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ORIGINAL PAPER

Effect of continuous Cd feeding on the performance of a nitrification reactor

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Abstract The inhibitory effect of Cd on nitrification was investigated in a continuous-flow system with enriched nitrifying bacteria. The maximum specific ammonium utilization rate and the half-saturation constant were found as 671 mg NH_4–N/g VSS day and 0.48 mg/l, respectively. In the case of continuous Cd input at 1 and 2.5 mg/l, nitrification was inhibited by 30% and 47%, respectively. Inhibition ranged from 20% to 40% and no further increase in inhibition was exhibited in new runs except at 10 mg/l influent Cd. At 10 mg/l influent Cd, specific ammonium utilization and nitrate production rates were inhibited by 90%. On the contrary, a serious nitrite accumulation was not observed during this period. When Cd feeding was stopped, recovery from inhibition was observed after 37 day which was seen by the improvement in ammonium utilization and nitrate production rates. A shift in microbial population from the initial *Nitrosomonas* sp. to the Cd-tolerant *Nitrosospira* sp. was observed in the recovery period from severe Cd inhibition. After the domination of *Nitrosospira* species, redosing at 10 mg/l and then at 15 mg/l did not affect the performance as before.

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Abbreviations

Cd_{bio}	Biosorbed Cd, mg Cd/g MLSS
Cd_{volt}	Labile Cd concentration in bulk solution, measured by voltammetry (Cd^{2+} and Cd in weak complexes), mg/l
K_I	Inhibition coefficient, mg/l
K_{S,NH_4-N}	Half-saturation constant for ammonium, mg/l
$q$$NH_4$–N	Specific ammonium utilization rate, mg NH_4–N/g VSS day
$q$$NO_3$–N	Specific nitrate production rate, mg NO_3–N/g VSS day
q_{max,NH_4-N}	Maximum specific ammonium utilization rate, mg NH_4–N/g VSS day
q_{max,NO_3-N}	Maximum specific nitrate production rate, mg NO_3–N/g VSS day
$q_{max,NH_4-N,app}$	Apparent maximum specific ammonium utilization rate, mg NH_4–N/g VSS day
S	NH_4–N in bulk solution, mg/l

Introduction

Studies demonstrated that short-term batch assays may not adequately reflect the response observed in continuous-flow reactors which are subject to prolonged

toxic exposure (Hu et al. 2004). The behavior of heavy metals in real wastewater treatment systems should therefore be examined by long-term experiments in continuous-flow systems. The results will possibly provide an insight into real systems in the determination of threshold concentrations, optimum operational conditions and design of biological reactors. Most importantly, the behavior of heavy metals under prolonged exposure times, acclimation, adaptation, shifts and changes in bacterial community can best be investigated in continuous-flow experiments.

A constant input of heavy metal may not affect biological treatment (Yetiş and Gökçay 1989). Acclimatized sludges maintain their high removal capacities even if they are exposed to high concentrations of heavy metals such zinc, cadmium and mercury (Neufeld and Hermann 1975). Resistance of biological treatment systems to metal toxicity may be greatly enhanced by proper acclimation. Acclimation usually involves the use of alternative pathways, which are not disrupted (or least disrupted to a lesser degree) by the presence of heavy metals. This process is, nevertheless, limited and the cell may not be able to further acclimation at relatively higher concentrations, resulting in complete containment of biological activity (Gikas and Romanos 2006). During acclimation, either resistant organisms are selected and/or microbes are metabolically adapted to the metal. In the case of metabolic adaptation, microorganisms are thought to synthesize new enzymes to substitute the metal-inactivated ones or create alternative shunt pathways (Yetiş and Gökçay 1989).

Nitrification is the key process for biological nitrogen removal in many wastewater treatment plants (WWTP). Nitrifying bacteria are very sensitive to inhibitory compounds and most of the time inhibition causes the washout of nitrifiers from treatment plants. The recovery from inhibition requires a very long time due to the slow growth rates of nitrifiers. Although the toxicity of heavy metals in nitrification processes has been studied in numerous works (Gökçay and Yetiş 1991; Mazierski 1995; Madoni et al. 1999; Lee et al. 1997), there is a lack of information on the response of nitrifying bacteria after prolonged exposure to heavy metals. Most of the experiments on heavy metal toxicity to nitrifiers were carried out in batch reactors with short contact times and also with unacclimated microorganisms (Gökçay and Yetiş 1991; Hu et al. 2002). Furthermore, the main reason of observing lower inhibitory levels in continuous flow systems compared to batch systems has not been fully clarified yet.

In this study, the inhibitory effect of long-term Cd input was evaluated in a continuous-flow nitrifying reactor. The inhibitory characteristics of Cd in continuous-flow and batch nitrification systems were compared. Most importantly, results of molecular methods were used to find the relationship between the changes in microbial diversity and the response of the enriched nitrifying culture to heavy metal inhibition. By this way, the link between inhibition and nitrifying species was delineated.

Materials and methods

Experimental set-up

In order to investigate the effects of Cd on nitrifying bacteria only, the experimental studies were carried out in a continuous-flow nitrification system enriched in terms of nitrifiers. A completely mixed 20 l aeration tank was connected by a plastic tube to a 10 l settling tank. The reactor was operated at sludge retention time (SRT) of 20 day and hydraulic retention time (HRT) of 1.0 day. One liter of the mixed liquor was daily removed from the aeration tank to achieve the desired SRT. A peristaltic pump was utilized to recycle the settled sludge at a flow rate of 10 l/day. The reactor was aerated and completely mixed by two circular aquarium air stone diffusers which are connected to an air pump (Millipore Inc.). Since the activity of nitrifying bacteria is considerably influenced by environmental conditions, temperature, pH, and dissolved oxygen (DO) were constantly controlled. The pH of the reactor was controlled by a pH control unit. One molar sodium bicarbonate ($NaHCO_3$) and 0.5 M hydrochloride acid (HCl) solutions were automatically added to the aeration tank to maintain the reactor pH at 7.5 ± 0.2 and to supply the required inorganic carbon. The temperature within the activated sludge unit was maintained constant at 25°C by the aquarium heater. DO levels were kept around 6–7 mg/l and complete mixing inside the reactor was provided by aeration.

Medium

The feed was synthetically prepared with de-ionized water and had the following composition: 50–

250 mg/l $(NH_4)_2SO_4$ as ammonium source, 50 mg/l $MgSO_4 \cdot 7H_2O$, 5 mg/l $MnSO_4 \cdot H_2O$, 2.56 mg/l $CaCO_3$, 10 mg/l $FeSO_4 \cdot 7H_2O$, 8.1 mg/l K_2HPO_4. This solution was devoid of organic carbon to disable heterotrophic growth and enhance the dominance of nitrifiers in the sludge. All chemicals were supplied from Merck KGaA, Germany.

Experimental procedure

The experimental period consisted of the following two phases:

Phase I: In the absence of Cd, the biokinetic parameters were determined by operating the continuous-flow reactor at various influent ammonium loadings (190–600 mg NH_4–N/g VSS. day). The influent ammonium was changed to a new value upon attainment of steady-state at each initial ammonium concentration.

Phase II: Cd was continuously fed and its level was gradually increased in each run. The influent ammonium concentration was chosen as 200 mg/l NH_4–N during this phase to provide substrate independent removal. Increments in Cd feeding were adjusted such that less than 50% decrease took place in ammonium utilization or nitrate production rates. The influent Cd loading to the system was increased in increments of 2 mg/l Cd. When the decreases in ammonium utilization rate (qNH_4–N) and nitrate production rate qNO_3–N) were higher than or close to 50%, Cd feeding was stopped and the system was fed with NH_4–N only. At each influent Cd concentration, the reactor was operated for at least 14 day. This period was necessary to reach steady-state conditions and to acclimate nitrifying bacteria to Cd. The operating conditions in Phases I and II are shown in Fig. 1.

Determination of nitrifier activity

Throughout the experimental period, samples were daily taken from the feed tank, effluent collection tank (composite samples) and from the aeration tank (grab sample). NH_4–N, NO_2–N and NO_3–N concentrations were then measured. Ammonium utilization and nitrate production rates calculated by Eqs. 1 and 2 were used for controlling the efficiency and performance of the system.

$$qNH_4\text{–}N = \frac{Q(NH_4\text{–}N_{inf} - NH_4\text{–}N_{eff})}{V \cdot X} \quad (1)$$

$$qNO_3\text{–}N = \frac{Q(NO_3\text{–}N_{eff})}{V \cdot X} \quad (2)$$

where NH_4–N_{inf}, NH_4–N_{eff} are the influent and effluent ammonium concentrations, respectively, mg/l, NO_3–N_{eff} is NO_3–N concentration in grab and composite samples, mg/l, Q is the influent flow rate, l/day, X is the biomass concentration, mg/l, V is the volume of the aeration tank, l. Inhibition at a particular Cd dose was found by the daily calculation of ammonium utilization rate relative to the previous steady-state ammonium utilization rate determined in the absence of Cd. Then, the arithmetic mean of these daily inhibition values was reported as a singular value for that particular Cd dosing period and the 95% confidence interval was added.

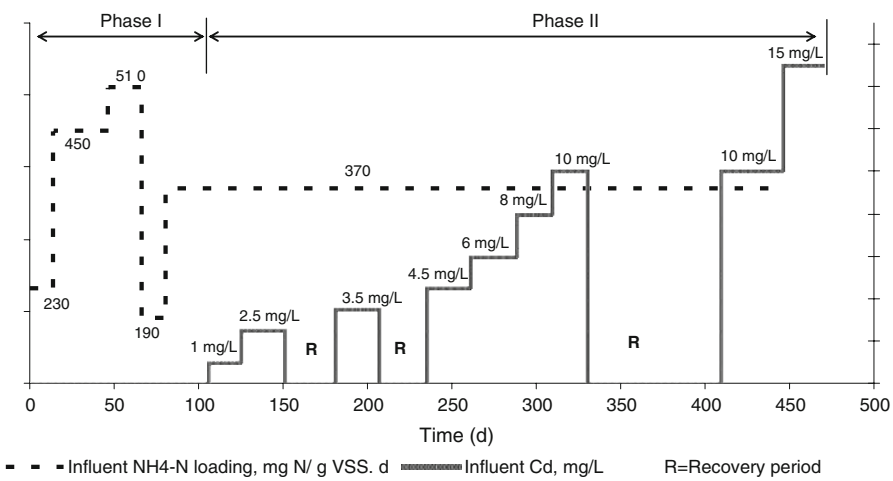

Fig. 1 The operating conditions in Phases I and II

For interpretation of results, the concentration of the free ammonia (NH_3–N) concentration was calculated at all conditions by using Eq. 3.

$$NH_3-N = \frac{NH_4-N}{1+10^{(9.25-pH)}} \quad (3)$$

The half-saturation constant $\left(K_{S,NH_4-N}\right)$ and maximum ammonium utilization rate $\left(q_{max,NH_4-N}\right)$ and maximum nitrate production rate $\left(q_{max,NO_3-N}\right)$ were estimated by nonlinear regression.

Cd concentration was measured in samples taken from the feed tank, inside the reactor and from the secondary clarifier. Labile Cd (Cd_{volt}) in the bulk solution (mixed liquor) was measured by voltammetry in samples filtered through 0.45 μm syringe filters (Millipore Corp., USA).

Analyses

Both total (Cd_T) and labile Cd (Cd_{volt}) concentrations were measured by voltammetry using the VA 797 Computrace, Metrohm Inc. which was operated using the Differential Pulse Polarography (DPP) in the Dropping Mercury Electrode (DME) mode. Operational conditions were as follows: start:end potential: −0.2:−0.7 V, initial purge time: 400 s, pulse amplitude: 0.0505 V, pulse time: 0.4 s, voltage step: 0.00595 V, sweep rate: 0.0149 V/s, peak potential: -0.585 ± 0.08 V, electrolyte solution: 3 mol/l KCl. The total metal in the sample was determined after adjusting the pH < 2 with ultrapure HCl and digestion by Ultraviolet (UV) photolysis using the UV digester (Metrohm Inc.). In digestion, for the destruction of inert metal complexes, 0.5 ml concentrated HCl and 0.5 ml of 30% H_2O_2 were added to 10 ml of samples. Samples were irradiated with UV at 80–85°C for 4 h and then measured with voltammetry. NH_4–N concentrations were analyzed using the Nessler Method with Hach DR/2000 spectrophotometer. NO_2–N concentrations ranging from 0 to 150 mg/l were analyzed by Ferrous Sulfate Method (Method 8153) with Hach DR/2000 spectrophotometer by using Nitriver 2 powder pillows. NO_2–N concentrations ranging from 0 to 0.3 mg/l NO_2–N were analyzed by Diazotization Method (Method 8057) with Hach DR/2000 spectrophotometer by using Nitriver 3 powder pillows. NO_3–N concentrations were analyzed by Cadmium Reduction Method (Method 8039) with HACH DR/2000 spectrophotometer by using Nitra Ver5 powder pillows.

The DO concentrations and pH were measured using inoLab Oxi 730 meter (WTW, Germany) and inoLab-1 pH meter (WTW, Germany), respectively. Mixed liquor suspended solids (MLSS) and volatile suspended solids (VSS) analyses were performed using the Method 2540E in Standard Methods (APHA, AWWA, WPCF 1998).

Results and discussions

Nitrification efficiency in the absence of Cd

In the first phase, the continuous-flow reactor was operated at various ammonium loadings as shown in Fig. 1 to assess the nitrification efficiency and find the biokinetic parameters. In this period, NH_4–N, NO_2–N, NO_3–N and MLVSS concentrations were daily measured as shown in Fig. 2. At all NH_4–N loadings, 98% of the influent ammonium was removed except in the transition phase in which the efficiency dropped to 91% for 1 day only. The effluent NH_4–N concentration on Day 61 increased to 92 mg/l as a result of pH drop to 4 which was due to a failure in pH control system. In parallel to NH_4–N increase, nitrite oxidation decreased gradually. For a certain period, free ammonia concentration ranged from 2.01 to 0.05 mg/l which resulted in nitrite accumulation with a maximum NO_2–N concentration of 110 mg/l. The recovery from free ammonia inhibition lasted 6 day. Another nitrite accumulation was observed during the transition period when the applied ammonium loading increased from 190 to 370 mg NH_4–N/g VSS. day. The NH_4–N removal ceased for a certain period of time. However, nitrite accumulations in the first and second stages differed from each other. In literature, the threshold free NH_3–N concentration for nitrite oxidation inhibition was reported as 0.1 mg/l (EPA 1993). In our system, only 50% inhibition was observed around 1 mg/l free NH_3–N, which is 10 times higher than the reported value. For 0.16 mg/l NH_3–N, the decrease in nitrite oxidation was around 14%. On the other hand, in the second case, nitrite oxidation inhibition started at a much lower free ammonia concentration of around

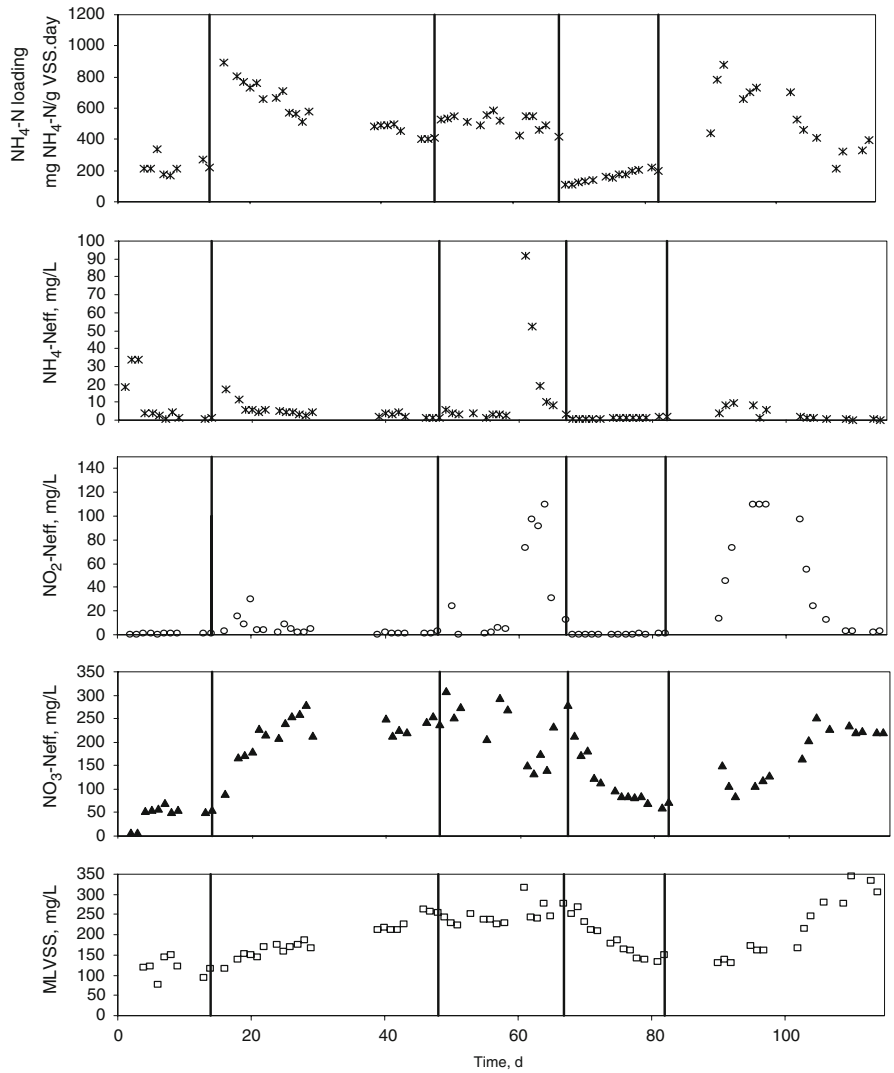

Fig. 2 Daily effluent NH_4–N, NO_2–N, NO_3–N and MLVSS measurements in Phase I (without Cd)

0.07 mg/l. The inhibition was around 50% at 0.16 mg/l free NH_3–N in this period. Nitrite oxidation recovered after the MLVSS concentration in the reactor reached a level around 300 mg/l. These findings suggest that the threshold free ammonia concentration was possibly related to the quantity of nitrite oxidizers in a nitrifying culture. If the quantity of nitrite oxidizers is high enough to resist free ammonia, nitrite oxidation proceeds until to a certain level of free ammonia concentration. For a better judgment, the quantity of nitrite oxidizers in these two different periods should be compared. In both periods, the inhibition due to free ammonia was reversible.

Estimation of biokinetic parameters in the continuous-flow reactor

Steady-state ammonium utilization and nitrate production rates were first determined in the absence of Cd at each influent NH_4–N loading. The runs at 50 and 200 mg/l influent NH_4–N were conducted twice to check the repeatability of results. Even in different time periods (Day 1 and 66 for 50 mg/l, Day 47 and 106 for 200 mg/l), almost the same results were obtained under the same influent conditions indicating no change in nitrifier activity and fraction. The half-saturation constant for ammonium utilization, K_{S,NH_4-N}, q_{max,NH_4-N} and q_{max,NO_3-N} were estimated

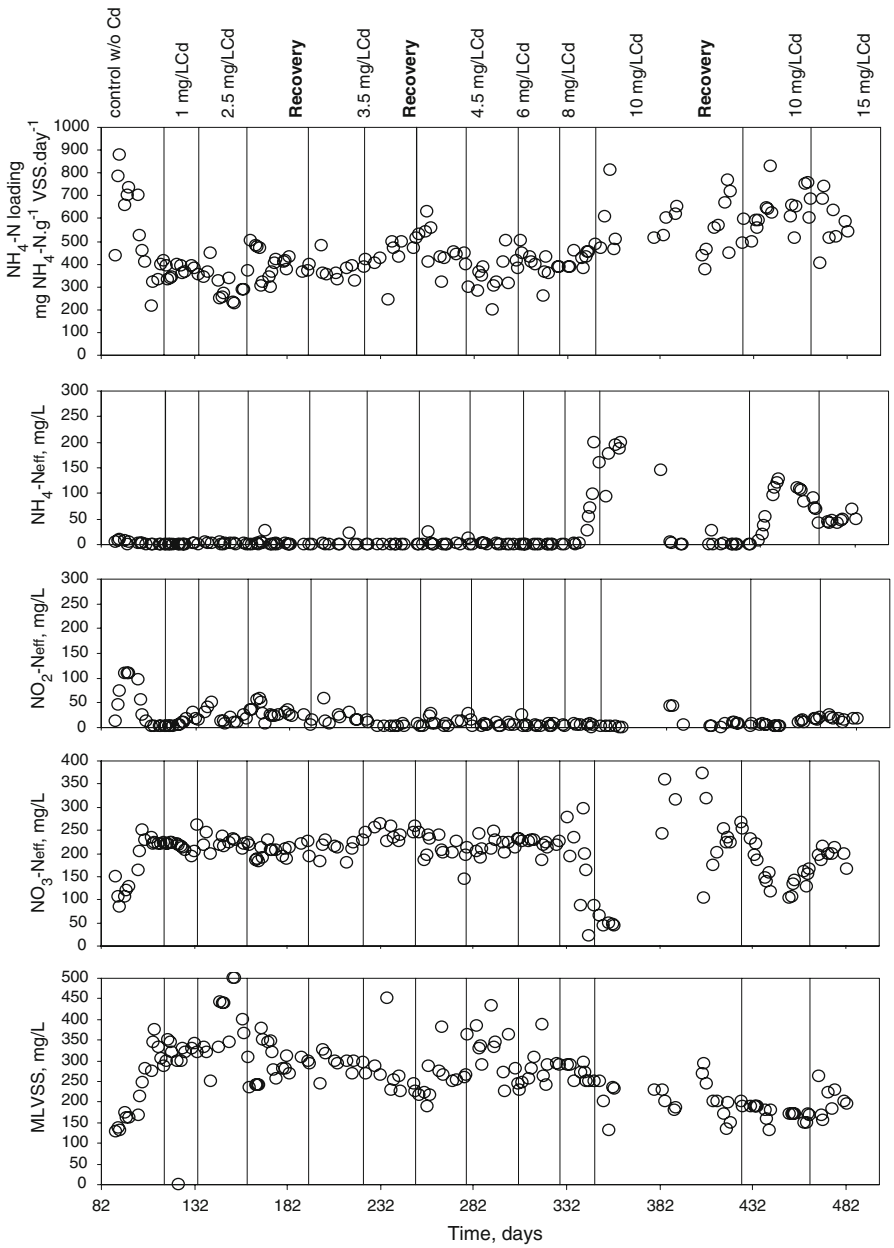

Fig. 3 Daily ammonium loadings, effluent NH_4–N, NO_2–N, NO_3–N and bulk MLVSS measurements in Phase II with Cd

by nonlinear regression method using the Graph PAD package program and found as 0.48 mg/l, 671 mg NH_4–N/g VSS day and 685 mg NO_3–N/g VSS day, respectively.

Effects of Cd on ammonium utilization and nitrite oxidation

To determine the changes in ammonium utilization and nitrate production rates in the presence of Cd, the continuous-flow system was operated at various influent Cd concentrations as shown in Fig. 1. In this period, NH_4–N, NO_2–N, NO_3–N and MLVSS were daily measured as shown in Fig. 3. Steady-state ammonium utilization and nitrate production rates at various influent Cd are shown in Fig. 4. Labile Cd (Cd_{volt}) concentrations in the bulk (mixed liquor) are given in Fig. 5.

Although addition of 1 mg/l of Cd initially decreased the specific ammonium utilization and

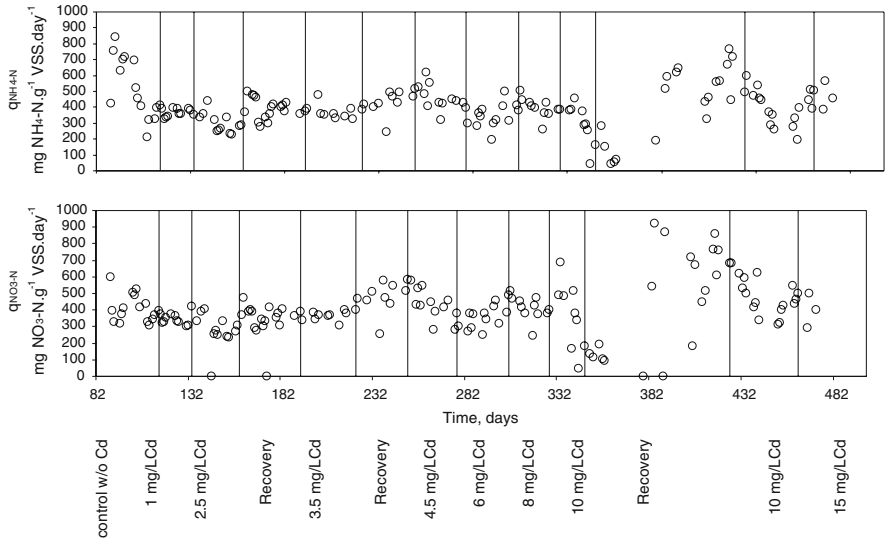

Fig. 4 Ammonium utilization (upper figure) and nitrate production rates (lower figure) in Phase II with Cd

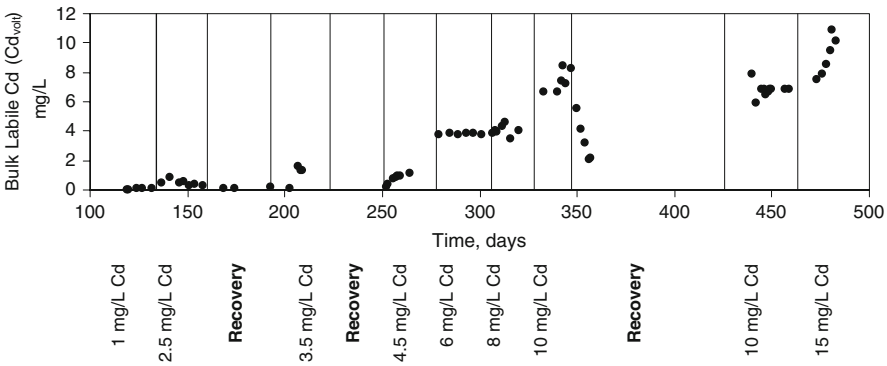

Fig. 5 Bulk labile Cd (Cd_{volt}) concentrations in Phase II

nitrate production rates by approximately 30%, after some while there was no measurable impact and there were essentially no changes in effluent pH, NH_4–N, NO_2–N and NO_3–N compared to baseline (before Cd addition) effluent concentrations. Ammonium removal was approximately 90% throughout the continuous feeding at 1 mg/l Cd. After the influent Cd concentration was increased to 2 mg/l, the specific ammonium utilization rate decreased to 269 mg NH_4–N/g VSS day corresponding to 47% inhibition. Under these conditions, the continuous feeding of Cd was stopped to see if any recovery took place. Nitrification recovered only partially by about 20%.

Inhibition ranged from 20% to 40% and no further increase in inhibition was exhibited in new runs except at 10 mg/l influent Cd (Fig. 6). At 10 mg/l Cd, the effluent ammonium concentration increased gradually to 200 mg/l which was accompanied by a

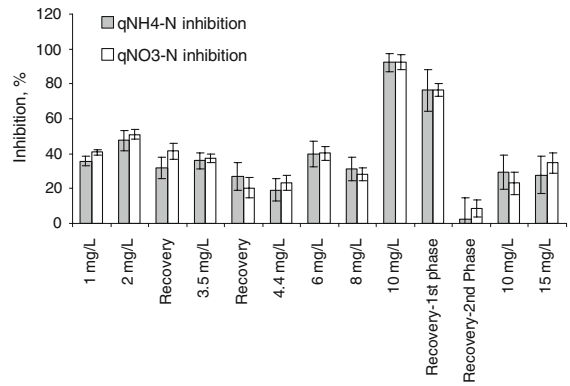

Fig. 6 Inhibition in the rates q_{max,NH_4-N} and q_{max,NO_3-N} at various Cd loadings (the error bars represent 95% confidence interval)

decrease in effluent nitrate concentration to 22 mg/l. The inhibition in the rates q_{NH_4-N} and q_{NO_3-N} was 90%. On the contrary, a serious nitrite accumulation

was not observed during this period which indicates that ammonia oxidizers are more sensitive than nitrite oxidizers as shown in previous studies (Lee et al. 1997). Continuous feeding of 10 mg/l Cd was stopped and the change in inhibition was then followed. The recovery from inhibition lasted about 37 day which was longer compared to other recovery periods. More interestingly, higher nitrate production rates were observed near the end of this recovery period. After attainment of recovery, 10 mg/l Cd was continuously fed to the system for the second time. Contrary to the first case, there was only 20% decrease in ammonium removal efficiency and 50% decrease in the rates q_{NH_4-N} and q_{NO_3-N} (Fig. 6). In the final step, the influent Cd concentration was increased to 15 mg/l and no change was observed in the level of ammonium oxidation inhibition.

The inhibition of nitrite oxidation was below 30% for all Cd dosings, although the free ammonia concentration increased to a maximum of 5 mg/l. In our previous batch experiments much higher inhibition levels were observed at these free ammonia concentrations (Semerci and Çeçen 2007). The relatively low inhibition at such a high free ammonia concentration could be the result of acclimation of nitrite oxidizers to free ammonia. This was also consistent with previous findings (Jianlong and Ning 2004).

Mechanism of heavy metal inhibition

Heavy metals are noncompetitive inhibitors as concluded in most of the studies (Beg et al. 1982; Lewandowski 1987; Mazierski 1995; Hu et al. 2002; Ren and Frymier 2003). In the presence of noncompetitive inhibitors, the specific ammonium utilization rate in the continuous nitrification system can be predicted by Eq. 4.

$$q_{NH_4-N} = \frac{q_{max,NH_4-N,app}S}{K_{S,NH_4-N} + S} = \frac{q_{max,NH_4-N}S}{(1 + \frac{I}{K_i})(K_{S,NH_4-N} + S)} \quad (4)$$

The q_{max,NH_4-N} and K_{S,NH_4-N}, were determined in the first phase of the study. The inhibitor concentration I was expressed in terms of bulk labile Cd rather than free or biosorbed Cd since inhibition was shown to depend on labile metal concentration (Semerci and Çeçen 2007; Semerci 2007). The continuous-flow

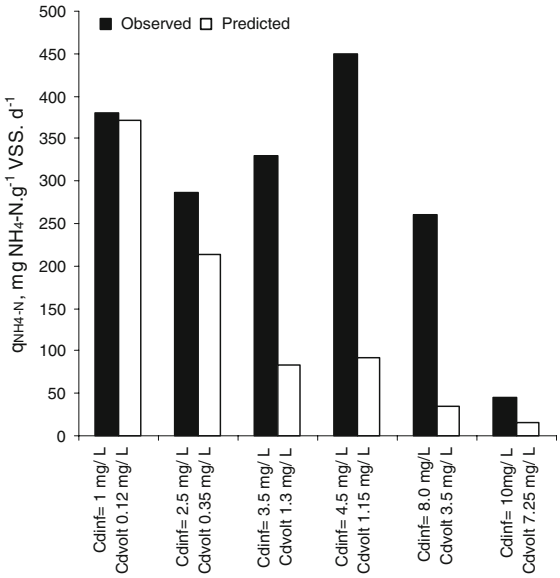

Fig. 7 Comparison of the observed and predicted ammonium removal rates (q_{NH_4-N}) at various labile Cd concentrations in the reactor

reactor was initially filled with the same sludge as in previous batch experiments. Therefore, in the present continuous-flow study the observed q_{NH_4-N} at various bulk labile Cd concentration were compared with the ones predicted according to the noncompetitive inhibition model (Fig. 7). For prediction of results, the inhibitor coefficient (K_i) was taken from our previous batch experiments (Semerci and Çeçen 2007) which was expressed in terms of labile Cd. As seen, in the initial periods of Phase II (1 and 2 mg/l), the observed q_{NH_4-N} values were very close to the predicted rates and decreased with Cd concentration. However, between 3.5 and 10 mg/l influent Cd, the observed q_{NH_4-N} values were considerably higher than the predicted ones, thus the inhibitory effect of Cd was lower than predicted (Fig. 7). The potential reasons for this observation could be explained by the changes occurring in microbial community which was investigated extensively in another paper using *amoA* and 16S rRNA gene based molecular methods (Mertoglu et al. 2008). The operational period can be divided into three phases in terms of the changes in nitrifying species according to the results of the molecular methods. The first phase covers the operation of the reactor in the absence of Cd and operation with continuous feeding at 1 and 2.5 mg/l influent Cd. *Nitrosomonas* sp. *clone Y34*, *Uncultured*

Nitrosomonas sp. c*lone Y35* and *Nitrosomonas eutropha* were the active species during this phase (Mertoglu et al. 2008). The second phase was started with the addition of 3.5 mg/l Cd. *Nitrosomonas*. sp. *clone Y34* and *Unc. Nitrosomonas*. sp. *clone Y35* disappeared and *N. eutropha* and *Nitrococcus mobilis* became dominant in the system during this phase (Mertoglu et al. 2008). There was a great difference in this period between observed and predicted q_{NH_4-N} as explained before. Furthermore, the observed inhibition throughout this period was inconsistent with previous batch experiments. The reason of inconsistency seems to be the change in microbial species since the biokinetic parameters in batch experiments had been determined with a biomass in which *Nitrosomonas*. sp. *clone Y34*, *Unc. Nitrosomonas*. sp. *clone Y35* and *N. eutropha* were dominant.

In the third phase, a major change occurred in the diversity of ammonia oxidizers due to the severe inhibition caused by 10 mg/l Cd (Fig. 6). Metal tolerant ammonia oxidizing *Nitrosospira* species became dominant as a result of severe inhibition as shown in the other paper (Mertoglu et al. 2008). As shown in Fig. 6, the recovery period consisted of two phases. During the first phase, inhibition continued for a certain period of time despite the stop of continuous Cd feeding. *N. eutropha* and *N. mobilis* were still present during this phase (Mertoglu et al. 2008). In the second phase, recovery was observed as indicated by an increase in q_{max,NH_4-N} and q_{max,NO_3-N}. These rates increased even to higher values than before Cd addition (Fig. 4). Since *Nitrosospira* sp. has high substrate affinity with low K_{S,NH_4-N} (Schramm et al. 1999), observation of higher rates is an expected result. A new Cd input of 10 mg/l and then another dosing at 15 mg/l Cd did not cause the expected inhibition.

Toxicity of heavy metals in biological systems was related to the factors such as type, concentration and the form of soluble metal (cation or complex), history of the activated sludge (acclimated or nonacclimated), operational parameters (pH), microorganism concentration, mean cell residence time and the presence of other compounds in wastewater (Tyagi et al. 1991; Zarnozsky et al. 1994). Since there are too many factors, differences may exist in published results. Due to the complexity of the process, more investigation is necessary in order to compare the results obtained by using different methods and generalize the heavy metal toxicity assessment procedure. Also, the stage of a culture and characteristics of microorganisms affect the concentration of metals necessary to inhibit biological systems. Jönsson et al. (2001) conducted inhibition tests with activated sludge originating from treatment plants that mainly received domestic wastewater and found out that this sludge was more sensitive to inhibitors than sludges from treatment plants with a considerable industrial load. The difference in results was attributed to the adaptation of the sludge to small amounts of a variety of toxic substances. In our case, microbial community shifted from *Nitrosomonas* and *Nitrosococcus* to *Nitrosospira* sp. rather than becoming adapted to toxic compound. In another study, *Nitrosospira* were found to be the predominant microorganism in habitats exposed to high toxic metal concentrations in soil media (Stephen et al. 1999). In the study of Tsai et al. (2005), *Nitrosomonas communis* was the predominant microorganism in the class of Betaproteobacteria before Cd addition. However, nitrifiers disappeared as a result of Cd inhibition. A comparison of inhibition data with previous studies could not be done since microbial composition was not investigated in most of the studies.

Conclusions

Experiments were carried out in a continuous-flow nitrifying system to simulate real activated sludge systems. The purpose was to monitor the performance and microbial changes in the nitrifying culture as a result of prolonged exposure to the toxic metal Cd.

Exposure of nitrifying bacteria to 1–2.5 mg/l of Cd resulted in moderate inhibition of nitrification. The inhibition during this period could be predicted with the model developed from batch experiments. Increases in influent Cd concentration to 3.5 mg/l and further to 8 mg/l did not increase the degree of inhibition. This was unexpected and also not consistent with previous batch experiments. It was seen that acclimation had occurred by the selection of heavy metal resistant species. Depending on the level of metal feeding, sensitive nitrifying species disappeared and heavy metal resistant bacteria became dominant in the sludge. As shown in detail in a complementary study (Mertoglu et al. 2008), at

moderate inhibition levels, *Nitrosomonas*. sp. *clone Y34*, *Unc. Nitrosomonas* sp. *clone Y35* species were present, but these species disappeared then. Instead, *N. eutropha* and *N. mobilis species* became dominant in the sludge. *Nitrosospira* sp. was found to be the most resistant species (Mertoglu et al. 2008). When this species became dominant in the system, only 50% inhibition was observed at 10 and 15 mg/l Cd.

The results of continuous-flow experiments also showed that activity of nitrifying bacteria could change as a result of shifts in microbial community. The great difference between the inhibition levels reported in various studies (Lee et al. 1997; Gerneay et al. 1997; Hu et al. 2002) for the same metal may be attributed to the existence of different nitrifying species in each system. This study is a good example on the importance of incorporation of molecular tools into heavy metal inhibition studies. These results also indicate that in future studies it would be better to report EC_{50} values along with the composition of the biomass.

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References

APHA, AWWA, WPCF (1998) Standard methods for the examination of water and wastewater, 20th edn. American Public Health Association, Washington

Beg SA, Siddiqi RH, Ilias S (1982) Inhibition of nitrification by arsenic, chromium, and flouride. J Water Pollut Control Fed 54(5):482–488

EPA (1993) Nitrogen control manual. EPA/625/R-93/010, US EPA, Washington, DC

Gernaey K, Verschuere L, Luyten L, Verstraete W (1997) Fast and sensitive acute toxicity detection with an enrichment nitrifying culture. Water Environ Res 69:1163–1169. doi:10.2175/106143097X125911

Gikas P, Romanos P (2006) Effects of tri-valent (Cr(III)) and hexa-valent (Cr(VI)) chromium on the growth of the activated sludge. J Hazard Mater B133:212–217. doi:10.1016/j.jhazmat.2005.10.023

Gökçay CF, Yetiş Ü (1991) Effect of chromium (VI) on activated sludge. Water Res 25(1):65–73. doi:10.1016/0043-1354(91)90100-5

Hu Z, Chandran K, Grasso D, Smets BF (2002) Effect of nickel and cadmium speciation on nitrification inhibition. Environ Sci Technol 36(14):3074–3078. doi:10.1021/es015784a

Hu Z, Chandran K, Grasso D, Smets BF (2004) Comparison of nitrification inhibition by metals in batch and continuous-flow reactors. Water Res 38:3949–3959. doi:10.1016/j.watres.2004.06.025

Jianlong W, Ning Y (2004) Partial nitrification under limited dissolved oxygen conditions. Process Biochem 39:1223–1229

Jönsson K, Aspichueta E, de la Sota A, la Jansen JC (2001) Evaluation of nitrification-inhibition measurements. Water Sci Technol 43(1):201–208

Lee Y-W, Ong S-K, Sato C (1997) Effects of heavy metals on nitrifying bacteria. Water Sci Technol 36(12):69–74. doi:10.1016/S0273-1223(97)00730-0

Lewandowski Z (1987) Behaviour of biological reactors in the presence of toxic compounds. Water Res 21(2):147–153. doi:10.1016/0043-1354(87)90043-1

Madoni P, Davoli D, Guglielmi L (1999) Response of SOUR and AUR to heavy metal contamination in activated sludge. Water Res 33(10):2459–2464. doi:10.1016/S0043-1354(98)00455-2

Mazierski J (1995) Effect of chromium (Cr(VI)) on the growth rate of activated sludge. Water Res 29:1479–1482. doi:10.1016/0043-1354(94)00315-X

Mertoglu B, Semerci N, Guler N, Calli B, Cecen F, Saatci A M (2008) Monitoring of population shifts in an enriched nitrifying system under gradually increased cadmium loading. doi.:10.1016/j.jhazmat.2008.03.056

Neufeld RD, Hermann ER (1975) Heavy metal removal by acclimated activated sludge. J Water Pollut Control Fed 47(2):310–329

Ren S, Frymier PD (2003) Kinetics of the toxicity of heavy metals to luminescent bacteria. Adv Environ Res 7(2):537–547. doi:10.1016/S1093-0191(02)00022-9

Schramm A, de Beer D, den van Heuvel JC, Ottengraf S, Amann R (1999) Microscale distribution of populations and activities of *Nitrosospira* and *Nitrospira* spp. along a macroscale gradient in a nitrifying bioreactor: quantification by in situ hybridization. Appl Environ Microbiol 65(8):3690–3696

Semerci N (2007) Effect of heavy metal speciation on nitrification inhibition. PhD Dissertation, Boğaziçi University, İstanbul

Semerci N, Çeçen F (2007) Importance of cadmium speciation in nitrification inhibition. J Hazard Mater 147(1):503–512. doi:10.1016/j.jhazmat.2007.01.041

Stephen RJ, Chang Y-J, Macnaughton SJ, Kowalchuk GA, leung KT, Flemming CA (1999) Effect of toxic metals on indigenous soil β-Subgroup proteobacterium ammonia oxidizers community structure and protection against toxicity by inoculated metal-resistant bacteria. Appl Environ Microbiol 65(1):95–101

Tsai YP, You SJ, Pai TY, Chen KW (2005) Effect of cadmium on composition and diversity of bacterial communities in activated sludges. Int Biodeterior Biodegradation 55:285–291. doi:10.1016/j.ibiod.2005.03.005

Tyagi RD, Couillard D, Tran FT (1991) Analysis of final settling tank in relation to control of metal inhibition in the activated sludge process. Can J Chem Eng 69:534–543

Yetiş Ü, Gökçay CF (1989) Effect of nickel (II) on activated sludge. Water Res 23(8):1003–1007. doi:10.1016/0043-1354(89)90174-7

Zarnozsky L, Derco J, Kuffa R, Drtil M (1994) The influence of cadmium on activated sludge activity. Water Sci Technol 30(11):235–242