

EVALUATION OF THREE DIFFERENT PURITIES OF CRAB-SHELL FOR THE REMEDIATION OF MINE IMPACTED WATER¹

Kristopher M. Korte, Caroline E. Newcombe, and Rachel A. Brennan²

Abstract: Crab-shell chitin has been shown to effectively reduce SO_4^{2-} , enhance alkalinity, and remove metals from mine impacted water (MIW) at the bench scale and in field trials. To date, this research has been conducted using inexpensive, raw crab shell (ChitoRem™ SC-20), which in addition to chitin, contains CaCO_3 and residual protein. All three of these components contribute to MIW treatment simultaneously, and are therefore difficult to uncouple. In this experiment, three different purities of crab shell (SC-20, SC-40, and SC-80) and limestone were tested for their ability to remediate natural MIW from an abandoned coal-mine in central Pennsylvania. The goals of this project were: 1) to compare the efficiency of metals and sulfate removal between different purities of chitin; and, 2) to begin to uncouple the contributions of chitin, protein, and calcium carbonate when raw crab shell chitin is used for the treatment of acid mine drainage.

Sacrificial batch microcosms containing natural MIW, stream sediment, and either SC-20, SC-40, SC-80, or limestone were established in duplicate and incubated at room temperature for up to 117 days. The most complete and rapid metals removal was observed with SC-20, followed by SC-40, SC-80, and limestone. SC-20 removed more than 99% of Al, Fe, and Zn and more than 98% of dissolved Mn. SC-40 exhibited similar metals removal, but at slower rates. SC-80 and limestone were not effective at removing Mn. Alkalinity production followed similar trends, with SC-20 surpassing the other substrates with a total alkalinity of 1175 mg/L as CaCO_3 after 117 days. Elevated NH_4 production was observed at early times only with SC-20, indicating that it is residual protein, not chitin, releasing this nutrient. It is likely that the rapid dissolution of CaCO_3 from the crab shell, coupled with NH_4 release and biological SO_4^{2-} reduction all contributed to elevated alkalinity values and consequently superior metals removal with SC-20. Preliminary geochemical modeling suggests that the probable mechanisms for metals removal with SC-20 include precipitation of Al and Fe oxides/hydroxides and manganese carbonates, as well as physical adsorption onto components of the crab shell.

Additional Key Words: chitin, CaCO_3 , protein, acid mine drainage, metals, bioremediation

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Introduction

The bane of the mining industry, mine impacted water (MIW) is a complex, toxic mixture of SO_4^{2-} , acidity, and metals, requiring biological, chemical, and physical treatment steps for thorough remediation. Crab-shell chitin has been shown to biologically reduce SO_4^{2-} , chemically enhance alkalinity, and physically remove metals from mine impacted water (MIW) at the bench scale (Daubert and Brennan, 2007) and in field trials (Venot et al., 2008). To date, this research has been conducted using inexpensive, raw crab shell (ChitoRem™ SC-20), which in addition to chitin, contains CaCO_3 and residual protein. All three of these components contribute to MIW treatment simultaneously, and are therefore difficult to uncouple. We believe, however, that the fermentation of the protein in the SC-20 is responsible for the release of a short, but elevated burst of NH_4 at the beginning of treatment. Although an ideal nutrient for stimulating microbial growth, excess NH_4 may be toxic to aquatic life. To remove NH_4 from this process, two potential solutions are available: NH_3 stripping of the treated effluent, or the use of a higher purity chitin (without protein) in the reactive zone. Higher purities of crab shell chitin are commercially available in which the protein and/or calcium carbonate have been chemically removed: ChitoRem™ SC-40 contains chitin and CaCO_3 ; and ChitoRem™ SC-80 contains chitin only. As the level of chitin purification increases, so does the cost: from \$0.50 - \$0.75/lb + freight for SC-20, to \$5/lb + freight for SC-80 (JRW Bioremediation, LLC, 2008). In contrast, spent mushroom compost, which is currently the most commonly used substrate for passive MIW treatment systems in Pennsylvania, retails for approximately \$50 per ton (Dietz, 2006). However, we have demonstrated that spent mushroom compost is not as effective as chitin for the removal of many metals, especially manganese (Robinson-Lora and Brennan, 2008), which minimizes the importance of the initial capital cost savings it offers.

The goals of this project were: 1) to compare the efficiency of MIW treatment using SC-20 compared to other, higher purity chitin substrates; and, 2) to begin to uncouple the contributions of chitin, protein, and calcium carbonate when raw crab shell chitin is used for the treatment of acid mine drainage.

Materials and Methods

Chemicals

Varying purities of chitin derived from Dungeness crab were used in this experiment: ChitoRem™ SC-20, SC-40, and SC-80 (JRW Bioremediation, LLC, Lenexa, KS). The number

designations in these substrates refer to the approximate percent by weight of chitin present (i.e., 20, 40, and 80% chitin). The approximate compositions of these grades of chitin are provided in Table 1. The ChitoRem™ was used as received from the vendor: no pretreatment was performed. The particle size of the ChitoRem™ varied, but was generally < 1 mm. Limestone chips were used in control bottles as a source of CaCO₃. To prepare the chips, limestone rocks (Prairie Central, Champaign, IL) were broken using a mortar and pestle and then sieved to an 18 – 45 mesh (0.35 – 1 mm) particle size. Ultra High Purity (UHP) nitrogen gas (MG Industries, Malvern, PA) was used to degas the MIW and microcosm bottles during preparation.

Table 1. Approximate percent by weight compositions of the three purities of chitin used in the microcosm experiments.

	SC-20	SC-40	SC-80
Chitin	20 - 25	40 - 45	> 85
Protein	25 - 35	0	0
Calcium carbonate	30 - 50	40 - 45	3
Moisture	< 10	< 10	< 10

Source Water & Sediment Collection

MIW 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 no headspace. To serve as an inoculum source for the microcosm experiments, unexposed, saturated sediment was collected from a stream bank near the site in nonsterile 50-mL centrifuge tubes. After collection, both water and sediment samples were immediately placed on ice and transferred to The Pennsylvania State University and refrigerated at 4 °C. The following day, the water was degassed with UHP nitrogen in the collection vessel for approximately 90 minutes to ensure low dissolved oxygen conditions (~0.40 mg/L final DO) prior to setting up the microcosms.

Microcosm Preparation

Sacrificial microcosm tests were conducted to evaluate the individual contributions of chitin, protein, and CaCO₃ to acid mine drainage treatment when crab shells are used as a substrate. This was done by testing varying purities of crab-shell chitin: SC-20 (chitin + protein + CaCO₃); SC-40 (chitin + CaCO₃); SC-80 (chitin only); and limestone (CaCO₃) only. Twenty (20)

replicate bottles were established for each grade of chitin and for limestone (i.e., 10 duplicates each). A set of 20 bottles with sediment only were also established as a negative control. Homogenized stream sediment (bacterial source) was transferred in 0.5-g aliquots to 100 replicate 160-mL glass serum bottles. To each bottle, 0.25-g of ChitoRem[®] (either SC-20, SC-40, or SC-80) or 0.25-g limestone chips were added, as appropriate. The bottles were degassed with UHP nitrogen, filled with 100-mL of degassed AMD water, and sealed with 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 1 hour before each sampling point, the bottles to be sacrificed were gently shaken by hand in an effort to homogeneously mix the dissolved components, and allowed to settle. Duplicate bottles of each treatment condition were periodically sacrificed over a period of 117 days and the water analyzed for pH, NH₃, acidity, alkalinity, anions, and dissolved metals. Periodically during the experiment, water from sacrificed SC-20 bottles was aerated with a fine-bubble diffuser to determine the time required for NH₃ stripping under natural conditions.

Analytical Procedures

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 NH₃, SO₄²⁻, and metal analysis. Alkalinity and hot acidity were determined by titration as described in Standard Methods (APHA, 2005). An Accumet basic AB15 pH meter coupled with pH (Thermo-ORION pH probe) and NH₃ (ISE ORION 9512) electrodes were used for all measurements. Before SO₄²⁻ and metals analyses were conducted, frozen water samples were thawed and filtered (0.45µm). Sulfate was measured at room temperature using an Ion Chromatograph (IC, Dionex DX-100) equipped with an Ionpac AS4A column and a carbonate-bicarbonate elluent (APHA, 2005). Dissolved metals were preserved in HNO₃ and measured using an Inductively Coupled Plasma emission spectrometer (ICP, Leeman Labs PS3000UV) at the Materials Characterization Laboratory at The Pennsylvania State University.

Results

Changes in pH, acidity, alkalinity, and calcium

Over the course of the experiment, the pH in microcosms containing crab-shell chitin logarithmically approached stable pH values in approximately 30 days (Fig. 1). Purities of crab-

shell containing CaCO_3 (i.e., SC-20 and SC-40), reached final pH values of 7.8, whereas highly purified crab-shell devoid of CaCO_3 (SC-80) reached a maximum pH of 6.15. Microcosms containing only limestone had a similar pH profile to that of SC-80, and control bottles containing sediment only maintained a relatively steady acidic pH, with a final value of 4.2 (Table 1). As expected with this pH increase, acidity decreased rapidly and linearly while alkalinity conversely increased (Fig. 2 and 3, Table 1). The greatest decrease in acidity and greatest increase in alkalinity was observed for SC-20, followed by SC-40, SC-80, and limestone (Figs. 2 and 3). Acidity remained at an average of 167.5 mg/L as CaCO_3 in the controls (Table 1). Because alkalinity is immeasurable below pH 4.5, the alkalinity in the controls remained effectively zero for the duration of the experiment. The dissolution of CaCO_3 from the substrates is evident by observed increases in dissolved Ca for all treatments, with the highest increase observed for SC-20, followed by SC-40, limestone, and SC-80. Calcium production peaked after approximately 7 days of incubation with SC-20 (182 mg/L) and 14 days with SC-40 (155 mg/L), and then returned to background levels (105 mg/L) after 30 days. A lower, but more sustained concentration of calcium was achieved with limestone, averaging 119 mg/L throughout the experiment.

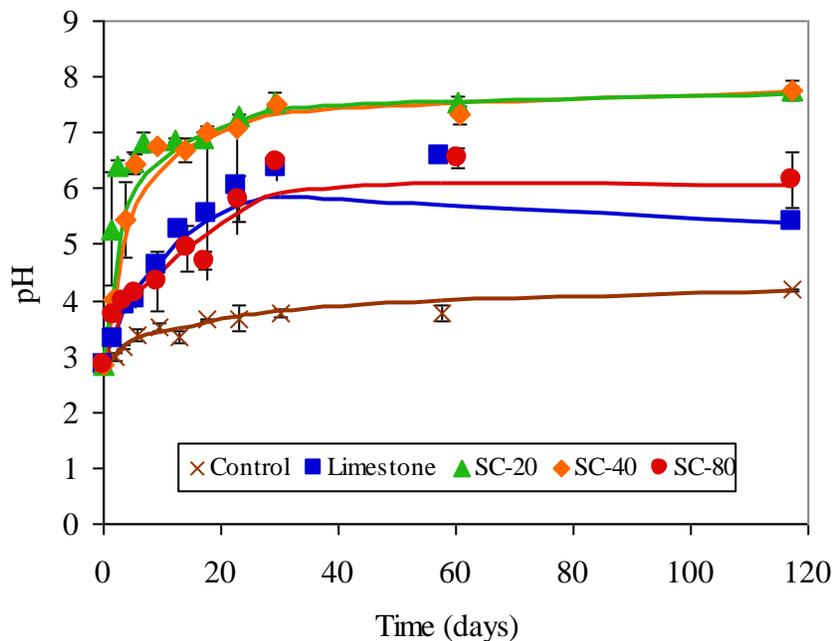


Figure 1. Effect of crab-shell purity on pH in MIW microcosms over time. Data points are duplicate averages; error bars represent one standard deviation; lines are running averages of interpolated data.

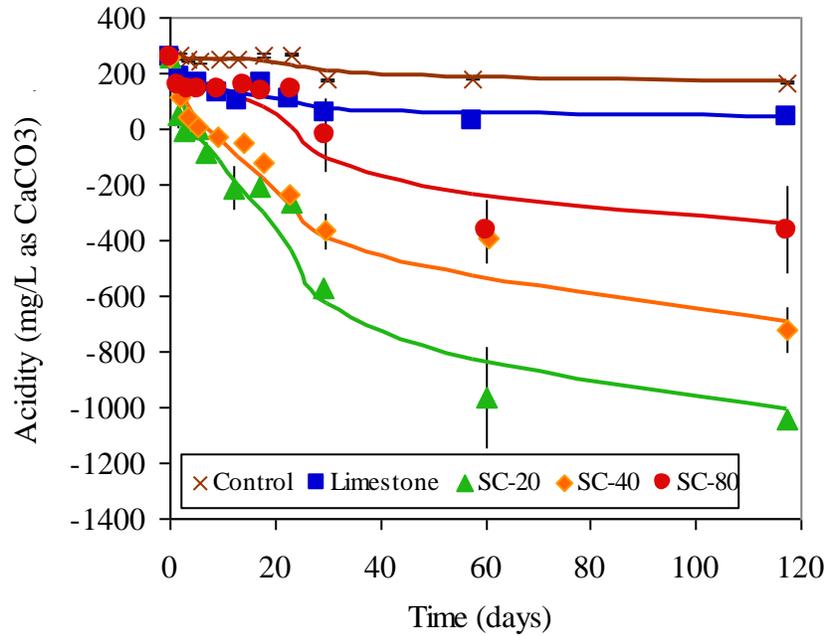


Figure 2. Acidity in MIW microcosms treated with varying purities of crab-shell chitin over time. Data points are duplicate averages; error bars represent one standard deviation; lines are running averages of interpolated data.

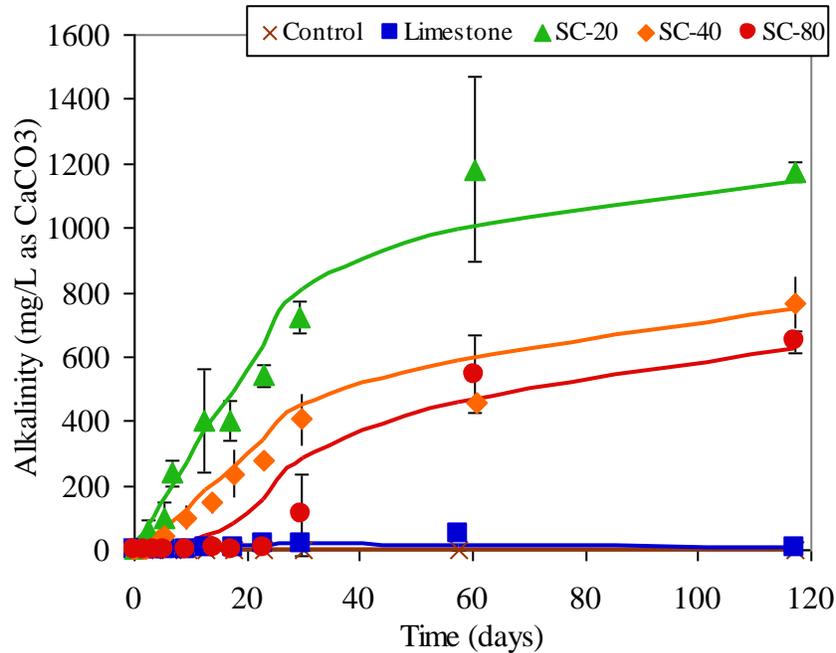


Figure 3. Alkalinity in MIW microcosms treated with varying purities of crab-shell chitin over time. Data points are duplicate averages; error bars represent one standard deviation; lines are running averages of interpolated data.

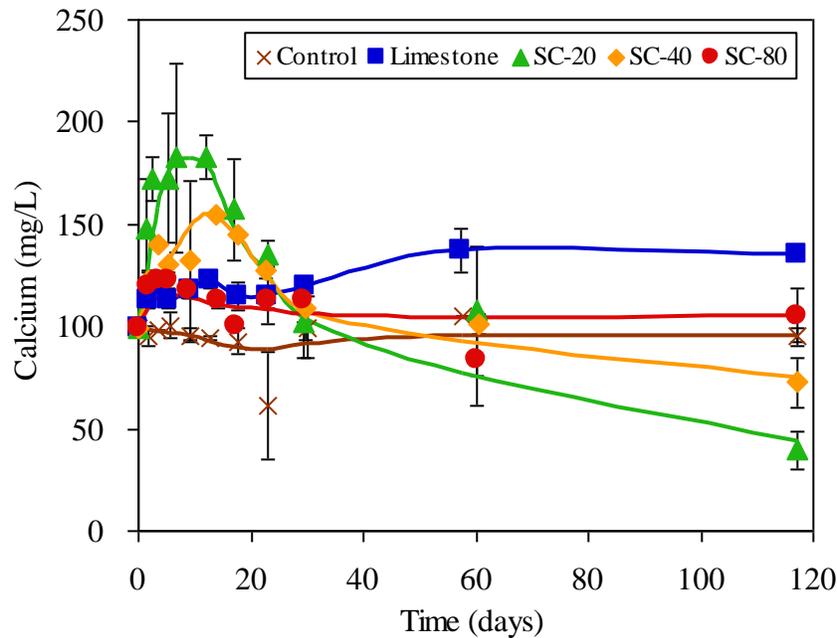


Figure 4. Dissolved calcium ions in MIW microcosms treated with varying purities of crab-shell chitin over time. Data points are duplicate averages; error bars represent one standard deviation; lines are running averages of interpolated data.

Table 2. Water chemistry data from microcosm tests showing the effectiveness of different purities of crab-shell chitin in treating MIW sampled from Kittanning Run in Altoona, Pennsylvania. Values shown are duplicate averages taken on the final sampling point at t = 117 days, except for the Raw Water which was measured at t = 0.

	Raw water	Control, no chitin	Limestone	SC-20	SC-40	SC-80
pH	2.84	4.19	6.57	7.75	7.76	6.15
Hot acidity (mg/L as CaCO ₃)	258.2	167.5	27.1	-1043.5	-718.6	-361.4
Alkalinity (mg/L as CaCO ₃)	0.0	0.0	47.9	1175	767.9	645.6
Aluminum (mg/L)	10.9	9.78	<0.05*	<0.05*	<0.05*	0.18
Iron (mg/L)	18.9	42.5	13.0	<0.05*	<0.05*	1.90
Zinc (mg/L)	0.59	0.89	<0.05*	<0.05*	<0.05*	<0.05*
Manganese (mg/L)	19.8	17.6	21.2	0.32	1.30	15.7
Calcium (mg/L)	99.0	95.2	135.0	44.0	75.0	105.6
Ammonia (mg/L as N)	0.0	0.0	0.0	57.7	12.1	29.9
Sulfate (mg/L)	1480.3	1247.6	890	9.0	187.3	11.2

*Below detection limit

Metals

In general, as pH increased, dissolved metal concentrations in microcosms treated with chitin decreased (Table 2). The profile shown for Al (Fig. 4) is typical for most of the metals, including Fe (Fig. 5) and Zn (Table 2), with SC-20 exhibiting the fastest removal, followed closely by SC-40, and later by SC-80 and limestone. For Al, Fe, and Zn, SC-20 reduced dissolved concentrations to below their detection limit (0.05 mg/L) within 2 – 3 days, whereas the same removal took 5 days for SC-40, and 30 – 60 days for SC-80. Although treating with limestone effectively removed Al and Zn to below the detection limit within 30 – 60 days, it was less effective with iron, leaving 13.0 mg/L remaining after 117 days. Manganese removal followed a different trend altogether (Fig. 6). SC-20 and SC-40 removed 98.4% and 93.4% of the initial Mn, respectively, but it took 117 days. Manganese removal was not effective with SC-80 or limestone, however (Fig. 6). Control bottles that did not contain crab-shell chitin or limestone demonstrated little or no reduction in dissolved metals concentrations (Table 2).

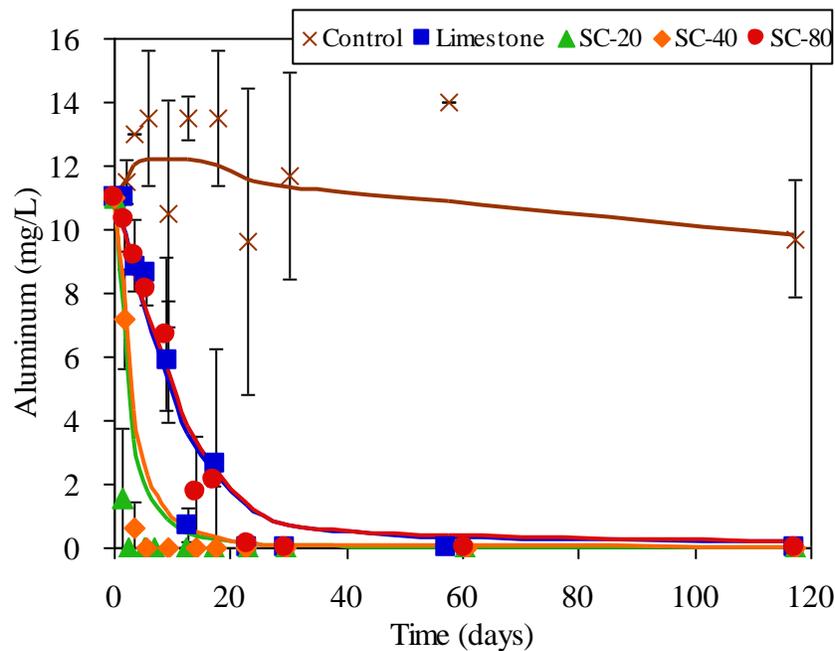


Figure 4. Dissolved Al in MIW microcosms treated with varying purities of crab-shell chitin over time. Data points are duplicate averages; error bars represent one standard deviation; lines are running averages of interpolated data.

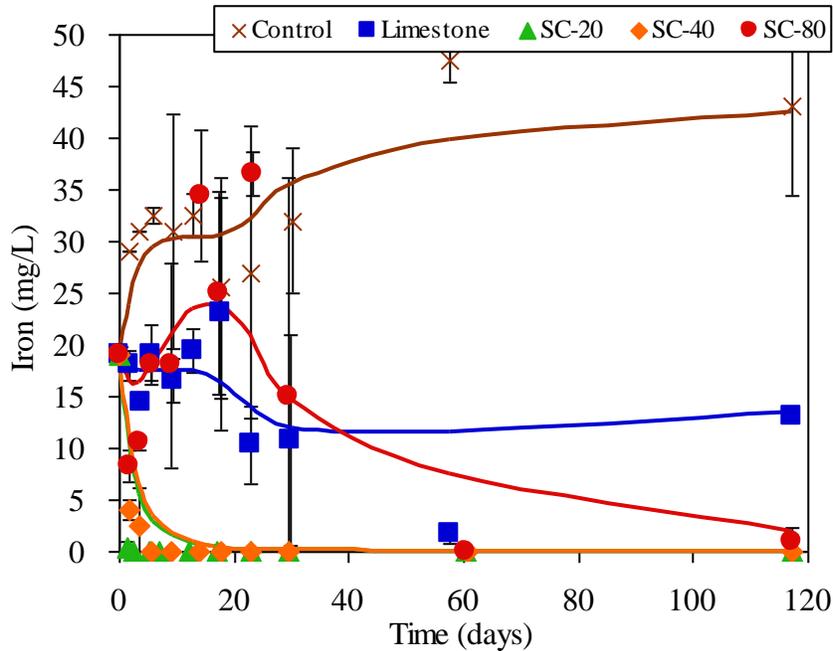


Figure 5. Dissolved iron in MIW microcosms treated with varying purities of crab-shell chitin over time. Data points are duplicate averages; error bars represent one standard deviation; lines are running averages of interpolated data.

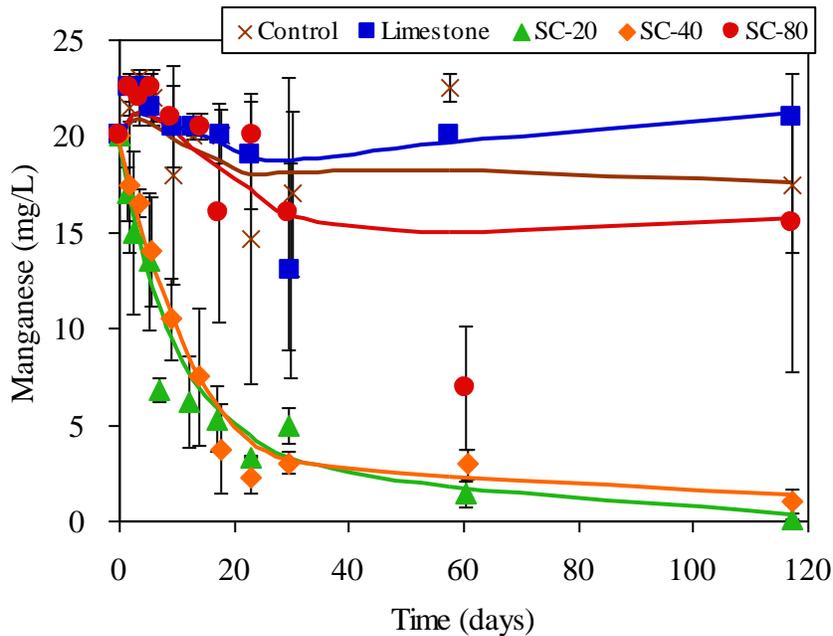


Figure 6. Dissolved manganese in MIW microcosms treated with varying purities of crab-shell chitin over time. Data points are duplicate averages; error bars represent one standard deviation; lines are running averages of interpolated data.

Sulfate and ammonia

Sulfate concentrations in microcosms treated with chitin were observed to decrease over time in comparison to controls (Fig. 8), with the fastest and most complete reduction observed for SC-20, followed by SC-40, SC-80, and limestone. An accumulation of black precipitate, presumably ferrous sulfide, was also noted in bottles containing chitin. Although not directly measured in this experiment, a corresponding increase in sulfide concentrations during SO_4^{2-} reduction in the presence of chitin has been noted previously in our laboratory (Robinson-Lora and Brennan, 2008). These observations indicate that conditions in the microcosms were sufficiently reducing to allow biological sulfate reduction to occur.

Ammonia production was most pronounced in microcosms containing the protein-rich SC-20 (Fig. 9), reaching a maximum value of 95 mg/L as N after 12 days, and then decreasing to an average 45.3 mg/L as N for the remainder of the experiment. Ammonia levels in bottles containing protein-stripped SC-40 remained relatively stable at an average concentration of 7.6 mg/L as N. Ammonia concentrations in bottles containing SC-80 were initially below detection for the first 23 days, and then increased to an average 19.2 mg/L as N for the latter part of the experiment.

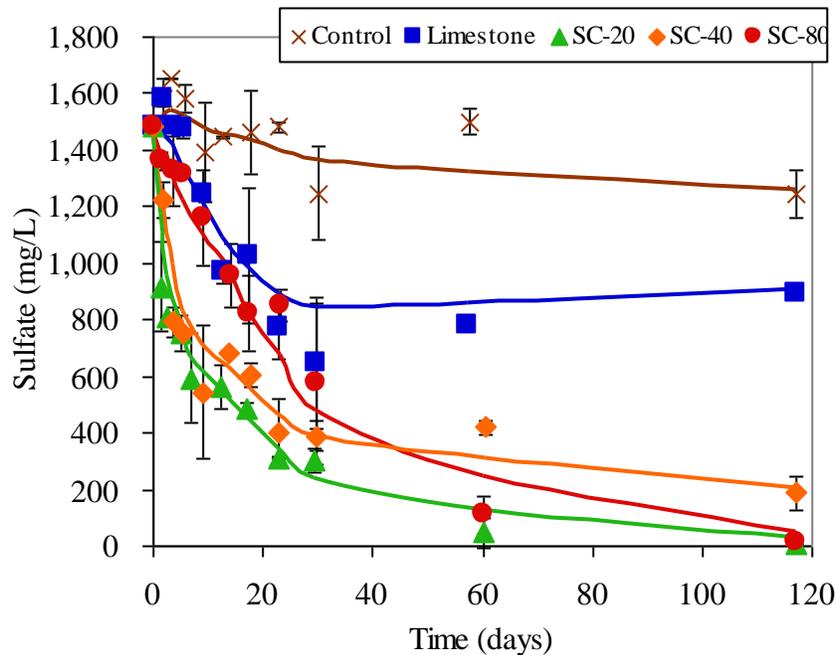


Figure 8. Sulfate in MIW microcosms treated with varying purities of crab-shell chitin over time. Data points are duplicate averages; error bars represent one standard deviation; lines are running averages of interpolated data.

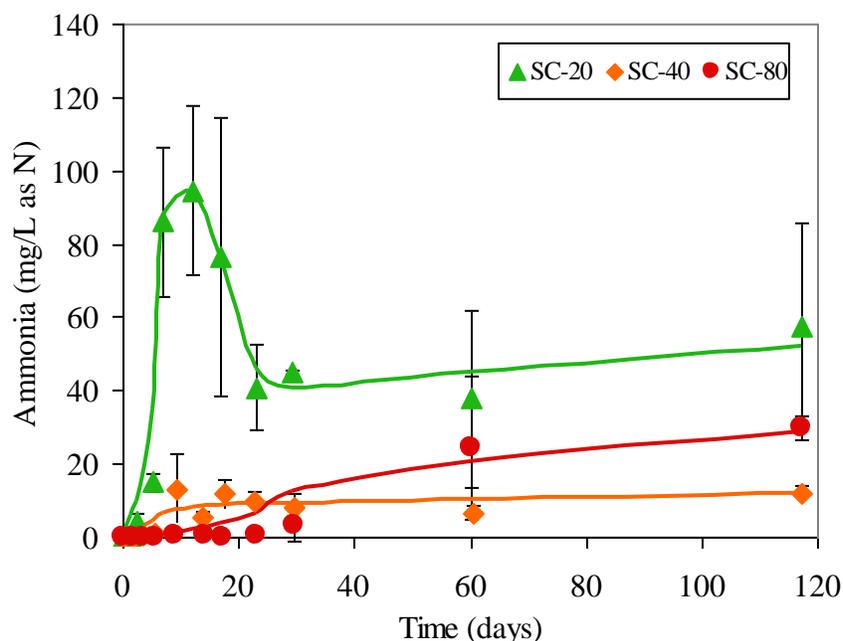


Figure 9. Ammonia in MIW microcosms treated with varying purities of crab-shell chitin over time. Ammonia concentrations were below detection in microcosms treated with limestone and in the controls (not shown). Data points are duplicate averages; error bars represent one standard deviation; lines are running averages of interpolated data.

Discussion

The dominant changes in water chemistry during the course of treatment in this experiment were likely caused by the dissolution of CaCO_3 from the crab shells and from limestone, and to a lesser extent, by the activity of SO_4^{2-} reducing bacteria (SRB). As CaCO_3 is rapidly released from the SC-20, SC-40, and the limestone chips, it reacts with the acidic MIW to form CO_2 , which in turn reacts with more CaCO_3 to form bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) ions. The high rate of CaCO_3 dissolution (Fig. 4) combined with the release of NH_4 from protein (Fig. 9) likely contributed to the high alkalinity observed in the SC-20 bottles (Fig. 3). Lower alkalinity in the limestone controls relative to the SC-40 bottles may be due to lower rate of CaCO_3 dissolution from the limestone chips relative to the rapid rate of CaCO_3 dissolution from the crab shell. Although the same mass of limestone chips and crab shells were added to the microcosms, and the overall particle size of limestone chips was approximately the same as that of the crab shell pieces, the limestone may have had a smaller surface area to volume ratio, and therefore was not able to dissolve as much CaCO_3 as the crab shell.

Another contributor to changes in water chemistry in this experiment is the activity of SRB. As SRB reduce SO_4^{2-} to hydrogen sulfide, HCO_3^- is formed (Luptakova. and Kusnierova, 2005). For every mole of SO_4^{2-} reduced, one mole of alkalinity is produced, with a corresponding increase in pH. The combined dissolution of CaCO_3 from the crab shell and limestone, the release of NH_4 from protein, and the formation of HCO_3^- by SRB, all increased the pH and alkalinity of the water, which contributed to the precipitation of metals.

Metals

In general, as the pH rose during the course of treatment, dissolved metals concentrations decreased. The solubilities of Al, Fe, Zn, and Mn cations and the complexes they form are dependent on the pH of the system. Generally, as the pH increases, the solubility of metal compounds decreases, forcing them to precipitate out of solution. Very rapid and similar rates of metals removal were observed for SC-20 and SC-40, indicating that the rapid increase in pH and dissolution of CaCO_3 from the crab shell was likely facilitating the precipitation of Al, Fe, and Mn. Geochemical modeling performed in our laboratory indicates that the likely precipitating minerals for Al, Fe, and Mn in the early stages of treatment with crab-shell chitin are hercynite/diaspore ($\text{FeAl}_2\text{O}_4/\text{AlOOH}$), hematite/magnetite ($\text{Fe}_2\text{O}_3/\text{Fe}_3\text{O}_4$), and rhodochrosite (MnCO_3), respectively. At later times, when sulfate reducing bacteria became active, Fe is most likely precipitating as pyrite (Fe_2S).

The lag in metals removal for SC-80 is reflective of its lack of CaCO_3 and the time required to establish a strong microbial community capable of reducing sulfate and producing alkalinity. In the case of SC-80, more research will need to be done to determine whether metals precipitated as sulfides or carbonates. The similarity in SC-80 and limestone metal-removal behavior may just be a coincidence, since these two materials do not share any similar characteristics.

Another potential mechanism for metals removal is sorption. Chitin itself is an excellent physical sorbent, and has been shown to be effective for removing metals like Al, arsenic, Cr, Cu, Fe, Mn, Ni, and Zn from aqueous solutions, especially at low pH (Hawke et al., 1991; McAfee et al., 2001; Franco et al., 2004; Vijayaraghavan et al., 2005). Our previous work indicated that Fe sorption to crab-shell chitin was likely occurring at early times, while pH was still low and before alkalinity and sulfide were high enough to allow for Fe removal as a precipitate (Daubert and Brennan, 2008). Ongoing sorption studies and geochemical modeling

in our laboratory are focused on quantifying the mechanism(s) for metals removal in crab-shell-supported MIW treatment systems.

Ammonia

The elevated spike in NH_3 from SC-20 at early times confirms that it is being released from the easily-fermentable protein component, rather than the chitin component, of the crab shell. The gradual increase in NH_3 production from the SC-80 at latter times may be due to the fermentation of chitin. Our preliminary data (not shown) indicates that NH_3 can be stripped from solution at circum-neutral pH, suggesting that air sparging may be a viable solution for removing high NH_3 concentrations from waters that have been treated with SC-20. After the protein has been removed from the SC-20 through fermentation, air sparging may be discontinued. In future work, a cost analysis comparing the less expensive SC-20 combined with air sparging versus treatment with a more expensive, refined grade of crab-shell chitin, will be developed to help guide the selection of the most appropriate substrate for MIW treatment.

Conclusions

In MIW microcosms treated with varying purities of crab-shell chitin and limestone, the following observations are noted:

- SC-20 (chitin + protein + CaCO_3) promotes the most rapid removal of acidity, SO_4^{2-} , and metals.
- SC-20 and SC-40 (chitin + CaCO_3) are approximately equivalent in terms of metals removal.
- SC-80 (chitin only) and limestone (CaCO_3) require much longer incubation times for the removal Al, Fe, and Zn, and are not effective for the removal of Mn.
- It is the protein, not chitin, in SC-20 that is responsible for elevated NH_3 levels during the initial phases of treatment. Air sparging effectively removes this NH_3 without further pH adjustment.

The results of this investigation indicate that SC-20 crab-shell chitin shows promise as an alternative substrate for MIW remediation due to its treatment efficiency, availability, and low

cost. A cost analysis should be conducted to determine the relative efficiency of MIW treatment using SC-20 with air sparging vs. SC-40.

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