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Effects of seawater salinity on nitrite accumulation in short-range nitrification to nitrite as end product

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Abstract: The effect of seawater salinity on nitrite accumulation in short-range nitrification to nitrite as the end product was studied by using a SBR. Experimental results indicated that the growth of *nitrobacteria* was inhibited and very high levels of nitrite accumulation at different salinities were achieved under the conditions of 25—28℃, pH 7.5—8.0, and the influent ammonia nitrogen of 40—70 mg/L when seawater flow used to flush toilet was less than 35% (salinity 12393 mg/L, Cl^- 6778 mg/L) of total domestic wastewater flow, which is mainly ascribed to much high chlorine concentration of seawater. Results showed that high seawater salinity is available for short-range nitrification to nitrite as the end product. When the seawater flow used to flush toilet accounting for above 70% of the total domestic wastewater flow, the removal efficiency of ammonia was still above 80% despite the removal of organics declined obviously(less than 60%). It was found that the effect of seawater salinity on the removal of organics was negative rather than positive one as shown for ammonia removal.

Keywords: seawater salinity; short range nitrification-denitrification to nitrite as the end product; nitrite accumulation; organics removal efficiency; ammonia removal efficiency

Introduction

Short-range nitrification-denitrification to nitrite as the end product is defined as the nitrification is controlled by oxidation of ammonia to nitrite as the end product without further oxidation into nitrate, and then the nitrite is reduced into N_2 under anoxic conditions at the presence of organic carbon. Present techniques to realize nitrification-denitrification to nitrite as the end product include separation and immobilization of pure species of *nitrosomonas* (Tanaka, 1996), temperature control as does in SHARON process (Hellinga, 1998), free ammonia concentration control (Turk, 1989), and dissolved oxygen concentration control as does in OLAND process (Bernet, 2001). However, the inhibitions of some ions and toxic materials to the growth of *nitrosomonas* and *nitrobacteria* involved in nitrification are often ignored. The effects of inhibitory factors on the growth of *nitrosomonas* are different from those on *nitrobacteria* due to their different growth characteristics. In comparison with *nitrosomonas*, *nitrobacteria* are much sensitive to outside environmental changes. Hence, its growth is inhibited at the initial stages of presence of toxic materials. As a result, nitrite could accumulate due to the inhibition of nitrite oxidation.

Utilization and exploitation of seawater resource have attracted much attention in many coastal cities. Using seawater to flush toilet is one of important measures to save freshwater resource. Seawater used to flush toilet usually accounts for some 30% of the total domestic wastewater flow. The introduction of seawater into municipal wastewater treatment system must affect biological treatment process, especially biological nitrogen removal. Despite several studies (Chaarls, 1999; Wang, 2000; Woolard, 1995) on nitrogen removal from brine wastewater, the effects of seawater salinity on nitrogen removal of wastewater treatment systems are unclear. Furthermore, the effects of seawater salinity on nitrite accumulation have not been reported yet. It is very important for realizing toilet flushing with seawater to study the effects of seawater salinity on nitrite accumulation in the short-range nitrification-denitrification to nitrite as the end product.

1 Materials and methods

1.1 Experimental equipment

The schematic diagram of experimental equipment is shown in Fig.1. Two 38 L circular SBR reactors with conform bottoms are used. The reactor is equipped with an air compressor and a mechanical mixer for mixing domestic wastewater with seawater. Its height and diameter are 70 and 30 cm respectively. A rotameter and a thermostat are used to regulate the aeration and to maintain a desired temperature. In addition, the pH and ORP probes and sensor are installed as well.

1.2 Experimental methods

The domestic wastewater used in this study was taken from Maidao Wastewater Treatment Plant in Qingdao, Shandong Province, and the inoculated activated sludge from

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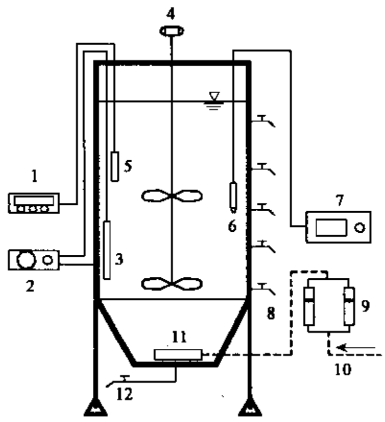

Fig.1 Schematic diagram of experimental equipment

1. ORP probe; 2. temperature controller; 3. temperature sensor; 4. mechanical mixer; 5. ORP sensor; 6. pH sensor; 7. pH probe; 8. sample; 9. rotameter; 10. compressed air; 11. microspore aerator; 12. waste sludge

the secondary settling basins of Orbal oxidation ditches in Laixi Wastewater Treatment Plant. The characteristics of influent (raw wastewater) and experimental conditions are shown in Table 1.

Table 1 Characteristics of influent and experimental conditions

Parameter	Range
COD	300—450 mg/L
NH_4^+-N	40—70 mg/L
NO_3^--N	10—15 mg/L
NO_2^--N	< 1 mg/L
Temperature	25—28℃
DO	3—4 mg/L
pH	7.5—8.0
MLSS	3—4.5 g/L

The analytical methods and instruments used in the experimental study are shown in Table 2.

Table 2 Analytical methods (APHA, 1992) and instruments for the experiment study

Parameter	Analytical methods and instruments
COD	Standard method (APHA)
NH_4^+-N	Nessler's reagent colorimetric method
NO_3^--N	Thymol colorimetric method
NO_2^--N	N-(1-naphthyl)-diethylamine colorimetric method
Temperature	YSI model 50B
DO	YSI model 50B
pH	Hanna model pH211

The purpose of this study is to find out the effects of seawater salinity on nitrite accumulation in nitrification and compare the effects of seawater salinity on the removal of ammonia nitrogen with those on organics removal with the variation of seawater flow percentage of the total domestic wastewater flow (seawater salinity) at certain ranges of temperature and pH.

2 Results and discussion

2.1 Effects of seawater salinity on nitrite accumulation

Seawater is a complex liquid system, which consists of many kinds of minerals and organics. Saturation dissolved oxygen concentration of seawater reduces due to high salinity. The COD_{Cr} of seawater used in this study is 0.47—1.63 mg/L. The compositions of freshwater and seawater are shown in Table 3.

Table 3 Compositions of freshwater and seawater

Unit: mg/L

Parameter	Na^+	K^+	Ca^{2+}	Mg^{2+}	SO_4^{2-}	Cl^-	Salinity
Seawater	10770	399	412	1290	2712	19354	35186
Freshwater	6.3	2.3	15	4.1	11.2	7.8	120
Mixed water (35% seawater)	3773	141	153	454	956	6778	12393

The main ions of seawater that might affect microorganisms of wastewater are K^+, Na^+, Ca^{2+}, Mg^{2+}, SO_4^{2+}, and Cl^- which are indispensable to biologic growth except chlorine ion. At low concentration, they promote biologic growth. However, at high concentration, they give rise strong inhibition. The concentration of Cl^- of seawater is 2481 times that of freshwater and 7.13 times the concentration of SO_4^{2-} of seawater. Thus, seawater salinity is mainly composed of chlorine and sodium concentrations. With the addition of seawater into wastewater, the level of its salinity affect water activity, which results in the change of water osmotic pressure.

Different microbes have different biologic responses to the changes of water osmotic pressure. Nitrification includes two steps fulfilled by two kinds of autotrophic microorganisms, *nitrosomonas* and *nitrobacteria*. The first step refers to ammonia oxidation to nitrite by *nitrosomonas*, and then nitrite is oxidized to nitrate by *nitrobacteria*. *Nitrosomonas* and *nitrobacteria* have different biologic characteristics and endurable power to the variation of outside environment. In order to observe the effects of seawater salinity on nitrite accumulation in nitrification, two parallel tests were carried out in respective reactors, i.e. the domestic wastewater with no seawater in one reactor, and with 35% seawater added into another reactor.

The inoculated activated sludge was taken from the secondary clarifiers of Orbal oxidation ditches in Laixi WWTP. At the early period of the tests, the concentration of nitrate was about 15 mg/L and nitrite was less than 1 mg/L. Tests were begun at 12th August 2002. The reactor already operated for 4 d prior to the addition of seawater to keep the original characteristics of raw activated sludge. 35% seawater was added in the reactor from 17th August at the medium temperature of 25—28℃, pH 7.5—8.0, and the ammonia nitrogen concentration of the influent 40—70 mg/L. The operation cycle of SBR was 10 h, and the filling, aeration, sedimentation, and withdraws times were 0.5 h, 7 h, 1.5 h and 1 h respectively. The increase of nitrite concentration was found just on the day when seawater was added. Then the nitrite concentration increased rapidly on the second day, nitrite nitrogen concentration accounting for 40% of the total concentration of [NO_2^--N + NO_3^--N]. On the third day, the ratio percentage [NO_2^--N / (NO_2^--N + NO_3^--N)] reached more than 90%. It showed that nitrification to nitrite as the end product came into effect by nitrite accumulation due to the inhibition of *nitrobacteria* growth. This was attributed to the addition of seawater. The 35% seawater was added on 30th August. During this period, the experimental results were as follows: the nitrite ratio, over 95%; NO_2^--N, 11—16 mg/L; NO_3^--N, below 1 mg/L; the ammonia nitrogen concentration of the effluent was below 5 mg/L and the removal efficiency of ammonia nitrogen was over 90%. The results are shown in Fig.2.

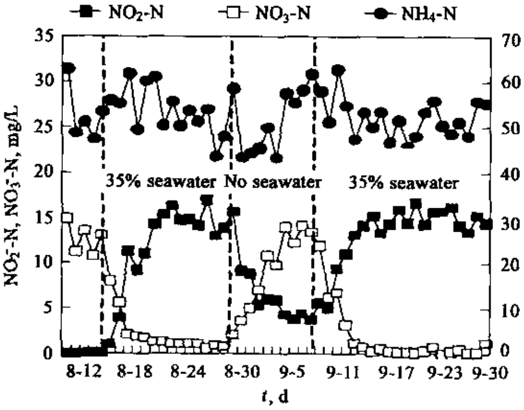

Fig.2 The variation of ammonia, nitrite and nitrate nitrogen concentration with operation time at different salinities

The addition of seawater was stopped on 1st September to study the effects of seawater salinity on nitrite accumulation. From then on, the concentration of nitrite nitrogen declined gradually, while that of nitrate nitrogen increased slowly. 9 days late, on 9th September, the nitrate nitrogen concentration was more than the nitrate nitrogen concentration which showed that innitrification seawater salinity had insignificant effect on *nitrosomonas*. 35% seawater was added again into the reactor from 10th September, and then the nitrite concentration increased rapidly. The concentration of nitrite was more than that of the nitrate on the second day. It showed that seawater salinity has significant effect on nitrobacteria growth and *nitrosomonas* has some endurable power to the variation of seawater salinity. At this stage, the nitrite ratio kept at above 95% with NO_2^--N, 13—17 mg/L and NO_3^--N, below 1 mg/L.

The effects of seawater salinity on nitrite accumulation are discussed in terms of the characteristics. Both *nitrosomonas* and *nitrobacteria* use inorganic carbon compound such as CO_2, CO_3^{2-}, HCO_3^- as carbon source and obtain energy from the oxidation reactions with NH_3, NH_4^+, and NO_2^-. They are autotrophic and absolute aerobic bacteria with low yield coefficient, low growth rate and long generation cycle. Its growth can be inhibited by changes of environment. The biologic characteristics of *nitrosomonas* and *nitrobacteria* are quite different form each other, which are shown in Table 4.

Table 4 Biologic characteristics of *nitrosomonas* and *nitrobacteria*

Parameter	*Nitrosomonas* (ellipse or bar shape)	*Nitrobacteria* (ellipse or bar shape)
Bacteria size, μm	1 × 1.5	1 × 1.5
Generation cycle, h	8—36	12—59
Trophic type	Autotrophic	Autotrophic
Type of bacteria	Strictly aerobic	Strictly aerobic
The most ratio growth rate, μm/h	0.04—0.08	0.02—0.06
Yield coefficient, *Y*	0.04—0.13	0.02—0.07
Saturation constant *K*, mg/L	0.6—3.6	0.3—1.7

It was found by our observation that *nitrobacteria* were easier to be inhibited than *nitrosomonas*. For example, at 30—35℃, the difference between the growth rate of *nitrobacteria* and *nitrosomonas* gave rise selection pressure, which resulted in nitrite accumulation due to the inhibition of *nitrobacteria* growth. This has been proved by SHARON process. When the pH or the ammonia concentration was high, the nitrite accumulation also occurred at the presence of high free ammonia. Some experimental results showed that the activity of *nitrobacteria* was inhibited when the free ammonia concentration was more than 1 mg/L; the activity of *nitrosomonas* was inhibited only when the free ammonia concentration was more than 5 mg/L (Anthonisen, 1976; Chaarls, 1998). Hence, it could be found that seawater salinity inhibited the growth of *nitrobacteria*, thus preventing nitrite from further oxidation. As a result, the nitrite accumulation occurred.

2.2 Comparison of effects of seawater salinity on organics removal efficiency and those on ammonia removal efficiency

Tests were carried out to compare the effects of seawater salinity on organics removal efficiency with ammonia nitrogen removal efficiency. The variations of COD, ammonia, nitrite and nitrate in the operation period are shown in Fig.3. At nitrification stage, the ratio of nitrite to the sum of nitrate and nitrite was kept at very high levels all the time (above 95%) with the maximum concentration of nitrite nitrogen 26.06 mg/L and the ammonia removal efficiency above 90%, which indicated the possibility of realizing nitrification to nitrite as the end product in the salinity range of seawater used for toilet flushing (in mixing with tap water at a radio of 7:13 and salinity of some 12393 mg/L). Fig.4 shows the variation of nitrite in one nitrification and denitrification cycle at different salinities. The concentration of nitrite was almost similar at the end of nitrification when the seawater percentage of domestic wastewater was 20%, 35%. However, the nitrite concentration declined remarkably when the seawater percentage was 70%. Thus, high salinity had inhibition effect both on the *nitrobacteria* growth and on the *nitrosomonas* growth. Fig. 5 shows that the variation of ammonia nitrogen concentration at different salinities which indicated that seawater salinity has slight effect on ammonia removal. Fig.6 shows the variation of COD_{Cr} in one cycle at different salinity. Fig. 7 shows the variation of COD_{Cr} and ammonia of the effluent at different seawater percentages of domestic wastewater(20%, 35% and 70%), from which it was found that the seawater salinity had slightly effect on the removal of COD when the seawater percentage of domestic wastewater was less than 35%. However, the organics removal efficiency declined sharply(less than 70%) when the seawater percentage of domestic wastewater was more than 70%. COD of the effluent was more than 150 mg/L while the removal efficiency of ammonia still kept above 80% without remarkable variation.

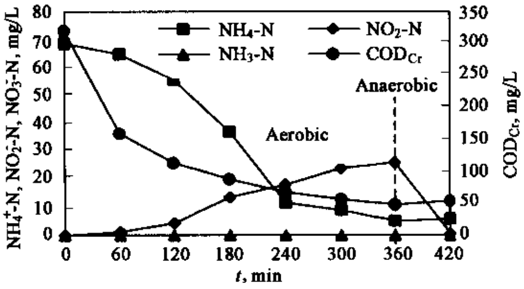

Fig.3 Variation of COD, ammonia, nitrite and nitrate nitrogen concentration in one nitrification-denitrification cycle at salinity of 35% seawater percentage of the domestic wastewater; influent COD, 318.9 mg/L; ammonia nitrogen concentration, 68.29 mg/L

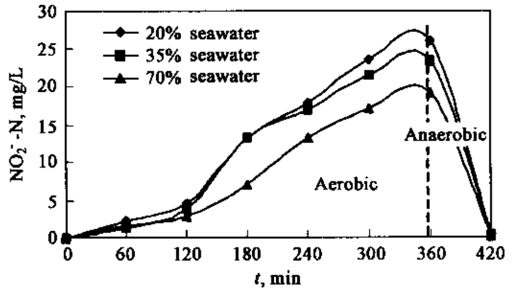

Fig. 4 Variation of nitrite nitrogen concentration in one nitrification and denitrification cycle at different seawater percentage(or different salinity)

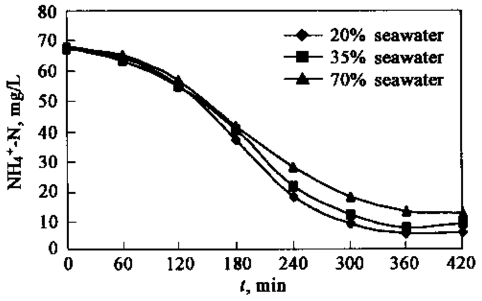

Fig.5 Ammonia concentration variations in one cycle at different salinities

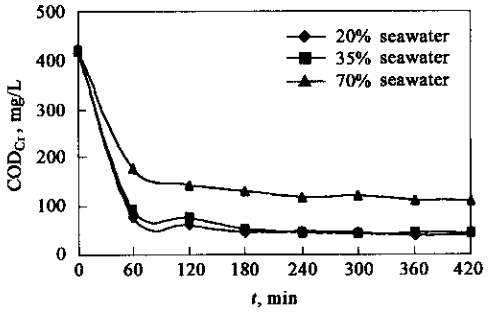

Fig.6 Variation of COD_{Cr} concentration in one cycle at different salinities

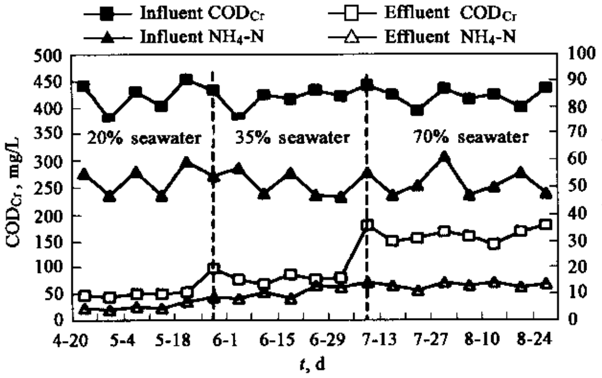

Fig.7 Variations of COD_{Cr} and ammonia concentration at seawater percentage of 20%, 30% and 70%

2.3 Effects of seawater salinity on activated sludge characteristics in nitrification ended to nitrite

The effects of seawater salinity on degradation of domestic wastewater organics and nitrification were verified by microorganism characteristics of the activated sludge system. The result of microscope observations of the settled activated sludge used as inoculated sludge from Orbal oxidation ditch process of municipal wastewater treatment plant has shown that its biologic community was abundant with many kinds of filamentous bacteria, zoogloea and protozoa. The micrograph of activated sludge zoogloea of wastewater without seawater is shown in Fig. 8. The activated sludge flocs are large and loose and zoogloea in it is close. With the increased of seawater mixing percentage the biologic community changed significantly. The number of protozoa and filamentous bacteria declined gradually, and almost disappeared when the seawater mixing percentage reached 70% of the domestic wastewater. The zoogloea became much tight, as shown in Fig. 9. The floc became fine and more compact. The degradation of wastewater organics depends on integrated effects of wastewater microorganisms. The number of microbial genera of activated sludge reduces because of the increased salinity. Thus, the organics removal rates declined. However, in activated sludge system, *nitrobacteria* are only 5% of the total microorganism number. The effects of salinity on autotrophic bacteria are much less than those on heterotrophic bacteria. Some halophilic bacteria like *nitrosamonas* and *nitrobacteria* can survive with the presence of seawater and nitrite. They change their endurable power with the increase of salinity. And the removal efficiency of ammonia can still keep high at high salinity. This provides a favored condition for nitrogen removal through short-range nitrification-denitrification ended to nitrite. The addition of seawater into the domestic wastewater changes settling characteristics of sludge. Its settling performances become better and better with the increased seawater percentage. Sludge bulking seldom occurred under high salinity in this study.

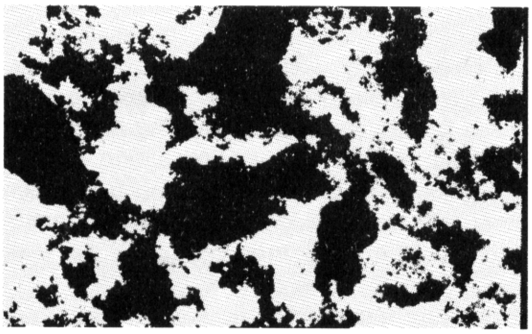

Fig. 8 Micrograph of activated sludge zoogloea in wastewater without seawater (10 ×)

Fig. 9 Micrograph of activated sludge zoogloea in wastewater with mixing of 70% seawater (10 ×)

In this study, the effects of seawater salinity on nitrite accumulation in the short-range nitrification ended to nitrite were investigated. Actually, there are many factors that affect nitrite accumulation of nitrification to nitrite as the end product such as temperature, pH, DO, ammonia concentration, free ammonia concentration, and sludge age. The interaction of these factors is the key to keep steady nitrite ratio in the nitrification ended to nitrite. It was found that the ammonia concentration of the effluent increased due to the decreased nitrite concentration and nitrite ratio when the temperatures reduced. Hence, higher temperatures are favorable for nitrification to nitrite as the end product. Besides the organics and ammonia nitrogen concentrations of domestic wastewater are higher in Qingdao area. These might cause the inhibition on the growth of nitrobacteria to some degree due to high initial free ammonia concentration in the study. However, before and after the addition of seawater the concentration of nitrite showed that the effect of seawater salinity on nitrite accumulation is positive.

3 Conclusions

The growth of *nitrobacteria* was inhibited and a very high level of nitrite accumulation was achieved with the ratio of nitrite to the sum of nitrite and nitrate above 95% under the conditions of ammonia concentration 40—70 mg/L, pH 7.5—8.0, temperature 25—28℃ and the salinities of the mixed seawater/tap water at 35% seawater percentage. The high chlorine concentration was responsible for the following results. It showed that seawater salinity is favorable for nitrification. The effects of seawater salinity on the ammonia removal is positive, while that on organics removal is negative, and the autotrophic bacteria for nitrification were more adaptive than heterotrophic bacteria for organics removal. When seawater mixed percentage of domestic wastewater was more than 70%, the organics removal efficiency declined sharply and the ammonia removal efficiency still keep above 80%. Chlorine concentration is a factor to realize nitrification to nitrite as the end product in item of four known ones, i. e. temperature, DO, free ammonia concentration and pH.

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