Nitrification Process of Lianhua Stream in Xiamen, Fujian Province

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Abstract. The nitrification rate, ammonia oxidation and nitrite oxidation potential in surface water, and sediment of Lianhua Stream were studied. Laboratory microenvironment experiments show that the nitrification rate at the interface between sediment and overlying water is much higher than that in surface water, with the nitrification rate $0.08834 - 0.1591 \text{mg(L.d)}^{-1}$ and $0.0001 - 0.0025 \text{mg(L.d)}^{-1}$ respectively. The potential of ammonia oxidation and nitrite oxidation of sediment in Lianhua Stream is also significantly higher than that of surface water. The total ammonia oxidation potential of sediment in Lianhua Stream is $8.396 - 13.247 \text{mg.(L.d)}^{-1}$, while the total nitrate oxidation potential is $3.522 - 9.322 \text{mg.(L.d)}^{-1}$. The high concentration of ammonia nitrogen promotes the activity of ammonia oxidative bacteria, which leads to the potential for high ammonia oxidation. Meanwhile, the high rate of ammonia oxidation and low rate of nitrite oxidation in the water body contribute to the accumulation of nitrites in the water.

1. Introduction

Nitrogen is active in the process of biogeochemical transformation and migration in the stream, especially the nitrification. Nitrification is the only natural process in aquatic ecosystem that can convert $\text{NH}_4^+$ into $\text{NO}_3^-$, which is closely related to the source and concentration of $\text{NO}_3^-$[1][2]. Therefore, understanding the mechanism of nitrification process and its influencing factors in the stream can completely explain the nitrification process and its source of nitrogen in the source basin, which is helpful to further reveal the mechanism of nitrogen migration and transformation in the stream.

The concept of nitrification was proposed in 1983 and was defined as the process of converting toxic $\text{NH}_3$ to non-toxic $\text{NO}_3^-$ by autotrophic nitrifying bacteria[3][4]. From the reaction equation of nitrification, it can be seen that nitrification in water is a biochemical process affected by multiple factors. In 1985, Richardson proposed that nitrification rate[5][6] can be affected by controlling environmental factors such as DO and $\text{NH}_4$ concentration.

It is found that the nitrification rate increases with the increase of DO. When the DO reaches a certain concentration, the nitrification rate tends to be stable. With the increase of pH, the nitrification rate increases correspondingly, while when the pH reaches 7.5, the nitrification rate decreases sharply. At the same time, temperature affects the growth of nitrifying bacteria and indirectly affects the nitrification intensity. When the temperature rises, the biological reaction activity increases until the optimal critical temperature is reached. When the temperature is exceeded, the biological enzyme will be destroyed to a certain extent, so the biological activity will decrease[7][8].
In this study, the nitrification of overlying water and sediment in Lianhua Stream in Xiamen was studied. The nitrification rate in water and sediment were measured by indoor microenvironment experiment, and then the total ammonia and total nitrite potential nitrification rate were determined. The main influencing factors of nitrification were analyzed, and the limiting steps and occurrence mechanism of nitrification in water were discussed.

2. Materials and methods

In this study, Lianhua stream, a typical headwater stream in Tongan District of Xiamen, China, which belongs to a tributary of Xixi River. In this study, surface water and sediment samples were collected from the upper reaches to the lower reaches of Lianhua stream in December 2016 and 2017. Surface water samples were collected by water collector and sediments were collected by cylindrical mud collector. Conductivity, pH, temperature, DO were measured by portable measuring instrument. Water samples and sediments were transported back to the laboratory for follow-up treatment. After filtered by 0.45 μm membrane, the concentration of NO\textsubscript{3}\textsuperscript{-}N, NH\textsubscript{4}\textsuperscript{+}-N and NH\textsubscript{3}-N were determined and calculated according to national standards.

2.1. Microenvironment experiment

In order to determine the nitrification intensity of surface water and sediment, the collected water samples and sediments were cultured in the laboratory. The specific experimental methods are as follows: the water sample and sediment were loaded into the conical bottle of 250ml respectively, and the bottle is wrapped with sealing film and avoid the light. the conical bottle was put into the constant temperature oscillator at a rotating speed of 80 rpm and a temperature of 20 degrees. Flow of water bodies of removal of the photosynthesis of phytoplankton was simulated in natural states. The oscillator was cultured under ventilated conditions for 4 days. NH\textsubscript{4}\textsuperscript{+}-N, NO\textsubscript{3}\textsuperscript{-}-N and NO\textsubscript{2}\textsuperscript{-}-N concentration was determined by sampling at the same time every day.

Under dark conditions, the change of ammonia nitrogen concentration is the result of the interaction of ammonia and nitrification. The related studies show that the amination in surface water can be ignored [9]. Therefore, without nitrification inhibitor in this microenvironment test, the nitrification intensity in water sample can be calculated by determining the change of ammonia nitrogen concentration in water sample.

2.2. Determination of the ammoxidation and nitrite oxidation potential rate

Nitrification in water includes two steps: ammonia oxidation and nitration oxidation. In order to further study the main influencing factors and related mechanisms of nitrification, the ammonia and nitrite oxidation potential nitrification rate were determined.

2.2.1 Determination of ammonia oxidation and nitrite oxidation potential nitrification rate in Surface water samples

Determination of total ammonia oxidation potential: 100ml water samples were taken into 150ml polyethylene flask and added with nitrate oxidation inhibitor NaClO\textsubscript{3} and NH\textsubscript{4}\textsuperscript{+}-N solution. Under the dark condition of 20 degrees for 24 hours, the ammonia oxidation potential was calculated according to the difference of nitrate nitrogen content before and after culture.

Determination of total nitrite oxidation potential: 100ml water samples were cultured in 150ml polyethylene flask with ammoxidation inhibitor propenyl thiourea (ATU) and nitrous nitrogen solution for 24 hours under dark conditions of 20 degrees. The nitrite oxidation potential was calculated according to the difference of nitrite nitrogen content before and after culture.

Grading determination of the potential of ammonia oxidation and nitrite oxidation: after the original water sample was filtered by a filter membrane with a pore size of 2 microns, the potential of ammonia oxidation and nitrite oxidation was determined according to the above method, and the free-state activity is expressed as F (<2.0. μm). The particle state activity is recorded as F (> 2.0μm) [10][11].
2.2.2 Determination of ammonia oxidation and nitrate oxidation potential in sediment samples

12.5g fresh sediment was taken into 250ml conical bottle, and 100ml overlying water sample filtered by 0.2 μm filter membrane was added. The subsequent of the determination steps of ammonia oxidation and nitrite oxidation potential were the same as 2.2.1.

3. Results and analysis

3.1. The nitrification rate

The nitrification rate of sediment-water interface at each sampling point in Lianhua steam was lower, at a range of 0.0001 - 0.0025 mg(L.d)^{-1}, and the nitrification rate in sediment overlying water was relatively high, which is 0.08834-0.1591 mg(L.d)^{-1}. From upstream to downstream, it basically shows an upward trend (Table 1).

| Sample Sites | Surface water / mg(L.d)^{-1} | Sediment / mg(L.d)^{-1} |
|--------------|-----------------------------|-------------------------|
| A            | 0.0001±0.0001               | 0.0834±0.0011           |
| B            | 0.0002±0.0001               | 0.0939±0.0009           |
| C            | 0.0003±0.0002               | 0.1080±0.0021           |
| D            | 0.0003±0.0001               | 0.1064±0.0006           |
| E            | 0.0025±0.0002               | 0.1495±0.0013           |
| F            | 0.0002±0.0001               | 0.0856±0.0022           |
| G            | 0.0004±0.0003               | 0.1591±0.0014           |

3.2. Results of ammonia oxidation and nitrite oxidation potential nitrification rate

3.2.1 Ammonia oxidation and nitrite oxidation potential nitrification rate of surface water

As shown in Table 2, the total ammonia oxidation potential nitrification rate in the surface water body of Lianhua stream were 0.431 - 0.871 mg(L.d)^{-1}, while the total nitrate oxidation potential rate were 0.164 - 0.251 mg(L.d)^{-1}. The total ammonia oxidation potential was a little higher than the total nitrate oxidation potential (T-test, P < 0.05), and the ratio was 1.31.

| Sample sites | Total ammonia oxidation mg(L.d)^{-1} | Total nitrate oxidation mg(L.d)^{-1} |
|--------------|--------------------------------------|-------------------------------------|
| A            | 0.545±0.031                          | 0.193±0.024                         |
| B            | 0.441±0.042                          | 0.192±0.045                         |
| C            | 0.821±0.034                          | 0.182±0.033                         |
| D            | 0.431±0.038                          | 0.207±0.025                         |
| E            | 0.871±0.012                          | 0.164±0.016                         |
| F            | 0.764±0.021                          | 0.251±0.050                         |
| G            | 0.601±0.013                          | 0.205±0.027                         |

The ammonia oxidation potential and nitrate oxidation potential were separate into free state activity and particle activity, F (< 2.0 μm) for free state activity, F (> 2.0 μm) for particle state activity. The particle activity was obtained from the total ammonia oxidation potential and nitrate oxidation potential of raw water minus the free state activity. The ammonia oxidation potential of particles in Lianhua Stream were 0.276 - 0.552 mg(L.d)^{-1}, accounting for 53.6% - 70.4% of the total ammonia oxidation potential, compared to the dissolved ammonia oxidation potential with 0.196 - 0.402 mg(L.d)^{-1}, so the particle ammonia oxidation was dominant. The oxidation potential of
particulate nitrite was 0.089 - 0.125 mg(L.d)^{-1}, which accounted for 37.5% - 47.5% of the total oxidation potential of nitrite, and the oxidation of particulate nitrate was dominant (Table 3).

**Table 3.** Size-fractionated of the total ammonia and nitrate oxidation potential rate in Lianhua Stream

| Sample sites | Total ammonia oxidation potential /mg(L.d)^{-1} | Total nitrate oxidation potential /mg(L.d)^{-1} |
|--------------|-----------------------------------------------|-----------------------------------------------|
|              | F(<2μm)           | F(>2μm)           | F(<2μm)           | F(>2μm)           |
| A            | 0.220±0.021       | 0.349±0.030       | 0.093±0.011       | 0.125±0.027       |
| B            | 0.203±0.022       | 0.368±0.030       | 0.075±0.013       | 0.098±0.029       |
| C            | 0.210±0.029       | 0.276±0.043       | 0.059±0.089       | 0.089±0.029       |
| D            | 0.321±0.026       | 0.450±0.028       | 0.076±0.020       | 0.096±0.021       |
| E            | 0.196±0.025       | 0.350±0.032       | 0.081±0.016       | 0.089±0.027       |
| F            | 0.233±0.028       | 0.552±0.036       | 0.056±0.017       | 0.094±0.031       |
| G            | 0.402±0.026       | 0.464±0.035       | 0.066±0.017       | 0.099±0.028       |

3.3.2 Ammonia oxidation and nitrate oxidation potential at the interface of sediment overlying water

As shown in Table 4, the total ammonia oxidation potential of sediment in Lianhua Stream is 8.396 - 13.247 mg(L.d)^{-1}, while the total nitrate oxidation potential is 3.522 - 9.322 mg(L.d)^{-1}. The total ammonia oxidation potential was significantly higher than the total nitrate oxidation potential (t-test, p < 0.05), and the ratio was 1.24.

**Table 4.** Ammonia and nitrite oxidation potentials in sediment of Lianhua Steam

| Sample sites | Total ammonia oxidation potential /mg.(L.d)^{-1} | Total nitrate oxidation potential /mg(L.d)^{-1} |
|--------------|-----------------------------------------------|-----------------------------------------------|
| A            | 9.927±0.010                                   | 7.966±0.015                                   |
| B            | 11.672±0.021                                  | 8.014±0.023                                   |
| C            | 13.247±0.110                                  | 9.322±0.021                                   |
| D            | 11.020±0.013                                  | 8.323±0.014                                   |
| E            | 9.716±0.020                                   | 3.522±0.010                                   |
| F            | 8.396±0.014                                   | 3.564±0.011                                   |
| G            | 10.570±0.030                                  | 8.220±0.033                                   |

4. Discussion

According to the results of microenvironment experiments, it can be seen that the nitrification rate in the surface water of each sampling point in Lianhua Stream is relatively lower than that in sediment. It proves that the nitrification and biological absorption intensity of simple water bodies are weaker than that of sediment-water interface, in which suspended particles are involved.

It has also been confirmed that nitrifying bacteria are suspended in water or attached to and grown on sediment surface[9][12], and the oxygen concentration gradient and diffusion boundary at sediment-water interface determine the activity of nitrifying bacteria. The results show that the reaction processes of nitrification and denitrification generally occur at the sediment-water interface. Because there is no sediment in the simple water body, and the number of bacteria, fungi and microorganisms is also small, so the nitrification and denitrification rate is nonactive.

Although the nitrification rate in the surface water is low, but it has higher ammonia oxidation and nitrite oxidation potential. When the water body is deep and the water volume is large, the total nitrification amount will also be very considerable. Compared with the nitrification rate of upstream and downstream, it can be found that the nitrification rate of downstream is basically higher than that of upstream. The reason for this phenomenon may be that the environmental factors of different sampling sites are different, such as the process of exogenous input, the resuspension of water and sediment, the change of DO and so on.
It is known from Table 5 that the concentration of $\text{NH}_4^+$-N in the upper and lower reaches of the stream is quite different, the concentration in the downstream is a little higher than that in the upstream, and the corresponding nitrification rate in the downstream is also higher than that in the upstream. Therefore, there is a certain relationship between $\text{NH}_4^+$-N concentration and nitrification rate, but it is not a linear correlation.

**Table 5. Basic parameters in surface water and sediment in Lianhua Stream**

| Sample sites | Tem. | pH  | DO  | $\text{NO}_3^-$-N | $\text{NH}_4^+$-N | $\text{NH}_3$-N | $\text{NO}_2$-N |
|--------------|------|-----|-----|-------------------|-------------------|----------------|----------------|
|              |      |     |     |       water        |       sediment    |       water     |       sediment  |
| A            | 18.9 | 7.07| 9.24| 1.53±0.051        | 0.59±0.018       | 15.59±2.01     | 0.123±0.010    |
| B            | 18.9 | 7.48| 5.33| 1.45±0.012        | 0.66±0.026       | 14.23±1.34     | 0.13±0.008     |
| C            | 17.6 | 7.25| 9.00| 1.74±0.023        | 0.68±0.037       | 17.13±1.02     | 0.18±0.012     |
| D            | 17.6 | 6.80| 6.02| 1.80±0.034        | 0.69±0.045       | 12.38±2.10     | 0.17±0.007     |
| E            | 17.8 | 7.05| 6.58| 1.86±0.036        | 0.68±0.044       | 16.28±2.38     | 0.17±0.008     |
| F            | 18.4 | 7.47| 6.35| 1.85±0.047        | 0.62±0.037       | 13.12±1.57     | 0.18±0.011     |
| G            | 18.9 | 6.83| 7.05| 1.87±0.052        | 0.73±0.208       | 11.74±1.04     | 0.17±0.013     |

Some studies show that nitrification rate is also affected by pH, DO, and water temperature. It can be seen from Table 5 that the pH, DO, and water temperature of each sampling point is obviously different, so it is necessary to consider the influence of each environmental factor on nitrification rate, and the correlation between each environmental factors and nitrification rate needs to be studied in subsequent experiments.

According to the results of ammonia oxidation and nitrite oxidation potential, it can be seen that the ammonia oxidation and nitrite oxidation potential of the surface water body of Lianhua Stream is significantly lower than that of sediment (Table 2 and 4). This may be related to the difference of biomass and population structure of nitrifying bacteria. Nitrification consists of two steps: ammonia nitrogen is firstly oxidized to NO$_3^-$; under the action of ammonia oxidizing bacteria, and then NO$_2^-$ is oxidized to NO$_3^-$ under the action of nitrite oxidizing bacteria. Ammonia nitrogen is the substrate of ammonia-oxidizing bacteria. High concentration of ammonia nitrogen will increase the biomass of ammonia-oxidizing bacteria, which will promote the ammonia oxidation process, resulting in a higher ammonia oxidation rate. By comparing the ammonia nitrogen concentration of surface water with that of sediment overlying water (Table 5), it can be found that the ammonia nitrogen concentration in sediment overlying water is high, which is an important reason for the high ammonia oxidation potential of sediment. In addition, the higher ammonia oxidation potential can provide sufficient substrate for the process of nitrate oxidation, which leads to a higher process of nitrate oxidation.

Compared with the total nitrate oxidation potential nitrification rate, the partial correlation analysis of total ammonia oxidation potential and nitrite concentration was carried out by SPSS software. The results showed that there was a significant positive correlation between the total ammonia oxidation potential rate and the concentration of nitrite in Lianhua stream ($r \geq 0.814$, $p < 0.05$, $n \leq 7$), which indicated that high rate ammonia oxidation and low rate nitrite oxidation promoted the accumulation of nitrite in Lianhua Stream.

**5. Conclusion**

The results of laboratory microenvironment experiments show that the nitrification rate in sediments is higher than that in surface water, and the nitrification rates are 8.34-15.91 mg/(m$^2$.d) and 0.01-0.25 mg/(m$^2$.d). It can be concluded that the nitrification of stream ecosystem mainly occurs at the sediment-water interface. The potential nitration rate of ammonia oxidation and nitrate oxidation in sediments is also significantly higher than that in surface water. It may be related to the difference of biomass and population structure of nitrifying bacteria between them, and further experimental demonstration is needed. The high concentration of ammonia nitrogen in the water body of Lianhua...
stream promotes the activity of ammonia-oxidizing bacteria, which leads to high ammonia oxidation potential.

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