ANAEROBIC BIOREMEDIATION OF ACID MINE DRAINAGE USING EOS[1]

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Abstract: Recent laboratory and field studies have shown that injection of Edible Oil Substrate (EOS®) into the subsurface can provide an effective, low-cost alternative for the enhanced anaerobic bioremediation of a variety of pollutants including acid mine drainage (AMD). EOS® is prepared from a mixture of slowly biodegradable emulsified oil (e.g. soybean oil) and easily biodegradable substrates. As AMD impacted water flows thought the treated zone, EOS® stimulates growth of iron and sulfate reducing bacteria, increasing the pH, reducing sulfate, and immobilizing iron, copper, nickel, zinc and related toxic metals. All materials used in the process are Generally Recognized As Safe (GRAS), food-grade materials (21 CFR 184.1400) for *in situ* application.

The impact of EOS® treatment on AMD was evaluated in both batch and flow-through column experiments. Batch microcosms were constructed with AMD generating spoils from a former coal mine in Sequatchie Valley, TN, simulated acid mine drainage, and a small liquid inoculum from an anaerobic treatment wetland. Sulfate declined from 1,800 mg/L to 10 mg/L, pH increased from 2.6 to 6.4 and iron was precipitated in a 2:1 molar ratio with sulfate removal. These results demonstrate that EOS® addition can be very effective in treating AMD and the initial pH does not significantly inhibit microbial growth.

Laboratory columns were also packed with mine spoils and received a one time treatment of EOS® with a microbial inoculum. Simulated AMD was then pumped through the columns with a four-day hydraulic retention time. During passage through the EOS® treated columns, pH increased from less than 3 to near 6, SO_4 was reduced by 75%, and aluminum, copper and zinc were reduced to below the analytical detection limit. In this system, effluent dissolved iron concentrations appear to be controlled by the amount of dissolved sulfide available for precipitation.

Additional Key Words: Sulfate reducing bacteria, heavy metals immobilization, in situ acid rock remediation

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Introduction

A variety of anaerobic bioremediation processes have been shown to be effective for treatment of acid mine drainage (AMD) including engineered bioreactors, treatment wetlands, and permeable reactive barriers (Hammack et al., 1998; Skousen et al., 1998; Vile and Wieder, 1993; Benner et al., 2002). In this paper, we report on a new approach for in-situ treatment of AMD using emulsified edible oils. In this process, an oil-in-water emulsion with small uniformly sized droplets is prepared using an edible oil (typically soybean oil), edible surfactants, and high energy mixing. The soybean oil provides a slow release organic substrate to support long-term anaerobic treatment of iron and sulfate. Easily biodegradable soluble substrates can also be included to generate rapid, initial growth of the required bacteria. Once prepared, the emulsion is injected into the ground where the oil droplets are immobilized as a thin coating on the sediment and rock surfaces. This immobilized oil forms a subsurface treatment zone where sulfate is reduced to sulfide, pH increases and many metals precipitate including iron, aluminum, copper, zinc and other chalcophilic metals (e.g. Pb, Cd, Hg).

Blowes and his colleagues have used a similar approach for AMD treatment in permeable reactive barriers (PRBs). 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 iron and sulfate with a resulting increase in pH and immobilization of heavy metals. Extensive laboratory, pilot and full-scale demonstrations have shown this approach can be very effective in controlling AMD (Ludwig et al., 2002; Waybrant et al., 2002). A full-scale PRB has been in operation for several years at the Nickel Rim mine site near Sudbury, Ontario and continues to reduce AMD concentrations by over 1000 mg/l SO_4 and 250 mg/l Fe (Benner et al., 1999). Scientifically, the permeable reactive barrier approach has been demonstrated to be very effective. However, PRBs have not been widely adopted by the mining industry, presumably because of the substantial costs for barrier construction and the difficulty of installing barriers in at typical mine sites.

Edible oil emulsions can be used to form PRBs, similar to those proposed by Blowes. However, installation costs for edible oil barriers are significantly lower than typical PRBs installed by conventional trenching, especially when the treated zone is deep below ground surface. 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. This allows use of emulsions for AMD treatment in a variety of configurations including barriers, aerial treatments, and direct injection into spoil piles and mine tailings.

In this work, we conduct batch microcosm and flow through column studies to evaluate the use of soybean oil emulsions for treatment of acid mine drainage.

Environmental Impacts

Coal and hard rock metal mining (including gold, copper, lead and zinc) can result in significant surface and groundwater contamination. Coal and other important metal ores are found associated with sulfide deposits. During mining operations, these ores and related sulfide minerals [pyrite (FeS_2), pyrrhotite (FeS), chalcopyrite ($CuFeS_2$) and enargite (Cu_3AsS_4)] are exposed to oxygen and water, resulting in the formation of large amounts of sulfuric acid and dissolution of heavy metals including iron, manganese, copper, cobalt, cadmium, nickel, and zinc [Figure 1].

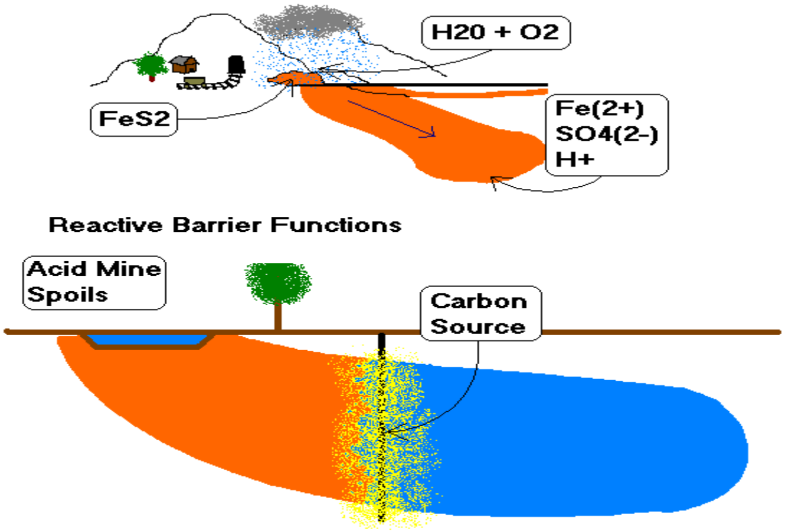

Figure 1: Acid mine drainage formation and treatment

Effective control technologies are needed to manage this tremendous environmental and economic problem. However many of these sites are very large and located in remote areas. Control technologies for these sites must be low-cost, simple to implement and require little or no ongoing maintenance.

Anaerobic bioremediation processes may be used to reduce sulfate and immobilize heavy metals in the subsurface if a carbon and energy source is available to drive iron and sulfate reduction. Hydrogen and low molecular weight organic acids can be produced from the fermentation of a variety of organic substrates including compost, manure, sugars, oils, and organic rich sediments. Iron reducing bacteria (IRB) and sulfate reducing bacteria (SRB) use the hydrogen (or organic acids) to reduce ferric iron (Fe^{3+}) and sulfate (SO_4^{2-}). Hydrogen sulfide (H_2S) produced from sulfate reduction (equation 1) is then available for precipitation of ferrous iron (Fe^{+2}) and heavy metals (e.g., Cu, Co, Cd, Pb, Ni, and Zn).

$$SO_4^{2-} + 4\ H_2 \rightarrow H_2S + 2\ H_2O \text{ and } 2\ OH^- \qquad \textbf{(1)}$$

Experimental Methods

Microcosm experiments were conducted to evaluate the use of soybean oil as a substrate for sulfate reducing bacteria under different environmental conditions. Microcosms for each treatment were constructed in triplicate in 240 mL serum bottles fitted with a thick rubber stoppers and aluminum crimp seals to exclude oxygen and contained 190 mL simulated AMD, 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. Two types of AMD were evaluated. The standard AMD consisted of 20 mM $FeSO_4$ solution titrated to pH 3.0. Neutralized AMD was prepared by titrating the standard AMD to pH = 7 with 1.0 M sodium bicarbonate solution to simulate the effects of natural buffering during flow through uncontaminated aquifer material.

Saturated flow through column experiments were then conducted to evaluate the potential for AMD treatment in permeable reactive barriers 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 hydraulic retention time (HRT) of ~ 5 days. 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 33-mL Edible Oil

Substrate (EOS®) and 25 mL of inoculum from the microcosms diluted to 100 mL with the iron sulfate influent solution. EOS® is a proprietary organic substrate containing emulsified edible oil, easily biodegradable substrates and bacterial nutrients and is specially formulated for enhancing in-situ anaerobic bioremediation processes (www.EOSRemediation.com). One acidic and one neutralized column remained untreated as no added carbon controls. Figure 2 illustrates the general setup for the columns.

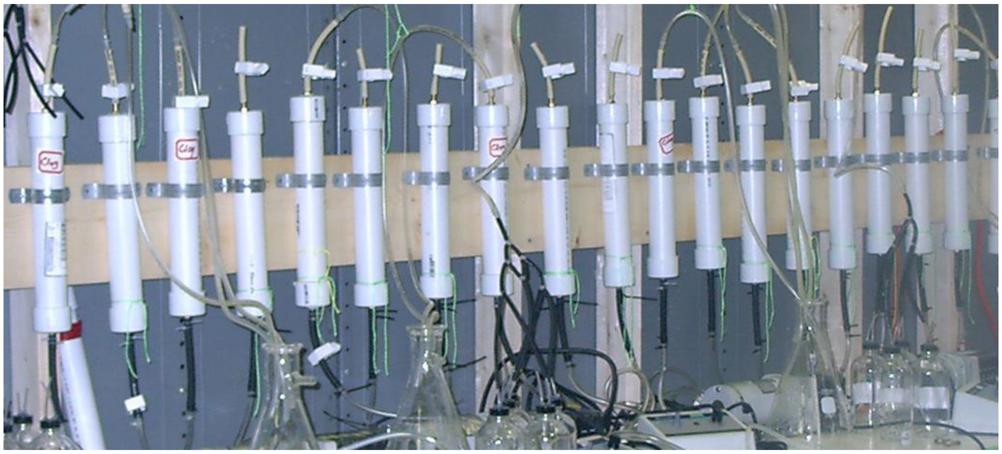

Figure 2: Intermittent flow columns

Results

An initial series of microcosm experiments were conducted to evaluate the ability of soybean oil to stimulate iron and sulfate reduction under the harsh conditions typical of acid mine drainage. Coal mine spoils were obtained a former mine in Sequatchie Valley, TN for use in the acid mine drainage studies. Experimental treatments included:

- Live control (100 mg/L $(NH_4)_2HPO_4$, 25 mL inoculum but no added carbon)
    - Acidic AMD
    - Neutralized AMD
- Basic treatment (live control + 200 mg/L molasses, 200 mg/L yeast extract)
    - Acidic AMD
    - Neutralized AMD
- Soybean oil (basic treatment plus 2500 mg/L liquid soybean oil)

  - Acidic AMD
  - Neutralized AMD
- Autoclaved killed controls

Fig. 3 shows the observed variation in dissolved sulfate, dissolved iron and pH in the different microcosms. Values shown are the average of triplicate incubations. There was no significant change in the sulfate, iron or pH levels in the killed control, acidic live control and neutralized live control. The basic treatment resulted in a moderate increase in pH but no significant change in sulfate or iron, presumably due to the small amount of organic substrate added. In contrast, the basic treatment plus soybean oil resulted in 100% reduction in sulfate and increase in pH to over 6 in both the acidic and neutralized treatments. Sulfate reduction was somewhat more rapid in the neutralized bottles, despite the low pH due to the added mine spoils. The low pH of the microcosms did not significantly impact treatment in any bottle.

Iron was removed from the acidic soybean oil treatments at a ratio of 1 mole iron per two moles sulfur indicating precipitation as an iron disulfide or as a mixture of FeS and S^0. However, since these incubations were prepared with a very high ratio of iron to sulfate (1:1), sulfate was depleted first, leaving ~ 5 moles/L Fe remaining in the aqueous phase.

The microcosm results indicated that emulsified liquid soybean oil could provide a very effective treatment for AMD. To further evaluate this process, six columns were packed with coal mine spoils similar to the material used to construct the AMD microcosms. Four columns (2 acidic AMD and 2 neutralized AMD) received a one-time treatment of EOS® with an inoculum from the microcosms. One acidic and one neutralized column remained untreated as no added carbon controls.

Fig. 4 shows monitoring results from the columns receiving an acidic AMD influent (pH ~ 2.6 to 2.8). In all live carbon amended columns, there is a dramatic increase in pH and substantial reduction in sulfate and dissolved iron. Copper and zinc remained below detection in the effluent of the carbon amended columns but increased to high levels in the untreated column. The only metal with low removal efficiency was manganese. This is believed to be due to the relatively high aqueous solubility of manganese sulfide. As in the microcosms, the low pH did not significantly inhibit the bacterial consortia responsible for sulfate reduction. Again the metals removal was partially limited by sulfate availability in the column influent.

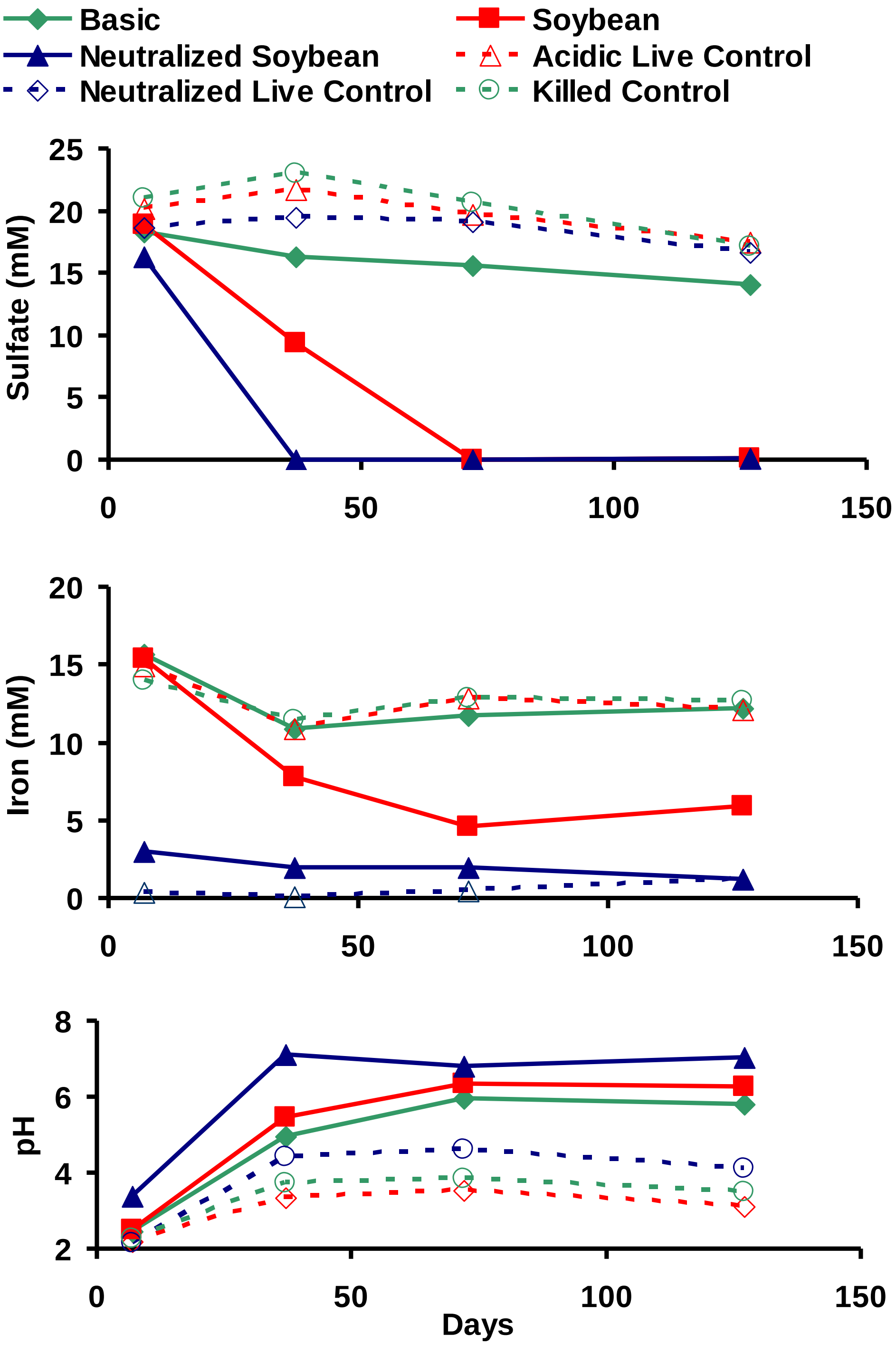

Figure 3: Variation in dissolved sulfate, iron, and pH in AMD microcosms

After 130 days operation, the column influent was changed in one neutralized column and one acidic column (A6) to evaluate conditions more representative of typical acid mine drainage and examine the response to higher concentrations of aluminum, copper, manganese and zinc. The new influent was prepared with $FeSO_4$ (2 mM), $MnSO_4$ (5 mM), $CuSO_4$ (1 mM), $AlK(SO_4)_2$ (4 mM), $ZnSO_4$ (1 mM) and $CaSO_4$ (7 mM). Figure 5 shows the variation in influent and effluent concentrations with time for the acidic AMD column, A6.

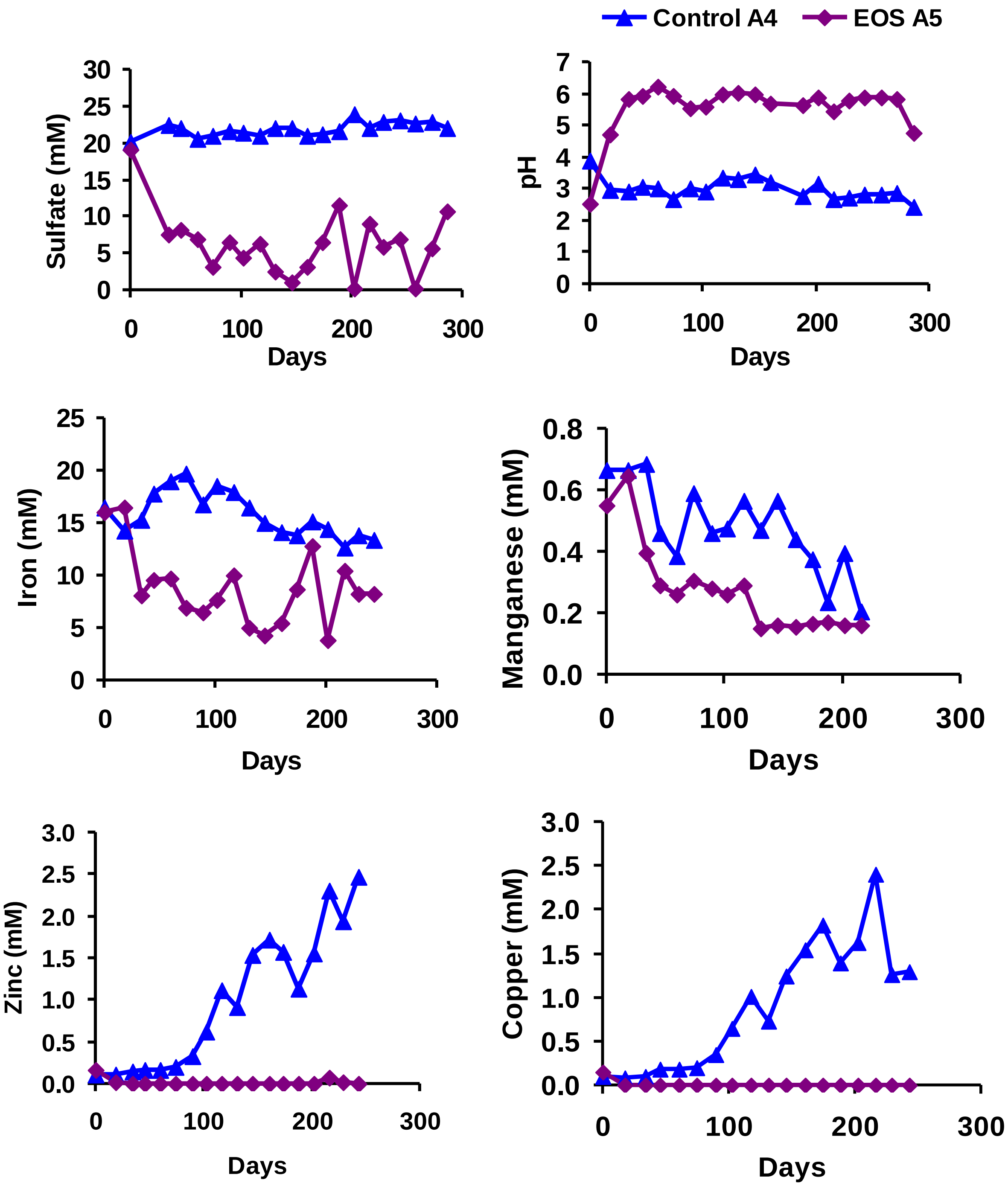

Figure 4: Treated versus untreated effluent in acidic AMD columns.

Column A6 continued to provide very good treatment after the influent was modified to include higher concentrations of aluminum, copper, zinc and sulfate. pH increased from 2.8 to 5.8 during passage through the column and aluminum concentrations were below the analytical detection limit in all effluent samples. Sulfate concentrations were reduced by over 75% and copper and zinc concentrations remained very low, despite the increased concentrations in the column influent. The only contaminants that did not show good removal were iron and manganese. The low manganese removal efficiency is presumably due to the high aqueous solubility of manganese sulfides (Table 1). The cause of the low iron removal efficiency is unknown, but is probably not related to solubility constraints since iron sulfides are orders of magnitude less soluble than manganese sulfides.

Table 1: Calculated Aqueous Solubility in the Presence of 0.01 mM Sulfide (S^{-2})

Solid Precipitate	Log Solubility Product[1]	Aqueous Solubility (M)
MnS	-10.5	$10^{-5.5}$
FeS	-18.1	$10^{-13.1}$
ZnS	-24.7	$10^{-19.7}$
CuS	-36.0	$10^{-31.0}$

[1] Solubility products are from Bodek et al., 1988.

There are a variety of factors that could limit sulfate removal efficiency in the emulsion treated columns. In the laboratory, the short hydraulic residence time (5 days) and small amount of added substrate may have limited sulfate removal. However in the field, contact times would be much higher (typically 1 to 2 months) and substrate would be added in excess of the minimum requirements. None-the-less, the laboratory columns provided very good sulfate removal (20 mM or 1925 mg/L) with a high pollutant to substrate efficiency. To date, over 0.7 grams of sulfate have been removed per gram of concentrated EOS® and sulfate removal efficiency continues to remain high.

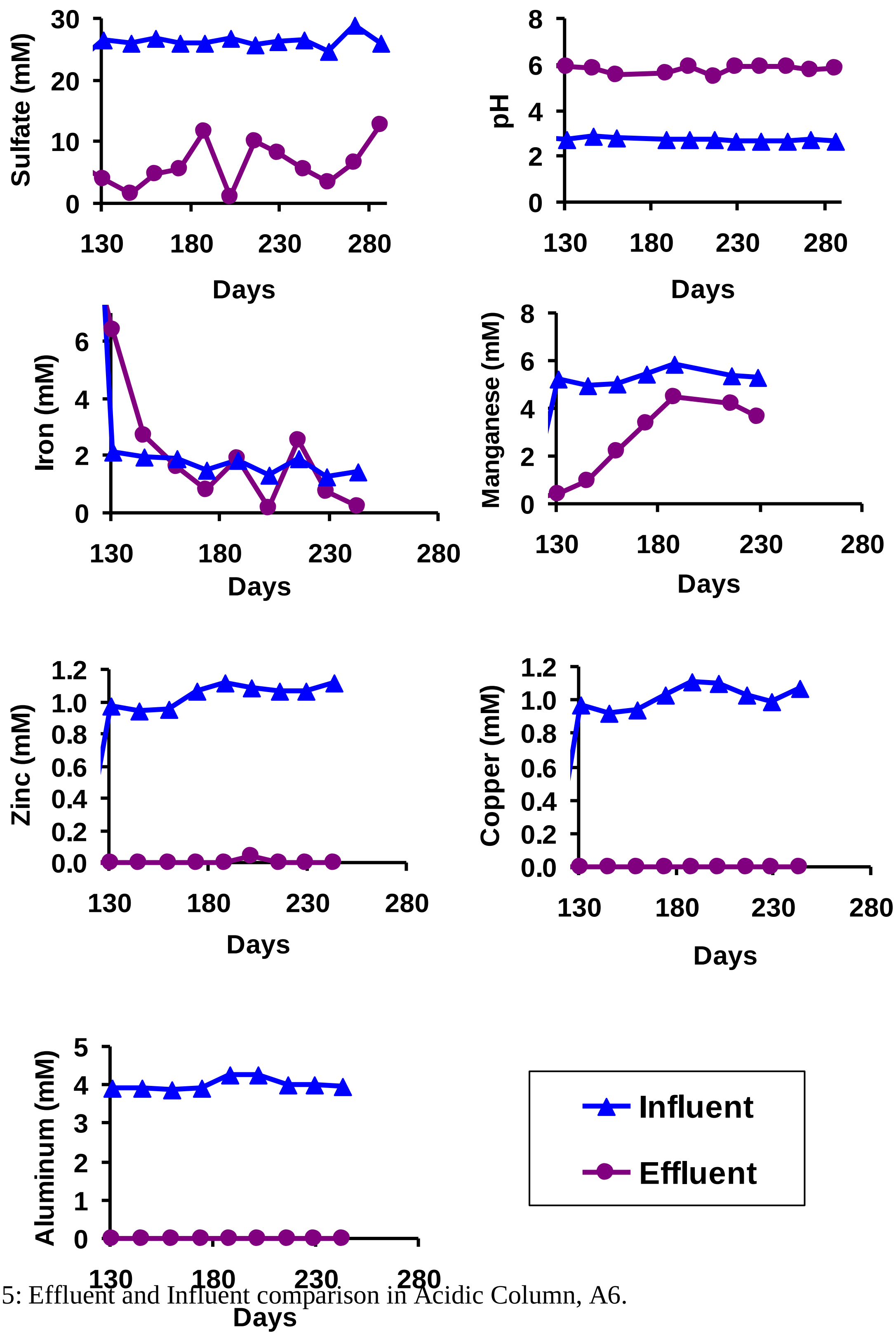

Figure 5: Effluent and Influent comparison in Acidic Column, A6.

A variety of studies have also shown that the activity of sulfate reducing bacteria (SRB) can be inhibited by high levels of dissolved sulfide. Okabe et al. (1994) reported that H_2S levels as low as 2 mM can inhibit SRB growth in batch and continuous cultures. Sulfide toxicity was probably not an issue in our work, since sulfide concentrations were below 0.02 mM (0.6 mg/L) in the effluent of each column. These low sulfide levels in the column effluent indicate that sulfide produced during sulfate reduction is being immobilized in the column, although the mineral form of the precipitate has not been defined.

The high concentrations of metals in the column influent (108 mg/L Al, 64 mg/L Cu, 262 mg/L Zn) could also have inhibited microbial growth. Utgikar et al. (2002) reported that levels of 4-20 mg/L Cu or 20-40 mg/L Zn were toxic to *desulfovibrio* strains and a mixed culture of SRB. However in our work, the high metals concentrations were probably rapidly reduced during passage through column by precipitation as insoluble metal sulfides. None-the-less, Utgikar et al. (2001) have shown that precipitation of heavy metal sulfides outside the cell wall can create a barrier between reactants and necessary enzymes for sulfate reduction, reducing the rate of sulfate reduction.

Conclusions & Recommendations

Liquid soybean oil emulsion treatments were very effective in stimulating anaerobic biodegradation of acid mine drainage. The low initial pH of the AMD did not significantly inhibit sulfate reduction in either batch microcosms or flow through column experiments. A one time addition of EOS® and a microbial inoculum effectively reduced sulfate and heavy metals in the column effluent for over 7 months with a 5 day hydraulic residence time. Monitoring will continue to identify the factors limiting dissolved iron removal efficiency and to estimate the amount of pollutant that can be removed per mass of added substrate.

The application of EOS® to groundwater, mine spoils, or tailing impoundments could provide a cost effective and environmentally safe approach for treatment of acid mine drainage. However, further research is needed to evaluate the costs and benefits of this approach under realistic field conditions.

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