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TECHNICAL ARTICLE

Manganese and Sulfate Removal from a Synthetic Mine Drainage Through Pilot Scale Bioreactor Batch Experiments

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Abstract Mine drainage is a significant problem in the Appalachian Plateau due to elevated metal and solute concentrations. Most metals may be removed by oxidation/precipitation or natural buffering, but Mn is more difficult to remove due to its higher solubility. Some mine drainages in southeastern Kentucky have average sulfate and Mn concentrations exceeding 1,300 and 30 mg L^{-1}, respectively. Manganese does not readily form sulfidic minerals, and MnS precipitation following sulfate reduction has not proven to be a promising pathway for permanent Mn immobilization. Our study involved batch experiments with five different organic carbon sources in combination with five inorganic substrates to treat a simulated mine drainage with pH 6.2 and Mn and sulfate concentrations of 90 and 1,500 mg/L, respectively. The Mn removal capacity varied widely between treatment mixtures, from <10 to 100%. Sulfate removal showed a similar divergence, ranging from <10 to >80%. The most effective treatment was provided by the biosolids or wood mulch amendments in combination with the creek sediment. Sulfate reduction levels were not stoichiometrically matched with MnS formation, suggesting that the prevalent Mn removal mechanisms were sorption and precipitation as oxide, oxyhydroxide and carbonate, rather than Mn-sulfide phases.

Keywords Bioreactor · Manganese removal · Mine drainage · Sorption · Sulfate reduction · Sulfate/sulfide · Precipitation

Introduction

The mountaintop removal and valley fill method is a common surface mining approach in Eastern Kentucky (Kentucky Office of Mine Safety and Licensing 2005). When the overburden is exposed to surface weathering, pyrite oxidation often produces acidic mine drainage (Pond 2004; USEPA 2002), unless the native geology has substantial buffering capacity. Neutralization of the acidity results in the natural precipitation of most of the soluble metals. However, Mn has a much higher solubility than other metals, sometimes resulting in solutions with high concentrations of Mn and sulfate at circumneutral pH levels (Stumm and Morgan 1996).

Research using sulfate reduction to facilitate metal and sulfate removal has found widely varying efficiencies ranging from 40 to 99% (Benner et al. 1999; Champagne et al. 2005; Chockalingam and Subramanian 2006; McIntire and Edenborn 1990; Sass et al. 2001; Waybrant et al. 1998). Several variables impact the efficacy, including the initial SO_4^{2-} concentration, temperature, residence time (Waybrant et al. 2002), pH, redox potential (Eh), and the carbon source (Gibert et al. 2002). Factors such as the particle size of the organic matter, the labile: recalcitrant C ratio, and the availability of N and P are also very important (Gibert et al. 2002; Waybrant et al. 2002). Most metal sulfides are fairly insoluble and stable (Stumm and Morgan 1996). However, there are several mechanisms occurring in a fully functioning treatment system that are difficult to separate, such as metal sulfide precipitation,

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oxide, hydroxide and/or carbonate formation, simple sorption, and physical filtration (Neculita et al. 2007; Wildeman and Updegraff 1997). In addition, removal mechanisms may change over time. Initially, sorption seems to predominate until sorption sites are saturated (Gibert et al. 2005; Machemer and Wildeman 1992). As the system matures and microbial populations flourish, metal sulfide precipitation begins to dominate.

However, very little Mn has been permanently immobilized in compost wetlands or sulfate reducing bioreactors, particularly at low pH (Hallberg and Johnson 2005). Of the many metals and metalloids commonly found in mine drainage, Mn is one of the more difficult to remove due to the complexity of the interactions governing Mn solubility. Manganese precipitation is inhibited if the Fe: Mn ratio is too high (Karathanasis and Barton 1997; Nairn and Hedin 1993) and Mn oxide precipitates will dissolve if Fe^{2+} concentrations are too high (Evangelou 1998; Stumm and Morgan 1996). In addition, most other metals preferentially complex with sulfides before Mn does (Stumm and Morgan 1996). Research has shown widely variant success in Mn removal. Tabak et al. (2003) successfully removed 95% of the Mn load from AMD, while Machemer and Wildeman (1992) were only able to see a temporary decrease in Mn concentrations. Machemer and Wildeman (1992) also observed a relationship between metal and SO_4^{2-} concentrations over time, suggesting that following the saturation threshold stage, sulfate reduction could be a potential metal removal mechanism. However, of the four metals tested (Mn, Zn, Cu, Fe), Mn-sulfides were the least likely precipitates, as expected by their K_{sp} values.

Several coal mine sites in southeastern Kentucky have mine drainages with Mn and sulfate levels exceeding 30 and 1,200 mg/L, respectively (Fisher et al. 2007; Karathanasis and Barton 1997; Pond et al. 2008). The objective of this study was to evaluate the efficiency of various inorganic and organic substrates for the removal of Mn by sulfate reduction in a simulated synthetic mine drainage using laboratory bench-scale bioreactors. Information from these experiments will be used for designing field-scale treatment bioreactors to treat high Mn and SO_4 drainages at southeastern Kentucky mine sites.

Materials and Methods

Research Materials

The five organic amendments used in the study consisted of corn mash from a bourbon distillery, soybean oil, wood mulch, sorghum syrup, and biosolids. The five inorganic substrates included limestone, marble, creek sediment, polished river gravel, and sand. The key factors for choosing the substrates and amendments were their availability and cost. Ideally, the treatment material would be inexpensive or free, and available in large quantities on demand. Wood mulch was selected because of previously discovered concerns about the longevity of substrate (Karathanasis and Barton 1997), while the corn mash was chosen as a potentially ideal source of readily available nutrients. Soybean oil had been previously shown to be effective in Mn removal (Phifer et al. 2001), and the sorghum syrup was used as a high sugar material, which is produced in abundance locally. The marble and limestone were selected as substrates because of the potential for carbonate dissolution and removal of Mn by $MnCO_3$ formation. The sand was used to allow sustainable permeability and surface reactivity of the bioreactor over a long lifespan. The creek sediment was chosen because of the diversity in sediment size, the inherent sulfate reducing microbial population, and as a potential source of nutrients. The polished river gravel was chosen as a substrate because of its different mineralogical composition (dominated by quartz) compared to the mixed mineralogy of the creek sediment.

Combining the substrates and the amendments created 25 different duplicated treatments for use in the batch experiments. The following combinations were tested: creek sediment and biosolids, creek sediment and corn mash, creek sediment and mulch, creek sediment and soybean oil, creek sediment and sorghum syrup, and creek sediment without an amendment. A control treatment, consisting of no substrate or amendment was also used. The source, composition, and selected properties of the materials are listed in Table 1.

A synthetic mine solution was used in the experiments rather than the actual mine drainage to simplify the treatment chemistry and minimize interference from other components (i.e. Fe). The synthetic mine drainage had a pH of 6.2 and contained approximately 90 mg L^{-1} Mn and 1,500 mg L^{-1} SO_4^{2-}. Reagent grade manganese sulfate ($MnSO_4$) and calcium sulfate dihydrate ($CaSO_4{\cdot}2H_2O$) were used as the Mn^{2+} and SO_4^{2-} sources (Fisher Scientific). The mixture was tested in a batch experiment using an acid-washed glass 1 L suction flask (Fig. 1). The substrates and amendments were mixed in each flask on a 10:1 mass to mass ratio. The amendments were added on an oven-dried mass basis, except for the oils. The density ratio of the oils was used to determine the requisite masses of each. The mine drainage was added on an equivalent (1:1) mass/mass or mass/volume ratio to the substrate. In order to enforce and maintain an anaerobic environment in the flasks, purified nitrogen gas was flushed through the system continuously at a rate of $\approx$ 0.014 m^3/h. A gas trap was installed on each flask and filled with sodium thiosulfate to allow the nitrogen gas to leave the flask without allowing oxygen to enter the system.

Table 1 Substrate and amendment characteristics

Sample	Source	Composition	pH (S.U.)	CEC (meq/100 g)	Total P (%)	Total N (%)
Biosolids	Winchester, KY wastewater treatment plant	<1.00 mm, air-dried	11.5	3.8	0.76	1.75
Corn mash	Local bourbon distillery	Mash (40% water)	6.3	12.7	0.63	5.21
Mulch	Univ. farm wood chip waste	<1.00 mm, air dried	8.3	53.2	0.93	2.14
Sorghum syrup	Retail store	Liquid	NA	NA	0.03	0.07
Soybean oil	Retail store	Liquid	NA	NA	0.00	0.00
Creek sediment	University research forest stream bed	Mixture of gravel, sand, silt, and clay	6.2	0.188	–	–
Limestone	Local limestone quarry	10 mm mean diameter	7.8	0.080	–	–
Marble	Garden store	20 mm mean diameter	8.5	0.018	–	–
River gravel	Garden store	5–30 mm diameter	4.8	0.039	–	–
Sand	Garden store	1–5 mm diameter	9.3	0.049	–	–

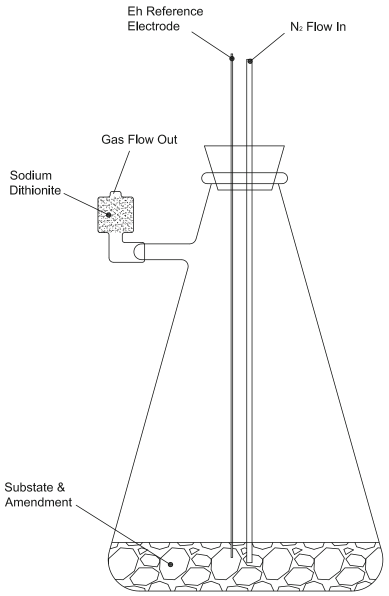


Fig. 1 Schematic diagram of the batch experimental setup

Material Analysis and Preparation

The materials used in the experiments were tested for cation exchange capacity (CEC) with ammonium acetate at pH 7, 1:1 solid/water paste pH, total nitrogen (N) and phosphorus (P) by Kjeldahl digestion, and particle size analysis for the creek sediment by the pipette method (USDA-NRCS 1996).

Sample Collection and Analysis

A sample was extracted from the flask daily for 3 weeks (21 days) or until the major constituents reached a stable concentration, and was analyzed for Eh, pH, Mn, and sulfate. The Eh and pH were measured using a Fisher Scientific Accumet AP62 pH/mV meter immediately after sample collection, using an Accumet pH probe and a silver/silver chloride Mettler Toledo InLab redox probe, with values adjusted to reflect a hydrogen reference electrode. A subsample pipetted from each flask was filtered through a 0.45 µm membrane filter and preserved in 1-N hydrochloric acid for Mn and SO_4 analysis. Manganese levels were tested using a Solaar M5 Thermo-Elemental atomic absorption spectrometer. Sulfate-sulfur concentrations were analyzed by a variation of APHA Method 4,500-SO_4^{2-} E (APHA 1998), using a Molecular Devices Versamay tunable plate reader at 450 nm (D’Angelo et al. 2001).

Mineralogical Analysis

Following the completion of each batch experiment, the matrices were dried in an oven at 55°C and sieved through a 150 µm sieve to maximize particle size homogeneity. The mineralogy of the matrices was determined by thermogravimetric analysis (using a DuPont Instruments 951 thermogravimetric analyzer) and X-ray diffraction (with a Philips PW 1729 X-ray generator and a Philips PW 1840 diffractometer using a cobalt tube as the x-ray source) (Karathanasis and Barton 1997). Nitrogen gas was used to flush the sample container and prevent mineral oxidation.

Statistical Analysis

The statistical analysis of the data was performed using a 9.1 version of the SAS program. Final sulfate, Mn, and redox levels were tested to determine differences. Since the majority of the treatments were not directly comparable (because neither the substrates nor the amendments were the same), the analysis was based on each substrate and

Table 2 Mean redox potential (mV) and pH for each combination of substrate and amendment; values represent the mean of duplicated treatments

	Creek sediment		Limestone		Marble		River gravel		Sand	
	Redox	pH	Redox	pH	Redox	pH	Redox	pH	Redox	pH
Amendment										
Biosolids	−17	12.1	88	11.6	−189	11.7	−306	11.4	−69	11.3
Corn mash	−80	5.6	−56	6.5	−87	5.6	0	6.2	56	5.8
Sorghum syrup	−147	5.1			−37	6.1	64	5.1	58	4.5
Soybean oil	−75	6.1	230	6.0	174	8.1	−152	6.2	252	5.7
Wood mulch	−294	8.3	−320	8.1	−232	8.3	−249	8.3	−302	8.4
None	−1	7.2	17	7.4	−49	6.7	−21	6.5	−19	6.5

each amendment. The “PROC MIXED” procedure with the LS-Means statement was used for the analysis based on the nature of the data and the correlation between each sampling point, or day, with those coming before and after it. The PROC MIXED procedure does not control Type-II error; thus, due to the large number of comparisons for each data set, the maximum probability allowed to indicate significant differences was 0.001 ($\alpha = 0.001$). The Satterthwaite approximation was used to calculate the degrees of freedom for the analysis.

Results and Discussion

Material Characteristics

Individual substrate and amendment characteristics are summarized in Table 1. The mean Eh and pH of each treatment are presented in Table 2. The biosolids were lime-stabilized and thus were strongly alkaline. The corn mash and the creek sediment were slightly acidic and the limestone was slightly alkaline, while the sand, marble, and mulch were moderately basic. River gravel was the single substrate or amendment, and was acidic, with a pH of 4.8. The three solid matrixes were similar in P and N content, ranging from a low of 0.76% to a maximum of 0.93%, though the corn mash had more than twice the nitrogen concentration than either the wood mulch or the biosolids. Both the sorghum syrup and the soybean oil had very low or no N and P, as both elements are removed during processing to increase the longevity of the oils (Z. Christensen, personal communication, Feb. 3, 2008). The marble, sediment, and limestone had nearly equivalent CEC, while the exchange capacity of the river gravel was negligible.

Manganese Removal

Most of the treatments caused a significant decrease in Mn concentration over the trial period (Figs. 2, 3). The biosolids and wood mulch amendments showed the greatest potential to remove Mn, as all of the combinations involving either of those amendments achieved a significant removal rate. The treatments without the addition of an amendment also produced a significant decrease in Mn concentration over time. In contrast, the soybean oil, sorghum syrup, and corn mash each caused a significant change in only two of the five treatments. All of the substrates except the river gravel caused a significant decrease in Mn concentration in four or five of the six treatment combinations. Thus, with respect to Mn, river gravel appeared to be the least effective substrate and the mulch and biosolids treatments the most effective amendments. The remaining amendments and substrates showed varying Mn removal efficiencies without a clearly definable order of effectiveness.

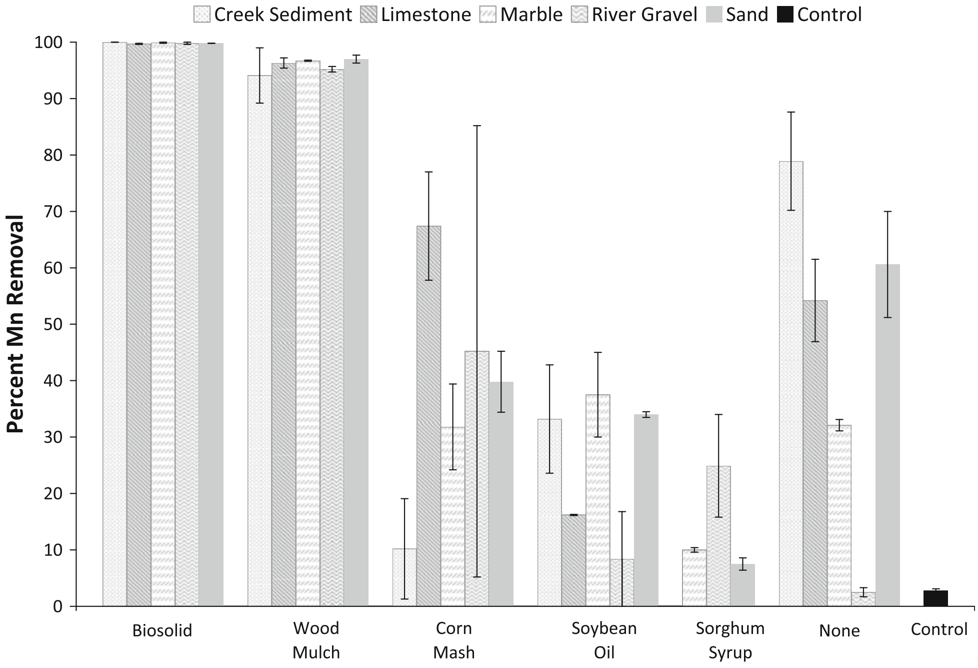
Removal by Amendment

Each of the organic amendments had a distinctive capacity to remove Mn from solution. The most effective amendments were the biosolids and the wood mulch, while the least effective treatments were the sorghum syrup and the soybean oil.

Biosolids

The biosolids treatments removed 100% of the Mn from solution (Fig. 2); however, the biosolids were lime-stabilized, with a resultant treatment pH always greater than 10 and generally higher than 11. Equilibrium speciations by MinEQL + indicated the potential for Mn oxy-hydroxide precipitation under oxidized conditions and carbonates and/or sulfides under reducing conditions. Mineralogical analysis of the biosolids treatments consistently indicated the presence of rhodochrosite ($MnCO_3$) and manganite ($MnO(OH)$). Previous research has found it generally takes a period of several days to develop sulfate-reducing conditions (Cocos et al. 2002; URS 2003; Waybrant et al. 1998). Consequently, the removal of the Mn within the first

Fig. 2 Percent total Mn removal as a function of amendment. *Error bars* indicate ± 1SD from the mean of two replicates



24 h most likely occurred through precipitation of oxy-hydroxides due to the high pH or formation of rhodochrosite as a result of the substantial concentrations of carbonates in the biosolids.

Wood Mulch

The wood mulch treatments were almost as effective as the biosolids treatments, removing 94–97% of the Mn from solution (Fig. 2). However, as with the biosolids treatments, the majority of the Mn was removed within the first 24 h, indicating a removal mechanism other than sulfate reduction. The pH of the mulch treatments generally stabilized slightly above 8 (Table 2). While this is considerably lower than the pH of the biosolids treatments, it is high enough to favor Mn oxy-hydroxide precipitation at the relevant Eh range (Stumm and Morgan 1996). The creek sediment-mulch combination was the only treatment showing noticeable amounts of MnS and rhodochrosite. Given a CEC of approximately 50 meq/100 g, the mulch amendment would also provide sufficient sorption capacity to bind Mn from the synthetic mine solution.

Corn Mash

Even though the corn mash had a high CEC (Table 1) and the highest levels of N of any of the amendments, only two of its treatment combinations showed a significant decrease in Mn concentration (Fig. 2). The river gravel-corn mash and marble-corn mash treatments realized removal efficiencies of 45.2 and 31.8%, respectively, but the standard deviation was very high between replicates (40%). The creek sediment—corn mash treatment only achieved a treatment efficiency of approximately 10%, suggesting that the corn mash may not be the optimal organic source for a successful treatment amendment.

Soybean Oil and Sorghum Syrup

Laboratory and field scale research at the Savannah River site found that soybean oil was an effective amendment to facilitate sulfate reduction (Phifer et al. 2001). However, our findings indicated neither the sorghum syrup nor the soybean oil to be consistently effective in reducing Mn concentrations (Fig. 2). The highest Mn removal rate for the sorghum and soybean oil amendments was 25 and 37.5%, respectively. Both amendments were extremely low in total N and P, which are essential for bacterial growth (Gibert et al. 2002; Waybrant et al. 2002). The research at the Savannah River Site found it necessary to add rock phosphate and commercial fertilizer to the aquifer to provide the requisite nutrients for the sulfate reducing bacteria to flourish (Phifer et al. 2001). Without either of these key nutrients, sulfate-reducing bacteria were unable to convert the sulfate to sulfide in significant quantities to allow the majority of the Mn to precipitate. In addition, both the syrup and the oil had very low sorption capacity to bind Mn (Table 1). Chemical speciation by MinEQL + indicated

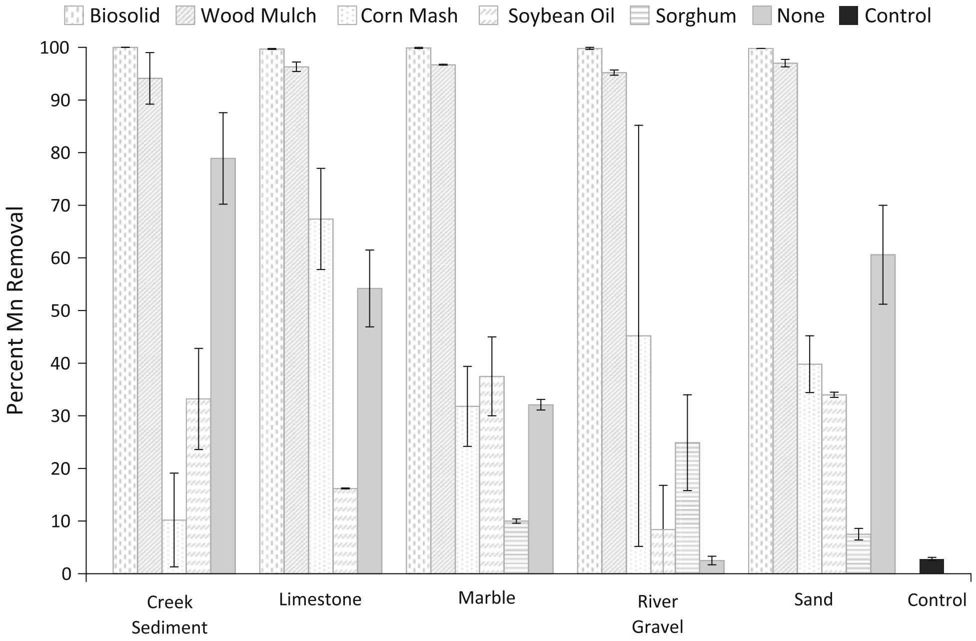


Fig. 3 Percent total Mn removal as a function of substrate. *Error bars* indicate ± 1SD from the mean of two replicates

that the Mn decrease observed in the various treatments may have been the result of MnS precipitation, the sorption capacity of the substrates, or oxidation. Trace amounts of MnS and Mn-hydroxide were detected by XRD analysis. Regardless of the cause for moderate Mn removal in these systems, neither sorghum syrup nor soybean oil appeared to be as effective as the biosolids or the mulch amendments in removing Mn from the synthetic mine drainage.

Removal by Substrate

Each substrate was tested as a control without the addition of any organic amendment. The most effective substrate used in the study was the creek sediment, which removed 79% of the Mn from solution, while the least effective substrate was the polished river gravel, with only 2.5% removal efficiency (Fig. 3). Of the five substrates used in the experiment, river gravel was the least effective when combined with each of the organic amendments. Four of the six river gravel treatments did not show significant Mn removal. Significant differences among all biosolids and wood mulch treatments implied that the controlling factor in the river gravel batches was the organic amendment rather than the substrate. Manganese removal by substrate without an organic amendment was correlated to the total CEC for each treatment with a moderate relationship ($r^2 = 0.53$) at the 0.16 probability level. The correlation coefficient decreased ($r^2 = 0.27$) when Mn removal was compared to the CEC for all treatments, but the significance increased greatly, to less than 0.01. These results indicate that simple sorption is a substantial source of Mn removal in these systems. However, it fails to account for the entirety of the Mn removed, thus implicating other removal mechanisms. This is particularly applicable to substrates with very low sorption capacity (Table 1).

River Gravel

The low CEC and specific surface area of the river gravel most likely reduced some of the inherent benefits of a mineral substrate by minimizing attachment points for bacteria, resulting in the lowest Mn removal efficiency. In addition, river gravel was the only substrate or amendment to have a strongly acidic pH, too acidic to allow Mn-oxide or oxy-hydroxide formation.

Limestone/Marble Chips

The limestone and marble treatments without an organic amendment provided significant Mn removal rates of 54 and 32%, respectively, possibly due to carbonate or hydroxide precipitation (Fig. 3). Both the limestone and the marble chips were chosen for evaluation in this portion of the experiment because of the likelihood for minor dissolution, which could then allow for the formation of $MnCO_3$. Indeed, mineralogical analysis confirmed the presence of small amounts of rhodochrosite and manganite in the treatments. Speciation by MinEQL + also suggested solution saturation with $MnCO_3$. Although autocatalytic formation of Mn-oxides and oxy-hydroxides is not favored below pH 8.0 (Lovett 1992), it is possible that the extremely high concentrations of Mn (1.8 mM) may have

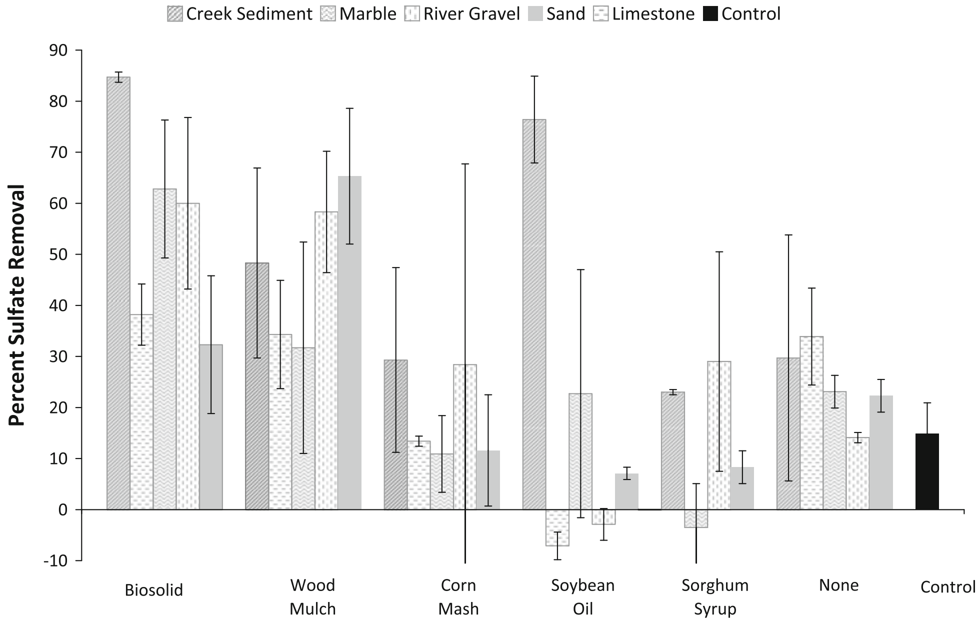


Fig. 4 Percent total sulfate removal as a function of amendment. *Error bars* indicate ± 1SD from the mean of two replicates

induced some Mn-precipitation or co-precipitation in these treatments.

Sand

The sand was the second most effective substrate in Mn removal following the creek sediment (Fig. 3). Apparently, the smaller particle size of the sand particles provided a significantly higher specific surface area for increased microbial attachment and ion exchange. Of the substrates used in this research, the sand also had the highest pH (9.26). This pH may have contributed to the formation of Mn-hydroxides and possibly rhodochrosite. The smaller pores of the sand substrate may have also allowed sulfate reducing bacteria to more readily control their growth microenvironment than the marble and limestone substrates (Skousen et al. 2000; Zagury et al. 2005). Mineralogical analysis of the matrices following the treatment confirmed the presence of rhodochrosite and manganite in both the corn mash-sand and biosolids-sand combinations. The remaining sand combinations did not show any changes in mineralogy.

Creek Sediment

The creek sediment provided the best potential for Mn removal of the five substrates (Fig. 3). The sediment consisted of a mixture of gravel (>2 mm), sand and silt (2 − 0.002 mm), fine size particles (<0.002 mm), and organic matter. Inorganic components of the sediment consisted of limestone, shale, and sandstone materials. As a result, the sediment possessed many of the properties of the limestone substrate, such as the presence of carbonates and the inherently high buffering capacity. The combination of the autochthonous organic matter and the large specific surface area of the sediments also resulted in an ideal environment to support microbial communities. The sediment also had the highest CEC of the five substrates (Table 1), most likely due to the combination of fine size particles and organic matter at all stages of decomposition. Sulfate-reducing bacteria require attachment points and a source of carbon and nutrients, generally found where a diverse organic source serves as an optimum environment for microbial sulfate reduction (Boudreau and Westrich 1984; Gibert et al. 2002; Waybrant et al. 2002). Thus, the creek sediment matrix could serve as the ideal substrate due to diversity in particle sizes, organic material, microbial populations, mineralogy and chemistry.

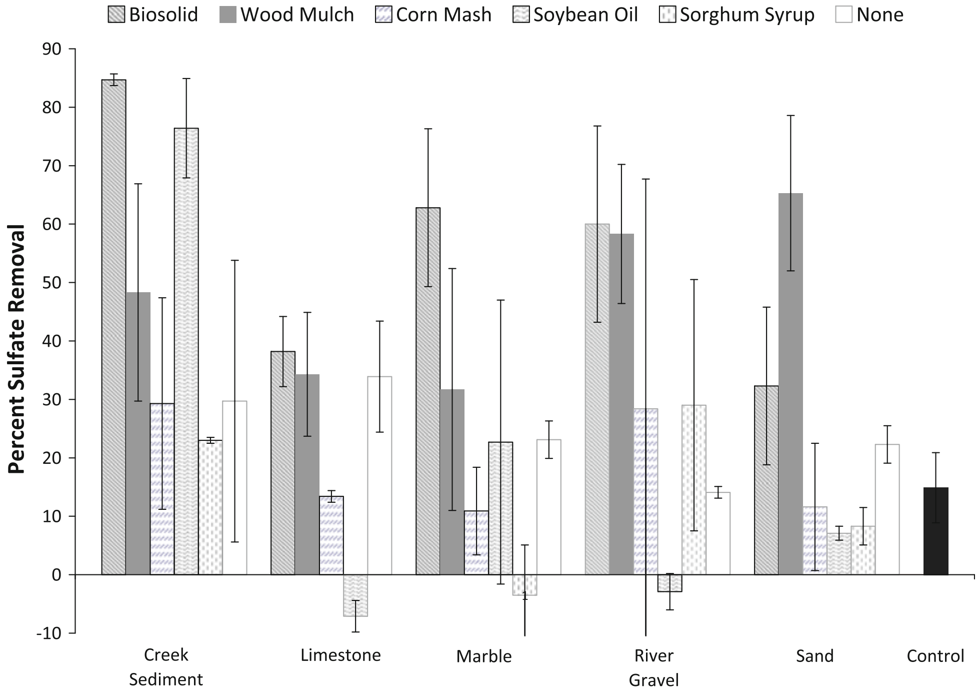
Sulfate Removal

Only the biosolids treatments were able to consistently attain a significant reduction in sulfate concentrations during the treatment period (Figs. 4, 5). Surprisingly, the control treatment resulted in a reduction of 15% of the initial sulfate concentration. Each amendment and substrate produced at least one treatment with a statistically significant sulfate concentration reduction over the treatment period, except for the corn mash treatments. The differences between substrates and amendments are further explored below.

Removal by Amendment

Several of the treatments had high variations between replicates for sulfate removal, with standard deviations

Fig. 5 Percent total sulfate removal as a function of substrate. *Error bars* indicate ± 1SD from the mean of two replicates



averaging around 10% and ranging from a low of 0.5% to a maximum of nearly 40% (Fig. 4). Two of the five amendments did not show any statistically significant differences between the substrate combinations due to high variation between replicates. The corn mash-river gravel and corn mash-creek sediment treatments had average sulfate removal efficiencies approximately three times that of other treatments (Fig. 4), but their high standard deviations (18–39%) accounted for the lack of significant differences between the treatments. Wood mulch, like the corn mash, was the other amendment not showing statistical differences between treatments, usually due to high standard deviations (10–20%). However, the mulch did consistently decrease SO_4^{2-} concentrations during treatment.

The biosolids and wood mulch were the only amendments that consistently contributed to sulfate removal (Fig. 4). The sorghum syrup, soybean oil, and treatments without an amendment each produced one combination with a significantly different sulfate concentration after the treatment period, while the wood mulch produced two successful treatment combinations. The syrup and soybean oil were only effective in combination with the creek sediment, suggesting that the sediment, rather than the organic amendment, was the determining factor in sulfate reduction. Of all the organic amendments used, the biosolids were most likely to have native sulfate reducing bacteria, accounting for the substantial sulfate reduction during the treatment period.

Soybean oil added to the creek sediment contributed to a 76% reduction in sulfates, whereas in the sand, river gravel, and limestone treatments, there was an increase in sulfate during the trial period. Soybean oil lacked the N and P required for bacterial proliferation (Gibert et al. 2002; Waybrant et al. 2002) and is known to significantly reduce sulfate concentrations due to its carbon density (Phifer et al. 2001). Two soybean oil treatments resulted in an increase of sulfate concentrations, two others had moderate decreases in the sulfate concentration over the treatment period, and the final treatment had a significant reduction in sulfate concentration. This variability may have been caused by the diversity in microbial communities between the substrates. Sulfate-reducing bacteria are incapable of using large organic molecules and require other microbes to dissolve them into smaller, more soluble compounds (Skousen et al. 2000; URS 2003). The creek sediment, which was the substrate that produced the largest sulfate decrease when used in conjunction with the soybean oil, may have been the most efficient in breaking down the fatty acids in the oil to smaller compounds due to its naturally diverse microbial population. Consequently, the creek sediment treatment was capable of degrading the soybean oil and using it as a carbon source for sulfate reduction, whereas the other substrates were incapable of doing so.

Removal by Substrate

Each substrate produced at least one combination with significantly reduced sulfate concentrations after the treatment period (Fig. 5). Two of the substrates, limestone and marble chips, were only effective in combination with the biosolids amendment. As shown previously, the biosolids amendment consistently resulted in decreased sulfate concentrations; thus, the limestone and marble chips provided no benefit. The most beneficial substrate was the creek sediment (Fig. 5). The primary sulfate removal mechanisms from these systems appeared to be sulfate reduction. The total amounts of sulfate (7.5 mM) and Mn (1.8 mM) removed were not stoichiometrically consistent with a viable MnS formation, suggesting that Mn removal was mainly due to carbonate or oxide/oxy-hydroxide precipitation. The sediment contained a diverse population of natural microbial communities, including sulfate-reducing bacteria, more so than any other substrate, thus explaining the better sulfate removal efficiency.

The sand resulted in significantly lower sulfate concentrations, both without an amendment and with the addition of wood mulch (Fig. 5). As discussed previously, the wood mulch may have supported native sulfate-reducing bacterial communities. The small particle size of the sand may have been an ideal matrix for bacteria as they are capable of maintaining a suitable micro-environment in the interstitial spaces. A similar occurrence may have developed in the biosolids treatment, but the variability between replicates (13.5%) inhibits consistent interpretations. The river gravel exhibited high SO_4^{2-} removal capacity, in contrast to the Mn removal capability, probably due to different environmental preferences of the microbial communities involved. Four of the six treatment combinations removed greater than 30% of the initial sulfate concentration, but with considerable variability between replicates.

Conclusions

The most effective treatment combinations for Mn removal were those using biosolids or wood mulch as the amendment, particularly in combination with the creek sediment substrate. Similarly, sulfate removal was best in the creek sediment—biosolids combination, but without a clear second choice. The sorghum syrup and soybean oil were inconsistent in their capacity to induce sulfate reduction, likely due to low N and P levels. Therefore, fertilizer addition may increase SO_4^{2-} removal by those amendments. Wood mulch and the treatments with no amendment were similar in most aspects to each other, showing moderate Mn removal capacities, which were clearly less than the sediment and biosolids treatment. Substrate particle size and chemistry appeared to be an important aspect in the success or failure of a treatment. For example, the sand matrix induced more favorable conditions for microbial establishment and proliferation, while carbonate dissolution products from limestone and marble facilitated $MnCO_3$ formation. The creek sediment encompassed both of these aspects, as well as native microbial communities suitable for large organic molecule decomposition and sulfate reduction. Thermodynamic indicators suggested that no single removal mechanism (sorption, sulfide, oxide, or carbonate formation) was capable of removing all the Mn from solution in these treatments. Although significant sulfate removal was attained by certain amendment-substrate combinations, Mn-sulfide precipitation appeared to be of minor importance as a pathway for Mn removal in most treatments. Thermal and XRD analyses of treated substrates suggested sorption and precipitation as oxides, oxy-hydroxides, and carbonates to be the prevailing Mn removal mechanisms. Although the reported removal efficiencies may shift due to seasonal changes, the batch experiments suggested the most favorable treatment combination to be the creek sediment-biosolids mixture. Because the density of the material may inhibit percolation of the mine drainage solution through the substrate and considering the volume of biosolids required, a half and half mixture of wood mulch-biosolids amendment with creek sediment should be appropriate.

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