A LABORATORY INVESTIGATION OF PASSIVE REMEDIATION OF ACID MINE DRAINAGE USING CHITIN FROM CRAB SHELLS

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Abstract

Acid mine drainage (AMD) is a serious problem for many areas of the world, but particularly in Pennsylvania, where over 250,000 acres of abandoned coal mines and mine waste containing pyritic rocks leach sulfuric acid into surrounding waters. The acidity of these waters often becomes very high, promoting the dissolution of metals like iron, aluminum, and manganese. The combined acidity and metals content of AMD waters pose a serious threat to humans and wildlife. Using currently available treatment technologies, it would cost over $15 billion to remediate the approximately 3,000 miles of streams affected by AMD in Pennsylvania.

This research explores the possibility of using chitin, a naturally occurring, inexpensive polymer found in the shells of crustaceans, like crabs and shrimp, as an alternative substrate for the passive treatment of AMD. Chitin’s natural properties make it an ideal candidate for stimulating AMD treatment: upon fermentation it releases volatile fatty acids and ammonia which can support the growth of sulfate reducing bacteria, the key organisms involved in AMD remediation. In addition, the calcium carbonate found naturally in crab shells can serve as a buffer and increase alkalinity. Furthermore, chitin is inexpensive and naturally abundant as a waste product of the fishing industry.

Sacrificial microcosm tests were conducted to evaluate the ability of chitin from crab shells to reduce the acidity and dissolved metals concentrations in AMD water collected from Kittanning Run in Altoona, Pennsylvania. In the presence of chitin, pH increased from 3.21 to 6.79, acidity decreased from 192 to -114 mg/L, and alkalinity increased from 0 mg/L to over 200 mg/L, in just 9 days. Corresponding to this increase in pH, dissolved aluminum and iron concentrations were reduced by more than 95% and manganese concentrations were reduced by 81%. In addition, sulfate concentrations were observed to decrease from 489 to 308 mg/L, confirming the activity of sulfate reducing bacteria. The results of this work demonstrate for the first time the effectiveness of chitin as an alternative substrate for AMD remediation.
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Introduction and Background

Acid Mine Drainage Overview

Acid mine drainage (AMD) is a serious problem that threatens waterways in many areas of the world, and particularly in Pennsylvania, where more than 250,000 acres of abandoned coal mines and mine waste, containing pyritic rock (iron disulfide, FeS$_2$) leach sulfuric acid into surrounding waters (PADEP, 2000). The increasing acidity of these waters promotes the dissolution of metals like iron, aluminum, and manganese. The main reaction that governs AMD is shown in equation 1:

\[
4FeS_2(s) + 15O_2 + 14H_2O \rightarrow 4Fe(OH)_2(s) + 8SO_4^{2-} + 16H^+ \quad (1)
\]

The overall chemistry of AMD is catalyzed by certain extremophile bacteria called \textit{Acidithiobacillus ferrooxidans} and is aided by several other bacteria, archaea, and eucarya (Baker and Banfield, 2003). In most cases, the primary oxidizer is not actually molecular oxygen, but ferric iron. Furthermore, this reaction is multi-stepped and contains both oxygen-dependent and oxygen-independent steps (Johnson and Hallberg, 2005). The first step is the initial oxidation of pyrite with oxygen to form ferrous sulfate and two protons (H$^+$) per mole of oxidized pyrite (equation 2).

\[
2FeS_2(s) + 7O_2 + 2H_2O \rightarrow 2Fe^{2+} + 4SO_4^{2-} + 4H^+ \quad (2)
\]

The rate determining step is the oxidation of ferrous iron to ferric iron, which proceeds slowly under acidic (pH 2-3) conditions, and only slightly faster at higher pHs (equation
3). This reaction also depends heavily on the presence of *A. ferrooxidans* (Baker and Banfield, 2003), and it consumes hydrogen ions (i.e., protons).

\[
4Fe^{2+} + O_2 + 4H^+ \rightarrow 4Fe^{3+} + 2H_2O
\]  

(3)

Ferrous iron may be hydrolyzed in the third step to form solid ferric hydroxide, known as yellowboy, as well as hydrogen ions (equation 4).

\[
4Fe^{3+} + 12H_2O \rightarrow 4Fe(OH)_3(s) + 12H^+
\]  

(4)

Named for its bright color, yellowboy creates a film of metal precipitate on the bottom of streams and other affected waterways, and can vary from yellow to orange, and even to bright red in some cases. However, formation of the precipitate is pH dependent and yellow boy will only occur if the pH is above 3.5 (PADEP, 2006). Therefore, waters may be severely disabled water by AMD but lack the telltale sign of yellowboy.

The final step is what allows AMD reactions to be regenerating and circular. Additional pyrite and ferric iron react to form more ferrous iron and hydrogen ions (equation 5), to start the process over again.

\[
FeS_2(s) + 14Fe^{3+} + 8H_2O \rightarrow 15Fe^{2+} + 2SO_4^{2-} + 16H^+
\]  

(5)

The nature of the net reaction produces acid (in the form of hydrogen ions), sulfate ions, and depending on the conditions, oxidized iron or yellowboy (PADEP, 2006). Because of the decreased pH, other minerals, including metals, also dissolve in the water. This increases the net acidity further, according to equation 6:
Acidity $= [H^+] - [HCO_3^-] - 2[CO_3^{2-}] - [OH^-]$ \hspace{1cm} (6)

Conversely, alkalinity is conventionally defined as shown in equation 7:

Alkalinity $= [HCO_3^-] + 2[CO_3^{2-}] + [OH^-] - [H^+]$ \hspace{1cm} (7)

A moderate amount of alkalinity in water is necessary to resist future pH changes, and promote a healthy ecosystem for surface waters. At least 20 mg/L as CaCO₃ is required to support freshwater biota, but 75 mg/L as CaCO₃ is recommended to effectively buffer a surface water system (Deas, et al., 1999).

The highly acidic conditions created by acid mine drainage reactions are toxic to wildlife, insects, plants, and microorganisms living in or near contaminated water, and can severely harm the ecosystem. Although there are certain kinds of plants and animals that can resist changes in water chemistry, most cannot. Large fish begin to die at pH 6.5, can no longer hatch eggs at pH 5.0, and all fish die at pH 3.0 (EPA, 2006). Insects disappear at pH 2.0. Furthermore, the coating of yellow boy along streambeds and vegetation damages the aquatic environment and makes survival for plants and small animals difficult. It suffocates and prevents light from reaching plants and algae, as well as hinders the movement of smaller animals. Because of the interconnectedness and highly interdependent nature of an ecosystem, these losses in wildlife can seriously threaten other animals that feed on affected organisms (EPA, 2006).

Not only is the immediate vicinity of acid mine drainage negatively affected by contamination, but AMD disturbs areas farther downstream, where creeks and runoff
enter lakes and rivers, or where AMD contaminates groundwater that supplies potable water. In areas affected by AMD, drinking water treatment must be modified and consequently becomes more expensive.

Lake and river areas are also where humans play, work, and live. AMD’s harsh chemistry is not only dangerous for humans, but can damage concrete and metal structures, increasing construction and repair costs. Moreover, AMD has noticeably deterred the population from outdoor recreation. The Pennsylvania Fish and Boat Commission report an annual loss of approximately $67 million on fisheries and recreation due to AMD (EPA, 2006).

**Acid Mine Drainage Treatment Options**

Many possible remediation options have been proposed and implemented to solve the problem of AMD. Conventional, ex-situ treatment options have long been the first step in attempts at remediation. These typically involve active chemical neutralization through the addition of one or more of the following: lime (calcium oxide), calcium carbonate, sodium carbonate, or sodium hydroxide, among others. Chemical neutralization generates a lot of sludge in the form of metal precipitates that must be removed from the water. Furthermore, the neutralizing agent only attacks the acidity and dissolved metals by raising the pH through chemical addition. It fails to address the source of the problem, which is that bacterial activity has deteriorated to the point that it can no longer reverse acidity or decrease sulfate concentrations (Johnson and Hallberg, 2005). Other ideas for AMD treatment revolve around the concept of preventing acidic
drainage from occurring at all through “source control” techniques. This method of
treatment works by depriving the pyritic rock of water and oxygen, thereby preventing
the circular, acid-producing reactions (equations 1 – 5) from occurring. Source control
techniques include flooding and sealing underground mines, storing mine waste in
impermeable waste heaps, blending mineral wastes, and coating the rock with a sealant;
however, all of these have numerous practical disadvantages (Johnson and Hallberg,
2005). All of these treatment solutions continuously require a large amount of energy, as
well as human and material resources. Using such technologies, the Pennsylvania
Department of Environmental Protection (PADEP) estimates that it would cost over $15
billion to remediate the 2,400 miles of impaired streams, 250,000 acres of abandoned
surface mines, and over 8,000 abandoned oil and gas wells in Pennsylvania alone
(PADEP, 2000).

Passive, in situ solutions have become a much more popular alternative to these energy-
and cost-intensive treatments. Passive treatments provide low-tech solutions and
generally involve the flow of contaminated water through or over some kind of solid that
will treat the water. These include limestone channels and drains, aerobic and anaerobic
wetlands, vertical flow wetlands, and permeable reactive barriers, all of which require
little maintenance once installed (Johnson and Hallberg, 2005).

The passive treatment of acid mine drainage through permeable reactive barriers (PRBs)
is the most-recent solution (Gilbert, et al., 2003). A PRB is a trench constructed in the
flow path of contaminated water, which is filled with a mixture of organic substrate and
sometimes limestone gravel. PRBs are the only solution that simultaneously reduce sulfate, increase alkalinity, and remove metals through precipitation. To achieve this, PRBs utilize the activity of sulfate-reducing bacteria (SRB) that exist naturally in most aquatic environments. These bacteria are chemoorganotrophic in that they oxidize organic compounds for energy and cell synthesis, and they work anaerobically to reduce sulfates to hydrogen sulfide, as shown in equations 8, 9, and 10:

\[
4H_2 + SO_4^{2-} + H^+ \rightarrow HS^- + 4H_2O \tag{8}
\]
\[
CH_2O(s) + SO_4^{2-} \rightarrow HS^- + HCO_3^- \tag{9}
\]
\[
Me^{2+} + HS^- \rightarrow MeS(s) + H^+ \tag{10}
\]

where \( CH_2O \) represents an organic substrate, and \( Me \) represents a metal cation (Luptakova. and Kusnierova, 2005). This series of reactions consumes sulfate and hydrogen ions, and creates alkalinity in the form of \( HCO_3^- \), but requires an organic food source for the bacteria to survive. Hydrogen sulfide also reacts with dissolved metals to form metal sulfides, which precipitate and fall out of solution. Metal sulfides are far more resistant to low pH than zero valent metals, which resolubilize if water conditions become acidic again (U.S. Army Corps of Engineers, 2001).

The ideal organic substrate for AMD treatment is the subject of much research. Experimental investigations have used leaf mulch, wood chips, sawdust, sewage sludge, paper industry sludge, various animal manures, waste cellulose, municipal compost, mushroom compost, and many other organic wastes as substrates to support AMD remediation (Waybrant, et al., 1998, 2002; Gilbert, et al., 2003; Chang, et al., 2000;
Cocos, Et al., 2002). The chosen substrate must sustain treatment and therefore steadily degrade over time, as well as be bulky enough to trap metal sulfides (Chang, et al., 2000), yet permeable enough to allow for normal water flow. Generally, none of the above choices perform adequately for various chemical and practical availability reasons. For example, it has been shown that the activity of SRB is often limited by a lack of available nitrogen (Waybrant, et al., 2002), resulting in passive treatment systems that are designed overly large to compensate for low sulfate reduction rates. A substrate that can increase SRB activity by providing sufficient nitrogen, and in so doing decrease the size and cost of the system, is needed to efficiently and effectively treat AMD.

**Chitin**

Chitin is the second-most abundant biopolymer on earth after cellulose, and is a naturally occurring polysaccharide that exists in the exoskeleton of insects and crustaceans, as well as the cell walls of fungi, microorganisms, and plants (Felse, et. al., 1999). However, most chitin used for large-scale applications is derived from crab and shrimp waste from the shellfish industry (JRW Bioremediation, LLC.). Its chemical formula is C₈H₁₃NO₅, and its chemical structure is shown in Figure 1.
Figure 1. The chemical structure of two chitin monomers connected by a $\beta$-1,4-linkage.

Chitin and its derivatives have a strong positive charge, which gives it many useful applications (Felse et al., 1999). Commonly used as a water coagulant, the positive charge on chitin, or a chitin derivative such as chitosan, neutralizes the negative charge of suspended colloids and bacteria in the water, allowing them to coalesce and settle out of solution. This binding ability also allows chitin to be useful in consumer products such as cosmetic powders and moisturizers, toothpaste, contact lenses, synthetic sponges, and diapers, among many others (University of Delaware, 2006). Furthermore, chitin’s antimicrobial properties and its biocompatibility with animals and humans make it valuable in the healing of burns and wounds, and the subject of numerous studies on other potential medical applications (Felse et al., 1999).

Most importantly, chitin appears to be an ideal substrate for passive AMD remediation. Upon fermentation it breaks down to form volatile fatty acids (VFAs) and ammonia, which serve as a substrate and nitrogen source, respectively, for SRB (Vera et al., 2001; Brennan et al., 2006). Since chitin is 6-7% nitrogen, it possesses the ideal carbon to nitrogen ratio for such bacterial growth (Harkness et al., 2003). Different purities of
chitin are available for purchase, from the least refined (SC-20) to the most refined (SC-80), ranging in cost from $1 to $6 per pound, respectively (JRW Bioremediation, LLC.). Besides being the least expensive alternative, another advantage of using SC-20, which is 20% pure chitin by weight, is that it also contains approximately 40% calcium carbonate (CaCO₃) by weight. Calcium carbonate forms much of the crustacean exoskeleton (i.e. crab shell) and counteracts acidity upon dissolution, increasing the healthy alkalinity of the water and raising the pH. As the pH increases, dissolved minerals, and metals in particular, precipitate out of solution. Furthermore, chitin retains its permeability during degradation (Brennan, 2003), which is an essential attribute of substrates used in passive reactive barriers.

Not only is chitin inexpensive, naturally occurring, and readily available, there is evidence that it is a feasible substrate for use in remediation activities. Chitin has successfully served as a bacterial substrate for the remediation of water contaminated with chlorinated solvents (Brennan et al., 2005; Sorenson et al., 2002) and nitrate (Robinson-Lora and Brennan, 2005), in both batch and column experiments and in the field. There is also evidence that chitin can adsorb metal ions to its surface. It has been shown that chitin can adsorb cadmium and lead from sea water, and that microfungi having chitin and chitin derivatives in their cell walls uptake heavy metal ions, including silver, zinc, lead, copper, nickel, cobalt, cadmium, iron, and chromium (Felse and Panda, 1999). The metal cation uptake is a direct result of electrostatic attraction to the amine groups on the polysaccharide (de Oliveira Franco, et al. 2004), as well as the availability of amine and hydroxyl sites for chelation with metal ions (Kartal and Imamura, 2005).
Purpose and Hypotheses

The purpose of this thesis is to assess the potential of using chitin as a substrate for bacterial remediation of acid mine drainage. Because of chitin’s natural properties, it is theorized that results from this study will show that chitin effectively treats AMD waters by: 1) supporting the activity of sulfate reducing bacteria; 2) increasing the pH and alkalinity of the water; and 3) removing dissolved metals from solution. This research will explore these hypotheses by answering the following questions:

- Will chitin support the activity of sulfate reducing bacteria?
- Will chitin reduce acidity and increase alkalinity of AMD water? How quickly and to what extent?
- Will chitin reduce the concentration of dissolved metals in AMD water? How quickly and to what extent?

These questions will be answered by completing a series of sacrificial batch microcosm experiments on AMD waters and sediments treated with chitin. Treated and untreated water samples will be analyzed for pH, acidity, and alkalinity as well as dissolved aluminum, iron, and manganese concentrations. Sulfate concentrations will be measured for the purpose of demonstrating the activity of SRB over time in the bottle systems.
**Materials and Methods**

All experimental water was collected from Kittanning Run in Altoona, Pennsylvania. Kittanning Run is on the EPA’s Total Maximum Daily Loads list (Clean Water Act, Section 303d) due to its acid mine drainage conditions (EPA, 2000). The area in which Kittanning Run flows is scarred by large areas of mine waste piles and by abandoned deep and surface mines. Kittanning Run and another nearby stream are part of the Altoona Water Authority reservoir complex located near the Horseshoe Curve rail line, as shown in Figures 2 and 3. Treatment of these waterways would benefit the water supply and help restore wildlife to the ecosystem. The Pennsylvania Department of Environmental Protection has secured financial support to treat the AMD in the region, but declares Kittanning Run “difficult to restore” due to the multitude of unreclaimed mines and the severity of AMD symptoms in the stream and watershed (PADEP, 2001).
Figure 2. Map illustrating the area of Altoona, PA, where AMD water and associated sediments were collected for this study (PADEP: eMapPA, 2004). Circle designates the sampling area (see Figure 3).
Water was collected from a concrete-lined basin approximately 2.7 miles downstream of the nearest mine, as shown in Figures 2 and 3, by slowly submersing 1-gallon polyethylene jugs and avoiding aeration. The jugs had been thoroughly cleaned with Alconox Liqui-Nox® soap and water, dilute sulfuric acid, and a DI rinse. After collection, the water samples were refrigerated with minimal headspace until needed. Soil samples were collected in nonsterile 50-mL centrifuge tubes from a nearby marsh, shown in Figure 3. The samples were chosen for their proximity to yellow boy and for their dark color in an effort to obtain sediment with a high SRB content. These too were kept refrigerated and used within 1-12 days of collection.

Three rounds of sacrificial, batch microcosm experiments were conducted over the period of one year. Each round of bottle tests necessitated the collection of new samples to
ensure reduced conditions and an active microbial population in the water and sediment, respectively. Before each experiment, collected water was degassed in the collection vessel with nitrogen for approximately 90 minutes to ensure low dissolved oxygen (DO) conditions (approximately 0.40 mg/L final DO). In addition, all serum bottles were degassed with nitrogen gas for 10 minutes after adding sediment and chitin (as described below) to remove oxygen from the headspace. The serum bottles were then filled with 100-ml of degassed AMD water and sealed with butyl rubber stoppers and aluminum crimp tops. The bottles were shaken by hand to distribute the sediment and then incubated in the dark at room temperature until analysis. Each batch test was operated sacrificially: duplicates of control and experimental bottles were set up and expended for sampling at regular time intervals. Bottles were shaken by hand and then allowed to settle for approximately 24 hours before sacrificing in an effort to homogeneously mix the dissolved components of the bottle system. Control bottles held soil and water from the impaired stream, while the active bottles held the same soil and water, as well as chitin. In addition, the first batch contained 0.01 g of limestone in all bottles for supplementary buffering. The limestone was later determined to be unnecessary and eliminated from future experiments. The first two batch experiments confirmed the potential for chitin’s use as an SRB substrate in the treatment of AMD, and helped to estimate the rates of sulfate reduction so that the timing of future experiments could be improved. Results from these experiments can be seen in Appendix A.

The third, and final, batch experiment was run in December 2005 for nine consecutive days. Sample water and soil was collected from Kittanning Run on December 7, 2005,
with an air temperature of 26.1°F. Microcosms were setup on December 8, 2005 as follows. Control bottles contained 0.5-g soil and 100-mL water, while experimental bottles contained 0.5-g soil, 0.25-g SC-20 grade chitin, and 100-mL water. Duplicate controls were sacrificed and sampled on the first day (t = 1), and then every 2 to 4 days until the last day of sampling (t = 9). Duplicate active 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 siphoned from the bottle and frozen in 20-mL plastic (t = 1 - 2) or glass (t = 2 - 9) scintillation vials until metal, fatty acid, and sulfate concentrations could be analyzed. Before performing any of these three analyses, the water samples were filtered using 0.45μm nylon filters to remove any suspended soil or chitin particles from solution.

Acidity and alkalinity measurements were performed using titration methods (Clesceri, et al., 1998), and the results calculated as mg CaCO₃/L. Alkalinity was measured by titrating undiluted samples to pH 4.5 using 0.02N sulfuric acid (H₂SO₄). Acidity was measured using the hot peroxide titration method and 0.02N hydrogen peroxide (NaOH). Appendix B contains full procedures for each of these methods. An Accumet basic AB15 pH meter coupled with an Orion 915600 pH electrode was used for all measurements.

Metal analyses were conducted by the Penn State University Materials Characterization Laboratory, using a Leeman Labs PS3000UV inductively coupled plasma emission spectrophotometer (ICP), capable of measuring concentrations as low as 0.05 mg/L.
Concentrations of three of the most prominent AMD cations in this water, aluminum, iron, and manganese, were measured simultaneously from 5-mL water samples.

Sulfate concentrations were measured using a DX-100 ion chromatograph (IC) with a Dionex IonPac 4 AS4A SC column and a carbonate-bicarbonate eluent. Analysis was run at 20°C and a flow rate of 2 mL/min.

**Results and Discussion**

Over the course of nine days, following the addition of chitin, the pH in the active microcosms rose steadily from an initial value of 3.21 to close to neutral with a final average value of 6.79. Figure 4 shows how control bottles maintained a relatively steady acidic pH, while those being treated with chitin logarithmically approached neutral pH.
As expected, with this pH increase, acidity decreased rapidly and linearly while alkalinity conversely increased (Figures 5 and 6). Acidity remained at an average of 158.3 mg/L as CaCO$_3$ for control samples. Because alkalinity is immeasurable below pH 4.5, the alkalinity in the controls remained effectively zero for the duration of sampling, and are therefore not shown in Figure 6. These changes in water conditions were likely caused by both the activity of SRB and the slow dissolution of calcium carbonate from the crab shells. The SRB act to reduce sulfates to hydrogen sulfide, and in doing so create bicarbonate (HCO$_3^-$) as shown in equations 8 and 9 (Luptakova. and Kusnierova, 2005). The calcium carbonate reacts with acid to form carbon dioxide, which in turn reacts with more calcium carbonate to form bicarbonate (HCO$_3^-$) and carbonate (CO$_3^{2-}$) ions. This
increases the buffering capacity of the water, allowing it to neutralize free hydrogen ions as shown in equation 7.

Figure 5. Acidity of AMD water in microcosms containing chitin, compared to controls without chitin over time. Data points are duplicate averages; error bars represent one standard deviation.
Figure 6. Alkalinity increase of AMD water in microcosms containing chitin, compared to controls without chitin over time. Data points are duplicate averages; error bars represent one standard deviation.

The trends shown in Figures 4, 5, and 6 indicate the establishment of a healthier water and the potential for a healthier ecosystem overall after treatment with chitin. High alkalinity content is important for resisting the pH-decreasing effects of future acid influx to the waters.

Additionally, as pH increased, dissolved iron, aluminum, and manganese concentrations in microcosms treated with chitin decreased, as shown in Figures 7, 8, and 9, respectively. Dissolved metals dropped to less than 0.03% of starting concentrations (below detectable limits, 0.05 mg/L) for iron and aluminum, and to 19% of the starting concentration for manganese. Control bottles that did not contain chitin demonstrated little or no change in dissolved aluminum and manganese concentrations. Dissolved iron
did decrease by about 75% in the controls, but was still approximately 5 mg/L more concentrated in dissolved iron than the active, chitin-containing samples. Decreasing iron concentrations in the control bottles may have been due to the small increase in pH from 3.21 to 3.4, which may have been enough to stimulate FeS to precipitate out of solution. The low solubility of iron sulfide (FeS) at low pHs is shown in Figure 10.

Figure 7. Dissolved iron concentrations of AMD water in microcosms containing chitin, compared to controls without chitin over time. Data points are duplicate averages; error bars represent one standard deviation.
Figure 8. Dissolved aluminum concentrations of AMD water in microcosms containing chitin, compared to controls without chitin over time. Data points are duplicate averages; error bars represent one standard deviation.

Figure 9. Dissolved manganese concentrations of AMD water in microcosms containing chitin, compared to controls without chitin over time. Data points are duplicate averages; error bars represent one standard deviation.
The overall decrease in metals concentrations observed in this experiment can be attributed to decreasing solubility of metals as the pH rose toward neutral. The solubilities of iron, aluminum, and manganese cations and the complexes they form are dependent upon the pH of the system. Generally, as the pH increases, the solubility of metal compounds decreases. In AMD water, the likely complexes for iron, manganese, and aluminum are FeS, MnS, and Al(OH)$_3$, respectively (Sullivan et al., 1988). Figure 10 illustrates the solubility of these three metal complexes as a function of pH. The solubility product constants ($K_{sp}$) at 25°C are $6.3 \times 10^{-18}$ M$^2$ for FeS, $2.5 \times 10^{-13}$ M$^2$ for MnS, and $1.3 \times 10^{-33}$ M$^4$ for Al(OH)$_3$ (McQuarrie and Rock, 1984).

![Figure 10. Solubilities of FeS, MnS, and Al(OH)$_3$ as a function of pH (raw data from McQuarrie and Rock, 1984).](image)

These solubility trends correlate directly to observations from this study. As shown in Figure 10, it is expected that FeS would precipitate out of solution first, followed by Al(OH)$_3$, and then MnS, with increasing pH. As demonstrated in Figure 11, iron,
aluminum, and manganese did indeed precipitate out of solution in that order in this experiment, as the pH increased from 3.2 to 6.8 due to treatment with chitin.

Figure 11. Dissolved metals concentrations and pH in AMD microcosms treated with chitin over time. Data points are duplicate averages; error bars represent one standard deviation.

Additionally, as previously discussed, chitin has been shown to adsorb metals onto its surface, which may be occurring here. To determine the exact location of the metals as they disappear from solution would require further analysis (for example, scanning electron microscopy (SEM) could be used to examine the surface of the chitin and sediment particles for precipitated metal complexes). Nevertheless, this experiment shows an efficient dissolved metals removal rate when chitin is used as a substrate for AMD treatment.
A positive indication of SRB activity, sulfate concentrations in microcosms treated with chitin were observed to decrease over time in comparison to controls, as shown in Figure 12; however, a significant lag occurred before sulfate reduction began.

![Figure 12. Sulfate reduction in AMD microcosms treated with chitin, compared to controls without chitin. Lines represent the running average of the data points. Data points are duplicate averages; error bars represent one standard deviation.](image)

This lag in sulfate reduction is likely a function of bacterial activity and acclimation to microcosm conditions. For this experiment, soil samples containing the bacteria were collected from a frozen marsh area and refrigerated overnight before being returned to room temperature. As a comparison, soil for microcosms in the second batch experiment were collected on a 72°F afternoon and displayed a reduction in sulfate levels almost immediately upon the addition of chitin (Figure 19 in Appendix A). The bacteria in the third batch experiment were possibly dormant and needed time to reactivate and repopulate before they could begin successfully degrading sulfate. Additionally, the high sulfate concentrations in these water samples may have produced a difficult environment
for the sulfate-reducing organisms to operate efficiently. Figure 12 suggests that the SRB did not entirely acclimate until around day 7.

Overall, chitin demonstrated successful remediation of this AMD water by efficiently increasing pH and alkalinity, and reducing acidity, dissolved metals, and sulfate concentrations, as summarized in Table 1.

Table 1: Summary of findings for batch microcosm tests of chitin-treated AMD waters

<table>
<thead>
<tr>
<th>Measured parameter</th>
<th>Controls, no chitin (t=0)</th>
<th>Controls, no chitin (t=9)</th>
<th>Actives, chitin (t=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>3.21</td>
<td>3.27</td>
<td>6.79</td>
</tr>
<tr>
<td>Acidity (mg/L as CaCO3)</td>
<td>192.3</td>
<td>164.4</td>
<td>-114.1</td>
</tr>
<tr>
<td>Alkalinity (mg/L as CaCO3)</td>
<td>0.0</td>
<td>0.0</td>
<td>235.13</td>
</tr>
<tr>
<td>Aluminum (mg/L)</td>
<td>14.0</td>
<td>16.0</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td>Iron (mg/L)</td>
<td>21.0</td>
<td>5.2</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td>Manganese (mg/L)</td>
<td>12.0</td>
<td>12.5</td>
<td>2.3</td>
</tr>
<tr>
<td>Sulfate (mg/L)</td>
<td>489.3</td>
<td>471.1</td>
<td>303.2</td>
</tr>
</tbody>
</table>

*Below detection limit

The successful application of chitin must also be practically and economically feasible. Assuming a maximum flow rate of 1.6 million gallons per day in Kittanning Run in Altoona, PA (EPA, 2000), a porosity of 40%, and a required hydraulic residence time of 9 days for full remediation, as found in this experiment, a permeable reactive barrier would need to be 139,500 m³. An average depth of 8 m and width of 10 m would require the barrier to be 1743 m long, or 1.1 miles. A linear-scale up from this experiment, in which 0.25 g of chitin was used per 100 mL of water, this barrier would need to be filled with 348,750 kg of SC-20 grade chitin, which would cost $767,250, based on the price of $1 per pound. However, it is unlikely that all of the chitin was consumed in this
experiment, so the actual cost might be considerably less. For example, if the assumption is made that only 0.01 g of chitin is required to treat 100 mL of water with a residence time of 22 days, as in the first batch of microcosm experiments (Appendix A), then the barrier would need to be 4263 m long (2.6 miles), filled with only 34,100 pounds of SC-20 grade chitin for a materials cost of $75,020. Calculations can be found in Appendix C. Of course, these calculations are approximations only and would need to be refined based on the results of further research, as recommended in the next section.

**Conclusions and Recommendations**

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

- pH rose from 3.21 to 6.79.
- Acidity fell from over 150 mg/L to less than -100mg/L.
- Alkalinity rose from nonexistent to over 200 mg/L.
- Dissolved iron and aluminum fell to less than 0.05 mg/L from 21 mg/L and 14 mg/L respectively, and dissolved manganese fell from 12 mg/L to 2.3 mg/L.
- Sulfate concentrations decreased from nearly 500 mg/L to close to 300 mg/L.

The results of this experiment and those conducted previously demonstrate the efficiency and extent of treatment that chitin provides as a substrate for the passive remediation of acid mine drainage. The efficiency at which treatment occurred establishes chitin’s potential for use at the field scale. Furthermore, chitin shows promise as an attractive
substrate due to its inexpensiveness, considerable availability, and the small mass required to treat a relatively large volume of water.

These positive results should be confirmed by running additional microcosm experiments, using AMD water with different ranges of acidity and metals. In addition, volatile fatty acid concentrations should be analyzed in the systems to determine what types of chitin fermentation products are available as substrates to SRB in the chitin-AMD system.

A positive confirmation of these results would warrant a transition to continuous flow column experiments, and if those are successful, then tests should progress to the field to examine chitin’s potential as a sole substrate for passive AMD remediation. In addition, the effectiveness of chitin as a sole substrate should be directly compared to the effectiveness other substrates, such as spent mushroom compost, and possibly tested as a fractional amendment to those substrates. A practicable workup to the field in the form of a small permeable chitin barrier would help to evaluate chitin’s longevity and effectiveness at reducing acidity at a large scale.
Acknowledgements

I would like to extend many thanks to the Department of Civil & Environmental Engineering at Penn State University for their support, and particularly to Dr. Rachel Brennan and Mary Ann Robinson Lora, for all their assistance with this research and for patiently supporting me throughout the project. Thank you also to Christine Olmeda for all her work sampling water in Altoona with me, and her never-ending encouragement, Dr. Brian Dempsey for his guidance in choosing the right AMD sampling point, as well as his loan of wader boots, and Henry Gong at the Materials Characterization Lab for conducting the metals analyses. Finally, and most of all, thank you to those of you that gave me the encouragement and understanding I needed. My parents, Tom and Nancy, my brothers, Mark and Steve, my boyfriend, Bryan, my roommates, Susan and Sam, and my peers in chemical engineering, thank you.
References


JRW Bioremediation, LLC. Lenexa, Kansas.


Appendix A

*Previous Batch Experiments*

**Batch 1**

Soil and water samples were collected in February 2005 from Kittanning Run and experiments were run February 9, 2005 to March 3, 2005. Duplicate active sample bottles contained 100- mL AMD water, 0.01-g limestone gravel, 0.5-g soil, and 0.01-g SC-20 grade chitin. Control bottles contained 100-mL AMD water, 0.01-g limestone gravel, and 0.5-g soil. pH, acidity, and metals concentrations were measured over the course of 22 days using the methods described previously, and are shown in Figures 13, 14, and 15. Fatty acid concentrations were determined using a high performance liquid chromatograph (HPLC). A Waters 2695 separations module and a Waters 2996 photodiode array detector were used with a mobile phase of 0.005 M sulfuric acid and a flow rate of 0.6 mL/min. However, the fatty acid analysis showed no substantive buildup of fatty acids in the system.
Figure 13. pH neutralization of AMD water in microcosms containing chitin, compared to controls without chitin over time for first microcosm tests. Data points are duplicate averages; error bars represent one standard deviation.

Figure 14. Acidity neutralization of AMD water in microcosms containing chitin, compared to controls without chitin over time for first microcosm tests. Data points are duplicate averages; error bars represent one standard deviation.
Figure 15. Dissolved metals concentrations in AMD water in microcosms containing chitin compared to controls without chitin over time for first microcosm tests. Data points are duplicate averages; error bars represent one standard deviation.
Batch 2

Samples were collected October 4, 2005 from Kittanning Run with an air temperature of 72°F, and were run from October 16-21, 2005. Sample bottles contained 100-mL AMD water, 0.5-g soil, and 0.25-g SC-20 grade chitin. Controls contained 100-mL AMD water and 0.5-g soil. pH, acidity, alkalinity, sulfate concentrations, and metals concentrations were measured over the course of 5 days, as shown in Figures 16 – 20.

Figure 16. pH neutralization of AMD water in microcosms containing chitin, compared to controls without chitin over time for second microcosm tests. Data points are duplicate averages; error bars represent one standard deviation.
Figure 17. Acidity neutralization of AMD water in microcosms containing chitin, compared to controls without chitin over time for second microcosm tests. Data points are duplicate averages; error bars represent one standard deviation.

Figure 18. Alkalinity of AMD water in microcosms containing chitin over time for second microcosm tests. Controls remained at 0 mg/L for all 5 days. Data points are duplicate averages; error bars represent one standard deviation.
Figure 19. Sulfate concentrations of AMD water in microcosms containing chitin, compared to controls without chitin over time for second microcosm tests. Data points are duplicate averages; error bars represent one standard deviation.

Figure 20. Metals concentrations of AMD water in microcosms containing chitin, compared to controls without chitin over time for second microcosm tests. Data points are duplicate averages; error bars represent one standard deviation.
Appendix B

Acidity and Alkalinity Titration Methods
(Clesceri, et al., 1998)

Alkalinity

Measurements must be performed within two days of sampling. These measurements can only be performed on those samples with pH 4.5 or greater. Samples with pH less than 4.5 can skip to acidity methods.

1) Decant approximately 65-mL of sample water into a 100mL beaker, recording the exact volume of water.
2) Titrature (while stirring) with 0.02N sulfuric acid to end point pH 4.5.
3) Record the exact volume of acid transferred to the sample.

$$\text{Alkalinity} \cdot \left(\frac{mg}{L \ as \cdot CaCO_3}\right) = \frac{A \cdot N \cdot 50000}{V}$$

where A is the mL of sulfuric acid used to titrate to pH 4.5, N is the normality of the acid (should be 0.02), and V is the mL of initial sample.

Continue to acidity methods to get the pH below 4.0.

Acidity

1) Decant approximately 65-mL of sample water into a 100-mL beaker, recording the exact volume of water.
2) If the pH is greater than 4.0, add 0.02N sulfuric acid in 1.0-mL increments to lower the pH to 4.0 or less. If not, skip to 3.
3) Add 7 drops of hydrogen peroxide using a transfer pipet (~1 drop/10 mL of sample)
4) Heat the sample to boiling and continue boiling for 2-4 minutes in the hood.
5) Add 2 drops of hydrogen peroxide.
6) Cool the sample to room temperature and immediately titrate with 0.02N sodium hydroxide to pH 8.2.

$$\text{Acidity} \cdot \left(\frac{mg}{L \ as \cdot CaCO_3}\right) = \frac{(A \cdot B - C \cdot D) \cdot 50000}{V}$$

where A is the mL of sodium hydroxide used to titrate to pH 8.2, B is the normality of the sodium hydroxide (should be 0.02), C is the mL of sulfuric acid used to titrate to pH 4.0, D is the normality of the acid (should be 0.02), and V is the mL of initial sample.
Appendix C

Calculating Permeable Reactive Barrier Dimensions

Hydraulic retention time, $\theta$, can be defined as the number of days required for chitin to fully remediate the AMD water in the bottle system.

$$\theta = \frac{Volume}{FlowRate} = \frac{Volume}{Velocity \times A_x}$$

where the velocity is the superficial Darcy velocity, and $A_x$ is the cross-sectional area of the flow path.

However, because a permeable reactive barrier increases the velocity of the stream, the linear velocity, which takes into account the porosity ($p$) of the medium through which the water flows, must be used for calculations.

$$LinearVelocity = \frac{Velocity}{Porosity}$$

and

$$\theta = \frac{Volume}{LinearVelocity \times A_x}$$

For example, in the third microcosm experiment samples exhibited a residence time ($\theta$) of 9 days, and assuming a cross sectional area of 80 m$^2$, an uninhibited flow rate of 6.2×10$^6$ L/day (velocity of 77.5 m/day), and a porosity of 40%, the required volume is 55800 m$^3$, as shown below.

$$LinearVelocity = \frac{77.5m/day}{0.4} = 193.75m/day$$

$$9days = \frac{Volume}{(193.75m/day) \times (80m^2)} \implies Volume = 139,500m^3$$

A depth of 8 m was assumed in order to accommodate fluctuations in stream depth and AMD infiltration to groundwater. A width of 10 m was assumed as the average span of the stream.

Calculating Chitin Requirements for a Given Treatment Efficiency

Chitin requirements were calculated by the following formula.
\[ \text{Mass of chitin} = \text{AdjustedFlowRate}\left(\frac{m^3}{\text{day}}\right) \times \theta [\text{day}] \times \frac{\text{Mass of chitin in bottle}[\text{kg}]}{\text{Volume of water in bottle}[m^3]} \]

For example, in the third microcosm experiment, 0.25 g of chitin was used per 100 mL of water, \( \theta \) was 9 days, and the flow rate adjusted to the linear velocity of the stream was 15500 m\(^3\)/day.

\[ \text{Mass of chitin} = 15500 \frac{m^3}{\text{day}} \times 9 \text{days} \times \frac{2.5 \times 10^{-4} \text{kg}}{0.100 L} = 348,750 \text{kg} \]
Appendix D

Pictures—Sampling from Kittanning Run in Altoona, PA
Linda N. Daubert
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EDUCATION

The Pennsylvania State University
Bachelor of Science in Chemical Engineering
Minors in Environmental Engineering and French
Honors in Environmental Engineering
Thesis: A Laboratory Investigation of Passive Remediation of Acid Mine Drainage Using Chitin from Crab Shells
Thesis Supervisor: Dr. Rachel Brennan

EXPERIENCE

Penn State Department of Civil & Environmental Engineering, University Park, PA
January 2005-May 2006
Environmental Engineering Laboratory Research
Supervisor: Dr. Rachel Brennan

IBM Microelectronics, Burlington, VT
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Pre-professional Environmental Engineer
Supervisor: Janet Doyle, Timothy Baechle

State College Area Family YMCA, State College, PA
June-November 2003
Membership Service Representative
Supervisor: Darlene Smith

State College Area High School, State College, PA
October 2001-May 2002
Symphonic Band President
Supervisor: Richard Victor

PROFESSIONAL MEMBERSHIPS

Tau Beta Pi
Phi Eta Sigma

AWARDS & HONORS

1st place Poster Presentation in Engineering at Undergraduate Research Exhibition (2006)
Engineering Student Intern of the Year Nominee (2005)
College of Engineering Scholarship (2002-2006)
Academic Excellence Scholarship (2002-2006)
Dean’s List (2002-2006)
COMMUNITY & EXTRACURRICULAR ACTIVITIES

Tau Beta Pi—PA Beta Chapter

Initiation Chair (2005-2006); Public Relations Chair (2005)

International Journeys Story Hour

Co-president (2004-2006)

Coordinate and present international culture and stories to children at a local library

Engineering Mentor to underclassmen

PRESENTATIONS

“A Laboratory Investigation of Passive Remediation of Acid Mine Drainage Using Chitin.”

Poster presentation, Penn State Undergraduate Research Exhibition, 2006.

LANGUAGE PROFICIENCY

French (spoken and written)