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Hydrometallurgy 56 (2000) 127–144

hydrometallurgy

www.elsevier.nl/locate/hydromet

Biological removal of ammonia and nitrate from simulated mine and mill effluents

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Received 11 November 1998; received in revised form 30 September 1999; accepted 7 November 1999

Abstract

Although nitrification/denitrification processes have been used to remove ammonia and nitrate from municipal and industrial processes, there are few reported results on the removal of these ions from mine effluents. Ammonia and nitrate are present in the effluent due to the widespread use of ammonium-nitrate-fuel oil (ANFO) as a blasting agent, and the use of other nitrogen-containing reagents in the mill. Unlike other process effluents, there is very little nutrient content in mine effluents. The objective of this work was to develop a microbial process for the removal of ammonia from simulated mine effluents. Biologically, this is accomplished in two steps. In the first step, ammonia is oxidized to nitrate, and in the second step, nitrate is reduced to dinitrogen gas. Ammonia oxidation (nitrification) was tested using both continuously stirred tank reactors and trickling filters. The stirred tank reactor was chosen for the combined system because it produced final effluents with lower ammonia concentrations. For nitrate reduction (denitrification), a packed bed reactor operated in the upflow mode was tested with methanol being used as a carbon and energy source. The nitrate profiles within the denitrification reactor showed an exponential decay, a characteristic of plug flow conditions. A simulation model was developed for the denitrification reactor which described performance as a function of feed nitrate (NO_3) and methanol concentrations (MeOH) and retention time (RT): Productivity (mg NO_3/l per hour) = $0.722(NO_3) + (MeOH)[1.416 - 0.024\ (MeOH) + 0.0011(NO_3) - 0.776(RT)]$. The methanol to nitrate ratio required for denitrification was 0.86:1. When the two processes were set up in series, it was successful in removing ~90% of the nitrogen.

Keywords: Ammonia; CSTR; Nitrate; Mine effluents; Nitrification; Denitrification; Packed bed reactor

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0304-386X/00/$ - see front matter
PII: S0304-386X(99)00088-2

1. Introduction

1.1. Sources and environmental effects of ammonia

Mine and mill effluents can contain appreciable quantities of ammonium and/or nitrate ions. The discharge of appreciable quantities of ammonia to receiving waters has adverse environmental effects because it is toxic to fish and other aquatic organisms. Both ammonia and nitrate are nutrients for aquatic plants, which could also result in the production of algal blooms and would contribute to the eutrophication of receiving waters.

Ammonium-nitrate-fuel oil (ANFO) is widely used as a blasting agent; nitrogen-containing reagents such as amines are used in the mill and ammonium sulphate is used to elute uranium from ion exchange (IX) resins. Ammonium hydroxide is also used to precipitate uranium and ammonia is used as a lixiviant in copper and nickel hydrometallurgy.

Ammonium ions are in equilibrium with dissolved ammonia as expressed by the following equation:

$$NH_4^+ + OH^- \leftrightarrow NH_3 + H_2O.$$

Ammonia in solution is also in equilibrium with gaseous ammonia. The ammonia concentration, relative to the ammonium ion concentration, increases with increasing temperature and pH. Unionized ammonia (NH_3) is the toxic form while the ammonium ion is relatively non-toxic [1]. The total ammonia concentration in mine effluents varies from 10 to 40 mg/l. The concentration of nitrate in mine effluents is site specific and varies widely from 25 to 300 mg/l.

1.2. Nitrification

Sequential biological ammonia oxidation and nitrate reduction reactions are commonly used to remove nitrogen from both municipal and industrial wastewaters [2]. Nitrification, the biological oxidation of ammonia to nitrate, is a two-step process in which nitrite is formed as an intermediate. The microorganisms responsible for effecting the transformation are *Nitrosomonas* and *Nitrobacter* as shown below [3]:

$$55NH_4^+ + 76O_2 + 109HCO_3^- \rightarrow 54NO_2^- + 104H_2CO_3 + 57H_2O + C_5H_7O_2N\ (\textit{Nitrosomonas})$$

$$400NO_2^- + NH_4^+ + 4H_2CO_3 + HCO_3^- + 119O_2 \rightarrow 400NO_3^- + 3H_2O + C_5H_7O_2N\ (\textit{Nitrobacter})$$

According to the reactions above, approximately 4.3 g of O_2 and 8.64 g HCO_3^- are needed per gram of ammonia–nitrogen that is oxidized to nitrate. Although not shown here, small quantities of phosphorus are also needed to support bacterial growth.

Nitrifying bacteria are subject to inhibition by a variety of organic compounds [4] although other studies have concluded that most organic compounds have minimal effects on nitrification [5,6]. High concentrations of ammonia and nitrous acid have also

been shown to affect nitrification [7]. In most situations, any accumulation of nitrite is transient since the specific growth rate of *Nitrobacter* is larger than that of *Nitrosomonas*. Incomplete nitrification with nitrite accumulation has been observed [7] when the *Nitrobacter* sp. is more severely inhibited. The optimal pH range for nitrification is 7.5–8.6 [3]. It is important that there be sufficient alkalinity in the wastewater to balance the acid produced by nitrification, otherwise a reduction in the pH could adversely affect nitrification. At temperatures below 10°C, the bacterial metabolism decreases significantly, and below 4°C, it stops completely [8]. Dissolved oxygen concentrations above 1 mg/l are needed for nitrification to occur. The nitrification step is much slower and more prone to upset than the denitrification step [9,10]. Frequently, industrial wastes do contain compounds that are inhibitory to the nitrifying bacteria [11].

1.3. Denitrification

Denitrification, the biological reduction of nitrogen oxides to dinitrogen gas, is also referred to as nitrate respiration and is mediated by a number of bacterial genera [12], among them are *Pseudomonas*, *Paracoccus*, *Flavobacterium*, *Alcaligenes*, and *Bacillus* spp. The nitrate ion is reduced to dinitrogen gas by the following pathway [12]:

$$2NO_3^- \rightarrow 2NO_2^- \rightarrow 2NO \rightarrow N_2O \rightarrow N_2.$$

Actively denitrifying cultures are frequently a mixture, in which the overall denitrifying activity is the result of several species, each of which mediates one or more steps in the reaction sequence. Although the denitrifying bacteria are aerobic microorganisms, they can utilize oxidized nitrogen compounds as terminal electron acceptors in place of oxygen. Low oxygen concentrations or the absence of oxygen are required for denitrification to occur [13,14], although some strains of denitrifying bacteria are capable of denitrification in solutions saturated with oxygen and can use both electron acceptors simultaneously [15]. Denitrification usually requires the addition of an external carbon source to act as an electron donor [16]. Methanol is normally used due to its low cost and low biomass yield. The stoichiometry for the process when methanol is used as the carbon source is [17]:

$$NO_3^- + 1.08CH_3OH + 0.24H_2CO_3 \rightarrow 0.06C_5H_7NO_2 + 0.47N_2 + 1.68H_2O + HCO_3^-.$$

Theoretically, for every 1.0 g of nitrate consumed, 0.56 g of methanol is needed, producing 0.10 g of new biomass and 0.81 g of alkalinity, which results in a pH increase. The optimal pH is between 7 and 8; values below 6.5 result in a significant reduction in the denitrification activity of the bacteria. As in the case for nitrification, a minimal amount of phosphate is required. Bulk organic matter in seawater has been shown to have a molar C:N:P ratio of 106:16:1 [18,19]. This figure, known as the Redfield ratio, can also be used as a good approximation for the elemental composition of microbial biomass. Converting this ratio to a weight basis results in a ratio of 41.1:1 for C:P (w:w). Other values that use slightly higher carbon content have also been used in metabolic models of phosphorus removal in treatment systems [20]. However, two other factors complicate the phosphorus budget: some bacteria such as *Acinetobacter*

and *Pseudomonas* spp. can accumulate phosphorus as polyphosphates in amounts up to 10% of their total weight [21], and some phosphate may precipitate as calcium phosphate species. The potential for using biological denitrification to remove nitrate from mine effluents was reviewed by Sanmugasunderam et al. [22]. Mine waters generally lack the phosphate required by the denitrifiers, thus, supplements are usually necessary.

1.4. Nitrification / denitrification

Two possible sequences for a nitrification/denitrification plant can be used [23]. The first, nitrification followed by denitrification, usually requires the addition of an external carbon source to act as electron donor for denitrification [16]. The second, which utilizes denitrification followed by nitrification in which a large portion of the nitrified effluent is recycled back to the first reactor, has several advantages: organics in the effluent can be used as a carbon source and organics that might be toxic to the nitrifiers are removed in the first stage. The denitrification–nitrification sequence has the disadvantage of producing an effluent containing appreciable nitrate concentrations and may not be feasible if the effluent is low in metabolizable organics as in the case of mine effluents.

1.5. Reactors

The types of reactors that can be used for nitrification and denitrification are the fluidized bed reactor [24,25], the Continuously Stirred Tank Reactor (CSTR) [26,27], the packed tower [2], suspended sludge [28], the aerated submerged fixed film (ASFF) reactor [29], and the Rotating Biological Contactor (RBC) [30,31]. The most popular systems for large-scale wastewater nitrification are the CSTR, the packed towers, and the RBC [2]. The packed tower, the CSTR, and the fluidized bed are used for denitrification. In the packed tower, the feed is pumped upflow through a column filled with stone or synthetic packing. Periodic back flushing with gas and/or liquid is required to prevent solids buildup on the packing. The fluidized bed reactor is similar to the packed bed in that the wastewater feed passes upward through packing at a sufficient velocity to fluidize the packing media.

The RBC consists of a series of discs on which the biomass grows; the discs are partially submerged in a trough and mounted on a rotating shaft. The shaft alternately contacts the biomass with the wastewater and the atmosphere for adsorption of oxygen. The RBC was designed for aerobic processes. A series of RBCs is used at the Homestake Mine in Lead, South Dakota to degrade cyanide and thiocyanate and to nitrify the ammonia resulting from the degradation of those compounds [32]. Several other innovative systems have been developed. The LINPOR™ process uses an activated sludge reactor containing open pore plastic cubes. Nitrification occurs on the surface of the cubes and denitrification occurs within the pores in the interior of the cubes [33]. Rogalla and Bourbigot [34] developed an upflow biofilter system that utilized a granular support system with aeration introduced at a point one third of the height of the column. The upper zone of the reactor was oxic and the lower zone was anoxic, so that both nitrification and denitrification would occur in one reactor. A high efficiency system for

nitrification, using hollow fibres in a reactor was developed by Brindle and Stephenson [35]. The hollow fibres were used to transfer oxygen to the reactor and the nitrifying bacteria adhered to the external surface of the fibres. Reverse osmosis (RO) and nanofiltration (NF) membranes have been investigated in a laboratory study for the removal of ammonia and nitrate from synthetic and actual mine effluents [36]. Both membranes were successful at removing ammonia complexes from mine effluents but the NF membrane was less effective than the RO membrane at neutral pH values [36]. However, the cost of RO and NF membranes makes them unsuitable for large-scale applications.

1.6. Objective

The objective of this study was to evaluate, at the laboratory scale, the feasibility of biological nitrification/denitrification for the removal of ammonium nitrogen from simulated mine effluents. The treatment system employed a CSTR for nitrification followed by a packed bed reactor operated in the upflow mode for the denitrification phase. The effectiveness of the CSTR was compared to a trickling filter bed for the nitrification step.

2. Materials and methods

2.1. Nitrification

Review of available data from mine and mill operations suggests that ''typical'' treated, untreated, and pre-concentrated effluents have the approximate compositions listed in Table 1. Synthetic media were thus made to simulate these compositions. Nitrification was tested in two reactor configurations. Since nitrification is an aerobic process, the two configurations that were tested were the CSTR and the trickling filter reactor. The CSTR is known to provide the lowest ammonia concentration in the effluent but is somewhat more expensive to run and more sensitive to process upsets than the trickling filter reactor system. Use of the trickling filter reactor will produce higher ammonia concentrations in the effluent, but the process is relatively simple to control and inexpensive to operate.

Table 1
Typical ammonia containing mine effluents

Type	Flow (m^3/h)	NH_3 (mg/l)	pH	SO_4^{2-} (mg/l)	Fe (mg/l)	Zn (mg/l)	Hardness[a]
Clean	240	25	8	500	0.5	0.1	700
Dirty	240	25	7	2000	500	500	200
Concentrate	24	200	7	5000	1000	1000	1000

[a]As $CaCO_3$ equivalent, mg/l.

Nitrification was first tested in two 19.5-l stainless steel CSTRs (R1 and R2) connected in series. Air was sparged into the tanks at a flow rate of 2.4 l/min and agitation was maintained at 1000 rpm with Lightnin® TS2010 mixers. A heater was available for temperature control but most of the tests were performed at room temperature. Initially, each reactor was filled with 16 l of a modified nitrifier medium [37] containing the following per liter: 3.0 g $(NH_4)_2SO_4$, 216 g Na_2HPO_4, 11.2 g KH_2PO_4, 1.6 g $MgSO_4$. $7H_2O$, 8.0 g $NaHCO_3$, 0.23 g $FeCl_3 \cdot 6H_2O$, and 0.3 g $CaCl_2 \cdot 7H_2O$. To this liquid, 750 ml of liquid effluent from an activated sludge reactor operated at Wastewater Treatment Plant in Gloucester, Ontario, was added. This latter solution served as the inoculum since it was known to contain large numbers of nitrifying bacteria. The pH of the combined solution was approximately 8.0. The solutions were recirculated until sufficient nitrifying bacteria were present to remove 50% of the original ammonia in the feed. After 2 weeks, the circuit was run continuously. Under continuous operation, an ammonia solution containing 0.377 g/l $(NH_4)_2SO_4$ and a nutrient solution containing 4 g/l $NaHCO_3$, 13.5 g/l Na_2HPO_4, 0.7 g/l KH_2PO_4, 0.1 g/l $MgSO_4 \cdot 7H_2O$, 0.014 g/l $FeCl_3 \cdot 6H_2O$, and 0.011 g/l $CaCl_2 \cdot 2H_2O$, with pH 8.44 were fed separately to R1 by two variable speed pumps. The ratio of ammonia solution to nutrient solution was 10:1. A timer controlled the pumps so that additions were made initially for a period of 90 s every 10 min, and then continuously as the inoculum became established. Liquid transfer from R1 to R2 was by gravity overflow.

Nitrification tests were also performed with trickling filter reactors packed with glass, granite, and ceramic. The glass rings (0.953 cm ID × 0.953 cm long), granite (+0.635–0.953 cm), and ceramic (+0.653–0.953 cm) packing were placed in plexi-glass columns (5.63 cm ID × 60 cm long). The total reactor volumes were 1494 ml, which was used to calculate the retention time. To colonize the reactors, some solution from the continuously stirred tank reactors was circulated through the columns for 5–10 days. In order to compare the performance of the column packing materials, the columns were operated with similar flow rates. For the trickling filter reactors, the nominal consumption rate (reported as milligram of ammonia consumed per litre of total reactor volume per day) is also affected by packing volume and wetted area (liquid/gas throughput).

2.2. Denitrification

The denitrification reactor was a plexi-glass column (5.63 cm diameter, 74 cm high) packed with 2.73 kg of gravel (+0.653–0.953 cm). The total volume of the reactor was 1494 ml and the liquid or void volume was 950 ml. The reactor was operated in an upflow mode in order to maintain anaerobic conditions. Two columns with the same internal dimensions were used, one with sampling ports (distance from inlet: 4, 11, 20, 36, 54, 61.5, and 67 cm) located at varying locations in order to obtain profiles of pH and nitrate as well as other chemical species within the reactor and one without sampling ports. A soil inoculum was used to colonize the column with a population of denitrifying bacteria. The initial feed contained: 800 mg/l nitrate; 400 mg/l methanol; 1000 mg/l KH_2PO_4; 500 mg/l NaCl, and 500 mg/l $MgSO_4$. The pH was adjusted to 7.8 with $Ca(OH)_2$. The reactor was run for approximately 1 week under each set of

experimental conditions with samples taken every 2 or 3 days. When the concentration of nitrate for three samples was within 10% of each other, it was assumed that equilibrium was achieved and the experimental conditions were changed. To obtain the empirical model for denitrification, a three-level factorial design (randomized) was used. The following values were used for each variable: nitrate; 100, 300 and 500 mg/l; methanol; 0.125, 0.375, and 0.625 ml/l; phosphate; 2.0, 6.0, and 10.0 mg/l. During the experiments, there was a continual buildup of biomass, causing some plugging. It was therefore necessary to pass compressed air (10 psig) for 5–10 s through the column to remove some of the excess biomass every 2 weeks.

2.3. Nitrification / denitrification

The overall process, including nitrification and denitrification, was performed by placing the modules in series. A schematic showing the overall process is shown in Fig. 1. There was an intermediate storage or holding tank between the second nitrification reactor outflow and the packed bed inlet. It was found that there could be significant holding time in this tank and denitrification would occur in the tank. Data are shown for the constituents that were consumed in this tank before the solution was fed to the packed bed. Initially, methanol was added to the storage tank outflow in a mixing tank and the outflow of this tank was fed to the packed bed. However, it was found that substantial denitrification took place in this vessel, and as a result, it was difficult to evaluate the performance of the packed bed itself. After 344 days of operation of the CSTRs, the tank was removed and the methanol fed directly through a ‘‘T’’ connection into the feed line to the packed bed.

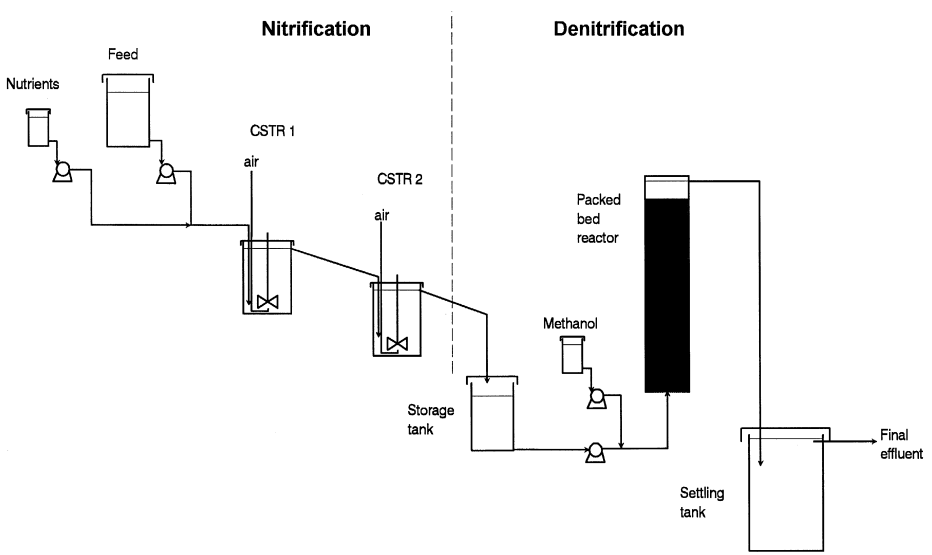

Fig. 1. Schematic of combined nitrification/denitrification circuit.

2.4. Analyses

Microbial populations were characterized by taking samples from the influent, the effluent, and the various ports in the column. The samples were plated on 10% trypticase soy agar [38] and enumerated 72 h later. Bacterial colonies from the agar plates were randomly selected and tentatively identified by Gram stain, either the oxyferm or enterotube identification system (Roche Diagnostics, Div. Hoffmann-La Roche, Nutley, NJ).

The Eh and pH were measured on samples collected at the column outlet using a Fisher Accumet pH meter. An ion-selective electrode (Fisher Scientific) was used to measure the ammonia concentration. Sulphate, nitrate, and nitrite analyses, were performed with a Dionex High Performance Liquid Chromatograph (HPLC); sulphate was analysed with a conductivity detector; and nitrate and nitrite with a UV detector. All samples were passed through 0.2-μm filters before analysis. Calcium analysis was performed by Inductively Coupled Plasma (ICP) Spectroscopy on acidified (HCl) solutions.

Data were analysed using Statgraphics (Statistical Graphics), a statistical applications software.

3. Results and discussion

3.1. Nitrification

Fig. 2A, B, C shows the results of nitrification over the first 150 days of continuous operation in the CSTRs. The graph shows the inlet and outlet ammonia concentrations, outlet nitrite and nitrate concentrations, and the total hydraulic retention time in the two 19.5-l CSTRs as a function of the cumulative run time. It was seen that biological oxidation of ammonia started to occur soon after the process was made continuous (day 0). The effluent from the CSTR in the first 19 days contained high concentrations of nitrite (100–350 mg/l) and almost no nitrate. This may be due to the reactor containing lower numbers of the *Nitrobacter* bacteria, which are responsible for the conversion of nitrite to nitrate. After 19 days, the nitrate concentration increased and the nitrite concentration decreased as the oxidation of ammonia to nitrite became the limiting reaction. Up to 80 days, the performance of the reactor varied due to the adjustments that were made in the retention time and the feed concentrations. To increase the biological population, the reactor was run in batch mode for 2 days. After 85 days, the reactor was run continuously again with a feed containing approximately 100 mg/l ammonia and a total retention time of 80 h. For the next 60 days, the reactor was run with a feed containing, on the average, 106 mg/l ammonia, while the total retention time was slowly reduced from 81 to 38 h. During this time, the effluent from the reactor contained an average of 3 mg/l ammonia, 329 mg/l nitrate, and 1 mg/l nitrite. The productivity of the reactor reached a maximum of 98 mg NH_3/l per day.

Nitrification was also examined in trickling filter reactors with different packing materials. A comparison of the outlet concentrations for the three different packing

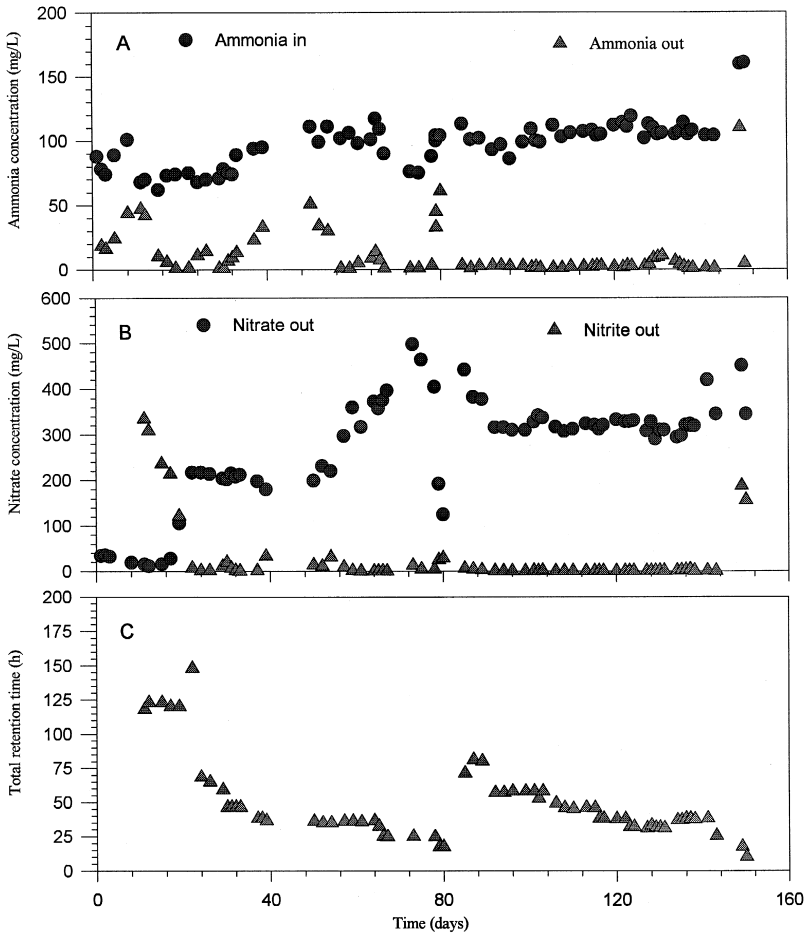

Fig. 2. Performance of the stirred tank nitrification reactor. (A) Ammonia concentration in the feed and final effluent. (B) Nitrate and nitrite concentration in the final effluent. (C) Total retention time.

materials used in the trickling filters is shown in Table 2. The flow rates were gradually increased in steps of 0.45 ml/min on a 7–12 day cycle. The reactor packed with granite exhibited the most consistent performance for nitrification. At flow rates of 0.54, 1.0, and 1.4 ml/min, its nominal consumption rates were 40, 80, and 119 mg NH_3/l per day. A summary of the average performances of the different packings is given in Table 2. The following conclusions can be drawn: the performance of the glass packing is poor in comparison to all other packing materials; at intermediate retention times of 4.5 h, the rate of ammonia removal is 210 mg NH_3/l per day. The granite packing is slightly better than the glass packing in all operating ranges. Colonization occurs more rapidly and stability is better. Typical performance at retention times of 4.8–4.0 h is about 250 mg NH_3/l per day with 50–85 mg/l of ammonia removed from the feed. The ceramic packing took about a month to colonize, resulting in poor performance at the beginning.

Table 2
Summary of average nitrification performance using trickling filter reactors with different packing materials

Days	Feed NH_3 (mg/l)	Exit NH_3 (mg/l)	Retention time[a] (h)	Rate[a] (mg NH_3/l per day)
Glass				
0–21	111	103	1.75	110
23–29	101	83	3.69	117
30–63	90	26	24.00	64
66–81	99	22	17.14	108
83–133	105	62	4.53	228
Ceramic				
0–25	95	54	18.46	53
31–46	104	42	6.00	248
49–60	103	20	4.44	449
63–77	84	4	4.29	448
79–87	123	75	2.07	557
123–171	39	19	2.89	166
Granite				
0–22	79	20	42.86	33
24–49	95	10	20.00	102
55–70	104	47	5.71	240
73–84	103	65	4.29	213
87–101	84	28	4.29	313
103–111	123	73	2.11	569
147–195	39	8	2.67	279

[a]Retention time and rates are based on the total reactor volume of 1494 ml.

However, once the ceramic packing was colonized, consistently higher ammonia oxidation rates occurred; at a retention time of 4–6 h, removal rates of 400 mg NH_3/l per day and 70–90 mg NH_3/l per day of ammonia were observed. At a flow rate of 1.8 ml/min, the consumption rates were 162 and 152 mg NH_3/l per day for the granite and ceramic packings.

Most comparisons of packing media for trickling filters have focused on the removal of biological oxygen demand (BOD) and total suspended solids (TSS) removal [39]. High BOD inhibits nitrification, primarily due to competition for oxygen between the nitrifiers and heterotrophic bacteria [40,41]. Mine effluents are low in soluble organic compounds, thus, competition for oxygen is not critical. The surface area for microbial colonization and the ability of the nitrifiers to adhere to the surface will have the greatest effect on nitrification. Two factors have been shown to be important in cell adhesion: hydrophobicity of the cells and support surfaces [42]. However, in some cases, hydrophilic surfaces and electrokinetic potential are more important [43,44]. The initial difficulty in colonizing the ceramic may be due to the hydrophilic nature of the ceramic surface, but once the surface is colonized, the large surface area could support high numbers of nitrifiers, and thus, significant nitrification rates. Productivities of 400 mg NH_3/l per day were obtained using a CSTR containing plastic beads as a solid support [45]. Higher productivities have been observed with other reactor configurations [46,47].

A productivity of 1100 mg NH_3/l per day was obtained with a draft tube fluidized bed reactor [46] and a productivity of 2200 mgNH_3/l per day with a CSTR that was continuously sparged with pure oxygen and contained reticulated foam beads as a solid support [47]. The final effluent from the CSTRs contained lower ammonia concentrations, and thus, was used in subsequent tests.

3.2. Denitrification

3.2.1. Bacterial distribution

After the startup of the denitrification reactor, samples were taken to determine the bacterial distribution. The results are provided in Table 3. The short retention times in the experiment did not allow a population gradient to develop in the aqueous phase. Most of the bacterial activity was due to microorganisms adhering to the packing material; however, it is very difficult to obtain a measure of the bound biomass without dismantling the reactor. Most of the bacteria found in the samples tested were Gram-negative and belonged to the following genera: *Pseudomonas*, *Acinetobacter*, *Alcaligenes*, and *Achromobacter* spp. Bacteria belonging to these genera are known to be denitrifiers. Also of interest was the variety of denitrifiers that were isolated, which implies that a varied and robust population existed in the reactor. Thus, the packed bed reactor should have sufficient resilience to adjust to major changes in feed composition.

3.2.2. Denitrification variation along the length of the reactor

Results from one experimental run with the ported column where the methanol and nitrate were nearly all consumed are shown in Fig. 3. The variations of nitrate, methanol, and phosphate concentrations, and the pH along the length of the reactor are shown on this graph. In this case, the feed contained 109 mg/l nitrate, 112 mg/l methanol, 14 mg/l phosphate, and was at a pH of 7.6. The retention time at 60 cm was 17 min. It was seen that as denitrification proceeds, both the nitrate and methanol concentrations decrease almost identically, the final concentrations were 19 and 13 mg/l for nitrate and methanol, respectively. The observed nitrate and methanol profiles are characteristic of plug flow in the reactor. The phosphate concentration was reduced only marginally, being 10 mg/l after 60 cm. The pH initially decreased, which is possibly

Table 3
Bacterial numbers at various locations in the packed bed reactor

Location from inlet (cm)	Colony forming units $\times 10^5$
0.0	35
4.0	51
11.0	20
20.0	26
54.0	26
Effluent	41

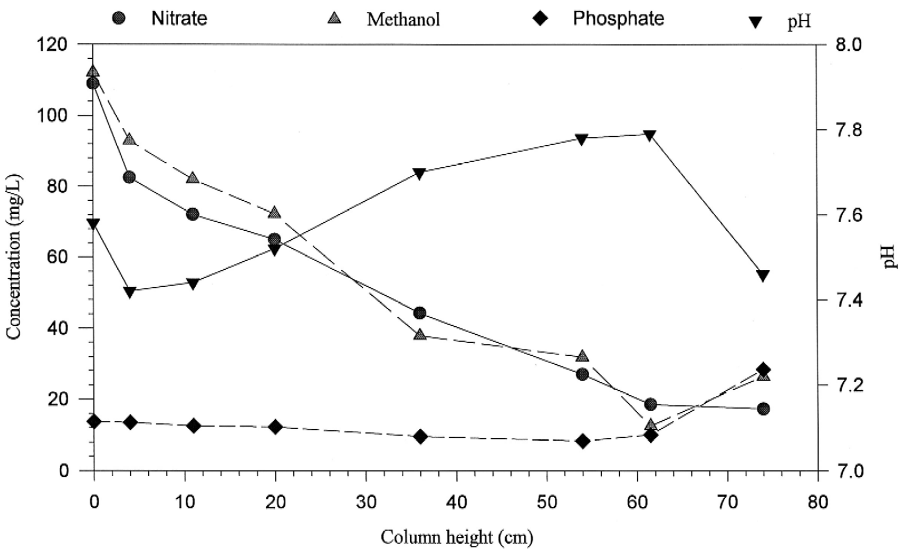

Fig. 3. Denitrification along the length of the packed bed reactor.

due to aerobic activity, but as denitrification proceeded, it produced alkalinity according to the stoichiometry presented previously, resulting in an increase in the pH.

3.2.3. Reactor performance:

Fig. 4 shows the performance of the denitrification reactor at 22°C over a period of 60 days. The graph shows the inlet and outlet nitrate concentrations as well as variations

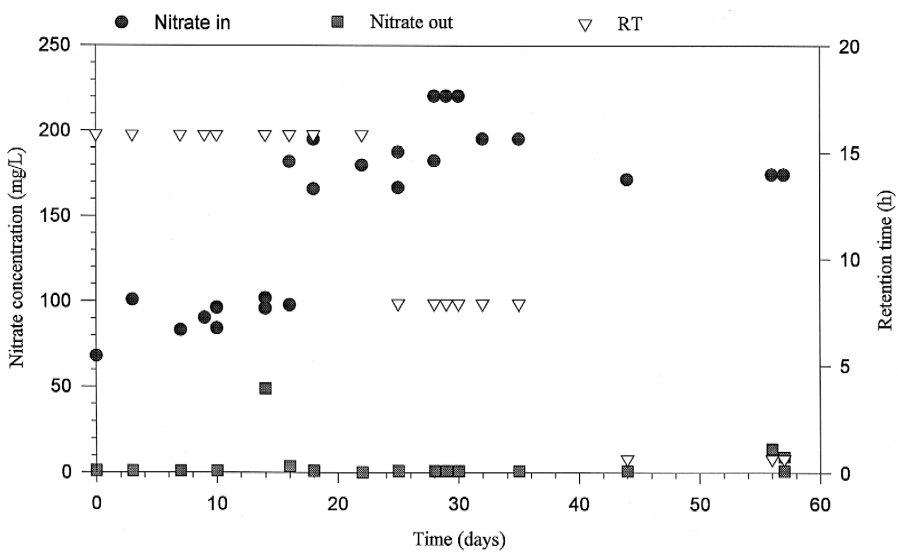

Fig. 4. Denitrification in the packed bed reactor.

in the retention time over this period of time. Initially, feed containing approximately 100 mg/l nitrate was fed to the reactor with a retention time of 15.8 h; the reactor was able to consume all of the nitrate under these conditions. After 15 days, the feed nitrate was increased to 200 mg/l; there was still little or no nitrate in the effluent. After 25 days, the retention time was then decreased by a factor of two and the nitrate concentration was still below 2 mg/l. After a further reduction in the retention time to less than 1 h, the nitrate concentration in the effluent increased to about 10 mg/l. It subsequently decreased to below 2 mg/l as the biological activity in the column increased. The fact that the column was able to compensate for such large changes in the feed nitrate and the retention time indicates that there was excess denitrification capacity in the column under these experimental conditions.

3.2.4. Determination of nutrient requirements

The main nutrients required for denitrification are carbon and phosphorus, which are available as methanol and phosphate. To determine the quantities that were required for complete removal, a number of tests were performed at various concentrations of nitrate, methanol, and phosphate, as well as at different retention times. The ranges of inlet nitrate concentrations tested were 51–405 mg/l, which are typical of the concentrations encountered in the mine effluents. The concentrations of the other variables tested were 29–529 mg/l methanol, 12–61 mg/l phosphate, and retention times of 0.30–1.14 h (83 separate data points were collected). The amount of methanol and phosphate used as a function of the amount of nitrate consumed in these tests is shown in Fig. 5. From the graph, there appears to be a linear relationship between methanol and nitrate consumption. The line that is shown was fitted to the data by linear regression, with a slope of

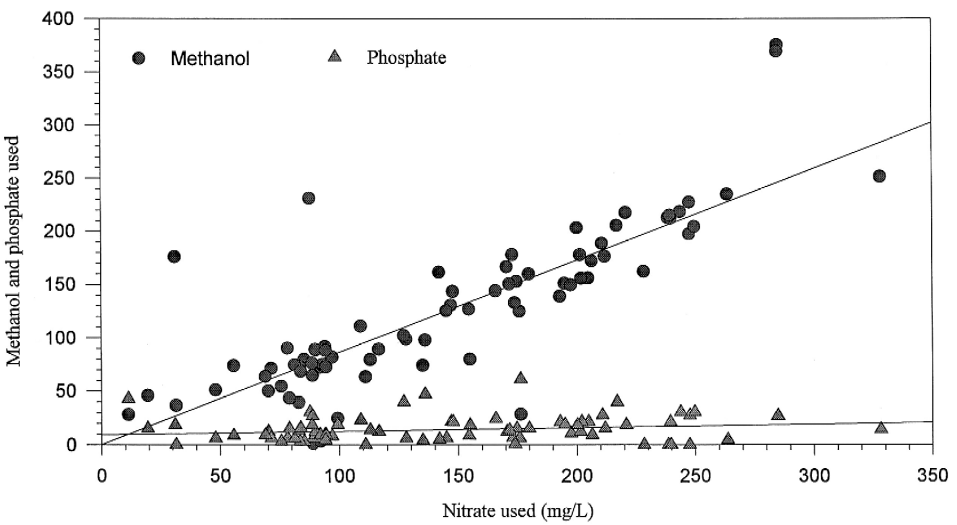

Fig. 5. Methanol and phosphate consumption in a packed bed denitrification reactor.

0.86. This is higher than the theoretical value of 0.56 that was predicted from the stoichiometry. This may be due to methanol, being consumed for aerobic respiration producing CO_2 or a higher biomass yield than that indicated by the stoichiometry. Phosphate consumption was relatively constant as a function of the nitrate removed; the average phosphate consumption varied from 0 to 43 mg/l but did not appear to be limiting.

In order to determine the effect of these variables on the performance of the reactor, an empirical equation was fit to the data. A stepwise variable selection procedure (Statgraphics) was used to obtain a model that has a small set of significant variables. It

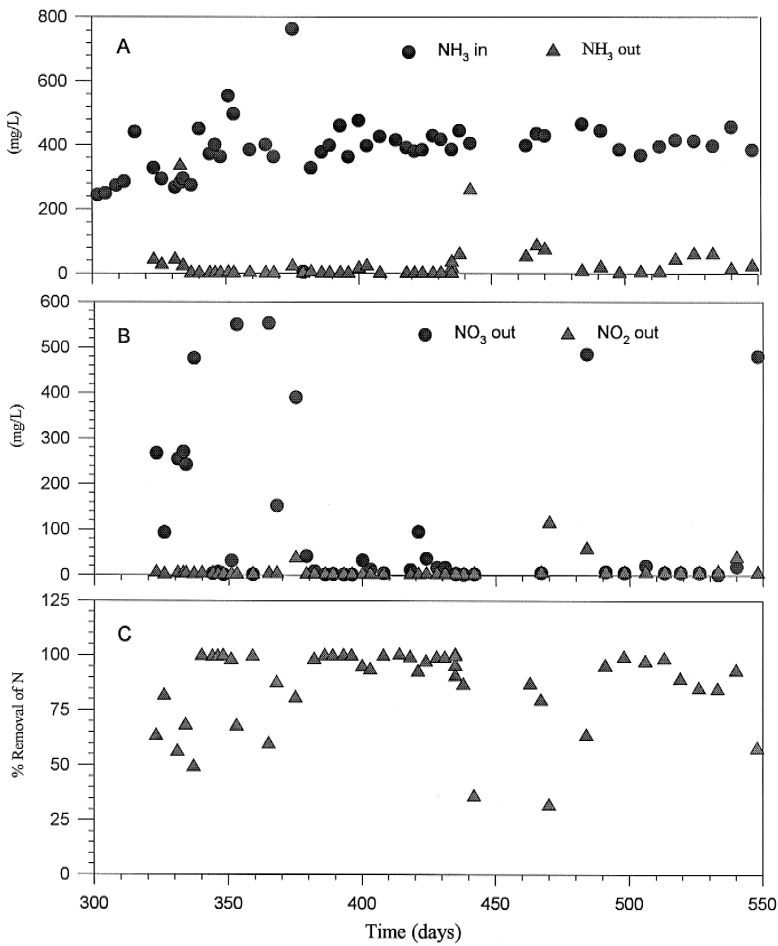

Fig. 6. Performance of the combined nitrification denitrification process for the removal of ammonia. (A) Ammonium concentration in the feed and final effluent. (B) Nitrate and nitrite concentration in the final effluent. (C) Percentage removal of nitrogen for the overall process.

relates the reactor productivity to the inlet concentrations of nitrate, methanol, and phosphate, and the retention time in the reactor, as follows:

$$\text{Reactor productivity}\,(\text{mg/l per hour}) = 0.722(\text{NO}_3) + (\text{MeOH})\left[1.416 - 0.0024(\text{MeOH}) + 0.0011(\text{NO}_3) - 0.776(\text{RT})\right],$$

where: Reactor productivity = (NO_3 in the inlet − NO_3 in the outlet)/retention time (mg/l per hour); NO_3 = inlet concentration of nitrate (mg/l); MeOH = inlet concentration of methanol (mg/l); RT = retention time (h).

The correlation coefficient was 0.97. Even though the equation is empirical, it provides a good estimate of the data in the range of the variables tested. The phosphate concentration did not significantly affect the productivity at the 95% confidence level. The amount of phosphate in these tests was present in excess amounts.

This equation enables one to predict the amount of nitrate that will be present in the effluent from a packed bed reactor given the inlet nitrate and methanol concentrations and a reactor retention time. It is important to keep in mind that this equation is valid only in the experimental range tested.

3.3. Nitrification / denitrification

To test the overall biological process for ammonia removal, the nitrification and denitrification modules were connected in series (Fig. 1). The performance of the overall circuit is shown in Fig. 6A, B, C. Fig. 6A shows the ammonia concentration in both the feed and final effluents. After a short incubation period, the overall process is able to remove nearly all of the ammonia from a feed that contains approximately 400 mg/l ammonia. The nitrate and nitrite that are produced as a result of the nitrification process are converted to nitrogen gas in the denitrification reactor (Fig. 6B). During the first 75 days, this second process did not operate well as evidenced by the fluctuating nitrate concentrations in the final effluent; but after day 375, the nitrate concentration was reduced to near zero. The amount of nitrogen that is removed in the overall process is shown in Fig. 6C. Other than several points during the first 75 days, nearly 100% of the nitrogen is removed by the combined nitrification/denitrification process tested here.

4. Conclusions

The results given here show that nitrification/denitrification processes can be successfully used to treat mining effluents. In the nitrification process, ammonia was oxidized to nitrate in CSTRs and trickling filter reactors. It was noted that the final effluent quality was better in the CSTRs so they were used for further experiments. A packed bed reactor operated in the upflow mode was found to be suitable for the removal of nitrate from simulated mine effluent. An effluent that is low in nitrogen with very short retention times can be obtained with this system. The critical parameters for denitrification are nitrate concentration, temperature, and flow rate (retention time). The

ratio of methanol consumed to nitrate consumed was 0.86:1. An empirical model was successfully fit to the data and enabled the productivity and final effluent concentrations to be calculated if the inlet nitrate and methanol concentrations and retention time are known. By employing an integrated process comprising nitrification and denitrification, high ammonia removal efficiencies can be obtained.

The kinetics of denitrification were an order of magnitude more rapid than nitrification, and the denitrification was also more robust. To improve the reliability and reaction rate of the nitrification process, either additional work on improving the trickling filter system should be done or the biological process should be replaced with a chemical one. Future work can also be directed towards scaling up the process to pilot scale with real mine effluents to be tested.

Acknowledgements

The authors gratefully acknowledge the thorough reviews of the manuscript by J. Skeaff, C. Hogan, and J. Nadeau of the Environment Laboratory of Natural Resources, Canada.

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