SUBSTRATE DEGRADATION AND METAL REMOVAL PERFORMANCE OF A 1,500-GALLON SULFATE-REDUCING BIOREACTOR FOR MINING-INFLUENCED WATER TREATMENT[1]

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Abstract: The successful long-term operation and sustainability of passive systems for the treatment of mining influenced water (MIW) affected by metals and acidity has been challenged by a lack of understanding on organic substrate design. Metabolic activity of sulfate-reducing bacteria (SRB) in bioreactors produces H_2S that precipitates dissolved metals, and it is the main mechanism that removes metals from solution. Fermentation products of the organic substrate provided support the SRB indirectly. The relation between changes in bioavailable carbon (C) in the substrate, SO_4^{2-} reduction and metal removal was thus studied.

The systems under investigation are two identical 1,500-gallon tanks filled with volume fractions of 0.7 walnut shells, 0.25 corn stover, and 0.05 manure. The tanks have an operating volume of approximately 1,100 gallons, receive influent water at a nominal rate of 1 L/min, and have an estimated hydraulic residence time of about 1.5 days (based on 50% porosity). The bioreactors receive mining influenced water from the National Tunnel in Black Hawk, CO that has a typical metals concentration of 35mg/L Fe, 8mg/L Zn, and 0.6mg/L Cu; pH is circumneutral at 5.5 ±1.0.

To assess bioavailable C in the substrate, hot water and acid soluble C and lignin-to-cellulose ratios were periodically analyzed. Preliminary chemical analysis of the substrate suggests that only the hot water extractable fraction (organic acids and labile polysaccharides) changed during the first three quarters of operation. The lignin-to-cellulose ratio during the same period was relatively unchanged, suggesting that the rate of organic substrate depletion was low. Sulfate reduction was variable but sufficient to promote efficient removal of Cu and Zn. After the first 12 months of operation, the average percentages for metal removal in the duplicate bioreactors were >99% for Cu and Zn and 70% for Fe, the latter ranging between 40 and 95%.

The low rate of substrate depletion suggests the substrate mixture may provide long-term support of the sulfate-reducing consortium. The bioreactors were able to successfully remove Cu and Zn but Fe removal was problematic. This suggests that extent of SO_4^{2-} reduction was not sufficient to effectively remove iron in the National Tunnel MIW.

Additional Key Words: walnut shell, corn stover, solid phase substrate analysis, metal concentrations

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Introduction

In efforts to mitigate some of the environmental degradation caused by the generation of mining influenced water from abandoned mine sites, passive treatments have been widely studied and implemented throughout the US (for a recent review, see Cohen 2006; Wildeman et al. 1994). Passive systems include sulfate-reducing reactors, environments in which bacterial metabolism reduce sulfate to sulfide. This process precipitates dissolved metals and generates alkalinity, thus neutralizing the acidity of impacted waters (Kaksonen et al., 2006).

To better understand the bacterial activity of passive treatment systems, upon which sulfate-reducing systems rely, Buccambuso et al. (2007), examined the functional microbial communities within bioreactors. Design and performance optimization have also been studied by Zaluski et al. (2006). In addition, several bench-scale studies on substrate selection have been conducted (Figueroa et al. 2007, Place et al. 2005, Seyler et al. 2003), but much remains unknown about the performance of field-scale systems. In this study, substrate degradation and metal removal capabilities of two identical 1,500-gallon bioreactors was examined over a 12-month period. The conceptual design of the bioreactors was presented in Zaluski et al. (2006). The bioreactors design provided a section for removal of most of the Fe as $Fe(OH)_3$ precipitates and a section for SO_4^{2-} reduction. The reactor design also included substrate in replaceable bags to facilitate future maintenance. Thus, inorganic components such as limestone were not included because of weight issues. The reactors receive water from the National Tunnel adit in Black Hawk, CO. The metals concentration of the Influent water is 35mg/L Fe, 8mg/L Zn, and 0.6mg/L Cu, with circumneutral pH at 5.5 ±1.0.

To assess the extent of substrate degradation and sustainability, several parameters were analyzed: organic and inorganic carbon ratios, organic acids and starch contents, and cellulose and lignin fractions. Combustion of a small mass of sample is used to estimate general changes in the organic carbon content in the substrate, and thus it provides a quick and simple way to detect whether carbon is being degraded. To detect more specific changes in the availability of energy sources in the substrate, hot water and acid extractions are performed; the soluble masses of these experiments are organic acids—the carbon fraction more easily biodegradable—and cellulosic material, respectively. The present study tracked changes in substrate composition over time. By observing the rate of carbon depletion we can better understand how sustainable certain substrates may be.

In addition to substrate composition, the concentration of SO_4^{2-} and metals were analyzed. The metal removal ability of reactors was expected to be related to the extent of SO_4^{2-} reduction within the reactors. Sulfate reduction along with substrate utilization, may give a clearer understanding of sulfate-reducing bacteria activity and system design requirements. Bioreactor metal removal performance is strongly dependent on successful sulfate-reduction, which is in turn dependent on the availability of electron donors to the microbial community. Whether the selected substrate can support an active microbial community of SO_4^{2-} reducers to promote metal removal was the objective of this study. This paper is a progress report on the data collected in the first year of the study. A final report that incorporates the final data set and associated statistical analysis will be presented in a future publication.

Methods

Methods I: Substrate Analysis

Previous to filling up the reactors with substrate, two slotted (1/4-slots) PVC pipes of 2" (inner diameter) were placed in each reactor approximately 1-ft from the side-wall and midway between the influent and effluent points (Fig. 1). The columns extend the height of the tank and have slots along the side to allow sacrificial bags to be in close contact with the rest of the substrate and thus be representative of the system. Nylon mesh bags containing 40 g of dry substrate mixture (70% walnut shells, 25% corn stover, and 5% manure), and nylon mesh bags with 10 grams of corn stover. Corn stover is a “waste” material that consists of the stalks and leaves of the corn plant that has a relatively large fraction of “easily degradable carbon” compared to wood. Sacrificial bags containing “corn stover only” were also placed into the columns at the beginning of the experiment. Bags were removed every 120-180 days to analyze the substrate and track its compositional changes. Of the total 52 bags, 24 contain 40 g of the “mixture” substrate and 28 are filled with 10 g of “corn stover only” material. A total of 19 bags have been removed so far and complete analyses have been performed on 12 of them.

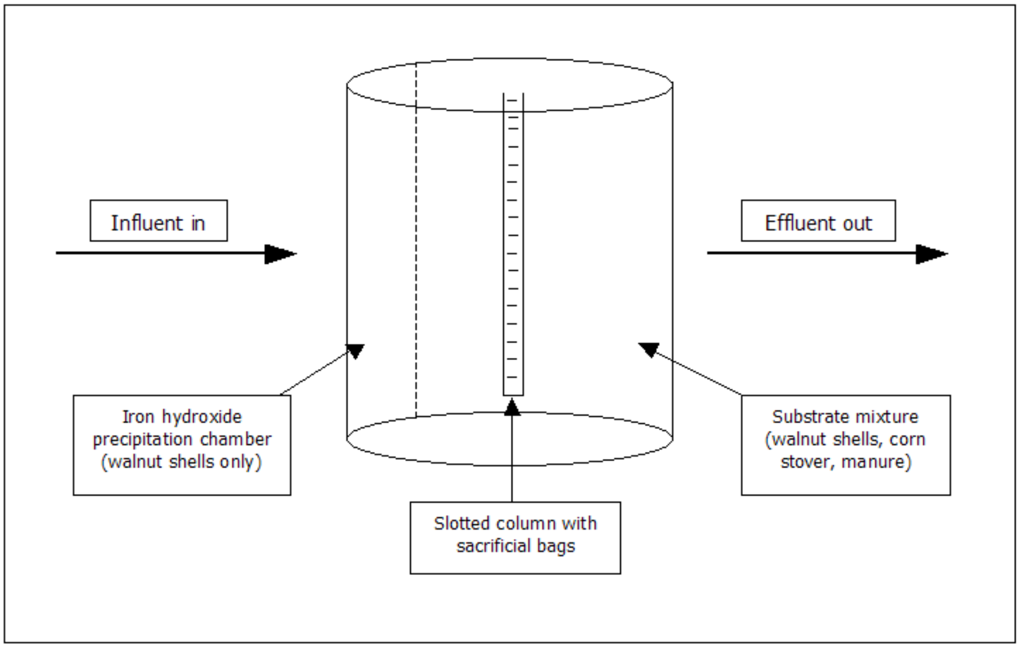

Figure 1 Schematic diagram of a bioreactor showing direction of water flow, precipitation chamber, and one of the two 2-in. diameter pipes mid-way in the reactor, where sacrificial bags are placed.

Analyzed parameters

Below is an outline of the five parameters utilized to track carbon fraction changes in the substrate. Analyses were performed in the following order:

(1) Gross weight of the substrate (dry) was measured in sacrificial mesh bags; comparing the weight of the bags periodically suggests whether the material degrades significantly over time;

(2) Organic and inorganic content of substrate were monitored to indicate whether there were changes in the ratio of the *total* degradable carbon that can potentially support microbial activity;

(3) Organic acids and starch contents, including oligo-, polysaccharides and fructans, were monitored to observe potential changes in the ratio of the *more easily* degradable carbon with respect to the total carbon in the substrate. Because the breakdown of organic acids requires little energy, this fraction of the substrate was utilized first and it supplies byproducts to fuel other metabolic reactions carried out by other microbial populations in the reactors.

(4) Cellulose and hemicellulose content was used to track changes in the ratio of these two forms of carbon that may be *less readily* available for microbial metabolism; and

(5) Lignin content is the most recalcitrant and therefore least available form of organic carbon; it has been shown by others to change negligibly over time (Gibert et al., 2004). Although monitored in this experiment, lignin ratios are not discussed in this paper.

Description of methods

To determine the gross weight changes of the substrate (Parameter #1) over time, sacrificial bags (with initial mass of 40 or 10 g) were dried at 105 ˚C and weighed.

Organic content of the above substrate sample (Parameter #2) was measured by combusting 0.3-0.5 g of the dried sample at 550˚C for 2 hours; organic C is the fraction of sample that volatilizes, and the portion of sample that remains is the inorganic matter.

Starch and organic acid content of the substrate in sacrificial bags, parameter #3, was determined by performing a *hot water extraction* (TAPPI Test Method). Two grams of the solid phase substrate, dried in parameter #1, were placed in a flask, to which 100 ml of boiling water is then added. The mixture is covered and placed in a boiling water bath for 3 hours. The mixture is then filtered under a vacuum through a pre-weighed fritted crucible. The crucible is dried at 105˚C for 12 hours. The material in the crucible is the hot water-insoluble fraction; subtracting the initial from the final mass gives the *hot water-soluble fraction*, which is the organic acid and starch content.

Parameter #4 is measured by performing an acid digestion on the remainder of the hot water-insoluble sample in step #3. Three milliliters of 72% (by weight) H_2SO_4 is added to 0.3 g of sample; the mixture is placed in a 30˚C water bath for 2 hours. The acid hydrolyzes the cellulosic material but does not change lignin composition. The mixture is filtered, weighed, and dried as in step #3. The fraction of sample that remains in the crucible is *acid-insoluble*, or the lignin content—parameter #5. Similar to parameter #3, the acid-soluble, or cellulosic fraction, is determined by subtracting initial from final mass. Figure 2 is a schematic diagram of the substrate analysis. For a more complete description of Parameters #4 and #5, refer to Place et al. (2005) and references therein.

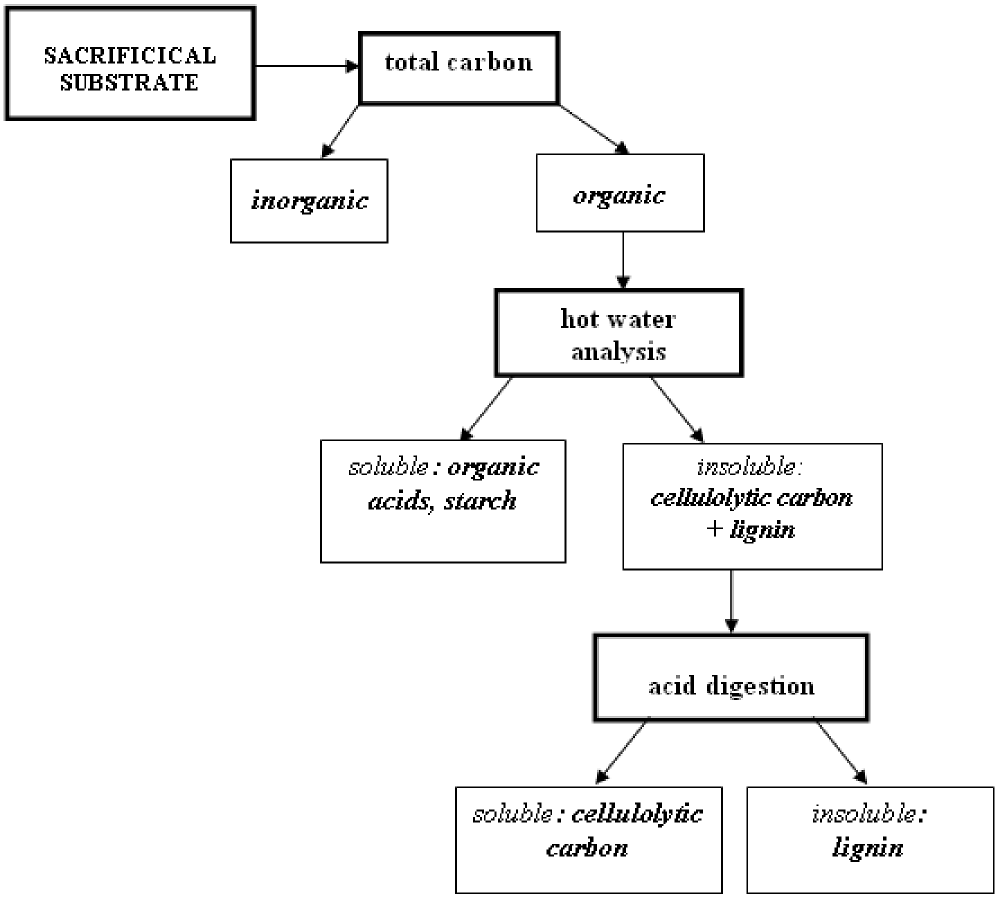

Figure 2 Methods used to determine substrate degradability. Processes are in bold letters; fractions are in italics.

In the 4th sampling event, substrate analysis of a “corn stover only” sacrificial bag was performed. Analyzing the compositional changes of the “mixture substrate” with that of “corn stover only substrate” provided a comparison of relative organic carbon degradation in these two materials, if any. Walnut shells provide physical structure to the mixture and thus ensure a stable hydraulic conductivity within the reactors; because they are mainly composed of cellulose and lignin, however, this material is not a significant source of energy for microbes as the relatively low cellulose to lignin ratio suggests this material is recalcitrant to microbial breakdown. In contrast, corn stover is composed mainly of simple carbon molecules (e.g., organic acids, starch, polysaccharides) and is therefore more accessible to biodegradation. Since there is a higher ratio of simple carbon in “corn stover only” bags, it was expected that “corn stover only” samples would degrade more rapidly than bags containing a mixture of all three substrates; furthermore, a measurable decrease in organic carbon, and hot water-soluble and acid-soluble fractions as also expected.

Prior to analyzing the substrate, collected samples were sieved and divided into two fractions: greater and smaller than 125μm. Weights were recorded to document the proportion of each fraction in the sampled substrate. It is hypothesized that the ratio of organic carbon differs between the two fractions and that in particles smaller than 125μm there would be a higher ratio of inorganic carbon. Analyses were performed in sets of duplicates or triplicates; percentages are the average of samples collected from both Cell 1 and Cell 2.

Methods II: Field Parameters

Temperature, oxidation-reduction potential, and dissolved oxygen concentrations were measured weekly for influent and effluent waters. Temperature was measured with a Conductivity TDS meter (Orion, Model 124). Oxidation-reduction potential was measured with a pH/Eh meter (Beckman, Φ11 pH meter). A Dissolved Oxygen Test Kit was used to determine dissolved oxygen concentrations (Hach, Cat. No. 1469-33).

Methods III: Bioreactors' Metal Removal Performance

Samples of treated effluent water from Cells 1 and 2 and of influent water were collected weekly during the twelve-month period and analyzed by Inductively Coupled Plasma—Atomic Emission Spectrometry (ICP-AES) at the Colorado School of Mines. Prior to analysis, collected field samples were passed through a 0.45μm filter and preserved with concentrated HNO_3.

Results and Discussion

Results and Discussion I: Substrate Degradation

The gross dry weights of the sacrificial bags were 40 g for the corn stover/walnut shell/manure mixture and 10.0 g for the corn stover only bags. The average dry weight of the sacrificial bags collected in each quarter is presented in Table 1. The dry weight of the sacrificial bags was the same as the initial bag for the 1st and 2nd quarters based on a student t-test. However, the mass of the 3rd quarter and 4th bags were significantly different from the initial values with a greater than 95% confidence. The corn stover bag lost a greater fraction of its initial mass that the mixture bags, which was expected.

Table 1. Gross dry weight of sacrificial bags collected at each quarter and range of depths for the bags analyzed at each sampling event. Between 3 and 6 bags were removed at each event.

Sampling Event	Content	Average mass (g)	Standard Deviation	Range of depths of bags in column (m)
Initial	mixture	40.31	n/a	n/a
1st	mixture	40.68	0.23	0.23-0.77
2nd	mixture	39.91	1.67	0.41-0.86
3rd	mixture	38.1	0.61	0.51-0.94
4th	corn stover*	6.75	1.72	0.84-1.1

*Initial mass of corn stover bag was 10 g.

Table 2 summarizes the average organic carbon content of four sacrificial bags, plus initial mixture and initial corn stover. The initial materials represent the baseline chemical conditions of the substrate. Data for fractions greater and smaller than 125µm are presented. As expected, there appears to be a difference in organic carbon content between fractions. However, there was no measurable change in the organic content of the substrate mixture for the > 125µm over the first three quarters. The small changes in total mass to date make it challenging to detect differences in organic content with our methods.

For the < 125µm fraction, there was a difference between the average organic fraction of the Initial and the 1st sampling event (91% compared to 85%) but because of the standard deviation these values are statistically not different. The change in the average carbon content between the initial material and the subsequent quarters is not significantly different until the third quarter. While the average organic content appears to be the same in the 1st, 2nd and 3rd quarter, it is not until the 3rd quarter that the standard deviation is small enough for a statistical difference with the initial material.

Table 2 Organic carbon content of sacrificial bags placed in sulfate-reducing bioreactors and removed for chemical analysis. Standard deviations (±) are next to average percentage values.

	Average organic carbon (%)	
Sampling Event	<125 µm fraction	>125 µm fraction
Initial	91±0.1	97±0.2
1st **Nov. '06 – March '07**	85±5.7	95±2.3
2nd **Nov. '06 – May '07**	84±2.7	96±0.8
3rd **Nov. '06 – Aug. '07**	85±2.2	97±0.8
4th **Nov. '06 – Dec. '07** ***(corn stover only)****	82±1.2	94±0.4

*Initial corn stover values are: <125µm fraction = 0; >125µm fraction = 99±0.2%.

Table 3 below shows hot water-soluble results for the first 3 sampling events. The data suggest that there was a decrease in the amount of availability of organic acids and starches in the substrate. The hot water soluble fraction during the 3rd quarter was statistically lower than the 1st and 2nd quarter fractions. The decrease in hot water-soluble carbon may reflect the existence of a "healthy" microbial population that was obtaining energy from the provided substrate. Considering that sulfate-reducing bacteria rely on the activity of fermenters and cellulolytic bacteria for the initial digestion of complex carbon molecules, it is of importance, from a microbiological perspective, to be able to track changes in the relative masses of these energy sources. The significance is two-fold: it may suggest that there is microbial activity, upon which sulfate-reducing bioreactors rely, and, consequently, that carbon sulfate reduction may be occurring and thus bioreactors are functional. The change in carbon mass over time will be compared to the corresponding SO_4^{2-} reduction rate for the complete study period in a future publication to examine the efficiency of carbon utilization by SO_4^{2-} reducers.

Table 3 Hot water-soluble fractions through time of sacrificial "mixture" bags containing walnut shells, corn stover, and manure from sulfate-reducing bioreactors. Standard deviations are next to average percentage values. *Initial corn stover hot water soluble fraction was 13.0±0.9%.

Sampling event	**Average *hot water soluble* carbon—organic acids and starches (%)**
Initial Mixture	8.7 ± 0.3
1st **Nov. '06 – March '07**	8.4 ± 0.7
2nd **Nov. '06 – May '07**	6.1 ± 0.5
3rd **Nov. '06 – Aug. '07**	3.0 ± 0.2
4th **Nov. '06 – Dec. '07** *(corn stover only)**	5.3 ± 0.9

Lastly, preliminary results from the substrate fraction that is acid-soluble, or the cellulosic material, for the 1st and 2nd sampling events were found to be similar at 60% ±1.5. The relatively constant content of the acid-soluble fraction may reflect a very slow degradation of the cellulosic substrate by the microbial community.

Results and Discussion II: Field Parameters

Water temperatures, shown in Fig. 3, appear slightly higher for the effluent water contained in the bioreactors, especially during the spring and summer months. The 1500-gallon bioreactors appeared to mitigate freezing problems that were associated with smaller (55-gallon) bioreactors also treating National Tunnel MIW over the same winter period in 2006/2007 (Buccambuso et al. 2007).

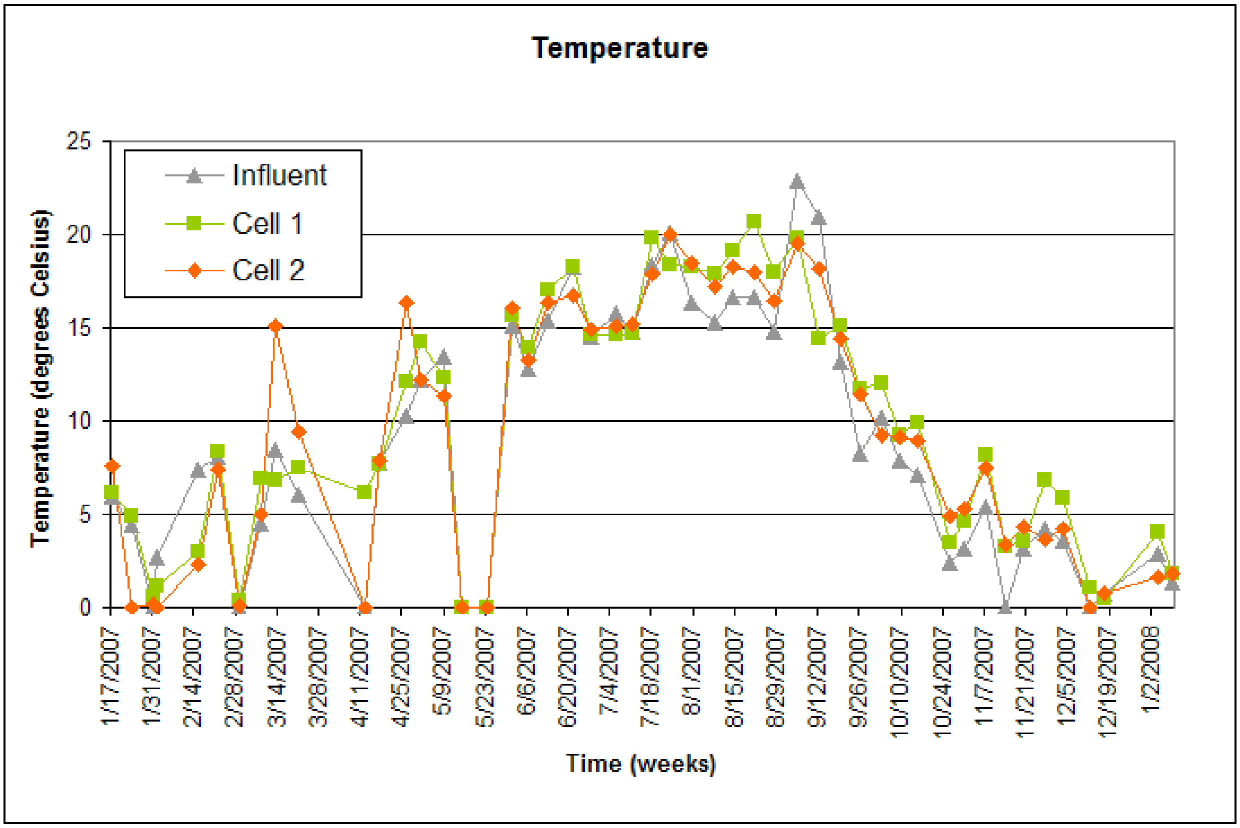

Figure 3 Weekly temperatures of influent and effluent waters.

ORP results for the first 12months of the study are shown in Fig. 4. Reactors became anaerobic relatively quickly (as indicated by negative ORP), about 6 weeks after their set up. Compared to Cell 1, Cell 2 appears to have sustained a more reductive environment throughout. This may explain the overall higher sulfate-reduction in that cell. While Cell 1 had an anaerobic environment overall, it also experience unintentional loading of oxidized iron that may have increased the ORP and impacted sulfate-reduction.

Cell 1 and 2 effluent values for dissolved oxygen were consistently 0 mg/L, while influent dissolved oxygen was always greater than 1 mg/L. Influent dissolved oxygen ranged from 1 to 2 mg/L. Results are not shown graphically. Absence of dissolved oxygen is expected since Eh measurements suggested that the bioreactor environment was anaerobic.

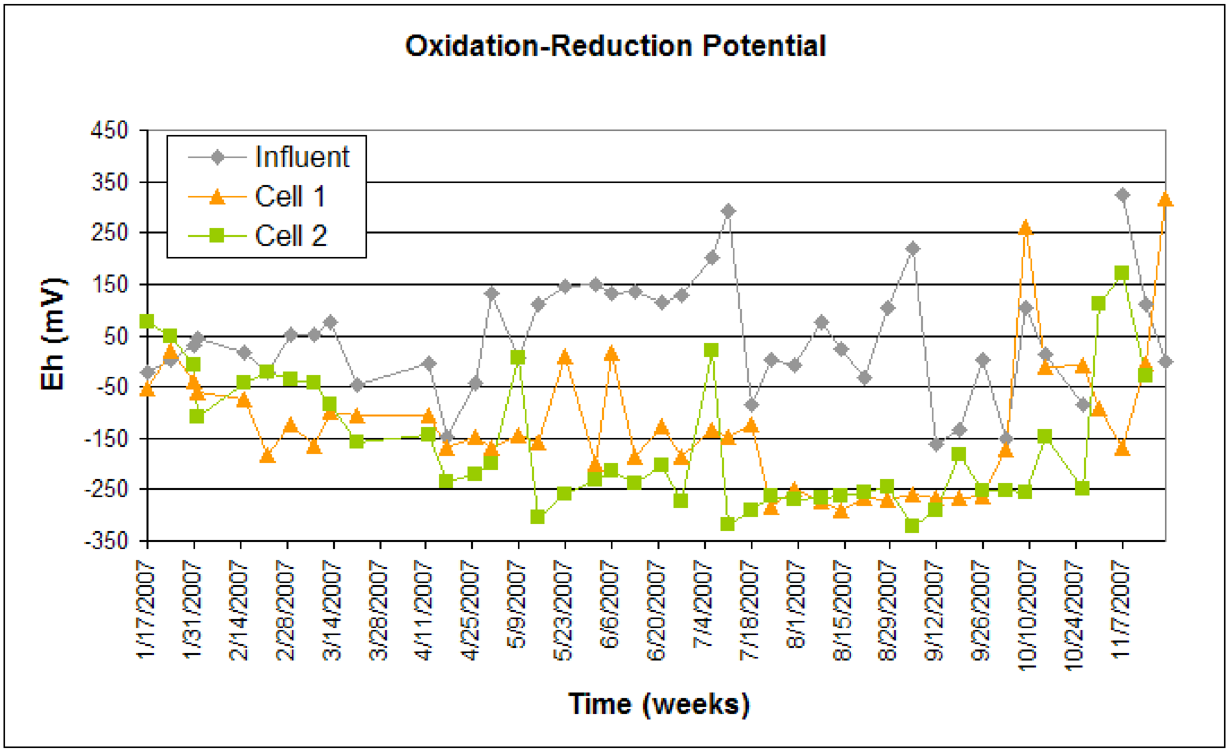

Figure 4 Weekly ORP values for influent and effluent waters.

Results and Discussion III: Bioreactors' Metal Removal Performance

Results for Cu, Zn, Fe, and Sr analyses are presented in the following graphs.

Effluent metal concentrations were typically lower than the influent metal load values. Copper concentrations are shown in Fig. 5. Initial Cu effluent in Cell 2 appears higher than the influent concentration, which may be explained by the release of metals originally present in the substrate within the reactors. For all metals, there was a spike in influent concentration from early May and through mid-June that slowly tapers off over the next several months; this increase was likely due to the mobilization of additional dissolved metals during the spring runoff. While both Cell 1 and Cell 2 removed Cu similarly for the first eight months of the bioreactors operation, Cell 1 has two spikes of Cu in the effluent water (at mid-August and early October) that were higher than the copper concentration in the influent water. The effluent copper spikes may be explained by several operational malfunctions in Cell 1. Although the cells are duplicates, Cell 1 experienced iron hydroxide clogging events more frequently in the iron hydroxide precipitation zone of the reactor. These clogging events resulted in higher head loss in Cell 1 relative to Cell 2 and disruption of water flow.

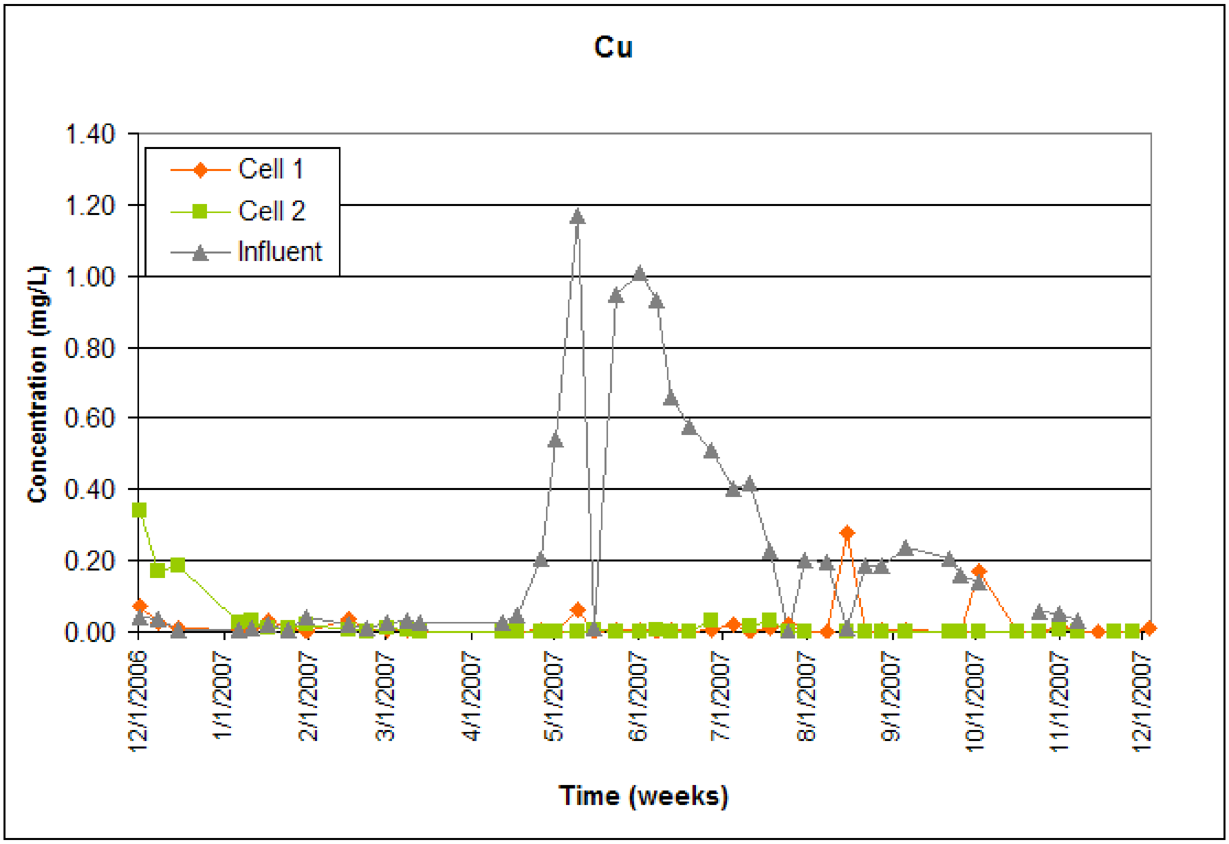

Figure 5 Weekly influent and effluent copper concentrations in the passive sulfate-reducing bioreactor systems for the National Tunnel discharge in Black Hawk, CO.

Zinc concentrations of the effluent waters, shown in Fig. 6, were lower than the influent. Unlike Cu, Zn removal was consistently removed throughout the monitoring of the bioreactors. Cell 1 was able to produce an effluent zinc concentration of less than 0.1 mg/l in early January 2007. In contrast, Cell 2 did not achieve similar zinc removal efficiency until May 2007. Zinc concentrations were typically less than detection even after the influent spike associated with the spring runoff. Both Zn and Cu sulfides' low solubility constants (2.91×10^{-25} and 1.28×10^{-36}, respectively) may explain the successful removal of these two metals as sulfides (Davis and Cornwall 1998).

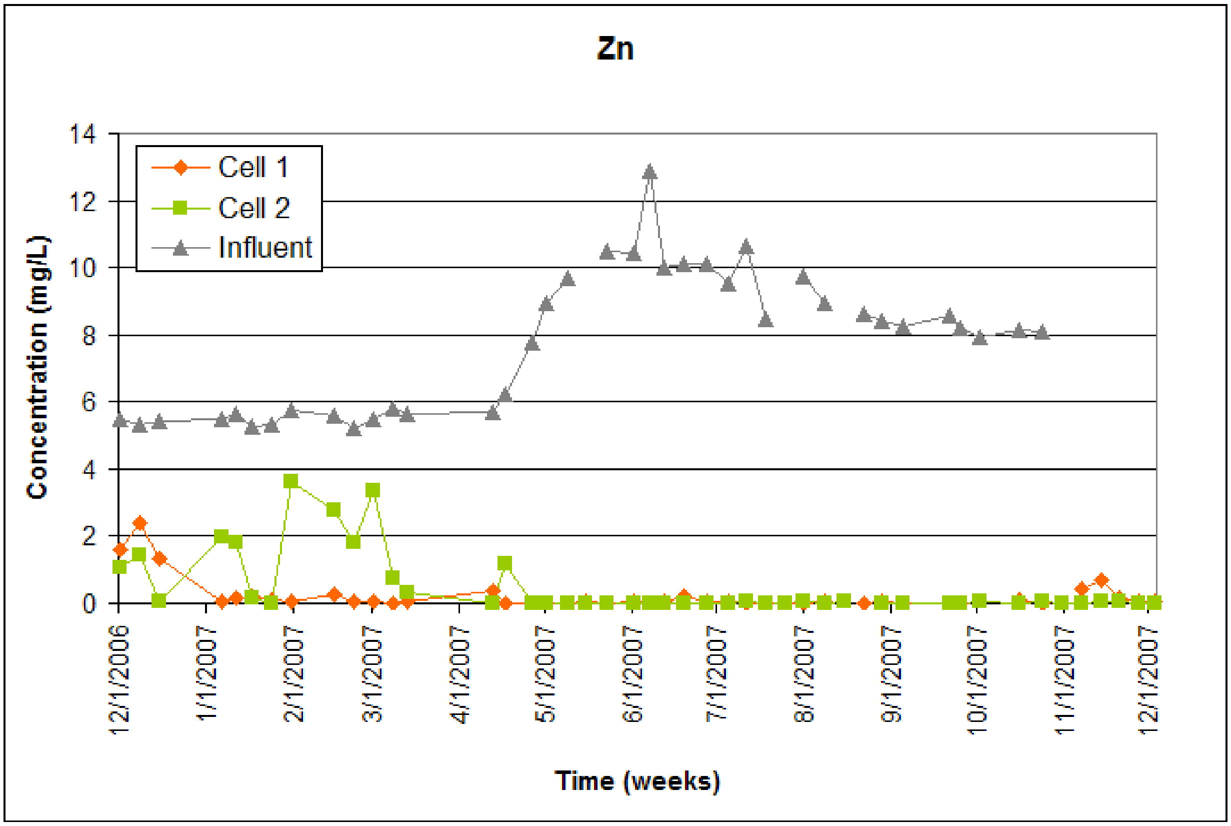

Figure 6 Weekly influent and effluent zinc concentrations in the passive sulfate-reducing bioreactor systems for the National Tunnel discharge in Black Hawk, CO.

Iron removal, as shown in Fig. 7, was partially effective, but not to the same extent as Cu and Zn. For Fe, it was also noticeable that removal trends were not the same for the duplicate cells. Six months elapsed before both Cell 1 and 2 effectively removed most of the Fe in the influent water for a brief period. The effluent of both cells was variable throughout the 12-months operation reported herein. Cell 1 in particular appeared to have the most difficulty in decreasing Fe concentrations for a prolonged period of time. Lastly, for the last month of data presented for Cell 1 in Fig. 4, there was a spike in Fe levels. One reason for the higher concentration of Fe in the treated water than in the influent may be the release of Fe^{2+}. The Fe influent to the bioreactors was predominantly Fe^{3+}. The precipitation and subsequent accumulation of this metal as $Fe(OH)_3$ in the bioreactor, made Fe^{3+} available as an alternative electron acceptor in the anaerobic environment. Additionally, Fe^{3+} may be acting as a competing electron acceptor with SO_4^{2-}. The increase in the concentration of Fe in the effluent may reflect the reduction of Fe^{3+} that has entered the substrate matrix in the bioreactors. Essentially, the electron from the organic substrate was diverted to iron reduction, a more energetically favorable reaction. The organic substrate required to reduce Fe^{3+} was not considered in the original design.

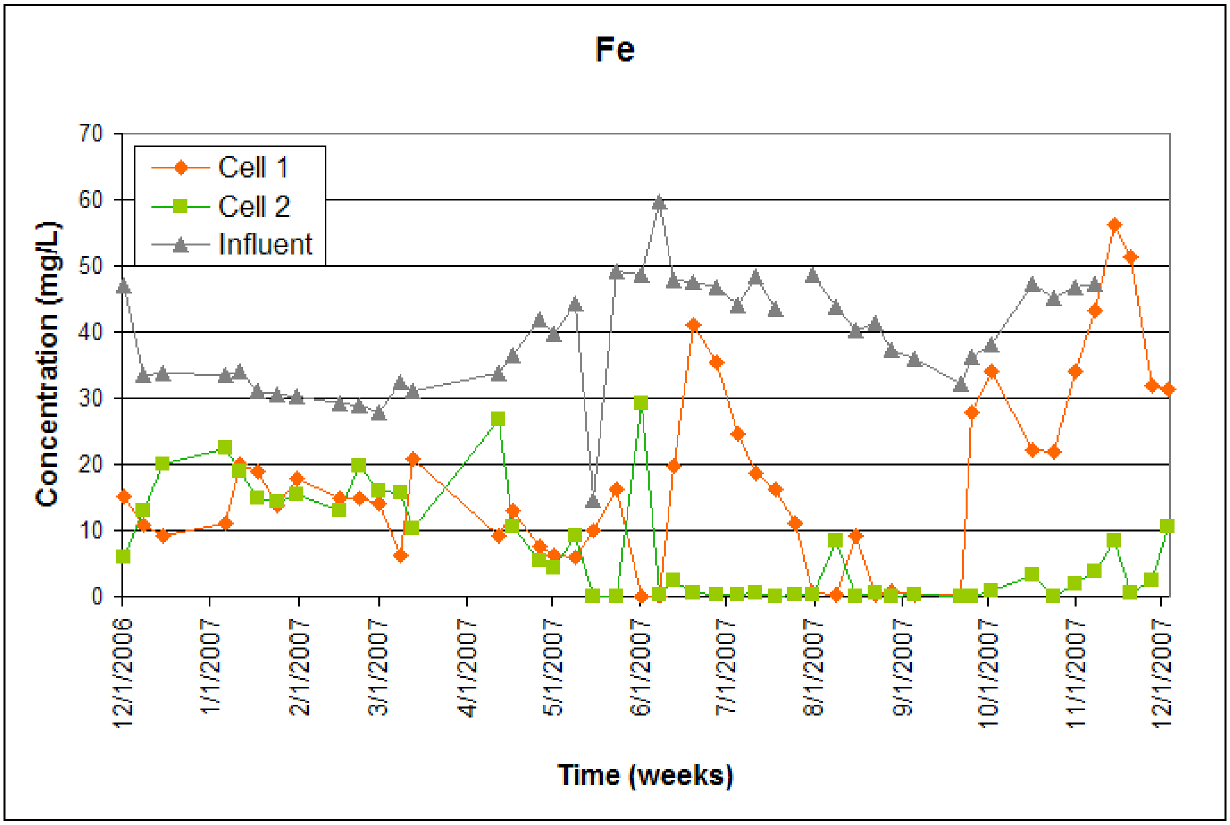

Figure 7 Weekly Influent and Effluent iron concentrations in the passive sulfate-reducing bioreactor systems for the National Tunnel discharge in Black Hawk, CO.

Changes in S concentrations are shown in Fig. 8. Sulfate removal was measurable from the first month of the reactors operation at 2 to 10% of the influent value. Differences of this magnitude are not visible at the scale shown in Fig. 5. The removal of Cu, Zn, and Fe as shown by in the previous graphs also suggests the production of sulfide. A higher removal percentage of influent SO_4^{2-} concentration was noted after the reactors had been working for 6 months. However, this may be related to the increasing tendency of the effluent valve in the reactors to clog with time. Thus, the effective hydraulic residence time was increased. A flow rate of one-liter-per-minute coupled with a 10% decrease in influent SO_4^{2-} concentration (typical value 290 mg/L as S) results in a SO_4^{2-} reduction rate of 0.3 mol S/m3-d, a typical design value recommended by Gusek (Gusek and Conroy, 2007). Further, bench-scale column studies with National Tunnel MIW and a similar corn stover and walnut shell mixture produced a SO_4^{2-}

reduction rate of 0.3 mol S/m3-d after one month of operation (Figueroa et al. 2007). Because the flow rate was variable after the first months of operation, the effluent SO_4^{2-} concentration only serves as a qualitative indicator of the SO_4^{2-} reduction rate. Analysis of the flow data in conjunction with SO_4^{2-} removal is in progress and will be reported in a future publication. As it was the case with Fe, Cell 2 removed sulfur more consistently than Cell 1, although during the last 3-4 months of operation, neither reactor decreased sulfur concentrations significantly. Particularly during the last month of sampling, both effluents seem to show a spike in the concentration of S; this potential trend may suggest that SO_4^{2-} reduction is declining due to colder temperatures. It is also possible that this is a seasonal occurrence and that SO_4^{2-} reduction will resume in the warmer months; SO_4^{2-} reduction appears to be more significant in the spring and summer (see Fig. 8). Monitoring of these reactors is ongoing and more data on their performance will be forthcoming.

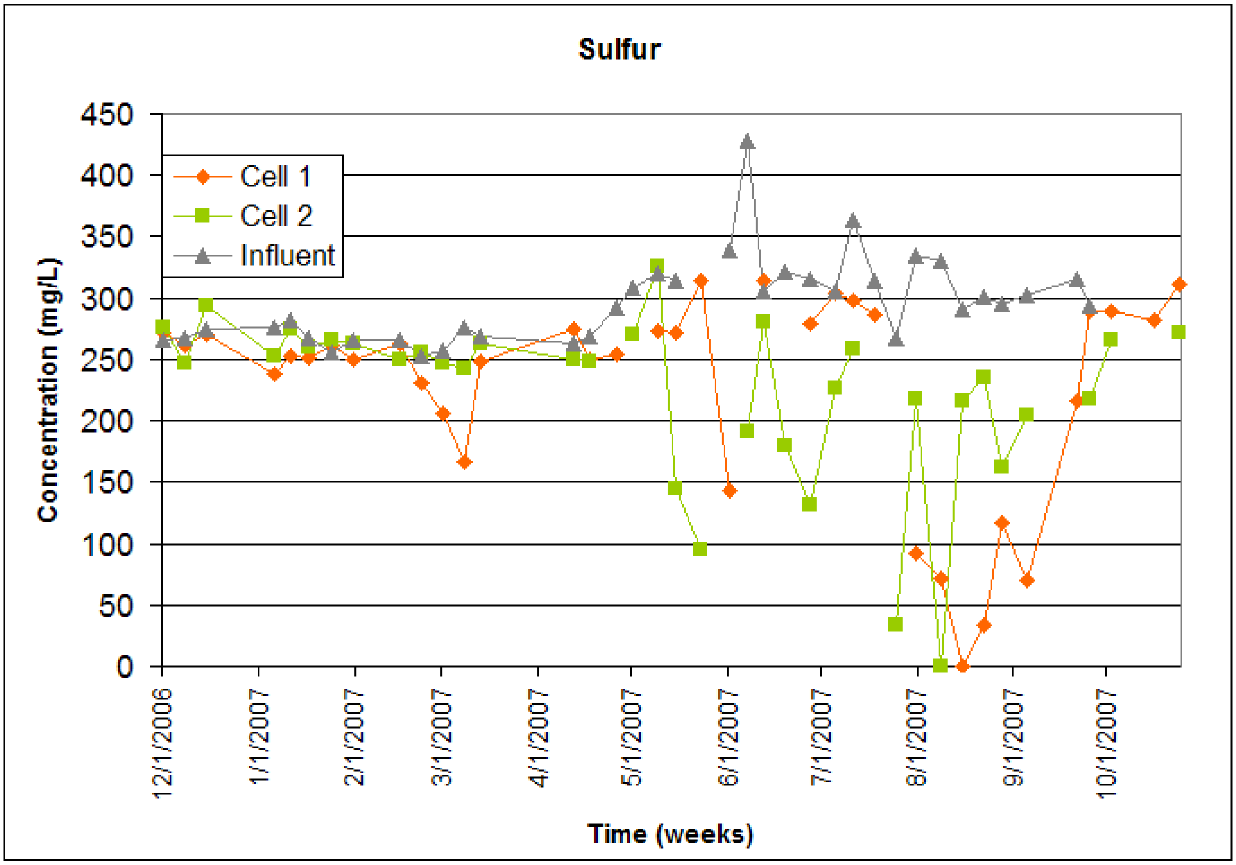

Figure 8 Weekly Influent and Effluent sulfur concentrations in the passive sulfate-reducing bioreactor systems for the National Tunnel discharge in Black Hawk, CO.

Conclusions and Recommendations

Data from substrate degradation analyses, which show a small but measurable change in organic carbon through time, suggest that the substrate was slowly biodegraded. Only the hot water soluble fraction (labile organics) exhibited a statistically significant decrease. This fraction appears to have decreased more quickly after 6 months of reactors' operation (see Table 3). Organic acids and starches, which are the basic energy sources of sulfate-reducers, do not seem to have been depleted; however the competition for organic substrate between Fe and SO_4^{2-} reduction may impact future SO_4^{2-} reduction. It is possible that the current content of corn stover in the mixture, at 25%, does not provide a sufficient amount of degradable organic and that a higher ratio of this material in the mixture would allow for concurrent Fe and SO_4^{2-} reduction. Greater availability of organic acids should improve iron removal as the additional sulfide could then participate in the precipitation of Fe^{2+} as ferrous sulfide.

The small changes in SO_4^{2-} concentration during most of the first 12-months made it difficult to determine SO_4^{2-} reduction rates. However, the continued removal of Zn and Cu, suggests that a minimum rate of SO_4^{2-} reduction was sustained. The variability in effluent iron may also indicate that SO_4^{2-} reduction did not provide sufficient biogenic sulfide sufficient to remove Fe as suggested by graphs of Fe and sulfate-sulfur removal.

Lastly, the authors suggest examination of the microbial communities in the reactors in order to know which metabolic types of microorganisms were present during the different periods of the reactors' operation (initial set up, spring runoff, warmer months, etc.). DNA was extracted from substrate samples and archived for this purpose. More importantly, this information may help determine whether the types of microorganisms that support SO_4^{2-} reducers (fermenters and cellulolytic bacteria) are active, and when. To this end, it may be helpful to examine the microbial composition of bioreactors over time, and to evaluate that information in the context of reactors' sulfate-reduction performance.

The removal of metals in aquatic environments through biologically mediated SO_4^{2-} reduction is related to carbon degradation in anaerobic systems. The release of soluble organic matter occurs slowing in anaerobic environments, which highlights the importance of long-term studies. Understanding the processes that drive the passive remediation of mine-influenced waters is vital for their successful design and operation; therefore, the long-term monitoring of the chemical and physical changes that take place in these reactors is one of the most effective ways to optimize the efficiency and sustainability of passive systems.

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