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Technical Article

Nitrification in Constructed Wetlands Treating Ochreous Mine Water

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Abstract. A survey of ochreous discharges from former coal mines in the UK indicated ammonia contamination at 2-5 mg/L in water from flooded shafts of depths >400-800 m. Although significant, this was much less than historically observed in working mines. No correlation was observed between ammonia and iron concentrations. However, ammonia was removed to some extent in constructed wetlands designed primarily to remove iron.

A mechanistic study of wetland removal of ammonia from mine water indicated the main process to be bacterial nitrification, similar (despite great differences in operating conditions) to that occurring in many wastewater treatment works. The study was based on water containing 4-5 mg/L ammonia and some 12-27 mg/L iron from the abandoned Woolley mine in Yorkshire. Notwithstanding relatively high salinity and short residence time, most of the ammonia entering the wetlands was, at least initially, converted to nitrate. Field measurements showed that the conversion efficiency was increased at lower flow rates, higher temperature, and longer flow stabilisation, which are all consistent with bacterial action. Subsurface flow conditions were simulated in column studies, using pre-sterilised gravel and mine water taken from the wetland cells; two strains of bacteria commonly associated with nitrification in domestic wastewaters, *Nitrosomonas europaea* and *Nitrobacter agilis*, were able to reproduce the 89% ammonia oxidation observed in the wetlands. It was concluded that the high degree of aeration, neutral pH, and nutrient content of the mine water greatly favoured nitrification. Although more saline and lower in biochemical oxygen demand than organic wastewater, nitrification was not inhibited.

Key words: Ammonia removal; constructed wetlands; iron removal; mine water; nitrification; United Kingdom

Introduction

After gaining popularity in domestic and agricultural wastewater treatment, constructed wetlands are now often employed for mine water remediation. These constructed wetlands are designed primarily to remove iron and manganese and to neutralise acidity (Hedin 1997; Younger 2000). However, a recent study (Demin et al. 2002) indicates a capability to remove ammonia, a common contaminant in water from deep coal mines (Lowe 1997). The main objective of this research project was to ascertain if nitrification was responsible, as occurs in domestic wastewater. A further objective was to study links (if any) between ammonia and iron behaviour. For these purposes, a detailed study was carried out at a site in Yorkshire and a survey was made of representative mine waters in the UK.

Study Sites

The UK Coal Authority identified 41 sites at which processes to remove iron and to neutralise acid-mine discharges are already operating or under development (Figure 1). The sites are located in five geographical areas: Scotland, Wales, Lancashire, Yorkshire/Derbyshire, and Northumberland/Durham. We visited 21 of these sites in the course of this work.

The Woolley mine site was studied in detail because its composition was typical for northern England (Lowe 1997) and because its initial levels of ammonia (about 4.3 mg/L) and iron (about 27 mg/L) were cause for concern. About 120 L/s of water from about 100 km of abandoned coal workings in the local area (Laine 1999) are treated by aeration and sedimentation systems and constructed wetlands (Figure 2). The wetland system comprises a 1.1 ha surface flow wetland and 4 pea-gravel subsurface flow research wetland cells.

The surface flow wetland (introduced in June 1995) occupies a former agricultural field and is densely vegetated with *Scirpus lacustris* (true bulrush), *Typha latifolia* (bulrush), *Typha angustifolia* (lesser bulrush), *Phragmites australis* (common reed), *Iris pseudacorous* (flag iris), *Phalaris arundinacea* (reed canary grass), and *Juncus effusus* (soft rush). Initially, the wetland efficiently removed iron to < 1 mg/L but did not remove ammonia. Four subsurface flow cells were therefore added in 1998 to test whether this would provide more efficient ammonia removal. Each cell is 20 m long, 14 m wide, and 1 m deep and filled with pea gravel to a depth of 0.4 m. Cells 1 and 2 were initially planted with approximately 1200

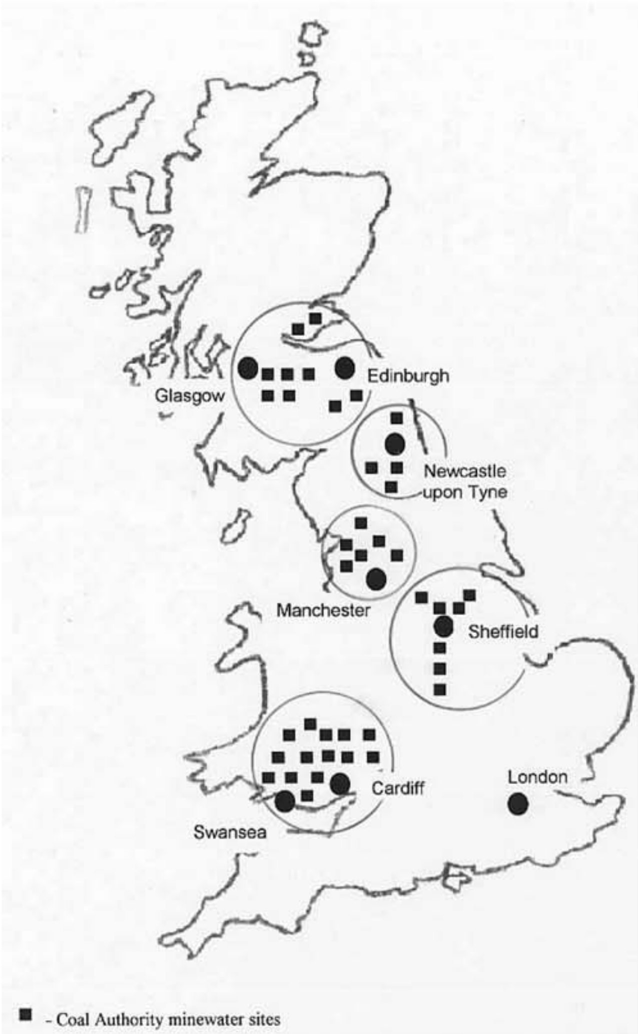

Figure 1. Geographic distribution of the Coal Authority mine water sites (2001)

Scirpus lacustris, cells 3 and 4 with about 800 *Phragmites australis*. The plants in Cells 2 and 4 were mulched with peat to a depth of about 0.01 m (Demin et al. 2002).

The flow of mine water through the surface flow wetland was fairly constant at about 120 L/s. However, the pumps were turned off each day from 17.00 to 20.00 to reduce electricity usage. The maximum flow rate for each cell was about 10 L/s. Thus, the four subsurface flow wetland cells together were able to treat about 30% of the total flow of water through the surface flow wetland.

Methods

Chemical and physical techniques

Ammonia (reported as mg/L N) and iron concentrations were measured using a portable Hach DR/2010 spectrophotometer. Biochemical oxygen demand (BOD) was determined using a Hach

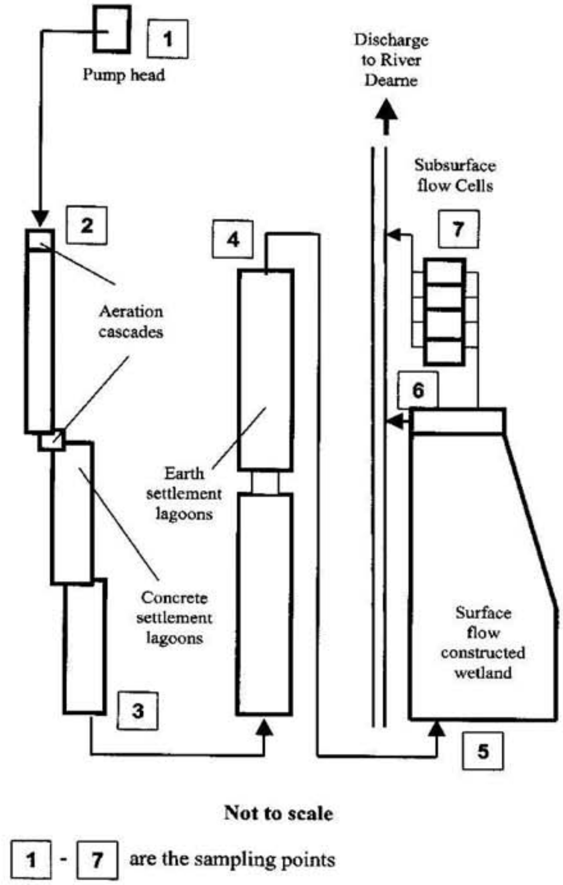

Figure 2. Sampling points at the Woolley mine water treatment system: (1) pump head; (2) outlet from the first aeration cascade; (3) outlet from the concrete settlement lagoons; (4) outlet from the earth settlement lagoons; inlet (5) to and outlet (6) from the surface flow wetland; and combined outlet of the four subsurface flow cells (7)

BODTrack. Portable probes (WTW, Germany) were used to measure pH, dissolved oxygen, conductivity, and temperature in the wetlands.

The volume of flow in the surface flow wetland was estimated by depth measurements at a rectangular slot (installed at the wetland weir exit), using:

$$Q = 1.7\, h^{3/2}\, a \qquad (1)$$

where Q is the flow rate (m^3/s), h is the water depth in the slot (m), and a is the slot width (m).

Microbiological techniques

Starter cultures of *Nitrobacter agilis* and *Nitrosomonas europaea* were prepared by inoculating 0.1 mL of freeze-dried stored culture into 10 mL of C/N-sufficient medium, as described in the supplier's instructions (NCIMB Ltd., Aberdeen, UK). The cultures were incubated at 26°C and 100 r.p.m. for 24 hours. Subcultures were stored in sterile cotton-plugged conical flasks at 4°C and subsequently used to inoculate fresh medium. All media and components were autoclaved for 30 minutes at 121°C at +1 bar, and all handling procedures were carried out aseptically.

Laboratory wetland simulations

Laboratory subsurface wetland simulations were carried out using a glass column 500 mm in height and 75 mm internal diameter in conjunction with a 101U/R peristaltic feed pump (Watson-Marlow Ltd., UK), allowing flow rates from 0.1 to 2.0 L/hour.

Statistics

One-way analysis of variance (ANOVA) was used to evaluate statistical differences of the sets of experimental data (Helsel and Hirsch 1992; Sincich et al. 1999). All chemical analyses were performed in triplicates and their average values were compared.

Results and Discussion

Table 1 shows the data on flow rate and iron and ammonia contamination for 21 inactive mines. Iron was reduced to < 1-2 mg/L except at Silkstone, where the wetland was not yet fully established. Ammonia was similarly tempered to < 1 mg/L, except at Polkemmet, where the small natural wetland employed had insufficient capacity. All visited sites were contaminated with iron but only deeper mines (400-800 m) contained much ammonia. Ammonia in these mine waters ranged from 2.3 to 5.0 mg/L. Increased concentrations of ammonia in deep mine waters have been observed before (Lowe 1997), and are believed to result from the breakdown of proteinaceous materials under anaerobic conditions. Conversely, iron concentrations were not correlated with mine depth. Thus, there was no apparent relationship between iron and ammonia content.

Iron and ammonia removal at the Woolley mine

During 3 years of monitoring at the Woolley mine site, a decline was observed in iron content (at the pump head) from approximately 27 mg/L in the end of 1998 to about 12 mg/L at the end of 2001 as a result of changes underground. However, pump head ammonia concentrations remained relatively stable throughout the period of study at approximately 4.3 mg/L. Typical iron and ammonia removal data through the treatment scheme are shown in Figure 3.

Table 1. Summary data on the visited mine water sites

	Mine Site	Type	Flow rate, L/s	Total Fe, mg/L		NH_3-N, mg/L	
				In	Out*	In	Out*
			SCOTLAND				
1	Monktonhall	Deep	30	67	1.8	2.5	0.3
2	Polkemmet	Deep	85	68	1.0	5.0	3.0
3	Kames	Shallow	15	17	1.0	2.0	0.3
			WALES				
4	Taff Merthyr	Shallow	60	12	1.9	0.6	0.1
5	Morlais	Deep	120	47	-	2.3	-
6	Pontlanfraith 1	Shallow	55	25	-	1.8	-
7	Pontlanfraith 2	Shallow	0.3	2.5	-	0.5	-
8	Rhymney	Shallow	200	11	-	0.5	-
9	Blaenavon	Shallow	17	23	-	0.8	-
10	Six Bells	Deep	35	45	-	3.3	-
11	Lindsay	Shallow	15	53	-	1.1	-
12	Craig Y Aber	Shallow	20	23	-	1.2	-
13	Ynysarwed	Shallow	17	160	-	1.0	-
			ENGLAND (YORKSHIRE AND DERBYSHIRE)				
14	Woolley	Deep	120	12	0.5	4.3	0.2
15	Bullhouse	Shallow	40	56	0.1	0.9	0.7
16	Fender	Shallow	28	10	0.2	0.7	0.2
17	Silkstone	Shallow	5	17	4.8	1.3	0.6
			ENGLAND (LANCASHIRE)				
18	Old Meadows	Shallow	60	20	0.7	1.5	0.3
			ENGLAND (DURHAM AND HORTHUMBERLAND)				
19	Edmondsley	Shallow	4	16	0.2	1.4	0.4
20	Acomb	Shallow	10	40	-	1.8	-
21	Lambley	Shallow	20	6	-	0.2	-

* - outflow data are given only for operational mine water treatment systems

Most of the iron was removed in the concrete and earth settlement lagoons by oxidation, precipitation, and sedimentation (as ochre). Residual iron was removed by filtration in the wetlands. However, ammonia was not removed in the aeration/ sedimentation system. This confirms that ammonia is not removed by simple chemical oxidation.

The conditions existing in the Woolley wetland system were, in principle, favourable for nitrification (Barnes and Bliss 1983; Cooper et al. 1996). The mine water was essentially fully aerated (50-100% of normal saturation), the pH was 7.6-8.1, temperature normally varied from 7-20°C, conductivity (without temperature compensation) was 5.0-8.0 mS/cm (which indicates very hard water), and the BOD was relatively low (30-50 mg/L). Nevertheless, as reported earlier (Demin et al. 2002), at the beginning of the operation in 1995, the surface flow wetland did not remove ammonia. Ammonia removal was first observed in July 1998. At that stage, the wetland removed about 75% of the ammonia in summer and about 50% in winter. This difference was attributed to a decrease of microbial activity at lower temperatures and seasonal plant dieback. At the same time, the subsurface flow cells removed ammonia both in summer and winter and demonstrated 65% higher ammonia-removing ability than the surface flow wetland (3.8 $g/m^2/day$ compared to 2.3 $g/m^2/day$).

To understand the effects of the flow rate in the surface flow wetland on the rate of ammonia removal, we conducted a 24-hour experiment on 29/30th July 1999. The data are shown in Figure 4. Flow was measured at the discharge from the wetland into the receiving ditch. As the subsurface flow cells were completely drained, no measurements were performed there. At the beginning of the experiment (12:00), the outflow from the wetland was about 100 L/s. This then decreased from shutdown to a minimum of about 25 L/s after 5 hours (i.e., 2 hours after the pumps had been switched on again) and returned to a full flow somewhere between midnight and 05:00 the next day.

Thus, the anticipated delayed reaction of the wetland outlet was substantial: although the pumps were off for only 3 hours, the wetland flow was affected for at least twice that period. The outlet ammonia levels decreased from about 0.9 mg/L (at 100 L/s) at noon to about 0.2-0.4 mg/L (at 25-35 L/s) between 20:00-24:00 before increasing to 1.2 mg/L at 06:00 (at 100 L/s) the next day.

Two effects appeared to be operative. The first was that the wetland was able to remove more ammonia (mg/L) at lower flow rates; increased residence time

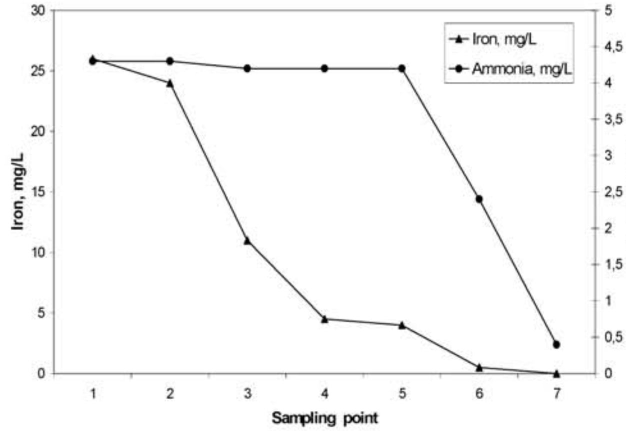

Figure 3. Iron and ammonia removal in the Woolley mine water treatment scheme (1999)

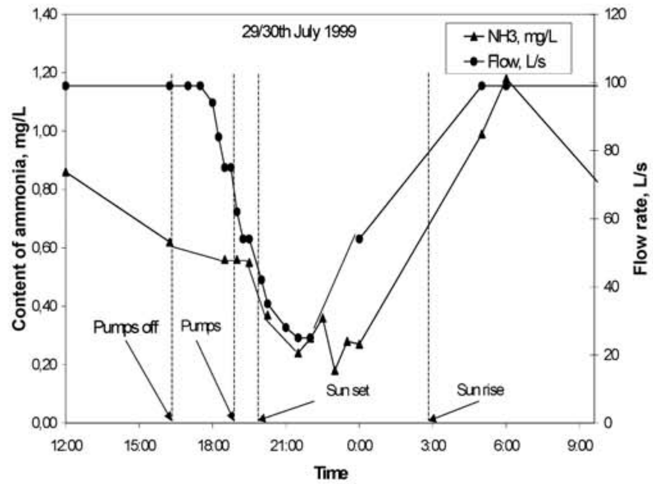

Figure 4. Daily variations of flow rate and ammonia concentration in the surface flow wetland

provided longer reaction times. The second effect was an increased efficiency during the day at full flow, which was surprising since it has been suggested that light might inhibit the activity of nitrifying bacteria (Painter 1970). This can probably be explained by the increased temperature of the mine water during the day (from 16.5 to 20°C), since nitrification is a temperature-dependent process (Barnes and Bliss 1983). It is also likely that the bacteria needed time to adapt to the higher flow rate in the wetland.

During the first half of 2000, a steady increase in ammonia removal (with a greater portion of the ammonia removed in the inlet zone) was observed in the surface flow wetland. The rate of ammonia removal stabilised at an average of 3.60 mg/L in summer and 3.43 mg/L in winter. Thus, about 90% of the ammonia was removed: 10% was consumed by the plants, 25% was measured as nitrate, and 55% was assumed to be converted via nitrate to nitrogen gas. We explained this maturation effect as a result of ochreous sludge build-up (during the previous 5 years of operation) in the inlet zone of the surface flow wetland (Demin et al. 2002). Low-density ochreous sludge with high hydraulic conductivity provided a suitable substrate for bacterial colonisation and changed the hydraulic regime in the inlet of the wetland into a partially subsurface flow regime.

Laboratory-scale nitrification study

Previous experiments suggested that a microbial mechanism was largely responsible for ammonia removal, as observed in sewage treatment wetlands, where it is known that autotrophic forms of bacteria are the primary agents in nitrification (Demin et al. 2002; Sharma and Ahlert 1997). The strains of ammonia-oxidising bacteria most commonly found in wastewater treatment plants are *Nitrosomonas europaea* and *Nitrobacter agilis* (Tyrrell et al.1997). Nitrification is conveniently represented (U.S. EPA 1975) by:

$$NH_4^+ + 1.5O_2 \rightarrow 2H^+ + H_2O + NO_2^- \quad (2)$$

$$NO_2^- + 0.5O_2 \rightarrow NO_3^- \quad (3)$$

These reactions are believed to serve as energy-yielding reactions for *Nitrosomonas* and *Nitrobacter*.

Laboratory work was carried out (using a simulation of a subsurface flow cell) to establish if a population of these bacteria could reproduce the effects at Woolley. A preliminary experiment was run for 14 days in the column filled with autoclaved and dried pea gravel (originating from Cell 4 of the Woolley wetland system) and ammonia feed solution (approximately 4 mg/L solution of $(NH_4)SO_4$ at pH 7.7 and dissolved oxygen concentration of about 80% of normal saturation) pumped through the column continuously at a flow rate of 0.5 L/h (which gives a similar residence time as in a real subsurface flow wetland cell at 6 L/s). After the adsorption capacity of the gravel was reached (approximately 3 days from the start of experiment), practically no further ammonia removal was observed.

Nitrifying bacteria (*Nitrosomonas europaea* and *Nitrobacter agilis*) were introduced into the column and ammonia feed solution was pumped through under similar conditions. Both types of bacteria had been revived and introduced into the column at the same time and left for 21 days with 10% standard C/N-sufficient medium to acclimatise before the pumping started.

As the bacteria are autotrophic, the feed solution was expected to obtain enough CO_2 from the air while dripping into the column, based on the solubility of CO_2 in water (900 mL CO_2/L of water at 20 ºC) (Weast 1984) and that about 99% of the CO_2 is dissolved (Umbreit et al. 1957). Micronutrients were not added, as the amount of nutrients initially introduced into the column with the C/N-sufficient media was believed to be sufficient for the experiment. The feed solution was pumped at 3 different flow rates (0.5, 1.0, and 1.5 L/hr) continuously for 6 days at each flow rate. Ammonia removal data are shown in Figure 5.

After the introduction of bacteria, the column removed 2.08±0.25 mg/L, 1.69±0.11 mg/L, and 1.24±0.08 mg/L (about 46, 38, and 27%) of ammonia at flow rates of 0.5, 1.0, and 1.5 L/hr, respectively. As can be seen from Figure 5, the dependence of the rate of ammonia removal upon the flow rate was linear.

To compare whether artificially introduced bacteria were able to remove equal amounts of ammonia from ammonia feed solution as the bacteria living in the fresh gravel would remove from fresh Woolley mine water, we conducted a similar experiment with fresh gravel and mine water. The results are shown in Figure 6. The column filled with fresh gravel removed 3.59±0.10 mg/L, 3.46±0.05 mg/L, and 3.27±0.08 mg/L (86, 82, and 78%) of ammonia at flow rates of 0.5, 1.0, and 1.5 L/hr, respectively. The dependence of the rate of ammonia removal on the flow rate was also almost linear. In both cases, the conditions in the columns were reasonable for bacteria (pH about 7.6-7.8, dissolved oxygen concentration about 80%, and temperatures of 18-23 C°). Therefore, either the introduced bacterial population was different from the bacterial population existing in the wetland or some essential components necessary for successful growth of the nitrifying bacteria were missing in the ammonia feed solution.

To enhance the artificially introduced bacterial population, 25% (by volume) of the gravel in the column was replaced with fresh gravel from Cell 4. The flow of ammonia solution was continued for 45 days at a rate of 0.5 L/hr. Since it takes about 3 weeks for the nitrifying bacteria to get established (Demin et al. 2002), we expected some increase in ammonia removal in the column after this time. The experimental data are shown in Figure 7. One-way ANOVA analysis of the sets of ammonia-removal data in the column before and after 25% of the gravel was replaced showed that they were not statistically different ($p=0.42$ at $\alpha=0.05$). Thus, it appears that the introduction of a natural bacterial population by replacement of 25% of the gravel did not improve the rate of ammonia removal in the column.

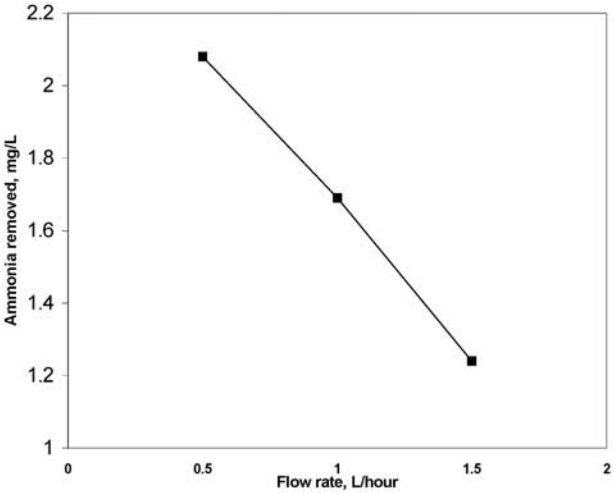

Figure 5. Ammonia removal in the column filled with sterilised gravel, introduced bacteria (*Nitrosomonas europaea* and *Nitrobacter agilis*) and synthetic ammonia feed solution

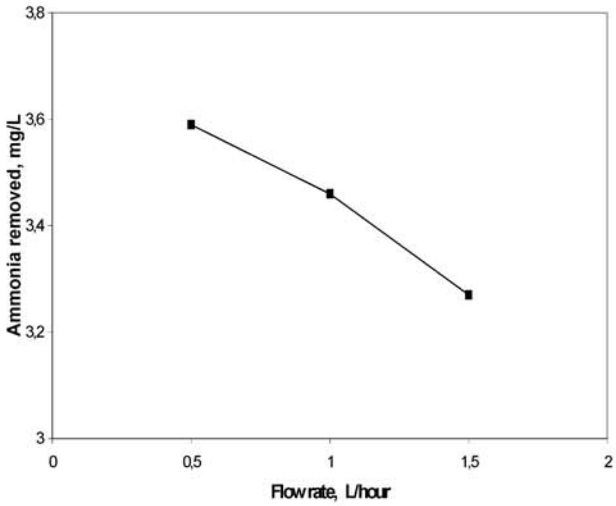

Figure 6. Ammonia removal in the column filled with fresh gravel and fresh mine water removed

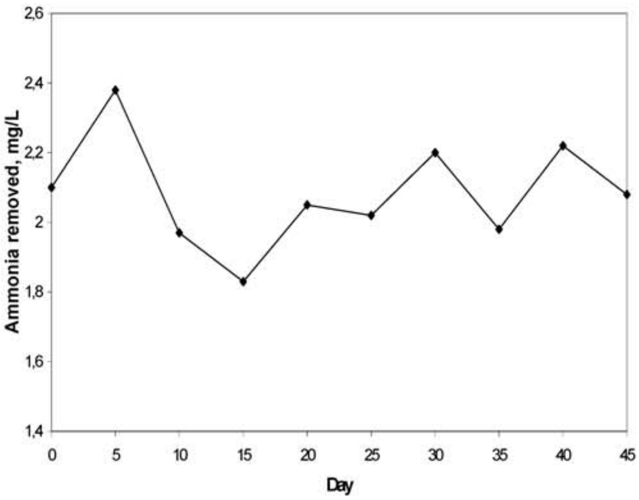

Figure 7. Ammonia removal in the column with 25% fresh gravel

The second hypothesis was that some essential components necessary for successful growth of the bacteria were missing in the synthetic feed solution. To test this hypothesis, we conducted another experiment using sterilised gravel with the same introduced nitrifying bacteria (*Nitrosomonas europaea* and *Nitrobacter agilis*) and sterilised mine water from the inlet into the surface flow wetland. The column was filled with sterilised mine water and left for 7 days. Then sterilised mine water was pumped through the column for 30 days at a flow rate of 0.5 L/hr. Figure 8 shows the rate of ammonia removal. Four weeks after the introduction of the bacteria, the ammonia removal rate in the column was about 89% (similar to the 86% removal rate observed in the column with fresh gravel and fresh mine water at the flow rate 0.5 L/hr). This is almost twice as high as the removal rate in the experiment with sterilised gravel, introduced bacteria, and the ammonia feed solution (46% at a flow rate of 0.5 L/hour).

Presumably, this large difference in ammonia removal was due to the availability of inorganic carbon in the mine water and the pH buffering by the bicarbonate (about 420 mg/L as $CaCO_3$). Bicarbonate ions neutralise the acidity produced by nitrification. It has been shown experimentally (U.S. EPA 1993) that 6.0-7.4 mg of bicarbonate alkalinity (as $CaCO_3$) are required to neutralise the hydrogen ions produced by the oxidation of 1 mg of ammonia to nitrate. The (*Nitrosomonas europaea* and *Nitrobacter agilis*), and sterilised mine water neutralisation reaction also produces additional CO_2 for bacterial growth.

Conclusions

The study confirmed that discharges from many underground coal mines in the UK are contaminated with iron and ammonia, although the parallel

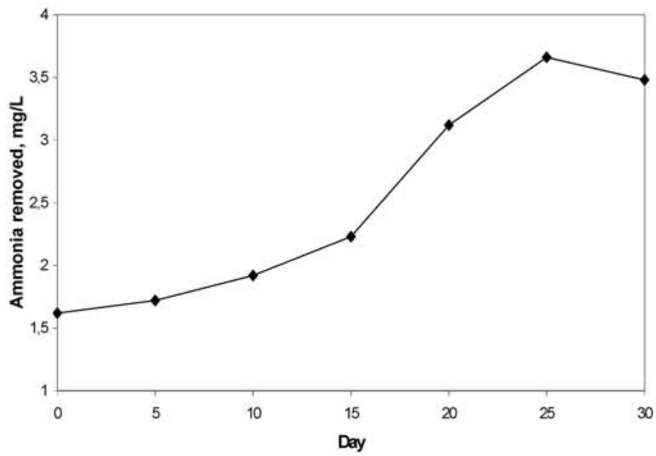

Figure 8. Ammonia removal in the column with sterilised gravel and introduced bacteria

occurrence of these species has no apparent link. The most polluting of the discharges are being systematically remediated under the auspices of the Coal Authority, primarily to remove iron as ochre. Wetland systems constructed to filter residual ochre overflowing from primary sedimentation also remove small concentrations of ammonia, once they are sufficiently mature. Iron and ammonia concentrations meet legal discharge requirements after appropriate wetland treatment.

Field and laboratory experiments were consistent with ammonia removal by microbial oxidation. At the Woolley treatment system, such removal was strongly dependent on conditions, such as flow rate, and nitrate was only formed significantly in the surface and subsurface flow wetlands. Controlled laboratory simulations of subsurface flow conditions showed that *Nitrosomonas europaea* and *Nitrobacter agilis* (bacteria normally associated with ammonia oxidation in municipal wastewaters) efficiently reproduced the field results. Thus, despite great contrasts in operating conditions, nitrification based on essentially the same microbial activity could account for ammonia removal in both mine water and municipal wastewater treatments.

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