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The effects of continuous Cu(II) exposure compared with a shock dosage of Cu(II) on pollutant removal in activated sludge

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ABSTRACT

In order to determine how Cu(II) exposure affects activated sludge, we investigated pollutant removal and recovery of removal efficiency after continuous exposure to Cu(II) or a shock dosage of Cu(II) in a sequencing batch reactor (SBR) system. The continuous exposure experiment, involving 20 mg L^{-1} Cu(II), showed that the removal efficiency for chemical oxygen demand (COD) initially decreased rapidly to 7%, but conspicuously recovered to 78% by the 50th day and then dropped to nearly zero by the 160th day. The removal of NH_3-N was even more conspicuously inhibited and failed to recover during the experimental period. The continuous dosage of activated sludge with 20 mg L^{-1} of Cu(II) for nearly 160 d rendered the SBR almost completely unable to remove pollutants. In the shock dosage (batch) experiment, the removal of both COD and NH_3-N was inhibited after shock dosages of 10, 20, and 40 mg L^{-1} Cu(II). However, after 24 h, the removal efficiency of COD at each shock dosage recovered to 81, 60, and 52%, respectively; NH_3-N removal efficiency recovered to 78, 74, and 45%, respectively.

Keywords: Activated sludge; Cu(II); COD; NH_4^+; Recovery capability

1. Introduction

Copper, in the form of Cu(II), is widely used in metallurgy, chemistry, battery manufacturing, and electroplating, which releases a large amount of copper into aquatic environments or wastewater treatment plants. Trace concentrations of copper are necessary for micro-organisms to grow, but excessive amounts of copper cause inhibitory or even toxic effects to microbes [1–3]. When wastewater containing more than ~10–20 mg L^{-1} Cu(II) flows into biological wastewater treatment systems, such as activated sludge (AS) systems in sewage treatment plants, it negatively affects the system's stability and pollutant removal efficiency [4].

Many researchers have investigated biosorption [5,6] and the removal of heavy metals [7]. Others have focused on the effect of Cu(II) on the removal of pollutants, oxygen uptake rates, and respiration in AS [4,8]. In previous studies, the shock dosages of Cu(II), at various concentrations, have been added in continuous or batch reactors. However, most studies have investigated that the toxicity of Cu(II) shock dosages have used batch reactors with only trace levels of the metal [9,10] and over a short period (usually <1 week) [2,11,12]. Additionally, in AS systems, heavy metals are not biodegradable and can only be absorbed by

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sludge and transformed into sediments or complexes. Therefore, heavy metals accumulate in the system and cause continuous toxicity effects [13]. The performance of micro-organisms in AS under long-term (>100 d) exposure to high levels of Cu(II) has rarely been documented, and there are even fewer studies comparing the effects of chronic and acute Cu(II) toxicity on AS.

Furthermore, most studies have concluded that a certain concentration of Cu(II) harms the micro-organisms in AS, but few studies have investigated the ability of AS to recover its pollutant removal capabilities after Cu(II) is no longer applied. Lee et al. found that by adding 5 mg L^{-1} Cu(II) into a lab-scale continuous-flow AS reactor for 21 d, nitrification was inhibited [14]. After that, nitrification recovered when Cu(II) was no longer added. However, the tested Cu(II) concentration was relatively low, at less than 10 mg L^{-1}. Therefore, it is necessary to study the effect of Cu(II) on the ability of AS to remove pollutants and recover from Cu(II) toxicity, when it is continuously dosed at a high concentration of Cu(II) over a long time.

This study investigated the effects of chronic Cu(II) toxicity on AS by continuously exposing AS to Cu(II) and then measuring the removal of chemical oxygen demand (COD) and NH_3-N in a sequencing batch reactor (SBR) for approximately 160 d. In addition, through a Cu(II) shock dosage experiment, we performed a comparative study on the acute toxic effects of different concentrations of Cu(II) (0, 10, 20, and 40 mg L^{-1}) on the sludge system. Thus, we explored different inhibitory effects and the ability of AS to recover its pollutant removal activity under both long- and short-term exposure to Cu(II).

2. Materials and methods

2.1. Continuous Cu(II) exposure in a SBR

Two SBRs were used in this study. One reactor was used as the control reactor (CR), and the other reactor (the experimental reactor; ER) was dosed with copper. Each reactor had a 4.0-L working volume and was operated at 20℃ on a 12-h cycle. Each cycle included 0.5 h of synthetic wastewater feed, 6 h of aeration, 2 h of settling, 0.5 h of decanting, and 3 h of idling. The sludge retention time was maintained at approximately 16 d.

The seed sludge was obtained from a local sewage treatment plant in Shanghai, China, and was acclimated with synthetic wastewater using sodium benzoate (C_6H_5COONa) as the carbon source, ammonium chloride (NH_4Cl) as the nitrogen source, and monopotassium phosphate (KH_2PO_4) as the phosphorus source, with COD maintained in the range of 650–820 mg L^{-1}. The COD:N:P ratio of the wastewater was maintained at 100:5:1, and necessary mineral nutrients were added in the influent. During the aeration period, dissolved oxygen (DO) was maintained at 2.0–2.5 mg L^{-1}, the pH was maintained at 6.5–8.0, and the concentration of mixed liquor suspended solids (MLSS) was maintained at 2,000-3,000 mg L^{-1}. The concentrations of COD and NH_3-N in the influent and effluent were measured regularly. When the removal efficiency of COD and NH_3-N in the system reached the steady state, 20 mg L^{-1} Cu(II) was added into the ER. No Cu(II) was added to the other SBR (the CR).

2.2. Batch experiment with a shock dosage of Cu(II)

The AS used in the batch experiment was taken from the continuous experiment reactor (SBR, at steady state), but the batch experiment was performed using reactors with 1.0-L working volumes. At the beginning of the batch experiment, 1 L of mixed liquor was taken from the continuous SBR and immediately poured into a batch reactor. After settling for 30 min, the supernatant of the mixed liquor was drained, and the synthetic water was added to the batch reactor such that the initial COD and NH_3-N concentrations were 600 mg L^{-1} and 40 mg L^{-1}, respectively. The pH, DO, temperature, and other parameters were adjusted to match those of the continuous exposure experiment. Next, different Cu(II) concentrations (0, 10, 20, and 40 mg L^{-1}) were added into different batch reactors. Parallel aeration was performed continuously over a 24-h period in all four reactors using an air pump. Effluent samples were collected and measured for COD and NH_3-N throughout the experiment.

2.3. Analysis methods

The COD, NH_3-N, MLSS, pH, and temperature were analyzed according to the standard methods [15].

2.4. Data analysis

In the Cu(II) batch experiment, the inhibition degree (ID) was calculated using the concentrations of COD and NH_3-N in the reactors at different sampling times, according to the following formula [12].

$$\text{Inhibition degree (ID)} = (C_b - C_s)/C_b \times 100\% \quad (1)$$

where C_b is the difference in concentration (mg L^{-1}) of a substrate between the initial time and time t in the CR (without Cu(II) shock dosage) and C_s is the difference in concentration (mg L^{-1}) of a substrate between the initial time and time *t* in an experimental batch reactor (containing a Cu(II) shock dosage).

3. Results and discussion

3.1. Effect of continuous Cu(II) exposure on AS in SBR

During the 160-d experimental period, 20 mg L^{-1} Cu(II) was continuously added to the ER's influent stream, starting on the 42nd day. This experiment allowed us to investigate the effect of continuous Cu(II) exposure on the ability of AS to remove pollutants. The removal efficiency of COD and NH_3-N from wastewater is usually used to indicate the changes in the biological activity of AS in the presence of heavy metals [8,16–18]. Therefore, by determining the concentrations of COD and NH_3-N over a long period, we were able to compare the continuous toxicity of Cu(II) on AS in both the CR and ER.

3.1.1. The effect of continuous Cu(II) exposure on the removal of organic pollutants in SBR

Fig. 1 shows the long-term effect of Cu(II) on COD removal. Fig. 1(a) shows that the efficiency of COD removal remained higher in the CR than in the ER during the 160-d experiment, with an average of 95% removal (range: 89–98%). Before Cu(II) addition, the average COD concentration in the ER was 37.84 mg L^{-1}, giving a removal efficiency of 94%. This indicates that, once acclimated, the AS in both the ER and CR was able to remove organic pollutants effectively. When 20 mg L^{-1} Cu(II) was added to the ER on day 42, as shown in Fig. 1(b), the effluent COD drastically increased from 38.8 mg L^{-1} to 696 mg L^{-1}. Fourteen days later, the COD was 768 mg L^{-1}, and the COD removal efficiency was reduced to only 2%. These results demonstrate that the micro-organisms in the ER were strongly inhibited, which then prevented them from degrading organic compounds efficiently. After the 89th day following the start of the experiment, a noticeable recovery in COD removal appeared in the ER, such that the effluent COD declined to 142.6 mg L^{-1} and the removal efficiency reached a maximum of 78%. This recovery probably arose because the cultivated micro-organisms in the ER had gradually adapted to the cupric environment. Another probable reason for the recovery is that continuously dosing copper may have significantly changed the microbial community structure, and the micro-organisms containing copper-resistance genes survived [19]. However, approximately 20 d later, the effluent COD increased again, and the COD removal efficiency finally decreased to almost zero. These results suggest that the ability of AS to degrade organic pollutants in a SBR was seriously inhibited by long-term, continuous exposure to 20 mg L^{-1} Cu(II). Although COD removal in the ER recovered 47 d after starting the Cu (II) exposure, continuous exposure to Cu(II) eventually hindered the ability of AS to degrade pollutants in the SBR.

3.1.2. The effect of continuous Cu(II) exposure on NH_3-N removal

As shown in Fig. 2, effluent NH_3-N was less than 10 mg L^{-1} in the CR during the 160-day experiment, and the average concentration of NH_3-N in the ER effluent before adding Cu(II) was 3.7 mg L^{-1}. On the second day after the onset of Cu(II) exposure, the effluent NH_3-N increased to 23.7 mg L^{-1}, and after 80 d, the effluent NH_3-N fluctuated around 30 mg L^{-1}, which was higher than the NH_3-N concentration in the influent stream. Studies by Gerardi [20] and Juliastuti et al. [12] have shown that nitrobacteria are more sensitive to the medium and to variations in inhibitory conditions than heterotrophic bacteria are. Cu(II) disrupted the bacterial cells' osmotic pressure, which caused some cells to rupture, such that NH_3-N was not removed effectively by the micro-organisms. Furthermore, NH_3-N concentration in AS increased as a result of the ruptured cells.

In contrast to the observed recovery of COD removal, we found that the nitrobacteria-mediated NH_3-N removal failed to recover during the experimental period. After the onset of Cu(II) exposure, the removal of NH_3-N in the ER plummeted, showing a removal efficiency of less than 10%. In addition, the concentrations of nitrate and nitrite were both below the detectable limits, indicating that the metabolisms of nitrobacteria and nitrite bacteria were more seriously inhibited by continuous Cu(II) exposure than those of other aerobic, carbon-metabolizing bacteria.

3.2. Effect of Cu(II) shock dosages on AS pollutant removal ability in batch experiments

In order to determine the effect of different concentrations of a shock dosage of Cu(II) on the ability of AS to remove pollutants, we performed a comparative experiment in which a single dosage of Cu(II) was introduced into a batch reactor with acclimated AS.

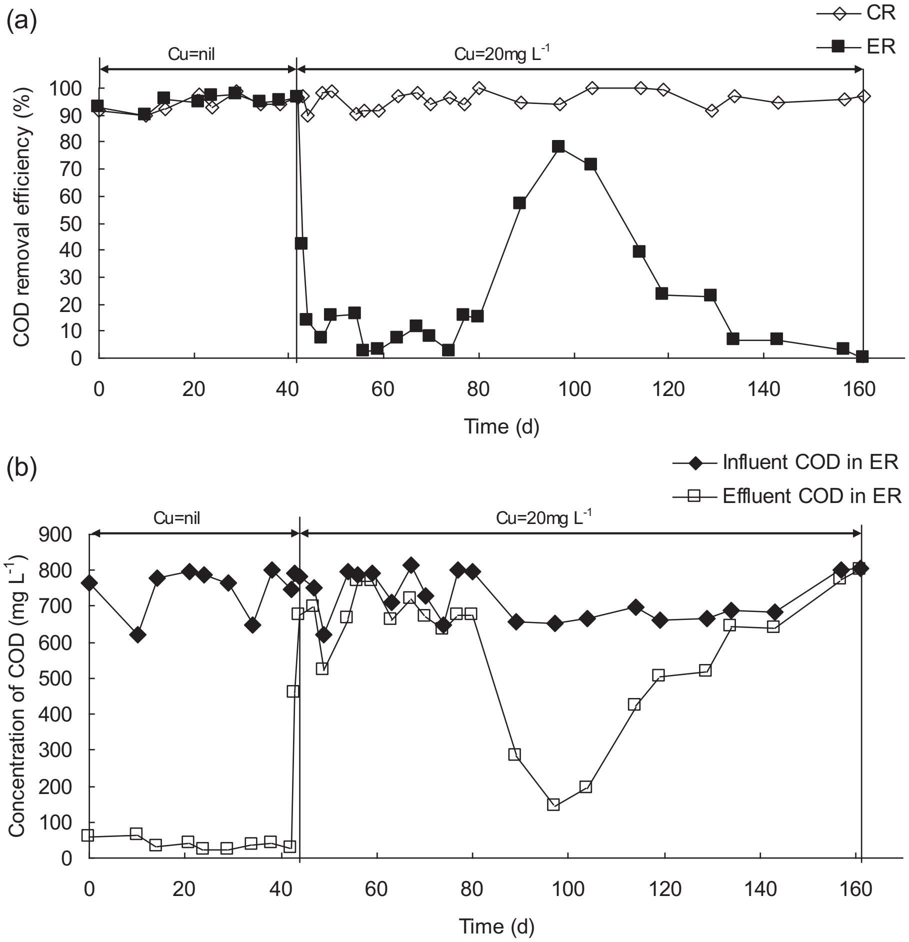

Fig. 1. Effect of Cu(II) on COD removal (a) the comparison of COD removal efficiency between CR and ER; (b) effect of Cu(II) on COD removal ability of AS system.

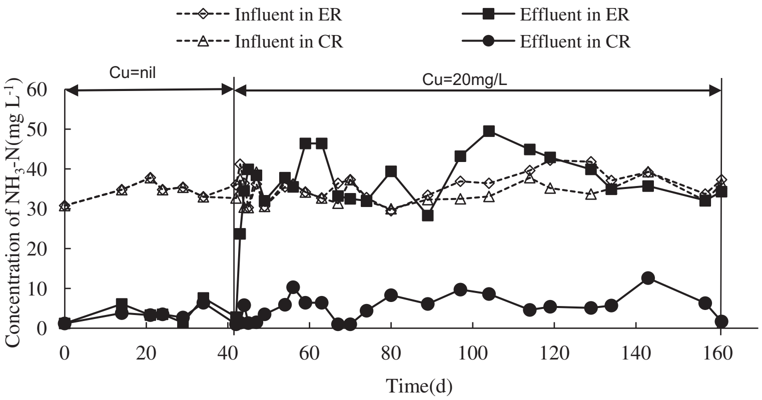

Fig. 2. Variation of concentration of influent and effluent NH$_3$-N in CR and ER.

3.2.1. Effect of Cu(II) shock dosage on pollutant removal

Fig. 3(a) shows that in each batch reactor, the efficiency of COD removal increased rapidly within 0.5 h, reaching more than 50% efficiency. This probably occurred because Cu(II) was adsorbed by the sludge floc in an early phase of the experiment, such that the Cu(II) exerted little toxicity on micro-organisms in the sludge [21,22]. Meanwhile, the biomass adsorbed some organic pollutants, leading to a rapid rise in the efficiency of COD removal. In the reactor that did not receive Cu(II), the effluent COD tended to be constant, maintaining between 92 and 96% efficiency for 2 h after the experiment began. As for the other three reactors, which were given shock dosages of Cu(II), the effluent COD increased slightly at 7.5 h after adding the Cu(II) dose. This may have occurred as a result of the acute toxicity of Cu(II), which causes some bacterial cells to rupture and stimulates some to secrete extracellular polymeric substances, leading to the observed increase in COD [23].

After 8 h of operation, the efficiency of COD removal in Cu(II)-containing reactors slightly increased. In the reactor with 10 mg L^{-1} Cu(II), the COD removal efficiency increased to 81.5% (at 11 h) and remained at 80% until 24 h. This finding indicates that AS markedly recovered its ability to degrade pollutants. The efficiency of COD removal in reactors containing 20 and 40 mg L^{-1} Cu(II) also recovered, to 60.3 and 52.5%, respectively, indicating that AS recovers differently in different systems. While this experiment was only conducted for 24 h, extending the exposure may allow micro-organisms to further adapt

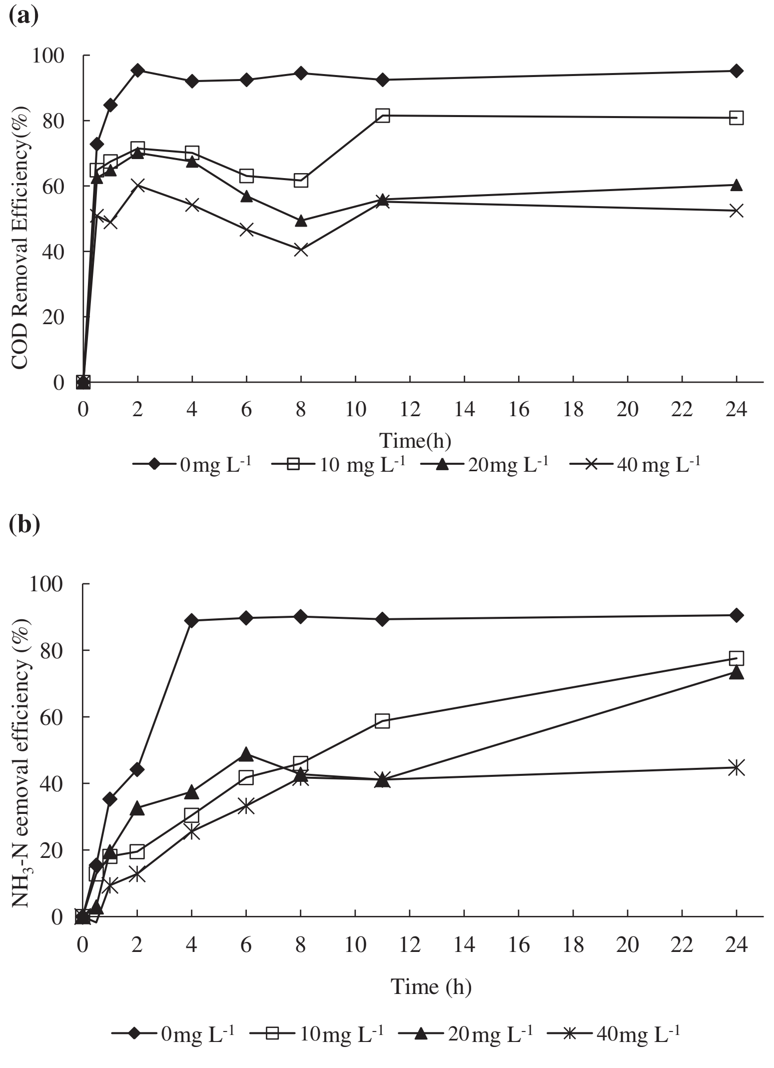

Fig. 3. Effect of different Cu(II) dosages on COD (a) and NH_3-N (b) removal ability in batch reactors.

to certain metals [24]. The results of our experiment also demonstrate that micro-organisms acclimated in a heavy-metal medium may eventually adapt to a cupric environment with continuous aeration. However, we found that the capacity of AS to recover its COD removal activity was different depending on how much Cu(II) was added during the shock dosage.

In the batch reactor without Cu(II), during the first 4 h of the experiment, the efficiency of NH_3-N removal significantly increased and then remained at 90%, as shown in Fig. 3(b). Conversely, the effluent COD in the same reactor became stable after 2 h rather than 4 h required for NH_3-N removal to stabilize. This indicates that nitrification occurred after carbonization. At 4 h, the batch reactor without Cu(II) reached almost 90% efficiency for NH_3-N removal. However, the efficiencies of NH_3-N removal in the reactors given shock dosages of Cu(II) were only 20%–40%, indicating that the ability of AS to degrade NH_3-N is severely inhibited after being exposed to a dose of Cu(II). After 24 h of operation, the efficiency of NH_3-N removal reached 77.6, 73.5, and 44.8% in reactors dosed with 10, 20, and 40 mg L^{-1} Cu(II), respectively.

While the efficiency of NH_3-N removal was severely hindered after the Cu(II) shock dosages, we did find that AS was able to recover some capacity for NH_3-N removal. Based on the Cu(II) shock dosages, we found that the efficiency of NH_3-N removal followed a trend at 24 h: $10 > 20 > 40$ mg L^{-1} Cu(II) (Fig. 3(b)). The NH_3-N removal efficiency decreased with increasing concentrations of Cu(II). Four hours after starting the experiment, the batch reactor without Cu(II) showed almost 90% NH_3-N removal. However, the batch reactors containing Cu(II) removed NH_3-N slowly, with 20–40% removal at 4 h, clearly indicating that the ability of AS to degrade NH_3-N was inhibited.

Six hours after the experiment began, the effluent concentration of NH_3-N slowly decreased in the reactor containing 10 mg L^{-1} Cu(II), and its removal efficiency was 78% at 24 h, recovering to 85.7% of that in the reactor without Cu(II). The efficiency of NH_3-N removal was consistently below 50% in the reactor containing 20 mg L^{-1} Cu(II) during the first 11 h, but it recovered to 74% at 24 h, which was 81.2% of that in the reactor without Cu(II). For the reactor containing 40 mg L^{-1} Cu(II), the acute toxicity of the dosage inhibited the removal of NH_3-N, resulting in an efficiency of 40% after 8 h, which was only half the removal obtained in the reactor without Cu(II). The different recovery capabilities of AS exposed to different Cu(II) shock dosages, with respect to NH_3-N removal, probably occurred because some nitrobacteria may have had a tolerance to Cu(II) and hence successfully degraded NH_3-N [25]. In addition, Tsai et al. found that some nitrobacteria, like Bacteroidetes, harbor a tolerance for certain heavy metals [24]. Our experiment indicated that there exists a certain threshold for sludge tolerance of Cu(II). The ability of AS to remove NH_3-N was inhibited, but was able to recover, when it was stressed with a shock dosage of 10 and 20 mg L^{-1} Cu(II); it failed to recover when a shock dosage of 40 mg L^{-1} Cu(II) was applied.

3.2.2. Assessing and comparing the effects of Cu(II) on the ability of AS to remove pollutants

The toxicity of heavy metals is a key factor that influences the ability of AS to remove pollutants. Generally, a quantitative toxicity assessment of AS in terms of its ability to remove pollutants can be obtained by calculating the ID [2,12].

As shown in Fig. 4(a), in the batch reactors given shock dosages of 10, 20, and 40 mg L^{-1} Cu(II), ID tended to increase continuously for the first 8 h and reached a peak at 8 h. After 11 h, the ID of the reactor containing 10 mg L^{-1} Cu(II) was below 15%. ID values exceeding 30% were found in the reactors containing 20 and 40 mg L^{-1} Cu(II). The degree of toxicity to AS caused by shock dosages of Cu(II), as measured by the ability of the AS to remove COD, can be described as follows: $40 > 20 > 10$ mg L^{-1}.

Previously, it has been reported that the toxicity to AS associated with continuous heavy metal exposure differs from the toxicity arising from a shock dosage of a heavy metal [26]. In our experiment, we found that on the 97th day of the continuous Cu(II) exposure experiment, COD removal efficiency in the ER reached a peak value of 78% after 6 h of aeration in that day's working period, compared with 83.2% in the CR. Such a recovery was similar to that seen in the batch reactor containing a shock dosage of 10 mg L^{-1} Cu(II). With a 20-mg L^{-1} shock dosage of Cu(II), the COD removal efficiency was already inhibited and therefore reached only 57% at 6 h. These results show that AS could remove COD better when Cu(II) exposure occurred over a long period of time compared to when Cu(II) exposure occurred as a short-duration shock dosage. Even though the aeration time was extended to 24 h in the batch reactor containing 20 mg L^{-1} Cu(II), the COD removal efficiency only recovered to 60%, which was less than the 78% COD removal obtained in the ER in the continuous exposure experiment. This indicates that after long-term exposure to Cu(II), micro-organisms in the ER recovered better and could remove more COD than in the batch experiment. However, continuing to expose AS to Cu(II) eventually caused permanent destruction of the AS system.

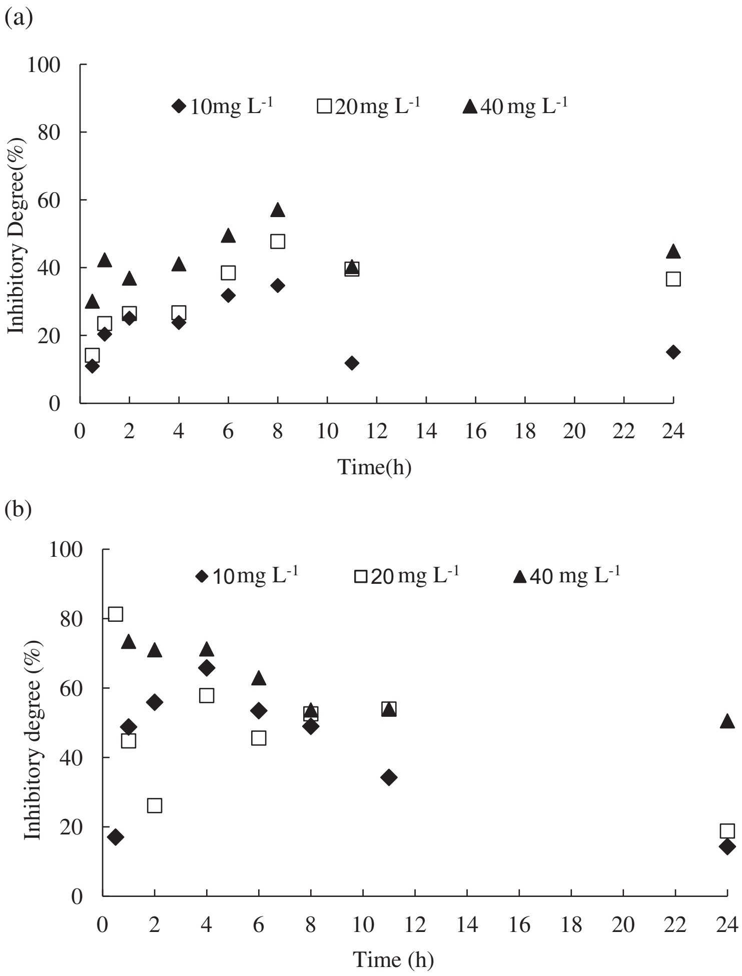

Fig. 4. Inhibition degree of COD (a) and NH_3-N (b) removal with different Cu(II) dosages.

In the reactor containing the 10-mg L^{-1} Cu(II) shock dosage, the ID increased to 65% in the first 4 h and then decreased to nearly 20% at 24 h (Fig. 4(b)). It shows that both the initial toxicity to micro-organisms caused by the shock dosage and the recovery of NH_3-N removal were remarkable. The ID values for the reactors containing 10, 20, and 40 mg L^{-1} Cu(II) were similar at 8 h, but prior to this time, their ID values differed. With a shock dosage of 40 mg L^{-1} Cu(II), the AS was strongly inhibited, and its ability to recover was relatively poor, yielding an average ID of 68.5%. The average ID related to NH_3-N removal during the 24-h experiment was 1.5–2 times greater than that of COD removal at the same shock dosage. This may have occurred because nitrobacteria are autotrophic bacteria, which are relatively fewer in number than heterotrophic bacteria in AS systems. Furthermore, nitrobacteria grow slowly, which magnify their negative effects and make them more susceptible to being overcome by heterotrophic bacteria [27].

Kim et al. discovered that in sludge exposed to 5, 15, and 30 mg L^{-1} Cu(II) for 9 h, the nitrification ID values were 26.4, 52.1, and 86.4%, respectively [28]. After 8 h in our shock experiment, the ID values were 49, 52.5, and 53.9% in reactors containing shock dosages of 10, 20, and 40 mg L^{-1} Cu(II), respectively. Therefore, the differences in nitrification ID values observed here were not as distinct as in previous studies.

4. Conclusions

In a 160-d experiment involving continuous Cu(II) exposure in a long-term operating SBR, we found that the ability of AS to degrade organic pollutants was seriously inhibited by continuous exposure to 20 mg L^{-1} Cu(II). Although the AS recovered the ability to remove COD in the ER 47 days after the onset of Cu(II) exposure, continuous exposure to Cu(II) severely hindered the ability of AS to degrade pollutants in the SBR. The ability of AS to remove NH_3-N was even more severely inhibited, given that the efficiency of NH_3-N removal was below 40% and failed to recover during the experimental period. In the Cu(II) shock dosage experiment, when AS was exposed to shock dosages of 10, 20, and 40 mg L^{-1} Cu(II), the sludge was able to tolerate Cu(II) toxicity to some extent. However, the capacity for AS recovery was different depending on the shock dosage applied: $10 > 20 > 40$ mg L^{-1} Cu(II) for COD removal and $10 > 20$ mg L^{-1} Cu(II) for NH_3-N removal; AS failed to recover after a shock dosage of 40 mg L^{-1} Cu(II). Moreover, in the shock dosage experiment, the inhibition degrees for NH_3-N removal were all greater than those for COD removal, and the degree of toxicity before recovery, measured by the ability of AS to remove COD, according to shock dosage, was $40 > 20 > 10$ mg L^{-1}.

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References

[1] F. Dilek, Ü. Yetis, Effects of heavy metals on activated sludge process, Water Sci. Technol. 26 (1992) 801–813.

[2] D.J. 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.

[3] W. Lo, L.M. Ng, H. Chua, P.H.F. Yu, S.N. Sin, P.K. Wong, Biosorption and desorption of copper (II) ions by *Bacillus* sp., Appl. Biochem. Biotechnol. 107 (2003) 581–592.

[4] P. Gikas, Single and combined effects of nickel (Ni(II)) and cobalt (Co(II)) ions on activated sludge and on other aerobic microorganisms: A review, J. Hazard. Mater. 159 (2008) 187–203.

[5] K. Pakshirajan, T. Swaminathan, Biosorption of lead, copper, and cadmium by *Phanerochaete chrysosporium* in ternary metal mixtures: Statistical analysis of individual and interaction effects, Appl. Biochem. Biotechnol. 158 (2012) 457–469.

[6] Y. Wu, J. Zhou, Y. Wen, L. Jiang, Y. Wu, Biosorption of heavy metal ions (Cu^{2+}, Mn^{2+}, Zn^{2+}, and Fe^{3+}) from aqueous solutions using activated sludge: Comparison of aerobic activated sludge with anaerobic activated sludge, Appl. Biochem. Biotechnol. 168 (2012) 2079–2093.

[7] S. Kalyuzhnyi, M. Gladchenko, A. Epov, V. Appanna, Removal of chemical oxygen demand, nitrogen, and heavy metals using a sequenced anaerobic–aerobic treatment of landfill leachates at 10–30˚C, Appl. Biochem. Biotechnol. 109 (2003) 181–195.

[8] S.A. Ong, P.E. Lim, C.E. Seng, M. Hirata, T. Hano, Effects of Cu(II) and Cd(II) on the performance of sequencing batch reactor treatment system, Process Biochem. 40 (2005) 453–460.

[9] S. Aslan, B. Gurbuz, Influence of operational parameters and low nickel concentrations on partial nitrification in a submerged biofilter, Appl. Biochem. Biotechnol. 165 (2011) 1543–1555.

[10] S.N. Sin, H. Chua, W. Lo, P.H.F. Yu, Effects of trace levels of copper, chromium, and zinc ions on the performance of activated sludge, Appl. Biochem. Biotechnol. 84–86 (2000) 487–500.

[11] M. Gutiérrez, J. Etxebarria, L. de las Fuentes, Evaluation of wastewater toxicity: Comparative study between Microtox® and activated sludge oxygen uptake inhibition, Water Res. 36 (2002) 919–924.

[12] S.R. Juliastuti, J. Baeyens, C. Creemers, D. Bixio, E. Lodewyckx, The inhibitory effects of heavy metals and organic compounds on the net maximum specific growth rate of the autotrophic biomass in activated sludge, J. Hazard. Mater. 100 (2003) 271–283.

[13] H. Chua, F.L. Hua, Effects of a heavy metal (zinc) on organic adsorption capacity and organic removal in activated sludge, Appl. Biochem. Biotechnol. 57–58 (1996) 845–849.

[14] Y.W. Lee, Q. Tian, S.K. Ong, C. Sato, J. Chung, Inhibitory effects of copper on nitrifying bacteria in suspended and attached growth reactors, Water Air Soil Pollut. 203 (2009) 17–27.

[15] APHA (American Public Health Association), Standard Methods for the Examination of Water and Wastewater, nineteenth ed., American Public Health Association, American Water Works Association, Water Environmental Federation, Washington, DC, 1995.

[16] A. Santos, E. Alonso, P. Riesco, Influence of cadmium on the performance of an activated SBR sludge treatment, Environ. Technol. 26 (2005) 127–134.

[17] S. Sirianuntapiboon, M. Boonchupleing, Effect of biosludge concentration on the efficiency of sequencing batch reactor (SBR) system to treat wastewater containing Pb^{2+} and Ni^{2+}, J. Hazard. Mater. 166 (2009) 356–364.

[18] L.G. Zhang, J. Yin, L. Liu, Characterization of metabolic activities of waste-activated sludge from the SBR process, J. Environ. Sci. Health. A 44 (2009) 752–757.

[19] J. Li, Y. Liu, T. Zhang, L. Wang, X. Liu, R.H. Dai, The effect of Ni(II) on properties of bulking activated sludge and microbial analysis of sludge using 16S rDNA gene, Bioresour. Technol. 102 (2011) 3783–3789.

[20] M.H. Gerardi, Nitrification and Denitrification in the Activated Sludge Process, Wiley, New York, NY, 2002.

[21] P. Battistoni, G. Fava, M.L. Ruello, Heavy metal shock load in activated sludge uptake and toxic effects, Water Res. 27 (1993) 821–827.

[22] J.P. Chen, D. Lie, L. Wang, S. Wu, B. Zhang, Dried waste activated sludge as biosorbents for metal removal: Adsorptive characterization and prevention of organic leaching, J. Chem. Technol. Biotechnol. 77 (2002) 657–662.

[23] S.F. Aquino, D.C. Stuckey, Soluble microbial products formation in anaerobic chemostats in the presence of toxic compounds, Water Res. 38 (2004) 255–266.

[24] T. Ginn, S.S. Sengor, S. Barua, J. Moberly, B. Peyton, Metal toxicity effects on microbial growth and

degradation, in: Slovenia and U.S. Workshop on Environmental Science and Engineering, Ljubljana, 2006, pp. 39–40.

[25] Y.P. Tsai, S.J. You, T.Y. Pai, K.W. Chen, Effect of cadmium on composition and diversity of bacterial communities in activated sludges, Int. Biodeterior. Biodegrad. 55 (2005) 285–291.

[26] G. Renella, A.M. Chaudri, P.C. Brookes, Fresh additions of heavy metals do not model long-term effects on microbial biomass and activity, Soil Biol. Biochem. 34 (2002) 121–124.

[27] B.E. Rittman, P.L. McCarty, Environmental Biotechnology Principals and Applications, McGraw-Hill, New York, NY, 2001.

[28] K.T. Kim, I.S. Kim, S.H. Hwang, S.D. Kim, Estimating the combined effects of copper and phenol to nitrifying bacteria in wastewater treatment plants, Water Res. 40 (2006) 561–568.