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Inhibition of the activities of activated sludge in a sequencing batch reactor by high-strength ammonium nitrogen

Y.H. Ding*, H.Q. Ren, Q. Wang

State Key Laboratory of Pollution Control and Resources Reuse, School of the Environment, Nanjing University, Nanjing 210093, China
Email: yhding@nju.edu.cn

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ABSTRACT

Two sequencing batch reactors were applied to investigate the activated sludge activities by high-concentration ammonia. The results indicated that the activity of nitrifying bacteria was much more susceptible than that of organics-utilizing bacteria and more severely inhibited by high-concentration ammonia. DHA in one SBR time cycle was increased to a maximum at first and then gradually decreased to a minimum value, which indicated that the activities of organics-utilizing bacteria could be maintained stably when fed with plenty of organic nutrients under high-concentration ammonia, but both the removed load of COD and NH_4-N in activated sludge acclimated under high-concentration ammonia were higher than that under normal conditions, and the DHA declining rate of sludge acclimated under high-concentration ammonia was slower. This means that the activated sludge acclimated under high-concentration ammonia was also able to obtain strong resistance to inhibition by high-concentration ammonia, and the ratio of nitrifying bacteria to heterotrophic bacteria was increased gradually.

Keywords: Activities; Activated sludge; Sequencing batch reactor; High-strength ammonia

1. Introduction

In general, ammonia is one of the critical components in many kinds of wastewater and is regarded as one of the key pollutants to cause damages to water quality, such as eutrophication and black-odor of water body. So it is obligatory for the residual ammonia in the treated wastewater to satisfy the Integrated Wastewater Discharge Standard (GB 8978-1996). To some degree, it is easily degraded by biochemical processes, for example, ammonia can be effectively absorbed and assimilated by nitrifying bacteria in conventional sludge process, because its concentration is usually below 50 ppm, which is appropriate for the bacteria survival.

However, some kinds of industrial wastewaters from chemical/pharmaceutical industries, landfill leachate and [1,2] contain high-concentration ammonia, that is inhibitive and toxicant to biological activities. Therefore, many kinds of physicochemical reactions are explored to remove high-concentration

*Corresponding author.

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ammonia from wastewater. For example, ammonia can be effectively precipitated and removed from wastewater as MAP (magnesium ammonium phosphate (MAP), with an appropriate stoichiometric ratio (e.g. $Mg/NH_4/PO_4 = 1:1:1$), or blown off as ammonia gas by air stripping, with an appropriate pH value of 9–11. The residual ammonia in treated wastewater can be easily absorbed or degraded by a subsequent biochemical process. Although physicochemical reactions are regarded as a highly effective method to deal with wastewater containing high-concentration ammonia, this method has some negative impacts to the environment, for example, the residual chemical sludge and ammonia gas is produced or released in large amounts by this physicochemical reaction process [3]. Thus, it is necessary to deal with disposal of residual chemical sludge or absorb and purify ammonia gas. These physicochemical reaction processes demand relatively high treating costs and strict operation.

Therefore, as compared with physicochemical reaction processes, biochemical processes, such as conventional activated sludge process, have some obvious advantages, for example, ammonia is absorbed and changed into nitrite, nitrate, and nitrogen gas, or assimilated into cell tissues. All these produced components are much more compatible with the environment than residual chemical sludge or ammonia gas. However, the biochemical processes are usually destroyed by strong inhibition from high-concentration ammonia, and this results in an obvious decrease in all kinds of pollutants in wastewater, including ammonia and other organic compounds.

Researchers found that the DHA (dehydrogenase activity) value of organics-utilizing bacteria is decreased rapidly by high-concentration ammonia, as the COD (chemical oxygen demands) removal efficiencies are decreased. It seems that the activities of organics-utilizing bacteria are strongly inhibited by high-concentration ammonia [4]. The characteristics of nitrification, ammonium oxidation enzyme, and microbe activities were investigated with pure bacteria culture, activated sludge [5–7], and many other biological systems, such as membrane bioreactor, anoxic/oxic, anaerobic/anoxic/oxic processes, etc., because it takes totally 4.57 mg O_2 to oxidize 1 mg ammonia to nitrite nitrogen, and to add extra carbon sources to realize denitrification with pH adjustment, so that many biochemical processes are costly, with low ammonia removal efficiencies—less than 50% [8,9].

But little was known about the inhibitive and resistive characteristics of activated sludge in SBR (sequencing batch reactor) with high-concentration ammonia [10,11]. At present, SBR, characterized by simple configuration, little volume, automatic control and high removal efficiencies for nitrogen, organics, and phosphorus, is frequently used to treat municipal or industrial wastewater, and therefore, it is important to study the inhibition and resistance of activated sludge to high-concentration ammonia in SBR.

In these experiments, two series of SBR reactors and ten series of flasks are used to acclimate the activated sludge and investigate the inhibition and resistance of activated sludge to high-concentration ammonia in SBR.

2. Materials and methods

2.1. Experimental set-up

Two series of sequencing batch reactors, called run 1 and run 2, respectively, are made of acrylic plastic (8 mm thick), with a working volume of 3.5 L. One SBR time cycle is 24 h, it takes 20 s to fill, 16 h to aerate, 4 h to settle, 20 s to dispose, and 4 h to idle orderly, an automatic control assembly, composed of time relays and a water-level controller are employed to maintain the SBR system to run stably. Fig. 1 shows the schematic system of SBR. The activated sludge was supplied by a chemical wastewater treatment plant in Wuxi city of Jiangsu province.

2.2. Synthetic wastewater

High-concentration ammonia wastewater is prepared according to some chemical properties of municipal and industrial wastewater. Run 1 and run 2 are supplied with ammonia concentration of about 50 mg/L and 400 mg/L, respectively, other components include 600 mg/L glucose, 50 mg/L $MgSO_4$, 50 mg/L $CaCl_2$, 100 mg/L $NaHCO_3$, 100 mg/L NaCI, and 100 mg/L KH_2PO_4, respectively, and the trace elements are added as well.

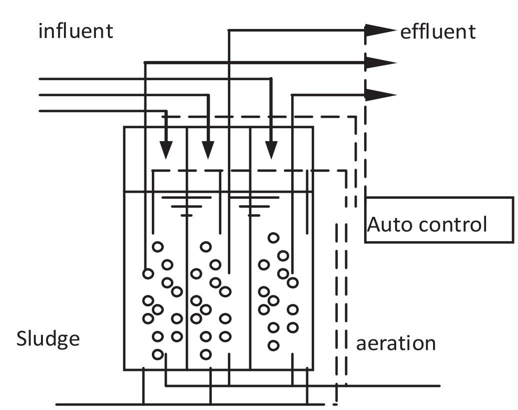

Fig. 1. A schematic representation of sequencing batch reactors.

2.3. Analytical methods

The measurement of COD (chemical oxygen demands) and NH_4-N is conducted according to the Standard Methods [12]. A Japan Shimadzu spectrophotometer UV1700 is used. DHA (dehydrogenase activity) is measured by the TTC methods, including triphenyl formazan (TPF) produced from the reduction of 2,3,5-triphenyltretrazolium (TTC). About 10 ml activated sludge is added into 15-ml centrifugal tubes, centrifuged for 10 min, and added to 10 ml distilled water. Then, 2 ml activated sludge is taken from the centrifugal tubes to 20-ml centrifugal tubes. To each tube, 2 mL 3% TTC, 2 ml 0.1 mol/l glucose, and 2 mL tris-HCI are added. Then, the samples are mixed on a vortex, and the tubes are closed and incubated in the darkness for 16 h at 37℃. After the incubation, 2 drops H_2SO_4 (98%) are added into each tube to check the reactions. Five milliliter of toluene is added. The samples are shaken for 5 min and siphoned out organic layer to analyzed for DHA. For calibration, seven standards of TTC (20, 40, 60, 80, 100, 120, and 140 μg/ml) are prepared, corresponding to the TF values of 0.018, 0.036, 0.054, 0.072, 0.09, and 0.108 mg TF/mL. The red-colored complexes are measured within 1 h using the same spectrophotometer as for DHA at 492 nm [13].

The sludge oxygen utilization rate (SOUR) is determined by calculating the O_2 respiration rate of microbes. In this process, about 300 ml of activated sludge is sucked out into a DO bottle (250 ml) and settled down for 30 min, then siphoned out upper water, and filled with aerated synthetic wastewater (50 mg/L NH_4-N). Finally, a rubber plugged with an DO probe is used to airproof the DO bottle. The mixed liquor sludge is agitated by an electromagnetic beater, and simultaneously, the DO value with time is recorded, and the SOUR is expressed by mg O_2/(g MLSS·h). When glucose is contained in the synthetic wastewater, the SOUR is regarded as contributed to all the microbes in the activated sludge; however, when no glucose is contained in the synthetic wastewater, the SOUR is mainly contributed to the nitrifying bacteria [14]. The inhibiting resistance of activated sludge by high-concentration ammonia is carried as follows: 5 portions of 50 ml mixed suspended sludge are taken from run 1 and run 2, respectively, settled for 30 min and siphoned out the upper separated water layer, then the sludge is introduced into 300-ml flasks, ammonium nitrogen with concentrations of 50, 200, 350, 600, and 1,000 mg/L is added, aerated for 16 h, and idled for 1 h. Finally, the series of sludge is analyzed for index of DHA, COD, and NH_4-N, respectively.

3. Results and discussions

3.1. COD removal and DHA of activated sludge

The activated sludge in run 1 and run 2 was acclimated at similar operational parameters, but there was an obvious difference of added ammonia concentration between run 1 and run 2. The ammonia concentration in run 1 and run 2 was about 50 and 500 mg/L, respectively. Then, differences of COD removal efficiencies between run 1 and run 2 were observed. Fig. 2 shows that the COD removal efficiency of run 1 was maintained above 83.81%; however, run 2 was at 54.16%. In other words, if the ammonia concentration was smaller than 50 mg/L, no obvious and negative influence was found on the activities of organics-utilizing bacteria; therefore, the COD removal efficiency was commonly high. But if the ammonia concentration was raised up to the level of hundreds ppm, an obvious decrease in the COD removal efficiency was observed. The results indicated

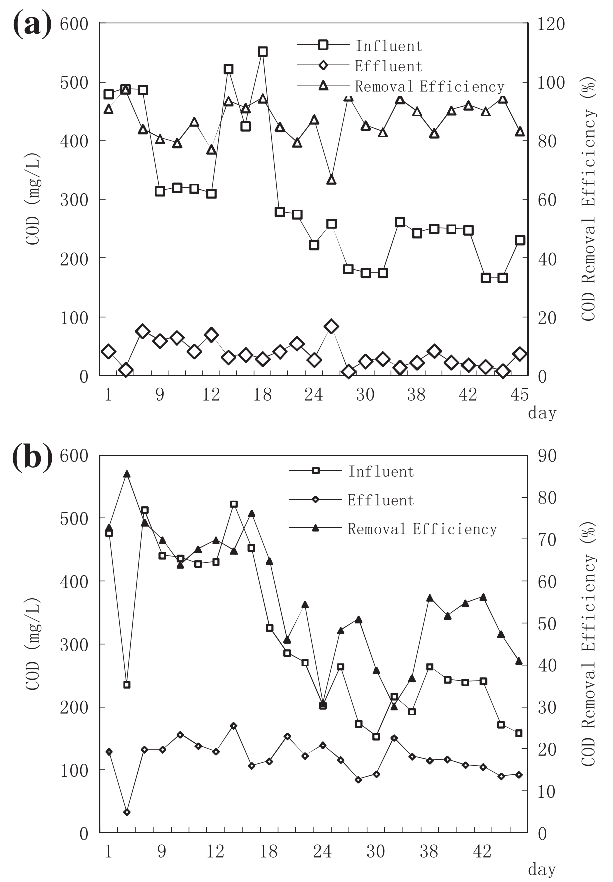

Fig. 2. COD removal performance (a). run 1; (b). run 2 (variation of the influent and effluent COD concentration, and removal efficiencies).

that the activities of organic-utilizing bacteria in activated sludge were inhibited severely by high-concentration ammonia.

DHA is usually used to represent the enzyme activities of bacteria. Fig. 3 shows that the average DHA value of run 1 was 12.92 mg TF/[g MLSS h], which was much higher than 5.60 mg TF/[g MLSS h] of run 2. Therefore, it is possible to reflect the activity changes of organics-utilizing bacteria by DHA value, that is, the DHA curve can be used to express directly the inhibition to activities of activated sludge by high-concentration ammonia.

As SBR is operated by time cycles, in each time cycle, it is natural for all kinds of bacteria in activated sludge to accommodate and survive in aerobic, anaerobic, and anoxic environment, so that its COD and DHA curves are very peculiar. Fig. 4 shows that the COD concentration for run 1 and run 2 was reduced from 454.40 and 465.07 mg/L in the beginning to 27.73 mg/L and 106.67 in the end, respectively, while the performance of DHA was different from COD. At first, the DHA value of the activated sludge in run 1 increased from 13 to 14 mg TF/[g MLSS h] and then declined gradually to 2.8 mg TF/[g MLSS h]. The DHA value of sludge in run 2 increased from 5.6 to 6.7 mg TF/[g MLSS h] at first and then declined gradually to 0.8 mg TF/[g MLSS h]. The results indicated that the COD concentration decreased continuously from a maximum to a minimum value in each time cycle. The DHA changes were different from COD—it rose up to a maximum value in the beginning for hours, then decreased continuously to a minimum value in each time cycle. This can be explained by the fact that when bacteria in activated sludge were supplied with plenty of organic nutrients, the inhibition of the activity of organic-utilizing bacteria by high-

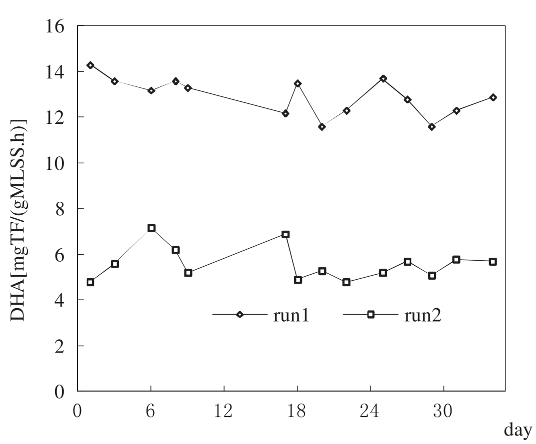

Fig. 3. DHA performance of activated sludge in run 1 and 2.

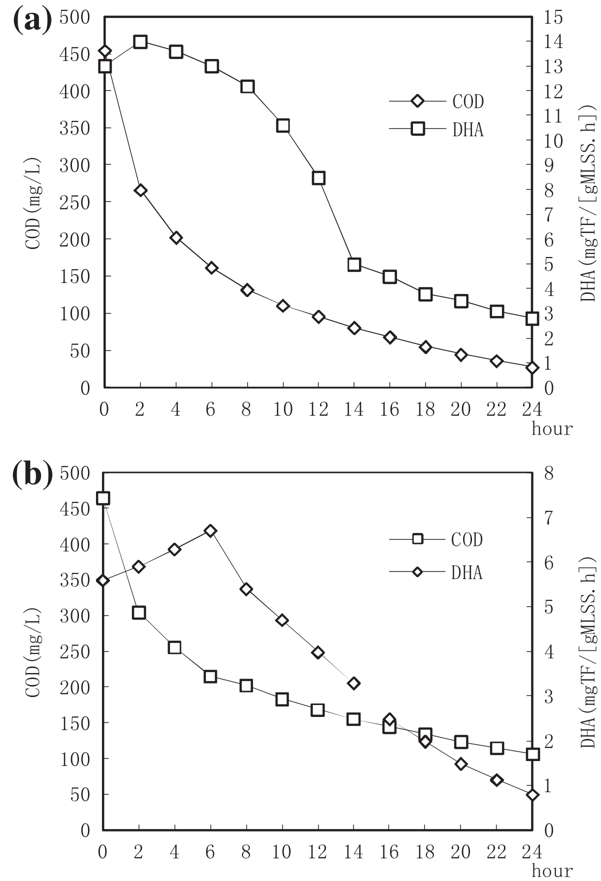

Fig. 4. COD and DHA performance in one cycling time (a). run 1; (b). run 2 (variation of COD concentration of liquid and variation of DHA of activated sludge in the reactor).

concentration was not obvious, but when the organic nutrients were exhausted, the activity of organic-utilizing bacteria was inhibited severely by high-concentration ammonia. So the activities of organics-utilizing bacteria increased to an maximum value when fed with high-concentration levels of organics, then declined when organics concentration decreased sharply. On the other hand, the activity of bacteria in the activated sludge acclimated by high-concentration ammonia was inhibited much more severely than that by the normal concentration level of ammonia.

3.2. NH_4-N removal

The ammonia concentration in municipal wastewater is commonly less than 50 mg/L. No obvious negative influence is found on the nitrification process, but if the ammonia concentration is enhanced to hundreds ppm, an obvious decline in NH_4-N removal efficiency occurs. Fig. 5 shows that the NH_4-N removal efficiency of run 1 was 99.82% in average and

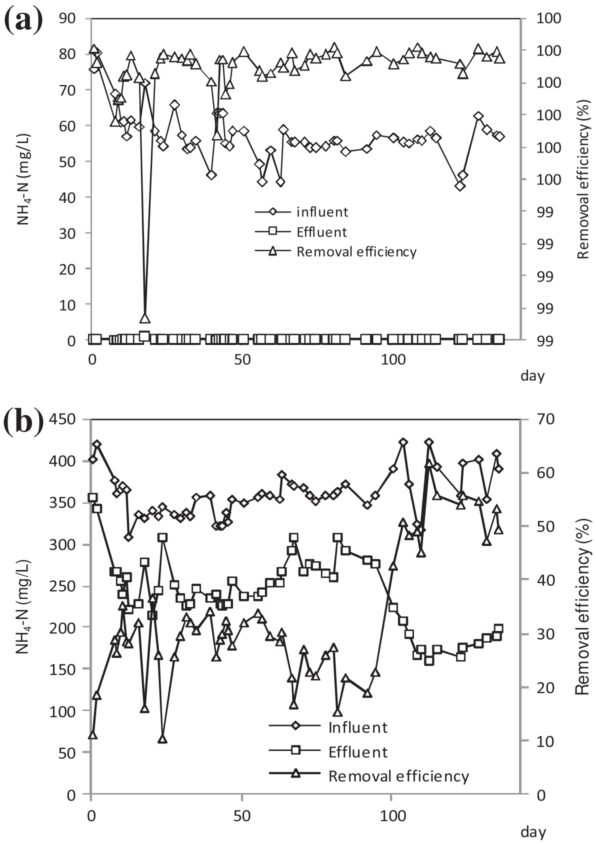

Fig. 5. NH_4-N removal performance (a). run 1; (b). run 2 (variation of the influent and effluent NH_4-N concentration and removal efficiencies).

that of run 2 was 33.24%, as the ammonia concentration of run 1 was about 50 mg/L, but that of run 2 was 400 mg/L.

The results indicate that the activities of nitrifying bacteria in activated sludge were also inhibited severely if the ammonia concentration was enhanced rapidly.

Although both activities of organics-utilizing bacteria and nitrifying bacteria in the activated sludge were inhibited by high-concentration ammonia to some degree, the activity of nitrifying bacteria was much more susceptible than that of organics-utilizing bacteria. This indicates that wastewater containing high-concentration ammonia and organic compounds was able to be treated by SBR to remove ammonia and organic compounds partly. This experimental result is very significant, because it indicates that it was possible for the high-concentration ammonia wastewater to be treated by combined physical/chemical or biochemical processes directly.

3.3. Resistance to the inhibition of activated sludge by high-concentration ammonia

It was possible for the sludge activities to be expressed instantly by SOUR(n) (SOUR of nitrifying bacteria) and SOUR(h) (SOUR of heterotrophic bacteria, or organics-utilizing bacteria). Fig. 6 shows that the total SOUR of nitrifying bacteria and heterotrophic bacteria in run 1 was much higher than that of run 2, but the ratio of SOUR(n) to SOUR(h) in run 2 increased gradually and higher than that in run 1. These results indicate that the SOUR of nitrifying bacteria in run 2 increased gradually, and the resistance of nitrifying bacteria to inhibition by high-concentration ammonia increased gradually also.

Although the activities of activated sludge were inhibited by high-concentration ammonia, it showed obvious resistance to that inhibition, the NH_4-N removal efficiency of run 2 increased slowly despite inhibition by high-concentration ammonia. In the series of experiments on resistance, the series of NH_4-N concentration was planed at about 50, 200,

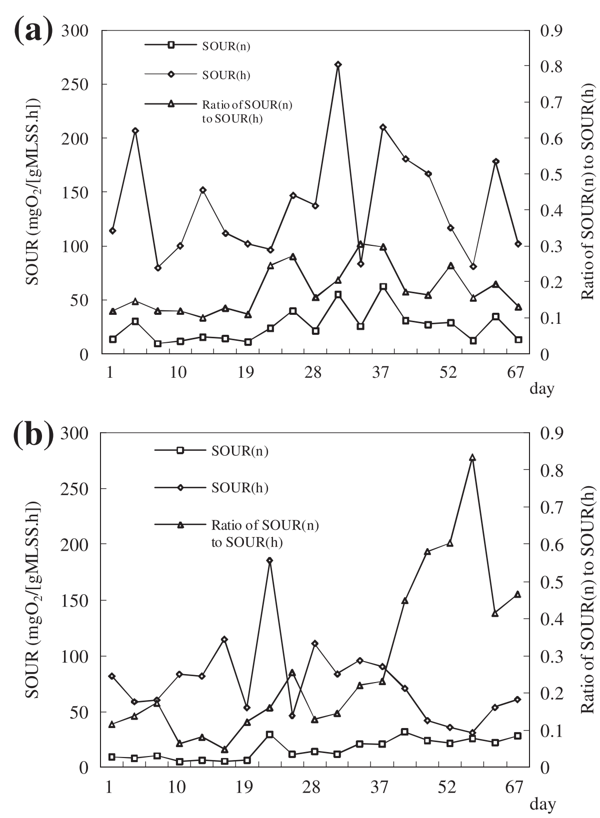

Fig. 6. SOUR performance (a). run 1; (b). run 2 (SOUR of nitrifying bacteria [SOUR(n)], SOUR of heterotrophic bacteria [SOUR(h)], and its ratio).

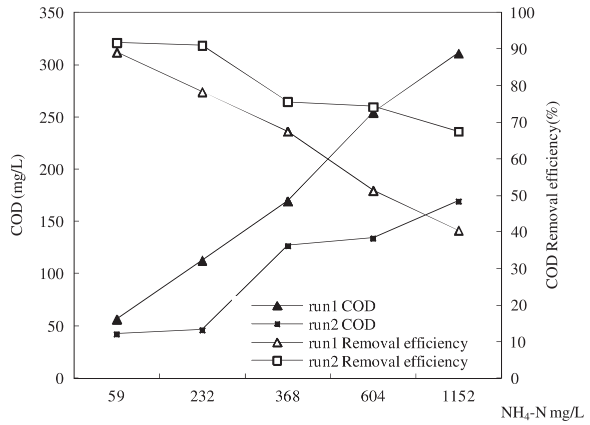

Fig. 7. COD removal efficiencies affected by the concentrations of ammonium nitrogen (the sludge was taken from run 1 and run 2, respectively).

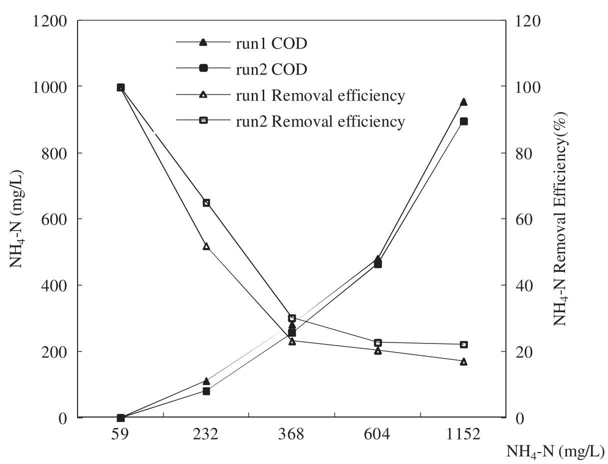

Fig. 8. NH_4-N removal efficiency affected by the concentrations of ammonium nitrogen (the sludge was taken from run 1 and 2, respectively).

350, 600, and 1,000 mg/L, respectively, its accurate concentration measured was 59, 232, 368, 604, and 1,152 mg/L ,respectively. Figs. 7 and 8 show that both COD and NH_4-N removal efficiencies of activated sludge taken from run 2 were much higher than those of run 1, when the NH_4-N concentration added was enhanced from 59 to 1,152 mg/L. Viewing the curving slope of Fig. 9, DHA declining rate of run 1 was faster than that of run 2. Fig. 10 shows that the NH_4-N load removed by activated sludge in run 2 was much higher than that of run 1, and therefore, it is reasonable that if the activated sludge was acclimated under the condition of high-concentration ammonia for a long time, and it was possible to obtain a strong resistance to the inhibition. The experimental result indicated that it is possible to apply SBR to treat wastewater containing high-concentration ammonia.

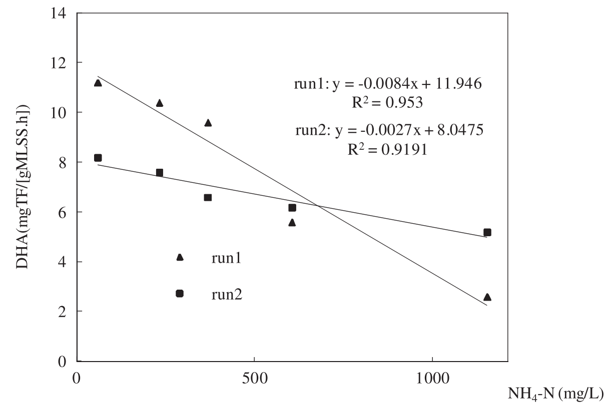

Fig. 9. DHA affected by the concentrations of ammonium nitrogen (the sludge was taken from run 1 and run 2, respectively).

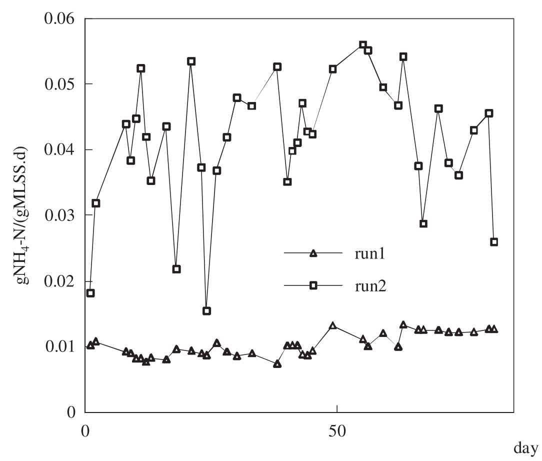

Fig. 10. NH_4-N load removed by activated sludge.

4. Conclusion

All the experimental results indicated that although both the activities of organics-utilizing bacteria and nitrifying bacteria in activated sludge were inhibited by high-concentration ammonia to some degree, the activity of nitrifying bacteria was much more susceptible than that of organics-utilizing bacteria. The activity of bacteria acclimated by high-concentration ammonia was inhibited much more severely than that by normal concentration level of ammonia.

In one SBR time cycle, when bacteria in activated sludge was supplied with plenty of organic nutrients, the inhibition of the activity of organic-utilizing bacteria by high concentration was not obvious, but when the organic nutrients was exhausted, the activity of organic-utilizing bacteria was inhibited severely by high-concentration ammonia.

The DHA and resistance experiments values indicated that the activities of organics-utilizing bacteria were able to be maintained stably when fed with plenty of organic nutrients and be inhibited obviously by high-concentration ammonia when organic nutrients was exhausted. The results of experiments on resistance also showed that the activated sludge acclimated under high-concentration ammonia was also able to obtain an strong resistance to the inhibition by high-concentration ammonia, and the ratio of nitrifying bacteria to heterotrophic bacteria was increased gradually either.

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