PILOT-SCALE EVALUATION OF SOLID AND LIQUID PHASE ORGANIC SUBSTRATES USED IN BIOCHEMICAL REACTORS FOR THE TREATMENT OF MINING INFLUENCED WATER[1]

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Abstract.   Sulfate-reducing biochemical reactors (BCRs) were installed to provide a basis for substrate selection for a final treatment remedy for mining influenced water (MIW) from the National Tunnel adit. Black Hawk, CO.   The MIW was characterized by elevated metal and SO_4^{2-} concentrations as well as pH fluctuations from 4.8-6.4.   Three pairs of BCRs were installed in 2006, each of which contained a different mixture of solid or liquid-phase substrates.   The above-ground BCRs partially froze during the first winter, prompting a redesign of the hydraulic system in May 2007 and the installation of heating and insulation in preparation for the following winter in October 2007.   During the hydraulic system re-design, a fourth pair of reactors that contained CHITOREM$^{®}$ SC-20 chitin complex was added to provide data on an alternative solid-phase substrate. Results from 2007 suggest that nearly all of the reactors removed greater than 95% of the Fe and Zn and 50-95% of the sulfate from the MIW during September through December 2007. Copper removal was typically above 95%. In addition, chitin complex reactors demonstrated high Mn removal (average removal was 80%) and high alkalinity (average of 4200 mg/L as $CaCO_3$) during the same period.   The high alkalinity was partially due to high concentrations of ammonium (up to 450 mg/L as nitrogen).   Operational challenges due to winter conditions in November and December 2007 caused significant temperature and flow fluctuations; however, effective removal of metals was still observed.   The BCRs will be monitored through summer 2008 and the data will be evaluated in late-summer 2008 to determine which substrates (CHITOREM$^{®}$ SC-20, ethanol or solid phase organic mixture) are best suited for long-term treatment of the National Tunnel MIW by BCRs.

Additional Key Words: Acid mine drainage, chitin complex, limestone dissolution, manganese removal, sulfate-reducing bioreactors, water treatment

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Introduction

The National Tunnel drains mining influenced water (MIW) to the north fork of Clear Creek in Black Hawk, Colorado. The National Tunnel is within the Clear Creek/Central City (CC/CC) Superfund Site. The National Tunnel MIW exhibits water quality values presented in Table 1. These elevated metal concentrations and low pH contribute directly to conditions toxic for aquatic life and exceed the EPA ambient aquatic standards (Table 1, U.S. EPA 2004). Under the Record of Decision for the CC/CC Superfund (CERCLA) site, passive water treatment has been selected as the remedy. Biochemical reactors (BCRs) that promote sulfate reduction are one type of passive treatment system capable of reducing metals and increasing pH. In 2006, a system of pilot-scale BCRs was installed through a joint venture among Colorado School of Mines (CSM), the U.S. Environmental Protection Agency (EPA) Office of Research and Development's Engineering Technical Support Center (ETSC) and Golder Associates. The hypothesis is that BCRs would provide effective, long-term treatment of National Tunnel MIW.

Table 1- Measured target parameter ranges and treatment objectives

Criteria	2006 measured influent range (mg/L dissolved,)	2007 measured influent range (mg/L dissolved,)	Effluent Treatment objective (mg/L total recoverable)[1]
Calcium	210-380	180-280	N/G[2]
Copper	< 0.1	<1.1	0.01
Iron	40-50	40-65	1
Manganese	16-20	20-30	1
Zinc	5.1-6.8	4.6-14.3	0.1
Sulfate	780-900	810-1380	N/G[2]
pH	6.0-6.6	4.8-6.4	6.5-8.5
Temperature	6.2-17.1	4.6-9.2	N/G[2]

[1] Current National Ambient Water Quality Criteria (U.S. EPA, 2004)
[2] Not a goal for this study

The BCRs supported active sulfate reducing bacterial communities (Prieto et al. 2008) and demonstrated the removal of metals including Fe, Cu, and Zn from National Tunnel MIW over the course of six months in 2006 (Buccambuso et al. 2007). Biogenic sulfide and elevated alkalinity were presumed to be responsible for metal removal in the BCRs, primarily by raising MIW pH to circum-neutral values and subsequent precipitation of metals in carbonate and sulfide forms. The initial BCRs were installed as three pairs, each pair containing a different

substrate mixture to provide a basis for selection of a substrate for final treatment remedy. The goal was to provide a carbon source for the SO_4^{2-}-reducing bacteria either directly (ethanol) or indirectly (wood, hay and corn stover). Testing of the three substrate mixtures: 1. ethanol (liquid-phase), 2. hay plus wood and 3. corn stover plus wood (both solid-phase) resulted in differential MIW treatment in the BCRs.

The operation of these BCRs will continue at least until the summer of 2008, however, several improvements were made to the BCR systems in 2007 to address hydraulic and winterization problems present in the previous system. Piping and layout were improved to provide regular influent flow to the BCRs and reduce clogging in the piping by iron-hydroxide precipitates. In order to provide year-round treatment, heating systems were also installed to ensure that BCRs and their influents and effluents did not freeze during cold months. Reactor contents were minimally disturbed during the series of improvements and a fourth set of reactors was added which contained CHITOREM® SC-20, a chitin complex. Previous results from microcosm studies suggested that CHITOREM ® SC-20 was a good substrate for SO_4^{2-} reducing bacteria (Daubert and Brennan 2007). The BCRs containing this substrate were designated CHTN reactors. The EPA Engineering Technical Support Center and JRW Bioremediation are currently evaluating CHITOREM ® SC-20 in bench-scale and column studies.

This paper reflects data collected through December 2007 and will discuss the metal removal efficiency and continued operation of the original BCR treatment systems and CHTN reactors in addition to the effects of physical modifications made to the system. The intent of this paper is to report on the progress of the BCR substrate assessment for treatment of the National Tunnel MIW. The performance evaluation of the liquid (ethanol-fed) BCR, two solid phases organic BCRs and CHTN BCRs will continue until at least summer 2008. The extensive data set will be amenable for a detailed assessment of the four types of BCR substrate and will be presented in future publications. A detailed examination into the Mn removal mechanisms will be presented in a companion paper (Venot et al., 2008).

Methods

Initial and Current Configurations

The discharge from the National Tunnel adit flowed through a 12-inch diameter pipe to the north fork of Clear Creek. The pipe outlet was located adjacent to the Mill Street Bridge in

Blackhawk, Colorado, under which the pilot-scale BCRs were located. Initial installation of the treatment system occurred in June 2006 (Table 2). National Tunnel drainage was fed to the reactors composed of 210L plastic drums. The ethanol fed BCRs were designated ETOH 1 and 2, the hay plus wood BCRs were designated HYWD 1 and 2 and the corn stover plus wood BCRs were designated CSWD 1 and 2. The numbers were assigned to the BCR label to differentiate the duplicate BCRs from each other.

Table 2. Operational timetable for biochemical reactors

Date	Event
05/16/06	ETOH, HYWD, and CSWD reactors installed in "Y" configuration with constant-head system, flow of 34.0L/day
10/04/07	Flow reduced to 11.3L/d
11/22/06	Last sampling of reactors before winterization problems
04/17/07	Reactors thaw, sampling resumes
05/09/07	Last sampling of reactors in initial configuration
05/16/07	Reactors reconfigured with hydraulic improvements, chitin reactors added, and flow set to 22.7L/d
05/23/07	First sampling of revised reactor configuration
10/19/07	HYWD 4 reactor began leaking from effluent hose connection
10/24/07	Winterization system installed on ETOH and HYWD reactors
10/31/07	Power to reactor heating stopped, reactors cooled
11/07/07	Chitin 8 reactor accidentally drained during week and was refilled
11/17/07	Winterization system installed on CSWD and Chitin reactors and power resumed
11/30/07	Power to reactor heating was cut, influent and effluent pipes froze, and no flow to reactor
12/01/07	Power resumed to reactor heating system, influent and effluent pipes thaw, and flow resumed

A 20L bucket on top of each drum provided a constant-head feed. The first pair of 20L buckets on top of the ETOH reactors was fed in series after which a split in the delivery piping fed each of two series of 20L buckets atop the HYWD and CSWD reactors (Fig. 1). In the default position the valves were closed. The water flowed through the chain of 20L buckets and then to the north fork of Clear Creek (no flow to the BCRs). The BCRs received inflow from the constant-head buckets which emptied into the reactors when the valves were open. The time-controlled valves were initially set to deliver six 5.7-liter increments of MIW per day. At this flow rate, the retention time was calculated to be 1.5-2.5 days based on an estimated pore volume of 0.3-0.5 v/v. By September, 2006, sulfate removal had steadied at only ~25% of measured influent values at this retention time. Flow was subsequently reduced to two 5.7-liter increments

of MIW per day in October of 2006 to promote higher rates of SO_4^{2-} removal (Table 2). Retention time increased to 4.5-7.5 days at this lower flow rate.

The influent flowed downward through the packed substrates and samples were collected by lowering the effluent hose. This system maintained saturation of the substrates and the addition of an overflow port allowed for a constant water level in each reactor. A 5.08 cm. (2-in.) diameter well screen pipe running vertically through the center of each reactor served for substrate sampling described later in this paper (Buccambuso et al. 2007).
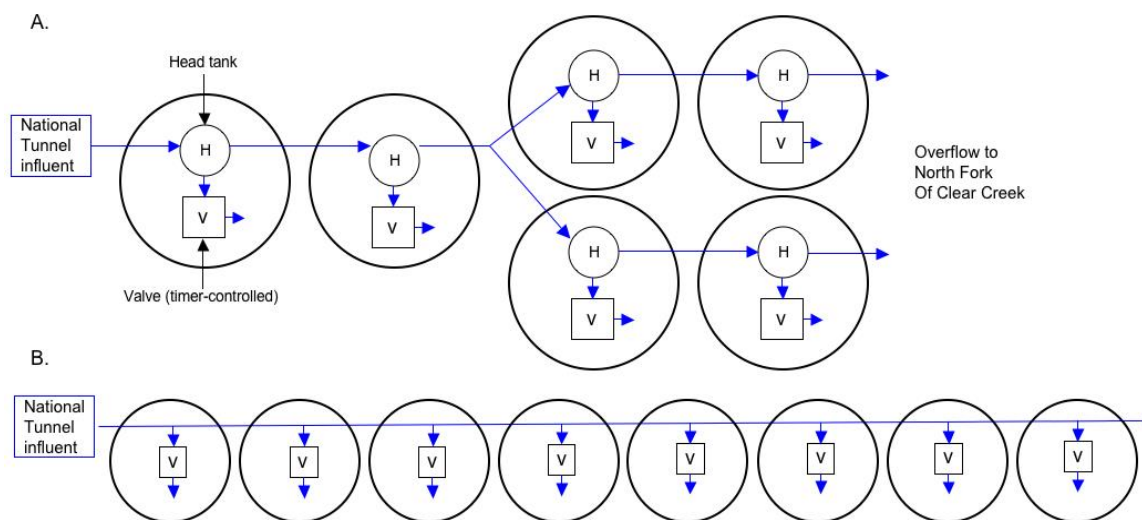
Figure 1. Configuration of influent flow to: (A) 2006 reactor configuration and (B) 2007 reactor configuration (drawing not to scale).

The ethanol feed system was modified in May 2007 from the 2006 operation. Each ethanol fed BCR received approximately 700 milliliters (ml) per week of 50% ethanol/50% water. Timer-controlled valves provided daily 100 ml additions of ethanol solution from a pair of 3.8 liter bottles to the first pair of reactors in the series. The ethanol bottle was manually refilled weekly; the distributed ethanol volume was measured, and was adjusted accordingly to insure consistent ethanol flow. A timed test using a graduated cylinder and stopwatch was used to demonstrate that ethanol flow was nearly identical for half to completely full ethanol feed bottles. The ethanol was fed into the BCRs adjacent to the point were the MIW entered the BCRs at the surface of the of the limestone support media.

In the May 2007 revision of the reactor installation, National Tunnel water was continuously fed through a 7.5m (approx.) section of pipe along which eight evenly spaced "T" junctions led

to valves (timer-controlled) that fed individual reactors (typical configurations shown in Fig. 2). The valves were programmed for total daily additions of 23L of MIW to the initial six BCRs and the additional two CHTN reactors. This increased retention time to 2.2-3.7 days based on the previously used pore volume estimate. The flow to each reactor was set by adjusting the open time period of the valve. The flow from a hose connected to each timer was measured with a bucket and stop watch to determine the time period required to deliver 5.7L per cycle. The valves for the series of reactors open in succession such that no two valves were open at the same time, insuring equal flow to each. The configuration of the reactor overflow and sampling ports remained unchanged.
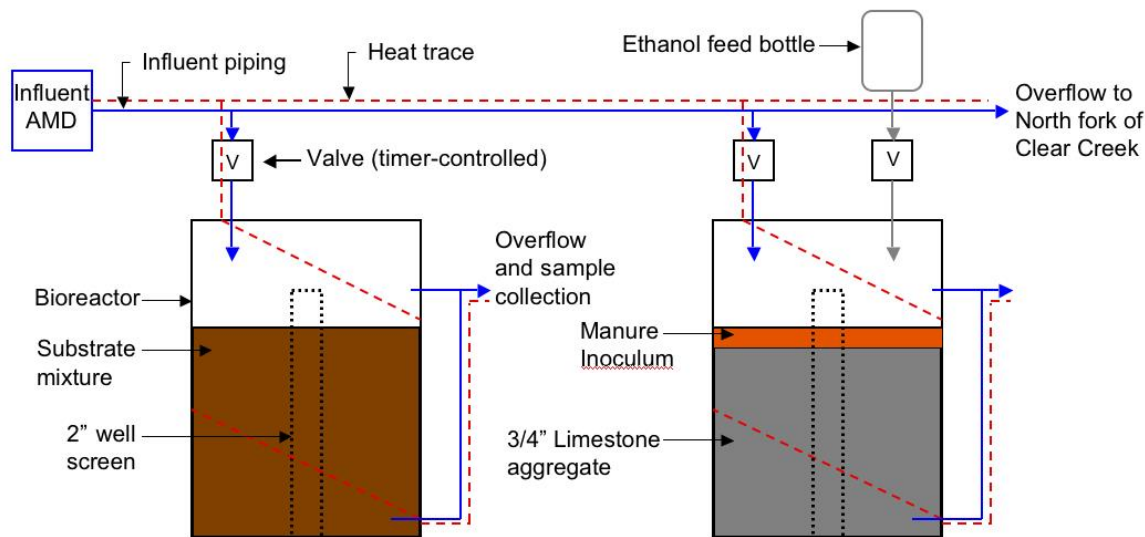
Figure 2. Current biochemical reactor test configuration for solid phase (left) and ethanol-fed, liquid phase (right)

In addition to the hydraulic system modifications, Raychem™ heat trace tape (purchased from Grainger Industrial Supply, www.grainger.com) was installed in October 2007 to prevent freezing issues that occurred during the previous winter. The heat trace tape was run along the entire length of the main influent pipe with "T" splices (to each valve) allowing a length of heat tape to run down the piping to each reactor, around each reactors three times, and up each effluent hose. All exposed heat trace along PVC pipes and hoses was covered with standard foam pipe insulation whereas fiberglass water heater insulation sheets (7 cm thick) were wrapped around timers and reactors.

Composition of Solid and Liquid Phase Reactor Substrates

With the exception of the ETOH BCRs, the initial composition of the substrates was not altered during modification of the treatment system in 2007. Iron filings added to the top of the ETOH reactors in 2006 were removed in 2007 when it was determined that the Fe filings were not necessary for maintenance of reducing conditions in the reactor. The surface of the ETOH reactor limestone was minimally disturbed during this procedure. The composition of the original reactors in addition to that of the two CHTN reactors is displayed in Table 3. All substrates were packed into the reactors on-site. The solid phase organic substrate BCRs were first packed with a 3 to 4-inch thick layer of ¾-inch aggregate, permeable limestone base that allowed for effluent drainage collection from the bottom of the reactors. The limestone aggregate was followed by the addition of organic substrate mixed with limestone sand and horse manure inoculum to a total volume of 170L. Ethanol-fed BCRs were packed solely with ¾-inch aggregate limestone then topped with a layer of horse-manure inoculum and the iron filings, resulting in a total volume of 190L. The percent composition of the packing in each reactor was calculated using the known density of the substrates (Buccambuso et al. 2007). Finally, the well screen pipe installed in each BCR served for periodic collection of mesh bags filled with the respective substrate used in each reactor for microbial community molecular work and substrate degradation assays.

Table 3. Solid and liquid-phase reactor substrate composition by weight percent

Component	Liquid-phase substrate		Solid phase substrate					
	ETOH 1	ETOH 2	HYWD 3	HYWD 4	CSWD 5	CSWD 6	Chitin 7	Chitin 8
Wood Chips	0%	0%	50%	50%	35%	35%	0%	0%
Corn Stover	0%	0%	0%	0%	30%	30%	0%	0%
Hay	0%	0%	10%	10%	0%	0%	0%	0%
Chitin	0%	0%	0%	0%	0%	0%	0%	0%
Horse manure	1%	1%	0%	0%	0%	0%	0%	0%
Cow Manure	0%	0%	10%	10%	15%	15%	0%	0%
Limestone	98%	98%	30%	30%	20%	20%	0%	0%
Chitorem® SC-20	0%	0%	0%	0%	0%	0%	7%	7%
Pea Gravel	0%	0%	0%	0%	0%	0%	50%	50%
Sand	0%	0%	0%	0%	0%	0%	43%	43%
Iron Filings	1%	1%	0%	0%	0%	0%	0%	0%

The CHTN reactors were packed with a mixture of sand, pea-gravel, and CHITOREM® SC-20 (Table 3). CHITOREM ® SC-20 chitin complex was provided by JRW Bioremediation, Lenexa, KS. This crab-shell based substrate had previously demonstrated potential in bioremediation of MIW (Daubert and Brennan 2007). CHITOREM ® SC-20 is composed of crushed crab shell (< 1 mm in nominal size) with a chemical composition of approximately 20% chitin, 40% chitin-

bound calcium carbonate, 30% protein, and less than 9% nitrogen. The sand and gravel mixture was necessary to insure even distribution and suspension of the CHITOREM® SC-20 particles throughout the reactor and to maintain hydraulic conductivity.

Sampling Strategy and Methods

The experimental strategy remained unchanged from 2006 (Table 4). In addition, as of September 26, 2007, reactor temperature was sampled directly from the center of the reactor using a temperature probe. Weekly collections of influent and effluent samples for the field and laboratory tests as described in Table 5 remained unchanged. Collection of solid-phase samples for microbial community and substrate analysis will be continued in 2008.

Table 4. Sampling frequency and analytes monitored

Analyte or Analyte group	Frequency of Samples or Measurements	Sample Collection or Measurement Method	Sample container type/size	Field or Lab[1]	Preservation
Cu, Fe , Zn, Ca, Mg, Mn, S	Weekly	Grab, filtered	15 ml plastic vial (CSM)	L	Nitric acid, room temperature
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	Direct, reactor and ambient[3]	NA	F	NA

[1] Where measurement/analysis was performed, field (F) or lab (L).
[2] Alkalinities were titrated in the field, unless the ambient temperature caused the digital titrator solution to freeze, in which case the samples were stored on ice and titrated upon arrival in the lab.
[3] As of September 26, 2007, temperature was measured directly from the reactor and ambient air rather than a grab-type sample.

Table 5. Methods for Solution Analysis

Analyte or Analyte group	Measurement Method	Equipment	Reporting Units	Detection Limit
Cu, Fe , Zn, Ca, Mg, Mn S[1]	EPA 6010B ICP-AES	Perkin Elmer ICP-AES Model 3000	mg/L	In mg/L Ca = .0073 Cu = .0042 Fe =.0056 Mg = .001 Mn = .001 S = .041 Zn = .0034
Alkalinity	EPA 310.1	HACH digital titrator	mg/L as $CaCO_3$	± 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 conductivity	EPA 120.1	Conductivity/ temperature meter with probe	microsiemen	± 1 microsiemen
Temperature	--	Temperature meter with probe	Celsius	± 0.1 o C

[1] Sulfate measured as 3x sulfur

Results and Discussion

A lack a lower level of replication in the performance was observed between paired reactors in 2007 than during the first year of operation. As a result, the measured parameters for the reactors in each pair will be presented individually rather than as an average. Performance assessments of the four reactor types are based primarily on a comparison of influent and effluent metal concentrations with discussion of pH, alkalinity, and sulfate concentrations.

Modifications to the biochemical reactor installation

The complexity of the original reactor piping and head-tank system caused several issues that have been largely resolved with the new layout (Figs. 1 and 2). The most important of which was the simplified piping that provided fewer places for ice to accumulate and cause complete or partial blockages in the system. Even when the influent valves, and influent and effluent hoses of the reactors froze during power shortages in 2007, the influent water still flowed through the main distribution pipe, bypassed the reactors, and exited into the north fork of Clear Creek,

(Table 2). Additionally, heating and insulating the entire system prevented winterization problems that were experienced with the initial reactor installation.

Temperature

The temperature of a reactor without heating and/or insulation is directly dependent on influent and ambient air temperatures. It would be expected that the temperature of a BCR should be near that of the influent water with positive and negative influences depending on ambient air temperature and hydraulic retention time. If low enough, ambient air temperatures could sufficiently cool the reactors to cause freezing in the feed system plumbing and BCRs. This was evident from the first year of winter operation in December 2006 and unusually cold conditions in October and November 2007 that caused freezing issues with the revised reactors (Fig. 3). The reactor temperatures dropped below the influent temperature because of intermittent power to the heating system at the end of October 2007 (Table 2 and Fig. 3). Once the intermittent power problem was resolved, the heating system proved capable of maintaining reactor temperatures above 6° C and thus prevented freezing of the influent, reactor, and effluent. Measured temperatures varied greatly between reactors. At the completion of this study, the effect of reactor temperature on SO_4^{2-} reduction rates will be evaluated.
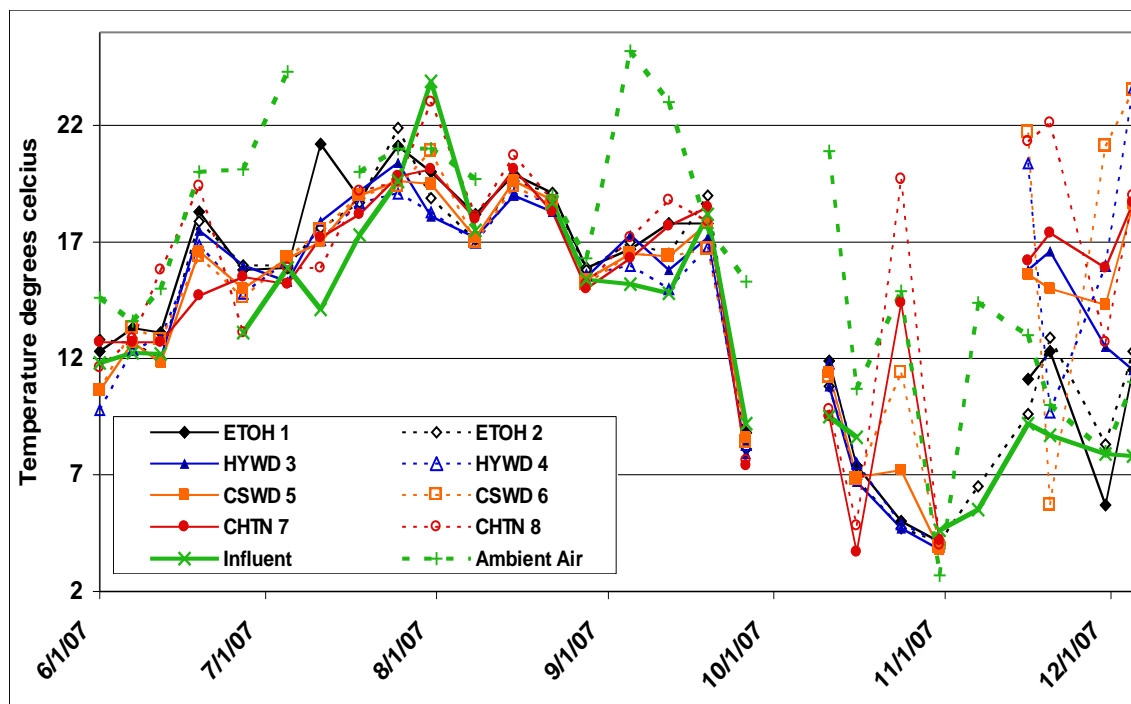
Figure 3. Temperature with time for National Tunnel influent, BCR effluent, and ambient air.

<u>Biochemical reactor pH and alkalinity</u>

A reduction in National Tunnel influent pH was observed during July and August in 2007 relative to July and August 2006. The effluent pH of the HYWD and CSWD BCRs was lower than the influent in July and August 2006. However, in July and August 2007, the effluent pH of the HYWD and CSWD BCRs was higher than the influent even though the influent pH was more than 0.5 pH unit lower than the same period in 2006 (Fig. 4). These data suggest that the more mature solid-phase substrate BCRs had better pH neutralizing capabilities. In contrast, the ETOH BCRs exhibited the opposite relative pH effect during the same period. The ETOH effluent pH in July and August 2006 was greater than 6.6 but was less than 6.5 in July and August 2007 when influent pH was lower. The effluent alkalinity over the same period for all of these reactors was perhaps more stable and slightly higher in 2007. In the future, statistical analysis will be required determine the significance of these preliminary observations.

The apparent improvement in pH neutralizing capacity of the HYWD and CSWD BCRs is potentially due to several factors:

1. a reduction of organic-acid producing fermentations as the substrates age, a phenomenon noted by Buccambuso et al. (2007);

2. a reduction in the amount of MIW fed post May 2007 (23 L/d vs. 34 L/d during the first 4 months of operation in 2006); or

3. increased SO_4^{2-} reduction.

Potential mechanisms for increasing pH and alkalinity in ETOH reactor effluents are sulfate reduction and limestone dissolution. A statistical analysis of the alkalinity and pH data in conjunction with SO_4^{2-} reduction and Ca production is required to understand the variation in pH neutralization and alkalinity production between 2006 and 2007.

The CHTN reactors typically produce the largest increase in pH and alkalinity. Factors contributing to the ability of the CHTN BCRs to increase pH and alkalinity include:

1. dissolution of chitin bound $CaCO_3$;

2. SO_4^{2-} reduction and

3. NH_4 production from protein degradation.

The collection of NH_4 data in conjunction with pH, alkalinity, SO_4^{2-} and Ca data will be required to elucidate the mechanisms responsible for pH and alkalinity increases in the CHTN BCRs.
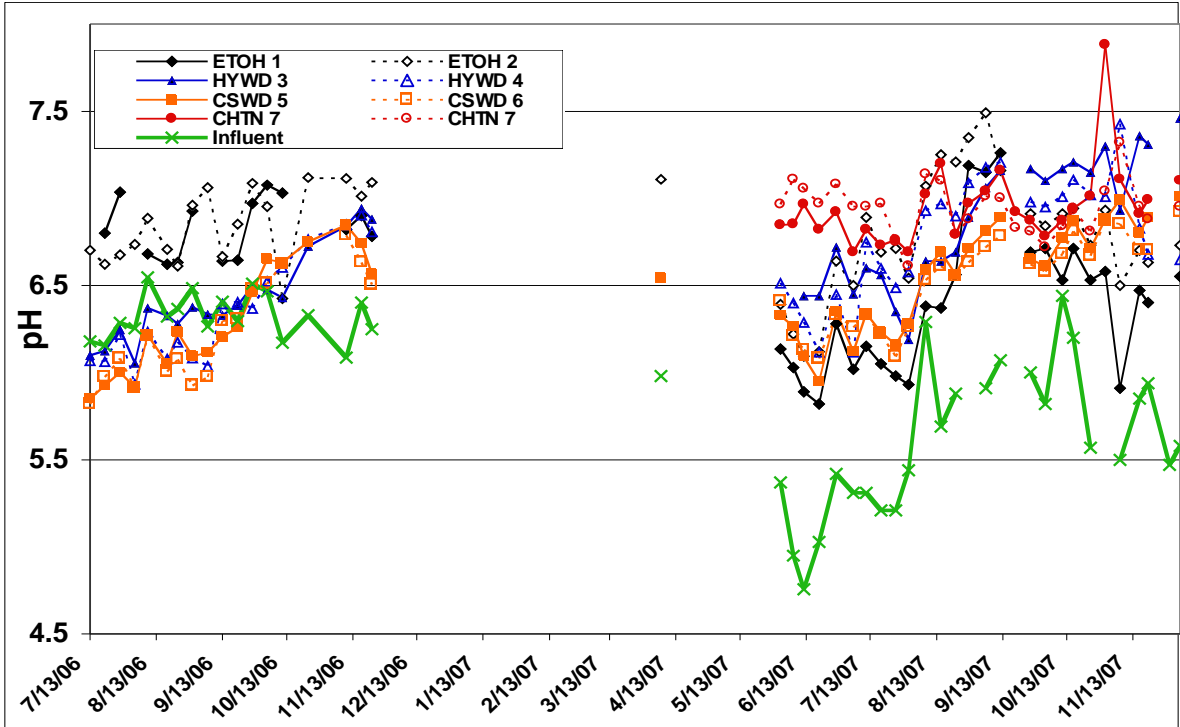
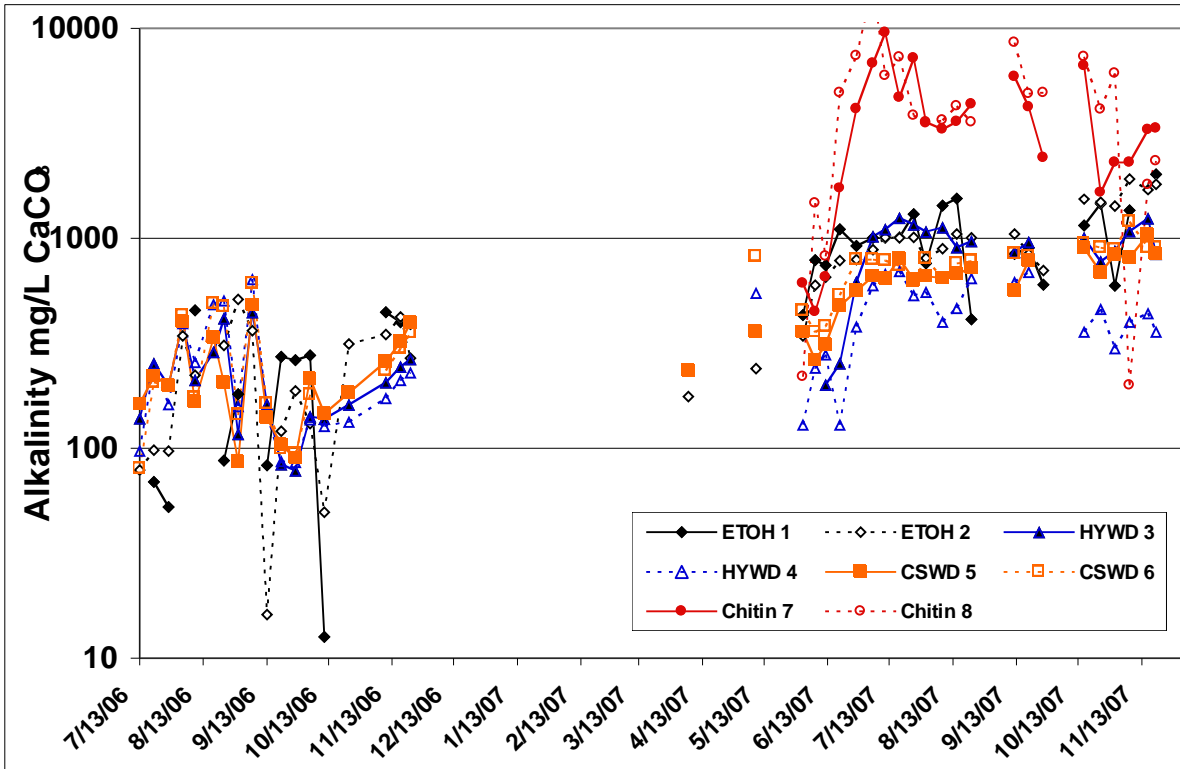Figure 4. pH with time for National Tunnel influent and bioreactor effluent

Figure 5. Alkalinity with time for National Tunnel bioreactor effluent
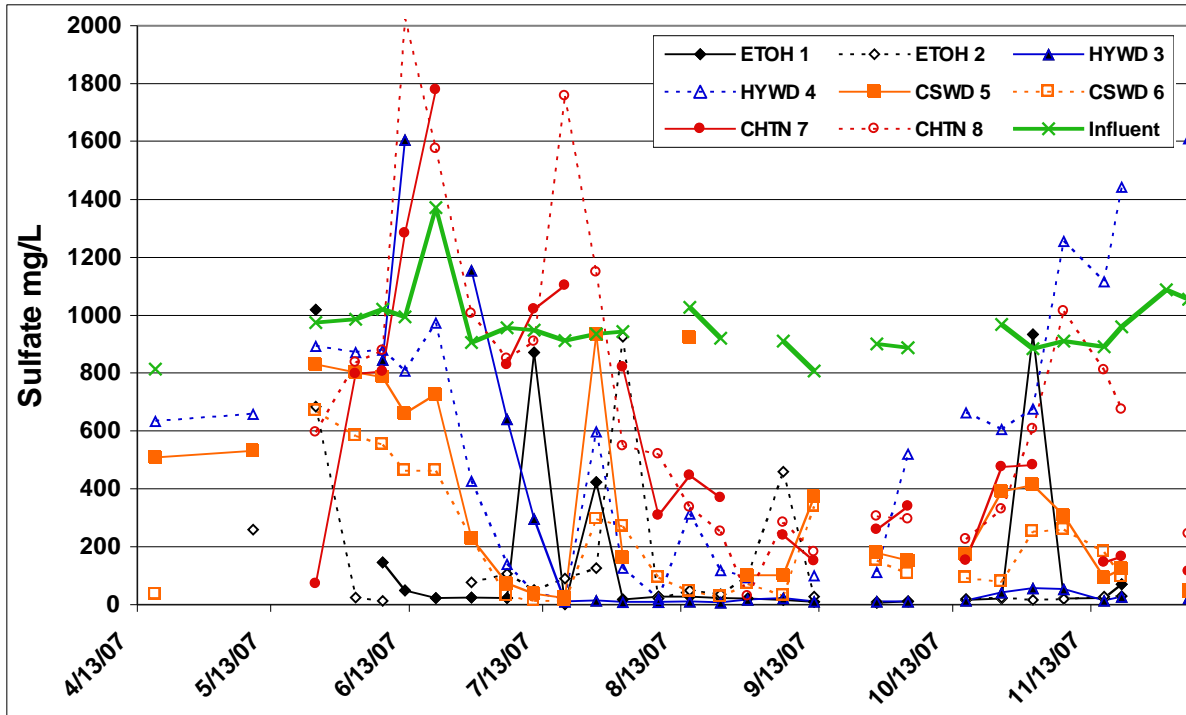
Figure 6. Sulfate concentration with time for National Tunnel influent and bioreactor effluent for updated reactor installation
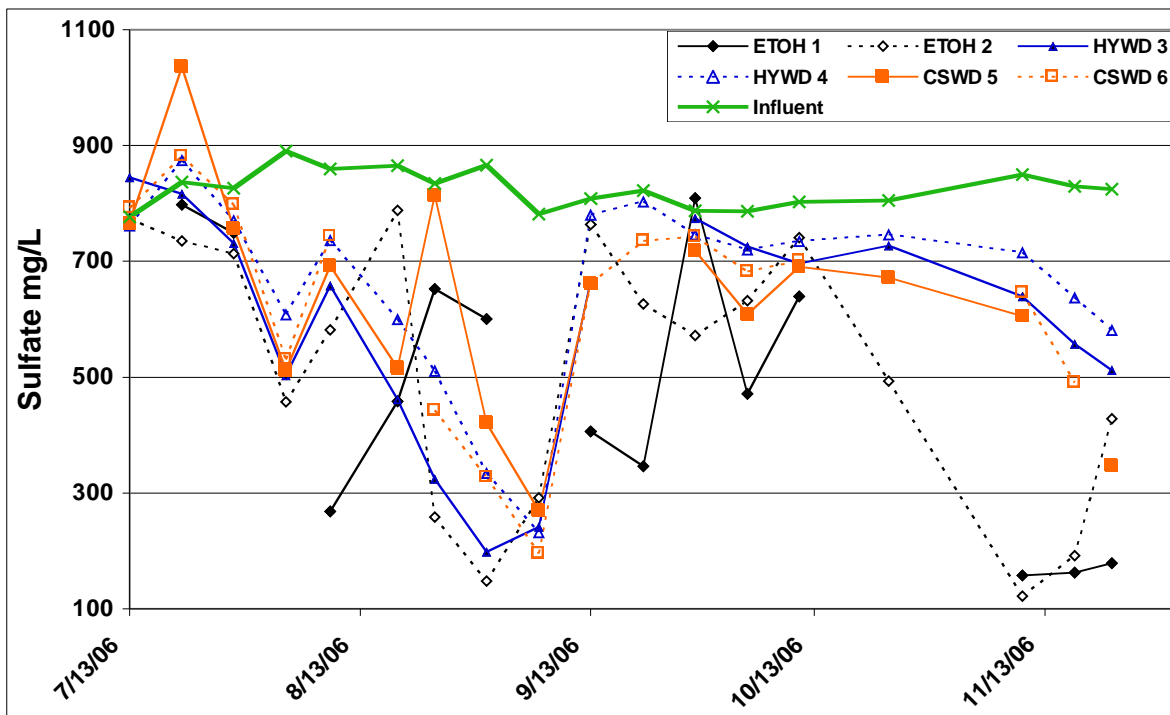

Figure 7. Sulfate concentration with time for National Tunnel influent and bioreactor effluent for initial reactor installation

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Cu, Fe, Zn, Mn, and sulfate removal

The effluent metal concentrations suggest that all the BCRs are capable of meeting the current National Ambient Water Quality Criteria (U.S. EPA, 2004) for Cu and Zn. Within three months of CSWD, HYWD, and ETOH BCR start up in both 2006 and 2007, effluent concentrations of Cu below the detection limit (0.0042 mg/L) and Zn typically < 0.1 mg/L were measured. The same is true for the CHTN reactors after a similar length start-up period. The removal of these metals seems largely independent of observed variations in SO_4^{2-} reduction and pH.

Iron removal increased slowly after reactor start-up in 2006 and 2007 and demonstrated greater variability (Fig. 8) than the removal of Cu, Zn and Mn. During the 2007 start-up period, CSWD 5, ETOH 2, and HYWD 3 reactors demonstrated Fe concentrations much greater than influent (between 06/13/07 and 08/13/07), suggesting that accumulated Fe precipitates were released into the effluent. The disturbance from freezing and moving the BCRs may have contributed to reoxidation of the Fe, resuspension of iron into solution or iron desorption from BCR substrates into the effluent.

A similar but less dramatic leaching of Mn occurred from mid- May through mid-July 2007 in CHTN7, ETOH 1, ETOH2, and HYWD 3 BCRs (Fig. 8). The cause of these leaching events and the reason(s) for which leaching occurred in specific reactors is not apparent as alkalinity, pH, and SO_4^{2-} removal were greater than or equal to values demonstrated during periods of elevated Fe and Mn removal (10/20/07-12/13/07) from the initial reactors. Additionally, significant Fe and Mn leaching occurred from ETOH 1 during October and November 2007, potentially related to a drop of pH to near-influent levels during that period. Continued measurements will be necessary to ascertain if Fe and Mn removal resumes in this reactor.

A comparison of sulfate removal from 2006 (prior to reactor freezing) and 2007 suggests that SO_4^{2-} removal in the former did not reach the extent demonstrated in the latter (Figs. 6 and 7). After the 3 month startup period in 2007, all the BCRs were able to remove at least 50% of the influent SO_4^{2-} from August to late October. HYWD4 developed a leak in October 2007 after a freezing event and effluent total sulfur eventually exceeded the influent SO_4^{2-} as S concentration in November and December of 2007. Freezing effects also were likely responsible for effluent total S spikes between October and November in the 2007 BCRs. We postulate that the freezing physically disrupts the precipitated metal sulfides with metal sulfide nanoparticles becoming re-

suspended in the effluent. This will be verified in future samplings by performing total S analysis in addition to the current dissolved S analysis.

Although removal rates are relatively consistent for individual BCRs from October to November 2007, it is not possible to determine which reactor pair demonstrates the best SO_4^{2-} reduction because of variability between reactors in each pair and the absence of measured flow data. Monthly validation of the flow to the BCRs to be measured in spring and summer of 2008 will be used to evaluate the specific SO_4^{2-} reduction rate and will permit verification of hydraulic retention times. There is no indication that the retention time decrease to 2.2-3.7 days from 4.5-7.5 days negatively affected SO_4^{2-} removal in the 2007 reactors. The data demonstrates that maximal SO_4^{2-} removal rates for all 2007 reactors reached approximately 50% (Fig. 6), whereas 2006 BCR sulfate removal peaked at 32% of influent values.
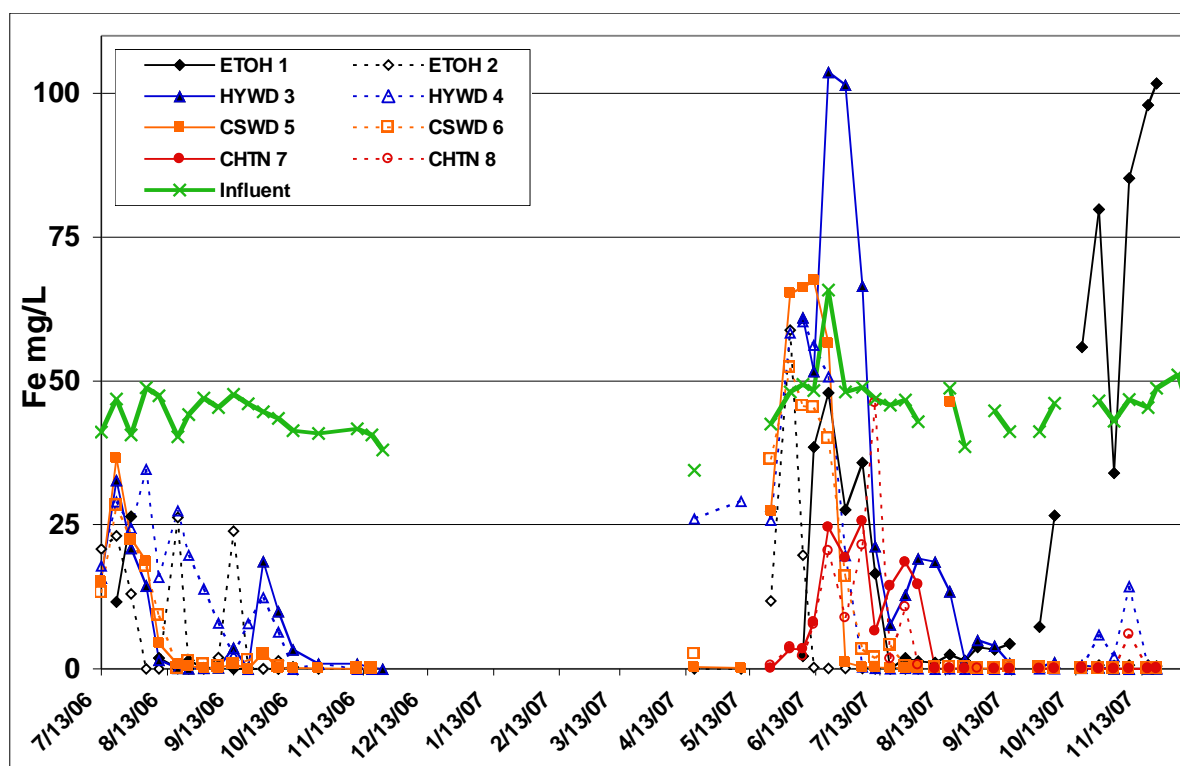
Figure 8. Fe concentration with time for National Tunnel influent and bioreactor effluent

Elevated Mn removal by CHTN reactors

Mn removal to below 5 mg/L from an influent concentration >20 mg/l was demonstrated by CHTN 7 and CHTN 8 BCRs (Fig. 9). This was particularly impressive when compared to the

initial six reactors that typically demonstrated removal of no more than half of influent Mn concentrations. This extent of Mn removal was closely matched by only HYWD 3 BCR. In comparison, HYWD 4 BCR reached a Mn concentration of 12 mg/L in September 2007. However, a leak developed in the reactor effluent fitting of this reactor and the effluent Mn concentration rose to above the influent level in December 2007 (Fig. 8). The ability of CHTN reactors to remove Mn below 5 mg/L was noteworthy because MnS_2 and other sulfide precipitates do not readily form in the presence of other metals. Additionally, the conditions for Mn (II) oxidation were unfavorable due to the presence of Fe(II), reducing conditions and pH < 8 (Hallberg et al. 2007). In previously completed studies, Mn sorption to materials typically within BCRs was generally weak (Neculita et al. 2007).

The exact cause of Mn removal is uncertain but it is likely that alkalinity resulting from the dissolution of chitin-bound $CaCO_3$ played an important part by promoting the formation of $MnCO_3$. This was supported by an apparent relationship between alkalinity and Ca concentrations in the CHTN reactor effluents (Fig. 10). However alkalinity-balance calculations suggest that calcite dissolution did not contribute all of the measured alkalinity; other factors such as ammonia generated from CHTN protein may have been involved. Ammonium measured in a single set of grab samples (using the HACH NH_4-Nitrogen, High Range, Test 'N Tube reagents with a DR890 Colorimeter) from the CHTN reactors in November 2007 was as high as 450 mg/L (reported as nitrogen) (Venot et al. 2008).
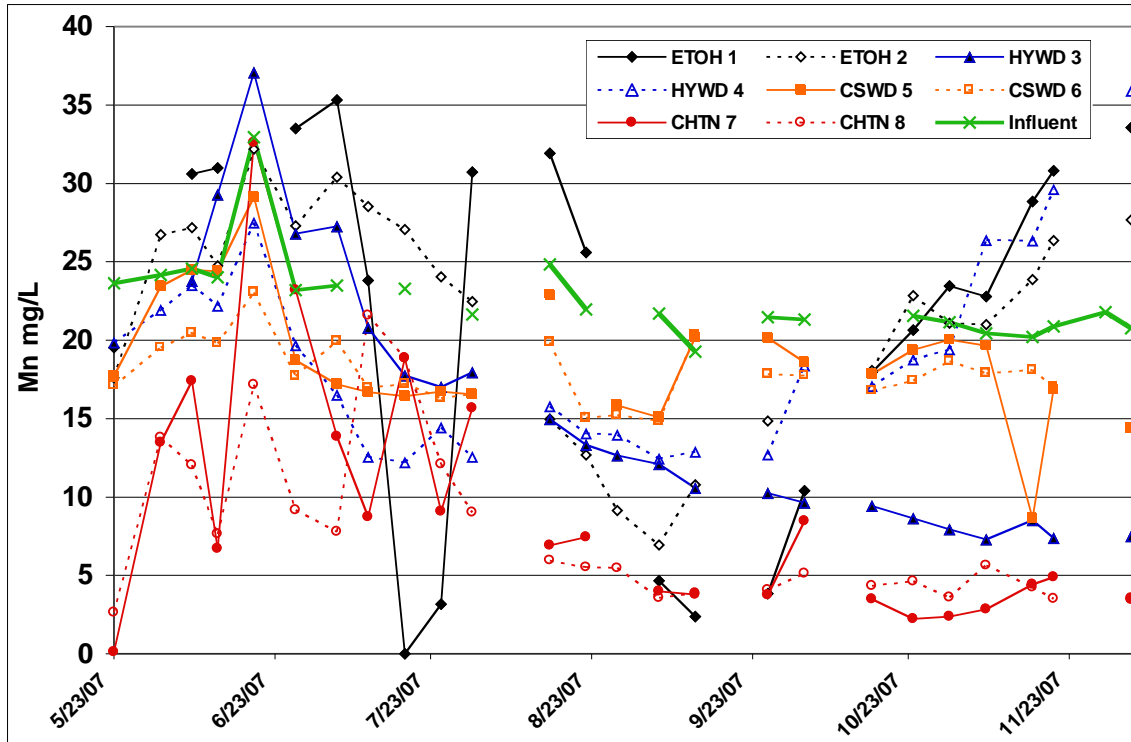
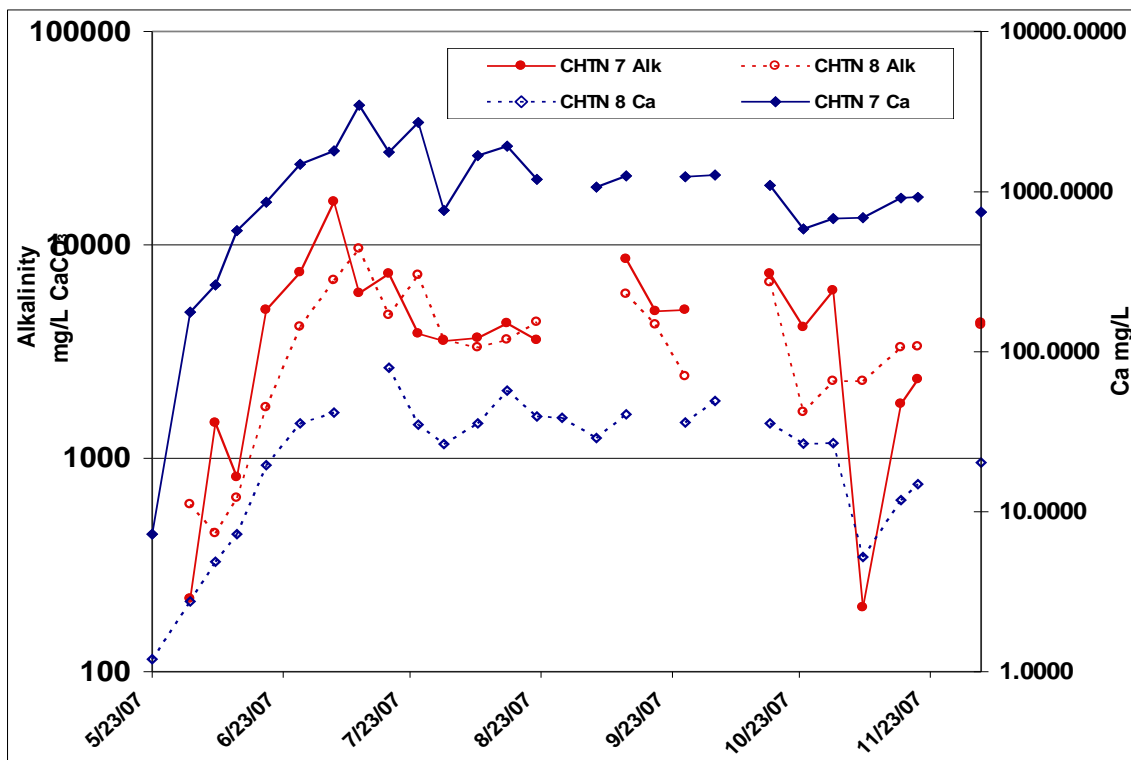Figure 9. Mn concentration with time for National Tunnel influent and bioreactor effluent

Figure 10. CHTN BCR alkalinity and Ca concentration with time for National Tunnel influent and bioreactor effluent

Conclusion

Overall, the reactors have demonstrated metal removal up to 99% and pH neutralization of MIW from the National Tunnel drainage over the course of 18 months. In general, these abilities have persisted through periods of flow and temperature fluctuations caused by freezing temperatures and power outages. The updated reactor installations have demonstrated several notable characteristics in 2007:

- Improved flow and temperature control were maintained during winter weather. However, temperature and flow fluctuations were likely causes of variations in SO_4^{2-} and Fe removal.
- Effective removal of Cu and Zn was observed despite temperature and flow fluctuations.
- Preliminary observations suggest that a decrease in retention time did not prevent effluent pH (except ETOH1), alkalinity (except HYWD4), and SO_4^{2-} removal values from exceeding those of the 2006 reactors
- Freezing and/or draining of reactors appeared to cause spikes in reactor effluent metal concentrations after flow was reestablished.

Hydraulic and winterization improvements made to the reactor system have allowed the system to operate through the winter, and operation will continue through summer 2008. Components that control flow will be monitored more frequently in spring and summer of 2008 to allow for better differentiation of reactor treatment capacity. The data collected through 2007 suggest the following preliminary conclusions about the performance and sustainability of the four types of BCRs tested for MIW treatment:

- All BCRs were able to remove up to 99% of Cu and Zn after a 3-month start-up period in 2007.
- ETOH BCRs demonstrated variability in the removal of Fe and Mn. Both BCRs exhibited poor Mn removal and even showed a release of Mn after the first freezing event. In contrast, Fe removal was high in ETOH 2 but low in ETOH 1. ETOH 1 exhibited a release of Fe after the first freezing event in October of 2007.
- HYWD BCRs did not demonstrate duplicity of performance. HYWD 3 provided more consistent and higher SO_4^{2-} removal and Mn removal than HYWD 4.
- CSWD 5 and 6 reactor results demonstrated a high level of duplicity. These BCRs had the least Mn removal.

- CHTN reactors demonstrated noteworthy Mn removal likely due to high alkalinity and other unknown factors at the expense of significant NH_4 production.

Acknowledgements

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