Controls on zinc uptake from circum-neutral mine drainage in freshwater biofilms

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Abstract This paper presents results from in vitro flask and flume experiments using freshwater biofilms sourced and cultivated from a mine-impacted stream in North Yorkshire, UK. Flask experiments showed the rapid uptake of Zn from the water column into biofilms. This uptake was not light dependant and suggests chelation of Zn by negatively charged functional groups in extracellular polymeric substances (EPS) secreted within the biofilm are the key sink for Zn. Long term monitoring of flume systems also showed area adjusted removal rates up to 1.2 g Zn $m^{-2}d^{-1}$ which is consistent with many other biologically-mediated treatment systems.

Keywords Mine water, Biofilm, Remediation, Zinc, Mesocosm

Introduction

Although the terms acid mine drainage (AMD) and acid rock drainage (ARD) prevail in the literature, many metal mine discharges are of circum-neutral pH owing to the buffering of acidity generated by sulphide weathering by carbonate-rich country rock. Over 6 % of rivers in England and Wales have recently been identified as having metal pollution at levels exceeding aquatic life standards due to discharges from metal mines (Mayes *et al.* 2009). Assessments of the pH distribution of such discharges suggest 85 % have a pH between 6 and 8, while around 5 % have pH <4 (Mayes *et al.* 2010; Jones *et al.* 2013). The most common metal pollutants in these circum-neutral discharges are Zn > Pb > Cd > Cu (Jarvis and Mayes 2012). Many researchers are currently investigating low-cost technologies to strip such mobile metals from waters, notably Zn, given its ubiquity and toxicity to aquatic life at modest concentrations (Alabaster and Lloyd 1980). These passive technologies encompass bioreactors, sorbent media as well as low-cost reactants based on recycled industrial by-products (Gandy and Jarvis 2012, Mayes *et al.* 2011, Warrender *et al.* 2011). While all have shown promise in various settings, the perennial ambitions to develop treatment technologies that can ally effective metal removal with low residence time, small land footprint and treatment longevity still remain elusive for many circum-neutral metal mine waters.

In recent years, studies on biofilms have found they are capable of storing large inventories of trace metals (Rogerson *et al.* 2008). Biofilms are communities of cyanobacteria, sulphate reducing bacteria and photosynthetic diatoms, held together in a matrix of extracellular polymeric substances (EPS). The biofilm EPS is formed as a product of bacterial metabolic reactions, and although the EPS components can be highly variable dependent on what bacterial strains are present, they typically comprise of polysaccharides and proteins, nucleic acids, lipids and humic substances (Sutherland 2001). EPS molecules contain the ionisable function groups, carboxyl, phosphoric, amine and hydroxyl, all of which give the EPS a net negative charge (Hullebusche *et al.* 2003). This negative charge allows the EPS to sequester cationic metals (Me^{2+}) from the water column. The EPS effectively protects an ecosystem, inhabited by cyanobacteria and photosynthetic microbes, governed by processes that are independent of those occurring in the bulk water column (Bisset *et al.* 2008). During daylight hours photosynthetic microbes utilise carbon dioxide which increases pH, while during night time hours when respiration predominates carbon dioxide is released and thus the pH lowers. Bisset *et al.* (2008) observed that despite water column fluctuations in pH of between 7.8 and 8.9, the pH at the biofilm surface was 9.4 in the light and 7.8 in the dark. The implications of this internal pH change are two-fold. Internal changes in pH can result in chelated metals being released from the EPS (Hullebusch *et al.* 2003). However, in alkaline streams the increased pH and chelation of Ca^{2+} by the biofilm can promote carbonate precipitation within the film, a potential sink for other divalent metals (Me^{2+}), and a process that would not occur otherwise by thermodynamic means alone (Rogerson *et al.* 2008). Recent field assessment of biofilm Zn concentrations in circum-neutral streams draining areas of former Pb and Zn mining in North Yorkshire, UK, showed median Zn concentrations of 1800 mg/L (range: 800–4100 mg/L), a bioconcentration factor of over 3500 from the water column under baseflow conditions (Jones *et al.* 2013). There is therefore considerable opportunity to use biofilm as a tool to remediate areas that are affected by mine drainage. However, further research is needed to elucidate the pathways for metal uptake (*e.g.* biomineralization versus sorption) and permanence of metal removal in biofilms. This paper aims to assess these processes through in-vitro experiments of biofilms cultured from mine-impacted streams. This serves both to improve our understanding of biolfims as a control on contaminant transport in mine-affected streams, but also to underpin approaches to harness biofilms for polishing treatment applications metal mine discharges.

Methods

Batch experiments

Static batch experiments are a useful tool for answering a specific question, and were suitable to compare the effects that separate treatments to the biofilm had on metal concentrations in solution. For each experiment using an agitated flask method, 150 mL of mine water was placed into a cleaned 250 mL conical flask with 5 g of biofilm. Biofilm was collected on the day of the experiment and transported in such a way that would avoid any denaturing of the biofilm quality. Biofilm and water was collected from Dukes Level mine discharge (Lat 54°04′43N, Long 1°57′41W), a major mine drainage level with ambient Zn concentrations of 950 $\mu g\ L^{-1}$ and major ion chemistry characterised by $Ca^{2+}–HCO_3^-–SO_4^{2-}$-dominated waters. Each experiment was left for 21 days in a controlled environment laboratory which was set up to simulate summer conditions in the UK (ambient air temperature set at 18 °C ±1 °C, with 8 hours UV light/d). Treatments were applied to the flasks to observe the different effects of photosynthesis on metal uptake. Each flask was wrapped in a material to alter the amount of UV light that passed through to the biofilm. This was done on a four treatments that ranged through full light (FL: 6.82 kLux), high light (HL: 4.83 kLux), low light (LL: 2.64 kLux) and no light (NL: 0 kLux) exposures. After the incubation period, biofilm flasks where sampled for aqueous metal concentration. A filtered (0.45µm) 14 mL sample of water was taken and acidified immediately in preparation for trace metal analysis by a Perkin Elmer OES optima 5300DV Inductively Coupled Plasma Optical Emission Spectrometer. A blank and standard suite where analysed every 15 samples to check instrument calibration, and wavelengths were selected in accordance with standard methods (USEPA Method 200.7).

Flume Experiments

Continuous flow flume experiments were adapted from previous work by Pedley *et al.* (2009), with the objective of observing long-term cycling of biogeochemical parameters, and assessing the influences on trace metal mobility in a controlled environment. The novelty of this system is built around the culture and long term maintenance of biofilm communities collected from environmentally relevant field sites; in this case metal mine impacted streams in North Yorkshire, UK. The flume experiments had a recirculating design permitting the experiment to proceed under controlled environmental conditions with no external influence. The flume consists of a 50cm long, 7 cm wide polycarbonate gutter, lined with carbonic fabric and small rocks to aid colonisation and, given the flow rates applied, increase water turbulence throughout the vertical water column (Fig. 1). Gutter was housed in a purpose built Perspex flume box. Flow was driven by a Waston Marlow 300 series tube pump, with four 313D pump head attachments (Watson Marlow, Falmouth, UK), running at 55 rpm, through peristaltic tubing (1.6 mm wall, 4.8 mm bore). The mesocosm was subjected to a 8:16 day:night light cycle (light: Thorn Lopak 250 W HPS-T sodium lamp), in a windowless, air conditioned laboratory (ambient air temperature 18 °C ± 1 °C).

Two flumes where colonised with biofilm (sourced as per flask experiment), and one was sterilised before the experiment with an 10 % acid rinse (HNO_3), ethanol rinse and finally ultrapure (18 Ω) water rinse, and left uncolonised to act as control flume. Electrodes where placed in the flumes to constantly monitor pH (Pinpoint pH monitor, American Marine Inc.) and conductivity (Pinpoint Conductivity Monitor – Freshwater Hardness, American Marine Inc.). 14 mL water samples were taken every three days to establish initial trends of total metal removal in the system. Water analysis proceeded as per flask experiments. Non-parametric statistical tests were applied to discontinuous percentage data in Minitab v. 15. Geochemical analysis is undertaken using PHREEQC Interactive 2.18.3 and the WATEQ4F database.

Results and discussion

Batch experiments

Static batch experiments show biofilm is capable of high levels of metal uptake, with an average metal uptake of 80 %, and a maximum of 99 % removal. Control data (empty flask and water) show Zn is still being removed, most likely due to sorption onto glassware or precipitation from the water column (Fig. 2). However, the removal of Zn is significantly lower (Kruskal Wallis: H: 20.4, d_f: 4, $p<0.001$) in con-

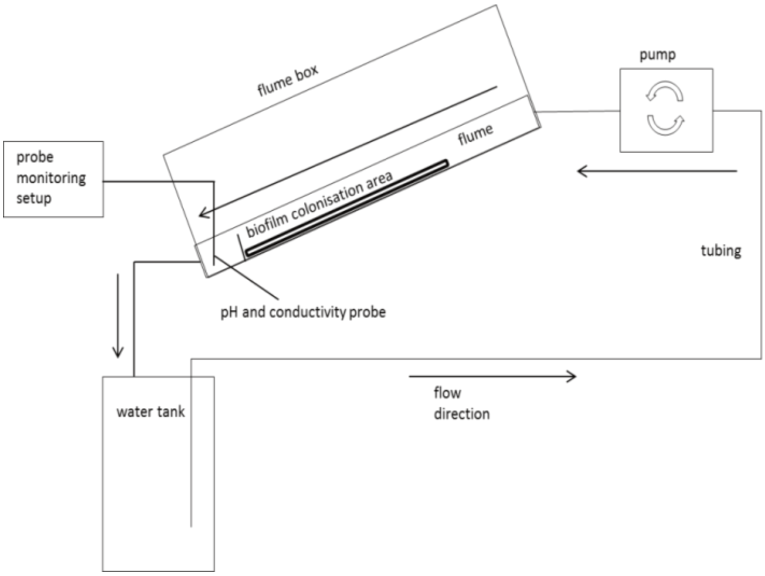

Fig. 1 *Schematic diagram of continuous flow experimental apparatus*

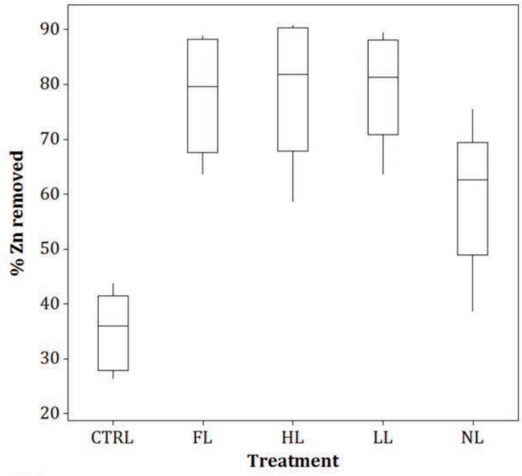

Fig. 2 *% Zn removal in batch experiments*

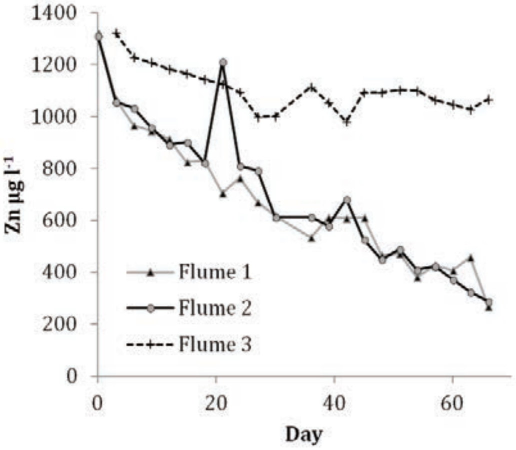

Fig. 3 *Zn removal over time in mesocosms*

trol treatments (median of 36 % Zn removal) than in the biofilm occupied flasks (median 74 % Zn removal) suggesting that biofilm is responsible for the bulk of Zn removal from the water column (Fig. 2). There is no significant difference in median Zn removal rates between the light treatments and those with no light present (Kruskal Wallis: H: 6.69, d_f: 3, p = 0.08). However, while the data suggests that metal uptake is not affected by changes in light intensity (*i.e.* metal removal continues with no light present), it is important to note the small volumes of water used and the amount of zinc in the sample vessel was finite.

Other studies have shown how divalent metal uptake into biofilms is chemoselective via chelation of divalent metals by extracellular polymeric substances (EPS); a process which usually favours ions those with a low charge density (z_i^2/r_i ratios; Rogerson *et al.* 2008). The replenishment of EPS would require a light source in order to keep the phototrophic component of the biofilm alive and its structural integrity maintained over long periods of time. By the end of the experiment, the NL biofilm is completely degraded and is in a fine "powdery" form, whereas the biofilms FL, HL, and LL are still functioning as normal with its structural integrity maintained. The removal of Zn from the NL flask suggests it is possible that metal removal occurred before biofilm degradation in the NL flask. This uptake in the absence of growth is consistent with chelation of ions within the EPS matrix as opposed to direct uptake within photosynthesising organisms.

Flume experiments

The flume experiments proceeded for 66 days and Fig. 3 shows the systematic decline in dissolved Zn in the water column, with 78 % of initial Zn removed by the biofilm. The control flume (with sterilised biofilm – shown as dashed lines in Fig. 3) show initial Zn removal, which levels off after around 3 weeks. Both the active (non-sterilised) flumes (number 1 and 2) show Zn removal which continues until the end of the trial, albeit at slightly lower rates with time. These patterns are again consistent with the abiotic removal of metals from the water column by EPS in the sterilised treatments and the production of new EPS in active flumes maintaining removal over the longer term: a total of ≈ 9.5 m^3 of water was circulated through each flume. The abrupt spike in flume 2 after day 22 is likely to be due to entrainment of a small amount of organic debris in the sample. Observations of water pH highlight the micro-environment created by the biofilm which would be conducive to greater sorption of Me^{2+} and also potentially shift saturation of key mineral phases (notably $ZnCO_3$, $CaCO_3$ and

pH	$CaCO_3$	$ZnCO_3$	ZnO (a)
Mine water (7.16)	-1.03	-1.13	-2.10
Flume (daytime: 9.18)	1.71	-1.98	0.29
Flume (nighttime: 7.87)	0.00	-0.98	-0.16

***Table 1** Saturation indices for selected phases under different pH conditions: ambient mine water pH, biofilm daytime pH and biofilm nighttime pH.*

amorphous Zn oxides) towards solid phase precipitation. Water pH of the original mine water ranges from 6.38 to 7.71 (mean of 7.16; *n* = 15), while the mean daytime pH obtained from sensors within the biofilm is 9.18 (range 7.81-9.70, *n* = 245). This is consistent with the observed geochemical micro-environment in other calcifying biofilm mesocosms (*e.g.* Bissett *et al.* 2008) where daytime pH values far higher than those common in the bulk water column are apparent due to photosynthetic carbon utilisation. Table 1 highlights the differences in saturation state for some relevant phases based on differences in water column and biofilm pH alone, without taking into account potential concentration of divalent metals by EPS chelation. It is apparent that the biofilm pH leads to waters supersaturated in amorphous zinc oxide and calcite, which could play a key role in direct Zn uptake, or through sorption or co-precipitation onto calcite (*e.g.* Zachara 1991). The differences in daytime and nighttime pH are also stark, highlighting a key potential control on diel cycling of Zn in the water column (Nimick *et al.* 2011).

Management implications

Previous attempts to remove Zn from mine discharges have had mixed results. The adaptation of anoxic limestone drains to encourage smithsonite ($ZnCO_3$) precipitation has been investigated at field scale and although showing initial promise, removal rates of 10 – 30 % were a product of the difficulty of maintaining narrow geochemical operating conditions under field conditions (Nuttall and Younger 2000). Much research effort has also gone in to developing bioreactors or sorbent-based systems for mine water removal. Although sulphate-reducing bioreactors have typically been focussed on highly acidic mine waters (where alkaline media and action of sulphate reducing bacteria help buffer the pH), there have been successful applications of bioreactors for circum neutral waters. For example Gandy and Jarvis (2012) report average area-adjusted Zn removal rates of 0.4 g $m^{-2}day^{-1}$ in pilot field systems in northern England. However, maintaining efficient flow distribution through dense reactors and concerns over the waste status of spent organic and metal-rich substrate remain issues to be overcome. Sorbents, or reactive media are another option that have been explored by many workers. Generally sorbents are capable of high metal removal rates, up to 99 % removal of zinc) which initially sounds promising, however, this level of uptake can only be maintained for small volumes of water (≈ 200 L), as functional sites for metals to sink to are saturated, the removal efficiency drops (Warrender *et al.* 2011). Furthermore, while modest volumetric removal rates have been maintained for reasonable timescales in field sorbent-based systems, these effects have been ascribed to the colonisation of biofilm communities on the surface of the sorbent media (*e.g.* Mayes *et al.* 2009b). The advantage of the biofilm is that it is a community of living organisms, so the EPS which they create, is constantly being replenished. This effectively means that sites for metal uptake are constantly being produced, resulting in higher efficiencies being maintained. Comparisons of preliminary data here with other mine water treatment schemes reveal similar metal removal rates in terms of area adjusted removal rates. The area adjusted removal rates rely on a calculation between residence time (the time taken for water to flow through the flume system) and flow (L/s), which takes into account the metal load removed per unit surface area of biofilm per day ($g\ m^{-2}d^{-1}$). The mean removal rates for the flumes was $0.16\ g\ m^{-2}d^{-1}$ (min = 0.13, max = $0.20\ g\ m^{-2}d^{-1}$) which was achieved with a 9.6 minute mean residence time. Pilot and field studies utilising algal mats have been seen to achieve a similar order of magnitude of Zn removal with removal rates of $0.47\ g\ m^{-2}d^{-1}$ and $0.25\ g\ m^{-2}d^{-1}$ respectively observed (Kalin 1998). The removal rates documented here are also well above the range of those quoted for wetland systems (*e.g.* Gillespie *et al.* 1999, Song *et al.* 2001).

Conclusions and further work

Biofilm holds the potential to be a low cost, passive remediation tool. Biofilms are ubiquitous in circum-neutral pH, metal-rich waters and play a crucial role in the diel and seasonal cycling of trace elements (*e.g.* Nimick *et al.* 2011). The laboratory studies here highlight how natural biofilm communities can be readily cultured and maintained under controlled conditions in the laboratory. Under these conditions, zinc removal was consistent and long-lived; the production of EPS appears to replenish sites for metal uptake within the biofilm. Zn removal rates are consistent with many other systems, but it is the low residence time and longevity of treatment without maintenance that are most promising for developing biofilms into a treatment option. Harnessing these low-energy biological processes in remote locations, where modest Zn concentrations impact headwater streams of otherwise high amenity value, could provide a new approach in mine water treatment. Future work is concentrating on elucidating these uptake mechanisms in greater detail through a range of solid phase assessments, assessing the permanence of Zn removal, and evaluating the scope for engineering systems at full scale. The latter will also include laboratory evaluation of synthetic EPS for Zn removal. Given the evidence here suggests that the Zn uptake is only indirectly controlled by photosynthetic processes and it is the EPS production that maintains effective removal through chelation of Me^{2+}, there may be considerable scope for enhancing metal removal with synthetic EPS. Determining these specific metal uptake pathways and optimal operating conditions are going to be crucial to underpin field development of effective biofilm-based treatment systems in the future.

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