Microbial communities in passive remediation systems at three abandoned coal mine sites in the United Kingdom

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Abstract

Mining leaves a legacy of potential long-term environmental impact, including ferruginous (and often acidic) waters that are generated within and flow from underground workings, and surface-located waste materials. Passive remediation systems, utilising constructed wetlands, have been widely used in recent years to treat coal mine waters. These utilise the abilities of microorganisms to catalyse redox transformation of iron and sulfur, and to generate alkalinity. The microbial communities in constructed wetlands at three contrasting passive mine water treatment schemes in the United Kingdom were investigated in the present study: (i) a RAPS (Reducing Alkalinity Producing System) to treat net acidic mine water; (ii) a constructed wetland used to treat brackish coal mine drainage; (iii) a passive system used to simultaneously treat coal mine water and secondary treated wastewater effluent. Microorganisms present in water and sediment samples were identified using a combined approach involving cultivation-based and culture-independent methods. While bacteria known to catalyse redox transformations of iron and sulfur were identified in all three sites, the indigenous microbial communities were strongly influenced by the chemistries of the influent mine waters, with bacterial species found in marine waters and those typically found in sewage detected in sites (ii) and (iii), respectively. The diversity of bacteria in mine waters appeared to become increasingly diminished as the water flowed through the passive systems. Archaeal communities in the three sites were dominated by methanogenic species, and some ammonia-oxidizing archaea were also identified. Laboratory experiments demonstrated that the microorganisms present in the sediments were able to remobilise iron (via reductive dissolution of the ferrihydrite-like minerals that accumulated at the water/sediment interface) under conditions of oxygen limitation. This suggested that microbially-catalysed cycling of iron between the ferrous and ferric states is a typical feature of these constructed wetlands, which has implications on their net efficiencies as remediation systems.

Key words: coal mine, passive mine water treatment, bacteria, archaea, molecular biology

Introduction

Waters draining coal and metal mines can have widely different chemical characteristics. One of these, which is often used to delineate many mine waters, is pH. This is a major factor, as it has a high influence on the solubilities of (cationic) metals, rates of (abiotic) oxidation of iron, and on the microflora and macro-organisms that can live in mine waters. At its point of discharge, mine waters frequently contain little or no dissolved oxygen, and the dominant (or even exclusive) form of soluble iron present is ferrous. Oxygenation of mine water streams by diffusion, mass transport and oxygenic photosynthesis creates conditions favourable to the diverse species of bacteria and archaea that can obtain energy from the dissimilatory oxidation of ferrous iron.

Mine waters can be colonized by a wide diversity of microorganisms. Prokaryotic microorganisms (archaea, and particularly bacteria) are found in greater numbers of cells and species (i.e. biodiversity) than eukaryotes, and these have been the focus of a large body of applied and fundamental research.

Microorganisms have a major impact on the behaviour and cycling of metals in the environment. This can be mediated by a variety of opposing mechanisms, including assimilation/adsorption, precipitation/dissolution, oxidation/reduction, and methylation/dealkylation (Johnson 2006). For example, redox changes can result in the production of metal ions that are either more or less soluble under prevailing environmental conditions, leading to their spontaneous precipitation or dissolution. Microbially-catalysed formation of minerals ("biomineralisation") is particularly important in the case of mine water treatment, as this can "lock-up" metals (e.g. iron, manganese and other transition metals) and metalloids (such as arsenic) in forms where they are no longer available to the biosphere, and therefore non-toxic. However, all such biominerals can be subject to dissolution if conditions (e.g. pH, oxygen status) of their local environment changes, for example if anoxic sediments are exposed to air, e.g. as a consequence of lower water levels in constructed wetlands. This means that engineered systems used to immobilise metals and metalloids present in influent mine waters require careful long-term management to avoid the problem or re-release of toxins.

The Coal Authority manages issues related to historic coal mining in the United Kingdom (UK), including pollution caused by mine waters, as well as regulating current mining activities. The Coal Authority operates over 70 mine water treatment schemes (MTWSs) across the UK, the majority of which are passive treatment systems, often using constructed wetlands. Passive systems are perceived as low cost operations although they have limitations (e.g. Johnson and Hallberg 2002). Where mine waters are net acidic, passive MWTSs require an additional source of alkalinity as part of the remediation protocol. This may be derived from microbiologically-catalysed reactions (e.g. sulfate reduction, at pH <7) or, at least in part, from the dissolution of basic minerals. This is the case with SAPS (successive alkalinity-producing systems) and RAPS (reducing and alkalinity producing systems; Younger et al. 2003).

Microbial activity is key to the success of passive mine water treatment. Bacteria and other microorganisms catalyse redox transformations of metals, such as iron and manganese, and of sulfur; these reactions have a major influence on the effectiveness of passive treatment operations. It is pertinent, therefore to understand the composition and dynamics of microbial communities that colonise constructed wetlands used to treat mine waters. However, there have been relatively few reports on the microbial communities that colonise these constructed sites (Johnson et al. 2002). The current study has investigated the microbial communities in water and sediments at three passive MWTSs located at abandoned coal mine sites in England and Wales, and has examined the potential for the cycling of iron in these anthropogenic environments.

Methods

Sampling

Based on data provided by the Coal Authority, three contrasting mine sites in England and Wales were selected for this study: (i) Tan-y-Garn, located adjacent to the Cathan river in Garnswllt (51°46'10.9"N, 3°59'9.42 W; Wales), where the treatment system uses a RAPS (Reducing Alkalinity Producing System) technology; (ii) Horden, located in a coastal area of Co. Durham (54°46'18.02 N, 1°18'36.92 W; England) where the MWTS is used to treat brackish coal mine drainage; (iii) Lamesley, near the Team river in Co. Durham (54°54'18.92 N, 1°35'58.62 W; England), which was the first constructed wetland in the UK to be used for the simultaneous treatment of coal mine water and secondary-treated wastewater effluent. Water samples from the three sites above described were collected in October 2014 at different locations within the passive MWTSs, as described below.

Water samples were taken at Tan-y-Garn MWTS at the (i) inflow (water flowing into the RAPS system); (ii) mid-flow (water draining the RAPS); (iii) outflow (water draining the final reed-bed), (fig.1). In addition, two solid samples were removed (for laboratory analysis and experimental work) from the RAPS system itself (fig.1). These were: (i) ochreous material (ferric iron-rich), which had accumulated on the surface of the RAPS, and (ii) residual black organic compost material, taken from about 15 cm depth within the RAPS. A similar sampling regime was adopted at Horden MWTS to that used at Tan-y-Garn (3 water samples taken from similar points, plus two solid (ochre and compost) samples from the primary wetland (fig. 1). Sampling at the Lamesley co-treatment site differed from the approach used at the other two sites. Unfortunately, the site had suffered a serious act of vandalism the previous night, resulting in the temporary cessation of mine water pumping. On the day of sampling only secondary treated wastewater (sewage water) was being discharged into the site. It was therefore deemed to be inappropriate to use the same sampling protocol to that used at Tan-y-Garn and at Horden; only a single water sample was taken (that draining the final reed-bed; fig.1). However, ochre and compost samples were removed from the first reed-bed as before, as these were considered not be have been significantly impacted by the temporary cessation of mine water input.

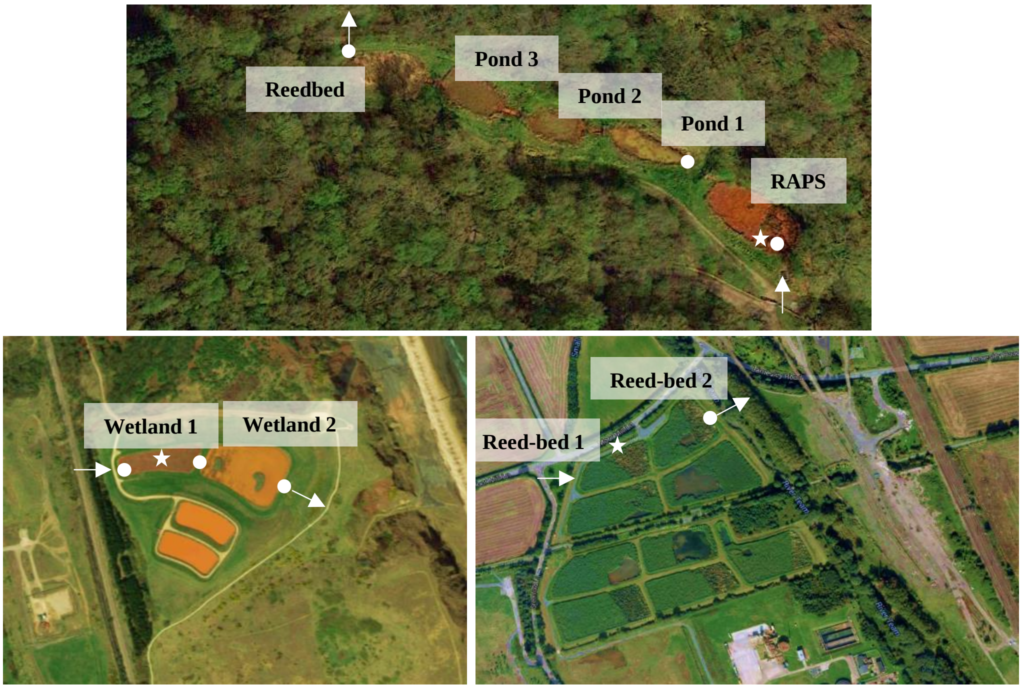

Figure 1. *Aerial view of sampling at water (circles) and sediment (stars) samples were taken at the Tan-y-Garn (top), Horden (bottom left) and Lamesley (bottom right) sites. Arrows indicate water flow. Aerial photographs taken from Google Earth.*

Physico-chemical analysis

Temperature (T), redox potential (ORP), pH value, conductivity (SC) and concentration of dissolved oxygen (DO) in all water samples were measured in situ using a Multi-parameter System (YSI 556 MPS). Laboratory analyses of sulfate, iron, dissolved organic carbon (DOC), and transition metals were carried out 2-4 days after sampling on cold-stored acidified, filtered mine waters. Sulfate was measured using a Dionex IC25 ion chromatograph with an Ion Pac AS-11 column equipped with a conductivity detector. Ferrous iron and total iron concentrations in water samples were determined using the Ferrozine method (Stookey 1970). Transition metals were measured with a Dionex-320 ion chromatograph fitted with an IonPAC® CS5A column and an AD absorbance detector. Concentrations of DOC were measured using a LABTOC DOC analyzer (Pollution and Process Monitoring, UK).

Microbiological analysis

Cultivation-based microbiological analysis of water and sediment samples was restricted to looking for the presence of acidophilic bacteria that catalyse redox transformations of iron. Water samples (50 µL) and sediment samples from the three mine sites were spread onto a variety of solid overlay media designed to promote the growth of different physiological groups of acidophilic microorganisms (Johnson and Hallberg 2007). The inoculated plates were incubated aerobically at 30°C for up to 4 weeks. Colonies were identified as iron-oxidizers (ferric iron-encrusted) or potential iron-reducers, and their identities confirmed by amplifying and sequencing their 16S rRNA genes.

Biomolecular analysis of indigenous microbial populations

In order to avoid problems endemic to cultivation-based analysis of microbial populations, a biomolecular approach was used based on the amplification of a target gene (the 16S rRNA gene) using the DNA present in microbial biomass collected on membranes by filtering water samples, or present in ochre and compost samples taken from the RAPS, wetland 1 or reed-bed 1 in each of the test sites (Tan-y-Garn, Horden and Lamesley, respectively). This involved two separate but complimentary analyses:

(i) community profiles, using T-RFLP (terminal restriction enzyme fragment length polymorphism); (ii) construction and analysis of clone libraries using amplified 16S rRNA genes.

DNA was extracted from the mine water filtrates and sediment samples using MoBio "ultraclean soil DNA isolation kits", following the manufacturer's instructions. Terminal restriction enzyme fragment length polymorphism (T-RFLP) analysis of amplified genes was carried out to assess the microbial diversity of samples as described by Kay et al. (2013), and the T-RFs (terminal restriction fragments) identified by comparing them with those in the databank maintained at Bangor University.

Clone libraries were constructed to identify bacterial and archaeal composition of one sample from each mine site. The most diverse sample at each sites, as determined by the complexities of their T-RFLP profiles, were selected for library construction. For bacterial clone libraries these were: (i) Tan-y-Garn, mid-flow (for both bacterial and archaeal clone libraries); (ii) Horden, mid-flow (bacterial) and inflow (archaeal); (iii) Lamesley, outflow (bacterial and archaeal). The protocols followed during the construction of the clone libraries are described in Falagán et al. (2014).

Mesocosms set up

Samples of ochre and compost were suspended, separately, in pH 7 basal salts solution to produce homogeneous slurries, 1 mL of which were dispensed into sterile foam-bunged 20 mL universal bottles (i.e. each bottle received 1 mL of ochre slurry and 1 mL of compost slurry from a particular wetland site). Different volumes of glucose were added, equivalent to 0, or 10, 100 or 1000 mg dissolved organic carbon (DOC)/L, and the volumes in each bottle were made up to 10 mL with sterile pH 7 basal salts solution. Each test was carried out in duplicate (24 bottles in total). Samples were removed for analysis of ferrous iron (using the Ferrozine assay; Stookey 1970) and then the bottles were placed in jars within which anaerobic environments were generated using the AnaeroGen system (Oxoid, UK). The jars were incubated for 2 weeks at 30°C, after which they were opened, visually examined and photographed, and samples removed again to determine concentrations of soluble ferrous iron.

Results

Physico-chemical analysis of mine water samples

Data obtained in the field and in the laboratory on the chemical analysis of mine waters in different locations in the three sites are shown in table (tab. 1).

Table 1 *Physico-chemical data from the three sites sampled. Units: E_H (mV), T (°C), SC (mS/cm), DO (%), DOC, metals and sulfate (mg/L). I, inflow; M, mid-flow; O, outflow; <, below detection limit; n.a., not analysed.*

	pH	E_H	T	SC	DO	DOC	Fe^{2+}	Fe^{3+}	Fe_{total}	SO_4^{2-}-S	Mn	Zn	Co
							Tan-y-Garn						
I	5.14	327	11.0	0.397	21	3.24	37.4	2.8	40.2	72	2.7	0.3	2.3
M	6.46	256	11.1	0.470	45	2.00	11.2	<	11.2	45	1.4	0.4	0.3
O	5.80	394	10.9	0.367	68	2.34	<	<	<	47	<	<	<
							Horden						
I	6.45	282	14.1	35.29	88	1.11	21.2	16.2	37.4	1020	2.9	0.3	1.0
M	6.99	203	14.1	31.10	88	0.24	6.1	3.9	10.1	917	2.7	0.1	0.2
O	7.48	238	13.5	34.98	90	0.23	1.7	6.7	8.4	949	2.8	0.1	<
							Lamesley						
I	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	<	0.9	0.9	n.a.	n.a.	n.a.	n.a.
M	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
O	7.15	336	12.9	1.27	68	6.87	<	0.8	0.8	22	0.3	<	<

Microbial community compositions

Few isolates were obtained from the water and sediment samples. Only two acidophilic bacteria were obtained, a strain of the recently-described species "*Acidibacillus ferrooxidans*", which catalyzes both the oxidation and reduction of iron (isolated from the compost collected at Horden), and strains of the iron-reducing heterotrophic acidophile *Acidiphilium* sp. which were isolated from all the mine sites.

Other bacterial isolates obtained from Tan-y-Garn and Lamesley sites were either known pathogens (e.g. *Rhanella aqualitis*) or closely related to pathogenic bacteria.

Tan-y-Garn MWTS

The T-RFLP profiles obtained from amplified bacterial 16S rRNA (fig. 2) showed that the bacterial community in the mine water that flowed into the site was highly heterogeneous, but became increasingly less diverse as the water flowed through the passive system. Over 40% of the total T-RFs in water draining the site was accounted for by a single T-RF (and therefore possibly a single bacterial species; 226 ± 2 nt; HaeIII digests) and three other T-RFs of 10-15% relative abundance each, were also present. Bacteria closely related to species known to catalyze the dissimilatory reduction of ferric iron (*Geobacter psychrophilus*), oxidation of sulfur and reduction of nitrate (*Sulfuricella denitrificans*), methylotrophs (methane/methanol-oxidizers) and anaerobic aromatic compound-degraders (*Syntrophorhabdus* sp.) were detected. Over 50% of the T-RFs for archaea in the mine water samples were accounted for by a single T-RF (213 ± 2 nt; HaeIII digests) although analysis of the sequences of clones obtained of archaea indicated a more heterogeneous archaeal community. Species closely related to those identified as ammonium-oxidizers (*Nitrosopumilus maritimus*) and methanogens (e.g. *Methanomassiliicoccus luminyensis*) were detected, and corresponded to dominant T-RFs in T-RFLP profiles.

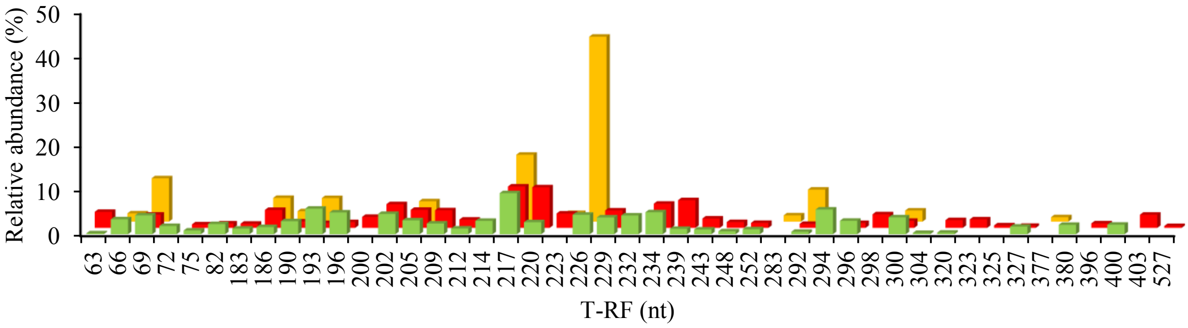

Figure 2 *T-RFLP profiles of HaeIII-digested bacterial 16S rRNA genes obtained from DNA extracted from harvested biomass from water flowing into the RAPS (green bars), water flowing out the RAPS (red bars) and water flowing out the final reed-bed (yellow), at the Tan-y-Garn MWTS.*

Horden MWTS

As with Tan-y-Garn, the bacterial community became increasingly less diverse as the water flowed through the wetland, though in this case this was due to the increasing dominance of a single T-RF of 192 ± 2 nt (HaeIII digests) which accounted for 65% of the total in the water discharged from the site. Analysis of the sequences of clones obtained, showed that most of the clones were related to bacterial species found in marine environments (e.g. *Desulfuromusa ferrireducens*). The bacterial communities at the Horden site were shown to be the most metabolically diverse of those in the three sites investigated. Bacteria identified included those closely related to species known to catalyze the dissimilatory reduction of ferric iron and of manganese (IV) (*Geobacter bemidjiensis*), oxidation of sulfur (*Pelobacter carbinolicus*), ammonium oxidation (*Nitrosomonas europaea*), sulfate reduction (*Desulfuromonas machiganensis*) and nitrate reduction (*Tistrella bauzanensis*). However, in contrast to the bacterial communities, analysis of the sequences of archaeal clones obtained from samples from the Horden site showed that most were closely related to isolates known to be methanogenic (e.g. *Methanomassiliicoccus luminyensis, Methanothermococcus okinawensis, Methanobacterium aarhusense*), and only one related to an ammonium-oxidizer (*Nitrosopumilus maritimus*).

Lamesley MWTS

In contrast to waters sampled at similar points at Tan-y-Garn and Horden, where species diversity appeared to be relatively limited, the T-RFLP profile from Lamesley treated water discharge implied a highly heterogeneous bacterial community, though T-RFs of 192 ± 2 nt and possibly 226 ± 2 nt (the dominant peaks in Horden and Tan-y-Garn, respectively) were also present, albeit in small relative percentages. Analysis of the sequences of clones showed the presence of pathogenic bacteria (*Legionella* spp.) and nitrate reducers (*Aquabacterium commune*), as well as others. Archaea closely related to species known to be methanogens (e.g. *Methanomassiliicoccus hollandica, Methanosarcina* spp.), ammonium-oxidizers (*Nitrososphaera viennensis*), thermophilic (*Ignisphaera aggregans*), and sulfur-reducers (*Hyperthermus butylicus*) were detected.

Mesocosm experiments

The concentration of ferrous iron in the Tan-y-Garn mesocosms prior to being incubated under anaerobic conditions was 0.02 mM (~1 mg/L). However, concentrations increased by over two orders of magnitude in all of the incubated mesocosms, whether or not they were amended with glucose. Final concentrations of ferrous iron were quite similar in non-amended Tan-y-Garn mesocosms and in those containing 10 or 100 mg DOC-glucose/L, though these were significantly higher in both mesocosms that had been amended with 1000 mg DOC-glucose/L (fig. 2). The concentration of ferrous iron in the Horden mesocosms prior to being incubated under anaerobic conditions was 0.009 mM (~ 0.5 mg/L). As with the Tan-y-Garn samples, concentrations of ferrous iron increased in all of the Horden mesocosms, though final concentrations were far lower than with the Tan-y-Garn samples. Also in contrast to Tan-y-Garn, the addition of glucose did not appear to have a major impact on final ferrous iron concentrations, and the smallest of these were found in mesocosms that had been amended with the largest concentration (1000 mg DOC-glucose/L) used (fig. 2). The concentration of ferrous iron in the Lamesley mesocosms prior to being incubated under anaerobic conditions was the lowest of the three MWTSs (0.004 mM; ~0.2 mg/L). Concentrations of ferrous iron increased as a consequence of being incubated under anaerobic conditions with final concentrations being intermediate between those found in Horden and Tan-y-Garn mesocosms. As with the Horden mesocosms and in contrast to Tan-y-Garn, the addition of glucose did not appear to have a major impact on final ferrous iron concentrations (fig. 2), and the smallest of these were found in mesocosms that had been amended with 100 mg DOC-glucose/L.

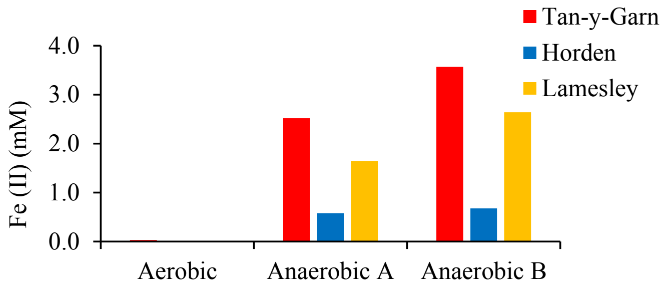

Figure 2 *Comparison of the amounts of ferrous iron generated in mesocosms prepared using compost and ochre from the three MWTS (no glucose was added). Concentrations of soluble ferrous iron present in replicate mesocosms generated after two weeks incubation under anaerobic conditions are shown in comparison to those at the start of the experiment ("aerobic")*

In contrast to iron reduction, evidence for microbially-catalyzed dissimilatory reduction of sulfate was only observed in mesocosms amended with glucose. Visual examination of mescosms following anaerobic incubation showed that in virtually all cases where glucose had been added, the color of the sediment had changed from brown to jet black, a clear indication that amorphous FeS/hydrotroilite ($FeS.nH_2O$) had formed, presumably via bacterial reduction of sulfate in the water to hydrogen sulfide which reacts with ferrous iron at circum-neutral pH ($Fe^{2+} + HS^- \rightarrow FeS + H^+$). Confirmation of biomineralization of iron sulfide came from addition of acid to the blackened sediments, which resulted in a rapid evolution of H_2S.

Discussion

Given the circum-neutral pH of the mine waters being treated at the three sites investigated in the present study, the possibility of detecting acidophilic microorganisms might be expected to be remote. However, given the heterogeneous nature of ochres and composts samples and the known abilities of many species of acidophilic bacteria and archaea to catalyze redox transformations of iron and sulfur, trying to detect some of these microorganisms in the three MWTSs was considered to be a worthwhile exercise. Two representative species of acidophilic bacteria, both known to reduce ferric iron and one know to oxidize ferrous iron, were isolated from the sites. These bacteria did not show up in T-RFLP profiles, which suggests that their numbers were small relative to other bacteria.

Microbial communities within the three MWTS sites were strongly influenced by the chemistry of the water entering the treatment systems. Tan-y-Garn was dominated by bacteria known to be involved in the iron and sulfur cycles. Horden microbial communities were dominated by bacterial species related to those found in marine environments. Lamesley was dominated by bacteria typically found in sewage. Archaeal communities in the three sites were dominated by methanogenic species and archaea involved in the nitrogen cycle (ammonium oxidation, nitrate reduction). Significantly, bacterial diversity in mine waters at both Tan-y-Garn and Horden appeared to become increasingly restricted as the water flowed through the treatment systems.

Large amounts of iron were remobilized by incubating mixtures of composts and ochres under oxygen-free atmospheres. Addition of glucose had little impact on net iron reduction. One of the interesting observations was that, although large amounts of ferrous iron were remobilized (presumably via microbially-catalyzed reductive dissolution of ferrihydrite and other ferric iron minerals present in the accumulation of ochreous materials as the wetlands aged), this varied significantly between samples from the three wetland sites. The reason for this may be due to differences in the nature of the compost materials or the ochres (or both). The former is considered to be more likely, implying that the residual compost at Tan-y-Garn is far less recalcitrant than that at Horden, and that the Lamesley compost falls between the two, where wastewater effluent provides a second source of carbon. Differences in ochre mineralogy cannot be ruled out however, as the susceptibility of ferric iron minerals to reductive dissolution is known to vary with their degrees of crystallinity, with amorphous ferric iron hydroxide being more readily reduced than ferrihydrite, which is in turn more susceptible to reductive dissolution than is goethite etc., (Bridge and Johnson 2000).

The observation that oxygen limitation can result in rapid remobilisation of iron in constructed wetlands used to remediate ferruginous mine waters has important implications in terms of the net efficiencies of these as remediation systems. Anoxic conditions are likely to prevail at the compost layer and during the summer months due to the higher temperatures and growth of wetland plants. The interface of the compost and ochre layers is therefore likely to be an intense zone of redox transformations and cycling of iron between the ferrous and ferric states. This necessarily cuts down the efficiency of the constructed wetlands as sites for iron precipitation, though the fact that concentrations of ferrous iron in waters draining all three sites were still relatively small (though the data for Lamesley were compromised by the site problems on the day of sampling) implies that the sites were working effectively overall. It is conceivable, however, that by eliminating or greatly lowering the potential for biological remobilization of iron, the footprints of the sites could be greatly reduced while at the same time maintaining their net effectiveness. The fact that bacteria known to catalyse the reductive dissolution of ferric iron minerals (*Geobacter* and *Acidiphilium*) as well as others that catalyse the oxidation of ferrous iron (e.g. *Acidibacillus*) provides further evidence that iron cycling is a dynamic process in these environments. The presence of wetland plants is likely to promote iron reduction and remobilization, due to oxygen uptake by plant roots and their additional input of organic carbon (readily metabolizable exudates and lysates, as well as more recalcitrant dead plant matter).

Ferrous iron generated by microorganisms could itself be biomineralized (as FeS) as a consequence of bacterial sulfate reduction, which also requires anoxic conditions. However, data in table (tab. 1) show that concentration of sulfate in waters flowing into and out of the MWTS sites are very similar, indicating that the reduction of sulfate to sulfide is at best a minor process in these environments. It is interesting in this respect that bacterial sulfate reduction was only observed in those mesocosms to which glucose had been added, suggesting that, although sulfate-reducing bacteria were present, most of the DOC present in the wetlands could not be metabolized by these microorganisms.

Conclusions

The microbial communities in the three MWTS examined were determined primarily by their water chemistries; Tan-y-Garn was dominated by bacteria implicated in the iron- and sulfur-cycles; brackish waters at Horden were dominated by salt-tolerant bacteria, the organic carbon-rich waters at Lamesley had large numbers of copiotrophic bacteria (and pathogens). Archaeal community seems less influenced by the water chemistries; methanogens and other archaea involved in the nitrogen cycle were present in the three mine sites.

Iron-metabolising bacteria were identified in relatively small numbers at the three sites. Iron-oxidising bacteria are likely to have only minor impact on net removal of Fe in these circum-neutral pH waters, though they may be important at the micro-aerobic interface between the compost and ochre layers in the wetlands. Bacteria that catalyse the reduction of iron appear to be more abundant and presumably more active. The organic carbon required by these bacteria may come from (i) the compost itself; (ii) exudates from the roots of *Phragmites* and other wetland plants (iii) wastewater effluent in Lamesley MWTS, and (iv) surrounding vegetation. Inputs of organic matter from will tend to promote the reductive dissolution of ferric iron minerals, which may have a negative impact on the net efficiency of iron removal. Effective management of organic carbon sources could improve the efficiency of iron removal in passive mine water treatment. For example, this could imply separating treatment system elements based on non-biological oxidation and precipitation of iron from constructed wetlands used downstream for polishing the mine waters.

Acknowledgements

The authors would like to thank the EU Research Fund for Coal and Steel MANAGER project for partly funding this research and the UK Department of Energy and Climate Change for providing the remainder of the funding. The Coal Authority would also like to acknowledge the assistance provided by Northumbrian Water Ltd as joint operators of the Lamesley co-treatment site.

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