Substrate and biogeochemical design considerations for sulfate reducing bioreactors

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Abstract This paper examines the effect of organic substrate on zinc removal, microbial community structure and geochemistry of sulfate reducing bioreactors. Eight columns supplemented with limestone have been receiving mining influenced water for 6 months. Columns containing Ponderosa Pine chips performed poorly (<50 % zinc removal) while columns containing alfalfa, walnut shells or Ponderosa Pine sawdust removed over 99 % of influent zinc. Phylogenetic characterization of microbial DNA showed significant differences in diversity with substrate. Geochemical modeling indicated that limestone dissolution should lead to $ZnCO_{3(s)}$ precipitation. Zinc removal in the eight columns appears to be a complex process of biotic and abiotic reactions.

Keywords sulfate reducing bioreactor mine water treatment

Introduction

Sulfate reducing bioreactors (SRBRs) are an inexpensive and simple, yet elegant way to remove metals from mining influenced water (MIW) via metal sulfide precipitation. An organic substrate mixture such as wood and hay is used as a carbon and energy source by a complex microbial community responsible for the reduction of sulfate to sulfide. This sulfide then complexes with metal ions forming insoluble precipitates. However, the apparent simplicity of SRBRs belies the complexity of the biogeochemistry that is responsible for metal removal. Design of SRBRs must incorporate organic and inorganic substrate selection and, geochemical considerations, along with physical design parameters. While pine chips are a common and inexpensive component of SRBR's, terpenes found in pine resin can inhibit microbial activity. Ponderosa Pine in particular has been associated with inhibitory concentrations of terpenes (Himejima *et al.* 1992). Field experience has shown that SRBRs often fail to efficiently remove metals or experience premature failure, indicating that improved design guidelines are needed.

The goal of this paper is to present our preliminary evaluation of substrate and biogeochemical considerations relative to the performance of 8 large-scale columns operated under field conditions by Freeport-McMoRan (FCX). Results to date support multiple concurrent mechanisms of zinc removal and varied extent of zinc removal.

Methods

MIW

The columns were fed mining influenced water (MIW) collected from a FCX mine site and stored in a large tank. The MIW had a pH ≈ 6.5 with the following major constituents: sulfate, 5200 mg/L; chloride, 30 mg/L; calcium, 520 mg/L; magnesium, 720 mg/L; sodium, 180 mg/L and zinc, 160 mg/L. The alkalinity of the MIW was essentially zero.

Column Construction and Operation

The columns were constructed from 6″ (0.15 m) I.D. plastic pipe with an active substrate containing length of 40″ (1 m). Five liquid phase and three solid phase sampling ports were installed along the length of the col-

Column	Woodchip %	Sawdust %	Alfalfa %	Walnut Shells %	Limestone %	Total Mass (kg)	Pore Volume (L)
C1	50	10	10	-	30	3.9	14.3
C2	-	35	35	-	30	3.7	14.2
C3	35	-	35	-	30	3.6	13.9
C4	35	35	-	-	30	5.2	13.7
C5	-	-	70	-	30	3.5	12[a]
C6	-	70	-	-	30	6.2	13.1
C7	70	-	-	-	30	3.9	15.1
C8	-	-	-	70	30	14.4	12.7

[a] Approximate. Column began to leak while filling.

***Table 1**. Column content by mass fraction and approximate pore volume of each column.*

umn. The columns were packed with various combinations of Ponderosa Pine woodchips, Ponderosa Pine sawdust, alfalfa hay or crushed walnut shells (Table 1). All of the columns contained 30 % limestone by mass with a particle size between 4.8 and 6.3 mm. The columns were then filled with MIW and the inoculum mixture described below. The initial MIW and inoculum mixture was then recirculated through the columns for circa 2 months. The columns were then switched to down-flow mode with a flow rate of 0.4 L/d (hydraulic residence time between 30 to 38 days). The flow rate for columns C2, C5, C6 and C8 was increased to 0.8 L/d 92 days after flow was initiated.

Organic Substrate

The Ponderosa Pine woodchips and sawdust were both sourced from a lumber mill in Santa Clara, NM. The woodchips were collected about 5 years ago. For the last 4 years, the woodchips were stored outside in a plastic 55-gallon (208 L) drum. The sawdust was procured in early 2012 where it had been sitting for a few months at the sawmill in an area that was outside and partially exposed. Trees for both materials were sourced from the same geographical region with no major changes in material or location in the last 10 years as part of a thinning operation that only cuts younger trees. The alfalfa was grown near Safford, AZ. The origin of the crushed walnut shells is unknown, other than being grown in the United States of America. No attempt was made to sterilize the organic substrates.

Inoculum

A sulfate reducing enrichment culture grown on sulfate and ethanol obtained from the University of Arizona was used to inoculate five separate microcosms, each containing one of the organic substrates used in the columns. Because the organic materials were not sterilized, they likely contained additional microorganisms beyond the inoculum. These microcosms were then incubated under sulfate reducing conditions. A mixture of the liquid phase from these five microcosms was then used to inoculate the columns.

Sampling

Liquid samples for DNA extraction were collected from the middle liquid sampling ports 41 days after pumping was initiated. These samples were collected under a flow of nitrogen to maintain anaerobic conditions. Liquid effluent samples were collected periodically for metals analysis.

Analytical

Metals were analyzed by inductively coupled plasma emission atomic spectroscopy (ICP-AES) using a Perkin Elmer Optima Model 3000. Alkalinity was measured in the field by titration. Sulfide was measured using a HACH test kit (part number 223801). Oxidation-reduction potential (ORP) and pH were measured on-site using potentiometric probes.

DNA Processing

DNA was extracted from aqueous phase samples collected from the middle sampling port of each column. Ten mL of effluent was filtered with 0.2 micron polycarbonate filters and bead beaten for 1.5 minutes, DNA was extracted using a phenol chloroform protocol, reducing the contaminants such as humics and other PCR inhibitors. The DNA was then processed by high-throughput pyrosequencing by a commercial laboratory (Engencore) and analyzed using the quantitative inferences into microbial ecology pipeline (QIIME) and the Greengenes database.

Results

Metal Removal and Effluent Chemistry

The major contaminant of concern in the selected MIW is zinc. Fig. 1 displays the effluent zinc concentration in each column as a function of elapsed time after pumping was initiated. No data was presented until after one pore volume of water had been pumped through the columns. The zinc concentration in columns containing pine chips increased to above 100 mg/L by day 118. The rest of the columns achieved zinc effluent concentrations of less than 1 mg/L by day 118. Fig. 2 presents alkalinity data for the same time period. The decreasing trend in alkalinity for columns C2, C5, C6 and C8 after day 90 corresponds with the increased pump-rate that occurred at that time. Other factors may also be affecting alkalinity.

Microbiology

Based on the liquid phase DNA samples collected on day 40 of column operation, species diversity as measured by the number of species per number of sequences per sample, was greatest in C6 (sawdust only) and lowest in C7 (woodchips only). The species diversity in C6 was 4 – 5 times greater in C6 relative to C7. No clear trend has been identified between the microbial species present and zinc removal efficiency. No microbial density measurements have been performed.

Geochemical Modeling of Metal Removal Mechanisms

Geochemical modeling (Visual MINTEQ, Gustafsson 2011) indicates that the influent MIW is slightly over-saturated with respect to gypsum ($CaSO_4(s)$) at 25 °C. Modeling predicts that at equilibrium, 58 mg/L calcium and 138 mg/L sulfate should precipitate to form 196 mg/L gypsum. In fact, several MIW samples that were not diluted or acidified before transport to CSM contained precipitate. This

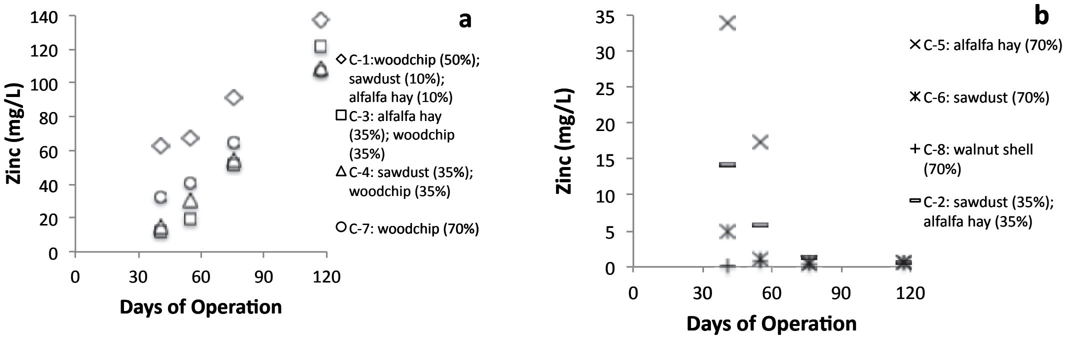

Fig. 1 *Zinc concentration in column effluent as a function of time elapsed after pumping commenced. a) displays data from columns that contained pinewood chips. b) has only the columns that contained no pine woodchips.*

Column Number and Contents	Zn (mg/L)	pH	DO[1] (mg/L)	ORP[2] (mV)	Alkalinity[3] (mg/L)	Ca^{2+} (mg/L)	S^{2-} (mg/L)
C-1: woodchip (50%); sawdust (10%); alfalfa (10%)	137	6.6	2.3	87	150	670	0
C-2: sawdust (35%); alfalfa hay (35%)	0.7	6.8	0.3	-258	470	740	55
C-3: alfalfa hay (35%); woodchip (35%)	122	6.5	1.7	84	330	770	0
C-4: sawdust (35%); woodchip (35%)	109	6.5	1.8	82	160	670	0
C-5: alfalfa hay (70%)	0.6	6.5	0.6	-181	520	840	59
C-6: sawdust (70%)	0.6	6.9	1.8	30	200	740	1
C-7: woodchip (70%)	107	6.7	2.5	79	120	600	0
C-8: walnut shell (70%)	0.5	6.8	0.5	-181	480	740	33

[1]Dissolved Oxygen, [2]Oxidation-reduction potential, [3]Measured as mg/L CaCO$_3$

***Table 2**. Column effluent chemistry on day 118 of column operation.*

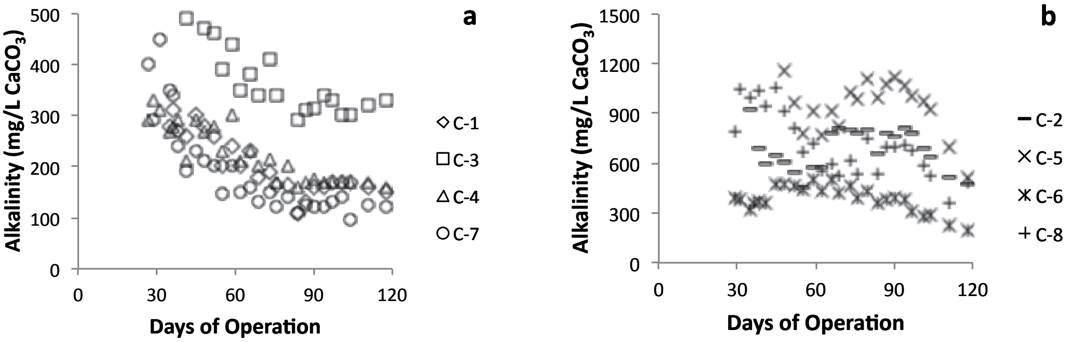

***Fig. 2** Alkalinity in column effluent as a function of time elapsed after pumping commenced. Fig. 2a displays data from columns that contained pinewood chips. Fig. 2b has only the columns that contained no pine woodchips.*

also occurred in some column effluent samples that were not diluted upon collection.

Analysis of ICP data from the supernatant of a sample containing precipitate before and after the precipitate was dissolved by acid showed both calcium and sulfur mass decreasing by 33 % when precipitation occurred. Interestingly, the concentrations of zinc, magnesium and sodium also decreased (circa 30 % by mass), indicating that these ions had co-precipitated with the gypsum. It is likely that these elements were co-precipitated because geochemical modeling indicated that none of these three ions (Na, Zn and Mg) were over-saturated.

As the MIW contacts limestone in the columns, the limestone will dissolve releasing calcium and carbonate. Substantial limestone dissolution is occurring in all of the columns except C7 as indicated by increased calcium concentration in the effluent. Additionally, the process of sulfate reduction produces carbonate. The increased calcium causes the system to be even more over saturated with respect to gypsum, increasing the likelihood of gypsum precipitation in the column and presumably co-precipitation of other cations. Gypsum has been observed in water samples taken from the column side-ports. In addition, the increased CO_3^{-2} concentration may lead to the over-saturation as well as precipitation of zinc carbonate ($ZnCO_{3(s)}$). The solubility of zinc carbonate is strongly dependent on total carbonate concentration and pH (higher pH leads to lower solubility because more of the total carbonate is in the form CO_3^{2-}).

To model $ZnCO_{3(s)}$ precipitation, the amount of carbonate produced in the columns must be estimated. The following two methods were used. Alkalinity titrations were used assuming that all of the alkalinity in the water samples is due to carbonate and that the titration captures all of the carbonate. There are two major sources of error in this assumption: 1) other sources of alkalinity are likely present, notably, organic acids, leading to over-estimation and 2) the starting pH of the titrations is near the first pK_a of carbonic acid so not all of the carbonate will be captured in the titration, leading to under estimation of total carbonate. Another estimate of carbonate was made by looking at the amount of calcium produced in the column, using the assumption that all of the calcium is coming from the dissolution of limestone (1 mole CO_3^{-2} for each mole Ca). This method could lead to under-estimation due to: 1) any carbonate produced via microbial metabolism will be missed because no calcium is produced and 2) precipitation of gypsum in the column will cause under-estimation of calcium produced.

The modeling predictions in Table 3 were made assuming the total carbonate produced in the columns ranged from 120 to 800 mg/L (as $CaCO_3$) as indicated by the two methods described in the above paragraph. Zinc and sulfate concentrations of 160 mg/L and 5200 mg/L, respectively, were used. The sulfate affects the precipitation of zinc carbonate by forming soluble zinc-sulfate complexes that act to increase zinc solubility. The effluent pH of the columns ranged from 6.5 to 6.9 on day 118, which would result in between 21 to 97 % of the zinc being removed by zinc-carbonate precipitation (assuming equilibrium), depending on the carbonate concentration.

pH	Carbonate (mg/L $CaCO_3$)	Zinc Precipitated (%)
6.0	120	0
6.0	800	56
6.5	120	21
6.5	800	91
7.0	120	55
7.0	800	98

***Table 3.** Zinc precipitation ($ZnCO_{3(S)}$) predicted by Visual MINTEQ with 160 mg/L zinc and 5200 mg/L sulfate.*

The production of organic acids in the column could increase zinc solubility by forming soluble zinc organic-acid complexes. However, Visual MINTEQ predicts this effect to be small. If 200 mg/L of both acetic acid and propionic acid were present, this would decrease the precipitation of zinc at pH 6 when 716 mg/L carbonate is present by approximately 1 %.

If sulfide is present, geochemical modeling predicts that essentially all of the sulfide produced will form zinc-sulfide precipitate (spharelite, $ZnS_{(s)}$) until all of the zinc is consumed. Because zinc sulfide is over 10 orders of magnitude less soluble than zinc carbonate, as long as excess dissolved sulfide is present, any zinc carbonate present should be converted to zinc sulfide.

Discussion

The Ponderosa Pine woodchips used in these columns appeared to affect initial microbial diversity as indicated by the microbial DNA data. Zinc removal after 4 months of operation is also low in the columns containing woodchips, although it is not entirely clear if this is due to reduced sulfide production (less zinc sulfide precipitation) or because the pH and alkalinity are lower in these columns, which would result in less zinc carbonate precipitation. The temporally increasing zinc concentration in columns C1, C3, C4 and C7 corresponds with the decreasing alkalinity in these columns. These trends and the modeling results in Table 3 suggest that zinc carbonate precipitation may be the primary zinc removal mechanism in the woodchip containing columns; the decreasing alkalinity may explain the increasing zinc concentration. However, zinc sulfide production in these columns cannot be discounted with the available data. The decreasing alkalinity may be due either to less limestone dissolution as well as decreasing sulfate reduction. It is difficult to estimate limestone dissolution from the calcium concentration because of gypsum precipitation in the columns. It is possible that both zinc removal mechanisms are occurring simultaneously.

The higher alkalinity in the columns without woodchips suggests that microbial activity is higher in these columns relative to the woodchip containing columns. This is also supported by the detection of sulfide in the non-woodchip containing columns; as mentioned previously, sulfate reduction results in the production of alkalinity. The enhanced zinc removal in columns C2, C5, C6 and C8 would most likely be due to zinc sulfide precipitation due to the lower solubility of zinc sulfide relative to zinc carbonate. The higher alkalinity in these columns would, however, make zinc carbonate formation more likely in the case that sulfide is completely consumed.

Interestingly, the Ponderosa Pine sawdust does not seem to have the same inhibitory effect as pine-chips. It is possible that the inhibitory compounds have leached out of the sawdust due to increase mass transfer rates resulting from the much greater specific surface area of the sawdust. Sealed methanogenic batch incubations performed by CSM with these two materials both exhibited relatively low biodegradability (data not shown), suggesting that the sawdust is also inhibitory in a sealed system where potentially inhibitory compounds cannot be flushed away.

Geochemical equilibrium modeling suggests potential zinc removal mechanisms but the predictions must be interpreted judiciously. One reason is that the modeling assumes equilibrium when predicting the formation of precipitates. However, the kinetics of precipitation can be slow and real systems are often not in equilibrium. Another reason is that, prediction of the quantity of precipitates relies on knowing the total amounts of the constituent reactants present. If precipitation is occurring in the columns, the measured aqueous concentrations of the relevant elements may not reflect the total concentrations in the system. Regardless, geochemical modeling provides valuable insight as demonstrated by the potential for zinc carbonate precipitation in the columns.

A more complete story on the substrate and biogeochemical considerations for sulfate reducing bioreactor design is forthcoming. Future plans include additional collection of liquid phase and solid phase samples from the still operating columns. This will allow comparison between liquid phase and biofilm microbial community structure. The identity of mineral precipitates on the solid phase substrate will be examined by scanning electron microscopy – energy dispersive x-ray spectroscopy (SEM-EDS) and presumed metal forms based on sequential extractions (*e.g.* Tessier *et al.* 1979). The relative amount of solid phase organic matter degradation will also be evaluated. The relationship of the microbial community with geochemistry and organic matter changes are expected to allow us to improve our design procedure for SRBRs treatment mining influenced water.

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