

Sustainable electron donor amendments with potential for faster and less expensive mine waste (bio)remediation

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Abstract

Metals and metalloids from mine waste present a unique set of challenges for in situ or ex situ remediation. Ionic strength, pH, and metal/metalloid concentration are outside of typical ranges encountered in freshwater environments, and because of this combined biological-chemical remediation can be inefficient, expensive, or completely ineffective. Many microbial remediation processes for mine waste are predicated on stimulating microbial Fe(III) and/or Mn(IV) reduction, because these cells influence the combined microbial-chemical processes that: a) directly reduce toxic metals/metalloids, b) increase the pH to precipitate non-redox-active metals, and c) generate biogenic-reactive ferrous iron to chemically reduce toxic metals/metalloids. Stimulating these cells requires engineered amendments of electron donor(s). The problem is that the current commercially available “long-term, slow-release” electron donor(s) are derived from soybean oil and are purely lipid based. Lipids are exceedingly poor electron donor(s) for microbial metal reduction, and as such the use of lipid electron donors has failed many more times than it has been successful, and it is an extremely expensive technology. In addition, lipids can saponify during in situ process with calcium and magnesium ions, and partially fermented lipid combined to form a wax that impedes wells and groundwater flow. These electron donor(s) have been used commercially merely because good alternatives that are “long-term” have not been developed.

We have developed a technology based on amendment of novel electron donor(s) derived from animal co-products, which far outperform conventional electron donors for stimulating metal reduction. These co-products are comprised of waste generated in the animal rendering process such as feathers or bone. This technology will lead to faster remediation times at considerably lower cost. Batch incubations with aquifer material and/or sediments were designed and run to mimic in situ conditions. The rendered animal co-product electron donors stimulated simultaneous fermentation, Fe(III) reduction, and alternate metal/metalloid reduction via combined microbial-chemical processes, while emulsified vegetable oil generated primarily methane. Data suggest the protein content and nitrogen released accelerated metal reduction, and it is the protein content that promoted the rapid onset of metal-reducing microbial activity, while the remaining animal (not soybean) lipid content sustained it. Price per ton of these electron donors is in the USD 1000 – USD 1300 range, while similar lipid based amendments are in the USD 6,000 to USD 7,000 per ton range. This is a more effective and more cost efficient technology for metal/metalloid mine waste remediation.

Keywords: *bioremediation, metals, metalloids, electron donor, site remediation*

1 Introduction

Metal, metalloid, sulfate and nitrate (mine waste) bioremediation is still a widely used and critical aspect of environmental engineering; it is very often the preferred strategy by both federal and state regulatory agencies (Moran, Zogorski, and Squillace 2007). However, field strategies still rely on outdated data that prescribe over-addition of lipid-only high molecular mass electron donors (such as vegetable oils) with or without bioaugmentation into sites with pH values outside the range of metabolism for metal reducing microbial families. It is very rarely the lack of cells, but incorrect conditions from misguided electron donor amendment that prevent complete reduction (which is defined as the stoichiometric production of the end

products of the electron acceptor in solid or aqueous form). In addition, the current technologies are not sustainable; adding excessive electron donor leads to methanogenesis, while similar or better rates can be developed using targeted concentrations of electron donor and combined biological-chemical reactions. Mining wastes that are electron acceptors are amongst the most prominent and problematic groundwater contaminants in the United States and abroad (Siegrist et al. 2006). Mine waste contamination accounts for a number of all active hazardous waste sites (Nakano et al. 2000). Many compounds are toxic, potentially carcinogenic, and tend to partition between the solid and aqueous phases readily such that a small release can have a very long lifetime in situ (Aulenta, Majone, and Tandoi 2006; Henry and Bennett 2001; Lee et al. 1995).

One biological process that has become almost the industry standard for dissolved, anoxic groundwater plumes is stimulating native or added microorganisms (Aulenta, Majone, and Tandoi 2006; Moran, Zogorski, and Squillace 2007; Pelle et al. 2002) to reduce the contaminant of interest, either directly via microbial respiration or indirectly via biogenic ferrous iron. An electron donor is added to stimulate microbial activity (native and/or added), and the contaminants are reduced to either non-toxic or immobile endpoints. The organisms involved may or may not conserve energy for growth via these reactions, but the compounds involved are used as electron acceptors in either direct or operationally defined co-metabolic reactions (Maymo-Gatell, Anguish, and Zinder. 1999; Maymo-Gatell, Gossett, and Zinder. 1997; Maymo-Gatell, Nijenhuis, and Zinder 2001). Numerous organisms have been demonstrated to catalyze the primary processes driving these reactions, which are Fe(III) and Mn(IV) reduction (He et al. 2005; Duhamel and Edwards 2006). In addition, all metal, sulfate, and nitrate reducing reactions consume protons, which raises the pH of groundwater and precipitates other non-redox-active metals.

In all cases this type of bioremediation is stimulated by adding electron donors that will simultaneously promote the processes necessary to reduce the contaminants. Molecular hydrogen is generated by fermenters, and most strategies to date have relied on adding fermentable substrates including lactate, high molecular mass sugars (e.g. Molasses), and most frequently emulsified vegetable oil (EVO). The issue to date has been over-addition, or adding more electron donor than is required. The other issue is delivery – i.e., not targeting the correct cells because the electron donors are broadly distributed rather than targeted to specific hydrogeologic locations where the cells, contaminants, and ancillary geochemical conditions are ideal for the required activity.

All approaches to date have been “macro” – deliver as much amendment as possible in the hope that the right microbial populations will be stimulated along the way. Our approach is theoretically and mechanistically different. We have been investigating rendered animal co-products as electron donors for three (3) years under a program called the Animal Co-Products Research and Education Center (ACREC), which is a subdivision of the Fats and Proteins Research Foundation (FPRF). Rendered animal co-products are all materials left over in the meat industry (pork, beef, and poultry), that does not become human foodstuff (Alvarez, Drummond, and Mullen 2018; Aspevik et al. 2017; Haas et al. 2007; Leaphart et al. 2012; Mekonnen, Mussone, and Bressler 2016; Nelson and Schrock 2006; Parini and Cantini 2008, 2009; Ramirez et al. 2012; Seidavi, Zaker-Esteghamati, and Scanes 2019). It is cooked at extreme temperatures and pressures to be “rendered” until is pathogen and impurity free. The high value materials become animal feed (primarily dog and cat food). However, the lowest value materials including feather meal, meat and bone meal, tallow, and poultry by-product, have no reasonable market for use; they are often disposed of in landfills (Aspevik et al. 2017; Mekonnen, Mussone, and Bressler 2016; Parini and Cantini 2008). While they may not be a good substrate for mammals – they are excellent substrates for microorganisms!

These co-products are combinations of protein, lipid, iron and free amino acids; the proportions change depending on the feedstock (Alvarez, Drummond, and Mullen 2018; Haas et al. 2007; Nelson and Schrock 2006; Parini and Cantini 2008; Ramirez et al. 2012; Seidavi, Zaker-Esteghamati, and Scanes 2019). This combination of biomolecules is ideal for stimulating microbial activity, much more so than singular molecules such as lipids alone (Del Mundo and Sutheerawattananonda 2017; Long and Borden 2006). From a bioremediation perspective they are also ideal. One ton of feather meal costs USD 1000 – USD 1300, whereas one pound of EVO substrate can cost USD 3.00 to USD 3.50 (making it USD 6000 to USD 7000 per ton). As the

data below demonstrate – the co-products are as effective or more effective than EVO, while being fractions of the cost. Cost is often the difference between a stakeholder choosing the best remedial option, or not. The research to date has become a patent-pending electron donor technology under US Patent # 16/454,995. The data below demonstrate its effectiveness with metals, metalloids, and other electron acceptors in contaminated aquifer material.

There are three mechanisms that drive bioremediation of mine wastes by these electron donors. The first is direct respiration of reducible contaminants to either less toxic or less mobile forms. This is the process that promotes nitrate and sulfate reduction (which are residuals of nitric and sulfuric acid used in leaching of minerals from rock), and several redox active metals and metalloids such as U(VI), Cr(VI), and Se(VI). The second mechanism is indirect reduction via biogenic Fe(III). Fe(III) reduction is the most dominant anaerobic respiration on the planet, and ferrous iron both aqueous and adsorbed is a strong reductant for a number of metals and metalloids. Cr(VI) is also an example of this mechanism such that there are two simultaneous processes promoting Cr(VI) reduction. Hexavalent chromium is both acutely toxic and carcinogenic, and it is an element that must be mined in the mineral phase (as such of importance to the mining industry). The third is Fe(III) and Mn(IV) reduction mediated pH increases, that co-precipitate non-redox-active metals such as zinc, copper, and cadmium. Mine waste is typically low to very low pH because of acid leaching, and Fe(III)/Mn(IV) reduction consumes protons and raises pH over time, effectively resetting the pH of the water/groundwater and precipitating metals by changing the speciation of aqueous metals to metal oxides, which precipitate directly or co-precipitate on other surfaces. In addition, in comingled plumes with chlorinated solvents these electron donors are very effective for remediation of trichloroethylene.

Contaminated aquifer material has been used in all experiments; both in laboratory and field experiments. The material was not buffered or amended with media or cells; it was just aquifer material plus electron donors (to mimic site conditions). Several different sites have been tested in screening experiments; one is an industrial site in South Carolina, and another is an industrial site in California. Co-products were added at concentrations that reflect a 1:1 stoichiometry of electron donor to electron acceptors, and then scaled to determine the impact on increasing concentration. We tested the co-products versus several control electron donors, which have been reported in the literature and that are currently vended in the remediation marketplace (Harkness and Fisher 2013). While these data have not yet been replicated with mine waste directly – bioremediation is one technology that has been reported for mining wastes both aqueous and solid. As such, these data can be interpreted to suggest that similar amendments in mining waste(s) will promote metal and organics reduction, and likely promote processes that detoxify or sequester metals and metalloids in situ.

2 Methodology

2.1 Laboratory batch incubations

Batch experiments, described below, were constructed using previously reported strict anoxic techniques (Wei and Finneran 2009). Briefly, aquifer sediment samples were homogenized in an anoxic glove bag filled with N₂ and CO₂ and hydrogen (80:20 vol %). 10 g sediment was dispensed into each serum bottle (30 ml), and 10ml groundwater was added into the bottle in order to saturate the sediment. The bottles were sealed with thick butyl rubber stoppers and crimped. Once the sealed bottles were removed from the glove bag, the headspace was flushed with (hydrogen and CO₂ free) nitrogen gas, which had passed through a heated, reduced copper filled glass tube to remove any traces of oxygen.

Electron donor co-products tested included: feather meal (FMed) (80% protein, balance lipid plus amino acids), meat and bone meal (MBM) (70% protein, balance lipid plus iron and amino acids), poultry by product (60% protein, balance lipid and amino acids), and dissolved air flotation skimmings (DAF) (~50% protein, balance lipids). Control electron donors included acetate, lactate, and emulsified vegetable oil. Electron donors were added at stoichiometry to all known electron acceptors. Rendered animal co-product electron donors were added as solids; the remaining electron donors were added as aqueous amendments.

The various electron acceptors (metals, metalloids, nitrate, sulphate, or chlorinated solvents) were quantified over time to determine the rate and extent of reduction. Electron donors were not re-amended in any bottles, and all experiments were conducted versus unamended control incubations. Batch incubations are pictured below in Figure 1.



Figure 1 Photo of batch incubations with aquifer material and overlying groundwater; note that the incubations are not slurried (i.e. lower volume of water than solids)

2.2 Field incubations

The pilot test consisted of nine injection wells spaced approximately six (6) feet apart, with an operationally defined radius of influence of four (4) feet. Each well was screened at three depths referred to as shallow, intermediate, and deep. In addition, there were eight monitoring wells. Four of the wells are in the shallow and moderate depth saprolite, and the others were in the deeper saprolite below the proposed pilot treatment volume to assess downward migration of amendments and/or contamination.

All amendments (electron donors and bromide tracer) were added as a combined suspension by gravity feed; a pump was only be used when gravity feed could not deliver the necessary volume. The goal was to deliver a theoretically uniform electron donor concentration throughout the entire treatment volume. Electron donors were only re-amended if necessary, which was determined using ongoing data analyses. The electron donor mass was distributed evenly amongst the nine (9) total injection wells, at three (3) depths per injection well, for a total of 27 injection points. As such, the mass added to each injection point during the initial injection event will between 80-100 kg. Each injection point (27 total) will receive 1000 liters (L) of amendment solution (264 US gallons). The solution concentrations expected during the initial injection event following fermentation were acetate at 12.4 mM (1,013.1 mg/L), formate at 48.6 mM (3,310 mg/L), and lactate at 8.2 mM (918.9 mg/L). The concentration of bromide (calculated as the sodium salt) tracer in the amendment solution added at each point was 0.97 mM (100 mg/L); this concentration was sufficient to monitor contaminant changes due to dilution, dispersion, or adsorption.

The amendment solution was maintained in nine (9) separate 1m³ polycarbonate totes, or polycarbonate totes of comparable size that is large enough to hold 740L of volume. The amendments were suspended into site water. The Totes were placed at each well preferably above the ground surface. Each tote had a valve connected to dedicated tubing that was consecutively placed into the deep, intermediate, and shallow injection levels. The tubing was raised between each well depth to the appropriate screened level within each well. Each section of tubing was used for only one injection event at a single well nest, and then disposed of as waste. The injection wells remained covered and locked between injection events. The effluent port from each tote will have a quick connect fitting that was used to connect the tote effluent port to the dedicated tubing housed in each well.

The amendment volumes (1000 L at nine wells) were added by depth, with the deepest 9 injection depths amended first, the intermediate 9 injection depths amended second, and the shallowest 9 injection depths amended last. This was to minimize flow interruptions caused by mounding, which could impinge on lower depths from higher depths if the order were reversed. Each tote was allowed to drain under gravity until

empty. Once all nine totes were empty for a specific depth series, the valves were closed, and the next depth series was prepared and amended. The totes were not decontaminated between depths; a mild water rinse sufficed. Field injections are shown in Figure 2 below.



Figure 2 Field injection totes emplaced around well array for in situ microbial Fe(III), Mn(IV), and Cr(VI) reduction; field totes contained soluble electron donors (low molecular mass organic acids) derived from fermentation of higher molecular mass electron donors

2.3 Analytical methods

Table 1 Analytical techniques used within all tasks

Analyte	Analytical Method	Reference
TCE and daughter products	Gas chromatography with flame ionization detection (GC-FID)	(Loffler, Tiedje, and Sanford 1999)
Total bioavailable iron (solid/liquid)	Ferrozine Assay	(Lovley and Phillips 1986; Lovley and Phillips 1987)
Metals in solids/solution	Inductively coupled plasma-mass spectrometry (ICP-MS)	(Crompton 2001)
Organic acids, alcohols, lipids	GC-FID	(Ezeji, Qureshi, and Blaschek 2007b, 2007a; Ezeji, Qureshi, and Blaschek 2004)
CO ₂ ; CH ₄ ; H ₂	GC with thermal conductivity detection or reduction gas detector (for H ₂), and GC-GPC (for radiolabeled substrates)	(Kwon and Finneran 2008; Hatch and Finneran 2008)
Ammonium/nitrate sulfide/sulfate	Cline assay (sulfide) and Modified Berthelot reaction (ammonium), Ion chromatography	(Rhine et al. 1998; Simpson 2001)
pH	Standard calomel electrode probe	N/A

3 Results

3.1 Laboratory batch incubations

Hexavalent Cr(VI) is reduced by a variety of mechanisms. Figure 3 demonstrates the various microbiological and chemical pathways by which Cr(VI) is reduced to Cr(III), which is an innocuous end product. Cr(III) is present in multivitamins, and is non-toxic at low concentrations. Only Cr(VI) is regulated in groundwater. All co-products tested stimulated Cr(VI) reduction including DAF skimmings (Figure 4). Cr(VI) was not reduced in unamended incubations, but was completely reduced in under 40 days when stoichiometric DAF was added (based on 110 mg/L as chemical oxygen demand (COD)). Data suggested that Cr(VI) was reduced directly in

this case, as there was no Fe(II) generated in solution following Cr(VI) reduction. As was demonstrated in the field data presented below – Fe(III) reduction and Cr(VI) reduction are linked and when Fe(III) is present it accumulates following Cr(VI) reduction.

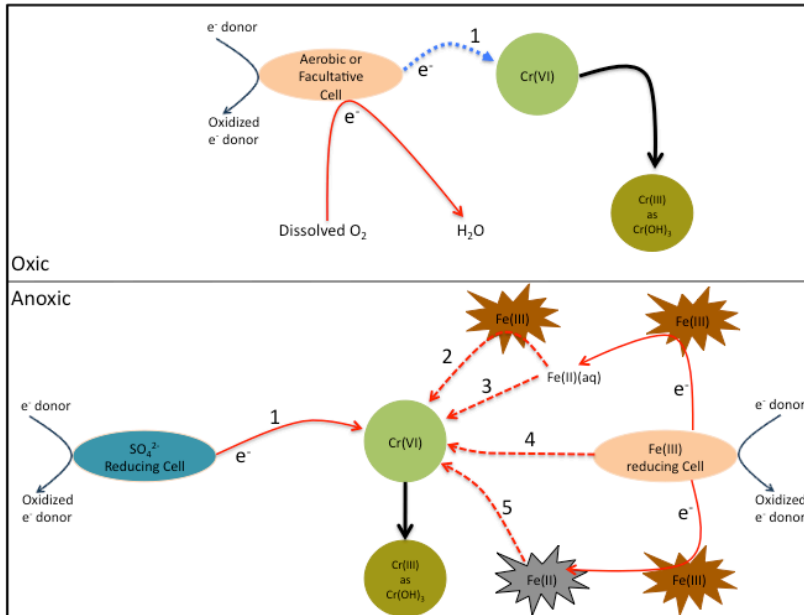


Figure 3 Multiple pathways for Cr(VI) reduction via microbial and chemical reduction; note most pathways are predicated on Fe(III) reduction and Fe(II) electron transfer

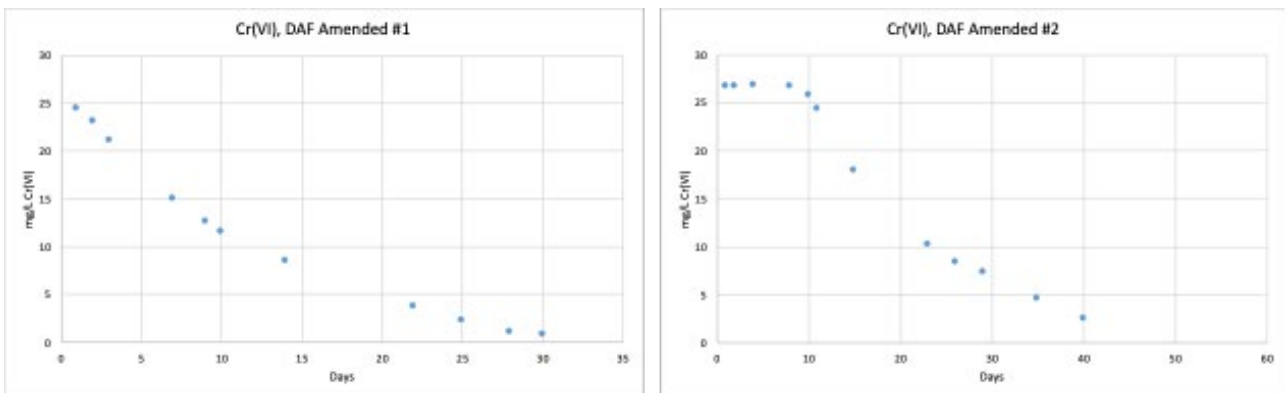


Figure 4 Cr(VI) reduction in aquifer material incubations amended with DAF skimmings

Uranyl carbonate (U(VI)) is a common mine waste constituent, and it often co-occurs with nitrate that has been derived from using nitric acid as part of the mineral leaching procedures. In electron donor amended aquifer material in which the predominate low molecular mass organic acid generated by co-product fermentation was acetate, U(VI) was reduced to the immobile U(IV) form concurrently with Fe(III) reduction and following nitrate reduction (Figure 5). Nitrate was present at almost 300 mg/L, which is a reasonable concentration following a mine waste discharge. However, it was reduced in under 25 days, while U(VI) was reduced in under 40 days. In all cases nitrate reduction preceded U(VI) reduction, with ammonium as the dominant terminal product.

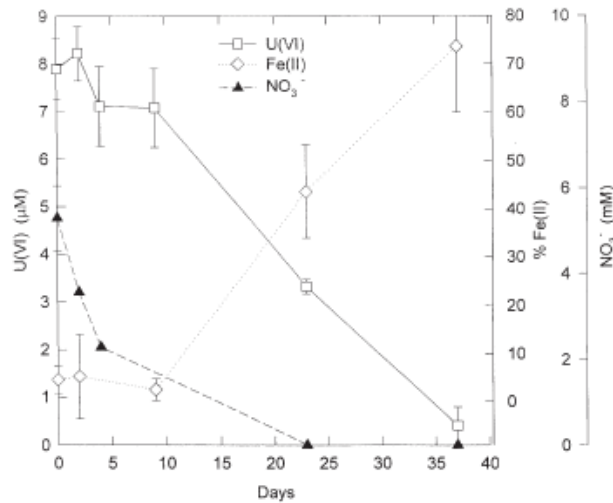


Figure 5 Nitrate, Fe(III), and U(VI) reduction in aquifer material incubations amended with electron donors that ferment to generate predominately acetate; results are the mean of triplicate incubations and bars indicate one standard deviation

Chlorinated solvents may be co-mingled with metals depending on the site activity and the discharges associated with machines and other equipment. All co-products tested promoted TCE reduction to ethene (the innocuous end product). However, feather meal was particularly effective and promoted TCE reduction at rates and to an extent that was comparable to lactate, which is generally considered the most efficient electron donor for complete dechlorination. Feather meal was more effective than emulsified vegetable oil, which is the dominant electron donor in the remediation marketplace now.

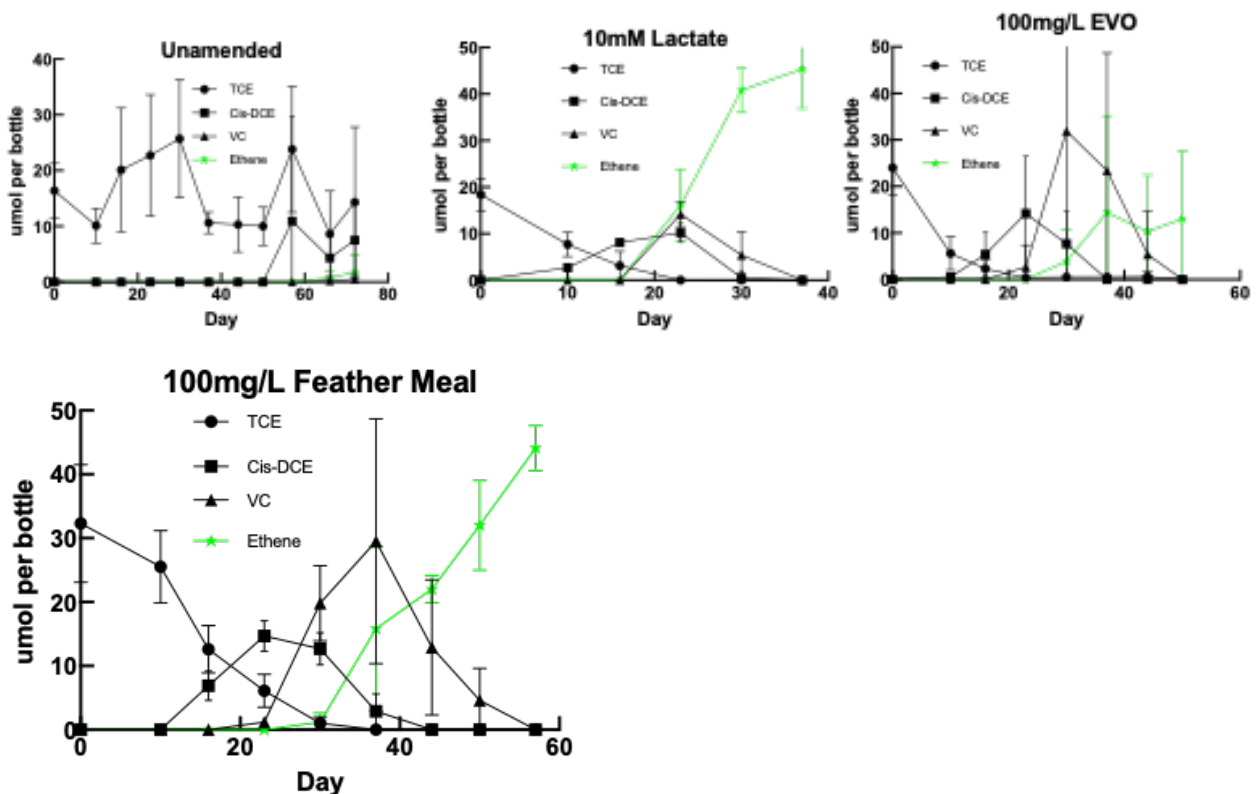


Figure 6 Trichloroethylene (TCE) reduction in aquifer material incubations amended with lactate, emulsified vegetable oil, and feather meal versus unamended incubations; results are the mean of triplicate incubations and bars indicate one standard deviation

3.2 Field pilot tests

As indicated in Figure 3 there are multiple pathways for Cr(VI) reduction, and many are linked to Fe(III), Fe(II), and microbial Fe(III) reduction. The confounding factor is if Mn(IV) is present as an electron acceptor as well. All known Mn(IV) reducers are also Fe(III) reducers, as such these processes are also linked. However, Mn(IV) is a “better” electron acceptor because is more electronegative and all electrons generated as ferrous iron will be chemically re-oxidized by Mn(IV) and therefore no ferrous iron electrons will be directed to reduced Cr(VI) until the Mn(IV) is depleted.

When electron donor was added to the aquifer (field amendment) there was a progression of metal reduction starting with Mn(IV) reduction (Figure 7). Fe(III) was concurrent as evidenced by the increase in aqueous Cr(VI), which was due to adsorbed Cr(VI) being liberated. However, ferrous iron did not accumulate (immediately re-oxidized by Mn(IV)) and therefore no Cr(VI) was reduced until the Mn(II) started to plateau, indicating that Mn(IV) was nearly depleted. Once the Mn(IV) was depleted, both Fe(II) accumulated, and Cr(VI) was completely reduced. At peak 110 mg/L Cr(VI) was completely reduced to non-detect levels.

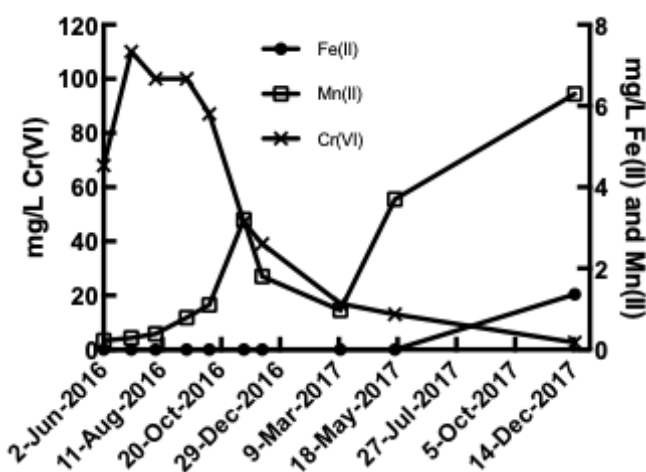


Figure 7 Fe(III), Mn(IV), and Cr(VI) reduction in aquifer material (field) following electron donor amendment

The pH in the same groundwater was increased over time from acidic (pH 4.0) to basic or even alkaline. The pH of the aquifer material amended in this pilot test increased in all wells, but in the most active Fe(III) reducing wells the pH increased from less than 4.0 to over 7.0 (Figure 8). This will be critical for any metals that are not redox-active, but may precipitate as the pH shifts from acidic to basic to alkaline (such as copper, zinc, and cadmium).

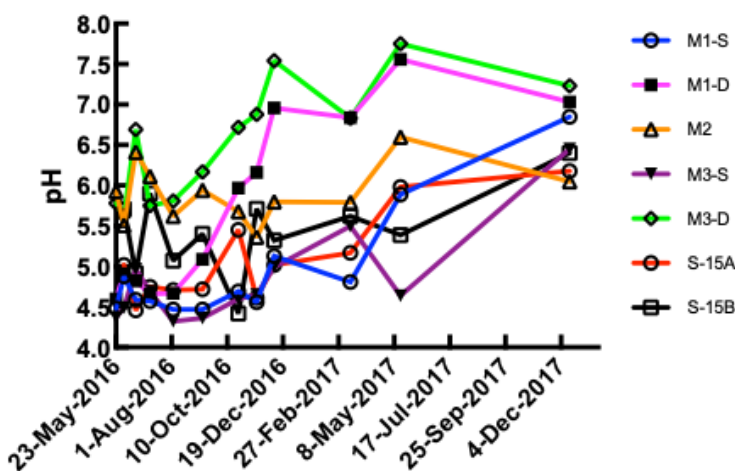


Figure 8 pH increases mediated by Fe(III) reduction in wells amended with electron donors

4 Discussion

Bioremediation is one available approach for metals, metalloids, and radionuclides that has been well reported in aquifer material, soil, and sediment (Lloyd, 2003). While data are limited in mine waste, regarded as a more extreme environment, bioremediation has been either suggested or applied for mine wastes amongst all metals and metalloids reported (Lloyd, 2003).

The data reported above are a compendium of experiments in both the laboratory and field in which metal reduction was the primary microbial processes stimulated by the electron donor amendments, which in this case were high molecular mass compounds derived from rendered animal co-products or low molecular mass organic acids developed during fermentation of these high molecular mass compounds.

Direct microbial reduction or combined microbial-chemical reduction via ferrous iron are the most common applications for metals, metalloids and radionuclides, or organic compounds that may be co-mingled in these wastes. As indicated above compounds such as Cr(VI) and U(VI) can be directly reduced by electron donor amendment, the low molecular mass organic acids derived from fermentation of rendered animal co-product electron donors promoted this activity on a time scale of days to weeks in laboratory incubations, and weeks to months in field applications. These data are well supported in the literature, in which microbial Cr(VI) or U(VI) reduction were stimulated with only electron donor amendment (Lovley, 1995). Translating these data to mine waste will require amendments that can promote metal reduction in high concentration materials, as well as low pH material. However, the data above suggest pH can be influenced by the same processes.

Fe(III) reduction increases the pH by consuming protons. The reaction below demonstrates this:



Low pH is a typical situation in mining wastes, because of the array of acids utilized to leach minerals from geologic media. Stimulating metal reduction can increase the pH from acidic to neutral, at which point more microbial populations can and will develop and become active, which will increase overall bioremediation potential. The data above and in prior reports suggests this pH effect can be stimulated with electron donor amendment (Lovley, 1995). The electron donors suggested here (rendered animal co-product electron donors) promoted this activity in low pH aquifer material, and that suggests that low pH mine waste would also be susceptible to this approach.

5 Conclusion

Electron donors are critical for in situ bioremediation. The appropriate electron donor can promote an array of reactions that ultimately facilitate contaminant reduction which can lead to detoxification, immobilization, or both. Mine waste is unique in that all bioremediation strategies will be predicated on Fe(III) reduction, which is the dominant metal-reducing respiratory process catalysing both microbiological and chemical reactions to simultaneously reduce the mine waste metals, metalloids, oxyanions, and possibly organic co-contaminants. Therefore, electron donors that specifically promote Fe(III) and metal reduction are necessary for efficient and cost-effective bioremediation. While mine wastes are generally more “extreme” environments than those reported here, the microbial processes can be stimulated in both. As such it is reasonable to suggest that electron donors that specifically target metal reducers can find a role in mine waste remediation, particularly those that can be applied on a large scale while reducing cost compared to the currently available amendments.

While low molecular mass organic acids and alcohols are effective, they are not cost-effective in that they need to be continuously added and the starting specialty chemicals are relatively expensive. Soybean oil-based electron donors are ineffective for promoting metal reduction, in most cases lipid-only electron donors promote methanogenesis at the expense of all other microbial processes. The rendered co-product electron donors are a balance between faster oxidizing compounds akin to low molecular mass organic acids, and longer-term electron donors like lipid-only soybean oil derived compounds.

Rendered co-products contain protein, which hydrolyses and ferments to generate several smaller molecules including amino acids, ammonium, and peptide short polymers. All of these protein derived compounds promote metal reduction effectively, and microbial respiration both fast and long-term is facilitated by proteins. Once the proteins stimulate activity, the lipid portion of the co-product electron donor can sustain microbial activity via fermentation to low molecular mass organic acids, alcohols, and molecular hydrogen. Some co-product electron donors have free amino acids and/or ferric iron as part of the molecular structure, which specifically promote metal reduction. These are solid phase compounds that can be applied several ways: permeable reactive barrier (PRB), direct push borehole backfilling, surface overlay, or for surface water applications in-line tile drain baffles in which the water flows through (for river systems with metal contamination). The cost is relatively inexpensive for large applications in which many tons of electron donor (or other reactive amendment) are required. This works to the advantage of mine waste sites, which are generally large and have higher volumes of water to be treated.

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