GROWTH INHIBITION OF *CELLULOMONAS FLAVIGENA* INDUCED BY COPPER AND ZINC: DETERMINATION OF TOXICITY THRESHOLDS AND THE COMBINED EFFECTS OF COPPER AND ZINC ON THE GROWTH OF A CELLULOLYTIC-FERMENTING BACTERIA[1]

A. Ruhs[2], L. Figueroa[2] and T. R. Wildeman[2]

Abstract. The effective use of anaerobic passive treatment systems (APTS), such as sulfate-reducing bioreactors, to treat acid mine drainage will help to mitigate water contamination from mines located in remote areas as well as cut current treatment costs. One drawback to APTS has been an observed decline in long-term performance. Several environmental factors, such as initial metals concentration and temperature, may contribute to observed declines in sulfate reduction carried out by the microorganisms. APTS contain a complex microbial ecosystem, and metal toxicity could be indirectly affecting sulfate-reduction by inhibiting other important microbes. Previous research has found that organisms capable of degrading cellulose (cellulolytic-fermenters) are dominant within a sulfate reducing bioreactor (Pruden et al., 2005), and their ability to produce viable substrates for the sulfate-reducing bacteria is the rate-limiting step in sulfate reduction (Logan, 2003). This investigation examines the individual effect of zinc and copper, and then the combination of both metals, on a pure culture of cellulolytic fermenters, specifically *Cellulomonas flavigena* (ATCC 482).

C. flavigena exhibited 50% growth inhibition between a copper concentration of 0.00188 mM and 0.0038 mM. Further investigation of growth inhibition within this range of copper concentrations is currently underway. Glucose consumption was also much less in the copper containing bottles, as expected due to the low biomass observed in these bottles. The pH remained relatively constant throughout the experiments, staying within an optimal microbial growth range between pH 6 and 7. Possible changes in solution phase metals concentration and organic acid production throughout the experiments were monitored, and these results will be available during the conference. Concentrations of Zn(II) at 0, 0.125, and 0.25 mM were also examined to determine the effects on cell growth for *C. flavigena*. The results for zinc toxicity and a binary metal mixture will be presented at the conference.

Additional Key Words: Anaerobic Passive Treatment System, sulfate reducing bioreactor

---

[1] Paper was presented at the 2006 Billings Land Reclamation Symposium, June 4-8, 2006, Billings MT and jointly published by BLRS and ASMR, R.I. Barnhisel (ed.) 3134 Montavesta Rd., Lexington, KY 40502.

[2]Alison Ruhs, Graduate Research Assistant, Environmental Science and Engineering, Colorado School of Mines, Golden, CO 80401. Linda Figueroa, Associate Professor, ESE, CSM. Tom Wildeman, Professor, Chemistry and Geochemistry, CSM, Golden, CO 80401.