

Chapter 18

**Nitrogen Isotope Fractionation and Its Significance in
Biogeochemical Processes Occurring in Marine
Environments**

Eitaro WADA

*Mitsubishi-Kasei Institute of Life Sciences, Minamiooya, Machida,
Tokyo 194, Japan*

ABSTRACT Natural abundance of ^{15}N in biogenic substances was investigated with special reference to marine plankton samples.

Simple model on the nitrogen isotope fractionation was developed for the processes of assimilation, fixation and denitrification.

The occurrence of nitrogen isotope fractionation was generally found out in the process of both ammonium and nitrate assimilation by marine diatoms and marine algal flagellates. In boreal areas the ^{15}N abundance for plankton is regulated by the nitrogen isotope fractionation during nitrate assimilation, while ^{15}N abundance for N_2 -fixing blue-green algae showed characteristic low $\delta^{15}\text{N}$ values close to that of atmospheric nitrogen. Small isotope fractionation in the process of N_2 -fixation is responsible for the latter fact. ^{15}N abundance provides three plankton groups according to the form of inorganic nitrogenous compounds used as nitrogen source for primary production.

Nitrogen isotope fractionation was studied during the decomposition of *Trichodesmium erythraeum*, *Chaetoceros* sp. and *Calanus plumchrus* collected in the western North Pacific Ocean. It was found that $\delta^{15}\text{N}$ values for particulate matters were highly variable and closely correlated with the reproduction of bacterial

biomass and/or coagulation of dissolved organic nitrogen.

Significance of nitrogen isotope fractionation factor as a ecological parameter was figured out in marine environments along individual metabolic pathway involving N_2 -fixation, assimilation, decomposition, and denitrification.

18.1 INTRODUCTION

Nitrogen is cycled in the ocean in a complex manner. Various kinds of biochemical processes are involved and the extent of nitrogen isotope fractionation varies depending upon the kinetic mode of individual metabolic reactions. Miyake and Wada (1967) were the first to report the $^{15}N/^{14}N$ ratios of nitrogenous compounds occurring in marine environments. $\delta^{15}N$ of marine biogenic nitrogen relative to atmospheric nitrogen is +7‰ on an average, and tends to increase along the food chain. ^{15}N abundance in pelagic plankton is apparently related to the form of inorganic nitrogen used for their growth (Wada and Hattori, 1976). Cline and Kaplan (1975) have shown that nitrate in the oxygen-depleted waters of the eastern tropical North Pacific Ocean is enriched with ^{15}N up to 18‰. Large isotope fractionation of nitrogen associated with denitrification was emphasized.

The nitrogen isotope fractionation by microorganisms have been investigated in laboratory experiments. Hoering and Ford (1960) showed that isotope fractionation of nitrogen hardly occurs in the molecular nitrogen fixation by *Azotobacter* spp. On the other hand, the fractionation factor up to 1.03 was obtained in denitrification and nitrate reduction to nitrite by *Pseudomonas*, *Bacillus* and *Alcaligenes* (Wellman et al., 1963; Cook et al., 1973), by an unidentified marine denitrifier (Miyake and Wada, 1971), and by *Serratia marnorubra* (Miyazaki, 1971). In order to explain the isotope effect in the chemical reductions of nitrate and hydroxylamine, Brown and Drury (1967) presented a two-atom model in which the cleavage of an N-O bond is rate-limiting. Rees (1973) developed a steady state kinetic model for sulphur isotope fractionation in bacterial sulfate reduction. A similar model was also applied to the nitrogen isotope fractionation in nitrate assimilation by marine diatoms (Wada and Hattori, 1978).

The present communication provide additional data on ^{15}N abundance in nitrogenous materials occurring in marine environments and on nitrogen isotope fractionation in decomposition of pelagic plankton, assimilation of

inorganic nitrogen by phytoplankton and bacterial denitrification. Using these data, together with the data available in the literature, the mode and extent of nitrogen metabolism in different marine environments are discussed.

18.2 MATERIALS AND METHODS

Samplings of plankton and sediments, and procedures for measurement of nitrogen isotope ratios were described elsewhere (Wada and Hattori, 1976; Wada et al., 1975). Nitrogen isotope ratio ($^{15}\text{N}/^{14}\text{N}$) was determined using a Hitachi RMU-6R mass spectrometer fitted with a dual inlet system and a double collector for radiometry. Nitrogen isotopic composition is expressed in terms of $\delta^{15}\text{N}$ as defined by the following equation:

$$\delta^{15}\text{N} (‰) = \frac{(^{15}\text{N}/^{14}\text{N})_{\text{sample}} - (^{15}\text{N}/^{14}\text{N})_{\text{standard}}}{(^{15}\text{N}/^{14}\text{N})_{\text{standard}}} \times 1000$$

Two sets of reagent grade ammonium sulfate with $\delta^{15}\text{N}$ of -3.4 and $1.3‰$ relative to atmospheric nitrogen were used as running standards. The precision of $\delta^{15}\text{N}$ measurement was $\pm 0.3‰$.

Algal culture and decomposition experiments were carried out in the same ways as described previously (Wada and Hattori, 1978; Miyake and Wada, 1971). *Phaeodactylum tricornutum*, *Chaetoceros* sp. and *Dunaliella tertiolecta* were grown on the ASP-2 medium of Provasoli (1957) with some modification and *Cricosphaera carterae* on the CS medium of Blankly (1971) without soil extract.

Kinetic isotope effect of nitrogen was expressed in terms of the fractionation factor, α , which was defined as the ratio of the rate constant k for lighter isotope (^{14}k) to that for heavier one (^{15}k). So far as the progress of the reaction is limited less than 5%, this can be approximated by the ratio of nitrogen isotope ratio of reactant [$R_r = (^{15}\text{N}/^{14}\text{N})_r$] to that of product [$R_p = (^{15}\text{N}/^{14}\text{N})_p$]:

$$\alpha = \frac{^{14}k}{^{15}k} = \frac{R_r}{R_p} = 1 - \frac{\delta^{15}\text{N}_p}{1000}$$

18.3 RESULTS

Table 18.1 summarizes $\delta^{15}\text{N}$ values for marine plankton, suspended

Table 18.1

Summary of $\delta^{15}\text{N}$ measurements in particulate matter and in sedimentary organic matter.
 $\delta^{15}\text{N}$ values were normalized to atmospheric nitrogen (0.0‰).

Cruise and station number	Date of sampling	Location		NO_3^- (μg at N/1)	$\delta^{15}\text{N}$ (‰)	Remarks
		Lat.(N)	Long.(E)			
KT69-3	1969/3/14	Off Manazuru, Sagami Bay		3.0	6.5	Copepoda ¹⁾
KH71-3	1971/6/30	28°30'	145°00'	0.1	2.5	Miscellaneous ²⁾
	7/23	44°	154°	12.5	1.9	<i>Chaetoceros</i> sp. ²⁾
	7/24				3.4	<i>Chaetoceros</i> sp. ²⁾
	7/23				4.4	<i>Calanus plumchrus</i> ²⁾
19	7/24				5.0	<i>Calanus plumchrus</i> ²⁾
KH72-1	1972/5/16	22°05'	125°04'	—	7.8	<i>Gonostoma</i> sp. ³⁾
					4.4	<i>Acantheephyra</i> sp. ³⁾
KH73-1	1973/1/16	20°01'	140°00'	0.0	2.6	Miscellaneous ²⁾ 10 m
					2.0	20 m
					1.8	30 m
14	1973/1/30	22°00'	125°52'	0.6	2.2	Miscellaneous ²⁾
KT73-10	1973/8/4	Near Ohshima		0.0	4.1	<i>Trichodesmium</i> sp. ²⁾
KH74-3	1974/8/5	31°18'	128°35'	0.1	-2.0	<i>Trichodesmium erythraeum</i> ²⁾
	8/5				0.4	<i>Trichodesmium</i> sp., green colonies

Table 18.1 Continued

Cruise and station number	Date of sampling	Location		NO ₃ ⁻ (μg at N/1)	$\delta^{15}\text{N}$ (‰)	Remarks
		Lat. (N)	Long. (E)			
B10	8/5				-1.6	<i>Trichodesmium</i> sp., mixture of white and green colonies
B10	8/5				-1.5	<i>Trichodesmium</i> sp., brown colonies
KT78-15 A7	1978/9/9	34°00'	140°20'		6.4	Particulate-N collected with Whatman CF/C glass fiber filter
KT74-2 2	1974/1/12	Off Hayama, 78 m depths Sagami Bay			4.1 4.6 12.0 4.7 5.5	Organic matter in sediments 0-5 cm 5-10 cm 10-15 cm 15-20 cm 20-25 cm

1) Plankton specimen collected with 0.09 mm mesh NORPAC net.

2) Plankton specimen collected with 0.09 mm MTD net.

3) Collected with ORI net (0-2,000 m oblique tow).

detrital matter and bottom sediments collected on cruises of the University of Tokyo operated R.V. Tansei-maru (KT) and Hakuho-maru (KH). The values for phytoplankton in the boreal areas of the North Pacific fall within a range ever reported (Miyake and Wada, 1967; Wada et al., 1975; Wada and Hattori, 1976), and are lower than those for ambient nitrate (cf. Fig. 18.5). The occurrence of nitrogen isotope fractionation associated with nitrate assimilation by phytoplankton is suggested. Except one example (a 10–15 cm deep sediment from Sagami Bay), $\delta^{15}\text{N}$ values for zooplankton, detritus and sediments are similar. Low $\delta^{15}\text{N}$ values close to that for atmospheric nitrogen have been reported with respect to marine nitrogen fixing blue-green algae, *Trichodesmium* spp. (Wada and Hattori, 1976). This was confirmed by the present investigation. For reference, the available data on ^{15}N abundance in marine biogenic substances are depicted in Fig. 18.1.

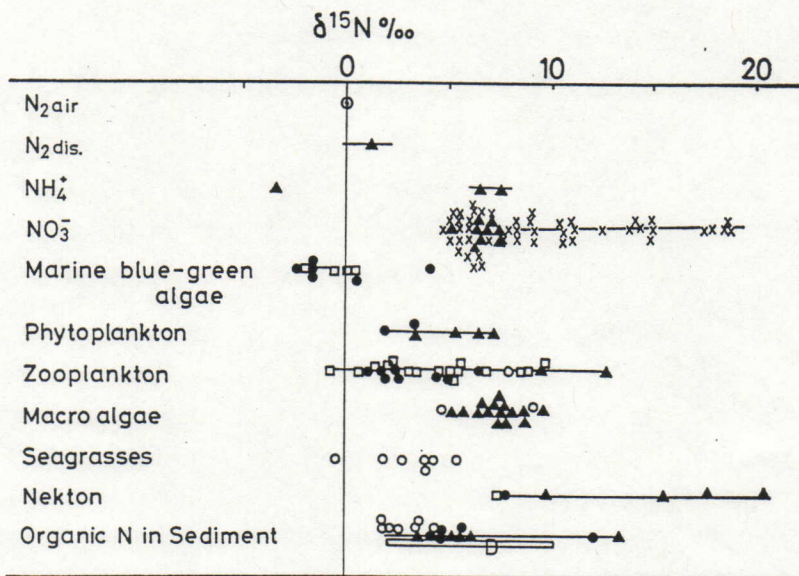


Fig. 18.1 Summary of $\delta^{15}\text{N}$ in the biogenic nitrogen bearing substances in the North Pacific Ocean. Data from the seagrass community at the coastal areas of Gulf (○) were included.

● present data, ▲ Miyake and Wada (1967), × Cline and Kaplan (1975)

□ Wada and Hattori (1976), ◻ Peters et al., (1978)

○ Wada et al. (manuscript in preparation).

Wada and Hattori (1978) have demonstrated that the isotope fractionation in nitrate assimilation by a marine diatom, *Phaeodactylum tricornutum*, is inversely correlated with its growth. This was also the case, when

Table 18.2

Nitrogen isotope fractionation in the assimilation of inorganic nitrogenous compounds by marine microalgae.

$\delta^{15}\text{N}$ values were normalized to that of substrate used as nitrogen source.

N-source (mole/l)	Incubation time (days)	Cell-number (cells/ml)	$\delta^{15}\text{N}$ (‰)	Remarks
<i>Chaetoceros</i> sp.				
NO_3^- 2.5×10^{-3}	0	4.0×10^4		Shaking culture
	17	8.7×10^5	-0.4	
<i>Phaeodactylum tricornutum</i>				
NH_4^+ 1.8×10^{-3}	0	4.3×10^4		Shaking culture
	21	3.7×10^6	-7.0	
	0	4.3×10^4		
	21	3.2×10^6	-7.9	
	52	5.2×10^6	-9.6	
NO_2^- 0.5×10^{-3}	0	2.8×10^4		Shaking culture
	15	3.5×10^6	-2.5	
	30	1.0×10^7	-1.4	
NO_3^- 1.2×10^{-2}	0	1.4×10^4		Shaking culture
	9	8.5×10^6	-9.7	
	0	1.4×10^4		
	14	4.7×10^6	-14.5	
	1.0 $\times 10^{-2}$	0	2.2×10^6	
1.5	9.4×10^6	-6.6		
4.5	2.4×10^7	-7.5		
<i>Dunaliella tertiolecta</i>				
NH_4^+ 5.0×10^{-3}	0	—		Stagnant culture
	36	7.3×10^5	-5.4	
NO_3^- 1.0×10^{-2}	0	—		Stagnant culture
	13	2.4×10^6	-12.6	
	35	4.3×10^6	-14.8	
<i>Cricosphaera carterae</i>				
NH_4^+ 5.0×10^{-3}	0	—		Stagnant culture
	31	—	-3.2	
NO_3^- 1.0×10^{-2}	0	—		Stagnant culture
	30	—	-7.4	

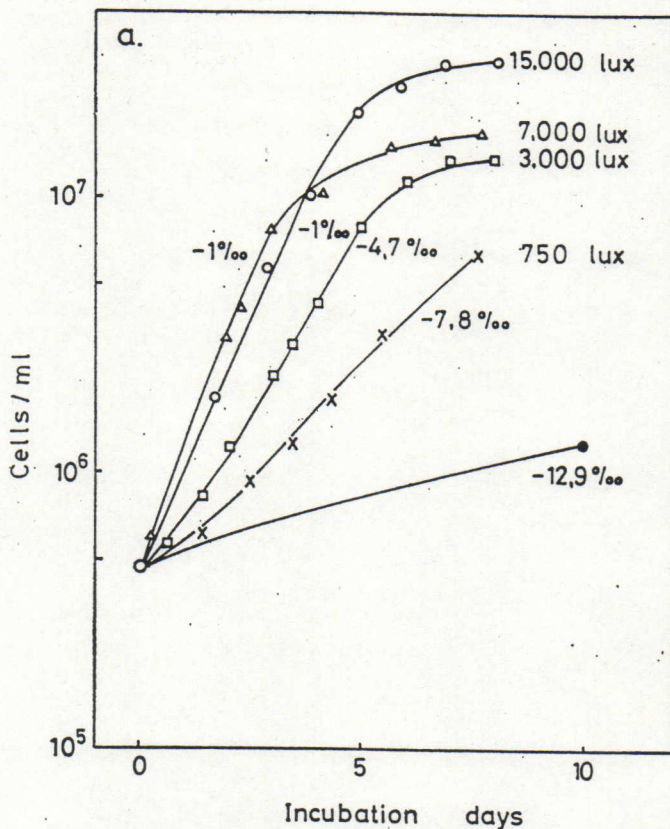


Fig. 18.2a

ammonium was used as a sole source of nitrogen (Fig. 18.2). Similar nitrogen isotope effects on assimilation of nitrate and ammonium were observed with marine planktonic algae other than diatoms (Table 18.2).

When dead cells of *Chaetoceros* sp. and *Calanus plumchrus* were suspended in filtered sea water and incubated together with natural population of bacteria collected from beach sand, particulate nitrogen rapidly decreased accompanied by increase in $\delta^{15}\text{N}$ in residual materials (Fig. 18.3 a, b). In a later period of incubation, particulate nitrogen (PON) slightly increased and $\delta^{15}\text{N}$ decreased down to 1.5‰. This can be attributed to bacterial growth using soluble nitrogenous compounds. Delwiche and Steyn (1970) showed that bacteria preferentially utilize $^{14}\text{NH}_4^+$ over $^{15}\text{NH}_4^+$. When the incubation was further extended, $\delta^{15}\text{N}$ for particulate matter increased again. The degradation of bacterial cells must be responsible for this increase. The results of the decomposition experiment with *Scenedesmus* sp. reported by

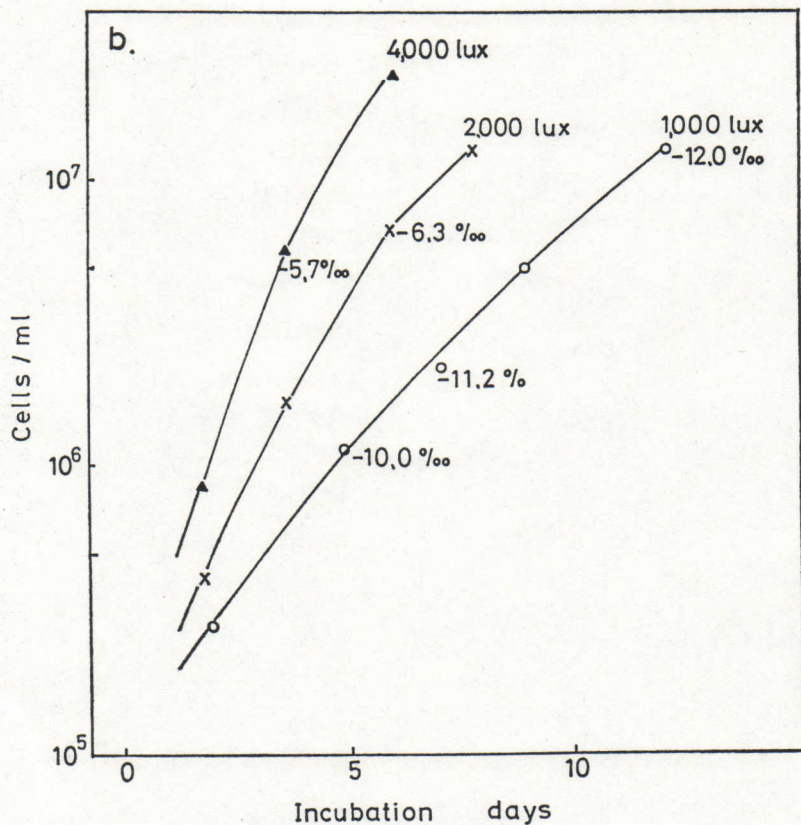


Fig. 18.2b

Fig. 18.2 Variation of $\delta^{15}\text{N}$ in *Phaeodactylum tricornerutum* during growth under different conditions ($22 \pm 2^\circ\text{C}$).

- a 1×10^{-2} mole/l of nitrate was used as nitrogen source.
 b 5×10^{-3} mole/l of ammonium was used as nitrogen source.

Miyake and Wada (1971) can be explained along this line.

When *Trichodesmium* cells were incubated, ^{15}N values of residual materials varied to some extent, but they were 1.2‰, on an average, higher than that of the starting materials (Fig. 18.3 c).

Our preliminary observation showed that apparent isotope fractionation factor of denitrification by a marine denitrifier isolated from brackish lake, Hamana, decreased from 1.012 to 1.002 when KNO_3 concentration was reduced from 2% to 0.2% and concentrations of both peptone (5 g/l) and yeast extract (1 g/l) were kept constant (Table 18.3).

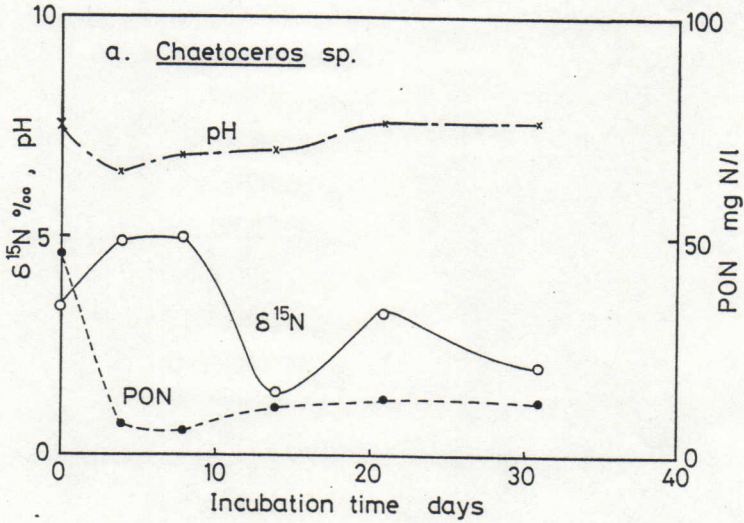


Fig. 18.3a

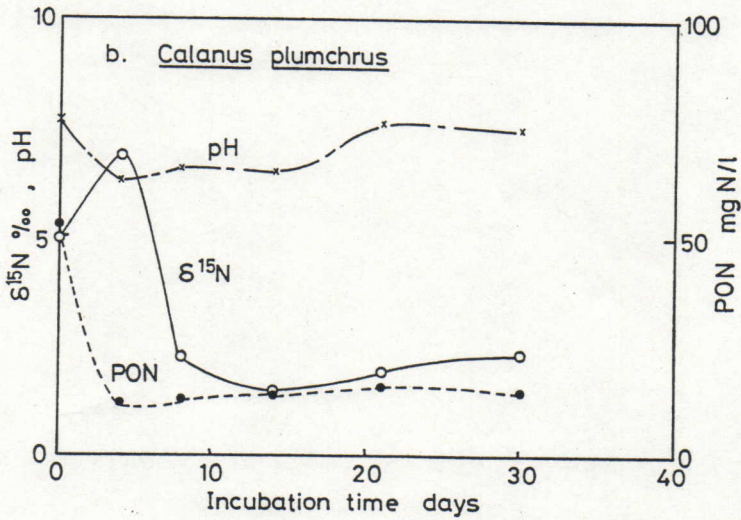


Fig. 18.3b

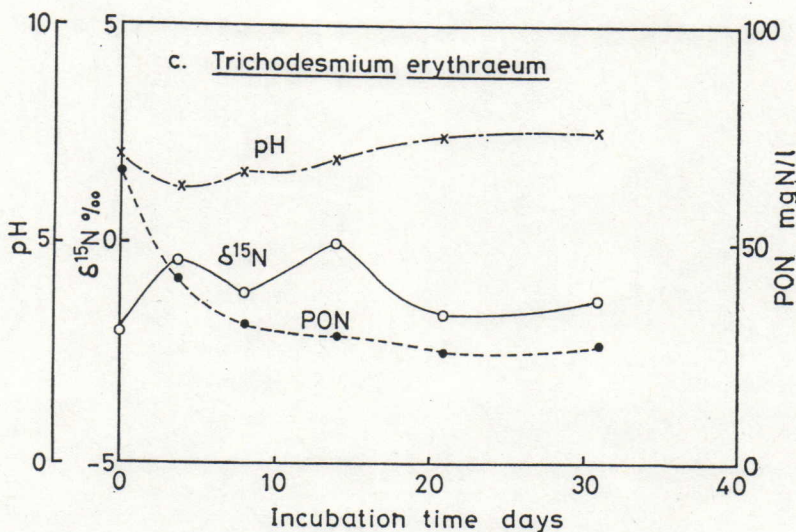


Fig. 18.3c

- Fig. 18.3 Variation of $\delta^{15}\text{N}$ during the decomposition of natural plankton samples. Indicated amount of plankton samples were placed into 7 l of the surface seawater collected from Kuroshio area near Izu-Islands. Before incubation seawater was filtered with glass fiber filter (Whatman GF/C) and suspension of seawater with the beach sand collected at Shikinejima Island was put as a microbial source. Incubation was carried out in 10 l of polyethylene bottles at $19 \pm 1^\circ\text{C}$ with air bubbling one hour per day. At intervals, 500 to 1,000 ml of samples were withdrawn and the residual materials were collected on the glass fiber filter (GF/C). $\delta^{15}\text{N}$ values were normalized to atmospheric nitrogen (0.0‰).
- a *Chaetoceros* sp., collected at Stn. 19 of KH71-3 cruise.
 - b *Calanus plumchrus*, collected at Stn. 19 of KH71-3 cruise.
 - c *Trichodesmium erythraeum*, collected at Stn. B10 of KH74-3 cruise.

18.4 DISCUSSION

18.4.1 THEORETICAL CONSIDERATION ON BIOCHEMICAL NITROGEN ISOTOPE EFFECTS

A large isotope fractionation in chemical reactions takes place when a bond to the isotopic atom is formed or broken in or before a slow step of reaction sequence. In microbial systems, the extent of kinetic isotope frac-

Table 18.3
Nitrogen isotope fractionation in various biochemical processes.

Reaction	Apparent isotope fractionation factor	Remarks
Assimilation		
$\text{NO}_3^- \rightarrow \text{Organic-N}$	1.000 - 1.019	<i>Phaeodactylum tricornutum</i> Wada and Hattori, 1978
$\text{NO}_2^- \rightarrow \text{Organic-N}$	1.000 - 1.0025	<i>Chaetoceros</i> sp. Present date
$\text{NH}_4^+ \rightarrow \text{Organic-N}$	1.000 - 1.010	<i>Dunaliella tertiolecta</i>
		<i>Cricosphaera carterae</i>
Nitrification		
$\text{NH}_4^+ \rightarrow \text{NO}_3^-$	1.000 - 1.021	Marine nitrifier Miyake and Wada, 1971
Nitrate reduction		
$\text{NO}_3^- \rightarrow \text{NO}_2^-$	1.000 - 1.029	<i>Serratia marinorubra</i> Miyazaki, 1971
	1.011 - 1.017	Natural soil Blackmer and Bremner, 1977
	1.0065 - 1.019	Natural soil Chien et al., 1977
Denitrification		
$\text{NO}_3^- \rightarrow \text{N}_2$	1.000 - 1.021	Marine denitrifier Miyake and Wada, 1971
	1.04	The eastern tropical Cline and Kaplan, 1975
	1.014 - 1.023	North Pacific Ocean
	1.020	Natural soil
	1.017	<i>Pseudomonas</i> sp. Blackmer and Bremner, 1977
	1.002 (0.2% KNO_3)	<i>Pseudomonas</i> sp. Wellman et al., 1968
	1.012 (2.0% KNO_3)	Marine denitrifier Delwiche and Steyn, 1970
		Koike and Wada, unpublished
N_2 fixation		
$\text{N}_2 \rightarrow \text{NH}_4^+$	0.991 - 1.004	<i>Azotobacter</i> spp. Hoering and Ford, 1960
	0.999 - 1.008	<i>Anabaena cylindrica</i> Minagawa and Wada, 1978

Table 18.3 Continued

Reaction	Apparent isotope fractionation factor	Remarks
Decomposition		
Organic-N \rightarrow Dissolved organic-N	0.999 - 1.002 1.000 - 1.002	Miyake and Wada, 1971
	0.998 - 1.002	<i>Scenedesmus</i> sp.
	0.995 - 1.002	<i>Trichodesmium erythraeum</i>
	0.998 - 1.006	<i>Chaetoceros</i> sp.
	mean 1.000	<i>Calanus plumchrus</i>
Organic-N \rightarrow NO ₃ ⁻	0.991 - 1.000	Natural soil
Amino acid synthesis		Rennie et al., 1976
		Rat for non-essential amino acid synthesis
		Gaebler et al., 1966

tionation of elements such as carbon, nitrogen and sulfur is likewise primarily regulated by an enzymic reaction step in which a bond to the isotopic atom is formed or broken (Harrison and Thode, 1953; Park and Epstein, 1960; Wada and Hattori, 1978).

In this treatise, we mainly deal with the following processes: (i) assimilation of nitrate and ammonium by phytoplankton, (ii) reduction of nitrate by bacteria, (iii) reduction of dinitrogen, or nitrogen fixation, by planktonic blue-green algae, and (iv) decomposition of organic nitrogenous substances by bacteria. In the assimilation of nitrate, nitrate is first reduced and ammonium formed is in turn incorporated to produce amino acids and other organic nitrogenous compounds. The first three processes (i, ii and iii) are closely conjugated with electron transport system of respiration or photosynthesis, and, in many cases, rates of overall reactions are controlled by supply of reductant (NADH or NADPH) or energy (ATP) coupled to the electron transport system. In such situations, the following reaction scheme can be commonly applied:



transport across cell membrane



where k 's refer to rate constants, S_i and S_o , substrate or reactant (NH_4^+ , NO_3^- or N_2) in and out of cells, E , free form of key enzyme, ES , enzyme-substrate complex, and P , reaction product. k_2 is a function of electron flux through electron transport system or a function of ATP flux.

In a steady state, we obtain

$$-\frac{d[S_o]}{dt} = k_o^+[S_o] - k_o^-[S_i] \quad (4)$$

$$\frac{d[S_i]}{dt} = \frac{d[E]}{dt} = \frac{d[ES]}{dt} = 0 \quad (5)$$

and

$$\frac{d[P]}{dt} = k_2[ES] \quad (6)$$