Geomicrobiology Journal, 22:141–149, 2005

ISSN: 0149-0451 print / 1362-3087 online
DOI: 10.1080/01490450590945979

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Passive Treatment of Mn-Rich Mine Water: Using Fluorescence to Observe Microbiological Activity

K. L. Johnson
School of Engineering, University of Durham, Durham, DH1 3LE, United Kingdom

A. Baker
School of Geography, Earth and Environmental Sciences, University of Birmingham, Birmingham, B15 2TT, United Kingdom

D. A. C. Manning
School of Civil Engineering and Geosciences, University of Newcastle, Newcastle upon Tyne, NE1 7RU, United Kingdom

Conventionally, limestones have been used in passive mine water treatment systems. Limestones with the highest proportion of calcite are recommended since they have the greatest long-term alkalinity generating potential. Manganese is present in mine waters and needs to be removed in order to comply with environmental quality standards. This paper compares seven different Permian carbonate rocks, both limestone and dolomite, in their ability to promote manganese oxidation in real mine waters over an 8-h period. The substrates are characterised using thermogravimetric analysis, X-Ray diffraction and scanning electron microscopy. Fluorescence spectrophotometry is used to monitor any changes in the dissolved organic matter concentration in the water as manganese is removed. We determine that there is no statistically significant correlation between manganese removal and the proportion of calcite or between manganese removal and substrate surface roughness. Fluorescence spectrophotometry demonstrates that there is a distinct change in the observed spectra in the water during manganese removal. There is a positive and statistically significant correlation between manganese removal and the production of a tyrosine-like substance (up to ∼150 ppb in 8 h), which fluoresces at 270–280 nm excitation wavelength and 300–310 nm emission wavelength, suggesting that microbial activity is an important factor in promoting manganese removal within dolomite passive treatment systems. It may be possible to use fluorescence spectrophotometry to monitor for microbial activity in passive treatment systems.

Received 15 June 2004; accepted 22 February 2005.
The work was supported by the U.K. Engineering and Physical Sciences Research Council (grant numbers 98316317 and GR/R34332/01). The author would also like to thank Mr. Paul Allison of Sherburn Minerals Ltd. for supplying the dolomite which was used in the experiments. In addition, many thanks to Elisa Lopez-Capel, Kath Liddle, David Barber, and Malcolm Slater for their work on the characterisation of the substrates.
Address correspondence to Karen L. Johnson, School of Engineering, University of Durham, Durham, DH1 3LE, United Kingdom. E-mail: Karen.johnson@durham.ac.uk

Keywords dolomite, fluorescence spectrophotometry, limestone, manganese, mine water, oxidation, passive treatment, tyrosine

INTRODUCTION

Background

Dissolved manganese is an ubiquitous contaminant in mine waters and landfill leachates commonly present at concentrations from 1–200 μM. It causes major problems for the water industry as it stains laundry and can cause major pipe blockages at concentrations of >1 μM. Manganese removal has always been regarded as problematic whether using either passive or active treatment. However, there is very little research into passive manganese removal despite increasing interest from regulatory bodies in the light of recent changes in European water quality legislation. The UK Environment Agency has recently reduced the Environmental Quality Standard (EQS) for manganese to 0.03 mg/L ($\sim$0.6 μM) dissolved manganese (annual average) in order to comply with European Directives. Since this new EQS is actually lower than the UK Drinking Water Inspectorate's Maximum Permitted Concentration Value of 0.05 mg/L ($\sim$0.9 μM), there has been renewed interest in manganese removal and successful manganese treatment technology is in increasing demand.

Manganese Oxidation

Abiotic manganese oxidation is auto-catalytic and is regarded as a two-step process. In the first step Mn^{2+} is sorbed onto the manganese oxide or oxyhydroxide surface with concomitant partial oxidation of Mn^{2+} to Mn^{3+} and in the second rate-limiting step the disproportionation of Mn^{3+} to Mn^{4+} occurs (Morgan and Stumm 1964). The rate of abiotic manganese

oxidation has been summarised by Morgan and Stumm (1964) by equation 1, which shows the rate to be dependent on both the concentration of Mn^{2+} ions and the amount of manganese oxide present:

$$d[Mn^{II}]/dt = k_0[Mn^{II}] + k_1[Mn^{II}][MnO_2]$$

where

$$k_o = 4 \times 10^{12}\ M^{-3}[O_2 \cdot Aq][OH^-]^2 \quad [1]$$

and

$$k_1 = 10^{18}[O_2 \cdot Aq][OH^-]^2$$

Junta and Hochella (1994) characterised the role that mineral surfaces play in the heterogeneous oxidation of Mn^{2+}. They showed that the oxidation of Mn^{2+} begins through adsorption onto 'steps' on mineral surfaces present in solution. It appears that it is the geometric character, more than the composition, of the immediate surface that plays a significant role in controlling the rate of oxidation during the early stages of the reaction. After the initial oxidation of adsorbed Mn^{2+} at the mineral surface, the newly formed site becomes the most reactive site for continuation of the adsorption-oxidation process.

Manganese oxidation is kinetically slow because of the high activation energy required for Mn oxide precipitation (Crerar and Barnes 1974). Mn oxide formation does not readily occur without either highly oxidising and/or high pH (above pH 9) conditions (Sikora et al. 2000). There are many catalysts for manganese oxidation although none are as effective as manganese oxides themselves. Many authors have investigated the catalytic effects of clay minerals on manganese oxidation (for example Reddy and Perkins 1976; Potter and Rossman 1979; Ostwald 1984; Yavuz et al. 2003). Reddy and Perkins (1976) found that under alternate wetting and drying conditions, illitic clay was capable of fixing significant quantities of manganese either by physical entrapment or precipitation. Potter and Rossman (1979) also proposed that clay minerals (illite and montmorillonite) are necessary for the formation of certain manganese precipitates such as desert varnish.

In addition, there are numerous strains of bacteria which increase manganese oxidation rates (the rate being dependent on which species of bacteria is involved; e.g., Zhang et al. 2002) by as much as five orders of magnitude (Nealson 1983). The degree of influence that bacteria have on manganese oxidation has proved difficult to determine, as the majority of metabolic inhibitors which are used to prevent biotic activity in control experiments also influence abiotic manganese oxidation rates (Shiller 2004).

Despite these problems, it is widely assumed that manganese oxidation occurring at circum-neutral pH is biologically catalysed (Zhang et al. 2002). Many of the manganese-oxidisers that have been identified thrive in low organic carbon environments but there is as yet no definite proof that these bacteria are actually gaining energy from the process of manganese oxidation (e.g., Tebo et al. 2004). Other suggestions for what biological role manganese oxidation has to play include the breakdown of refractory organic matter into more labile fractions which would be easily accessible to microorganisms (Sunda and Kieber 1994). Therefore it would seem essential to be able to monitor both biological processes and organic matter transformations that might be taking place during passive manganese treatment. One suitable technique is that of fluorescence spectrophotometry, which has the ability to simultaneously measure both the refractory organic matter (humic and fulvic-like fluorescent fractions) as well as microbially produced organic matter (protein-like fluorescent fractions).

Fluorescence Spectrophotometry

Fluorescence has been widely used to investigate the nature of organic matter in soils, fresh water, marine and groundwater (e.g., Coble et al. 1990; Mopper and Schultz 1993; Baker and Genty 1999; Baker 2001; Baker and Spencer 2004) and to monitor the evolution of dissolved organic matter from geological samples (Claret et al. 2003) but never before for monitoring manganese removal processes in passive treatment systems. Fluorescence occurs when molecules which have been exposed to a high-energy light source release energy in the form of light. The emitted fluorescent light is at a much longer wavelength than the excitation wavelength. In natural waters, it is usually organic acids (humic and fulvic-like) and two amino-acid populations (tyrosine-like and tryptophan-like) which cause fluorescence (Coble et al. 1998; Baker and Genty 1999; Baker and Inverarity in press). Typically in fresh waters, humic and fulvic-like substances are the predominant fluorescent material, with excitation maxima in the range of 300 to 360 nm and emitted light in the range 400 to 480 nm.

In contrast, in sewage effluents and farm wastes, in the terrestrial environment, and in surface marine waters, the highest fluorescence intensities observed are attributed to the aromatic amino acids tryptophan (excitation wavelength 270–280 nm and emission wavelength 340–360 nm) and tyrosine (excitation wavelength 270–280 nm and emission wavelength 300–310 nm) (Determann et al. 1998; Parlanti et al. 2000; Baker 2001, 2002). Of the two, tyrosine-like fluorescence is rarely observed in the freshwater environment. One of the authors (AB) has never observed significant tyrosine-like fluorescence levels in uncontaminated freshwater samples, but does observe this fluorescence centre in untreated human and farm excreta (Baker 2002, unpublished data), suggesting it may be related to recent biological activity. This agrees with observations of tyrosine-like (and tryptophan-like) fluorescence in marine waters, which is observed to be closely associated with active algal blooms and freshly produced organic matter (Mopper and Schultz 1993; Mopper et al. 1995; Tanoue 1995; Determann et al. 1998; Coble et al. 1998) and has been reproduced in laboratory algal growth experiments (Parlanti et al. 2000).

Fluorescence spectrophotometry has the capability of simultaneously detecting fulvic-like, tryptophan-like and tyrosine-like fluorescence centres, without sample treatment such as concentration, and with a rapid analysis time (minutes). In addition, fluorescence spectrophotometry has very low detection

limits that for fulvic-like and protein-like fluorescence centres is typically at the ppb level. For this reason, fluorescence may provide a suitable technique for determining whether manganese removal is microbially mediated in the earliest stages of passive manganese removal when microbial communities may be present at levels difficult to monitor using alternative techniques.

Passive Treatment

Passive treatment uses natural materials to promote natural chemical and biological processes for the treatment of contaminated water (Younger et al. 2002). The most common method for removing dissolved manganese from solution is via the precipitation of manganese oxides. The factors and mechanisms controlling manganese oxidation are redox dependent. Oxidation and precipitation of manganese is favoured by high pH (low H^+) and high Eh (low e^-). Sikora et al. (2000) state that 0.29 and 0.15 mg/L of dissolved oxygen are required to oxidise and precipitate 1mg/L of manganese precipitated as MnO_2 or MnOOH, respectively.

This suggests that manganese oxidation should proceed when concentrations of dissolved oxygen and Mn^{2+} are at levels commonly found in river water and some other natural systems (Hem 1980). The distribution of manganese between the hexa-aqua ion, inorganic and organic complexes, and in association with colloids is determined by environmental variables (Chiswell and Mokhtar 1986), but the most common state of dissolved manganese in rivers is Mn^{2+}(Hem 1980), and it is apparent that there are kinetic restrictions at play. However, at circumneutral pH, manganese oxidation will occur under oxic conditions in the presence of a suitable catalyst such as manganite (MnOOH).

Manganese removal passive treatment systems are usually placed at the end of the treatment process stream, so that they receive waters from which all of the iron has already been removed as it is usually difficult to remove any manganese in the presence of ferrous iron (Nairn and Hedin 1993). The manganese removal unit process itself often consists of oxic rock filters, hosting algal and/or bacterial consortia which create high-pH microniches within which the precipitation of manganese oxyhydroxides and oxides occurs (e.g., Phillips et al. 1995; Hamilton et al. 1999). For the algae in such systems to photosynthesise effectively, unobstructed daylight and low influent turbidities are necessary. They are, therefore, subject to marked seasonal (and diurnal) variations in performance efficiency.

In the United States, the "Pyrolusite Process" (Vail and Riley 2000), a patented bioremediation method, has been successfully used to treat manganiferous waters. In this system, a limestone gravel reactor is inoculated with manganese-oxidising bacteria which are chosen site-specifically. However recent work (Rose et al. 2003) suggests that special inoculation may not be necessary.

All of these existing systems require relatively large areas of land as they must be shallow in order to ensure sufficient infiltration of light and/or dissolved oxygen. A new enhanced bioremediation method operates as a subsurface flow gravel bed (Johnson 2003a, 2003b). The provision of air at depth by a passive aeration system and the use of a catalytic substrate help overcome the slow kinetics which are usually associated with manganese oxidation and most importantly allows deeper systems to be built where large areas of land are not available.

It is important to note that in all of these systems the nature of the substrate becomes less important with time as the substrate surface becomes covered with manganese oxyhydroxide deposits and these take over as the main catalyst within the system. The main function of the substrate then becomes the provision of a steadfast surface for the attachment of the biofilm/manganese oxyhydroxide deposits. It has up till now been tacitly assumed that limestone is the best substrate for promoting manganese oxidation in such systems, as high pH is widely regarded as the most important factor in manganese oxidation and calcite-rich limestone has the greatest long-term alkalinity generating potential. However the occurrence of natural manganese dendrites on the surface of dolomite drew our attention to the possible catalytic capabilities of dolomite with respect to manganese oxidation. The extensive literature on the occurrence of manganese deposits in association with carbonates is reviewed by Roy (1997). McBride (1979) suggests that laboratory precipitated manganese oxides may have a preference for magnesite over calcite but there is little information available as to why this should be the case.

This paper describes the application of fluorescence spectrophotometry to trials using seven Permian dolomitic limestones from NE England (Raisby and Hartlepool Anhydrite Formations) in batch experiments in order to further understand the role of microbial processes in manganese removal from contaminated mine waters.

Substrate Characterisation

The Permian carbonates used were all collected from aggregate quarries in the Durham-West Hartlepool district in northeast of England and are from one of two formations, the Raisby Formation and the Hartlepool Anhydrite Formation (see Table 1). The Permian lies above the Carboniferous coal measures of the Durham coalfield and it is most probable that all of the strata would have been exposed to manganese-bearing groundwaters in situ. Manganese dendrites are clearly visible on bedding planes and joints within dolomites from the lower parts of both Hart and Witch Hill quarries. Quartzite gravel was used as a noncarbonate substrate for control purposes. The substrates were collected as 20 mm clasts directly from the quarries and characterised in the laboratory both hydrochemically, mineralogically, and geometrically (in terms of surface roughness). No microbiological analyses were carried out on the substrates or the water as these resources were not available within the project.

Bulk Chemical Analyses

In order to ascertain the pH upon dissolution, 0.5 g of the ground substrates was added to 20 mL of deionised water, shaken

TABLE 1
Permian limestones and dolomites used in manganese removal batch experiments

Name	Location, UK Ordnance Survey Grid Reference	Brief description
[1]Coxhoe limestone	Coxhoe, NZ 340 355	Hard crystalline grey
[2]Lower Hart dolomite	Hart, NZ 475 345	Friable yellow coloured
[2]Upper Hart dolomite	Hart, NZ 475 345	Friable cream coloured
[1]Lower Witch Hill dolomite	Witch Hill, NZ 345 397	Friable cream coloured
[1]Upper Witch Hill dolomite	Witch Hill, NZ 345 397	Friable cream coloured
[1]Lower Crime Rigg dolomite	Crime Rigg, NZ 344 418	Friable cream coloured
[1]Upper Crime Rigg dolomite	Crime Rigg, NZ 344 418	Friable yellow coloured

[1]Raisby Formation.
[2]Hartlepool Anhydrite Formation.
In each case the rock is identified according to the quarry source, and is subdivided where appropriate according to its location within the quarry (upper or lower levels).

vigorously and left for 12 h. The pH of the deionised water used was originally 5.65. The pH of the resulting solution was measured using a Camlab MY/6P Ultrameter. The substrates were also analysed for trace metals using the Aqua Regia method except for the limestone from Coxhoe quarry, for which XRF data was provided by the quarry owner (Tarmac Ltd).

Mineralogical Analyses

0.3 g of ground sample was subjected to thermogravimetric analysis using a Netzsch STA 449C Jupiter TG-DSC instrument, heating under a carbon dioxide atmosphere to a temperature of 1000°C. Observed weight losses were recalculated to give the calcite and dolomite contents.

All rock substrates were analysed by XRD, using a Phillips X'Pert Pro diffractometer, fitted with an X'Celerator and a secondary monochromator. Each sediment sample was analysed as a randomly oriented powder sample in a circular aluminium cavity mount. The samples were then scanned from 7–70° programmed to a nominal step size of 0.033 degrees and a time step of 100 s.

Surface Roughness Characteristion

Secondary electron images of the air-dried substrate surface were obtained using a Hitachi S2400 scanning electron microscope fitted with an Oxford Instruments ISIS X-ray detection and analysis system. The images were then analysed using image analysis to calculate the mean and standard deviation of the intensity of all the pixels in greyscale image. Assuming that substrates with a greater surface roughness would have a greater range of pixel intensity than those with smoother surfaces, the image intensity standard deviation was used as a proxy for the substrate's surface roughness.

Manganese Removal Experiments

The substrates (20 mm size) were washed with de-ionised water and subsequently air-dried. 250 ml Duran glass bottles were filled to the 100 ml mark with substrate and topped up to 200 ml with mine water. The bottles were then fitted with a bung which allowed aeration using an aeration pump (Peak Aquatics AP-1001) on its highest setting. The systems were aerated for eight hours and manganese concentrations were measured every hour. Three replicates were carried out for each substrate.

Net-alkaline mine water from a recently closed (December 1998) fluorite mine (Frazer's Grove Mine in the North Pennines, United Kingdom; Johnson and Younger 2002) was used in the laboratory experiments. The water was circum-neutral (pH$\sim$7). The chemical composition of the mine water varied with time due to groundwater rebound in the area of the mine (see Johnson and Younger 2002 for more detailed geochemical analysis). During the experimental period, values of bicarbonate alkalinity were between 120–160 mg/L equivalent $CaCO_3$, 15–30 mg/L (270–540 μM) Mn, 5–10 mg/L (89–178 μM) Fe and 5–10 mg/L (77–154 μM) Zn. The water was stored for 72 h prior to use to allow the iron to precipitate out to ensure that ferrous iron could not interfere with the manganese oxidation process. After this period of storage the water was then siphoned from the container and iron and manganese concentrations were measured using a Unicam 929 atomic absorption spectrophotometer. Analytical precision was ± 2 μM.

One substrate, dolomite from the lower levels of Hart quarry, was then used in further experiments to assess the value of fluorescence spectrophotometry as an indicator of microbial activity associated with manganese removal. Six (single) experiments were set up as listed below:

- Control 1: aerated de-ionised water with no substrate;
- Control 2: aerated de-ionised water with Lower Hart dolomite;
- Lower Hart dolomite and mine water not aerated;
- Lower Hart dolomite and mine water aerated;
- Lower Hart dolomite and mine water plus 0.2 g MnO_2 (catalyst) not aerated;
- Lower Hart dolomite and mine water plus 0.2 g MnO_2 (catalyst) aerated.

Manganese dioxide (Sigma-Aldrich, 99.99%) was used in order to catalyse manganese oxidation. Manganese concentrations were measured and the dissolved organic matter was also monitored every hour for 8 h using fluorescence spectrophotometry.

TABLE 2
Percent manganese removal for various substrates at 2 and 6 h plus mineralogical data calculated from TG-DSC

Substrate name	% Mn removed at 2 hrs (+/− SD)	% Mn removed at 6 hrs (+/− SD)	% calcite from TG-DSC	% dolomite from TG-DSC	% non-carbonate from TG-DSC	pH (50 mL DIW with 0.5 g substrate)	Surface area equivalent (SD from image analysis)
Coxhoe limestone	14 (4)	22 (2)	97.2	0.0	2.8	9.42	38.8
Lower Hart dolomite	78 (12)	94 (4)	60.4	26.9	12.7	9.50	53.4
Upper Hart dolomite	53 (1)	76 (13)	5.9	92.4	1.7	9.53	50.0
Lower Witch Hill dolomite	38 (8)	62 (9)	2.7	90.6	6.7	9.61	53.1
Upper Witch Hill dolomite	45 (4)	69 (4)	20.1	77.0	2.9	9.83	46.7
Lower Crime Rigg dolomite	61 (5)	79 (3)	11.0	87.6	1.4	9.30	41.0
Upper Crime Rigg dolomite	56 (5)	77 (7)	13.5	83.3	3.2	9.79	58.3
Quartzite gravel	24 (n/a)	31 (n/a)	n/a	n/a	n/a	8.75	28.2

Fluorescence Spectrophotometry

Fluorescence spectrophotometry provides information concerning the role of microbial communities in the process of manganese oxidation. The eight substrates (Table 1; single 20 mm clasts) were washed gently in deionised water and air-dried. One clast was placed in 50 mL of deionised water for 24 h and then fluorescence spectrophotometry was used to investigate the nature of any organic matter which had evolved from the substrates. The clasts were also washed in sodium hypochlorite, thoroughly washed in deionised water, and the analysis repeated in order to ascertain whether the observed fluorescence was associated specifically with the presence of surface organic matter.

A Perkin-Elmer Luminescence Spectrometer (Model LS50B) was used to obtain fluorescence spectra using the software FLWinLab to interpret the results. Techniques used were identical to those described in Baker (2001, 2002). Briefly, the excitation wavelengths were set to run from 200 to 400 nm in 5 nm steps and emitted fluorescence detected between 280 and 500 nm at 0.5 nm steps. Results were displayed as Excitation Emission Matrices (EEM) and the intensity of the tyrosine-like peak and any other fluorescence centres recorded. To calibrate the results obtained, the Raman band of deionised water was regularly analysed at 348 nm excitation: mean peak height was 16.90 ± 0.76 ($n = 15$).

RESULTS AND DISCUSSION

Manganese Removal Experiments

The percentage manganese removed at both 2 and 6 h for the substrates is given in Table 2. Manganese removal from solution was greatest for the Lower Hart dolomite and least for the Coxhoe limestone, at both 2 and 6 h. There is no statistically significant correlation between the proportion of manganese removed in the batch experiments and the calcite or dolomite content of the substrate. The Lower Hart dolomite is in fact the least pure carbonate with 12.7% (by weight) of noncarbonate material (quartz and kaolinite) and the Coxhoe limestone has the highest calcite content (Table 2).

The pH of the substrates in de-ionised water is also listed in Table 2. There is no statistically significant correlation between pH (of solution containing 0.5 g substrate in 20 mL deionised water) and percent manganese removed in the batch experiments.

Table 3 shows trace element concentrations present in the Permian limestones as determined following digestion using Aqua Regia. There is no statistically significant correlation between trace element concentrations and percent manganese removed in the batch experiments.

Figure 1 shows secondary electron images (×200) of the surfaces of quartzite gravel (Figure 1a) and the Upper Crime

TABLE 3
Trace element concentration (mg/kg) in Permian limestones as determined following digestion using Aqua Regia

Substrate name	Fe	Mn	Zn	Na	K	Ba	Sr	Al	Cr	Cd	Cu	Pb	Ni	Co	As	Hg
Lower Hart dolomite	5550	266	291	155	284	63	400	1516	0	0	0	0	0	0	61	8
Upper Hart dolomite	584	85	20	221	232	31	128	10	0	0	0	0	0	0	91	12
Lower Witch Hill dolomite	2462	228	41	364	380	43	234	1278	0	0	0	0	0	0	90	12
Upper Witch Hill dolomite	2114	177	41	272	261	49	177	536	0	0	0	0	0	0	83	12
Lower Crimerigg dolomite	3550	371	52	242	353	65	189	1326	0	0	0	0	0	0	92	12
Upper Crimerigg dolomite	4592	413	24	278	315	239	314	604	0	0	0	0	0	0	90	13

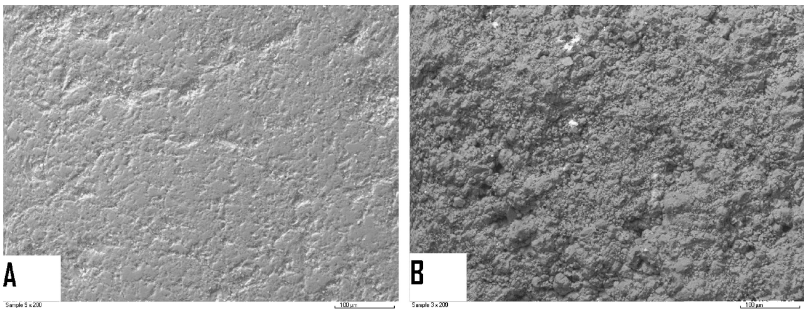

FIG. 1. Secondary electron images (×200) of the surface of quartzite gravel (A) and the Upper Crime Rigg (B) dolomite, respectively.

Rigg dolomite (Figure 1b), which had the lowest and highest standard deviation respectively in image greyscale intensity and were therefore categorised using the image analysis process as the smoothest and roughest surfaces.

There is a statistically insignificant relationship between surface roughness measured by SEM/image analysis methods (Spearman's $R^2 = 0.38$) and the percent manganese removed (see Table 2).

Fluorescence Spectra

The EEM plots obtained from the fluorescence spectrophotometry showed that very little fluorescent dissolved organic matter was initially present on the surface of the substrates. Fulvic and humic-like peaks were below detection limit (approximately 40 intensity units). The highest fluorescence intensity was in the region of 270–280 nm excitation wavelength and 300–310 nm emission wavelength, that attributed to tyrosine-like fluorescence (Table 4). Tyrosine standards (Sigma) produced a fluorescence centre in the same optical location and detection limits were ∼10 ppb level (a fluorescence intensity peak of 100 units correlating with 32 ppb). Table 4 shows that all of the carbonate substrates showed this tyrosine-like fluorescence intensity peak although it was not observed for the quartzite gravel.

TABLE 4
Tyrosine-like fluorescence intensity peak present on the surface of the substrates

Substrate	After treatment with sodium hypochlorite (intensity units)	Before treatment with sodium hypochlorite (intensity units)
Lower Hart dolomite	14	178
Upper Hart dolomite	0	141
Witch Hill Lower dolomite	0	126
Witch Hill Upper dolomite	5	118
Crimerigg Lower dolomite	3	186
Crimerigg Upper dolomite	2	103
Coxhoe limestone	0	45
Quartzite gravel	0	0

Small differences in peak intensity probably relate to surface area variations, as each clast, although nominally the same size (20 mm diameter), has a different surface area as a consequence of differences in surface roughness. However, it is interesting to note that the Coxhoe limestone (which removed the least manganese in the batch experiments) has the lowest tyrosine-like fluorescence intensity peak. In contrast, the Lower Hart dolomite, (which removed the most manganese in the batch experiments) has the second highest tyrosine-like fluorescence intensity peak. The ability to generate the tyrosine-like substance appears to be related specifically to the substrate surface, because it was largely removed in all cases when the substrates were washed in sodium hypochlorite solution (see Table 4). There is a statistically significant positive correlation (Spearman's $R^2 = 0.80$) between the tyrosine-like fluorescence intensity peak and the ability of the substrates to promote manganese oxidation as is shown in Figure 2. Despite these observations, it cannot be assumed that the dolomite surface is the only source of tyrosine-like fluorescence in these experiments. An unknown proportion of the observed tyrosine-like fluorescence may be produced through abiotic reactions. Further experiments were carried out to measure the evolution of the tyrosine-like fluorescence intensity peak in relation to manganese removal using the Lower Hart dolomite.

Tyrosine-like fluorescence increases linearly with time (Figure 3). Control 1, the laboratory blank which consisted of aerated de-ionised water with no dolomite, demonstrated no statistical increase in tyrosine-like fluorescence over the eight hour period. Control 2, which consisted of aerated deionised water with dolomite, in contrast demonstrated that the dolomite substrate may play a role in the evolution of the tyrosine-like fluorescence since this increases with time with the substrate present. The other experiments use mine water rather than deionised water and also emphasise the importance of aeration in the generation of tyrosine-like fluorescence. Tyrosine-like fluorescence is produced at a greater rate when both substrate and aeration are present. Both control experiments also suggest that dissolved manganese and its subsequent removal may be associated with the production of tyrosine-like fluorescence since production is greater when the dolomite is aerated in mine water rather than in deionised water.

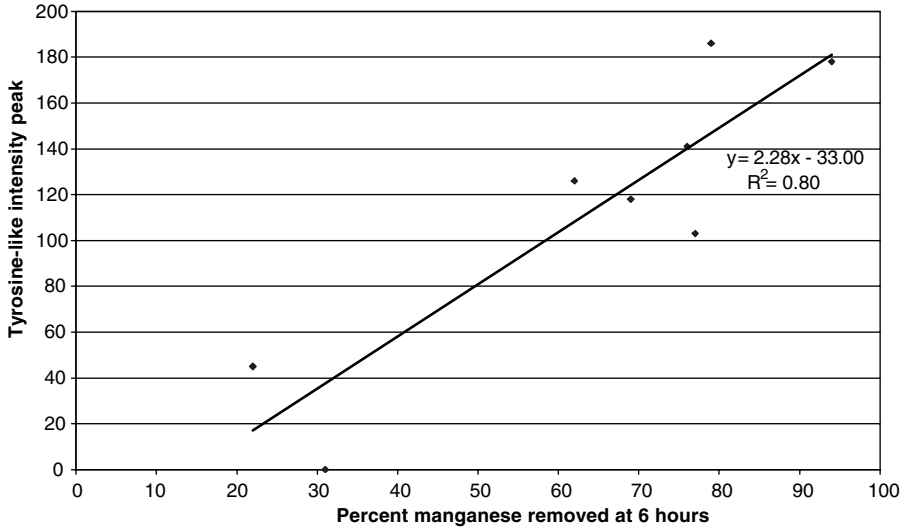

FIG. 2. Tyrosine-like fluorescence intensity peak (from Table 4) versus percent manganese removed at 6 h (from Table 2) for the various carbonate substrates.

Figure 4 shows the natural logarithm of the normalised manganese concentration (ln ($[Mn^{2+}]/[Mn^{2+}]_0$)) against time. The linear distribution of the data indicates that the reaction is likely to be first order with respect to reduction in Mn^{2+} concentration. More manganese is removed in the presence of substrate and aeration, and also when the catalyst MnO_2 is present. The batch experiment which consisted of aerated dolomite and MnO_2 in mine water shows the greatest manganese removal.

Figure 5 compares Mn removal with changes in tyrosine-like fluorescence. In each experiment there is a linear correlation between the production of tyrosine-like fluorescence and the removal of manganese from solution. This is not surprising since we know that both processes are enhanced by the presence of aeration and substrate. The data points fall into two groups; firstly those experiments that are not aerated which have gradients of approximately 0.22 and secondly those that are aerated which have a gradient of 0.06. With aeration, almost 4 times as much tyrosine-like fluorescence is produced for a given Mn loss, when compared to experiments without aeration. In addition, Figure 5 shows that the percentage increase in tyrosine-like substance is greater after 8 h in the experiments where MnO_2 is present, compared to when it is not. Since MnO_2 leads to greater manganese removal, this is consistent with the hypothesis that more tyrosine-like fluorescence is produced when more manganese is removed.

Overall, the amount of tyrosine-like fluorescence in the water increases as manganese is removed in the batch experiments. Since the tyrosine-like substance is known to be present initially on the surface of the substrates, it seems likely that at least part of the increase in tyrosine-like fluorescence intensity in water (with time) is due to desorption of the molecule

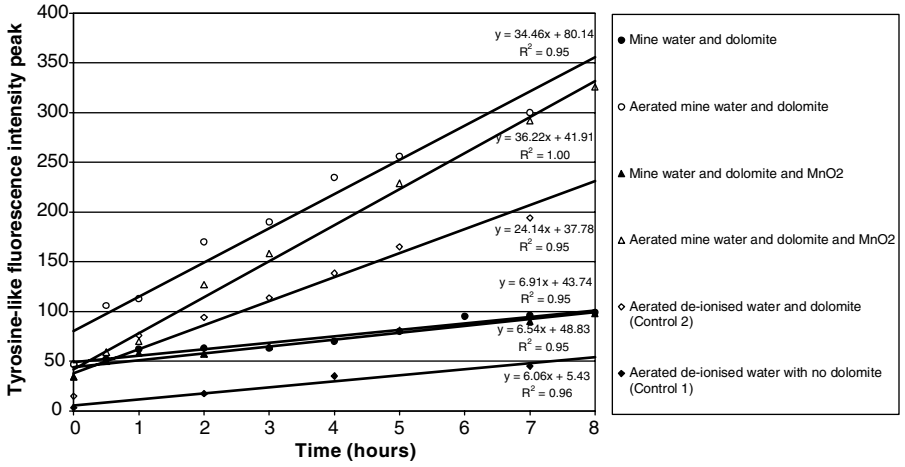

FIG. 3. Tyrosine-like fluorescence intensity peak vs. time in manganese removal batch experiment using Lower Hart dolomite.

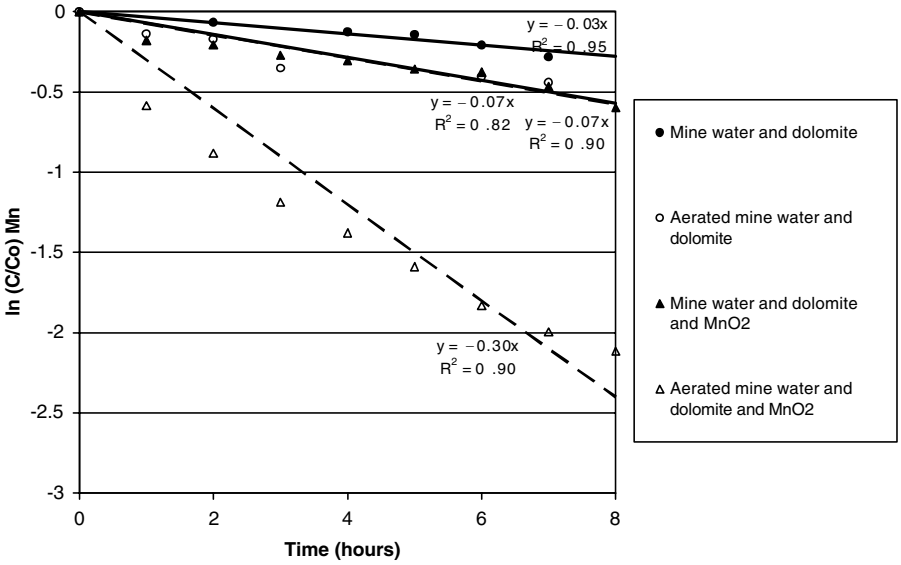

FIG. 4. Manganese removal rates versus time in four batch experiments using Lower Hart dolomite.

from the substrate surface. The production of tyrosine-like fluorescence intensity increases with aeration and in the presence of the dolomite substrate. Abiotic production of tyrosine (via possible oxidative transformations of dissolved organic material involving MnO_2) is unlikely as humic-like and fulvic-like fluorescence intensities were below detection limits in the mine water. Therefore it seems probable that a significant proportion of the tyrosine-like fluorescence is being produced microbially. If the tyrosine-like fluorescence is indicative of microbiological activity, fluorescence spectrophotometry may provide a quick and easy method for tracking activity in batch experiments of this sort. These findings then also provide direct evidence that microbial processes are involved in manganese oxidation.

CONCLUSIONS

Seven carbonate rocks from the Permian Raisby Formation and Hartlepool Anhydrite Formation have been used in trials to compare their effectiveness as different substrates for the removal of manganese from net-alkaline minewaters using model passive treatment systems. The sample with the highest calcite content (limestone from Coxhoe) was the least effective substrate for the promotion of manganese oxidation. There is no statistically significant correlation between percentage manganese removal and the following substrate characteristics:

- pH of a solution containing 0.5 g substrate with 20 mL de-ionised water;
- percentage calcite content of the substrate;

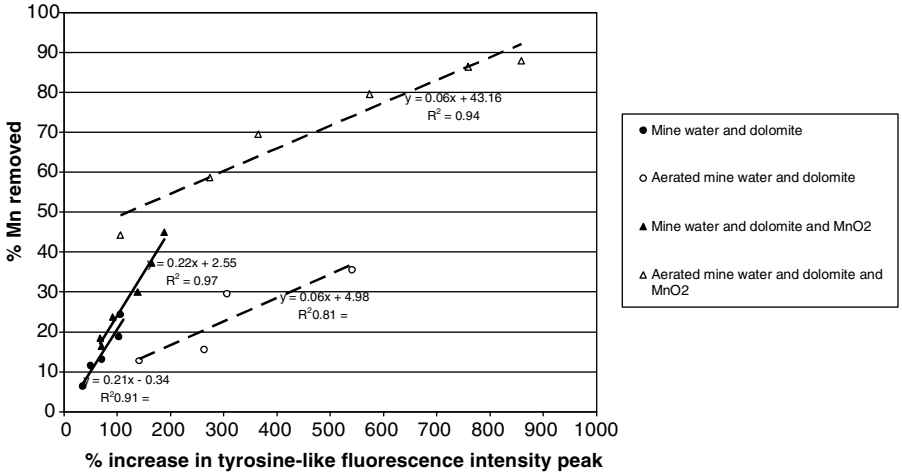

FIG. 5. Percent manganese removed versus percent increase in tyrosine-like fluorescence intensity peak in the 8-h manganese removal batch experiments using Lower Hart dolomite.

- percentage dolomite content of the substrate or;
- percentage of any other trace metal in the substrate.
- surface roughness as measured by image analysis techniques.

Correlations have been observed between the proportion of manganese removed from solution and the measured intensities of tyrosine-like fluorescence. It is considered that increased levels of tyrosine-like fluorescence reflect increased microbial activity in those experiments that have greatest removal of manganese (with aeration and in the presence of an MnO_2 catalyst). This finding highlights the potential importance in further experiments of using measurements of tyrosine-like fluorescence as an indicator of microbial activity.

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