Influence of Chemical Oxygen Demand Concentrations on Anaerobic Ammonium Oxidation by Granular Sludge From EGSB Reactor[1]

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Objective To investigate the effect of chemical oxygen demand (COD) concentrations on the anaerobic ammonium oxidation (ANAMMOX). **Methods** An Expanded Granular Sludge Bed (EGSB) reactor was used to cultivate the granular sludge and to perform the ANAMMOX reaction in the bench scale experiment. NH_4^+-N and NO_2^--N were measured by using colorimetric method. NO_3^--N was analyzed by using the UV spectrophotometric method. COD measurement was based on digestion with potassium dichromate in concentrated sulphuric acid. **Results** When the COD concentrations in the reactors were 0 mg/L, 200 mg/L, 350 mg/L, and 550 mg/L, respectively, the NH_4^+-N removal efficiency was 12.5%, 14.2%, 14.3%, and 23.7%; the removal amount of NO_2^--N was almost the same; the nitrate removal efficiency was 16.8%, 94.5%, 86.6%, and 84.2% and TN removal efficiency was 16.3%, 50.7%, 46.9%, and 50.4%, moreover, the COD removal efficiency was 85%, 65.7%, and 60%; the COD removal rate was 27.42, 61.88, and 97.8 mg COD/(h • L). **Conclusion** COD concentrations have a significant influence on anaerobic ammonium oxidation by granular sludge.

Key words: Anaerobic ammonium oxidation; Granular sludge; Nitrogen removal; COD

INTRODUCTION

The phenomenon of anaerobic ammonium oxidation was originally discovered in a denitrifying fluidized-bed reactor treating effluent from a methanogenic reactor in the 1990s[1]. Anaerobic ammonium oxidation (ANAMMOX) process is a strictly anaerobic denitrification process, in which ANAMMOX autotrophic bacteria directly oxidize ammonium to dinitrogen gas using nitrite as electron acceptor. In recent years, the anammox process has received great attention and lots of researches on this field have been done[2-18].

van de Graaf *et al.*[3] reported that ANAMMOX is a biological oxidation process by using the ^{15}N-labelled compounds and its optimum electron acceptor is hydroxylamine (NH_2OH) produced by nitrite acid. Once there are excess hydroxylamine and ammonium, hydrazine accumulates temporarily and finally converts to dinitrogen gas. This autotrophic process allows over 50% of the oxygen to be saved and no organic carbon source is needed.

Many factors have inhibitory effects on anammox process, such as substrate, pH and temperature. Jetten *et al.*[4] showed that ANAMMOX reaction occurs between 20℃ and 43℃ and the optimal temperature is 40℃. Many kinds of organic compounds might control or accelerate this process. van de Graaf *et al.*[3] discovered that various organic compounds have important controls on the ANAMMOX. The investigators found that 1-5 mmol/L acetate, 1 mmol/L glucose or 1 mmol/L fructose has negative influences on ANAMMOX in the batch experiments carried out with wastewater.

Recently, the research has been focused on the high ammonium wastewater. Jetten *et al.*[4] used the coordinate process (SHARON-ANAMMOX) to treat high ammonium wastewater from sludge digestion tank and found that the total nitrogen (TN) loading rate is about 0.8 kg/(m^3·d) and 53% TN (39% NO_2^-, 14% NO_3^-) participating in the conversion. The effluent of SHARON process is taken as the influent of ANAMMOX. Nitrate is completely removed and ammonium remains in the ANAMMOX reactor with the control of nitrite. The removal efficiency of ammonium is about 83%. Dongene *et al.*[5] also used the coordinate process to treat the high concentration (1000-1500 mg NH_4^+-N/L) ammonium wastewater

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and after two years running, more than 80% ammonium-N is converted to dinitrogen gas. Siegrist *et al.*[6] utilized sequencing batch reactor (SBR) to treat high ammonium concentration of leachate and attained the higher ammonium removal efficiency. They also analyzed the potential mechanism of ammonium removal and found that 70% ammonium can be removed by ANAMMOX process.

The objective of this research was to investigate the effect of COD concentration on ANAMMOX reaction.

MATERIALS AND METHODS

Influent and Seeding Sludge

Synthetic wastewater used in this experiment consists of $(NH_4)_2CO_3$, $NaNO_2$, $CaCl_2$, $MgSO_4$, dextrose, trace element solution I (including EDTA, $FeSO_4$) and trace element solution II (including EDTA, $ZnSO_4 \cdot 7H_2O$, $CoCl_2 \cdot 6H_2O$, $MnCl_2 \cdot 4H_2O$, $CuSO_4 \cdot 5H_2O$, $Na_2MoO_4 \cdot 2H_2O$, $NiCl_2 \cdot 6H_2O$, $NaSeO_4 \cdot 10H_2O$, H_3BO_3). The COD concentrations in the media were different and adjusted to 0 mg/L, 200 mg/L, 350 mg/L, and 550 mg/L, respectively. All chemicals obtained were of analytical grades.

Anaerobic sludge from brewery wastewater treatment plant was used as seeding sludge. Its concentration in the reactor after inoculation was about 3.3 g/L.

Reactor

EGSB reactor was used as the ANAMMOX reactor. The reaction zone was 2.3 L and settling volume was 2.46 L. The reactor was operated under anoxic condition and hydraulic retention time (HRT) was maintained for 1.20 days. ANAMMOX phenomenon was obviously detected in EGSB reactor and the removal efficiency of ammonium, nitrite and nitrate was 46.5%, 98.9%, and 85.1%, respectively.

Experimental Procedure

Anaerobic granular sludge from EGSB reactor was used. Fifty mL sludge and the synthetic wastewater were added into 500 mL serum bottles, while continuously sparged with oxygen-free nitrogen for 2-3min to create the anaerobic environment. The bottles were then tightly stoppered, after being flushed with nitrogen gas using rubber stopper and statically incubated at 37℃ (the temperature at which the EGSB reactor was operated). The samples were taken at certain intervals.

Sampling and Analysis

Before sampling, the serum bottles were vigorously shaken and allowed to settle for 2 min. Liquid samples were collected using 20 mL disposable syringes with 18G12 cm needles. The pH value in each sample was measured. The samples were then acidified using 2 mL H_2SO_4/L and stored in a refrigerator until analysis was conducted.

COD, NH_4^+-N, NO_2^--N, NO_3^--N, TSS were measured according to standard methods established by the American Public Health Association[19]. NH_4^+-N and NO_2^--N were measured by the different colorimetric methods. NO_3^--N was analyzed by the UV spectrophotometric method. COD measurement was based on digestion with potassium dichromate in concentrated sulphuric acid for 2 h at 150℃.

RESULTS

Removal of NH_4^+-N at Different COD Concentrations

The influent concentration of NH_4^+-N was maintained at about 172.8 mg/L and the COD concentrations were adjusted to 0 mg/L, 200 mg/L, 350 mg/L, and 500 mg/L in four reactors, respectively, in order to investigate the influence of COD concentrations on the ammonium removal. The results are depicted in Fig. 1.

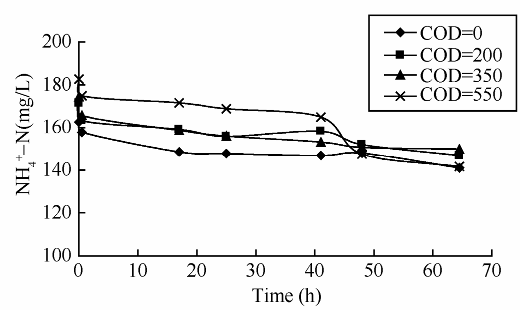

FIG.1. Effect of COD concentrations on NH_4^+-N removal.

Fig. 1 shows that when the COD concentrations were 0, 200, 350, and 550 mg/L, the NH_4^+-N removal efficiency was 12.5%, 14.2%, 14.3%, and 23.7%, respectively. The COD concentration in the fourth reactor (550 mg/L) was similar to that in EGSB reactor, so the NH_4^+-N removal efficiency was the highest, accounting for 23.7%.

From Fig. 1 we could see that the different influent COD concentrations had significant influence on the NH_4^+-N removal, especially at the beginning of reaction. Moreover, the lower the influent COD concentration was, the more the NH_4^+-N amount was removed. To further investigate the ammonium removal in the initial phase, relative short-term (9 h) experiments were carried out and the results are shown in Fig. 2.

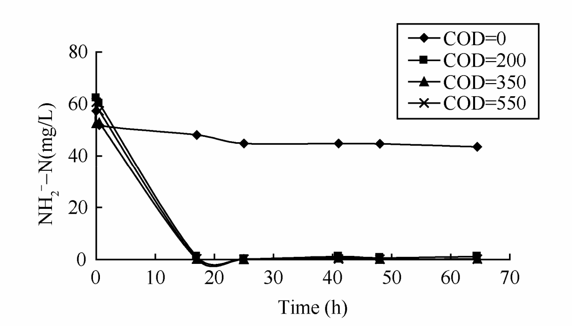

FIG. 2. NH_4^+-N removal at different influent COD concentrations (9 h).

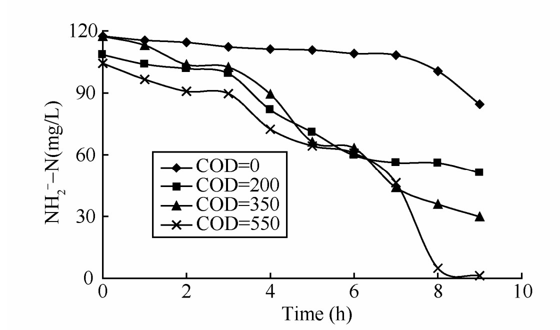

FIG. 4. Nitrite removal at different influent COD concentrations (9 h).

Removal of NO_2^--N at Different COD Concentrations

The influence of COD concentrations on nitrite removal was studied. The influent concentration of NO_2^--N was about 58.2 mg/L. The COD concentrations were 0 mg/L, 200 mg/L, 350 mg/L, and 500 mg/L, respectively. Nitrite removal was carried out with the excess of ammonium, the results are depicted in Fig. 3.

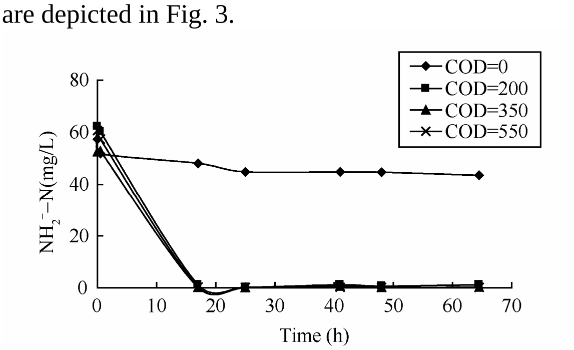

FIG. 3. Effect of COD concentrations on NO_2^--N removal.

Fig. 3 indicates that when there was no COD in the influent, the removal amount of NO_2^--N was 6.9 mg N. When the influent COD concentrations were 200, 350, and 550 mg/L, respectively, the removal amount of NO_2^--N was almost the same, being about 30.0 mg N, suggesting that nitrite could be removed through ANAMMOX when there was no COD in the influent; and the excessive amount of nitrite was removed through the traditional denitrification using the organic carbon sources as electron donors.

The nitrite removal at beginning of the reaction was studied and the results are shown in Fig. 4.

During the first 9 hours of reaction when there was no COD in the influent, the amount of NO_2^--N removed was 11.5 mg N. That is to say, 11.5 mg N was removed through ANAMMOX reaction. When the influent contained organic carbon sources, the NO_2^--N removal rate increased with the increase of COD concentration.

Removal of NO_3^--N at Different COD Concentrations

Nitrate removal was performed at different COD concentrations in order to investigate the influence of COD on NO_3^--N reduction. The influent nitrate concentration was maintained at about 82.0 mg/L, and the influent COD concentrations were adjusted to 0 mg/L, 200 mg/L, 350 mg/L, and 550 mg/L, respectively. The experimental results are shown in Fig. 5.

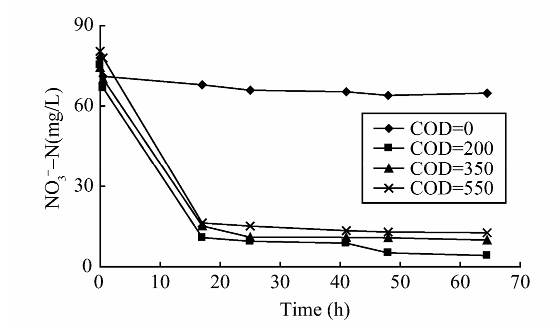

FIG. 5. Effect of COD concentrations on NO_3^--N removal.

Fig. 5. demonstrates that when the influent COD concentrations were 0 mg/L, 200 mg/L, 350 mg/L, and 550 mg/L, respectively, the removal efficiencies of NO_3^--N were 16.8%, 94.5%, 86.6%, and 84.2%. The removal mechanism of nitrate was similar to that of nitrite, that is to say, the NO_3^--N reduction was fulfilled by ANAMMOX reaction when there was no COD in the reactor. When there was COD in the reactor, the NO_3^--N was removed through ANAMMOX reaction in combination with traditional denitrification.

The NO_3^--N removal in the initial phase of the reaction is shown in Fig. 6. The NO_3^--N removal rate might be controlled by organic carbon sources with different influent COD concentrations. The higher the influent COD concentration was, the stronger the inhibition on the NO_3^--N removal rate.

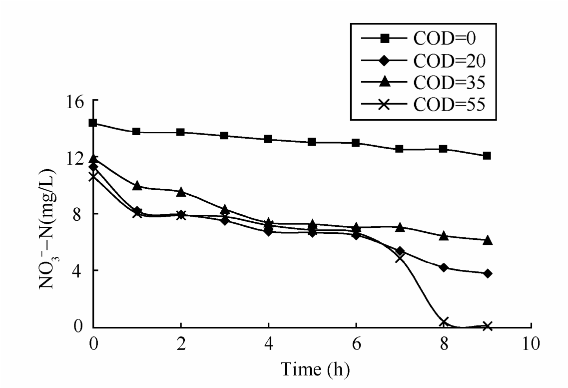

FIG. 6. Nitrate removal at different influent COD concentrations (9 h).

TN (Total-Nitrogen) Removal at Different COD Concentrations

The effect of COD concentrations on total nitrogen removal is depicted in Fig. 7.

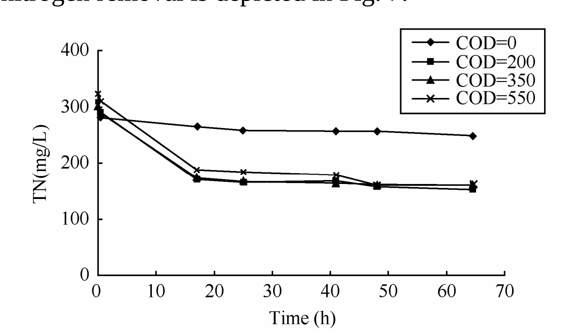

FIG. 7. Effect of COD concentrations on TN removal.

Fig. 7 demonstrates that the TN removal in four reactors was 24.3, 78.4, 70.7, and 81.6 mg N, respectively, and the corresponding removal efficiencies were 16.3%, 50.7%, 46.9%, and 50.4% when the influent COD concentrations were 0 mg/L, 200 mg/L, 350 mg/L, and 550 mg/L.

Removal of COD

The removal of COD during the ANAMMOX reaction are studied by measuring the COD concentrations in the influent and effluent, the results were depicted in Fig. 8.

When the influent COD concentrations were 200 mg/L, 350 mg/L, and 550 mg/L, respectively, the removal efficiency was 85%, 65.7%, and 60%. The higher the influent COD concentration was, the lower the removal efficiency was. Addition of organic carbon sources had a significant influence on the reactor performance. Heterotrophic bacteria in the anaerobic granular sludge could remove the organic carbon sources, both the removal efficiency and rate

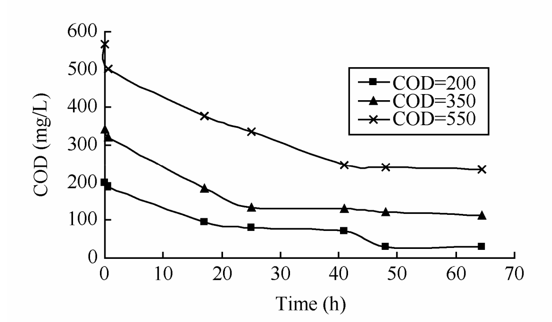

FIG. 8. Removal of COD.

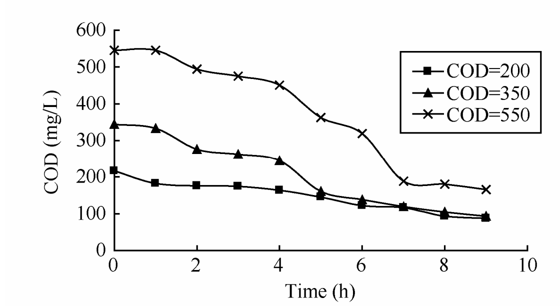

FIG. 9. COD removal during the first 9 hours.

were steady. The COD removal during the first 9 hours is shown in Fig. 9.

It could be seen from Fig. 9 that the higher influent COD concentrations were, the faster the anaerobic granular sludge removed COD. When the influent COD concentrations were 200 mg/L, 350 mg/L, and 500 mg/L, respectively, the removal rate was 27.42, 61.88, and 97.8 mg COD/(h • L).

DISCUSSION

In terms of the above results and analysis, ANAMMOX bacteria exist in the anaerobic granular sludge, which uses nitrite as electron acceptor and ammonium as electron donor to produce nitrogen gas. When there is no COD in the reactor, the rate of NH_4^+-N removal is the fastest in initial phase of the reaction. At the same time, the concentration of COD could inhibit the removal of NH_4^+-N, and the higher the influent COD is, the lower the NH_4^+-N removal efficiency at the beginning. The reason might be that the heterotrophic bacteria in the anaerobic granular sludge compete for nutrients with the autotrophic bacteria, which control the proceeding of ANAMMOX. Many investigations indicate that heterotrophic bacteria have the stronger competition capability and adaptability to the conditions than autotrophic bacteria. Our results obtained here are consistent with these reports. The existence of organic carbon sources has remarkable effects on the removal rate of NH_4^+-N.

Mulder *et al.*[1] and Graaf *et al.*[3] have shown the following stoichiometrical equations:

$$5NH_4^+ + 3NO_3^- \rightarrow 4N_2 + 9H_2O + 2H^+$$

$$G_0' = -297\ kJ/mol\ NH_4^+ \quad (1)$$

$$NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O$$

$$G_0' = -358\ kJ/mol\ NH_4^+ \quad (2)$$

From Figs. 1, 3, and 5, we could calculated the removal proportion of ammonium-N, nitrite-N to nitrate-N was 1:0.64:0.61. If ANAMMOX reaction proceeded according to the equations (1) and (2), the ammonium could not be removed so much.

Graaf *et al.*[10] concluded the stoichiometry of ANAMMOX reaction from the FBR(Fluidized Bed Reactor) as following:

$$NH_4^+ + 1.31NO_2^- + 0.0425CO_2 \rightarrow 1.045N_2 + 0.22NO_3^- + 0.0425CH_2O + 1.87 H_2O + 0.09OH^- \quad (3)$$

The stoichiometry of combined catabolic and anabolic reactions was determined by Strous *et al.*[11], which was as follows:

$$NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- + 0.13H^+ \rightarrow 1.02N_2 + 0.26NO_3^- + 0.066CH_2O_{0.05}N_{0.15} + 2.03\ H_2O \quad (4)$$

Equation (4) indicates that the reaction can produce a small quantity of nitrate with the control of nitrite and the excess of ammonium.

During the whole reaction period in our study, the effect of influent COD concentrations on the ammonium-N removal was considerably obvious. The higher the influent COD concentration was, the more remarkable the inhibitions were, especially at the first phase of the experiments. The removal rate and amount of ammonium, nitrite and nitrate were controlled by the COD concentrations in the reactor. Moreover, the higher the influent COD concentration was, the lower the COD removal efficiency was. At beginning of the reaction, the higher the influent COD concentration was, the higher the COD removal rate was and the more significant the inhibitory effect on ANAMMOX reaction were.

Recently studies on ANAMMOX have been focused on the treatment of wastewater with high ammonium-N concentrations (such as landfill leachate), therefore there are more challenges when using ANAMMOX process in the practical wastewater treatment. Research of the influence of COD concentrations on ANAMMOX reaction is of great significance.

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