

LABORATORY AND FIELD-SCALE EVALUATION OF LOW-PH FE(II) OXIDATION AT SEVERAL SITES IN PENNSYLVANIA¹

Trinh C. DeSa², Juliana F. Brown and William D. Burgos

Abstract. The Hughes Borehole is located in the bituminous coal region of south-central Pennsylvania and drains an underground mine complex of ca. 2,950 ha. The borehole discharge averages approximately 63 L/s of acidic (pH 4, 230 mg/L acidity) mine drainage that contains elevated concentrations of Fe(II) (100 mg/L) and numerous trace metals. Long-term monitoring of the site showed that biological Fe(II) oxidation occurred without human intervention and produced a 0.6 ha iron mound up to 2 m deep. On-mound channel reactors along with laboratory-scale “gutter” reactors were constructed to determine conditions that can exploit biological Fe(II) oxidation for passive treatment. Dissolved Fe(II) was much more efficiently oxidized from gutter reactors that contained iron mound sediment than ones without any sediment. Residence times of 5-10 h were shown to oxidize ~100% of dissolved influent Fe(II) and remove 75% of dissolved total Fe. The Lower Red Eyes discharge (pH 4, 1,420 mg/L acidity, 540 mg/L Fe(II)) is also located in south-central Pennsylvania and is unique in that it emerges in the middle of a state forest and produces a spectacular series of iron terraces without ever being “captured” by any adjacent stream. Similar laboratory-scale experiments were conducted with intact sediment “chunks” to measure the kinetics of Fe(II) oxidation at various locations on the iron mound. Residence times of 10 h were shown to oxidize 55% of dissolved influent Fe(II) and remove 27% of dissolved total Fe. Rates of Fe(II) oxidation were not strongly correlated to dissolved oxygen but were affected by the water column height overlying the sediment chunk. While the water chemistry was distinctly different at these two sites, both showed that Fe(II) oxidation was strongly dependent on hydraulic residence time. Therefore, a consistent design approach may be feasible for sites that wish to exploit low-pH Fe(II) oxidation for treatment.

Additional Key Words: acid mine drainage, metal oxidation, Fe(II) oxidation

¹ Paper was presented at the 2010 National Meeting of the American Society of Mining and Reclamation, Pittsburgh, PA *Bridging Reclamation, Science and the Community* June 5 - 11, 2010. R.I. Barnhisel (Ed.) Published by ASMR, 3134 Montavesta Rd., Lexington, KY 40502.

² Trinh C. DeSa is an Environmental Engineer I with ARCADIS, Inc., Newtown, PA 18940, Julian F. Brown is a M.S. student in Environmental Engineering at Penn State, William D. Burgos is a Professor of Environmental Engineering, Department of Civil and Environmental Engineering, The Pennsylvania State University, University Park, PA 16802.

Proceedings America Society of Mining and Reclamation, 2010 pp 190-203

DOI: 10.21000/JASMR10010190

<http://dx.doi.org/10.21000/JASMR10010190>

Introduction

Acid mine drainage (AMD) causes significant harm to the environment. The northern Appalachian Plateau of the eastern United States contains more than 8,000 km of streams that are affected by drainage from abandoned coal mines (Boyer and Sarnoski, 1995). Many of these streams are located in Pennsylvania where approximately half of the discharges are acidic, having a $\text{pH} < 5$ (Brady et al., 1998). AMD impacts 45 of the 67 counties in Pennsylvania.

Cost-effective treatment technologies for the remediation of AMD are needed. In Appalachia, the removal of iron is probably the most important aspect of AMD treatment. Biological low-pH Fe(II) oxidation can improve the performance of conventional passive limestone treatment systems. Limestone dissolution neutralizes acidic water and promotes the precipitation of Fe(III) (hydr)oxides (Cravotta and Trahan, 1999; PADEP, 1999). Unfortunately, these Fe(III) oxides chemically coat limestone surfaces (commonly called “armoring”), limiting further limestone dissolution and neutralization capacity, and, more importantly, hydraulically clog the limestone bed (PADEP, 1999; Rose et al., 2004; Weaver et al., 2004).

To limit armoring and clogging, dissolved Fe may be removed from AMD before waters are neutralized with limestone. In the pH range typical of acidic Appalachian mine drainage (2.5 - 4.5) (Cravotta et al., 1999), the abiotic oxidation of Fe(II) is kinetically limited, but hydrolysis and precipitation of Fe(III) will still occur (Fig. 1). Under such conditions, acidophilic Fe(II)-oxidizing bacteria (Fe(II)OB) may catalyze Fe(II) oxidation, allowing for the oxidative precipitation of Fe from AMD discharges at low-pH (Kirby et al., 1999; Johnson and Hallberg, 2003; Nicormat et al., 2006; Nengovhela et al., 2004; Williamson et al., 2006). This water may then be neutralized using limestone before it is released into nearby streams. The spatial separation of iron oxidation and precipitation from alkalinity addition would improve the operation and maintenance of limestone beds and reduce the associated costs. This spatial separation can be achieved by promoting low-pH Fe(II) oxidation across natural iron mounds (commonly found immediately downstream of emergent acidic mine discharges) before conveying the water to a limestone treatment bed.

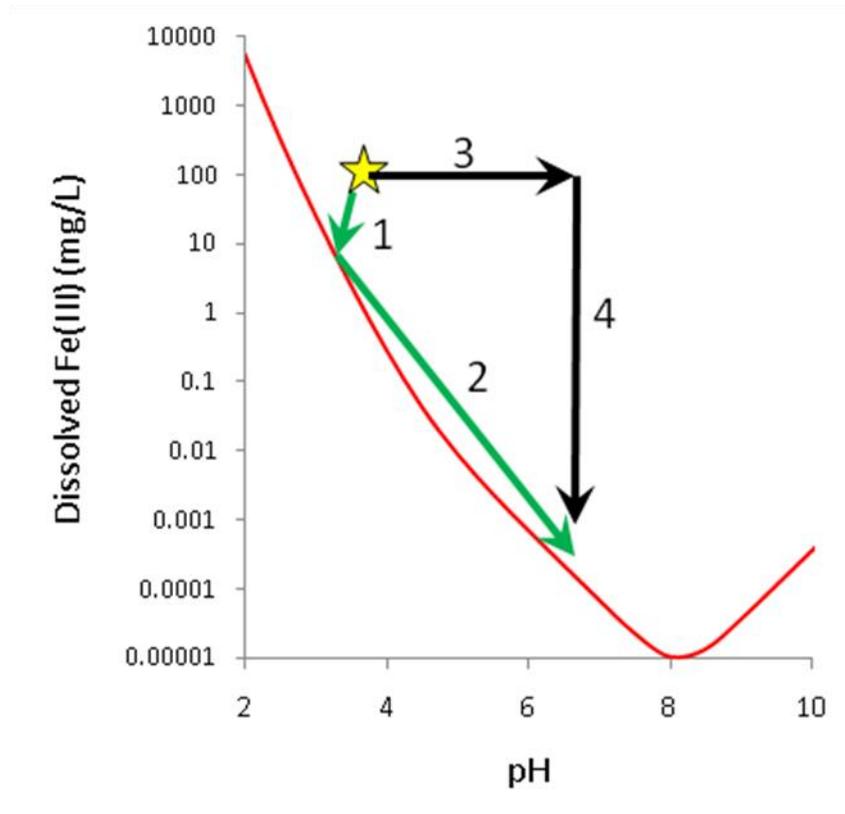


Figure 1. Low-pH Fe(II) oxidation with respect to the solubility of Fe(III). The red line represents the solubility of $\text{Fe}(\text{OH})_3(\text{s})$ as a function of pH. The star represents a hypothetical AMD discharge at pH 4.0 with 100 mg/L Fe(II). Line 1 represents the reaction path followed during biological low-pH Fe(II) oxidation. The pH decreases because of the precipitation of $\text{Fe}(\text{OH})_3(\text{s})$. Line 2 represents the reaction path when the water discharged from the iron mound is conveyed through a limestone bed. Lines 3 and 4 represent the combined processes of alkalinity addition, abiotic Fe(II) oxidation, and Fe(III) precipitation. A considerably greater load of Fe will be precipitated in the limestone bed via this conventional treatment scheme.

The overarching goal of our studies of low-pH Fe(II) oxidation has been to better understand the biogeochemistry of these systems such that this naturally occurring process can be exploited for passive treatment of AMD (Lucas 2008; Senko et al. 2008; DeSa 2009). The specific objectives of this research were to conduct laboratory experiments in flow-through, open channel, “gutter” reactors using iron mound sediments and water from Hughes Borehole to evaluate the effect of residence time on low-pH Fe(II) oxidation and Fe removal, and to conduct similar laboratory experiments in flow-through, sediment “chunk” reactors using intact sediment pieces removed from the Lower Red Eyes iron mound.

Material and Methods

Field Sampling

The emergence of Hughes Borehole is surrounded by a chain link fence. The water flows under the fence (2.5 m downstream of borehole), through a rectangular weir, across the existing iron mound (essentially uncontrolled), and discharges off the “toe” of the iron mound (60 m downstream of borehole) into a small tributary before flowing into the Little Conemaugh River (0.61 km downstream of borehole). The channel downstream of the weir is relatively deep (ca. 0.6 m) with fast moving water and captures the majority of the flow from the borehole. A smaller portion of the water is conveyed as shallow (ca. 1 cm) sheet flow across the iron mound surface. The deep channelized flow likely has a much shorter hydraulic residence time across the mound (ca. minutes to travel to toe) as compared to the sheet flow regions (ca. tens of minutes).

The fence and toe locations were chosen for long-term monitoring of geochemical changes across the iron mound (July 2008 to July 2009). Field measurements included electrical conductivity, dissolved oxygen, and pH and temperature using portable meters. Water samples were filtered (0.2- μm) into sterilized, acid washed centrifuge tubes and chemically preserved in the field with HCl (for Fe(II) and Fe(III)), or HNO₃ (for metals by ICP-AES), or H₂SO₄ (for total organic carbon analysis). Samples for sulfate were neither acidified nor filtered. Water samples were transported to the laboratory on ice and refrigerated at 4 °C until analyzed. Sediment samples were collected from the top 2-cm of the iron mound at several locations with sterile spatulas and placed into sterile whirl-pak bags.

Lower Red Eyes is a low flow (0.44 L/s) AMD site located in Somerset County, PA, USA. The AMD discharge is the result of surface mine runoff that collects in one pond and then travels underground to emerge at a previously drilled exploratory borehole within the Prince Gallitzin State Forest. A series of iron terraces formed on fallen trees and small and large pools make up the entire transect (Fig. 2). After flowing through the forest for nearly 150 m, the discharge seeps back into the ground. This site is unique to the majority of observed AMD sites, which flow into a nearby stream causing the geochemical gradient to be disrupted. As a result, the entire Fe(II) oxidation and Fe(III) precipitation process can be studied and the entire range of pH and dissolved oxygen (DO) can be measured in the field. Upstream and downstream terrace and pool features were selected as sampling points and field measurements were conducted as

mentioned above. Water and sediment samples were also collected in the same manner as those at Hughes Borehole.



Figure 2. Photos of iron mound formed downstream of Lower Red Eyes discharge. A) Detail of pools and terraces. B) Upstream view towards emergence (located between men).

Laboratory “Gutter” Reactors

Flow-through, open channel, laboratory-scale “gutter” reactors were constructed with iron mound sediments from Hughes Borehole in square PVC tubes (2.5 cm x 2.5 cm x 91 cm; $w \times h \times l$) (Fig. 3B). “Sediment reactors” contained 100 g of air dried sediment evenly packed along the bottom of the tubes, while “Control reactors” contained no sediment. Inlet weirs were designed to maintain a 0.64 cm water column height in each reactor. The gutter reactors had a slope of 1.3 cm/91 cm (0.014) to allow for gravity flow. A 19 L glass feed tank containing water from Hughes Borehole (fence location, unfiltered) was sealed with a rubber stopper and constantly purged with N_2 gas to minimize any oxidation of Fe(II). A peristaltic pump was used to convey water from the feed tank to the four inlet weirs, and the same pump was used to collect the channel effluents into waste containers. Samples were collected from the influent and effluent ends of the gutter reactors.



Figure 3. A) Picture of the channel reactors built on the Hughes Borehole iron mound. B) Picture of laboratory-scale “gutter” reactors showing the feed tank in the background and the four open-channel, flow-through reactors in the foreground.

Experiments conducted in the gutter reactors allowed longer residence times than were possible with the on-mound channel reactors. Experiments were conducted sequentially at residence times of 10, 5, 2, and 1 hour, starting at the longest residence time and finishing at the shortest residence time. The flow rates in each reactor for these residence times were 0.207, 0.415, 1.04, and 2.08 mL/min, respectively and were verified by gravimetric analysis. The flow rates were set to achieve the selected residence times based on the assumed water volume in the gutter reactor.

For select experiments, coir landscaping mats (Rolanka) were added to the gutter reactors to provide additional surface area. 10 g of coir mat was added to each sediment reactor. The coir mat was cut to 2 cm wide and 0.64 cm inches in depth, to fit within the constraints of the reactors and was placed on top of the sediment.

Laboratory “Chunk” Reactors

Flow-through “chunk” reactors were constructed in the laboratory to mimic field conditions at Lower Red Eyes. The reactors consisted of two plastic storage containers (13 cm x 8 cm x 6 cm) in series connected with Tygon L/S 14 tubing. “Sediment reactors” contained either terrace sediments or pool sediments (5 – 140 g) and “Control reactors” contained no sediment. Water was collected at the emergence of Lower Red Eyes and filter sterilized (0.2 μm) prior to use in the reactors. Site water was stored in a 19 L glass feed tank and constantly purged with N_2 . A peristaltic pump was used to pump water from the feed tank into the first reactor, from the

first reactor into the second reactor, and from the second reactor into a waste container at a constant flow rate to maintain a volume of 175 mL and a water column height of 0.64 to 1.9 cm water in each reactor. Three residence times (10, 5, and 2 hours) were studied sequentially. Residence times referred to the total time in the reactor series.

Reactors were sampled at the initial start (time = 0), and after 1, 2, 4, and 6 residence times. At the influent and effluent of each reactor, 1 mL of sample was collected and centrifuged at 13,400 rpm for 2 minutes to separate any precipitates. Total dissolved Fe and Fe(II) were measured from the sample. The pH of the remaining supernatant was measured with a Thermo Orion 550A benchtop pH meter and semi-micro Thermo Orion pH probe. Dissolved oxygen was also measured at each sampling point using an Extech ExStik II DO field meter.

Analytical Methods

Dissolved ferrous iron, Fe(II), was measured by the ferrozine assay (Stookey 1970) after centrifugation (2 min at 13,400 rpm). Dissolved total iron was also measured by the ferrozine assay after all iron was reduced by hydroxylamine-HCl. Fe(II) and total Fe concentrations were measured by absorbance at 562 nm using a Shimadzu – UV-1601 Spectrophotometer. Dissolved ferric iron, Fe(III), was calculated as the difference between total Fe and Fe(II). pH was measured in the supernatant using a Thermo Orion 550A benchtop pH meter with a semi-micro pH probe. Dissolved oxygen (DO) was measured directly in the gutters using a Cole-Parmer benchtop dissolved oxygen meter. Metals were measured by inductively coupled plasma atomic emission spectrometer (ICP-AES) using a Perkin-Elmer Optima 5300 ICP-AES. Acidity was calculated indirectly based on pH and measured concentrations of dissolved Fe(II), Fe(III), Mn, and Al according to Kirby and Cravotta (2005). Sulfate was measured by the HACH SulfaVer 4 method using a HACH DR/2800 spectrophotometer. Total organic carbon (TOC) and total nitrogen (TN) samples were analyzed using a Shimadzu TOC-VCSN Total Organic Carbon Analyzer and Shimadzu TNM-1 Total Nitrogen Measuring Unit, respectively. Lithium metaborate fusion was used to dissolve solid sediment samples, then ICP-AES was used to measure elemental content. Sediment samples were air dried, mixed lithium metaborate, heated to 1000 °C for 30 min, and then diluted into nitric acid prior to ICP-AES analysis. Metal oxide percentages were based on the original weight of sample.

Results and Discussion

We have been studying biological low-pH Fe(II) oxidation since October 2005, at the request of OSM and PADEP BAMR personnel. We have focused our studies on AMD discharges that have produced large iron mounds with no human intervention. We have had two projects funded by BAMR to characterize the microbial communities and mineral precipitates found on these iron mounds. Our first project focused on Gum Boot Run in McKean County, Pennsylvania and the Fridays-2 discharge near Hollywood, Pennsylvania in Clearfield County. These two discharges were initially designated as “positive” and “negative” control sites, respectively, with respect to the extent of Fe(II) oxidation measured in the field. The most surprising and significant finding from these studies was that the microbial communities and the rate of Fe(II) oxidation (measured in laboratory batch reactors) were the same in certain locations on both iron mounds (Lucas, 2008; Senko et al., 2008). In other words, the designations of “positive” and “negative” control were not appropriate with respect to the potential for low-pH Fe(II) oxidation. Furthermore, it is quite likely that biological low-pH Fe(II) oxidation will occur to varying extents downstream of any low-pH Fe(II)-rich discharge. This research project began in October 2005 and was completed in August 2007.

Our second project focused on the Hughes Borehole located near Portage, Pennsylvania in Cambria County (Table 1). The objectives of this second project were to conduct laboratory experiments in flow-through, open channel, “gutter” reactors using iron mound sediments and water from Hughes Borehole to evaluate the effect of residence time on low-pH Fe(II) oxidation and Fe removal, to conduct similar field experiments in open channels constructed on the Hughes Borehole iron mound, and to monitor the effective removal of Fe across the whole iron mound for one year. Using the gutter reactors (Fig. 3B), we developed a laboratory assessment protocol where we incrementally decreased the hydraulic residence time to determine what minimum residence time was required for an acceptable level of treatment (DeSa et al., 2010). We started with a 10 h residence time and continued until a pseudo-steady state condition had been reached with respect to the effluent Fe(II) concentration. We started with the longest residence time first in order to acclimate the sediment microbes to the laboratory conditions. The residence times were then sequentially decreased to 5 h, 2 h, and 1 h. When operated at a 10 h residence time, the pseudo-steady state $[\text{Fe(II)}]_{\text{out}}/[\text{Fe(II)}]_{\text{in}}$ ratio dropped to 0.03 (i.e. 97 % of the influent Fe(II) was oxidized) (Fig. 4). The consumption of Fe(II) and dissolved oxygen (not

shown), and the drop in pH are consistent with biological oxidation of Fe(II) followed by the precipitation of Fe(OH)₃(s). When operated at a 5 h residence time, the pseudo-steady state [Fe(II)]_{out}/[Fe(II)]_{in} ratio increased to 0.10. When operated at 2 h and 1 h residence times the pseudo-steady state [Fe(II)]_{out}/[Fe(II)]_{in} ratios increased further to 0.31 and 0.76, respectively. At these shortest residence times, the effluent pH also increased, consistent with less precipitation of Fe(OH)₃(s).

Table 1. Water chemistry of Fridays-2, Hughes Borehole, and Lower Red Eyes discharges.

	Fridays-2	Hughes Borehole	Lower Red Eyes
Flow (gpm)	36 N=1	300-2,000	
Temp (°C)	9.8 ± 0.1 (9.7-10.0) N=4	12.7 ± 0.6 N = 30	9.4 ± 0.7 N = 7
DO (mg/L)	0.36 ± 0.43 (0-0.97) N=4	1.10 ± 0.80 N = 21	0.59 ± 0.70 N = 5
Conductivity (mS)	0.772 ± 0.001 (771-772) N=2	1.08 ± 0.1 N = 23	4.36 ± 0.16 N = 7
pH	4.1 ± 0.2 (3.9-4.4) N=4	3.96 ± 0.25 N = 29	4.04 ± 0.25 N = 7
Dissolved Fe (mg/L)	45.2 ± 3.4 (42-51) N=6	88.5 ± 0.33 N = 7	537.24 ± 21.6 N = 5
Dissolved Fe(II) (mg/L)	--	100.2 ± 17.4 N = 25	543.03 ± 18.22 N = 5
Al (mg/L)	20.0 ± 2.4 (17-22) N=6	8.19 ± 0.39 N = 7	43 ± 1.15 N = 3
Mn (mg/L)	1.3 ± 0.1 (1.2-1.4) N=6	2.42 ± 0.02 N = 7	113 ± 2.89 N = 3
SO₄²⁻ (mg/L)	316.3 ± 90.3 (252.5-380.2) N=2	573 ± 63 N = 5	2992 ± 368 N = 12
Zn (mg/L)	--	0.27 ± 0.01 N = 7	13 ± 1.53 N = 3
Acidity (mg CaCO₃/L)	--	228 ± 4.9 N = 7	1419 N = 1

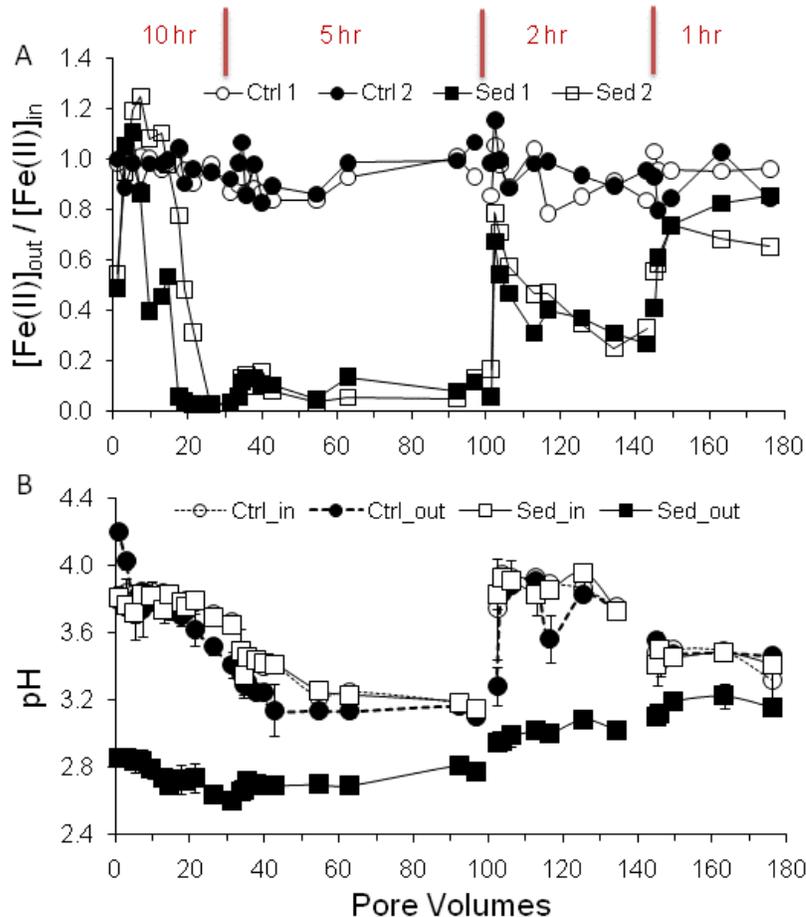


Figure 4. Low-pH Fe(II) oxidation in laboratory flow-through “gutter” reactors operated at variable hydraulic residence times. Experiments began at a 10 h residence time that was incrementally reduced to 5, 2, and 1 h (delineated in A). Sediment reactors (Sed) contained sediment from Hughes Borehole and Control reactors (Ctrl) contained no sediment. For the normalized results in A, influent Fe(II) concentrations ranged from 60 to 100 mg/L.

These data suggest that a hydraulic residence time of 5 – 10 h across the Hughes Borehole iron mound (as shallow sheet flow) could provide significant passive treatment – where close to 100% of Fe(II) could be oxidized and 75% of the total Fe could be removed from solution. We also conducted field experiments in the on-mound channel reactors but because of the diameter of the influent pipe intake structure, the slope of the mound, and the length of the channels, we could only test a narrow range of relatively short residence times (2 – 33 min).

We have also begun research at a site referred to as Lower Red Eyes near Ogletown, Pennsylvania in Somerset County (Table 1). We were shown this site by OSM and PADEP BAMR personnel. The emergent discharge has an average pH of 4.0 and dissolved

concentrations of iron, manganese, aluminum, and sulfate of 520, 115, 45, and 3200 mg/L, respectively. This discharge is unique in that it emerges in the middle of a state forest and produces a spectacular series of iron terraces without ever being “captured” by any adjacent stream. At almost every other site, the iron mounds are terminated (along with any fortuitous water treatment) due to discharge into the receiving stream. The emergent discharge also has much higher acidity and Fe(II) as compared to other sites we have studied. To maintain the biophysical environment of the terrace and pool sediments, which we believe exerts some control on natural Fe(II) oxidation, we cut out intake “chunks” of sediments and placed them in flow-through reaction vessels (vs. re-packed sediments as done with Hughes Borehole). We conducted otherwise identical variable-residence time experiments using sediments and water from Lower Red Eyes and obtained very similar results (Fig. 5) (Brown and Burgos, 2009). Again, we feel these results are very encouraging because we may now be able to demonstrate that the same design approach for sizing low-pH Fe(II) oxidation systems can be applied to most sites regardless of AMD chemistry.

We measured the rates of Fe(II) oxidation using sediment chunks collected from a variety of locations on the Lower Red Eyes iron mound. Our goal was to determine if one particular physical environment (e.g., terraces vs. pools) or geochemical environment (e.g., pH 2.5 vs. pH 3.5) allowed for faster rates of Fe(II) oxidation. If this was true, than a low-pH iron mound could be manipulated or engineered to provide the ideal conditions for Fe(II) oxidation and Fe removal. We found that, on a surface-area normalized basis, sediments collected from pools oxidized Fe(II) at faster rates as compared to sediments collected from terraces (data not shown). In these flow-through experiments, the dissolved oxygen concentrations dropped the most in reactors where the Fe(II) oxidation rate was the greatest. Thus, the rates of Fe(II) oxidation were not well correlated with the dissolved oxygen concentrations.

We also measured the rates of Fe(II) oxidation with a single sediment chunk where we systematically increased the overlying water height. We found that the rate of Fe(II) oxidation decreased significantly when the water column height was increased from 1 cm to 2 cm to 3 cm (data not shown), even though the dissolved oxygen concentrations were never limiting. Our interpretation of these results is that the shallow sheet flow for a 1 cm water column depth somehow stimulate biological Fe(II) oxidation. Furthermore, even though sediments from pools were shown to most rapidly oxidize Fe(II), this would not likely happen if the pool depth were

allowed to increase over 1 cm. Therefore, based on all our results to date, we believe an iron mound with very shallow pools should provide the best conditions for low-pH Fe(II) oxidation.

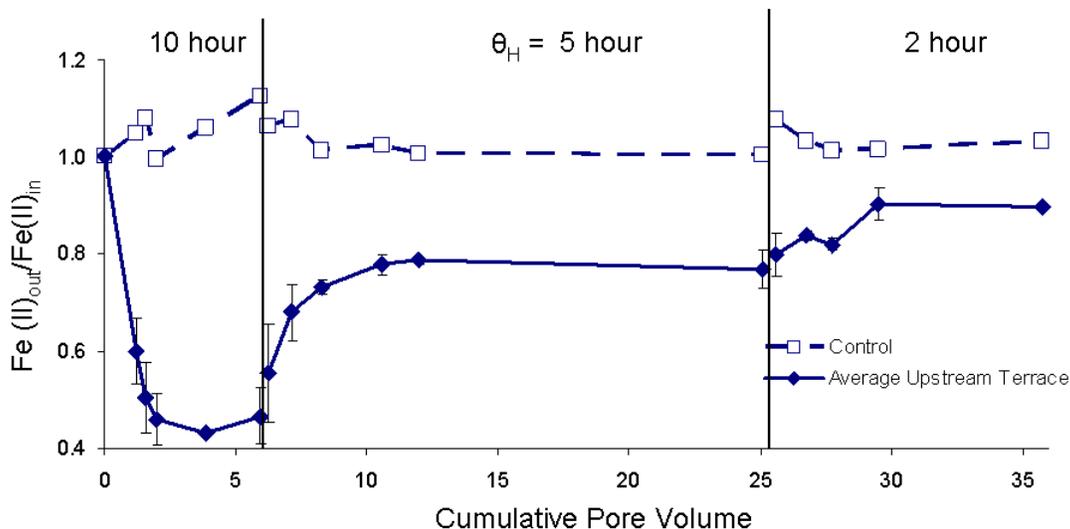


Figure 5. Low-pH Fe(II) oxidation in laboratory flow-through “chunk” reactors operated at variable hydraulic residence times. Experiments began at a 10 h residence time that was incrementally reduced to 5, and 2 h. Duplicate reactors contained intact chunks of terrace sediment collected from Lower Red Eyes and Control reactors contained no sediment. For these normalized results, influent Fe(II) concentrations ranged from 450 to 550 mg/L.

Low-pH Fe(II) oxidation can be exploited for passive treatment of AMD. A hydraulic residence time of 5 – 10 h of shallow sheet flow across an existing iron mound could oxidize >95% of influent Fe(II), and remove >70% of total Fe (at Hughes Borehole). Physical modifications of the iron mound to increase surface area for microbial growth and/or Fe precipitation could further enhance performance. The precipitates found on these iron mounds were essentially pure iron with little to no metal cation incorporation. These minerals could prove valuable for industrial reuse applications.

Acknowledgements

We thank Brent Means from the US Office of Surface Mining, and Malcolm Crittenden from the Pennsylvania Department of Environmental Protection for directing us to the Lower Red Eyes discharge. This work was funded by the Pennsylvania Department of Environmental Protection, Bureau of Abandoned Mine Reclamation by grant. J.F. Brown and W.D. Burgos

were also provided support from the National Science Foundation under Grant No. CHE-0431328.

Literature Cited

Boyer, J., and Sarnoski B (1995) 1995 Progress Report—Statement of mutual intent strategic plan for the restoration and protection of streams and watersheds polluted by acid mine drainage from abandoned coal mines. Philadelphia, PA, US EPA, appendix, <http://www.epa.gov/reg3giss/library.htm>

Brady K.B.C, Kania T, Smith W.M, and Hornberger R.J (1998) Coal mine drainage prediction and pollution prevention in Pennsylvania. Pennsylvania Dept of Environmental Protection, Ch 4, accessed 18 Jan 2010, <http://www.dep.state.pa.us/dep/deputate/minres/districts/cmdp/main.htm>

Brown J.F., and Burgos W.D. (2009) Unraveling the Biogeochemical Relationships at an Acid Mine Drainage Site. Poster Presented at the American Society for Microbiology General Meeting, Philadelphia, PA, May 2009.

Cravotta C.A., and Trahan M.K., (1999) Limestone drains to increase pH and remove dissolved metals from acidic mine drainage. *Appl Geochem* 14: 581-606 [http://dx.doi.org/10.1016/S0883-2927\(98\)00066-3](http://dx.doi.org/10.1016/S0883-2927(98)00066-3)

Cravotta C. A. III, Brady K.B.C., Rose A.W., and Douds J.B. (1999) Frequency distribution of the pH of coal-mine drainage in Pennsylvania. Water-Resources Investigations Report (United States Geological Survey) 99-4018A, USGS Toxic Substances Hydrology Program. **1**, 313-324.

DeSa, T.C. (2009) Promoting Low-pH Biological Iron(II) Oxidation of acid mine drainage at Hughes borehole: field and laboratory experiments. PhD Diss, The Pennsylvania State Univ, University Park, PA, USA, 120 pp.

DeSa T.C., Brown J.F., and Burgos W.D. (2010) Laboratory and field-scale evaluation of low-pH Fe(II) oxidation at Hughes Borehole, Portage, Pennsylvania. *Mine Water and the Environment*, Accepted December 2009. <http://dx.doi.org/10.1007/s10230-010-0105-5>.

Johnson D.B., and Hallberg K.B., (2003) The microbiology of acidic mine waters. *Res Microbiol* 154: 466-473 [http://dx.doi.org/10.1016/S0923-2508\(03\)00114-1](http://dx.doi.org/10.1016/S0923-2508(03)00114-1)

- Kirby C.S., and Cravotta CA (2005) Net alkalinity and net acidity 1: Theoretical considerations. *Appl Geochem* 20: 1920-1940 <http://dx.doi.org/10.1016/j.apgeochem.2005.07.002>
- Kirby C.S., Thomas H.M., Southam G., and Donald R., (1999) Relative contributions of abiotic and biological factors in Fe(II) oxidation in mine drainage. *Appl Geochem* 14: 1101-1101 [http://dx.doi.org/10.1016/S0883-2927\(98\)00071-7](http://dx.doi.org/10.1016/S0883-2927(98)00071-7)
- Lucas M.A. (2008) The comparison of two acid mine drainage sites in central Pennsylvania: field site characterizations and batch reactor experiments. PhD Diss, The Pennsylvania State Univ, University Park, PA, USA, 172 pp.
- Nengovhela N.R., Strydom C.A., Maree J.P., and Greben H.A. (2004) Chemical and biological oxidation of iron in acid mine water. *Mine Water Environ* 23: 76-80 <http://dx.doi.org/10.1007/s10230-004-0042-2>
- Nicormat D., Dick W.A., and Tuovinen O.H. (2006) Assessment of the microbial community in a constructed wetland that receives acid coal mine drainage. *Microbial Ecol* 51: 83-89 <http://dx.doi.org/10.1007/s00248-005-0267-z>
- PA DEP (1999) The science of acid mine drainage and passive treatment. Bureau of Abandoned Mine Reclamation, Pennsylvania Dept of Environmental Protection, accessed Jan 18, 2010, <http://www.portal.state.pa.us/portal/server.pt?open=514&objID=588922&mode=2>
- Rose A.W., Bisko D., Daniel A., and Bower M.A., 2004 An "autopsy" of the failed Tangascootak #1 vertical flow pond. *Proceedings America Society of Mining and Reclamation*, 2004 pp 1580-1594, <http://dx.doi.org/10.21000/JASMR0401580>,
- Senko J.M., Wanjugi P., Lucas M., Bruns, M.A., and Burgos W.D. (2008) Characterization of Fe(II) oxidizing bacterial activities and communities at two acidic Appalachian coalmine drainage-impacted sites. *ISME J* 2: 1134-1145 <http://dx.doi.org/10.1038/ismej.2008.60>
- Stookey L.L. (1970) Ferrozine – a new spectrophotometric reagent for iron. *Anal Chem* 42: 779–781 <http://dx.doi.org/10.1021/ac60289a016>
- Williamson M.A., Kirby C.S., and Rimstidt J.D. (2006) Iron dynamics in acid mine drainage. 7th International Conference on Acid Rock Drainage, 2006 pp 2411-2423 <http://dx.doi.org/10.21000/jasmr06022411>