

Short Communication

Passive Remediation of Acid Mine Drainage
Using Crab Shell Chitin

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ABSTRACT

Streams contaminated by acid mine drainage (AMD) are complex environmental systems which require biological, chemical, and physical treatment steps for thorough remediation. In this work, a novel concept for treating AMD was investigated, in which the biological reduction of acidity, chemical enhancement of alkalinity, and physical sorption of metals occurred simultaneously using one multifunctional substrate: chitin from crab shells. Sacrificial microcosm tests were conducted to evaluate the ability of chitinous material from crab shells to reduce acidity and dissolved metals concentrations in AMD water collected from Kittanning Run in Altoona, Pennsylvania. In the presence of SC-20 grade crab shell chitin, pH increased from 3.21 to 6.79, acidity decreased from 192 to -114 mg/L, and alkalinity increased from 0 to 235 mg/L, in just 9 days. Corresponding to this increase in pH, dissolved iron and aluminum concentrations were reduced by more than 99% and manganese concentrations were reduced by 81%. In addition, sulfate concentrations were observed to decrease from 489 to 303 mg/L, confirming the activity of sulfate reducing bacteria. Physical adsorption of iron to chitin, chemical precipitation of aluminum hydroxide (Al(OH)₃), and biologically induced manganese sulfide (MnS) precipitation were the likely mechanisms of dissolved metals removal in this system. The results of this work demonstrate for the first time the effectiveness of chitin as an alternative substrate for AMD treatment.

Key words: acid mine drainage; abandoned mine drainage; AMD; passive treatment; chitin; crab shell; bioremediation; mining waste

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INTRODUCTION

ACIDIC, METAL-LADEN DRAINAGE from abandoned coal mines throughout the world can pose a serious threat to humans, wildlife, and exposed structures. The excessive cost to remediate surface waters impacted by acid mine drainage (AMD) has spurred interest in passive anaerobic treatment technologies, such as vertical flow wetlands and permeable reactive barriers. These systems simultaneously increase alkalinity and remove metals by supporting the growth of sulfate reducing bacteria, which require an organic substrate to survive. Unfortunately, the slow-release, fermentable substrates most suitable for use in these systems are typically deficient in nitrogen, which limits the activity of sulfate-reducing bacteria (Waybrant *et al.*, 2002). Consequently, passive treatment systems are often overdesigned to compensate for low sulfate reduction rates. A nitrogen-containing substrate, such as chitin, is required to increase the activity of sulfate reducing bacteria, and thereby decrease the size and cost of passive AMD treatment systems.

Chitin, which is derived from the shells of crustaceans such as crab and shrimp, is the second most abundant biopolymer on earth after cellulose (Beaney *et al.*, 2005), with several gigatons being produced annually (Howard *et al.*, 2003). The chemical formula of chitin is $C_8H_{13}NO_5$ (containing 6–7% nitrogen), giving this material a nearly ideal carbon-to-nitrogen ratio for bacterial growth (Harkness *et al.*, 2003) and making it an attractive slow-release substrate for treating contaminated water systems where nitrogen may be limiting. In addition to releasing nitrogen, chitin has been shown to produce hydrogen, acetate, and a variety of other fatty acids during its fermentation (Vera *et al.*, 2001), confirming that it is a viable substrate for sulfate-reducing bacteria, the key organisms in biological AMD treatment. Chitin is also an excellent physical sorbent, especially at low pH, and has been shown to remove metals like aluminum, arsenic, chromium, copper, iron, manganese, nickel, and zinc from aqueous solutions (Hawke *et al.*, 1991; McAfee *et al.*, 2001; Franco *et al.*, 2004; Vijayaraghavan *et al.*, 2005). Furthermore, crab shell chitin retains its permeability during degradation (Brennan, 2003), which is an essential attribute of substrates used in passive reactive barriers.

Since chitin is an abundant waste product of the shellfish industry, availability is not limited and costs are low: the least refined grade of crab shell chitin, SC-20 (~20% pure chitin), retails for approximately \$1 per pound (JRW Bioremediation, LLC, Lexena, KS). An advantage of SC-20 is that it contains approximately 40% calcium carbonate ($CaCO_3$) by weight, which can serve as a buffer and increase the alkalinity of treated waters. In contrast, spent mushroom compost, which is currently the most

commonly used substrate for passive AMD treatment systems, retails for approximately \$50 per ton (Dietz, 2006, personal communication); however, spent mushroom compost is not a significant source of nitrogen or calcium carbonate and has limited longevity, which may outweigh the initial cost savings benefits it offers. The purpose of this preliminary investigation was to evaluate the ability of crab shell chitin to serve as an alternative substrate for AMD remediation.

EXPERIMENTAL PROTOCOLS

AMD water was collected from Kittanning Run in Altoona, Pennsylvania, approximately 2.7 miles downstream of the nearest coal mine, by slowly submerging polyethylene jugs into the stream while avoiding aeration and capping with minimal headspace. Soil samples were collected in nonsterile 50-mL centrifuge tubes from a nearby marsh. After collection, both water and soil samples were refrigerated at 4°C. The following day, the water was degassed with nitrogen in the collection vessel for approximately 90 min to ensure low dissolved oxygen (DO) conditions (~0.40 mg/L final DO). During this time, 0.5-g soil and 0.25-g SC-20 grade crab shell chitin (equivalent to ~0.05-g pure chitin and ~0.1-g $CaCO_3$) were added to 20 replicate 160-mL glass serum bottles, and the headspace degassed with nitrogen for 10 min to remove oxygen. Control bottles without chitin were also established. The degassed AMD water was then transferred to each bottle anaerobically in 100-mL aliquots, and the bottles were sealed with butyl rubber stoppers and aluminum crimp tops. The bottles were shaken by hand to mix the sediment and chitin, and then incubated in the dark at room temperature until analysis. Approximately 24 h before each sampling point, the bottles to be sacrificed were shaken again by hand in an effort to homogeneously mix the dissolved components. Duplicate bottles were sacrificed daily until changes in pH and acidity stabilized.

Acidity, alkalinity, and pH measurements were conducted immediately when the bottles were sacrificed, and the remaining sample water was pipetted from the bottle and frozen at -20°C for later anion and metal analysis. Alkalinity and hot acidity were determined by titration as described in Standard Methods (Clesceri *et al.*, 1998). An Accumet basic AB15 pH meter coupled with an Orion 915600 pH electrode was used for all measurements. Before anion and metals analyses were conducted, frozen water samples were thawed and filtered (0.45 μ m). Chloride, nitrate, and sulfate concentrations were measured at room temperature using a DX-100 ion chromatograph (IC) with a Dionex IonPac 4 AS4A SC column and a car-

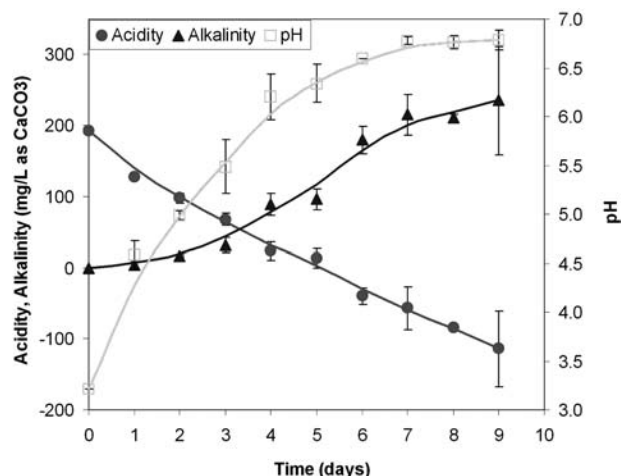


Figure 1. Acidity, alkalinity, and pH in AMD microcosms treated with chitin over time. Data points are averages of duplicate microcosms; error bars represent one standard deviation; lines are running averages.

bonate–bicarbonate elluent. Metal analyses were conducted by the Penn State University Materials Characterization Laboratory, using a Leeman Labs PS3000UV inductively coupled plasma emission spectrophotometer (ICP).

RESULTS

Over the course of 9 days, the pH in microcosms containing chitin logarithmically approached neutral pH from an initial value of 3.21 to a final average value of 6.79 (Fig. 1). Control bottles without chitin maintained a relatively steady acidic pH, with an average value of 3.3 (Table 1). As expected, with this pH increase, acidity de-

creased rapidly and linearly while alkalinity conversely increased (Fig. 1 and Table 1). Of the 1,000 mg/L CaCO_3 that was theoretically available (~ 0.1 -g crab shell CaCO_3 was added to each 100-mL of AMD water) less than 35% was consumed in neutralizing the acidity and adding to the alkalinity in this experiment. In controls without chitin, acidity remained at an average of 158.3 mg/L as CaCO_3 (Table 1). Since alkalinity is immeasurable below pH 4.5, the alkalinity in the controls remained effectively zero for the duration of the experiment.

As pH increased, dissolved iron, aluminum, and manganese concentrations in microcosms treated with chitin decreased (Fig. 2). Dissolved metals dropped to less than 0.03% of starting concentrations (below detectable limits, 0.05 mg/L) for iron and aluminum, and dropped to 19% of the starting concentration for manganese (Table 1). Control bottles that did not contain chitin demonstrated little or no change in dissolved aluminum and manganese concentrations (Table 1). Dissolved iron did decrease by about 75% in the controls, but was still approximately 5 mg/L more concentrated than the active, chitin-containing samples.

Chloride ion concentrations peaked after 1 day of treatment with chitin and remained approximately four times higher in bottles containing chitin than in the controls (Table 1), indicating the rapid dissolution of a chloride-bearing salt from the surface of the crab shell. Nitrate reduction occurred rapidly and linearly within the first 5 days of treatment with chitin, whereas significant sulfate reduction did not begin until after day 7 (Fig. 3). Sulfate reducing bacteria usually require several days to become active in anoxic systems, so this lag is not unexpected. During the course of treatment, sulfate concentrations were reduced from 489 to 303 mg/L (37%); however, this occurred only over the last 2 days of the experiment. If the experiment had been allowed to continue, greater sul-

Table 1. Chemistry of raw AMD water sampled from Kittanning Run in Altoona, Pennsylvania and that of batch Microcosms showing the effectiveness of chitin treatment.

	Raw water (t = 0)	Control, no chitin (t = 9 days)	Chitin (t = 9 days)
pH	3.2	3.3	6.8
Hot acidity (mg/L as CaCO_3)	192.3	164.4	114.1
Alkalinity (mg/L as CaCO_3)	0.0	0.0	235.1
Aluminum (mg/L)	14.0	16.0	<0.05 ^a
Iron (mg/L)	21.0	5.2	<0.05 ^a
Manganese (mg/L)	12.0	12.5	2.3
Chloride (mg/L)	5.17	6.21	23.9
Nitrate (mg/L)	1.58	0.80	0.66
Sulfate (mg/L)	489.3	471.1	303.2

Values shown are duplicate averages; ^abelow detection limit.

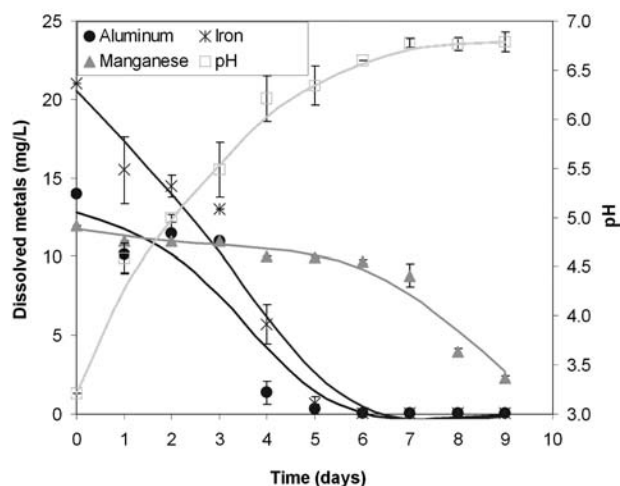


Figure 2. Dissolved metals concentrations and pH in AMD microcosms treated with chitin over time. Data points are averages of duplicate microcosms; error bars represent one standard deviation; lines are running averages.

fate reduction may have been observed since the mass of pure chitin (500 mg/L) added to the microcosms was theoretically enough to reduce 237 mg/L of sulfate (i.e., 0.5-g sulfate reduced per gram of pure chitin) according to the following reaction adapted from Luptakova and Kusnierova (2005):

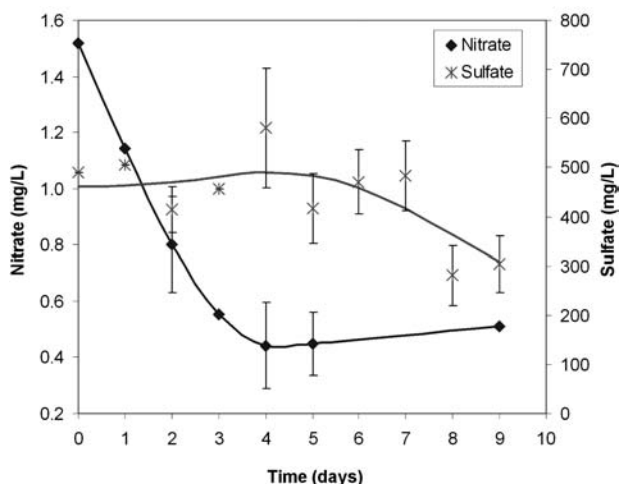
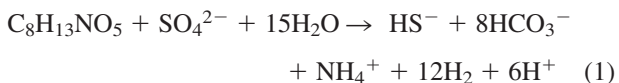


Figure 3. Reduction in nitrate and sulfate concentrations in AMD microcosms treated with chitin over time. Data points are averages of duplicate microcosms; error bars represent one standard deviation; lines are running averages.

DISCUSSION

The changes in AMD water conditions during the course of treatment with chitin were likely caused by a combination of chemical, physical, and biological activity. It seems probable that the initial increase in pH and alkalinity was caused by the rapid dissolution of calcium carbonate from the crab shells reacting with acid, as has been noted by others (Lee *et al.*, 2004). Sulfate reducing bacteria likely also contributed to alkalinity through the formation of bicarbonate (HCO_3^-) (Luptakova and Kusnierova, 2005) when they began to reduce sulfate to hydrogen sulfide after 7 days. These combined chemical and biological reactions worked together to increase the buffering capacity of the water in this experiment.

Metals removal in this system could be due to a number of mechanisms, but is most likely due to a combination of physical sorption at low pH early in the experiment followed by precipitation at higher pH later in the experiment. Dissolved iron concentrations were reduced immediately upon the addition of chitin prior to the reduction of sulfate, indicating that iron may have physically adsorbed onto chitin. It has been previously documented that chitin has a high affinity for iron, with maximum sorption observed at pH 4.0 (Franco *et al.*, 2004). Although some minor sorption of aluminum to chitin may have occurred within the first 3 days of the experiment, aluminum concentrations dropped quickly

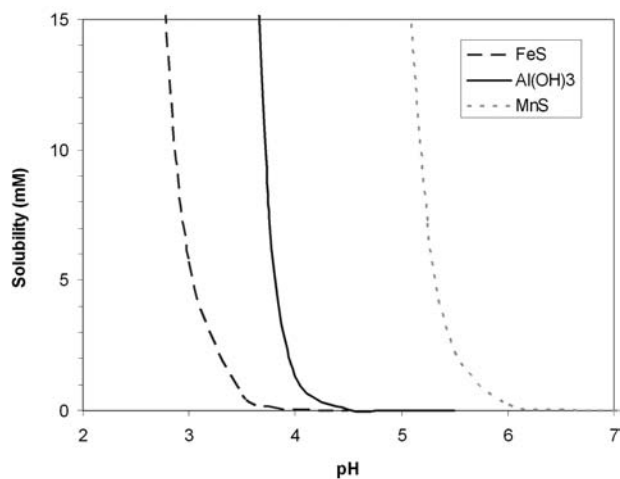


Figure 4. Solubilities of FeS, MnS, and $\text{Al}(\text{OH})_3$ as a function of pH based on their solubility product constants (K_{sp}) at 25°C: $6.3 \times 10^{-18} \text{ M}^2$ for FeS; $1.3 \times 10^{-33} \text{ M}^4$ for $\text{Al}(\text{OH})_3$; and $2.5 \times 10^{-13} \text{ M}^2$ for MnS (raw data from McQuarrie and Rock, 1984). Note that the majority of iron is believed to have sorbed to the chitin in this experiment rather than precipitated as FeS; however, FeS is shown here for completeness.

on the fourth day of the experiment as the system approached $\text{pH} = 6$, indicating that aluminum may have precipitated out of solution as aluminum hydroxide $[\text{Al}(\text{OH})_3]$ (Sullivan *et al.*, 1988). Manganese, however, was not significantly reduced until sulfate reduction began after 7 days, indicating that manganese was likely precipitated as manganese sulfide (MnS) (Sullivan *et al.*, 1988). Based on their solubility product constants (K_{sp}) (McQuarrie and Rock, 1984), it is expected that with increasing pH, $\text{Al}(\text{OH})_3$ would precipitate out of solution first followed by MnS (Fig. 4). This ordering is consistent with the observations made in this experiment as the pH increased from 3.2 to 6.8 due to treatment with crab shell chitin (Fig. 2). The mechanisms of metals removal from a chitin-supported environment are complex, however, and should be examined further to improve the design of AMD treatment systems.

SUMMARY

In AMD microcosms treated with chitin, the following changes were observed over the course of 9 days:

- pH increased from 3.21 to 6.79.
- Acidity decreased from 192 to -114 mg/L
- Alkalinity increased from 0 to 235 mg/L.
- Sorption of iron to chitin, and precipitation of aluminum and manganese were the likely mechanisms of dissolved metals removal in this system. Dissolved iron and aluminum concentrations were reduced by more than 99% (from 21 mg/L and 14 mg/L, respectively, to less than 0.05 mg/L), and dissolved manganese was reduced by 81% (from 12 to 2.3 mg/L).
- The activity of sulfate reducing bacteria was indicated by a 37% decrease in sulfate concentrations (from 489 to 303 mg/L); however, greater sulfate reduction may have been observed with longer experimental time.

The results of this preliminary investigation provide a "proof of concept" for AMD treatment using chitin. Chitin shows promise as an alternative substrate for AMD remediation due to its multifaceted treatment efficiency, availability, and low cost. Additional tests should be performed to determine the longevity of chitin so that the efficiency and cost of AMD treatment can be estimated. The effectiveness of chitin for treating AMD water with different acidity and metals concentrations should also be evaluated at both microcosm and column scales before proceeding to field demonstration.

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