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Study on Denitrification Performances of Anaerobic Ammonium Oxidation and Its Influencing Factors in an Up-flow Anaerobic Sludge Bed Reactor

Yong Li and Rui Zhao[1]*

Faculty of Geosciences and Environmental Engineering, Southwest Jiaotong University, Chengdu - 611 756, People's Republic of China.

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This study uses the tamed anaerobic ammonium oxidation (Anammox) sludge through being inoculated in an up-flow anaerobic sludge bed (UASB) reactor to treat the simulated wastewater in order to investigate the effects of anaerobic ammonia nitrogen removal and to find out the main influencing factors of Anammox. The experimental results show that the most suitable NH4⁺-N and NO_2^--N concentration loads for Anammox bacteria are both 220mg/L, with the hydraulic retention time of 4h, temperature of 35°C, as well as PH of 8.0, and the corresponding removal rates of NH_4^+-N0NO_2^--N and TN are 97%, 98.5%, 88%, respectively. It is suggested that the UASB reactor has a highly efficient performance of denitrification, which provides a useful insight for further engineering applications.

Key words: Anaerobic ammonium oxidation, Up-flow anaerobic sludge bed, Anammox bacteria, Denitrification, hydraulic retention time, PH, Temperature.

Anaerobic ammonium oxidation (Anammox) is a biological process where two kinds of nitrogen are directly converted into dinitrogen gas by microbes with NH_4^+ as electron donor and NO_3^-/ NO_2^- as electron acceptor under anaerobic or anoxic conditions[1-4]. This technique has made breakthrough in the basic theory of conventional nitrification denitrification biological nitrogen removal, and provides a simple and economic nitrogen removal way for low C/N ratio of wastewater[5].

International academics have studied the important characteristics of anammox bacteria[6], which also arouses great attention to domestic scholars. In our experiment, an up-flow anaerobic sludge bed (UASB) reactor is used to inoculate tamed anammox sludge. The denitrification performances of anammox reaction and its influencing factors are identified to find out the activity of anammox bacteria and its stable operating conditions.

MATERIALS AND METHODS

Experimental wastewater and sludge

The experimental wastewater is composed of basic and trace elements, containing NH_4^+-N and NO_2^--N. The basic element components are: $NaHCO_3$ of 0.5g/L, KH_2PO_4 of 0.02 g/L, $MgSO_4$ of 0.3g/L, $CaCl_2$ of 0.15 g/L, NH_4Cl and $NaNO_2$ dosing as needed. The culture medium of trace element has two types as I and II, with both dosages of 1ml/L, and its corresponding compositions are shown in table1. The pH of the culture medium is

* To whom all correspondence should be addressed.
E-mail: ruizhao@home.swjtu.edu.cn

adjusted by HCl and NaOH solutions. The reactor inoculated sludge is red anammox granular sludge with a diameter of 2 to 7mm, which is already tamped in the Lab.

Table 1. Trace element compositions of the influent

Components	ρ/(mg·L^{-1})	Components	ρ/(mg·L^{-1})
	I	$MnCl_2 \cdot 4H_2O$	0.99
$FeSO_4$	5.0	$CuSO_4 \cdot 5H_2O$	0.25
EDTA	5.0	$NiCl \cdot 6H_2O$	0.19
	II	H_3BO_3	0.014
EDTA	5.0	$NaMoO_4 \cdot 2H_2O$	0.22
$ZnSO_4$	0.204	$NaSeO_4 \cdot 10H_2O$	0.21
$CoCl_2 \cdot 6H_2O$	0.24		

EXPERIMENTAL

Devices and Methods

The experiment employs UASB as a reactor, and this device is made of organic glass with the height of 150 cm, inner diameter of 15 cm, an effective volume of 15L and an insulating interlayer outside. It is placed in the dark shadows of the Lab to avoid the inhibition of anammox bacteria. The influent is continuously pumped into the bottom of the reactor by using the peristaltic pump (BT100-1F). Through sludge bed, it then comes out from the top of the reactor. Gases are generated and discharged through a three-phase separator. The diagram of UASB reactor is shown in Fig.1

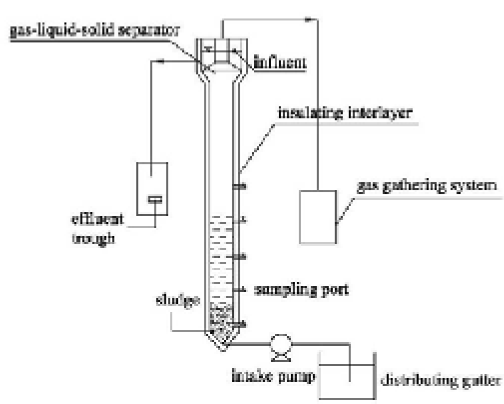

Fig. 1. The structure of UASB reactor

The tamped anammox sludge of 0.75L is inoculated in a UASB reactor, which is about 4cm high of the reactor and accounts for about 5% effective volume. The Mixed Liquor Suspended Solids (MLSS) is 99.2g/L, and Mixed Liquor Volatile Suspended Solids (MLVSS) is 59.7g/L, whilst their ratio of MLVSS/ MLSS is 0.602. The NH_4^+-N and NO_2^--N concentrations of the initial influent are both 70mg/L. When the reactor is stable for operation, NH_4^+-N and NO_2^--N concentrations are gradually raised by a gradient of 50mg/L to reach the maximum concentration loads. Thus, the concentration variations of NH_4^+-N, NO_2—N, TN of the influent and effluent as well as NO_3^--N of the effluent are monitored to determine the denitrification performances of the reactor, anti-shock loading capability and the growth of anammox bacteria. In addition, the influences of hydraulic retention time (HRT) (2-8h), pH value (6.0-9.0) and temperature (20-40°C) on anammox are also investigated.

Analytical Methods

In this experiment, Nessler's reagent spectrometry is utilized to determine the concentration of NH_4^+-N, N-(1-Naphthyl)-ethylenediamine spectrometry to determine the concentration of NO_2—N, ultraviolet spectrophotometry to determine NO_3-N, alkaline potassium persulfate digestionultraviolet spectrophotometry to determine the concentration of TN [7]. The temperature and pH are measured by the Starter 3C acidometer.The parameters, such as pH, the concentrations of NH_4^+-N, NO_2^--N and NO_3-N of the influent and effluent are measured every day, to monitor the operation conditions of the reactor for further adjustment.

RESULTS AND DISCUSSION

Anammox Nitrogen Removal

The nitrogen removal effect is measured by control of the NH_4^+-N and NO_2^--N concentrations of the influent. Previous studies indicate that the suitable temperature and pH for anammox bacteria is 30 to 40°C and 6.7 to 8.3[8-11]. Accordingly, the temperature of the reactor for the experiment is determined as 35°C, the pH of the influent as 7.5, initial hydraulic retention time (HRT) as 24h. Once the reactor has been stable for operation, HRT is changed into 8h, and the initial NH_4^+-N and NO_2^--N concentrations of the influent gradually increase by 50mg/L periodically. The variation of the NH_4^+-N and NO_2^--N of both the influent and the effluent; the NO_3^--N and TN concentrations of the effluent are shown in Fig.2. Moreover, the removal rates of NH_4^+-N0NO_2^--N and TN are shown in Fig.3.

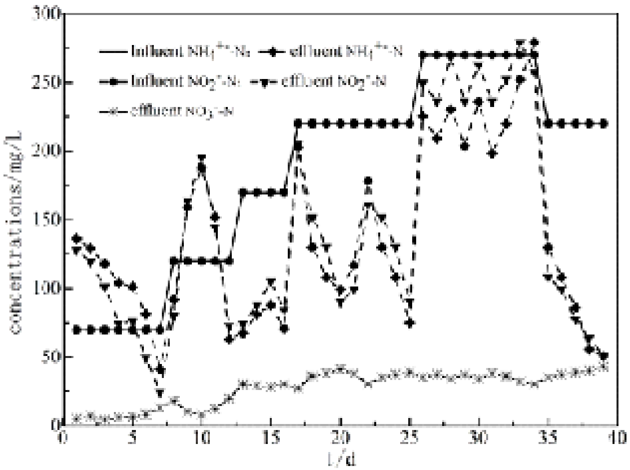

Fig. 2. Concentrations vs. time for NH_4^+-N0NO_2^--N and NO_3^--N of the influent and the effluent

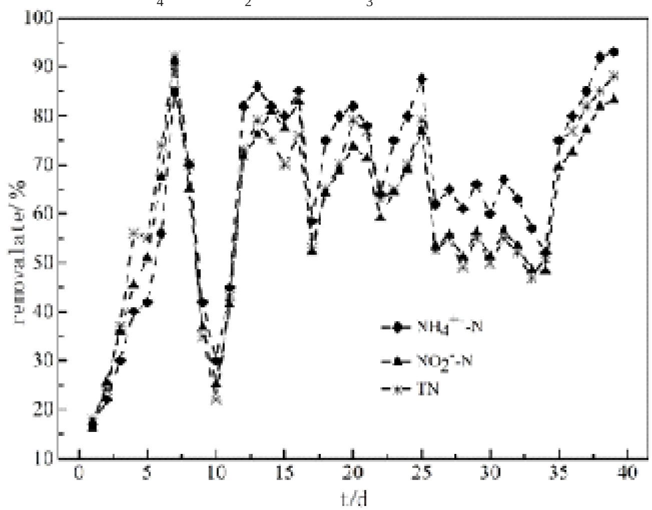

Fig. 3. Removal rates vs. time for NH_4^+-N0NO_2^--N and TN

As shown in Fig.2 and Fig.3, the activity of anammox sludge is decreased at the first three days of inoculation, because the sludge has been stored in refrigerator for a period of time. Moreover, the sludge may be exposed to oxygen thus to result in slight pollution of anammox bacteria. For this reason, the NH_4^+-N and NO_2^--N concentrations of the effluent are 49mg/L and 40.6mg/L respectively, and their corresponding removal rates are 30 % and 42%. However, the NH_4^+-N and NO_2^--N concentrations of the effluent are 10.64 mg/L and 2.1 mg/L with their corresponding removal rates of 84.8% and 97.1% on the 7th day. Especially, the removal rate of TN is 90.95%. In the meantime, mole ratios of the removed NH_4^+-N, NO_2^--N and the generated NO_3

$^{-}$-N is 1:1.15:0.23, which is close to the empirical anammox stoichiometric molar ratios of 1:1.32:0.26 [12-13]. Thus, it is suggested that the activity of inoculated sludge is recovered after seven days of operation. When the NH_4^+-N and NO_2^--N concentrations of the influent both increase to 120mg/L on the 8th day, their concentrations of the effluent are 36mg/L and 30mg/L, respectively. On the 12th day, the NH_4^+-N and NO_2^--N concentrations of the effluent are 21.6mg/L and 26.4mg/L with their corresponding removal rates at 82% and 78%. When the NH_4^+-N and NO_2^--N concentrations of the influent both continually increase to 170mg/L on the 13th day, their concentrations of the effluent are 23.8mg/L and 27.2mg/L with corresponding removal rates of 86% and 84%. On the 14th day, the NH_4^+-N and NO_2^--N concentrations of the effluent are 30.69mg/L and 34mg/L with corresponding removal rates of 82% and 80%. On the 16th day, the NH_4^+-N and NO_2^--N concentrations of the effluent are 25.5mg/L and 32.3mg/L with corresponding removal rates at 82% and 80%. When the NH_4^+-N and NO_2^--N concentrations of the influent both increased to 220mg/L on the 17th day, their concentrations of the effluent are increased to 91.3mg/L, 92.4mg/L, respectively. On the 25th day, the NH_4^+-N and NO_2^--N concentrations of the effluent are decreased to 27.5mg/L and 35.2mg/L with corresponding removal rates at 87.5% and 84%. At this time, the height of anammox bacteria is 6cm within the reactor, which is increased by 2cm, and the mole ratios of removed NH_4^+-N and NO_2^--N to the generated NO_3^--N are1:0.96:0.202. Although the mole ratios are fluctuated in contrast with the 7th day, they are in a suitable rage of matrix ratio (the ratio of NO_2^- toNH_4^+) as 0.95 to 1.2 [14]. When the NH_4^+-N and NO_2^--N concentrations of the influent both increase to 270mg/L on the 27th day, their concentrations of the effluent are increased to102.6mg/L and114.75mg/L with corresponding removal rates at 62% and 57.5 %. On the 34th day, the NH_4^+-N and NO_2^--N concentrations of the effluent are still as high as 129.6mg/L,118.8mg/L, respectively, and the corresponding removal rates are 52% and 56%. The removal rate of TN is 48.4%, which is possibly due to the strong inhibitory effect of the high NO_2^--N concentration to anammox bacteria, indicating that the concentration load of anammox bacteria on NH_4^+-N and NO_2^--N is increased to maximum. On the 35th day, the NH_4^+-N and NO_2^--N concentrations of the influent are both decreased to 220mg/L. On the 39th day, the NH_4^+-N and NO_2^--N concentrations of the effluent are 15.4 mg/L and 15.4 mg/L with the corresponding removal rates at 93% and 93%, and the removal rate of TN is 84%. The effluent is recovered to normal, and mole ratios of removed NH_4^+-N and NO_2^--N to generated NO_3^--N are 1:1:0.21, in accordance with the empirical anammox bacteria matrix ratio[14]. Therefore, the most suitable NH_4^+-N and NO_2^--N concentrations of the influent are both 220mg/L. In addition, the height of anammox bacteria increase to 6cm, and the volume increase by 0.35L, indicating that the activity of anammox bacteria is comparatively high.

Influences of HRT on Anammox Reaction

The reactor is set under the conditions where the NH_4^+-N and the NO_2^--N concentrations of the influent are both 220.mg/L, the temperature is 35°C, and pH is 7.5. As the HRT is changed to 8h, 6h, 4h, 2h respectively, the reactor is monitored to investigate the influences of HRT on anammox reaction. The reactor operates stably for 5 days when the HRT is fixed, and the NH_4^+-N, NO_2^--N and TN concentrations of the influent and effluent are monitored every day. With different HRT values, the removal rates of NH_4^+-N, NO_2^--N and TN are shown in Fig.4. When the HRT is 8h, the average volume load of the reactor is 1.64kg/ (m^3·d) calculated in TN. The average NH_4^+-N and NO_2^--N concentrations of the effluent are 15.4mg/L and 15.4 mg/L, and average removal rates of NH_4^+-N, NO_2^--N and TN are 93%, 93%, 83%, respectively. When HRT is reduced from 8h to 6h, the average volume load of the reactor increases from 1.64kg/ (m^3·d) to3.28kg/ (m^3·d). The average NH_4^+-N and NO_2^--N concentrations of the effluent are 9.9mg/L and 7.26mg/L, and the average removal rates of NH_4^+-N, NO_2^--N and TN are 95.5%, 96.7% , 86%, respectively. When HRT is reduced from 6h to 4h, the average volume load of the reactor is increased from 3.28kg/ (m^3·d) to 6.56kg/ (m^3·d). The average NH_4^+-N and NO_2^--N concentrations of the effluent are 11mg/L and 8.8mg/L, and the average removal rates of NH_4^+-N, NO_2^--N and TN are 95%, 96%, 85%, respectively. When the HRT is reduced from 4h to 2h, the average volume load of the reactor is increased from 6.56kg/ (m^3·d) to 13.12kg/ (m^3·d). The average NH_4^+-N and NO_2^--N concentrations of the effluent are 75.9mg/L and 71.72mg/L, and average removal rates of NH_4^+-N, NO_2^--N and TN are 65.5%067.4% and 58%. Based on the above results, the most suitable HRT for anammox reactor is considered to be 4h, and the maximum average volume load is 6.56kg/ (m^3·d).

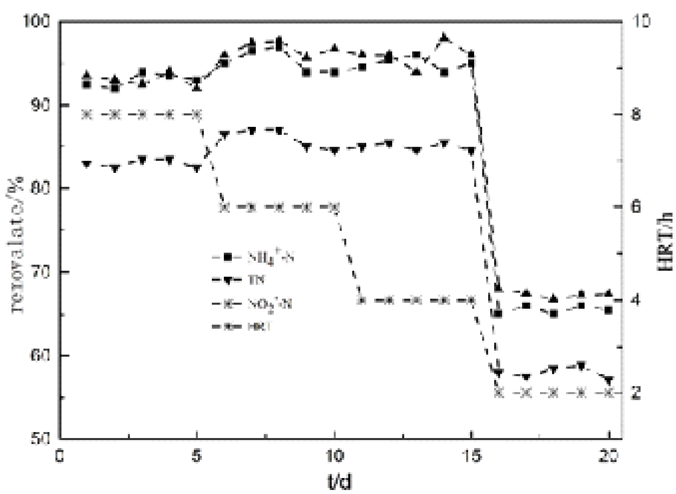

Fig. 4. Removal rate vs. HRT for NH_4^+-N0NO_2^--N and TN

Influences of Temperature on Anammox Reaction

The reactor is set under the following conditions where HRT is 4h, the pH is about 7.5 and NH_4^+-N and NO_2^--N concentrations of the influent are both 220mg/L. As the temperature is changed to 20°C, 25°C, 30°C, 35°C, 40°C, respectively, the reactor is monitored to investigate the influences of temperature on anammox reaction. When the reactor operates stably for 5 days with the temperature being fixed with the same value, the NH_4^+-N, NO_2^--N and TN concentrations of the influent and effluent are monitored every day. The average removal rates of NH_4^+-N, NO_2^--N and TN at different temperatures are shown in Fig.5. As shown in Fig.5, the temperature has a great impact on anammox reaction. When the temperature is 20°C, the average NH_4^+-N and NO_2^--N concentrations of the effluent are 73.7mg/L and 69.52 mg/L, and the average removal rates of NH_4^+-N, NO_2^--N and TN are 66.5%068.4% and 61.4%. When the temperature is increased from 20°C to 25°C, the average NH_4^+-N and NO_2^--N concentrations of the effluent are 48.84 mg/L and 46.86 mg/L, and the average removal rates of NH_4^+-N0NO_2^--N and TN are 77.8%, 78.7% and 70%. When the temperature is increased from 25°C to 30°C, the average NH_4^+-N and NO_2^--N concentrations of the effluent are 20.9 mg/L and 18.7 mg/L, and the average removal rates of NH_4^+-N, NO_2^--N and TN are 90.5%, 91.5 % and 81.4%. When the temperature is increased from 30°C to 35°C, the average NH_4^+-N and NO_2^--N concentrations of the effluent are 11 mg/L and 8.8 mg/L, and the average removal rates of NH_4^+-N, NO_2^--N and TN are 83%, 80 % and 73%. When the temperature is increased from 35°C to 40°C, the average NH_4^+-N and NO_2^--N concentrations of the effluent are 37.4 mg/L and 44 mg/L, and the average removal rates of NH_4^+-N, NO_2^--N and TN began to slightly decrease to 83%, 80 % and 73%. When the temperature is increased from 40°C to 42°C, the average NH_4^+-N and NO_2^--N concentrations of the effluent are 101.2 mg/L and 88 mg/L, and the average removal rates of NH_4^+-N0NO_2^--N and TN dramatically decrease to 55%, 60 % and 51%. Consequently, the suitable temperature range for anammox reaction is 30°Cto 35°C, and the most suitable temperature is 35°C. When the temperature is above 40°C, the nitrogen removal of anammox reaction shows the least effect.

Influences of PH on Anammox Reaction

The reactor is set under the following conditions where HRT is 4h, temperature is 35°C, and NH_4^+-N and NO_2^--N concentrations of the influent are both 220mg/L. When the pH is adjusted to 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, respectively, the

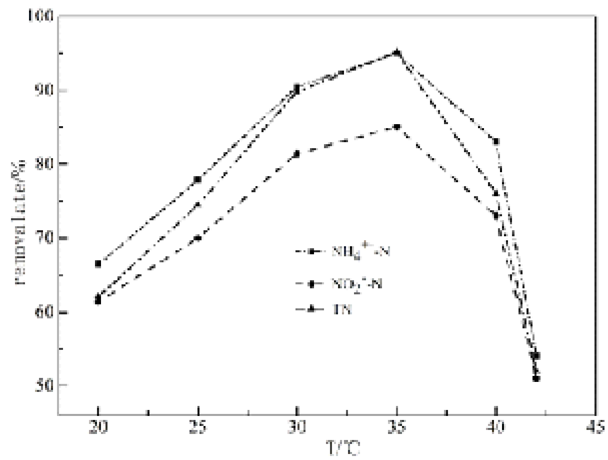

Fig. 5. Removal rates vs. temperature for NH_4^+-N0NO_2^--N and TN

reactor is monitored to investigate the influences of pH on anammox reaction. When the reactor operates stably for 5 days with the temperature being fixed with the same value, the NH_4^+-N, NO_2^--N and TN concentrations of the influent and effluent are monitored every day. The average removal rates of NH_4^+-N, NO_2^--N and TN with different pH are shown in Fig.6. When the pH is 6.0, the anammox reaction is inhabited so that the removal effect is the least. When the PH increases from 6.0 to 6.5, the denitrification effect of the reactor is not obvious and the average removal rate of NH_4^+-N is increased from 44% to 60.5%. The average removal rate of NO_2^--N is increased from 46% to 62%, and the average removal rate of TN is increased from 36% to 51%. When the PH is increased from 6.5 to7.0, the denitrification effect of the reactor is improved, and the average removal rates of NH_4^+-N, NO_2^--N and TN are increased to 87%, 89% and 78%. When the PH is 8, the average denitrification effect is the best, and the average removal rates of NH_4^+-N, NO_2^--N and TN are reached maximum, which are 97%, 98.5% and 88%. When the PH is 8.5, the reaction shows alkaline, so that the denitrification effect is decreased, and the average removal rates of NH_4^+-N, NO_2^--N and TN are decreased to 90%, 92% and 80%. When the PH is 9, the denitrification effect is decreased dramatically, so that the average removal rates of NH_4^+-N, NO_2^--N and TN are decreased to 50%, 55.5% and 40%. Therefore, the suitable PH range for anammox microbes is 7.0 to 8.5, and the most suitable PH is 8.

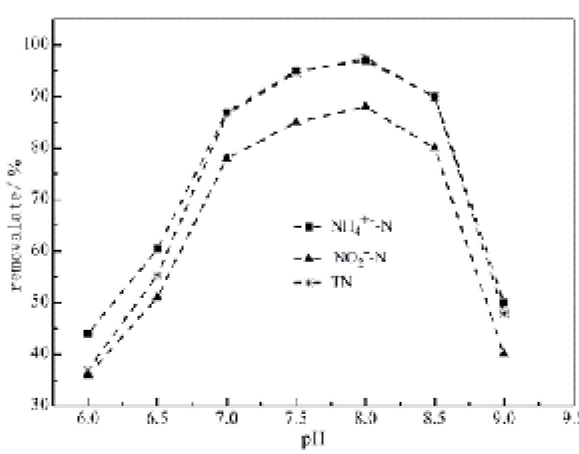

Fig. 6. Removal rate vs. pH for NH_4^+-N0NO_2^--N and TN

CONCLUSIONS

1. The tamped anammox sludge inoculated in a UASB reactor is cultivated to test the denitrification effects of anammox reaction. The volume load is raised by gradually increasing the NH_4^+-N and NO_2^--N concentrations of the influent. When the NH_4^+-N and NO_2^--N concentrations are both 220 mg/L, the removal effects are relatively stable with their removal rates at 93%. When the NH_4^+-N and NO_2^--N concentrations are increased to 270 mg/L, the activity of anammox bacteria is inhabited to indicate that the denitrification effect is the least. Consequently, the most suitable NH_4^+-N and NO_2^--N concentrations for anammox bacteria are both about 220mg/L.
2. When the HRT is 2h and the average volume load of the reactor is 13.12kg/ (m^3·d), the activity of anammox bacteria is inhibited, thus the denitrification shows the least effect. When the HRT is 4h, and the average volume load of the reactor is 6.56 kg/ (m^3·d), the removal rates of NH_4^+-N, NO_2^--N and TN are high, which shows the best denitrification effect in the experiment. Hence, the most suitable HRT for anammox reactor is 4h, whilst its maximum average volume load is 6.56kg/ (m^3·d).
3. The temperature has a great impact on anammox reaction. When the temperature is as low as about 20°C, the removal shows a least effect. As the temperature increases, the denitrification effect is also increased. When the temperature is 35°C, the removal rates of NH_4^+-N0NO_2^--N and TN are 95%, 96% and 85%. When the temperature is more than 35°C, the denitrification effect of anammox reaction is reduced.
4. The PH has an obvious influence on anammox reaction. When the PH is 6 to 7, the anammox activity is inhibited. When the PH is increased to 8, the removal effect is the best, whilst the average removal rates of NH_4^+-N0NO_2^--N and TN are increased to the maximum, which are 97%, 98.5% and 88%. When the PH is more than 8.5, the denitrification effect is reduced.

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