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Kinetic Parameters of the Transformation of Nitrogen-Containing Compounds in Natural Water

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Abstract—Kinetic parameters of ammonification, nitrification, and nitrate consumption by aquatic organisms has been determined by the laboratory modeling. The studied reactions represent the closed cycle of the nitrogen-containing compounds transformation in natural waters.

Keywords: Ammonification, nitrification, nitrate consumption, ammonium chloride, glycine, reaction rate, reaction half-time.

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Natural waters contain various nitrogen compounds: inorganic (ammonia, ammonium salts, nitrites, and nitrates) and organic, being in turn subdivided into macromolecular (polypeptides, nucleic acids, and nucleoptoteins) and of low molecular weight (amino acids, amines, amides, and urea).

The significant part of the nitrogen-containing compounds passes into water in the course of dead organisms degradation, the other source being lifetime secretion of the aquatic organisms, atmospheric precipitation, industrial, agricultural, and domestic wastewaters. The main cause of decrease of inorganic nitrogen compounds concentration is their consumption by aquatic organisms, majorly by phytoplankton and bacteria. The dynamics of concentrations and ratios of the nitrogen forms depends on the rate of a set of chemical and biochemical processes: ammonification, nitrification, nitrates consumption by phytoplankton, and denitrification.

The mentioned biochemical processes can be represented by the following scheme:

$$N_{\mathrm{org}} \xrightarrow{k_1} \mathrm{NH}_4^+ \xrightarrow{k_2} \mathrm{NO}_2^- \xrightarrow{k_3} \mathrm{NO}_3^+ \xrightarrow{k_4} N_{\mathrm{org}}, \quad (1)$$

with k_i being the rate constant of the respective process: k_1, ammonification; k_2, first stage of nitrification; k_3, second stage of nitrification; and k_4, nitrate consumption.

In this work the results of experimental determination of the rate constants of nitrogen transformation in the presence of ammonium chloride or glycine. Furthermore, the calculation of the rate constant of nitrates consumption is demonstrated.

EXPERIMENTAL

Water used throughout the work was sampled February 10, 2010 in Petrozavodsk (Lososinka river). The concentration of some inorganic nitrogen compounds (NH_4^+, NO_3^-, and NO_2^-) as well as total nitrogen N_{tot} was determined at the sampling date using the certified procedures [1–3]. The sample was divided into three parts, 1.5 L each. One of them was incubated at 19ºC without any additives. 1.5 mL of ammonium chloride solution (0.1 g/L) was added to the second specimen; hence, the ammonium concentration was increased by 0.1 mg of N per L. 8 mg of glycine was added to the thirds specimen, hence, N_{org} was increased by 1 mg/L. All the specimen were incubated in dark, in contact with air. The nitrogen forms concentrations were determined at regular intervals. The total duration of the experiment was of 40 days.

RESULTS AND DISCUSSION

The initial concentrations of the determined nitrogen forms in the natural water were as follows (in

Concentration of different nitrogen forms in the samples, mg of N per L^{-1a}

Date	no. 1 (no additives)			no. 2 (addition of NH_4Cl)			no. 3 (addition of glycine)		
	NH_4^+	NO_2^-	NO_3^-	NH_4^+	NO_2^-	NO_3^-	NH_4^+	NO_2^-	NO_3^-
10.02.2010	0.060	0.002	0.060	0.160	0.002	0.060	0.060	0.002	–
15.02.2010	0.028	0.004	–	0.046	–	–	0.269	0.005	–
18.02.2010	0.025	–	–	0.087	0.002	–	0.699	0.004	–
19.02.2010	–	0.003	–	-	0.002	–	–	0.004	–
25.02.2010	0.023	0.002	–	0.095	0.002	–	0.813	0.002	0.120
01.03.2010	0.022	0.001	0.110	0.091	0.001	0.050	–	0.004	–
03.03.2010	0.028	0.004	–	0.049	0.004	–	0.920	0.008	–
09.03.2010	0.015	0.003	–	0.040	0.006	–	0.760	0.032	
15.03.2010	0.015	0.005	–	0.010	0.005	–	0.170	0.015	
22.03.2010	0.014	0.001	0.004	0.012	0.001	0.090	0.011	0.001	1.210

[a] "–" No data.

mg of N per L): NH_4^+ 0.06, NO_2^- 0.002, NO_3^- 0.06, N_{tot} 0.63, and N_{org} 0.51.

The determined concentrations of various nitrogen forms as function of the analysis date are tabulated.

Kinetics of Nitrogen Transformations in Pure Natural Water

As seen from the tabulated data, ammonium ions concentration was the highest in the initial sample. After 5 days, it was decreased to approximately half of the initial value and then remained constant till day 21. Then, the second stage of ammonium concentration decrease took place, the concentration dropped down to 0.015 mg of N per L and remained at that level till the experiment end (day 40).

Concentration of NO_2^- was quite low, in the range of 0.001–0.005 mg of N per L, throughout the whole experiment, the value being typical of natural Karelia water. No noticeable maximum at the concentration profile were revealed.

NO_3^- concentration was determined at days 19 and 40. At day 19, it was of 0.05 mg of N per L higher than initially, due to transformation of N_{org}, NH_4^+, and NO_2^- into NO_3^- according to the scheme (see above). However, at day 40, nitrate ions were practically absent in the sample, likely, due to reverse transformation into N_{org} [4].

The N_{org} concentration was calculated at days 19 and 40; at day 19, it was decreased by 0.16 mu of N per L with respect to the initial value. Then, at day 40, the N_{org} concentration was practically the same. Likely, at the initial stage the organic nitrogen was efficiently decomposed, whereas towards the end of experiment that reaction was slowed down, and the accumulation of newly formed N_{org} via nitrates consumption compensated for the initial decrease..

Unfortunately, the results of that reference experiment could not be used for determination of the rate constants following the procedure described in [5], as no maxima at NH_4^+ profile was detected. Therefore, the rate constants were determined from the additional experiments, performed in the presence of either extra ammonium chloride or extra glycine.

Kinetics of Nitrogen Transformations in Natural Water with Ammonium Chloride Additive

Kinetics of NH_4^+ and NO_2^- concentrations change in specimen no. 2 are shown in Fig. 1.

The initial concentration of ammonium ions was of 0.06 + 0.1 = 0.16 mg of N per L. As seen from Fig. 1a, at day 5 the ammonium concentration was decreased, and then at day 15 it reached the local maximum, 0.095 mg of N per L. The observation of the local maximum allowed calculation of the rate constants

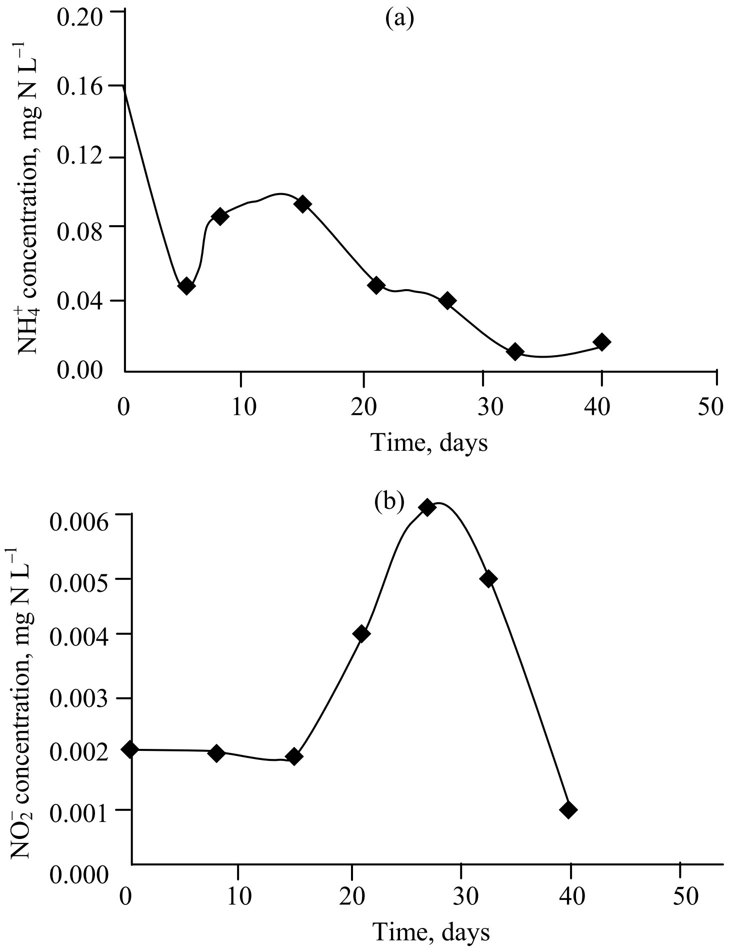

Fig. 1. Kinetics of (a) NH_4^+ and (b) NO_2^- concentration change; experiment with NH_4Cl added.

according to the equations set (2) [5]. In particular, the ammonification and first stage of nitrification rate constants could be determined under assumption that the reactions were strictly consecutive and the first order kinetics was held. Literature data revealed that the assumptions were reasonable [6].

$$[N_{\text{amm}}]_{\max} - [N_{\text{amm}}]_0 = [N_{\text{org}}]_0 \left(\frac{k_2}{k_1}\right)^{\frac{k_2}{k_1 - k_2}},$$
$$t_{\max} = \frac{\ln \frac{k_2}{k_1}}{k_2 - k_1}, \qquad (2)$$

where $[N_{\text{amm}}]_{\max}$, highest ammonium concentration; $[N_{\text{amm}}]_0$, initial ammonium concentration; $[N_{\text{org}}]_0$, initial concentration of organic nitrogen; $t_{\max}$, moment at which the highest ammonium concentration was reached.

The concentrations at day 5 were taken as the initial values, as ammonium concentration was the lowest at that point. By substituting the respective values in Eq. (2) with experimental numbers, the rate constants were estimated to be of $k_1 = 0.032$ day^{-1}, $k_2 = 0.226$ day^{-1}. Thus, k_2 (corresponding to the first stage of nitrification) was 7 times higher than k_1 (ammonification). That was in general in line with the results reported previously [7, 8]: k_1 of 0.01–0.04 day^{-1} and k_2 of 0.22–0.62 day^{-1}; k_2 was always higher than k_1.

From k_1 and k_2 values, the half-periods of the respective reactions were calculated as

$$\tau_{1/2} = \frac{\ln 2}{k}.$$

The results were as follows: $\tau_{1/2}$ (ammonification) = 21.7 day, $\tau_{1/2}$ (first stage of nitrification) = 3.1 days.

Fron Fig. 1b it is to be seen that nitrite ions concentration was almost constant till day 15, but it reached the maximum of 0.006 mg of N per L at day 21.

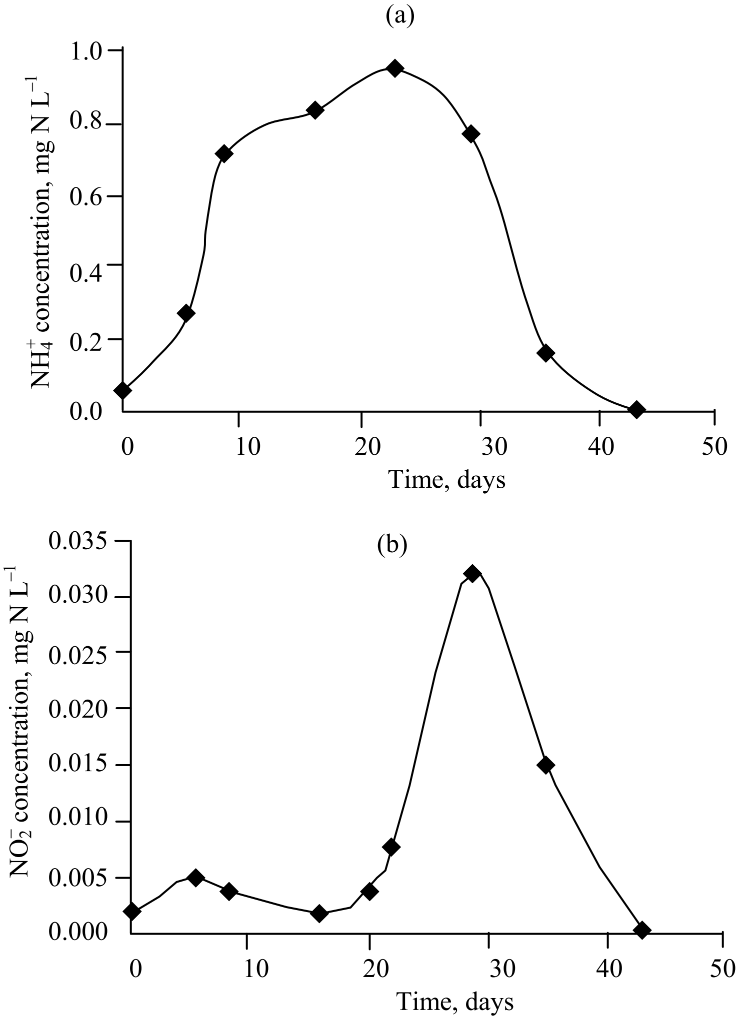

Fig. 2. Kinetics of (a) NH_4^+ and (b) NO_2^- concentration change; experiment with glycine added.

The observed maximum at nitrite concentration profile allowed calculation of the rate constant of the second stage of nitrification k_3 according to Eq. (3) [5].

$$[NO_2^-]_{max} - [NO_2^-]_0 = k_1k_2[NO_{org}]_0 \left[\frac{e^{k_1t}}{(k_1-k_2)(k_3-k_1)} \frac{e^{k_2t}}{(k_1-k_2)(k_3-k_2)} \frac{e^{k_3t}}{(k_1-k_3)(k_2-k_1)}\right],$$

where $[NO_2^-]_{max}$, highest nitrite concentration; k_1, ammonification rate constant; k_2, rate constant of the first stage of nitrification; $[NO_2^-]_0$, initial nitrite concentration; t, moment at which the highest nitrite concentration was reached; $[N_{org}]_0$, initial concentration of organic nitrogen.

After substituting with the numerical values, k_3 = 2.35 day^{-1}.

Thus, the second stage of nitrification was 10.4 times faster than the first stage. That coincided with the referenced data [9] and explained the generally low concentration of nitrite in natural water: they were rapidly oxidized to NO_3^-. The half-period of the second stage of nitrification was of 0.3 day.

Kinetics of Nitrogen Transformations in Natural Water with Glycine Additive

Kinetics of NH_4^+ and NO_2^- concentrations change in specimen no. 2 are shown in Fig. 2.

As seen from Fig. 2a, ammonium ions concentration was increasing sharply at the initial stage of the experiment, no lag period was observed. Indeed, glycine is an easily digestible organic compound, and the microorganisms do not need time to adopt themselves to the changed conditions. The highest ammonium concentration in the presence of glycine was reached at day 21, being of 0.92 mg of N per L. That was 61% f the initial total organic nitrogen. At day 40, at the end of the experiment, the ammonium ions practically vanished due to oxidation into nitrate.

Using the Eqs. (2) and the respective numerical values, the rate constants were calculated: $k_1 = 0.077$ day^{-1}, $k_2 = 0.027$ day^{-1}. In contrast to the previous experiment, in the presence of glycine k_1 was higher than k_2. That was explained by the increased concentration of organic nitrogen and therefore its higher consumption rate. The ammonification rate constant was 2.4 times higher than that in the experiment 2 (the initial organic nitrogen concentration was about 3 times higher than that in the experiment 2). The calculated half-periods of the reactions were as follows: $\tau_{1/2}$ (ammonification) 9 day, $\tau_{1/2}$ (first stage of nitrification) 25 day. From the position of the maximum at nitrite concentration profile (Fig. 2b) and having known k_1 and k_2, the rate constant of the second stage of nitrification was calculated using Eq. (3): $k_3 = 0.76$ day^{-1}; the half-period of that stage was of 0.9 day. From the reaction rate constants, the second stage of nitrification was faster than the other stages n the both experiments. The first stage of nitrification should therefore be the rate-limiting step; that was in line with the available reference data.

Nitrate ions concentration was initially smoothly increasing in the presence of glycine, to reach 0.12 mg of N per L at day 19 and 1.21 mg of N per L (74% of the total nitrogen) at day 40. Hence, nitrates are predominant product of nitrogen-containing compounds transformation in natural water and in model systems. Total nitrogen concentration at day 40 was of 1.47 mg of N per L, organic nitrogen concentration was of 0.25 mg of N per L.

From the tabulated results, the average ammonification rate (the increase of ammonium concentration per time up to reaching the highest concentration) could be calculated: $v = \Delta\chi/(t_0 - t_{\max})$. In the experiment 2, $v = 0.005$ mg N (L day)$^{-1}$, in the experiment 3, $v = 0.041$ mg N (L day)$^{-1}$.

The major results of the experiments were as follows. Whereas in the case of pure natural water the kinetic parameters of nitrogen transformation could be hardly determined, in the presence of additional ammonium chloride or glycine the rate constants could be determined by using the theory of consecutive first-order reactions. The rate constant of ammonification was in line with the reference data, thus, the additional nitrogen compounds introduced into the system did not influence much the kinetic parameters of the studied reactions. In the presence of glycine the ammonification rate constant was higher than the first stage of nitrification rate constant, in contrast to the experiment with ammonium salt used as additive. However, the second stage of nitrification was the fastest in the both experiments.

The obtained results allow description of the cyclic transformation of the nitrogen compounds depending on the certain conditions, in particular, to model the effect of the artificial introduction of one of the forms of nitrogen into the natural system.

Calculation of Nitrate Consumption

From analysis of nitrogen forms distribution in more than 730 of Karelian water objects [10] it followed that the fractions of NH_4^+, NO_3^-, and (in particular) NO_2^- were quite low, whereas organic nitrogen prevailed. The exceptions were some deep stratified objects, such as Ladozhskoe and Onezhskoe lakes: in those cases, nitrate concentration was comparable to that of organic nitrogen. Without strong external stimuli, the ratio of the nitrogen forms in Karelian objects was almost stationary; therefore, the reaction rates in the cyclic process (1) should be close: $V_1 = V_2 = V_3 = V_4$, with V_1, V_2, V_3, and V_4 being rates of ammonification, first nitrification stage, seconf nitrification stage, and nitrate consumption. As all the reactions were of the first order, $k_1C_1 = k_2C_2 = k_3C_3 = k_4C_4$, with k_1, k_2, k_3, and k_4 being the respective rate constants and C_1, C_2, C_3, and C_4 being the observed concentrations of N_{org}, NH_4^+, NO_2^-, and NO_3^-, respectively. Hence, having known the experimental concentrations of nitrogen forms and one of the rate constants, other rate constants could be derived.

From the above reported experiments the values of k_1 and k_2 or k_2 and k_3 were determined simultaneously. Therefore, the k_4 rate constant could be calculated, describing nitrate consumption. From the average ammonification rate constant (under conditions of Karelian water objects), 0.035 day^{-1} [8], and the respective average value of N_{org}, 0.51 mg of N per L [10], the ammonification rate was calculated: $V_1 = k_1C_1 = 0.0154$ mg N (L day)$^{-1}$. Similarly, for the first stage of nitrification, $V_2 = 0.016$ mg N (L day)$^{-1}$. Therefore, the mean rate of nitrogen compounds transformations under stationary conditions was of 0.015 mg N (L day)$^{-1}$. Then, $k_4 = V_1/C_4 = 0.015/0.01 = 1.5$ day^{-1}. As nitrates consumption is generally affected by the photosynthetic activity of phytoplankton, the latter rate constant is the most variable of all the discussed ones. In particular, in the case of large and deep objects, such as Ladozhskoe, Onezhskoe, and Segozero lakes, nitrates concentration is as high as 0.25 mg of N per L, therefore, $k_4 = 0.015/0.25 = 0.06$ day^{-1}, much low than in the cases of other Karelian water objects.

To conclude, the combination of analysis of natural objects, the model laboratory experiments, and the theoretical analysis allowed determination of kinetic parameters of single stages of nitrogen compounds transformation in the natural waters.

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