was demonstrated by S16 rRNA analysis and cultivation of indigenous bacteria. It could be shown that the physicochemical condition of the site is closely correlated with the bacterial activity. There is no known merely inorganic process able to cause the extreme iron concentrations observed at this site (cf. Fig.2). Redox conditions measured in the piezometers after bailing indicate the close

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correlation with the Fe(III)/Fe(II) boundary as well as with the E_H-pH limits of natural aqueous systems. The correlation is especially close in a E_H-pH region defined by iron-oxidizing bacteria (Baas-Becking et al. 1960).

Thus, the involvement of bacteria in the oxidation of metal sulphides is well established, as reviewed by Johnson and Hallberg (2003). In the present study, iron-oxidizing bacteria have been identified from field sampling. Thus, their involvement in the generation of ground waters with extremely high Fe and sulfate concentrations is evident for the site under study. Microbial or biotic oxidation is dominant at low pH due to the activities of a variety of microorganisms. *Acidithiobacillus ferrooxidans* (previously known as *Thiobacillus ferrooxidans*) was the first such bacterium isolated (Temple and Colmer, 1951) and was shown to oxidize Fe(II) in culture conditions. Until the 1980s this organism was considered to dominate AMD generation and has been widely employed commercially in bioleaching of metals. The emphasis on this organism was a result of its relative easy isolation and culture.

Molecular techniques have shown a wide variety of microorganisms to be present in AMD, e.g. *A. ferrooxidans* and *Leptospirillum ferrooxidans*). In situ, consortia of acidophilic microbes may be essential for efficient generation of AMD and bioleaching (Rawlings et al., 1999). A number of studies have estimated microbial oxidation rates of pyrite in cultural conditions. Olson (1991) reported abiotic rates of 2.5×10^{-9} mol/m^2 s and biotic rates of 8.6×10^{-8} mol/m^2 s in an interlaboratory comparison with *A. ferrooxidans*. In this study with pH 2-3 and temperature of 25 °C the biotic rates were on average 34 times the abiotic rates. Edwards et al (1998) using *L. ferrooxidans* from Iron Mountain, California obtained higher rates (1.0×10^{-5} to 1.4×10^{-6} mol/m^2 s in biotic and 1.3×10^{-6} to 2.8×10^{-7} mol/m^2 s abiotic) at pH 0.7 and 42 °C. In this case the biotic rates were only 5 to 8 times higher. Rates depend on the organism, cultural conditions and estimates of pyrite surface area. These experiments demonstrate the ability of microorganisms to enhance pyrite oxidation under controlled conditions.

A recent publication (Tyson et al., 2004) describes the sequences of five genomes from one biofilm at the Iron Mountain/California site. This is the first example of community genomics where sequencing of microorganisms living together in nature has been carried out. How far such studies can go to help understanding the role of the microorganisms is unclear. It is still fair to conclude that the roles of microorganisms detected in acidic mine sites remains mostly unknown since their culture and characterization has not been possible.

Classical microbiology techniques involving isolation and culture of microorganisms are inadequate to describe the diversity and functioning of microorganisms present in AMD. However, such an approach does indicate whether particular functional types (e.g. iron oxidizers) are present. Counts of bacteria as described in this paper are very likely to underestimate the numbers of any functional group as some microorganisms will not grow in the selected cultural conditions. Also, particular taxa such as *A. ferrooxidans* will survive in a variety of conditions and will change their functional role. At present, a combination of techniques as in this study serves to show that a variety of living microorganisms are present in contaminated groundwater of a mine site and that taxa known to carry out functions such as iron oxidation are widely dispersed in such waters and remain active.

REFERENCES

Edwards, K.J, Bond, P.L., Gihring, T.M. and Banfield, J,F, (2000) An archael iron-oxidizing extreme acidophile important in acid mine drainage. *Science*, 287, 1796-1799.

Edwards, K.J., Gihring, T.M. and Banfield, J.F. (1999). Sasonal variations in microbial populations and environmental conditions in an extreme acid mine drainage environment. *Applied and Environmental Microbiology*, 65, 3627-3632.

Edwards, K.J., Schrenk, M.O., Hamers, R. and Banfield, J.F. (1998). Microbial oxidation of pyrite: Experiments using microorganisms from an extreme acidic environment. American Mineralogist, 83, 1444-1453.

Fyson A, Gelbrecht J. (2004) Kontrollierte Eutrophierung: Nährstoffzugaben und Induktion mikrobieller Stoffumsetzungen durch erhöhte Primärproduktion. In: *Nixdorf B, Deneke R (eds.) Grundlagen und Maßnahmen zur biogenen Alkalisierung von sauren Tagebauseen*, Weissensee Verlag, Berlin., pp. 89-104

Hallberg, K.B and Johnson, D.B. (2001). Biodiversity of acidophilic microorganisms. *Advances in Applied Microbiology*, 49, 31-84.

Johnson, D.B. (1995) Selective solid media for isolating and enumerating acidophilic bacteria. Journal of Microbiological Methods 23, 205-218.

Johnson, D.B., Rolfe, S., Hallberg, K.B. and Iversen, E. (2001) Isolation and phylogenetic characterisation of acidophilic microorganisms indigenous to acidic drainage waters at an abandoned Norwegian copper mine. *Environmental Microbiology* 3: 630-637.

Johnson, D.B. and Hallberg, K.B. (2003). The microbiology of acidic mine waters. Research in Microbiology, 154, 466-473.

Kalin, M., (2001) "Closure with Ecological Engineering of a Remote Cu/Zn Concentrator: Overview of 10 years R & D Field Program" *Proceedings of the Fourth International Symposium on Waste Processing and Recycling in Mineral and Metallurgical Industries, MET SOC 40th Annual Conference of Metallurgists of CIM*, Toronto, August 26-29, pp. 521-533.

Kalin, M 2004a Slow progress in controlling acid mine drainage (AMD): A perspective and a new approach", *Peckiana*, Staatliches Museum für Naturkunde Görlitz, (3), pp.101-112.

Kalin, M., (2004b), Passive Mine Treatment: The Correct Approach, *Ecological Engineering: The Journal of Ecotechnology*, (22:4-5), pp. 299-304.

Lau, P.C.K., H. Bergeron and M. Kalin, 2001 "Bacterial Consortia in a Ground Water Plume from acid Generating Tailings" *Proceedings of the Fourth International Symposium on Waste Processing and Recycling in Mineral and Metallurgical Industries, MET SOC 40th Annual Conference of Metallurgists of CIM,* Toronto, August 26-29, 2001, pp. 371-379.

Meinrath G., Hurst S., Gatzweiler R. (2000) Aggravation of Licensing Procedures by Doubtful Thermodynamic Data. Fresenius Journal of Analytical Chemistry 368, 561 - 566

Meinrath G. (2001) Measurement Uncertainty of Thermodynamic Data. Fresenius Journal of Analytical Chemistry, 369, 690 – 697.

Meinrath G., May P.M. (2002) Thermodynamic Prediction in the Mine Water Environment. Mine Water and the Environment, 24 – 35.

Meinrath G., Merkel B., Ödegaard-Jensen A., Ekberg C., (2004) Sorption of Iron on Surfaces: Modelling, Data Evaluation, and Measurement Uncertainty. Acta Hydrochimica et hydrobiologica 32, 154 – 160.

Ödegaard-Jensen A, Ekberg C., Meinrath G., (2004). LJUNGSKILE: A computer program for assessing uncertainties in speciation calculations. Talanta, 907 – 916.

Olson, G.J. 1991. Rate of pyrite bioleaching by Thiobacillus ferrooxidans: Results of an interlaboratory comparison. *Applied and Environmental Microbiology,* 57, 642-644.

Rawlings, D.E., Tributsch, H. and Hansford, G.S., (1999). Reasons why '*Leptospirillum*'-like species rather than *Thiobacillus ferrooxidans* are the dominant iron-oxidizing bacteria in many commercial processes for the bioremediaition of pyrite and related ores. *Microbiology,* 145, 5-13.

Temple, K.L. and A.R. Colmer. 1951. The autotrophic oxidation of iron by a new bacterium: Thiobacillus ferrioxidans. J. Bacteriol. 62, 605-611.

Tyson, G.W., Chapman,J., Hugenholtz, P., Allen, E.E., Ram, R.J., Richardson, P.M., Solovyev, V.V., Rubin, E.M., Rokhsar, D.S. and Banfield, J.F. 2004. Community structure and metabolism through reconstruction of microbial genomes from the environment. *Nature*, 428, 37-43.

Yu JY, Heo B, Choi JK, Cho JP, Chang HK (1999) Apparent solubilities of schwertmannite and ferrihydrite in natural waters polluted by mine drainage. *Geochimica Cosmochimica Acta* 63, 3407 - 3416