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EFFECTS OF HEAVY METALS ON NITRIFYING BACTERIA

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ABSTRACT

Laboratory evaluations were conducted to study the toxic responses of heavy metals such as copper and nickel of an autotrophic culture of strict nitrifiers (*Nitrosomonas* sp. and *Nitrobacter* sp.) in continuous flow stirred tank reactors (CSTR).One of the CSTRs was operated as a suspended growth (SG) system while the other was operated as an attached & suspended growth (A&SG) system. Nitrification inhibition in a SG and A&SG systems was investigated with the emphasis on the effect of shock loading of copper and nickel. As a result of the copper and nickel test, *Nitrosomonas* sp. was found to be equally or more sensitive than *Nitrobacter* sp. However, a higher influent nickel concentration of 50 mg/L was needed to cause a similar percent inhibition of ammonium oxidation than the copper concentration of 5 mg/L. A geochemical equilibrium speciation model, MINTEQA2/PRODEFA2, was used to compute the concentrations of various chemical species present in the wastewater for both systems. The high correlations of $Cu(NH_3)_4^{+2}$ and $Ni(NH_3)_4^{+2}$ with percent inhibition were found and it was thought that they were probably the species responsible for the inhibition of ammonia oxidation. © 1997 IAWQ. Published by Elsevier Science Ltd

KEYWORDS

Attached growth; copper; inhibition; media; nickel; nitrification; speciation; suspended growth.

INTRODUCTION

The presence of toxic substances such as metals and even ammonia itself exert a significant impact on the performance of nitrifying bacteria which will in turn affect the nitrogen balance in the environment or in an engineered system. In fact, due to the sensitivity of nitrifying bacteria to certain chemicals, nitrifying bacteria are being considered as possible bioassay organisms in assessing toxicity levels of aqueous systems (Wang and Reed, 1984). Although the general toxic effects of heavy metals on nitrification are well-known, specific toxicological responses of nitrifying bacteria have not been well understood.

Sato *et al.* (1986a,b, 1988) have studied the effects of copper and nickel on the growth of pure cultures of *Nitrosomonas europaea* in batch system. Using a chemical speciation model, they found that the inhibition in the growth of *Nitrosomonas europaea* was highly correlated to the amine compounds of copper and nickel. In this study, research on the effects of copper and nickel on nitrifiers will be extended using a mixed culture of nitrifiers in a continuous flow SG reactor and A&SG reactor. An attempt will be made to identify

the various chemical species in the dissolved phase that are responsible for the inhibition of ammonia oxidation.

MATERIALS AND METHODS

Biological reactor system

Three identical continuous flow units were built, each having a hydraulic capacity of 11.5 L. Each unit has two compartments: an aeration section (7.3 L) and a settling section (4.2 L). The aeration compartment was equipped with feeding and air supply devices. Air was supplied uniformly to the aeration section at a rate of 1. 4 L/min. through two diffuser stones.

Reactor 1 was a SG vessel which was used as a control and fed only nutrient feed solution. Reactor 2 was also a SG unit but fed with a toxic compound. Reactor 3 was a suspended & attached growth (BIO-POR^R) unit in which biomass support media were submerged. Cylindrically shaped porous material, UKIDAMA^R, was used as support media. The solid media are made of polypropylene non-woven fabric material which provide a large effective surface area for biological growth and large volume of interstitial space for passage of liquid and air. The three laboratory plants were operated in parallel under controlled conditions. The feed solution was an inorganic nutrient solution containing approximately 50 mg/L of ammonium as N. Stock chemical solutions were prepared with deionized water from an E-Pure deionized water system (Barnstead, 3-Module E-Pure) The quantity of various compounds used to make up 45 litres of synthetic wastewater is shown in Table 1. All experiments were conducted at a constant temperature of 25°C.

Table 1. Composition of synthetic wastewater

Compound	Stock conc. (g/L)	Quantity used for 45 L(mL)	Final conc. (mg/L)
$(NH_4)_2{\cdot}SO_4$[(a)]	47.17	225.0	235.9
$MgSO_4{\cdot}7H_2O$	100.00	22.5	50.0
$MnSO_4{\cdot}H_2O$	10.00	22.5	5.0
$CaCl_2{\cdot}2H_2O$	7.50	22.5	3.8
KCl	14.00	22.5	7.0
$NaH_2PO_4{\cdot}H_2O$	26.65	22.5	13.3
$FeSO_4{\cdot}7H_2O$	20.00	22.5	10.0
Na_2CO_3[(b)]	53.00	225.0	265.0
$NaHCO_3$[(b)]	42.00	225.0	210.0
$CuCl_2{\cdot}2H_2O$[(c)]	1.34	450.0	13.4
$NiSO_4{\cdot}6H_2O$[(d)]	22.39	450.0	223.9

(a) Provides 50 mg/L ammonia-nitrogen.
(b) 1.0 M Buffer solution.
(c) Provides 5 mg/L of copper, and used for copper test.
(d) Provides 50 mg/L of nickel, and used for nickel test.

Experiment with copper

1.34 grams of $CuCl_2{\cdot}2H_2O$ was dissolved in 1 litre of deionized water and 450 millilitres of the solution was added to 45 litres of synthetic wastewater. Synthetic wastewater containing approximately 5 mg/L of Cu^{+2} was fed continuously until nitrification was reduced by approximately 90%. At this juncture, wastewater without copper was fed to both systems until nitrification was fully recovered.

Experiment with nickel

Nickel stock solution was prepared with 22.39 grams of $NiSO_4 \cdot 6H_2O$ in 1 litre of deionized water. 450 millilitres of the solution was added to 45 litres of synthetic wastewaters for both suspended growth and attached & suspended growth systems. Synthetic wastewater containing 50 mg/L of Ni^{+2} was fed to both systems until nitrification was reduced by approximately 90%. At this point, wastewater without nickel was fed to the both systems until there was no visible impact shown in nitrification.

Computer simulation: geochemical equilibrium speciation

A geochemical equilibrium speciation model, MINTEQA2/PRODEFA2, was used to compute the concentrations of various chemical species present in the wastewater of the reactor systems. In an effort to determine the chemical species that exhibit toxicity effects to nitrifying bacteria, the concentrations of various species computed by MINTEQA2/PRODEFA2 were regressed against the percent inhibition as:

$$\% \text{ Inhibition} = \frac{\text{Total } NH_3 \text{ in the effluent}}{\text{Averaged total } NH_4^+\text{-N converted to } NO_3^-\text{-N}} \times 100$$

The approach to determine the possible chemical species causing toxic effects is based on two assumptions. The first assumption is that the toxic effects on the nitrifiers were attributed to the chemical species in the dissolved phase. In the case of dissolved metal concentrations, the various chemical species are easily computed based on equilibrium equations and constants. For the second assumption, concentrations of various chemical compounds, other than those of the target metal, nitrogen and carbon species were assumed to be minimally removed in the reactors. The concentrations of these compounds used in the MINTEQA2/ PRODEFA2 model were concentrations prepared for the feed solution.

RESULTS AND DISCUSSION

The experiments were designed to simulate and monitor the response of stable nitrification systems to a sudden continuous step-wise input of heavy metals. The first phase of the experiments was to cultivate nitrifying organisms and to equilibrate the systems. A steady-state condition was assumed to be achieved when 99% removal of the influent ammonia was consistently obtained in the reactors. The second phase of the experiments with heavy metalwas performed to study the effects of copper and nickel on nitrification.

The second phase of the experiment consisted of dosing the reactors with toxic chemicals. First, copper was added as copper chloride to the aeration section of SG and A&SG reactors through feeding devices, while control reactor was operated with only feed solution and suspended biological solids. After first adding copper to SG and A&SG, copper did not give any visible impact to the systems and there were essentially no changes in pH, alkalinity, NH_4^+, NO_2^-, and NO_3^- in the effluent. After approximately 60 hours, the copper concentration in the reactor reached about 5 mg/L. At this concentration, nitrification appeared to be partially suppressed, indicated by a drop in effluent nitrate and increase in ammonium concentrations. Further evidence for the inhibition due to copper was shown as increase in pH and alkalinity. As expected, less significant inhibition was exhibited in the A&SG reactor than the SG reactor. The complete and continuous nitrification was always obtained in the control. No further increase in toxicity was exhibited until the copper concentration increased up to 25 mg/L (Fig. 1). At 30 mg/L, however, sharp increases of ammonium and corresponding decreases in nitrate concentration occurred in the effluent from the SG unit. The A&SG reactor showed no apparent increase in inhibition at this level. Very low nitrite concentration was maintained in all the units. This indicates that *Nitrosomonas* sp. is equally or more sensitive than *Nitrobacter* sp. to copper, thus the first-stage nitrification is the rate-limiting step under stressed conditions with copper.

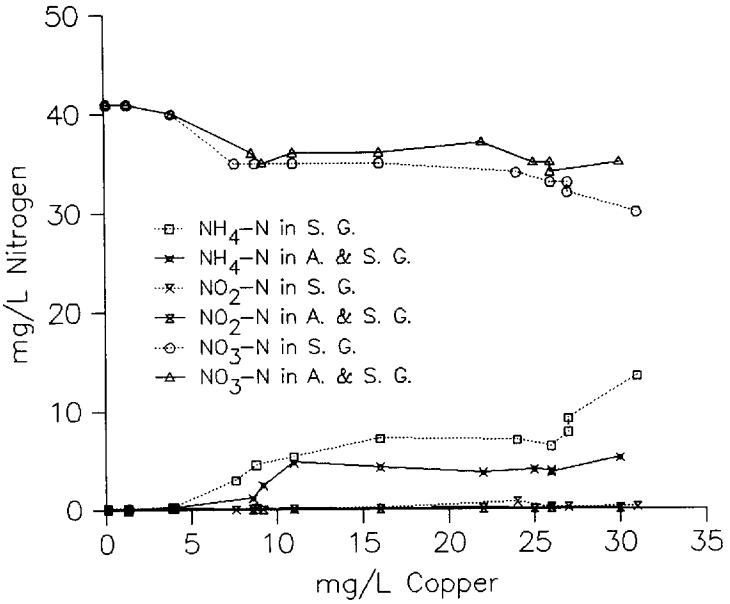

Figure 1. Change in concentrations of inorganic nitrogen species as a result of a copper input.

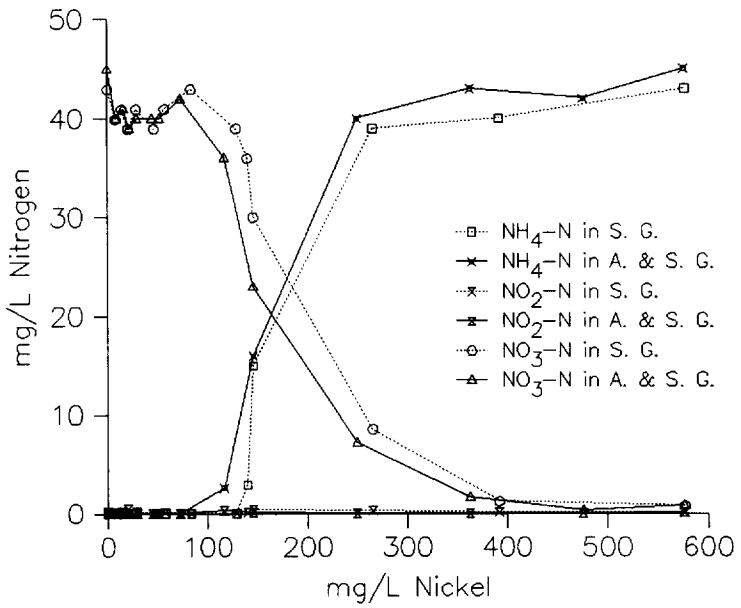

Figure 2. Change in concentrations of inorganic nitrogen species as a result of a nickel input.

As shown in Fig. 2, there was no visible inhibition of nitrification to both SG and A&SG systems until the total nickel concentration in the reactors was approximately 100 mg/L. When the nickel concentration was approximately 150 mg/L, inhibition of ammonia oxidation was about 35 and 38% in SG and A&SG units,

respectively. At least 94% of ammonia oxidation inhibition was observed in both reactors when the nickel concentration in the reactor increased to 250 mg/L. This lack of inhibition at low concentrations followed by a sharp increase in inhibition at a higher concentrations may imply a threshold toxic concentration for nickel. Similar threshold concentration observation were made on nitrifying bacteria by Sato *et al.* (1986a,b, 1988) and on the growth of *Mercenaria Mercenaria* by Calabrese *et al.* (1977). On the other hand, low nitrite concentration was maintained in all the units until the addition of nickel has ended. This indicates that *Nitrosomonas* sp. was more sensitive to nickel than *Nitrobacter* sp. in both SG and A&SG reactors. At all times, complete and continuous nitrification was observed in the control reactor.

Concentrations of ammonia species and 21 copper species were computed using MINTEQA2/ PRODEFA2 for the copper test. A quick glance at the computed concentrations indicates that concentrations that were greater than 10^{-10} moles/L were those of ammonium (NH_4^+), cupric triamine ($Cu(NH_3)_3^{+2}$), and cupric tetraamine ($Cu(NH_3)_4^{+2}$). Of all the species computed using MINTEQA2/PRODEFA2, concentrations of only three species, NH_4^+, NH_3 (aq.), and $Cu(NH_3)_4^{+2}$, showed a positive relationship with the percent ammonia oxidation inhibition in both SG and A&SG systems. All other chemical species had negative correlation, i. e. , decrease in percent inhibition was observed for an increase in the species concentration.

Although NH_4^+ is highly correlated to percent inhibition, it may not be the cause. Ionized ammonia(NH_4^+) has been found to be non-toxic or much less toxic than unionized ammonia. The high correlation of NH_4^+ to percent inhibition may be an artifact because the percent inhibition was calculated based on the concentration of total NH_3 in the effluent of reactor. Anthonisen *et al.* (1976) examined the effects of ammonia and nitrous acid on nitrification and observed that the range of free ammonia concentrations that begin inhibition of *Nitrosomonas* sp. were 10 to 150 mg/L. Neufeld et. al. (1980) observed that unionized ammonia begins to inhibit nitrification at a concentration of 10 mg/L. The general inference that can be drawn from these studies with regards to unionized and ionized ammonia toxicity is that unionized ammonia begins to inhibit nitrification at concentrationss greater than 10 mg/L. The unionized ammonia concentrations in the reactors as computed by the MINTEQA2/PRODEFA2 model were all less than 10 mg/L throughout the study period. If ammonia is inhibitory at concentration above 10 mg/L as shown by previous work, then one may infer that, for this study, ammonia may not have been inhibitory because of the low concentrations in the reactors. Therefore, the presence of $Cu(NH_3)_4^{+2}$ may be the cause for the inhibition of *Nitrosomonas* nitrifying activity.

On the other hand, concentrations of two ammonia species and 18 nickel species were computed using MINTEQA2/PRODEFA2 for nickel test in both reactors. Only three species, NH_4^+, NH_3 (aq.), and $Ni(NH_3)_4^{+2}$, showed positive trends between the species concentrations and percent inhibition of *Nitrosomonas* sp. activity in both systems. All other species had negative correlations. As explained in the copper test, the high correlations of NH_4^+ and NH_3 (aq.) with percent inhibition may be an artifact due to the computed percent inhibition calculated from the total ammonia concentrations. Therefore, it is possible that the presence of $Ni(NH_3)_4^{+2}$ may result in the inhibition of ammonia oxidation by *Nitrosomonas* bacteria.

CONCLUSIONS

Nitrosomonas sp. was observed to be more sensitive to copper and nickel than *Nitrobacter* sp. A&SG growth system was found to be more resistant to step inputs of copper and equally sensitive to step inputs of nickel than SG system. *Nitrosomonas* sp. was found to be gradually inhibited by copper while there appears to be a threshold nickel concentration in which the activity of *Nitrosomonas* sp. was severly inhibited. From the experimental results, nitrifying bacteria was found to be more sensitive to copper than nickel.

Of all the chemical species determined by MINTEQA2/PRODEFA2, $Cu(NH_3)_4^{+2}$ was probably the species responsible for the inhibitory effects on ammonia oxidation in both SG and A&SG systems. Similarly the tetraamine species of nickel, $Ni(NH_3)_4^{+2}$, was found to be highly correlated to percent inhibition of ammonia oxidation and was also probably responsible for the inhibitory effects in both SG and A&SG systems.

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