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Comparison of the efficiency of chitinous and ligneous substrates in metal and sulfate removal from mining-influenced water

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Abstract

Mining-influenced water (MIW) remediation is challenging, not only due to its acidity and high metal content, but also due to its presence in remotely located mine sites with difficult surrounding environments. An alternative to common remediation technologies, is the use of sulfate-reducing bacteria (SRB) to achieve simultaneous sulfate reduction and metal removal in on-site anaerobic passive systems. In these systems, the organic carbon source (substrate) selection is critical to obtaining the desired effluent water quality and a reasonable treated volume. In this study, we evaluated the use of two different substrates: a chitinous product obtained from crushed crab shells, and a more traditional ligneous substrate. We put the substrates, both with and without water pretreatment consisting of aeration and pH adjustment, in anaerobic experimental columns. The treatment with the chitinous substrate was more effective in removing metals (Al, Cu, Fe, Cd, Mn, Zn) and sulfate for a longer period (458 days) than the ligneous substrate (78 days) before suffering Zn breakthrough. The reactors fed with pretreated water had longer operational periods and lower metals and sulfate concentrations in the effluent than those with untreated influent water. Zn was consistently removed to levels <0.3 mg/L for 513 days in the chitinous substrate columns, while levels <0.3 mg/L were maintained for only 140 days in the ligneous substrate pretreated column. The highest sulfate removal rates achieved in this study were in the range of 5–6 mol/m³/d for the chitinous substrate and 1–2 mol/m³/d for the ligneous substrate. Overall, the chitinous substrate proved to be more efficient in the removal of all the aforementioned metals and for sulfate when compared to the ligneous substrate. This could be the determinant when selecting a substrate for passive systems treating acidic MIW, particularly when Zn and Mn removal is necessary.

Keywords

acid mine drainage; zinc; manganese; sulfate reducing bacteria; anaerobic bioreactors; sulfate reduction rate

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1. Introduction

Acidic mining-influenced water (MIW), which is formed due to the biochemical reaction of sulfide minerals in active and inactive mining sites, has been widely found to increase metals and sulfate contaminants in streams and groundwater (Hiibel et al., 2011; Klein et al., 2014; Pinto et al., 2011). Several metals (e.g., Fe, Cu, Al) found in MIW can be removed by precipitation induced by pH neutralization and aeration, but other metals (e.g. Zn, Mn) are more difficult to remove by these processes (Medírcio et al., 2007; Nuttall and Younger, 2000), and may require alternative treatments, such as membrane filtration, ion exchange, adsorption, etc. (Fu and Wang, 2011). These alternative treatments are usually more expensive than traditional treatment systems, and demand the input of chemicals, energy, and supervision for the effective removal of contaminants. Therefore, we pursued the use of a passive system that requires fewer monetary and material resources for metal removal.

Anaerobic bioremediation is one of the passive treatment technologies commonly used to precipitate and separate metals as metal sulfides using sulfate reducing bacteria (SRB) (Al-Abed et al., 2017). Under anaerobic bioremediation, metals removal occurs by 1) precipitation as sulfides, 2) precipitation as carbonates or hydroxides (a consequence of the increase of pH), and 3) adsorption onto the substrates and onto the biomass. In addition to metal adsorption, substrates play other important roles: as carbon and nitrogen sources for biomass growth, as an inoculum source, as air/water exchanging porous medium, and as a neutralizing agent (Neculita and Zagury, 2008). Hence, the substrate amount and composition directly affect the removal efficiency and lifetime of a bioreactor.

The selection of simple and easily degradable substrates, those that have short-chain low molecular weight (e.g., methanol, ethanol, lactate, acetate, etc.), has been successfully tried for sulfate and metals removal. But not all SRB species are able to oxidize lactate or ethanol to CO₂ (Zagury and Neculita, 2007), and they usually demand high amounts of substrate in an active system. Hence, these are unattractive for passive systems, which typically utilize complex substrates such as sawdust, wood chips, hay, alfalfa, manure, or combinations of these without a continuous supply of substrate. In order to oxidize these complex substrates and to produce short-chain carbon compounds (e.g., acetate, etc.), SRBs rely on acidogens and methanogens as they cannot oxidize them by themselves (Neculita et al., 2007).

The use of a mixture of materials, rather than a single material substrate, usually yields better efficiencies due to synergism (Zagury and Neculita, 2007). Manure is frequently used in these mixtures because of its nutrient content, matrix complexity, degradability and low cost, but it is not efficient as a single substrate material because it tends to generate clogs in the system as it is compacted during the operation. Hence, combinations of easily available carbon (as manure) and ligneous materials (e.g., wood chips, hay, sawdust, etc.) are popular choices of substrate to obtain a combination of degradability and porosity in Sulfate Reducing Bioreactors (SRBRs).

Substrates based on ligneous materials have been found by some investigators to be ineffective for Mn removal. For example, ligneous materials combined with calcium carbonate and urea were reported to be ineffective in removing Mn (Zagury et al., 2006;

Zagury and Neculita, 2007). However, Mn removal by precipitation and adsorption in SRBRs has been reported at a pH range of 7.0 to 10 using a ligneous substrate with manure (Logan et al., 2005) and a combination of ligneous substrate, spent mushroom compost, and manure (Vasquez et al., 2016).

Due to these findings, we looked to emerging substrates, such as chitin-based products (crushed crab shells, etc.), as a viable option in SRBRs (Daubert and Brennan, 2007; Robinson-Lora and Brennan, 2010; Venot et al., 2008). Specifically, we looked at crushed crab shells, a chitin-based product, because of its composition: 40% calcium carbonate (neutralizing agent), 30% protein (carbon source), 20% chitin (N-acetylglucosamine polymer that serves as a solid support for the biomass), 7% moisture, and 3% ash (Pinto et al., 2011). The physical, particulate form of crushed chitin needs to be addressed for effective use in SRBRs, as it tends to mat and have very low permeability/hydraulic conductivity after being wetted. Therefore, it needs to be mixed with sand to obtain greater porosity and have the desired range of hydraulic conductivity in SRBRs (Al-Abed et al., 2017; Robinson-Lora and Brennan, 2009).

In pilot-scale chitinous substrate bioreactors with metal and sulfate-laden influent, Venot et al. (2008) found successful removal of Cu (0.03 in the influent to 0.002 mg/L in the effluent), Zn (4.67 to 0.02 mg/L), and Mn (19.3 to 3.77 mg/L). Robinson-Lora and Brennan (2010) compared a crab shell product with lactate to spent mushroom compost (SMC) in sacrificial batch microcosms. They concluded that crushed crab shells were more efficient in metal and SO_4^{2-} removal and in acidity neutralization. Both substrates removed Al, but only the chitinous substrate removed Fe and Mn (Mn removal reached a 73% removal rate likely precipitating as rhodochrosite).

Neculita and Zagury (2008) evaluated maple wood chips, maple sawdust, composted poultry manure, and leaf compost as substrates in anaerobic batch reactors reporting effective removal rates (92–99.8%) for Fe, Ni, Cd, Zn, and Mn. The authors also found that higher C/N ratios and dissolved organic carbon (DOC)/ SO_4^{2-} ratios provided greater sulfate and metal removal. Since it is difficult to compare substrate efficiencies with different variables, Zn and Mn removal efficiencies in chitinous and ligneous substrates should be compared using similar bioreactors and influent water.

The primary objective of this study was to evaluate the long-term effectiveness of a chitin (crushed crab shells) substrate compared to traditional ligneous (wood chips, hay, and manure) substrates on Zn, other metals (Al, Cu, Fe, Cd, Mn), and sulfate removal in MIW under anaerobic column bioreactor conditions. The secondary objective includes the evaluation of aeration and neutralization water pretreatment on the removal of the mentioned contaminants.

2. Materials and Methods

2.1. Mine water collection and pretreatment.

Water collected by the investigators from the Formosa Superfund site in Oregon was used in this investigation. The Untreated Formosa Water (UFW) was collected at the mine site,

transported, and stored under a nitrogen blanket at room temperature using the methods described by Al-Abed et al. (2017). The mine water was pretreated in the laboratory in 15 L batches using mechanical stirring (IKA Model RW20 stirrers, Wilmington, NC) and continuous air purging for 30 minutes. At the 15th minute, ~14.3 mL of 10 N NaOH was added to the bucket for neutralization. After those 30 minutes, the water was allowed to settle for 15 minutes, sand filtered, and labeled as Pretreated Formosa Water (PFW). The metal content was measured before and after the pretreatment (see Table 1).

2.2. Columns fill materials.

The chitinous substrate filling material was comprised of 140 g SC-20 (crushed crab shells product Chitorem SC-20®, JRW Bioremediation LLC, Lenexa, KS) and 460 g quartz sand (Global Drilling Suppliers, Inc., Cincinnati, OH). The lignocellulose substrate filling material was comprised of 252 g ash tree wood chips (locally collected), 17 g hay (locally collected), and 4 g cattle manure (obtained from an Ohio farm). Each component of filling material was characterized for moisture (ASTM Method D2216–10) and elemental composition by Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES) analysis (EPA Method 6010B) after acid digestion using EPA Method 3051. Total Organic Carbon (TOC) was measured using a Shimadzu TOC-VCPH analyzer equipped with a SSM-5000A solid sample module.

2.3. Column bioreactor design, set-up and operation.

Six plexiglass columns (length 101 cm, inner diameter 3.81 cm, volume 1.15 L) were operated in parallel (Fig. S11) with upward flow configuration. The column fillings were selected to compare the substrate performance: Columns 1, 3, and 5 (chitinous columns) contained a sand/SC-20 (3:1) mixture, while Columns 2 and 4 (lignocellulosic columns) contained a wood chips, hay, and cattle manure mixture. Column 6 was filled with sand only (Inert Control). The substrate amounts were determined to keep similar total carbon levels (32 g) in Columns 1–5 (see carbon content of these materials in Table S11).

Three influent reservoirs (11-L glass bottles) were kept in an anaerobic chamber (Coy Laboratory Products, Grass Lake, MI) under continuous stirring conditions (~160 rpm) using PC-410D Corning Plates. Columns 1 (Untreated Chitin) and 2 (Untreated Ligneous) were fed with UFW. Columns 3 (Pretreated Chitin) and 4 (Pretreated Ligneous) were fed with PFW. Columns 5 (Chitin Abiotic Control) and 6 (Inert Control) served as the abiotic controls and were fed with UFW containing sodium azide (FWSA – 1.4 g NaN₃ in 11 L UFW).

After filling, Columns 1–4 were inoculated with 20 mL of an SRB-containing biomass obtained from an SRBR operating in Colorado. Inoculated Columns 1–4 remained stagnant for a period of nine weeks to allow biomass growth prior to the operation. Columns 5 and 6 were filled at the end of the stagnant period, and influent pumping started simultaneously for all.

The operational period started with a flowrate of 80 mL/day, corresponding to a hydraulic retention time (HRT) of ~100 h (calculated for the chitinous columns based on the result of a tracer study). After 113 days of successful Zn removal, the flowrate was increased to 160

mL/day (HRT of 50 h) to determine if that change affected removal efficiencies. There was a gap in data collection of one week at the 441st day due to a facility shutdown. The oxidation reduction potential (ORP) was monitored inside the columns using ORP probes and a Prober-PH8 interface connected to a computer obtaining measurements every 5 minutes. The effluent was collected in Tedlar bags (3-L) from a port near the top of the columns.

The influent and effluent of the columns were analyzed weekly for pH (EPA Method 9040B), alkalinity (EPA Method 310.1), dissolved metals by ICP-AES, anions (including sulfate by EPA Method 300.0 using a Dionex ICS-2000 Ion Chromatograph, Thermo Scientific, MA), sulfide (HACH Method 8131), and ammonia-N (HACH Method 10200 using a HACH DR890 Colorimeter, Hach, Loveland, CO). Visual Minteq 3.1 (Gustafson, 2013) was used to estimate aqueous phase speciation of the columns' aqueous phase.

Additionally, the generated gases were collected through a venting port at the top of each column into a Tedlar bag. The gas volume was measured using a graduated syringe; gas composition was determined using Agilent 6890 GC-TCD (Agilent Technologies, Inc., Loveland, CO).

3. Results and Discussion

3.1. Materials and Formosa water characterization.

Particle size distribution, total carbon content and elemental composition of the chitinous product SC-20 and the sand used in this study have been reported previously (Al-Abed et al., 2017). In brief, the percent organic carbon content in the substrate materials was 41.8%, 37.2%, 19.2%, and 4.89% for wood chips, hay, SC-20, and manure, respectively. The C/N ratio of the chitin polymer was calculated as 6.86 and 1.40 for the protein in crab shell, and considering their composition, the C/N ratio of the chitinous and ligneous substrates were calculated to be 3.58 and 371, respectively. The pH of the UFW was acidic (2.94), and it contained high amounts of Fe, Zn, and sulfate (Table 1). The applied pretreatment increased the pH to ~6.6 and reduced the Al, Cu, and Fe concentrations considerably, but the impact was marginal for Zn removal.

3.2. Formosa water pretreatment.

Since the acidity of the Formosa MIW could prevent metal removal by inhibiting SRB growth, the addition of an alkaline material (e.g., limestone) to the substrate becomes necessary to neutralize the acidity and to cause iron and aluminum precipitation. Wildeman et al. (2014) reported that Fe and Al could interfere with Zn removal in SRBRs. In this study, MIW aeration and neutralization were performed as a pretreatment to compare metal removal performance with the chitinous substrate that naturally contains CaCO₃. The PFW pH was 6.67, and the dissolved oxygen (D.O.) concentration was 4.47 mg/L; Al, Cu, and Fe concentrations were low (<1 mg/L) (Table 1). Zn was partially removed in the Pretreatment process, falling from 73.9 mg/L to 57.3 mg/L from UFW to PFW by precipitation as Zn₄(OH)₆(SO₄) according to Visual Minteq. The Mn concentration decreased minimally from 1.53 mg/L to 1.42 mg/L.

The main metal removal mechanism in this process was precipitation as hydroxides, since NaOH was used for pH adjustment. Despite this positive effect of the pretreatment in the influent characteristics, it is often difficult to perform these operations in seasonally inaccessible mine sites because they require an active system, involving an energy source and/or a reagents supply. Hence, in those sites, it is desirable to have a passive system to perform the entire treatment using a substrate that includes a neutralizing agent (e.g. chitinous materials), the addition of a neutralizing agent (e.g. limestone) into the substrate, or the use of a material with buffering capacity as substrate (e.g. crushed crab shells) to provide a suitable pH for SRB within the system.

3.3. Operational and measured variables.

Zinc and manganese were the target contaminants to be removed in this study. But Zn breakthrough determined the end of the experiments, and was reported after effluent concentrations of >1 mg/L were observed. The variables during the operational period were as follows:

3.3.1. Experimental operational period and zinc removal.—The initial operational period of 119 days with a flowrate of 80 mL/day (100-h HRT) resulted in the successful removal of sulfate, Zn, and other metals in all columns, except for the Inert Control Column (Figures 1a-c and 2a). During the second phase, with a flowrate of 160 mL/day (50-h HRT), sulfate and metal removal occurred in all columns, except in the Inert Control and Untreated Ligneous Columns. The column with the longest operational period with untreated influent (before the Zn breakthrough) was the Untreated Chitin Column (458 days) followed by the Chitin Abiotic Column (161 days). The SRB activity not only increased the operational period, but it also increased the sulfate removal rates (SRR), although Zn concentrations in the effluent were similar in both columns (<0.3 mg/L).

There are several processes that explain the better removal performance obtained with SRB activity: sulfide generation allowing metal precipitation as sulfides, metal adsorption onto the biomass, polymer degradation which causes greater substrate surface available for adsorption, protein and calcium carbonate release into the system to promote biomass growth, and acidity neutralization (Al-Abed et al., 2017; Sheoran and Sheoran, 2006). The adsorption of Mn and Zn onto the chitinous product SC-20 was studied and reported in Pinto et al. (2011). Zinc formed sphalerite (ZnS) in the chitinous columns as reported in Al-Abed et al. (2017). The Untreated Ligneous Column did not have the substrate buffering capacity to neutralize the influent, and it soon consumed the alkalinity generated during the stagnant period and had an early breakthrough (78 days) with Zn concentrations of 0.27–1.24 mg/L in the effluent. ZnS was also formed in the ligneous substrate columns according to Visual Minteq. Apparently, the higher pH and higher sulfide availability obtained in the chitinous columns allowed higher Zn precipitation, hence the observed lower effluent concentrations.

As expected, the columns fed with pretreated influent had longer operational periods for both substrates mostly as a consequence of the higher pH in the system. The Pretreated Ligneous Column removed Zn to concentrations between 0.074 and 0.931 mg/L for 344 days, and the Pretreated Chitin Column had an operational period of 513 days with Zn

concentrations in the effluent ranging from 0.008 to 1.088 mg/L. The pretreated influent, with higher alkalinity, favored suitable conditions for the biomass to thrive, generating higher amounts of sulfide and also a higher ratio of HS^- to S^{2-} formation with the subsequent higher metal sulfide precipitation. Additionally, hydroxide and carbonate precipitation occurred at higher rates at this higher pH, according to Visual Minteq simulations.

3.3.2. Manganese.—Manganese is considered a recalcitrant metal (Medírcio et al., 2007) and may be adsorbed or may precipitate as carbonate or sulfide depending on the SRBR chemistry (Al-Abed et al., 2017). Manganese was marginally removed during the pretreatment (1.53 mg/L in the UFW decreased to 1.42 mg/L in the PFW), likely by coprecipitation with Al, Cu, and Fe. The Chitin substrate columns successfully removed Mn and suffered Mn breakthroughs earlier than Zn breakthroughs (Figures 1a-b) at days 126, 161, and 400 in the Chitin Abiotic, Untreated Chitin, and the Pretreated Chitin Columns, respectively. The column order for Mn breakthrough was similar to Zn breakthrough.

In the chitin columns, Mn most likely precipitated as alabandite (MnS), as reported by Al-Abed et al. (2017). The Mn content in the manure contributed to the observation of high Mn concentrations in the effluent of both ligneous columns. After 175 days in the Pretreated Ligneous Column, Mn had similar concentrations in the influent and effluent, showing no removal, although a small surge was observed in the Pretreated Ligneous Column between days 113 and 154, probably solubilizing some of the precipitated or adsorbed Mn. Hence, Mn was not removed in the ligneous columns, in contrast to the chitinous columns. Therefore, according to these results, if Mn is a target metal in a system, chitinous products are a better option than ligneous substrates.

3.3.3. Cadmium.—The Untreated Ligneous Column and the Chitin Abiotic Column had Cd breakthroughs starting at days 154 and 211, respectively, while both chitin biotic columns removed Cd to below the detection limit of 0.002 mg/L throughout the experiment (Fig. 1c). Cd removal is known to occur by precipitation as sulfide and by adsorption onto the substrate and onto the biomass (Al-Abed et al., 2017; Fu and Wang, 2011; Neculita et al., 2007), which agrees with our observations.

3.3.4. Aluminum, copper, and iron.—The removal of Al, Fe, and Cu is important, because they often compete with other metals (e.g., Zn and Mn) for the adsorption sites and for the S^{2-} ions for precipitation, and they are usually present in higher concentrations in MIW. Under reducing conditions in the experiments, Al could have precipitated as the hydroxide in all columns, but it solubilized again whenever the pH was <5 (Fig. SI2).

Copper was removed to below the method detection limit of 0.007 mg/L in all of the columns throughout the experimental period, except for the Chitin Abiotic Column, which suffered Cu breakthrough starting at day 281 (Fig. SI3). Cu most likely precipitated as CuS in the bioactive columns because it did not solubilize when the pH decreased, but probably as the hydroxide in the Chitin Abiotic Column because it solubilized at low pH values. In the Chitin bioactive columns, Visual Minteq estimated that CuS formed for 100% of the available Cu^{2+} ions. Adsorption could have also contributed to Cu removal as previously

reported (Neculita et al., 2007; Pinto et al., 2011). Regardless, sulfide generation by the biomass played an important role for Cu removal and would increase sludge stability.

Iron was removed to below the method detection limit of 0.105 mg/L by precipitation in all of the columns (Fig. S14) as pyrite (FeS) according to Visual Minteq. Fe suffered an early breakthrough (at the 28th day) in the Untreated Ligneous Column and later (253th day) in the Untreated Chitin Column. This could be due to the pyrite solubilizing when the pH decreased <5 (Al-Abed et al., 2017). Given that Cu and Fe were together in the system with sulfur, the formation of chalcopyrite (CuFeS₂) was also possible, as suggested by Visual Minteq.

However, these three metals precipitated during the pretreatment; this is evident in the fact that their influent and effluent concentrations were below reporting limits in the columns with PFW influent. As a result, in the pretreated columns, there was no evaluation for the removal of these metals.

3.3.5. Sulfate and sulfate removal rates.—Sulfate reduction is linked to metal removal efficiency in SRBRs, hence substrate evaluations are usually performed based on their ability to promote sulfate reduction. Sulfate concentrations in UFW and FWSA were steady at ~2,000 mg/L; while the PFW sulfate concentration was steady at ~1,000 mg/L (Fig. 2a). This reduced concentration in the PFW was probably due to precipitation as gypsum and to volatilization as H₂S.

The Chitin Abiotic Column demonstrated significant sulfate removal with effluent concentrations of ~700 mg/L in days 21–91 and then slowly increasing up to 1,050 mg/L by day 421. Sulfate removal in this abiotic column could be due to sulfate adsorption onto the chitinous substrate or due to precipitation as gypsum, barite, or ettringite fostered by the high Ca content and the presence of Ba in the influent. The Untreated Chitin Column had low sulfate effluent concentrations (~250 mg/L) during the initial 100-h HRT period; it then increased up to ~350 mg/L in the initial 50-h HRT period, and it then gradually increased up to ~900 mg/L by the time of Zn breakthrough. The Untreated Ligneous Column started with a very low effluent sulfate concentration, but after a few weeks of stability, suffered an early breakthrough (sulfate breakthrough occurred at 105 days, while zinc breakthrough occurred at 119 days), as previously discussed. The Pretreated Chitin Column had low sulfate concentrations in the initial period (~395 mg/L) and later stabilized at concentrations in the range of 800–900 mg/L. The Pretreated Ligneous Column also had low concentrations in the initial period (~400 mg/L), but later sulfate reached a plateau at ~900 mg/L.

According to Visual Minteq, sulfate precipitation does not occur at the conditions present within the ligneous columns, hence sorption and sulfate reduction to sulfide fostered by microbial activity could be responsible for the observed sulfate removal. Since the Abiotic Chitin and the Untreated Chitin columns had similar influent (except for the addition of sodium azide to FWSA), the difference in sulfate concentration between them provides the means to quantify the contribution of the biomass to sulfate reduction (Fig. 2b). This difference was higher in the first 154 days, a little longer than the 100-h HRT period, with values of 300–700 mg/L (15–35% of the influent sulfate) and then declined to 40–200 (2–

20% of the influent sulfate). The higher difference in the initial period with lower HRT suggests that the biomass was not able to increase activity to the necessary rate to maintain the SRR levels.

The Untreated Chitin and Chitin Abiotic Columns were the two columns with the highest SRR (Fig. 2c), and these rates were higher while operating at 50-h HRT, in which a maximum of 6.11 mol/m³/d was reached in the Untreated Chitin Column and 5.80 mol/m³/d in the Abiotic Chitin Column. The maximum SRR achieved in the Untreated Chitin and Chitin Abiotic Columns are higher than the highest SRR reported in the review by Hao et al. (2014), which corresponds to 3.84 mol/m³/d in the study of Gibert et al. (2004) that used sheep manure at 216-h HRT. Overall, the chitinous substrate could be very efficient in sulfate removal, which can be translated into longer operational periods or reduced blueprints with respect to ligneous substrate alternatives or ligneous/manure mixtures.

3.3.6. Sulfide.—A fraction of the influent sulfate was reduced to soluble sulfide in the aqueous effluent, another fraction was reduced to H₂S and moved to the gaseous phase, a third fraction precipitated as sulfides or sulfates (which fosters metal removal), and yet another fraction remained as soluble sulfate in the columns' effluent. Pretreated Chitin had the highest measured sulfide concentrations with a peak at 79 mg/L in day 56; it had concentrations >10mg/L observed for the first 161 days (Fig. SI5).

The second highest sulfide concentrations were observed in the Untreated Chitin Column with concentrations >10 mg/L detected for 140 days. The other columns had sulfide concentrations <1 mg/L, except in weeks 1–4 in which all columns had concentrations >1 mg/L, except for the Inert Control Column which kept sulfide concentrations <0.04 mg/L throughout the experimental period.

The observed high sulfide concentration periods can be associated with higher SRB activity influencing metal removal. Sulfide generation was noticeably greater in the chitinous substrate columns than in the ligneous substrate columns, confirming their capacity to increase metal removal efficiency.

3.3.7. pH and alkalinity.—The optimum pH for SRB to thrive is 5 to 8, and pH <5 results in lowering sulfate reduction rates and increasing sulfide salts solubility (Neculita et al., 2007). The CaCO₃ alkalinity in the SC-20 efficiently neutralized the acidic influent pH in the chitinous substrate columns, but no alkaline material was added to the ligneous substrate columns, and the alkalinity could increase only by the effect of microbial activity.

The UFW pH was acidic (~2.44, Fig. 3a) with zero alkalinity, while the PFW pH was ~6.5 with 63 mg/L CaCO₃ alkalinity. UFW was effectively neutralized in all chitinous substrate columns, with pH being higher in the Pretreated Chitin Column than the Untreated Chitin Column, which was higher than the Chitin Abiotic Column (Fig. 3a). Alkalinity peaked at the beginning of the operational period in all chitinous substrate columns and then decreased to low levels (Fig. 3b), probably due to depletion of the CaCO₃ in the substrate, as indicated by low Ca concentrations in the solid residues (Table SI2). The alkalinity observed in the initial period in the Untreated Ligneous Column was soon depleted to 63 mg/L as CaCO₃

indicating that the substrate could not sustain SRB activity, and most of the salts and biomass were exhausted by day 49. The Pretreated Ligneous Column was able to sustain the SRB activity for a longer period, since alkalinity was added in the pretreatment. However, despite having a neutral influent, the pH decreased in this column to <4 , probably because the low pH was not suitable to sustain SRB activity within the column.

This reduction in SRB activity, within the Pretreated Ligneous Column, resulted in higher effluent sulfate concentrations. This acidic condition and SRB reduction also eventually resulted in Zn breakthrough occurring earlier in this column than in those with chitinous substrate. For all columns, Zn breakthrough occurred immediately after pH decreased to <5 and CaCO_3 alkalinity was measured at 63 mg/L . It was clear that influent acidity neutralization is critical in SRBRs success in metal removal.

3.3.8. E_h .—Anoxic environments are the most favorable conditions for SRB growth, which implies higher SRR. Nevertheless, sulfate removal is possible even when SRBRs are operating under a positive E_h (Neculita et al., 2007; Reisman et al., 2003).

All columns except the Inert Control Column started with a reduction potential of approximately -200 mV after the stagnant period. The reduction potential of the Chitin Abiotic Column increased to a relatively stable potential of $\sim 200 \text{ mV}$ after 33 days, which was followed by an escalation to 476 mV toward the end of the operational period (Fig. 3c). Even at this high E_h , the Abiotic Column still removed sulfate at a significant rate, probably by adsorption and precipitation. The Untreated Ligneous Column had a continuous increase in E_h , likely influenced by the pH decrease, reaching 200 mV by the 100th day. After that, it remained in the $200\text{--}400 \text{ mV}$ range with no sulfate reduction, except for a short period (after the 200th day), in which a small reduction was observed, probably due to sorption. The Pretreated Ligneous Column remained with $E_h < 0 \text{ mV}$ until it suddenly increased up to 400 mV after the 300th day. In spite of the high E_h , this column reported some sulfate removal in this period, suggesting that the biomass had activity after the pH drop on day 175, and after the E_h increase on day 300. The Untreated Chitin Column had an early E_h increase, but it remained at $<100 \text{ mV}$ E_h and reported very high SRR, only comparable to the Chitin Abiotic Column. In the Pretreated Chitin Column, E_h increased from -200 mV to 130 mV with consistent sulfate removal throughout the experimental period.

Generally, keeping the reaction zone of an SRBR under reducing conditions is important to foster sulfate reduction. The substrate selection is an important factor to determine E_h . In our experiment, the chitin-based substrate showed lower E_h than the ligneous substrates, which could be important at some mine sites because this could mean that the reactors might operate at lower E_h generating effluent with lower metal content or allowing the flowrate to increase; thus, reducing the system's blueprint.

3.3.9. Ammonia.—Nitrogen can be released as a product of protein-rich chitinous material degradation in SRBRs and from ligneous substrates. Although, at a $\text{pH} < 9.3$, it will mostly remain as NH_4^+ (Takeno, 2005). Nevertheless, under anoxic conditions, it may also form ammonia, particularly at high pH, and the presence of ammonia in the water can contribute alkalinity to the system (Neculita et al., 2007).

Ammonia was measured as ammonia-N with the chloramine ammonia method, which increases the pH to 9.5 to ensure that all the nitrogen present would be read as ammonia. Hence, nitrogen could be present as ammonium and still be registered as ammonia-N.

Ammonia-N had a peak in the beginning of the experimental run with concentrations of 3,200 mg/L (7th day) in the Untreated-Chitin Column and 1,870 mg/L (7th day) in the Chitin Abiotic Column (Fig. 3d), possibly because of the high microbial activity degrading protein during the stagnant period. However, it decreased with time, reaching concentrations <5 mg/L in all the columns, except for the Untreated Chitin Column, which had ammonia concentrations in the range of 10–13 mg/L until the completion of the study.

While these concentrations are higher than the 1.9 mg total ammonia nitrogen/L used as the chronic criterion magnitude in the Aquatic Life Ambient Water Quality Criteria (U.S. EPA, 2013), most of the measured ammonia concentrations could be present as ammonium and not pose a risk for the environment. In spite of that, the aeration of the reactor's effluent would be recommended to warrant the ammonia removal from the effluent of SRBRs.

3.3.10. Gas composition in the effluent.—The composition of the gaseous and aqueous phases usually reflects the columns' biomass activity: H₂S and sulfide generation are associated with SRB activity, while methane content in the gaseous phase can be associated with methanogenic activity. The H₂S content in the gaseous phase was not measured, but the odor was apparent while collecting the gas phase for GC analysis in all columns.

The methane content (Fig. SI6) showed that methanogens had a period of high activity at the beginning of the experiment in both ligneous columns and a delayed period of high activity in the chitin bioactive columns. These results showed that methanogens and SRB activity was higher at the initial period of operation, but both microorganisms lowered their activity during the second part of the study with CH₄ having lower concentrations in the gas phase and lower sulfide concentrations in the aqueous phase preceding the 200th day of operation. This could be a sign that biomass growth lessened while operating at higher HRT, and at some point, the available carbon was the limiting factor for such growth. Apparently, the chitinous substrate columns had higher carbon available than the ligneous substrate columns, which explains the higher gas generation and observed longer operational periods.

3.4 Substrate comparisons.

The acidic UFW had a very different fate while feeding the Untreated Chitin and the Untreated Ligneous Columns. The Untreated Ligneous Column suffered an early and sudden pH decrease because this substrate did not have buffering capacity, and the alkalinity generated during the stagnant period was able to buffer the influent for only 56 days. Hence, the addition of limestone, or other alkalinity source, to neutralize the MIW acidity would be necessary to obtain better performance with ligneous substrates.

The chitinous substrate, naturally containing CaCO₃, was able to neutralize the influent's acidity, promote a suitable environment for biomass activity, and remove metals for 458 days. The Untreated Chitin Column was highly efficient in metal removal in spite of the high

Al, Fe, and Cu concentrations in the influent. The metal removal efficiency was improved (Zn consistently reached <0.2 mg/L), and the operational period was extended (513 days) in the Pretreated Chitin Column until the experiments were stopped, which happened before experiencing Zn breakthrough to ensure that ZnS remained in the solid residues as reported by Al-Abed et al. (2017).

The Pretreated Ligneous Column was able to operate for 337 days with a Zn effluent <1 mg/L, but the effluent pH decreased suddenly to 4.15 at day 175 (lower than the influent pH), causing poor Fe, Zn, and Mn removal rates. Overall, the chitinous substrate seemed to better promote sulfate reduction and metal removal in these experiments than the ligneous substrate.

If the MIW is to be treated with ligneous substrate, the addition of alkaline materials (e.g. limestone) into the substrate would be recommended rather than pretreating the influent because pretreatment requires the operation of an active system and because the addition of alkaline materials would probably obtain a better performance in metal removal and an extended operational period. Although the use of a chitinous substrate would give a better performance and extended period than the ligneous substrate, several factors need to be considered before selecting a particular substrate: the cost of the substrate, since the chitinous product usually would cost more than wood chips, hay, and manure, the desired final water quality, and if Zn and Mn are target contaminants. Another advantage of the chitinous substrate is the extended operational period due to its ability to gradually release protein and carbonates into the system as the chitin polymer is degraded (Al-Abed et al., 2017). This could be beneficial in certain inaccessible sites in which unsupervised operations are desired.

4. Conclusions

Sulfate reduction and zinc removal to levels <0.3 mg/L were successfully achieved using a chitinous product as the substrate in SRBR columns with acidic MIW influent during an operational period of 458 days. A ligneous substrate, with no alkalinity added, achieved sulfate reduction and Zn removal for only 70 days, probably because the biomass could not adapt to the low pH.

The influent MIW pretreatment, consisting of aeration and pH adjustment to 6.6 with NaOH, extended the operational periods of both substrates, to >513 days using the chitinous substrate and to 344 days with the ligneous substrate. The influent pH and the lower concentrations of Al, Cu, and Fe in the pretreated MIW were the determinants in obtaining the extended operational period. In the case of MIW treatment performed in a seasonally inaccessible site, as may be the case of abandoned mine sites, the pretreatment may not be the best option because it is not a passive system as it may require the addition of an alkalinity source within the substrate. Considering both substrate alternatives, the chitinous substrate could be an effective option for MIW treatment in passive systems treating acidic MIW, particularly when an extended operational period or Zn and Mn removal is desired.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

- We investigated metals and sulfate removal from MIW in anaerobic bioreactors.
- Two types of substrates were tested: chitinous and ligneous.
- The chitinous substrate generated effluent with lower metal and sulfate content.
- The chitinous substrate offered effective treatment over longer operational periods.
- The chitinous substrate could be an option when Zn and Mn removal is required

Fig. 1.

Zinc (a), manganese (b), and cadmium (c) removal in the experimental columns

Fig. 2.
Sulfate (a), sulfate difference between the Chitin Abiotic and the Untreated Chitin columns (b), and Sulfate Removal Rate (c) in the experimental columns.

Fig. 3. Measured pH (a), alkalinity (b), E_h (c), and ammonia-N (d) in the experimental columns. E_h was the only parameter measured through a probe inside the columns and not by collecting a sample.

Table 1

pH, metals, sulfate, and dissolved oxygen content in the Untreated Formosa Water (UFW) and Pretreated Formosa Water (PFW)

Parameter	Units	UFW	PFW
pH	pH units	2.94	6.67
Al	mg/L	17.8	0.057
As	mg/L	0.019	0.004
Ba	mg/L	0.010	0.05
Ca	mg/L	72.7	75.9
Cd	mg/L	0.267	0.242
Cu	mg/L	16.8	0.690
Fe	mg/L	106	0.357
Mg	mg/L	18.6	17.3
Mn	mg/L	1.53	1.42
Ni	mg/L	0.043	0.049
Pb	mg/L	0.078	<0.017
Zn	mg/L	73.9	57.3
Sulfate	mg/L	2620	770
Dissolved Oxygen	mg/L	1.43	4.47