ABSTRACT

Lindow, Nicholas L. Use of Soybean Oil and Soybean Products for Groundwater Bioremediation. (Under the direction of Dr. Robert C. Borden)

Recent laboratory and field studies have shown that injection of soybean oil and related materials into the subsurface can provide an effective, low-cost alternative for enhanced anaerobic bioremediation. The purpose of this laboratory study is to further evaluate the effectiveness of emulsified soybean products for the reduction and immobilization of nitrate, chromate, and acid mine drainage.

Batch microcosm screening studies were first conducted to evaluate the potential of several different substrates to enhance in-situ anaerobic biodegradation of nitrate. The substrates evaluated were food-grade materials, including molasses, liquid soybean oil, fully hydrogenated soybean wax, blown soybean oil, soy methyl ester, and mineral oil. With the exception of mineral oil, the tested substrates were all able to support nitrate degradation with the slowest degradation rates occurring for fully hydrogenated soybean wax.

Intermittent flow through soil column studies were also conducted to evaluate the substrate degradation rate, nitrate removal efficiency, and lifespan of different soybean treatments. The soybean oil treatment stimulated denitrification removed nitrate and nitrite concentrations below detection in the liquid and fully hydrogenated soybean wax treated columns without the addition of a denitrifying bacterial inoculum. No apparent benefits or disadvantages were observed for either treatment.

An identical study was conducted for chromate. Microcosm results were favorable, showing aqueous chromium (VI) removal even in no added carbon control microcosms. However,
soil column experiments yielded mixed results. No removal of influent chromium was observed in columns treated with liquid or fully hydrogenated soybean oil at high influent chromate concentrations. A second set of columns were tested with lower concentrations of chromate, and resulted in complete removal of effluent chromium to below detection.

Batch microcosms were also constructed with acid mine drainage (AMD) generating spoils from a former coal mine in Sequatchie Valley, TN. The bottles were amended with simulated acid mine drainage and a small liquid inoculum from an anaerobic treatment wetland. Several combinations of treatments were evaluated including easily degradable sugars (molasses), more slowly degradable oils (soybean), yeast extract, and partially neutralized with sodium bicarbonate buffer. Despite the low pH and presence of toxic metals, in-situ reduction of AMD was significantly enhanced with a soybean oil substrate. 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. Lesser removal of some iron and sulfate also occurred in bottles amended with only molasses and yeast extract. These results demonstrate that soybean oil addition can be very effective in treating AMD and the initial pH of the AMD is not a significant problem if an appropriate microbial inoculum is provided.

Laboratory soil columns packed with mine spoils were also studied for AMD degradation. Treated columns received a one time amendment of the commercially available soybean emulsion EOS® (Edible Oil Substrate) and were bioaugmented with the bacterial consortia developed from the microcosm studies. Simulated AMD was then pumped through the columns with a six to seven-day hydraulic retention time (HRT). During passage through the EOS® treated columns, pH increased from <3 to ~6, SO4 was reduced by 75%, and
aluminum, copper and zinc were reduced to below the analytical detection limit.

Amendments were later made to the influents to include greater concentrations of manganese, zinc, aluminum, copper, and sulfate with less iron. The effect of the added sulfate increased electron donor usage and effectively shortened the potential lifespan of the treatment.

Changes in permeability were monitored for all the column experiments to evaluate the potential for fouling of an edible oil barrier with biomass and/or inorganic precipitates. Significant loss in permeability was observed in most of the treated columns. A mass balance of the carbon added through soybean treatment and potential carbon use due to biological redox reactions is also evaluated for all columns. The rate of carbon use appears heavily reliant on the electron acceptor loading rate, but only gross estimates of treatment lifespan are possible due to high mass balance error.
USE OF SOYBEAN OIL AND SOYBEAN PRODUCTS FOR GROUNDWATER BIOREMEDIATION

By

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1. Introduction

Recent laboratory and field studies have shown that anaerobic bioremediation processes can be effective for in-situ treatment of groundwater contaminated with chlorinated solvents and suggest the potential for treatment of a wide variety of other contaminants including nitrate, perchlorate, acid mine drainage, and certain heavy metals. The chemistry behind the remediation of chromate, nitrate, and AMD is simple. For each process, the oxidized ions (nitrate, chromate, sulfate, and their intermediates) are reduced by bacteria through anaerobic respiration. Nitrate and sulfate are readily used as alternative electron acceptors. However, chromate may be toxic to naturally occurring bacterial populations even at low concentrations (McLean et al; 2000).

The most common approach for stimulating in-situ anaerobic bioremediation of contaminated groundwater has been to circulate water containing a dissolved, readily biodegradable organic substrate through the treatment zone. This approach has been very effective at some sites (Ellis et al., 2000; Martin et al., 2001; Major et al., 2002). However problems with clogging of process piping, injection and pumping wells may increase operation and maintenance costs. An alternative approach employed at some sites has been to distribute a ‘slow-release’ organic substrate throughout the treatment zone that can support anaerobic biodegradation of the target contaminants for an extended time period. Slow-release substrates include cellulose, chitin, Hydrogen Release Compound (HRC®) and certain edible oils. HRC is a polymerized ester that dissolves over time releasing lactate which can support anaerobic biodegradation of chlorinated solvents and other contaminants (Koenigsberg et al., 2000; Wu 1999). A variety of edible fats and oils have been shown to
support reductive dehalogenation including corn oil, hydrogenated cottonseed oil beads, and solid food shortening (Dybas et al., 1997), beef tallow, melted corn oil margarine, and coconut oil (Lee et al., 1998). Zenker et al. (2000) screened a variety of organic substrates to identify materials that would slowly biodegrade over time. Subsequent studies showed that both liquid soybean oil and semi-solid hydrogenated soybean oil could support complete dehalogenation of TCE to ethene in microcosms containing sediment from a chlorinated solvent impacted site. Studies by Hunter (2001; 2002) demonstrated that soybean oil could be used to stimulate anaerobic degradation of other problem contaminants including nitrate and perchlorate.

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 SO₄ 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.
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 with small uniformly sized droplets is prepared using an edible oil (soybean oil), edible surfactants, and high energy mixing. The soybean oil provides a slow release organic substrate to support long-term anaerobic activity. 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 to form a PRB where the oil droplets are immobilized as a thin coating on the sediment and rock surfaces. 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 treatment in a variety of configurations including barriers, aerial treatments, and direct injection into spoil piles and mine tailings. The immobilized oil forms a subsurface treatment zone for the reduction of sulfate, nitrate, or chromate.

The objective of this work is to evaluate the use of soybean oil and soybean-based products in the remediation of groundwater contaminated with nitrate, chromate, and AMD. Soybean products were chosen for their relative cost-effectiveness and for their low impact on the environment. All results were obtained in lab microcosms and soil columns. No field scale results have been gathered within the boundaries of this project.
2. Environmental Impacts

2.1 Nitrate

Groundwater in large areas of the United States contains excessive levels of nitrate from animal and human waste disposal. Agricultural practices and leaking hog lagoons have resulted in very high nitrate concentrations in groundwater. Elevated nitrate concentrations are also a major problem associated with land disposal of treated sludge from domestic wastewater treatment plants. High nitrate concentrations in groundwater are a health concern and can lead to water quality problems in surface water. The US Geological Survey found that nitrate concentrations exceeded the drinking water standard in over 15% of the wells sampled, indicating that millions of acres are contaminated with excess nitrate (Canter, 1997). The principal effects of nitrate contamination arise as a result of the conversion of nitrate to nitrite in the oral cavity and/or stomach. Nitrite oxidizes hemoglobin to methaemoglobin, which interferes with the transport of oxygen by the blood (methaemoglobinemia). Young children are especially susceptible to this condition, thus it has the synonym Blue Baby Syndrome (National Institute for Public Health and Environmental Protection, 1989).

Nitrate is stable in aerobic environments. However, under anoxic conditions, denitrification can reduce nitrate into nitrogen gas.

\[
\text{C}_{56.3}\text{H}_{99.6}\text{O}_{6.0} \text{(liquid soybean oil)} + 62.6 \text{NO}_3^- \text{(Nitrate)} + 62.6 \text{H}^+ \rightarrow \\
56.3 \text{CO}_2 + 31.3 \text{N}_2 \text{(Nitrogen Gas)} + 81.1 \text{H}_2\text{O}
\]

The bacteria responsible for this process (known as denitrifiers) are facultative anaerobes that can use nitrate as a terminal electron acceptor in their metabolic pathways. Soybean oil acts
as the electron donor and carbon source. Denitrifying bacteria are sensitive to pH and prefer neutral conditions. Denitrification is also inhibited by dissolved oxygen, even at low concentrations.

2.2 Chromium

Chromium (Cr(VI)) is highly mobile, acutely toxic, mutagenic, teratogenic, and carcinogenic material. Hexavalent chromium (Cr$^{6+}$) exposure has been associated with an increased risk of lung cancer (McLean, et al, 2000). Due to its health effects, chromium and its compounds have been placed on the priority list of toxic chemicals in the US, UK, and Canada (Cheung and Gu, 2003). Chromium may be released into the environment due to improper storage and disposal of chrome plating, wood preserving and alloy formation wastes. The removal of chromium from the environment has been studied extensively, with principal methods involving physiochemical treatments, biosorption, and biotransformation. Biotransformation is the reduction of high valence-state chromium to a lower valence state through microbiological processes. Trivalent chromium (Cr (III)) is immobile under alkaline to slightly acidic conditions, and 100 times less toxic than hexavalent chromium. Since the initial reports of bacterial chromate reduction in the 1970s, chromate (CrO$_4^{2-}$) reduction has been observed under aerobic and anaerobic conditions by strains of gram-positive bacteria and fungi (Palmer and Puls, pp 1994). Other strains of bacteria including pseudomonas and a variety of sulfate reducing bacteria are capable of biologically removing hexavalent chromium from wastewater and groundwater (Cheung and Gu, 2003). The exact pathway of reduction is not always certain, but is most likely a detoxification mechanism, an electron acceptor in cellular metabolism, or an extracellular, enzymatic reaction. In any case, a
soybean oil substrate could act as a carbon source or electron donor for bacterial mediated chromium reduction.

2.3 Acid Mine Drainage

Coal and hard rock metal mining (including gold, copper, lead and zinc) often results in extensive 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 (FeS₂), pyrrhotite (FeS), chalcopyrite (CuFeS₂) and enargite (Cu₃AsS₄)] are exposed to oxygen and water, resulting in the formation of large amounts of sulfuric acid and dissolution of heavy metals including iron (Fe), manganese (Mn), copper (Cu), cobalt (Co), cadmium (Cd), nickel (Ni), and zinc (Zn) [see Figure 2.1]. The low pH, high sulfate and metals concentrations of surface waters wreak havoc on aquatic life. In the United States there are thousands of active mines, and between 100,000 and 500,000 abandoned mines (USEPA, 1995). These mines are a major environmental issue with more than 50 mine sites on the Superfund National Priorities list. USEPA (2000) lists 2.2 million acres of polluted surface water resulting from mining operations in just eight states. In Pennsylvania alone, costs for control of AMD from former coal mines are estimated to be between $5 and 15 billion (PADEP, 1997).
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. Naturally occurring iron reducing bacteria (IRB) and sulfate reducing bacteria (SRB) use the hydrogen (or organic acids) to reduce ferric iron (Fe$^{3+}$) and sulfate (SO$_4^{2-}$) to pyrite (FeS$_2$). Shown below is a representative reaction for iron and sulfate
reduction using sulfate as the terminal electron acceptor. These reactions consume H⁺ causing a decline in acidity and increase in pH.

\[
\text{Fe}^{3+} + \text{H}^+ + 2 \text{SO}_4^{2-} + 7.5 \text{H}_2 \rightarrow \text{FeS}_2 + 8 \text{H}_2\text{O}
\]

In some AMD impacted aquifers, the amount of sulfate present exceeds that required for precipitation of the dissolved iron and the excess sulfate will be released as sulfide (S²⁻) through the following reaction.

\[
\text{SO}_4^{2-} + \text{H}_2 \rightarrow \text{S}^{2-} + 4 \text{H}_2\text{O}
\]

The excess sulfide will then react with other heavy metal ions forming insoluble precipitates as the pH increases.
3. Experimental Methods

The methods employed in our experiment were designed to mimic groundwater conditions typically seen in contaminated sites. Microcosm and soil column experiments were performed in the NCSU Environmental Engineering Lab. In all cases, care was taken to ensure the quality of results and the integrity of samples. Duplicate analyses were conducted on 10% of all samples to evaluate the reproducibility of the analytical results.

3.1 Analytical Materials

Sulfate, nitrate, nitrite, and acetate were analyzed following standard method 4110B: 'Ion chromatograph with chemical suppression of eluent conductivity' (Eaton et al; 1995). Typically samples were taken from sample ports at the influent and effluent of the columns using a 5-mL disposable syringe and diluted 1:9 with a carbonate eluent matrix for chromatograph separation. Samples were allowed to sit 24 hours to allow the dissolved iron to precipitate out of solution. The samples were then filtered using a 0.45 micron syringe filter and frozen until analyzed.

A standard lab analytical pH probe and meter were used for all for pH measurements. The meters were calibrated before and after every 15-20 samples using pH 4.0 and 7.0 buffers. 5-mL samples were analyzed immediately after sampling. Standard storage, handling, and calibration procedures supplied by the manufacturer were used.

Total dissolved organic carbon was analyzed using both the Dohrman Scientific DC-190 TOC analyzer and the Shimadzu TOC 5000A analyzer with autosampler. Standard
procedures were followed for both instruments using the protocol supplied by the manufacturers. Pure samples and manual injections were used for the DC-190, which measured total carbon (TC) and inorganic carbon (IC) to find the total organic carbon (TOC) in the sample. For the 5000-A, samples were diluted 7:1 with DI water prior to analysis. Samples were stored in 2.5-mL serum vials at 4°C until analyzed.

Dissolved oxygen was measured using Chemet DO ampoules. The detectable range for the Chemet kits was 0-1.0 mg/L of dissolved oxygen. Immediately after sampling, the tip of the ampoule was inserted directly into the syringe and carefully broken into the sample while pushing in the plunger. While somewhat difficult at first, the method limits exposure to atmospheric conditions.

Measurements of dissolved metals, and total sulfur were all performed on a Perkins Elmer Plasma II Ion Coupled Plasma Argon Emission Spectrometer (ICP-AES). Typically 9:1 dilutions were used in a 0.1 N HCl matrix for transport of the sample. Samples were filtered prior to dilution in acid through a 0.45 micron syringe filter. No refrigeration was necessary for storing the samples prior to analysis.

Soil samples from the columns were analyzed for carbon using a CHN elemental analyzer after being dried in a 105°C oven for 48 hours for estimates of remaining carbon in the system. Gas headspace measurements of methane were analyzed using gas chromatography.
3.2 Experimental Materials

Sediments used for the experiments included field sand from a local supplier (Caudle Sand and Rock, Raleigh, NC), acid mine spoils from a former coal mine in Sequatchie Valley, TN, and aquifer material from hand auger samples at the Raleigh Neuse River Waster Water Treatment Plant (NRWWTP). Sediments were passed through a No. 4 standard sieve (4.75 mm) before use in microcosms and column experiments to remove the larger particles.

The field sand was used in the nitrate and chromate intermittent flow columns because of its favorable permeability. The texture of the sand can be described as having a median grain size of 0.38 mm, a coefficient of uniformity (D₆₀/D₁₀) of 4.5, and 7% passing a No 200 sieve (75 µm). Detailed texture analyses were not performed on the other sediments. The acid mine spoils were used in both the microcosm and flow column experiments, and appeared to be a sandy loam after the larger particles were removed by the No 4 sieve. Relatively high permeability was observed in the column experiments for the AMD spoils. Sediment samples for the nitrate and chromate microcosm experiments were collected from 16 feet below grade at the Raleigh NRWWTP sludge application fields using a hand auger. This material consisted of a low permeability sandy-silt. It was not used in column experiments due to its low permeability. The soils used in each microcosm study are outlined in table 3.1.

The emulsions used for column treatments were prepared according to the procedure outlined by Coulibaly and Borden (2003) by blending 33% by volume soybean oil, 62% water, and 5% premixed surfactant (38% polysorbate 80, 56% glycerol monooleate GMO from Lambent Technologies, and 6% water) in a Waring Commercial blender at high speed for five
12 minutes. The wax emulsions were prepared using a fully hydrogenated soybean oil wax that was melted in a hot water bath. Hot water was used in the blending process as well, and negligible separation occurred post-mix. The AMD columns exclusively were treated with the commercially available edible oil substrate (EOS®). 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).

3.3 Microcosm Procedure

Microcosm experiments were conducted to evaluate the potential of several different soybean products to enhance biodegradation and/or immobilization of three major types of pollutants: (1) nitrate; (2) chromium; and (3) AMD. Products evaluated include liquid soybean oil, fully hydrogenated soybean oil, soy methyl esters, blown soybean oil, and molasses. The basic apparatus for these studies consisted of 160 or 240 mL serum bottles fitted with rubber stoppers and aluminum crimp seals to exclude oxygen. Each experimental treatment was prepared in triplicate with each bottle containing sediment, a solution of desired pollutant, and bacterial inocula where necessary.

Table 3.1 Experimental Conditions for Nitrate, Chromate, and AMD microcosms

<table>
<thead>
<tr>
<th>Bottle Vol</th>
<th>Sediment (Vol)</th>
<th>Liquid Vol</th>
<th>Contaminant Conc</th>
<th>Inoculated?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrate</td>
<td>160 mL NRWWTP soil (50 mL)</td>
<td>100 mL</td>
<td>18 mM KNO₃</td>
<td>No</td>
</tr>
<tr>
<td>Chromate</td>
<td>160 mL NRWWTP soil (50 mL)</td>
<td>100 mL</td>
<td>0.02 mM K₂Cr₂O₇</td>
<td>No</td>
</tr>
<tr>
<td>AMD</td>
<td>240 mL AMD spoils (25 mL)</td>
<td>190 mL</td>
<td>20 mM FeSO₄</td>
<td>Yes</td>
</tr>
</tbody>
</table>
A variety of substrates or environmental conditions can be evaluated in this manner to identify optimal conditions for bacterial growth. Both autoclaved, killed controls and live, no added carbon controls were included to observe background reduction. Incubations were typically monitored over time for changes in aqueous pollutant concentration, pH, dissolved organic carbon, and gas production.

3.4 Intermittent Flow Column Procedure

Liquid soybean oil, fully hydrogenated wax, and EOS® were selected based on the microcosm results for further evaluation in intermittent flow column experiments. The columns were designed to replicate the aquifer environment using sediment and slow influent flow rates. The basic apparatus consisted of 2” PVC pipes (4.1 cm interior diameter) cut to 30 cm lengths, packed with sediment, capped and fitted with brass fixtures. Packing the columns began with sheathing the bottom end with end caps fixed with PVC cement. Brass fixtures were inserted and sealed with silicon gel. A circular, fine synthetic mesh screen followed by synthetic fiber packing material was inserted into the bottom of the column to prevent sediment washout. Tubing was attached at the bottom and a small amount of water allowed to flow through and quickly clamped to prevent air bubbles. The sediment was packed wet with a rubber tamp for tight settlement of the material and to limit entrapped air. Once the columns were filled, the top end caps were fitted as the bottom end caps with synthetic cotton packing and a fine mesh. Figure 3.1 illustrates the packing procedure for all the intermittent flow columns.
Masterflex peristaltic pumps were used to intermittently pump an influent solution at 20 mL per day through each column. The pumps were activated daily by automatic timer for three minutes during which time the 20 mLs were pumped through each column. This procedure was necessary due to the desired low flow rate and limitations of the pumps. The effluent sampling ports were fitted with syringe connections where a 5-mL disposable syringe could be connected, the pumped turned on and samples obtained. The injection port was used to inject the soybean treatment solution. Figure 3.2 illustrates the general setup for the columns.
3.5 Carbon Mass Balance Procedure

One of the major benefits of soybean oil and wax emulsion treatments is the longevity of the carbon source. Mass balance calculations were performed to evaluate the longevity of each soybean treatment. The mass of carbon added to each column as soybean emulsion and the carbon released from each column was calculated based on effluent TOC, IC, and volatile solids (VS). Volatile solids are defined as the material burned off at 550°C. VS samples are first weighed, dried at 105°C for 48 hours, re-weighed, burned in a muffle furnace at 550°C for two hours, and then weighed once more. Using this procedure the moisture content and VS content can be determined for both liquid and solid samples. VS analyses are not appropriate for low concentration samples, due to the difficulty measuring the small differences in weight of low concentration samples. The amount of carbon added to the columns was estimated to be 77% of VS by weight (soybean oil is 77% carbon). TOC and IC samples were taken every two weeks to determine the long term carbon mass flux.
Biologically mediated redox reactions convert organic carbon to carbon dioxide and other oxidized organic compounds. The amount of carbon dioxide produced can be calculated from the total mass of nitrate, chromate, iron, copper, manganese, sulfate, and oxygen reduced based on the following balanced oxidation-reduction reactions (Sawyer, et al, 1994). These reactions assume the organic substrate is completely mineralized to carbon dioxide without any partially oxidized intermediates produced.

\[
\text{C}_{56.3}\text{H}_{99.6}\text{O}_{6.0} \text{ (liquid soybean oil)} + 106.6\text{H}_2\text{O} \rightarrow 56.3\text{CO}_2 + 312.8\text{H}^+ + 312.8\text{e}^- \quad (1)
\]

\[
\text{C}_{56.3}\text{H}_{108.6}\text{O}_{6.0} \text{ (fully hydrogenated soybean)} + 106.6\text{H}_2\text{O} \rightarrow
56.3\text{CO}_2 + 321.8\text{H}^+ + 321.8\text{e}^- 
\]

\[
\text{C}_{50.8}\text{H}_{90.9}\text{O}_{7.2} \text{ (EOS\textsuperscript{®})} + 94.4\text{H}_2\text{O} \rightarrow 50.8\text{CO}_2 + 279.7\text{H}^+ + 279.7\text{e}^- \quad (3)
\]

\[
\text{O}_2(\text{aq}) + 4\text{H}^+ + 4\text{e}^- \leftrightarrow 2\text{H}_2\text{O} \quad (4)
\]

\[
2\text{NO}_3^- + 12\text{H}^+ + 10\text{e}^- \leftrightarrow \text{N}_2 + 6\text{H}_2\text{O} \quad (5)
\]

\[
2\text{SO}_4^- + 19\text{H}^+ + 16\text{e}^- \leftrightarrow \text{H}_2\text{S} + \text{HS}^- + 8\text{H}_2\text{O} \quad (6)
\]

\[
\text{Fe(OH)}_{3(s)} + 3\text{H}^+ + \text{e}^- \leftrightarrow \text{Fe}^{2+} + 3\text{H}_2\text{O} \quad (7)
\]

\[
\text{Cr(VI)} + 3\text{e}^- \leftrightarrow \text{Cr(III)} \quad (8)
\]

\[
\text{Mn(III)} + \text{e}^- \leftrightarrow \text{Mn(II)} \quad (9)
\]

\[
\text{Cu(III)} + \text{e}^- \leftrightarrow \text{Cu(II)} \quad (10)
\]

In these reactions, the chemical formula for liquid soybean oil, hydrogenated soybean oil and EOS\textsuperscript{®} are determined assuming the average composition of substrate. Liquid soybean oil is assumed to contain 1.0 mole of glycerol, 0.75 moles of oleic acid, 1.56 moles of linoleic acid, 0.21 moles of linolenic acid, 0.12 moles of stearic acid, and 0.36 moles of palmitic acid.
Fully hydrogenated soybean oil is assumed to contain 1.0 mole of glycerol, 2.64 moles of stearic acid, and 0.36 moles of palmitic acid. EOS® contains 0.16 moles of lactic acid, 0.08 moles of yeast extract, 0.24 moles of polysorbate 80, 0.16 moles of glycerol mono oleate, and 2.36 moles of liquid soybean oil. Since no change in oxidation state occurs for zinc or aluminum, these metals were not included in the electron balance calculations. The following ratios in table 3.2 were used to calculate the mass of CO₂ produced for each respective ion reduced based on the balanced redox reactions.

<table>
<thead>
<tr>
<th>Electron Acceptor (EA)</th>
<th>Liquid Soybean g IC/g EA</th>
<th>Fully Hydrogenated Soybean g IC/g EA</th>
<th>EOS® g IC/g EA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxygen (MW 32)</td>
<td>0.990</td>
<td>0.962</td>
<td>1.000</td>
</tr>
<tr>
<td>Nitrate (MW 62)</td>
<td>0.639</td>
<td>0.621</td>
<td>0.644</td>
</tr>
<tr>
<td>Sulfate (MW 96)</td>
<td>0.660</td>
<td>0.641</td>
<td>0.666</td>
</tr>
<tr>
<td>Iron (MW 55.8)</td>
<td>0.142</td>
<td>0.138</td>
<td>0.143</td>
</tr>
<tr>
<td>Chromium (MW 52)</td>
<td>0.457</td>
<td>0.444</td>
<td>0.461</td>
</tr>
<tr>
<td>Manganese (MW 54.9)</td>
<td>0.144</td>
<td>0.140</td>
<td>0.145</td>
</tr>
<tr>
<td>Copper (MW 63.5)</td>
<td>0.125</td>
<td>0.121</td>
<td>0.126</td>
</tr>
</tbody>
</table>
4. Nitrate Results

Soil and groundwater were collected from the Neuse River Waste Water Treatment Facility in Raleigh, NC for use in the nitrate reducing microcosms. Each bottle was constructed in triplicate and contained 100 mL of groundwater, 250 mg/L nitrate as nitrogen, 50 mg of the target substrate to be evaluated, and 50 mL of moist sediment. A variety of carbon sources were evaluated for stimulating nitrate reduction including liquid soybean oil, blown soybean oil, fully hydrogenated soybean oil wax, mineral oil, soy methyl ester (SoyGold 1100) and molasses.

![Figure 4.1](image)

Figure 4.1: Effect of different carbon sources on nitrate and nitrite reduction in batch microcosms containing Raleigh WWTP sediment and groundwater.

The sum of nitrate and nitrite as total nitrogen in the experimental microcosms is shown in Figure 4.1. Blown soybean oil, liquid soybean oil, SoyGold 1100 and molasses stimulated denitrification. Molasses addition did not result in complete nitrate removal. The microcosms with molasses substrate received an average of 69 mg of pure molasses (more
than the target 50 mg). Molasses is primarily glucose (C₆H₁₂O₆) and contains 32.5% carbon by weight, so each microcosm was spiked with 22.4 mg of organic carbon capable of reducing 30 mg of nitrate as N. The mass of nitrate in each microcosm was approximately 25 mg as N, and TOC levels in the molasses microcosms were an average of 74 mg/L after 224 days, indicating sufficient electron donor for further denitrification. However, bioavailable organic carbon could have been depleted through other side reactions (iron and sulfate reduction and methanogenesis) although no measurements were taken that corroborate with this presumption. In contrast to molasses, blown soybean oil, liquid soybean oil and SoyGold 1100 (soy methyl ester) resulted in complete nitrate and nitrite removal from all microcosms in 350 days. The fully hydrogenated soybean oil wax resulted in some denitrification. However denitrification rates were significantly lower than for the other materials. There was no significant nitrate removal in the microcosms amended with mineral oil. Each bottle was amended with enough substrate to bring the organic carbon concentrations to 200-400 mg/L. However, TOC levels were lower than expected as reported in figure 4.2. Background TOC varies from 1-10 mg/L in killed and live controls.

![Figure 4.2: Variation in TOC concentrations in the Nitrate Reducing Microcosms](image-url)
Microbial activity or sorption prevented TOC levels from peaking during the first 100 days, but after sources of electron acceptor were depleted, microbial activity slowed and the TOC levels built up in the blown soybean, SoyGold 1100, and molasses amended microcosms. Biomass turnover could also have resulted in the peak of TOC on day 224.

Other parameters measured included nitrite and sulfate. Only the fully hydrogenated soybean microcosm produced significant nitrite concentration in one of the three triplicates. The nitrite concentration steadily rose to a peak of 328 mg/L after 401 days. The other two fully hydrogenated soybean amended microcosms produced no detectable nitrite (detection limit = 5 mg/L). Sulfate was consistently at or below the detection limit of 5 mg/L.

Based on the microcosm results, liquid soybean oil and the hydrogenated soybean oil wax were selected for further study in intermittent flow columns. The columns were constructed as described in the column packing procedure and packed with field sand from a local quarry. The influent to each column contained 4.0 mM potassium nitrate (56 mg/L NO₃-N) and 100 mg/L calcium chloride solution. The columns were operated for two weeks without any substrate to establish equilibrium conditions. Column ‘N2’ was treated with 100 mL of 8% soybean oil-in-water emulsion, column ‘N3’ was treated with 100 mL of 8% hydrogenated soybean oil wax emulsion, and column ‘N1’ was left untreated as a control. No inoculum was added due to the natural abundance of denitrifying bacteria. The following results include an illustration of the influent and effluent nitrate concentrations, iron, dissolved oxygen, pH, acetate, TOC, and IC for each column. Graphs are also included representing amounts of CO₂ that could be produced from each electron acceptor and the measured IC
levels. Acetate concentrations are shown in units of mg/L as C for comparison with TOC and IC results.

Figure 4.3 shows the influent and effluent concentrations versus time in the control column, N1. Nitrate concentrations remained high in the effluent at ~60 mg/L as N. Dissolved oxygen was present in the effluent at ~6 mg/L and no dissolved iron was found, indicating oxidizing conditions were maintained throughout the duration of the experiment.
Figure 4.3: Control Column (N1) results: (a) nitrate, dissolved iron, DO and pH in column influent; (b) nitrate, dissolved iron, DO and pH in column effluent; (c) acetate, TOC, and IC as mg/L C in column effluent; and (d) potential IC production.
There was little or no difference between influent and effluent DO, nitrate, and iron so the potential IC production was negligible. Experimental IC measurements show up to 8 mg/L IC was produced over the initial 18 days, presumably due to oxidation of background organic carbon.

Column N2 was allowed to equilibrate with the influent nitrate solution for two weeks and then treated with 100 mL of 8% soybean emulsion. The first sample period represents day zero on the following graphs, and the emulsion injection occurred on day four. No inoculum, yeast extract, or lactic acid was used, and successful denitrification was achieved. Figure 4.4 shows that within one week of soybean oil treatment, nitrate was removed to below detection and has remained below detection for four months. Nitrite also remained below detection for the remainder of the experiment.
Figure 4.4: Liquid soybean treated column (N2) results: (a) nitrate, dissolved iron, DO and pH in column influent; (b) nitrate, dissolved iron, DO and pH in column effluent; (c) acetate, TOC, and IC as mg/L C in column effluent; and (d) potential IC production. Emulsion injection occurred on day 4 and TOC values peaked at 1400 mg/L on day 32 of sampling.
The liquid soybean emulsion treatment led to a sharp increase in the solubilized Fe (II), and dissolved oxygen in the effluent dropped to non-detect due to the strongly reducing conditions in the column. The effluent pH increased slightly from the control to ~6.5 due to the consumption use of hydrogen ions in denitrification as shown in equation (4). The potential rate of inorganic carbon production indicates a sharp peak around day 30 due to a higher flow rate of 28 mL/d. By day 60 the flow was reduced to the target rate of 20 mL/d. The potential IC production in mg/d varies with flow rate. After day 75, the inorganic carbon production stabilized around 0.8 mg/d of IC released. When combined with the organic carbon released by the column, the carbon consumption rate at the end of the experiment was ~1.4 mg/d.

The large initial peak of potential IC is due to the higher flow rates at the beginning of the experiment. From day zero to day 132, the measured IC values are less than the potential values. However as effluent organic carbon concentrations declined, the IC levels increased and the majority of the carbon was released as IC, not TOC. Theoretically the bacteria should use the substrate inefficiently when it is in abundance and more completely when starved for electron donor. This is also reflected in the graph of TOC, acetate, and IC. Initially inefficient substrate breakdown by the bacteria leads to carbon released from the system as acetate and possibly other mobile long chain organic carbon compounds (not measured). But as the column equilibrates and TOC concentrations dip, the bacterial metabolism becomes more efficient at breaking down the remaining organic carbon and producing CO₂. This translates into effective theoretical predictions into carbon released from the system and makes it possible to estimate the remaining lifespan of the substrate.
treatment. The release of dissolved iron seems to correspond with TOC release. Organic compounds could chelate with iron and flush it out of the system, or as the bacteria become more starved for electron donor, consumption of secondary electron acceptors such as Fe (III) may decline. However the decline in dissolved iron could also be due to depletion of readily reducible iron oxides.

The final column in the nitrate study is N3, treated with emulsified fully hydrogenated soybean wax. Emulsion injection occurred on day 4. The results are shown in figure 4.5.
Figure 4.5: Fully hydrogenated soybean wax treated column (N3) results: (a) nitrate, dissolved iron, DO and pH in column influent; (b) nitrate, dissolved iron, DO and pH in column effluent; (c) acetate, TOC, and IC as mg/L C in column effluent; and (d) potential IC production. Emulsion injection occurred on day 4 and TOC values peaked at 1300 mg/L on day 18 of sampling.
Nitrate was reduced to below detection by two weeks after emulsion injection and remained below detection for the life of the experiment. The release of carbon was more gradual and sustained in the wax emulsion treated column than the liquid soybean oil column where we saw a large peak of TOC and rapid drop in the effluent. Patterns in the dissolved iron and oxygen levels were similar, representing highly reduced conditions. Iron and TOC levels also dropped as IC levels rose. The calculated carbon balance through the columns revealed inconsistencies between the potential and measured values for effluent IC. The measured IC concentrations are lower than the potential values, until the latter part of the experiment. This may be due to incomplete oxidation of organic substrates, or due to loss of IC during sample collection and storage. There is no way to determine if CO₂ was lost during processing. However the high acetate levels in the column effluent during the early portion of the experiment indicate that soybean was not completely converted to IC. The figure comparing acetate levels as compared to IC and TOC breakthrough for the column clearly shows elevated acetate levels in the column effluents during the initial stages, and increased IC levels in the latter stages as carbon sources begin to diminish and bacteria demonstrate greater efficiency of carbon substrate use. The TOC data should be more accurate and mass balances do show consistencies between potential and measured TOC. The initial peak of potential IC production is due to higher flow rates during the start up period of the study.

The column treated with fully hydrogenated soybean wax emulsion, N3, illustrated similar nitrate removal, but more residual carbon distribution. For the month after treating the columns with emulsion, the volatile solids were measured in the effluent of each column. The control column released very little VS (72.3 mg as carbon). The liquid soybean
emulsion treated column released significant VS; about 22.9% of the injected carbon was released in the first 2 weeks of monitoring (1443 mg as carbon). As expected the fully hydrogenated soybean emulsion was less mobile, and a greater percentage remained within the soil column after treatment; only 3.6% of the injected carbon was released (238 mg as carbon). The liquid soybean and fully hydrogenated wax treated columns received essentially the same amount of emulsion. However, more of the liquid soybean oil emulsion was discharged in the column effluent. Figure 4.6 shows a comparison of cumulative total carbon (TC) released from the liquid soybean oil and fully hydrogenated wax treated columns. The liquid soybean oil treated column released high initial levels of TC suggesting that this material will spread further down gradient in the subsurface. The wax emulsion treatment may require less frequent replenishment.

![Graph showing cumulative total organic carbon in nitrate column effluent](image)

**Figure 4.6: Cumulative total organic carbon in nitrate column effluent**

An overall carbon balance for the three columns is shown in Table 4.1. The total carbon added through treatment is shown with the initial loss based on our volatile solids analysis over the first two weeks after injection. The initial TOC release reflects an extended initial carbon loss before the treatment equilibrates. The remaining carbon was measured by the
The dry weight of soil samples taken at the end of the experiment. Remaining carbon is expressed as the mean of three samples and shows the analytical error based on two standard deviations. The mass balance error may be in a small part due to carbon trapped in the synthetic screens or the small amount of soil in the end caps of the columns that was not analyzed.

Table 4.1: Carbon Balance for Nitrate Columns

<table>
<thead>
<tr>
<th></th>
<th>N1</th>
<th>N2</th>
<th>N3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg C</td>
<td>mg C</td>
<td>%</td>
</tr>
<tr>
<td>Carbon Injected</td>
<td>0</td>
<td>6324</td>
<td>100.0%</td>
</tr>
<tr>
<td>Initial VS Release</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(day 0-14)</td>
<td>72</td>
<td>1443</td>
<td>22.8%</td>
</tr>
<tr>
<td>Initial TOC Released</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(day 14-47)</td>
<td>4</td>
<td>941</td>
<td>14.9%</td>
</tr>
<tr>
<td>Secondary TOC Released</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(day 48-187)</td>
<td>1</td>
<td>106</td>
<td>1.7%</td>
</tr>
<tr>
<td>Measured IC Released</td>
<td>2</td>
<td>69</td>
<td>1.1%</td>
</tr>
<tr>
<td>Potential IC Released</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Remaining Carbon</td>
<td>0 (± 33)</td>
<td>2238 (± 313)</td>
<td>35.4% (± 5.0%)</td>
</tr>
<tr>
<td>Missing Carbon</td>
<td>0</td>
<td>1426 (± 313)</td>
<td>22.5% (± 5.0%)</td>
</tr>
<tr>
<td>Carbon Consumption</td>
<td>0.0</td>
<td>1.3</td>
<td>1.9</td>
</tr>
<tr>
<td>Rate (day 48-187)</td>
<td></td>
<td>(mg/d)</td>
<td></td>
</tr>
</tbody>
</table>

The balance indicates that 20-40% of the added carbon could not be accounted for. Based on remaining carbon and carbon consumption rates measured over the last 140 days of the
experiment, it is possible to calculate potential treatment lifespan. The following table 4.2 shows the total longevity of the treated columns including the experimental period.

Table 4.2: Potential lifespan of nitrate columns

<table>
<thead>
<tr>
<th>Column ID</th>
<th>N2</th>
<th>N3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>Liquid Soybean</td>
<td>Fully Hydrogenated Soybean</td>
</tr>
<tr>
<td>Carbon Remaining (mg)</td>
<td>2238</td>
<td>3035</td>
</tr>
<tr>
<td>Carbon Consumption Rate (mg/d)</td>
<td>1.3</td>
<td>1.9</td>
</tr>
<tr>
<td>Lifespan (years)</td>
<td>5.2</td>
<td>4.9</td>
</tr>
</tbody>
</table>

The resulted show that the liquid soybean oil treatment and the fully hydrogenated soybean treatment would have lasted 5.2 and 4.9 years respectively including the experimental operating time. The experimental treatment sizes were grossly designed to last the duration of the test, and field scale treatments could release carbon for many years.

Another principle concern for soybean treatment barriers is the loss of permeability due to emulsion injection. The concept of a permeable reactive barrier system relies on the groundwater flowing through the barrier and not around it. Excessive loss in hydraulic conductivity could lead to problems in design, and thus the permeability of the soil columns was measured before treatment and at the end of the experiment. The results are displayed in table 4.3. The control column experiences a negligible loss, and remained around 0.005-0.002 cm/s as seen in the other two columns before treatment. Column N2 treated with
liquid soybean emulsion resulted in a factor of 10 loss in permeability. This can be expected due to soybean oil’s hydrophobic nature and tendency to fill large voids in the sediment where normally water would flow through. The fully hydrogenated soybean treatment in column N3 resulted in a larger loss in permeability by a factor of 100. This could significantly hinder the ability of contaminated groundwater to flow through the system and be treated. As the soybean wax cools, it will harden and remain in the sediment where it blocks flow paths for water. Biological growth could also be a factor in permeability loss as well as the formation of nitrogen bubbles in sediment pores from denitrification. Certainly the effect of soybean treatment on the permeability must be taken into account for the design of a PRB.

Table 4.3: Permeability Losses in Nitrate Reducing Columns

<table>
<thead>
<tr>
<th>Column ID</th>
<th>K (Before Emulsion) Measured 3/21/03</th>
<th>K (Final) Measured 10/7/03</th>
<th>K final/ K initial (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N1 (control)</td>
<td>5.2E-3 (cm/s)</td>
<td>2.0E-3 (cm/s)</td>
<td>38.5</td>
</tr>
<tr>
<td>N2 (liquid soy)</td>
<td>5.8E-3 (cm/s)</td>
<td>3.3E-4 (cm/s)</td>
<td>5.7</td>
</tr>
<tr>
<td>N3 (wax soy)</td>
<td>4.8E-3 (cm/s)</td>
<td>3.6E-5 (cm/s)</td>
<td>0.8</td>
</tr>
</tbody>
</table>

The extent and longevity of the loss in permeability is not certain and may not be able to be extrapolated to field conditions. If the soybean treatment is the major cause for permeability loss, we would see an initial loss and slow recovery in hydraulic conductivity over time. If bioactivity is clogging the soil pores, then the permeability could gradually decrease with cellular growth. With just these two measurements it is impossible to know the trend in
permeability changes. The loss permeability could have also affected the flow rate through the columns, as seen in the decline of potential IC production graphs previously. For full scale systems a method for increasing flow pressure towards the PRB may have to be implemented, such as a sheet pilings funnel.

**Summary of Nitrate Results**

Complete and sustained denitrification was observed in both the liquid soybean oil and fully hydrogenated soybean wax emulsion treated columns. This experiment successfully demonstrated the potential application of emulsified soybean oils into groundwater systems contaminated with nitrate. By incorporating liquid soybean and solid soybean wax emulsions, the longevity and reducing capabilities of the treatment area can be controlled. The average HRT for the columns was 5 days, so the results show a rapid nitrate removal rate. Potential problems associated with the treatment are the release of reduced iron which may or may not be of major concern, and the loss of permeability which may only be a temporary issue and not applicable to field conditions. The overall benefits include the in-situ approach, low environmental impact, and effective removal of nitrate.

The error in the carbon balance analyses could be in part due to the formation of calcite (CaCO₃, log K = -8.3) within the column (Bodek et al; 1988). To calculate the mass of carbonate removed by this reaction, the following formula was used:

\[ [Ca] \times [CO_{3}^{2-}] = 10^{-8.3} \]

Calcium was being pumped to the column as 100 mg/L CaCl₂ (0.9 mM). However the amount of carbonate removed at these concentrations is minimal.
5. Chromate Results

Chromium (Cr) can be immobilized in aquifer sediments by converting the highly soluble, toxic form, Cr [VI], to the less toxic, much less mobile form, Cr [III], under anaerobic conditions. The nitrate reducing microcosms from our initial experiment formed the basis for our chromate reducing microcosms. Since successful denitrification was achieved, the microcosms were expected to provide a good consortium of anaerobic bacteria to reduce chromate. The nitrate microcosms were incubated for 401 days and then spiked with 0.02 mM K$_2$Cr$_2$O$_7$ and brought to the original water level. Thus day 401 for the nitrate microcosms is day zero for the chromate microcosms. The killed control was autoclaved twice, and all bottles were sampled the next day. The measured chromium concentration was varied, and the amendment concentration of 2 mg/L Cr was not observed in any bottle. Oddly the killed control yielded one of the lowest levels. Other bottles that apparently fully reduced chromate to below detection were the liquid and blown soybean oil treated bottles. The other treatments, including the live control exhibited gradual removal of soluble chromium, and led us to believe that chromium could be readily removed from the system given even low carbon and electron donor sources. The nature of chromium removal in the microcosms is unclear, but could also be sorption to the sediment. Figure 5.1 shows results from the microcosm study.
Three columns were constructed and operated to evaluate the potential application of soybean products for chromium treatment. The experimental protocol followed the same basic procedures as used in previous studies. Three replicate columns were packed with field sand and operated with an influent solution of 0.1 mM Cr. One column was then treated with 100 mL of 11% emulsified liquid soybean oil and a second column received 100 mL of 11% emulsified soybean oil wax. These columns were then operated for approximately two months while monitoring for changes in dissolved oxygen, chromium and TOC.

Effluent chromium concentrations were very similar in the soybean oil emulsion, soybean oil wax emulsion and untreated columns with essentially 100% breakthrough of the influent chromium indicating negligible removal. Effluent dissolved oxygen concentrations were also very high in all three columns indicating no significant biological activity in any of the columns. These results suggest that the chromium solution was toxic to microorganisms in the columns preventing the development of anaerobic conditions. These results illustrated in

![Graph showing chromium concentrations over time](attachment:chromate_microcosm_study.png)

Figure 5.1: Results of chromate microcosm study. The microcosms consist of the previous nitrate study spiked with 0.02 mM potassium dichromate
Figure 5.2 indicate that when high levels of toxic heavy metals are present, stimulation of anaerobic bioremediation processes using soybean oil (or any other material) may be difficult. Significant sorption of chromium to column sediment was observed, and effluent concentrations did not equilibrate with the influent for well over one month despite the 5 day HRT. The graph does not show the initial two week equilibration period.

![Figure 5.2: Chromate concentration in the initial column experiment. Treatment injection occurred on day 6 and failure to observe chromate reduction after 2 months terminated the study.](image)

To develop a better method for stimulating chromium immobilization using soybean oil, separate field sand columns were constructed and allowed to equilibrate with 4.0 mM KNO₃ and 100 mg/L CaCl₂ influent solutions. Liquid soybean and fully hydrogenated soybean emulsions were prepared and injected into two separate columns, with a third as a control. The columns were monitored until complete denitrification was observed in the treated columns. These columns were to serve as pre-stressed environments, where a consortium of bacteria has established themselves prior to chromate introduction. After one month, significant denitrification occurred in the two treated columns, and the influent solution was changed to 0.05 mM potassium dichromate (K₂Cr₂O₇) solution with 50 mg/L CaCl₂.
Figures 5.3-5.6 illustrate the results of the second chromium experiment. Analytical measurements were similar to the nitrate columns. The graphs show the variation in chromate, nitrate, iron, dissolved oxygen in the influent and effluent over time, TOC, IC, and acetate in mg/L as C in the effluent, and potential IC production from the different electron acceptors. The ampoules used for detection of dissolved oxygen are not accurate when the sample contains chromate, so no influent DO measurements are shown.

Results from the control column C1 are shown in figure 5.3. The high nitrate and dissolved oxygen concentrations in the effluent show that reducing conditions did not develop. However, when low concentrations of chromium (5.5 mg/L) were introduced, breakthrough was not observed until the final 2 weeks of the experiment, and only at 50% of the influent concentration. The mechanism for chromium removal is uncertain.
Figure 5.3: Control column (C1) results: (a) nitrate, dissolved iron, DO and pH in column influent; (b) nitrate, dissolved iron, DO and pH in column effluent; (c) acetate, TOC, and IC as mg/L C in column effluent; and (d) potential IC production. Solution change occurred on day 49.
The exact reason for chromium removal in the control column is not certain, but could be due to ion exchange or sorption on the clay rich sediment. Low levels of dissolved iron were also mobilized, indicating reducing conditions. Although the field sand control column used in our experiment removed low levels of chromate, it could still remain immobilized in the soil in its more toxic hexavalent state. The initial peak in potential IC production is due to the presence of nitrate in the influent solution.

Column C2 was treated on day 6 with emulsified liquid soybean oil and results show significant denitrification activity after one month. The influent solution was then switched to 0.05 mM potassium dichromate and 50 mg/L calcium chloride. Figure 5.4 shows the results of the experiment.
Figure 5.4: Liquid soybean treated column (C2) results: (a) nitrate, dissolved iron and pH in column influent; (b) nitrate, dissolved iron, DO and pH in column effluent; (c) acetate, TOC, and IC as mg/L C in column effluent; and (d) potential IC production. Emulsion injection occurred on day 6. Solution change to Cr occurred on day 49.
Chromium was never detected in the column effluent above the 0.5 mg/L detection limit. Redox conditions are believed to be strongly reducing due to the low dissolved oxygen and presence of reduced iron in the effluent. Initial TOC levels were high, but only 4.4% of the added carbon passed out of the column as measured TOC over the entire course of the experiment. From the few acetate measurements made on this column, the majority of this TOC is believed to be composed of soybean oil degradation products including acetate and long chain fatty acids. The graph representing the conversion of electron acceptors to CO$_2$ shows that chromate at such low levels does not dominate the bacterial metabolism. The results show promising chromate removal and indicate that emulsified soybean oil as a viable option for PRB construction.

The third column, C3, was treated with emulsified fully hydrogenated soybean oil, and yielded similar results to those seen in column C2. The initial nitrate level was reduced to non-detect in the column effluent, representing the healthy growth of anaerobic organotrophs, before the influent was changed over to a 5.5 mg/L chromium and 50 mg/L calcium chloride solution. Figure 5.5 shows the results from that experiment.
Figure 5.5: Fully hydrogenated soybean treated column (C3) results: (a) nitrate, dissolved iron and pH in column influent; (b) nitrate, dissolved iron, DO and pH in column effluent; (c) acetate, TOC, and IC as mg/L C in column effluent; and (d) potential IC production. Emulsion injection occurred on day 6. Solution change occurred on day 49.
These results show that the fully hydrogenated soybean oil can also support both
denitrification and chromate reduction, with almost identical results as in the liquid soybean oil treated column. The only major difference could come from the carbon balance. Again initial TOC levels were high and steadily decreased. Only 4.4% of the injected carbon left the column as TOC. The low electron acceptor influent solution also resulted in low CO₂ production and the possibility of methanogenesis and fermentation as primary soybean uses, as reflected by the three methane measurements shown in table 5.3.

Table 5.1 shows the balance of carbon for the two treated chromium columns, C2 being the liquid soybean treated column and C3 the fully hydrogenated soybean treated column.
Table 5.1: Carbon Balance for chromate reducing columns

<table>
<thead>
<tr>
<th></th>
<th>C1 mg</th>
<th>C2 mg</th>
<th>C3 mg</th>
<th>%</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Carbon Injected</strong></td>
<td>0</td>
<td>6884</td>
<td>5947</td>
<td>100.0%</td>
<td>100.0%</td>
</tr>
<tr>
<td><strong>Organic Carbon Released (day 0-14)</strong></td>
<td>34</td>
<td>530</td>
<td>280</td>
<td>7.7%</td>
<td>4.7%</td>
</tr>
<tr>
<td><strong>TOC Released (day 15-104)</strong></td>
<td>9</td>
<td>225</td>
<td>178</td>
<td>3.3%</td>
<td>3.0%</td>
</tr>
<tr>
<td><strong>TOC Released (day 105-196)</strong></td>
<td>6</td>
<td>81</td>
<td>70</td>
<td>1.2%</td>
<td>1.2%</td>
</tr>
<tr>
<td><strong>Measured IC Released (196 days)</strong></td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>0.0%</td>
<td>0.0%</td>
</tr>
<tr>
<td><strong>Potential IC Released (196 days)</strong></td>
<td>19</td>
<td>60</td>
<td>55</td>
<td>0.9%</td>
<td>0.9%</td>
</tr>
<tr>
<td><strong>Final Sediment Carbon Content</strong></td>
<td>0</td>
<td>2983</td>
<td>2641</td>
<td>43.3% (± 8.1%)</td>
<td>44.4% (± 1.9%)</td>
</tr>
<tr>
<td><strong>Mass Balance Error</strong></td>
<td>0 (± 132)</td>
<td>3005 (± 556)</td>
<td>2709 (± 111)</td>
<td>43.7 (± 8.1%)</td>
<td>45.6 (± 1.9%)</td>
</tr>
<tr>
<td><strong>Carbon Consumption Rate (mg/d)</strong></td>
<td>0.1</td>
<td>0.9</td>
<td>0.8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The carbon added through treatment accounts mass of carbon injected. The amount that exited the column effluent over the initial two weeks of operation is accounted for as volatile solids samples taken from the column effluent daily. Less than ten percent of the injected treatment migrated from the columns in the initial two week span. Very low percentages for both measured and potential inorganic carbon were released from the column due to the low levels of electron acceptors in the flow through solution. Measured IC in the treated columns showed very little difference from the control column. TOC concentrations were also very low. The final carbon consumption rate was calculated as the cumulative IC and TOC.
released over the final 91 days of the experiment. The following table presents a theoretical lifespan for each treated column with the final sediment carbon mass including the 196 days of the experiment.

Table 5.2: Potential lifespan for the treated chromate reducing columns

<table>
<thead>
<tr>
<th>Column ID</th>
<th>C2</th>
<th>C3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>Liquid Soybean</td>
<td>Fully Hydrogenated Soybean</td>
</tr>
<tr>
<td>Final Sediment Carbon Content (mg)</td>
<td>2983</td>
<td>2641</td>
</tr>
<tr>
<td>Carbon Consumption Rate (mg/d)</td>
<td>0.9</td>
<td>0.8</td>
</tr>
<tr>
<td>Total Lifespan (years)</td>
<td>9.7</td>
<td>9.6</td>
</tr>
</tbody>
</table>

Treatment carbon mass was grossly designed to last the duration of the experiment and does not represent a full-scale treatment lifespan. Due to the low electron acceptor flux through the column, the carbon consumption is relatively low, and the treatment longevity is greater than seen in the nitrate reducing columns. To account for much of the missing carbon, the production of methane could have been a major factor. Overall methane production is not known because methane was not routinely monitored. Table 5.2 shows the results from three headspace analyses for the chromate columns taken during the latter stages of the test, showing low concentrations of methane production in the treated columns. Carbon use by methanogens could explain the disparity between injected carbon, used carbon, and the remaining carbon in the columns at the end of the experiment.
Table 5.3: Methane production in the chromate columns

<table>
<thead>
<tr>
<th>Date</th>
<th>C1 (mg/L)</th>
<th>C2 (mg/L)</th>
<th>C3 (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>11/11/03</td>
<td>&lt; 0.001</td>
<td>0.37</td>
<td>1.83</td>
</tr>
<tr>
<td>11/25/03</td>
<td>&lt; 0.001</td>
<td>1.07</td>
<td>2.64</td>
</tr>
<tr>
<td>12/11/03</td>
<td>&lt; 0.001</td>
<td>2.29</td>
<td>3.08</td>
</tr>
</tbody>
</table>

Permeability loss due to treatment was also monitored in the three chromate columns, with the results shown in table 5.3. Changes in permeability are important in PRB design, and show significant loss in the two treated columns. A two-fold increase was measured in the control column. The losses in the treated columns are explained by soybean oil’s hydrophobic nature and clogging of preferred flow paths and/or the biological growth caused by the carbon substrate availability clogging sediment pores. Interestingly, the two treated columns had identical losses in permeability which could mean the loss is due to biological clogging and not the soybean treatment. Had the permeability loss been due to the soybean oil we would have expected differences between the wax and liquid soybean treatments.

Table 5.4: Permeability changes in the Chromate Reducing Columns

<table>
<thead>
<tr>
<th>Column ID</th>
<th>K (Before Emulsion) Measured 7/10/03</th>
<th>K (After Emulsion) Measured 1/15/04</th>
<th>K final/ K initial (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1 (control)</td>
<td>1.24E-3 (cm/s)</td>
<td>2.78E-3 (cm/s)</td>
<td>224%</td>
</tr>
<tr>
<td>C2 (liquid soy)</td>
<td>1.10E-3 (cm/s)</td>
<td>4.05E-5 (cm/s)</td>
<td>3.7%</td>
</tr>
<tr>
<td>C3 (wax soy)</td>
<td>1.03E-3 (cm/s)</td>
<td>3.84E-5 (cm/s)</td>
<td>3.7%</td>
</tr>
</tbody>
</table>
Summary of Chromate Results

The results from the chromate flow through columns indicate favorable removal capacity for low levels of chromate. The initial experiment showed that higher heavy metals concentrations could inhibit biological growth, making an emulsified soybean PRB an ineffective remediation technology. Results from the second column experiment using lower levels of chromate indicated that if strong biological growth is already present, a soybean oil substrate could be used to reduce and immobilize chromium in groundwater flow through columns. Again the possible drawbacks are iron mobilization and permeability loss, both of which are dependent on aquifer conditions. Another problem associated with the soil column experiment was the high sorption capacity of the field sand used in the columns. An extended period of time lapsed before effluent chromium was detected in the control column despite the 5 day HRT, and the sorption could have been a major mechanism in the removal of chromium even in the treated columns. The positive immobilization of chromium does provide promising results.
6. AMD 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 environmental conditions typical of acid mine drainage. Coal mine spoils were obtained from a former coalmine in Sequatchie Valley, TN for use in the acid mine drainage studies. Each treatment was constructed in triplicate containing 190 mL simulated acid mine drainage and 25 mL of moist sediment. An adapted sulfate reducing bacterial inoculum was obtained from a Successive Alkalinity Producing System (SAPS) pond in Pennsylvania. Experimental treatments included:

- Neutralized live control (no added carbon)
- Neutralized basic treatment (soluble substrates, i.e. molasses, yeast extract, nutrients)
- Neutralized basic treatment plus 2500 mg/L liquid soybean oil
- Acidic live control (no added carbon)
- Acidic basic treatment (soluble substrates)
- Acidic basic treatment plus 2500 mg/L liquid soybean oil
- Autoclaved killed control

The acidic basic treatment consisted of 190 mL of a 20 mM FeSO$_4$ solution titrated to pH 3.0 with H$_2$SO$_4$, 200 mg/L molasses, 200 mg/L yeast extract, 100 mg/L dibasic ammonium phosphate [(NH$_4$)$_2$HPO$_4$] and 25 mL inoculum. Live controls received the inorganic nutrients and microbial inoculum but no added molasses or soybean oil. For the neutralized treatments, the simulated AMD was titrated with 1.0 M sodium bicarbonate solution to pH = 7.0 to simulate the effects of natural buffering during groundwater flow through uncontaminated aquifer material.
Figure 6.1 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. In contrast, the soybean oil addition resulted in 100% reduction in sulfate and increase in pH to over 6 in both the acid and neutralized treatments. Sulfate reduction was somewhat more rapid in the neutralized bottles. However the low pH of the microcosms did not significantly impact treatment in any bottle. Iron was removed from the acid soybean oil treatments at an approximate ratio of 1 mole iron per two moles sulfur indicating iron is precipitated as an iron disulfide with the same chemical composition as pyrite.
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. Synthetic AMD (20 mM FeSO₄ acidified to pH~3 or buffered with sodium bicarbonate to pH~5) was intermittently pumped 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. One acidic and one neutralized column remained untreated as no added carbon controls.

Figure 6.2 shows monitoring results from the neutralized control column A1. By adding a sodium bicarbonate buffer to the influent solution it was hoped that extremely low pH levels would be avoided and more biologically favorable conditions would be created for microbial growth. However, pH dropped to ~3.2 during passage through the untreated control column as displayed in the following graphs. Sulfate and total metals concentrations remained at or above influent concentrations and very little inorganic or organic carbon was released, despite the high organic content of the soil used in packing the columns. The carbon balance graph shows the possibility of some sulfate reduction activity corroborated by low TOC levels in the effluent.
Figure 6.2: Neutralized control column (A1) results: (a) sulfate, iron, manganese, zinc, copper, aluminum, and pH in column influent; (b) sulfate, iron and pH in column effluent; (c) manganese, zinc, copper, and aluminum in column effluent; (d) TOC, IC and acetate as mg/L C in column effluent; and (e) potential IC production.
Low levels of dissolved manganese, zinc, and copper were released from the sediment in the control column, producing an effluent typical of AMD groundwater plumes.

In the neutral EOS amended column A2, there is a dramatic reduction in sulfate and increase in pH. There was also a dramatic drop in dissolved iron which was the only metal in the column influent. Copper and zinc remained below detection (0.5 mg/L detection limit) in the effluent. Dissolved manganese was higher in the column effluent than influent, presumably due to the relatively high aqueous solubility of manganese sulfide. Figure 6.3 illustrates column A2 experimental results.
Figure 6.3: EOS treated neutralized column (A2) results: (a) sulfate, iron, manganese, zinc, copper, aluminum, and pH in column influent; (b) sulfate, iron and pH in column effluent; (c) manganese, zinc, copper, and aluminum in column effluent; (d) TOC, IC and acetate as mg/L C in column effluent; and (e) potential IC production. Emulsion injection occurred on day zero.
Sulfate was the main electron acceptor and accounts for the majority of the carbon dioxide in the effluent measured as IC. Acetate and TOC concentrations show strong correlations reflecting biological breakdown of the injected EOS treatment. However, the IC concentrations in the effluent are consistently lower than expected. Metal carbonates could precipitate as the pH rises, removing IC from solution. The low pH could also result in degasing CO\textsubscript{2} from the effluent prior to sample collection. The treatments show beneficial pH changes and sulfate and metals removal efficiency of 50-75%. After 300 days of operation there is evidence of reduced biological activity. However some activity may continue until the carbon use reaches the control state of 0.1 mg/d. The last sample measurements indicate carbon use of 2.5 mg/d.

Column A3 is a duplicate neutral EOS treated flow through soil column of A2. However after 130 days operation, the column influent was changed to evaluate conditions more representative of typical acid mine drainage and examine the microbial response to higher concentrations of aluminum, copper, manganese and zinc. The new influent was prepared with FeSO\textsubscript{4} (2 mM), MnSO\textsubscript{4} (5 mM), CuSO\textsubscript{4} (1 mM), AlK(SO\textsubscript{4})\textsubscript{2} (4 mM), ZnSO\textsubscript{4} (1 mM) and CaSO\textsubscript{4} (7 mM). Figure 6.4 shows the variation in effluent concentrations with time for the neutralized AMD column A3, treated with EOS substrate.
Figure 6.4: EOS treated neutralized column (A3) results: (a) sulfate, iron, manganese, zinc, copper, aluminum, and pH in column influent; (b) sulfate, iron and pH in column effluent; (c) manganese, zinc, copper, and aluminum in column effluent; (d) TOC, IC and acetate as mg/L C in column effluent; and (e) potential IC production. Emulsion injection occurred on day zero. Influent change occurred on day 131.
Column A3 saw similar results as its duplicate A2 with a good rise in pH and over 50% removal of sulfate and metals content in the effluent. The amendments made on day 130 increased the sulfate load on the column, along with the heavy metals concentration. This addition did not seem to inhibit microbial activity, but rather boost carbon use, as reflected in the second graph. In effect, the increased electron acceptor load exhausted the carbon resources of the treatment quicker than shown in the unamended column A2. By the end of the experiment, the carbon use rates were comparable to the control column and sulfate concentrations in the effluent matched influent levels. The carbon added through treatment for this column apparently had been burned up by the microbial activity after 300 days. Increased sulfate levels and metals concentration could explain the increased carbon use.

Column A4 was a second control column without the addition of a sodium bicarbonate buffer, with effluent graphs shown in figure 6.5. This would be the acidic control column, representing AMD flow through low alkaline conditions. The effluent contained higher dissolved metals and a lower pH than the neutral control column due to additional metals from minerals in the sediment.
Figure 6.5: Acidic control column (A4) results: (a) sulfate, iron, manganese, zinc, copper, aluminum, and pH in column influent; (b) sulfate, iron and pH in column effluent; (c) manganese, zinc, copper, and aluminum in column effluent; (d) TOC, IC and acetate as mg/L C in column effluent; and (e) potential IC production.
The results from the acidic control column show that a lower pH will increase metals loading. Without the carbon treatment, there was virtually removal of sulfate, and IC, TOC, and acetate remained below detection for most of the experiment.

Column A5 was treated with EOS and fed an acidic influent solution of 20 mM FeSO₄ for the duration of the experiment. Effluent variations are shown in figure 6.6. The carbon treatment resulted in a sustained pH increase to near neutral conditions, substantial sulfate reduction, and precipitation of over 50% of the influent iron. Copper and zinc were non-detect in the effluent, but manganese was released in the effluent due to the high aqueous solubility of manganese sulfide. 15-20 mM of sulfate was removed for over seven weeks of sampling.
Figure 6.6: EOS treated acidic column (A5) results: (a) sulfate, iron, manganese, zinc, copper, aluminum, and pH in column influent; (b) sulfate, iron and pH in column effluent; (c) manganese, zinc, copper, and aluminum in column effluent; (d) TOC, IC and acetate as mg/L C in column effluent; and (e) potential IC production. Emulsion injection occurred on day zero.
Sulfate was the major oxygen source for potential CO$_2$ formation. The measured IC never reached the potential values, and TOC concentrations do not account for the disparity. The low IC concentration in the effluent may be due to precipitation of metal carbonates and/or degassing of CO$_2$. Carbon use rates began to decline toward the end of the experiment along with the extent of sulfate reduction. As in the microcosms, the low pH did not significantly inhibit the bacterial consortia responsible for sulfate reduction. The total metals reduction was approximately 10-15 mM, much less than sulfate removal. The metals removal may have been limited by the availability of reduced sulfide in the effluent solution.

The last AMD study was column A6, an acidic EOS treated column with the same metals amendment on day 130 as column A3. The concentrations of copper, zinc, manganese, aluminum, and sulfate in the influent were increased while the iron concentration was reduced. These changes did not reduce microbial activity and the positive effects of the EOS treatment were again reflected by increased pH levels, high sulfate removal, and the precipitation of most metals. Figure 6.7 shows the results from column A6.
Figure 6.7: EOS treated acidic column (A6) results: (a) sulfate, iron, manganese, zinc, copper, aluminum, and pH in column influent; (b) sulfate, iron and pH in column effluent; (c) manganese, zinc, copper, and aluminum in column effluent; (d) TOC, IC and acetate as mg/L C in column effluent; and (e) potential IC production. Emulsion injection occurred on day zero. Influent changed on day 131.
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 zinc, copper, and aluminum concentrations were below the analytical detection limit in all effluent samples. Iron and manganese remained high after the influent change most likely due to solubility issues, but levels were much lower than the control column. The increased sulfate levels in the flow solution seemed to increase the carbon use rate, and higher levels of IC were measured after the amendment. Carbon use remained high at the end of the experiment, above 2 mg C/d so the lifespan of the treatment had not yet been exhausted. However increased sulfate levels in the effluent indicate a reduction of biological activity.

By tracking the carbon in and out of each treated column, it may be possible to estimate the lifespan of the EOS treatment. The following table 6.1 shows the summary of our carbon balance for all 4 treated columns. This information could prove useful for gross estimates of a PRB lifespan.
Table 6.1: Carbon balance for neutralized AMD columns A1-A3.

<table>
<thead>
<tr>
<th></th>
<th>A1</th>
<th></th>
<th>A2</th>
<th></th>
<th>A3</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg</td>
<td></td>
<td>mg</td>
<td></td>
<td>mg</td>
<td></td>
</tr>
<tr>
<td>Carbon Injected</td>
<td>0</td>
<td></td>
<td>13815</td>
<td>100.0%</td>
<td>13815</td>
<td>100.0%</td>
</tr>
<tr>
<td>Organic Carbon Released (day 0-14)</td>
<td>142</td>
<td>2174</td>
<td>15.7%</td>
<td>2165</td>
<td>15.7%</td>
<td></td>
</tr>
<tr>
<td>TOC Released (day 15-89)</td>
<td>25</td>
<td>362</td>
<td>2.6%</td>
<td>213</td>
<td>1.5%</td>
<td></td>
</tr>
<tr>
<td>TOC Released (day 90-302)</td>
<td>21</td>
<td>56</td>
<td>0.4%</td>
<td>166</td>
<td>1.2%</td>
<td></td>
</tr>
<tr>
<td>Measured IC Released (302 days)</td>
<td>0</td>
<td>285</td>
<td>2.1%</td>
<td>345</td>
<td>2.5%</td>
<td></td>
</tr>
<tr>
<td>Potential IC Released (302 days)</td>
<td>43</td>
<td>1681</td>
<td>12.2%</td>
<td>1346</td>
<td>9.7%</td>
<td></td>
</tr>
<tr>
<td>Final Sediment Carbon Content</td>
<td>0</td>
<td></td>
<td>0</td>
<td></td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Mass Balance Error</td>
<td>0</td>
<td>10189</td>
<td>73.8%</td>
<td>9925</td>
<td>71.8%</td>
<td></td>
</tr>
<tr>
<td>Carbon Consumption Rate (mg/d)</td>
<td>0.1</td>
<td>1.6</td>
<td>2.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(day 91-302)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 6.2: Carbon balance for acidic AMD columns A4-A6.

<table>
<thead>
<tr>
<th></th>
<th>A4 mg</th>
<th>A5 mg</th>
<th>A6 mg</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon Injected</td>
<td>0</td>
<td>13815</td>
<td>100.0%</td>
<td>13815</td>
</tr>
<tr>
<td>Organic Carbon Released (day 0-14)</td>
<td>3</td>
<td>3738</td>
<td>27.1%</td>
<td>2010</td>
</tr>
<tr>
<td>TOC Released (day 15-89)</td>
<td>13</td>
<td>98</td>
<td>0.7%</td>
<td>188</td>
</tr>
<tr>
<td>TOC Released (day 90-302)</td>
<td>16</td>
<td>71</td>
<td>0.5%</td>
<td>90</td>
</tr>
<tr>
<td>Measured IC Released (302 days)</td>
<td>0</td>
<td>9</td>
<td>0.1%</td>
<td>75</td>
</tr>
<tr>
<td>Potential IC Released (302 days)</td>
<td>182</td>
<td>1719</td>
<td>12.4%</td>
<td>1800</td>
</tr>
<tr>
<td>Final Sediment Carbon Content</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8480</td>
</tr>
<tr>
<td></td>
<td>(± 2363)</td>
<td>(± 194)</td>
<td>(± 1.4%)</td>
<td>(± 9603)</td>
</tr>
<tr>
<td>Mass Balance Error</td>
<td>0</td>
<td>8189</td>
<td>59.3%</td>
<td>1247</td>
</tr>
<tr>
<td>Carbon Consumption Rate (mg/d)</td>
<td>0.1</td>
<td>0.4</td>
<td>0.8</td>
<td></td>
</tr>
</tbody>
</table>

The carbon added through treatment was measured as volatile solids injected into the column. The amount exiting the column was measured with VS samples for the first two weeks of operation. Only 15% passed out of the column during this period for most of the columns. The measured versus potential inorganic carbon values are presented, with the potential values representing more realistic estimates due to the low measured IC in the effluent samples. The over all TOC released resulted in no more than 3% of the total carbon
injected over the duration of the experiment. The remaining carbon was measured from
dried soil samples. The deviation in dried sample results made estimations of remaining
carbon difficult, and error in the carbon balance was expected due to the complex chemistry
involved with acid rock drainage flow. The formation of carbonate precipitates consistently
would make IC values lower than expected. Siderite would be a major carbonate compound
with the elevated levels of dissolved iron. The dissolution of siderite is as follows (Bodek,
1988):

\[ \text{FeCO}_3 (s) \leftrightarrow \text{Fe}^{+2} + \text{CO}_3^{-2} \; ; \; \log K = -10.68 \]

Assuming 25°C and zero ionic strength, the solubility constant for the formation of siderite
could be calculated based on the total inorganic carbon and pH of the effluent. The
following formula was used for calculation of the solubility product for siderite.

\[ \log SP = \log \left\{ \left[ \text{Fe}^{+2} \right] \left[ \text{CO}_3^{-2} \right] / 10^{-10.68} \right\} \quad (11) \]

In order to calculate the presence of carbonate in solution, the concentration of total
inorganic carbon, \([\text{H}_2\text{CO}_3^*]\), was assumed to be equivalent to the measured concentrations of
IC. For calculations, the concentration of IC was assumed to be at the detection limit of 0.5
mg/L in instances where no IC was detected. Using the values of \(\text{pKa}_1 = 10^{-6.35}\) and \(\text{pKa}_2 = 10^{-10.33}\), the following formulas were utilized (Bodek et al;1988):

\[ [\text{HCO}_3^-] = 10^{6.35} \times [\text{IC}] / [\text{H}^+] \]

and \([\text{CO}_3^{-2}] = 10^{-10.33} \times [\text{HCO}_3^-] / [\text{H}^+] \]

The following graphs illustrate the solubility product with soluble iron over time in each
column.
Figure 6.8: The log of solubility product for the dissolution of siderite in the treated AMD columns A2, A3, A5, and A6. The solution change effected column A3 and A6 after day 130 by removing high iron levels. The solubility assumes 25°C and zero ionic strength.
The results show that the effluent of all treated columns was usually at saturation for siderite. When the log[SP] was greater than zero, indicating supersaturated conditions, dissolved iron concentrations were lower. It is more apparent in the neutralized columns A2 and A3 because a bicarbonate buffer was added to neutralize the influent solution. The formation of siderite could explain the error in carbon mass balance calculations by decreasing IC in solution.

The longevity of the column is presented in the table 6.3. The final sediment carbon content is zero for most columns, so the lifespan is just the experimental duration.

<table>
<thead>
<tr>
<th>Column ID</th>
<th>A2</th>
<th>A3</th>
<th>A5</th>
<th>A6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Final Sediment Carbon Content (mg)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8480</td>
</tr>
<tr>
<td>Carbon Consumption Rate (mg/d)</td>
<td>1.6</td>
<td>2.4</td>
<td>0.4</td>
<td>0.8</td>
</tr>
<tr>
<td>Total Lifespan (years)</td>
<td>0.8</td>
<td>0.8</td>
<td>0.8</td>
<td>29.8</td>
</tr>
</tbody>
</table>

Total lifespan for column A6 is a gross over estimation. Carbon use should have been higher if not for the previously mentioned difficulties measuring effluent carbon. Measurements of effluent sulfate concentrations did show decreased sulfate removal toward the final sampling period, indicating the end of treatment lifespan. Elevated electron acceptor loading should have increased carbon consumption.
Permeability was also monitored during the AMD experiment, before emulsion treatment, directly after emulsion treatment, and at the terminus of the study. By taking these three measurements, we should be able to compare the permeability loss due to the emulsion and that of biological growth. Table 6.3 displays the findings from our permeability tests.

Table 6.4: Permeability monitoring for AMD columns

<table>
<thead>
<tr>
<th>Column ID</th>
<th>K (Before Emulsion) Measured 1/27/03</th>
<th>K (After Emulsion) Measured 3/09/03</th>
<th>K (final) Measured 1/07/04</th>
<th>K final/K initial (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1 (control)</td>
<td>2.74E-2 (cm/s)</td>
<td>7.15E-2 (cm/s)</td>
<td>3.67E-2 (cm/s)</td>
<td>134%</td>
</tr>
<tr>
<td>A2 (treated)</td>
<td>3.2E-2 (cm/s)</td>
<td>2.61E-2 (cm/s)</td>
<td>4.9E-3 (cm/s)</td>
<td>15%</td>
</tr>
<tr>
<td>A3 (treated)</td>
<td>2.0E-2 (cm/s)</td>
<td>3.27E-2 (cm/s)</td>
<td>8.6E-3 (cm/s)</td>
<td>43%</td>
</tr>
<tr>
<td>A4 (control)</td>
<td>2.41E-2 (cm/s)</td>
<td>0.1181 (cm/s)</td>
<td>3.54E-2 (cm/s)</td>
<td>147%</td>
</tr>
<tr>
<td>A5 (treated)</td>
<td>1.37E-2 (cm/s)</td>
<td>5.54E-2 (cm/s)</td>
<td>1.82E-2 (cm/s)</td>
<td>133%</td>
</tr>
<tr>
<td>A6 (treated)</td>
<td>1.71E-2 (cm/s)</td>
<td>4.5E-2 (cm/s)</td>
<td>1.8E-3 (cm/s)</td>
<td>11%</td>
</tr>
</tbody>
</table>

From our findings the EOS treatment caused slight loss in permeability as compared to the initial measurements and the final test. However, it is believed to be biological activity that is responsible for the loss due to the fact that directly after the emulsion injection the permeability for all columns remained constant or increased, probable due to the mobilization of fine particles by the surfactants in EOS. It was not until the final test that significant permeability loss was recorded. The treated columns all showed slight permeability decreases with the exception of column A5. Column A3 which saw the highest microbial activity also displayed the highest drop in hydraulic conductivity. These finding
clearly show the permeability loss due to soybean treatments are a biological phenomenon for the sediment tested in this experiment.

Throughout the study a black precipitate was observed in the effluent of the treated columns. On day 243, filtered and unfiltered samples were collected from the effluent of each column and analyzed by ICP. Small amounts of the precipitate were also analyzed by ICP after dissolution in 0.1 N HCl. The results from those tests are shown below in table 6.5.

Table 6.5: Analysis of black precipitate observed in effluent of treated columns. Results include filtered, unfiltered, and acidified solid samples. ‘Difference’ is the difference between the filter and unfiltered samples. Sulfate samples were collected separately on the same date. Other aqueous sulfur present is the difference between the filtered total sulfur and sulfate measurements.
Concentrations of total dissolved sulfur were greater than sulfate indicating that aqueous sulfur materials were present other than sulfate. Filtration also removed much larger ratio of sulfur from the effluent than was present in the black precipitates, suggesting the formation of pure sulfur or other non-sulfide precipitates. Acid extractions of the black precipitates reveal a mix of manganese and iron sulfides in a one-to-one ratio between total metals and sulfur. Further tests on the black precipitate by X-ray diffraction show an amorphous, poorly crystalline solid with an indefinite chemical composition.

**Summary of AMD Results**

The results for all columns show significant reduction in sulfate concentrations, increases in pH to near neutral conditions, and the precipitation of heavy metals. The only metals that did not show excellent removal were iron and manganese. The low manganese removal efficiency is presumably due to the high aqueous solubility of manganese sulfides (Table 6.4). The cause of the lower 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 (Bodek, et al., 1988).

<table>
<thead>
<tr>
<th>Solid Precipitate</th>
<th>Log Solubility Product</th>
<th>Aqueous Solubility (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MnS</td>
<td>-10.5</td>
<td>$10^{-5.5}$</td>
</tr>
<tr>
<td>FeS</td>
<td>-18.1</td>
<td>$10^{-13.1}$</td>
</tr>
<tr>
<td>ZnS</td>
<td>-24.7</td>
<td>$10^{-19.7}$</td>
</tr>
<tr>
<td>CuS</td>
<td>-36.0</td>
<td>$10^{-31.0}$</td>
</tr>
</tbody>
</table>
There still remains a question as to why 100% sulfate removal was not achieved. There are a variety of factors that could limit the sulfate removal efficiency in the emulsion treated columns. In the laboratory, the short hydraulic residence time (6-7 days) and small amount of added substrate may have limited removal efficiency. 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 excellent sulfate removal (15-20 mM) with a very high pollutant to substrate efficiency. The overall ratio of substrate added to sulfate reduction is roughly 10:1 on a molar basis. The columns were treated with 900 to 1000 mmoles of carbon and 70 to 100 mmoles of sulfate were removed from each column overall.

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 H₂S 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 indicate that sulfide produced during sulfate reduction is being somehow immobilized in the column, although the exact mechanism of sulfur retention is not known.

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.

Despite not reaching complete sulfate and heavy metals removal, the EOS treatment exhibited beneficial sulfate reduction, near neutral pH conditions in the effluent, and reduced dissolved metals concentrations. This technology could prove to be more cost efficient and environmentally beneficial than conventional limestone treatments.
7. Conclusions & Recommendations

The findings of our study have extended the capabilities of soybean products in the bioremediation field. Soybean oil has already been shown to biodegrade a number of groundwater pollutants including chlorinated solvents. Liquid soybean oil and hydrogenated soybean oil wax were very effective in stimulating anaerobic biodegradation of nitrate, low levels of chromate, and acid mine drainage. In the work conducted under this project, we were not able to conclusively immobilize chromium in concentrations in excess of 0.05 mM with soybean treatment, presumably due to the toxicity issues. Other soy-based substrates including blown soybean oil and soy methyl esters can also be used to simulate contaminant biodegradation. However these materials do not appear to have any major advantages over the standard soybean oil products.

Potential problem areas of the process include the mobilization of reduced iron and permeability losses. The iron release may prove to be unimportant especially in aquifers with low iron mineral deposits. Reduced iron is not necessarily a dire problem either except for the formation of rust once oxidized, a problem tackled by drinking water treatment plants and well-water homeowners already. The permeability issue could be more concerning, because the design of permeable reactive barriers relies heavily on the hydraulic conductivity of the aquifer. A major loss in permeability could cause contaminated waters to avoid the treatment area by flowing around it. This could effectively negate the effect of the PRB on the contaminant plume. In order for a soybean emulsion PRB to be effective the permeability through the treatment zone must be such that the contaminant plume can flow
through. Temperature, sediment chemistry, electron acceptor load, and hydraulic retention time could also affect the performance of the PRB.

Electron acceptor load seems to be a very important aspect on the lifespan of the soybean treatment. Based on the carbon balance estimates from the three studies, a higher electron acceptor load rates result in accelerated carbon consumption rates. The chromate, nitrate, and AMD studies each had a consecutively higher carbon consumption rate and increasing electron acceptor load rate. The carbon balance provided a gross estimate of treatment lifespan. By taking the overall carbon consumption rates previously calculated and comparing them throughout the three column experiments, we can observe the hypothetical trend. The electron acceptor loading on each column is represented by the potential IC production rate, which includes all electron acceptors measured (nitrate, chromate, sulfate, oxygen, iron, manganese, and copper). Carbon consumption and potential IC production are illustrated per area of the column for comparison. The following figure 7.1 illustrates the trend.

![Carbon consumption rates versus potential IC production](image_url)

**Figure 7.1:** Carbon consumption rates versus potential IC production. Each point represents one column from all 8 treated columns. The rates are per area of the column (0.001 m³).
The trend is difficult to prove based on these experimental results. The measured carbon consumption rates for the AMD columns are underestimations due to the difficulty measuring effluent carbon. The chromate and nitrate column results should be greater indicators due to the better mass balance outcome. Further study and better measurements of carbon consumption are necessary.

Soy products in PRBs have various applications for remediation in groundwater, mine spoils, or tailing impoundments, and provide a cost effective and environmentally safe approach for treatment. The approach is low impact because it is in-situ, and nothing but food-grade components are used in the treatment. Field demonstrations should now be conducted to evaluate the costs and benefits of this approach under realistic field conditions.
9. References


9. Appendix

Table 9.1: Results of final sediment carbon analysis and calculation of hydraulic retention time for the nitrate reducing columns

<table>
<thead>
<tr>
<th>Column ID</th>
<th>% C</th>
<th>Dry Weight Soil (g)</th>
<th>Final Carbon (g)</th>
<th>Ave</th>
<th>St Dev</th>
<th>Volume (mL)</th>
<th>Soil Dens (g/mL)</th>
<th>n</th>
<th>Ave Flow (mL/d)</th>
<th>HRT (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N1 a</td>
<td>0.04</td>
<td>569</td>
<td>0.228</td>
<td>0.209</td>
<td>0.033</td>
<td>306.70</td>
<td>1.86</td>
<td>0.30</td>
<td>18.6</td>
<td>4.9</td>
</tr>
<tr>
<td>N1 b</td>
<td>0.04</td>
<td>569</td>
<td>0.228</td>
<td>0.171</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>N1 c</td>
<td>0.03</td>
<td>569</td>
<td>0.228</td>
<td>0.209</td>
<td>0.033</td>
<td>306.70</td>
<td>1.86</td>
<td>0.30</td>
<td>18.6</td>
<td>4.9</td>
</tr>
<tr>
<td>N2 a</td>
<td>0.47</td>
<td>568.7</td>
<td>2.673</td>
<td>2.445</td>
<td>0.346</td>
<td>306.70</td>
<td>1.85</td>
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<td>20.8</td>
<td>4.4</td>
</tr>
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<td></td>
<td></td>
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</tr>
<tr>
<td>N2 c</td>
<td>0.36</td>
<td>568.7</td>
<td>2.616</td>
<td>2.047</td>
<td></td>
<td></td>
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</tr>
<tr>
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<td>306.70</td>
<td>1.86</td>
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<td>17.5</td>
<td>5.3</td>
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<td>0.64</td>
<td>569</td>
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<td>3.243</td>
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<td>569</td>
<td>3.243</td>
<td>3.243</td>
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<td></td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 9.2: Results of final sediment carbon analysis and calculation of hydraulic retention time for the chromate reducing columns

<table>
<thead>
<tr>
<th>Column ID</th>
<th>% C</th>
<th>Dry Weight Soil (g)</th>
<th>Final Carbon (g)</th>
<th>Ave</th>
<th>St Dev</th>
<th>Volume (mL)</th>
<th>Soil Dens (g/mL)</th>
<th>n</th>
<th>Ave Flow (mL/d)</th>
<th>HRT (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1 a</td>
<td>0.04</td>
<td>568.1</td>
<td>0.227</td>
<td>0.189</td>
<td>0.066</td>
<td>306.70</td>
<td>1.85</td>
<td>0.30</td>
<td>17.1</td>
<td>5.4</td>
</tr>
<tr>
<td>C1 b</td>
<td>0.04</td>
<td>568.1</td>
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<td></td>
</tr>
<tr>
<td>C1 c</td>
<td>0.02</td>
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<td>0.227</td>
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<td>0.62</td>
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<td>3.450</td>
<td>3.172</td>
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<td>306.70</td>
<td>1.81</td>
<td>0.32</td>
<td>17.8</td>
<td>5.4</td>
</tr>
<tr>
<td>C2 b</td>
<td>0.52</td>
<td>556.5</td>
<td>2.894</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C2 c</td>
<td>0.57</td>
<td>556.5</td>
<td>2.894</td>
<td>3.172</td>
<td></td>
<td></td>
<td></td>
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<td>C3 a</td>
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<td></td>
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</tr>
<tr>
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<td>2.775</td>
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</table>
Table 9.3: Results of final sediment carbon analysis and calculation of hydraulic retention time for the AMD reducing columns

<table>
<thead>
<tr>
<th>Column ID</th>
<th>% C</th>
<th>Dry Weight Soil (g)</th>
<th>Final Carbon (g)</th>
<th>Ave</th>
<th>St Dev</th>
<th>Volume (mL)</th>
<th>Soil Dens (g/mL)</th>
<th>n</th>
<th>Ave Flow (mL/d)</th>
<th>HRT (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1 a</td>
<td>0.96</td>
<td>478.7</td>
<td>4.596</td>
<td>6.319</td>
<td>3.581</td>
<td>306.70</td>
<td>1.56</td>
<td>0.41</td>
<td>18</td>
<td>7.0</td>
</tr>
<tr>
<td>A1 b</td>
<td>2.18</td>
<td>478.7</td>
<td>10.436</td>
<td>6.319</td>
<td>3.581</td>
<td>306.70</td>
<td>1.56</td>
<td>0.41</td>
<td>18</td>
<td>7.0</td>
</tr>
<tr>
<td>A1 c</td>
<td>0.82</td>
<td>478.7</td>
<td>3.925</td>
<td>6.319</td>
<td>3.581</td>
<td>306.70</td>
<td>1.56</td>
<td>0.41</td>
<td>18</td>
<td>7.0</td>
</tr>
<tr>
<td>A2 a</td>
<td>1.47</td>
<td>481.5</td>
<td>7.078</td>
<td>5.601</td>
<td>1.287</td>
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<td>0.41</td>
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<td>306.70</td>
<td>1.56</td>
<td>0.41</td>
<td>17.8</td>
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<td>5.094</td>
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</tr>
<tr>
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<td>4.198</td>
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<td>0.41</td>
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</tr>
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<td>9.108</td>
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<tr>
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<td>306.70</td>
<td>1.48</td>
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