

## CONTRIBUTION OF SULFUR REDUCING BACTERIA IN AN ARTIFICIAL WETLAND TREATING AMD<sup>1</sup>

R. Guy Riefler<sup>2</sup>, Ben Stuart, and Jeremy Krohn

**Abstract.** In 2002, a multi-cell vertical-flow wetland system was installed to treat acid mine drainage (AMD) discharging from a flooded abandoned coal mine in Coshocton, Ohio that generates on average 60 lb/d of acidity, 17 lb/d of iron, and 5 lb/d of aluminum. Within a year, the cells began emitting a strong sulfide odor and filling with a thick black sludge, primarily where effluent from a vertical-flow wetland mixes with the mine outfall. We have recently completed a two-year investigation on the operation of the wetland system and the source of the sludge. Tracer tests showed a significant reduction in retention time in the cells and alkalinity generation in the wetlands system was insufficient during the winter season.

The solids content of the sludge was 6.5% with 42% of the solids identified as the volatile fraction (58% fixed), indicating it was biological in nature with a very high mineral content. Further analysis revealed concentration of iron at 4,745 mg/L and total sulfur at 10,595 mg/L. From this and other evidence it was concluded that the mineral portion of the sludge was primarily FeS. Most probable number analysis, revealed a sulfate reducing bacteria population in the sludge sample of  $6.22 \times 10^6$  per gram. However, laboratory grown sulfur reducing bacteria cultures did not form a thick, viscous sludge but rather a fine, powdery residue of black FeS. Further attempts to isolate and culture the culpable bulking bacteria were unsuccessful. Terminal restriction fragment length polymorphisms (T-RFLP) analysis has revealed a diverse microbial community, and we are currently building a clone library to sequence and identify major contributors to that community.

Influent and effluent loadings were compared in each cell of the wetland system to determine the sources of alkalinity. A mass balance was performed on each cell using monthly acidity and alkalinity analyses and flow rates to determine the alkalinity production in each cell of the wetland system. Surprisingly, alkalinity generation was greatest in the cells filled with sludge. Alkalinity is generated in vertical-flow wetlands primarily by limestone dissolution, and although sulfur reduction is known to be present, its contribution to alkalinity production is usually considered insignificant. Using stoichiometry increases in calcium concentration due to limestone dissolution were converted to alkalinity increases, and using the mass balance approach the contribution of alkalinity production due to limestone generation was determined. Similarly, decreases in sulfate concentration were stoichiometrically converted to alkalinity concentrations, and the contribution of sulfur reducing bacteria to alkalinity production was determined. Total alkalinity production matched well with the sum of alkalinity due to limestone dissolution and alkalinity due to sulfur reducing bacteria activity indicating consistency in the approach. Over half of the alkalinity produced in the wetland system was due to sulfur reduction. This study indicates the importance of the often overlooked sulfur reducing bacteria in vertical-flow wetlands and demonstrates the value of a mass balance approach to identify the contributions of different processes.

---

<sup>1</sup> 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.

<sup>2</sup>R. Guy Riefler, Ph.D., Assistant Professor of Civil Engineering; Ben Stuart, Assistant Professor; and Jeremy Krohn, Master of Science Student, Department of Civil Engineering, Ohio University, Athens, Ohio 45701.