Use of carbon additions to enhance zinc removal from mine drainage in short residence time, flow-through sulfate-reducing bioreactors

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Abstract

The effectiveness of liquid carbon additions to enhance zinc removal in laboratory-scale short hydraulic residence time (19 hours) compost bioreactors receiving synthetic mine water with a high influent zinc concentration (45 mg/L) was investigated. The unique combination of short hydraulic residence time, high strength wastewater and carbon additions was designed to investigate the potential for application of this approach to mine water treatment. Effective removal of zinc could not be sustained by sulfate reduction and / or other attenuation processes, in the presence of such elevated zinc concentrations, without carbon supplementation. Propionic acid addition resulted in improved and sustained performance (mean zinc removal 99%). Comparison of bioreactors receiving continuous propionic acid with those in which carbon addition ceased after a period of time demonstrated distinct differences in the microbial communities. The addition of propionic acid promoted the activities of sulfate reducing bacteria with the compost substrate becoming a net sink for sulfate, which led to efficient zinc removal via bacterial sulfate reduction. Upon cessation of propionic acid addition, carbon limitation resulted in oxidising conditions and the growth of sulfur oxidising bacteria with the compost substrate becoming a net source of sulfate, compromising zinc removal by bacterial sulfate reduction. These research findings show the potential for modest liquid carbon additions to compost-based passive treatment systems to enhance rates of metal attenuation in a short hydraulic residence time, enabling remediation of highly polluting mine drainage at sites with limited land availability.

KEYWORDS: Zinc, mine drainage, compost bioreactor, carbon addition, sulfate reducing bacteria, residence time

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1. Introduction

Low pH, high metal concentration mine discharges are among the most ecologically damaging effluent types world-wide. In the UK zinc is particularly prevalent in drainage from abandoned metal mines with over 50% of the total zinc flux to freshwaters of England and Wales attributed to such pollution (Mayes et al. 2013). Although the majority of discharges in the UK are characterised by relatively low zinc concentrations (see Figure S1), a limited number of highly contaminated (up to 45 mg/L zinc) discharges cause severe ecological damage and are therefore a target for remediation. Such heavily polluted mine drainage is also well documented elsewhere in the world (e.g. Castillo et al., 2012; Mosley et al., 2015; Strosnider et al., 2011, 2013).

Compost bioreactors utilising bacterial sulfate reduction (BSR) are a favoured approach to metal mine drainage remediation (Gandy et al. 2016; LaBar and Nairn 2018; Neculita et al. 2007; Vasquez et al. 2016). However, limitations of such low-energy passive systems, in many locations, include their large footprint and uncertainty regarding their effectiveness in treating high metal concentrations. Many UK discharges occur in remote upland locations, such as in northern England and western Wales, where availability of flat land for treatment systems is limited (see Mayes et al., 2009). Attenuation of zinc in low-cost, low maintenance passive systems with a short hydraulic residence time (HRT) to enable a small footprint is therefore favoured. Whilst many investigations into the potential of compost bioreactors for mine drainage remediation have used systems in which HRT is measurable in days (Biermann et al. 2014; Cruz Viggi et al. 2010; Di Luca et al. 2011; Song et al. 2012; Strosnider et al. 2011, 2013), recent research has demonstrated successful removal of zinc in a HRT of less than 14.5 hours (Gandy et al. 2016). In the research reported here the limitations of compost bioreactors, particularly for the remediation of highly contaminated UK discharges, were investigated together for the first time. Short residence time passive bioreactors receiving high zinc concentration mine water operated continuously for two years, with controlled testing of the benefits of carbon additions to enhance performance.

The principle of BSR is that the reduction of sulfate by sulfate reducing bacteria (SRB) under anaerobic conditions, using a carbon source (represented as CH_2O) as an electron donor, generates sulfide (reaction (1)), which in turn reacts with metals to precipitate metal sulfides (e.g. zinc sulfide, reaction (2)).

$$2CH_2O + SO^{2}_{4}^{-} \rightarrow H_2S + 2HCO^{-}_{3} \quad (1)$$

$$H_2S + Zn^{2+} + 2HCO^{-}_{3} \rightarrow ZnS(s) + 2H_2O + 2CO_2 \quad (2)$$

The choice of carbon source is important to sustain the long-term efficiency of treatment (Costa et al. 2009). Simple organic compounds that are easily degradable, such as carboxylic acids or alcohols, are used by SRB as carbon and energy sources (Gibert et al. 2004; Martins et al. 2009). In laboratory cultures, lactate is the most common carbon source used by SRB

but would be prohibitively expensive to employ in full-scale treatment systems (Costa et al. 2009). Different types of compost are therefore frequently used to provide a long-term source of carbon (Neculita et al. 2007). These more complex organic sources are far less costly than proprietary carbon sources, are widely available, and often have physical characteristics that make them suitable for use in flow-through water treatment systems.

Whilst a number of researchers have explored the benefits of carbon addition to compost-based treatment systems (e.g. Costa et al. 2009; Dvorak et al. 1992; Mayes et al. 2011; Nielsen et al. 2018), these studies are based on either a high HRT (greater than 24 hours) or a comparatively low zinc concentration (less than 20 mg/L). In the research reported here the focus is on the combination of relatively short HRT treatment systems, since their absolute size is a key constraint to wider deployment of the technology in the UK, and waters containing a high zinc concentration. The extent to which the microbial communities key to metal attenuation are influenced by carbon addition under short HRT conditions, and in turn whether they can sustain bacterial sulfate reduction sufficiently to maintain effective zinc removal, is specifically investigated.

As compost bioreactors are driven by SRB activity an improved understanding of their microbial community diversity and function is critical for long-term performance (Hiibel et al. 2008). Several studies have demonstrated a relationship between system performance and microbial community (e.g. Baldwin et al. 2015, 2016; Drennan et al. 2016, 2017). Engineering design and system operation should thus be configured to ensure optimum activities of the SRB that are responsible for remediation. Enhancement of microbial communities in short HRT bioreactors subjected to high influent zinc concentrations has not previously been investigated.

This study, using laboratory scale upflow column experiments, aims to (1) evaluate the effectiveness of liquid carbon additions on zinc immobilisation in short HRT (19 hours) compost bioreactors receiving a high influent zinc concentration (45 mg/L), (2) assess the responses of a microbial community to such metal and carbon additions, (3) determine whether microbial responses favourable to the immobilisation of metals can be engineered in enhanced passive treatment systems receiving carbon additions.

2. Materials and methods

2.1. Experimental configuration

Two sets of laboratory-scale continuous upflow bioreactors (internal diameter 105 mm, length 500 mm) were operated in triplicate. Limestone gravel (diameter < 10 mm) was placed at the base of each bioreactor (depth 40 mm) and overlain by a reactive substrate (depth 400 mm) comprising British Standards Institution (BSI) Publicly Available Specification (PAS) 100 compost (45% v/v), wood chips (45% v/v) and activated sludge from a municipal wastewater treatment plant (10%). A 25 mm cover of water ensured that the substrate remained

saturated (Figure S2). The substrate was sourced from a decommissioned pilot-scale bioreactor that treated zinc-rich, circumneutral mine water for 2 years (Gandy et al. 2016). This substrate was selected as it was known to have supported BSR previously, but via treatment of a relatively low strength wastewater (mean pH 7.74 and 2.32 mg/L Zn; Gandy et al. 2016) unlikely to invoke any inhibitory effects. Samples from across the entire depth and length of the bioreactor were thoroughly mixed before placement of 3,530 cm^3 in each laboratory bioreactor. The substrate was saturated with synthetic mine water to facilitate the calculation of porosity (0.48 – 0.51) and estimate hydraulic residence time. A Watson-Marlow 300 series peristaltic pump was set up to give a mean residence time of 19 hours (mean flow-rate 1.6 ml/min).

2.2. Bioreactor operation

Synthetic mine water (mean 45 mg/L Zn, 156 mg/L SO_4, pH 4.1, Table S1), produced by dissolving laboratory-grade salts in deionised water, was passed upwards through the bioreactors for 755 days. The pH was controlled by addition of <10 mL of 1% H_2SO_4 to the mine water. This water quality was representative of an actual mine water discharge in northern England (see Table S1 for details).

Propionic acid (13.4M) addition to one set of three bioreactors (1A, B, C) commenced on day 234 at a rate of 1 ml per 35 L influent water. The other set of three bioreactors (2A, B, C) operated as a control and continued to receive synthetic mine water only. On day 511 propionic acid addition to one bioreactor (1A) ceased.

2.3. Water sampling and analysis

Samples were collected at fortnightly intervals in polypropylene bottles from the influent mine water and the effluent of each bioreactor with more intense (weekly) sampling immediately after propionic acid addition commenced. Flow rate was measured on each sampling occasion by measuring the volume of effluent water collected over a specified time. Measurements of water temperature, pH, oxidation-reduction potential (ORP) and electrical conductivity in the influent and effluent waters were recorded using a pre-calibrated Myron L 6P Ultrameter. Total alkalinity was determined using a Hach digital titrator with 0.16 N sulfuric acid and bromcresol-green methyl-red indicator. Two 30 ml aliquots were acidified with 1% v/v concentrated nitric acid, one following filtration (0.45 µm cellulose nitrate filters) for total and filtered cation analysis. A 30 ml aliquot was filtered and left unacidified for anion analysis. Samples were stored at 4 °C prior to analysis. Cation analysis was performed using a Varian Vista-MPX Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES). Anion concentrations were determined using a Dionex DX320 Ion Chromatograph (IC).

2.4. Substrate sampling and geochemical analysis

Substrate samples were collected from all bioreactors at the end of the trial. In the bioreactors that received propionic acid (1A, B, C), two samples were collected, at approximate depths of 220 mm (middle of reactors) and 310 mm (bottom of reactors), in pre-washed (analytical grade nitric acid, 10% v/v) polypropylene bottles which were filled with water from within the bioreactors. One sample was stored at minus 80°C, prior to microbial analysis, and the other at minus 20°C, prior to geochemical analysis. An additional sample was collected at an approximate depth of 90 mm (top of reactors) for microbial analysis only. In the control bioreactors (2A, B, C), two samples were collected at an approximate depth of 220 mm (middle of reactors) and stored as above prior to geochemical and microbial analysis. Samples were allowed to defrost in an anaerobic cabinet before analysis. Geochemical analysis followed the Acid Volatile Sulfide – Simultaneously Extracted Metals (AVS-SEM) method of Allen et al. (1991) with the exception that H_2S was purged from the sample for 3 hours to ensure that all AVS was recovered, as recommended by Standard Method 4500-S^{2-} J (APHA, 2005). Metals analysis was undertaken as for water samples. A control sample of the original mixed substrate was subjected to the same analysis.

2.5. Microbial analysis

Twelve 16S rRNA PCR amplicon libraries were sequenced comprising three (top, middle and bottom) depths for each of Set 1 bioreactors (A, B, C) and an additional three samples from the middle of each one of the three control bioreactors. All bioreactor substrate samples were collected at the end of the trial (see Supporting Information (SI) for a more detailed methods description). Briefly, amplicons of 16S rRNA gene fragments (V4/V5 region) were PCR amplified with barcode-ligated amplification primers from DNA extracts. Amplicons were then pooled and sequenced using the Ion PGM™ sequencing platform. Sequence libraries for each sample were assembled and analysed using the QIIME2 analysis pipeline (Caporaso et al., 2010). A principal components analysis (PCA) of sample diversities was generated using the STAMP v2 software package (Parks et al., 2014). Phylogenetic trees of key representative sequences and their BLAST derived close relatives were generated in MEGA7 (Kumar et al., 2016).

3. Results and discussion

3.1. Zinc and sulfate removal

There was no significant difference between the concentrations of total zinc and filtered zinc in the effluent throughout the trial (Mann-Whitney U test; $p > 0.05$ for replicates 1A, 1B and 1C). Therefore, all values reported here are total zinc concentrations.

Effective removal of zinc (removal efficiency consistently > 90%) occurred in all bioreactors during the first 90 days of the trial, but effluent zinc concentrations increased in all three

bioreactors between 90 and 230 days (Figure 1(A)). Initially there was evidence of a decrease in sulfate concentration between influent and effluent in all bioreactors (Figure 1(B)). Based on a molar ratio of sulfate to zinc of 1:1 (Reactions (1) and (2)), calculation of the predicted effluent zinc concentration, assuming zinc removal only as a sulfide precipitate via BSR (using the difference in influent and effluent sulfate concentration), indicates that actual effluent zinc concentrations during the first 230 days of operation were much less than predicted in all bioreactors (see Figure S3). Processes other than zinc sulfide precipitation (e.g. sorption) were therefore contributing to zinc attenuation during this period.

As effluent zinc concentrations increased during the first 230 days of the trial there was a corresponding decrease in mean percentage sulfate reduction (defined as the difference between influent and effluent sulfate concentrations), from 20% to 3.6% (Figure 1(B)). This indicated that effective removal of the high influent zinc concentration (mean 45 mg/L) could not be sustained by sulfate reduction and / or other attenuation processes. Other studies have reported zinc to be toxic or inhibitory to SRB at such concentrations (Poulson et al. 1997; Utgikar et al. 2002, 2003), although Castillo et al. (2012) and Falk et al. (2018) found that bacterial communities later recovered due to the proliferation of more metal-resistant species. Whilst toxicity was not studied specifically in these trials, there is no direct evidence that the elevated zinc concentration was toxic or inhibitory to sulfate reduction.

Upon commencement of propionic acid addition on day 234 effluent zinc concentrations decreased substantially in all three replicates, from a mean of 22.3 mg/L to < 0.5 mg/L (mean removal efficiency 99.1%) by day 427 (Figure 1(A)). There was no significant difference in zinc concentration between replicates during the period of propionic acid addition to all bioreactors, between days 235 and 511 (Mann-Whitney U test; $p > 0.05$ for all replicates). A corresponding increase in percentage sulfate reduction, which was sustained at a mean of 41% (Figure 1(B)), indicates that the SRB responded to the supplementary carbon with the result that the rate of attenuation of zinc as its sulfide increased. Like zinc, there was no significant difference in sulfate concentration between the three replicates (Mann-Whitney U test; $p > 0.05$ for all replicates). Between days 235 and 511 predicted effluent zinc concentration, assuming only precipitation as its sulfide via BSR, was very close to actual effluent zinc concentration (Figure S3), suggesting that BSR was the key zinc attenuation process during this phase of the trials.

After propionic acid addition to bioreactor 1A ceased on day 513, effluent zinc concentration immediately increased (Figure 1(A)), with removal efficiency < 1% by the end of the trial. A substantial decrease in percentage sulfate removal also occurred with effluent sulfate concentrations higher than influent sulfate concentration at times (as shown by negative values in Figure 1B)). These findings suggest that the presence of an easily available electron donor is the limiting factor for sulfate reduction in such systems. Similar observations have been made by others following cessation of methanol addition (Bilek 2006; Mayes et al. 2011) and depletion of lactate (Zhang and Wang 2014). Zinc removal efficiency in bioreactors 1B

and 1C, which continued to receive propionic acid, remained > 95% until the end of the trial and percentage sulfate removal was sustained at 30 - 40%. This suggests that the deteriorating performance of the bioreactors up to Day 230 of the trial was due to insufficient labile carbon to maintain high rates of BSR. In the control bioreactor set, which did not receive propionic acid, effluent zinc concentrations steadily increased until stabilising at around 37 mg/L (mean removal efficiency 17.1%) (Figure 1(A) and Figure S4). Likewise, percentage sulfate removal progressively decreased throughout the trial indicating that SRB activity was limited in these control bioreactors (Figure 1(A) and Figure S5).

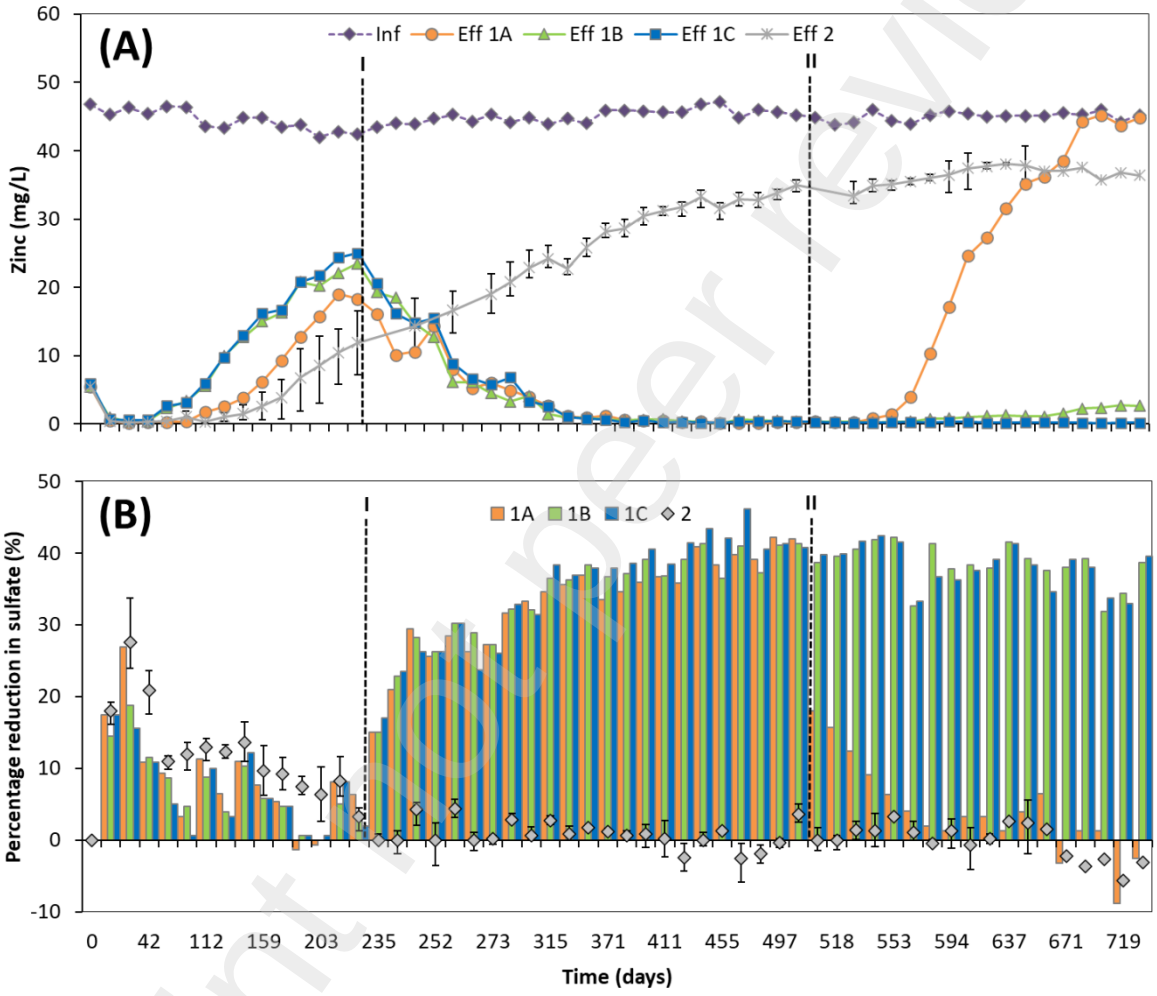

Figure 1. Effect of propionic acid addition on total zinc removal and sulfate reduction in laboratory-scale bioreactors. (A) Influent and effluent total zinc concentrations in bioreactors receiving propionic acid (Eff 1A, Eff 1B, Eff 1C) and mean effluent total zinc concentration in bioreactors receiving no propionic acid (Eff 2). (B) Percentage reduction in sulfate concentration in bioreactors receiving propionic acid (1A, 1B, 1C) and mean percentage reduction in sulfate concentration in bioreactors receiving no propionic acid (2). Error bars represent the range of results from triplicate samples. Vertical dashed lines refer to: (I) commencement of propionic acid addition; (II) cessation of propionic acid addition to reactor 1A.

3.2. Alkalinity, pH and Eh

Changes in pH, Eh and alkalinity concentration between influent and effluent (Figure 2) were consistent with variations in zinc and sulfate removal. Effective buffering of the acidic influent water occurred throughout the trial with an influent mean pH of 4.1 consistently elevated to an effluent pH of 6.29 – 7.92, which is optimal for SRB activity (Neculita et al. 2007). The only notable deviation was in bioreactor 1A, 68 days after propionic acid addition had ceased, when effluent pH decreased from a mean of 7.34 to a mean of 6.41 for the remainder of the trial (Figure 2).

Influent and effluent Eh values were also consistent with conditions that favoured BSR and zinc removal as its sulfide. Eh decreased between influent (mean 382 mV) and effluent (mean 196 mV) in all biroeactors, with a marked decrease in effluent Eh at commencement of propionic acid addition (Figure 2). Although strongly anaerobic conditions did not appear to become established within the bioreactors, the effluent Eh measurements reported here likely overestimate the actual Eh values within the pore waters. The low flow rates of the bioreactors necessitated an extended period of sample collection and it is possible that oxidising conditions became re-established within the samples before Eh was measured. Furthermore, Eh measurements made on effluent waters are likely not reflective of those in the bulk compost.

Effluent alkalinity concentration initially decreased in all bioreactors before increasing upon commencement of propionic acid addition, indicating enhanced alkalinity generation due to BSR (reaction (1)) together with continued calcite dissolution from the limestone gravel (Figure 2). Upon cessation of propionic acid addition effluent alkalinity concentration decreased sharply in bioreactor 1A, compared to reactors continuing to receive propionic acid (1B and 1C), albeit effluent alkalinity was beginning to decrease in all bioreactors (Figure 2).

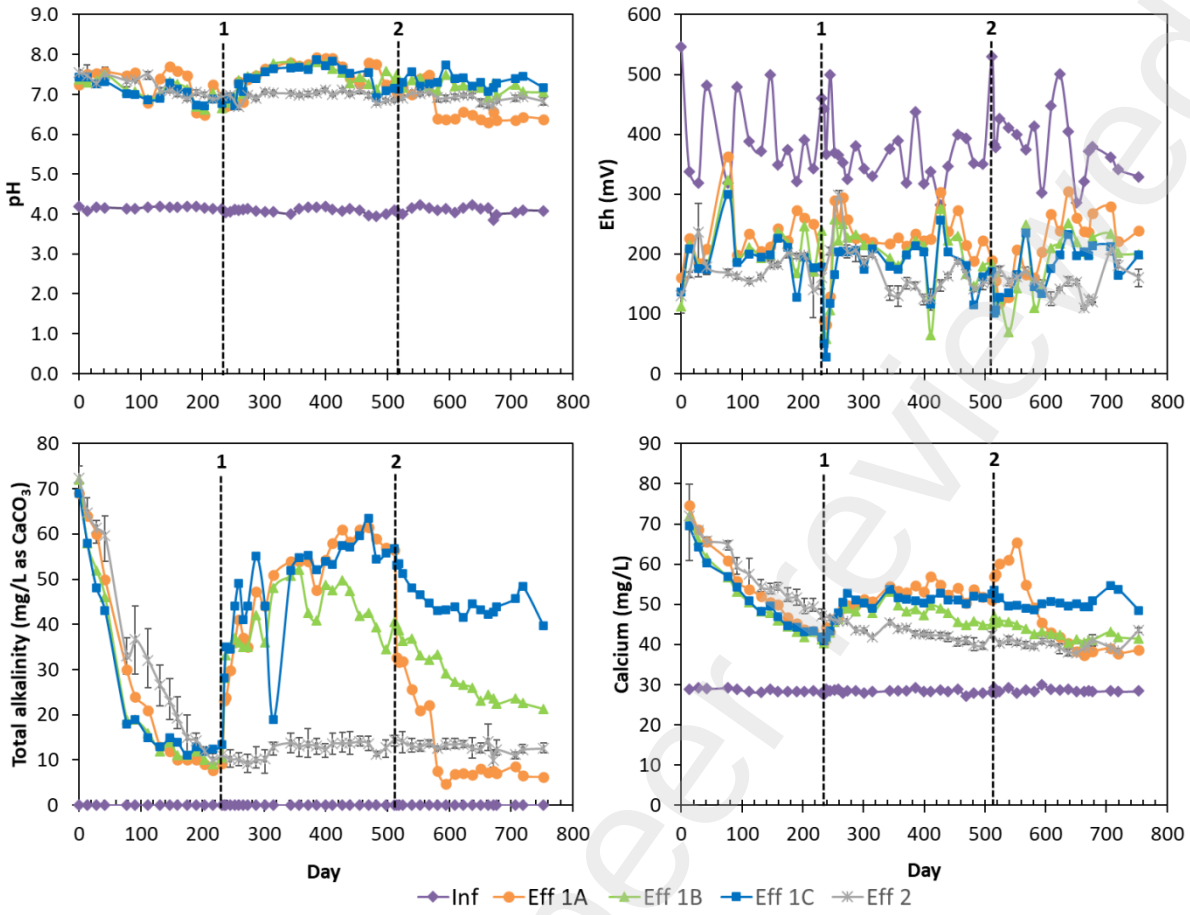

Figure 2. Influent and effluent pH, Eh, total alkalinity and total calcium concentration in bioreactors receiving propionic acid (Eff 1A, Eff 1B, Eff 1C) and mean effluent pH, Eh, total alkalinity and total calcium concentration in bioreactors receiving no propionic acid (Eff 2). Error bars represent the range of results from triplicate samples. Vertical dashed lines refer to: [1] commencement of propionic acid addition; [2] cessation of propionic acid addition to reactor 1A.

3.3. Substrate geochemical analysis

Sampling and analysis of the substrates was undertaken at the end of the trial to investigate metal attenuation processes. The determination of acid volatile sulfides (AVS) and simultaneously extracted metals (SEM) has previously been used effectively to assess the role of BSR as a zinc removal mechanism (Gandy et al. 2016; Jong and Parry 2004; LaBar and Nairn 2018). Figure 3 shows that substantial accumulation of both AVS and zinc occurred in the bioreactors receiving propionic acid. This is consistent with the observed decreases in zinc and sulfate between the influent and effluent waters (Figure 1) and implies that ZnS was the main sink for zinc within these bioreactors. Despite having already accumulated some AVS and zinc during its emplacement in a pilot-scale flow through bioreactor treating zinc-rich water (Gandy et al. 2016), the original compost substrate contained much lower concentrations of zinc (81 mmol/kg) and AVS (148 mmol/kg) (Figure 3). Solid phase zinc

concentrations in two of the bioreactors receiving propionic acid were higher in the bottom layer (1B 1,895 mmol/kg; 1C 1,117 mmol/kg) than in the middle layer (1B 1,106 mmol/kg; 1C 745 mmol/kg); in bioreactor 1A, concentrations in the bottom and middle layers were similar (Figure 3). Conversely, the AVS concentrations were higher in the middle layer (mean of the three bioreactors 1,410 mmol/kg) than in the bottom layer (mean 878 mmol/kg) (Figure 3). They also showed little variation between the three bioreactors at equivalent depths (SD = ± 78 mmol/kg in middle layer; SD = ± 53 mmol/kg in bottom layer) compared to zinc concentrations (SD = ± 191 mmol/kg in middle layer; SD = ± 510 mmol/kg in bottom layer). Higher zinc concentrations in the bottom layer can be attributed to vigorous BSR close to where the influent water entered the bioreactors, due to relatively high zinc and sulfate concentrations. Gandy et al. (2016) and LaBar and Nairn (2018) also noted vertical variations in metal removal with the highest concentrations found closest to the influent ends of the systems. No notable difference in either zinc or AVS concentration was observed between bioreactor 1A, in which propionic acid addition ceased on day 511, and the other bioreactors receiving propionic acid, albeit the zinc concentration in the middle layer of this bioreactor was slightly higher than that in the bottom layer. Concentrations of both AVS (939 mmol/kg) and zinc (473 mmol/kg) were substantially lower in the control bioreactor that did not receive propionic acid. Nevertheless, the accumulation of some ZnS, particularly in the early stages of the trial, has resulted in higher concentrations than in the original substrate.

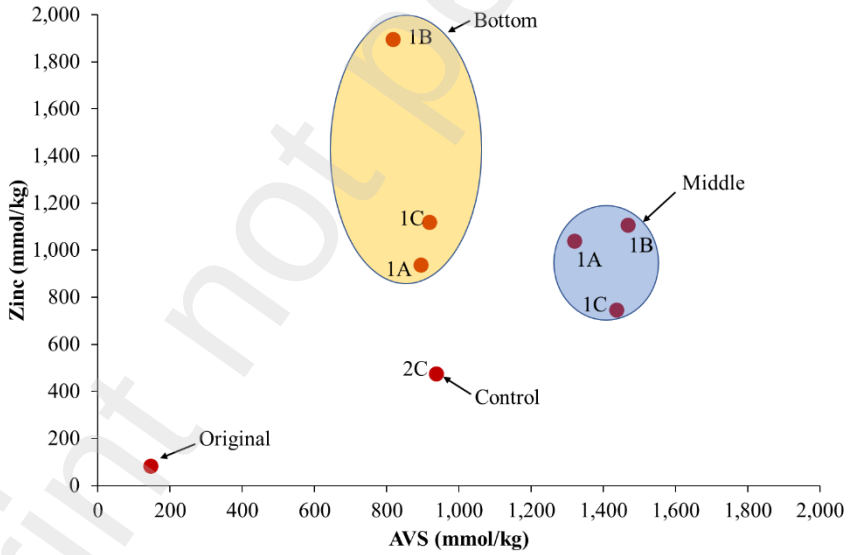

Figure 3. Concentrations of Acid Volatile Sulfide (AVS) and zinc in substrate from laboratory-scale bioreactors receiving propionic acid (1A, 1B, 1C), from a control bioreactor receiving no propionic acid (2C) and in the original substrate.

The molar ratio of AVS:Zinc in the BSR process is 1:1 (Reactions (1) and (2)) and can be used to indicate the predominant metal removal mechanism. A molar ratio > 1 demonstrates an excess of sulfide present within the substrate and implies that metals mainly exist in the form of sulfide minerals (Vasquez et al. 2016). If the molar ratio is < 1 other attenuation

mechanisms, such as adsorption and binding to organic matter, must play an important role in metal attenuation. The AVS:Zinc ratio is > 1 (mean 1.51) in the middle layer of all bioreactors, including the control which received no propionic acid (1.98), which suggests that sufficient sulfide was available to immobilize all of the zinc present as a sulfide. In the bottom layer, however, the AVS:Zinc ratio is < 1 (mean 0.74), albeit close to unity in bioreactors 1A (0.96) and 1C (0.82). Therefore, other attenuation mechanisms must also have taken place in this area of the bioreactors, which is consistent with previous findings (Gandy et al. 2016; Neculita et al. 2008).

3.4. Substrate microbial analysis

Community analysis revealed some common features in the libraries consistent with the compost bioreactor origin of the substrate (Gandy et al. 2016). Specifically, putatively sulfate reducing bacteria (SRB) accounted for 7.9% ± 0.4 (average ± SE) of sequences. These SRB had 100% sequence homology with those recovered from natural or engineered anaerobic sulfate reducing systems (Figure S6) and taxa identified indicated a dominance of H_2 utilising SRB autotrophs (see SI for a more detailed discussion). Likewise, syntrophic bacterial partners putatively responsible for fermentative degradation of compost material to supply SRB substrates were also common features. Close relatives of these dominant taxonomic groups (Candidatus *Caldatribacterium*, the family *Anaerolineacae* and the family *Spirochaetaceae*) were also identified previously in natural or engineered anaerobic environments (Figure S7).

Despite these common features, a spatial analysis (PCA) of the compost bioreactor communities (Figure 4) provided useful mechanistic insights into differences in processes and conditional changes. For instance, regardless of depth, all communities from the two bioreactors continuously receiving propionic acid from Day 231 to Day 753 (1B and 1C) clustered together. Contrastingly, depth resolved communities from reactor 1A, in which propionic acid addition ceased on Day 511, were separated not just from the 1B and 1C communities but also from each other. This spatial separation most likely reflected selection by development of a redox gradient within 1A through the absence of propionic acid-driven oxygen consumption, increase in compost Eh and consequent re-oxidation of sulfides accumulated during propionic acid feeding. This redox gradient was evidenced by a substantial enrichment of putatively oxidative chemolithotrophic bacteria (Figure S8), namely, *Sulfurifustis*, Gammaproteobacterial MBMPE27 group, and *Gallionellaceae* spp. in the bottom (i.e. closest to the inlet) and middle sections of the column (see SI for a more detailed discussion). Growth of putative sulfur oxidizers was consistent with effluent compositions after cessation of propionic acid addition (Day 511), from which point bioreactor 1A transitioned from a net sulfate sink to a net source towards the end of the trial (Figure 1(B)). Control reactor communities did not substantially enrich for oxidative chemolithoautotrophs as in reactor 1A, or cluster with reactors 1B and 1C, because without any propionic acid feeding they did not either develop permanently low Eh conditions (as in 1B and 1C) or accumulate reduced sulfur sufficient to sustain oxidative chemolithoautotrophic growth (AVS

levels in all the controls were considerably lower than the middle sections of the 1A, B and C bioreactors).

A further inference made from these community composition patterns was that toxicity due to elevated Zn concentrations in the influent was not a key constraint on bacterial activity compared to carbon limitation (previously noted above) and changing redox. High influent Zn concentrations, which did not change throughout operation, clearly had no effect on the growth of other functional groups present in the bioreactor compost i.e. the putative sulfur oxidising bacteria *Sulfurifustis*, which responded with growth on cessation of propionic acid addition.

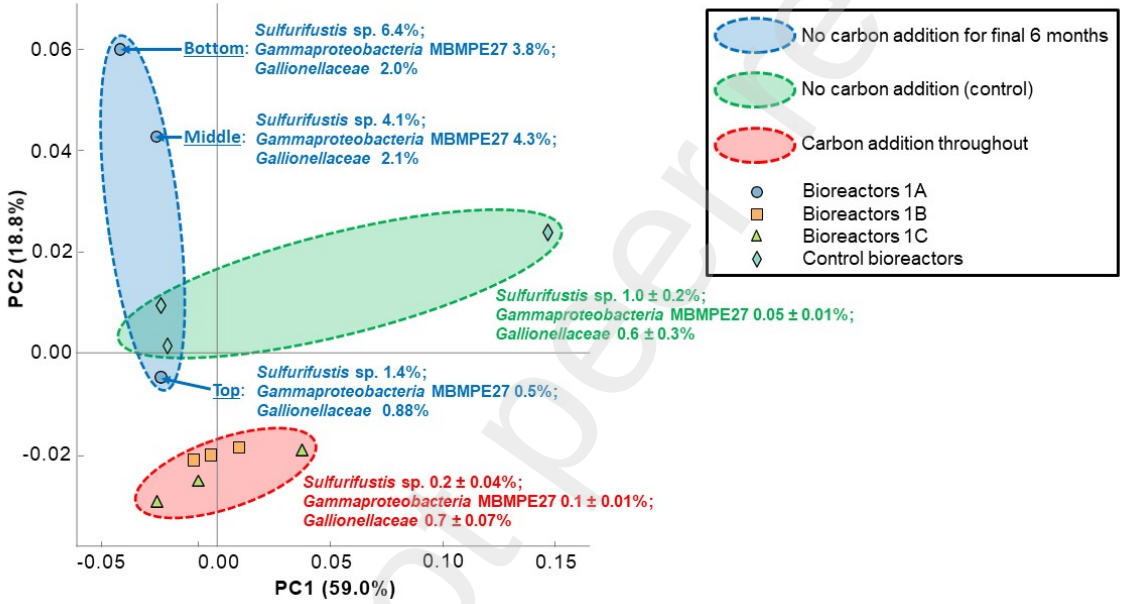

Figure 4. A Principal Component Analysis (PCA) based on amplicon sequence variant (ASV) frequencies within 16S rRNA gene sequencing libraries constructed from the compost bioreactors. Samples from the top, middle and bottom of the 1A (blue circles), 1B (orange squares) and 1C (green triangles) column bioreactors are shown, plus samples from the middle of the three control reactor columns (cyan diamonds). Ellipses are drawn around three data groups: the 1A samples which stopped receiving propionic acid for the last six months of reactor operation; a group comprising the 1B and 1C samples which received propionic acid throughout; and the control reactors which did not receive carbon additions. Mean % ± SE contribution of specific taxonomic groups related to sulfide and iron oxidation are provided for two of the circled groups (1B + 1C and control). Individual sample values presented for the 1A group data to illustrate bottom to top progression of changes observed in this bioreactor.

4. Conclusions and implications

Liquid carbon additions to compost-based passive systems, harnessing bacterial sulfate reduction (BSR), offer the potential to enhance rates of attenuation of zinc (mean of 99% zinc removal during carbon addition) in short HRT treatment units receiving high strength wastewater. For the passive units investigated here, deterioration in treatment performance with respect to zinc was due to available carbon limitation, which was overcome by the addition of propionic acid as a carbon source. Other divalent contaminant metals (e.g. lead, cadmium, copper) could potentially be removed too, given the lower solubility products of their sulfides, thus broadening scope for deployment of such low carbon technologies at sites with high strength wastewaters but restricted land availability.

Laboratory-scale systems receiving continuous propionic acid and those in which propionic acid addition ceased after a period of time induced distinct differences in the microbial communities in the composts of the respective systems, which are indicative of the dominant processes occurring in relation to metal removal. Addition of propionic acid favoured the activities of the SRB and their syntrophic partners present in high proportions in the compost substrate, inducing a net sink for sulfate via BSR and hence efficient Zn removal. Upon cessation of propionic acid addition, the resulting carbon limitation increased the substrate oxidation potential (as evidenced by the growth of sulfur oxidising bacteria), which compromised zinc removal (as ZnS) via BSR and resulted in a system that was a net source of sulfate. Hence, by such an intervention it is possible to engineer the microbial community and its overall function to enhance treatment with respect to metal removal from the contaminated mine water.

The laboratory-scale research described here used a compost commonly available in the UK, a laboratory-grade liquid carbon addition (propionic acid), and a synthetic mine water representing an actual low pH mine water discharge in the UK. The experiments ran for approximately two years. Shortened tests of this type, using different composts, liquid carbon sources and mine waters, would be a useful precursor to design and installation of any pilot- or full-scale system at which liquid carbon addition might be anticipated as a requirement, especially given the large investment overall to construct a full-scale treatment system. Such tests would also provide better understanding of the range of liquid carbon sources deployable for this purpose, and also contribute to better design guidance for enhanced passive treatment.

Passive treatment has been defined as using only naturally-available energy sources in systems that require infrequent but regular maintenance (Younger et al. 2002). Use of liquid carbon additions in full-scale treatment systems would be a departure from this definition. However, scaling from the experiments described here to the volume of liquid carbon required for actual mine water discharges reveals that energy requirements could be very modest. In our experiments propionic acid was dosed at a rate of 1 mL per 35 L of synthetic

mine water. Scaling to treatment of a mine water discharge with a flow-rate of 10 L/s, as an example, the same dose rate would equate to using 24.7 L of liquid carbon per day, or approximately 9 m^3/year. This is a relatively small amount in terms of a full-scale wastewater treatment system, and at a dose rate of approximately 1 L/hour the use of small-scale renewable energy systems to control dosing should be feasible. A key research priority is the identification and reliability testing of waste liquid carbon sources as an alternative to proprietary laboratory chemicals, to strengthen the sustainability case for enhanced passive systems for treatment of metal-contaminated wastewaters in short HRT systems.

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Supplementary materials

Supplementary material associated with this article can be found in the online version.

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