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Technical Article

Anaerobic Bioremediation of Acid Mine Drainage using Emulsified Soybean Oil

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Abstract. Batch incubation and flow-through column experiments were conducted to evaluate the use of emulsified soybean oil for in situ treatment of acid mine drainage. Addition of soybean oil, soluble substrates, and a microbial inoculum to the batch incubations resulted in complete depletion of SO_4, 50% reduction in Fe, and an increase in pH to >6. A one time injection of emulsified soybean oil, lactate, yeast extract, and a microbial inoculum stimulated SO_4 and metal ion reduction for ≈300 days in laboratory columns packed with mine tailings receiving influent solutions with a pH ≈3 and ≈5. In all emulsion treated columns, SO_4 and Fe were reduced, pH increased to >6, and Al, Cu and Zn removal efficiency was 99% or greater. Cu, Fe, Mn and Zn were removed as metal sulfides and/or carbonates with removal efficiency decreasing with increasing metal sulfide solubility. The low pH and high heavy metals concentrations did not significantly inhibit biological activity. However, SO_4 removal with associated precipitation of metal sulfides may have been limited by the short hydraulic retention time (6-7 days) of the columns. There was a significant hydraulic conductivity loss in one of the four treated columns, indicating that hydraulic conductivity loss may be an issue under certain conditions.

Key Words: biological sulfate removal; sulfide production; metal sulfide precipitation

Introduction

Acid mine drainage (AMD) is an enormous economic and environmental problem. In the United States, there are thousands of active mines, and between 100,000 and 500,000 abandoned mines (U.S. EPA 1995). There are more than 50 mine sites on the Superfund National Priorities list and over 890,000 ha (2.2 million acres) of polluted surface water resulting from mining operations in just eight states (MSE 2000). In Pennsylvania alone, costs for control of AMD from former coal mines are estimated to be over $15 billion (PA DEP 2005).

Effective control technologies are needed to manage this tremendous environmental and economic problem. However many sites are very large and located in remote areas. Control technologies must be low-cost, simple to implement, and require little maintenance. Anaerobic bioremediation processes can be used to reduce sulfate (SO_4) and immobilize heavy metals in the subsurface if a carbon and energy source is available to drive SO_4 reduction. Hydrogen and low molecular weight organic acids can be produced by the fermentation of a variety of organic substrates including compost, manure, sugars, oils, and organic rich sediments. Naturally-occurring SO_4 reducing bacteria (SRB) use the hydrogen (or organic acids) as an electron donor and SO_4 as an electron acceptor, consuming H^+ and producing hydrogen sulfide (H_2S) in the following reaction:

$$2\,H^+ + SO_4^{2-} + 4\,H_2 \rightarrow H_2S + 4\,H_2O$$

Sulfide produced in this reaction will react with a variety of metals ions (e.g. Fe, Ni, Zn, Cd, Pb, Cu, Hg), forming less soluble precipitates. Three general approaches have been use to bring biodegradable organic carbon into contact with the AMD to stimulate SO_4 reduction: (1) engineered bioreactors; (2) treatment wetlands; and (3) permeable reactive barriers.

Engineered bioreactors have been successfully used to treat AMD using dissolved and solid substrates. Dissolved substrate reactors are continuously fed a readily biodegradable aqueous substrate (Hammack et al. 1998; Quan et al. 2003; Tsukamoto et al. 2004) that supports sulfate reduction with associated precipitation of metals as low solubility metal sulfides. Solid substrate systems typically contain compost, manure, saw dust or other solid materials that slowly hydrolyze over time releasing low molecular weight fatty acids and H_2 that also support sulfate reduction (Chang et al 2000; Cheong et al 1998; Drury 1999; Dvorak et al. 1992). Use of soluble substrates can result in high removal efficiencies with hydraulic retention times (HRTs) between 6 and 12 hours. Solid substrate bioreactors could have lower operating costs due to the low cost of the organic substrate supplied. However, HRTs for these systems may need to be much higher (5-20 days) to provide sufficient time for hydrolysis of the complex organics (Chang et al 2000; Cheong et al 1998; Drury 1999; Dvorak et al. 1992). Both soluble and solid substrate systems can achieve high sulfate and metal removal efficiencies under ideal conditions.

However, soluble substrate systems require significant process control. Solid substrate systems may be simpler to operate, but removal efficiencies can decline with time due to changes in composition of the solid substrate.

Anaerobic wetlands can also be used effectively for AMD treatment (Skousen et al. 1998). In these systems, the mine water is passed through a subsurface treatment zone with large amounts of organic material from decaying vegetation or biomass introduced during wetland construction. In subsurface, water-saturated conditions, naturally occurring bacteria quickly deplete the available oxygen and then use Fe(III) and SO_4 as alternative electron acceptors for respiration. During this process, protons are consumed (increasing pH) and sulfide is produced, causing Fe and toxic heavy metals to precipitate. Over 400 wetlands have been constructed to treat AMD, with this approach being most popular in the eastern U.S. (Vile and Wieder 1993).

Permeable reactive barriers (PRBs) for AMD treatment are based on a similar approach. In the PRB process, a trench is excavated across an AMD plume and backfilled with a biodegradable organic material (typically compost, manure, etc.) and a pH buffer (typically limestone). The organic material provides a carbon source to stimulate reduction of Fe and SO_4 with a resulting increase in pH and immobilization of heavy metals. Extensive laboratory, pilot-scale, and full-scale demonstrations have shown that this approach can be very effective in controlling AMD (Ludwig et al. 2002; Waybrant et al. 2002).

In this paper, we report on a new approach for in-situ treatment using emulsified edible oils. In this process, an oil-in-water emulsion is injected into the treatment zone to provide a carbon and energy source for anaerobic bacteria. A concentrated emulsion can be prepared at an off-site manufacturing facility from an edible oil (soybean oil), edible surfactants, and other more easily biodegraded soluble substrates. The soybean oil provides a slow release organic substrate to support long-term anaerobic activity. The easily biodegradable substrates generate rapid, initial growth of the required bacteria. High energy mixers are used to generate small, uniform size oil droplets (typically ≈ 1 μm) for easy distribution in the subsurface. The emulsion concentrate is diluted with water on-site using a simple proportional feed system and injected into the subsurface to form a PRB where the oil droplets are immobilized on the sediment and rock surfaces. Methods for distributing edible oil emulsions in saturated sediments have been presented by Coulibaly and Borden (2004), Coulibaly et al. (accepted), and Jung et al. (accepted).

Figure 1 illustrates one approach for treating AMD seeps discharging from a tailings impoundment. Emulsified oil would be injected through a row of temporary injection wells or direct push points located a short distance up-gradient of the pond embankment. Since the oil droplets are roughly the size of a single bacterial cell, they can migrate considerable distances through most sediments. However, the oil droplets will stick when they collide with sediment surfaces. Typically, most oil droplets are immobilized on the sediment within a few days after emulsion injection. Over the first 50 to 100 days, the soybean oil (a triglyceride) is expected to hydrolyze, releasing glycerol (an alcohol) and long-chain fatty acids (LCFAs). Glycerol is very soluble and relatively easy to biodegrade, so this material will be quickly consumed by SRB and other anaerobic microorganisms. The LCFAs are much less soluble in water. We hypothesize that most LCFAs initially sorb to sediment surfaces, and then are slowly fermented via beta oxidation, releasing acetate and H_2. The acetate and H_2 is then used for sulfate and/or metals reduction. As AMD migrates through the emulsion treated zone, the acetate and H_2 are used to reduce SO_4 to sulfide, precipitating heavy metals within the tailings pile. In this approach, the SO_4 removal rate will likely be controlled by the rate of LCFA fermentation to acetate and H_2. Treatment efficiency could be controlled by injecting more emulsion to form a larger treatment zone with a longer hydraulic residence time (HRT) for LCFA fermentation. Costs for this approach are expected to be lower than other alternatives since there are no above-ground facilities to construct. Metals sulfide precipitates would be deposited within the tailings pile so there would not be any residuals requiring disposal. In addition, emulsified oils can be effectively distributed to virtually any location that can be reached by a drill rig, including both unconsolidated material and fractured rock. In this work, we evaluated the use of soybean oil emulsions for in situ bioremediation of AMD in batch incubations and flow-through columns packed with mine tailings.

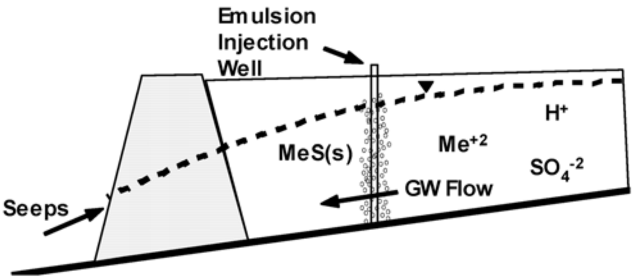

Figure 1. Injection of emulsified edible oil into a tailings impoundment, forming a permeable reactive barrier, to stimulate sulfate (SO_4^{-2}) reduction and precipitation of metal sulfides (MeS(s))

Methods

An initial series of incubations was conducted to evaluate the ability of soybean oil to stimulate Fe and SO_4 reduction under the harsh environmental conditions typical of AMD. Batch incubations for each treatment were constructed in triplicate in 240 mL serum bottles fitted with thick rubber stoppers and aluminum crimp seals to exclude oxygen. Each bottle contained 190 mL of 20 mM $FeSO_4$ solution titrated to pH 3.0 with H_2SO_4, 25 mL mine spoils, the organic substrate to be evaluated, and a bacterial inoculum from a successive alkalinity producing system (SAPS) pond in Pennsylvania. The mine spoils were obtained from a former coal mine in Sequatchie Valley, TN and were passed through a No. 4 standard size sieve to remove larger material prior to use. Incubations were monitored with time for changes in pH, SO_4, and Fe.

Saturated, flow-through column experiments were then conducted to evaluate the potential for AMD treatment in PRBs generated with edible oil emulsions. PVC columns (30 cm by 4.5 cm dia.) were wet packed with sieved mine spoils and compacted with a rubber tamp to limit entrapped air. Synthetic AMD was intermittently pumped upward through the columns at a flow rate of 20 mL per day, resulting in an average HRT of 6-7 days. The HRT was estimated based on the total porosity (40 - 44%). After allowing the columns to equilibrate with the AMD influent, four of the columns (2 acidic and 2 neutralized) received a one-time treatment of 50 mL of emulsion, then 3 mL of inoculum from the batch incubations, followed by an additional 50 mL of emulsion. The emulsion was prepared diluting 33% EOS® concentrate with 67% neutralized column influent. One acidic and one neutralized column remained untreated as controls.

EOS® is a proprietary organic substrate containing emulsified soybean oil (60%), food-grade surfactants (10%), lactate (4%), and yeast extract (2%) and is prepared using high energy mixing to generate small, uniform oil droplets (≈1 µm diameter) with a negative surface charge to reduce droplet capture by sediment surfaces (EOS Remediation, Raleigh, NC). Since the oil droplets are already dispersed in a continuous water phase, the emulsion is very easy to disperse in water and can be mixed in the field with a simple proportional feed system. The EOS®/neutralized AMD mixture had a pH≈5 due to the lactic acid present in the EOS®.

The influent and effluent from each column were periodically monitored for pH, SO_4, H_2S, acetate, total carbon (TC), inorganic carbon (IC), and dissolved metals [Al, Cd, Cr, Cu, Fe, Pb, Mn, and Zn]. Cd, Cr, and Pb were below detection in all samples. We monitored pH with a standard probe and meter. H_2S was periodically monitored using CHEMetrics colorimetric ampoules with a detection limit of 0.1 mg/L. SO_4 and acetate were analyzed by ion chromatography using a Dionex 2010i ion chromatograph and suppressed conductivity detector. TC and IC were analyzed using Shimadzu 5000A or Rosemount Dohrmann DC-190 TOC analyzers following manufacturers' instructions. Dissolved metals samples were passed through a 0.45 µm syringe filter immediately after collection and then diluted 9:1 with 0.1 N HCl as a preservative prior to analysis. Dissolved metals analyses were performed on a Perkin Elmer Plasma II Ion Coupled Plasma Argon Emission Spectrometer (ICP-AES) with an effective quantitation limit of 0.05 mg/L. After 10 months of operation, the columns were taken apart and analyzed for carbon content using a CHN elemental analyzer to estimate the remaining amount of organic substrate.

Batch Incubation Results

An initial series of batch incubations was conducted to evaluate the ability of soybean oil to stimulate SO_4 reduction under the harsh environmental conditions typical of AMD. Experimental treatments included:

- Autoclaved (killed) control
- Live control (100 mg/L $(NH_4)_2HPO_4$ and 25 mL inoculum with no added carbon)
- Soluble substrate (100 mg/L $(NH_4)_2HPO_4$, 25 mL inoculum, 200 mg/L molasses, and 200 mg/L yeast extract)
- Soybean oil (100 mg/L $(NH_4)_2HPO_4$, 25 mL inoculum, 200 mg/L molasses, 200 mg/L yeast extract, and 2500 mg/L liquid soybean oil

Figure 2 shows the observed variation in SO_4, Fe, and pH in the different batch incubations. Values shown are the average of triplicate incubations. In comparison to the killed and live controls, the soluble substrate treatment resulted in a moderate increase in pH, a small decrease in SO_4, and no significant change in Fe. While SO_4 removal was only about 320 mg/L, this is more than sufficient to neutralize the acidity present as H^+. The limited SO_4 removal in the soluble substrate treatment was due to the small amount of substrate provided. At the start of these incubations, the theoretical chemical oxygen demand (COD') to sulfate ratio was 0.22, which is considerably below the theoretical requirement for reduction of SO_4 to H_2S.

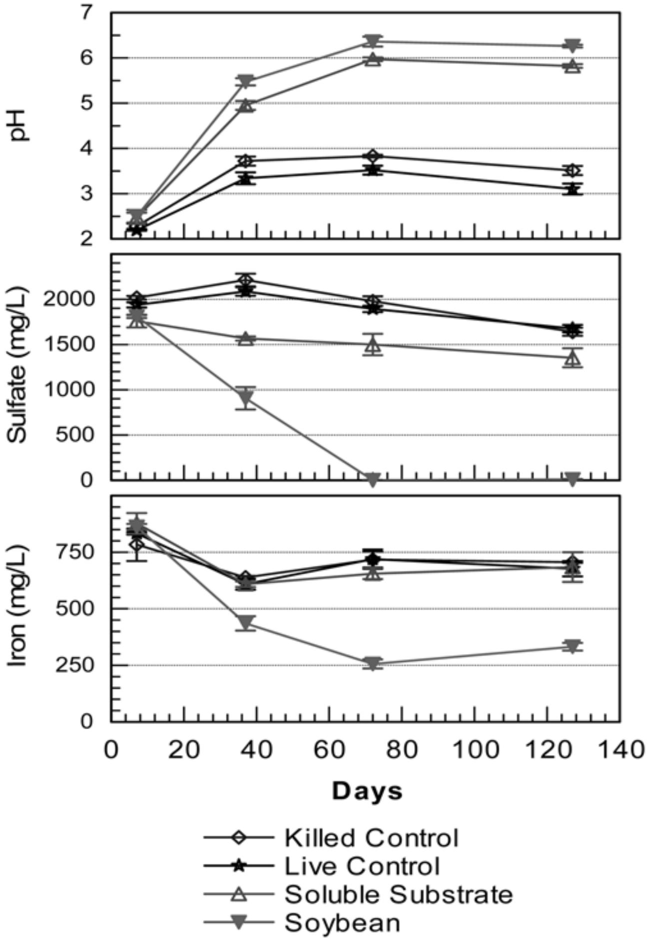

Figure 2. Variation in pH, dissolved sulfate, and iron in batch incubations; error bars are ±1 standard deviation of triplicate incubations

In the soybean oil treatment, pH increased to >6, SO_4 was depleted, and Fe decreased by ≈50%. The more extensive removal in the soybean oil treatment was due to the much greater amount of organic substrate available for biodegradation (initial COD'/SO_4 = 3.8). This preliminary screening study indicates that liquid soybean oil could potentially be used to simulate Fe and SO_4 reduction, even under the low pH conditions commonly associated with AMD.

Column Effluent Results

To evaluate the potential for in situ treatment of AMD, six columns were packed with coal mine spoils similar to the material used to construct the batch incubations. Influent solutions designed to represent acidic AMD (20 mM $FeSO_4$ acidified to pH ≈3) and partially neutralized AMD (20 mM $FeSO_4$ buffered to pH ≈5 with $NaHCO_3$) was intermittently pumped through each column at a flow rate of 20 mL per day, resulting in an average HRT of 6 to 7 days. This HRT is comparable to retention times in solid substrate bioreactors (Chang et al 2000; Cheong et al 1998; Drury 1999; Dvorak et al. 1992). After allowing the columns to equilibrate with the AMD influent, four of the columns (2 acidic and 2 neutralized) received a one-time treatment of a commercially available emulsified oil product (EOS®) and a microbial inoculum. One acidic and one neutralized column remained untreated as no added carbon controls.

Figure 3 shows results from the neutralized (N1) and acidic (A1) control columns. During passage through the neutralized control column, there was little change in Fe or SO_4 levels and very little inorganic carbon (IC) or organic carbon (OC) was released. However, there was a 1 - 2 pH unit drop during passage through the neutralized control column with significant leaching of Al, Cu, Mn, and Zn from the coal mine spoils. The decrease in pH was likely associated with oxidation of metal sulfides in the mine spoils by oxygen present in the column influent. SO_4, Fe, pH, IC, and OC levels in the acid control column effluent were similar to the influent.

Monitoring results from the neutralized (N2) and acidic (A2) columns amended with emulsified soybean oil are shown in Figure 4. In the neutralized emulsion amended column, pH increased from ≈5 to just over 6, with a concurrent reduction in Fe and SO_4. Cu and Zn were below detection (< 0.01 mM) in the effluent. Sulfide was only monitored occasionally. However, when samples were analyzed, H_2S was consistently below the analytical detection limit of 0.002 mM (0.1 mg/L) (data not shown). In addition, there was no detectable sulfide odor in gas released from the column. Mn was higher in the column effluent than in the influent, presumably due to the relatively high aqueous solubility of MnS at pH ≈6. OC concentrations were relatively high over the first 100 days and then stabilized at 10-30 mg/L. IC concentrations in the effluent were low throughout most of the experiment, possibly due to precipitation of metal carbonates and/or degassing of CO_2 during sample collection. The emulsion treatment resulted in beneficial changes in pH and SO_4, with an Fe removal efficiency of 50-75%. SO_4 removal efficiency was highest shortly after emulsion injection when OC concentrations were highest, and then gradually declined as effluent OC levels decreased, indicating SO_4 reduction to sulfide was limited by the amount of dissolved organic carbon. Fe concentrations in the column effluent closely correlated with SO_4, suggesting that Fe removal was limited by sulfide production. After 300 days of operation, there was evidence of reduced biological activity, presumably due to depletion of the available substrate. Methane (CH_4) levels remained low throughout the experiment (<0.1 mg/L), indicating substrate consumption by methanogens was not significant.

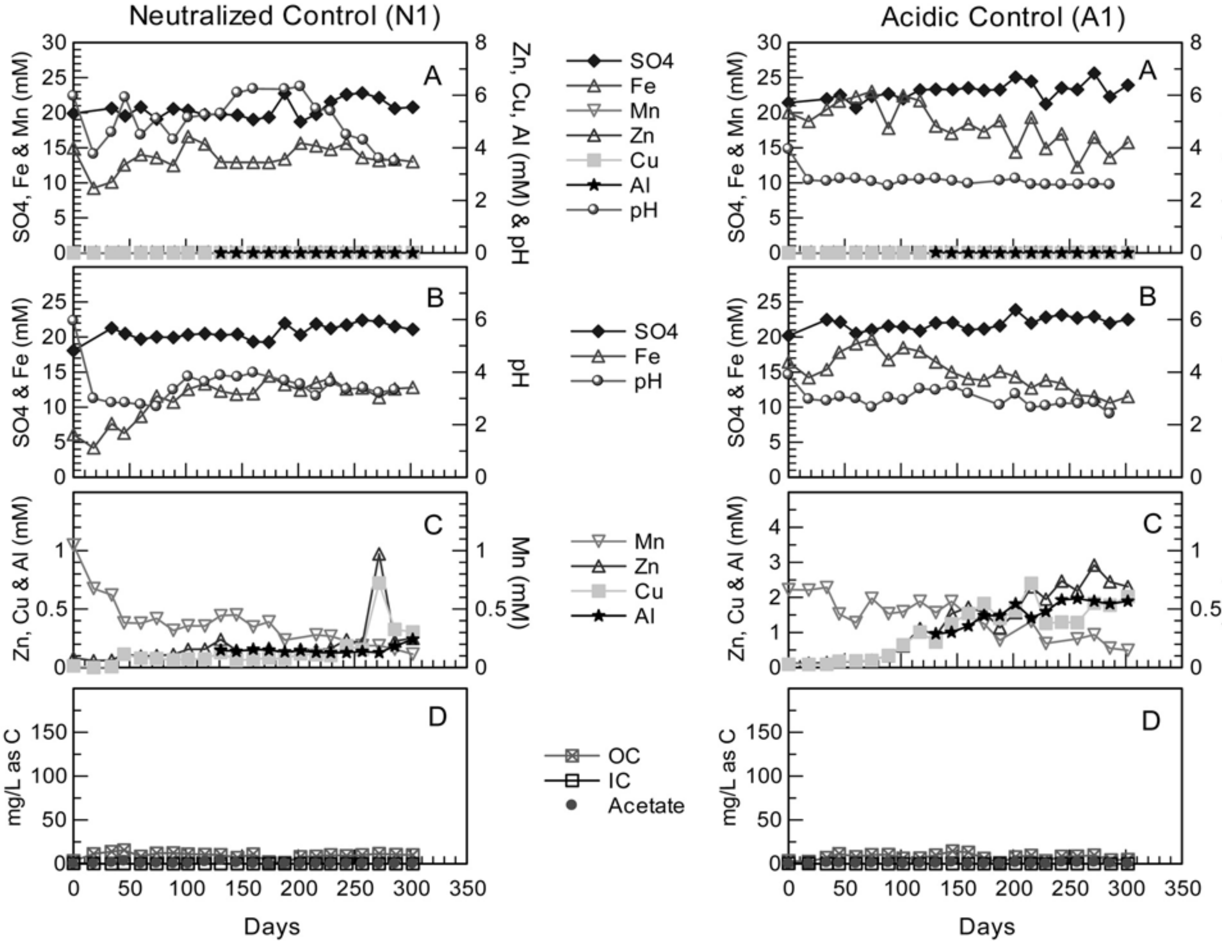

Figure 3. Monitoring results from neutralized control (N1) and acidic control (A1) columns (no added carbon): (A) SO_4, Fe, Mn, Zn, Cu, Al, and pH in column influent; (B) SO_4, Fe and pH in column effluent; (C) Mn, Zn, Cu, and Al in column effluent; and (D) OC, IC and acetate as mg/L C in column effluent

Results in the acidic emulsion amended column (A2) were similar to the neutralized amended column. However, less soluble organic carbon was released shortly after emulsion injection, presumably due to slower growth of microorganisms that initially hydrolyze soybean oil. The emulsion treatment resulted in a large increase in pH, from ≈2.7 to ≈5.8, and a decrease in Fe and SO_4. Again, H_2S remained below detection and Fe removal closely correlated with SO_4 removal, suggesting that Fe precipitation was limited by sulfide production. After the onset of SO_4 reduction, Cu and Al were below detection. However, Zn was occasionally detected. Mn was observed in the column effluent throughout the experiment at levels comparable to the untreated control columns. The low Mn removal efficiency is presumably due to the high aqueous solubility of MnS under mildly acidic conditions. In contrast to the neutralized columns, OC concentrations were relatively constant in A2, varying from 10 to 50 mg/L, with a significant fraction of the OC present as acetate. Again, CH_4 levels were low (<0.1 mg/L).

Figure 5 also shows monitoring results for neutralized (N3) and acidic (A3) columns amended with emulsified soybean oil. The initial construction and operation of these columns was identical to columns N2 and A2 (Figure 4). However, after 130 days of operation, the influent to these columns was altered to provide higher concentrations of Al, Cu, Mn, and Zn. The new influent solutions were prepared with 2 mM $FeSO_4$, 5 mM $MnSO_4$, 1 mM $CuSO_4$, 4 mM $AlK(SO_4)_2$, 1 mM $ZnSO_4$, and 7 mM $CaSO_4$, then titrated to pH ≈3 with H_2SO_4, (acidic) or pH ≈5 with $NaHCO_3$ (neutralized).

Over the first 130 days, results from N3 were very similar to its duplicate, N2. SO_4 and Fe were reduced with a concurrent increase in pH in response to emulsion addition. There was also an initial pulse of OC released, then OC concentrations stabilized at 50 – 100 mg/L. After the change in influent solution at 130 days, Fe concentrations in the column effluent dropped to below 0.01 mM (<1 mg/L), Mn concentrations increased, and removal efficiencies varied between 90 – 100% for Fe and 40 – 80% for Mn.

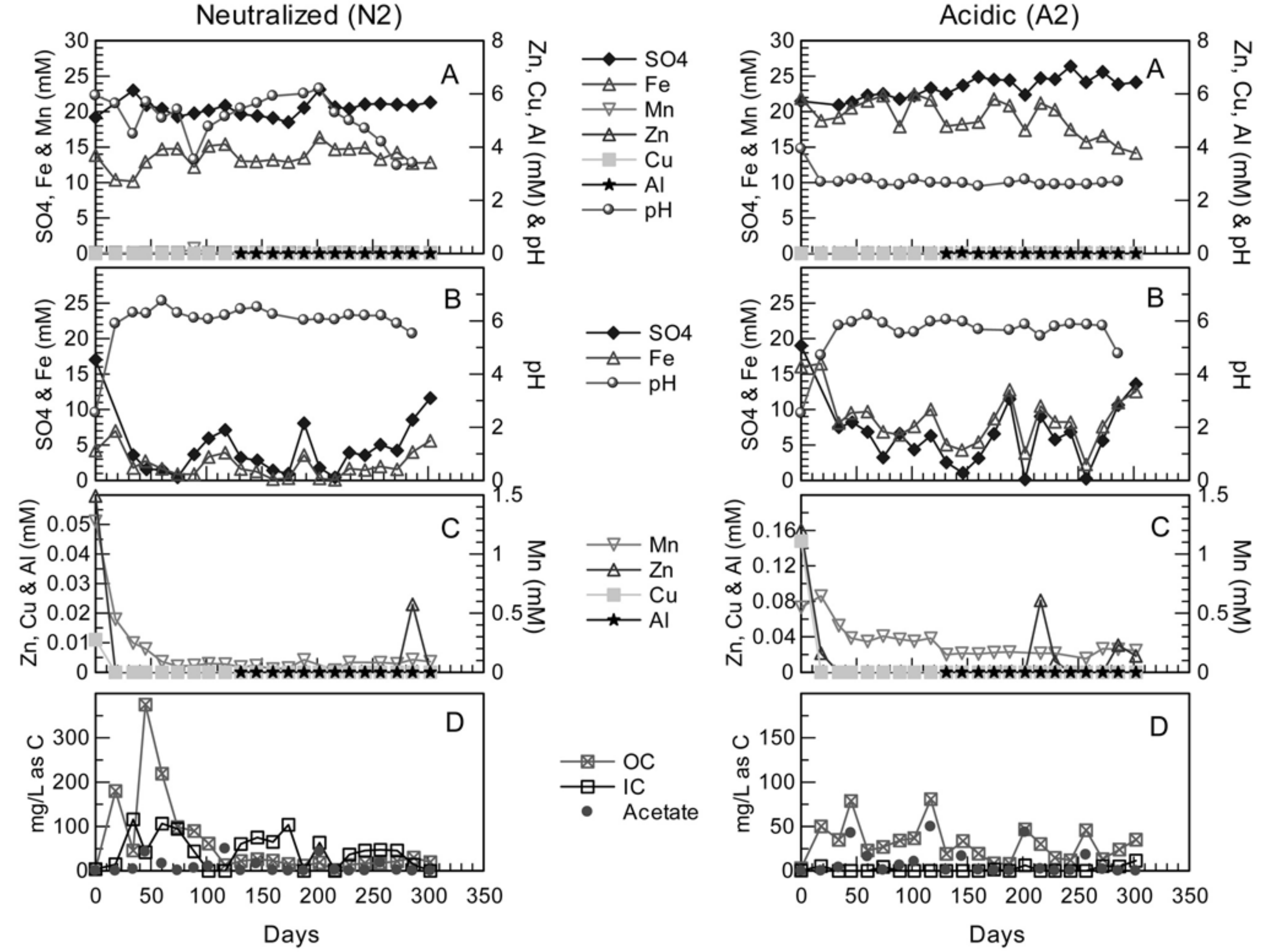

Figure 4. Monitoring results from neutralized (N2) and acidic (A2) columns treated with soybean oil emulsion: (A) SO_4, Fe, Mn, Zn, Cu, Al, and pH in column influent; (B) SO_4, Fe and pH in column effluent; (C) Mn, Zn, Cu, and Al in column effluent; and (D) OC, IC and acetate as mg/L C in column effluent; emulsion injection occurred on day zero

Al and Cu were consistently below detection (< 0.01 mM) in the column N3 effluent. Zn was detected in one sample at 0.01 mM. Towards the end of the experimental period, OC declined and SO_4 removal dropped below 30%, suggesting that the bioavailable carbon had been substantially depleted. H_2S and CH_4 levels were low throughout the experiment.

Over the first 130 days, pollutant removal in A3 generally matched column A2 results. The pH increased in response to microbially mediated Fe and SO_4 reduction. At 130 days, pollutant removal efficiency changed significantly with the change in influent solution. Al and Cu were reduced to below detection (>99% removal) and Zn was reduced by 97% to >99%. However, Fe removal efficiency declined and Mn concentrations increased. By the end of the experiment, SO_4 removal was minimal, suggesting substantial depletion of the organic substrate.

Throughout the column study, a black precipitate was observed in the effluent of the treated columns. This precipitate was collected from the effluent of N2, N3, and A3 on day 243, analyzed by X-ray diffraction, and then dissolved in 0.1 N HCl for analysis by ICP for Al, Cu, Fe, Mn, Zn, and total sulfur content (Table 1). Unfortunately, it was not possible to collect enough precipitate from column A2 for analysis. X-ray diffraction indicated the solid was an amorphous, poorly crystalline material. Elemental analysis indicated a sulfur to total metals molar ratio between 1:0.86 and 1:1.28. The molar ratio of sulfate to total metals removed in the columns varied between 1.5:1 and 2.4:1, suggesting that a portion of the SO_4 was converted to partially reduced sulfur species with intermediate valence states (e.g. thiosulfate, elemental and organic sulfur, etc.). This is consistent with effluent monitoring results on a few samples, which showed total dissolved sulfur concentrations between 120% and 260% of the sulfate concentration.

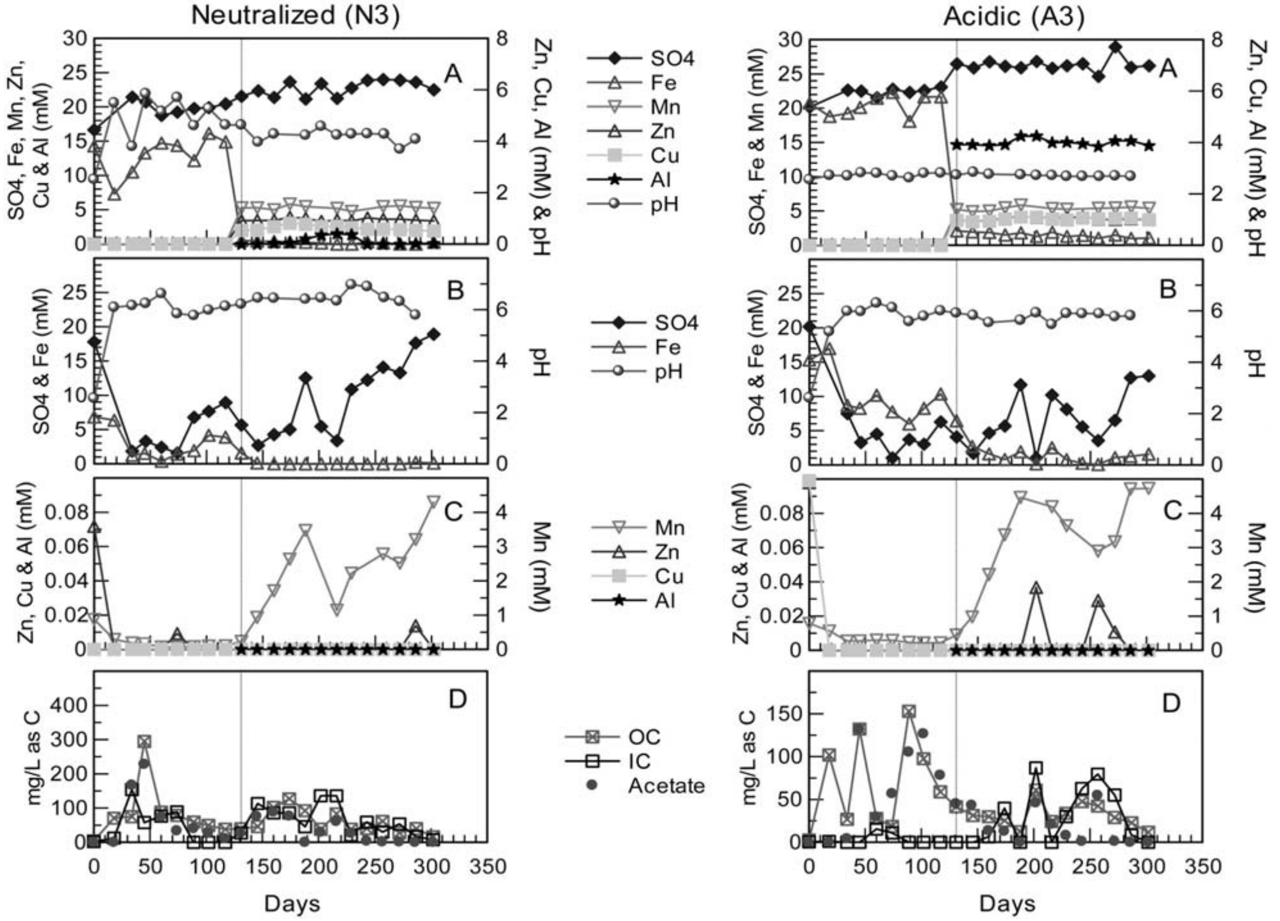

Figure 5. Monitoring results from neutralized (N3) and acidic (A3) columns treated with soybean oil emulsion: (A) SO_4, Fe, Mn, Zn, Cu, Al, and pH in column influent; (B) SO_4, Fe and pH in column effluent; (C) Mn, Zn, Cu, and Al in column effluent; and (D) OC, IC and acetate as mg/L C in column effluent. Emulsion injection occurred on day zero. Influent change occurred on day 131

Thiosulfate, organic sulfur and/or small colloidal particles of elemental sulfur would not have been detected with our standard monitoring techniques.

Carbon Mass Balance

A mass balance of carbon injected and released from each of the treated columns is presented in Table 2. In the first two weeks after injection, 12 – 25% of the injected carbon was released from the columns, representing emulsion that had not yet adsorbed to the tailings. This effect has been observed in prior column and sandbox studies (Coulibaly and Borden 2004; Coulibaly et al. accepted; Jung et al. accepted). The relatively large amount of emulsion released from the tailings columns is due to the large amount of emulsion injected, the coarse texture of the tailings, and the low surface area available for oil droplet

Table 1. Molar ratios of Al, Cu, Fe, Mn, and Zn to total sulfur (S) in black precipitate produced from neutralized (N2, N3) and acidic (A3) columns

	Al	Cu	Fe	Mn	Zn	S
(N2)	0.088	0.0	0.886	0.014	0.012	1.0
(N3)	0.032	0.0	0.543	0.273	0.011	1.0
(A3)	0.178	0.021	0.397	0.632	0.055	1.0

Table 2. Carbon mass balance for emulsion treated columns

	N2		N3		A2		A3	
	mg	%	mg	%	mg	%	mg	%
Carbon injected	12,020	100	12,020	100	12,020	100	12,02	100
Organic C released, day 0-14	1,770	15	1,741	14	2,947	25	1,476	12
Organic C released, day 15 – 302	418	3	379	3	169	1	278	2
Inorganic C released, day 0 - 302	285	2	345	3	9	0	75	1
Potential inorganic C produced (0 - 302 days)	1,464	12	1,227	10	1,502	12	1,672	14

attachment. Field studies have shown that essentially all of the oil droplets attach to solid surfaces as injected emulsion comes in contact with sediment during downgradient migration, (Zawtocki et al. 2004). Long term column and field studies indicate that once the droplets attach, they are immobile, until converted to more soluble compounds by microbial activity (Long and Borden, accepted; Zawtocki et al. 2004).

Over the next 288 days of operation, 1 to 3% of the injected substrate was released from the columns as OC, with most of the carbon present as acetate. Only 0 to 3% of the injected substrate was released from the columns as IC. The low IC production is surprising given the large amount of sulfate reduced. Potential IC production was estimated based on the amount of SO_4 removed in each column (influent minus effluent), assuming reduction of SO_4 to H_2S. Reducing equivalents required to drive these reactions were assumed to be provided by fermentation of the injected substrate to CO_2 and H_2. Details of the calculation procedure were provided by Lindow (2004). Monitoring results indicate methane production was not significant (data not shown).

Potential IC production was much greater than the IC actually measured in the column effluents. This large difference could be associated with sampling losses and/or precipitation within the column. Gas bubbles were frequently observed in the column effluent. However, it was not possible to accurately measure the composition or volume of gas released with the available equipment. Effluents from the neutralized columns were often supersaturated with siderite, suggesting that some of the IC may also have been removed from solution by precipitation in the column.

Potential IC production was equivalent to 10 - 14% of the injected substrate, suggesting that a substantial portion of the injected oil was eventually consumed in biotransformation reactions. Triplicate samples of the tailings were removed from each column and analyzed at the end of the experiments. Total carbon content varied from 0.8 to 5.4%, with an average of 1.42% by weight. There was no significant difference in carbon content between the treated and control columns. The absence of a detectable difference in carbon content is not surprising, given the small amount of material initially injected (≈0.8% by weight), the large amount of material depleted by flushing and biodegradation, and the variability in analytical results.

Hydraulic Conductivity Results

Head loss through the columns was monitored to evaluate the effects of emulsion injection, biological activity, and metals precipitation on the hydraulic conductivity of the tailings used to pack the columns (Figure 6). Prior to emulsion injection, the hydraulic conductivity varied from 0.014 to 0.032 cm/s due to minor variations in column packing. After emulsion injection, the measured hydraulic conductivity had increased slightly in three columns (N3, A2, and A3) and decreased in one column (N2). The small changes that were observed are the same magnitude as hydraulic conductivity changes observed in the two untreated columns (N1 and A1) and the initial column to column variability, indicating emulsion injection did not have a measurable impact on tailings hydraulic conductivity. After 304 days of operation, the measured hydraulic conductivity increased in one column (A2) and decreased in three columns (N2, N3, and A3), but the hydraulic conductivity change in columns N2 and N3 was within the natural variation in the measurements. The reason for the greater hydraulic conductivity loss in column A3 is not known.

Discussion

A one time injection of emulsified soybean oil, lactate, yeast extract, and a microbial inoculum stimulated sulfate and metal ion reduction for ≈300 days in laboratory columns packed with mine tailings and receiving influent solutions with moderate to low pH and elevated SO_4, Al, Cu, Fe, Mn, and Zn. Average removal efficiencies over the last 160 days of operation in the four treated columns are shown in Table 3. Fe, Mn, Cu, and Zn removal appears to be primarily due to formation of metal sulfides, with removal efficiency decreasing with increasing metal sulfide solubility. Fe removal was probably limited by sulfide availability as sulfide was below detection (<0.002 mM) in the effluent of every column and Fe removal decreased when SO_4 removal decreased (i.e. lower sulfide production). In addition, columns N2 and N3 were frequently supersaturated with siderite

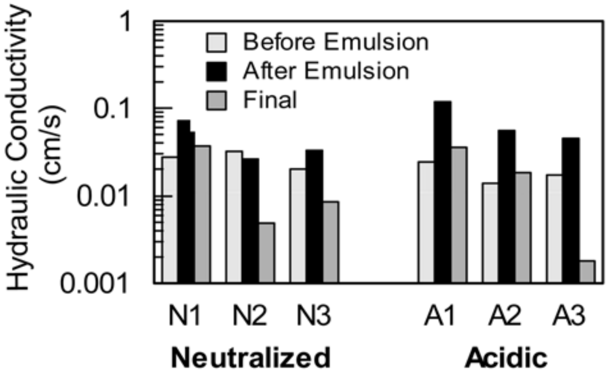

Figure 6. Hydraulic conductivity of each column before emulsion injection, immediately after emulsion injection, and after 304 days of operation (final)

Table 3. Removal efficiency in emulsion treated columns over the last 160 days of operation

	SO_4	Al	Mn	Fe	Zn	Cu
Potential metal sulfide			MnS (pink)	FeS	α-ZnS	CuS
Sulfide solubility product[1]			$10^{-10.5}$	$10^{-18.1}$	$10^{-24.7}$	$10^{-36.1}$
Neutralized (N2)	79%	NP[2]	NP	87%	NP	NP
Neutralized (N3)	56%	>99%	54%	77%	>99%	>99%
Acidic (A2)	75%	NP	NP	56%	NP	NP
Acidic (A3)	73%	>99%	36%	17%	99%	>99

[1] From Smith et al. (2001); [2] NP – not present in column influent

(Log Ksp = -10.68, Kotrly' and Sucha 1985), indicating that some portion of the Fe may have been removed as an iron carbonate. Al removal appeared to be due to the increase in pH during treatment.

In the batch incubations amended with soybean oil, molasses, and yeast extract, SO_4 was reduced to below detection in 72 days. However, in the flow-through columns, SO_4 removal efficiencies were in the range of 60 to 80%. The lower removal efficiency in the columns could be due to: (a) sulfide and/or heavy metal toxicity; or (b) the low levels of bioavailable substrate and short hydraulic retention time of the columns (6-7 days).

Okabe et al. (1995) reported that a sulfide concentration of 7.8 mM (250 mg/L S) reduced the cell yield of SRB by half at pH = 7. However, sulfide toxicity was probably not an issue in our work, since sulfide concentrations were below 0.002 mM (0.1 mg/L) in the effluent of each column. Utgikar et al. (2001) reported that levels of 0.17-0.19 mM (10.5-12 mg/L) Cu and 0.25-0.31 mM (16.5-20 mg/L) Zn were toxic to a mixed culture of SRB. In our work, the high metals concentrations were probably rapidly reduced during passage through the column by precipitation as insoluble metal sulfides, reducing toxicity effects.

Prior work with engineered bioreactors (Hammack et al. 1998; Quan et al. 2003; Tsukamoto et al. 2004) has shown that sulfate can be rapidly removed with a short HRT when a readily biodegradable, soluble substrate is present. However, when slowly biodegradable solid substrates are used, HRTs must be significantly longer to allow sufficient time for hydrolysis of the complex organics (Chang et al 2000; Cheong et al 1998; Drury 1999; Dvorak et al. 1992). Using a mathematical model to simulate sulfate reduction, Drury (2000) found that higher HRTs are required for solid substrates that are replaced less frequently. For example, if the solid substrate is replaced once every two years, a 14 day HRT may be acceptable, but if the replacement frequency is once every four years, a 25 day HRT would be required. Ideally, we would like inject additional emulsion once every few years. Consequently, longer HRTs may be required to allow sufficient time for microorganisms to ferment the long-chain fatty acids to acetate and H_2, with subsequent use of the acetate/H_2 for sulfate reduction. Some additional factor of safety may also be needed to account for non-uniform emulsion distribution and non-uniform water flow through the tailings. Fortunately, emulsions can be easily distributed in most sediment, forming thick PRBs with HRTs of 60 to 180 days.

Conclusions and Recommendations

The batch incubations showed that soybean oil can be used to stimulate SO_4 and metals reduction with associated precipitation of heavy metals. Flow-through column experiments demonstrated that a single emulsified oil injection could be used to reduce SO_4 and heavy metals for over 300 days, using 10-14% of the injected oil to support contaminant reduction. The high ratio of SO_4 removed to emulsified oil suggests that this approach may be cost effective at some sites. However, there were significant variations in removal efficiency between metals and for different experimental conditions.

Although these results are promising, additional research is needed to better understand the factors controlling pollutant removal efficiency and the ultimate fate of precipitated metals. Preliminary evidence suggests that metals can be removed within the tailings piles as both sulfide and carbonate precipitates. However, once the injected organic carbon has been depleted, the pH could drop, resulting in metal remobilization. To develop reliable designs for in situ barriers, we need to understand the effects of influent water chemistry and HRT on sulfate removal rates and the different factors controlling hydraulic conductivity loss. Field-scale pilot tests are needed at mine sites to identify factors that may limit actual treatment performance.

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References

Chang IS, Shin PK, Kim BH (2000) Biological treatment of acid mine drainage under sulphate-reducing conditions with solid waste materials as substrate. Water Res 34 (4): 1269-1277

Cheong YW, Min JS, Kwon KS (1998) Metal removal efficiencies of substrates for treating acid mine drainage of the Dalsung mine, South Korea. J Geochem Exploration 64 (1-3): 147-152

Coulibaly KM, Borden RC (2004) Impact of edible oil injection on the permeability of aquifer sands. J Cont Hydrol 71: 219– 237

Coulibaly KM, Long CM, Borden RC (accepted) Transport of edible oil emulsions in clayey-sands: 1-D column results and model development. J Engr Hydrol

Drury WJ (1999) Treatment of acid mine drainage with anaerobic solid-substrate reactors. Water Environ Res 71 (6): 1244-1250

Drury WJ (2000). Modeling of sulfate reduction in anaerobic solid substrate bioreactors for mine drainage treatment. Mine Water and the Environment 19 (1): 18-28

Dvorak DH, Hedin RS, Edenborn HM, and McIntire PE (1992) Treatment of metal-contaminated water using bacterial sulfate reduction: results from pilot-scale reactors. Biotech Bioengin 40 (5): 609-616

Hammack RW, De Vegt AL, Schoeneman AL (1998) The removal of sulfate and metals from mine waters using bacterial sulfate reduction: pilot plant results, Mine Water and the Environment 17: 8-27

Jung Y, Coulibaly KM, and Borden RC (accepted) Transport of edible oil emulsions in clayey-sands: 3-D sandbox results and model validation, J Engr Hydrol

Kotrly' S, Sucha L (1985) Handbook of Chemical Equilibria in Analytical Chemistry, John Wiley & Sons, New York, NY, 414 pp

Lindow NL (2004) Use of soybean oil and soybean products for groundwater bioremediation, MS thesis, Civil Eng, North Carolina State Univ, Raleigh, NC, 82 pp

Long CM, Borden RC (in press) Enhanced reductive dechlorination in columns treated with edible oil emulsion. J Contaminant Hydrology

Ludwig RD, McGregor RG, Blowes DW, Benner SG, Mountjoy K (2002) A permeable reactive barrier for treatment of heavy metals. Ground Water 40 (1): 59-66

Smith RM, Martell AE, Motekaitis RJ (2001) NIST critically selected stability constants of metal complexes. U.S. National Institute of Standards and Technology, Standard Reference Data Program, Gaithersburg, MD, 6 computer disks

MSE Technology Applications (2000) Mine waste technology program 2000 Annual Report, Butte, MT, 74 pp

Okabe S, Nielsen PH, Jones WL, Characklis WG (1995) Sulfide product inhibition of Desulfovibrio desulfuricans in batch and continuous cultures. Water Research 29 (2): 571-578

PA DEP (2005) A status report on the environmental legacy of coal mining in Pennsylvania. Pennsylvania Dept of Env Protection, Harrisburg, PA, http://www.dep.state.pa.us/dep/DEPUTATE/MINRES/BAMR/Documents/status_report_1.htm

Quan ZX, La HJ, Cho YG, et al., (2003) Treatment of metal-contaminated water and vertical distribution of metal precipitates in an upflow anaerobic bioreactor. Environ Tech 24 (3): 369-376.

Skousen J, Rose A, Geidel G, Foreman J, Evans R, Hellier W (1998) A Handbook of Technologies for Avoidance and Remediation of Acid Mine Drainage. National Mine Land Reclamation Ctr, Morgantown, WV, 131 pp

Tsukamoto TK, Killion HA, Miller GC (2004) Column experiments for microbiological treatment of acid mine drainage: low-temperature, low-pH and matrix investigations. Water Res 38 (6): 1405-1418

U.S. EPA (1995) Profile of the metal mining industry: sector notebook project. U.S. Environmental Protection Agency 310R95008, Washington, DC, 127 pp

Utgikar VP, Chen BY, Chaudhary N, Tabak HH, Haines JR, Govind R (2001) Acute toxicity of heavy metals to acetate-utilizing mixed cultures of sulfate-reducing bacteria: EC100 and EC50. Environ Toxicol and Chem 20 (12): 2662-2669

Vile MA, Wieder RK (1993) Alkalinity generation by Fe(III) reduction versus sulfate reduction in wetlands constructed for acid mine drainage treatment. Water Air Soil Poll 69: 425-441

Waybrant KR, Ptacek CJ, Blowes DW (2002) Treatment of mine drainage using permeable reactive barriers: Column experiments. Environ Sci Technol 36 (6): 1349-1356

Zawtocki C, Lieberman MT, Borden RC, Birk GM (2004) Treatment of perchlorate and 1,1,1-trichloroethane in groundwater using edible oil substrate (EOS$^{®}$). Proc, 4th International Conf on Remediation of Chlorinated and Recalcitrant Compounds, Monterey, CA, 7 pp

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