Use of propionic acid 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. Effective removal of such elevated zinc concentrations could not be sustained by sulfate reduction and / or other attenuation processes without carbon supplementation. Propionic acid addition resulted in improved and sustained performance by promoting the activities of sulfate reducing bacteria, leading to efficient zinc removal (mean 99%) via bacterial sulfate reduction. In contrast, cessation of propionic acid addition led to carbon limitation and the growth of sulfur oxidising bacteria, compromising zinc removal by bacterial sulfate reduction. These research findings demonstrate the potential for modest liquid carbon additions to compost-based passive treatment systems to engineer microbial responses which enhance rates of zinc 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 (Olías et al. 2020). 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. Whilst zinc is an essential trace element, it can be toxic to humans and ecosystems (Wei et al. 2020) and such discharges are therefore a target for remediation. 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 (Mayes et al. 2011). 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 (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 (Gandy et al. 2016). 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 residence time chosen for these trials (mean 19 hours) was based on the results of preliminary trials (unpublished results, Newcastle University) and was operationally defined as short with the key requirement being that it was less than 24 hours to make it applicable to the remediation of UK discharges.

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)).

$$2CH_2O + SO_4^{2-} \rightarrow H_2S + 2HCO_3^- \quad (1)$$

$$H_2S + Zn^{2+} + 2HCO_3^- \rightarrow ZnS(s) + 2H_2O + 2CO_2 \quad (2)$$

The choice of carbon source is important to sustain the long-term efficiency of treatment (Xu and Chen 2020). 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. However, the long-term efficiency of traditional compost-based treatment systems is limited by the supply of readily available carbon (Tsukamoto et al. 2004). This is particularly pertinent in the UK given limited land availability and system sizing constraints which necessitate a short HRT. To extend system lifetime and to stimulate microbial sulfate reduction the supplementation of the compost substrate with additional carbon sources has been successfully applied (e.g. methanol (Mayes et al. 2011), glycerol (Santos and Johnson 2017), molasses (Nielson et al. 2018), ethanol (Costa et al. 2009), acetate (Yildiz et al. 2019) and lactate (Zhang and Wang 2014)). Propionic acid was chosen as a carbon additive in the research reported here as, together with propionate, it has been recognised as an effective carbon source for SRB (Qian et al. 2019; Virpiranta et al. 2021; Xu and Chen 2020) and in preliminary trials using a range of carbon sources (unpublished results, Newcastle University) it proved the most effective at zinc removal.

Whilst improved treatment efficiency has been demonstrated by carbon supplementation, previous studies were 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 by hand at the base of each bioreactor (depth 40 mm) and overlain by a reactive substrate (depth 400 mm), sourced from a decommissioned pilot-scale bioreactor that treated zinc-rich, circumneutral mine water for 2 years (Gandy et al. 2016). The substrate comprised 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%). Activated sludge, which contains high concentrations of organic matter (Peng et al. 2017), has previously been shown to be an effective carbon source for SRB (Virpiranta et al. 2021). A 25 mm cover of water ensured that the substrate remained saturated (Figure S2). 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 a measured volume of synthetic mine water and porosity calculated according to the ratio of mine water volume to substrate volume. A Watson-Marlow 300 series peristaltic pump was set up to give a mean flow-rate of 1.6 ml/min, which, based on a calculated porosity of 0.48 to 0.51, equated to a mean residence time of 19 hours.

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 (Table S2) 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 each 35 L batch of mine water which, at such a low concentration, had an immeasurable impact on the sulfate concentration of the synthetic 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 (Figure S3). Processes other than zinc sulfide precipitation (e.g. sorption) were therefore contributing to zinc attenuation during this period. Others have similarly reported additional processes to be taking place (e.g. Gandy et al. 2016) whilst Neculita et al. (2008) attributed metal removal to a combination of metal hydroxide precipitation and sorption to the compost substrate.

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 from the microbial community composition patterns discussed below 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). Minor deviations in effluent zinc concentration can be attributed to operational issues. 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 such 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. Effective attenuation of both zinc and sulfate during periods of carbon addition to sulfate reducing bioreactors has previously been reported by others. Mayes et al. (2011) observed almost complete removal of zinc during a phase of methanol addition whilst Neilsen et al. (2018) reported up to 90% zinc removal when using molasses as a carbon source, albeit the HRT was 2 weeks which is considerably longer than that in the study reported here. Similarly, Costa et al. (2009) achieved over 90% zinc removal with the addition of both ethanol and wine wastes but in a HRT of 8 days.

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)). This decline in BSR upon cessation of propionic acid addition suggests that the microbial community adapted rapidly and 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).

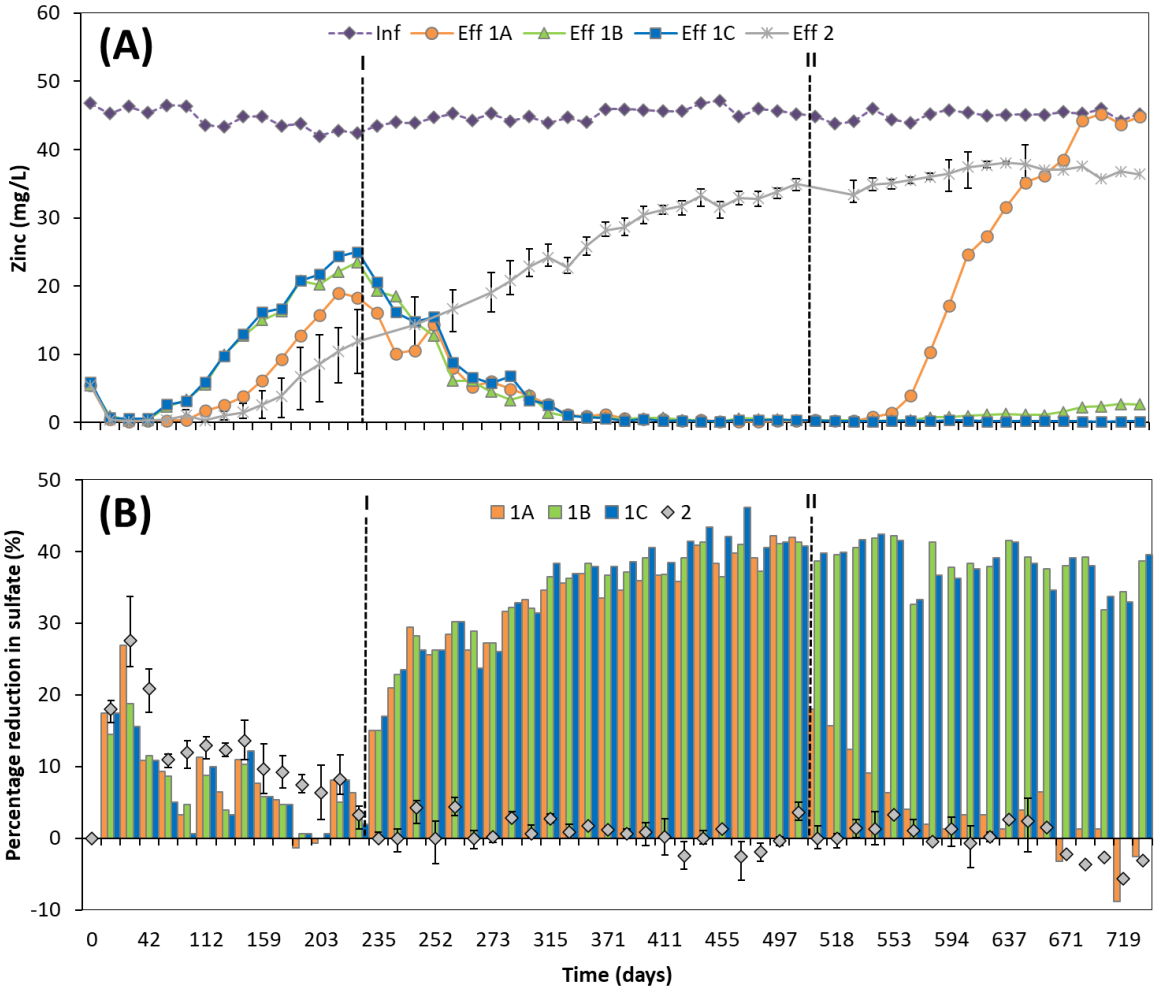


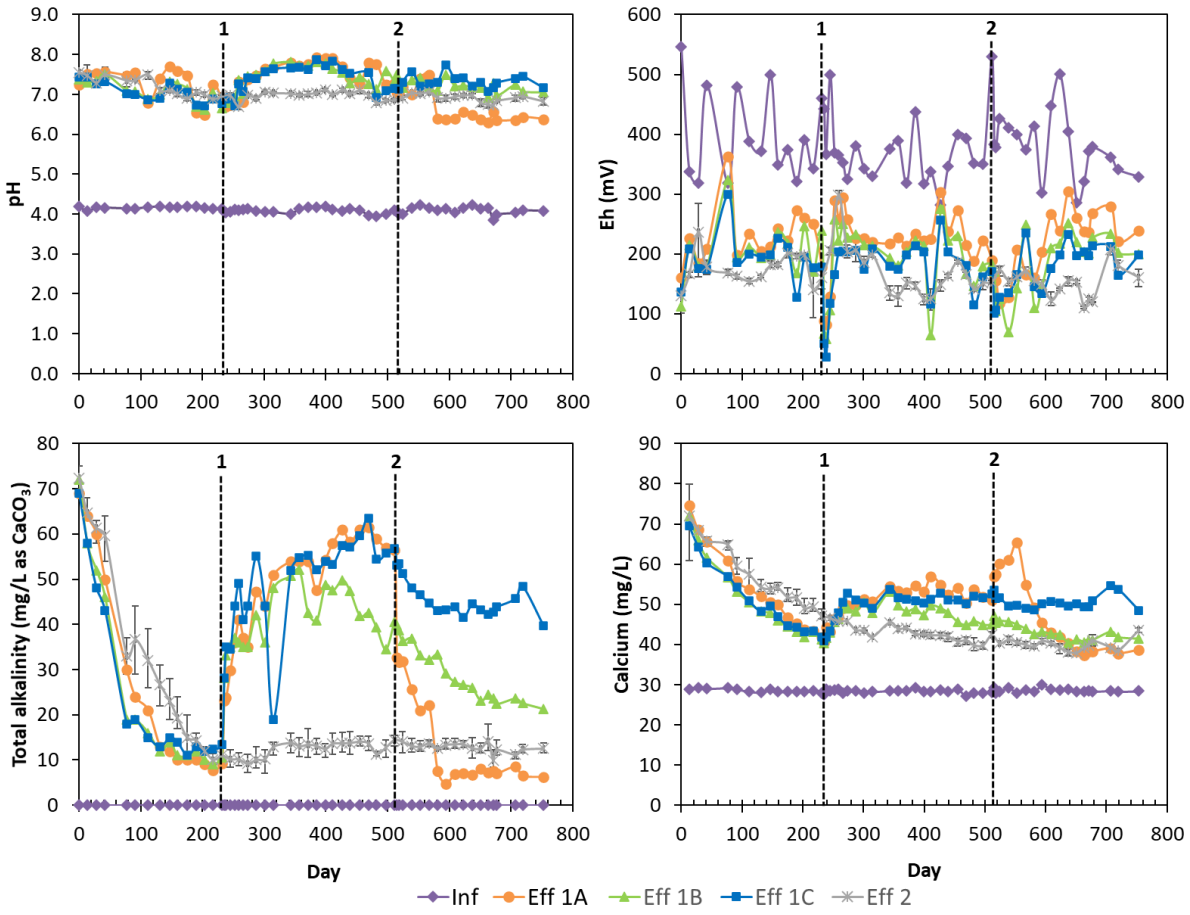
Figure 1. Effect of propionic acid addition on total zinc removal and sulfate reduction in laboratory-scale bioreactors. (A) Influent and effluent total zinc concentrations in bioreactors receiving propionic acid (Eff 1A, Eff 1B, Eff 1C) and mean effluent total zinc concentration in bioreactors receiving 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 (Xu and Chen 2020). 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, as observed by others (e.g. Mayes et al. 2011) during carbon additions, 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). Mayes et al. (2011) also noted increased alkalinity due to enhanced sulfate reduction during methanol addition. 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).



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

293 *3.3. Substrate geochemical analysis*

294 Sampling and analysis of the substrates was undertaken at the end of the trial to investigate
295 metal attenuation processes. The determination of acid volatile sulfides (AVS) and
296 simultaneously extracted metals (SEM) has previously been used effectively to assess the role
297 of BSR as a zinc removal mechanism (Gandy et al. 2016; Jong and Parry 2004; LaBar and Nairn
298 2018). Figure 3 shows that substantial accumulation of both AVS and zinc occurred in the
299 bioreactors receiving propionic acid. This is consistent with the observed decreases in zinc
300 and sulfate between the influent and effluent waters (Figure 1) and implies that ZnS was the
301 main sink for zinc within these bioreactors. Despite having already accumulated some AVS
302 and zinc during its emplacement in a pilot-scale flow through bioreactor treating zinc-rich
303 water (Gandy et al. 2016), the original compost substrate contained much lower
304 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.

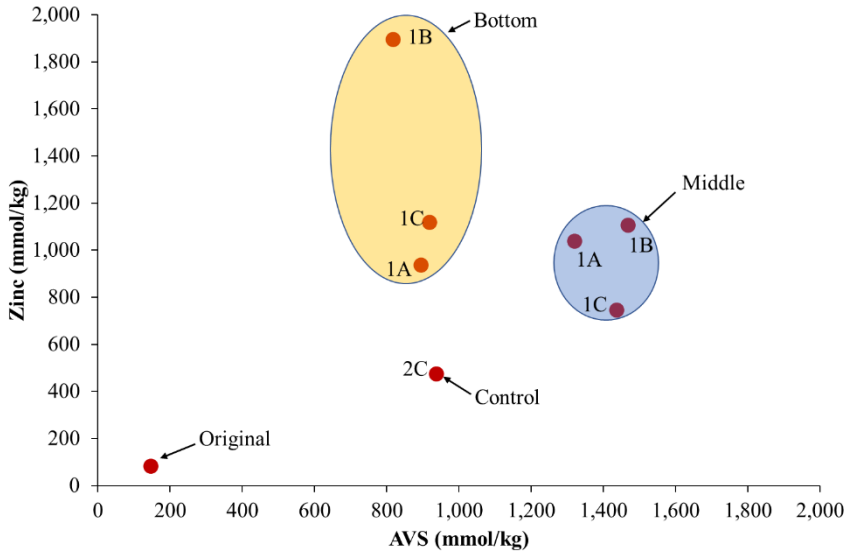

Figure 3. Concentrations of Acid Volatile Sulfide (AVS) and zinc in substrate from laboratory-scale bioreactors receiving propionic acid (1A, 1B, 1C), from a control bioreactor receiving no propionic acid (2C) and in the original substrate.

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 zinc concentrations in the influent was not a key constraint on bacterial activity compared to carbon limitation (previously noted above) and changing redox. High influent zinc 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.

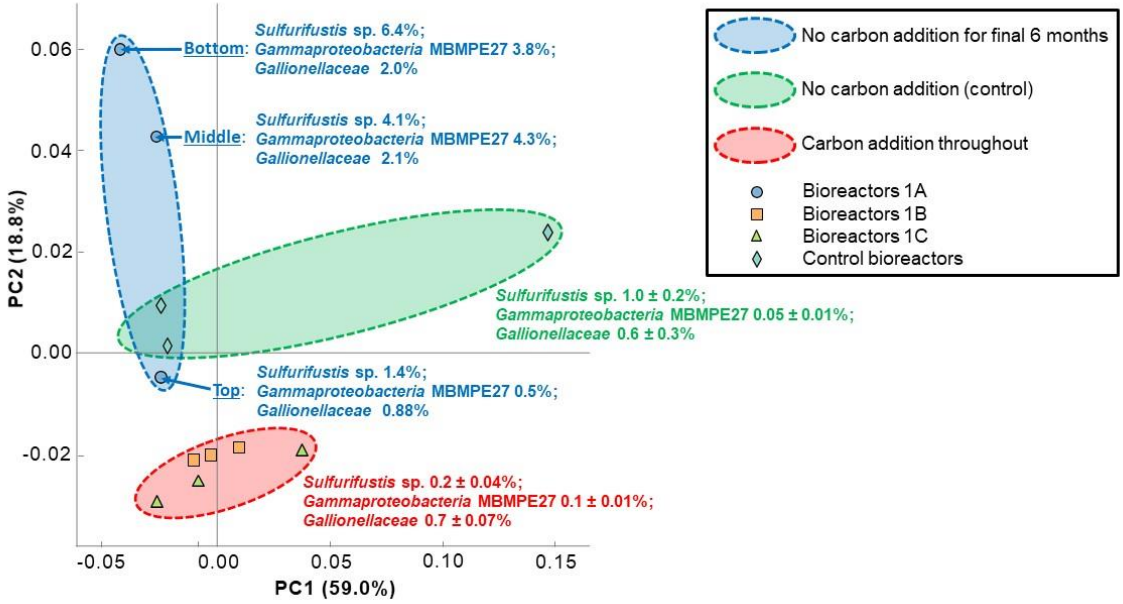

Figure 4. A Principal Component Analysis (PCA) based on amplicon sequence variant (ASV) frequencies within 16S rRNA gene sequencing libraries constructed from the compost bioreactors. Samples from the top, middle and bottom of the 1A (blue circles), 1B (orange squares) and 1C (green triangles) column 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.

3.5. Implications

Sustained zinc removal from high strength wastewater in short HRT sulfate-reducing bioreactors, as required in the UK due to limited land availability and associated treatment unit size constraints, necessitates carbon supplementation. Use of liquid carbon additions in full-scale treatment systems would be a departure from the definition of passive treatment as using only naturally-available energy sources in systems that require infrequent but regular maintenance (Younger et al. 2002). However, the volume of liquid carbon required could be very modest. A dosing rate of 1 mL propionic acid per 35 L of synthetic mine water in the laboratory experiments trialled here equates to 24.7 L per day, or approximately 9 m^3/year, for treatment of a mine water discharge with a flow-rate of 10 L/s, as an example. 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. Given such low volumes of propionic acid required, this would have minimal impact on overall treatment costs.

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 operated 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 contribute to better design guidance for enhanced passive treatment.

4. Conclusions

An evaluation of the effectiveness of liquid carbon additions on zinc immobilisation in laboratory-scale short HRT (19 hours) compost bioreactors receiving a high influent zinc concentration (45 mg/L) showed that enhanced rates of zinc attenuation (mean of 99% zinc removal) are possible. Without supplementation, available carbon limitation led to a deterioration in treatment performance, with respect to zinc. This was overcome by the addition of propionic acid which acted as an electron donor for the reduction of sulfate by SRB and led to enhanced zinc removal as a sulfide.

The different responses of the microbial communities in systems receiving continuous propionic acid addition and in systems in which propionic acid addition ceased after a period of time are indicative of the dominant processes occurring in relation to metal removal. Addition of propionic acid favoured the activities of SRB and their syntrophic partners present in high proportions in the compost substrate, inducing a net sink for sulfate via BSR and hence efficient zinc 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.

These research findings demonstrate the potential for microbial responses favourable to the immobilisation of zinc to be engineered in enhanced passive treatment systems receiving carbon additions. Even modest liquid carbon additions to compost-based passive treatment systems can increase rates of metal attenuation in a short HRT, enabling remediation of highly polluting mine drainage at sites with limited land availability, typical of abandoned mine sites in the UK. Given the lower solubility products of the sulfides of other divalent contaminant metals (e.g. lead, cadmium, copper), these metals could potentially be removed too, thus broadening scope for deployment of such low carbon technologies at sites with high strength wastewaters but restricted land availability. 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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