CHEMICAL ENGINEERING TRANSACTIONS
VOL. 62, 2017

A publication of AIDIC
The Italian Association of Chemical Engineering
Online at www.aidic.it/cet

Guest Editors: Fei Song, Haibo Wang, Fang He

ISBN 978-88-95608- 60-0; **ISSN** 2283-9216

DOI: 10.3303/CET1762224

Bio-geochemical Circulation of Nitrogen in Lakes and the Role of Microorganisms

Lihui Zhang

Department of Biology, Changchun Normal University, Changchun 130032, China
zhanglihui_91@163.com

In this paper, the nitrogen forms of lacustrine sediments and their roles in biogeochemical cycles were studied. The effects of nitrogen on water-sediments - physical, chemical and biological processes in submerged plant systems, and understanding the physiological and ecological effects and feedback mechanism of submerged macrophytes on eutrophic water bodies can provide a scientific basis for lake endogenous pollution control. The in situ experiments were used to analyze the spatial distribution of ammonia nitrogen concentration, total organic carbon concentration, nitrification activity and the number of ammonia-oxidizing bacteria in Shahe reservoir and the Yangwa depression. Combined with the pollution characteristics and actual characteristics of the two sections, it is found that the concentration of nitrogen and the nitrifying active water at the two gates of Shahe reservoir and Yangwa gate are similar.

1. Introduction

As important physical bases to develop industrial and agricultural production and maintain lives on the land, the freshwater lakes are closely related with human life and play a vitally important role in human life as well as in regional economic development.Being a complex biogeochemical process, the nitrogen cycle within the lake ecosystem includes: nitrogen input, nitrogen conversion in ecosystem and nitrogen.

Namely, creatures in the lakes can assimilate such inorganic nitrogen as ammonium and nitrate originating from atmosphere and surface water as well as some dissolved organic nitrogen there. Inorganic nitrogen are capable of nitrification and denitrification in different oxycline layers while organic nitrogen or degraded NH4+ can diffuse or deposit into sediment and form endogenous nitrogen in lakes. In the sediment part of organic nitrogen is degraded to NH4+ again while the rest becomes organic nitrogen pool. Generally speaking, assimilation, ammoniation of organic matter (degradation) and nitrification forms the important biogeochemical process of nitrogen in the lake ecosystem. Combined together organically, these 3 processes are cross linked and in complex coupling relationships with each other. More than often people study nitrification and denitrification with the 15N dilution technique by analyzing 15N ratio and temporal as well as spatial variation of δ15N .

2. Experiments

2.1 Water Quality Analysis

Use multi N/C 2100 TOC analyzer to analyze DOC and TN of the raw water. Then take 20ml raw water and filter with 0.45μm glass fiber filterable membrane. Determinate NH4+, NO3- and NO2- with ICS-900 ion chromatographic analyzer. Determinate the amount of 2 nitrogen forms (ammonium and nitrate) in each sample after BOD determination.

2.2 Sample DNA Extraction

Use the OMEGA Water DNA kit to extract total DNA suspended in water from the filterable membrane used to filter previously. Keep the DNA sample in refrigerator under the temperature of -20 □ (Baurmann and Feudel, 2004). Use BECKMAN analyzer to determinate density (μg/mL) and OD value of the DNA sample solution to be used for calculation of amoA gene copy number.

Please cite this article as: Lihui Zhang, 2017, Bio-geochemical circulation of nitrogen in lakes and the role of microorganisms, Chemical Engineering Transactions, 62, 1339-1344 DOI:10.3303/CET1762224

2.3 Extraction and Determination of Convertible Nitrogen

By adopting a certain method, sediment was converted in sequence to different grain size with nitrogen of 4 kinds, respectively ion-exchangeable nitrogen (IEF-N), weak-acid extractable nitrogen (WAEF-N), strong-alkali extractable nitrogen (SAEF-N) and strong-oxidation extractable nitrogen (SOEF-N).

2.4 NH_4-N Adsorption Kinetics Experiment

Weigh several batches of 0.5000g sediment dry sample, put into 100ml polyethylene centrifuge, add 50ml NH4Cl solution with density of 10mg/L and vibrate in room temp with speed of 200rpm. At a certain intervals (5min, 10min, 30min, 60min, 90min, 120min, 150min, 180min) take out the centrifuge, centrifugalize it for 10 minutes with speed of 500rpm, and filter the supernatant liquid with 0.45μm filterable membrane. Add water into the filtered liquid until it reached 50 ml, then determinate the amount of NH4-N in the extracted solution with the Nessler reagent photometric method. By subtracting the determinated amount from that of NH4-N in the raw water, we got the adsorption amount by the sediment. 3 parallel tests were done as above with relative error lower than 5%.

3. Biogeochemical Process of Nitrogen in the Lakes

3.1 Characteristics Analysis on Adsorption and Desorption of Ammoniacal-nitrogen by Surface Sediments in Lakes

When the concentration of ammonical-nitrogen in the solution lies within 0-120mg/L, the adsorption isotherm of ammonical-nitrogen by lake sediments is as shown in Figure 1, from which it can be seen that the adsorption amount of ammonia-nitrogen increased with the increase of NH4-N equilibrium concentration in the liquidoid. In low concentration area, adsorption amount was in linear relationship with the preliminary concentration of NH4-N but the amount did not see much difference. And in the high concentration area, the adsorption amount increased quite slowly but the amount differs obviously. These are consistent with previous studies.

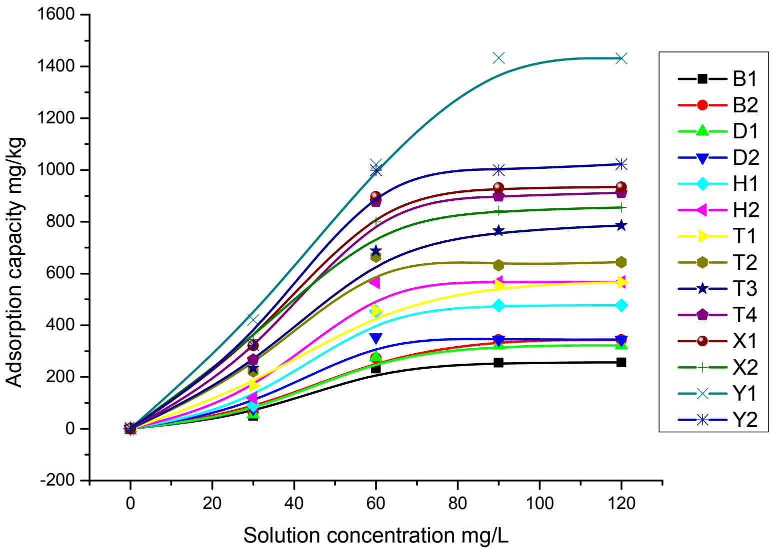

Figure 1: Adsorption isotherm of ammonical-nitrogen by lake sediments

Based on previous studies, Langmuir isotherm can fit well the isotherm of NH4-N adsorption by sediments. Langmuir Model:

$$\frac{C}{Q} = \frac{1}{Q_{\max}K} + \frac{C}{Q_{\max}} \quad (1)$$

In the formula, Q represents the adsorption amount of NH_4-N; Qmax, a capacity factor reflecting adsorption of NH4-N by the sediments, represents the maximum adsorption amount and indicates the capacity of NH_4-N in the sediments; C stands for the equilibrium concentration and K means the equilibrium adsorption coefficient. After fitting the test results with Origin Software, we have obtained adsorption parameters as shown in Table 1.

Table 1: Maximum adsorption amount (mg/kg) of NH4-N by lake sediments

Parameter	B1	B2	D1	D2	H1	H2	T1	T2	T3	T4	X1	X2	Y1	Y2
Qmax	555.56	344.83	294.21	516.32	1466.67	833.33	666.67	416.67	833.33	352.56	500	492.11	666.67	83.52
KL	0.09	0.08	0.35	0.07	0.07	0.46	0.24	0.31	0.92	0.03	0.74	0.69	0.14	0.07
R2	0.97	0.93	0.91	0.96	0.99	0.99	0.91	0.93	0.96	0.96	0.99	0.94	0.93	0.96

It can be known from Table 1 that the maximum adsorption amount of NH4-N by freshwater lake sediments in the middle and lower reaches of Yangtze River obtained a good result in fitting with the Langmuir Model, reaching to a significant level ($p<0.01$) and varying within a wide range between 294.11~1466.67mg/kg.

3.2 Exchange of Nitrogen at the Sediment-water Interface

Through simulative test we studied the inter-relationship between different parts of the sediment-water-plant system concerning the amount of different nitrogen forms.

Table 2: Relationship between various indicators for different treatment methods

		Interstitial water				Overlying water				Deposit sediment				
		TN	NH_4-N	NO_3-N	TON	TN	NH_4-N	NO_3-N	TON	TN	NH_4-N	NO_3-N	EN	MN
Interstitial	NH_4-N	0.71												
	NO_3-N	0.47	-0.06											
	TON	0.80	0.40	0.15										
Overlying	TN	0.9	0.75	0.41	0.78									
	NH_4-N	0.94	0.76	0.47	0.64	0.95								
	NO_3-N	0.77	0.42	0.78	0.40	0.80	0.85							
Deposit	EN	0.25	0.16	-0.30	0.58	0.27	-0.03	-0.19						
	MN	0.67	0.62	0.40	0.37	0.65	0.62	0.60	0.24					
	R-TN	0.69	0.60	0.11	0.63	0.67	0.63	0.41	0.28	0.69				
	S_t-TN	0.34	0.28	0.30	0.16	0.34	0.28	0.36	0.18	0.8	0.65			
	L-TN	0.60	0.61	0.06	0.48	0.59	0.50	0.31	0.37	0.80	0.95	0.79		
	P-TN	0.71	0.50	0.68	0.33	0.70	0.66	0.74	0.13	0.90	0.44	0.68	0.52	

Verifying result obtained from the Pearson product-moment correlation coefficient shows that, total nitrogen in pore water (PW-TN) is in obviously positive relations with ammonia-nitrogen in pore water (PW-NH4-N), total organic nitrogen in pore water (PW-TON), total nitrogen in overlying water (OW-TN), ammonia-nitrogen in overlying water (OW-NH4-N), nitrate in overlying water (OW-NO3-N), total nitrogen in sediments (S-TN), ammonia-nitrogen in sediments (S-NH4-N), mineralizable nitrogen in sediments(S-MN) and total nitrogen in roots (R-TN) ($p<0.01$ or $p<0.05$); PW-NH4-N is in obviously positive relations with OW-TN and OW-NH4-N ($p<0.05$); nitrate in pore water (PW-NO3-N) is in obviously positive relations with OW-NO3-N, S-MN, as well as total nitrogen in individual plant (P-TN), total nitrogen in roots (R-TN), total nitrogen in stems (St-TN) and total nitrogen in leaves (L-TN) of underwater plant watermilfoil ($p<0.01$ or $p<0.05$).

After cultivating watermilfoil with nutritional sediments, we have seen various nitrogen forms in overlying water above watermilfoil change with time as shown in Figure2. Since sampling day, OW-TN amount in Treatment 1, 2,4 and 5 decreased generally. Among them Treatment 2 and 4 reached the lowest OW-TN amount around 40 days after cultivation and then began to rise again. Treatment 3 saw a “single peak” change curve during the whole cultivation period with the peak at around 30 days after cultivation. OW-TN amount differs a lot among different treatment methods due to difference in nutritional sediments.

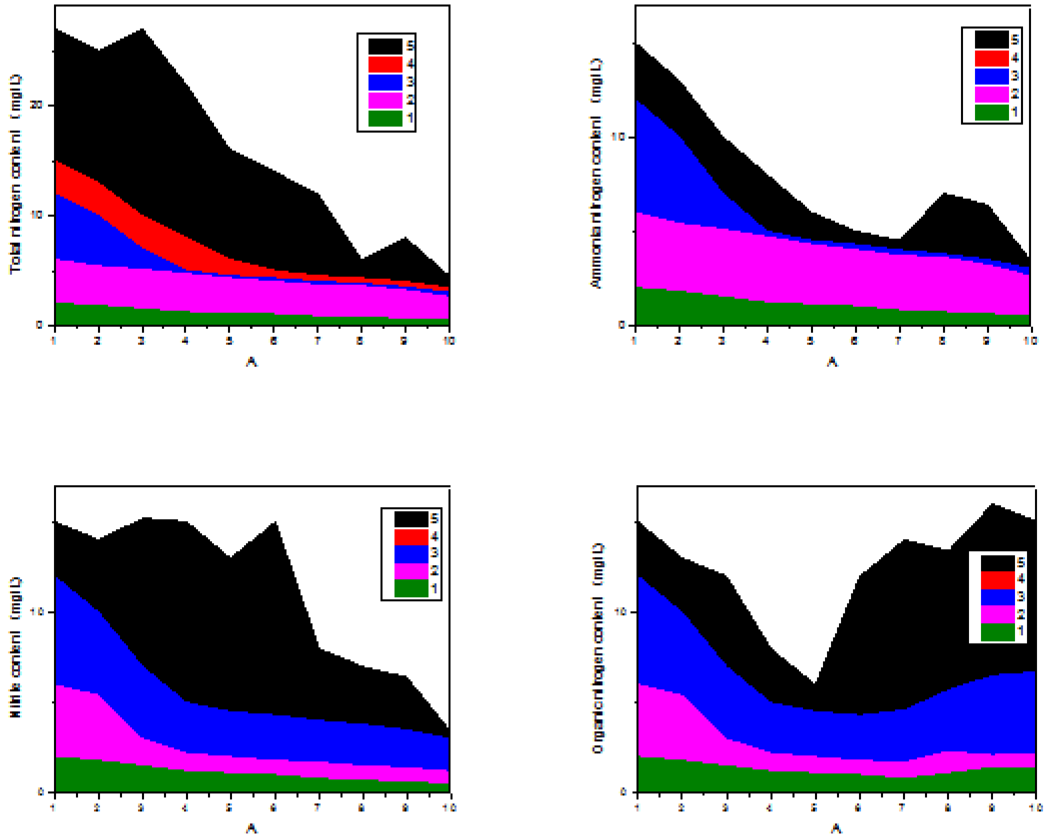

Figure 2: Variation of different nitrogen forms in the overlying water for different treatment methods

ON-OW amount varied in obviously different trends among different treatment methods. For Treatment 1, 2 and 3 it went in an uptrend generally while for Treatment 4 and 5, opposite with the first 3, it went in an uptrend at first and then in a downtrend. During preliminary stage of cultivation, Treatment 4 was the highest in nitrogen amount, then came Treatment 5, while Treatment 1, 2, 3 were the lowest. However, during the later stage of cultivation, Treatment 3 was the highest in nitrogen amount, then came Treatment 1 &2, while Treatment 4&5 were the lowest.

3.3 Spatial Variation Pattern of Ammonia-oxidation Bacteria in the Lakes

Through determination of amoA gene copy numbers in the water body of Shahe and Yangwa Gate sections, we've obtained the spatial variation pattern of ammonia-oxidation bacteria number in these 2 important sections of the North Canal (shown in Fig. 3). Copy number of ammonia-oxidation bacteria near the Shahe Gate was 1.84×108 copies/L-7.12×108 while that near the Yangwa Gate was 3.04×108-6.83×108 copies/L. These 2 sampling locations are different in pollution type, land use type and gate control law. Surrounded by residential area, the Shahe Gate is closed during sampling with a high percentage of total nitrogen in ammonia nitrogen while the Yangwa Gate, high in nitrate nitrogen due to impact of the Liangshui River in upstream, is surrounded by agricultural area and frequent in gate control. So the Yangwa Gate section had relatively higher nitrification activity and greater number of oxidized bacteria.
Water bodies before and after the Shahe and Yangwa Gates changed in different trends in terms of nutrient salt, nitrification activity and the oxidized bacteria number. The number of ammonia oxidized bacteria was high both inside and outside the Shahe Reservoir, but as it flowed downstream, the number decreased obviously. Meanwhile, the number was relatively smaller before the Yangwa Gate, but increased obviously 100m after the gate, which was consistent with the change pattern of nitrification activity. This indicates that particle and dissolved oxygen increased due to bigger water flow after the sluice was open, thus facilitating the increase of ammonia oxidized bacteria.

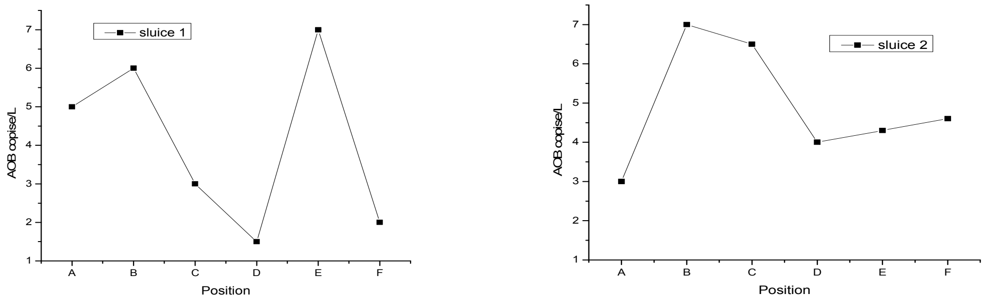

Figure 3: Spatial variation of ammonia-nitrogen, total nitrogen and DOC concentration in Shahe and Yangwa Gate sections

3.4 Denitrification Activity Determination of Embedded Microorganism Beads

The bacteria not embedded was high in denitrification activity and NZO production rate. NZO produced by bacteria not embedded in gaseous phase reached 645mmo/L 48h after cultivation while that produced by the bacteria embedded only reached 311mmo/L, which was only 48% of the former. This indicates after embedding microorganism activity decreased a lot (Figure 4).

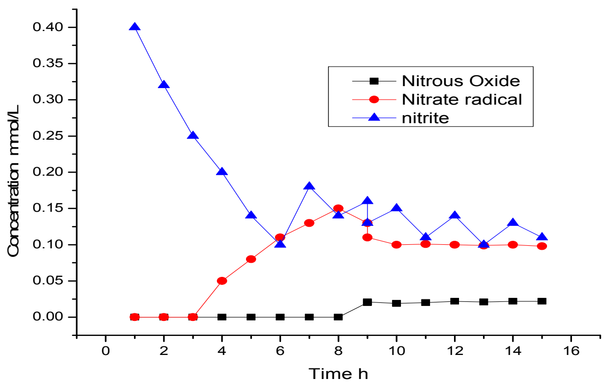

Figure 4: mmobilized microorganism's function to remove nitrate nitrogen from simulated water with continuous fed-batch

96h after cultivation, NZO produced by bacteria embedded and not embedded in gaseous phase reached respectively 1329mmol/L and 1562mmol/L, 23% and 32% of the total nitrate radical. Considering NZO dissolved in 50ml water, NZO produced through denitrification would have reached 66% and 92% respectively. After cultivation, there existed metal and nitrate accumulate in the solution as well as precipitation of starch from the immobilized beads. No generation of NZO was detected without bacteria comparison.

Soon after the test started, concentration of nitrate radical began falling, and on d5 it had fallen from 0.341 to 0.104mmol/L. Thereafter it saw no big difference. Nitrate was detected on 1.5d and reached 0.199mmol/L on d5, thereafter it kept between 0.1-0.18mmol/L, 30-50% of total nitrogen (0.341mmol/L). NZO was detected on d3 and its concentration kept between 0,021-0.032mmol/L, 12.32-18.76% of total nitrogen.

4. Conclusion

In this paper, the physical, chemical and biological processes of nitrogen in water-sediment-submerged plant systems were studied by environmental greenhouse simulation. The submerged plants promoted the migration and transformation of different forms of nitrogen at the sediment-water interface. It accelerated the mineralization of organic nitrogen, and the mineralized nitrogen content of sediments decreased significantly. The change of sediment structure also had some influence on different nitrogen migration and transformation.

The assimilation of lake nitrogen preferentially absorbs the light element so that the isotopic value of organic matter is low, while the inorganic nitrogen in the water is enriched with heavy element isotopic value. Root light elements are preferentially reacted with the remaining water in the nitrate-rich heavy isotope. In the nitrogen-restricted lakes, all the inorganic nitrogen is absorbed and assimilated to organic matter, then the organic nitrogen and inorganic nitrogen isotope values are same.

Acknowledgment

This work is supported by the Natural Science Foundation of China(NO.31570412), The project of 13th five-year science and technology research in jilin provincial education department (Ji jiao ke he zi (2016) No. 394).

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