COMPARING CHITIN AND ORGANIC SUBSTRATES ON THE NATIONAL TUNNEL WATERS IN BLACKHAWK, COLORADO FOR MANGANESE REMOVAL[1]

C. Venot[2], L. Figueroa[2], R.A. Brennan[3], T.R. Wildeman[4], D. Reisman[5], and M. Sieczkowski[6]

Abstract: The National Tunnel is a part of the Central City / Idaho Springs Superfund site. Because passive treatment is an important possibility for removal of contaminants from the water, the USEPA and the Colorado Division of Public Health and Environment (CDPHE) have been sponsoring a bench-scale study of different organic substrates for sulfate-reducing bioreactors (SRBRs). The substrates being tested include ethanol, woodchips and hay, woodchips and corn stover, and crab-shell chitin. After 6–18 months of operation, all of the reactors are showing significant amounts of sulfate reduction. In all of the reactors, Cu and Zn are removed to below their respective ambient water quality criteria of 0.010 and 0.100 mg/L. As is commonly observed in SRBRs, Mn removal is significantly less, with the exception of the chitin reactors. The reason for chitin's superior Mn removal may be the dissolution of calcite from the crab shell. In the chitin reactors, Ca has increased from 210 to 870 mg/L and alkalinity has increased from zero to up to 5,000 mg $CaCO_3$ / L. Furthermore, the pH of the effluent leaving the chitin systems averages 6.9. In most SRBRs, Mn is precipitated as $MnCO_3$ and significant removal does not occur until the pH is raised to between 7 and 8. This is the case in the other types of SRBRs being tested at the National Tunnel, as their Mn removal efficiencies have only approached 50 % at pH values ranging from 6.5 to 7.5. However, in the chitin reactors, 86% of the influent Mn is being removed from 21.5 mg/L to an average of 3 mg/L. The high removal is very similar to the removal of Mn in pulsed limestone beds that are maximized for the dissolution of calcite. These chitin reactors have been operating for six months while the other substrate reactors have been operating for over one year.

Additional Key Words: Water treatment, limestone dissolution, sulfate-reducing bioreactors

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[2] Christophe Venot, and Linda Figueroa, Div.of Environmental Science and Engineering, Colorado School of Mines, Golden, CO, 80401, e-mail: cvenot@mines.edu ,.[3]Rachel A. Brennan, Dept. of Civil & Environmental Engineering, The Pennsylvania State University, 213-C Sackett Building, University Park, PA 16802, .[4]Thomas Wildeman, Department of Chemistry and Geochemistry, Colorado School of Mines, Golden, CO, 80401, [5]David Reisman, USEPA, National Risk Management Research Laboratory, ETSC, Cincinnati, OH 45268, [6]Michael R. Sieczkowski, JRW Bioremediation, 14321 W. 96th Terrace, Lenexa, KS 66215.

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Introduction

Since 2006, the researchers at the Colorado School of Mines (CSM) in conjunction with the US Environmental Protection Agency (EPA) Office of Research and Development (ORD) and Region 8, and Golder Associates have been conducting comparison studies on how well different substrate recipes used in sulfate-reducing bioreactors (SRBRs) performed in removing contaminants from the National Tunnel water in Blackhawk, Colorado (Figueroa, et al., 2007, Buccambuso, et al., 2007). Pairs of reactors in a downflow configuration included an ethanol based configuration designed by the EPA and CSM, an organic substrate designed by EPA and Golder, and a corn stover substrate designed by CSM. In 2007, a pair of reactors was added to the configuration by EPA in collaboration with Pennsylvania State University and JRW Bioremediation that contained a chitin-based substrate. The chitin reactors performed quite differently than the other systems, and the purpose of this paper is to describe these differences and give some preliminary opinions on why these differences occurred.

Table 1 presents the typical range of concentrations of constituents found in the National Tunnel water. A key treatment objective is to remove metals from the water below their respective water quality criteria and standards. For the most part, all of the reactors succeed in that key objective. After a start-up period, the pair of chitin reactors also met the removal objectives for Cu and Zn. However, the effluents from these reactors have much higher concentrations of Ca and Mg as well as extremely high values of alkalinity, sometimes reaching above 7,000 mg of $CaCO_3$ per liter. In these chitin reactors, Mn is also removed to between 3 and 4 mg/L, whereas in most of the other reactors Mn removal is minimal. These properties suggested that the reactors may be precipitating a carbonate phase that is also removing Mn (Sibrell et al., 2007a, 2007b). Attempting to explain these high alkalinities and reasons for manganese removal is another objective of this paper. This is important because Mn is found in mining influenced waters from both coal- and metals-mining operations, and its removal in a passive system is quite difficult.

Table 1- Typical National Tunnel water composition. Concentrations are in mg/L except for pH.

Constituent	Conc. in mg/L	Treatment Objective in mg/L*
Aluminum	0.1 – 1.0	0.1
Iron	40 - 50	1.0
Manganese	20 - 25	1.0
Copper	0.2 – 1.0	0.01
Zinc	8 - 10	0.1
Calcium	200 - 250	Not applicable
Sulfate	900	Not applicable
Magnesium	50 - 80	Not applicable
pH	5.0 – 6.0	6.5 – 8.5

* Typical aquatic limits for Colorado watersheds (U.S. EPA, 2004).

Materials and Methods

Chitin Substrate Reactors

The chitin-based substrate used for the reactors was ChitoRem^{TM} SC-20 (crab-shell chitin) provided by JRW Bioremediation (Lenexa, KS). This material is used for the bioremediation of a broad range of contaminants including chlorinated solvents (Martin et al. (2004), Brennan et al. (2006)), perchlorate, nitrate (Robinson-Lora and Brennan, 2007), metals and mining influenced waters (Daubert and Brennan, 2007). The material is about 20 % actual chitin, 40 % $CaCO_3$, and 30 % protein. It is estimated that the nitrogen content is about 9 % and the P content is < 1 %. It is a brownish material of less than 1 mm in size. The material was packed into 120 liter non-metal barrels in a downflow configuration the same as the other reactors that were built in 2006 (Buccambuso, et al., 2007). The reactors were started in May of 2007 and were designed to receive 23 liters of National Tunnel water per day in 4 increments.

Sampling and Analysis

For the influent and effluent, field measurements include temperature, pH, alkalinity, specific conductance, oxidation/reduction potential (ORP); samples were taken and analyzed for, Zn, Cu, Mn total Fe, and total S. Tables 2 gives the sampling conditions and Table 3 gives the analytical information on the water constituents that were analyzed (Buccambuso, et al., 2007).

The water samples are analyzed for elemental concentrations using ICP-AES. Approximately 10 mL of filtered sample, using a 0.45 μm nitrocellulose filter, was acidified with

HNO_3. The samples were then analyzed on a Perkin Elmer Optima 3000 ICP-AES for the following 31 elements: Ag, Al, As, B, Ba, Be, Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, P, Pb, S, Sb, Se, Si, Sn, Sr, Ti, V, and Zn. All concentration results are given in mg/L.

Table 2. Sampling frequency and ambient water quality criterion or criteria analytes monitored.

Analyte or Analyte group	Frequency of Samples or Measurements	Sample Collection Measurement Method	Sample container type/size	Field or Lab[1]	Preservation
Cu, Fe , Zinc, Ca, Mg	Weekly	Grab, filtered	15 ml plastic v (CSM)	L	Nitric acid, no 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 conductance.	Weekly	Grab, unfiltered	NA	F	NA
Temperature	Weekly	Grab, unfiltered	NA	F	NA

1. Where measurement/analysis occurred: field (F) or lab (L).
2. Alkalinities were titrated in the field, unless the ambient temperature caused the digital titrator solution to freeze. Then the samples were 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	Not applicable
Alkalinity	EPA 310.1	HACH digital titrat	mg/L as $CaCO_3$	± 0.1 mg/L
pH	EPA 150.1	pH/mV meter with probe	s.u. units	± 0.1 s.u. units
Oxidation Reduction Potential	EPA 200.11	pH/mV meter with Ag/AgCl probe	mV	± 0.1 mV
Specific conductance	EPA 120.1	Conductivity/ temperature meter probe	microsiemen	± 1 microsiemen
Temperature	--	Conductivity/ temperature meter with probe	Celsius	± 0.1 $_{o}$C

During the ICP- AES analysis, an internal standard of Sc was used to correct for adjustments in sample uptake and plasma conditions. Also, concentration check standards were analyzed in the beginning of the run and after every 20 samples to monitor the stability of all analytical conditions. Results on collocated water samples show the relative standard deviation of a concentration value is about 5 % as long as concentration is 10 times the limit of detection (Hageman et al., 2005).

Results

Figures 1, 2, and 3 show the concentrations of Mn and Ca in mg/L and the alkalinity in mg $CaCO_3$/L plotted for each substrate. Besides the pair of chitin reactors, one of each of the other types of reactors is presented. The ETOH 2 is a reactor in which ethanol is fed into a limestone bed, the HYWD 3 reactor is a woodchip based substrate designed by Golder, and the CSWD 5 is a corn stover and wood chip based reactor designed by CSM (Buccambuso, et al., 2007). Table 4 gives the values measured on September 12, 2007, for the constituents of interest in the influent from the National Tunnel and the effluent from all eight reactors. The values are reported in mg/L as designated in Table 3. Although the substrate within each reactor pair is the same, as seen in Table 4, results between the two are often quite different. It is believed that this is because of differences in the amount of flow going into each reactor. Because of plugging by $Fe(OH)_3$ precipitates, controlling flow into the reactors has been difficult. The last three rows in Table 4 are an attempt to account for the alkalinity produced by the cells. In the first row, the alkalinity for each reactor effluent is assumed to be all due to bicarbonate and it is converted into mole per liter assuming that for each unit of $CaCO_3$ alkalinity there are 2 units of HCO_3^- alkalinity. Alkalinity could be generated from two sources: limestone dissolution and/or SO_4^{2-} reduction. In the second row, the alkalinity from the increase in Ca concentration in each reactor is given in moles per liter. In the third row, the decrease in SO_4^{2-} concentration is attributed to SO_4^{2-} reduction and it is assumed that 2 moles of bicarbonate are generated for each mole of SO_4^{2-} reduced.

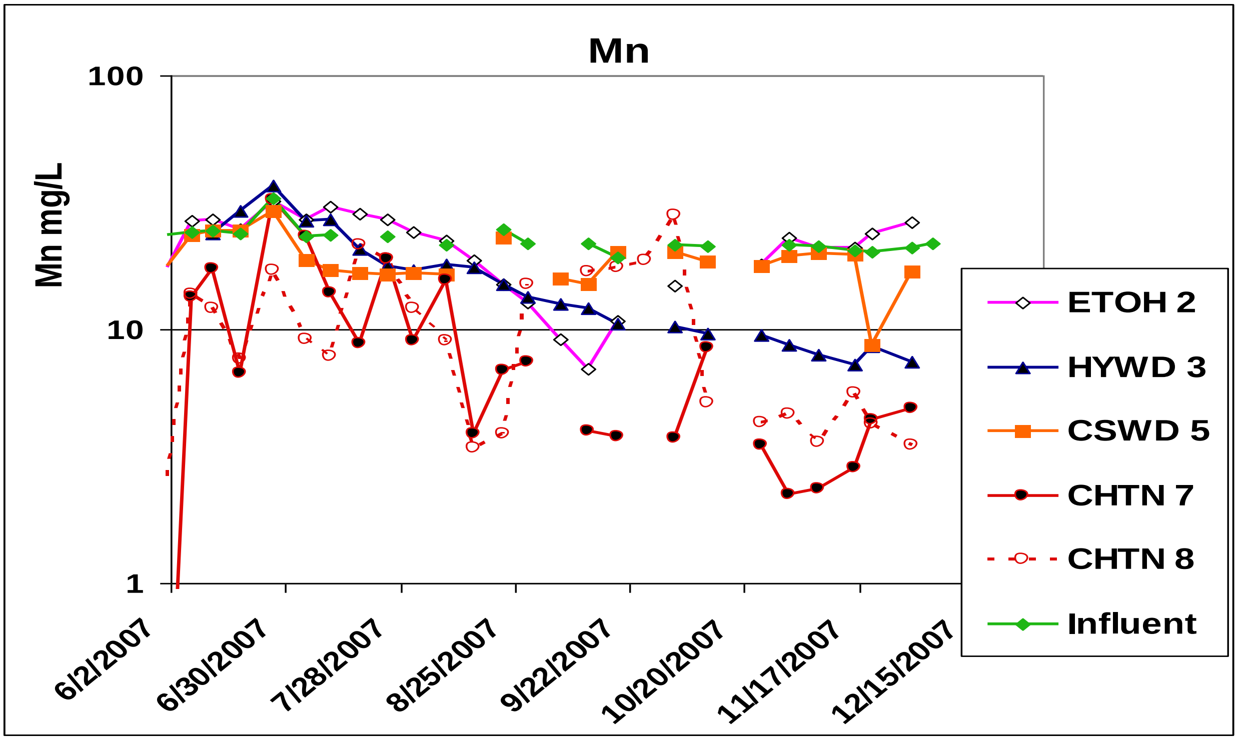

Figure 1. Concentration of Mn in mg/L for June to December of 2007.

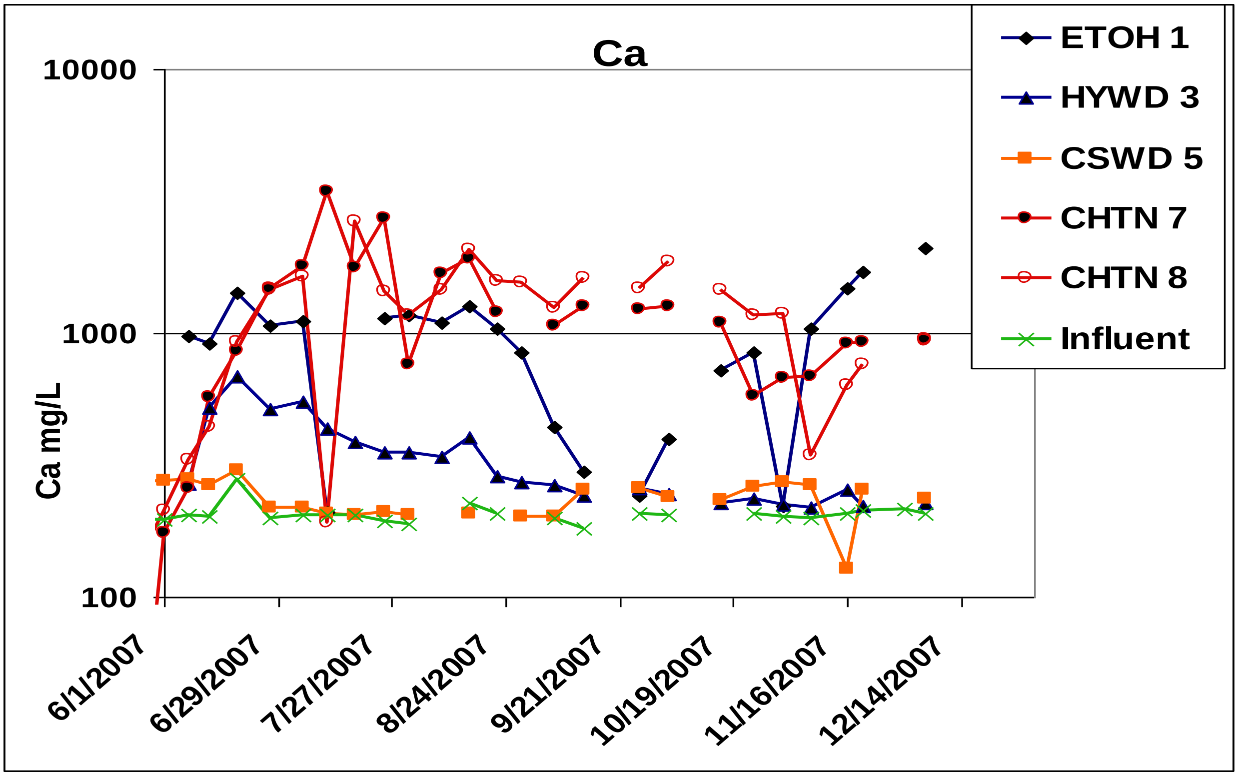

Figure 2. Concentration of Ca in mg/L for June to December of 2007.

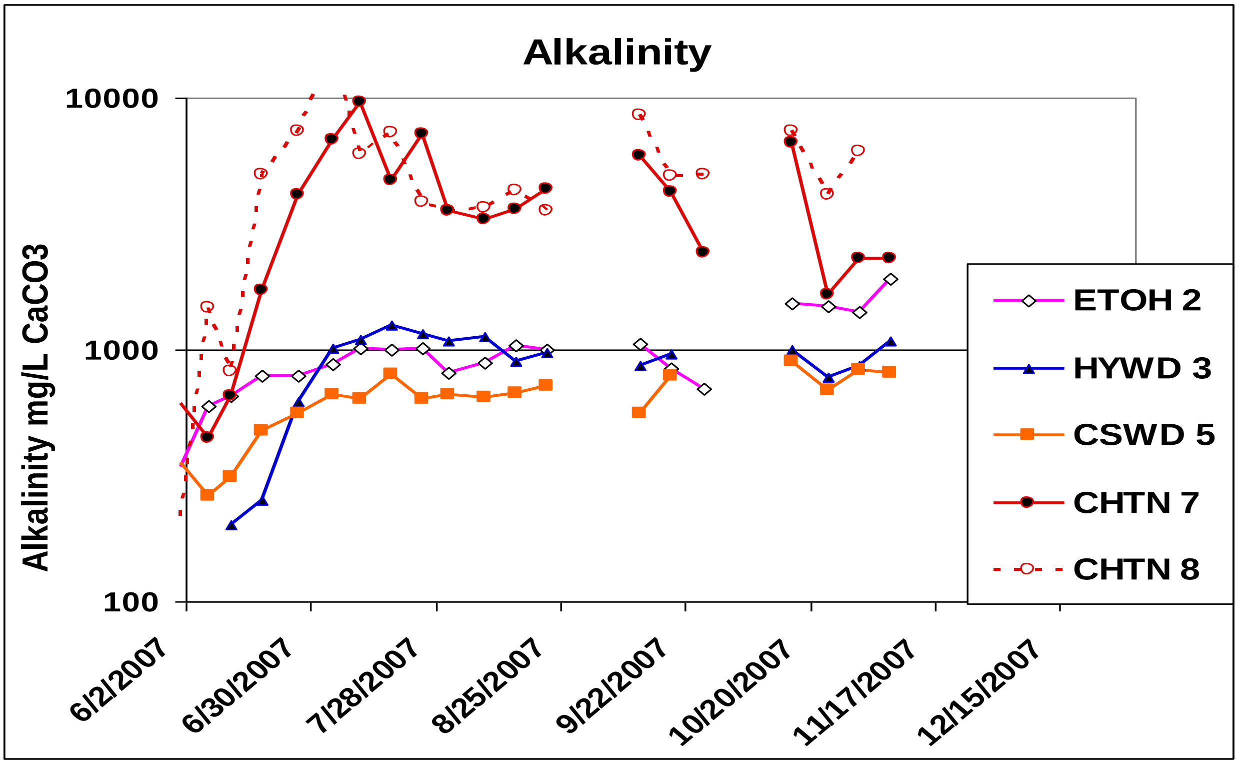

Figure 3. Alkalinity in mg $CaCO_3$/L for June to December of 2007.

Table 4. Aqueous constituents in the National Tunnel influent and in the effluent of the eight comparison reactors measured on September 12, 2007. Units for the values are given in Table 3. NA means not analyzed; DL means detection limit.

Constituent	**Infl**	**Eth 1**	**Eth 2**	**W/H 3**	**W/H 4**	**CS/W 5**	**CS/W 6**	**Chitin 7**	**Chitin 8**
Ca (mg/L) DL = 0.01 mg/L	181	297	454	241	182	254	282	1256	1609
Cu (mg/L) DL = 0.0009 mg/L	0.030	0.004	0.004	0.008	0.006	0.008	0.005	0.003	0.002
Fe (mg/L) DL = 0.002 mg/L	41.21	4.38	0.06	0.98	0.03	0.10	0.29	0.04	0.04
Mg (mg/L) DL = 0.0003 mg/L	53.9	41.9	51.3	47.3	51.9	60.6	63.5	NA	NA
Mn (mg/L) DL = 0.0003 mg/L	19.27	2.36	10.80	10.58	12.89	20.20	20.33	3.77	17.54
SO_4^{2-} (mg/L) DL = 0.33 mg/L	806	10	28	12	102	371	339	151	182
Zn (mg/L) DL = 0.0006 mg/L	4.67	0.02	0.01	0.01	0.01	0.02	0.01	0.02	0.02
pH	6.07	7.26	7.16	7.16	7.21	6.89	6.78	7.16	7.00
Temp (^{o}C)	14.8	17.8	16.5	15.8	15.0	16.4	16.4	17.7	18.8
Alkalinity (mg/L as $CaCO_3$)	0	840	1048	860	620	560	840	5880	8570
Alkalinity converted to HCO_3^- (moles/L)		0.017	0.021	0.017	0.012	0.011	0.017	0.118	0.171
HCO_3^- from Ca increase (moles/L)		0.003	0.007	0.002	0.000	0.002	0.003	0.027	0.036
HCO_3^- from $SO_4^=$ reduction (moles/L)		0.017	0.016	0.017	0.015	0.009	0.010	0.014	0.013

Discussion

Comparing the reactors in Table 4 reveals that all of the configurations remove Cu and Zn below aquatic criteria levels of 0.01 and 0.1 mg/L respectively (U.S. EPA 2004). All of the reactors remove Fe and add Ca, some to a greater extent than others. The chemical features that separate the chitin reactors from the others are consistent removal of more manganese (as seen in Fig. 1), usually higher concentrations of Ca in effluent (as seen in Fig. 2), and consistently higher alkalinities (as seen in Fig. 3). In the discussion that follows, the high alkalinities can be explained, however, the reason for more manganese removal is not clear.

Alkalinity Generation

When it was noticed that the alkalinities in the chitin reactors were sometimes approaching 10,000 mg $CaCO_3$/L, it was assumed that this was all due to large amounts of calcite dissolving from the crab shell. Indeed, the concentrations of Ca and Mg in the chitin reactors were often much higher than in the other reactors. These data led to the assumption that significantly more $MnCO_3$ was precipitating; a condition that was desired in the pulsed limestone bed reactor experiments completed in 2005 and 2006 (Sibrell et al., 2007a and 2007b). Attempts to balance the produced alkalinity with the bicarbonate formed through treatment reactions proved difficult. The last three rows in Table 4 are an example of an attempt at determining the sources of alkalinity. The following explains what was found.

Initially, the National Tunnel influent has a pH of between 5 and 6 and considerable iron in solution; thus, it has no alkalinity. In the reactors, alkalinity could have been produced from two known sources: calcite dissolution and SO_4^{2-} reduction. Limestone dissolution can be traced by the increase in the concentration of Ca. For each mole of Ca dissolved, one mole of HCO_3^- is released. This gives the bicarbonate concentration from the calcium concentration increase in the second of the bottom three rows. The following reaction reasonably describes SO_4^{2-} reduction (where CH_2O is the organic substrate in the reactor):

$SO_4^{=}$ (aq.) + 2 "CH_2O" → H_2S (aq.) + 2 HCO_3^- (aq.)

This reaction implies that for each mole of SO_4^{2-} reduced, 2 moles of bicarbonate are produced. Using this relationship, the SO_4^{2-} concentration decrease from the influent to the reactor effluent can be converted into bicarbonate alkalinity produced. This gives the bicarbonate concentration from SO_4^{2-} reduction in the bottom row of Table 4. Finally, the alkalinity measured in each reactor effluent is converted into bicarbonate concentration using the relation that each mole of $CaCO_3$ alkalinity is equivalent to 2 moles of bicarbonate alkalinity (Stumm and Morgan, 1996). This provides the bicarbonate concentration from alkalinity in the first of the bottom three rows.

For the first six reactors, calculation of the generated bicarbonate compares reasonably well with the bicarbonate found assuming only bicarbonate alkalinity. It is interesting to note that for these reactors, the majority of the bicarbonate comes from sulfate reduction. For the two chitin reactors, there is approximately three times more alkalinity than can be accounted by bicarbonate production; even though in these reactors, alkalinity from calcium dissolution is higher than that from sulfate reduction. Another possible explanation for the additional alkalinity could be NH_4 formation from protein degradation.

To verify this assumption, an NH_4 analysis was performed on samples from the effluent of the chitin reactors taken on November 20, 2007. The results are summarized in Table 5. The two balances are within 2 to 16 % of the total alkalinity, and the inclusion of NH_4 as part of the alkalinity balance does account for a significant amount of the total alkalinity in the reactor effluent.

Table 5. An alkalinity balance for the chitin reactors from the November 20, 2007, sampling.

Row No.	**Constituents**	**Chitin Reactor 7**	**Chitin Reactor 8**
1	Alkalinity (mg $CaCO_3$/L)	3340	2340
2	Alkalinity (moles of base / L)	0.0668	0.0468
3	Ammonia as nitrogen (mg/L)	450	220
4	Alkalinity from ammonia (moles/L)	0.0321	0.0157
5	Alkalinity from the increase in Ca (moles/L)	0.0177	0.0137
6	Alkalinity from sulfate reduction (moles/L)	0.0059	0.0165
7	Sum of alkalinities (moles/L) (Rows 4 + 5 +6)	0.0557	0.0459
8	Diff. btwn. measured and calculated alkalinities (Rows 2- 7)	0.0111	0.0009

Hence it is highly likely that the production of NH_4 in the chitin reactors may have been the cause of the increased alkalinity. However, this does not provide an answer to the higher removal of Mn, and the release of high concentrations of NH_4 into the watershed could be detrimental to aquatic organisms. The issue concerning the generation of excess NH_4 from bioreactors could also be a concern with those systems that use fish bone apatite (Ross et al., 2006) or soybean products (Lindow and Borden, 2004) as a substrate. Also, EPA ORD is addressing this issue by studying the use of aerobic polishing cells prior to discharge.

Manganese Removal

As seen in Fig. 1, the two chitin reactors indeed remove Mn to lower concentrations than do the other reactors. In SRBRs, Mn is in the +2 oxidation state, and the most insoluble form of Mn(II) in circum-neutral waters is $MnCO_3$ (Stumm and Morgan, 1996). It could also be argued that Mn is being adsorbed to the substrate. Machemer and Wildeman (1990), showed that in a passive treatment system that used a mushroom compost substrate, Mn will be adsorbed within the first six weeks, and that after that it can be released and Fe adsorbed. This was seen in the chitin reactors. Manganese concentrations in the effluents on May 23, 2007, one week after the chitin reactors were started, were 0.012 and 2.6 mg/L (data not shown). Then, as seen in Fig. 1, Mn concentrations in the effluents jumped to above 10 mg/L for about eight weeks before they gradually dropped to below 10 mg/L.

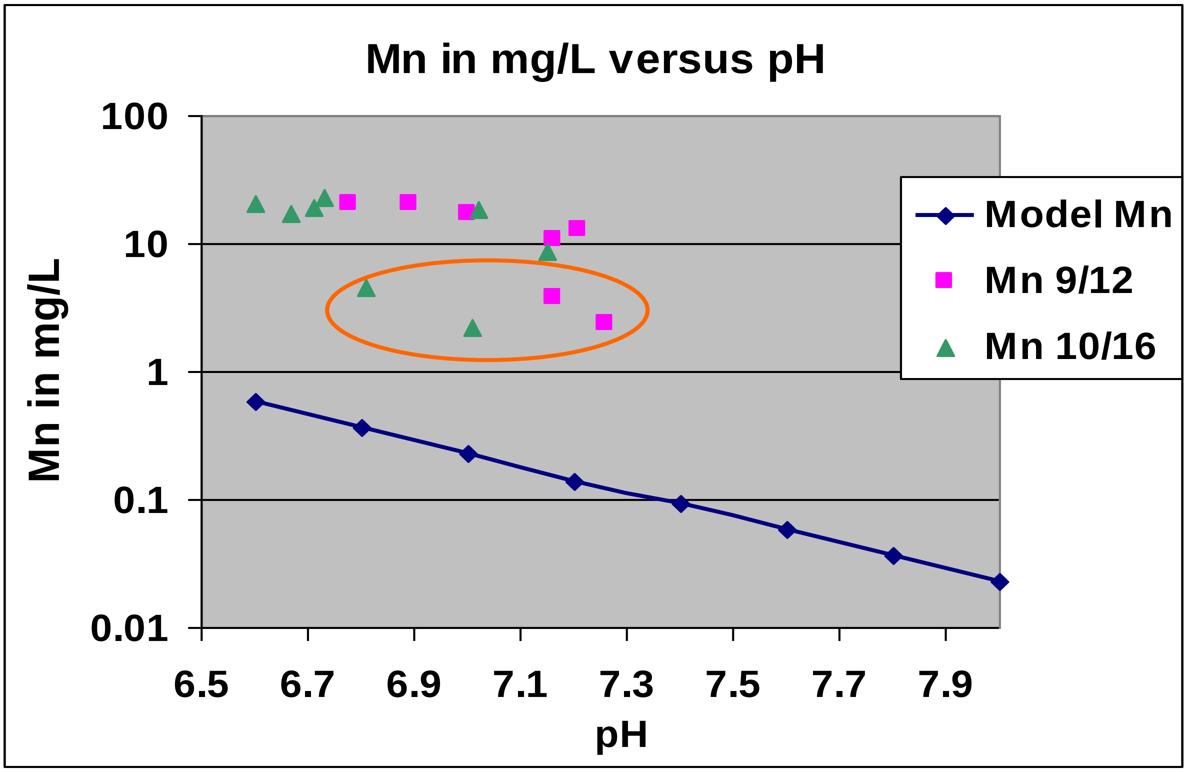

Figure 4. A graph of modeled and measured Mn versus pH for the reactors on September 12 and October 16, 2007. Mn(II) in mg/L is plotted on a logarithmic scale; a constant concentration of HCO_3^- of 0.020 moles /L was assumed. The circled points indicate the two chitin reactors.

Figure 4 is a plot of $Mn^{(II)}$ versus pH using equilibrium constant values from Stumm and Morgan (1996). To create this graph, a constant concentration of 0.02 moles/L was assumed for HCO_3^-. The model line assumes precipitation as a carbonate, and values for all eight reactors on September 12 and October 16 are also plotted. The graph highlights two interesting facts. First, the concentrations of Mn in all of the reactors are higher than the model concentration of Mn, which assumes that $MnCO_3$ is being precipitated. Second, the concentrations of Mn in the two chitin reactors are lower and closer to the model concentrations. This implies that with respect to this theoretical model of rhodochrosite precipitation, not only the two chitin reactors were supersaturated with respect to $MnCO_3$. Instead it appears that all the reactors were supersaturated with respect to $MnCO_3$ and by comparison, the chitin reactors become only slightly supersaturated before $MnCO_3$ was precipitated.

The fact that the model shows that Mn is supersaturated with respect to $MnCO_3$ in these reactors is not surprising. The calculations made to generate the model line in Fig. 4 assumed dilute solutions and the concentration of total ions is somewhere between 0.01 and 0.05 moles/L. The high concentration of total ions in these reactors would increase the concentration of slightly soluble ions (Stumm and Morgan, 1996). Also, the calculation used to generate the model assumed that no Mn complexes were formed, however, $Mn^{(II)}$ does form a neutral carbonate complex (Stumm and Morgan, 1996). These two factors are true for all eight reactors and so they do not explain the lower Mn concentrations in the chitin reactors. In Mn removal experiments reported by Sibrell et al. (2007b) on mine water from Doe Run, Mn was removed to a concentration of 0.06 mg/L at a pH of 7.7, and this is close to the model concentration line developed here.

To summarize, the following possibilities for the low concentrations of Mn in the chitin reactors can be eliminated:

- The high alkalinities are mostly due to ammonia and not bicarbonate, so this would not enhance removal.
- High concentrations of NH_4 in the chitin reactors would favor making soluble NH_4 complexes, which would render metals more soluble.
- With respect to model solubility calculations, all the reactors are supersaturated with respect to $MnCO_3$, so this does not appear to be a reason for better Mn removal in the chitin reactors.

- Adsorption does not appear to be a factor because, for the chitin reactors, the concentration pattern expected for adsorption was only seen in the first weeks of reactor operation.

Despite these results, the removal mechanisms for Mn in the chitin reactors remain unclear to the authors. In previous studies, this behavior has been noted in limestone bed reactors where Mn has been removed at pHs below 7 even though from a kinetic point of view this shouldn't occur (Wildeman and Gusek, 2005). As the authors have continued to look at Mn removal mechanisms in these systems, they have recognized that there is an interaction that occurs that is contrary to existing scientific knowledge. Determining just what is occurring is important because with respect to Mn, for coal mine discharges, the concentration limit is 2.0 mg/L; for the drinking water, the concentration limit is 0.3 mg/L, and typical aquatic limits are around 1.0 mg/L. Because Mn removal is essential in both metal mining and coal mining influenced waters, understanding the science of Mn aquatic chemistry is essential for passive treatment to consistently meet current water quality standards and criteria.

Conclusions

Although these chitin reactors have only been operating for less than six months, they have exhibited unexpected treatment results. These special properties in the effluent include:

- Better removal of Mn than other sulfate-reducing bioreactors,
- Higher dissolution of Ca from shell-bound calcite,
- Compared with other tested SRBRs, the release of NH_4 from the degradation of protein creates higher levels of alkalinity.

The mode and mechanism for the higher removal of Mn remain unclear.

References

Brennan, R. A., Sanford, R. A., and Werth, C. J. 2006. Biodegradation of tetrachloroethene by chitin fermentation products in a continuous flow column system. Journal of Environmental Engineering, 132 (6): 664 - 673. http://dx.doi.org/10.1061/(ASCE)0733-9372(2006)132:6(664).

Daubert, L. N., and Brennan, R. A. 2007. Passive remediation of acid mine drainage using crab shell chitin. Environmental Engineering Science, 24 (10): 1353 - 1358. http://dx.doi.org/10.1089/ees.2006.0199.

Buccambuso, E., A. Ruhs, L. Figueroa, J. J. Gusek, T. Wildeman, M. Holmes, and D. Reisman. 2007, Ethanol-fed or solid-phase organic sulfate reducing bioreactors for the National Tunnel drainage, Clear Creek/Central City Superfund Site? Proceedings America Society of Mining and Reclamation, 2007 pp 95-105. http://dx.doi.org/10.21000/JASMR07010095.

Figueroa, L., A. Miller, M. Zaluski, and D. Bless. 2007, Evaluation of a two-stage passive treatment approach for mining influenced waters, Proceedings America Society of Mining and Reclamation, 2007 pp 238-247. http://dx.doi.org/10.21000/JASMR07010238.

Hageman, P. L., K. S. Smith, T. R. Wildeman, and J. F. Ranville. 2005, Comparison of mine waste assessment methods at the Rattler mine site, Virginia Canyon, Colorado, Proceedings America Society of Mining and Reclamation, 2005 pp 470-486. http://dx.doi.org/10.21000/JASMR05010470

Lindow, N. L., and R. C. Borden. 2004, Anaerobic bioremediation of acid mine drainage using EOS, Proceedings America Society of Mining and Reclamation, 2004 pp 1192-1204 http://dx.doi.org/10.21000/JASMR04011192

Machemer, S. D and Wildeman, T. R. 1992. Organic complexation compared with sulfide precipitation as metal removal process from acid mine drainage in a constructed wetland". Jour. Contaminant Hydrology, v 9, pp 115-131. http://dx.doi.org/10.1016/0169-7722(92)90054-I.

Martin, J. P., Sorenson, K. S. Jr., Brennan, R. A., Sanford, R. A., Werth, C .J., Bures, G. H., Guest, G., and Fulton, R. 2004. Full-scale application of chitin for bioremediation of chlorinated solvents. Proceedings of the Remediation of Chlorinated and Recalcitrant Compounds: The Fourth International Conference, Monterey, CA.

Robinson Lora, M. A., and Brennan, R. A. 2007. Evaluation of chitin as an electron donor source for biological nitrate reduction: column studies. Proceedings of the In Situ and On-Site Bioremediation 9th International Symposium, Baltimore, MD, May 8.

Ross, C. S., R. B. Verburg, B. Stasney and L. McCloskey. 2006, Effective passive treatment of mine effluent using a phosphate reactive medium, 7th International Conference on Acid Rock Drainage, 2006 pp 1728-1752. http://dx.doi.org/10.21000/jasmr06021728.

Sibrell, P. L., M. A. Chambers, A. L. Deaguero, T. R. Wildeman, and D. J. Reisman. 2007a. An innovative carbonate co-precipitation process for the removal of zinc and manganese from mining impacted waters, Env. Eng. Sci.24, 881-895. http://dx.doi.org/10.1089/ees.2006.0126.

Sibrell, P. L., T. R. Wildeman, M. Deaton, and D. J. Reisman. 2007b, Treatment of metal-mine effluents by limestone neutralization and calcite co-precipitation, Proceedings America Society of Mining and Reclamation, 2007 pp 729-744 http://dx.doi.org/10.21000/JASMR07010729

Stumm, W., and J. J. Morgan. 1996. Aquatic Chemistry, 3rd Ed. Wiley-Interscience, New York, New York, 1022 pp..

U.S. EPA (U. S. Environmental Protection Agency). 2004. National Recommended Water Quality Criteria. United States Environmental Protection Agency. Office of Water. (4304T) Available at http://www.epa.gov/waterscience/criteria/wqcriteria.html

Wildeman, T. R., and J. Gusek. 2005. Mining influenced waters: their chemistry and treatment. U.S. EPA Acid Mine Drainage Workshop, Denver, CO, May 2005.