EVALUATING CRAB-SHELL CHITIN, LACTATE, AND SPENT MUSHROOM COMPOST FOR ACID MINE DRAINAGE REMEDIATION IN CENTRAL PENNSYLVANIA[1]

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Abstract: Acid mine drainage (AMD) is a major environmental consequence of mining activity. This is a recurring problem around the world, and especially in some specific areas of United States, including Pennsylvania. Remediation of AMD has three requirements: pH neutralization, reduction of sulfate concentrations, and removal of dissolved metals. The aim of this study was to evaluate the sustainable waste material, crab-shell-chitin, as a multifunctional substrate for AMD remediation. The performance of chitin was evaluated under different raw water characteristics (comparative sites test), and compared with lactate and spent mushroom compost (comparative substrates test).

Sacrificial, duplicate microcosms were prepared in serum bottles containing AMD water, sediment (microbial source), and the evaluated substrate. For the comparative sites test, three microcosm sets were prepared using AMD water from three different sites within central Pennsylvania, and all were treated with crab-shell-chitin. For the comparative substrates test, the AMD source was held constant and the substrate varied between crab-shell-chitin, sodium lactate, and spent mushroom compost. Microcosms were incubated in the dark, at room temperature, and under anoxic conditions for up to 50 days.

Crab-shell-chitin (ChitoRem™ SC-20) increased the pH from pH 3.0 – 3.5 to near neutral in 2 – 3 days. Increases in pH were much faster in the microcosms containing chitin than with the other substrates. In microcosms containing chitin, steady alkalinity generation and acidity removal were observed at average rates of 35 and -27 mg $CaCO_3$/L-d, respectively. The activity of SO_4^{2-} reducing bacteria was evident after 7 – 9 days of incubation, with reduction rates of 11.9 – 17.8 mg SO_4^{2-}/L-d. Similar changes in alkalinity, acidity, and SO_4^{2-} were also observed in lactate-containing microcosms, but they only started after 27 days. No alkalinity generation or sulfate reduction activity was observed in bottles containing spent mushroom compost. Aluminum and Fe removal was observed with all substrates, but it was much faster with chitin. Chitin was the only substrate able to partially remove manganese (>73%).

The results of this study indicate that crab-shell-chitin is a promising material to be used as a substrate or amendment for AMD remediation. Its slowly fermentable nature makes it a suitable electron donor source to support the activity of sulfate reducing bacteria. In addition, the rapid release of its built-in carbonate minerals effectively neutralize acidic waters and facilitate the precipitation of dissolved metals.

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Introduction

One of the most serious environmental concerns of mining activity is the production of acid mine drainage (AMD). This is a recurring problem around the world, especially in some specific areas of United States, including Pennsylvania (PADEP, 2000). AMD occurs when pyritic rocks and other sulfide minerals are exposed to the air and water. Atmospheric oxygen rapidly oxidizes pyrite releasing large amounts of H_2SO_4 and Fe^{3+}, which precipitates as $Fe(OH)_3$ or "yellow boy" (Equation 1). The high acidity of AMD-affected waters can also promote the dissolution of metals such as Zn, Cu, Cd, As, Ni, Pb, etc., from other minerals (Cocos et al., 2002, Gibert et al., 2003). The resulting acidic and metal-contaminated streams can adversely impact the environment.

$$4FeS_{2\,(s)} + 15O_{2\,(aq)} + 14H_2O_{\,(l)} \rightarrow 4Fe(OH)_{3\,(s)}\downarrow + 8SO_4^{2-}{}_{(aq)} + 16H^{+}{}_{(aq)} \quad \textbf{(1)}$$

Remediation of AMD requires three different processes: the addition of a neutralizing agent, the reduction of SO_4^{2-} concentrations, and the removal of dissolved metals. Traditional AMD treatment involves *ex-situ* chemical processes by the addition of an alkaline agent and/or sulfide to promote the precipitation of the metals as hydroxides, carbonates, or sulfides. Due to the very high cost of this type of treatment, alternative, *in-situ* and low-tech treatments have been investigated. They include the use of limestone channels and drains, aerobic wetlands, anaerobic vertical flow wetlands, and permeable reactive barriers (Johnson and Hallberg, 2005; Gibert et al., 2002). Anaerobic processes offer the advantage of allowing the three necessary processes for AMD remediation to occur almost simultaneously. They rely on the activity of sulfate-reducing bacteria (SRB), which consume an organic substrate to reduce sulfate to sulfide, and thereby facilitate the precipitation of metals as metallic sulfides (Equations 2 and 3):

$$CH_2O_{(s)} + SO_4^{2-}{}_{(aq)} + 6H^{+}{}_{(aq)} \rightarrow H_2S_{(aq)} + CO_{2\,(aq)} + 3H_2O \quad \textbf{(2)}$$

$$Me^{2+}{}_{(aq)} + H_2S_{(aq)} \rightarrow MeS_{(s)}\downarrow + 2H^{+}{}_{(aq)} \quad \textbf{(3)}$$

where CH_2O represents an organic substrate, and Me represent a divalent metal. The selection of this organic substrate has important impacts on both operational costs and the overall biological performance (Gibert et al., 2002). Numerous fermentable waste materials have been used as substrates with good results, and researchers have noticed that a mixture of various materials often leads to better performance (Neculita et al., 2007; Gibert et al., 2002). However, it has been also reported that low nitrogen availability often limits SO_4^{2-} reduction rates (Waybrant et

al., 1998). A C:N ratio between 10 and 16 has been proposed as optimum for SRB activity (Neculita et al., 2007; Cocos et al., 2002), with a maximum of 45–120 (Gibert et al., 2004). In addition, SRB require an environmental pH between 5 and 8 for efficient metabolism (Willow and Cohen, 2003). Although the SO_4^{2-} reduction reaction consumes some acidity, limestone chips have been added in both laboratory and field studies to fully satisfy this pH requirement (Neculita et al., 2007, Gibert et al., 2002).

An ideal substrate for passive AMD bioremediation will need to possess several characteristics to support SRB activity: buffering capacity, long-lasting provision of electron donor, and provision of required nutrients. Other practical considerations that need to be addressed in the selection of the substrate include the use of a bulky material with stable porosity to prevent major head losses and/or clogging, and slow degradability to ensure acceptable longevity (Johnson and Hallberg, 2005). Chitinous materials meet all of these requirements.

Chitin (a linear polysaccharide of N–acetylglucosamine) is, after cellulose, the second most abundant biopolymer in nature. It is naturally produced by arthropods (insects and crustaceans), mollusks, and fungi. Chitin is mainly extracted from crustacean shells, especially from crab and shrimp shells, which are waste products of the seafood industry. In its natural form, it is also associated with calcium carbonate, which provides strength to the shells (Percot et al., 2003). The chemical formula of chitin ($C_8H_{13}NO_5$) indicates a C:N ratio of 6.9, which makes it a suitable organic substrate for the growth of SRB. Recent studies have shown that the degradation of chitin creates reducing conditions that can promote and sustain anaerobic–reductive processes (Vera et al., 2001; Brennan et al., 2006). Chitin fermentation has been shown to produce volatile fatty acids (VFAs: acetic, butyric, propionic, etc.), some alcohols, and NH_4. These short chain organic compounds can be easily used by SRB as electron donors, while NH_4 is the preferred nitrogen source for bacterial growth (Rittmann and McCarty, 2001). Additionally, the presence of $CaCO_3$ can provide the required buffering capacity for the rapid recovery of alkalinity in acidic waters. The solid nature of chitin makes it easy to be delivered *in situ*. It also offers a suitable support for biofilm development, and at the same time, acts as a food source. In addition, its particle size and non-swelling nature help to maintain porosity and prevent clogging.

It has been shown that chitinous materials can be effectively used for AMD remediation (Daubert and Brennan, 2007), and this is likely due to its ability to simultaneously serve as an

electron donor source (VFAs), nitrogen source (NH_4), and neutralizing agent ($CaCO_3$) to sustain SRB activity. The aim of this study was to further evaluate the characteristics and performance of raw crab-shell-chitin for the remediation of AMD obtained from different sources, and to compare its performance to other leading substrates. Specifically, remediation rates using chitin were quantified under different raw water characteristics from three AMD sites (Comparative Sites Test) and compared with those obtained using spent mushroom compost and sodium lactate as alternative substrates (Comparative Substrate Test).

Materials and Methods

Chemicals

All chemicals used in this study were reagent grade or better. Sodium L-lactate salt ~98% (Sigma-Aldrich) was used as source of lactate. Ultra High Purity nitrogen gas (UHPNG, MG Industries, Malvern, PA) was used to purge samples. ChitoRem™ SC-20 (minimally processed crab shell), derived from Dungeness crab (JRW Bioremediation, LLC, Lenexa, KS), was used as the chitin source. According to the provider, the typical composition of this material is: 20 – 25% chitin, 35 – 50% protein, 25 – 35% mineral matter ($CaCO_3$), and <10% moisture. Before the experiments, the chitin used for Comparative Sites Test was dry- and then wet-sieved (with deionized water) using a 40 mesh sieve to remove fine particles. The remaining chitin was dried overnight at 50°C. No pretreatment was conducted for chitin used for Comparative Substrate Test. Spent mushroom compost was obtained from the Mushroom Test Demonstration Facility at The Pennsylvania State University, University Park. According to the provider, the original components of this composted material are straw-bedded horse manure, switch grass straw, poultry litter, distiller's grain, and gypsum.

AMD Water Sources

AMD-affected waters and associated benthic sediments (microbial source) used in Comparative Sites Test were obtained from three different streams within the Snow Shoe area (Centre County, PA): Beech Creek (BC), North Fork (NF), and Cherry Run (CR, Table 1). Water used for Comparative Substrate Test was collected from Kittanning Run in Altoona, PA and benthic sediments (microbial source) were collected from an adjacent, non-affected marsh. Before use, the water was purged with UHPNG for 2 hours to remove trace oxygen (final DO ≤ 0.5 mg/L). Anoxic water was sampled and analyzed for pH, SO_4^{2-}, and dissolved metals.

Sediments were drained, to remove excess water and to obtain a more solid material, and then measured for water content (Table 1).

Table 1. Sampling locations and initial characteristics of AMD water and sediments used in the microcosm tests.

	Comparative Sites Test			Comparative Substrate Test
	Beech Creek (BC)	North Fork (NF)	Cherry Run (CR)	Kittanning Run
Approximate sampling location (GPS)	41.08598°, -77.86725°	41.051015°, -77.96397°	41.05428°, -77.95562°	40.49781°, -78.47633°
		water		
pH	3.51	3.50	3.25	2.95
Alkalinity (mg/L $CaCO_3$)	0	0	0	0
Acidity (mg/L $CaCO_3$)	68	30	60	153
SO_4^{2-} (mg/L)	393	181	293	690
Al (ppm, MCL = 2)	1.7	2.9	1.6	10
Fe (ppm, MCL = 0.3)	9.3	7.4	1.2	10
Mn (ppm, MCL = 0.05)	5.8	3.5	2.3	15
		Sediment		
Moisture content (%)	27.7	51.0	39.0	31.9*

* Approximate sampling location for sediment: 40.49660°,-78.46230°
MCL: National Secondary Standard for drinking water.

Experimental Setup

Comparative Sites Test. For each sampling site, a set of 40 serum bottles (160 mL capacity, non-sterile) was prepared. All bottles were supplied with 1.0 g of sediment. To half of the bottles, 0.5 g SC-20 pre-washed chitin was added. The bottles with SC-20 chitin were labeled as "actives", and those without chitin were labeled as "controls". The bottles were purged for 10 minutes with UHPNG before and after adding 120 ml anoxic AMD water. The bottles were sealed with Teflon stoppers and aluminum crimp tops, manually shaken to ensure homogeneity, and incubated in the dark at room temperature (20 ± 1°C) for 20 days.

Comparative Substrates Test. For each evaluated substrate, a set of 20 serum bottles (160 mL capacity, non-sterile) was prepared. All bottles were supplied with 0.5 g sediment and 0.25 g of the substrate. An additional set of 14 bottles were prepared with sediment only to serve as controls. The bottles were then purged for 10 minutes with UHPNG before adding 100 ml of anoxic AMD water and then purged for 10 - 12 minutes more. The bottles were sealed with Teflon stoppers and aluminum crimp tops, manually shaken to ensure homogeneity, and incubated in the dark at room temperature (20 ± 1°C) for up to 50 days.

Sampling procedure. During the incubation period, duplicate bottles of each set of controls or actives were sacrificed periodically. Sampling frequency decreased as total incubation time increased. After each bottle was opened, samples were promptly tested for pH (<0.5 h), alkalinity, acidity, and sulfide (<4 h, sulfide was only measured in samples taken from the comparative sites test). Another portion of the sample was filtered (0.20 μm) before being stored for future analyses (4^oC for less than a week for anions and NH_4, and 4^oC with 0.2ml/L conc. HNO_3 for dissolved metals).

Analytical methods

Electrodes were used to measure pH (Accumet® BASIC, AB15 connected to a Thermo-ORION pH probe) and NH_4 concentrations (ISE ORION 9512). Alkalinity and acidity were measured by titrations with 0.02 N H_2SO_4 and NaOH, respectively, according to the procedure described in the Standard Methods (APHA, 2005). The titration end points were 4.5 and 8.3 for alkalinity and acidity, respectively. Concentration of anions was measured using an Ion Chromatograph (IC, Dionex DX-100), the analytical procedure for which is described elsewhere (APHA, 2005). The methylene blue spectrophotometric method (APHA, 2005) was used to measure the concentration of sulfide. Dissolved metal concentrations were measured by inductively coupled plasma emission spectrometry (ICP, Leeman Labs PS3000UV) at the Materials Characterization Laboratory at The Pennsylvania State University. Moisture content was determined gravimetrically, by weight loss after drying at 105°C (APHA, 2005).

The SRB population in the sediment inoculum used for the Comparative Sites Test was estimated following the most probable number method (MPN). Samples were cultured in Modified Baar's Medium, using acetate (not lactate) as a substrate, to ensure the existence of SRB capable of utilizing this compound, which is the main product of chitin degradation.

Sediment samples (0.5 – 1 g) were transferred using aseptic techniques to 50-ml sterile centrifuge tubes, containing 10 ml of sterile-anoxic $MgSO_4$ solution (1 mM). Tubes were put in an orbital shaker for 2 hours to gently homogenize the mixtures. Serial tenfold dilutions were then prepared, using sterile-anoxic $MgSO_4$ solution (1 mM). Freshly prepared Modified Baar's medium (4.5 ml; Atlas, 2005) was dispensed into 13-ml culture tubes while it was still warm to minimize oxygen inclusion. The MPN media tube was inoculated with 0.5 ml of the appropriate dilution. Triplicate serial dilutions were made to obtain a 10^2 – 10^7 dilution of the initial sediment-$MgSO_4$ solution mixture. The headspace of the culture tubes was flushed with UHP nitrogen and the tubes were incubated at 20°C for 30 days. The formation of a black precipitate (ferrous sulfide) was used as the indication of SRB activity.

The geochemical computer program PHREEQC (Parkhurst and Appelo, 1999) was used to estimate the saturation indexes (SI) of several Al, Fe, and Mn phases. To consider the occurrence of sulfide mineral, its concentration in the aqueous phase was calculated based on the deficit of SO_4^{2-} in the system, compared to the initial concentration.

Results

Comparative Sites Test

The addition of crab-shell-chitin caused a very rapid increase in pH in all actives bottles (Fig. 1A). After 2 days of incubation, measured pH values were already near to neutrality. Values continued increasing and reached a plateau around pH 7.5 after seven days of incubation. No significant changes were observed in the control bottles, except for the site CR, for which pH increased by only one unit on average. No significant differences were observed among the three evaluated sites ($\alpha = 0.05$).

In active bottles containing chitin, acidity was observed to steadily decrease over time while alkalinity was generated (Fig. 1B and 1C). At each sacrificial event, no significant differences were found in these parameters among the three evaluated sites ($\alpha = 0.05$). Average rates for alkalinity production and acidity removal corresponded to 37.9 and -27.5 mg $CaCO_3$ /L-d, respectively. Parallel to alkalinity, a steady increase in calcium concentration was observed during the first nine days of incubation, when a plateau was reached (data not shown). Average rate of increase in calcium concentration was 34.5 mg/L-d. Concentrations of calcium at the end of the test varied around 217 mg/L.

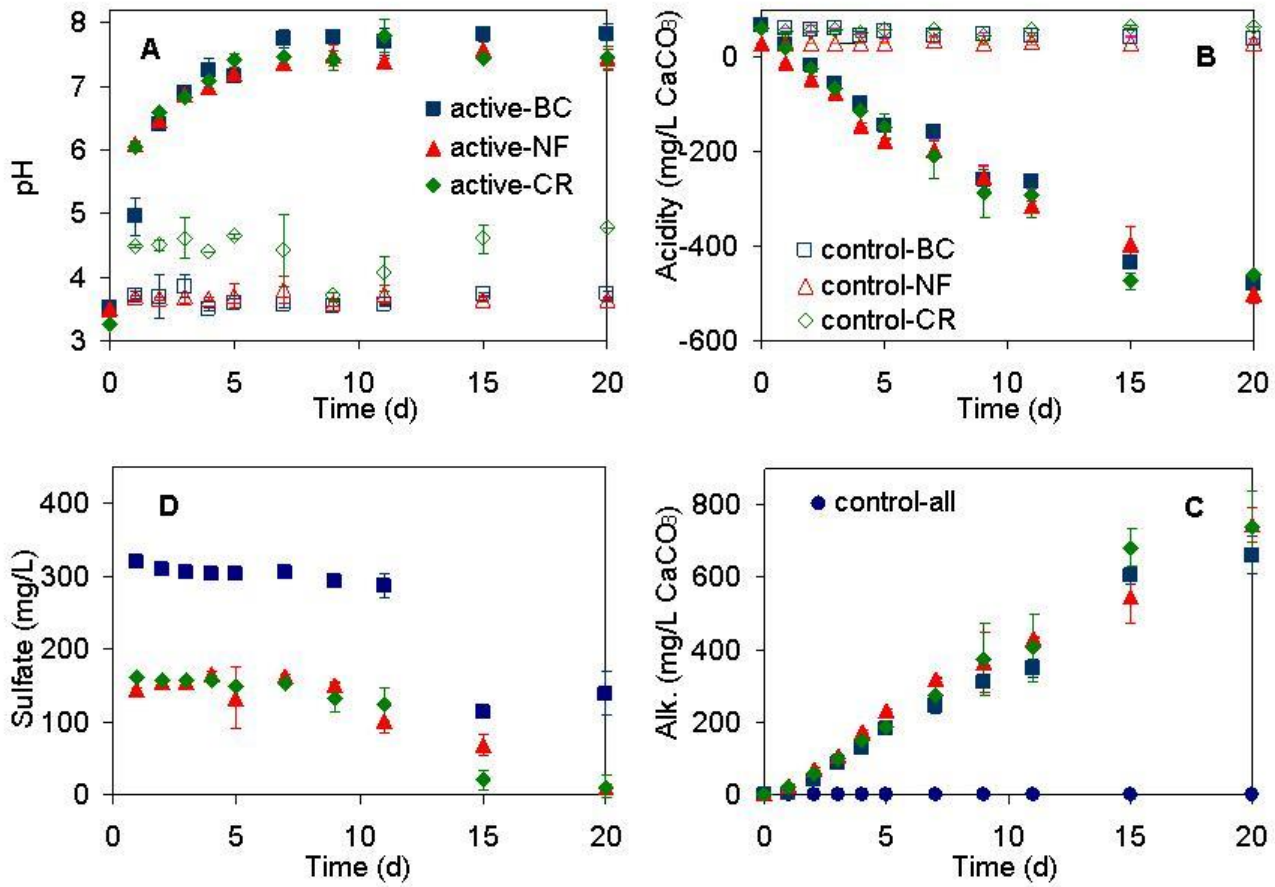

Figure 1. Changes in pH (A), acidity (B), alkalinity (C), and sulfate (D) over time in active (crab-shell chitin; closed symbols) and control (no chitin; open symbols) microcosms containing AMD water and sediment from three different locations in central Pennsylvania (BC = Beech Creek; NF = North Fork; CR = Cherry Run). Data points represent duplicate average measurements; error bars represent 1 standard deviation.

Ammonium accumulation started to be evident after 2 days of incubation in all active bottles, following a very similar pattern for all the evaluated sites. Its concentration steadily increased, reaching values between 48 and 68 mg N/L by the end of the test (data not shown). No NH_4 accumulation was observed in the control bottles.

Based on MPN analysis, an estimate of the initial SRB population in the sediment used as a microbial source was $14x10^3$, $12x10^3$, and $18x10^4$ per gram (on a dry basis) for sites BC, NF, and CR, respectively. The activity of the SRB started to be evident after seven days of incubation (Fig. 1D). This was characterized by a decrease in SO_4^{2-} concentration, an accumulation of sulfides, and the presence of black precipitates. Sulfate was almost completely exhausted in two of the three evaluated sites (>90% in NF and CR), while in the other site (BC) only about 60% of

the initial SO_4^{2-} was removed. Sulfate reduction rates, calculated using data from day 9 to day 20, varied from -11.9 to -16.5 mg SO_4^{2-}/L-d ($p \leq 0.01$). By the end of the test, accumulated sulfide concentrations ranged between 6.5 and 18.5 mg/L (data not shown). No significant changes were observed in the control bottles (data not shown).

Metal (Fe, Mn, Al) analyses were also performed during this microcosm test; however, results did not show a specific removal pattern. It is possible that micro-oxic conditions in the bottles at the beginning of the incubation led to Fe oxidation and precipitation, which may have also promoted the removal of other metals. Particle deposition was observed on the walls of the bottles, supporting this theory.

Comparative Substrates Test

A rapid increase in pH was observed in bottles containing chitin (from pH 3.0 to pH 6.5 in 3 days), compared with the other evaluated substrates (Fig. 2A). The addition of sodium lactate caused an almost immediate increase on pH of about two units; however, pH did not increase any further until after 27 days of incubation, reaching a maximum of pH 6.6 by the end of the test. Changes in bottles containing compost were much slower, reaching a maximum pH of 6.2 after 37 days, while no changes were observed in the control bottles.

Alkalinity generation and acidity removal was observed in the active bottles containing chitin and lactate. However, while these changes were almost immediate in the chitin bottles, changes in lactate bottles were only evident after 27 days of incubation (Fig. 2B). The rates of alkalinity generation for these two substrates were comparable, but the rate of acidity removal with lactate was about 50% of that obtained with chitin (Table 2). No alkalinity generation or acidity removal was observed in bottles containing spent mushroom compost or in the controls.

The addition chitin and spent mushroom compost caused a gradual increase in Ca concentrations (data not shown). In chitin microcosms, calcium concentrations reached a plateau around 235 mg/L after 15 days of incubation, while the maximum concentration of Ca in microcosms containing spent mushroom compost was 156 mg/l after 27 days. Rates of increase in calcium concentration corresponded to 10.6 and 2.4 mg/L-d, for chitin and spent mushroom compost, respectively.

Comparable rates of biological sulfate reduction were observed in bottles containing chitin and lactate (Fig. 2C and Table 2). However, the extent of the lag period was much shorter with chitin (9 d) than with lactate (27 d). Sulfate was completely removed in microcosms containing

chitin after 37 days of incubation. At the end of the 50 days of incubation, an average of 97 mg SO_4^{2-}/L was still detected in lactate-containing microcosms. No SO_4^{2-} reduction activity was observed in bottles containing spent mushroom compost or in the controls.

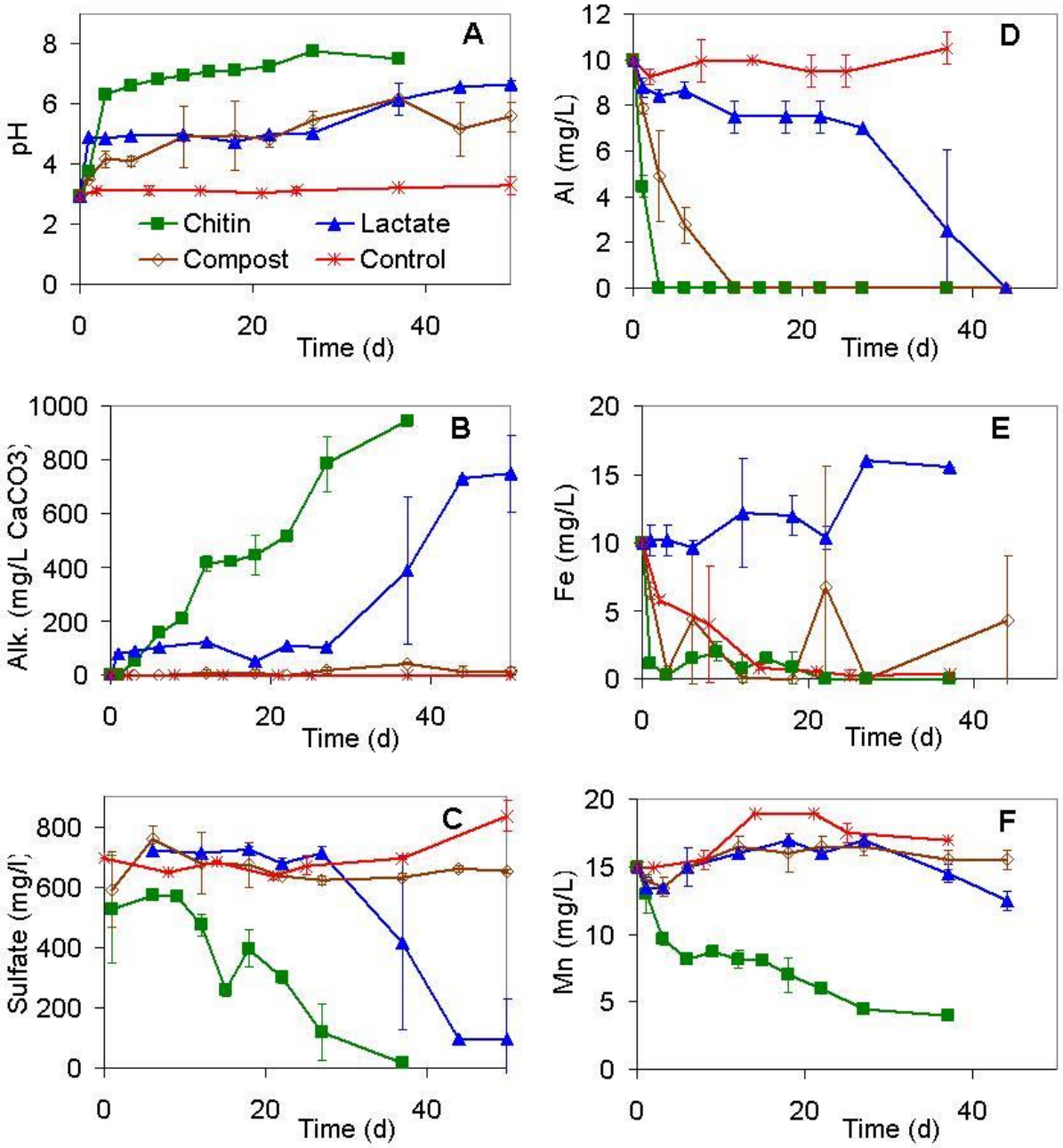

Figure 2. Changes in pH (A), alkalinity (B), sulfate (C), aluminum (D), iron (E), and manganese (F) over time in active microcosms containing AMD water and sediment and three different substrates; control microcosms had no substrate added. Data points represent duplicate average measurements; error bars represent 1 standard deviation.

Ammonium steadily accumulated in chitin-containing active microcosms during the first two weeks of incubation (data not shown). After 15 days, NH_4 concentrations fluctuated around 40 mg/L. Some NH_4 was also quickly released from spent mushroom compost after one day of incubation; however, its concentration did not exceeded 1 mg/L. No NH_4 was detected in microcosms containing sodium lactate or sediment only.

Table 2. Rates of alkalinity production, acidity removal, and sulfate reduction calculated during the comparative substrates tests.

Substrate	SC-20 Chitin	Sodium Lactate	Mushroom Compost
Alkalinity production			
Lag period, $t_{,initial}$ (d)	1	27	-
Rate (mg $CaCO_3$/L-d)	26.5	30.3	0
Acidity removal			
Lag period, $t_{,initial}$ (d)	1	27	-
Rate (mg $CaCO_3$/L-d)	-25.2	-12.7	0
Sulfate reduction			
Lag period, $t_{,initial}$ (d)	9	27	50
Rate (mg SO_4^{2-}/L-d)	-17.8	-24.8	0

Aluminum was completely removed by all substrates (Fig. 2D). However, removal was much faster with chitin than with compost or lactate (3, 12, and 37 days, respectively). Iron removal was observed in all bottles, except for those containing lactate (Figure 2E). Chitin was the only substrate able to partially remove Mn (~73%, Fig. 2F).

Discussion

Comparative Sites Test

In spite of the somewhat different characteristics of the three AMD water sources, the observed pH variation and the calculated rates of acidity removal and alkalinity generation were very similar for the three microcosm sets. In addition, when converted to meq/L-d, the rates of alkalinity and Ca increase are extremely similar (0.82 and 0.86 meq/L-d, respectively). This indicates that the dissolution of the chitin-associated carbonates present on SC-20 particles controlled the initial chemical changes inside the microcosms. After seven days of incubation, the pH stabilized around 7.5, which, according to the equilibrium of the carbonate system,

indicates the dominant presence of bicarbonates. Dissolution rates of calcium carbonates (calcite, dolomite) are pH dependent. Dissolution is very fast at low pH and its rate decreases about an order of magnitude with every unit increase in pH (Stumm and Morgan, 1996). Under acidic conditions, dissolution is mass transport limited, while under near-neutral pH, the rate is limited by surface area. This microcosm test was run under semi-stagnant conditions, with limited agitation and exposure of the surface of the chitinous materials. Therefore, it is likely that the observed alkalinity generation and acidity removal rates are slower than those that can be obtained under well-mixed or continuous-flow operation. Previous studies using different materials as substrates for AMD remediation have used an additional source of alkalinity such as limestone (Gibert et al., 2002). The presence of an alkalinity source, like limestone or the chitin-associated carbonates, can also produce changes in the AMD treatment system. As the pH increased, the water chemistry changes, affecting the equilibrium of the dissolved species. Therefore, some metals are expected to precipitate as the solubility limits of their hydroxides or carbonates are reached. The rapid recovery of pH and alkalinity obtained in the present study shows that chitinous materials are a promising and efficient source of neutralizing power for AMD remediation, and eliminate the need for an additional buffering agent. As incubation proceeded, the release of organic acids and the onset of SO_4^{2-} reduction likely played a more important role in the chemistry of the system. Organic acids drive a drop in pH while the SO_4^{2-} reduction reaction generates alkalinity.

Acclimation of SRB took about 7 days. In addition to circumneutral pH, these microorganisms usually need to be in a consortium with some fermenters to provide suitable substrate (simple, short chain organics). Since pH rapidly reached suitable values for SRB activity, this lag period is likely due to carbon and nutrient limitations at the beginning of the test, before the occurrence of chitin fermentation. Microcosms were not continuously stirred/mixed during incubation. This may have limited mass transport of substrate to the cells and could have also contributed to the delay in the activation of SRB. When taking into account the differences in the initial population added, the SRB activity was different for each site. Microcosms for the CR site had an initial SRB inoculum about an order of magnitude higher than the other two sites. However, the reduction rates obtained were relatively comparable. Similar rates of SO_4^{2-} removal were observed in previous batch studies, ranging between 3 to 109 mg/L-d, with a

similar acclimation time of around a week (Gibert et al., 2002). However, given the different conditions under which the tests were run, these rates cannot be directly compared.

Comparative Substrates Test

The changes in water quality observed in the chitin-containing microcosm set clearly resemble those observed during the comparative sites test. Despite the harsher initial conditions (i.e. lower pH, higher acidity, SO_4^{2-}, and metal concentrations), the remediation rates obtained during this test were very similar to those obtained during the comparative sites test. These similarities in pH, alkalinity, acidity, and Ca changes are, again, an indication that the dissolution of chitin-associated carbonate minerals plays a main role in the remediation process. Contrastingly, the initial pH changes observed in the lactate-containing microcosms were due to the dissolution of this salt itself. Simple equilibrium calculations show that when of 2.5 g/L of sodium lactate is added to a solution of pH 3, the expected new pH corresponds to 5.1. Indeed, the measured values during the first 27 days of incubation varied around pH 5.0. The later changes in pH values are associated with the beginning of biological SO_4^{2-} reduction. As indicated in Equation 2, one mole of alkalinity is generated per each mole of SO_4^{2-} that is reduced. Finally, changes in pH in those microcosms containing spent mushroom compost were much smaller and slower than with the other evaluated substrates. This indicates that, although compost has been shown to be a suitable substrate for AMD remediation (Gibert et al., 2002; Waybrant et al., 1998), its use requires that an external source of alkalinity be provided.

Previous research has shown lactate to be a superior substrate for SRB activity over other carbon sources, such as acetate or ethanol (Nagpal et al., 2000). However, in the present study, faster remediation was achieved chitin, the main fermentation product of which is acetate. In bottles containing either lactate or spent mushroom compost, three factors may have inhibited the SRB activity: low pH; lack of a buffering agent (i.e. limestone); and lack of a nitrogen source. With appropriate pH adjustments, it is likely that earlier changes in systems amended with lactate could be obtained. However, the soluble nature of this substrate requires a continuous supply, which would increase operating costs for continuous-flow site remediation.

Compared to the other substrates, chitin degradation generates the highest amounts of NH_4. This may indicate a potential drawback in the use of chitinous materials for passive treatment of AMD. However, a rapid nutrient delivery at the beginning of the process could be beneficial to stimulate bacterial activity and decrease the duration of the lag period. Chitin therefore can be

used in combination with other solid substrates. In this way, the process could benefit from the nutrient and buffering capacity of chitin, without diminishing the quality of the treated water.

Although the SO_4^{2-} reduction rates reported in this study are promising, it is important to remember that they were measured under controlled-laboratory conditions. For the *in situ* utilization of this substrate, differences in temperature, solid-to-solution ratio, hydraulic retention time, etc. will induce different acclimation times and reduction rates. In addition, changes in the substrate characteristics over time could also affect the performance of *in situ* treatments (Johnson and Hallberg, 2005; Neculita et al., 2007).

The calculated saturation indexes indicate that the probable mechanisms for Al and Mn removal are the precipitation of alunite ($KAl_3(SO_4)_2(OH)_6$) and rhodochrosite ($MnCO_3$), respectively. In the case of Fe, two mechanisms appear to be responsible for its removal: $Fe(OH)_3$ precipitation at the beginning of the test and iron sulfide precipitation once SRB became active.

Conclusions and Engineering Significance

The findings of this study demonstrate that chitinous materials can be used as an alternative substrate to support remediation processes. The obtained results indicate that, beyond its capacity to release electron donors for microbial activity, this material can play a major role in the neutralization of acidic streams. The addition of raw crab-shell-chitin alone:

- ✓ Increased the pH of AMD impaired waters to near-neutral values in less than three days.
- ✓ Promoted steady alkalinity generation and acidity removal, due to the dissolution of chitin-associated carbonate minerals.
- ✓ Rapidly stimulated the SRB activity, sustaining removal rates comparable with previous studies.
- ✓ Promoted a more efficient (i.e. faster and higher) removal of metals, compared to the other evaluated substrates.

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References

American Public Health Association (APHA), American Water Works Association (AWWA), Water Environment Federation (WEF). 2005. Standard Methods for the Examination of Water and Wastewater, 21th ed. Washington, DC.

Atlas, R. M. 2005. Handbook of Media for Environmental Microbiology. 2nd ed. CRC Press. Taylor & Francis Group. Boca Raton FL. pp 50. http://dx.doi.org/10.1201/9781420037487.

Brennan, R. A., Sanford, R. A., and C. J. Werth., 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)

Cocos, I. A., Zagury, G. J., Clement, B., and R. Samson. 2002. Multiple factor design for reactive mixture selection for use in reactive walls in mine drainage treatment. Water Research, 36(1):167-177. http://dx.doi.org/10.1016/S0043-1354(01)00238-X.

Daubert, L. N., and Brennan, R. A. 2007. Passive remediation of acid mine drainage using crab shell chitin. Environmental Engineering Science, 24 (10): 1475 - 1480. http://dx.doi.org/10.1089/ees.2006.0199.

Gibert, O., de Pablo, J., Cortina, J. L., and Ayora, C. 2004. Chemical characterization of natural organic substrates for biological mitigation of acid mine drainage. Water Research, 38:418-64196. http://dx.doi.org/10.1016/j.watres.2004.06.023.

Gibert, O., de Pablo, J., Cortina, J. L., and Ayora, C. 2003. Evaluation of municipal compost/limestone/iron mixtures as filling material for permeable reactive barriers for in-situ acid mine drainage treatment. Journal of Chemical Technology and Biotechnology, 78:489-496. http://dx.doi.org/10.1002/jctb.814

Gibert, O., de Pablo, J., Cortina, J. L., and Ayora, C. 2002. Treatment of acid mine drainage by sulfate-reducing bacteria using permeable reactive barriers: A review from laboratory to full-scale experiments. Reviews in Environmental Science and Biotechnology, 1:327-33. http://dx.doi.org/10.1023/A:1023227616422.

Johnson, D. B., and K. B., Hallberg. 2005. Acid mine drainage remediation options: a review. Science of the Total Environment, 338: 3-14. http://dx.doi.org/10.1016/j.scitotenv.2004.09.002.

Nagpal S., Chuichulcherm S., Livingston A., Peeva L. 2000. Ethanol utilization by sulfate-reducing bacteria: An experimental and modeling study. Biotechnology and Bioengineering, 70(5): 533-543. http://dx.doi.org/10.1002/1097-0290(20001205)70:5<533::AID-BIT8>3.0.CO;2-C.

Neculita, C. M., Zagury, G. J., Bussiere, B. 2007. Passive treatment of acid mine drainage in bioreactors using sulfate-reducing bacteria: Critical review and research needs. Journal of Environmental Quality, 36:1-16. http://dx.doi.org/10.2134/jeq2006.0066.

Parkhurst, D. L., Appelo, C. A. J. 1999. User's guide to PHREEQC (Version 2) – A computer program for speciation, batch-reaction, one-dimension transport and inverse geochemical calculations: U.S. Geological Survey Water-Resources Investigations Report 99-4259.

Pennsylvania Department of Environmental Protection (PADEP). 2000. Pennsylvania's Comprehensive Plan for Abandoned Mine Reclamation.

< http://www.depweb.state.pa.us/abandonedminerec/cwp/view.asp?a=1308&q=454835 >

Percot, A., Viton, C., Domard, A. 2003. Optimization of chitin extraction from shrimp shells. Biomacromolecules, 4:12-18. http://dx.doi.org/10.1021/bm025602k.

Rittmann, B. E., McCarty, P. L. 2001. Environmental Biotechnology: Principles and Applications. McGraw–Hill, New York, NY.

Robinson-Lora, M. A., Brennan, R. 2008. Evaluation of the Use of Crab Shells for Biological Denitrification. Submitted to Bioresource Technology.

Stumm, W., Morgan, J. J. 1996. Aquatic Chemistry: Chemical Equilibria and Rates in Natural Waters. Wiley – Interscience, New York, NY.

Vera, S. M., Werth, C. J., Sanford, R. 2001. Evaluation of different polymeric organic materials for creating conditions that favor reductive processes in groundwater. Bioremediation Journal, 5 (3): 169–181. http://dx.doi.org/10.1080/20018891079267.

Waybrant, K. R., Blowes, D. W., and C. J. Ptacek. 1998. Selection of reactive mixtures for use in permeable reactive walls for treatment of mine drainage. Environmental Science and Technology, 32:1972-1979. http://dx.doi.org/10.1021/es9703335.

Willow, M. A., Cohen, R. R. H. 2003. pH, dissolved oxygen, and adsorption effects on metal removal in anaerobic bioreactors. Journal of Environmental Quality, 32:1212-1221. http://dx.doi.org/10.2134/jeq2003.1212.