Diavik Waste Rock Project: Microbiological Succession in Waste-rock Testpiles

Brenda L. Bailey[1], David W. Blowes[1], W. Douglas Gould[2],Leslie Smith[3] and David C. Sego[4]

[1]Department of Earth and Environmental Sciences, University of Waterloo, Waterloo, ON, CANADA,blbailey@uwaterloo.ca, blowes@uwaterloo.ca
[2]Mining and Mineral Sciences Laboratories, Natural Resources Canada,Ottawa, ON, CANADA, dgould@nrcan.gc.ca
[3]Department of Earth and Ocean Sciences, University of British Columbia, Vancouver, BC, CANADA, lsmith@eos.ubc.ca
[4]Department of Civil and Environmental Engineering, University of Alberta, Edmonton, AB,CANADA, dave.sego@ualberta.ca

ABSTRACT
Sulfide mineral oxidation is catalyzed by bacteria including *Thiobacillus* species and *Acidithiobacillus* species and can result in the generation of acidic drainage. Experimental waste-rock piles (test piles) in a continuous permafrost region were studied to observe the microbial successions of *Thiobacillus* species and *Acidithiobacillus* species over time. On-going monitoring of the microbial community within the waste-rock test piles began in 2007. Most probable number techniques were used to quantify iron and sulfur oxidizing bacteria in effluent from test piles containing ~0.035 wt. % S and 0.058 wt. % S. The lower sulfurtest pile has maintained a near-neutral pH (ranged from 5.8 to 8) and has maintained a population of neutrophilic sulfur oxidizers. As air temperatures increased in spring and summer of each year, the measured pH in the higher sulfur test pile decreased from near-neutral in May to acidic conditions by October. During this transition, the populations of neutrophilic sulfur oxidizers decreased and the populations of acidophilic sulfur oxidizers and iron oxidizers increased.

Key Words: Arctic, waste rock, diamond mine

INTRODUCTION
Acid mine drainage (AMD) is generated during the oxidation of sulfide minerals and can be exacerbated by microbial activity. At mine sites, the extraction of an ore body generates waste rock that is typically stockpiled on the earth's surface. These stockpiles can range from tens of hectares in area and tens of meters high (Ritchie, 1994). Sulfide minerals in these waste rocks oxidize in contact with oxygen and water, and aerobic bacteria populations begin to grow.With a growing mining industry in northern Canada, examining the biogeochemical evolution of these waste rock stockpiles is important in understanding the rate of sulfide oxidation in regions with continuous permafrost.

Bacteria, such as *Thiobacillus* species and *Acidithiobacillus*species, are known to catalyze the oxidation of sulfur compounds and sulfide minerals leading to the generation of acidic drainage with high concentrations of SO_4 and dissolved metals (Nordstrom and Southam, 1997). Microbially mediated pyrite oxidation can occur directly or indirectly as described by Sand *et al*. (2001). Direct pyrite oxidation occurs through the attachment of a bacterium or cell to the sulfide mineral that solubilizesthe metal (Me), by an enzyme system utilizing oxygen as the oxidant, to sulfate and metal cations (Sand *et al*., 2001):

$$MeS + 2O_2 \xrightarrow{\text{bacteria}} MeSO_4 \quad (1)$$

Microbes can be indirectly involved in the oxidation of sulfide minerals by catalyzing the oxidation of Fe^{2+} to $Fe^{3+}$and S^0 to SO_4^{2-}:

$$MeS + 2Fe^{3+} \rightarrow Me^{2+} + S^0 + 2Fe^{2+} \quad (2)$$

The oxidation of Fe^{2+} is the rate limiting step in biotic pyrite oxidation (Singer and Stumm, *1970*). Microbially mediated pyrite oxidation by*Acidithiobacillusferrooxidans*, and related species,that catalyze Fe^{2+} oxidation in field studies is reported to be orders of magnitude faster than abiotic laboratory rates (Kirby *et al*, 1999).

$$FeS_2 + 14Fe^{3+} + 8H_2O \rightarrow 15Fe^{2+} + 2SO_4^{2-} + 16H^+ \quad (3)$$

The influence of temperature on sulfide oxidation rates can be described by the Arrhenius equation (Ahonen*et al*., 1992; Elberling et al., 2005):

$$\ln (k_2/k_1)=E_a(T_1-T_2)/(RT_1T_2) \quad \text{(Equation 1)}$$

where k_1 and k_2 are the rate constants at temperatures T_1 and T_2; E_a is the activation energy of the reaction; and R is the universal gas constant. Both abiotic and biotic sulfide mineral oxidation rates decrease with decreasing temperature and the oxidation of sulfide minerals during winter is assumed to be negligible.Seasonal temperature fluctuations, therefore, may influence the rate of release of sulfate from waste rock over time. However, studies have shown iron and sulfur oxidizing bacteria are present at low temperatures and can contribute to the oxidation of mine tailings in Arctic environmentsat temperatures as low as -11°C (i.e. Elberling*et al*., 2000; Leduc *et al*., 1993). The use of cover systems in Arctic environments to generate permafrost conditions in waste rock or mine tailings has been proposed as a method for limiting the generating of AMD (Dawson and Morin, 1996), yet limited information exists on the importance of bacterially-catalyzed oxidation of sulfide minerals in waste-rock stockpiles and their role in the generation of AMD in Arctic conditions.

This paper focuses on the biogeochemical succession within experimentalwaste-rockpiles(test piles) over four years, through the examinationof different groups of ironand sulfur oxidizing bacteria in terms of type and abundance, in a continuous permafrost region. Initial results from the test piles in 2007 and 2008 were previously discussed by Bailey *et al*. (2009). The microbial populations from 2007 through 2010 for the test piles are discussed herein. The effluent geochemistry corresponding to the sample locations examined in this microbial study are discussed by Bailey *et al*. in this volume (2012). The design and construction of the Diavik Waste Rock Research Facilities are described by Blowes *et al*. (2006) and Smith *et al*., (2009), the thermal regime within the test piles is described by Pham *et al*. (2011), air flow through the Type III test pile is described by Amos *et al*. (2009), and the hydrologyis described by Fretz *et al*. (2011).

Site description

The Diavik diamond mine is located 300 km northeast of Yellowknife, NWT in a continuous permafrost region (Figure 1). The mine site facilities were constructed on an island in Lac de Gras, an oligotrophic lake. This open pit and underground mining operation in the Slave geologic province will generate a 120 Mt waste-rock stockpile over the course of production. Host rocks consist mainly of granite and pegmatite lithologies containing irregular xenoliths of biotist schist. These host rocks are cut by diabase dikes. Diavik segregates the waste host rock based on sulfur content: Type I (<0.04 wt. % S), Type II (0.04 wt. % S to 0.08 wt. % S), and Type III (>0.08 wt. % S).

Research facilities

Three large scale waste-rock test piles were constructed as part of a multidisciplinary research program. Each test pile was constructed on a graded base, lined with a high density polyethylene geomembrane that captures and directs water to outflow drains (Figure. 2). Two test piles measure 15 m in height by 60 m by 50 m at the base. The first test pile consisted of Type I waste rock (0.035 wt. % S) and the second test pile Type III waste rock (0.058 wt. % S) (Figure 2).

The Type III test pile drains to two outflow drains, Type III north and Type III south basal drains. The third test pile was designed based on an approved reclamation concept and consists of Type III waste rock (0.082 wt % S) resloped and capped with a lower permeability till layer and freeze thaw layer (not discussed in this paper). Water directed to the base of the each pile flows through a heat traced drainage line to an instrumentation hut where water flows through a series of flow through cells (Figure 3). The first cell is used to collect water chemistry samples; the second cell takes continuous measurements of pH; and, the third cell continuous measurements of EC and temperature. Water is then directed to a tipping bucket to measure the flow rate.

Figure 1.Location of the Diavik Diamond Mine

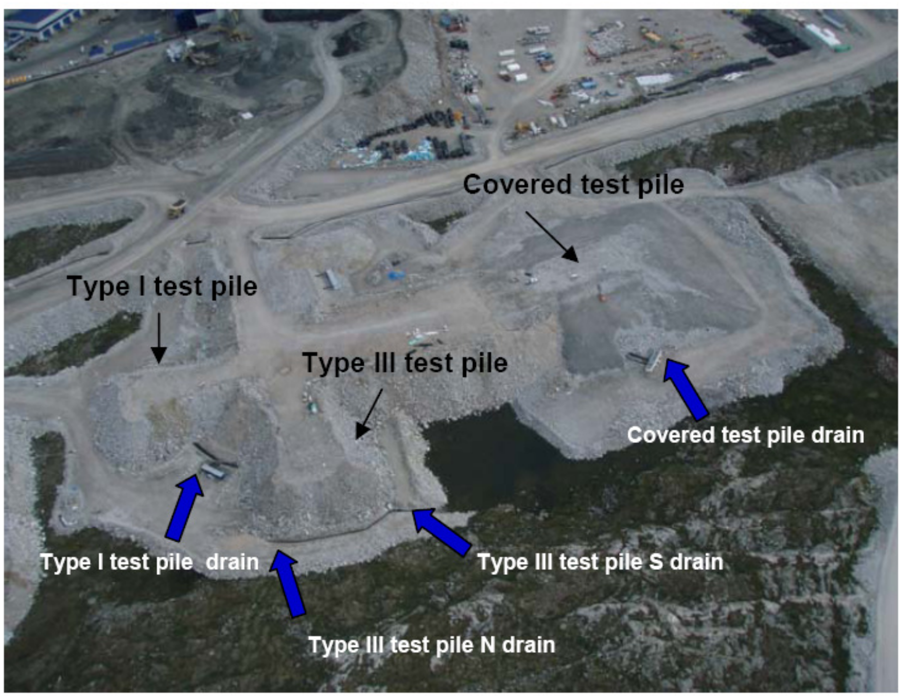

Figure 2. Test pile research facilities at Diavik

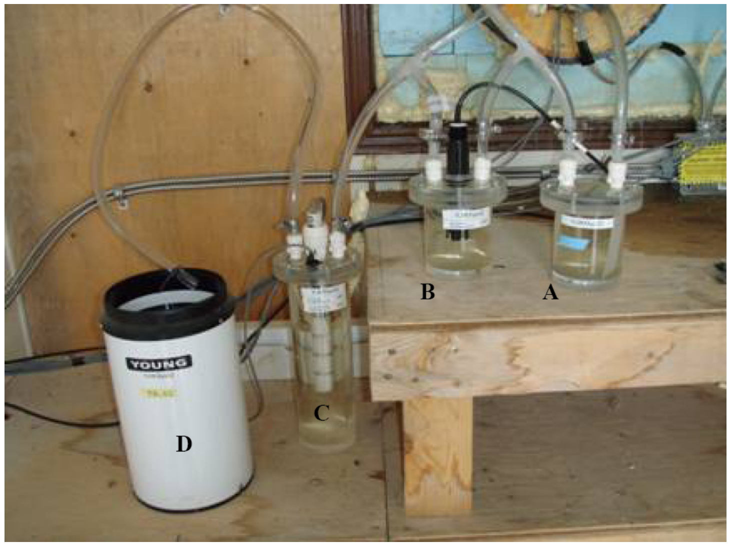

Figure 3. Flow through cell set-up a) water sampling cell b) pH measurements c) electrical conductivity measurements d) flow measurements

METHODS

Monitoring of the pore-water and effluent geochemistry from the test piles began in 2007 and is on-going. Three groups of iron and sulfur oxidizing microorganisms (*Acidithiobacillusferrooxidans* and related species, *Acidithiobaclluspthiooxidans*and related species, and *Thiobacillusthioparus*and related species) were monitored in effluent from the test piles and from *in situ* pyrite samples placed at varying depths within the test piles. Water samples were collected from flow-through cells using a sterile 60 mL polyethylene syringe (described in Bailey *et al*., this volume). Unfiltered, unpreserved samples were collected in 60 mL bottles. *In situ* pyrite samples consist of Nitex mesh bags (12 cm by 5 cm) filled with pyrite (*in situ* samples; Figure 4). Each *in situ* sample was sterilized prior to placement in the test pile (Figure 5). *In situ* samples were distributed at various depths in the test piles. Upon collection *in situ* bags were placed in polyethylene samples bottles.

Figure 4. a) *In situ* sample prior to placement in the test piles in autoclavable bag b) *In situ* samples extracted from the test piles.

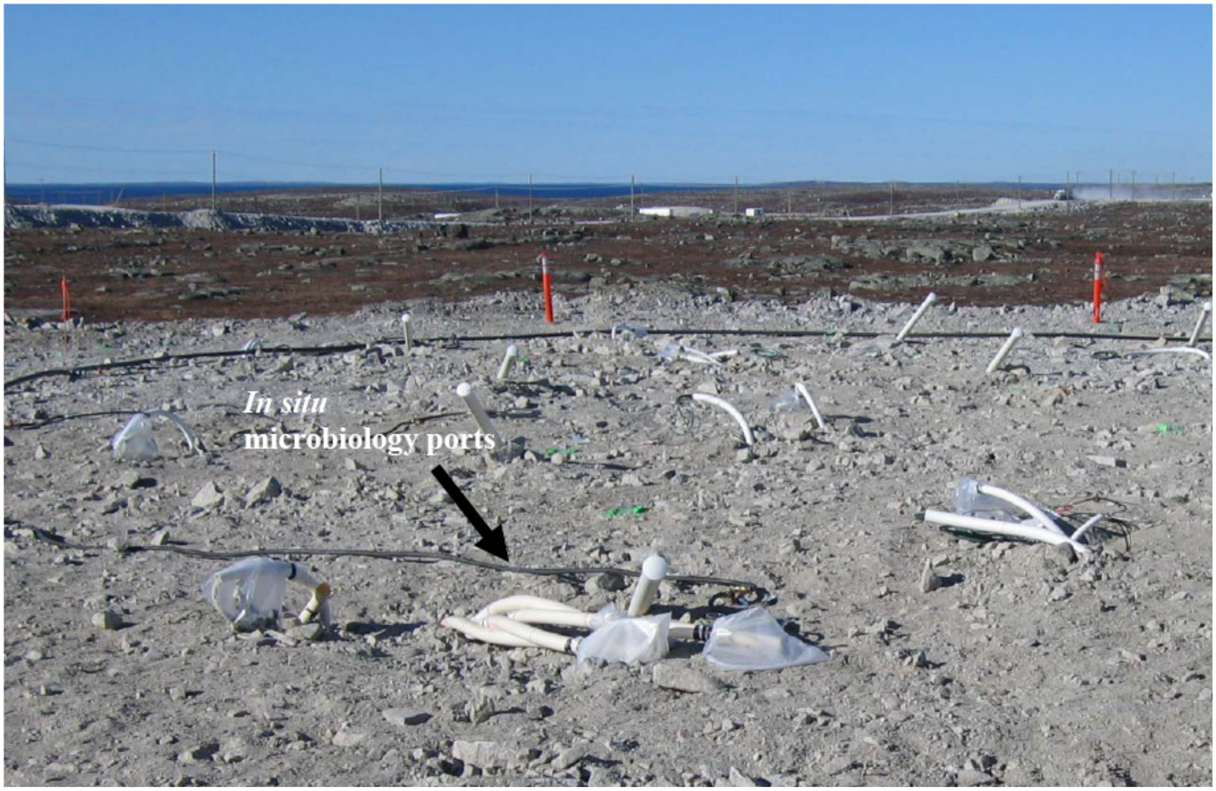

Figure 5.*In situ* microbiology ports on the top of the Type III test pile.

Both effluent and *in situ* samples were refrigerated at 4 °C until analysis at the University of Waterloo. Three types of microorganisms were studied using the most probable number method (Cochran, 1950), including neutrophilic S oxidizers (*T. thioparus* and related species), acidophilic S oxidizers (*A.thiooxidans* and related species), and acidophilic Fe oxidizers (*A. ferrooxidans* and related species). Detailed techniques and media were previously described by Hulshof *et al*., 2006.Positive results for iron oxidizing bacteria were determined by visual identification of Fe(III) (oxy)hydroxide precipitation (red colour) (Figure 6). The presence of sulfur oxidizers was determined by pH;pH 4.0 for acidophilic sulfur oxidizers and pH 6.8 for neutrophilic sulfur oxidizers.

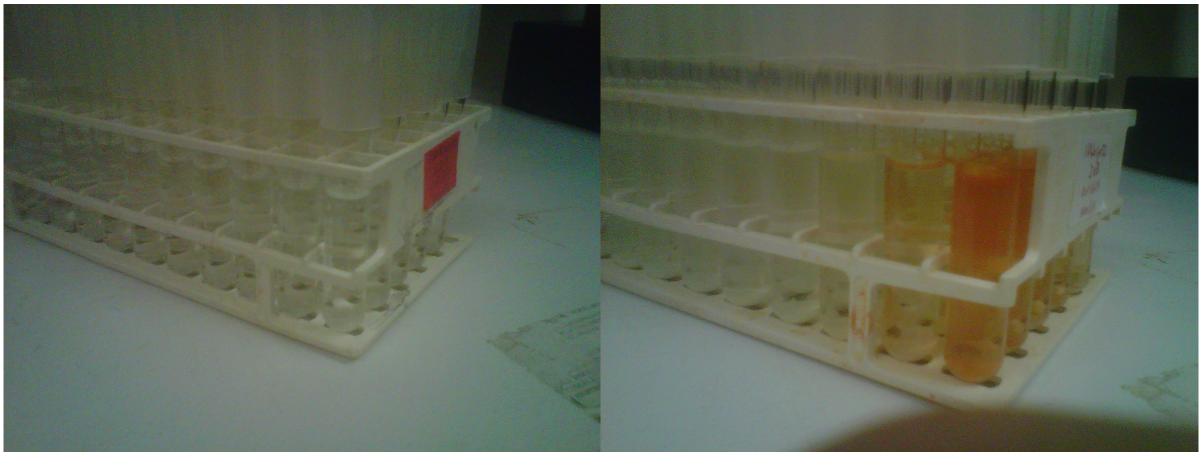

Figure 6. MPN test tubes for iron oxidizing bacteria (right) and control set (left).

RESULTS

***In situ* pyrite samples**

The *in situ* samples from the Type I test pile had a measurable population of acidophilicS oxidizers at 2 m and 12 m depths in October 2007 (Figure 7). In July 2008, the population was similar at the 2m depth, but decreased to below detection at 12 m. AcidophilicS oxidizers were not detected in July 2009 and July 2010, however, the population increased at the 4 m and 6 m depths in September 2010.Neutrophilic S oxidizers were detected in November 2006 and October 2007, and the population decreased to below detection in July 2008 and July 2009. A detectable population ofneutrophilic S oxidizers was observed at the 6 m depth in July 2010 and 4 m and 6 m depths in September 2010. No acidophilic iron oxidizers were detected in the Type I *in situ* samples.

The *in situ* samples from the Type III test pile had detectable neutrophilic S oxidizers at all depths in November 2006 (Figure 7). A detectable population of neutrophilicS oxidizers was observed in October 2007 at the shallow depths. In 2008, neutrophilic S oxidizers were detected at depths of 2 m, 4 m and 10 m in July and 4 m and 10 m in August. No neutrophilicS oxidizers were detected in 2009 and 2010. Acidophilic S oxidizers were only detected at 4 m and 6 m depths in October 2007 and July 2008. No acidophilic Fe oxidizers were detected in the Type III test pile *in situ* samples.

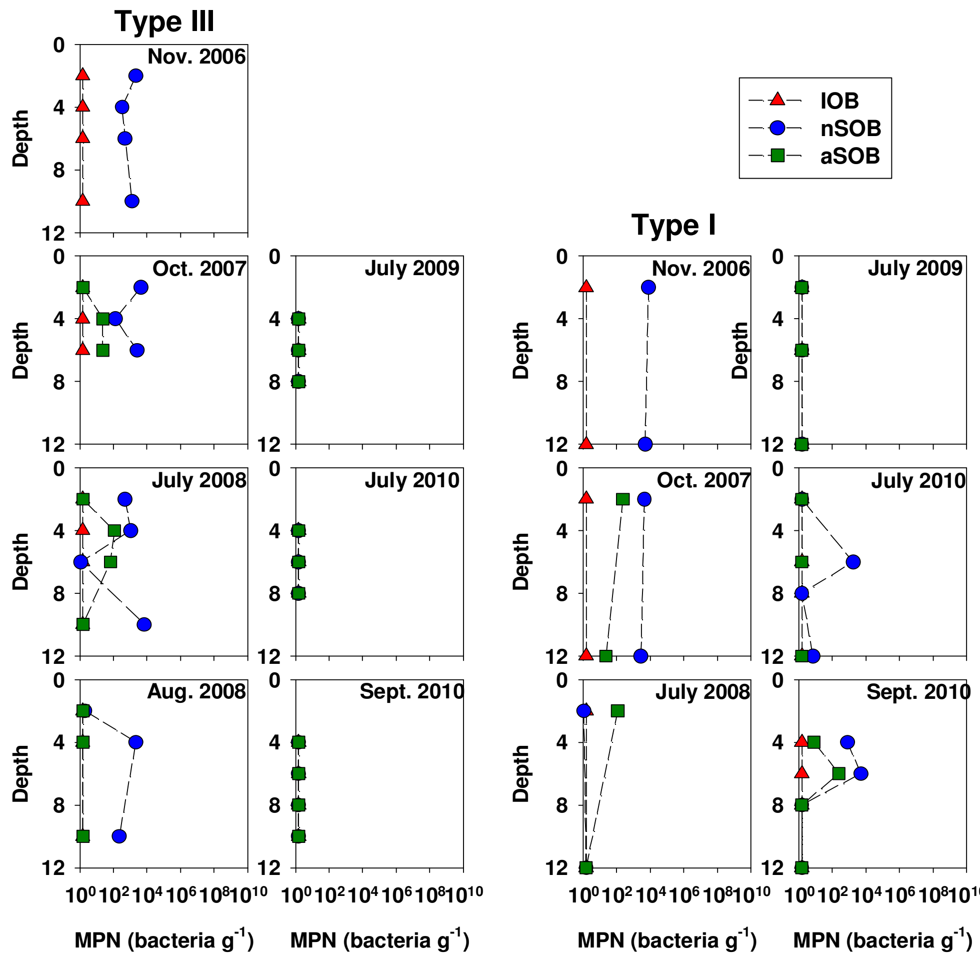

Figure 7. Populations of acidophilic Fe oxidizers (IOB), neutrophilic S oxidizers (SOBn) and acidophilic S oxidizers (SOBa) in the *in situ* samples collected from various depths in the Type I and Type III test piles.

Effluent samples

The basal drain effluent from the Type I test pile maintained a near neutral pH for the duration of this study. A population of neutrophilic S oxidizers was observed from 2009 through 2010, with the exception of one sample in September 2010 (Figure 8). Acidophilic S oxidizers were only detected at very low numbers (<10^2 bacteria/mL). The population of acidophilic Fe oxidizers was detected in June 2009 and late 2010, but at low numbers (<10^3 bacteria/mL).

The pH of the effluent from both of the Type III test-pile basal drains decreased from near neutral in May to acidic conditions by October each year. Neutrophilic S oxidizers were observed in both Type III test-pile basal drains in 2008 and early 2009 (Figure 8). Population numbers of neutrophilic S oxidizers decreased through 2009 concomitantly with pH. A similar trend was observed in 2010. Acidophilic S oxidizers were not detected in 2008; however, acidophilic S oxidizers were detected in 2009. The population of acidophilic Fe oxidizers increased with decreasing pH in the Type III test-pile basal drains each year.

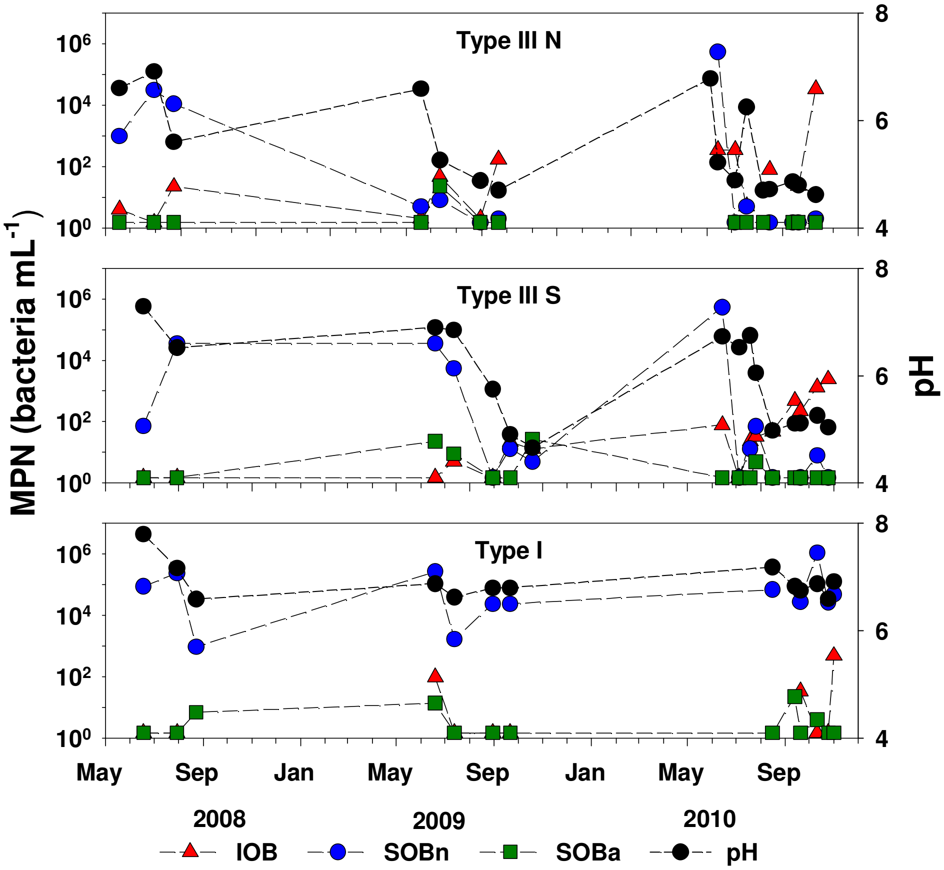

Figure 8. Populations of acidophilic Fe oxidizers (IOB), acidophilic S oxidizers (SOBa) and neutrophilic S oxidizers (SOBn) in the Type I test pile basal drain and Type III test pile north and south basal drains. The left axis represents MPN (bacteria/mL) and the right axis represents effluent pH.

DISCUSSION

A higher sulfide oxidation rate is expected when acidophillic Fe oxidizing bacteria are present, resulting in the release of sulfate, iron and dissolved metals. The initial results from this study showed the succession of bacteria populations with the evolution of effluent chemistry. Near neutral effluent in The Type I test pile corresponded to a population of neutrophilicspecies. Populations of acidophilic sulfur oxidizing bacteria and iron oxidizing bacteria were not the dominant species in the Type I test pile effluent. The Type III test pile effluent exhibited near neutral pH conditions after the piles thawed each spring.This neutral pH period was followed by a decrease to acidic conditions by the end of the field season. The evolving effluent in the Type III test pile was accompanied by changes in the microbial populations. Populations of acidophilic sulfur oxidizing bacteria and iron oxidizing bacteria were present each year. Continued monitoring and a more detailed analysis of the microbial population are required to further understand the biogeochemical evolution of these waste-rock test piles.

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REFERENCES

Ahonen, L., Tuovinen, O.H., 1992. Bacterial oxidation of sulfide minerals in column leaching experiments at suboptimal temperatures. Appl. Environ. Microbiol.58, 600– 606.

Amos, R.T., Blowes, D.W., Smith, L., Sego, D.C., 2009. Measurement of wind-induced pressure gradients in a waste rock pile.Vadose Zone Journal. 8, 953-962.

Bailey, B.L., Smith, L.J.D., Neuner, M., Gupton, M., Blowes, D.W., Smith, L., and Sego, D.C., 2009. Diavik Waster Rock Project: Early Stage Geochemistry and Microbiology. Securing the Future and 8th ICARD, June 22-26, 2009, Skellefteå, Sweden

Bailey, B.L., Blowes, D.W., Smith, L., and Sego, D.C. 2012. Diavik Waste Rock Project: Geochemistry of low sulfide content large-scale waste rock piles. 9th International Conference on Acid Rock Drainage, May 20-26, 2012, Ottawa, Canada

Blowes, D.W., Moncur, M.C., Smith, L., Sego, D., Bennett, J., Garvie, A., Gould, D., and Reinson, J., 2006. Construction of two large-scale waste rock piles in a continuous permafrost region.7th International Conference on Acid Rock Drainage.St. Louis, Mo.

Cochran, W.G. 1950. Estimation of bacterial densities by means of the "most probable number".Biometrics. 6, 105-116.

Dawson, R.F., Morin, K.A., 1996. Acid Mine Drainage in Permafrost Regions: Issues, Control Strategies and Research Requirements. MEND Project 1.61.2. Department of Indian and Northern Affairs Canada, Ottawa, Canada, pp. 1– 68.

Elberling, B., Schippers, A., Sand, W., 2000. Bacterial and chemical oxidation of pyritic mine tailings at low temperatures. Journal of Contaminant Hydrology. 41, 225–238.

Elberling, B., 2005. Temperature and oxygen control on pyrite oxidation in frozen mine tailings.Cold Regions Science and Technology. 41, 121-133.

Fretz, N., Momeyer, S., Smith., L., Blowes, D., Sego, D., Amos, R., 2011. Diavik Waste Rock Project: Unsaturated Water Flow. In Tailings and Mine Waste 2011 Proceedings, Vancouver, BC, 6-9 November, 2011.

Hulshof, A.H.M., Blowes, D.W., Gould, W.D. 2006. Evaluation of in situ layers for treatment of acid mine drainage: A field comparison. Water Research. 40, 1816-1826.

Kirby, C.S., Thomas, H.M., Southam, G., Donald, R., 1999. Relative contributions of abiotic and biological factors in Fe(II) oxidation in mine drainage. Applied Geochemistry. 14, 511–530.

Leduc, L.G., Trevors, J.T., Ferroni, G.D. 1993. Thermal characterization of different isolates of Thiobacillusferrooxidans. FEMS Microbiology Letters. 108, 189-194.

Nordstrom, D.K., Southam, G., 1997. Geomicrobiology of sulfide mineral oxidation.Reviews in Mineralogy. 35, 381-390.

Pham, N., Sego, D.C., Blowes, D.W., Amos, R.T., Smith, L., 2011b. Diavik Waste Rock Project: Thermal transport in a covered waste rock test pile. In Tailings and Mine Waste 2011 Proceedings, Vancouver, BC, 6 - 9 November, 2011

Ritchie, A.I.M., 1994. The waste rock environment, in: Jambor, J.L. and Blowes, D.W. (Eds.), Environmental Geochemistry of Sulfide Mineral Wastes, Mineralogical Association of Canada. Short Course, 22, pp. 201-245

Sand, W., Gehrke, T., Jozsa, P.G., Schippers, A., 2001. (Bio)chemistry of bacterial leaching-direct vs. indirect bioleaching. Hydrometallurgy. 59, 159–175.

Singer, P.C. and Stumm, W., 1970. Acid mine drainage: the rate determining step. Science. 167, 1121-1123.

Smith, L.J.D., Neuner, M., Gupton, M., Bailey, B.L., Blowes, D.W., Smith, L., and Sego, D.C. 2009. Diavik Waste Rock Project: From the Laboratory to the Canadian Arctic. Securing the Future and 8th ICARD, June 22-26, 2009, Skellefteå, Sweden