BIOLOGICAL SOURCE TREATMENT OF ACID MINE DRAINAGE[1]

Song Jin[2], Jeffrey S. Cooper, Paul H. Fallgren, Martin W. Stearns

Abstract: Acid mine drainage (AMD) originates from mining operations with oxidation of metal sulfides. The oxidizing process releases protons and generates acidity. A biological source treatment (BST) technique has been developed to address AMD at its source. The BST technique utilizes sulfate-reducing bacteria (SRB) and substrate amendments to establish an intracellular and/or extracellular biogeochemical formation on the surface of metal sulfide, shielding them from being oxidized. SRB transfer electrons from organic substrates to sulfate and form sulfide. This process consumes protons and raises pH, while dissolved metals are precipitated with sulfide. Laboratory studies have shown that effluent/solids from wastewater treatment plants contain adequate populations of SRB as the inoculum for BST applications. In laboratory studies, the pH of AMD samples increased from <4.0 to >7.0 and stabilized in the neutral pH range for more than 20 months with one treatment. A field pilot study of the BST technique was conducted at a reclaimed coal mine in central Tennessee. Results to date indicate a significant increase in pH in the testing area, stabilizing in the pH range from 6.0 to 7.0, from the initial acidic readings. Decreases in sulfate and increases in sulfide concentrations confirm valid sulfate-reducing activity. Electromagnetic surveys were conducted in the field pilot test to identify AMD source and assist in monitoring the performance. Data from both the laboratory and field studies demonstrate that BST process is an innovative technology that can be applied in suitable sites and offer a cost-effective and long-term treatment of AMD.

Additional Key Words: AMD, acid mine drainage, electromagnetic survey, metal removal, sulfate-reducing bacteria

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Introduction

Acid mine drainage (AMD) is a problem for large-scale mining operations in certain regions. AMD pollutes rivers and watersheds, resulting in a toxic environment for vegetative and aquatic life. AMD originates from the oxidation of metal sulfides, which release protons and sulfate and cause a rapid decline in pH. In addition, the increased acidity dissolves metals, leading to higher concentrations of potentially toxic metals in the AMD water.

Conventional passive treatments for AMD include lime neutralization, wetland, composite piles, and anaerobic bioreactors. All these processes are designed to increase pH and remove metals from the AMD. However, passive treatments have the drawback of long-term and inconsistent performance. The continued generation of AMD actually challenges the passive systems with an unlimited amount of influent AMD to treat, creating significant operational and economical obstacles.

Biological source treatment (BST) is a technique to address the AMD problem at the source by shielding acid-generating materials from being oxidized. The BST technique consists of the amendment of sulfate-reducing bacteria (SRB) in conjunction with selective substrates in the AMD source area. SRB in treating AMD effluent has been studied extensively (Canty, 1998; Chang et al., 2000; Dvorak et al., 1992; Elliot et al., 1998; Johnson and Hallberg, 2005; Johnson et al., 2002; Jong and Parry, 2003; Kim et al., 1999; Lyew et al., 1994; Machemer and Wildman, 1992; Tabak et al., 2003; Van Houten et al., 1994; Webb et al., 1998). Under anaerobic conditions, SRB can oxidize organic compounds and utilize sulfate as an electron acceptor. This process generates sulfide (S^{2-}) and consumes protons, which increases pH. The sulfide can combine with dissolved metals to form metal sulfide precipitates, eliminating metal toxicity in AMD. The mechanism is summarized in the following reaction:

$$2\ CH_3CHOHCOO^- + 3SO_4^{2-} + 2H^+ = 6HCO_3^- + 3H_2S \qquad \textbf{(1)}$$

However, these studies focus on SRB activities in wetlands, bioreactors or other designs targeting AMD effluent. Studies on using SRB at the source of AMD are limited to a few laboratory-scale projects (eg., Adams et al., 1995). In previous research, the BST technique relied on SRB to establish a biogeochemical formation on the surface of pyrite and other source minerals, shielding oxygen from oxidizing the materials and generating AMD (Fallgren and Jin, 2005; Jin et al., 2005). The instant effects of the reactions in the BST process increases pH of the AMD source. The products formed in the BST process also carry a long term effects in maintaining the pH in the neutral range, which is favored by SRB and other microbial populations. BST eliminates oxidation of AMD source materials from oxygen and water by formation of a film, mainly composed of microbial cells and extracellular polymers, on the metal sulfide surfaces (Christensen and Characklis, 1990; Jin et al., 2005). Recently, lipids have been detected and characterized on a pyrite surface, preventing pyrite from being oxidized (Zhang et al., 2003; and Zhang et al., 2006). Reductive minerals such as sulfide metals may be adsorbed to bacterial cell membranes or extracellular structures, and add to the "reductive barrier" function. The biogeochemical formation in the BST technique restricts diffusion of oxygen, creating an anaerobic barrier to oxidation (Characklis, 1990).

This study addressed both laboratory development of the BST technology and its application in a field pilot test. The laboratory studies were designed to understand the suitability of the technique under various conditions. These tests included determining the most effective dosage

of effluent/solid (ES) inoculum, monitoring the sulfate reducing activity, substrate screening to determine optimal, low-cost nutrient enhancements, determine the effectiveness of BST, and also determining the threshold pH at which the treatment could be applied (Fallgren et.al., 2005; Jin et al., 2005). The field pilot study was performed to validate the findings in the laboratory in a realistic setting. Results from the laboratory and field tests indicate that BST of AMD can be a long-term and cost-effective alternative to traditional treatment methods. Electromagnetic tools were also used to identify AMD sources and assist in post-treatment monitoring in the field.

Materials and Methods

The site under study was located on a closed mine site near Dunlap, TN. AMD water was collected from the location for analysis and for use in the laboratory studies. Samples were also taken from a nearby source of ES inoculum from a wastewater treatment plant (WWTP) in Dunlap, TN. This source was chosen because studies have reported that high numbers of SRB are present in ES (Lens et al., 1995; Manz et al., 1998; Schramm et al., 1999; Ingvorsen et al., 2003; Fallgren and Jin, 2005). All chemicals used for this study were reagent grade and purchased from Sigma-Aldrich (Milwaukee, WI), unless otherwise indicated. Dairy wastes (returned milk and spoiled ice cream) were obtained from a dairy farm in southern TN and as the substrate and nutrient source based on previous study (Fallgren and Jin 2005). Both ES and diary wastes were predetermined as pathogen free and permitted by the regulatory agency for this study.

Baseline characteristics were determined for the AMD and dairy wastes. Ion-selective electrodes were used to measure pH. Chloride (Cl^-), nitrate-nitrogen (NO_3^--N), and sulfate (SO_4^{2-}) were analyzed by ion chromatography on DIONEX DX-100 (Sunnyvale, CA). Ammonium-nitrogen (NH_4^+-N) was measured by indophenol blue colorimetric method on a Shimadzu UV-VIS spectrophotometer (Columbia, MD) by following the method described by Keeney and Nelson (1982). Dissolved organic carbon (DOC) was analyzed on a Shimadzu total organic carbon (TOC) analyzer (Columbia, MD). Sodium (Na), potassium (K), magnesium (Mg), and calcium (Ca) were measured by atomic absorption. Metals such as iron (Fe), arsenic (As), copper (Cu), nickel (Ni), and lead (Pb) were detected by inductively coupled plasma mass spectrometry. Biological activity reaction test (BART) tubes from HACH (Loveland, CO) were used to detect and quantify SRB populations in the samples.

Laboratory Studies.

Laboratory studies focused on microcosms constructed using triplicate 150-ml serum bottles under aerobic conditions. Each microcosm contained 100 ml of AMD water collected from the site and 1 g of crushed pyrite. All microcosms were incubated under room temperature (22 - 25^{o}C) in the dark.

ES Inoculum Dosage Test. The optimal amount of ES used as the SRB inoculum was determined by comparing different amounts of ES added to the microcosms containing adequate substrate (lactate). The amount of lactate added was based on stoichiometric calculations to deplete sulfate in the water sample solely through sulfate reduction. The amounts of ES inoculum applied were 0.1, 1.0, and 3.0 % wt of the volume of water sample. Sodium L-lactate was added to the final concentration of 1.0 % wt in all microcosms. Microcosms containing no substrate or inoculum served as controls. All microcosms were set up at an initial pH between

5.3 and 5.7. Sulfate-reducing activity was determined by the increased pH and the presence of metal sulfide precipitates.

Sulfate Reducing Activity Test. Sulfate reducing activities in the ES inoculum were monitored by measuring the changes in SO_4^{2-} concentration. A sulfidogenic medium containing a mineral salts solution (NaCl, $MgCl_2 \cdot 6H_2O$, KCl, $CaCl_2 \cdot H_2O$, and NH_4Cl), a phosphate buffer (KH_2PO_4 and K_2HPO_4) and a sulfate solution (Na_2SO_4; Colberg and Widdel, 1992) was used to selectively quantify sulfate reducing activity. The best-performing dosage, as determined by the previous test, was used in the microcosms. The same quantity of sulfate-reducing sediments from an anaerobic pond was used as an inoculum comparison. Control microcosms contained no inocula.

BST Tests. After successfully completing the ES inoculum dosage and sulfate reducing activity tests, additional microcosms were established to evaluate the overall effectiveness of BST. Based on a separate study on substrate screening, returned milk (dairy waste) was selected as the substrate (Jin, et al., 2005). Results from the sulfate reducing activity test indicated ES from an adjacent WWTP could successfully be used as an SRB inoculum.

In order to determine the effectiveness of various treatments, six microcosms were established. The suite of treatments including the microcosm contents are listed in Table 1. 100% stoichiometric dosage of substrate was determined based on the amount that can deplete sulfate solely through sulfate reduction. Different dosages of substrate were added into the corresponding treatments. Microcosms containing substrate only, ES inoculum only, and non-amendments microcosms served as controls. All microcosms were set up in triplicates and incubated in the dark under room temperature (22-25^{o}C) for 571 days. Aliquots from each microcosm was regularly collected and measured for pH.

Table 1. Treatments for Substrate Dosage Study.

Treatment ID	5% Stoichiometric Requirement Milk	100% Stoichiometric Requirement Milk	500% Stoichiometric Requirement Milk	3.0 % wt ES Inoculum
1				
2		■		
3				■
4	■			■
5		■		■
6			■	■

At the end the incubation period (571 days), pyrite was collected from the microcosms and observed under the scanning electronic microscopy (SEM). Structures formed on the surface of pyrite were photographed and measured.

Sulfate Reduction at Threshold pH Values. In order to determine the threshold pH at which BST could successfully be applied in raising and maintaining pH, a separate test was conducted. The same protocols as used in the BST tests as described above were applied in this study, except that initial pH values in the microcosms were adjusted by using sulfuric acid (H_2SO_4). The pH readings of the corresponding microcosms were adjusted to 4.0, 3.0, and 2.0. Microcosms were incubated for 226 days and pH was frequently measured in the aliquots collected from each microcosm.

SRB Activities in Other ES Sources. Preliminary screening indicates that SRB may ubiquitously exist in ES from various sources. ES samples were collected from WWTPs in Laramie,

Cheyenne (Wyoming), and Fort Collins (Colorado). Microcosms containing pyrite (3.0 % wt) and AMD water samples were established as described previously. These ES samples collected from different sources were inoculated and added to the microcosms. Returned milk was added at 1% wt final concentration in the microcosms as the substrate. AMD water collected from the Tennessee site was included as the control. Control microcosms were established at the same pH values as in other treatments, except that no ES or substrate was amended. The values of pH in the corresponding microcosms were pre-adjusted to 4.0, 3.0, 2.0, and no adjustment (2.8, control). BART methods (Hach, Colorado) were used to quantify SRB in the samples

Field Pilot Study. A field pilot study of BST in AMD treatment was initiated in a selected area on a closed mine site near Dunlap, Tennessee in January 2005. Monitoring work has been ongoing to evaluate the performance of the BST application in the study area.

Baseline Electromagnetic Survey. To select the pilot study area and BST injection locations, an airborne electromagnetic (EM) survey was performed at the mine site by Fugro Airborne Surveys (Houston, TX). Resolve 6 was used in the survey. It provides a six frequency system with horizontal coplanar coils capable of measuring the EM response at 400 Hz, 1500 Hz, 6400 Hz, 25000 Hz, 100000 Hz, and one coaxial coil pair at 3300 Hz (Fugro Airborne Surveys, 2006). The data was recorded from approximately 57 m with an electromagnetic sensor clearance of 30 m taking a reading every 0.1 seconds. The data from this survey provided a rapid assessment of the area and allowed for quick determination of possible AMD sources, although detailed information was not available due to the low accuracy settings of the survey. An area with existing monitoring wells and known discharge points was selected for the pilot study. Additional injection and monitoring wells were put in place to establish an injecting and monitoring network in the area (as shown in Fig. 1). Four-inch monitoring wells were installed to the bedrock. Wells were developed based on standard procedure before the injection. Groundwater level was measured before the pilot study started, upon the completion of well development until it was stabilized.

Figure 1. Field Application of BST.

Filed Pilot Study. After the placement of the injection and monitoring wells, ES inoculum and dairy wastes were injected gravimetrically. The dosages of ES inoculum and returned milk were precalculated based on the stoichiometric needs as described previously.

Pre-treatment sampling was conducted 1 week before the injection to establish a baseline. Post-injection groundwater samples were collected 2 weeks after the injection, upon stabilization of field parameters including pH, conductivity, and temperature. Groundwater samples were collected quarterly and analyzed in the laboratory for alkalinity, sulfide, sulfate and metals.

Field EM Survey. A field EM survey was conducted 6 months after the completion of the BST field application. Unlike the previous helicopter survey, this survey was conducted on the ground, focusing entirely on the area receiving treatments to evaluate the extent of the BST performance in the study area. Results from this survey provided increased resolution and details of the AMD profile changes in the treated area. This allowed for a more detailed conductivity map to be generated. This initial survey will serve as a benchmark for future ground surveys to demonstrate improvement in water quality in the testing area. Data obtained from a concurrent groundwater monitoring event were used to validate survey results.

Before the field EM survey began, a permanent grid was established to cover the pilot study area. Each point on the grid was a location where the conductivity of the ground was to be measured (in mS m^{-1}). The grid was made of seven lines in an approximate east-to-west fashion. Each line was separated by 30.5 m and each station on the line by 15.25 m. This resulted in a total of 150 measurement stations. Stations on the line were marked with wooden stakes and their locations were geospatially recorded using a Magellan (San Dimas, CA) Meridian Gold GPS.

With the grid in place, the optimal orientation and distance of the survey equipment best suited to map the AMD was determined. The instrument used was an EM34-3XL from Geonics Limited (Mississauga, Canada). Surveys were conducted in both the horizontal (vertical dipole) and vertical (horizontal dipole) orientations with coil separations of 10m (400 Hz), 20m (1600 Hz) or 40m (6400 Hz). This allowed for conductivity readings to be taken at different depths. Due to the variability in the instrument and depth to AMD from the surface, it was critical to run the survey in all six combinations (e.g. horizontal 10m and vertical 10m). It was also determined that the survey should be completed with the transmitting coil always to the east of the receiving coil, to make replication of the survey in the future simple.

Conductivity was measured at each station and recorded on an Allegro Cx data logger from Geonics Limited. At the end of each survey the data was uploaded to a computer for further processing. This entailed combining the conductivity data with the geospatial data of each station. This resulted in a file containing latitude (X), longitude (Y) and conductivity (Z). The conductivity (mS m^{-1}) was then converted to the standard unit of resistivity (ohm m^{-1}). The following equation (2) was used for this conversion:

$$\text{Resistivity (ohm m}^{-1}\text{)} = (1/\text{Conductivity (mS m}^{-1}\text{)}) \times 1000 \qquad \textbf{(2)}$$

The data was imported into the processing software, Surfer v. 6.04 Surface Mapping System by Golden Software, Inc. (Golden, CO). The grid method used to generate the contours in the map was Kriging, which was used because of its flexibility and its use of the data to look for global trends.

Results and Discussion

The baseline characterization of the samples are summarized in Tables 2 and 3.

Table 2. Baseline Parameters of the AMD Sample and Returned Milk

	pH	Cl^-, mg/l	NO_3-N, mg/l	SO_4^{2-}, mg/l	NH_4-N, mg/l	DOC, mg/l
AMD	5.14	3.58	< 0.10	1916.99	15.05	Not Detected
Returned Milk	4.51	1000.00	< 0.10	673.90	7.10	17510.00

Table 3. Baseline Cations and Metals in the AMD Sample and Returned Milk

	Na, mg/l	K, mg/l	Mg, mg/l	Ca, mg/l	Fe, mg/l	As, μg/l	Cu, μg/l	Ni, μg/l	Pb, μg/l
AMD	12.40	16.40	150.00	151.00	230.00	9.40	1.70	993.00	1.20
Returned Milk	480.00	137.00	100.00	1040.00	<10.00	<100.00	300.00	<100.00	100.00

Organic content in the AMD water was under the detection limit (0.08 mg/L). Ammonium was detected in the water at 15.5 mg/l, which is within the stoichiometric concentration range of supporting microbial activities based on the carbon availability. The water had a pH of 5.14, containing cations, metals and sulfate at elevated concentrations as expected. Returned milk is rich in dissolved organic carbon (DOC). Although it has a pH reading at 4.51, previous studies indicate that the pH factor can be rapidly eliminated by the enhanced sulfate reducing activity.

The ES inoculum dosage test indicates that an inoculation at 3.0 % wt is optimal to increase and sustain the pH (Fig. 2) within the neutral range. No lag time was observed in pH changes. Non-amended microcosms show a decline in pH to 2.5 within 35 days. The value of pH in microcosms amended with only substrate also decreased to 4.6. Microcosms amended with ES inoculum at the concentration of 0.1 % wt showed no significant pH change during the first 21 days, and pH decreased to 3.1 during the following 14 days, presumably due to the inadequacy of the innoculum. Microcosms amended with ES inoculum at the concentration of 1.0 % wt increased in pH to 7.1, but decreased to an acidic range after 14 days. This suggests that a certain dosage of ES inoculum inoculation is important to raise and maintain the pH in the neutral range in the AMD microcosms, though we anticipate the dosage to be site and inoculum specific.

Results from the sulfate reducing activity test demonstrate that the concentration of sulfate decreased 25 % in the ES inoculum-amended microcosms within 30 days (Fig. 3). This change is comparable to that in the pond sediment-amended microcosms, in which the concentration of sulfate decreased 21% during the same incubation period. Sulfate concentration in the control did not change observably, suggesting that sulfate reducing activity is responsible for the consumption of sulfate in the microcosms.

The results from the comprehensive BST microcosm study are summarized in Figure 3. Values of pH declined during the initial 7 days of incubation, probably due to the insufficiency of sulfate-reducing activities and the short term influence of acidity from the substrate at the establishment of the microcosms. Microcosms amended with the 100% and 500% stoichiometric amounts of substrate showed an increase in pH after 7 days and reached the neutral range of pH within approximately 3 months. During the extended incubation of the

microcosms, the values of pH in the substrate-only controls decreased to 3.3. As shown in Fig. 4, pH in the treatment samples increased to the neutral range in microcosms amended with ES inoculum and substrate (stoichiometric required amount and five times stoichiometric required amount) stabilized within the neutral pH range for 571 days of incubation.

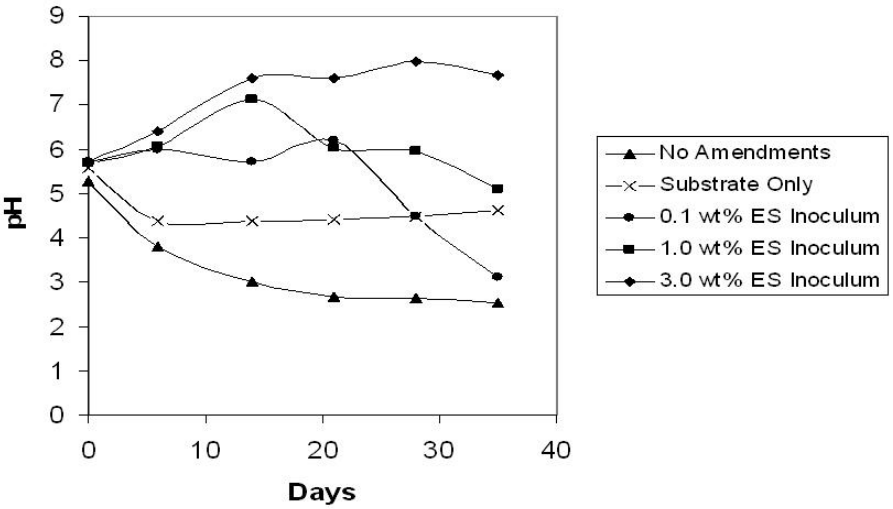


Figure 2. Dosages of ES Inoculum Inoculation in AMD Samples.

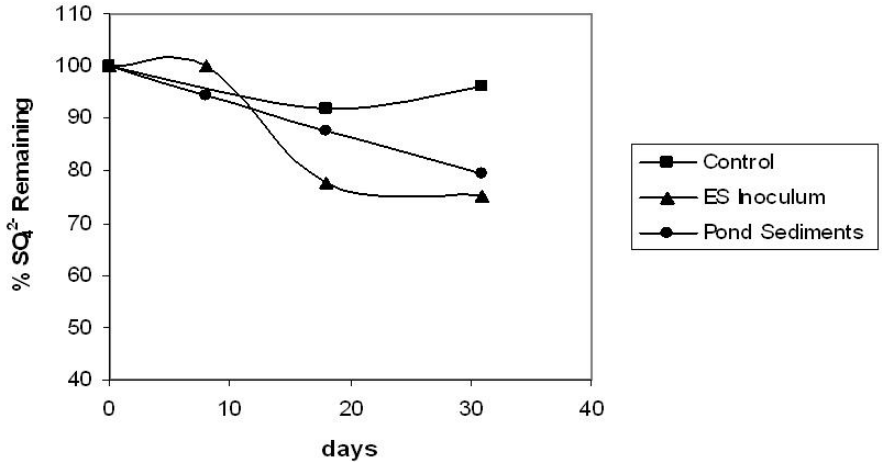


Figure 3. Sulfate-reducing Activities in the Microcosms

These findings suggest that the stoichiometric dosage of substrate is required to stimulate microbial growth and overcome the metal sulfide oxidation rate (AMD generation).

Results from the test of pH threshold vs SRB activity are summarized in Figure 5. Within 40 days of incubation, the pH increased to 6.6 in microcosms with pH pre-adjusted to 4.0. This pH was maintained during the remaining 186 days of incubation. Similarly, in microcosms with pH

pre-adjusted to 3.0, the pH increased to 6.1 within 40 days, and stabilized for the remaining duration of this study. In comparison, pH in the microcosms with pre-adjusted pH at 2.0 took 113 days to rise to 6.1, and stabilized for the remainder of the study. Results demonstrate that the SRB in the ES inoculum from the Dunlap location can tolerate pH as low as 2.0 without losing its metabolic activities, raising pH and maintaining it in the neutral range. This finding indicates that the ES inoculum may be an ideal SRB source to treat AMD in the field site, which typically has a pH from 3.0 to 5.0. It is also obvious that the higher initial pH is in favor of SRB activities, indicated by a shorter lag time shown.

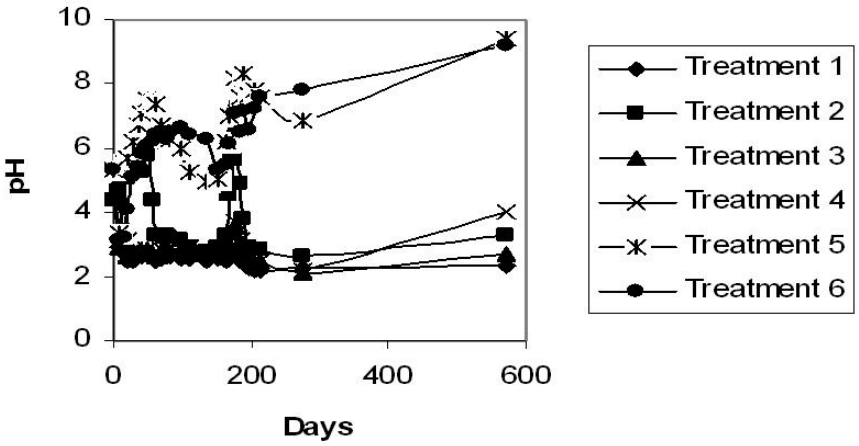


Figure 4. Stabilization of pH in BST Microcosms

As summarized in Table 4, results indicate that pH of the ES from other WWTPs were above 5.4, which is not expected to adversely affect the lower pH in the injecting locations. Ammonium is present at elevated concentrations in most ES, providing an essential nutrient for SRB activities.

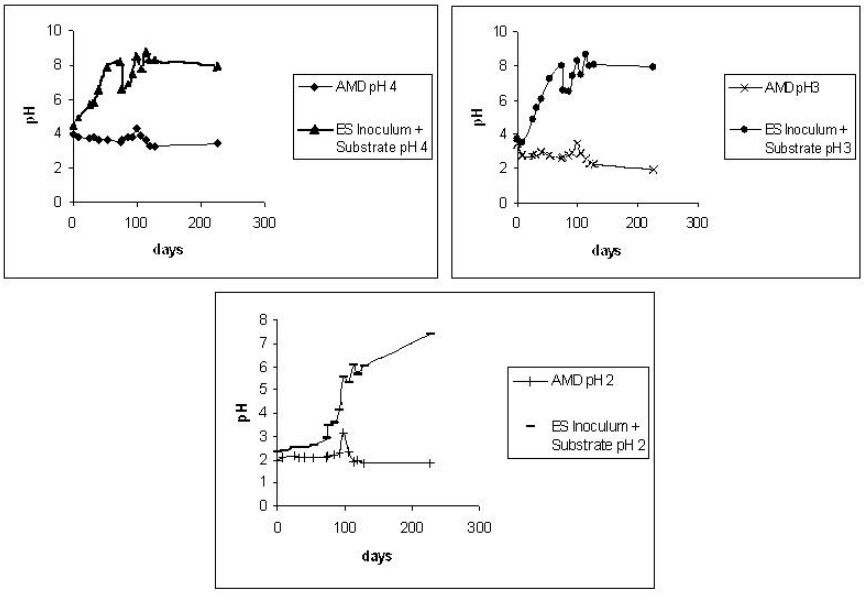


Figure 5. Sulfate Reduction in ES inoculum (Dunlap, TN) at different pH.

Table 4. Characterization of ES Inoculum.

Source Location	pH	NH4, mg/l	NO3, mg/l	SO4, mg/l	F, mg/l	Cl, mg/l	Br, mg/l	PO4, mg/l
1. ES Inoculum Laramie, WY	6.69	7.23	<0.10	1004.21	<0.10	113.83	<0.10	<0.10
2. ES Inoculum Cheyenne, WY	7.49	44.41	<0.10	<0.10	<0.10	203.55	<0.10	<0.10
3. ES Inoculum Fort Collins, CO	5.40	1291.72	62.87	63.74	<0.10	1085.51	<0.10	<0.10

Except for ES inoculum from Laramie (359,000 cfu SRB/ml), approximately 700,000 cfu SRB/ml were detected in ES inoculum samples from Dunlap, Cheyenne, and Fort Collins, indicating a ubiquitous existence of a high population of SRB in ES inoculum. ES inoculum from WWTPs appears to be a valid source for SRB used for AMD treatment.

The changes in pH values during the 88-day incubation period are summarized in Fig. 6. The pH readings rose into the neutral range (>6.5) in all microcosms amended with ES inoculum and returned milk substrate. The pH in the controls lagged in the acidic range. Surprisingly, initial pH does not seem to influence the rate of pH increasing in the microcosms amended with ES inoculum and milk. The pH in all amended microcosms increased into the neutral range within 40 days. Results from this study indicate that ES inoculum from different sources not only provide high populations of SRB, but also maintain the aggressive sulfate reducing activity that is tolerant to pH as low as 2.0.

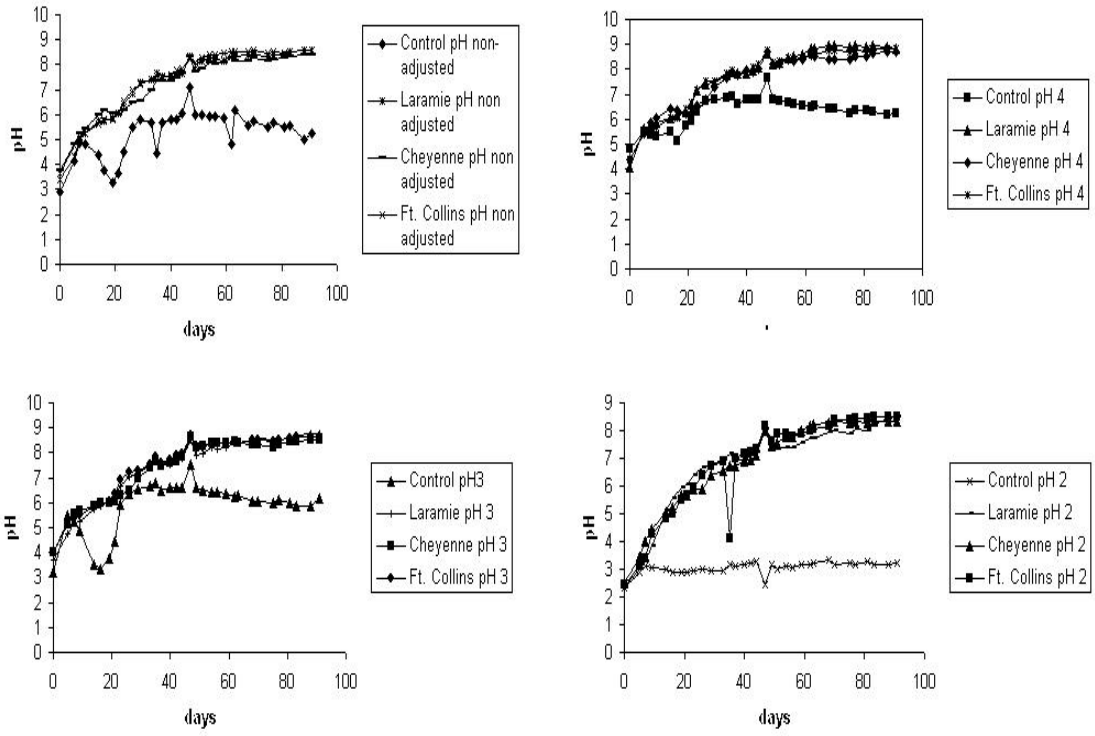


Figure 6. Effects of ES Inoculum from Different Sources on AMD Treatment.

In the study to confirm the existence of a barrier over the source material, a rich layer of biochemical film-like structure is observed on the pyrite surface. This material was analyzed by SEM and determined to be a mixture of biological and inorganic layered structure attached on the pyrite surface, as shown in Fig. 7. It is believed that this film structure serves as a barrier to oxygen and oxidation of pyrite. The impermeability and the reducing potential of this film may keep pyrite under the reduced condition; therefore eliminating the source of AMD generation. The integrity and longevity of this film apparently is important to the effectiveness of using the BST technique in AMD treatment. Data from our study has demonstrated that the film is stable and effective for an extended period of time after one application of BST. However, understanding of this structure is still lacking. Further studies to characterize the structure, its constitution and functions are warranted.

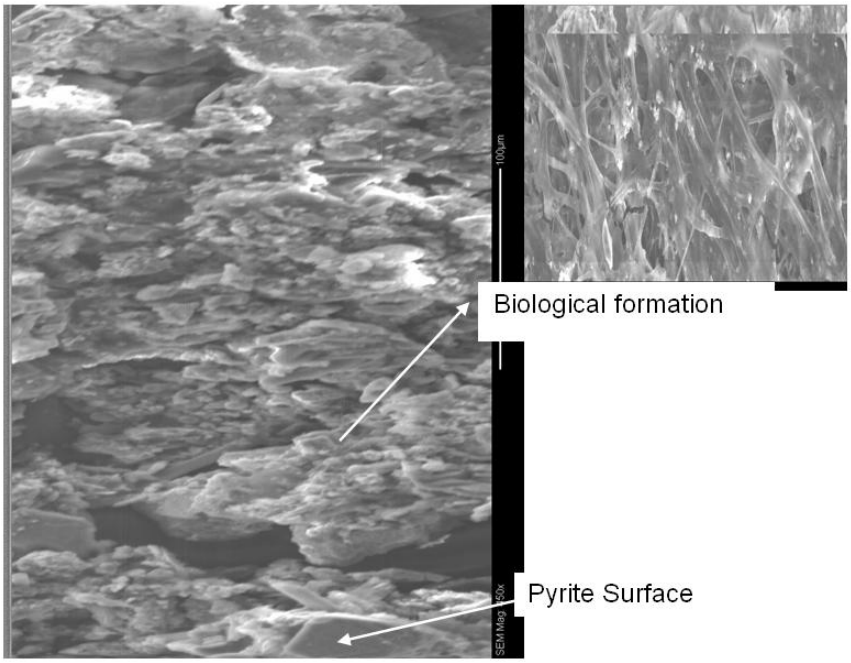
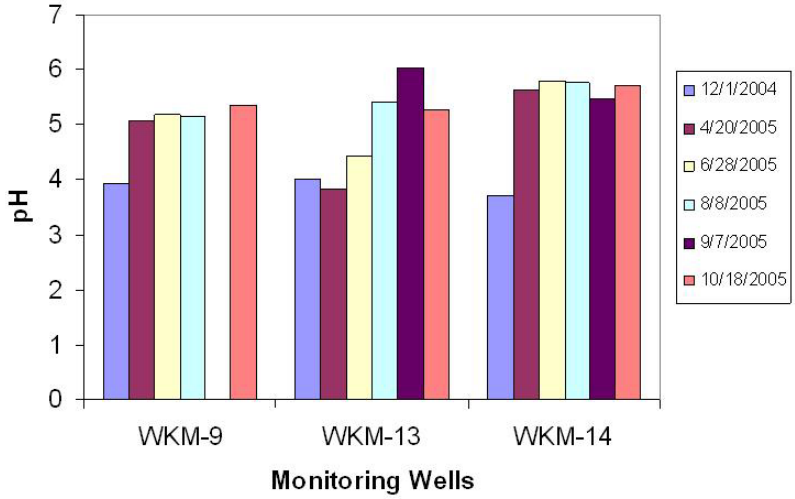
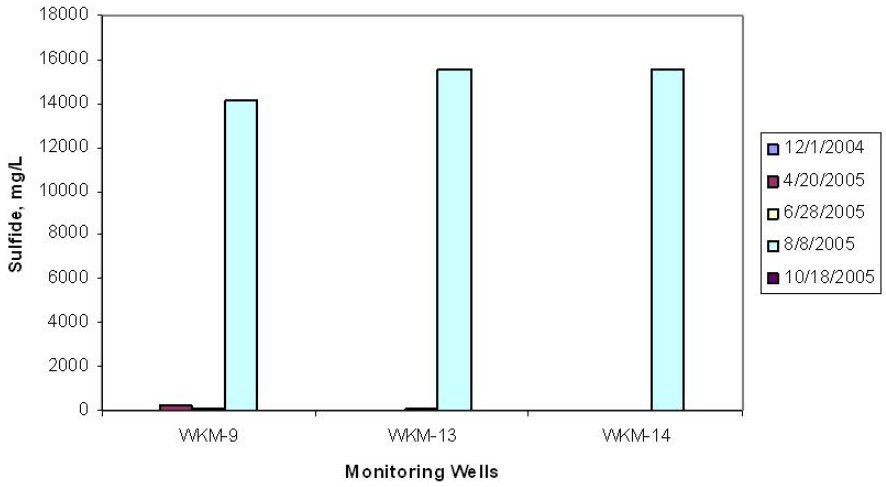


Figure 7. Biochemical Film Structure on AMD Source.

Field Pilot Study

Filed Pilot Application and Monitoring of BST. Values of pH measured in three monitoring wells (WKM9, 13 and 14) during the pre-injection and four post-injection monitoring events are presented in Fig. 8. In groundwater samples collected from these monitoring wells, pH increased by approximately 2 units into the pH 6.0 range (Fig. 8a). This increase in pH translates into a 100 magnitude decrease of acidity during the post treatment period. Correspondingly, S^{2-} concentration increased dramatically during the post treatment monitoring (see 8/8/05 data shown in Fig. 8b), supporting the observation of SO_4^{2-} reduction activity. A decline in S^{2-} concentration was observed in the most recent sampling event, presumably due to the precipitation of S^{2-} by metals in the groundwater (Figure 8b). This is consistent with the observation that a layer of bio-geochemical film has been formed on the sediment surface.





Figures 8a & 8b. Values of pH and S^{2-} in the Pilot Study Monitoring.

The rates of pH increase are slower than those observed in the laboratory study. Field heterogeneity and the dilution of the injected substrate and inoculum may be attributed to the lag. The inflow of fresh acid water from upgradient locations may also compromise the level of pH increasing in the pilot study area. This trend can be observed in the pH values summarized in Fig. 9, in which pH values in the downgradient locations tend to be increasing and stabilizing, unlike those in the locations upgradient of the injecting points. The plateau in pH trends in monitoring wells WKM-13 and 14 also reflect this factor. This factor can be eliminated by additional substrate amendments to the groundwater, so that the SRB activity can be enhanced to counter the pH decline. Another injection is proposed to enhance the SRB activities and offset the adverse influence from the upgradient acidic water flowing into the study area.

Existing data indicate that the influence of the injection on the downgradient locations is positive. Values of pH in well WKM-9 have been increasing. Further monitoring is ongoing to determine the trend of pH in this area.

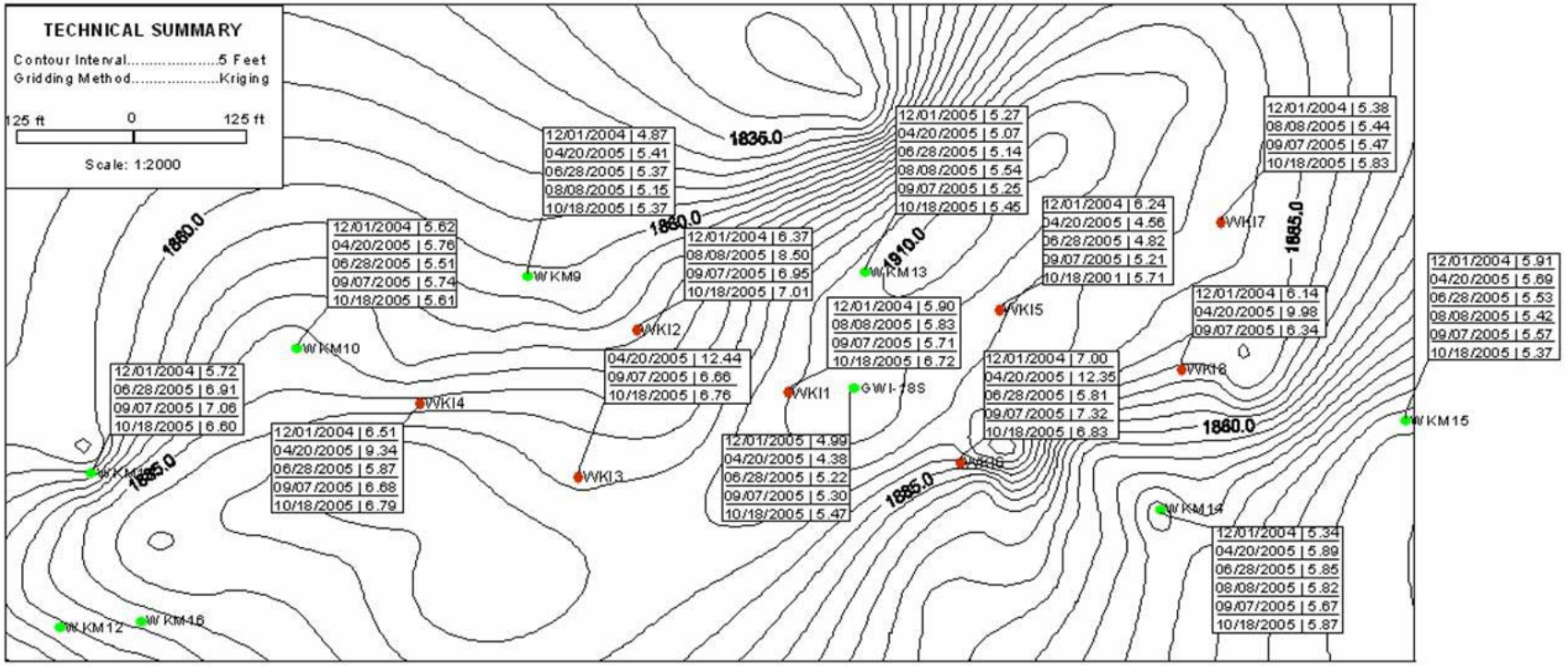


Figure 9. Summary of pH Values during the Post-injection Monitoring Events in the Pilot Study Area.

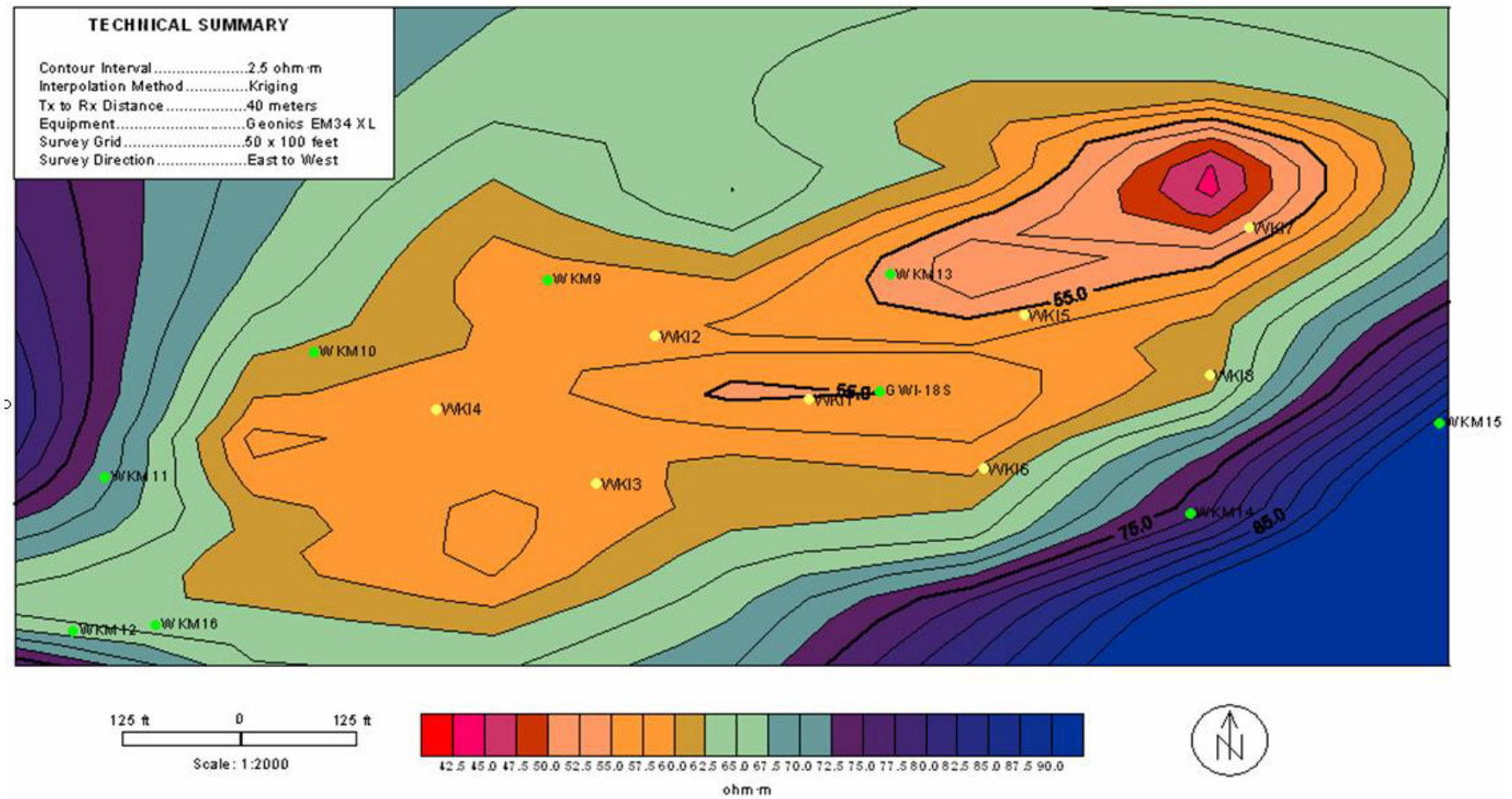


Figure 10. Contour map of apparent resistivity from 400 Hz vertical planar survey data.

Field EM Survey. All of the EM surveys generally correspond with the pH data obtained during the concurrent monitoring event. Additionally, observations that surface water in ponds to the northwest of the pilot study site were affected by AMD, indicating that they were down gradient from the source, were apparent on the EM surveys. However, it was found that the survey conducted using the longest, deepest penetrating wavelength (400 Hz) corresponded best to well data and field observations. Data from this survey are shown in Fig. 10. A radius of post-injection influence in the testing area is apparent in the contour map (Fig. 10). This evidence suggests that the EM method can be deployed as a non-invasive, efficient, above-ground screening tool to evaluate the performance of BST and that the influence of the BST extends beyond the immediate vicinity of the injection wells.

Conclusions

Results from the laboratory study conclusively demonstrate that the BST technique is effective in treating AMD. The data from the field pilot study indicate that the BST application has been successful to date in raising the pH of the AMD source areas, although further monitoring is still ongoing.

Readings of pH from wells down-gradient from the injection locations have demonstrated the effectiveness of BST in treating AMD at its source. The pH values have been increasing with time, indicating the sustaining SRB activity and the resulting elimination of AMD. Studies on ES inoculum from various sources show that SRB exists and carries the aggressive sulfate reducing activities when inoculated into the AMD source. Direct observation of the biochemical formation on the surface of AMD source materials supports our initial hypothesis that the formation of the "bio-barrier" plays a key role in "masking" the AMD source and eliminates the AMD generation from the source.

The deployment of BST process at sites with identifiable sources and accessibility was elaborated in this paper. Alternatively, the application of BST may be achieved through inflow water or installation of an in situ "biowall" that intercept the AMD effluent downstream, if the sources can not be pinpointed or site accessibility is limited. This mechanism of "masking" the AMD source eliminates the conventional passive treatment processes that have to be operated continuously. Data from the field study as of today support the laboratory results in demonstrating BST as an innovative, effective and economical technology in AMD remediation. Laboratory studies clearly show the long-lasting effectiveness of the BST process, although extensive field monitoring of wells and periodic EM surveys are warranted to offer adequate data to appraise the longevity and performance of this technology.

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