**Effect of alkalinity source on mechanisms of iron, manganese and zinc removal from acid mine drainage by sulfate-reducing bioreactors**

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**Abstract**

The purpose of this study was to investigate the effect of two different sources of alkalinity source on the mechanisms of metal removal in sulfate-reducing bioreactors. Four upward-flow sulfate-reducing bioreactors each containing a 23 L mixture of organic waste materials and either waste mussel shells or limestone as an alkaline amendment were tested at hydraulic retentions of 3.3 and 10 days to treat acidic mine drainage (pH 2.9, 30 mg/L Fe, 16 mg/L Mn, 5 mg/L Zn) for ten months. A combination of methods was used to examine the effect of alkalinity source on the fate of these metals. Consistent with the monitoring data of the effluent that showed circumneutral pH and low metal concentrations, higher concentrations of Fe, Zn and Mn were found in the spent than the initial substrate, with greater metal and acidity removal in l reactors containing mussel shells (at similar residence times). Sequential extraction procedures found that Fe was mainly in the oxidizable and the residual fractions, Zn in the reducible and residual, and Mn in the exchangeable, reducible and acid extractable fractions. SEM analyses confirmed the presence of pyrite in the substrate, and the use of PHREEQC supported the interpretation that precipitation of iron sulfide and oxyhydroxide minerals, manganese carbonates and zinc sulfide occurred within the substrate for both alkalinity sources. Adsorption edge experiments on the initial substrates confirmed the potential for Zn and Mn to adsorb onto organic materials. Alkalinity source greatly affected system performance with the mussel shell reactors outperforming limestone on a volumetric basis, with the inner surfaces of the mussel shells appearing to be important for greater ongoing alkalinity release, and the outer shells important as metal sorption sites not available in limestone reactors.

Keywords

Metal removal, mussel shell, sequential extraction procedure, adsorption, upward-flow.

1. **Introduction**

Acid mine drainage (AMD) is acidic, polluted water draining active and abandoned mining sites. It can contain high concentrations of metals (e.g. Fe, Al, Mn, Cu, Zn, Ni) and sulfates, with potentially significant environmental effects. Sulfate-reducing bioreactors (SRBRs) are a passive AMD treatment technique in which aqueous sulfide is produced through the microbial reduction of sulfate by sulfate-reducing bacteria (Eq. 1). Dissolved sulfide subsequently reacts with divalent metals (collectively denoted as M2+) present in the AMD to form highly insoluble metal sulfide minerals (Eq. 2) (Dvorak et al. 1992; Sheoran et al., 2010).

2CH2O + SO42- → 2HCO3- + H2S (1)

M2+ + H2S→ MS(S) + 2H+ (2)

Although the general processes responsible for the treatment of the metal-laden waters in SRBRs are now relatively well understood, and review articles have been published describing the state of knowledge (Sheoran, et al., 2010; Skousen et al., 2017), the exact metal removal mechanisms, and especially the environmental and design factors affecting them, remain difficult to generalize. Even though metal-sulfide precipitation is the targeted metal removal mechanism, other processes such as filtration, complexation, cation exchange, adsorption, (oxy)hydroxide precipitation and carbonate precipitation can also occur concurrently. Furthermore, the metal removal processes can change over time, and depend on several factors including the reactor design (e.g. flow design, organic substrate composition, microbial community, hydraulic retention time) and the AMD chemistry ([Gibert et al. 2002](#_ENREF_10); [Gusek 2005](#_ENREF_13)). For example, while adsorption may be an important removal process upon the start-up of a new engineered SRBR, sulfide precipitation could dominate later, once the sulfate-reducing bacteria are acclimated and the reducing conditions conducive to sulfate reduction are established ([Machemer and Wildeman 1992](#_ENREF_26); [Neculita et al. 2008](#_ENREF_28)). Passive SRBR systems are particularly challenging to understand because of the combination of biological, chemical, and physical processes, and because of the inherent variability in material properties, flow paths, and weather conditions.

A decrease in acidity and an increase in pH within SRBR are critical to achieving good performance. Studies have shown that the use of a specific alkalinity source (e.g., limestone) can be optimized by varying its particle size, or the freshness of its surface reactivity. In addition, a variety of non-mineral alkalinity sources have also been studied, with particular reference to the use of waste mussel shells (McCauley et al., 2009; Uster et al., 2015). Although previous research into the fate of target metals has examined a variety of influent chemistries, and a variety of changes to the use of a specific alkalinity source, there is insufficient understanding of the potential effect of widely varying alkalinity sources on mechanisms for metal removal while using the same influent chemistry.

In this study, the solids from the SRBR reactors with two alkalinity sources were destructively sampled, and chemical and mineralogical analyses of the spent substrate have been used to identify the metal-removal processes. Adsorption experiments have been used to evaluate the capacity of the organic substrate materials to adsorb Mn and Zn. Sequential extraction procedures (SEP) have been widely used to study the distribution and the speciation of metals in soils, sediments, and waste materials ([Hass and Fine 2010](#_ENREF_15); [Rao et al. 2007](#_ENREF_31)). Additionally, SEM equipped with an energy dispersive spectrometer (EDS) has proven to be successful in identifying solid-phase minerals in AMD treatment systems ([Gibert et al. 2005](#_ENREF_12); [Herbert et al. 2000](#_ENREF_17); [Neculita et al. 2008](#_ENREF_28)).. To provide additional insight into the potential mechanisms for metal removal, geochemical modelling can be used to consider potential mineral formation in SRBR. The combination of these methods has been used in this study to better elucidate the differences that alkalinity sources can have on metal removal mechanisms.

1. **Materials and Methods**

2.1 Sulfate-reducing bioreactors set-up and samples collection

In this study, upward flow SRBRs were tested to treat actual AMD sourced at an active coal mine located on the West Coast of the South Island of New Zealand. Four SRBR systems containing a reactive mixture (total reactive volume of 23.4 L) of bark (*Pinus radiata*), bark mulch (*Cordyline banksia*), compost and either waste mussel shells or limestone pieces as an alkaline material, were operated for a total of ten months (two continuous five-month periods separated by a two-month interruption period). The two-month interruption was intended to see if it would increase sulfate reduction and removal as suggested by Eger and Wagner (2002) and Whitehead et al. (2005). Details on the composition of the raw materials and on the mixtures in the reactors are provided in Tables 1 and 2.

Table 1. Physiochemical properties of materials used in reactors. Units of mg/kg of dry material except where noted. NM = not measured and assumed to be negligible.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Bark | Bark mulch | Compost | Mussel shell | Limestone |
| Moisture (%) | 40 | 18 | 51 | 13 | NM |
| Volatile solids (%) | 97 | 92 | 50 | 4.5 | NM |
| Bulk density (kg/m3) | 346 | 191 | 687 | 943 | 1400 |
| Ca | 0 | 0 | 803 | 365,000 | 364,000 |
| Mg | 546 | 55 | 2230 | 482 | 3750 |
| Fe | 7010 | 900 | 10,600 | 760 | 4230 |
| Al | 6050 | 930 | 8630 | 790 | 2780 |
| Mn | 170 | 92 | 680 | 28 | 220 |
| Zn | 53 | 36 | 490 | 11 | 23 |

Table 2. Substrate mixture composition and hydraulic parameters for four reactors.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Reactor ID | MS-S | MS-L | LS-S | LS-L |
| *Materials (% vol)* |  |  |  |  |
| Mussel shells | 30 | 30 | 0 | 0 |
| Limestone | 0 | 0 | 30 | 30 |
| Bark (*Pinus Radiata*) | 30 | 30 | 30 | 30 |
| Bark mulch (*Codyline banksia*) | 20 | 20 | 20 | 20 |
| Compost | 20 | 20 | 20 | 20 |
| *Mixed substrate parameters* |  |  |  |  |
| Hydraulic retention time (days) | 3.3 | 10 | 3.2 | 9.8 |
| Porosity | 0.51 | 0.51 | 0.49 | 0.49 |
| Total mixed mass (kg) | 13.1 | 13.1 | 16.4 | 16.4 |

The four systems have been tested to examine the influence of; (1) the alkaline material, using limestone (LS) or waste mussel shells (MS) tested separately, and (2) the hydraulic retention time (HRT) of 3 days (short, S) and 10 days (long, L). The four systems studied were mussel shell short HRT (MS-S), mussel shell long HRT (MS-L), limestone short HRT (LS-S), and limestone long HRT (LS-L). The detailed experimental set-up, as well as the water chemistry results of the flow-through treatability study, are described in Uster et al. (2014) and Uster (2015).

After ten months of operation, the influent AMD was stopped and the reactors were opened and drained under a high flow of nitrogen gas (BOC, 99.99 % purity). Spent substrate samples were manually retrieved using a plastic hand trowel from both the top and the bottom of the reactors (approximately 5 to 7 cm from the top and bottom sections of the reactive mixture). Samples from each of the four systems were collected and immediately placed in nitrogen-purged double-zip bags and frozen (-18 °C). Samples were taken in a way to be as representative of possible of the heterogeneous mixture in the reactors. While still frozen, approximately 200 g of samples were ground in an anaerobic bag to < 1 mm size fraction using an electric mill. Again, the samples were taken to provide a sample representative of the heterogeneous mixture of solids in each bag. The ground samples were thawed and mixed, and triplicate 2.5 g subsamples were used for the SEP described below.

2.2 Analytical methods

All chemical reagents used were analytical grade, and all glassware was previously acid-washed in 10 % HNO3 for a minimum of 24 h,and rinsed three times with tap water and three times with deionized water. All digestions were analyzed for Fe, Mn and Zn using ICP-MS (Agilent, 7500cx), according to Standard Methods 3125B ([APHA 2005](#_ENREF_1)). Quality assurance and quality control was achieved through the use of accredited standards, blanks, and replicated analyses which were within 10%. Duplicate analyses of total solids (TS) and volatile solids (VS) were performed on wet unground samples (Method 2540 G, APHA, 2005) to measure the moisture content and to obtain an indication of the organic content. TS and VS measurements were repeated until variation between duplicates was < 5 %. The Fe, Mn, and Zn content of the substrates, prior to use in the SRBR, were assessed following the APHA method 3030F (HNO3-HCl hot acid digestion). The pH meter (EDT RE-357Tx) was calibrated daily using pH 4, 7, and 10 buffer solutions. Alkalinity was measured according to APHA methods 2320B.

2.3 Sequential Extraction Procedure

Figure 1 provides an overview of the SEP. The methodology deviates from the original method from Jong and Parry (2004) for the extraction of fraction 3 and 6 only. Because the acid extractable step can also remove metals specifically sorbed on Mn-oxides (e.g. Mn-oxides are very sensitive to pH changes around 5, the pH of the sodium acetate extraction solution was adjusted to 5.5 instead of 5. Because of safety concerns, the residual fraction was obtained using a hot HNO3-HCl acid digestion (APHA method 3030F) instead of the hot perchloric acid digestion used by Jong and Parry (2004). SEP tests were conducted at room temperature (23 – 25 °C).

**Fig 1.** Sequential Extraction Procedure methodology (based on Jong & Parry, 2004).

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Between each step of the extraction, the solid-liquid samples were centrifuged at 5000 rpm (Hermle Z383) for 15 min. The supernatant was then carefully transferred in a centrifuge tube, filtered at 0.45 µm using a syringe filter, acidified to pH < 2 with concentrated nitric acid (Fisher, analytical grade 69 %), and stored at 4°C until metal analyses using ICP-MS. Before proceeding to the next extraction step, the solid residue was washed with 16 ml of deionized water and mechanically agitated for 10 min. The rinses were separated, filtered, and retained for analysis following the above mentioned procedure. For quality control purposes, each spent substrate sample was analyzed in triplicate to ensure the reproducibility of the methodology. In addition, triplicate digestions of each sample were performed following the same HNO3-HCl hot digestion as used for the residual fraction; these are called pseudo-totals to denote that small amounts of non-extractable metals could have been left after digestion (e.g., metals associated with silicates). A single blank containing all the reagents, but no spent substrate samples was also analyzed for each fraction, and the results were later subtracted from the spent substrate sample results.

In order to minimize oxidation and help preserve the metal speciation, the SEP experiment was conducted in a 500 L glove-bag (Sigma-Aldrich) filled with high purity nitrogen. When taken outside of the glove bag (for centrifugation or storage purposes), the samples were kept in tightly closed 50 ml centrifuge tubes wrapped with parafilm. Additionally, all extractant reagents were previously purged with high purity N2 for a minimum of 30 min, and all disposable materials were kept in the glove-bag for a minimum of 24 h prior to using them.

2.4 Adsorption Edge Determinations

An adsorption batch experiment was performed to gain additional insight into the organic mixture’s ability to retain Mn and Zn. Fresh bark, bark mulch and compost, in the proportion used in the reactors were sampled for the test. To minimize microbial activity during testing, the organic mixture was microwaved for 3 min at 1000 W, ground and sieved to obtain a particle size < 1 mm, and oven-dried at 55°C overnight to remove all moisture. This material was mixed and then sampled for the adsorption experiment.

The adsorption edge experiment was conducted over a pH range of 3 to 9 following the methodology from Bibby and Webster-Brown ([2006](#_ENREF_4)). A matrix solution of 0.1M NaNO3 was spiked with metal salts (Mn(NO3)2 and Zn(NO3)2) to obtain final concentrations of 5 mg/L Mn and 2 mg/L Zn. These concentrations and a solid to metal ratio of 1 g organic material per 100 ml of metal solutions were chosen to approximate 10 pore-water volumes when compared to the bioreactor flow-through experiment. 500 ml bottles containing the solid/liquid mix were kept overnight at 4°C to allow equilibrium and complete re-hydration of the solid surfaces. The solid/liquid slurry solutions were then carefully transferred in 2 L beakers and continuously agitated using a magnetic stirrer. The solution pH was lowered to 3 using diluted HNO3 and then slowly raised to pH 9 by incremental addition of diluted NaOH. 12 ml aliquots were taken at approximately each 0.5 pH unit increment and placed into 50 ml centrifuge tubes and immediately placed on a rotary shaker. After a 24 h period of constant agitation, the samples were centrifuged, the supernatant final pH verified, and 5 ml samples were filtered through 0.45µm syringe nylon filters, acidified to pH < 2 with concentrated HNO3 and analyzed for metal concentrations using ICP-MS. Two control experiments were also carried out to determine (1) the degree of metal removal through adsorption onto the containers walls and possible precipitation of metal oxyhydroxides; and (2) the release of metals from the organic mixture. Adsorption tests were conducted at room temperature (23 – 25 °C).

2.5 Solid phase analysis with SEM

SEM-EDS was used to characterize the spent substrate mineralogy. Samples were dried overnight at 105° prior to being manually disaggregated and mounted on plastic stubs and coated in approximately 25nm of carbon. Bark, bark mulch, mussel shells and limestone subsamples were looked at individually, and both the inner and outer sides of the mussel shell samples were analyzed. The SEM (JEOL JSM6100) was equipped with an Oxford Aztec silicon drift detector EDS, and morphological observations were performed at an accelerating voltage of 20 kV.

2.6 Geochemical modeling

The chemical speciation model PHREEQC version 3.0.6 ([Appelo and Parkhurst 1999](#_ENREF_2)) with the MINTEQV4 database was used to calculate the mineral saturation indices (SI) of selected mineral phases potentially precipitating in SRBRs. Input parameters used in the model were as follows: metals and sulfate from influent concentrations, oxidation-reduction potential (ORP) and temperature from pore-water measurements, and alkalinity and pH from effluent measurements. Values used in geochemical modeling are presented in Table 1. They were chosen to emulate the SRBR potential to precipitate metals within the substrate reactive mixture. The model mineral saturation results were then compared with the SEP results.

**Table 3.** Values used in geochemical modelling of expected precipitant formation in the four reactors. Values taken from Uster et al. (2014) and Uster (2015).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **Reactor ID** | **MS-S** | **MS-L** | **LS-S** | **LS-L** |
|  | **Source of Reactor Alkalinity** | **Waste Mussels** | **Waste Mussels** | **Limestone** | **Limestone** |
|  | **Residence Time** | 3 d | 10 d | 3 d | 10 d |
|  |  |  |  |  |  |
| **Water Source** | **Parameter (units)** |  |  |  |  |
| Effluent | pH | 5.9 | 6.7 | 4.1 | 5.9 |
|  | Alkalinity (mg/L as CaCO3) | 58 | 210 | 8 | 38 |
| Pore-water | Oxidation-reduction potential (mV) | -20 | -300 | 10 | -280 |
|  | Temperature | 17.1 | 17.1 | 17.1 | 17.1 |
|  | P (mg/L) | 5.9 | 33 | 5.2 | 12 |
| Influent | Fe (mg/L) | 60 | 60 | 60 | 60 |
|  | Al (mg/L) | 21 | 21 | 21 | 21 |
|  | Mn (mg/L) | 18 | 18 | 18 | 18 |
|  | Zn (mg/L) | 4.8 | 4.8 | 4.8 | 4.8 |
|  | S (mg/L) | 610 | 610 | 610 | 610 |

1. **Results**

3.1 SRBR effluent water quality

Results from the AMD treatability study (Table 4 and supplemental information) show that the SRBRs were effective at increasing pH and alkalinity, removing metals and, to a lesser extent, reducing sulfate concentrations ([Uster, 2015](#_ENREF_36)). Over the 10-month period, the effluent pH was constantly between 6 and 7.1 for reactors MS-S, MS-L, and LS-L, whereas system LS-S had effluent pH values between 5 and 6 from week 27 onwards. Over > 83% of Fe and Zn had been removed, at both 3 and 10 days HRTs, and using both alkalinity amendments (mussel shells or limestone). Mn had also been partially removed (median removal 20 - 55 %) by the substrate during weeks 6 to 20, but from week 20 to 40 (end of the test) its removal efficacy declined (median removal 6 to 31 %). The reactors operating at a longer HRT resulted in a significantly improved effluent quality for all monitored parameters. Additionally, SRBRs using mussel shell instead of limestone in the reactive mixture showed a better overall treatment efficacy, especially higher alkalinity generation and higher metal removal. However, no significant change in sulfate removal was observed between MS and LS.

**Table 4.** Median values for various water quality parameters over the 40 weeks of treatment (data from Uster, 2015). Influent data are provided in Table 7 in SI.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | MS-S | MS-L | LS-S | LS-L |
| Hydraulic residence time (d) | 3.3 | 10 | 3.2 | 9.8 |
| Alkalinity source | Mussel shells | Mussel shells | Lime-stone | Lime-stone |
| Fe removal (%) | 90.9 | 95.2 | 83.9 | 90.6 |
| Zn removal (%) | 99.5 | 99.7 | 92.3 | 96.1 |
| Mn removal (%) | 15.9 | 38.8 | 9.2 | 26.6 |
| Sulfate reduction (%) | 4.3 | 12.4 | 3.7 | 11.5 |
| pH (effluent) | 6.4 | 6.8 | 6.1 | 6.4 |
| Alkalinity (effluent; CaCO3 mg/L) | 179 | 315 | 64 | 150 |

3.2 Chemical analysis of substrates

Results from the hot acid digestions performed on the organic and alkaline materials prior to their use in the SRBRs (Table 1) indicated that metal contents were low in all materials, apart from Fe in the bark, the compost and the limestone. Because Fe and Zn were not expected to leach significantly from the substrates (SRBRs have high reported removal rates for these two metals), and because Mn was found in relatively high concentration only in the compost material, the results suggest that the organic and alkaline materials used in the SRBR substrates were unlikely to contribute high metal concentrations to the treated effluents.

3.3 Sequential extraction of substrates

The SEP results are shown in Fig. 2 and Fig. 3 (Table 6 in supplementary information shows top and bottom values, pseudo-total and blank results). The coefficient of variation of triplicate analyses for fractions 1 to 5 was found to be ≤ 12 % for all samples (n = 120), except three Zn samples in fraction 1 (coefficient of variation for Zn in fraction 1 was between 7-23 %), indicating that the methodology and the precision of the analytical and laboratory work were satisfactory. The residual step (fraction 6) had a much larger variation between triplicate analyses (usually between 20-30 %, but up to 85 % in one case). The metal losses during rinses performed in between each extraction step were substantial when compared to the total (i.e. extract plus rinse), with values constantly between 10-58 % for fractions 1 and 2 (water soluble species and exchangeable ions) and between 5-51 % for fractions 3 to 5. Based on this observation, the extracts and the rinses have been aggregated and considered together for the overall interpretation of the results (e.g. the rinse between the second and the third extraction step was aggregated with the results obtained for the second step and so on).

The blank results were overall very small compared to the metal extracted from the spent substrate samples. The only significant values ranged between 3-11 % for Fe in fractions 1, 2 and 3 and between 1.3-31 % for Zn in fractions 1 and 2. Considering that these fractions contained < 1 % and < 3 % of the total extracted amount of Fe and Zn, respectively, these findings have no impact on the interpretation of the results. The metal recovery (%) in the SEP, calculated as the sum of metal extracted in each fraction divided by the pseudo-total values ([Hullebusch et al. 2005](#_ENREF_19)), ranged between 90-300 % for Fe, 130-240 % for Zn, and 90 - 115 % for Mn. The higher values obtained for the sum of each extracted fraction compared to the pseudo-total values is a common issue associated with SEP and can be explained by several factors including low concentrations of some metals in the spent substrate samples (resulting in higher relative errors), imprecision of laboratory work, and redistribution of the metals into the remaining phases once a targeted phase has been extracted.

**Fig. 2** SEP results showing total concentration and partitioning of(A) Mn, (B) Zn, and (C) Fe in the spent reactive substrates. Results are average of top and bottom samples, and are reported in mg/kg (Mn, Zn) or in g/kg (Fe).



**Fig. 3** SEP results showing relative percentages (%) ofmetals in(A) MS-S, (B) MS-L, (C) LS-S, and (D) LS-L systems Results are average of top and bottom samples.



Based on the total extractable results (Fig. 2), the metal concentrations followed the same order in each sample (Fe > Mn > Zn), which is consistent with the relative influent metal concentrations. Based on the amount of metal found in each fraction, the relative partitioning of each element was found to be in the order of Mn > Zn > Fe for the water soluble, exchangeable and carbonate fractions, Zn > Mn > Fe for the reducible (Fe-Mn oxide) fraction, and Fe > Zn > Mn for the oxidisable (organic matter and sulfide) and residual fractions.

Fig. 2 shows a greater amount of Fe, and to a lesser extent a greater amount of Zn, in the final residual fraction in limestone systems compared to mussel shell systems. This observation does not represent additional Fe removal from the AMD. The main reason for the high Fe in the limestone systems is the higher Fe content in the limestone substrate,

3.4 Mineral phase identification

Figure 4 shows the results of SEM-EDS microanalyses (multiple localized scans with element mapping) performed on individual spent substrate materials (bark, bark mulch, mussel shells, limestone), with the inner and the outer sides of the mussel shells fragments examined separately.

**Fig 4.** Average results from multiple EDS element mapping scans (n = 5 to 10) showing contents (wt. %) of Fe, Mn, S & Zn on individual materials retrieved from the top parts of the reactors, with short and long HRT samples combined. Graph E shows Fe, Mn, Zn, and S on the inner and outer surfaces of the mussel shells.



The SEM-EDS results identified discrete iron sulfide and gypsum (CaSO4) minerals (Fig. 5). The average elemental composition of the iron sulfide mineral (wt. %) was 46 % S, and 38 % Fe (i.e. approximately a 1:2 molar ratio) suggesting pyrite (FeS2). Similar spherical precipitates containing Fe and S have been reported by other studies ([Gibert et al. 2003](#_ENREF_11), [2005](#_ENREF_12)). Although the precipitation of CaSO4 was not expected in the reducing conditions encountered within the SRBR substrates, its presence has previously been reported in the microenvironments within similar treatment systems ([Rose et al. 2007](#_ENREF_33)).

**Fig 5.** SEM-EDS element mapping of a fragment of bark in system LS-S showing discrete minerals of (A) iron sulfide, and (B) gypsum.



3.5 Adsorption of Zn and Mn onto organic materials

Results from the adsorption experiment showed that both Mn and Zn were effectively adsorbed onto the organic materials used in the SRBRs (Fig. 6).

**Fig 6.** Adsorption of (A) Mn and (B) Zn onto the organic SRBR substrate materials, for initial concentrations of 5 mg/L Mn and 2 mg/L Zn, and a suspended substrate concentration of 10g/L. Control experiments did not have substrate present.



Mn adsorption increased linearly with increasing pH, and at pH 7 up to 70 % of dissolved Mn was adsorbed. Even though no clear adsorption edge was visible, an approximate pH50 value of 4.5 can be derived. From the Mn-control experiment we found that up to pH 6.5 less than 20 % Mn was removed through adsorption onto the container walls or removed through precipitation (presumably as a manganese oxide), and only when the pH was raised above 7 were substantial amounts (63 %) of Mn removed via these mechanisms.

Zinc adsorption increased with increasing pH, with a distinct adsorption edge visible, and a pH50 value of 3.6. Between pH 3 and 5.5 almost 90 % of Zn was removed by adsorption; the Zn-control experiment showed > 20 % Zn removal only at pH >5.5.

3.6 Geochemical modeling

A comparison of saturation indices (SI) predicted with PHREEQC for low temperature stable mineral phases, and inferred mineral phases suggested from the SEP experiment, and using input values in Table 3, is presented in Table 5.

**Table 5**. Mineral phases anticipated by PHREEQC and SEP. Saturation indexes found by PHREEQC are shown, with those in bold having values > 0 indicating supersaturation, and those in italics have values < 0 indicating undersaturation. Shaded cells show cases where SEP indicated ≥ 9 % of the metal was associated with the corresponding mineral phase. Note that there was no extraction step in the SEP targeting phosphate minerals, and that (oxy)hydroxide and oxide phases are considered together in the SEP. Input values are in Table 3.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **Mineral phase** | **Formula** | **MS-S** | **MS-L** | **LS-S** | **LS-L** |
| **Fe** | Ferrihydrite | Fe(OH)3 | *-2.7* | *-9.7* | *-3.9* | *-8.3* |
|  | Goethite | α-FeO(OH) | **0.1** | *-6.9* | *-1.1* | *-5.5* |
|  | Hematite | Fe2O3 | **2.6** | *-11.4* | **0.1** | *-8.7* |
|  | Vivianite | Fe3(PO4)2∙H2O | **2.4** | *-8.0* | **0.0** | *-8.5* |
|  | Mackinawite | FeS  | *-10.2* | **0.2** | *-5.0* | **0.2** |
|  | Pyrite | FeS2 | *-7.4* | **9.2** | **2.3** | **12.2** |
|  | Greigite  | Fe3S4 | *-35* | **2.2** | *-15.2* | **5.2** |
| **Mn** | Rhodochrosite  | MnCO3 | *-0.5* | **0.5** | *-0.8* | *-0.1-* |
|  | Mn phosphate | MnHPO4 | **4.0** | **4.3** | **3.0** | **3.6** |
|  | Pyrolusite  | MnO2 | *-23* | *-30* | *-25* | *-28* |
|  | Manganite | MnOOH | *-11.4* | *-14.4* | *-12.6* | *-13.6* |
| **Zn** | Smithsonite | ZnCO3 | *-1.7* | *-9.7* | *-2.1* | *-9.5* |
|  | Zincite | ZnO | *-3.6* | *-11.8* | *-4.2* | *-11.7* |
|   | Sphalerite | ZnS | *-3.0* | **2.5** | **2.2** | **2.7** |

4 Discussion

4.1 Iron Removal Mechanisms

Despite the fact that the limestone originally contained more iron (mostly in the residual fraction 6), Fe was found to be mainly associated with the residual and organic/sulfide fractions in all four systems. We hypothesize that most Fe recovered from Fraction 5 (organic matter/sulfide) was associated with sulfide minerals rather than with the organic matter. The VS measurements on the post-treatment samples are 26 to 43 % of organic matter for mussel shell reactors, while the mixtures containing limestone had only 26 to 34 %. Although the methodology has clear limitations and probably slightly overestimates the true organic content (i.e. the mass loss during combustion also includes some mineral salts present in the spent substrates), these results suggest that most Fe recovered from Fraction 5 (organic matter/sulfide) was associated with sulfide minerals rather than with the organic matter, especially in the MS systems.

More Fe was associated with fraction 5 in MS systems (up to 35 %) compared to LS systems (10-15 %). It is possible that more Fe(II) was available in MS systems to react with the biogenic sulfides, and more Fe(III) was present in LS systems and removed as oxyhydroxides. This hypothesis is supported by the lower ORP values reported for MS reactors and the similar sulfate percentage removal reported for MS and LS systems (Table 1). Furthermore, the longer HRT systems resulted in additional Fe being removed in fraction 5, when compared to the short HRT; an observation which is again consistent with the higher sulfate reduction values reported for systems MS-L and LS-L.

The geochemical model predicted the precipitation of low temperature stable iron (oxy)hydroxides (e.g. goethite and hematite), and sulfides (greigite, mackinawite, pyrite). These results were mostly corroborated by the SEP results. While PHREEQC predicted oxyhydroxides to precipitate only in reactor MS-S (where the ORP values were higher), the SEP indicated that these minerals were found in both MS-S and MS-L. This difference is likely explained by the fact that aerobic microenvironments were also present in the MS-L system. On the other hand, pyrite was predicted and its presence confirmed by SEP in all four systems.

4.2 Manganese Removal Mechanisms

In MS systems, Mn was principally found in the exchangeable (30-33 %), the carbonate (32-36 %) and the Fe-Mn oxides fractions (21 %). In LS systems, Mn was associated in similar relative concentrations (approx. 20-30 %) with the exchangeable, carbonate bound and reducible fractions (Fig. 3). The removal of Mn through carbonate mineral precipitation (e.g. rhodocrosite) is supported by the fact that more Mn was associated with carbonates in the MS reactors compared to the LS systems, which is in accordance with the higher alkalinity concentrations measured in MS systems. Additionally, more Mn was removed with the carbonates in the top parts of short-HRT reactors for both alkalinity sources (Table 6 in SI), an observation that further supports the removal of Mn as rhodocrosite (i.e. pH, alkalinity and Ca concentrations were higher in the top sections of the upflow reactors compared to the bottom sections, due to the upward-flow design). It is also possible that the organic matter associated with the mussel shell (i.e. mussel flesh remains, and chitin-related proteins associated with the shells) provided additional adsorption sites, possibly explaining the greater proportion of Mn associated with the exchangeable fraction in MS systems compared to LS systems. It is also possible that higher-pH microenvironments close to the mussel shells or the limestone fragments allowed for the precipitation of Mn-oxides (e.g. birnessite), possibly explaining the 21 %, and 24-29 % of Mn recovered with fraction 4 in MS and LS systems, respectively. The removal of Mn through carbonate precipitation and adsorption has been recognized by others studies ([Robinson-Lora and Brennan 2010](#_ENREF_32); [Waybrant et al. 1998](#_ENREF_38)). This study’s findings are in contrast to the results of bioreactors from Neculita et al. ([2008](#_ENREF_28)) who found Mn to be almost entirely associated with the residual fraction, but in agreement with the those of Swash and Monhemius ([2005](#_ENREF_35)) who found most of the Mn to be in the carbonate fraction in constructed wetlands. In addition, the higher removals of Mn with longer residence times found in this study is consistent with a finding that precipitation of MnCO3 seems to be a kinetically regulated process. Lebron and Suarez ([1999](#_ENREF_24)) found that solutions supersaturated with respect to MnCO3 could remain metastable up to 72 h, supporting this finding.

Although PHREEQC suggested the precipitation of rhodochrosite only in system MS-L, the SEP results indicated that Mn carbonates precipitated in each of the four systems. Again, this difference is likely explained by the fact that the chemical inputs values were bulk geochemical parameters, and therefore precipitation in microenvironments close to the mussel shell or limestone fragments, where the pH could be significantly higher, would not be predicted by the model.

Considering that during the flow-through SRBR experiment the effluent pH was constantly between 6 and 7.1, and referring to Figure 6, we can assume that adsorption was an important mechanism for Mn removal, at least during the start-up period. This hypothesis is supported by both the SEP results (i.e. substantial amounts of Mn were retained in the exchangeable fraction), and the previous SRBR study (Uster et al, 2014), which showed that up to 80 % Mn was removed during the first few weeks of the flow-through experiment, followed by a constant decline. This was interpreted as a consequence of adsorption sites available for metal binding declining with time. Similar adsorption behavior for Mn has been reported in AMD systems ([Gibert et al. 2005](#_ENREF_12); [Webster-Brown et al. 2012](#_ENREF_39); [Younger et al. 2002](#_ENREF_41)) and in natural river environments ([Webster-Brown et al. 2012](#_ENREF_38)).

4.3 Zinc Removal Mechanisms

Most Zn in the MS reactors was associated with the Fe-Mn oxides (60-68%), and organic matter/sulfides fractions (14-24 %), while only 4-13 % was found in the residual fraction (Fig. 3). In LS reactors, Zn was mostly found in the residual fraction (45-57 %), the Fe-Mn oxide fraction (28-34 %) and only 11-13 % in the organic matter/sulfides phases. Although limestone originally contained approximately two times more Zn in the limestone compared to the mussel shell (Table 2), these results suggested that Zn was removed through adsorption onto and/or co-precipitation with Fe-oxides, as well as sulfide precipitation in both MS and LS systems. These findings are in agreement with other SEP studies which found most Zn to be associated with sulfides and Fe-Mn oxides ([Jong and Parry 2004](#_ENREF_22); [Neculita et al. 2008](#_ENREF_28)). Additionally, Wallman et al. ([1993](#_ENREF_37)) showed that zinc sulfides can dissolve in acidified reagents at pH 5 (e.g. due to the greater solubility of ZnS compared to other sulfide minerals). This implies that sulfide minerals, especially ZnS, could have dissolved in several steps of the extraction procedure used here, and not only in the oxidisable fraction, potentially leading to an underestimation of the true zinc sulfide amount.

Several studies have shown that Zn can adsorb onto organic matter, including living and dead biomass ([Azabou et al. 2007](#_ENREF_3); [Gibert et al. 2005](#_ENREF_12); [Webster-Brown et al. 2012](#_ENREF_39)). Additionally, numerous studies have shown that Fe- and Mn-oxides can adsorb high concentrations of metals such as Zn, Cu, or Cd ([Dyer et al. 2004](#_ENREF_9); [Jurjovec et al. 2002](#_ENREF_23); [Webster et al. 1998](#_ENREF_40)). Based on the SEP results of this study (Fig 2 and 3), the adsorption of Zn onto Fe and Mn oxyhydroxides appear to dominate, rather than binding to the organic matter.

Finally, the geochemical model predicted only zinc sulfides to precipitate (e.g. sphalerite and an amorphous ZnS phase), but results from the SEP indicated that Zn was associated with reducible fraction (Fe and Mn oxyhydroxides) also. Therefore, we can assume that both ZnS precipitation and additional removal through co-precipitation with and/or adsorption onto Fe-Mn oxides occurred.

4.4 Mussel Shell Reactivity

Figure 4E shows that more metals were retained on the outer side of the mussel shells compared to the inner side. These results indicate unique characteristics of this alkalinity source and merit discussion. The shells are comprised of three distinct layers: an inner shell layer (i.e. the inner side*, hypostracum*) primarily made of aragonite, a middle shell layer (*ostracum*) made of calcite and aragonite interbedded with proteins molecules, and an outer shell layer (i.e. the outer side, *periosctracum*) made of a nitrogen polysaccharide chitin (Cubillas et al. 2005). Therefore, considering that aragonite dissolves faster than calcite ([Cubillas et al. 2005](#_ENREF_7)), it is likely that the metals accumulating onto the inner side (i.e. aragonite layer) were continually released back in solution because of the faster dissolution. Additionally, the outer side (i.e. chitin layer) is likely to afford more adsorption sites compared to the inner aragonite layer. Further controlled experiments are warranted to further evaluate the reactivity of different parts of the mussel shell.

4.5 General Discussion

Similar to the SEP results, SEM-EDS microanalyses showed higher Fe concentrations than Mn or Zn concentrations, consistent with the influent AMD metal concentrations. The EDS scans indicated more metal accumulation in bioreactors operating at a short HRT (Fig. 4A, B, D). In contrast, the sulfur content of both the organic and the alkaline materials (Fig. 4C) showed that the longer HRT systems resulted in additional sulfur binding (presumably in the form of sulfides). These observations concurred with the water chemistry results, which showed that the longer HRT systems reduced more sulfate, but that the short HRT systems removed more metals from a mass balance perspective (i.e. the short HRT reactors received about 3 times the amount of AMD compared to the long HRT systems over the course of the 10 months flow-through experiment, Uster 2015). Short HRT systems also showed more Fe accumulation than S (on all materials and in all systems, apart from bark in MS-S), suggesting that sulfide precipitation was not the only removal pathway for Fe. In contrast, long HRT reactors either displayed similar Fe and S contents or additional S compared to Fe, suggesting that precipitation of iron sulfide was an important removal mechanism in these systems. This study did not include measurement of H2S gas production, limiting the ability to conduct a sulfur mass balance, which would be needed to better understand the loss of sulfide mineral precipitation with shorter residence times.

In this study, SEP showed that > 99 % Fe (the most abundant metal in the influent AMD) and > 88 % Zn were contained in the residual, reducible and oxidisable fractions, whereas Mn was mostly associated with the exchangeable and the carbonate fractions in MS systems (≥ 65%) and with the exchangeable, carbonate and Fe-Mn oxides fractions in LS systems (≥ 68%). These findings indicate that the immediate mobility and bioavailability of both Fe and Zn are lower than that of Mn, which is clearly more labile and could become more bioavailable if pH and ORP conditions in the spent substrate were to change. In addition, long HRT systems retained more Fe and Zn as sulfides (supported by the lower ORP and the higher sulfate reduction values), and more Mn as carbonates, which is consistent with the higher alkalinity values and the finding of Lebron and Suarez ([1999](#_ENREF_24)) which suggested that an HRT of ≥ 3 days was necessary to precipitate rhodocrosite. The results support a finding that longer residence times in passive treatment systems provide lower bioavailability and improved resilience, with significant benefits from increasing the residence time from 3 to 10 days.

While Fe and Zn sulfide mineral precipitation (the desired metal removal process) did occur, other removal mechanisms also took place, including the formation of Fe and Mn (oxy)hydroxide minerals, the adsorption of Mn and Zn onto organic matter and Fe- and Mn-oxides, precipitation of Mn carbonate, and, potentially, the formation of phosphorus precipitants.

The PHEEQC model predicted the precipitation of an iron phosphate (e.g. vivianite) in the two short HRT systems, and a Mn hydrogen phosphate (e.g. MnHPO4) in all four reactors. While the influent AMD phosphate concentration was constantly <1 mg/L, and the effluent concentrations were between 1 to 11 mg/L, the PO43- concentrations in the pore-waters (used for the modeling) ranged from 16 to 132 mg/L, clearly indicating release of phosphate from the SRBR substrate under reducing conditions. Others studies have also suggested the removal of Mn through precipitation or chemisorption with phosphates ([Robinson-Lora and Brennan 2010](#_ENREF_32); [Carliell-Marquet and Wheatley 2002](#_ENREF_6)). This hypothesis could not be verified via the SEP used in the present study because there is no extraction step that is selective for phosphate minerals

The reactors had five months of operation, then two months of rest, and then a further five months of operation. During this time, no influent or effluent occurred and the reactors were sealed. Because they were upflow reactors, the reactors were kept in anaerobic state easily during this time. Although unusual for a laboratory experiment, this on/off method of operation can happen with passive systems in the field where a wide variety of situations can occur that require cessation of operation (adverse weather, rerouting of AMD because of changing needs). In this case, the two-month interruption period was intended to see if metals (especially Mn) and sulfate removal rates would increase, and go back to the higher values observed during the first 5-month period of treatment. The results indicated no negative impact on treatment because of the cessation, but there is no clear indication that it has influenced the interpretation of data, nor that the interruption helped to increase the metals and sulfate removal rates.

Limitations of the current study include problems associated with suspected short-circuiting or preferential pathways development within the treatment system (i.e. reducing the HRT), and ingress of oxygen through the pore-water port during sampling events. These problems may have led to a less reducing environment within the SRBRs, and promoted the precipitation of (oxy)hydroxides instead of sulfide minerals.

1. **Conclusions**

Chemical and mineralogical analyses of the solid substrate were used to investigate metal removal mechanisms occurring in upward-flow sulfate-reducing bioreactors (SRBR) treating AMD over a 10-month period. Water chemistry results had previously indicated that Fe and Zn were effectively removed (≥ 80 %) in the SRBRs, and Mn partially removed (≤ 38 %). From a methodological point of view, SEP and geochemical modeling were more informative overall than SEM, which proved to be less effective in identifying metal-bearing mineral phases. Although frequent issues associated with SEP are difficult to assess (e.g. limited selectivity of the reagents, possibility of re-adsorption and re-distribution of the extracted metals, insufficiency of reagent if the metal concentrations are too high), the precision of the present methodology and laboratory work was found to be satisfactory.

The results, taken together, highlight the importance of the choice of alkalinity source in design of SRBR systems. In this study, the mussel shell reactors exhibited lower ORP, higher pH, and higher metal removal (at equivalent residence time). The contrast in the behavior of the inside and outside mussel shells demonstrate the subtle effects that alkalinity source can have on metal removal mechanisms: the inner surfaces of the shells appear to be key to ongoing alkalinity release, while the outer surfaces of the shells appear important as sorption sites to aid in metal removal.

The research also highlights the value of multiple methods for identifying metal removal mechanisms. In this study five different sets of data (water quality, SEP, adsorption, SEM-EDS, and PHREEQC modelling) were used to help elucidate mechanisms where each individual method on its own (with its own limitations) would be less valuable.

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**Supplementary materials**

**Table 6.** Sequential Extraction Procedure (SEP) results showing Fe, Mn and Zn partitioning in the spent reactive substrates. Results are expressed as mean of triplicate analyses and reported in mg/kg. Reagent blank values are already subtracted from the spent substrate sample values, and shown at the bottom of Table A-1. Pseudo-totals give total metals found from concentrated acid digestion. MS-S and MS-L indicate mussel shell systems short and long HRT; LS-S and LS-L indicate limestone systems short and long HRT, respectively. F1 (water soluble), F2 (exchangeable ions), F3 (acid extractable), F4 (reducible), F5 (oxidisable), F6 (residual), PT (pseudo-total).

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **SRBR** | **Sample location** | **Fractions** | **Fe (mg/kg)** | **Fe (%)** | **Mn (mg/kg)** | **Mn (%)** | **Zn (mg/kg)** | **Zn (%)** |
| MS-S | top | F1 | 0.86 | 0.02 | 21.98 | 3.5 | 0.92 | 0.4 |
|  |  | F2 | 4.52 | 0.10 | 247.69 | 39.1 | 1.09 | 0.5 |
|  |  | F3 | 19.97 | 0.42 | 189.95 | 30.0 | 8.65 | 4.0 |
|  |  | F4 | 553.47 | 11.8 | 116.23 | 18.4 | 128.80 | 60.1 |
|  |  | F5 | 1308.94 | 27.8 | 19.06 | 3.0 | 46.67 | 21.8 |
|  |  | F6 | 2814.17 | 59.9 | 38.44 | 6.1 | 28.10 | 13.1 |
|  |  | *Sum* | *4701.94* | *100* | *633.35* | *100* | *214.23* | *100* |
|  |   | *Pseudo-tot.* | *3189.31* | *147.4* | *784* | *80.8* | *165.6* | *129.3* |
|  | bottom | F1 | 1.79 | 0.03 | 18.43 | 3.1 | 0.49 | 0.2 |
|  |  | F2 | 4.92 | 0.09 | 163.32 | 27.3 | 0.35 | 0.2 |
|  |  | F3 | 19.22 | 0.36 | 199.79 | 33.4 | 2.69 | 1.4 |
|  |  | F4 | 719.09 | 13.6 | 141.24 | 23.6 | 113.23 | 58.0 |
|  |  | F5 | 1740.09 | 33.0 | 31.77 | 5.3 | 50.38 | 25.8 |
|  |  | F6 | 2787.01 | 52.9 | 43.43 | 7.3 | 28.20 | 14.4 |
|  |  | *Sum* | *5272.12* | *100* | *597.98* | *100* | *195.34* | *100* |
|   |   | *Pseudo-tot.* | *3757.29* | *140.3* | *444* | *134.8* | *120.1* | *162.6* |
| MS-L | top | F1 | 1.12 | 0.03 | 19.75 | 4.5 | 0.67 | 0.3 |
|  |  | F2 | 4.39 | 0.13 | 113.11 | 25.9 | 1.95 | 0.9 |
|  |  | F3 | 20.37 | 0.61 | 174.21 | 39.9 | 22.39 | 10.1 |
|  |  | F4 | 742.20 | 22.4 | 96.49 | 22.1 | 153.49 | 69.6 |
|  |  | F5 | 1205.33 | 36.4 | 16.00 | 3.7 | 28.73 | 13.0 |
|  |  | F6 | 1338.88 | 40.4 | 16.58 | 3.8 | 13.38 | 6.1 |
|  |  | *Sum* | *3312.28* | *100* | *436.13* | *100* | *220.59* | *100* |
|  |   | *Pseudo-tot.* | *3581.48* | *92.5* | *578* | *75.4* | *125.3* | *176.1* |
|  | bottom | F1 | 1.43 | 0.03 | 21.89 | 4.1 | 0.66 | 0.3 |
|  |  | F2 | 6.70 | 0.14 | 184.20 | 34.1 | 2.94 | 1.2 |
|  |  | F3 | 27.50 | 0.56 | 173.55 | 32.1 | 22.81 | 9.1 |
|  |  | F4 | 753.78 | 15.3 | 112.09 | 20.7 | 162.39 | 64.7 |
|  |  | F5 | 1630.41 | 33.2 | 18.86 | 3.5 | 41.15 | 16.4 |
|  |  | F6 | 2493.72 | 50.8 | 29.69 | 5.5 | 20.88 | 8.3 |
|  |  | *Sum* | *4913.53* | *100* | *540.28* | *100* | *250.83* | *100* |
|   |   | *Pseudo-tot.* | *3729.29* | *131.8* | *526* | *102.7* | *105.4* | *237.9* |
| LS-S | top | F1 | 1.26 | 0.01 | 16.08 | 2.1 | 10.33 | 2.5 |
|  |  | F2 | 2.98 | 0.01 | 174.13 | 23.1 | 4.03 | 1.0 |
|  |  | F3 | 17.45 | 0.08 | 185.84 | 24.7 | 18.73 | 4.5 |
|  |  | F4 | 859.29 | 3.8 | 231.64 | 30.8 | 125.79 | 30.4 |
|  |  | F5 | 2527.17 | 11.1 | 34.25 | 4.6 | 38.07 | 9.2 |
|  |  | F6 | 19310.07 | 85.0 | 110.27 | 14.7 | 217.27 | 52.5 |
|  |  | *Sum* | *22718.22* | *100* | *752.22* | *100* | *414.23* | *100* |
|  |   | *Pseudo-tot.* | *8501.44* | *267.2* | *734* | *102.5* | *231.2* | *179.2* |
|  | bottom | F1 | 2.71 | 0.01 | 13.16 | 2.4 | 4.41 | 1.0 |
|  |  | F2 | 3.41 | 0.01 | 127.03 | 23.2 | 3.71 | 0.8 |
|  |  | F3 | 15.80 | 0.07 | 98.93 | 18.1 | 21.82 | 4.8 |
|  |  | F4 | 820.24 | 3.4 | 96.16 | 17.6 | 173.37 | 38.2 |
|  |  | F5 | 1924.81 | 8.0 | 22.28 | 4.1 | 78.87 | 17.4 |
|  |  | F6 | 21194.43 | 88.5 | 190.03 | 34.7 | 171.37 | 37.8 |
|  |  | *Sum* | *23961.40* | *100* | *547.59* | *100* | *453.55* | *100* |
|   |   | *Pseudo-tot.* | *7749.79* | *309.2* | *352* | *155.5* | *217.8* | *208.3* |
| LS-L | top | F1 | 1.47 | 0.01 | 13.80 | 3.2 | 2.72 | 1.4 |
|  |  | F2 | 2.45 | 0.02 | 84.63 | 19.4 | 1.54 | 0.8 |
|  |  | F3 | 13.70 | 0.09 | 104.93 | 24.0 | 6.25 | 3.2 |
|  |  | F4 | 921.88 | 5.8 | 123.76 | 28.3 | 71.23 | 36.4 |
|  |  | F5 | 2384.35 | 15.0 | 26.09 | 6.0 | 23.29 | 11.9 |
|  |  | F6 | 12521.74 | 79.0 | 83.81 | 19.2 | 90.45 | 46.3 |
|  |  | *Sum* | *15845.59* | *100* | *437.03* | *100* | *195.48* | *100* |
|  |   | *Pseudo-tot.* | *5441.75* | *291.2* | *488* | *89.6* | *86* | *227.3* |
|  | bottom | F1 | 1.52 | 0.01 | 9.94 | 2.1 | 2.99 | 0.7 |
|  |  | F2 | 2.69 | 0.02 | 88.58 | 18.9 | 1.45 | 0.3 |
|  |  | F3 | 12.92 | 0.08 | 82.29 | 17.5 | 3.25 | 0.8 |
|  |  | F4 | 944.92 | 5.6 | 142.55 | 30.4 | 85.62 | 20.5 |
|  |  | F5 | 2453.11 | 14.6 | 29.89 | 6.4 | 41.64 | 9.9 |
|  |  | F6 | 13348.35 | 79.6 | 115.72 | 24.7 | 283.62 | 67.8 |
|  |  | *Sum* | *16763.52* | *100* | *468.98* | *100* | *418.56* | *100* |
|   |   | *Pseudo-tot.* | *4989.65* | *336.0* | *461.2* | *101.7* | *236.0* | *177.3* |
| Reagent blank | F1 | 0.08 |  | 0.02 |  | 0.14 |  |
|  |  | F2 | 0.33 |  | 0.59 |  | 0.11 |  |
|  |  | F3 | 0.77 |  | 0.2 |  | 0.1 |  |
|  |  | F4 | 4.35 |  | 0.39 |  | 1.32 |  |
|  |  | F5 | 0.71 |  | 0.08 |  | 1.16 |  |
|  |  | F6 | 4.28 |  | 0.09 |  | 2.20 |  |
|  |  | Sum | 10.53 |  | 1.37 |  | 5.02 |  |

Table 7. Median influent and effluent dissolved metal concentrations (mg/L) over ten months of bioreactor operation (Uster 2015).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **Influent** | **MS-S** | **MS-L** | **LS-S** | **LS-L** |
| Fe | 60 | 2.4 | 0.7 | 2.7 | 1.7 |
| Al | 21 | 0.1 | 0.04 | 0.4 | 0.1 |
| Mn | 18 | 13.5 | 9.9 | 14.8 | 11.8 |
| Zn | 4.8 | 0.02 | 0.01 | 0.4 | 0.2 |

Table 8. Area- and volume-adjusted dissolved metal removal rates over ten months of bioreactor operation (Uster 2015).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Mussel shell short HRT | Mussel shell long HRT | Limestone short HRT | Limestone long HRT | Published removal rates |
|  | Area-adjusted (g/m2/d) | Volume-adjusted (g/m3/d) | Area-adjusted (g/m2/d) | Volume-adjusted (g/m3/d) | Area-adjusted (g/m2/d) | Volume-adjusted (g/m3/d) | Area-adjusted (g/m2/d) | Volume-adjusted (g/m3/d) | Area-adjusted (g/m2/d) | Volume-adjusted (g/m3/d) |
| Al | 0.90 | 2.92 | 0.30 | 0.97 | 0.85 | 2.62 | 0.30 | 0.96 | 1.7-3.2a | - |
| Fe | 1.09 | 3.52 | 0.41 | 1.32 | 1.17 | 3.8 | 0.33 | 1.08 | 0.8b | - |
| Mn | 0.16 | 0.52 | 0.12 | 0.40 | 0.10 | 0.32 | 0.094 | 0.30 | 0.01b | - |
| Zn | 0.253 | 0.817 | 0.086 | 0.278 | 0.216 | 0.695 | 0.078 | 0.250 | 0.11d | 0.79f |

a = Hedin et al. ([1994](#_ENREF_67))

b = Heal and Salt ([1999](#_ENREF_66))

c = PIRAMID Consortium ([2003](#_ENREF_128))

d = Kadlec and Wallace ([2009](#_ENREF_87))

e = Ettner ([1999](#_ENREF_48))

f = Gandy and Jarvis ([2012](#_ENREF_51))