

## Microbial Effects on Nitrogen Circulation and Transformation on Water-sediment Interface of Source Water Reservoir

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Nitrogen circulation and transformation on water-sediment interface of Source Water Reservoir was studied through comparative experiment between sterilization incubation and non-sterilization incubation. The results showed that  $\text{NH}_4^+\text{-N}$  concentration in non-sterilization group continually increased and  $\text{NO}_3^-$  decreased by 97.7%. In the sterilization group, there was no obvious change except the increase caused by diffusion. The  $\text{S}^{2-}$  produced by sulfate reducing bacteria (SRB) weakened the denitrification. The content of TN and organic matter in the non-sterilization sediment samples decreased 26.28% and 18.37% respectively. After incubation, the quantity of ammonifiers, denitrifying bacteria and SRB increased greatly in the sediment of non-sterilization group. Therefore it can be concluded that microbes accelerated nitrogen circulation and conversion on water-sediment interface. Anaerobic condition is an important factor for  $\text{NH}_4^+\text{-N}$  release. SRB can inhibit denitrification, thus slow down the nitrogen removal rate of sediment.

### 1. Introduction

Internal pollution, caused by pollutants release from sediments, mainly happened on the water-sediment interface. Thus this interface was the most important environmental boundary layer for the mass exchange between water and sediment. Nitrogen (N), as the basic nutrient element for all vital movement, is also the notorious origin for eutrophication (Galloway, 2004). Violent biogeochemical cycle about nitrogen on the water-sediment interface has great influence on reservoir nutritional state and water quality (Wu, 1996). The nitrogen cycle is composed of multiple transformations of nitrogenous compounds, catalysed primarily by microbes (Jonathan, 2002). Nitrification and denitrification, occurring on the water-sediment interface and generating nitrite ( $\text{NO}_2^-\text{-N}$ ), were influenced by parameters such as DO, redox potential and so on. In these reactions, ammonium ( $\text{NH}_4^+\text{-N}$ ) and nitrate ( $\text{NO}_3^-\text{-N}$ ) changed their forms and transformed. The nitrite from different sources can join in nitrogen's circulation by kinds of biochemical processes such as nitrification, denitrification, and anaerobic ammonium oxidation (Carsten, 2006; Peter, 2003). Therefore nitrogen circulation and transformation on the water-sediment interface was a combination of multiform interrelated and interacted biochemical processes (Figure 1) (Zeng, 2007; Trimmer, 2003; Zehr, 2002). At present, research about this field mainly focuses on ocean, epilittoral zone and shallow lakes including the topics of nitrogen forms, each form's ecology significance and effect on nitrogen circulation, nitrification and denitrification' characteristics and influence factors (Lv, 2005; Chen, 2005; Wang, 2004; Tana, 2004; Casey, 2004). The study of this field focusing on source water reservoir was seldom reported. In this research, the nitrogen's circulation and conversion on water-sediment interface of source water reservoir was studied by comparative experiment between sterilization incubation and non-sterilization incubation.

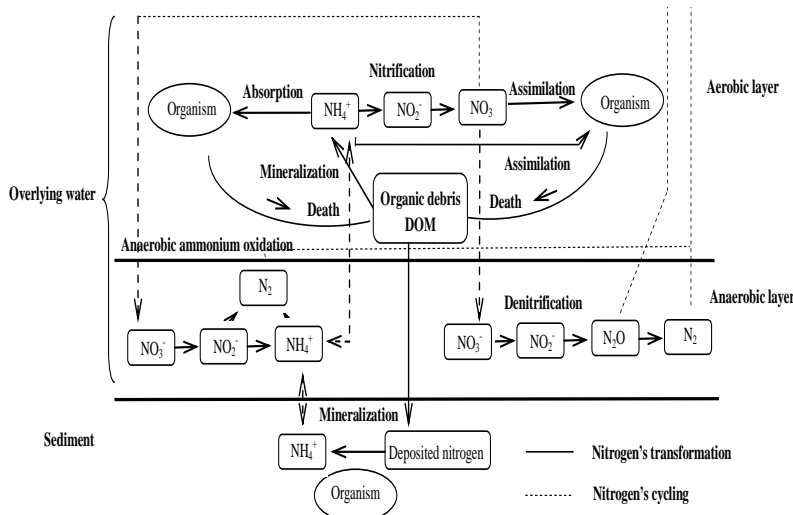


Figure 1: Nitrogen's circulation and transformation on the water-sediment interface

## 2. Materials and methods

### 2.1 Sampling

Water and sediments samples were collected from Shibiyanu Reservoir, which supplied source water for Xi'an City, China. Water was sampled 50cm upper the interface, then obtained through microporous filtering film (0.45  $\mu\text{m}$  pore size) and preserved at 4°C for further study. And sediments were collected by Peterson grab sampler and transported to the laboratory, then sieved to remove large pieces of stones and sand particles through Standard Testing Sieve. The sediment physicochemical properties were shown in Table 1. All the water and sediments samples were divided into two groups. One was non-sterilization group and the other was sterilization group treated by ultraviolet.

Table 1: Sediment physicochemical properties and the sterilization effect

TN(mg g <sup>-1</sup> )	TP(mg g <sup>-1</sup> )	Organic matter (%)	Moisture content (%)	Total bacterial count (cell g <sup>-1</sup> )		Sterilization rate (%)
				non-sterilization	sterilization	
1.168	0.813	3.296	42.649	$2 \times 10^4$	$1.5 \times 10$	99.93

### 2.2 Experiment design

The experiment was designed as two groups, non-sterilization group and sterilization group. Sediments (100ml for each incubator) were put into a series of brown wild-mouth glass incubators whose volume capacity and inradius were 250ml and 6cm. Each group contained two parallel series and each series contained 10 incubators which were number labelled consecutively. Overlying water (150ml for each one) was injected into each incubator slowly and carefully to avoid disturbing the sediments. Then all these incubators were airproofed by rubber plugs and avoid-light cultured at the temperature of 7~8°C. After some time, four incubators of same labelled number in both groups were opened and the overlying water qualities were tested. At the same time pore water of each incubators was obtained by centrifugation (4°C, 6000r, 30min) and tested using the same methods as used for overlying water. After centrifuged, the sediments were freeze dried and preserved at -20°C for further analysis. At the beginning of incubation the sampling was frequent, but gradually the sampling interval was timely protracted.

### 2.3 Chemical analysis

Analytical items and methods (State Environmental Protection Agency, 2002) about water samples were shown in Table 2.

The content of total phosphorus in sediments was tested by molybdate and L-ascorbic acid colorimetric method after Perchloric acid digestion (Li, 1983) and the content of organic matter in sediments was tested by potassium bichromate titrimetric method (State Environmental Protection Agency, 2002). The content of total nitrogen was tested by zinc cadmium reduction method after digested by alkaline potassium persulfate. The bacteria number involved in nitrogen circling was decided by MPN method.

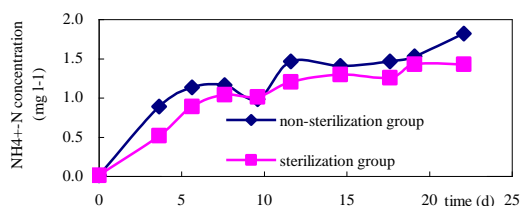
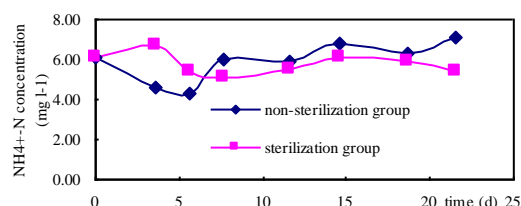
Table 2: Analytical items and method of water samples

Item	DO	NH <sub>4</sub> <sup>+</sup> -N	NO <sub>3</sub> <sup>-</sup> -N	TN	SO <sub>4</sub> <sup>2-</sup>
Method	HACH LED method	Nessler's reagent colorimetric method	Ultraviolet spectrophotometry	Ultraviolet spectrophotometry	Ion chromatography

### 3. Results and discussions

#### 3.1 Ammonification on the multi-phase

The NH<sub>4</sub><sup>+</sup>-N concentration in the overlying water of non-sterilization group increased gradually, while the NH<sub>4</sub><sup>+</sup>-N in the sterilization group fluctuated in a small range after reaching releasing balance (Figure 2). The changes of NH<sub>4</sub><sup>+</sup>-N concentration in the pore water of the two groups presented reverse regularities (Figure 3). After it reached releasing balance, which was caused by concentration gradient, about 6 days later, the NH<sub>4</sub><sup>+</sup>-N concentration in the pore water of the non-sterilization group increased slowly attributed to continuous ammonification. Contrarily in the sterilization group, the NH<sub>4</sub><sup>+</sup>-N concentration in the pore water decreased because of the releasing caused by concentration gradient. It was because that the increase of NH<sub>4</sub><sup>+</sup>-N concentration in the non-sterilization group was caused by microbes' vital movement. But in the sterilization group the increase of NH<sub>4</sub><sup>+</sup>-N in the overlying water was caused only by the concentration difference between overlying water and pore water. The fluctuation of NH<sub>4</sub><sup>+</sup>-N concentration in overlying and pore water of non-sterilization group was probably a coupling of ammonification in which NH<sub>4</sub><sup>+</sup>-N was generated and nitrification in which NH<sub>4</sub><sup>+</sup>-N was consumed. At the beginning, DO of the water-sediment interface (Figure 7) was high and this was favourable to nitrification. Therefore NH<sub>4</sub><sup>+</sup>-N concentration in overlying water was minimum 10 days later. Then DO in the incubators decreased gradually and the nitrification was inhibited, so NH<sub>4</sub><sup>+</sup>-N concentration increased gradually. NH<sub>4</sub><sup>+</sup>-N concentration minimum in the pore water appeared about 5 days later because DO in the pore water changed faster than it in the overlying water.

Figure 2: NH<sub>4</sub><sup>+</sup>-N concentration in the overlying waterFigure 3: NH<sub>4</sub><sup>+</sup>-N concentration in the pore water

#### 3.2 Nitrification and denitrification on the multi-phase

The coupling of nitrification and denitrification has been studied by many scholars (Luijn, 1996; Teissier, 2002). Denitrification happening in the sediments is an anaerobic reduction course, whose rate was closely related to the nitrate concentration in the sediment and water. But the nitrification, as one of the important source of nitrate, was an aerobic oxidation course. So the entire denitrification course was controlled by two kinds of completely reverse conditions. Therefore there is a strong coupling between nitrification and denitrification happening in the sediments.

Although initially it was comparatively high, DO in the incubators decreased quickly attributed to chemical and biological oxygen consumption (Figure 9). It was shown in Figure 4 that nitrate concentration in overlying water of the non-sterilization group decreased from 1.242 mg l<sup>-1</sup> at the beginning to 0.028 mg l<sup>-1</sup> 19 days later. The equivalent decrease in the sterilization group was not so great. At some special conditions, the sediments can adsorb nitrate in the overlying water. When nitrate in the water increased, the denitrification in the sediment anaerobic layer was strengthened and nitrate in the water was "inhaled" into the sediment anaerobic layer. Then nitrate in the water decreased. Contrarily when nitrate in the water decreased, the denitrification in the sediment anaerobic layer was weakened and nitrate diffusing from the overlying water into the sediments decreased. Accordingly nitrate accumulation in the overlying water made the nitrate concentration in overlying water maintain a certain stable value. Attributed to this transfer characteristic of nitrate, nitrate concentration in the microenvironment on the water-sediment interface can resist the disturbance coming from the changes of extraneous factors. Consequently at the beginning of the culture nitrate concentration in the overlying water was higher than the one in the pore water (Figure 5), so nitrate was "minus-released" and nitrate concentration in the pore water increased in a short time. Afterward nitrate concentration in the pore water of the non-

sterilization group decreased accompanied by denitrification. But this regularity in the sterilization group was not as obvious as in the non-sterilization group.

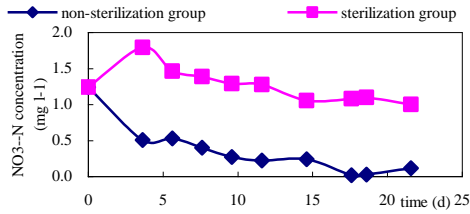


Figure 4:  $\text{NO}_3^-$ -N concentration in the overlying water

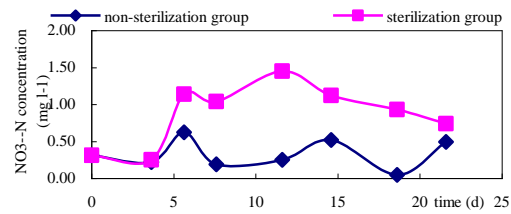


Figure 5:  $\text{NO}_3^-$ -N concentration in the pore water

The factors which can influence denitrification include nitrate concentration, carbon availability, temperature, DO, redox potential, inhibitor (such as  $\text{S}^{2-}$ ) concentration, pH, salinity, light, benthos disturbance, phytoplankton characteristics and so on (Xu, 2004). Sulfate concentration was tested in the experiment (Figure 6). The results showed that sulfate concentration in the non-sterilization group decreased by 60.61% (from  $10.99 \text{ mg l}^{-1}$  to  $4.22 \text{ mg l}^{-1}$ ) accompanied by DO decrease, while the equivalent decrease ratio in the sterilization group was only 30.88%. So it can be concluded that the sulfate-reducing bacterium (SRB) in the non-sterilization group consumed the sulfate in the overlying water and decreased its concentration in anaerobic conditions. And in this course, the inhibitor  $\text{S}^{2-}$  was produced which can decrease the denitrification rate and made the total nitrogen concentration increased as the organic matter was mineralized. As a result, nitrate concentration increased. And it was shown in Figure 7 that the oxygen consumption rate in the non-sterilization group was obviously higher than it in the sterilization group. So the changes about nitrogen in the non-sterilization group were more obvious than it in the sterilization group.

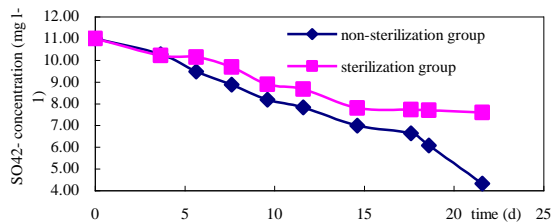


Figure 6:  $\text{SO}_4^{2-}$  concentration in the overlying water

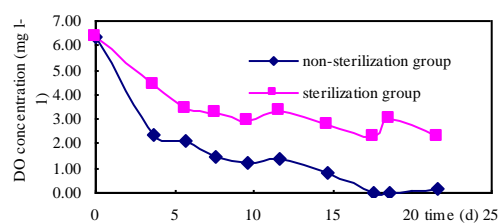


Figure 7: DO concentration in the overlying water

As shown in Figure 8-Figure 9, at the early stage of the culture experiment, the total nitrogen concentration increased a little without any fluctuation. This is because that besides the three kinds of inorganic nitrogen, there were some small molecule organic nitrogen released into the water attributed to organic matter mineralization. As DO was consumed, the accumulation of denitrification made the total nitrogen concentration in the overlying water decrease to minimum ( $0.597 \text{ mg l}^{-1}$ ) about 19 days later (Figure 8). Then the denitrification was inhibited, the total nitrogen concentration increased again.

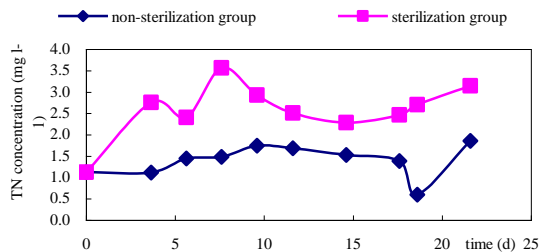


Figure 8: TN concentration in the overlying water

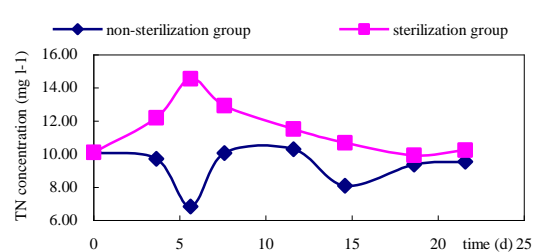


Figure 9: TN concentration in the pore water

### 3.3 Changes (total nitrogen TN, organic matter and number of bacteria involved in nitrogen cycling) of sediments

Sediments testing results (Figure 10 and Figure 11) showed that, content of total nitrogen and organic matter in the sediments of non-sterilization group both decreased after airproofed incubation. The decrease

percentages were 26.28% and 18.37% respectively. It was proved that the organic matter in the sediment was mineralized by microbes and nitrogen load was decreased by nitrogen cycling bacteria. But content of total nitrogen and organic matter in the sediments of sterilization group increased a little. It was possibly because that the treatment of sterilization made the organism debris deposited into the sediments which increased the content of total nitrogen and organic matter.

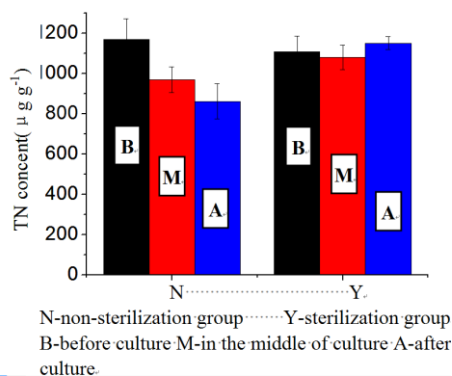


Figure 10: TN content of sediments

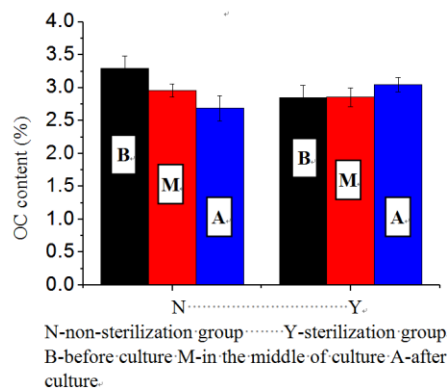


Figure 11: OC content of sediments

Testing results (Table. 3) of functional bacteria number showed that after airproofed incubation, the number of ammonifiers, denitrifying bacteria and SRB in the water-sediment interface of the non-sterilization group increased greatly. They increased by 784 times, 17 times and 51 times respectively. Each function bacteria effect on nitrogen circulation was proved. The increase of some nitrogen cycling bacteria and SBR indicated that these kinds of bacteria preferred anaerobic environment. Although it increased greatly after incubation, the ammonifiers were also in great quantity before incubation compared with other kinds of bacteria, which proved that ammonifiers can exist in great quantity both in aerobic and anaerobic conditions. At aerobic condition, the majority of  $\text{NH}_4^+\text{-N}$  was converted into nitrate by nitrobacteria, thus the increase of  $\text{NH}_4^+\text{-N}$  in the overlying water was not obvious. But at anaerobic condition, nitrification was weakened and  $\text{NH}_4^+\text{-N}$  produced by ammonifiers can not be completely converted into nitrate, so a great quantity of  $\text{NH}_4^+\text{-N}$  were released into overlying water. Therefore it can be concluded that anaerobic condition is an important factor for  $\text{NH}_4^+\text{-N}$  release.

Table 3: Bacteria numbers before and after culture (cells  $\text{g}^{-1}$ )

	ammonifiers	nitrobacterium	nitrosobacteria	denitrifying bacteria	SRB
Before the culture	$1.4 \times 10^3$	40	$4.5 \times 10^2$	$2.5 \times 10^2$	40
After the culture	$1.1 \times 10^6$	—	$4.5 \times 10^2$	$4.5 \times 10^3$	$2.1 \times 10^3$

#### 4. Conclusions

Research results showed that: (1) As the decrease of DO, the  $\text{NH}_4^+\text{-N}$  in non-sterilization group continually increased and the nitrate nitrogen ( $\text{NO}_3^-$ ) decreased by 97.7%. In the sterilization group, there was no obvious change except the increase caused by diffusion release. (2) The  $\text{S}^{2-}$  produced by SRB from  $\text{SO}_4^{2-}$  had weakened the denitrification. (3) The content of TN and organic matter in the non-sterilization sediment samples decreased by 26.28% and 18.37% respectively, which indicated that the organic matter was mineralized and the nitrogen load was reduced under microorganism effect. (4) At the end of incubation, the quantity of ammonifiers, denitrifying bacteria and SRB increased greatly. Anaerobic condition is an important factor for  $\text{NH}_4^+\text{-N}$ 's release.

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