ETHANOL-FED OR SOLID-PHASE ORGANIC SULFATE REDUCING BIOREACTORS FOR THE NATIONAL TUNNEL DRAINAGE, CLEAR CREEK/CENTRAL CITY SUPERFUND SITE?[1]

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Abstract: The U.S. Environmental Protection Agency (EPA) is planning to treat mining influenced water (MIW) from the National Tunnel Adit that discharges to North Clear Creek near the City of Blackhawk, Colorado. North Clear Creek is part of the Clear Creek/Central City Superfund Site, and the National Tunnel is a major contributor of contaminants to this tributary. The EPA would like to determine the trade-offs between two modes sulfate reducing bioreactor (SRBR). One is an ethanol-fed SRBR and the other is a solid substrate fed SRBR. Ethanol fed and solid substrate-fed SRBR were operated in parallel. The bioreactors were constructed from 55-gallon drums. The nominal hydraulic residence time for the bioreactors initially was approximately 3 days for the period of July through September 2006 and later increased to 9 days for the period of October through November 2006. Measurable sulfate removal (approximately 100 mg/L as S) was noted by the end of July for all bioreactors. Sulfate removal increased through August 2006 and on September 7, 2006 was 170 to 200 mg/L as S. Sulfate removal decreased to 50 to 100 mg/L by the end of September. This decrease coincided with a drop in MIW and ambient air temperatures. The flow was decreased by a factor of three at the beginning of October, which resulted in increased sulfate removal in October and November relative to the end of September. Removal of zinc was observed prior to the onset of sulfate reduction. There was some variability in removal of zinc but greater than 95% zinc removal was observed for all bioreactors after the end of July. Both types of SRBRs were capable of reducing zinc concentrations to below 0.1 mg/L. The effect of cold temperatures was greater in the solid phase substrate bioreactors than the ethanol fed bioreactors. This suggests that cellulolytic fermenters were affected to a greater extent than sulfate reducers by cold temperature because they indirectly provide the soluble substrate for the sulfate reducers.

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Introduction

The National Tunnel is located in Black Hawk, Colorado within the Central City/Clear Creek Superfund Site, Operable Unit 4. The discharge from the National Tunnel contains Zn (5 to 7 mg/L) above the EPA secondary maximum contaminant level for drinking water and well above toxic aquatic values for brook trout. The water also contains total Fe at 35 to 50 mg/L and Cu at 0.01 to 0.1 mg/L.

The Record of Decision for Operable Unit 4 of the Clear Creek/Central City Superfund site establishes passive treatment as the preferred alternative to treat mine drainage that flows from the National Tunnel into the North Fork Basin Clear Creek near the City of Blackhawk, Colorado. The first choice is specifically a passive system that relies on sulfate reduction to sulfide that targets the removal of Zn and Cu as metal sulfide precipitates.

There are two alternative methods for sustaining sulfate reducing bacteria (the producers of biogenic sulfide) in passive treatment systems. The most commonly used method utilizes solid phase organic wastes such as compost or wood chips as feed for a bacterial consortium that supports active sulfate reduction (Gusek et al. 2000). However, because there is easy access to the treatment site, an alternative method is to provide ethanol. Ethanol can be used directly by sulfate reducing bacteria to produce sulfide. Ethanol can be metered into the bioreactor using a constant head tank (Tsukamoto et al. 2004).

In addition, experiences at sites in Colorado and Wyoming (Reisinger et al. 2000, Farmer et al. 1995) suggest that special problems do occur when water at near freezing temperatures is treated in a passive treatment system. It appears that the system will operate reasonably well for a certain period of time and then, when there is a stress put upon the system, sulfate reduction substantially declines and subsequent efforts to reestablish sulfate reduction prove difficult.

The purpose of this study was to conduct a comparative treatability analysis of bench-scale sulfate reducing bioreactors (SRBRs) for the National Tunnel discharge located in Black Hawk, Colorado. This study focused on the ability of the ethanol and solid phase organic bioreactors to reduce influent Fe and Zn concentrations by $\geq$ 95% under field variations of mine water characteristics and temperature. This paper reports on the first six month of operation.

Methods

Six bench-scale SRBRs were constructed under the Mill Street Bridge in Black Hawk, Colorado on June 28 - 29, 2006. Water collected from the National Tunnel discharge was fed to the SRBR cells. Two SRBRs received a constant ethanol feed and four SRBRs were packed with two different solid phase organic substrate mixtures.

The mine water was fed to six mini-head tanks (5-gallon buckets). The first bucket tank received influent (approx. 1 gpm) from the National Tunnel discharge (left side of Fig. 1). The rest of the buckets received influent from hydraulic connection to the first bucket. The last two buckets in the hydraulic chain (right side of Figure 1) overflow the mine water to the creek. The overflow connections between the buckets and displacement volume (one gallon filled plastic bottle) were set to limit the volume in each bucket to approximately 1.5 gallons. The buckets were maintained full with an exchange of mine water approximately every 10 minutes. When the reactors were to be filled, a valve (timer-controlled) in the influent line shut off and isolated the buckets from new flow. Each bucket was equipped with a timer-controlled valve that opened as programmed to deliver about 1.5 gallons of mine water to each bioreactor for each cycle per day. Initially, the valve timers were programmed to deliver 1.5 gallons of water six-times per day. At the beginning of October 2006, the valve timers were reprogrammed to deliver water two-times per day.

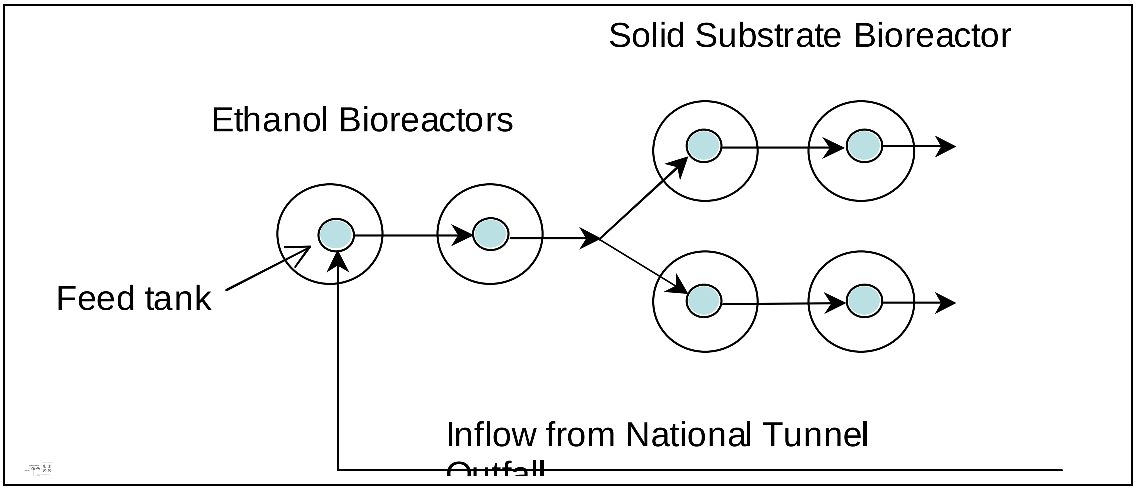

Figure 1. SRBR System Layout. Arrows show the flow of mine water through the bucket feed system (small circle) on top of the SRBRs (large circle).

<u>Ethanol Fed SRBRs</u>

The ethanol-fed SRBRs were comprised of two (2) 55-gallon drums plumbed in parallel. Each bioreactor was packed with approximately 50 gallons of 3/4” limestone. Flow to each reactor was controlled by a timer-controlled ball valve located at the outlet end of each reactor’s feed tank**.** Influent to the bioreactors entered at the top of the cell and flowed by gravity to the bottom. The drums were plumbed so that the bioreactors remain saturated throughout the test. The ethanol bioreactors were designated as Cells 1 and 2 for sampling purposes. A sketch of the system is shown below.

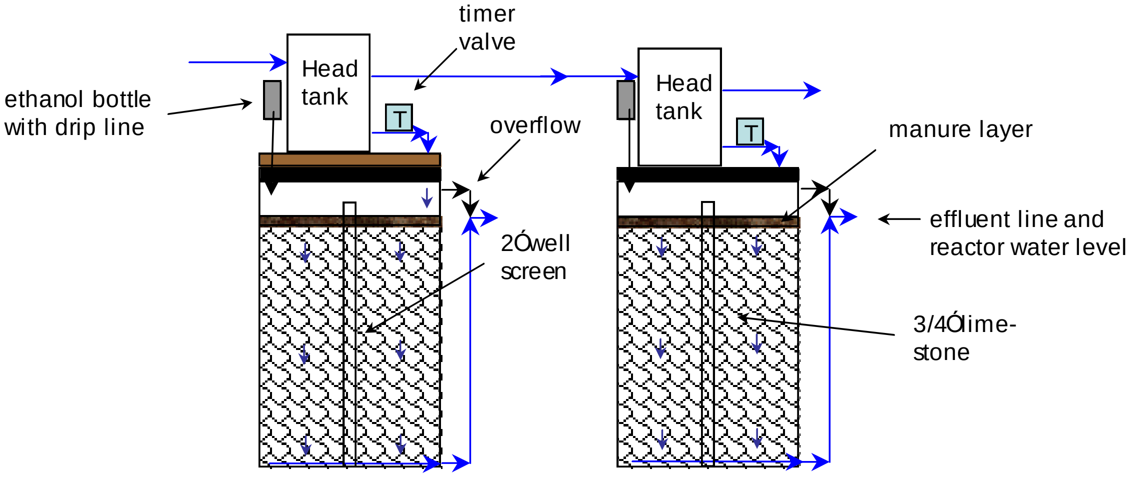

Figure 2. Ethanol Bioreactor Test Configuration

The ethanol SRBRs were inoculated with 5 lbs of horse manure layered on top of the limestone. Each of the ethanol cells have five removable limestone filled mesh bags inserted in a two inch diameter PVC well screen with 0.02 inch slots. These will be removed for microbial analysis at a yet to be determined date. After the removal of a mesh bag, the empty space will be filled with a replacement limestone filled mesh bag.

Solid Phase Substrate Mode

Four bench scale solid phase substrate cells treat the same water as the ethanol bioreactors. The mixing proportions of organic and inorganic components for the SRBR reactors are provided in the table below. The percentages of each component presented in Table 1 are weight percentages that add up to 100% for each mixture based on previous experience (Gusek et al 2000, Waybrant et al 2002). The total mass used in each SRBR cell was determined in the field based on the packed density of the mixture achievable in the field. The bench scale SRBR cells were also built from 55-gallon plastic drums; the drums were filled with reactive media to a volume of approximately 45 gallons.

Table 1 – Cell Mixture Ratios by As-Received Weight

Component	Cell 3	Cell 4	Cell 5	Cell 6
Wood Chips	50%	50%	35%	35%
Corn Stover	0%	0%	30%	30%
Limestone	30%	30%	20%	20%
Hay	10%	10%	0%	0%
Cow Manure	10%	10%	15%	15%

The solid phase bioreactor configuration is shown in Fig. 2. The flow path of influent and effluent mine water is the same as for the ethanol bioreactors (top to bottom). Each of the four solids phase substrate cells have five removable substrate filled mesh bags inserted in a two inch diameter PVC well screen with 0.02 inch slots. These will be removed for substrate and microbial analysis at a yet to be determined date. After a mesh bag is removed, the empty space will be filled with a replacement substrate filled mesh bag.

The SRBR cells were filled on June 29, 2006. At the conclusion of the test, the SRBR cells may be disassembled and physically inspected for conditions that could compromise long term operation such as precipitate accumulations.

Experimental Strategy

The overall sampling strategy of the bioreactors was to collect influent and effluent for analysis of performance indicators on a weekly basis. The bioreactors were set up for future internal sampling of the solid phase matrix. Solid phase samples will be assessed for microbial community composition and solid phase substrate composition. Samples for microbial analysis will focus on the solid phase, rather than the liquid phase, because of the general preference for microbes to attach to surfaces.

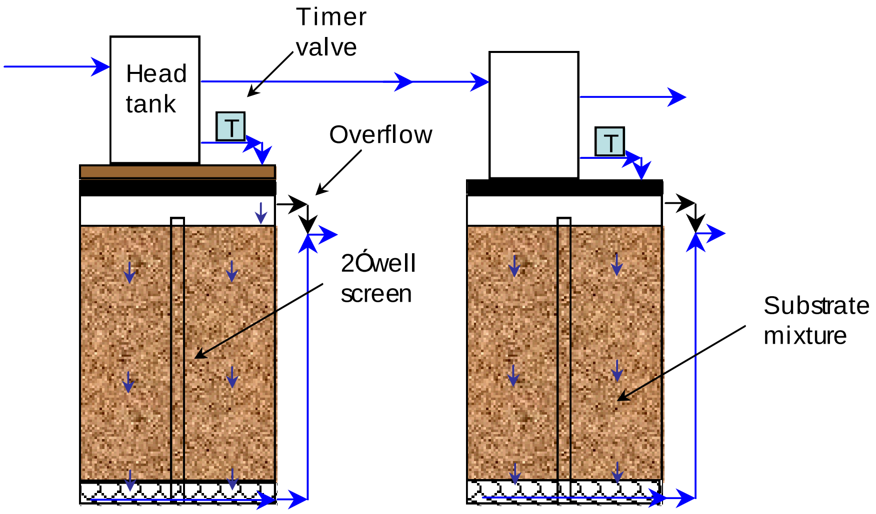

Figure 3. Solid Phase Bioreactor Test Configuration

Solution analysis

The influent and effluent samples were analyzed for temperature, pH, alkalinity, conductivity, ORP, Zn, Cu, total Fe, and total S.

Table 2. Sampling frequency and analytes monitored

Analyte or Analyte group	Frequency of Samples or Measurements	Sample Collection or Measurement Method	Sample container type/size	F or L[1]	Preservation
Cu, Fe , Zinc, Ca, Mg	Weekly	Grab, filtered	15 ml plastic vial (CSM)	L	Nitric acid, no ice CSM
Alkalinity	Weekly	Grab, unfiltered	NA	F[2]	NA
pH	Weekly	Grab, unfiltered	NA	F	NA
Oxidation Reduction Potential (ORP)	Weekly	Grab, unfiltered	NA	F	NA
Specific cond.	Weekly	Grab, unfiltered	NA	F	NA
Temperature	Weekly	Grab, unfiltered	NA	F	NA

[1] Where measurement/analysis will occur, field (F) or lab (L).

[2] Alkalinities will be titrated in the field, unless the ambient temperature causes the digitations titrator solution to freeze. Then the samples will be stored on ice and titrated upon arrival in the lab.

Table 3. Methods for Solution Analysis

Analyte or Analyte group	Measurement Method	Equipment	Reporting Units	Practical Limit
Cu, Fe , Zinc, Ca, Mg	EPA 6010B ICP-AES	Perkin Elmer Model 3000	mg/L	0.01 mg/L (lower limit) and ± 0.01 mg/L
Alkalinity	EPA 310.1	HACH digital titrator	mg/L as CaCO3	± 0.1 mg/L
pH	EPA 150.1	pH/mV meter with pH probe	s.u. units	± 0.1 s.u. units
Oxidation Reduction Potential (ORP)	EPA 200.11	pH/mV meter with Ag/AgCl probe	mV	± 0.1 mV
Specific cond.	EPA 120.1	Conductivity/ temperature meter with probe	microsiemen	± 1 microsiemen
Temperature	--	Conductivity/ temperature meter with probe	Celsius	± 0.1 ^{o} C

Results and Discussion

All SRBRs produced a slight increase in effluent pH (pH = 6.5 to 7) above the influent pH (6.2 to 6.5) after three months of operation. During the first three months, effluent pH from the solid phase substrate bioreactors was on average lower than influent pH suggesting that the bioreactors were dominated by fermentation reactions which produced organic acids that may lower pH. Influent Eh from the National Tunnel averaged -50 mV from July through November 2006. After the first month of operation the effluent Eh from all six SRBRs averaged -230 mV± -40 mV indicative of the establishment of a reducing environment within the bioreactors. Influent water temperature varied between 17 and 7°C. Bioreactor water temperatures were elevated above influent temperature for about the first 2.5 months of operation. The bioreactor temperatures dropped below the influent water temperature as ambient air temperature also declined, Fig. 4. Ambient air temperatures in Black Hawk ranged from average highs of 24^{o}C in July to 0^{o}C in December and average lows of 6^{o}C in July and –13^{o}C in December.

Alkalinity increases above the National Tunnel influent appear to be the combined effect of calcium carbonate dissolution and increasing alkalinity due to sulfate reduction, Fig. 5. The dissolution of calcium carbonate was expected to dominate effluent alkalinity in the first few months. The increase in alkalinity in October and November 2006 was consistent with relative increases in sulfate reduction during those months.

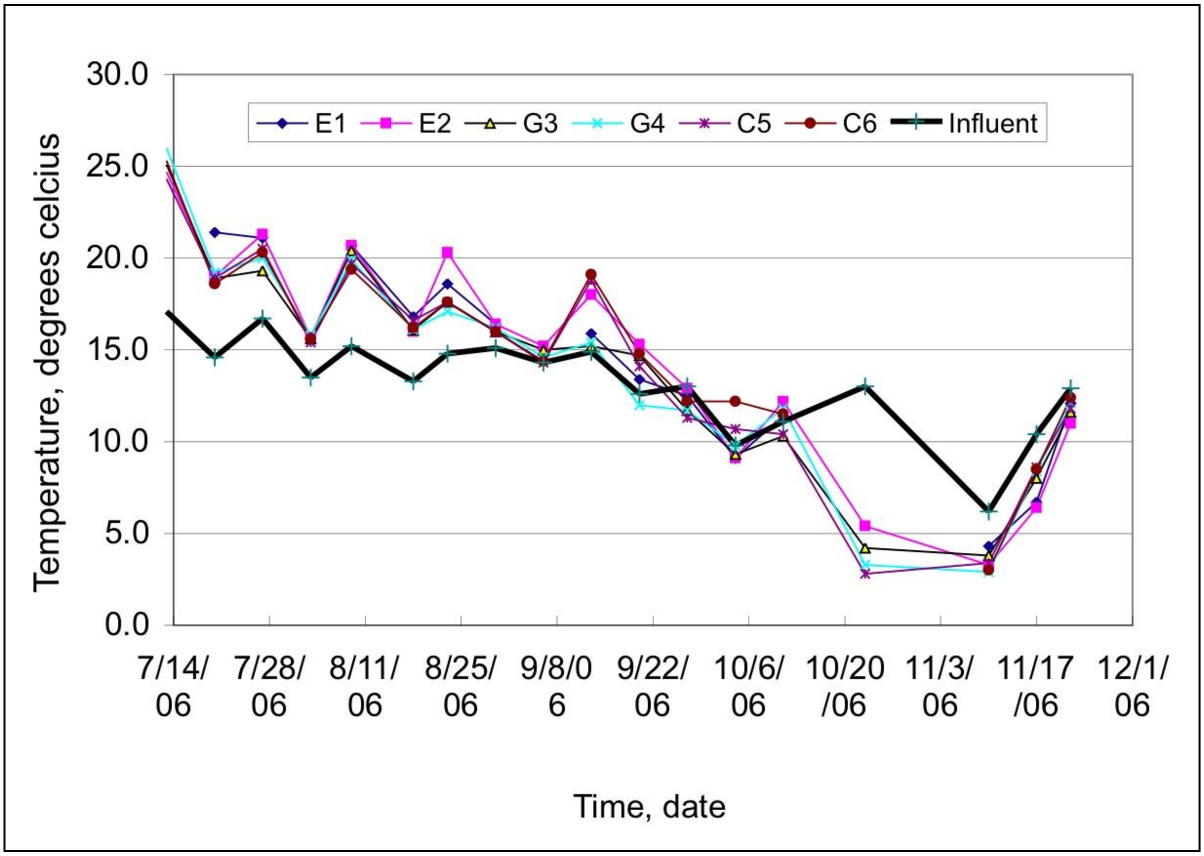

Figure 4. Temperature with time for National Tunnel influent and bioreactor effluent

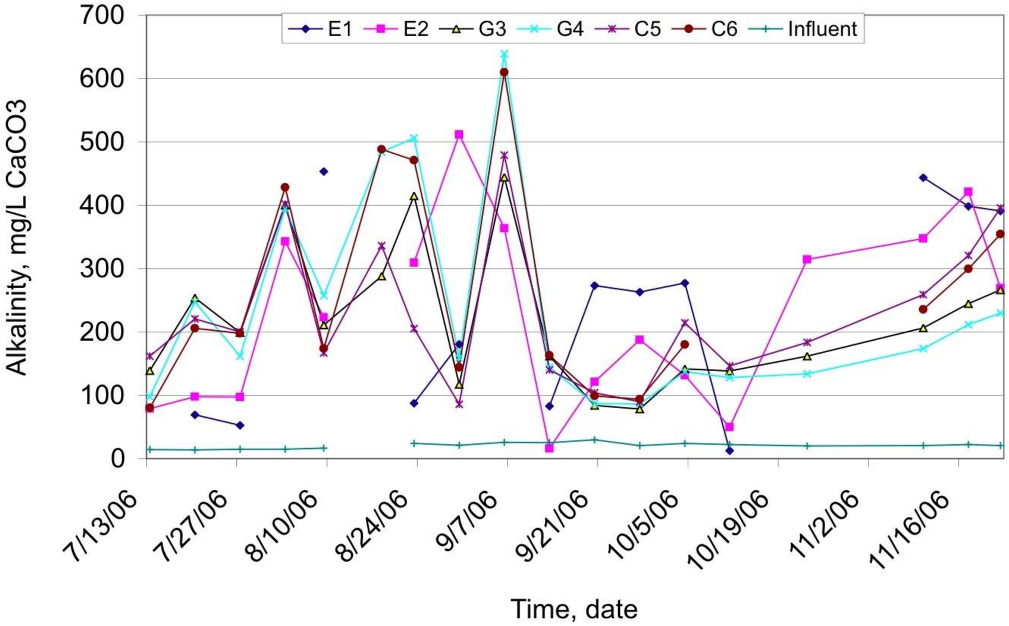

Figure 5 – Alkalinity with time for National Tunnel influent and bioreactor effluent

All bioreactors removed Zn consistently to below 0.2 mg/L after the first month of operation, Fig. 6. Zinc removal was constant even though the rate of sulfate reduction was quite variable,

Fig. 7. High rates of sulfate reduction were observed in the second and third months of operation. Lower rates of sulfate reduction in September coincided with reductions in influent water and ambient air temperature. Lower rates of sulfate reduction in September may also be due to the depletion of readily available organic substrates. High initial rates of sulfate reduction followed by a period of lower sulfate reduction and a subsequent recovery has been observed in laboratory columns subjected to constant temperatures (Waybrandt et al. 2002, Figueroa et al. 2004). The rate of sulfate reduction was also higher for ethanol fed reactors in November of 2006. This suggests that the cellulolytic fermenters are more inhibited by the colder temperatures than sulfate reducers. Logan et al. (2005) demonstrated that cellulose degradation is the rate-limiting step for solid phase substrate reactors treating mine drainage.

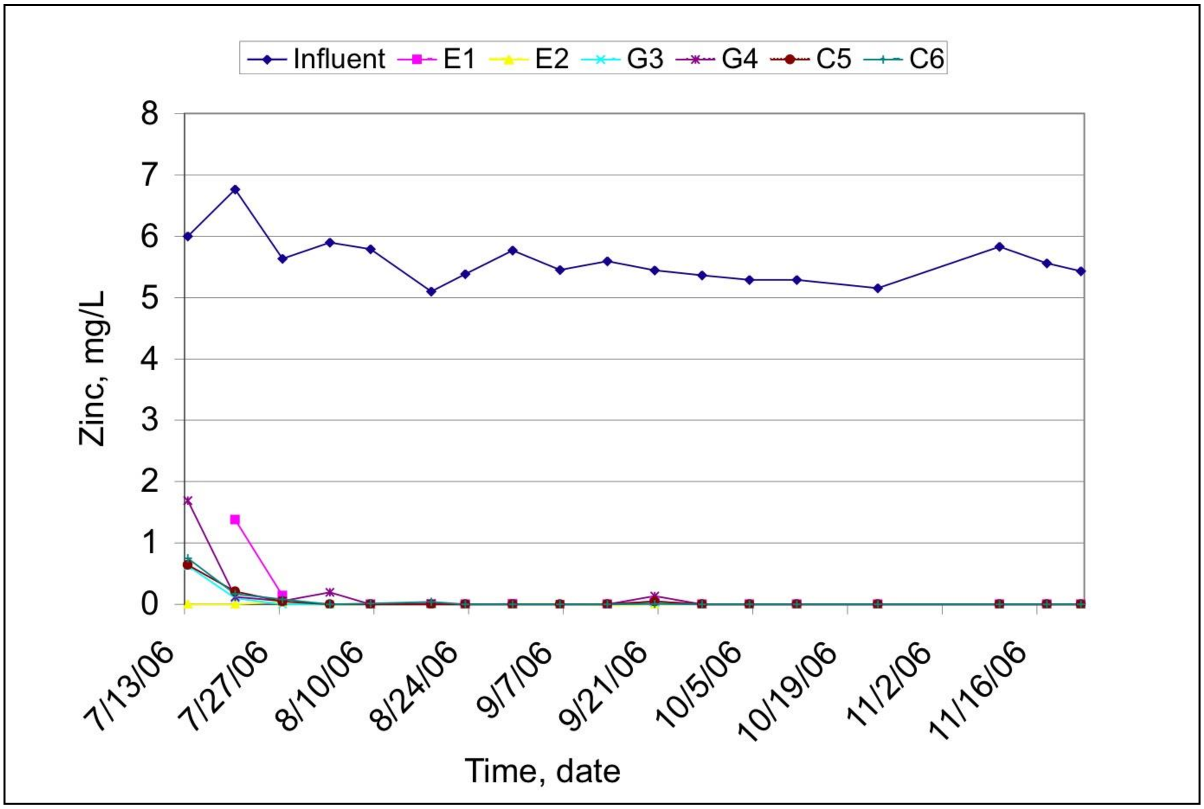

Figure 6. Zinc concentration with time for influent and bioreactor effluents.

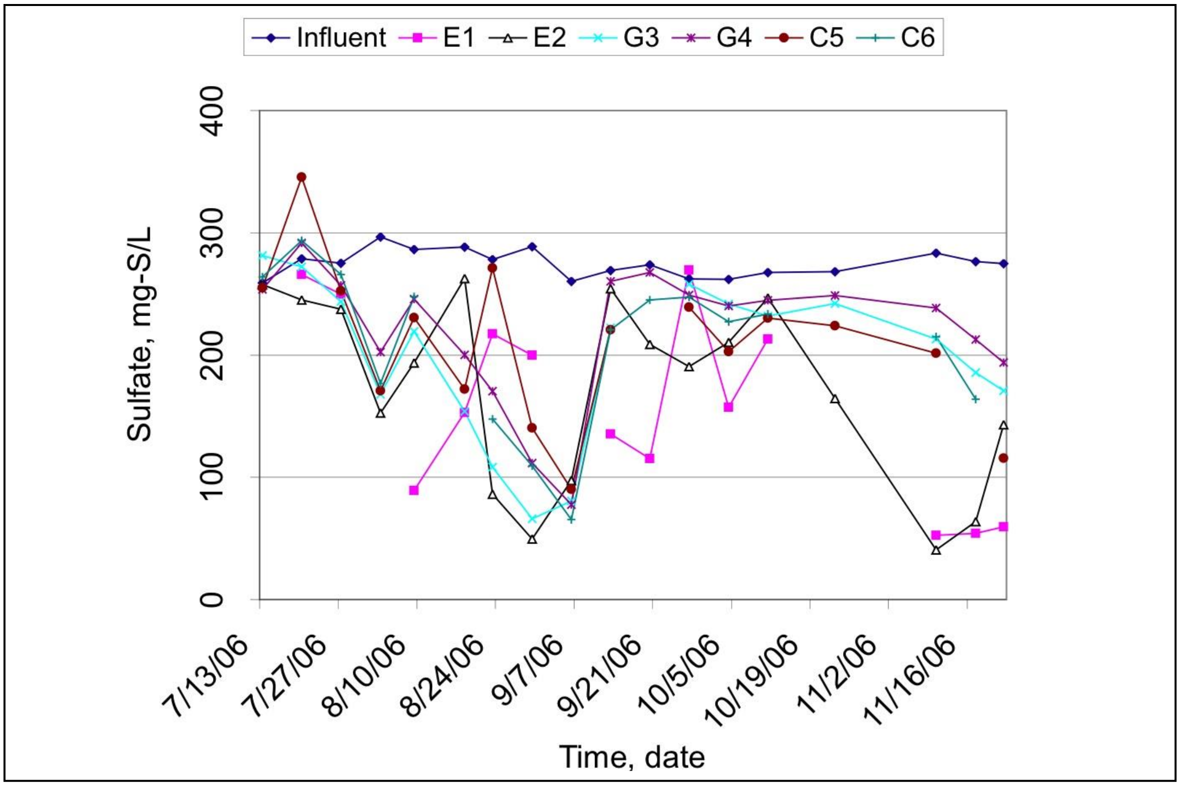

Figure 7. Sulfate with time for National Tunnel influent and bioreactor effluent

The removal of Fe (Fig. 8) in the SRBRs may be due to more than one mechanism. The first is precipitation of $Fe(OH)_3$ at the influent side (top) of the bioreactor. Previous analysis of the Fe^{3+}/Fe^{2+} iron composition of the National Tunnel drainage found that 90% of the Fe was Fe^{3+}. Interestingly, the Fe^{3+} particles were small and were able to pass through a 0.2 micron membrane filter (Butler 2005). The bioreactors surfaces will be examine in late Spring 2007 to check for Fe hydroxide precipitation. The Fe^{2+} iron portion of the mine water may be removed by Fe^{2+} sulfide precipitation. The other mechanism that could affect Fe^{3+} removal is the reduction of Fe^{3+} iron by iron-reducing bacteria. The produced Fe^{2+} iron could then be removed as a Fe^{2+} sulfide.

Conclusions

Both types of SRBRs were capable of reducing Zn concentrations to below 0.1 mg/L. The extent of sulfate removal with the onset of cold temperatures was affected to a greater extent in the solid phase substrate bioreactors than the ethanol fed bioreactors. This suggests that cellulolytic fermenters are affected to a greater extent than sulfate reducers by cold temperature because they indirectly provide the soluble substrate for the sulfate reducers. Ethanol fed bioreactors bypass the need for a cellulolytic fermenting population. Improved iron removal in October and November 2006 may be due to a layer of $Fe(OH)_3$ solids acting as a filter or microbial Fe reduction activity coupled with Fe sulfide precipitation.

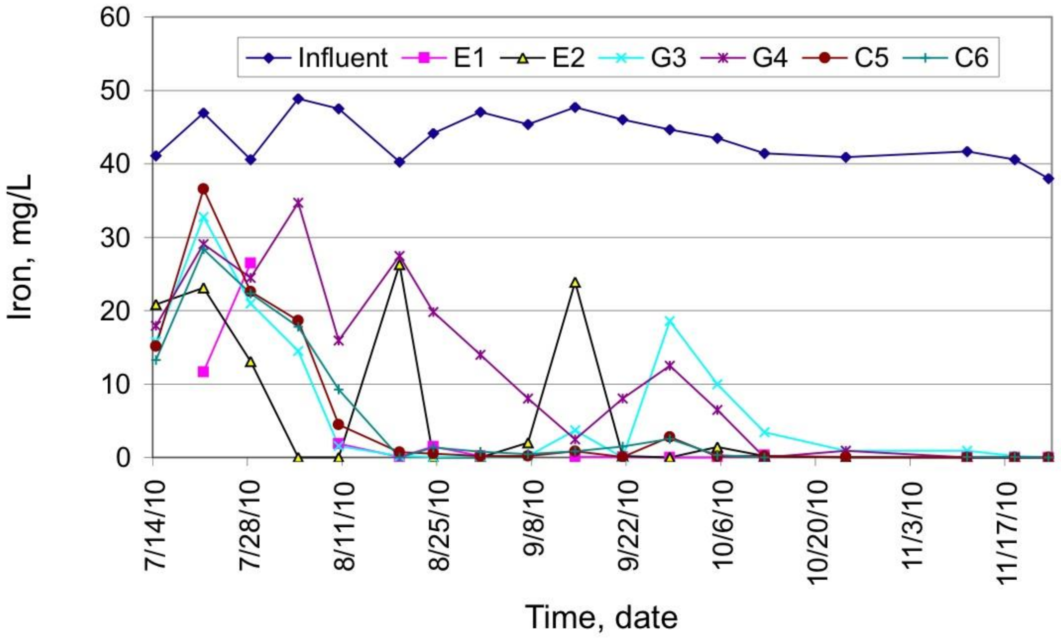

Figure 8. Iron concentration with time for National Tunnel influent and bioreactor effluent

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