The Nitrogen Cycle in the Sea

Yasuo MIYAKE* and Eitaro WADA**

Abstract

The main processes of nitrogen cycle in the sea, especially in the euphotic zone in the western North Pacific off Japan were investigated. In this area, the concentration of ammonia, nitrite and nitrate in the euphotic zone in summer were respectively 1-12, 0-0.4 and 0-7\mu g-atoms/l. The content of ammonia in surface water was about 5\mu g-atoms/l and decreased with depth. Nitrite showed maximum concentration at the layer of 50-100 m depth. Nitrate content increased with the depth showing the maximum of about 40\mu g-atoms/l below the oxygen minimum layer. The experiment to estimate the order of chemical reactions in the nitrogen cycle by using marine bacteria was carried out. The experimental result revealed that the reactions could be expressed as the first order reactions with the temperature dependency in the exponential form. A cycle model for the kinetic processes of nitrogen bearing reactions was presented by assuming that every reaction concerned was of the first order. By this model the ratios of contents of nitrite to ammonia and nitrite to nitrate were obtained as simple functions of reaction velocities.

The comparison was done between the theoretical and observed ratios of contents of these compounds. The vertical profile of nitrite was also discussed in view of water temperature, ammonia concentration and bacterial activity. With respect to the oxidation process, linear relationship between nitrate content and AOU was confirmed.

Introduction

In the geochemical study of nitrogen in the ocean, it is important to investigate its cycle. Many researchers (Cooper, 1937; von Brand et al., 1937, 1939; Vaccaro, 1963; Hamilton, 1964) studied on this problem along various ways of approach. However, up to now, little attempt has been made concerning quantitative evaluations for transformation of various nitrogen bearing substances in the sea.

In this paper, the main process in the nitrogen cycle in the sea, especially in the euphotic zone will be described where the transformation of nitrogen compounds is taking place actively.

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Method of observation

Sampling of water was made in the western North Pacific off Japan in August 1964 aboard the R. V. Ryofu-maru (1,200 tons) which belongs to Japan Meteorological Agency. The locations of the sampling are shown in Fig. 1. In this area, the two big oceanic currents, Oyashio and Kuroshio, encounter.

For ammonia determination, non-filtered samples were collected in 100 ml oxygen bottles. The bottles were covered with polyethylene sheets and stored in a freezer on a ship. The samples were used later to determine ammonia in the laboratory on land.

Ammonia was expelled from sea water by air distillation at pH 12 and was absorbed in 0.1 N sulfuric acid followed by colorimetry with Nessler’s reagent.

Nitrite and nitrate were determined aboard the ship within an hour after sampling with the methods of Barnes and Folkard (1951) and Mullin and Riley (1955) respectively.

Result of analysis

Analytical results are given in Table 1. The range in concentration of each form of inorganic nitrogen in sea water is as follows:

- nitrate-N; 0.0-40μg-atoms/l
- nitrite-N; 0.00-0.4μg-atoms/l
- ammonia-N; 0.0-14μg-atoms/l

A brief note on each nitrogen compound is given below.

Ammonia

The vertical distribution of ammonia is shown in Fig. 2. Average ammonia contents at the layer of 0-50 m, 50-1,000 m and 1,000-4,000 m in this region are 5, 2.5 and 0.5μg-atoms/l. In the air distillation method of ammonia determination, the analytical value may include not only free ammonia but also a part of weakly bound amide such as hexosamine.
Table 1. Vertical distribution of nitrogenous compounds at C stations.

<table>
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<th>Depth m</th>
<th>Temp. °C</th>
<th>NH₄⁺</th>
<th>NO₃⁻</th>
<th>NO₂⁻</th>
<th>NO₃⁻/NO₂⁻</th>
<th>NO₂⁻/NH₄⁺</th>
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Recently, Sagi (1966) presented smaller values of 0.0-1.0μg-atoms/l of ammonia at the depth below 100 m in the same region. He used phenol, sodium hypochlorite and nitroprusside as reagents. The discrepancy in analytical results may be due to the difference in the methods.

However, "ammonia" obtained by the air distillation method may also be useful in considering the oxidation process of nitrogen.

Nitrite

It is known that the concentration of nitrite shows maximum at a certain
Fig. 2. Vertical distribution of ammonia in the western North Pacific off Japan.

Fig. 3. Vertical distribution of nitrite in the western North Pacific off Japan.

Fig. 4. Vertical distribution of nitrate in the western North Pacific off Japan.
depth in shallow layers where a steep density gradient is observed. (Rakestraw, 1936). In our observation, though depths are a little different from area to area, the nitrite maximal layers are generally discovered in the range between 50 to 100 m depth at every location (Fig. 3).

**Nitrate**

In the sea nitrate is the most abundant nitrogen compound among others. Its occurrence is closely related to other nutrients, phosphate, silicate and dissolved oxygen. The concentration of nitrate increases with the depth and the maximum concentration is usually found just below the oxygen minimum. In Fig. 4 the vertical distribution of nitrate is shown.

**The experiment to estimate the order of chemical reactions involved in the nitrogen cycle**

Grill (1951) reported that the decomposition reaction of organic nitrogen to ammonia was regarded as the pseudo-first order reaction. An attempt to estimate the order of reactions of the oxidation processes has been carried out in our laboratory by using marine bacteria, nitrite and nitrate formers, which convert ammonia to nitrite, and nitrite to nitrate respectively.

The bacteria had been collected by Dr. Kawai in the Maizuru Bay on the coast of the Japan Sea. The composition of the media for bacteria culture was as follows: ammonia or nitrite, 30 mg-N/l; NaCl, 32 g/l; KH₂PO₄, 2 mg/l; EDTA-Fe, 0.2 mg/l and pH, 8.2 (with K₂CO₃).

It may be said that the velocity of reactions which take place in the presence of marine bacteria, is controlled by temperature (T), salinity, pH, the concentration of a reactant (C) and bacterial population.

According to Kimata, Kawai and Yoshida (1963), the effects of variation in pH and salinity on the activity of bacteria are very small in the existing range of these factors in the sea. It may be, therefore, enough to take account only for the effects of T, C and bacterial density on the reaction velocity.

The dependency of reaction velocity on concentration of reactant (C) was determined as follows. Two kinds of bacteria, nitrite and nitrate formers were incubated in glass flasks at a constant temperature (24±1°C) and the change with time in the nitrite concentration was observed.

When the reactions, ammonia to nitrite and nitrite to nitrate are of the first order reactions at constant temperature and with constant activity and density of bacteria, following equations will hold.

\[-\frac{dC}{dt}=kC\]  \hspace{1cm} (1)

\[C_t=C_0e^{-kt}\]  \hspace{1cm} (2)

\[C_t'=C_0(1-e^{-kt})\]  \hspace{1cm} (3)
where, \( C_0 \) is a concentration of reactant at the time \( t=0 \); \( C_t \) and \( C_t' \) are respectively concentrations of a reactant and a reaction product at the time \( t \); \( k \) is a velocity constant.

In the reaction, ammonia to nitrite, concentrations of ammonia and nitrite are respectively represented by \( C_t \) and \( C_t' \). In the reaction, nitrite to nitrate, \( C_t \) represents the nitrite concentration.

As shown in Figs. 5 and 6, the variations with time in nitrite concentration in two reactions, ammonia to nitrite and nitrite to nitrate, indicate that for a certain length of time these reactions may be regarded roughly as the first order reactions with different values of rate constants \( k \).

![Fig. 5. Relation between the oxidation of ammonia to nitrite by nitrite former and incubation time.](image)

![Fig. 6. Relation between the oxidation of nitrite to nitrite by nitrite former and incubation time.](image)

The above result of experiment suggests that the biochemical reactions in the sea in which alterations of inorganic nitrogen compounds take place may be regarded also as the first order reactions.

According to Kimura et al. (1963), the effect of temperature on the reaction velocity in the biochemical reaction, ammonia to nitrite, can be expressed as follows.

\[
-\frac{dC}{dt} = k'e^{\alpha T}
\]  

(4)

where \( C \) is the concentration of ammonia, \( k' \) and \( \alpha \) are constants, \( T \) is the temperature in the range from \( 0^\circ C \) to \( 30^\circ C \).

By combining the equations 1 and 4, the next equation which expresses the relationships between the reaction velocity, concentration and temperature can be deduced.

\[
-\frac{dC}{dt} = A \cdot C \cdot e^{\alpha T}
\]  

(5)

where \( A \) is a constant for a given activity and density of bacteria.
The cycle of nitrogen compounds in the sea

In order to explain the distribution of inorganic nitrogen compounds in the sea, a following cyclic model for the kinetic processes of reactions is presented.

\[
\text{Org.-N} \xrightarrow{K_1} \text{NH}_4^+ \xrightarrow{K_3} \text{NO}_2^- \xrightarrow{K_5} \text{NO}_3^-
\]

\[\begin{array}{c}
\uparrow \\
K_7 \\
\downarrow \\
K_9 \\
\downarrow \\
K_5 \\
\downarrow \\
K_4
\end{array}\]

In the above diagram, K is a rate constant for an individual step of the cycle. The reactions associated with biological actions are assumed to be irreversible.

In a closed system, the change with time in concentration of each nitrogen compound is expressed as follows.

\[
\frac{d(\text{Org.-N})}{dt} = K_7(\text{N}-\text{Org.-N}) - K_1(\text{Org.-N})
\]  
(6)

\[
\frac{d(\text{NH}_4^+)}{dt} = K_3(\text{Org.-N}) - (K_9 + K_4)(\text{NH}_4^+)
\]  
(7)

\[
\frac{d(\text{NO}_2^-)}{dt} = K_7(\text{NH}_4^+) - (K_9 + K_4)(\text{NO}_2^-)
\]  
(8)

\[
\frac{d(\text{NO}_3^-)}{dt} = K_9(\text{NO}_2^-) - K_4(\text{NO}_3^-)
\]  
(9)

\[
(\text{Org.-N}) + (\text{NH}_4^+) + (\text{NO}_2^-) + (\text{NO}_3^-) = \text{N}
\]  
(10)

At the steady state, the concentration of each form of nitrogen compound can be given as a function of each reaction velocity constant and the total concentration of nitrogen (N) as follows.

\[
(\text{Org.-N}) = \frac{K_7}{K_7 + K_5} \cdot \text{N}
\]  
(11)

\[
(\text{NH}_4^+) = \frac{K_7 \cdot K_3}{(K_7 + K_5)(K_5 + K_4)} \cdot \text{N}
\]  
(12)

\[
(\text{NO}_2^-) = \frac{K_7 \cdot K_1 \cdot K_4}{(K_7 + K_5)(K_5 + K_4)(K_4 + K_9)} \cdot \text{N}
\]  
(13)

\[
(\text{NO}_3^-) = \frac{K_7 \cdot K_1 \cdot K_2 \cdot K_9}{(K_7 + K_5)(K_5 + K_9)(K_9 + K_4)} \cdot K_4 \cdot \text{N}
\]  
(14)

From above equations, the following simple equations can be deduced by taking the ratio of a pair out of the four components.

\[
\frac{\text{NH}_4^+}{(\text{Org.-N})} = \frac{K_3}{K_9 + K_4}
\]  
(15)

\[
\frac{\text{NO}_2^-}{(\text{NH}_4^+)} = \frac{K_4}{K_5 + K_9}
\]  
(16)

\[
\frac{\text{NO}_3^-}{(\text{NO}_2^-)} = \frac{K_4}{K_9}
\]  
(17)
Discussion on the observed results in the sea

In the area studied, the concentration of ammonia, nitrite and nitrate in the euphotic zone in summer are respectively 1-14, 0-0.4 and 0-7 μg-atoms/l. The vertical distribution of the ratios, nitrite to ammonia and nitrite to nitrate and occurrence of the nitrite maximum in the euphotic zone will be discussed below.

Nitrite to ammonia ratio

Eq. 16 which gives the ratio of nitrite to ammonia concentration can be modified as follows.

\[
\frac{\text{NO}_2^-}{\text{NH}_4^+} = K_\text{s} \frac{1}{1 + \frac{K_\text{s}}{K_\text{a}}} \cdot \frac{K_\text{a}}{K_\text{s}}
\]  

(18)

where \(K_\text{a}/K_\text{s}\) is equal to \(\text{NO}_2^-/\text{NO}_3^-\) (Eq. 17) which ranges from 0.01 to 0.2 and \(K_\text{s}/K_\text{a}\) is considered to be around 1 or smaller than 1.

Eq. 18 can be, therefore, simplified as follows.

\[
\frac{\text{NO}_2^-}{\text{NH}_4^+} = \frac{K_\text{s}}{K_\text{a}}
\]  

(19)

where \(K_\text{a}\) and \(K_\text{s}\) are rate constants of the reactions, ammonia to nitrite and nitrite to nitrate.

As mentioned above, \(K_\text{s}\) and \(K_\text{a}\) can be expressed as follows.

\[
K_\text{s} = A \exp \cdot \alpha T
\]  

(20)

\[
K_\text{a} = B \exp \cdot \beta T
\]  

(21)

From the equations (20) and (21), the next equation is deduced.

\[
\ln \frac{\text{NO}_2^-}{\text{NH}_4^+} = \ln \frac{A}{B} + (\alpha - \beta)T
\]  

(22)

In the above equation, \((\alpha - \beta)\) is derived from the difference in temperature dependency of \(K_\text{s}\) and \(K_\text{a}\), and \(A\) and \(B\) may include various biological factors in which a population and activity of bacteria may be predominant. A constant \(\alpha\) is 0.10 according to the study by Kimata et al. (1963).

In Fig. 7, vertical variation of the ratio of nitrite to ammonia versus sea water temperature is given. In Fig. 8, relations of \(\log(\text{NO}_2^-/\text{NH}_4^+)\) and water temperature are shown. As shown in Fig. 8, the relation between \(\log(\text{NO}_2^-/\text{NH}_4^+)\) and temperature is approximately linear and a constant value of \((\alpha - \beta)\) of about \(-0.15\) (\(\beta\) of 0.25) can be obtained.

By using the values of \((\alpha - \beta)\) of \(-0.15\) and the ratio of nitrite to ammonia, the relation between water temperature and \(A/B\), which is expressed in the relative values to the surface value at the station C-1 can be obtained. The results show that the values of \(A/B\) can be roughly classified into two groups, 0.5 and 3.5 respectively for higher and lower temperatures. The ratio \(A/B\) is
considered to be related to the ratio of population and activity of nitrite and nitrate formers at each station.

Further studies are needed concerning the physico-chemical meanings of A and B or A/B, in other words, concerning the relations between reaction velocity of oxidation reactions for nitrite and nitrate formation and biochemical, catalytic activities.

*Vertical profile of nitrite concentration in the sea*

As mentioned above, nitrite shows the maximum value in its concentration from 50 to 100 m depth. The nitrite concentration is controlled by three factors as given in Eq. (22), i.e., A/B, ammonia concentration and water temperature.

*Fig. 8. Log ([NO$_3^-$]/[NH$_4^+$]) versus sea water temperature.*

When both the ratio A/B and ammonia concentration do not much vary vertically, there is no appreciable maximum in nitrite concentration. The nitrite profile at the station C-5 illustrates the above case. When values of A/B are different between the higher and lower temperature layers and the accumulation of ammonia occurs at a certain depth, a remarkable nitrite maximum appears by the effect of three factors. The nitrite profile at the stations C-1, 2 and 4 is regarded to be the latter case.

*Nitrite to nitrate ratio*

The ratio nitrite to nitrate concentration in the euphotic zone can be explained by Eq. 17 as follows.

$K_4$ in Eq. 17 might be proportional to the rate of the primary production.
The rate of primary production in regard to nitrogen can be expressed as $rM$, where $M$ is a biomass and $r$ is a function of temperature and light intensity. When the change in a biomass, $M$, is small throughout the euphotic zone and the concentration of nitrate in the same zone is also very small, it may be plausible to assume that the rate of the primary production is approximately proportional to nitrate concentration. The vertical variation of the primary productivity in the Kuroshio region was given by Saijo et al. (1962).

$K_s$ is the rate constant in the reaction of nitrite to nitrate. If the same type of temperature effect as in the reaction, ammonia to nitrite, is valid for the reaction, nitrite to nitrate, $K_s$ can be expressed in the form of $B \exp \beta T$, in which $B$ is a constant for a given density and activity of bacteria.

Thus, Eq. 17 can be rewritten as follows.

$$\frac{(NO_2^-)}{(NO_3^-)} = \frac{rM}{B \exp \beta T} \quad (23)$$

To compare vertical variation of the ratio of nitrite and nitrate in the euphotic zone at several stations, the relative values with respect to the surface value $(0.020$ on an average) are used. In Eq. 23, $M/B$ is assumed to be constant throughout the euphotic zone. As to the vertical variation of $r$, the values given by Saijo et al. (1962) are employed.

The vertical variation of $\exp \beta T$ is calculated by using a constant $\beta$ of 0.25 as above.

In Fig. 9, a comparison between the observation and calculation of the vertical profile of the ratio of nitrite to nitrate is given. As shown in the figure the calculation agrees fairly well with the observation.

![Fig. 9. The variation of the ratio of nitrite to nitrate versus depth.](image)

The main process of the nitrogen bearing reactions in intermediate and deep layers is the oxidation process.

With respect to nitrate, Richards (1965) and Sugiura et al. (1964) gave the relation between nitrate concentration ($\mu g$-atoms/l) and apparent oxygen utilization (A.O.U. ml/l) as follows:

$$\langle NO_3^- \rangle_{observed} = 5 \ A.O.U. + \langle NO_3^- \rangle_{preserved} \quad (24)$$

In Fig. 10, the relation between nitrate concentration and A.O.U. is shown in which a solid line is given by Eq. 24.

The concentrations of both nitrite and ammonia are very low in these layers. The observed ratios of nitrite to ammonia were 0.005 to 0.015 in the temperature range from 13 to 3°C. If the equation (22) holds in these layers,
nitrite to ammonia ratio should increase with depth. However, in these layers, little correlation between observed ratios and sea water temperature was found.

Generally, a few nitrite maximum layers are found in the sea. In the region near the station D, two nitrite maximum layers are found at the depth of around 50 m and 700 m. The occurrence of deeper maximum may also be mainly due to the vertical variation of the concentration of ammonia and the factor (A/B) as explained in the case for the euphotic zone.

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