*Desalination and Water Treatment*
www.deswater.com
doi: 10.1080/19443994.2014.893208

54 (2015) 1981–1989
May

Taylor & Francis
Taylor & Francis Group

# Long-term effect of Cr(VI) on ammonia-oxidizing and nitrite-oxidizing bacteria in an activated sludge system

Hongyang Zhang[a], Hongyan Zhai[a,*], Min Ji[a], Xiao Su[a], Zhenfeng Du[b], Jing Liu[c]

[a]*School of Environmental Science and Engineering, Tianjin University, Tianjin 300072, China, Tel. +86 27406057; Fax: +86 27406057; email: zhaihy@tju.edu.cn*
[b]*Tianjin Municipal Engineering Design & Research Institute, Tianjin 300051, China*
[c]*Tianjin University Research Institute of Urban Planning, Tianjin 300073, China*

Received 10 September 2013; Accepted 30 January 2014

ABSTRACT

Chromium (Cr) is a toxic compound in wastewater and can cause inhibitory effects on nitrification in a biological treatment system. However, different long-term inhibitory effects of Cr(VI) on ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB) were seldom investigated in activated sludge systems. In this study, the influences of 1−10 mg/L Cr(VI) on bioactivities and quantities of AOB and NOB in long-term inhibition and recovery periods were investigated. The transformation of inorganic nitrogen, fluorescence *in situ* hybridization assay for analyzing micro-organism population, and specific oxygen uptake rates of activated sludge were examined. Results show that the inhibitory effect of Cr(VI) on nitrification increased with the Cr(VI) loading concentration and running cycle. The inhibited nitrification can be gradually recovered in a recovery period despite of a high accumulation of Cr (including Cr(VI) and Cr(III)) in activated sludge. AOB were more sensitive than NOB to Cr(VI). AOB recovered fast both in activity and quantity. NOB recovered more slowly than AOB.

*Keywords:* Chromium; Nitrification; Nitrite-oxidizing bacteria; Ammonia-oxidizing bacteria; Activated sludge

## 1. Introduction

Chromium is widely used as raw material in petroleum refining, leather tanning, silver staining, and textile manufacturing. The chromium concentration in industrial wastewater is usually high. It was reported that the total chromium concentration of a primary settling effluent of tannery wastewater was as high as 40–65 mg/L [1]. Hexavalent chromium (Cr(VI)), which has been reported to be mutagenic and carcinogenic [2], is one of major stable forms of chromium. Cr(VI) exists in association with oxygen as chromate or dichromate in neutral wastewater. Cr(VI) is highly mobile and permeable through the bacterial cell membrane and therein is reduced by glutathione or other reducing matters to Cr(III) [3–5]. Cr(III) cannot be discharged out of the cell and further reacts with intracellular biomolecules to inhibit bioactivities of micro-organism [6,7]. An inefficient removal of Cr(VI) from industry wastewater usually results in a high Cr(VI) loading into municipal sewage systems and causes toxic effects on the biological treatment system.

*Corresponding author.

Nitrification, which involves a sequential conversion of ammonium ($NH_4^+$) to nitrite ($NO_2^-$), and further to nitrate ($NO_3^-$), is a controlling step in the biological nitrogen removal process. Nitrifying micro-organism include ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB). Generally, nitrifying micro-organisms in activated sludge appear to be more vulnerable to Cr(VI) than heterotrophic bacteria [8,9]. The influence of Cr(VI) on nitrification in activated sludge systems has been studied widely, which has been reviewed by Vaiopoulou and Gikas [9]. Vakova et al. [10] reported that a 1-h $EC_{50}$ value ($EC_{50}$ is an effective concentration, which caused a 50% inhibition on the respiration rates) of Cr(VI) on the biomass respiration activity was in a range of 40–90 mg/L. Madoni et al. [11] found that a 1-h exposure of activated sludge to a concentration of 83 mg/L Cr(VI) reduced the specific oxygen uptake uptake rate (SOUR) by 21.5%. Çeçen et al. [12] found that Cr(VI) had low biosorption in a nitrifying sludge system, and thus caused relatively low inhibition on nitrification with a 4 h-$IC_{50}$ of 83.61 mg/L and a 21 h-$IC_{50}$ of 38.97 mg/L ($IC_{50}$ is an impact concentration, which caused a 50% inhibition on $O_2$ and $CO_2$ consumptions). On the other hand, high biosorption and bioaccumulation of Cr(VI) by micro-organism [13–16] and high inhibitory effects have been widely reported. Stasinakis et al. demonstrated that 0.5 mg/L Cr(VI) reduced 74% of the ammonia uptake rate [5]. These controversial results from the previous studies are due to different experimental conditions, sludge concentrations, organic substances, and other factors.

Most of the studies on toxic effects of Cr(VI) on nitrification were mainly carried out by short-term shock loading batch assays. Only a limited number of studies investigated the inhibitory effects of a sustained Cr(VI) loading on nitrification and nitrifier activities. It was reported that a long-term Cr(VI) loading led to a prolonged inhibition of Cr(VI) on nitrification [5,17], and thus could result in failure in meeting the effluent discharge standards after the Cr (VI) loading. Stasinakis et al. [5] investigated the effects of continuous loadings of 0.5, 1, 3, and 5 mg/L Cr(VI) in a continuous-flow activated sludge reactor. They found that the inhibitory effect was significant on nitrification but minor on the organic substrate removal. Cr(VI) reduced the filament abundance and disperse growth of activated sludge. After termination of Cr(VI) addition, the ammonia removal rate recovered from 30% to 57% in 30 d. In their study, the increasing loading of Cr(VI) was conducted in one continuous-flow reactor, so the effects of different Cr (VI) concentrations cannot be separately compared. In another study, continuously feeding of 5, 10, and 25 mg/L Cr(VI) into sequencing batch reactor (SBR) systems decreased the $NH_4^+$-N removal efficiencies from 93.6–98.8% to 42.2, 24.1, and 20.3%, respectively [17]. After the termination of the Cr(VI) loading, the $NH_4^+$-N removal efficiencies improved slightly from 30 to 33%, 21.4 to 27.3%, and 19.1 to 24.2%, respectively, in 9 d [17]. The activity of an electron transport system was examined and showed a well correlation with the inhibitory rates of Cr(VI) on the substrate removal. However, the activity of the electron transport system is a specific indicator for respiration of aerobic micro-organisms [18]. The indicator only indicates the combined activities of heterotrophic aerobic bacteria and AOB, but cannot differentiate the inhibitory effects of Cr(VI) on the two species of bacteria. Moreover, few studies separately investigated the inhibition of Cr(VI) to AOB and NOB in an activated sludge system with a long-term Cr(VI) loading. AOB and NOB should have different sensitivities to Cr(VI), which could influence the inhibitory or recovery rates of nitrification. If the different responses of AOB and NOB to Cr(VI) were revealed, it could benefit researchers and engineers in developing effective recovery strategies for Cr(VI) inhibition.

Thus, the aim of this study was to reveal the different influences of Cr(VI) on AOB and NOB during a long-term inhibition and a recovery period in activated sludge systems. The bioactivities of AOB and NOB were examined by measuring the removal of ammonia, formation of nitrite and nitrate, SOUR values of ammoxidation, nitrite nitrogen oxidation, and heterotrophic bacteria in five identical SBRs. More important, the quantities of AOB and NOB were examined by fluorescence *in situ* hybridization (FISH) assays. Additionally, the distribution of Cr in the activated sludge system was examined.

## 2. Materials and methods

### 2.1. Synthetic domestic wastewater and activated sludge

A synthetic wastewater with 500 mg/L COD and 50 mg/L $NH_4^+$-N was prepared by dissolving the following chemicals (mg/L): glucose (483), $NH_4Cl$ (190), $KH_2PO_4$ (30), $NaHCO_3$ (205), $MgSO_4{\cdot}7H_2O$ (20), $FeSO_4{\cdot}7H_2O$ (2.5), $ZnSO_4{\cdot}7H_2O$ (0.25), $CaCl_2{\cdot}2H_2O$ (10), $CoCl_2{\cdot}6H_2O$ (0.05), and $MoO_3$ (1.50 μg/L) in ultrapure water. The pH of the influent was adjusted to $7.7 \pm 0.2$ using a sodium hydroxide solution. A Cr(VI)-contaminated wastewater was prepared by adding a proper volume of a $K_2Cr_2O_7$ stock solution (10.00 g/L as Cr(VI)) into the synthetic wastewater. Activated sludge was collected from an aerobic tank of a local sewage treatment plant, which employs a

$A^2/O$ treatment system with a solid retention time (SRT) of 22–33 d and a hydraulic retention time of 23 h. The collected activated sludge was screened through a 2-mm sieve to remove coarse particles before activation in lab.

### 2.2. SBR process

The schematic for one of the SBRs is shown in Fig. 1. The SBRs were operated sequentially in a 6-h cycle: influent filling (20 min), aeration (240 min), settling (75 min), and effluent withdrawal (15 min) (there was a 5-min idle time after influent filling and effluent withdrawal, respectively). During each cycle, 2.5 L of wastewater was treated. The mixed-liquor suspended solids (MLSS) were kept at around 6,000 mg/L by discharging excess sludge from reactors periodically and consequently a SRT of about 6 d was obtained in these reactors. The temperature and dissolved oxygen were kept at 25 ± 1℃ and above 2.0 mg/L, respectively, using a water bath. The sustained Cr(VI) loading experiment began when the effluent $NH_4^+$-N concentration was stable at less than 0.5 mg/L.

The five identical SBRs ran simultaneously. One of them, as a control reactor, was fed with the synthetic wastewater without Cr(VI) all through the test. For other four SBRs, it was divided into three phases: phase I (cycles 1−12), the four SBRs were fed with the synthetic wastewater without Cr(VI); phase II (cycles 13−57), the four SBRs were fed with the synthetic wastewaters containing Cr(VI) concentrations of 1, 3, 5, and 10 mg/L, respectively; and phase III (cycles 58−90), the four SBRs were fed with the synthetic wastewater without Cr(VI) again to simulate a long recovery period. The four SBRs were called as Cr(VI)-fed reactors in phase II and phase III, respectively, in section 3.

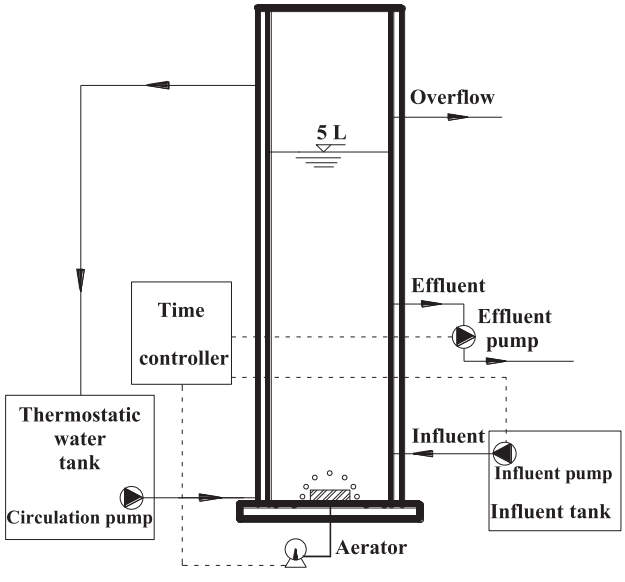


Fig. 1. Schematic of the SBR.

At the end of the aeration period in cycles 57 and 83, activated sludge from the five reactors was collected for analyses of SOUR, Cr distribution, and populations of AOB and NOB.

### 2.3. SOUR analyses

Ammoxidation SOUR ($SOUR_{NH4}$), nitrite nitrogen oxidation SOUR ($SOUR_{NO2}$), and heterotrophic SOUR ($SOUR_{org.C}$) were measured using a respirometer. Two 30-mL parallel samples of mixed liquor were collected from the five batch assay setups for measurement of SOUR in the ends of the phase II and phase III. The procedure for determinations of $SOUR_{NH4}$, $SOUR_{NO2}$, and $SOUR_{org.C}$ was based on a previous study [19]. An inhibition rate of micro-organism activity was calculated as following:

$$\text{Inhibition rate}\,(\%) = (SOUR_{control} - SOUR_{cr})/SOUR_{control} \times 100\% \quad (1)$$

where $SOUR_{control}$ was measured in the control reactor and $SOUR_{Cr}$ was measured in Cr(VI)-fed reactors.

### 2.4. Distribution of chromium in SBRs

In the activated sludge system, Cr(VI) could be reduced to Cr(III), which mainly deposits in activated sludge [7]. However, Cr(VI) and Cr(III) were not separately detected in this study, but simultaneously measured as Cr using a flame atomic absorption spectroscopy (Model WFX-130, Beijing Rui-Li Co., China). A 10-mL activated sludge solution was collected from the SBR and was directly digested according to a standard acid digestion method [20] for measurement of the total Cr concentration in the activated sludge solution. Another 50-mL activated sludge solution was collected and filtrated through a 0.45 μm membrane. Then, Cr in the filtrate was directly measured as soluble Cr. The left activated sludge on the membrane was collected and washed with a modified washing procedure [21,22]. Briefly, the collected activated sludge was re-suspended in a 30-mL washing solution (1 mmol/L EDTA, pH 7.0, and 0.1 mol/L NaCl), agitated at 150 rpm for 30 min, and centrifuged at 1,600 g for 5 min, followed by two cycles of washing, centrifugation, and supernatant removal. Then, the

washed sludge was digested with the standard acid digestion method for measurement of intracellular Cr. The concentration of surface-bound Cr was calculated by subtracting the soluble Cr and intracellular Cr from the total Cr concentration of the activated sludge solution.

### 2.5. Other analysis methods

In the FISH assay, samples fixation and hybridization steps were carried out according to a standard hybridization protocol [23] with two kinds of oligonucleotide probe, namely NSO1225 [24] (for AOB) and Ntspa662 [25] (for NOB), which are authorized to compose by Invitrogen Inc. Concentrations of $NH_4^+$-N, $NO_2^-$-N, $NO_3^-$-N, and COD were detected according to the standard methods [20]. MLSS and pH were measured with a MLSS analyzer (HACH Txpro-2) and a pH meter (HACH HQ-30d), respectively.

## 3. Results and discussion

### 3.1. Inhibition of nitrification with the sustained Cr(VI) loading

As shown in Fig. 2(a), the sustained Cr(VI) loading inhibited the nitrification performance, and the inhibitory effect became more and more significant as the loading concentration of Cr(VI) and running cycle increased. In the 1 mg/L Cr(VI)-fed reactor, the removal rate of $NH_4^+$-N began to decrease in cycle 35, and subsequently decreased to 48.5% in cycle 45. While in the 3, 5, and 10 mg/L Cr(VI)-fed reactors, the removal rates of $NH_4^+$-N began to decrease in cycles 15, 13, and 13, respectively, and subsequently decreased to 67.2, 60.0, and 49.9%, respectively, in later six cycles. In cycles 49−57, the removal efficiencies of $NH_4^+$-N were 43.0 ± 4.1% in the four Cr(VI)-fed reactors. The inhibition degree of Cr(VI) on the ammonia removal in this study is close to Cheng et al. [17] because of similar SBR systems and SRTs. The lowest Cr(VI) concentration used in Cheng's study was relatively high (5 mg/L Cr(VI)) [17], so the gradual increase of inhibitory effects caused by a low Cr(VI) loading (e.g. 1 or 3 mg/L Cr(VI)) was not observed.

The concentrations of effluent $NO_2^-$-N were all lower than 1.0 mg/L in the four Cr(VI)-fed reactors in phases I and II (Fig. 2(b)). The concentrations of $NO_3^-$-N in effluents dropped faster in the reactors with the higher Cr(VI) loading (Fig. 2(c)), and dropped to 1.3 ± 0.7 mg/L in cycles 49–57 in the four Cr(VI)-fed reactors.

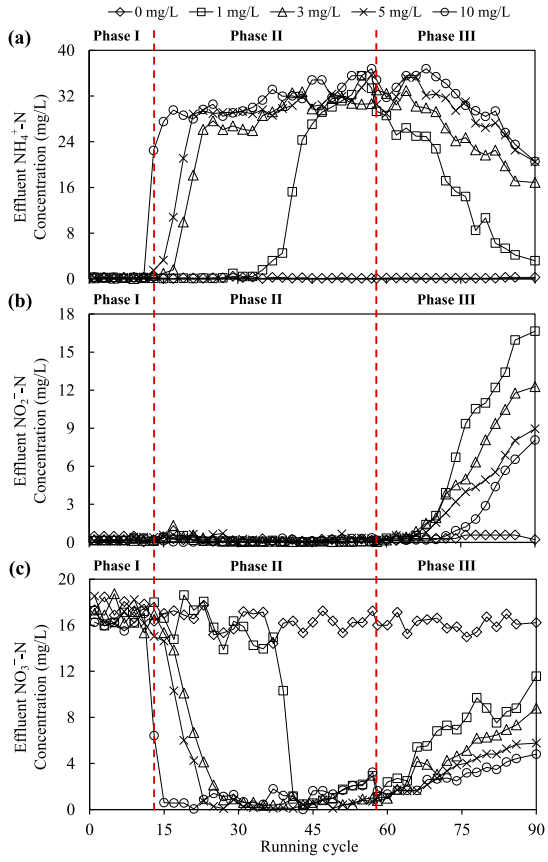


Fig. 2. Performance of the SBRs: (a) effluent $NH_4^+$-N concentration, (b) effluent $NO_2^-$–N concentration, and (c) effluent $NO_3^-$-N concentration effluents.

### 3.2. Recovery of nitrification after the sustained Cr(VI) loading

As shown in Fig. 2(a), after the termination of Cr(VI) loading, the $NH_4^+$-N concentrations in effluent decreased gradually in the four Cr(VI)-fed SBRs. This phenomenon agrees with the findings in literatures [17,26]. In the 1, 3, 5, and 10 mg/L Cr(VI)-fed SBRs, $NH_4^+$-N removal efficiencies recovered back to 95.0, 73.6, 66.2, and 65.4%, respectively, at cycle 90. An accumulation of $NO_2^-$-N was observed in the four SBRs, and significantly increased as the running cycle increased (Fig. 2(b)), while the $NO_3^-$-N concentration in effluent increased with the running time. Accordingly, the inhibitory effect of Cr(VI) on nitrification can be partially recovered and the recovery rates were faster in the reactors with lower Cr(VI) loadings. The accumulation of $NO_2^-$-N after the termination of Cr(VI) addition was seldom reported [5,9,16,17]. The accumulation of $NO_2^-$-N is a piece of important

information. It indicates that the activity of NOB was still in a low level while the AOB began to recover.

### 3.3. SOUR with and after the sustained Cr(VI) loading

Fig. 3(a) shows the values of $SOUR_{NH4}$ and $SOUR_{NO2}$ of activated sludge in cycle 57. The $SOUR_{NH4}$ and $SOUR_{NO2}$ in the control reactor were 0.147 and 0.107 mg $O_2$/(g MLSS min), respectively. The inhibition ratios of $SOUR_{NH4}$ in the 1, 3, 5, and 10 mg/L Cr(VI)-fed reactors were 93.9, 94.5, 94.6, and 95.6%, respectively, whereas the inhibition ratios of $SOUR_{NO2}$ were 61.0, 61.6, 72.0, and 85.1%, respectively. In cycle 83, $SOUR_{NH4}$ and $SOUR_{NO2}$ in the control reactor were 0.149 and 0.115 mg$O_2$/(g MLSS min), respectively. The inhibition ratios of $SOUR_{NH4}$ in the 1, 3, 5, and 10 mg/L Cr(VI)-fed reactors decreased and were 47.0, 46.3, 57.7, and 63.2%, respectively (Fig. 3(b)), whereas the inhibition ratios of $SOUR_{NO2}$ were 50.6, 54.1, 64.4, and 77.3%, respectively.

### 3.4. Effect of the sustained Cr(VI) loading on AOB and NOB populations

In the FISH assays, the activated sludge sample collected in cycle 83 from the control reactor was chosen as the control sample to examine the proportions of AOB and NOB. The relative proportions of AOB and NOB in total bacteria were statistically counted with an Image-Pro Plus software according to the FISH assay pictures (Figs. 4 and 5).The proportions of AOB and NOB in the control reactor in cycle 83 were 9.9 and 10.1%, respectively. In the 1, 3, 5, and 10 mg/L Cr(VI)-fed reactors, the proportions of AOB decreased to 2.3, 1.9, 1.5, and 1.4%, respectively, and the proportions of NOB decreased to 2.4, 1.5, 0.8, and 0.5% in cycle 57. In cycle 83, the proportions of AOB were 6.9, 5.3, 4.9, and 3.7% in the 1, 3, 5, and 10 mg/L Cr(VI)-fed reactors, respectively, and the proportions of NOB were 3.9, 3.9, 1.5, and 1.6%, respectively.

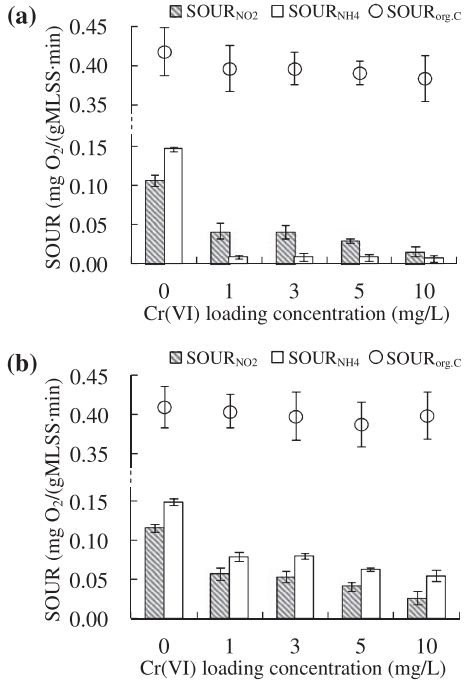


Fig. 3. Effects of Cr(VI) loading concentrations on $SOUR_{NO2}$, $SOUR_{NH4}$, and $SOUR_{org.C}$ in (a) cycle 57 and (b) cycle 83.

### 3.5. Distribution of Cr in the SBR system

As shown in Fig. 6, Cr was significantly accumulated inside of activated sludge in phase II. In the 1, 3, 5, and 10 mg/L Cr(VI)-fed reactors, total amounts of 17.5, 41.7, 63.5, and 156.2 mg/L Cr(VI) were detected, respectively. Of the total amount of Cr, 78, 87, 91, and 55% were inside the activated sludge and 19.6, 11.4, 8.5, and 44.2% were surface-bound. The total adsorbed amounts of Cr (including intracellular and surface-bound Cr) in activated sludge were 85.3, 204.5, 316.6, and 771.6 mg (cycle 57), which accounted for 73.5, 58.7, 54.5, and 68.6% of the total addition amount of Cr, respectively. Accordingly, the sorption uptakes of Cr by activated sludge in the four Cr(VI)-fed reactors were 3.1, 7.1, 11.7, and 25.8 mg/g MLSS, respectively.

In cycle 83, although the activated sludge in the four Cr(VI)-fed reactors had been fed with the synthesized wastewater without Cr(VI) for 26 cycles, high levels of Cr were still detected (Fig. 6). In the 1, 3, 5, and 10 mg/L Cr(VI)-fed reactors, 13.5, 35.5, 60.9, and 130.1 mg/L of Cr were remained in the system; 11.7, 33.0, 51.5, and 75.1 mg/L of Cr were inside activated sludge; 1.7, 2.2, 9.3, and 54.8 mg/L were surface-bound; the sorption uptakes of Cr by activated sludge were 2.4, 6.0, 10.6, and 22.6 mg/g MLSS, respectively. In comparison with the levels in cycle 57, 13.8, 8.8, 11.1, and 11.9% of the intracellular Cr were removed, respectively. Most of the Cr adsorbed into or onto the activated sludge were hard to be released back to water and very likely to have been reduced to Cr(III) [7].

### 3.6. Sensitivities of AOB and NOB to Cr(VI)

The results mentioned above show that AOB were more sensitive than NOB to Cr(VI). In the 1 mg/L Cr(VI)-fed reactor, the removal efficiency of $NH_4^+$-N

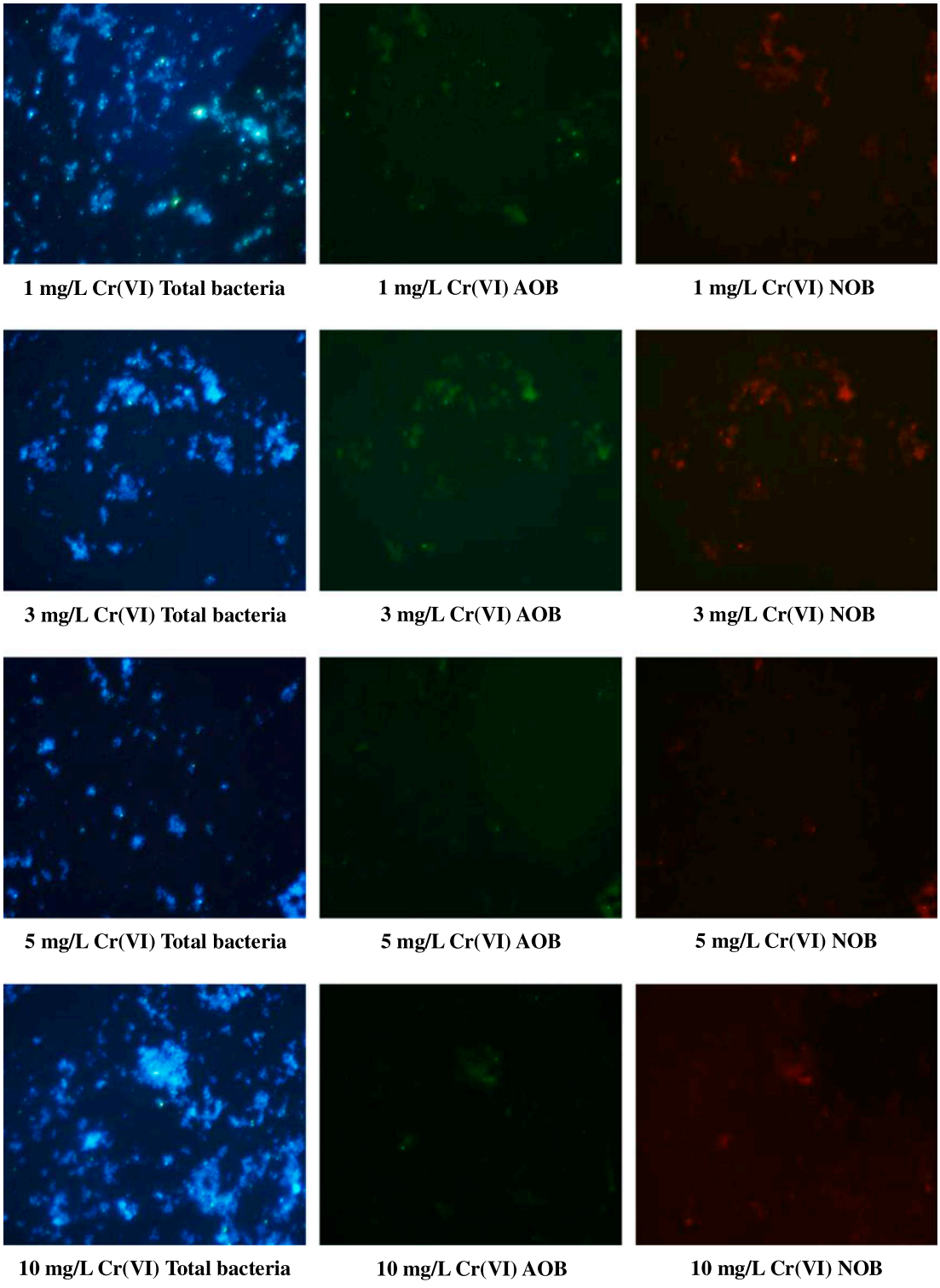


Fig. 4. FISH assay pictures of bacteria flora in SBRs with the different Cr(VI) loadings.

decreased from 99.3 to 93.9%, respectively, from cycle 33 to 37, and meanwhile the effluent concentrations of $NO_2^-$-N and $NO_3^-$-N changed little. It indicates that the transformation of $NH_4^+$-N to $NO_2^-$-N had been retarded, whereas the transformation of $NO_2^-$-N to $NO_3^-$-N had not been influenced. In other words, the

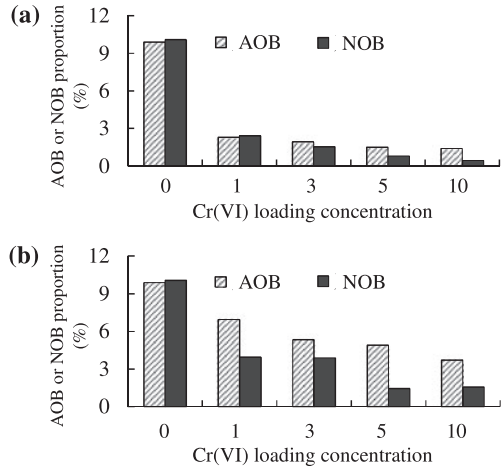


Fig. 5. Effects of Cr(VI) loading concentrations on the relative proportions of AOB and NOB in (a) cycle 57 and (b) cycle 83.

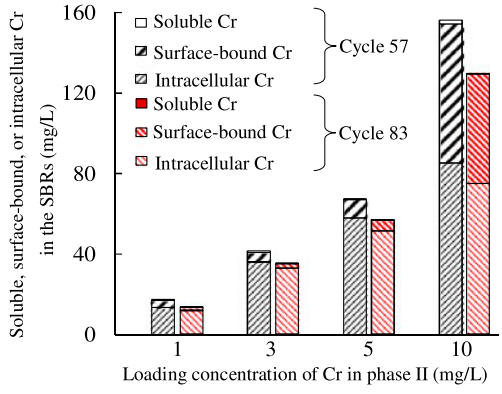


Fig. 6. Effects of Cr loading concentrations on the distribution and accumulation of Cr in the activated sludge system.

activities of AOB were inhibited earlier than NOB. In the recovery period, the accumulation of $NO_2^-$-N indicates that Cr(VI) caused different prolonged impacts on the activities of AOB and NOB. The recovery rates of AOB and NOB in the four Cr(VI)-fed SBRs were evaluated by determining apparent increasing rates of $NO_2^-$-N ($k_{NO2}$) and $NO_3^-$-N ($k_{NO3}$) using Eqs. (2–3):

$$k_{NO2} = dC_{NO2}/dt \quad (2)$$

$$k_{NO3} = dC_{NO3}/dt \quad (3)$$

where $C_{NO2}$ is the sum of concentrations of $NO_2^-$-N and $NO_3^-$-N, $C_{NO3}$ is the concentration of $NO_3^-$-N, and $t$ is the cycle number.

As shown in Fig. 7, the recoveries of AOB and NOB both included slow and rapid stages. In the slow recovery stage, activities of AOB and NOB recovered in close rates and thus no $NO_2^-$-N accumulation was observed. As the running cycle increased, activities of AOB ($k_{NO2}$ of 0.65−0.94 mg/(L cycle)) recovered faster than NOB ($k_{NO3}$ of 0.13−0.21 mg/(L cycle)), so accumulation of $NO_2^-$-N in effluent kept increasing in phase III.

The results of $SOUR_{NH4}$ and FISH further proved that AOB were more sensitive than NOB to the presence and absence of Cr(VI). The inhibition rates of $SOUR_{NH4}$ in the four Cr(VI)-fed reactors were all greater than 93% at the end of phase II, so the activities of AOB were almost suspended by the toxic effect of Cr(VI). However, 23, 19, 15, and 14% of the original AOB remained in the 1, 3, 5, and 10 mg/L Cr(VI)-fed reactors, respectively. The remaining portion of AOB, which lost activities, should be inactivated but still alive. Subsequently, AOB quickly recovered both in activity and quantity when the influent changed to wastewater without Cr(VI). For NOB, the change

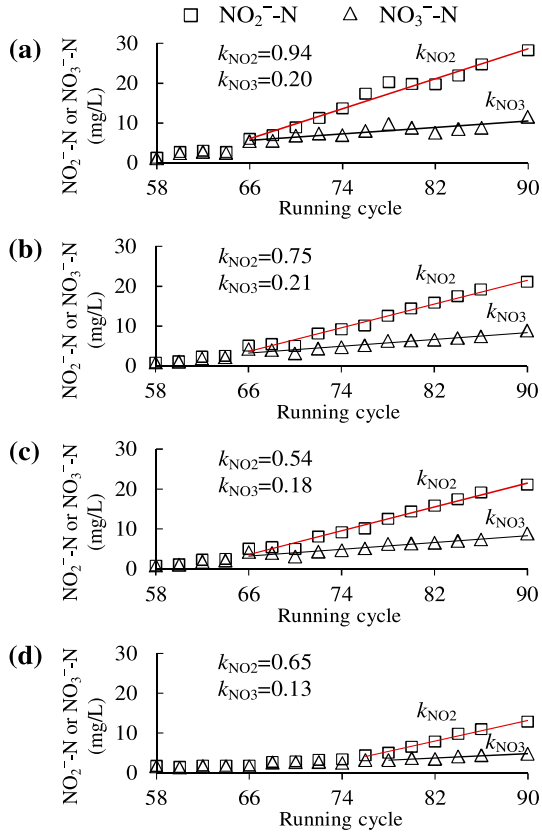


Fig. 7. Increasing rates of $NO_2^-$-N and $NO_3^-$-N in phase III in the SBRs inhibited by (a) 1 mg/L Cr(VI), (b) 3 mg/L Cr(VI), (c) 5 mg/L Cr(VI), and (d) 10 mg/L Cr(VI).

trends of $SOUR_{NO2}$ with the Cr(VI) loading concentration were similar to those of the relative population of NOB. It indicates that NOB lost their activities most likely due to the reduction of NOB population. As a result, NOB recovered with a slower rate and lower extend than AOB in phase III.

Another possible reason for the different sensitivities of AOB and NOB to Cr(VI) was the different spacial distribution characteristics of AOB and NOB in activated sludge. It was reported that AOB located mainly in the outer part of granular activated sludge, whereas NOB existed in the inner part [26,27]. The spacial distribution of AOB and NOB in amorphous activated sludge should be similar to that in granular activated sludge. Therefore, AOB contacted with Cr (VI) earlier than NOB and as a result, AOB responded more quickly than NOB to the loading of Cr(VI).

## 4. Conclusion

The inhibitory effect of the sustained Cr(VI) loading on nitrification increased with the loading concentration of Cr(VI) and running cycle. The inhibitory effect of Cr(VI) on nitrification can be recovered gradually despite a high accumulation of Cr(VI) in activated sludge. AOB were more sensitive than NOB to the presence and absence of Cr(VI). AOB recovered fast both in activity and quantity in the recovery phase, whereas NOB recovered more slowly than AOB.

Our findings suggested the different sensitivities of AOB and NOB to Cr(VI) should be considered in improving the nitrification efficiency after a shock or sustained loading of Cr(VI). Methods for activating AOB and enriching NOB in a nitrification-inhibited system could be effective to achieve a fast recovery.

## Acknowledgments

The authors are grateful for the financial supports by National Natural Science Foundation of China (51178302).

## References

[1] D. Orhon, E.A. Genceli, S. Sözen, Experimental evaluation of the nitrification kinetics for tannery wastewaters, Water SA 26 (2000) 43–50.
[2] J.G. Henry, G.W. Heinke, Environmental science and engineering, vol. 11, Prentice Hall, New Jersey, NY, 1989.
[3] D.H. Nies, S. Silver, Ion efflux systems involved in bacterial metal resistances, J. Ind. Microbiol. 14 (1995) 186–199.
[4] A.S. Stasinakis, N.S. Thomaidis, D. Mamais, M. Karivali, T.D. Lekkas, Chromium species behaviour in the activated sludge process, Chemosphere 52 (2003) 1059–1067.
[5] A.S. Stasinakis, N.S. Thomaidis, D. Mamais, E.C. Papanikolaou, A. Tsakon, T.D. Lekkas, Effects of chromium (VI) addition on the activated sludge process, Water Res. 37 (2003) 2140–2148.
[6] R. Bencheikh-Latmani, A. Obraztsova, M.R. Mackey, M.H. Ellisman, B.M. Tebo, Toxicity of Cr(III) to *Shewanella* sp. Strain MR-4 during Cr(VI) reduction, Environ. Sci. Technol. 41 (2007) 214–220.
[7] A.S. Stasinakis, N.S. Thomaidis, D. Mamais, T.D. Lekkas, Investigation of Cr(VI) reduction in continuous-flow activated sludge systems, Chemosphere 57 (2004) 1069–1077.
[8] D.J.B. Dalzell, S. Alte, E. Aspichueta, A. de la Sota, J. Etxebarria, M. Gutierrez, C.C. Hoffmann, D. Sales, U. Obst, N. Christofi, A comparison of five rapid direct toxicity assessment methods to determine toxicity of pollutants to activated sludge, Chemosphere 47 (2002) 535–545.
[9] E. Vaiopoulou, P. Gikas, Effects of chromium on activated sludge and on the performance of wastewater treatment plants: A review, Water Res. 46 (2012) 549–570.
[10] S. Vakova, J. Kupec, J. Hoffmann, Toxicity of chromium to activated sludge, Ecotoxicol. Environ. Saf. 42 (1999) 16–21.
[11] P. Madoni, D. Davoli, L. Guglielmi, Response of SOUR and AUR to heavy metal contamination in activated sludge, Water Res. 33 (1999) 2459–2464.
[12] F. Çeçen, N. Semerci, A.G. Geyik, Inhibition of respiration and distribution of Cd, Pb, Hg, Ag and Cr species in a nitrifying sludge, J. Hazard. Mater. 178 (2010) 619–627.
[13] L. Fude, B. Harris, M.M. Urrutia, T.J. Beveridge, Reduction of Cr(VI) by a consortium of sulfate-reducing bacteria (SRB-III), Appl. Environ. Microbiol. 60 (1994) 1525–1531.
[14] T. Srinath, T. Verma, P.W. Ramteke, S.K. Garg, Chromium (VI) biosorption and bioaccumulation by chromate resistant bacteria, Chemosphere 48 (2002) 427–435.
[15] D.J.B. Dalzell, S. Alte, E. Aspichueta, A. de la Sota, J. Etxebarria, M. Gutierrez, C.C. Hoffmann, D. Sales, U. Obst, N. Christofi, A comparison of five rapid direct toxicity assessment methods to determine toxicity of pollutants to activated sludge, Chemosphere 47 (2002) 535–545.
[16] A.M. Orozco, E.M. Contreras, N.E. Zaritzky, Cr(VI) reduction capacity of activated sludge as affected by nitrogen and carbon sources, microbial acclimation and cell multiplication, J. Hazard. Mater. 176 (2010) 657–665.
[17] L. Cheng, X. Li, R. Jiang, C. Wang, H. Yin, Effects of Cr(VI) on the performance and kinetics of the activated sludge process, Bioresour. Technol. 102 (2011) 797–804.
[18] C. Maurines-Carboneill, J.-J. Pernelle, L. Morin, G. Sachon, G. Leblon, Relevance of the INT test response as an indicator of ETS activity in monitoring heterotrophic aerobic bacterial populations in activated sludges, Water Res. 32 (1998) 1213–1221.
[19] J. Surmacz-Gorska, K. Gernaey, C. Demuynck, P. Vanrolleghem, W. Verstraete, Nitrification monitoring in activated sludge by oxygen uptake rate (OUR) measurements, Water Res. 30 (1996) 1228–1236.

[20] APHA, AWWA, WEF, Standard methods for the examination of water and wastewater, APHA, Washington, DC, 1998.
[21] Z. Hu, K. Chandran, D. Grasso, B.F. Smets, Impact of metal sorption and internalization on nitrification inhibition, Environ. Sci. Technol. 37 (2003) 728–734.
[22] T.M. Vasconcelos, F.M. Leal, Adsorption and uptake of Cu by *Emiliania huxleyi* in natural seawater, Environ. Sci. Technol. 35 (2001) 508–515.
[23] N. Yusof, M.A. Hassan, P.L. Yee, M. Tabatabaei, M.R. Othman, M. Mori, M. Wakisaka, K. Sakai, Y. Shirai, Nitrification of high-strength ammonium landfill leachate with microbial community analysis using fluorescence *in situ* hybridization (FISH), Water Sci. Technol. 29 (2011) 602–611.
[24] V. Degrange, R. Bardin, Detection and counting of *Nitrobacter* populations in soil by PCR, Appl. Environ. Microbiol. 61 (1995) 2093–2098.
[25] D.W. Han, J.S. Chang, D.J. Kim, Nitrifying microbial community analysis of nitrite accumulating biofilm reactor by fluorescence *in situ* hybridization, Water Sci. Technol. 47 (2003) 97–104.
[26] X. Shi, G. Sheng, X. Li, H. Yu, Operation of a sequencing batch reactor for cultivating autotrophic nitrifying granules, Bioresour. Technol. 101 (2010) 2960–2964.
[27] B. Zhang, Z. Chen, Z. Qiu, M. Ji, Z. Chen, Z. Chen, J. Li, X. Wang, J. Wang, Dynamic and distribution of ammonia-oxidizing bacteria communities during sludge granulation in an anaerobic aerobic sequencing batch reactor, Water Res. 45 (2011) 6207–6216.