ISSN 1070-3632, Russian Journal of General Chemistry, 2014, Vol. 84, No. 13, pp. 2656–2659. © Pleiades Publishing, Ltd., 2014.
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Kinetics Laws of Nitrification in Rybinsk Reservoir Waters

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Received July 30, 2014

Abstract—A new approach has been proposed for the evaluation of natural nitrification capacity of natural waters on the basis of experimental kinetic parameters of the transformation of nitrogen-containing compounds under aerobic conditions. The duration of the incubation period and rates of the transformation of various nitrogen-containing components, including ammonium ions, hydroxylamine, and nitrites, have been determined for the first time for different water columns and hydrological phases in the history of the Rybinsk reservoir. The nitrification capacity has been shown to be the most powerful factor ensuring self-purification of water basins from organic contaminations.

Keywords: nitrification, nitrogen, Rybinsk reservoir

DOI: 10.1134/S1070363214130258

INTRODUCTION

Transformations of nitrogen compounds play a key role in the life of water basins. The dynamics of the composition and ratio of different nitrogen compounds are determined by the rate of their bacterial degradation in the course of inner water circulation. In a simplified form, it may be represented by the following cyclic reaction sequence: $N_{org} \rightarrow NH_4^+ \rightarrow NO_2^- \rightarrow NO_3^- \rightarrow N_{org}$. Naturally, this scheme does not reflect all processes occurring in a water basin. There are various intermediate compounds among which hydroxylamine is one of the most important; it is formed in natural waters via two oppositely directed processes. On the one hand, hydroxylamine is produced by bacteria as a result of oxidation of ammonia and amines; on the other hand, it is formed as a metabolite in the reduction of nitrates. All these processes are mediated by microorganisms. The first step of nitrification is the oxidation of ammonia to the first stable nitrogen compound, hydroxylamine, which occurs on the surface of bacterial cells. Hydroxylamine then readily penetrates into the cell where it is oxidized to nitrite ion. While studying these processes, it is important to determine their rate and depth, which primarily depend on the accessibility of substrates for bacterial transformations.

Nitrification process is a potent factor responsible for self-purification of natural waters from organic substances. The goal of the present study was to determine kinetic parameters of the nitrification process and specific features of the transformations of ammonia into hydroxylamine and of hydroxylamine into nitrites in the Rybinsk reservoir, as well as estimate its potential nitrification capacity.

EXPERIMENTAL

Samples of water were withdrawn at standard Koprino, Mologa, Navolok, Izmailovo, Srednii Dvor, and Breitovo stations as shown in Fig. 1. The concentrations of nitrates, nitrites, and ammonium ions were determined according to the procedures described in [1]; hydroxylamine was determined by a modified GLC method [2], and total nitrogen was quantitated by the persulfate technique [3].

RESULTS AND DISCUSSION

The nitrification capacity was previously determined as the amount of nitrites and nitrates produced by nitrifying bacteria over a period of one month from ammonia added to natural water [4]. However, this method has substantial drawbacks. In particular, addition of 100 mg of an ammonium salt (ammonium magnesium phosphate) strongly changes the overall mineral content of water, which may impair to a

Fig. 1. Sampling map of the Rybinsk reservoir: (*1*) Koprino, (*2*) Mologa, (*3*) Navolok, (*4*) Izmailovo, (*5*) Srednii Dvor, (*6*) Breitovo.

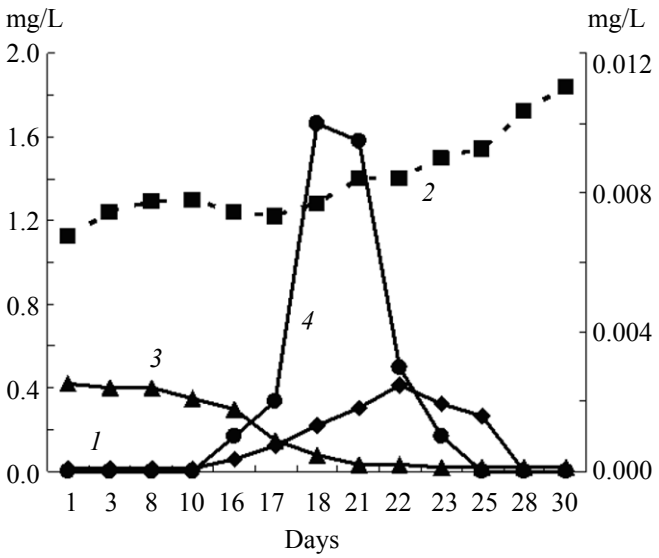

Fig. 2. Dynamics of the transformation of nitrogen-containing compounds at the Koprino station (experiment started on March 14; nitrates, ammonium ions, and nitrites refer to the left axis, and hydroxylamine, to the right axis): (*1*) NO_2^-, (*2*) NO_3^-, (*3*) NH_4^+, and (*4*) NH_2OH.

considerable extent the conditions for functioning of nitrifying bacteria. In this case, the concentration of minerals in water from the Rybinsk reservoir reaches a value of up to 4 g/L, so that the water becomes moderately saline. Furthermore, the addition of ammonium magnesium phosphate changes the ratio of the major ions, calcium and magnesium, and hence the pH value which is very important for waters with a low buffer capacity. It is proposed to define the nitrification capacity, or more accurately, natural potential nitrification capacity (NPNC) as the amount of nitrates formed in a natural water sample under standard conditions (20°C, protection from light) over a period sufficient for complete nitrification. This quantity includes both nitrates formed from mineral nitrogen originally present in a sample and a part of organic nitrogen subjected to ammonification. For the determination of NPNC water was sampled in different seasons at standard stations in the Rybinsk reservoir. Samples were placed into 20-L bottles which were kept for a month in the dark. A definite volume of water was withdrawn from the bottles at specified time intervals and analyzed for all inorganic nitrogen compounds, including hydroxylamine. Taking into account intermediate formation of hydroxylamine, the nitrification process can be represented by three consecutive first-order reactions: $NH_4^+ \rightarrow NH_2OH \rightarrow NO_2^- \rightarrow NO_3^-$. According to [5], the kinetics of this process may be described by the following system of three differential equations:

$$\frac{d[NH_4^+]}{dt} = -k_1[NH_4^+], \quad (1)$$

$$\frac{d[NH_2OH]}{dt} = k_1[NH_4^+] - k_2[NH_2OH], \quad (2)$$

$$\frac{d[NO_2^-]}{dt} = k_2[NH_2OH] - k_3[NO_2^-]. \quad (3)$$

Integration of this system under the initial conditions $t = 0$, $[NH_4^+] = [NH_4^+]_0$, $[NH_2OH] = [NH_2OH]_0$, $[NO_2^-] = [NO_2^-]_0$, $[NO_3^-] = [NO_3^-]_0$ gives

$$\frac{d[NH_4^+]}{dt} = -k_1[NH_4^+], \quad (4)$$

$$\frac{d[NH_2OH]}{dt} = k_1[NH_4^+]_0 e^{-k_1 t} - k_2[NH_2OH], \quad (5)$$

$$\frac{d[NO_2^-]}{dt} = \frac{k_1 k_2}{k_2 - k_1}\,[NH_4^+]_0(e^{-k_1 t} - e^{-k_2 t})k_3[NO_2]. \quad (6)$$

The concentration of $[NO_3^-]$ at any time can readily be calculated from the material balance equation

$$[NO_3^-] = [NH_4^+]_0 + [NH_2OH]_0 + [NO_2^-]_0 + [NO_3^-]_0 - [NH_4^+] - [NH_2OH] - [NO_2^-].$$

All experimental kinetic curves were fairly typical, and they conformed to generally accepted views.

The most characteristic data were obtained with the water sample withdrawn at the Koprino station on

Table 1. Natural potential nitrification capacity (NPNC) of water columns in the Rybinsk reservoir

Station	NPNC, μg/L	V_{nitr}, μg/L per day	N_{total}, μg/L	N_{org}, μg/L	% of mineralization
			October 1		
Koprino	93	3	936	257	28
Mologa	148	5	835	673	18
Navolok	289	10	1020	925	27
Izmailovo	165	6	882	755	20
Srednii Dvor	218	7	940	988	21
Breitovo	254	8	876	785	34
			October 26		
Koprino	76	3	928	358	10
Navolok	103	3	800	627	12
Breitovo	90	3	835	665	10
			March 14		
Koprino	526	10	1990	444	21
			May 24		
Koprino	321	10	1030	744	31
Mologa	171	6	966	758	17
Navolok	226	8	755	518	36
Izmailovo	197	7	966	710	22
Srednii Dvor	308	10	980	738	36
Breitovo	127	4	755	553	16
			July 26		
Koprino	181	6	708	504	12
Mologa	212	7	712	558	23
Navolok	147	5	650	535	20
Izmailovo	126	4	702	522	16
Srednii Dvor	156	5	675	462	24
Breitovo	213	7	722	606	26

Table 2. Rate constants (h^{-1}) of the reactions: $NH_4^+ \xrightarrow{k_1} NH_2OH \xrightarrow{k_2} NO_2^- \xrightarrow{k_3} NO_3^-$

Station	k_1	k_2	k_3
		October 1	
Koprino	0.002	0.04	0.005
Mologa	0.004	0.04	0.008
Navolok	0.004	0.05	0.010
Izmailovo	0.003	0.06	0.012
Srednii Dvor	0.003	0.04	0.009
Breitovo	0.004	0.05	0.015
		October 26	
Koprino	0.001	0.04	0.004
Navolok	0.001	0.04	0.002
Breitovo	0.0005	0.04	0.02
		March 14	
Koprino	0.016	0.06	0.004
		May 24	
Koprino	0.004	0.08	0.012
Mologa	0.002	0.07	0.005
Navolok	0.001	0.01	0.004
Izmailovo	0.002	0.03	0.005
Srednii Dvor	0.001	0.05	0.002
Breitovo	0.002	0.03	0.006
		July 26	
Koprino	0.002	0.01	0.003
Mologa	0.001	0.04	0.004
Navolok	0.002	0.06	0.005
Izmailovo	0.002	0.06	0.005
Srednii Dvor	0.002	0.06	0.005
Breitovo	0.004	0.09	0.008

March 14 (Fig. 2). The concentration of ammonium started to appreciably decrease only after 14 days, and hydroxylamine appeared almost simultaneously (1 μg N/L); the maximum concentration of the latter (10 μg N/L) was observed on the 18–21th day. The formation of NH_2OH was accompanied by increase in the concentration of nitrites from 0.01 to 0.4 mg N/L, which attained its maximum value on the 22th day when the concentration of hydroxylamine dropped down to zero again. Starting from this moment, the concentration of nitrites decreased in parallel with increase of the concentration of nitrates, whose maximum was observed on the 28th day. This nitrification pattern may be regarded as classical; analogous pattern was observed in almost all other experiments.

The obtained kinetic parameters displayed seasonal differences. In August, the nitrification lag phase in all parts of the Rybinsk reservoir was fairly short. The first step of the process started at an appreciable rate after 1 to 5 days and attained the maximum rate after 7 to 10 days. The third nitrification stage was accompanied by rapid accumulation of nitrates synchronously with the first phase up to the maximum nitrite concentration. After 15–20 days, the process entered the stationary phase, and the kinetic curve came to a plateau. The rate of nitrification with respect to nitrate formation was estimated at 4 to 10 μg/L per day, which is comparable with the data obtained by the radiocarbon method with the use of nitrapyrin as specific inhibitor of lithotrophic oxidation of ammonium ions for the Rybinsk reservoir in the early 1980s [6] (in August, the rate of nitrification was 17, 3, and 2 μg/L per day at the Koprino, Navolok, and Srednii Dvor statins, respectively), as well as for the lakes Drukshyai (4 to 8 μg/L) [7] and Sevan (2 to 16 μg/L) [8], by the isotope method [9] for the lake Grasmere (3 to 6 μg/L per day), and by the kinetic method [10] for the lakes Chuch'yarvi and Onezhskoe (12 and 20 μg/L per day, respectively). In autumn, the nitrification process in the Rybinsk reservoir slows down; therefore, the nitrogen pool deposited in organic matter and accessible to biota is smaller. In late October, the rate of nitrification was 3 μg/L per day at all three stations (Table 1).

The natural potential nitrification capacity of the Rybinsk reservoir varied from 76 to 556 μg/L (Table 1). In some periods, up to 36% of organic nitrogen could be converted into inorganic forms. The high ability for biochemical degradation indicates an essential role of this factor in the replenishment of water basins with nutrients in a form readily accessible to primary producers. This reduces nutrient deficiency in critical periods of biocenose functioning and is one of the most powerful factors ensuring self-purification of water basins from organic pollution.

The potential rates of bacterial transformations of nitrogen-containing components in Rybinsk reservoir differ at all steps of the nitrification process. The rate constants vary from 0.0005 to 0.09, and the rate of oxidation of hydroxylamine to nitrites is higher by an order of magnitude than the rates of its formation from ammonia and of oxidation of nitrites to nitrates (Table 2). Obviously, this is the reason why the concentration of hydroxylamine in natural waters is very small so that it generally cannot be detected. In all the examined cases, the rate-determining step is the transformation of ammonia into hydroxylamine, whose rate does not exceed the order of 10^{-2} mg/L.

CONCLUSIONS

(1) A method has been proposed for the assessment of nitrification capacity of natural waters by experimental determination of the kinetic parameters of aerobic transformations of nitrogen-containing compounds; the proposed method is free from some disadvantages intrinsic to other methods.

(2) Taking into account that up to 40% of nitrogen in organic matter can be converted into inorganic nitrogen compounds, nitrification capacity is one of the most potent factors ensuring self-purification of water reservoir from organic contaminations.

(3) The rates of bacterial transformations of nitrogen-containing components in water columns of the Rybinsk reservoir are different at all steps of the nitrification process, and the corresponding rate constants range from 0.0005 to 0.09.

(4) The kinetic parameters of the second nitrification step, formation of intermediate hydroxylamine, have been determined for the first time; the rate of this step is very small, so that it is rate-determining.

ACKNOWLEDGMENTS

This study was performed under financial support by the Russian Foundation for Basic Research (project no. 14-05-00346).

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