MICROBIAL POPULATIONS IN ACID SULFATE SOILS: POTENTIAL ROLE IN METAL AND ACID RELEASE

by

Wu, X.[1], Wong, Z. L.[1], Sten, P.[2], Engblom, S.[3], Österholm, P.[4] and Dopson, M.[1]

[1] *School of Natural Sciences, Linnaeus University, SE-391 82 Kalmar, Sweden*
[2] *Vaasa University of Applied Sciences, FI-65200 Vaasa, Finland*
[3] *Novia University of Applied Sciences, 65200 Vaasa*
[4] *Department of Geology and Mineralogy, Åbo Academy University, Åbo, Finland*

Soils containing metastable iron sulfides (FeS_n; n = 1.0–1.3) and pyrite (FeS_2) are found in the region around Vaasa, Finland. If the iron sulfides present in these "potential" acid sulfate soils (PASS), which contain sulfidic material, are exposed to air, oxidation reactions can mobilize acidity and metals, and they are subsequently termed "actual" acid sulfate soils (AASS) with sulfuric horizons. If uncontrolled, these reactions can have a major effect on the iron and sulfur cycles and cause significant environmental damage. The environmental pressure to identify occurrences of PASS is increasing due the requirement to drain land for residential housing, agriculture, or industry. Metastable iron sulfides in PASS may abiotically rapidly oxidize to form Fe^{3+}-containing minerals and elemental sulfur, as well as other inorganic sulfur compounds (Ward et al. 2004), while the subsequent oxidation of sulfur and FeS_2 may be catalyzed by indigenous microorganisms (Schippers & Sand 1999). The role of acidophilic microorganisms (pH optimum ≤5) in the formation of acidic, metal-laden solutions by catalyzing sulfide mineral dissolution is well documented (Dopson & Johnson 2012). Despite this, knowledge of the microbial populations in PASS and AASS and their potential role in metal and acid release is limited.

PASS and AASS were sampled from the Risöfladan experimental field, Vaasa, which contains around 0.5% sulfur as approximately 50% FeS_n (n = 1.0–1.3; metastable iron sulfides) and 50% FeS_2. Soil samples from a vertical depth profile at 30, 75, 127 and >180 cm beneath the surface were aseptically sampled (Fig. 1) and frozen until analysis for the presence of microorganisms.

The presence of microorganisms was investigated by genomic DNA extraction, amplification of the 16S rRNA gene and cloning into a plasmid vector (Dopson & Lindström 2004), and individual taxonomic units were identified by restriction fragment length polymorphism. Examples of unique species were then DNA sequenced. In addition, indigenous acidophilic microorganisms were enriched in the laboratory in stirred tank reactors containing 5% (wt/vol) PASS or AASS in sterile water at natural pH values as well as after adjustment to pH 3 (Dopson and Lindström 2004).

Biological leaching in stirred tank reactors demonstrated metal and acid release. The unoxidized fraction had an initial pH of 7.9 that decreased to 3.0 after

Fig. 1. Vertical profile of acid sulfate soil showing (from top to bottom) top soil, oxidized material, the oxidized/unoxidized boundary, and dark grey metal sulfides. Photograph courtesy of Rainer Rosendahl.

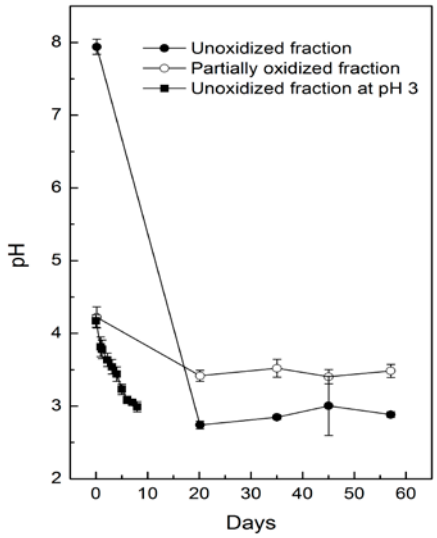

Fig. 2. pH decrease in stirred tank bioreactors of the unoxidized, partially oxidized, and unoxidized (at pH 3) fractions. The unoxidized fraction with an initial acidic pH was inoculated with an enrichment culture of microorganisms previously grown on PASS at low pH.

45 days of leaching, while the total iron increased from 22.4 to 30.7 mM. The partially oxidized fraction had an initial pH of 4.2 that decreased to 3.5 (Fig. 2), while the total iron also increased. Evidence for biological activity was observed via a decrease in the Fe^{2+} concentration, suggesting microbial oxidation of Fe^{2+} to Fe^{3+}. Finally, stirred tank reactors inoculated with an enrichment culture of indigenous microorganisms at pH 3 had a more rapid increase in total iron and decrease in pH (Fig. 2), as well as a decrease in the Fe^{2+} concentration from 2.2 to 1.2 mM after 8 days. This suggested that the partially oxidized ASS contains indigenous microorganisms capable of catalyzing metal and acid release at an acidic pH.

The microorganisms sampled from the PASS and AASS in the Risöfladan experimental field and in the stirred tank reactors will be identified by molecular phylogenetic methods and compared to the environmental conditions within the soil. In addition, the microorganisms will be evaluated for their ability to catalyze metal and acid release from PASS.

REFERENCES

Dopson, M. & Johnson, D. B. 2012. Biodiversity, metabolism and applications of acidophilic sulfur- metabolizing micro-organisms. Environ Microbiol (in press).

Dopson, M. & Lindström, E. B. 2004. Analysis of community composition during moderately thermophilic bioleaching of pyrite, arsenical pyrite and chalcopyrite. Microb Ecol 48, 19–28.

Schippers, A. & Sand, W. 1999. Bacterial leaching of metal sulfides proceeds by two indirect mechanisms via thiosulfate or via polysulfides and sulfur. Appl Environ Microbiol 65**,** 319–321.

Ward, N. J., Sullivan, L. A., Fyfe, D. M., Bush, R. T & Ferguson, A. J. P. 2004. The process of sulfide oxidation in some acid sulfate soil materials. Australian J Soil Res 42**,** 449–458.