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Screening and Application of Nitrifying Bacteria at Low Temperature

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***Abstract*—A method of screening nitrifiers at low temperature was presented in this paper: enrichment of nitrifiers at 12℃, bacterium isolation and purification, preliminary screening and determination of nitrification effect. According to the above method, two kinds of ammonia-oxidation bacteria and two kinds of nitrite-oxidation bacteria were screened and separated from soil in cold area of north China, with the ammonia removal efficiency of 53%~62% and nitrite removal efficiency of 31%~36%, respectively. Some ecological factors impacting nitrification effect were discussed and the results showed that the most suitable temperature for the bacteria was 25℃ and the most appropriated pH value was in slightly alkaline range (pH 7.0-8.0). In the stable operating conditions, over 90% removal rate for ammonia and 70% removal rate for total nitrogen could be observed. At the same time, relatively high COD removal rate could be reached stably at 90%, which means both organic matter and ammonia could be removed together in the same reactor. The ecological factors analysis indicated that neither nitrite bacteria nor nitrate bacteria would be significantly influenced in growth and activity at 12℃~15℃ and the system could be operated stably at pH value of 7.0-8.0.**

Keywords-Biological enhancement at low temperature; Nitrobacteria; Isolation; Acclimation

I. INTRODUCTION

With the growing phenomenon of nitrogen pollution in water, removal of nitrogen pollution in wastewater treatment has become a hot issue. Nitrifying bacteria played a major role within ammonia nitrogen removal in water. The most appropriate temperature was mostly 30~35 ℃ as nitrifying bacteria, but in the cold northern region, the low temperature was not conducive for nitrifying bacteria to play its normal ability on ammonia nitrogen removal [1]. Therefore, the research for nitrifying bacteria which was suitable for cold north regions with very low winter temperature was practical significance.

II. EXPERIMENTAL METHODS

A. Nitrite and Nitrate Bacteria Isolation

Sampling time was in February 2009, when the outdoor temperature is -4 ℃. Samples of soil and sediment was taken and stored at 4 ℃. Separation and purification method was used by flat-panel dash, slant inoculation and dilution separation [2].

The test was proceed in the volume of 20 L SBR small scale reactor, and gradually increasing the ammonia load, from 50 mg/L to 150 mg/L [3]. Nitrifying bacteria was enriched and acclimated by at 12 ℃ low temperature shaker. Followed by dilution and microscopy counts, the appropriate dilution of the solution was taken by 1 mL and inoculated in enrichment medium of nitrite and nitrate bacteria [4]. They were cultured for 2 to 3 weeks under the conditions of moisture and aerobic at 12 ℃.

Nitrifying bacteria was isolated in the form of plates, and different single-colony was selected for line separation. They were cultured for 2 to 3 weeks under the conditions of moisture and aerobic at 12 ℃ [5]. Repeat these steps until the ideal single colony was gained. 2 strains of cold-resistant nitrite bacteria and 2 strains of cold-resistant nitric bacteria with high activity were isolated in the experiments. They were inoculated on slant medium, and cultured for 48 h under moisture and aerobic conditions at 12 ℃, and finally, refrigeration at 4 ℃.

B. Nitrification Performance by Ecological Factors

The comparative study of nitrification performance within the isolated nitrifying bacteria strains was proceed under the pH conditions of 7.0, 7.5, 8.0 and 8.5 [6]. Pure strains of nitrite bacteria were inoculated in liquid medium for nitrite bacteria and cultured for 3 days under shaking and at 12 ℃. Then, 2mL broth was inoculated at liquid medium for nitrite bacteria with ammonia concentration of 180mg/L and initial pH of 7.0, 7.5, 8.0 and 8.5, cultured in 12 ℃ shaker. Every 2d, sample was taken and measured the ammonia concentration for calculating the ammonia removal efficiency. With experiments as above, changing the experimental temperature, the impact of nitrification performance by temperature was also investigated [7]. Thus, nitrification performance of nitric bacteria was tested by the same steps.

C. Construction of Low-temperature Bio-augmentation Bacterial Consortium and Small Scale Test Application

2 strains of heterotrophic bacteria, 2 strains of nitrite bacteria and 2 strains of nitric bacteria with high activity and low temperature adaptability isolated by experiments were inoculated with 2000 mg/L of activated sludge in sewage wastewater. By dosing the fillers of 40% volume, the common growth state of activated sludge and biofilm was achieved [8]. During the monitoring of water indicators, the water pH and dissolved oxygen concentration and other parameters were adjusted to ensure co-growth of heterotrophic bacteria and nitrifying bacteria at low temperature. Continuous culture to the sludge concentration of 3500 mg/L, then by the way of compound bacteria, they

were inoculated to the SBR reactor and united acclimation. Under continuous operation at 12 ℃ low temperature, its low-temperature performance was examined for 6 months, and the other SBR reactor with the same concentration of activated sludge was as a reference.

III. RESULTS AND DISCUSSION

A. *Nitrification efficiency under the impact of ecological factors*

1) *pH value*

In this study, nitrification efficiency of the strains were compared under the pH of 7.0, 7.5, 8.0 and 8.5. Pure strains of bacteria was inoculated in nitrite bacteria liquid medium at12 ℃ and cultured for 3 days under shaking. 2 mL bacilli was inoculated in nitrite bacteria liquid medium, the medium contained ammonia which concentration was 180mg / L and initial pH were 7.0, 7.5, 8.0 and 8.5, placed at 12 ℃under shaking, measured concentration of ammonia every 2 days and calculated the ammonia removal efficiency. Nitric acid bacteria use the same steps, the use of nitric acid bacterial culture medium and analysis nitrite removal efficiency.

When the pH value was 7.0, removal rate of ammonia nitrogen was the highest，up to 47%, with pH value higher, ammonia nitrogen removal rate would decrease. When the bacteria in the pH value of 8.0-8.5, nitrite removal rate was 29%, below this pH, activity would reduce.

2) *Temperature*

With the temperature higher, the removal rate of ammonia nitrogen would increase. The rate was highest at 25 ℃, respectively 62% and 36%. At the temperature of 5 ℃, the rates were both lower than 16%. The rates were respective 53-60% and 31 -34% at 12 ℃ or 15 ℃.

The nitrite and nitrate bacteria were filtered out and has a good nitrification performance at 15 ℃ , which can be applied to low-temperature treatment environments in northern region.

3) *Growth curve*

Nitrifying bacteria was inoculated in the liquid separation medium by 5% (v/v) inoculation under shaking at 15 ℃, the three repeated samples were taken every 24h, the culture medium was (take note of volume of the culture medium) filtered by filter paper for determination of nitrite and nitrate, and then filter paper was dried at 75 ℃ 40min, weighed dry weight which minuend the weight of the filter paper (dried), the result was dry weight of cell. With culture time as the abscissa and dry weight of cell as the vertical axis, growth curve was drawn (mg/mL) in figure 1.

B. *Small Scale Application of Low-temperature Bacteria Consortium*

1) *Comparison of the degradation capacity of COD*

The MLSS of SBR reactor was around 3500 mg/L, Hydrolic Retention Time (HRT) was 12Hr, COD concentration of 300 mg/L, 30-day experimental results shown in Figure 2.

As showed in figure 2, the effluent COD of Adding multiple microorganisms were significantly lower than the conventional activated sludge system. The effluent COD of adding complex bacterium kept 15~20 mg / L while effluent COD of the conventional activated sludge maintained at 40~50 mg/L. The COD removal efficiency of multiple microorganisms was significantly higher than conventional activated sludge system. COD removal efficiency of of adding complex bacterium maintained at 90~95% while conventional activated sludge maintained at 80 ~ 85%. Low temperature have significantly inhibit on conventional activated sludge, Sludge sedimentation and flocculation are worse, and the effluent COD volatile. The effluent COD of the SBR reactor which adding multiple microorganisms <30 mg/L, which stably achieved <Discharge standard of pollutants for municipal wastewater treatment plant GB18918-2002> class A level (50 mg/L).

2) *Comparison of ammonia removal*

The ammonia concentration of SBR reactor was around 50 mg/L. 30-day experimental results were shown in Figure 3.

As figure shown, in the Adding reactor which adding multiple microorganisms effluent ammonia concentration maintained at 2~4.5 mg/L while in conventional activated sludge system effluent ammonia concentration maintained at 7~9.5 mg/L. After adding multiple microorganisms, Ammonia nitrogen removal rate retained at 90~95%. Low temperature at 12 ℃, the system added multiple micro-organisms, removal of ammonia nitrogen was significantly strengthened and effluent ammonia concentration was 4.5 mg/L at the tops, which was better than the effluent standards of CLASS A (when temperature ≤12 ℃, he effluent standards was 8 mg/L).

3) *Comparative analysis of total nitrogen removal*

The total nitrogen concentration of SBR reactor was around 50 mg/L. 30-day experimental results were shown in Figure 4.

As shown in figure 4, the effluent total nitrogen of the reactor added multiple microorganisms maintained at 10~14.5 mg/L while in conventional activated sludge system effluent total nitrogen concentration maintained at 18~25 mg/L. TN removal which added multiple microorganisms maintained at 71~80% and at low temperature such as 12 ℃, the effluent total nitrogen was less than 14.5mg/L which was better than the effluent standards of CLASS A (when temperature ≤12 ℃, he effluent standards was 15 mg/L).

IV. CONCLUSION

Screening, acclimation and complex of nitrobacteria at low temperature and the complex bacteria application in pilot were studied systematically in the paper.

1) The study confirmed the feasibility of screening psychrotolerant bacteria from different cold natural

environment and application with complex bacteria in biological system in wastewater treatment plant.

2) The study showed that adding psychrotolerant bacteria to the biological system could effectively enhance treatment capacity at cold winter and ensure the effluent to achieve A1 standard stably.

3) It is the key of the technology engineering application to keep the dosing psychrotolerant bacteria stable in the system and become the dominant species.

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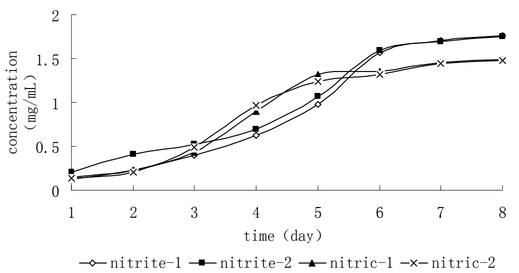

(Nitrite-1,2: Nitrite bacteria; Nitric-1,2: Nitric bacteria)
Figure 1. Nitrifying bacteria growth curve at 15 ℃

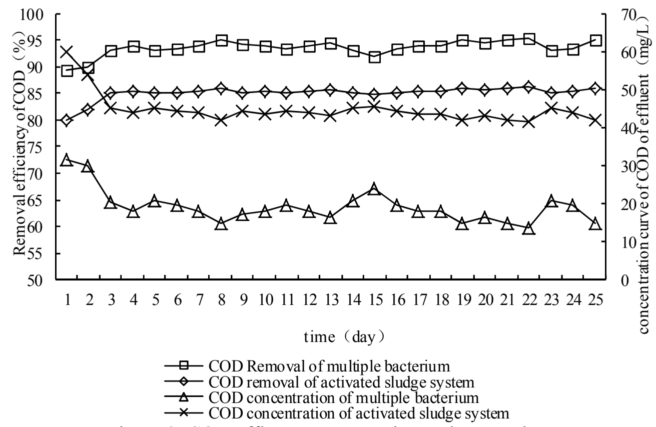

Figure 2. COD effluent concentration and removal rate

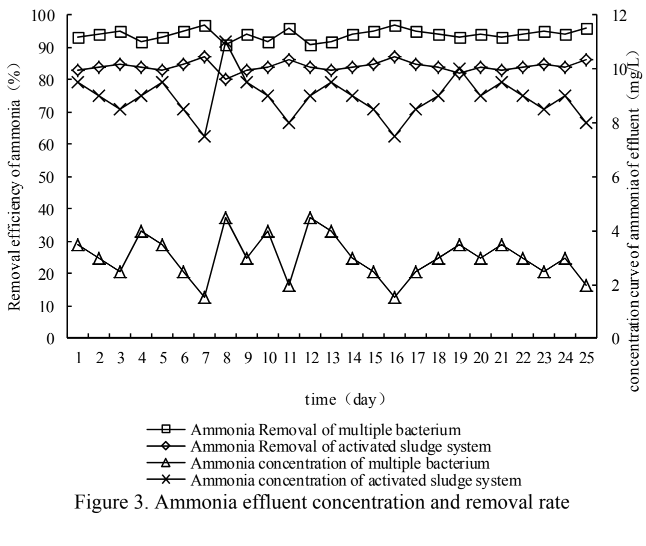

Figure 3. Ammonia effluent concentration and removal rate

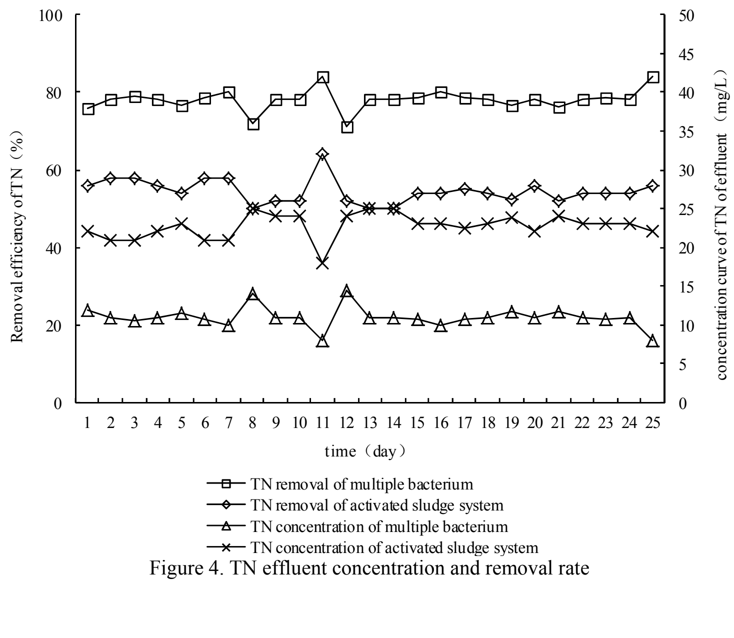

Figure 4. TN effluent concentration and removal rate