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Effect of high-strength ammonia nitrogen acclimation on sludge activity in sequencing batch reactor

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Abstract

The effect of high-strength ammonia nitrogen acclimation on sludge activity in sequencing batch reactor (SBR) was investigated. Two batch experiments, RUN1 and RUN2, were conducted with the influent ammonia nitrogen concentrations 60 and 500 mg/L, respectively. The sludges inoculated from RUN1 and RUN2 were used to treat a series of influent with ammonia nitrogen concentrations of 59, 232, 368, 604 and 1152 mg/L. It is found that the activated sludge acclimated to higher ammonia nitrogen concentrations revealed higher COD and NH_4^+-N removal efficiencies, and slower DHA decrease. The results confirmed that the activities of the bacteria in activated sludge in SBR were inhibited by high-strength ammonia nitrogen, whereas the activated sludge acclimated to high-strength ammonia nitrogen showed substantial resistance to inhibition by influents containing high levels of ammonia nitrogen.

Key words: activated sludge; sequencing batch reactor; high-strength ammonia nitrogen; dehydrogenase activity

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Introduction

Water pollution by ammonia nitrogen remains a serious environmental and public concern worldwide because it is an important nutrient that can cause eutrophication. Effluents containing ammonia nitrogen are primarily produced by the chemical industry, fertilizer production, landfills and piggery. Biological process has been used widely as one of the most promising methods to treat ammonia nitrogen wastewater. Several publications summarized the treatment feasibility by biological methods, such as MBR (membrane bioreactor), A/O MBR (anoxic/aerobic membrane bioreactor), SBR (sequencing batch reactor) (Xue et al., 2009; Galí et al., 2007; Hasar et al., 2009; Li and Zhao, 2001; Feng et al., 2007; Vázquez-Padín et al., 2009; Andreottola et al., 2001).

However, it is well known that high ammonia concentrations can not be remediated easily by conventional biological treatments. The reasons are that the removal efficiencies of COD (chemical oxygen demand) and NH_4^+-N decrease rapidly in response to high ammonia concentrations, and the DHA (dehydrogenase activity) of bacteria that can decline either. These decreases occur because high-strength ammonia nitrogen inhibits the activity of bacteria, including organics-utilizing bacteria and nitrifying bacteria (Ozturk et al., 2003), which subsequently affects the nitrification rates in post treatment systems. Overall, the main problem associated with the biological treatment of high strength industrial wastewater is that high concentrations of ammonia or nitrite can inhibit nitrification (Ilies and Mavinic, 2001). Limit research work has been documented on the detail regularities of the change and mechanisms in DHA and COD removal in the presence of ammonia-nitrogen at various levels.

The characteristics of nitrification, ammonia oxidation enzymes, and microbial activity in the presence of high concentrations of ammonia nitrogen have been thoroughly investigated using pure bacterial cultures and activated sludge (Li and Zhao, 1999; Dincer and Kargi, 2000). However little is known about the inhibitive and resistant characteristics of activated sludge exported to high concentrations of ammonia nitrogen (Campos et al., 2002; Spagni and Marsili-Libelli, 2009; Wang et al., 2007).

In the present study, SBR applied to nitrification and denitrification, was selected for wastewater treatment containing high strength ammonia nitrogen. DHA as a representative factor was examined for evaluating the bacteria activities. The study was conducted to compare the ability of SBRs to remove COD, NH_4^+-N and DHA from synthetic wastewater containing different levels of ammonia nitrogen. Additionally, we investigated the influence of different ammonia concentrations on the DHA, COD and ammonia levels to better understand the underlying change regularities and mechanism of microbial activities in the reactors.

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1 Materials and methods

1.1 Inoculum and synthetic wastewater

The inoculated sludge was supplied by a chemical wastewater treatment plant in Wuxi, Jiangsu Province, China. Synthetic wastewater was prepared based on the chemical properties of municipal and high strength industrial wastewater. The synthetic wastewater contained 250–600 mg/L COD, 1500 mg/L $NaHCO_3$, 50 mg/L KH_2PO_4, 50 mg/L $MgSO_4$, 50 mg/L NaCl, 50 mg/L $CaCO_3$ and trace elements. The inoculated sludges in two SBRs were acclimated to ammonia concentrations of 60 and 500 mg/L, respectively. All chemicals used were analytical reagent (AR) grade. Approximately 1.5 L of the supernatant was removed, and 1.5 L of the freshly prepared synthetic wastewater was fed.

1.2 Experimental setup

1.2.1 Operation of SBR experiments

Two SBRs (denoted RUN1 and RUN2) constructed of acrylic plastic (8 mm thick) with a working volume of 3.5 L were used (Fig. 1). The air flow inside the reactor was controlled by air pumps. The two reactors were operated with the same inoculated sludges. The inoculated sludges in RUN1 and RUN2 were acclimated to the synthetic wastewater contained about 60 and 500 mg/L ammonia, respectively.

The SBRs were operated at a cycle of 24 hr, each cycle comprised five phases: 20 sec to fill, 16 hr of aeration, 4 hr of settling, 20 sec to dispose and 4 hr to idle orderly. The systems were controlled using an automatic control system that consisted of time relays and a water level controller. The samples from SBRs were immediately filtered, and then analyzed for DHA, COD and ammonia.

1.2.2 Operation of batch experiments

Ten 300-mL reactors were performed in batch experiments to study the resistance to the inhibition of activated sludge by high-strength ammonia nitrogen. It was divided into two parts. In the first batch experiment, the inoculated sludges removed from RUN1 (60 mg/L NH_4^+-N) were put into five batch reactors, which allowed to settle for 30 min, the supernatant was then discarded, which named RUN1-1, RUN1-2, RUN1-3, RUN1-4, and RUN1-5. While in the second batch experiment, the inoculated sludges from RUN2 (500 mg/L NH_4^+-N) were put into batch reactors, which named RUN2-1, RUN2-2, RUN2-3, RUN2-4, and RUN2-5.

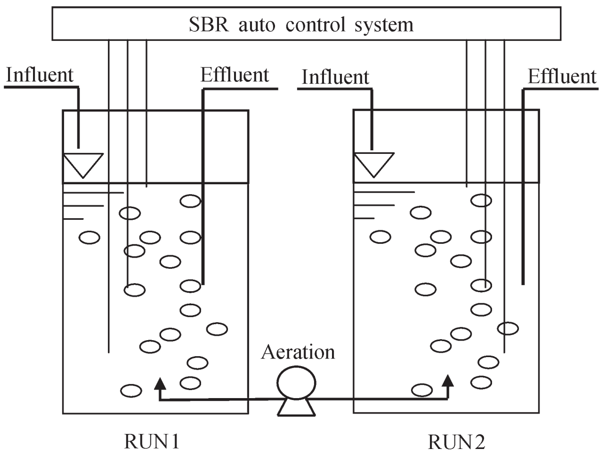

Fig. 1 Schematic of sequencing batch reactors (SBRs).

Synthetic wastewater was added to 300-mL batch-reactors containing ammonia concentrations of 59, 232, 368, 604 and 1152 mg/L, respectively. The same synthetic wastewater was treated, and operation parameters were as same as SBR. DHA, COD and ammonia were analyzed.

1.3 Analytical methods

The COD and ammonia concentrations were measured using a Shimadzu UV-1700 spectrophotometer (Japan) according to the Standard Methods (APHA, 1998).

DHA was measured using the TTC (triphenyl tetrazolium chloride) method, which is based on the production of triphenyl formazan (TPF) from the reduction of 2,3,5-triphenyltretrazolium (TTC). Briefly, 10 mL mixed sludge samples were added to 15 mL centrifuge tubes and then centrifuged at 4000 r/min for 10 min. The supernatant was then discarded and distilled water was added to give a final volume of 10 mL. Next, 2 mL of this sludge was mixed with 2 mL 5% TTC, 2 mL 0.1 mol/L glucose, and 2 mL Tris-HCl. The samples were then vortexed, and the tubes were closed and incubated in the dark for 16 hr at 37°C. After incubation, 2 drops of H_2SO_4 (98%) were added to each tube, followed by 5 mL of toluene. The samples were then shaken for 5 min and centrifuged at 4000 r/min for 10 min, after which the organic layer was removed. The absorbance of the organic layer at 492 nm was then measured using a spectrophotometer. DHA was then determined based on comparison with a standard curve generated from the analysis of seven standards containing 20, 40, 60, 80, 100, 120, and 140 μg/mL TTC corresponding to TF values of 0.018, 0.036, 0.054, 0.072, 0.09 and 0.108 mg TF/mL, respectively (Chaperon and Sauvé, 2008).

NH_4^+-N utilization rate (R) can be calculated according to Eq. (1):

$$R = \frac{(C_0 - C) \times V}{\text{MLSS} \times t} \tag{1}$$

where, C_0 (mg/L) is the concentration of influent ammonia nitrogen and C (mg/L) is the concentration of effluent ammonia nitrogen. V (L) is the volume of influent and t (day) is the reaction time.

2 Results and discussion

2.1 Acclimation of activated sludge by high-strength ammonia nitrogen in SBR

The activated sludges from RUN1 and RUN2 were acclimated by exposure to similar conditions. Figures 2 and 3 show the important parameters during the SBR operation in RUN1 and RUN2, respectively. As shown in Figs. 2a and 3a, the COD removal efficiency of RUN1 was maintained at greater than 83.81% throughout the experiment, while the removal of RUN2 was maintained at about 54.16%.

The activity of bacteria that utilize organic materials is commonly evaluated based on the DHA, because it reflects the enzymatic activity of the bacteria. Figures 2c and 3c provide a comparison of the DHA in the two reactors. The average DHA of RUN1 (12.92 mg TF/(g MLSS·hr)) was much higher than that of RUN2 (5.60 mg TF /(g MLSS·hr)), which indicates that no substantial inhibition of microbial activity was found with initial ammonia nitrogen 60 mg/L.

Comparing RUN1 and RUN2, the decreases of COD reduction and DHA had similar trends against the higher ammonia nitrogen concentration in the reactor. These results indicate that the activity of bacteria that utilize organic materials in the activated sludge was inhibited by high-strength ammonia nitrogen.

The concentration of ammonia nitrogen in municipal wastewater is generally less than 50 mg/L; therefore, there was almost no negative influence on nitrification in RUN1. However, inhibition of microorganisms activity that oxidize ammonia to nitrite was found to begin at

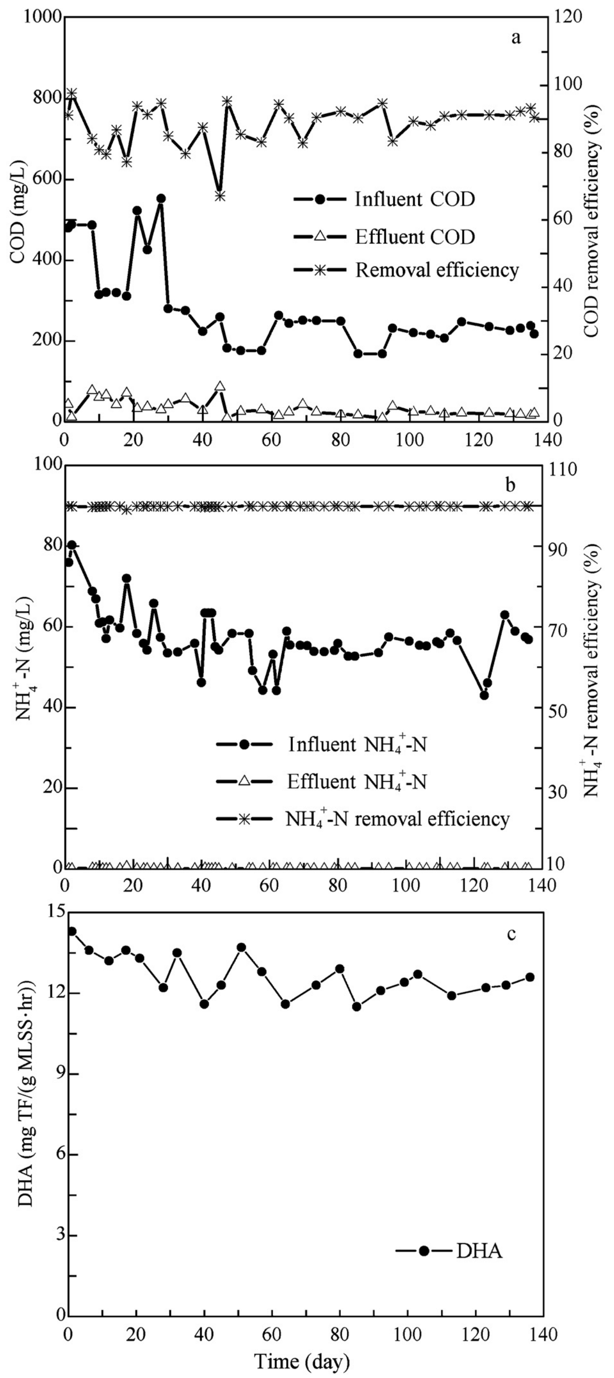

Fig. 2 Profiles of COD (a), NH_4^+-N (b), and DHA (c) in RUN1 (NH_4^+-N 60 mg/L).

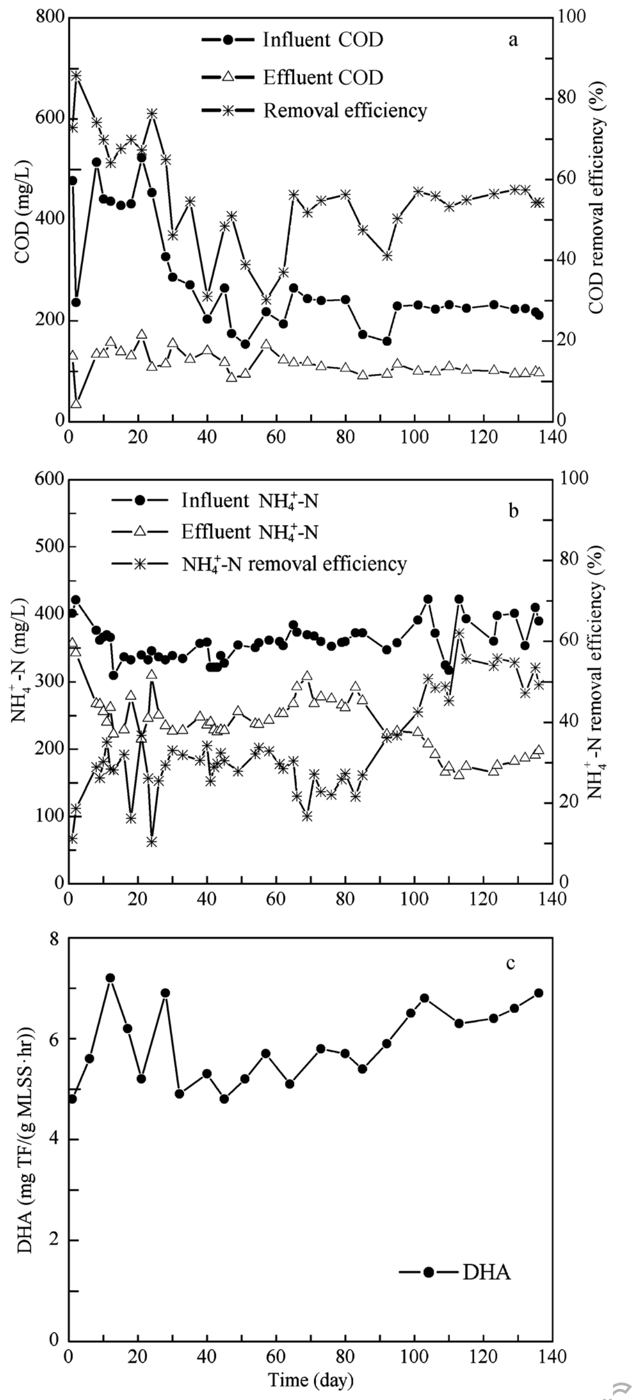

Fig. 3 Profiles of COD (a), NH_4^+-N (b), and DHA (c) in RUN2 (NH_4^+-N 500 mg/L).

ammonia nitrogen concentration of 150 mg/L in a system operated at 25°C and pH 8.0 (Chaperon and Sauvé, 2008).

As shown in Fig. 2b, at ammonia nitrogen in the influent about 60 mg/L, the average NH_4^+-N removal efficiency of RUN1 was 99.82%, indicating a good nitrification performance of SBR. Low ammonia nitrogen concentration can not affect the bacteria activity, which resulted in good removal performance of ammonia nitrogen. In RUN2 (Fig. 3b), at the beginning, average ammonia nitrogen removal efficiency was about 30%. From day 92, ammonia nitrogen removal efficiency improved gradually. The average removal efficiency was about 50%, and the maximum removal efficiency was 61.98%. Removal efficiency for ammonia nitrogen of RUN2 was ordinary, which was lower than the average NH_4^+-N removal efficiency of RUN1. These findings indicate that the increased ammonia nitrogen levels in wastewater led to a rapid decrease in the removal efficiency of NH_4^+-N, which suggests that the activities of nitrifying bacteria in sludge were inhibited by the rapid increase in NH_4^+-N concentration.

Throughout the whole SBR operational phase, we can see clearly from Fig. 4. Although the average NH_4^+-N removal efficiency of RUN1 was higher than that of RUN2, the NH_4^+-N utilization rate by activated sludge in RUN2 was higher than that of RUN1, indicating that when the activated sludge was acclimated to high-strength ammonia nitrogen it developed a strong resistance to the inhibition by ammonia nitrogen.

2.2 One circle operation in SBR

The SBR process was operated in cycles, the COD decline rate was continuous throughout the aeration, settling and idle stages. As shown in Fig. 5, the COD in RUN1 was reduced from 454.40 to 27.73 mg/L, while the COD in RUN2 was reduced from 465.07 to 106.67 mg/L. These results also indicate that the activity of bacteria that utilized organic materials in the sludge was inhibited by the high-strength ammonia nitrogen. The trend in the DHA differed from that of the COD. Specifically, the DHA in RUN1 increased from 13 to a maximum value of 14 mg TF/(g MLSS·hr), and then declined continuously to 2.8 mg TF/(g MLSS·hr). In RUN2, the DHA increased from 5.6 to 6.7 mg TF/(g MLSS·hr), and then it declined continuously to 0.8 mg TF/(g MLSS·hr). These findings indicate that it takes several hours for bacteria utilizing organics to adapt to high-concentrations of ammonia nitrogen, which may result in an increase in their activity. However, after their activity increases, the concentration of organic materials in the wastewater decreases, which is followed by a decrease in DHA.

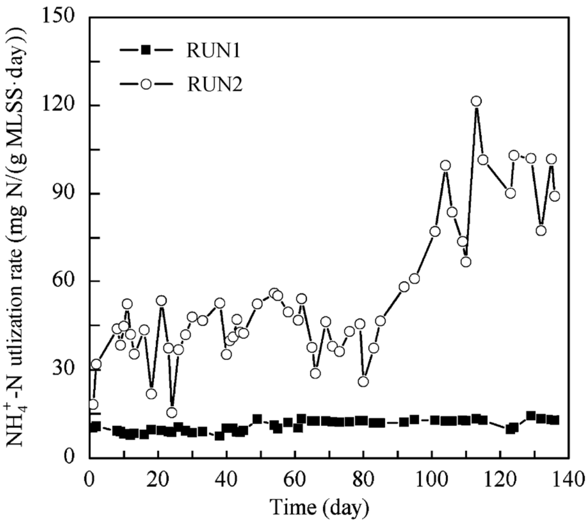

Fig. 4 NH_4^+-N utilization rate by activated sludge.

2.3 Effect of activated sludge by different concentrations of ammonia nitrogen in batch experiments

Five experiments were conducted at feed ammonia nitrogen concentrations of 59, 232, 368, 604 and 1152 mg/L, respectively. Figure 6a, b show the COD and ammonia nitrogen removal efficiencies and the utilization rate by activated sludge obtained under different feed ammonia nitrogen concentrations.

In the first batch experiment, from RUN1-1 to RUN1-5, the average effluent COD concentration increased from 56.6 to 311.2 mg/L and the effluent NH_4^+-N concentration increased from 0.12 to 1074.99 mg/L. In the second batch experiment, from RUN2-1 to RUN2-5, the average effluent

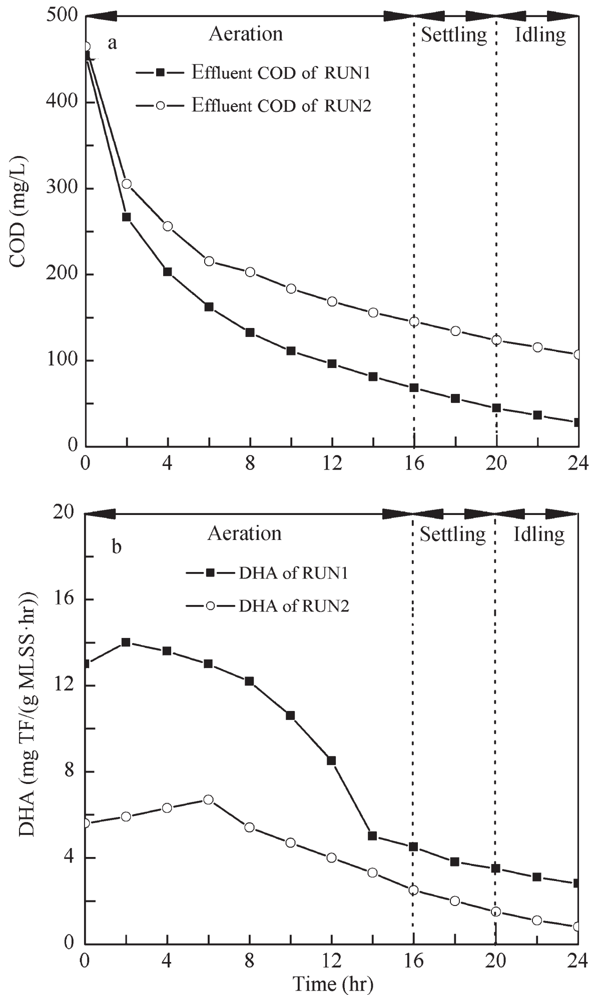

Fig. 5 COD (a) and DHA (b) in a single cycle operation in RUN1 and RUN2.

COD concentration increased from 42.4 to 169.8 mg/L and the effluent NH_4^+-N concentration increased from 0.07 to 955.36 mg/L. The variation in the influent ammonia nitrogen concentration showed a similar trend as was observed for the effluent COD and NH_4^+-N concentrations. The COD and NH_4^+-N utilization rate by activated sludge of RUN2 was higher than that of RUN1, which indicates that when the activated sludge was acclimated to high-strength ammonia nitrogen it developed a strong resistance to the inhibition by ammonia nitrogen. With higher utilization rate being observed for the second batch experiment than for the first batch experiment in response to all treatments. The high concentration of ammonia nitrogen affects microbial activity. As shown in Fig. 6c, the DHA of the first batch experiment decreased from 5.6 to 1.3 mg TF/(g MLSS·hr), while that of the second batch experiment decreased from 4.1 to 2.6 mg TF/(g MLSS·hr). The rate of first batch experiment decreased quickly. These findings suggest that there is a potential to apply biochemical reactor for the treatment of wastewater containing high-strength ammonia nitrogen by SBR.

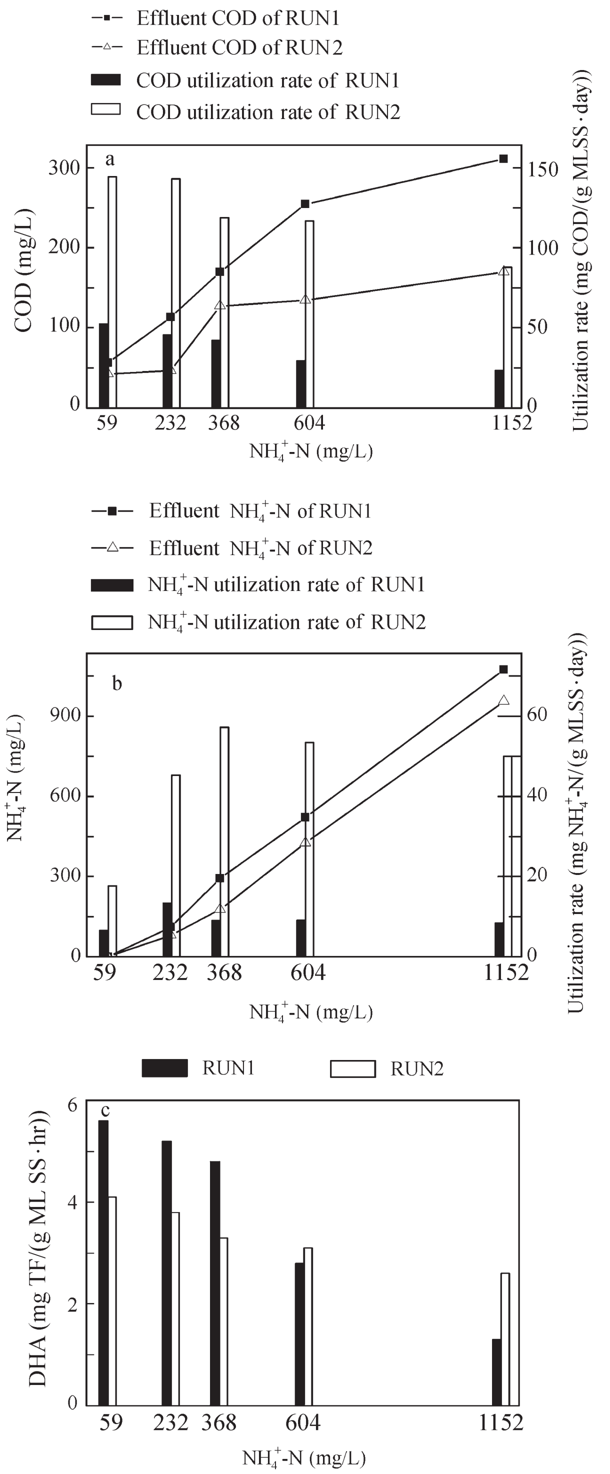

Fig. 6 Profiles of COD (a) (influent COD concentration in reactor 523 mg/L), NH_4^+-N (b), and DHA (c) affected by ammonia nitrogen.

3 Conclusions

Comparing two reactors with influent ammonia nitrogen concentrations of 60 and 500 mg/L, it demonstrated that the activity of organics-utilizing bacteria and nitrifying bacteria were inhibited by high-strength ammonia nitrogen. The findings indicated that the wastewater containing high organics and ammonia nitrogen concentrations could be subjected to biochemical treatment to reduce the level of organic compounds, followed by other combined processes to remove the ammonia nitrogen.

Although the activated sludge was inhibited by high-strength ammonia, the sludge showed obvious resistance to this inhibition following acclimation. Indeed, both the COD and NH_4^+-N removal efficiencies of activated sludge acclimated by exposure to high concentrations of ammonia nitrogen were higher than that of sludge that was not acclimated when the influent NH_4^+-N concentration increased from 59 to 1152 mg/L. In addition, the decrease in the DHA of activated sludge acclimated to high concentrations of ammonia nitrogen was slower than that of normal sludge. Taken together, these findings indicate that acclimation of sludge to high levels of ammonia nitrogen induced resistance to inhibition by wastewater containing high-strength ammonia nitrogen.

Acknowledgments

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