# THE ROLE OF PRECIPITATION AND BIOSORPTION IN THE ABIOTIC REMOVAL OF MANGANESE WITH CRAB-SHELL CHITIN[1]

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**Abstract.** This study evaluated manganese removal using crab-shells (SC-20) under abiotic, anoxic conditions. Tests were conducted to assess the contributions of each of the components of SC-20 (chitin, proteins, and minerals) to the observed changes. Manganese removal due to *precipitation* was evaluated in closed-system tests using raw or deproteinized SC-20. Manganese removal due to *adsorption* onto the organic components of SC-20 (chitin and protein) was evaluated under different pH conditions using sorption isotherms.

In closed-system precipitation tests with a contact time of 72 h and an initial manganese concentration ($Mn_o$) of 10 – 250 mg/L, removals of ~60 to >95% were obtained. Manganese removal decreased with increasing $Mn_o$ and could be attributed to the precipitation of rhodochrosite ($MnCO_3$) and/or $MnHPO_4$. Significant increases in pH (from pH 3 to pH 7.7 – 9.9) and alkalinity (from 0 to 55 – 525 mg $CaCO_3$/L) were also observed as a result of the dissolution of chitin-associated minerals. These changes appear to be promoted by the relatively large surface area and distinct composition of SC-20, including phosphates and soluble organic compounds.

The equilibrium of adsorption was well described by the Langmuir model. The maximum sorption capacity ($q_m$) depended greatly on the system's pH, with negligible sorption at pH < 5. At higher pH regimes, $q_m$ ranged from 0.165 to 0.981 for chitin. When both chitin and protein were present, $q_m$ increased 5 – 7 times, suggesting that chitin-associated proteins offer additional sorption sites for manganese.

Results demonstrate that Mn removal with SC-20 is achieved by a combination of the reactivity of chitin-associated minerals and the sorption capacity of chitin and its associated proteins. These characteristics could be easily exploited in polishing systems for the removal of the historically difficult Mn in mine impacted waters.

**Additional Key Words:** acid mine drainage, passive treatment.

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## Introduction

Manganese (Mn) is a common contaminant found in mine impacted waters (MIW) derived from coal and metal mining (Hallberg and Johnson, 2005). Manganese concentrations can vary considerably in untreated MIW, from < 1 mg/L to hundreds of mg/L, with an average of 10 – 25 mg/L (USEPA, 2008). Although it is typically found at lower concentrations in MIW and has a lower toxicity than most of its other metal co-contaminants, Mn still affects the appearance, taste, and odor of water. For these reasons, discharges from mining activities to surface water in the USA must comply with National Pollution Discharge Elimination System (NPDES) of the Clean Water Act, which limits the monthly average of dissolved Mn concentration in the effluent to 2 mg/L.

Numerous studies have reported promising results using waste materials like animal manure, vegetable compost, sawdust, and leaf mulch (Johnson and Hallberg, 2005; Gibert et al., 2002). However, among the possible contaminants found in MIW, Mn has been found notoriously difficult to remove (Johnson and Younger, 2005; Sibrell et al., 2007). Most treatment systems for Mn removal rely on its oxidation followed by its precipitation as manganese oxide ($MnO_2$). This requires highly alkaline conditions since abiotic and biological oxidation rates are slow for pH < 8.0. In addition, Mn oxidation can also be inhibited by the presence of iron (Fe, Johnson and Younger, 2005), the most prevalent metal contaminant in coal mining discharges. Under reducing conditions, partial Mn removal has been reported and attributed to its precipitation as rhodochrosite ($MnCO_3$, Waybrant et al., 1998).

Recent studies have shown the high efficiency of crab-shell chitin in removing Mn under reducing conditions in both laboratory and field tests (Daubert and Brennan, 2007; Korte et al., 2008; Robinson-Lora and Brennan, 2009a; Venot et al., 2008). The efficient acidity and metal removal has been attributed to the dissolution of chitin-associated carbonates, which are naturally present in the shells of crabs and other crustaceans to provide structural strength (Percot et al, 2003). In addition, adsorption has been identified as an important method of metal sequestration, especially under moderately acidic conditions. Although biosorption could be a transient phenomenon, results show a great sorption capacity for a variety of substrates, and some authors have recently suggested that it be used as the sole mechanism for remediation of MIW. Sorption onto the organic substrate or onto Al-Fe-(oxy)hydroxides has been observed at

initial stages of continuous treatment (Webb et al, 1998; Willow and Cohen, 2003; Gibert et al, 2005; Neculita et al, 2007). Promising results have been reported using inexpensive sorbents like fly ash, red mud, pine bark, bentonite, zeolites (Zoumis et al, 2000), palm fruit bunch, maize cob (Nassar et al, 2004), vegetable compost (Gibert et al, 2005), lignite (Mohan and Chander, 2006), and rice husk (Chockalingam and Subramanian, 2006). Utgikar et al (2000) also suggested applying metal adsorption as a preliminary step to decrease metal concentrations prior to biological sulfate reduction to prevent microbial inhibition due to metal toxicity.

The aim of this study was to evaluate the role of chitin and its associated minerals and proteins in the removal of Mn from MIW under abiotic and anoxic conditions. The performance of four different purities of crab shell (raw, deproteinized, demineralized, and demineralized/deproteinized) was assessed. This study was conducted in an effort to develop a better understanding of the mechanisms that drive the observed changes in systems treated with a complex material, such as crab-shell chitin, where oxidation of this metal is limited. We focused on the removal of Mn, since this metal has shown to be unusually difficult to remove in most conventional treatment systems.

## Materials and methods

Chemicals

All chemicals used in this study were reagent grade or better. Ultra High Purity argon gas (UHPAG) was purchased from MG Industries (Malvern, PA). ChitoRem® SC-20 (minimally processed crab shell), derived from Dungeness crab (JRW Bioremediation, LLC, Lenexa, KS), was used as an example of chitinous material. Raw SC-20 was rinsed with deionized water to remove readily soluble salts and dried overnight at 50°C. The obtained material (R-SC20) was sieved (No. 7 and 140) to remove big particles (>2.80 mm) and fines (< 0.106 mm). Three additional grades were obtained by removing chitin-associated proteins, or minerals, or both. Deproteination and demineralization were conducted according to protocols described in previous studies (Percot et al., 2003), using 1 N NaOH and HCl, respectively. The completion of the deproteination reaction was followed by measuring the absorbance of the supernatant at 280 nm, indicated by the attainment of a maximum plateau. The completion of the demineralization reaction was followed by measuring the pH of the supernatant until the pH remained acidic and stable after a new addition of the hydrochloric acid solution. Particles were

then washed with deionized water until neutrality of the rinsate, and dried and sieved as above to produce DP-SC20 (deproteinized material), DM-SC20 (demineralizaed material), and DMP-SC20 (demineralized and then deproteinized material).

<u>Characterization of particles</u>

The chemical composition of all solids was determined by lithium metaborate fusion, followed by ICP-AES analyses (Perkin-Elmer Optima 5300) at the Materials Characterization Laboratory at The Pennsylvania State University. Rock standards were used to calibrate the results. The total carbon and nitrogen of the obtained solids were analyzed by combustion, using a Fisons NA 1500 Elemental Analyzer, at the Agricultural Analytical Services Laboratory at The Pennsylvania State University. The surface area of the solids was measured by physical adsorption of $N_2$ and calculated using the BET (Brunauer, Emmett and Teller) method with a Micrometrics Instrument Corporation ASAP 2020. Mineralogical composition of R- and DP-SC20 was determined by powder XRD (Scintag, Inc., Cupertino, CA). The morphology of the solids were observed using a FEI Quanta 200 environmental scanning electron microscope (ESEM).

<u>Synthetic MIW (SMIW) solutions</u>

Synthetic MIW (SMIW) was used in all tests to limit the affect of other metals (likely present in natural MIW) on the results. SMIW solutions were prepared using $Na_2SO_4$ (1.4 g/L or 10 mM) as the background salt. Appropriate amounts $Mn(NO_3)_2$ were added to generate single metal solutions at the desired Mn initial concentrations. The initial pH was adjusted to the desired value by adding appropriate amounts of 1 N $H_2SO_4$ or 1N NaOH. Prior to use, solutions were deaerated with UHPAG for 2 – 3 h and equilibrated in an anaerobic chamber (Coy Laboratory Products, Inc., Grass lakes, MI) for at least 24 h.

<u>Manganese precipitation experiments</u>

Closed systems were used to evaluate the precipitation-driven Mn removal using R-SC20 and DP-SC20 under different conditions. Systems were prepared in glass centrifuge tubes, inside an anaerobic chamber. Filtered (0.2 μm), anoxic SMIW (40 ml) was mixed with predetermined amounts of R-SC20, DP-SC20, or limestone. A fixed solid load of 5 g/L (R-SC20 or DP-SC20 only), was used to treat SMIW with initial Mn ranging from 10 to 250 mg/L. Systems were prepared in triplicate, with duplicate blanks (without solid addition). Tubes were sealed and

continuously stirred on an orbital shaker for 72 h.  The 72-h contact time was chosen based on preliminary tests conducted in our laboratory to ensure that all chemical transformations in the systems were completed.  After the contact time was elapsed, the tubes were opened and the pH of the solution was measured inside the anaerobic chamber.  In addition, two subsamples were taken and filtered (0.2 μm).  One of the subsamples was promptly (<6 h) analyzed for alkalinity and the other was preserved with 1.5 ml/L conc. $HNO_3$ for analyses of dissolved metals and P.

Manganese sorption experiments

Manganese sorption onto DM- and DMP-SC20 was evaluated in closed systems, as a function of pH (pH 5 to pH 9) and Mn concentration (0.5 to 250 mg/L).  Systems were prepared in duplicate inside an anaerobic chamber, by mixing 20 ml of filtered (0.2 μm), anoxic SMIW with 0.1 g of DM- or DMP-SC20 in acid-washed glass vials.  Blank systems (without the addition of solids) were prepared in singlet.  As in the precipitation tests, vials were sealed and continuously stirred on an orbital shaker for 72 h, and then opened and the pH of the solution immediately measured inside the anaerobic chamber.  Samples were filtered (0.2 μm), diluted (1:10), and preserved in acidified-anoxic deionized water (2 ml/L conc. $HNO_3$) for Mn analyses.

Analytical methods

Electrodes were used to measure pH (Accumet® BASIC, AB15 connected to a Thermo-ORION pH probe).  Alkalinity was measured by titrations with 0.02 N $H_2SO_4$ according to the procedure described in Standard Method 2320B (titration end point pH 4.5, APHA, 2005).  Dissolved metal, sulfur, and phosphorus concentrations were measured by inductively coupled plasma emission spectrometry (ICP, Leeman Labs PS3000UV) at the Materials Characterization Laboratory at The Pennsylvania State University.  The ascorbic acid standard method 4500-P E (APHA, 2005) was used to measure the concentration of orthophosphate.

Statistical analyses of the collected data were performed using MINITAB® statistical software (Minitab Inc., State College, PA).  The geochemical computer program PHREEQC (Parkhurst and Appelo, 1999) was used to estimate the saturation indexes (SI) of several aluminum, iron, and manganese phases.  Equilibrium constants used in the calculation were taken from the MINTEQA2, version 4.0, database (USEPA, 1999).  The concentrations of nitrate released from the added $Mn(NO_3)_2$ was estimated based on the initial, measured Mn concentration.

<div align="center">

**Results**

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Characterization of solids

Results from the chemical characterization (Table 1) revealed calcium as the main component of R- and DP-SC20 (29 – 38% as CaO), followed by carbon (19 – 23 %) and moderate levels of magnesium (1.8 – 2.5% as MgO), phosphorus (3.7 – 5.0% as $P_2O_5$), and nitrogen (1.4 – 3.9 %). Other elements were present at trace levels (< 1%). In contrast, both DM- and DMP-SC20 are mainly composed of carbon and nitrogen, with almost negligible residues of chitin-associated minerals (Table 1). The measured carbon and nitrogen contents in DMP-SC20 are very close to the theoretical values for pure chitin ($C_8H_{15}O_5N$). The percentage of protein in DM-SC20 can be estimated based on its nitrogen content using Eq. 1 (Percot et al., 2003):

$$\%P = 6.25(\%N - 6.9) \tag{1}$$

where %P is the percentage of proteins, %N is the percentage of nitrogen, and 6.25 and 6.9 correspond to the theoretical percentage of nitrogen in protein and chitin, respectively. Therefore, the percentage of proteins in DM-SC20 corresponds to ~23%.

Table 1. Chemical composition and surface area of the solid materials.

| Sample | R-SC20 | DP-SC20 | DM-SC20 | DMP-SC20 |
|---|---|---|---|---|
| CaO (%) | 29 | 38.4 | 0.03 | 0.06 |
| C (%) | 22.8 | 18.9 | $46.2 \pm 0.8$ | $44.3 \pm 1.5$ |
| N (%) | 3.91 | 1.38 | $10.5 \pm 0.1$ | $6.9 \pm 0.1$ |
| $P_2O_5$ (%) | 3.58 | 4.63 | 0.39 | 0.06 |
| MgO (%) | 1.68 | 2.41 | 0.06 | 0.06 |
| SrO (%) | 0.30 | 0.37 | < 0.01 | < 0.01 |
| SiO2 (%) | <0.05 | 0.34 | <0.05 | <0.05 |
| $Na_2O$ (%) | 0.60 | 0.48 | 0.02 | 0.13 |
| $Al_2O_3$ (%) | 0.20 | 0.09 | 0.13 | 0.09 |
| $Fe_2O_3$ (%) | 0.07 | 0.04 | < 0.01 | < 0.01 |
| MnO (%) | 0.04 | 0.06 | < 0.01 | < 0.01 |
| $K_2O$ (%) | 0.09 | <0.05 | < 0.01 | < 0.01 |
| Surface area ($m^2$/g) | $13.9 \pm 0.1$ | $35 \pm 7$ | $2.0 \pm 1.1$ | $3.0 \pm 0.9$ |

A moderate porosity was measured in R- and DP-SC20 samples, with DP-SC20 having a much higher porosity than R-SC20 (Table 1). In contrast, a very limited and similar porosity was detected in those corresponding to DM- and DMP-SC20. SEM images reveal such differences in the porosity between materials containing chitin-associated minerals (R- and DP-

SC20) and those containing organic components only (DM- and DMP-SC20) (Fig. 1). SEM micrographs also reveal a somewhat similar structure of DM- and DMP-SC20, consisting of a series of stacked layers with limited visible porosity.

Calcite was identified by XRD as the major component of R- and DP-SC20 (data not shown). The source of phosphorus in these chitinous materials, determined after calcination of a sub-sample of R-SC20 (550 °C for 2 h), appears to correspond to hydroxylapatite (data not shown).
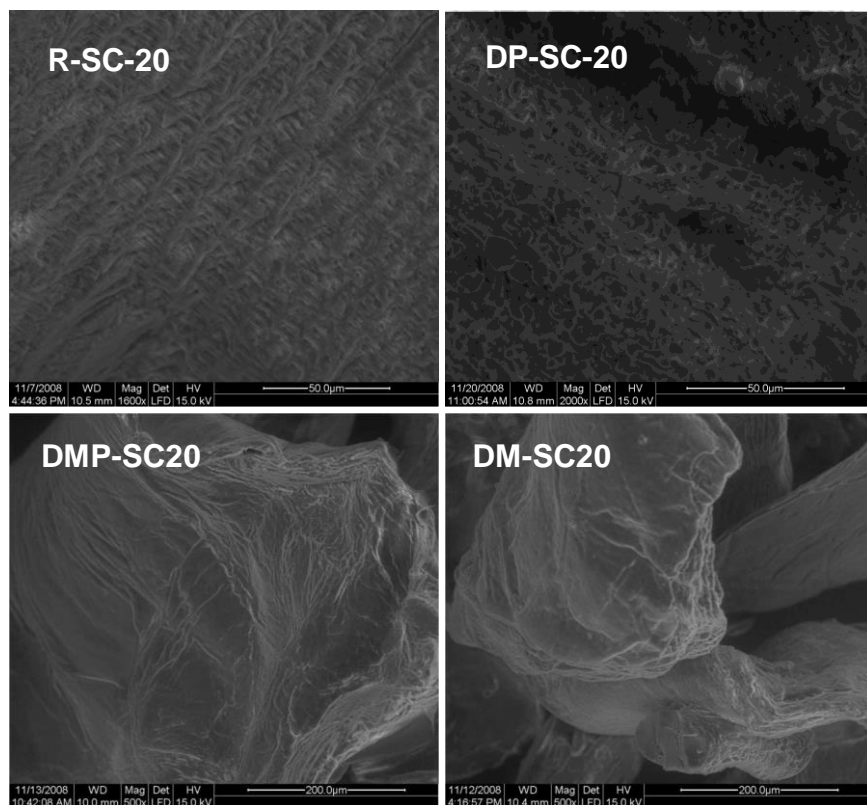


Figure 1. SEM micrographs R- SC20, DP-SC20, DM-SC20 and DMP-SC20.


Closed systems to evaluate total Mn removal due to precipitation

An inverse relationship exists between the initial manganese applied and the observed manganese removal after equilibration with the solids. In systems with initial Mn concentrations of 10 mg/L, >95% removal was achieved with a solid load of only 5 g/L. As the initial manganese concentration in the systems increased, its removal by both DP-SC20 and R-SC20 decreased (Fig 2A). For both solids, manganese removal decreased to ~60% when $Mn_o$ = 250 mg/L.

The dissolution of chitin-associated minerals resulted in significant increases in the pH and alkalinity of the system.  However, increases in the initial manganese concentration also affected the final observed values (Fig. 2B).  After the 72-hour contact time, the pH of systems increased from 3 to 9.5 – 9.9 when $Mn_o$ = 10 mg/L.  In contrast, the observed final pH only reached 7.7 – 7.9 when $Mn_o$ = 250 mg/L.  The opposite effect was observed for alkalinity, depending on the type of solid used (Fig. 2C).  While alkalinity drastically increased with increases of $Mn_o$ in R-SC20-treated systems (from 176 to 525 mg $CaCO_3$/L), a minor decrease was observed in DP-SC20-treated systems (from 83 to mg 55 $CaCO_3$/L).

Mineral dissolution from the evaluated materials also resulted in the accumulation of calcium and phosphorus by the end of the test.  Final Ca concentrations increased with increasing initial Mn concentrations (Fig. 2D).  Calcium concentrations were always much higher with R-SC20 (71 – 234 mg/L) than with DP-SC20 (23 – 41 mg/L).  Phosphorus concentrations did not appear to be affected by the initial concentration of Mn, but they did seem to be dependent on the type of solid used (Fig. 2E).  Higher P concentrations were always measured in R-SC20-treated systems (0.8 – 1.7 mg/L P) than in those treated with DP-SC20 ($\leq$ 0.2 mg/L P).  Measurements using the ascorbic acid spectrophotometric method confirmed that the speciation of the released phosphorus corresponds mainly to orthophosphates (data not shown).

Closed systems to evaluate total Mn removal due to sorption

The extent of manganese removal due to sorption was clearly affected by the type of solid, the pH of the solution, and the initial metal concentration (Fig. 3).  Sorption onto DM-SC20 clearly exceeded that observed for DMP-SC20, suggesting that chitin-associated proteins provide additional sorption sites.  For both types of solids, lower removals were observed as the initial metal concentration increased.  In addition, the extent of Mn uptake by both types of solids was observed to be very low under acidic conditions (pH ~5), and removal did not exceed 8%.  Higher amounts of Mn were removed by both types of solids as conditions changed from acidic to alkaline.  At pH ~9, Mn removal ranged between 26 to 91% for DM-SC20 and between 4 to 38% for DMP-SC20.
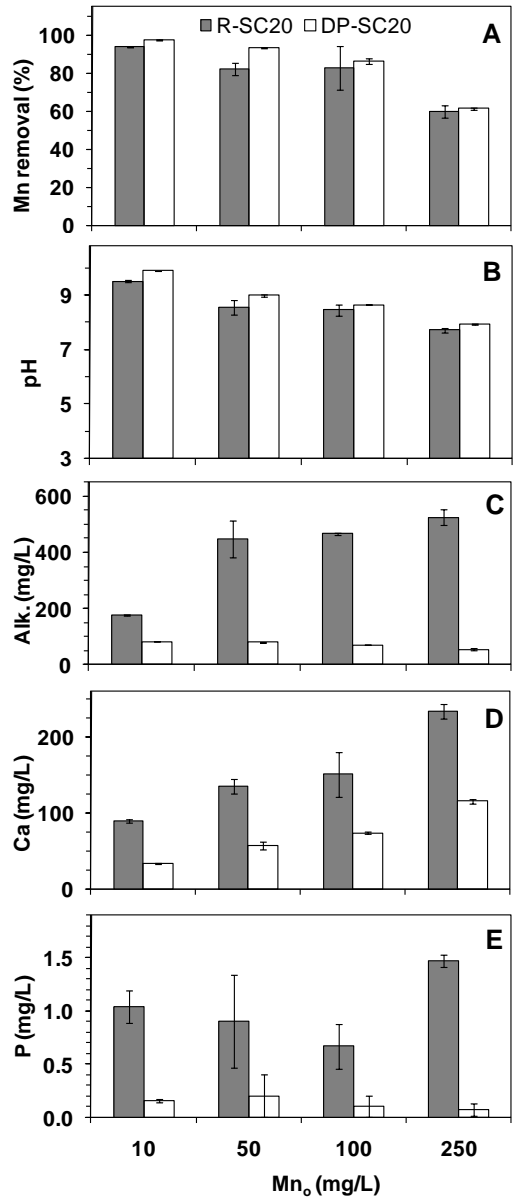
Figure 2. Manganese removal (A) and final pH (B), alkalinity (as $CaCO_3$) (C), calcium (D), and phosphorus (E) concentrations in SMIW systems treated with 5 g/L DP-SC20 and R-SC20. Initial pH = 3.0. Values represent triplicate averages; error bars represent one standard deviation.
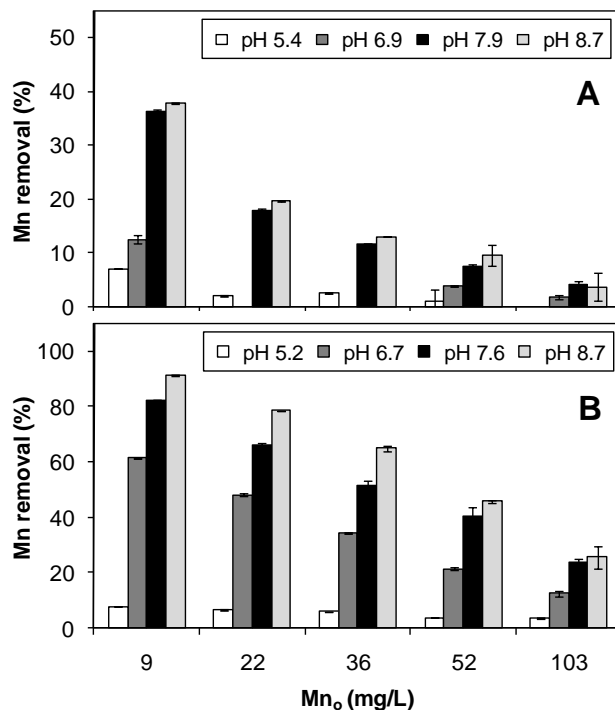
Figure 3. Manganese removal in SMIW systems treated with 5 g/L of (A) DMP-SC20 and (B) DM-SC20 at different pH values. Data points represent duplicate averages; error bars represent one standard deviation.

Manganese sorption isotherms were obtained by calculating the concentration of Mn sorbed onto the solid ($q_e$, mg/g) using Eq.2:

$$q_e = (C_o - C_f)/a \qquad (2)$$

where $C_o$ and $C_f$ are the initial and final concentration of Mn in solution (mg/L), respectively, and $a$ is solid load used (g/L). Sorption data were interpreted in terms of the Langmuir (Eq. 3) and the Freundlich (Eq. 4) sorption models:

$$q_e = q_m \frac{K_L C_e}{1 + K_L C_e} . \qquad (3)$$

$$q_e = K_F C_e^{1/n} \qquad (4)$$

where $q_e$ and $C_e$ are the equilibrium concentration of the adsorbate on the solid (mg/g) and in the liquid phases (mg/L), respectively; $q_m$ is the maximum adsorption capacity (mg/g) according to the Langmuir model; $K_L$, $K_F$, and $n$ are constants. The obtained correlation coefficients (Table 2) indicate a good agreement between the data and the two models. However, the Freundlich fitted curves result in higher standard errors ($s_\varepsilon$) and over-predict the sorption capacity of the solids at

high metal concentrations. The dependence of Mn uptake on pH is clearly reflected in the calculated maximum adsorption capacity ($q_m$). A 30-fold decrease in the hydronium ion concentration resulted in a 3-fold increase of $q_m$ under slightly acidic conditions (i.e. from pH 5.2 to pH 6.7). In contrast, under slightly alkaline conditions, a ten-fold decrease in the hydronium ion concentration (i.e. from pH 7.6 to pH 8.6) resulted in only a 10% increase of $q_m$. In addition, for the same pH regime, the adsorption capacities of DM-SC20 were 5.3 – 7.3 times higher than those calculated for DMP-SC20.

Table 2. Results obtained from the fitting of the isotherms to Langmuir and Freundlich adsorption models.

| Solid | pH | Langmuir model | | | | Freundlich model | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | $q_m$ | $K_L$ | $r^2$ | $s_e$ | $K_F$ | $n$ | $r^2$ | $s_e$ |
| DMP-SC20 | 5.4 | 0.165 | 0.085 | 0.995 | 0.011 | 0.008 | 1.268 | 0.849 | 0.036 |
| | 6.9 | 0.446 | 0.120 | 0.998 | 0.023 | 0.058 | 2.133 | 0.891 | 0.080 |
| | 7.9 | 0.900 | 0.307 | 0.857 | 0.054 | 0.198 | 2.631 | 0.871 | 0.201 |
| | 8.7 | 0.981 | 0.440 | 0.876 | 0.058 | 0.252 | 2.877 | 0.873 | 0.279 |
| DM-SC20 | 5.2 | 0.878 | 0.016 | 0.946 | 0.151 | 0.022 | 1.370 | 0.950 | 0.340 |
| | 6.7 | 3.271 | 0.133 | 0.980 | 0.395 | 0.353 | 1.920 | 0.913 | 1.046 |
| | 7.6 | 4.972 | 0.340 | 0.970 | 0.333 | 0.863 | 2.282 | 0.942 | 1.719 |
| | 8.6 | 5.437 | 0.439 | 0.963 | 0.298 | 1.279 | 2.697 | 0.908 | 1.473 |

## Discussion

Manganese removal due to precipitation

Oxidation is the most common strategy for Mn removal from aqueous systems. This requires high pH since abiotic and biological rates are slow for pH < 8.0. Previous researchers have also reported poor Mn removal in the presence of Fe (Johnson and Younger, 2005). In the present study, all experiments were performed in an anaerobic chamber. Therefore, Mn oxidation can be ruled out regardless of the high pH values reached at early times. In addition, Mn oxidation is usually characterized by the formation of black precipitates, which were not observed in the present study.

Calculated saturation indices indicate the possible precipitation of several minerals (Fig. 4). All systems appear to be supersaturated with respect to rhodochrosite ($MnCO_3$) and manganese

hydrogen phosphate ($MnHPO_4$). Manganese carbonates such as $MnCO_3$ and kutnahorite ($MnCa(CO_3)_2$) have been previously reported to occur under reductive conditions (Waybrant et al., 1998), as well as in aerated systems (Bamforth et al., 2006). On the other hand the precipitation of manganese carbonates is kinetically regulated, allowing rhodochrosite-supersaturated solutions (SI = 2 – 5) to remain metastable for at least 72 h (Lebron and Suarez, 1999); a time frame that is comparable with the residence time of many types of passive treatment systems (Ziemkiewicz et al., 2003). Therefore, it is possible that the poor Mn removal often observed in limestone systems is not due to insufficient alkalinity/carbonate provision, but rather to slow kinetics. In contrast, it is possible that the significantly higher concentrations of calcium and carbonates released from the chitin-associated calcite in R- and DP-SC20 treated systems led to their much higher levels of rhodochrosite-supersaturation. Such high supersaturation could be responsible for a faster precipitation of the newly formed Mn minerals.
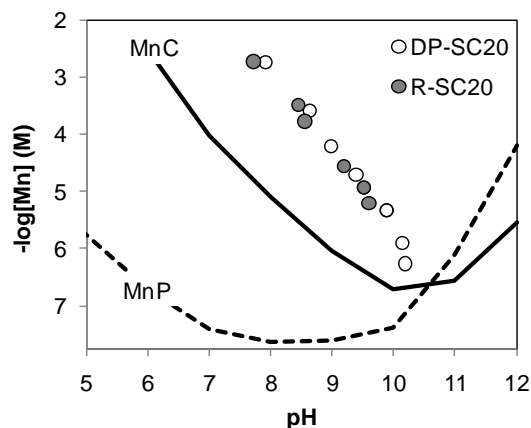


Figure 4. Relationship between final pH and final concentration of Mn in systems for precipitation tests (72 h contact time). Lines represent the concentration of the element in equilibrium with rhodochrosite (MnC) and MnHPO4 (MnP). Calculations for rhodochrosite assumed a total C concentration of 1 mM.

The alternative sink for manganese in the systems treated with either DP-SC20 or R-SC20 is precipitation as manganese hydrogen phosphate ($MnHPO_4$). This highly insoluble mineral has been reported to occur when hydroxylapatite is used for the control of acid drainage (Evangelou, 1995). Removal of Mn supported by the presence of phosphates has also been reported in anaerobic digestors via precipitation (Carliell-Marquet and Wheatley, 2002). In addition, the negatively charged phosphate group can also induce metal chemisorption. A recent study reported an increased adsorption capacity of rice husk for the removal of Pb, Cu, Zn, and Mn

after phosphate pretreatment (Mohan and Sreelakshmi, 2008).   However, geochemical calculations (Fig. 4), indicate that all systems were significantly far from the equilibrium with respect to $MnHPO_4$, despite the high Mn removal.   It is possible that phosphorus had preferentially precipitated as $Ca_4H(PO_4)_3$ limiting the formation of $MnHPO_4$.  For all systems, P concentrations were clearly limited by the precipitation of either of the considered phases (data not shown).  In addition, organic compounds can act as chelating agents, increasing the solubility of metals (Ahumada et al., 2001; Carliell-Marquet and Wheatley, 2002). Therefore, the VFAs and other organics released from the tested materials could also be the cause of the slightly lower Mn removal in systems treated with R-SC20.

Co-precipitation of Ca and Mn could also occur: Sibrell et al., (2007) reported significant removal of Mn via this mechanism in pulsed limestone beds, at pH values below 8.3.  Finally, another plausible explanation for Mn removal is the sorption of Mn onto the calcite surface, as has been previously reported (Zachara et al., 1991).

Manganese removal due to sorption

Manganese adsorption onto DMP- and DM-SC20 was found to be well described by the Langmuir model.  The characteristics of Langmuir adsorption can be interpreted using the dimensionless parameter, $R_L$, called the separation factor (Webber and Chakravorti, 1974).  This parameter is calculated using the Langmuir constant ($K_L$) and the initial concentration of the sorbate ($C_o$) as shown in Eq. 5:

$$R_L = 1/ (1 + K_L C_o) \tag{5}$$

Based on the calculated value, adsorption will be:  unfavorable for $R_L > 1$, linear for $R_L = 1$, favorable for $0 < R_L < 1$, and irreversible for $R_L = 0$.  For this study, $R_L$ values calculated for initial Mn concentrations between 0.5 and 250 mg/L were all between 0.01 and 0.99.  This indicates a favorable adsorption of Mn onto both types of solids.

For all the tested conditions, sorption capacities of DM-SC20 were always greater than those calculated for DMP-SC20.  This clearly suggests that the chitin-associated proteins present in DM-SC20 are greatly responsible for the observed removal of manganese.  In this case, a change from ~100% chitin (in DMP-SC20) to ~1:3 protein-to-chitin content in DM-SC20 leads to an increase of sorption capacity of 5.3 – 7.3 times.  This additional sorption capacity attributed to the chitin-associated proteins is due to the functional groups present in their constituent amino

acids. Several studies have reported the composition of chitin-associated proteins in crustaceans, by means of their amino acid residues (Shahidi and Synowiecki, 1991; Percot et al., 2003; Iijima, 2005). The exact structure of chitin-associated proteins remains somewhat unknown. However, studies report aspartatic and glutamatic acids, histidine, alanine, lysine, tyrosine, and arginine as common constituents of the evaluated organisms. Among these amino acids, two are acidic in character (aspartic and glutamic acids), each providing an extra carboxylic group, while other three are basic (histidine, lysine, and arginine), each providing an extra amine group. Under the conditions at which the tests were conducted (pH 5 – 9), it is expected that the carboxylic groups were deprotonized, while the amine groups were positively charged. It is also likely that, under slightly acidic conditions, the hydronium ions out-competed the manganese in solution, limiting the availability of binding sites. As the activity of the hydronium ions decreased under alkaline conditions, manganese was able to be sorbed onto the available carboxylic and amine groups.

Manganese adsorption onto several natural and synthetic sorbents has been previously evaluated. The reported maximum sorption capacities ranged between 2 and 30 mg/g (Nassar et al., 2004; Vaghetti et al., 2009; Vijayaraghavan et al., 2009). Exceptionally high values are reported for pecan nutshell (98 mg/g) and *Arthrobacter* sp. biomass (406 mg/g, Vaghetti et al., 2009). The occurrence of abundant negatively charged functional groups (i.e. carboxylic acids and phenol) on the surface of these two materials appear to explain their very high cation-sorption capacities. A direct comparison with the results obtained in this study cannot be made since all tests were conducted under different conditions. However, qualitatively speaking, the maximum Mn adsorption capacities of DMP- and DM-SC20 appear to be within the lower end of the reported range. This relatively poor Mn sorption of the evaluated chitinous mateirals also contradicts previous studies where metals like zinc, copper, cadmium, and lead were efficiently removed (Benguella and Benaissa, 2002; Rae and Gibb, 2003). However, according to the Irving – Wiliams order, $Mn^{2+}$ ions have a lower tendency to form complexes than the formerly mentioned transition metals (Stumm and Morgan, 1996). This could explain the relatively low Mn sorption observed here. Mn uptake was indeed the lowest in comparison to Pb, Cu, and Zn when using *Arthrobacter* sp. biomass as sorbent (Vaghetti et al., 2009). In the present study, the presence of elevated sulfate concentrations in the evaluated systems constitutes another factor that could have negatively influenced the Mn sorption. Sulfate was added to the SMIW in an effort to better imitate the typical conditions found in affected streams. However, sulfate-

manganese complexes are suspected to be formed in systems with high levels of sulfate. For example, for a solution with 1000 mg/L of sulfate and 10 mg/L of Mn, thermodynamic calculations indicate that ~27% will exists as $MnSO_{4(aq)}$ and ~73% as $Mn^{2+}_{(aq)}$. Therefore, the occurrence of manganese complexation could have partially inhibited its sorption onto the evaluated chitinous materials.

The present study was conducted to evaluate the individual contributions of chitin and its associated minerals and proteins to manganese removal when using a complex substrate such as crab shell particles. Results from sorption tests indicate that both chitin and protein are able to partially adsorb manganese. The presence of proteins significantly increases the sorption capacity of the material. For field applications, untreated crab-shell particles are the most cost-effective alternative. In this raw material, the proportion of protein-to-chitin has been reported as high as 1.2:1 (Robinson-Lora and Brennan, 2009b), which greatly exceeds the one reported here (~0.3:1). Consequently, higher manganese removal due to sorption onto the protein sorption sites could be expected. On the other hand, the acidic conditions commonly found in MIW might inhibit this sorption process. However, since chitin-associated carbonates have proven to be an excellent source of buffering capacity, the solution pH will rapidly increase. As a result, it can be expected that the chitin-associated proteins will contribute to the removal of manganese, once the pH of the solution is increased due to the dissolution of chitin-associated carbonates.

## Conclusions

Results from the present study demonstrate the important role of chitin-associated minerals (carbonates and phosphates) and proteins in the passive remediation of MIW using crab-shell chitin. Chitin-associated minerals appear to have a great reactivity, resulting in a fast generation of alkalinity and efficient removal of manganese. Although at a smaller scale, chitin-associated proteins represent an alternative sink of manganese due to their sorption capacity under alkaline conditions. In addition, the intricate and complex structure of the crab shells imparted by their three components (chitin + proteins + minerals) results in a relatively high surface area. These characteristics make crab-shell chitin an attractive alternative material for fast alkalinity generation and the treatment of historically difficult Mn-bearing MIW. More research is needed to evaluate the persistence and reversibility of the precipitation and sorption mechanisms, as well as the longevity of the raw material under continuous-abiotic conditions.

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