Project Report

Big Seven Mine Water:

Bench-Scale Testing of Anaerobic Bioremediation Using Local Substrates

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1 Project Description and Objectives

1.1 Problem Definition/Background

Mine Impacted Water (MIW), usually generated from mine sites, contains elevated levels of toxic heavy metals that can have an adverse impact on the environment. The major pathway involved in the generation of MIW is weathering of pyritic mineral ores, where sulfur is oxidized to sulfate followed by the release of metal ions. The low pH of the MIW generally leads to higher metal concentrations that are active and labile in the aqueous phase. Increased awareness of heavy metal toxicity and extent of water pollution throughout the country have stimulated regulators to investigate treatment processes which can remove heavy metals from MIW.

In mine water treatment (MIW), there is a preference for using passive remediation techniques because of potential for low cost and low maintenance, and reduced amounts of hazardous waste requiring disposal. Passive remediation using sulfate reducing bioreactors (SRBRs) is a promising approach to MIW treatment. SRBRs use heterotrophic or autotrophic anaerobes called Sulfate Reducing Bacteria (SRB), and offer the advantages of low operational costs and high metal removal even when operating with a low pH influent even in remotes sites, with no electricity and receiving heavy winter snow. The main mechanism of the SRBR process is the reduction of sulfate, usually present in high concentrations, to sulfide using a carbon source as the electron donor. Upon sulfate reduction, metal precipitation and co-precipitation occur via the formation of metal sulfides, hydroxides, and carbonates.

In most bioreactors, the inoculum sources of SRBs are the consortia existing at the remediation site. Sometimes, the addition of external inoculums are needed, and these come from sediment, manure, or other sources. In this case, the two selected substrates contained local materials only: 1) wood chips, hay, and horse manure for the ligneous substrate, and 2) Mackay Gulch soil, quartz sand, and horse manure for the soil-based substrate. The Mackay Gulch soil was collected from overburden material excavated at a mine tailings repository in Montana and was classified as sandy soil according to the particle size distribution performed at the Office of Research and Development laboratory.

The Big Seven Mine Site is the location of a previous gold/silver/lead/zinc mine in Cascade County, MT, which is part of the Carpenter Snow Creek Mining District Superfund Site in Montana. A discharging adit releases several gallons per minute of MIW into an unnamed tributary of Snow Creek, which is a tributary to Carpenter Creek. While there are several metal contaminants identified in the MIW from the Big Seven Mine Site (Table 1.1), zinc (Zn) has been selected as the main target contaminant to be removed, and was used to evaluate the efficiency of the substrates, but other metals like Al, Cu, Fe, Mn, Cd were also investigated in this study. The Big Seven water also contains a high concentration of sulfate, which is necessary for SRB activity.

Table 1.1 Metals Concentrations in Water Collected from the Big Seven Mine Site

Big Seven Mine Water		
Analytes	**Unit**	**Total Metals**
Aluminum	(mg/L)	2.01
Arsenic	(mg/L)	<0.00716
Cadmium	(mg/L)	0.0936
Calcium	(mg/L)	175
Chromium	(mg/L)	<0.0381
Cobalt	(mg/L)	0.407
Copper	(mg/L)	<0.00535
Iron	(mg/L)	117
Lead	(mg/L)	<0.228
Magnesium	(mg/L)	95.9
Manganese	(mg/L)	109
Nickel	(mg/L)	0.756
Potassium	(mg/L)	2.94
Selenium	(mg/L)	0.0626
Silver	(mg/L)	0.00660
Sodium	(mg/L)	4.77
Strontium	(mg/L)	0.453
Vanadium	(mg/L)	<0.0264
Zinc	(mg/L)	46.2
Sulfate as SO_4	(mg/L)	1300
pH	-	3.81
Alkalinity	(mg/L $CaCO_3$)	0.00

Note: Water was collected at the mine adit and characterized at the ORD laboratory.

1.2 Project Objectives

The primary objectives of this work were:

- To evaluate the removal of metals from the Big Seven MIW under anaerobic conditions using two column bioreactors with different substrates: the first constituted by wood chips, hay, and horse manure, and the second consisting of Mackay Gulch soil, sand, and horse manure.

2 Experimental

2.1 Experimental Design

The evaluation of Mn and Zn removal was performed using anaerobic columns. The two experimental columns received influent water at the same flowrate from the same reservoir, the only difference was the substrates in each column. No control columns or replicates were considered since the main project objective was to test the viability of the local materials as substrate. Approximately 60 L of Big Seven MIW was collected and shipped to the EPA/ORD Center Hill Facility in Cincinnati, OH for use in this study. The MIW and solid substrates were stored inside sealed containers at 4ºC until use in the experiment. The solid substrates were characterized for moisture content, elemental composition, and total organic carbon percentage.

The column mixtures were designed to contain 30 g of total organic carbon in each column, accounting for total organic carbon percentage and moisture content of each component of the substrate mixtures. Column 1 was loaded with a mixture of 28.39 g of wood chips, 48.76 g of hay, and 52.84 g of cattle manure (ligneous substrate), while Column 2 was loaded with a mixture of 26.92 g of Mackay Gulch soil, 576.93 g of quartz sand, and 261.55 g of horse manure (soil-based substrate). The sand was digested and washed to ensure it contained quartz sand of high purity. Therefore, the sand was used as an agent to ensure substrate porosity, and not considered part of the substrate. Ensuring porosity was important as most fine grain soils, like the McKay Gulch Soil, tend to ‘cake’ when wetted, and don’t allow water to permeate through the soil easily. The calculation results for determining the substrate mixtures are found in Table 2.1.

Table 2.1. Columns substrate content. The amounts were established based on total carbon content and moisture content of each material

Column 1						
Material	**TOC (%)**	**Required Carbon Mass (g)**	**TOC Material Mass (g)**	**Moisture Content (%)**	**Final Mass Added (g)**	**Substrate Content (w/w) (%)**
Wood Chips	48.0	12.0	25.0	11.9	28.4	22.0
Hay	41.7	12.0	28.8	41.0	48.8	38.0
Manure	41.6	6.00	14.4	72.7	52.8	41.0

Column 2						
Material	**TOC (%)**	**Required Carbon Mass (g)**	**TOC Material Mass (g)**	**Moisture Content (%)**	**Final Mass Added (g)**	**Substrate Content (w/w) (%)**
Mackay Gulch Soil	1.26	0.300	23.7	11.9	26.9	9.00
Manure	41.6	29.7	71.4	72.7	262	91.0

Sand	0.00	0.00	577	0.00	577	0.00

The substrate materials did not leach significant amounts of metals according to their elemental composition obtained through acid digestion (Table 2.2).

Table 2.2 Elemental composition of the substrate materials.

Sample		Wood Chips	Hay	Manure	Mackay Gulch Soil
Element	**Unit**	**Concentration**	**Concentration**	**Concentration**	**Concentration**
Arsenic	(mg/kg)	<1.35	<1.35	<1.35	<1.35
Cadmium	(mg/kg)	0.0931	<0.0710	0.297	1.24
Calcium	(mg/kg)	673	6100	5760	5470
Copper	(mg/kg)	<0.713	5.50	10.3	156
Iron	(mg/kg)	<5.66	90.8	587	38200
Lead	(mg/kg)	<42.9	<42.9	<42.9	286
Magnesium	(mg/kg)	125	1850	2380	11500
Manganese	(mg/kg)	2.24	1.38	11.4	50.8
Potassium	(mg/kg)	5.14	606	167	263
Sodium	(mg/kg)	0.216	313	46.7	151
Zinc	(mg/kg)	0.269	1.55	2.67	38.6

Metal removal efficiency was assessed by comparing the influent/effluent metal concentrations. The MIW was loaded into an 11-liter reservoir and placed inside an anaerobic chamber. The water was pumped from the reservoir to fill the columns from bottom to top. The columns were left idle to promote SRB growth for 6 weeks. Following this period, the MIW was pumped through the columns at a rate of ~1.1 L/week/column with the goal of achieving a hydraulic residence time of 48 hr. The water exited through the top of the column and was collected in Tedlar sampling bags. Samples of influent and effluent water were taken weekly to measure for pH, alkalinity, dissolved elements, dissolved oxygen, and sulfate concentration. During the course of the experiment, oxidation reduction potential (ORP) was monitored in-line and recorded every 15 minutes on a computer, the data was reduced with a daily average and converted to redox potential E_h.

At the conclusion of the 9-week experimental period, the columns were drained of the remaining aqueous solution. The solid residues were collected in three portions (top, middle, bottom) and freeze dried. The dried solids will be characterized for elemental composition.

2.2 Methods

2.2.1 Solids characterization

The solid substrates were subjected to initial characterization analyses detailed in QAPP L20963-QP-1-3, which included the following parameters:

- Moisture: Moisture content of the solid materials allowed to calculate the mass of each component to be added into the columns on a dry basis.
- Elemental composition: the elemental composition of the substrate materials was determined because these materials may leach some amounts of contaminants that may interfere with the metal removal results.
- Total Organic Carbon (TOC): determined the mass of each component was added onto each column to keep the desired carbon content in each substrate.

2.2.2 Aqueous phase weekly analyses

Weekly analyses were performed on the influent and effluent samples of the columns as described in QAPP L20963-QP-1:

- Aqueous pH: pH is a critical factor for the biomass to have a suitable environment to thrive. The desired range would be 5.5-7.5 in the effluent. The influent value was not adjusted and reflected the mine water pH.
- Alkalinity: Mine water alkalinity in the influent could be low, a substrate with buffering capacity would increase alkalinity in the effluent, but also microbial activity within the reactor would increase alkalinity.

- Dissolved Metals: This is an important parameter to measure the efficacy of the treatment. The performance of the reactor could be measured by a percentage removal of certain metal(s) of interest of by establishing an effluent desirable level to be maintained.
- Dissolved Oxygen (DO): The absence of oxygen in the influent reduces the transition zone in the reactor. The presence of oxygen inside of the reactor would generate stress for the biomass.
- Sulfate: Since the reactors remove metals by sulfate reduction to sulfates, this parameter is determinant in learning if the SRB are performing well and if the sulfate reduction was the main metal removal mechanism.

3 Results

3.1 Columns

The columns containing the Big Seven substrates and MIW were run for a total of 15 weeks. The columns sat idle for 6 weeks to allow for SRB growth. After that, MIW was pumped through the columns for 9 weeks. Sufficient growth is determined by visual inspection of the organic matter in the columns (organics turn black), and gas generation (usually H_2S) being present. Each week, the influent and effluent samples were tested for pH, alkalinity, dissolved oxygen (influent only), dissolved elements, and sulfates.

The porosity of the substrate is important because it allows a continuous operation without clogging the reactors. The porosity of the substrate was measured and used to calculate the hydraulic retention time (HRT) of the columns. Operational parameters and the measured porosity of the two experimental columns is reported in Table 3.1.

Table 3.1 Operational variables in the columns: flowrate, total volume, porosity, porous volume, and hydraulic retention time (HRT)

	Unit	Col 1	Col 2
Flowrate	(mL/h)	6.14	6.53
Total Volume	(L)	1158	1158
Average Porosity	(%)	70.6%	66.7%
Porous Volume	(L)	817	772
HRT	(h)	133	118

The HRT used in these columns is conservative (SRB reactors successfully removing Zn have operated in HRT of ~48 h in the ORD laboratory), and is meant to provide the biomass with low demand and generate high efficiency in metal removal.

3.1.1 Flow rate

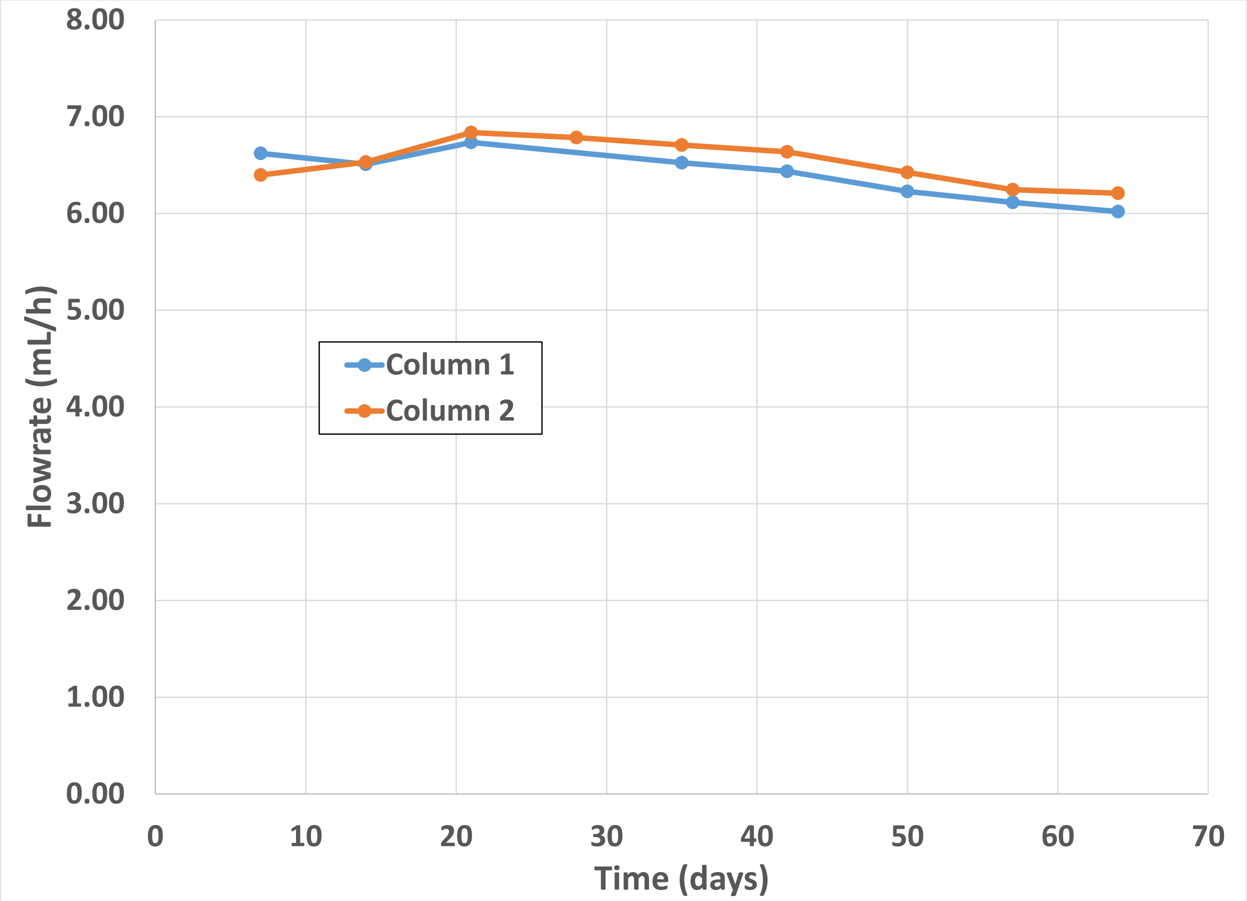


Figure 3.1 Weekly Flowrate Measurement

The flowrate remained constant around 6.0 to 6.8 mL/h during the experimental period in both columns.

3.1.2 pH Analysis

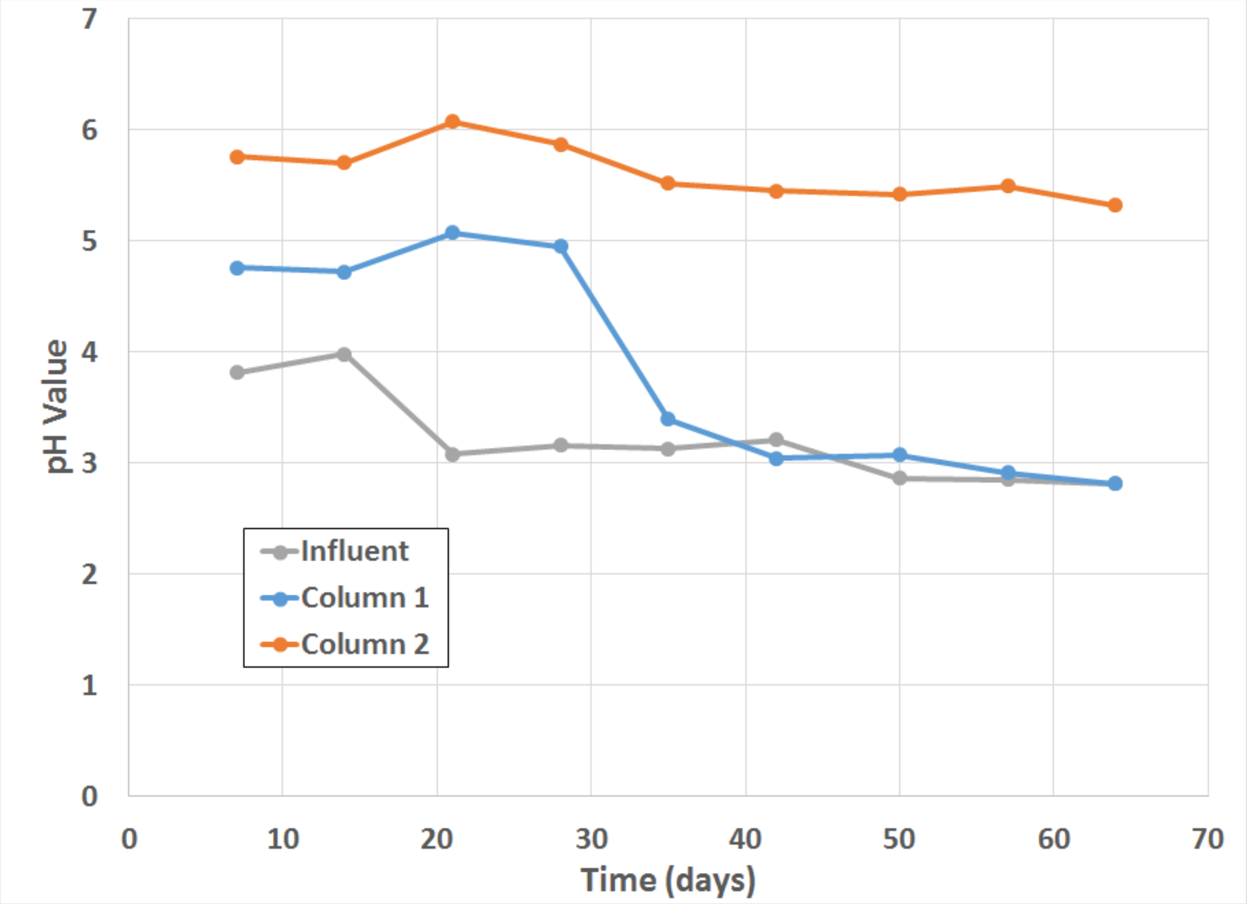
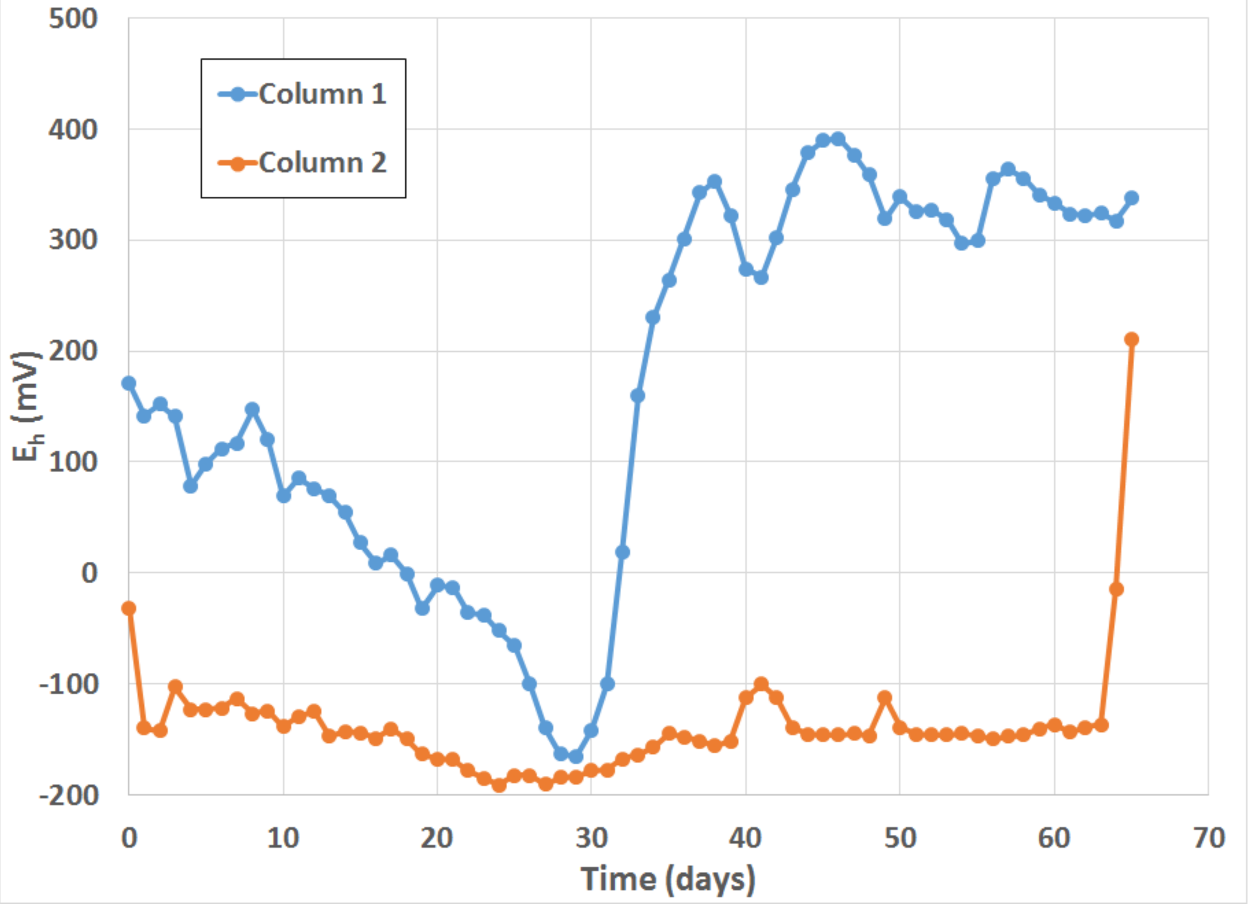


Figure 3.2 Weekly pH Measurement

Over the course of the column pumping, the influent solution and Column 2 effluent saw little change in pH value. This is in contrast to the effluent of Column 1, which showed a marked decrease in pH between weeks 4 and 5, implying that the buffering capacity of the wood chips, hay, and manure substrate was depleted by the 5th operational week.

3.1.3 Redox Potential (E_h)



Redox potential in Column 1 started in a positive value, but consistently decreased with time for 30 days, providing reducing conditions (<150 mV) for two days and then had a sudden increase up to 353 mV. Then the column remained under oxidizing conditions up to the end of the operational period. Column 2 had a start in -32 mV, next day was able to decrease down to -140 mV, but on the third day increased up to -102. After that it was consistently decreased for 24 days down to -191 mV. Column 2 was under reducing conditions until day 63, except for two brief episodes of E_h ~-100 mV. E_h was calculated by adding 202 mV to the values collected in an ORP probe located inside the column right before the effluent outlet.

3.1.4 Alkalinity

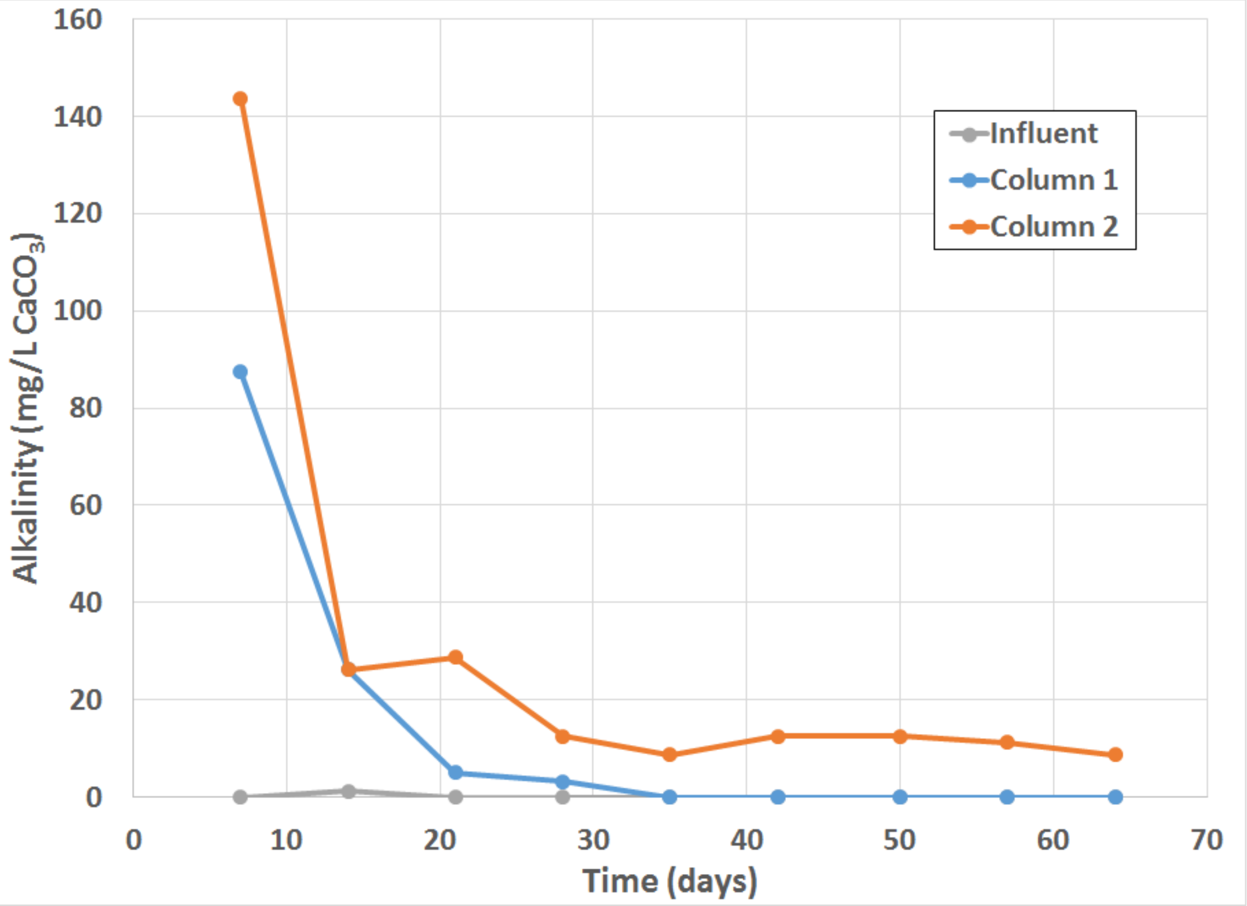


Figure 3.3 Weekly Alkalinity Measurement

Alkalinity decreased sharply in the effluent of Columns 1 and 2 at the onset of pumping. After week 2, alkalinity slowly decreased until Week 5, at which point Column 1 dropped below pH 3.9 (the target titration point for alkalinity) and did not demonstrate a measurable value for alkalinity. Likewise, Column 2 dropped to a minimum plateau of 8.75 mg/L $CaCO_3$, where the alkalinity remained equal or slightly higher for the remainder of the experiment. Probable causes of alkalinity in the reactor during the operational period include:

- A small amount of carbonate (TIC) in the Mackay Gulch soil (TC 1.34% = TIC = 0.04%, TOC = 1.3%) released from the soil in the reactor;
- SRB reduction of sulfate and oxidation of carbon, as the reaction produces bicarbonate.

The biomass initially present in Column 1 provided some buffering capacity for 4 weeks, but couldn't sustain the conditions after that.

3.1.5 Influent Dissolved Oxygen

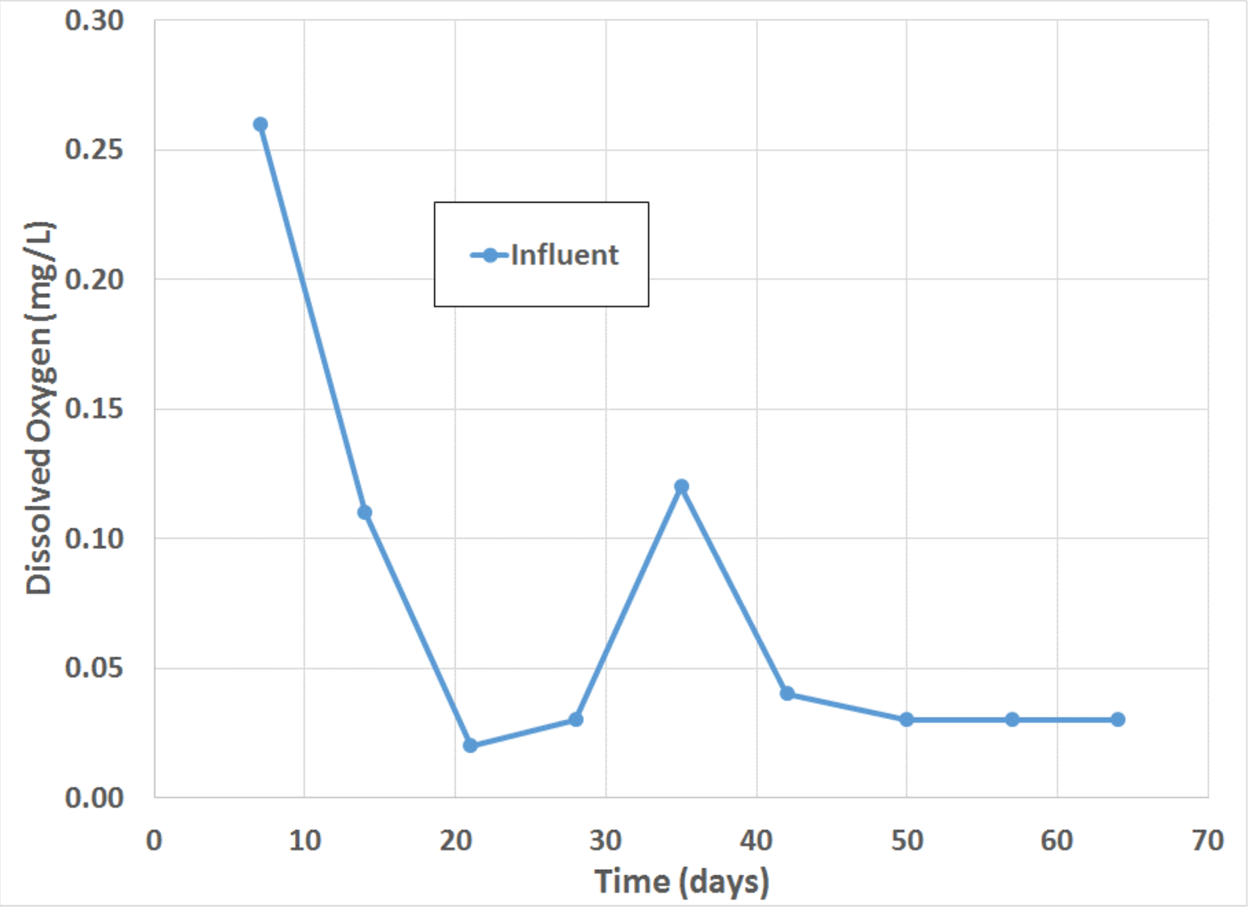


Figure 3.4 Weekly Dissolved Oxygen Measurement

The DO was higher during Week 1. This was likely caused by the incomplete equalization of the influent solution with the atmosphere of the anaerobic chamber. Despite this, the DO remained low during the entire investigation.

3.1.6 Sulfates

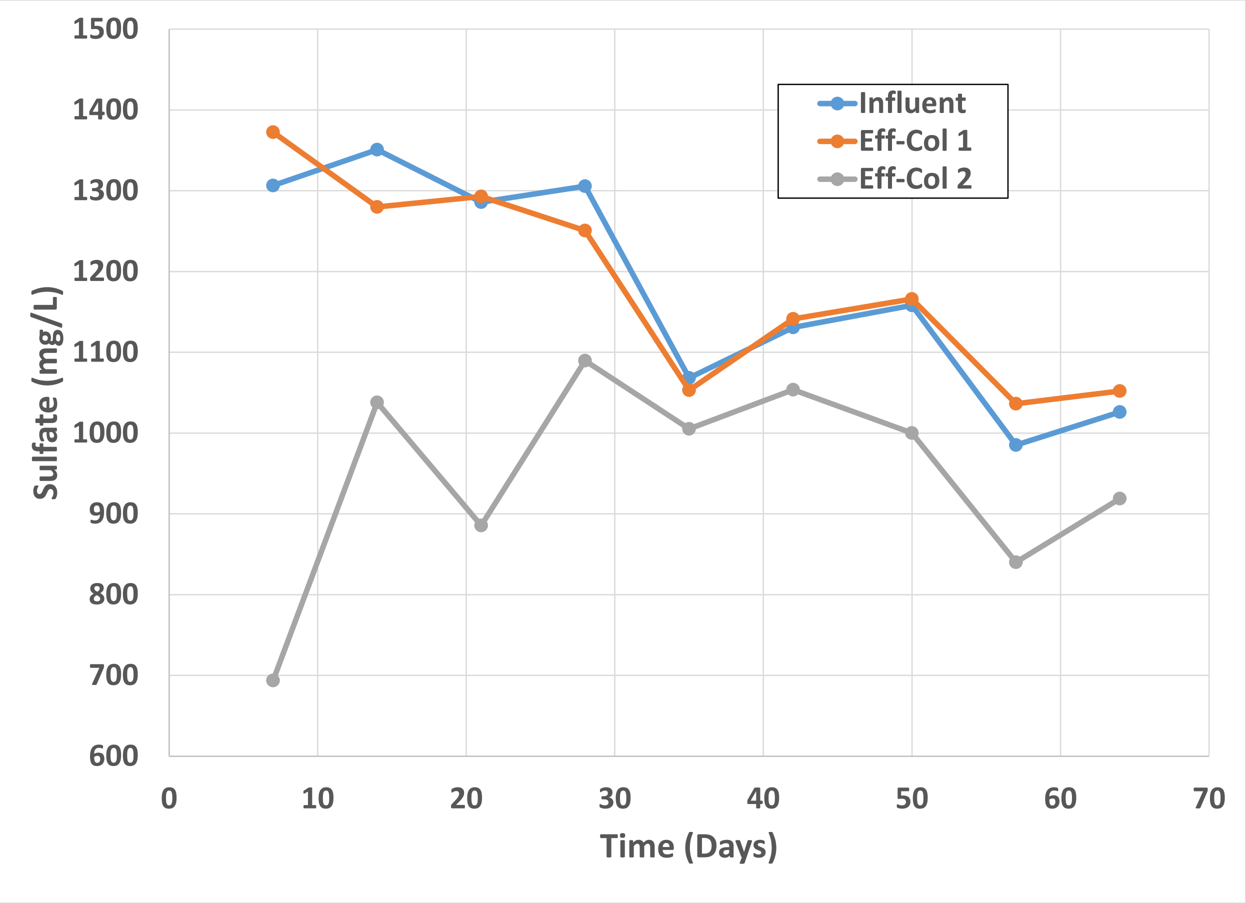


Figure 3.5 Weekly Sulfate Measurement

Column 1 achieved a very poor sulfate removal rate, it seems that the biomass needed increased pH to be able to keep a viable culture during the experiments. Column 2 started with good sulfate removal rates, but by week 4, the removal rate decreased significantly and did not recover. This substrate was exhausted after 9 weeks. It seems that water pretreatment (aeration and pH neutralization) could be desirable to sustain a longer operational period with these substrates that have a low buffering capacity.

3.1.7 Dissolved Metals

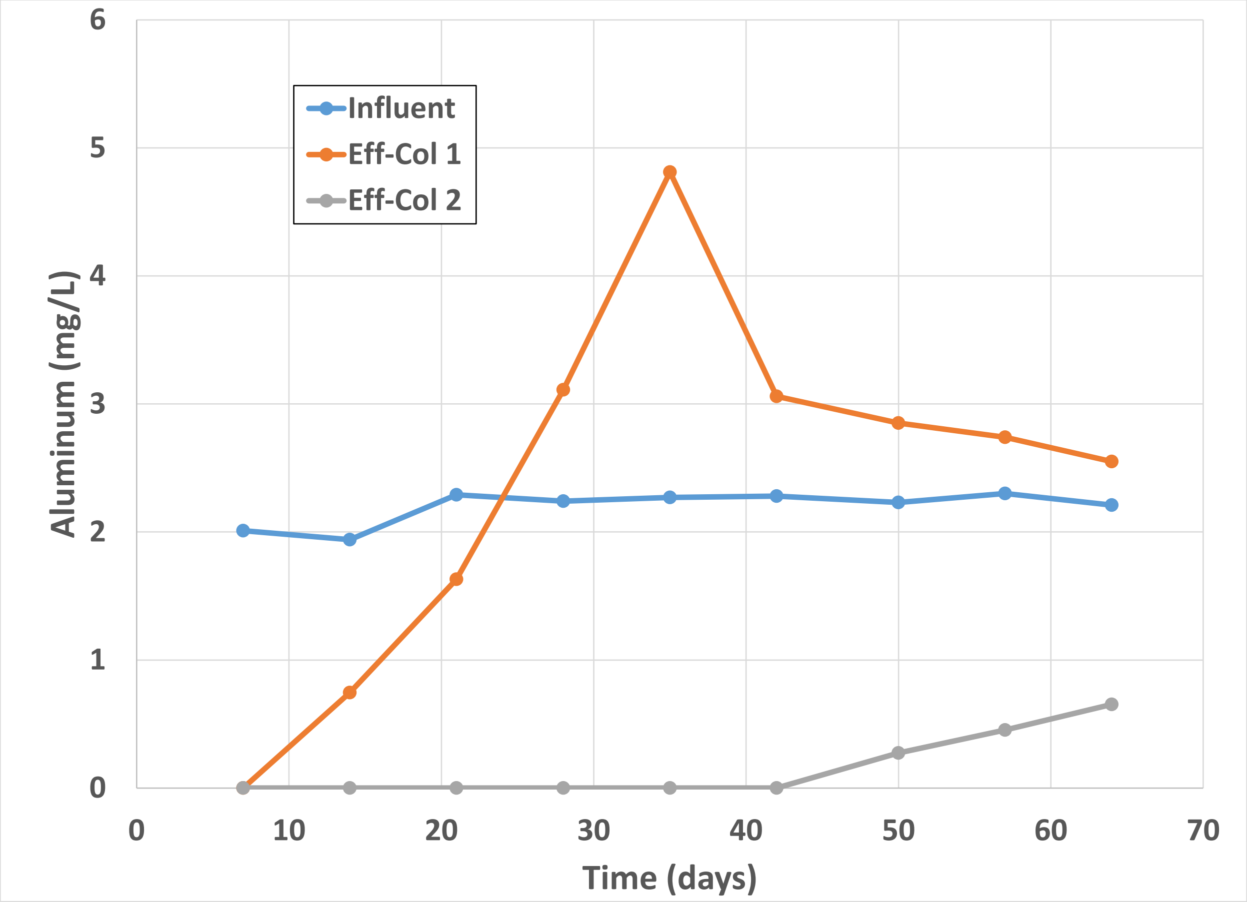


Figure 3.6 Weekly Aluminum Measurement

Aluminum was removed from Column 2 for 7 weeks, and then started to breakthrough. In Column 1, the breakthrough started on week 1.

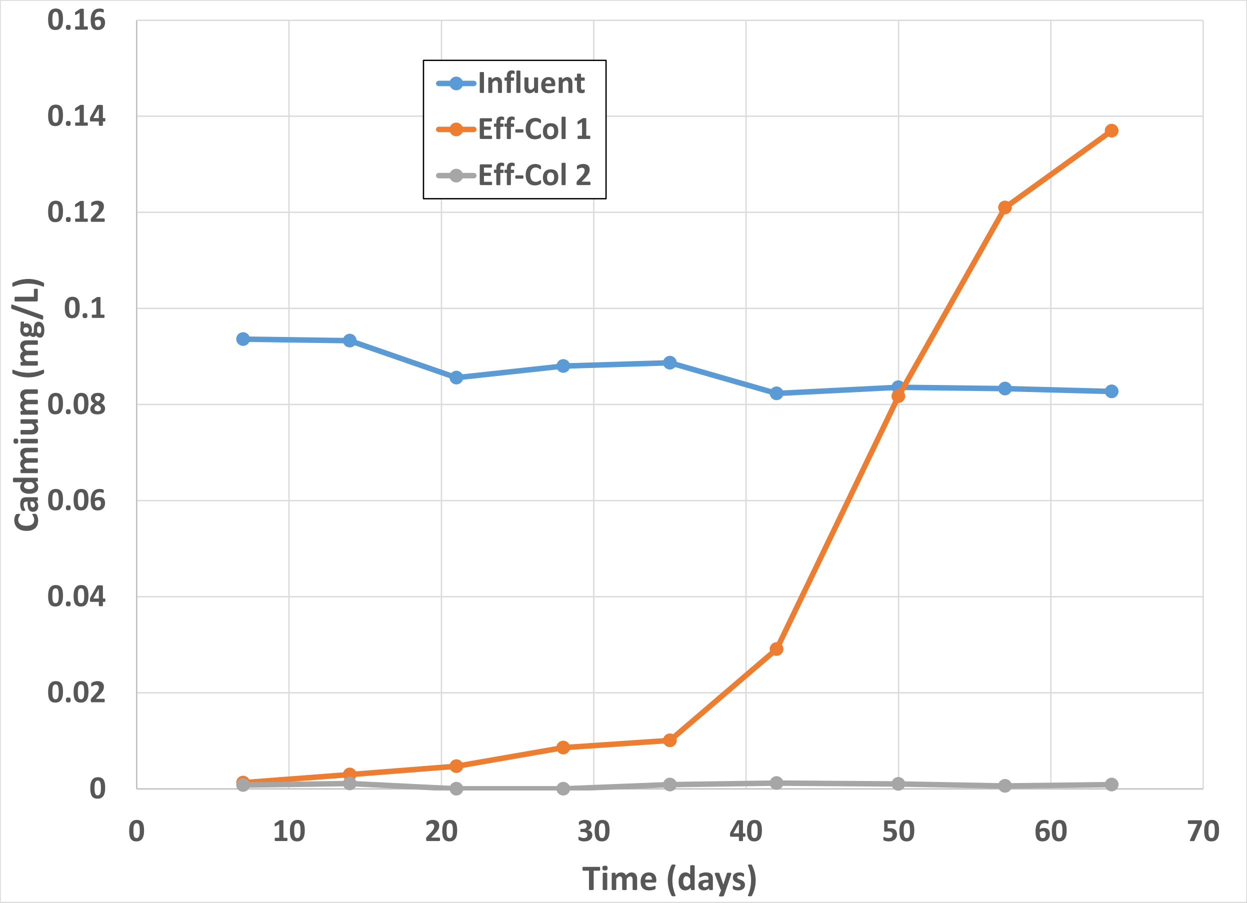


Figure 3.7 Weekly Cadmium Measurement

Cadmium removal was consistent in Column 2. Column 1 had one week of Cd removal and then experienced breakthrough.

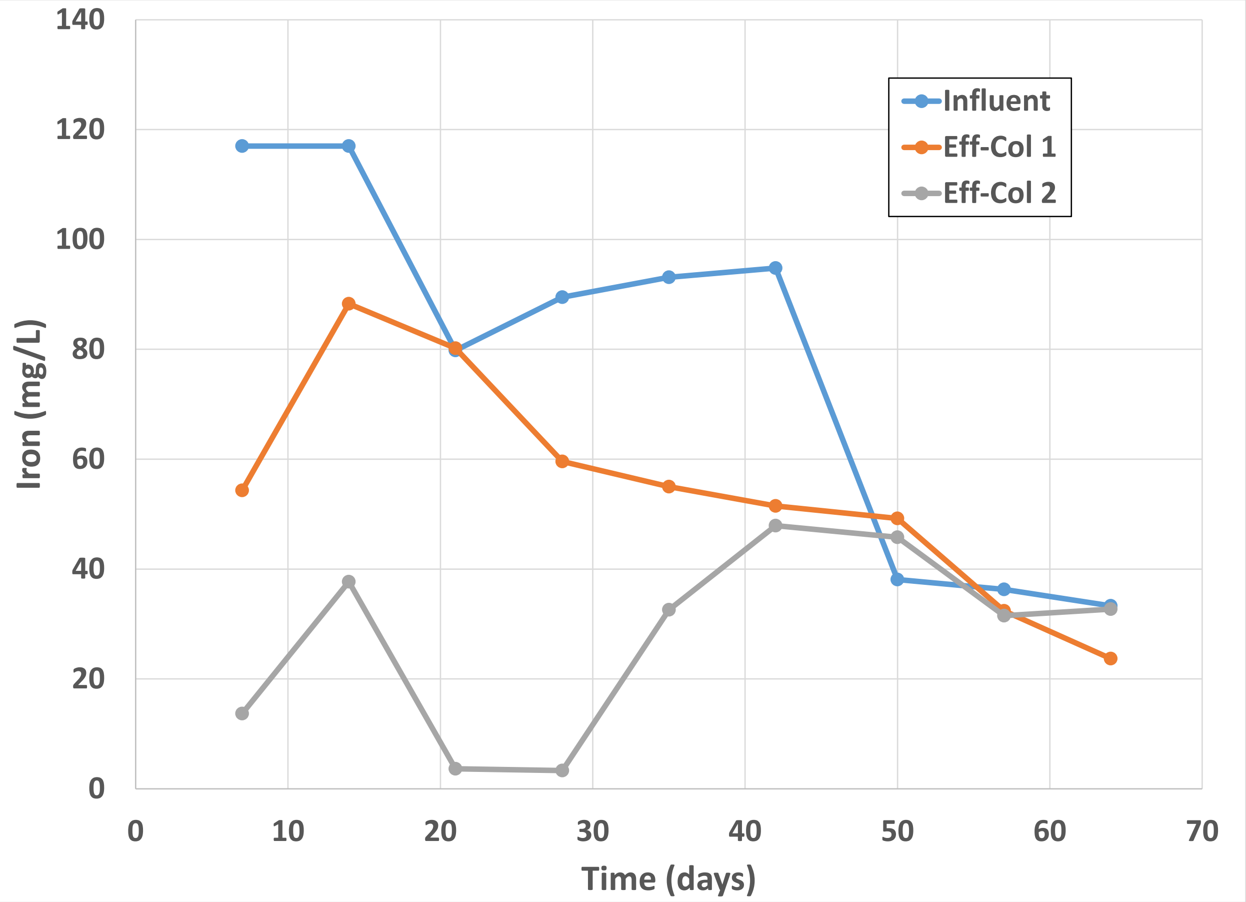


Figure 3.8 Weekly Iron Measurement

Iron was removed in Column 1 in the first 5 weeks, but later, even though the Fe concentration in the influent decreased (probably due to oxidation in the influent reservoir where precipitation occurred), Fe removal decreased to low levels.

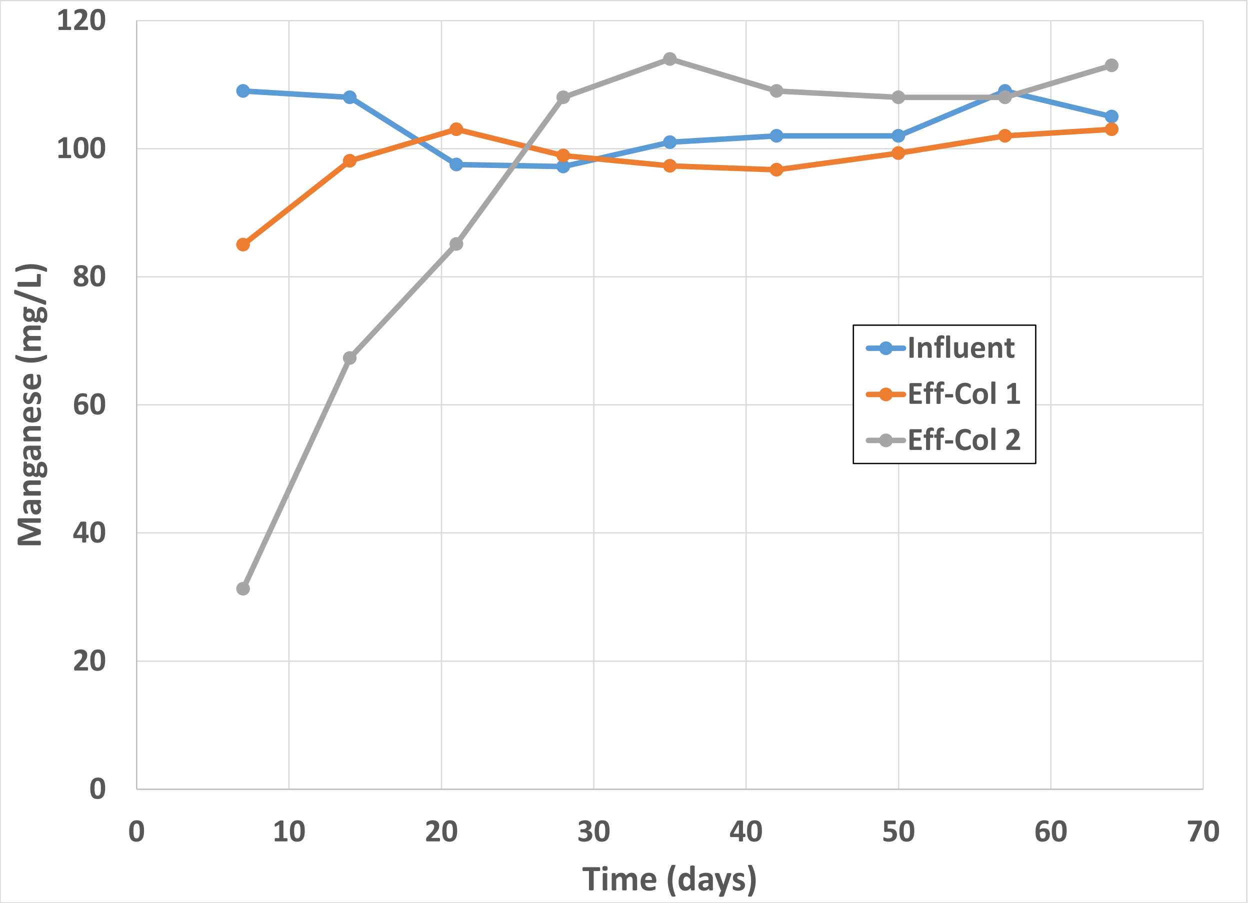


Figure 3.9 Weekly Manganese Measurement

Manganese was present in the Big Seven MIW at 100 mg/L. Column 2 showed a limited capacity to remove Mn during the first three weeks of column pumping. However, this was likely leached back into the water over the course of the remaining weeks. Column 1 removed a small amount of Mn in the first week; after that, there was no Mn removal.

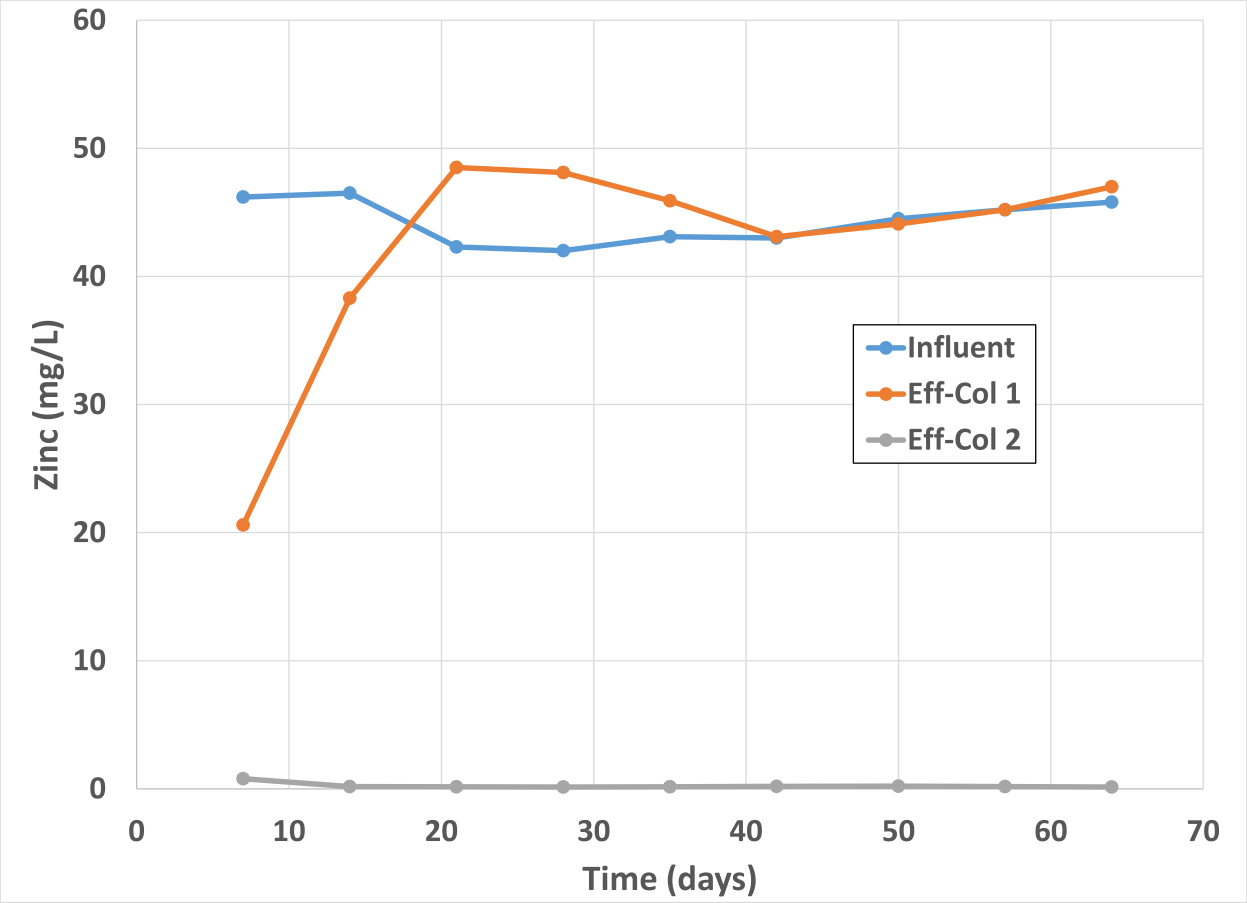


Figure 3.10 Weekly Zinc Measurement

The influent water had Zinc in concentrations of about 45 mg/L. Column 1 reduced Zn by about 50% during Week 1. However, this was leached back into the water up until Week 6 at which time, the Column 1 effluent maintained the same level of Zn as the influent. Column 2 was effective at removing and maintaining Zn at a concentration less than 0.2 mg/L from Week 2 through Week 9.

4. Conclusions and Recommendations

- Column 1 with wood chips, hay, and manure, did not achieve sulfate reduction and also failed to remove Zn.
- The substrate in Column 1 (wood chips, hay, and manure) had a poor performance and probably would need to have the pH neutralized before entering into the column to be able to provide some Zn removal.
- The substrate in Column 2 (Mackay Gulch soil and manure) performed better than Column 1. In spite of limited sulfate removal in Column 2, Zn removal was achieved during the 9 operational weeks. Nevertheless, within the same period, evidence suggests that the biomass (organisms) were exhausted. This was determined by reduced presence of the organisms (by visual determination) on the substrate. We predict that at the low pH in which the column was operated, it wouldn't be able to sustain Zn removal for

longer period than these 9 weeks under the conditions of the experiment. The efficiency of this substrate to remove sulfate and metals may be enhanced by adding another carbon source for the biomass to continue growth. The SRB would also benefit from a less acidic environment to be able to reduce sulfate. This environment could be provided by increasing the pH. It is predicted that an influent pH of ~6 should be sufficient to extend operational period using the Mackay Gulch and manure substrate.

- At this point, we can identify three (3) options to improve the longevity of the treatment with the Big Seven MIW as an influent. The 3 we have identified include (there may be others):

1. Changing the substrate to a high buffering substrate, like a chitin/sand substrate that was used in the ORD laboratory in previous experiments. The chitin product was SC-20 from JRW Bioremediation, which was quoted at a price of $1,800/ton) which achieved successful Zn removal for an operational period of 68 weeks with HRT of 45 h. For the Big Seven Mine, with 3 gpm of influent, operational time 365 days, and HRT of 45 hours, a chitin mass of 46 tons (83K $ at 1,800 $/ton) and a sand mass of 138 tons would be recommended.
2. Adding limestone to the tested substrates (wood chips-hay-manure and Mackay Gulch soil-manure) to increased their buffering capacity. This could be a cheaper option that would allow to extend the operational period, but probably not as long as the SC-20 would.
3. Implement a hybrid treatment system with two phases: aeration and pH adjustment used as pretreatment prior to the anaerobic Zn removal. However, if pretreatment is applied, a plan should be devised to deal with the generated sludge. Specifically, iron-containing sludge could potentially release iron and other metals if the sludge was exposed to weathering. In this case, the substrate mixture will need to be tested with the pretreated water to estimate performance for this hybrid alternative. Previous experiments with pretreated Formosa Mine water and wood chips, hay, and manure provided Zn removal of up to ~0.7 mg/L for 40 weeks and then experienced breakthrough. Zn concentrations did not reach the low levels that the chitin/sand substrate reached (<0.1 mg/L for 68 weeks) or the Big Seven with top soil and manure reached (~0.2 mg/L for 9 weeks).