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Importance of cadmium speciation in nitrification inhibition

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Abstract

In this study, the influence of Cd speciation on nitrification inhibition was investigated in batch suspended growth activated sludge systems which contain biomass enriched in terms of nitrifiers. For this purpose, parallel measurements of specific oxygen uptake rates (SOUR), ammonium utilization rates (q_{NH_4-N}) and Cd uptake were carried out. Cd speciation was adjusted with a strong complexing agent, ethylenediaminetetraacetic acid (EDTA). Free and biosorbed Cd concentrations were theoretically determined by using the MINEQL+ program and the Cd adsorption constant, whereas labile Cd was determined by voltammetric measurements. The presence of EDTA decreased nitrification inhibition by lowering the available Cd species and by preventing biosorption of Cd. Almost complete recovery from inhibition was attained by EDTA addition to nitrifying bacteria which were inhibited by Cd for a certain time. These results suggested that the sites sensitive to Cd were rather located on the surface of bacterial cell than inside. Nitrification inhibition depended on equilibrium concentrations of free (Cd^{2+}), labile (Cd_{volt}) and biosorbed Cd (Cd_{volt}) and did not correlate with the total Cd. The measurement of labile metal by voltammetry in inhibition studies is a promising approach since it is easy to apply in practice.

Keywords: Cadmium; Inhibition; Nitrification; Speciation; Voltammetry; Activated sludge; Biosorption

1. Introduction

The presence of heavy metals can adversely affect the operation of biological treatment processes [1,2] by accumulating to inhibitory concentrations. Several studies investigated the effects of heavy metals in biological systems alone or in combination with others [3–6]. A quite high variation is seen in the reported inhibitory range for metals, since different experimental conditions are existing in all studies (i.e. exposure time, type of buffer, pH, type and concentration of ligands). In addition to this, interpretation of results is based on different metal species such as total, labile, free or biosorbed metal. Under these circumstances, it is very difficult to compare the inhibitory concentration ranges.

Recent studies show that the bioavailability and toxicity of dissolved metals are strongly related to the free metal concentration rather than to the total [7–12]. The free ion activity model (FIAM) was applied to correlate toxicity and free metal ion [7,8,15]. Exceptions to the free ion activity model (FIAM) were discussed in detail by Campbell [9]. Degryse et al. [13] and Campbell et al. [14] showed that metals in the form of hydrated ions, labile organic and weak inorganic ligands such as chloride, sulfate, carbonate etc. may also contribute to toxicity by dissociating into the free form. Hence, besides the free metal ion, the importance of the labile metal concentration should also be considered in relating biological response (uptake or toxicity) to metal speciation. Similar to the FIAM, the biotic ligand model (BLM) was developed to investigate the relationship between the biological response and the metal adsorbed to sensitive sites at biological surfaces [12,15–18]. Much of the pioneering work on metal-organism interactions has been carried out with unicellular algae through the application of FIAM and BLM models alone or in combination. In biotreatment systems, soluble or total metal concentrations are usually measured by atomic absorption spectroscopy (AAS) and chemical equilibrium models such as MINEQL 4.5 or WHAM are used for the determination of free metal ion concentration or metal speciation. On the other hand, in the application of the BLM model in metal-surface complexation, the conditional stability constant has to be determined for the calculation of biosorbed

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Nomenclature

Cd_{bio}	biosorbed Cd concentration calculated with MINEQL+ (mg/L)
Cd_{volt}	Cd concentration measured in voltammetry (Cd^{2+} and Cd in weak complexes) (mg/L)
Cd^{2+}	free Cd concentration (mg/L)
I	% inhibition in q_{NH_4-N} or SOUR
I_{max}	maximum % inhibition in q_{NH_4-N} or SOUR
K	equilibrium constant or the conditional stability constant for binding of the metal to sensitive sites at the cell surface (L/g)
K_L	the Langmuir adsorption constant (mol/L)
K_M	Cd concentration in mg/L causing 50% reduction in q_{NH_4-N}
M	free Cd species (mg/L)
[M–X-cell]	concentration of the metal–bacterial surface complex (mol/L)
q_e	the adsorption density of free metal species at equilibrium (mol/g)
q_{max}	the maximum adsorption density of free metal species (mol/g)
q_{NH_4-N}	specific ammonium utilization rate, mg NH_4–N/g VSS h
q_t	the adsorption density of free metal species at time t (mol/g)
SOUR	specific oxygen uptake rate, mg O_2/g VSS min
{−X-cell}	unoccupied bacterial surface site (sensitive site) (g/L)

metal concentration. The use of labile metal concentration for the interpretation of inhibitory concentration could eliminate the necessities mentioned above.

Nitrification is the key process in the removal of ammonium from wastewater. Due to their susceptibility to toxic compounds, nitrifying bacteria have been mostly used in inhibition studies. Interestingly, few studies exist on the interaction of metals with nitrifying bacteria [7,8,19]. Comparison of these studies shows that there is no quantitative agreement on the effective concentration of metals that causes 50% inhibition (EC_{50}) in nitrification. In particular, the effect of metal speciation on nitrification has been rarely investigated [7,8,11].

Based on these considerations, the objectives of our study were:

- to investigate the relationship between heavy metal speciation and nitrification inhibition. The labile Cd is suspected to be the inhibitory form and consists of free Cd (Cd^{+2}) and Cd in weak complexes. Therefore, voltammetry was employed to differentiate between the total and the labile Cd. Cd was selected as the model heavy metal since it is the best known toxic heavy metal [20], susceptible to complexation and one of the mostly found in municipal and industrial wastewater treatment plants [21]. Cd speciation was adjusted with the use of the organic compound ethylenediamine tetraacetic acid (EDTA) which forms strong complexes with Cd [22]. Recovery from inhibition was studied by using EDTA as a washing agent.
- to model inhibition in terms of free, labile and biosorbed forms of Cd.

For these purposes, experiments were carried out in batch reactors with an activated sludge enriched in terms of nitrifiers. The inhibitory effect of Cd was examined by the measurement of specific oxygen uptake (SOUR) and ammonium utilization (q_{NH_4-N}) rates. Voltammetry was used for the determination of the labile Cd that includes free Cd (Cd^{2+}) and Cd in weak complexes. Theoretical speciation of Cd was calculated by MINEQL+ (version 4.5) [23] and compared with voltammetric measurements.

2. Materials and methods

2.1. Cd speciation

Voltammetry measures the free Cd^{2+} in solution. But in the time scale of voltammetric measurements [24], also weak Cd complexes may contribute to this measurement by dissociating into free form (Cd^{2+}). Throughout the paper Cd measured in voltammetry was thus shown as Cd_{volt} which is equivalent to labile Cd. Additionally, the MINEQL+ program (Version 4.5) was used to calculate the theoretical Cd speciation depending on total Cd, pH and concentrations of cations and anions in the solution. The flow diagram for the theoretical and voltammetric Cd speciation is presented in Fig. 1. Voltammetrically measured Cd concentrations were compared with those calculated by MINEQL at the original sample pH of 7.5.

In all nitrification reactors the bulk medium consisted of following compounds: 0.203 mM $MgSO_4 \cdot 7H_2O$, 0.03 mM $MnSO_4 \cdot H_2O$, 0.026 mM $CaCO_3$, 0.036 mM $FeSO_4 \cdot 7H_2O$, 0.047 mM K_2HPO_4, 15 mM $NaHCO_3$, 1.856 mM $(NH_4)_2SO_4$, $3CdSO_4 \cdot H_2O$ (2.28–3.42 mM). Comparison of voltammetric Cd measurements with theoretical MINEQL speciation is shown in Table 1 for a total Cd of 5, 10, 20 mg/L.

The most important Cd species is the free Cd^{2+} as predicted by MINEQL. On the other hand, Cd_{volt} was slightly higher than this value due to the presence of labile complexes with low stability constants, which may dissociate into free form. A possible ligand in the medium is the HCO_3^- ion, which is an inorganic carbon source for nitrifiers. As seen in Table 1, the presence of bicarbonate leads to a number of Cd-carbonate species of significant concentration with variable stability constants. At a constant Cd of 0.446 mM, the dissolved solid, otavite ($CdCO_3$)s was not formed in a HCO_3^- range from 0.111 to 7 mM. In any case, dissolved solids were absent in our system.

In many nitrification studies a phosphate buffer consisting of $H_2PO_4^-$ and HPO_4^{-2} ions is used for pH adjustment at a ratio of 1:4 [26], which is another important ligand for Cd. In such cases, the dissolved solid, $Cd(PO_4)_2$, has a potential to precipitate at approximately pH > 7.5, leading to a decrease in the free Cd in the bulk solution. Therefore, the MOPS [(3-(*N*-morpholino)propanesulfonic acid] buffer with a final

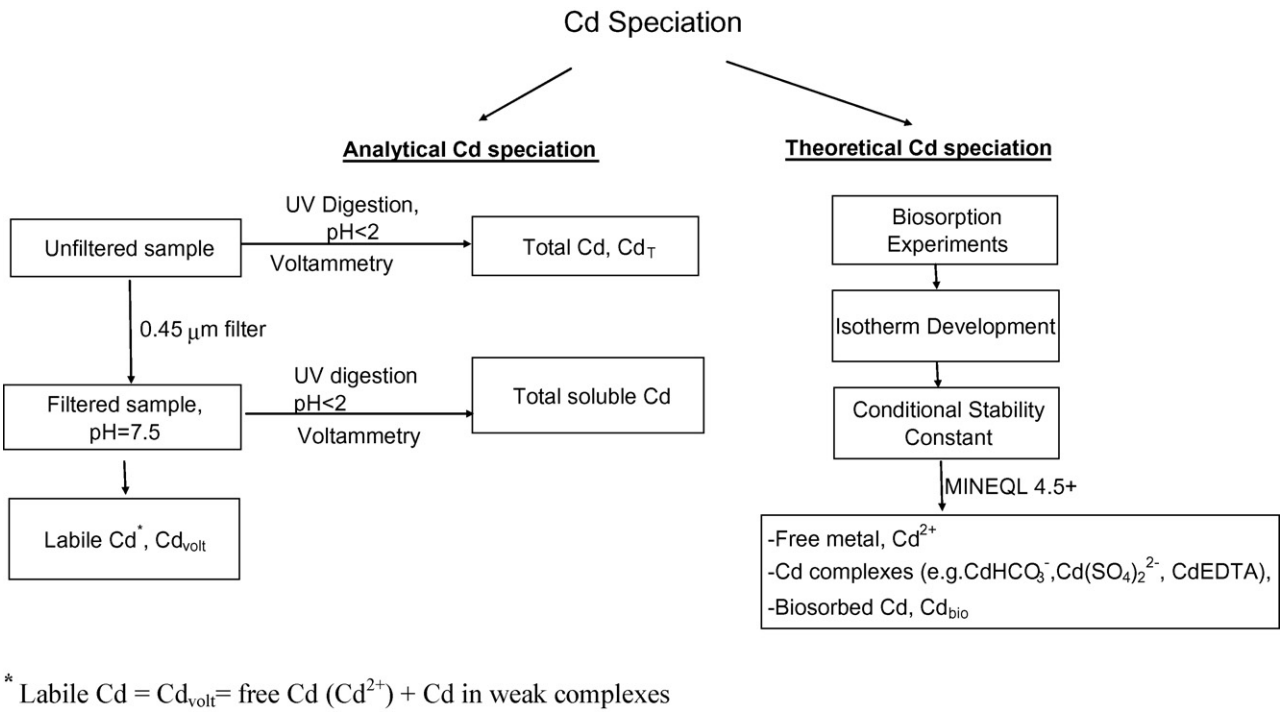

Fig. 1. Flow diagram of the Cd speciation procedure.

concentration of 20 mM was used in the adjustment of pH to 7.5 ± 0.2. This buffer does not form metal complexes [7,10,13] and also not interfere in voltammetry [13].

2.2. Determination of nitrification activity in the presence of Cd

2.2.1. Nitrifying sludge

The seed sludge enriched in terms nitrifiers was taken from another study [27]. It was then cultured in a 16 L batch reactor at a sludge age of 16 days. The reactor was daily fed on a fill-and-draw principle using the medium in Section 2.1. Aeration and mixing were accomplished by air supply. The bulk DO was about 3–4 mg/L. The initial NH_4^+–N concentration was about 250 mg/L. No organic carbon was supplied to the sludge in the enrichment period or in experimental runs. All experiments were carried out with sludge samples taken from this main reactor.

2.2.2. Nitrification runs

In batch reactors containing 300 ± 25 mg/L of MLVSS, both the specific ammonium utilization and oxygen uptake rates (SOUR) were determined in the presence and absence of Cd ranging from 0.0089 to 0.223 mM (1–25 mg/L). An activated sludge mixed liquor sample was taken from the main reactor and put into an 1000 mL aeration chamber 2 (Fig. 2). To this sludge, Cd was added at a determined concentration. Another activated sludge sample without Cd was set up as control (aeration chamber 1 in Fig. 2). The temperature of samples was kept at 25 °C. The samples were aerated by means of porous aquarium diffusers located at the bottom of the reactors and complete

Table 1
Cd species in the feed solution: comparison of MINEQL +4.5 and voltammetric results at pH 7.5 and pH < 2

Species	Stability constant, Log K [25]	Total Cd (mg/L), MINEQL results		
		5	10	20
Cd^{2+}	–	3.32	6.63	13.23
$CdOH^+$	−10.08	0.01	0.01	0.02
$CdHCO_3^+$	12.40	0.06	0.11	0.23
$Cd(CO_3)(aq)$	5.39	0.72	1.44	2.86
$Cd(CO_3)_2^{2-}$	7.22	0.02	0.04	0.07
$Cd(SO_4)_2^{2-}$	3.50	0.04	0.08	0.17
$Cd(SO_4)(aq)$	2.46	0.82	1.66	3.35
Total Cd according to MINEQL calculations, (mg/L)		4.99	9.97	19.93
Cd measured by voltammetry at pH 7.5 (Cd_{volt}) (mg/L)		4.06	8.39	16.65
Cd measured by voltammetry at pH < 2 (total Cd) (mg/L)		4.53	8.58	17.33

Composition of the medium 50 mg/L $MgSO_4{\cdot}7H_2O$, 5 mg/L $MnSO_4{\cdot}H_2O$, 2.58 mg/L $CaCO_3$, 10 mg/L $FeSO_4{\cdot}7H_2O$, 8 mg/L K_2HPO_4, 1260 mg/L $NaHCO_3$, 245 mg/L $(NH_4)_2SO_4$, 5–20 mg/L $3CdSO_4{\cdot}H_2O$.

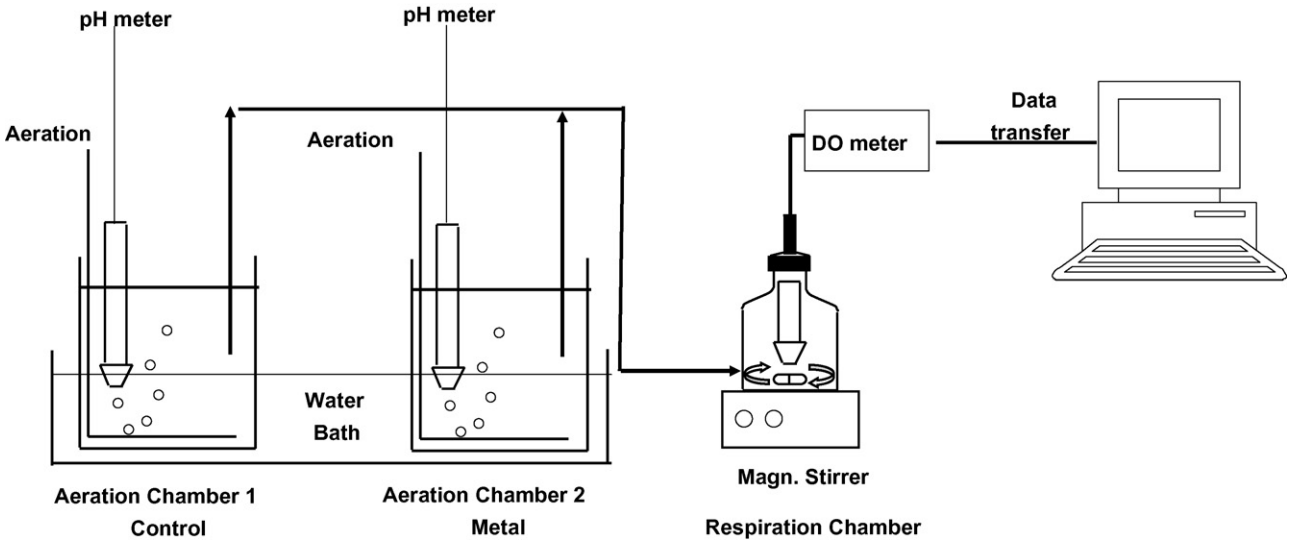

Fig. 2. Schematic presentation of experimental set-up.

mixing was provided. Ammonium and Cd were simultaneously added to the aeration chamber 2. Schematic presentation of the experimental set-up is shown in Fig. 2.

NH_4–N and Cd concentrations were measured in samples filtered through 0.45 μm syringe filters (Millipore Corp., USA) at the beginning and at specified time intervals over a period of at least 1 h. Ammonium utilization rates were determined from the slope of the concentration versus time plots for each Cd concentration. VSS concentrations of 300 ± 25 mg/L were measured in duplicate at the end of the tests for the calculation of specific ammonium utilization ($q_{NH_4–N}$) and specific oxygen uptake rates (SOUR).

For oxygen uptake rate measurements, at certain time intervals a portion of the mixed liquor was taken from the parent aeration chambers into a capped glass vessel (respiration chamber) with an exact working volume of 100 mL. The temperature of the vessel was constant at 25 °C during the measurement period. Complete mixing of the respiration chamber content was provided by a magnetic stirrer. A decrease in DO in the vessel due to the substrate oxidation and temperature of the solution during the test period were measured by a DO probe that has a high signal resolution and fast response (CellOx 325, WTW, Germany) and continuously recorded for 5 s intervals by a personal computer interfaced to the DO meter (WTW inoLab Oxi 730, Germany) (Fig. 2). OUR was calculated from the slope of the DO concentration versus time plots.

Inhibition was quantified in terms of the reduction in $q_{NH_4–N}$ and SOUR with respect to the control using Eq. (1):

$$I = \frac{\text{rate}_{\text{control}} - \text{rate}_{\text{metal}}}{\text{rate}_{\text{control}}} \times 100\% \quad (1)$$

In total, 25 runs were conducted. The initial NH_4–N in these experiments was chosen as 50 mg N/L and zero-order removal took place (data not shown) under uninhibited conditions. The bulk DO concentration in the aeration chambers was about 5–6 mg/L.

2.2.3. Effect of EDTA complexation on Cd Inhibition

EDTA may be present in nitrifying sludges forming strong complexes with metals and reducing the inhibition by decreasing the free metal ion concentration. The complexation of Cd^{2+} with EDTA is as follows:

$$[Cd^{2+}] + [EDTA] \leftrightarrow [CdEDTA] \quad (2)$$

The Cd–EDTA complex has a high stability constant of 18.20 and does not dissociate into free form. In additional nitrification runs, EDTA (0.08–0.1 mM) was used as an external chelating agent in order to determine the change in inhibition due to complexation. The next step was the recovery from inhibition by EDTA addition after exposure of sludge to Cd. In all runs, total and labile Cd concentrations were measured by voltammetry.

2.3. Cd sorption onto sludge

In accordance with batch runs, activated sludge (300 ± 50 mg/L) was contacted with 8.92×10^{-3} to 1.34 mM Cd (1–150 mg/L) and agitated by air for 24 h at a constant temperature of 25 °C. The pH varied in the range of 7.5 ± 0.2. After settlement of sludge, the metal in the supernatant was measured by voltammetry. Completion of sorption is achieved when the Cd concentration remains constant in the bulk liquid. Sorption of the Cd was completed in a rather short time of 30 min which is consistent with previous studies [28]. The metal uptake onto and/or into sludge was calculated as follows:

$$q_t = \frac{(C_0 - C_t)V}{M} \quad (3)$$

where q_t is the metal uptake per biomass at time t (mol metal/g biomass), C_0 and C_t are initial and final metal ion concentrations (Cd_{volt}, mol/L), respectively, M the dry biomass and V is the volume of the solution. At each equilibrium Cd concentration, q_e was determined and an isotherm was generated.

2.4. Analytical details

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 of the sample was determined after adjusting the pH < 2 with ultrapure HCl and digestion by 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. This procedure is also shown in Fig. 1. NH_4–N concentrations were analyzed using the Nessler Method with Hach DR/2000 spectrophotometer. The DO concentrations and pH were measured using inoLab Oxi 730 m (WTW, Germany) and inoLab-1 pH meter (WTW, Germany), respectively. MLSS and VSS analyses were performed using the Method 2540E in Standard Methods [26].

3. Results and discussion

3.1. Influence of total and initial labile Cd on ammonium utilization

The influence of total and initial labile Cd on specific ammonium utilization ($q_{NH_4–N}$) is presented in Fig. 3. The data in Fig. 3 are obtained from the same set of experiments. For most of the cases, the extent of inhibition increased as the total or initial labile Cd increased. The degree of inhibition reached 50% for a total Cd of 2–2.5 mg/L (0.0178–0.022 mM). A poor correlation was found between total Cd and inhibition, especially for EDTA added cases, as illustrated in Fig. 3a. For a total Cd of 15 mg/L (0.14 mM), inhibition ranged from 30 to 95.70%. On the contrary, the inhibition expressed in terms of $q_{NH_4–N}$ correlated better with the initial labile Cd (Fig. 3b).

3.2. Effect of Cd–EDTA complexation on nitrification

To assess the effect of CdEDTA complexation on nitrification, three batch reactors were operated in parallel and simultaneously NH_4–N, SOUR and Cd uptake measurements were carried out. The first reactor served as a control and contained biomass and ammonium only. The second reactor contained also a total Cd of 0.134 mM (15 mg/L), whereas the third one was fed with 0.134 mM (15 mg/L) Cd and 0.1 mM EDTA. The reactors were operated under identical conditions (pH 7.5, MLSS = 300 mg/L, MLVSS = 240 mg/L, $T = 25$ °C).

In the control reactor, NH_4–N utilization was linear ($R^2 = 0.98$) throughout the experiment period (Fig. 4a) which indicated zero-order removal. The average specific ammonium utilization rate was found as 49 mg NH_4–N/g VSS h. SOUR was initially 1.99 mg O_2/g VSS min and reached a maximum value of 3.0 mg O_2/g VSS min at 62 min. The corresponding NH_4–N utilization rate was 47 mg NH_4–N/g VSS h. The decrease in SOUR to 2.32 mg O_2/g VSS min at time 200 min could be due to nitrite accumulation which led to a lower oxygen consumption (Fig. 4b). This situation was also observed in previous batch experiments (data not shown).

As seen in Fig. 4a, in the presence of 15 mg/L of Cd alone, ammonium utilization was not linear and leveled off at some value. The $q_{NH_4–N}$ value decreased gradually within the experimental time and was calculated as 20, 20, 12, 10 and 8.7 mg NH_4–N/g VSS h at 0, 30, 120, 150 and 180 min. Reductions in $q_{NH_4–N}$ were 59, 59, 76, 79 and 82%, respectively. Preliminary experiments had shown that ammonium utilization followed a zero-order reaction at NH_4–N concentrations exceeding 5 mg/L (data not shown). Therefore, the decrease in $q_{NH_4–N}$ was the result of nitrification inhibition by Cd addition and could not be attributed to substrate limitations since the lowest NH_4–N concentration was 45 mg/L. Similarly, the specific oxygen uptake rates were reduced by inhibition and were found as 1.13, 0.72, 0.22 and 0.10 mg O_2/g VSS min at time 0, 38, 66 and 208 min (Fig. 4b). The corresponding percent reductions in SOUR were found as 51, 68, 91 and 96%. The reductions in SOUR were approximately 14% higher than those in $q_{NH_4–N}$. This can be attributed to the fact that SOUR represents the consumption of O_2 in both ammonium and nitrite oxidation steps [29].

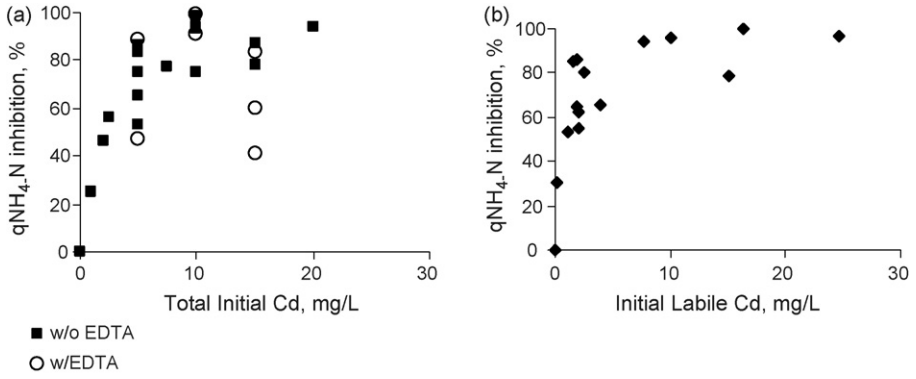

Fig. 3. Inhibition of specific ammonium utilization rate at various (a) total and (b) initial labile Cd (Cd_{volt}) concentrations.

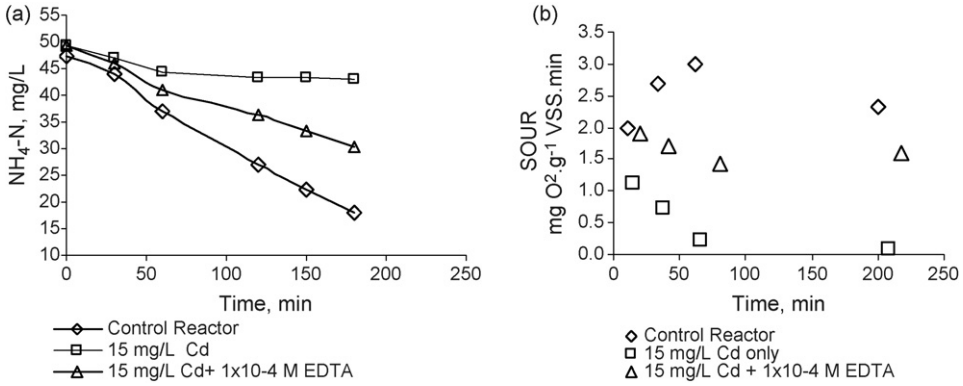

Fig. 4. Changes in nitrification activity with time in the presence and absence of Cd and EDTA, measured in terms of (a) ammonium utilization and (b) OUR profiles.

When Cd and EDTA were simultaneously added to the third reactor, there was a small reduction in q_{NH_4-N} and SOUR compared with the control reactor. The q_{NH_4-N} and SOUR did not change much with time as in the case of the second reactor. They decreased slightly to 28.8 mg NH4–N/g VSS h and 1.61 mg O_2/g VSS min (Fig. 4a–b). In the presence of Cd alone, the inhibition progressed with time due to uptake of Cd into/onto biomass. However, in EDTA added cases, ammonium utilization was linear and SOUR levels did not change much with respect to time (Fig. 4a–b).

In the case of Cd alone and simultaneous Cd and EDTA addition, Cd uptake was completed in 150 and 30 min, respectively, as reported in previous studies [28]. This also coincides with the change in reductions in q_{NH_4-N} and SOUR with time. The inhibition stayed constant after approximately 150 min in the first case whereas it reached a constant value after 30 min in the latter. These results support that there was a relationship between Cd uptake onto sludge and inhibition. Biologically active metal species are reported to be transported to the cell surface of any organism before they exert an effect [14]. Diffusion is the first transport step from the bulk solution towards the bacterial cell and is driven by the metal concentration gradient between the bulk solution and the cell wall. In EDTA added cases, only a slight change was observed in the percent reduction in SOUR and q_{NH_4-N} with time. This could be attributed to the decrease of the concentration gradient as a result of EDTA complexation with Cd species in bulk solution.

For a total Cd concentration of 15 mg/L, the relative decrease in q_{NH_4-N} and Cd uptake are shown as a function of EDTA concentration in Fig. 5. The Cd uptake and nitrification inhibition correlated with EDTA concentration.

EDTA is used as a washing agent to remove metals from bacterial surface [30]. EDTA was added to two parallel batch reactors, which had been contacted with Cd for 30 min (Fig. 6) to investigate the recovery from SOUR inhibition and the Cd species leading to inhibition. SOUR measurements were carried out at certain time intervals during the experiment period. Generally, in the present study, the use of EDTA removed the biosorbed Cd and thus led to a relief from inhibition as seen in Fig. 6a and b. Adsorbed Cd was quantitatively extracted at approximately 98% for a molar EDTA/Cd ratio of 1.82. Actually, the ratio was slightly lower than the effective EDTA concentration since also Ca, Mn and Mg in test medium form complexes with EDTA. Cd extraction from cell surface led to a noticeable recovery and the same SOUR level was reached as before Cd addition (Fig. 6a). Recovery from inhibition was abrupt with EDTA addition and the recovery rate was almost the same at different Cd concentrations as seen from Fig. 6. Extraction of adsorbed Cd was completed in an EDTA contact time of 2 min. As shown in Fig. 6b, almost no Cd remained on the biomass. It is a known fact that efflux of internalized metal does not occur when the contact time with EDTA is less than 5 min for different Cd loadings [30]. Our findings indicate that Cd sorbed to sensitive sites on the membrane surface (extracellular fraction) could be responsible for inhibition since only surface-bound Cd could be removed with EDTA extraction [30]. The recovery efficiency is highly related with the extraction efficiency, which in turn is dependent on the effective $EDTA_{eff}$/Cd ratio. This ratio should be at least 1:1 to achieve considerable extraction [30] since Cd forms 1:1 complexes with EDTA. Therefore, for practical purposes, it is likely that a nitrifying sludge exposed to Cd for a long time and retaining Cd may recover from inhibition by

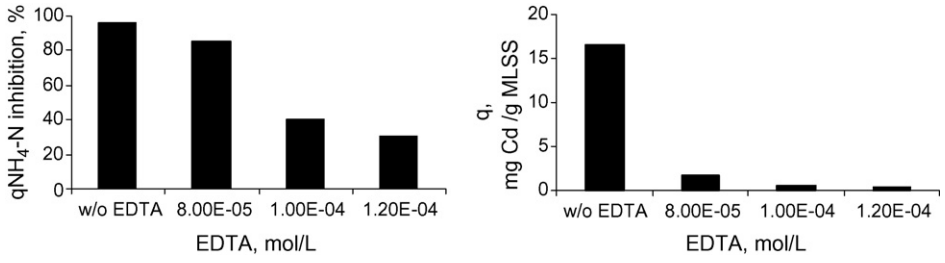

Fig. 5. Inhibition of q_{NH_4-N} and Cd uptake (q) as a function of EDTA concentration.

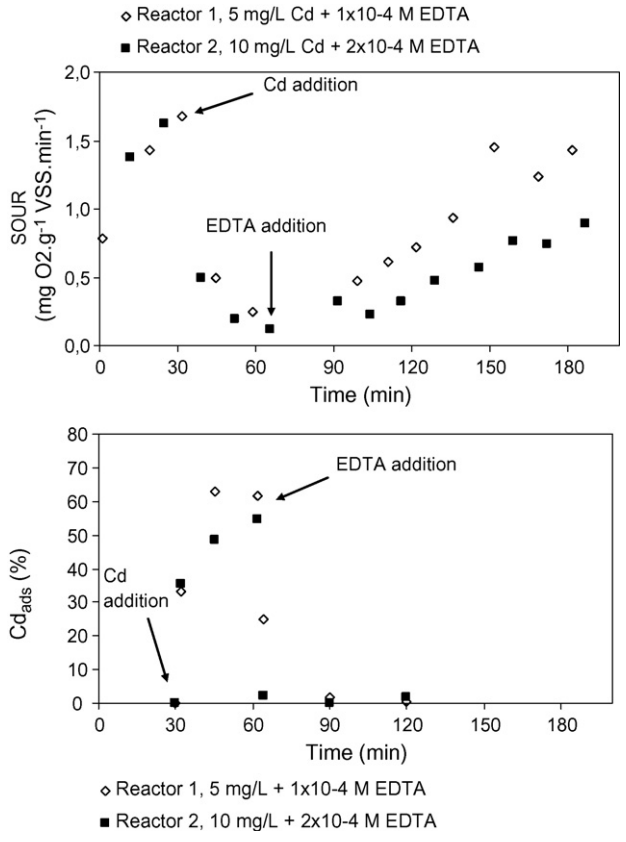

Fig. 6. Recovery of nitrification inhibition with EDTA addition.

EDTA addition. But, in real cases the Cd and EDTA are simultaneously present in a wastewater [21], therefore Cd is already complexed at the beginning and inhibition is not as severe as when Cd is alone.

3.3. Determination of conditional stability constants

Inhibition could be expressed in terms of both the free metal in the bulk medium and/or the biosorbed metal [12,16,18]. The biosorbed metal concentration can be calculated by equilibrium models such as MINEQL or WHAM [7,17], only if the adsorption constant (K_L) and concentration of binding site (y) are known.

Different methodologies exist to determine the conditional stability constant (K) and the concentration of binding site ($\{-X\text{-cell}\}$). These include measurement of metal internalization fluxes, metal loading and metal toxicity [18,28]. In the present study, the metal loading approach was applied using Langmuir adsorption isotherms (Fig. 7).

The interaction of a free surface site on the cell membrane, $-X$-cell, with a metal M^{Z+}, can be described as a surface complexation reaction as shown in Eq. (4) [16,28]:

$$M + -X\text{-cell} \overset{K}{\longleftrightarrow} M\text{–}X\text{-cell} \tag{4}$$

$$K = \frac{[M\text{–}X\text{-cell}]}{\{-X\text{-cell}\}[M]} \tag{5}$$

where M is the free metal species (mol/L), $-X$-cell the free surface site on the cell membrane (g/L), M–X-cell the metal–bacterial surface complex (mol/L) and K is the conditional stability constant (L/g).

A mass balance can be written for a bacterial surface in which the total mass, $\{X\text{-cell}\}_T$, is equal to the sum of unoccupied and occupied (complexed) sites by a metal [29]:

$$\{X\text{-cell}\}_T = \{-X\text{-cell}\} + \frac{[M\text{–}X\text{-cell}]}{y} \tag{6}$$

where $X\text{-cell}_T$ is the total bacterial (surface) mass (g/L) and y is the number of surface sites per unit mass of bacteria (mol/g). The Langmuir isotherm can be derived by substitution of Eq. (6) into Eq. (5). With some rearrangement, an explicit equation for [M–X-cell] can be obtained:

$$\frac{[M\text{–}X\text{-cell}]}{\{X\text{-cell}\}_T} = \frac{y[M]}{y/K + [M]} \tag{7}$$

The standard form of the Langmuir isotherm is as follows:

$$q = \frac{q_{max}[M]}{K_L + [M]} \tag{8}$$

where q is the adsorption density of free metal species (moles/g), q_{max} the maximum adsorption density of free metal species, (moles/g), [M] the equilibrium solution concentration of free metal species (mol/L), and K_L is the Langmuir adsorption constant (mol/L). Eqs. (7) and (8) are analog to each other and therefore the following relations can be found:

$$q = \frac{[M\text{–}X\text{-cell}]}{\{X\text{-cell}\}_T} \tag{9}$$

$$y = q_{max} \tag{10}$$

$$\frac{y}{K} = K_L \tag{11}$$

The Langmuir adsorption constant, K_L is related to the conditional stability constant K in Eq. (4) and is used in the MINEQL model. Both K and y can be calculated using Eqs. (10) and (11) if a Langmuir type isotherm can be derived from adsorption experiments, as done in Fig. 7.

Least square nonlinear regression analysis was applied to calculate the K_L and q_{max}, which were found as $10^{4.50}$ mol/L

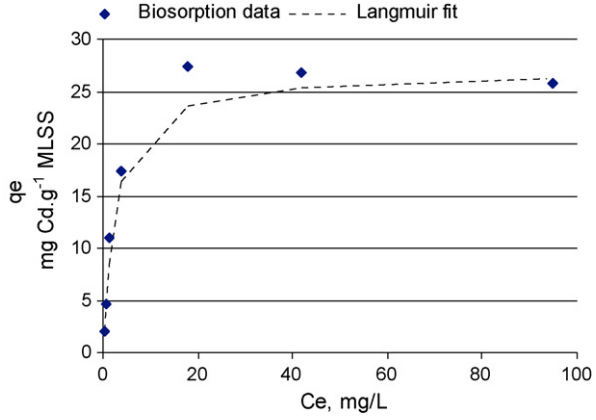

Fig. 7. Isotherm depicting the sorption of Cd onto biomass.

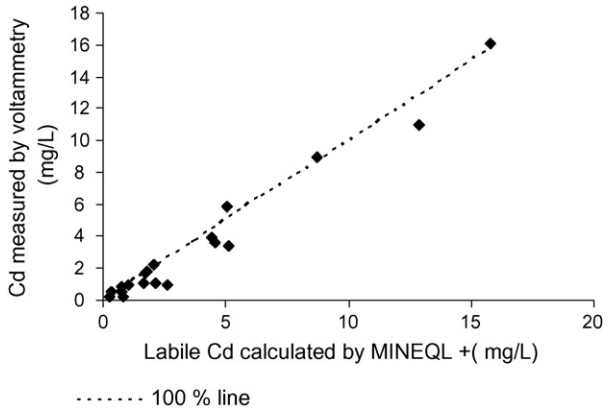

Fig. 8. Comparison of MINEQL and voltammetry results using the conditional stability constant (K_L).

($\log K_L = 4.50$) and 2.76×10^{-4} mol Cd/g cell, respectively. Then, the Langmuir adsorption constant (K_L) was incorporated into the MINEQL +4.5. Theoretical and analytical Cd speciation was done by following the flow diagram given in Fig. 1. As done in the present study, a comparative evaluation of K_L with two different methods, such as voltammetry and MINEQL, is often not made in literature. At low Cd concentrations consistent results were obtained with these methods (Fig. 8). The *t*-test was applied and the results were not significantly different from each other ($p > 0.05$) in the Cd range of 1–25 mg/L. As the initial Cd concentration increased (e.g. 50 mg/L), the difference between these two methods became statistically significant at equilibrium metal levels ($Cd^{2+} = 22.32$ mg/L in MINEQL, $Cd_{volt} = 42$ mg/L). Therefore, for calculation of biosorption at high Cd levels, the use of voltammetry is advised since it gives more reliable results than theoretical sorption predicted from chemical equilibrium.

3.4. Application of the free ion activity model (FIAM) and the biotic ligand model (BLM) in nitrification inhibition

In order to evaluate the effect of Cd speciation, the inhibition recorded in specific ammonium utilization rates (*I*) was correlated to different forms of Cd. Biological response (BR) (e.g. toxicity, uptake, growth) is directly proportional to the concentration of the surface complex, M–X-cell in the BLM model (Eq. (12)), whereas it is proportional to the activity of the free ion in solution, M, according to the FIAM model as shown in Eq. (13):

$$BR = k[\text{M–X-cell}] \quad (12)$$

$$BR = kK\{\text{X-cell}\}[\text{M}] \quad (13)$$

where *k* is constant of proportionality [16].

Both FIAM and BLM assume that the metal and its complexes in solution are in equilibrium with the metal bound to the surface. Therefore, equilibrium concentrations of different Cd forms were used in modeling. All data including EDTA, carbonate and phosphate complexation were considered. The inhibitory behavior with respect to different forms of Cd is shown in Fig. 9.

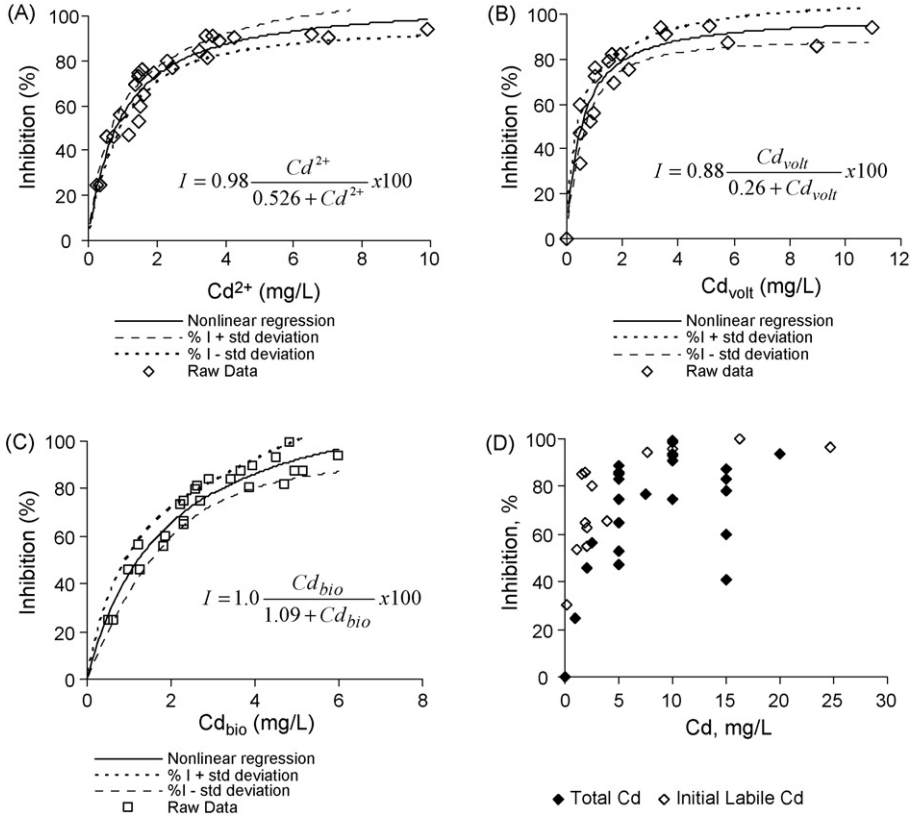

Fig. 9. Relationship between inhibition and different forms of Cd (a) equilibrium free Cd calculated by MINEQL, (b) equilibrium labile Cd measured with voltammetry, (c) equilibrium adsorbed Cd calculated with MINEQL and (d) initial total and labile Cd.

A saturation-type relationship as in Eq. (14) was used to correlate inhibition to the respective metal concentration, as done in other studies [31]:

$$I = \left(I_{\max} \frac{\mathrm{M}}{K_{\mathrm{M}} + \mathrm{M}} \right) \times 100\% \quad (14)$$

where $I_{\max}$ and I represent the maximum and observed % inhibition in q_{NH_4-N}, M represents metal concentration (either biosorbed Cd (Cd_{bio}), Cd measured by voltammetry (Cd_{volt}) or free Cd in MINEQL (Cd^{2+}), mg/L), K_M represents the Cd concentration in mg/L causing 50% reduction in q_{NH_4-N} (analog to EC_{50}).

Least square nonlinear regression was applied to find the model parameters in Eq. (14). Then, the maximum inhibition percentages, $I_{\max}$ and EC_{50} (or K_M) values were calculated for each Cd form as shown in Fig. 9a–c. Then, using these values, the model curves in Fig. 9a–c were drawn to compare experimental data with model results. Inhibition could be expressed with all three forms of Cd. This suggested that both the free ion activity model (FIAM) (Fig. 9a and b) and the biotic ligand model (BLM) (Fig. 9c) were applicable under the conditions of this study. In each case, the nonlinear regression had a high correlation coefficient ($R^2 = 0.95$) and the residuals were randomly distributed. As shown in Fig. 9d, inhibition did not correlate well with the total Cd and initial labile Cd (Cd_{volt}).

Here, the superiority of voltammetry is highlighted for Cd speciation in nitrification. Inhibition correlates best with voltammetric Cd results (Cd_{volt}) reflecting the labile Cd, both in the absence and presence of complexing agents. Additionally, this approach eliminates the need of assumptions in chemical equilibrium models. On the other hand, if atomic absorption spectroscopy (AAS) is used, the total soluble metal and not the free Cd concentration can be measured. Therefore, in order to apply the inhibition model basing on free and biosorbed Cd, using the equations in Fig. 9a and c, one should know the exact composition and pH of the water or wastewater. Most of the time, the determination of composition is difficult and time consuming. The stability constant of the organic matter-metal complexes and adsorption constant of Cd binding to sensitive sites should be known to calculate the free Cd ion or biosorbed Cd. On the other hand, the inhibition model based on Cd_{volt} (labile Cd concentration) is easy to use in practical applications. It only requires the measurement of the labile metal concentration with voltammetry.

4. Conclusions

The measurement methodologies applied in assessing heavy metal inhibition in biological systems should be carefully selected since physical and chemical speciation highly affects inhibition. A quite high Cd level may surprisingly lead to low inhibition in nitrification due to the complexing potential of Cd with inorganic and organic ligands. The diffusion of the metal to the sensitive sites could be retarded as a result of the decrease in the concentration gradient between the Cd in the bulk solution and on the surface of biomass. The complexation of metal may inhibit biosorption. In such cases, short-term activity measurement methods having a total duration of 5 or 10 min may lead to misleading information about the inhibitory values, especially for metals with slow uptake kinetics like Cd.

Inhibition was completely reversible when EDTA concentration was in excess compared to the metal concentration. This recovery from inhibition with EDTA addition supports the idea that rather Cd sorbed to sensitive sites on the membrane surface (extracellular fraction) could be responsible for nitrification inhibition. Both the FIAM and BLM models basing on the free metal ion activity and the biosorbed metal concentration were successfully applied in this study. A novelty in this study was to determine labile Cd analytically by voltammetry for nitrification inhibition. The good correlation between labile Cd and nitrification inhibition indicated that also weak Cd complexes could contribute to inhibition besides free Cd. This leads to the result that the labile metal concentration should also be considered in reporting inhibition levels. Therefore, the use of voltammetry is recommended in the assessment of the relationship between inhibition and metal speciation.

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References

[1] E.F. Barth, B.V. Salotto, G.N. McDermott, J.N. English, M.B. Ettinger, Effects of a mixture of heavy metals on sewage treatment processes, in: Proceedings of the 18th Ind. Waste Conf. Purdue, vol. 115, 1963, pp. 616–635.
[2] J. Wang, C.P. Huang, H.E. Allen, I. Poesponegoro, H. Poesponegoro, Effects of dissolved organic matter and pH on heavy metal uptake by sludge particulates exemplified by copper(II) and nickel(II): three variable model, Water Environ. Res. 71 (2) (1999) 139–147.
[3] Ü. Yetiş, C.F. Gökçay, Effect of nickel(II) on activated sludge, Water Res. 23 (8) (1989) 1003–1007.
[4] C.F. Gökçay, Ü. Yetiş, Effect of chromium(VI) on activated sludge, Water Res. 25 (1) (1991) 65–73.
[5] J. Mazierski, Effect of chromium(CrVI) on the growth rate of activated sludge, Water Res. 29 (1995) 1479–1482.
[6] F.B. Dilek, C.F. Gökçay, Ü. Yetiş, Combined effects of Ni(II) and Cr(VI) on activated sludge, Water Res. 32 (2) (1998) 303–312.
[7] H. Zhiqiang, K. Chandran, D. Grasso, B.F. Smets, Effect of nickel and cadmium speciation on nitrification inhibition, Environ. Sci. Technol. 36 (14) (2002) 3074–3078.
[8] F. Bramm, A. Klapwijk, Effect of copper on nitrification in activated sludge, Water Res. 15 (1981) 1093–1098.
[9] P.G.C. Campbell, Interactions between trace metals and aquatic organisms: a critique of the free ion activity model, in: A. Tessier, D.R. Turner (Eds.), Metal Speciation and Bioavailability in Aquatic Systems, John Wiley & Sons, Chichester, 1995, pp. 45–102.
[10] M.R. Twiss, O. Errécalde, C. Fortin, P.G.C. Campbell, C. Jumarie, F. Denizeau, E. Berkelaar, B. Hale, K.V. Rees, Coupling the use of computer chemical speciation models and culture techniques in laboratory investigations of trace metal toxicity, Chem. Speciation Bioavail. 13 (1) (2001) 9–24.
[11] Y.-W. Lee, S.-K. Ong, C. Sato, Effects of heavy metals on nitrifying bacteria, Water Sci. Technol. 36 (12) (1997) 69–74.
[12] C.S. Hassler, V.I. Slaveykova, K.J. Wilkinson, Some fundamental (and often overlooked) considerations underlying the free ion activity and biotic ligand models, Environ. Toxicol. Chem. 23 (2) (2004) 283–291.

[13] F. Degryse, E. Smolder, R. Merckx, Labile Cd complexes increase Cd availability to plants, Environ. Sci. Technol. 40 (3) (2006) 830–836.
[14] P.G.C. Campbell, O. Errécalde, C. Fortin, V.P. Hiriart-Baer, B. Vigneault, Metal bioavailability to phytoplankton-applicability of the biotic ligand model, Comp. Biochem. Physiol. Part C 133 (2002) 189–206.
[15] S. Huang, Z. Wang, Application of anodic stripping voltammetry to predict the bioavailable/toxic concentration of Cu in natural water, Appl. Geochem. 23 (8) (2003) 1215–1223.
[16] P.L. Brown, S.J. Marrkich, Evaluation of the free ion activity model of metal–organism interaction: extension of the conceptual model, Aquat. Toxicol. 51 (2000) 177–194.
[17] S. Jansen, R. Blust, H.P. Van Leeuwen, Metal speciation dynamics and bioavailability: Zn(II) and Cd(II) uptake by mussel (*Mytilus edulis*) and Carp (*Cyprinus carpio*), Environ. Sci. Technol. 36 (10) (2002) 2164–2170.
[18] V.I. Slaveykova, K.J. Wilkinson, Predicting the bioavailability of metals complexes: critical review of the biotic ligand model, Environ. Chem. 2 (2005) 9–24.
[19] K. Gernaey, L. Verschuere, L. Luyten, W. Verstraete, Fast and sensitive acute toxicity detection with an enrichment nitrifying culture, Water Environ. Res. 69 (1997) 1163–1169.
[20] D.H. Nies, Microbial heavy metal resistance, Appl. Microbiol. Biotechnol. 51 (1999) 730–750.
[21] C.A. Alder, H. Siegrist, W. Gujer, W. Giger, Behaviour of NTA and EDTA in biological wastewater treatment, Water Res. 24 (1990) 733–742.
[22] F.M.M. Morel, Principles of Aquatic Chemistry, Wiley–Interscience, New York, NY, 1983.
[23] W.D. Schecher, D.C. Mcavoy, MINEQL+: A Chemical Equilibrium Modeling System, Version 4.5 for Windows: Environmental Research Software, 1998.
[24] S. Tao, K.C. Lam, J. Cao, B. Li, Computer simulation of metal complex dissociation during free metal determination using anodic stripping voltammetry, Comput. Chem. 23 (1999) 61–68.
[25] EPA, MINTEQA2/PRODEFA2, A Geochemical Assessment Model for Environmental Systems: User Manual Supplement for Version 4.0, US EPA, National Exposure Research Laboratory, Ecosystems Research Division, Athens, Georgia, 1998 (revised September 1999).
[26] Standard Methods for the Examination of Water and Wastewater, 20th ed., APHA, AWWA, WEF, American Public Health Association, Washington, DC, USA, 1998.
[27] K.B. Alpaslan, F. Çeçen, Cometabolic degradation of TCE in enriched nitrifying batch systems, J. Hazard. Mater. B125 (2005) 260–265.
[28] P.O. Nelson, A.K. Chung, M.C. Hudson, Factors affecting the fate of heavy metals in the activated sludge process, J. WPCF 53 (8) (1981) 1323–1333.
[29] EPA, Nitrogen Control Manual, EPA/625/R-93/010 (1993), US EPA, Washington, DC, 1993.
[30] C.S. Hassler, V.I. Sloveykova, K.J. Wilkinson, Discriminating between intra- and extracellular metals using chemical extractions, Limnol. Oceanogr.: Methods 2 (2004) 234–247.
[31] Z. Hu, K. Chandran, D. Grasso, B.F. Smets, Impact of metal sorption and internalization on nitrification inhibition, Environ. Sci. Technol. 37 (4) (2003) 728–734.