Relationship Between Microbiologic Properties and Short-cut Nitrification and Denitrification in Constructed Rapid Infiltration System

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Shortcut nitrification is determined by metabolism intensity of ammonia oxidizing bacteria(AOB) and nitrite oxidizing bacteria(NOB). In the current study, the relationship between dissolved oxygen and shortcut nitrification process running effect is mainly discussed, the relationship between dissolved oxygen and biomass and metabolic activity of AOB and NOB is paid insufficient attention. Therefore, to discuss the influence of dissolved oxygen on short-cut nitrification from the perspective of biology, the relationship in the CRI system between the biofilm characteristic and nitric oxide in different wet-dry rates in the short-cut nitrification and denitrification process. The results show that when the ratio of wetting-drying-time is 1:1 and pH value is 8, accumulation of the nitrite nitrogen and short-cut nitrification and denitrification are happened in CRI system. The number of the nitrite bacteria is far more than the nitrobacteria. Accumulation of the nitrite nitrogen and short-cut nitrification and denitrification are happened are mainly due to unique structure characteristics and operation mode of the CRI system.

Key words: Microbiologic properties; short-cut nitrification and denitrification; ratio of wetting-drying-time; Constructed Rapid Infiltration system

Traditional sewage land treating systems suffer from several ubiquitous shortcomings and disadvantages, such as lower hydraulic load rates, lower wastewater treating capabilities per unit area, being easy to plug and so on¹⁻⁴. To overcome the hurdles mentioned above, Mr. Zhong Zuosheng and some other researchers in China University of Geosciences invented CRI based on the traditional rapid infiltration and the constructed wetland systems^{5,6}. Here is its working principle: the mixtures of 90% natural sands, 5% marble sands, 5% zeolite sands are filled in the rapid infiltration pond as the artificial filter material, then the rapid infiltration pond is fed wastewater once every six hours and the water flowing direction within it is vertical down; when wastewater goes through the filter layer, the biofilm will grow on the surface of the filter materials; then the biofilm and the filter medium will retain and adsorb those dissolved substances and suspended materials present in wastewater; meanwhile, the highly concentrated biofilm, attached to the high specific surface area of the filter material, rapidly depurates the pollutants in wastewater^{7.8}.

Currently, due to the simple operation, low engineering investment, low costs for operation and other advantages, CRI has been widely applied in projects and of important application value to the wastewater disposal in small-medium sized cities and towns in China. As practice proves,

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CRI is stable in operation, and most indicators of the effluent meet the (GB 18918-2002)⁹, and the removal rates to COD, BOD, SS and NH_3 -H are all above 85%, but the removal rate of TN is as low as 20% ¹⁰.

In recent years, biological technology of denitrification has been focused on in wastewater treatment field. The biological denitrification mainly comprises two processes, first of which is to transform the ammonia nitrogen into nitrate nitrogen through nitration, and the second step is to transform nitrate nitrogen into nitrogen through denitrogenation. In traditional biological denitrogenation, the final product of nitration is nitrate, and NO₂-N is the electron acceptor in the denitrogenation process. From the transformation process of nitrogen, that the ammonia nitrogen is oxidized into nitrate nitrogen comprises two different reactions catalyzed by two separate classes of bacteria, and the two reactions are separate. As for denitrifying bacterium, either nitrite or nitrate can be used as the final hydrogen acceptor, and thus the whole biological denitrogenation process can be completed through the route of $NH_4^+ \rightarrow NO_2^- \rightarrow N_2$. The denitrification technology through this route is defined as shortcut nitrification-denitrification biological nitrogen removal process. The process of the shortcut nitrification- denitrification biological nitrogen removal technique is less than traditional biological denitrogenation process for two steps, so it has the following advantages: 1) at the step of nitration, it can save about 25% of oxygen demand, and therefore reduce energy consumption; 2) at the step of denitrogenation, it reduces about 40% of organic carbonic resource and reduces the operation fee; 3) the denitrogenation speed of the NO₂⁻-N is higher than that of the NO₂⁻-N for 63%; 4) it reduces 50% of sludge; 5) the reactor volume can be reduced for 30%-40%. Therefore, this technique gets more and more attentionat home and abroad and has become the hot point in the research field of sewage biological nitrogen removal^[9-11].

The key to implement short-cut biological denitrogenation is how to control and terminate the ammonia oxidation process at the nitrosation stage. Most of current researches adopt the strategies of controlling dissolved oxygen (DO), free ammonia, temperature, pH value, operation method, etc. Comparatively speaking, controlling dissolved oxygen has following advantages: saving oxygen supply consumption, flexible and simple controlling method and being applicable to all kinds of sewages.

The short-cut nitrification is substantially determined by the metabolism act strength of ammonia oxidizing bacteria and nitrite oxidizing bacteria. Existing literatures mostly investigate the relationship between the operation effects of the short-cut nitration technique and dissolved oxygen and pay insufficient attention to research the regulation and control mechanism of short-cut nitration from the perspective of the biological amount of AOB and NOB and the perspective of metabolic activity. Therefore, this study researches the relationship in the CRI system between the biofilm characteristic and nitric oxidization in different wet-dry rates in the short-cut nitration and denitrification process, and aims to discuss the influence of dissolved oxygen on short-cut nitrification from the perspective of biology.

EXPERIMENTAL

Experimental device

A CRI simulation column was constructed in lab. The main body of the reactor was composed of a hard PVC pipe with 200 cm in height and an internal diameter of 21 cm; the filter material of the improved CRI consisted of 80% natural sand, 10% sponge iron, 5% marble sand and 5% zeolite sand. The filter layer height was 150 cm. The particle sizes and the relative contents of filler materials



improved CRI simulation column

Fig. 1. Thumbnail of the Experimental Installation

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were shown in Table 1. The CRI analog column was fed wastewater once every six hours. The water flowing direction within each reactor was vertical down. The thumbnail of the experimental installation was shown in Fig. 1.

Particle size	The relative contents (weight percent %)						
range (mm)	Natural sand	Sponge iron	Marble sand	Zeolite sand			
>2.0	11	7	15	16			
1.0-2.0	28	53	19	21			
0.56-1.0	35	26	24	19			
0.25-0.56	14	8	22	26			
<0.25	12	6	20	18			

Table 1. The particle sizes and the relative contents of filler materials

Methods

In order to be more close to the fact, the domestic wastewater was adopted as the experimental water sample. CRI started up with raw wastewater. Twenty days after the starting-up, the COD removal rate became stable, which indicated a successful membrane hanging. The hydraulic load is an important design and operational parameter in improved CRI. In this experiment, the ratio of wetting-drying-time is one of the most important process parameters. When the CRI analog column was mature, it went on operating under the ratio of wetting-drying-time of 1:3 and 1:1 respectively to study the nitrite accumulation in CRI and the influences factors.

RESULTS AND DISCUSSION

Accumulation of nitrate nitrogen in CRI

To penetrate deeply the removalnitrogen mechanism of CRI, this study examines the concentration of nitrite and nitrate nitrogen in CRI. The examined results are shown in figure 2 and figure 3.

Figure 2 shows when the ratio of wettingdrying-time is 1:1 and pH value is 8, the nitrite nitrogen at the 1.00m place is higher than the nitrate nitrogen in CRI. The proportion of nitrous nitrogen in the nitration product is more than 70%, which shows the internal space in the reactor has the accumulation of nitrite. The experiment result of figure 3 shows that when the ratio of wettingdrying-time is 1:1 and pH value is 8, the nitrite accumulation phenomenon is more evident than the case when the ratio of wetting-drying-time is 1:3 and pH value is 7, and that shows the nitrite accumulation in CRI has something to do with the wetting-drying-time and pH. The average nitric ammonia concentration in the effluent water of figure 3 is 6mg/L, and the average content of the nitric nitrogen is 13mg/L which is obviously higher than the nitric nitrogen, but when it is compared with the nitric nitrogen content, it reduces for about 10mg/L. Synthesizing the experiment results of figure 2 and figure 3, it can be inferred that there is accumulation of nitrite. Through analyzing the filler balancing relationship of the nitrogen-containing compound in the reactor, it can be inferred the ammonia nitrogen in the water seepage is oxidized first into nitrite, and then most of the trite nitrogen is directly transformed into gaseous nitrogencontaining compound flowing out by the means of denitrification, which means the CRI has shortcut nitration and denitrification.

Variation of nitrogen-containing compound in CRI

To verify the accumulation phenomenon and short-cut nitration and denitrification of nitrite in the aerated CRI, this study researches the variation of the concentration of the nitrogencontaining compound in the along the water flow, and the experiment result is shown in figure 4.

In figure 4, the reduction of ammonia nitrogen is correlated with the increase of nitrite and nitrate nitrogen. From 0-1.00m in CRI, the reduction of the ammonia nitrogen is rapidly reduced and the nitric nitrogen is rapidly increased, but the increasing rate of the nitrite is obviously lower than that of the reducing amount of the ammonia nitrogen. The concentration of the ammonia nitrogen at the 1m deep filtration layer

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Fig. 3. Variation of nitrate and nitrite content in effluent in CRI

reduces stably, and the concentration of the nitrite reaches the maximum value at the 1.00m, later the concentration variation of the nitrite takes on consistent tendency as the ammonia nitrogen. It can be inferred from figure 4 that the maximum removal function of CRI to the ammonia nitrogen is mainly concentrated on the deep filtration layer of 0-1.00m and shows minor ability to remove ammonia nitrogen at the deep filtration layer of 1.00-1.50m. The main product of the ammonia nitrogen is trite nitrogen which is not further oxidized into trate nitrogen. There is obvious nitrite accumulation in the reactor, which indicates the activity of the nitrobacteria is inhibited. Analyzed from figure 2 and figure 3 and compared the numerical relations of all kinds of inorganic nitrogen-containing compounds, the author found the reduction amount of the ammonia nitrogen is

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far more than the sum of the trite nitrogen and the nitrate nitrogen, and most of the reduced ammonia nitrogen is transgressed out in the form of gaseous nitrogen-containing compound.



Fig. 4. Profiles of nitrogen compounds in CRI

Analysis of microorganism activity

To further study the accumulation of the nitrite of CRI, this paper analyzes the amount of nitrite bacteria and nitrobacteria in different parts of the reactor and analyzes the linear variation

N			

Depth of filter(m)	0.25	0.50	0.75	1.00	1.25	1.50
Number of nitrosobacteria (/mg)	1.32×10^2	2.66×10 ³	4.01×10 ⁴	5.45×10 ⁶	3.35×10 ⁴	2.76×10^{3}
Number of nitrobacterium(/mg)	0.81×10^2	1.78×10 ²	2.23×10 ³	4.12×10 ³	2.34×10 ²	1.22×10^{2}

Table 2. Spatial distribution of nitrifying bacteria in CRI

features of biomembrane oxygen consuming content in the reactor. In the measurement of the biomembrane oxygen consuming content, a certain amount of ammonia nitrogen and nitrite are added with redistilled sterile water, after sterile oxygenizing, the mixed material is gained as the culture medium. Meanwhile, the oxygen consuming content of the endogenous respiration is corrected. The measured result mainly reflects the respiration oxygen consuming content of the ammonia oxidizing bacteria and the nitrobacteria. The results are shown in table 2.

The experiment result in table 2 shows the number of the nitrite bacteria is far more than the nitrobacteria. The number of the nitrite bacteria is increasing along the flow. At the 1m thickness of the filler, the number of the nitrite bacteria is decreased a little. The number of the nitrobacteria is small and stable in variation, only increases slightly at the bottom of the filler layer but the amplitude is less than an order of magnitude. Researches show that the oxygen consumption content in the internal biomembrane of the CRI reaches the maximum value at the position of the 1.00m filler thickness, while the result in figure 1 indicates the number of the nitrite bacteria in that position is the maximum. Therefore, it is clear that the oxygen consumption content variation is consistent with that of the nitrite bacteria, which means the number and the activity of the nitrite bacteria in the reactor show obvious advantages, and that's why the phenomenon of nitrite accumulation happens.

Discussion on mechanism of nitrite accumulation in CRI

The nitrite accumulation phenomenon and the excellent denitriding efficiency of the nitrite indicate that short-cut nitration and denitrification appears in the CRI. The unique structure and operation features of CRI are the fundamental cause for its short-cut nitration and denitrification denitriding. CRI utilizes particulate filler as the medium and carrier for filtration interception and biological oxidation. The water seepage flows downward along the filler but becomes local eddy flow in the filler gap, and on the whole, the adherent biomembrane on the filler surface of each unit has the concentration gradient distribution of substrate and dissolved oxygen, and such distribution provides conditions for different ecological forms of microorganisms taking on predominant econiche in the different parts within the biomembrane, that is to say, different microorganisms metabolic predominance regions exist there. On the upper region of the reactor, there are rich organic substrates and adequate dissolved oxygen. The multiplication speed of the heterotrophic bacteria is quite fast, while the multiplication speed of the ammonia oxidizing bacteria is low, so is its growing speed, what's more, its ability in gaining oxygen is weaker than that of the heterotrophic bacteria. For that reason, the quick-proliferative heterotrophic bacteria can occupy the surface of the biomembrane, while the metabolic predominant region of the ammonia oxidizing bacteria is located in the shallower area of the internal biomembrane. Oxidizing the nitrite into nitrate is implemented by the nitrite-oxidizing bacteria which are actually the nitrobacteria. The nitrobacteria forms a mutualism relationship with the ammonia oxidizing bacteria, with the metabolic product of the ammonia oxidizing bacteria as the substrate of the nitrobacteria. In the activated sludge technique, as there is adequate oxygen supply, the nitrobacteria and the ammonia oxidizing bacteria can form mixed predominant zones. However, in the biomembrane system, the oxygen gaining abilities for heterotrophic bacteria and ammonia oxidizing bacteria are better than that of the nitrobacteria, so the metabolic predominant zone of the nitrobacteria can only exist in the zone where the concentrations of the nitrite and dissolved oxygen are thick and the concentrations of the organism and the ammonia nitrogen are sparse. When the bulk-phase dissolved oxygen is low, the diffusion resistance of the biomembrane is large, the concentrations of the ammonia nitrogen and the organism are thick and oxygen supply is limited, the metabolic activity of the nitrobacteria would be inhibited, and thus the ammonia oxidation products are directly used in the denitrification process by the denitrifying bacteria.

CONCLUSION

- (1) When the ratio of wetting-drying-time is 1:1 and pH value is 8, accumulation of the nitrite nitrogen and short-cut nitrification and denitrification are happened in CRI.
- (2) Accumulation phenomenon of the nitrite nitrogen in CRI is further confirmed by analyzing the spatial distribution of nitrogen compounds, amounts of nitrosation bacteria and nitrifying bacteria and biological membrane oxygen consumption.
- (3) Accumulation of the nitrite nitrogen and short-cut nitrification and denitrification are happened in CRI are mainly due to unique structure characteristics and operation mode of the CRI.

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