Nitrogen Recycling Mechanism within the Thallus of *Laminaria japonica* (Phaeophyceae) under the Nitrogen Limitation*

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The nitrogen status in the thallus of *Laminaria japonica* Areschoug under nitrogen limitation is divided to two phases, slight and serious N-deficient phases. This is done by judging from the quantitative storage levels of the soluble nitrogen in the tissues. The slight N-deficient phase is represented by the consumption of soluble nitrogen compounds in the storage pools. After the exhaustion of the nitrogen storage pools, the nitrogen status changes from a slight N-deficient phase to a serious N-deficient phase. Then catabolism was progressed and NH_4^+ was accumulated as a catabolite under the serious N-deficient phase. The treatment of L-methionine-DL-sulfoximine, an inhibitor of glutamine synthetase (GS), resulted in a considerably higher NH_4^+ accumulation within the tissue segments rather than non-treatment. This fact suggests the presence of a nitrogen recycling mechanism that the NH_4^+ produced from catabolic processes is actively re-assimilated by GS in the apical tissues. In the natural environment, the blade length was negatively correlated to an NH_4^+ content in apical tissues along the thallus during the serious N-deficient phase. This suggests that the nitrogen recycling mechanism may be an important role for survival of the seaweed in the period of N-deficiency in seawater. In addition, this mechanism may be closely related to the erosion of apical tissues that is called "Suegare" in Japan. Furthermore, the inducing mechanism of "Suegare" is also discussed considering with the nitrogen recycling mechanism in the thallus under the serious N-deficiency.

Key words: *Laminaria*, nitrogen, assimilation, catabolism, senescence

Since the relationship between the growth of Laminariales and the ambient inorganic nitrogen concentration was reported,¹⁾ several investigations have been carried out on the growth limitation accompanying inorganic nitrogen deficiency in the summer season.^{2–6)} Under the nitrogen limitation, it was suggested that the growth was maintained by the consumption of the nitrogen storage pools, such as NO_3^- and amino acids.^{2–6)} However, there is no direct evidence for the utilization of these pools within the tissues under the nitrogen deficiency. The storage pools are exhausted after about one month.⁷⁾ Therefore the amount in the pools does not seem to satisfy the nitrogen demand of the thallus under the nitrogen deficiency from late spring until the renewal period. Probably, the other source contributes to the nitrogen demand under the nitrogen deficiency. From this point of view, comprehensive investigations on the nitrogen metabolism under the nitrogen limitation is helpful to understand senescence of the apical part of thallus in summer. The erosion of apical tissues, so-called "Suegare" in Japanese, is one of the important subjects for the artificial management of the cultivation in summer months.

The object of the present study is to get direct evidence for the utilization of nitrogen storage pools under nitrogen limitation. We propose the nitrogen acquirement mechanism under the nitrogen limitation, and discuss the

significance of the proposed mechanism for the growth and survival of the thallus in the natural environment.

Materials and Methods

The test samples, sporophytes of *Laminaria japonica* Areschoug were collected in Minamikayabe, Hokkaido from January to August, 1991. Laboratory experiments were carried out using first-year plants of sporophyte in January and April, 1991. These samples were carried to the laboratory keeping them in plastic bags and maintained for one day in a tank filled with nitrogen poor seawater less than $1\ \mu\text{M}$ as total inorganic nitrogen at 10°C under the 2000 lux (L:D = 16:8). Before the experiments, tissue segments ($5 \times 5\ \text{cm}$) were cut at several portions along the thallus. Microorganisms on the surface of tissue segments were repeatedly removed by soft brushing, wiping and washing with nitrogen poor seawater. After the segments were pre-cultured for 24 h in the same condition as mentioned above, mucus from segments was removed by wiping with sterilized gauze and rinsed with filtered seawater before the experimental cultures. Seawater used in pre-culture and experimental culture was filtered with a glass fiber filter (Whatman GF/F) and autoclaved at 120°C for 20 min. Pre-culture was conducted in 100–500 ml glass bottle filled with nitrogen poor seawater under the agitating (120 rpm) by using a magnetic stirrer. The experimental culture was carried out in the same condition to the pre-culture as described above.

In the first experiment, the used tissue segments were collected from the central portions along the thalli grown to 50 cm in January and 4 m in April, respectively. Some segments were cultured for 10–12 days under the nitrogen limitation less than $1\ \mu\text{M}$ of inorganic nitrogen. These segments were periodically collected and used for the analysis of internal nitrogen contents. To investigate the utilization of storage pools under the nitrogen

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limitation directly, the segments were incubated in 500 ml of seawater and enriched with $60 \mu\text{M}$ - ^{15}N - NH_4Cl (99.0 atom%) which the uptake and assimilation activities were saturated. After the 3 h, the half of segments were supplied to ^{15}N analysis. The other half of the segments were moved into ^{15}N -free medium and recultured for 3 h more under the nitrogen limitation. Then these segments were taken up and used for ^{15}N analysis. The ^{15}N contents in insoluble and soluble nitrogen pools were determined after the extraction by 10 ml of 80% chilled ethanol (4°C) for 24 h. The ^{15}N contents in soluble organic, $\text{NO}_2^- + \text{NO}_3^-$ and NH_4^+ fractions were separated and determined by the method described by Mizuta and Maita.⁸⁾

In the second experiment, the effects of *L*-methionine-*DL*-sulfoximine (MS), an inhibitor of glutamine synthetase, to the NH_4^+ content within the tissue segments were examined. Then the change of internal NH_4^+ was compared between the MS-treatment and non-treatment. The MS was added to 5 mM concentration⁹⁾ in each nitrogen poor seawater. After 5 days, the NH_4^+ content was measured. The influence of microorganisms on the surface of the segments was examined using antibiotics, Penicillin-G (800 mg/ml) and Streptomycin (200 mg/ml).^{10,11)} To understand the differences among the tissue segments along the thallus, four tissue segments were incubated for 5 days. These segments were collected from 0–5, 10–15, 30–35, 40–50 cm portions of the stipe-blade transition along the thallus grown to 50 cm in January. The several nitrogen contents (NH_4^+ , $\text{NO}_2^- + \text{NO}_3^-$, and soluble organic nitrogen) were measured after the experimental culture. The MS-treatment was simultaneously done on the same condition and compared to non-treatment specimens.

To understand the nitrogen metabolism of the thallus under the natural nitrogen limitation, sporophytes were collected once a month from January to August. After the blade length was measured, the apical tissue was submitted to the analysis of inorganic nitrogen contents (NH_4^+ , $\text{NO}_2^- + \text{NO}_3^-$).

The content of ethanol insoluble nitrogen was measured by the Kjeldahl method after the vacuum dryness in a desiccator. Ethanol soluble fraction was filtered with a grass fiber filter (Whatman GF/F) and the filtrate was submitted to the analysis of total soluble nitrogen and inorganic nitrogen contents (NH_4^+ , $\text{NO}_2^- + \text{NO}_3^-$). The soluble nitrogen content was measured using a nitrogen analyzer (Sumigraph N-200, Sumitomo Chemical Industry Co.). Inorganic nitrogen contents were analyzed using an autoanalyzer (Technicon AutoAnalyzer II) according to Strickland and Parsons.¹²⁾ Amino acids in the soluble nitrogen fraction were determined by the post column fluorescence derivation with *o*-phthalaldehyde using HPLC (KL-5, Shimadzu-Seisakusho Co.). Isotopic enrichment (^{15}N atom%) was determined using a ^{15}N analyzer (NOI-5, Statron) after Kjeldahl digestion as described by Fiedler and Proksch.¹³⁾ The ^{15}N content in each nitrogen fraction was calculated by the equation:

$$N_{(\text{allocation})} = \frac{\text{Excess atom\%}}{R} \cdot \frac{N_p}{W}$$

where, $N_{(\text{allocation})}$ denotes ^{15}N allocated into each fraction ($\mu\text{g N} \cdot \text{g}^{-1}$ wet wt); excess atom%, (final atom% ^{15}N) – (measured natural abundance ^{15}N); W , wet weight of sample segments (g); R , ^{15}N enrichment of dissolved nutrients (99.0 atom%); N_p , nitrogen content in each nitrogen fraction (μg), respectively. The nitrogen content in the NH_4^+ fraction was calculated by subtracting the ^{15}N in the organic nitrogen and $\text{NO}_2^- + \text{NO}_3^-$ fractions from that in the soluble nitrogen pool.

Results and Discussion

In the first laboratory experiment, the changes of several nitrogen contents within the tissue segments cultured under nitrogen limitation for *L. japonica* collected in April are shown in Fig. 1. Insoluble-N content in the tissue maintained for the initial 4 days, and gradually decreased after that. Soluble-N content dropped sharply for the initial 2 days and gradually decreased after that. From the changes of these nitrogen contents, this experimental period was divided into two phases, initial phase continued the first 4 days and the last 8 days. The first phase was defined as a slight N-deficient period when soluble nitrogen content was enough to the nitrogen demand for several metabolism. However, the last phase was characterized by the gradual

Fig. 1. The changes of insoluble and soluble nitrogen contents (A) and three soluble nitrogen pools (B) within the tissue segments of *Laminaria japonica*.

The segments were collected from the central portion along the thallus in April. The vertical bars indicate the standard deviations for 4–5 samples.

Table 1. Changes of major amino acids within the tissue segments of *Laminaria japonica* under the nitrogen limitation ($\mu\text{g} \cdot \text{g}^{-1}$ dry wt)

Time (days)	Asp + Asn	Serine	Glu + Gln	Proline	Alanine	Lysine
0	0.77 (0.20)	0.02 (0.03)	3.83 (0.88)	0.21 (0.47)	2.39 (0.74)	0.20 (0.11)
2	0.61 (0.27)	0.02 (0.04)	2.67 (1.29)	n.d.*	1.64 (0.89)	0.28 (0.08)
8	0.14 (0.02)	0.04 (0.04)	0.08 (0.09)	n.d.	n.d.	0.22 (0.17)
12	0.17 (0.02)	0.03 (0.00)	0.12 (0.05)	n.d.	n.d.	0.19 (0.01)

* n.d.: not detected.

The used samples were collected from the central portion along the thallus in April. The number in parenthesis indicates the standard deviation for 2–5 samples.

decrease of insoluble-N content. This indicates that the insoluble-N is decomposed and consumed to satisfy the nitrogen demand of the tissues after the soluble-N is almost exhausted. Therefore, this phase was defined as a serious N-deficient period. However, the large amount of the nitrogen produced by the decomposition of insoluble-N seemed to be excreted to the medium because the decomposed nitrogen did not exist in the tissue as soluble nitrogen. The change of total soluble-N content was brought by the decrease of amino acid content. Ammonium content gradually decreased for the slight N-deficient phase and was maintained at a low level for the serious N-deficient phase. Nitrite + nitrate was not detectable after 2 days. Although the contents of amino acid after 4 and 6 days were not measured, the content of amino acids seems to have disappeared before 8 days, judging from the change of soluble nitrogen content. The composition of amino acids changed remarkably from the slight N-deficient to the serious N-deficient period (Table 1). Glutamine + glutamic acid, asparagine + aspartic acid and alanine decreased remarkably with time. Proline and alanine disappeared in the serious N-deficient period reflecting with

Table 2. Changes of ^{15}N in each fraction of tissue segments of *Laminaria japonica* ($n=2$) during 3 h incubation in ^{15}N -free seawater after $^{15}\text{NH}_4\text{Cl}$ -uptake incubation for 3 h

Sample no.	Incubation time in ^{15}N -free SW (hours)	Insoluble pool	Soluble pool	Soluble fractions		
				org. N	$\text{NO}_2^- + \text{NO}_3^-$	NH_4^+
A	0	35.8	64.2	36.6	1.3	26.3
	3	40.2	59.8	33.5	2.1	24.3
B	0	28.6	71.4	44.3	3.8	23.3
	3	42.9	57.1	36.9	2.3	17.9

The amounts of ^{15}N in tissue segments are expressed as percentages to the total uptake ^{15}N . The used samples were collected from the central portion along the thallus in April.

Fig. 2. The effects of L-methionine-DL-sulfoximine treatment to NH_4^+ content within the tissue segments ($n=4-5$) of *Laminaria japonica*. The used samples were collected from the central portion along the thalli grown to 50 cm in January (A) and 4 m in April (B). The vertical bars indicate the standard deviations for 4-5 samples.

relative decrease of amino acid pool. Serine and lysine were relatively constant during this experiment. These results indicate that the main component of useful amino acid pool is composed of glutamine + glutamic acid, asparagine + aspartic acid and alanine, as reported for forced *L. japonica*.⁵⁾

Table 2 shows the changes of ^{15}N content distributed in each nitrogen fraction of tissue segments under the slight N-deficient period after ^{15}N -uptake incubation. The ^{15}N content in the insoluble-N pool of tissue segments increased after 3 h under a ^{15}N -free condition. On the other hand, the ^{15}N content in the soluble-N pool decreased. The ^{15}N decrease was brought by the allocation of ^{15}N from the soluble organic-N and NH_4^+ pools to the insoluble pool. Thus, the utilization of the soluble-N storage pool is directly indicated in the slight N-deficient condition. Although it is known that the NO_3^- is a major pool for *L. japonica*,⁶⁾ the NO_3^- accumulation as a storage pool was not observed in this study. The NO_3^- pool was probably already exhausted before the experiments.

The effects of MS-treatment to NH_4^+ content in the tissue segments are shown in Fig. 2. The NH_4^+ content in control groups was relatively constant through the experiment. However, the NH_4^+ content increased by the MS-treatment. The content after 10 or 12 days culture was about

Fig. 3. The differences of NH_4^+ accumulation by L-methionine-DL-sulfoximine treatment among the tissue segments along the thallus of *Laminaria japonica* in January.

The mean NH_4^+ content was expressed as the value for 5 days incubation under the nitrogen limitation in the figure. Vertical bars indicate the standard deviations for 4 samples. Arrows indicate the NH_4^+ content at the initial start of the experiment.

four times higher than the initial content in the thalli in January and April. This shows that the NH_4^+ accumulates due to the inhibition of glutamine synthetase (GS) for NH_4^+ assimilation. The difference of NH_4^+ content between MS-treatment and non-treatment means the amount of re-assimilated NH_4^+ by GS. It has been suggested that the accumulated NH_4^+ is derived from the catabolic processes because NH_4^+ uptake and NO_2^- reduction are negligibly small. The NH_4^+ may occur through the photorespiratory process¹⁴⁾ and the decomposition of organic nitrogen compounds, such as guanine¹⁵⁾ and arginine¹⁶⁾ in higher plants and unicellular green algae. Therefore, the re-assimilated NH_4^+ (%) was estimated by the equation:

$$\text{Re-assimilated } \text{NH}_4^+ (\%) = \frac{\text{Accumulated } \text{NH}_4^+ - \text{Reduced } N}{\text{Decreased insoluble } N \text{ \& soluble organic } N} \times 100$$

where, Accumulated NH_4^+ denotes the NH_4^+ amounts accumulated by MS-treatments; Reduced N , the reduced nitrogen amounts of $\text{NO}_2^- + \text{NO}_3^-$ in non-treatments; Decreased insoluble & soluble organic-N, the decrease of insoluble and soluble organic nitrogen contents for non-treatment. When the re-assimilated NH_4^+ was estimated using the NH_4^+ contents after 10-12 days incubation, about 35 and 27% to NH_4^+ produced by catabolic processes were re-assimilated by GS within the thalli. The residue seems to be excreted to the ambient seawater. The NH_4^+ accumulation by MS-treatment is not influenced by microorganisms because NH_4^+ accumulation was only brought down by about 18% by the antibiotics treatment.

The differences of MS-treatment to NH_4^+ content among the tissue segments along the thallus is shown in Fig. 3. Generally, NH_4^+ content is significantly higher (t -test; <0.05) in apical part (ca. $2 \mu\text{g N} \cdot \text{mg}^{-1}$ dry wt) than basal tissues ($0.9 \mu\text{g N} \cdot \text{mg}^{-1}$ dry wt). In the two basal tissues, the change of NH_4^+ content was not recognized in MS- and non-treatment after 5 days. On the other hand, the NH_4^+

content in apical tissues increased and reached to about three times higher (ca. $6\text{--}7\ \mu\text{g N}\cdot\text{mg}^{-1}$ dry wt) than that of the initial content. The result is caused by the more active catabolism than the assimilation. The active NH_4^+ accumulation by MS-treatment was observed in apical parts. This shows that the catabolic activities are more active in apical tissues than the basal meristematic tissues. It is also suggested that the re-assimilation activity of NH_4^+ by GS increased towards apical mature tissues. In relation to the sporophytes of *L. digitata*, GS activity increased to mature tissues along the thallus.¹⁷⁾ The GS pattern¹⁸⁾ based on NH_4^+ uptake was also observed for *L. japonica*. These reports support that the N-recycling mechanism is more active in mature tissues.

In the natural nitrogen deficient condition, the relationships between the blade length and the inorganic-N contents (NH_4^+ , $\text{NO}_2^- + \text{NO}_3^-$) within the apical tissue is shown in Fig. 4. The nitrogen environment and nitrogen status of the

Fig. 4. The relationship between the growth and the inorganic nitrogen contents for *Laminaria japonica* cultivated under natural conditions off Minamikayabe, Hokkaido.

The left side shows the relationship between the blade length and $\text{NO}_2^- + \text{NO}_3^-$ content in the slight N-deficient period. The right side shows the relationship between the blade length and NH_4^+ content in the serious N-deficient period. Each nitrogen content was measured using the apical tissues along the thallus.

thallus of cultivated *L. japonica* under natural conditions was reported in detail by Mizuta et al.⁷⁾ In the slight N-deficient condition from March to May,⁷⁾ the blade length is significantly ($p < 0.01$) correlated to the $\text{NO}_2^- + \text{NO}_3^-$ content. This result supports the hypothesis that the $\text{NO}_2^- + \text{NO}_3^-$ largely contributed to the rapid growth.²⁾ On the other hand, the blade length significantly ($p < 0.01$) shows the negative correlation to NH_4^+ content under the serious N-deficiency from April to August. This suggests that the "Suegare" is closely connected to the NH_4^+ accumulation and the decomposition of insoluble organic nitrogen. The "Suegare" phenomenon was generally said to occur when the water temperature increased above 22°C .¹⁹⁾ However, this phenomenon was recognized even if the ambient seawater temperature was below 20°C .⁷⁾ It was also reported that "Suegare" was also observed in younger sporophyte.²⁰⁾ Therefore, it seems that the temperature is not a main factor that induces to "Suegare" in summer months. It is generally known that nitrogen limitations lead to the decrease of chloroplast volume,²¹⁾ pigments such as chlorophyll,²²⁻²⁵⁾ nitrogen compounds such as nucleic acids²⁶⁾ and photosynthetic activity.²¹⁾ These observations appear to be consistent with the characteristics of "Suegare" tissues. Moreover, it has been suggested that the manure nutrients containing nitrogen compounds may prevent the decrease of blade length.¹⁸⁾ From these observations, the schematic proposal model for nitrogen recycling mechanism and "Suegare" phenomenon under the severe N-deficiency is shown in Fig. 5. The nitrogen starvation acts as a trigger of the catabolism induction and then leads to the N-recycling mechanism. The N-recycling mechanism is composed of catabolism and assimilation processes. When the catabolism is prominent when compared with assimilation, the large amount of NH_4^+ is accumulated in tissues. The amount of catabolite may influence the physiological processes within the tissues. Particularly, NH_4^+ treatment at high concentration is known to be toxic^{27,28)} on macroalgal growth. Therefore, the high accumulation of NH_4^+ may be inhibitory to several physiological processes and then the tissue cells result in death. At the same time, temperature may act as the promoter of catabolic activities. These processes result in

Fig. 5. The Schematic model showing the nitrogen recycling mechanism and "Suegare" phenomenon in *Laminaria japonica* under serious nitrogen deficiency.

the "Suegare" phenomenon. Moreover, the re-assimilated compounds in apical tissues are probably transported to basal tissues by the long-distance transport mechanism²⁹⁾ and incorporate to macromolecules for survival.

In summary, it is directly indicated that the stored soluble nitrogen such as amino acid is consumed after entering the N-deficient condition. When the N-storage pools are exhausted, the insoluble nitrogen may be largely contributed to satisfy the nitrogen demand of tissues by the catabolic processes. In addition, the thallus of *L. japonica* have the higher catabolic activity in apical parts. It was estimated that about 30% of the decomposed nitrogen is re-assimilated by GS through NH_4^+ . This nitrogen recycling mechanism may contribute to the growth and survival of whole plants in kelp beds under the nitrogen limitation. Furthermore, the activation of catabolic processes may result in severe erosion of apical tissues in summer months.

References

- 1) W. A. P. Black and E. T. Dewar: Correlation of some of the physical and chemical properties of the sea with the chemical constitution of the algae. *J. Mar. Biol. Ass. U.K.*, **28**, 673–699 (1949).
- 2) A. R. O. Chapman and J. S. Craigie: Seasonal growth in *Laminaria longicruris*: relationships with dissolved inorganic nutrients and internal reserves of nitrogen. *Mar. Biol.*, **40**, 197–205 (1977).
- 3) S. O. Asare and M. M. Harlin: Seasonal fluctuations in tissue nitrogen for five species of perennial macroalgae in Phode Island Sound. *J. Phycol.*, **19**, 254–257 (1983).
- 4) I. German, L. D. Druhl, and U. Hoeger: Seasonal variation in total and soluble tissue nitrogen of *Pleurophycus gardneri* (Phaeophyceae: Laminariales) in relation to environmental nitrate. *Mar. Biol.*, **96**, 413–423 (1987).
- 5) K. Oishi and N. Kunisaki: Free amino acid composition of acceleratedly cultured Makombu, *Laminaria japonica*, at different growing stages. *Bull. Japan. Soc. Sci. Fish.*, **36**, 1181–1185 (1970) (in Japanese).
- 6) M. Yokoyama and Y. Sanbonsuga: On a seasonal variation in nitrate-N content of *Laminaria* during forced cultivation. *Bull. Hokkaido Reg. Fish. Res. Lab.*, **44**, 67–72 (1979) (in Japanese).
- 7) H. Mizuta, Y. Maita, and M. Yanada: Seasonal changes of nitrogen metabolism in the sporophyte of *Laminaria japonica* (Phaeophyceae). *Nippon Suisan Gakkaishi*, **58**, 2345–2350 (1992).
- 8) H. Mizuta and Y. Maita: Effects of nitrate supply on ammonium assimilations in the blade of *Laminaria japonica* (Phaeophyceae). *Bull. Fac. Fish. Hokkaido Univ.*, **42**, 107–114 (1991).
- 9) P. G. Haxen and O. M. A. Lewis: Nitrate assimilation in the marine kelp, *Macrocystis angustifolia* (Laminaria: Phaeophyta). *Bot. Mar.*, **24**, 631–635 (1981).
- 10) R. S. Quatrano: Phizoid formation in *Fucus* zygotes: dependence on protein and ribonucleic acid syntheses. *Science*, **162**, 468–470 (1968).
- 11) J. McLachlan, L. C.-M. Chen, and T. Edelstein: The culture of four species of *Fucus* under laboratory condition. *Can. J. Bot.*, **49**, 1463–1469 (1971).
- 12) J. D. H. Strickland and T. R. Parsons: A practical handbook of seawater analysis. *Fish. Res. Bd. Can. Bull.*, **167**, 1–310 (1972).
- 13) R. Fiedler and G. Proksch: The determination of nitrogen-15 by emission and mass spectrometry in biochemical analysis: A review. *Anal. Chem. Acta*, **78**, 1–62 (1975).
- 14) A. J. Key, I. F. Bird, M. J. Cornelius, P. J. Lea, R. M. Wallsgrove, and B. J. Milfin: Photorespiratory nitrogen cycle. *Nature (London)*, **275**, 741–743 (1978).
- 15) R. Pettersen: Control by ammonium of intercompartmental guanine transport in *Chlorella*. *Z. Pflanzenphysiol. Bd.*, **76**, 213–223 (1975).
- 16) W. P. Wheeler and G. C. Stephens: Metabolic segregation of intracellular free amino acids in *Platymonas* (Chlorophyta). *J. Phycol.*, **13**, 193–197 (1977).
- 17) I. R. Davison and W. D. P. Stewart: Studies on nitrate reductase activity in *Laminaria digitata* (Huds.) Lamour. I. Longitudinal and transverse profiles of nitrate reductase activity within the thallus. *J. Exp. Mar. Biol. Ecol.*, **74**, 201–210 (1984).
- 18) H. Mizuta: The physiological and ecological study for nitrogen acquiring mechanism on the sporophyte of *Laminaria japonica*. Doctoral thesis, University of Hokkaido, 1992, p. 103 (in Japanese).
- 19) H. Tokuda: in "The Resources and Cultivation of Seaweeds" (ed. by H. Tokuda, M. Ono, and H. Ogawa), Midorishobou, Tokyo, 1989, pp. 35–59 (in Japanese).
- 20) S. Kawashima: Taxonomic and distributional remarks on the laminariaceous algae (Laminariales, Phaeophyceae) of Japan—6. *Aquabiology*, **50**, 190–193 (1987) (in Japanese).
- 21) I. Levy and E. Gantt: Development of photosynthetic activity in *Porphyridium purpureum* (Rhodophyta) following nitrogen starvation. *J. Phycol.*, **26**, 62–68 (1990).
- 22) A. R. O. Chapman, J. W. Markham, and K. Lüning: Effects of nitrate concentration on the growth and physiology of *Laminaria saccharina* (Phaeophyta) in culture. *J. Phycol.*, **14**, 195–198 (1978).
- 23) K. T. Bird, C. Habig, and T. DeBusk: Nitrogen allocation and storage patterns in *Gracilaria tikvahiae* (Rhodophyta). *J. Phycol.*, **18**, 344–348 (1982).
- 24) A. Lingnell and M. Pedersen: Nitrogen metabolism in *Gracilaria secundata* (Harv.). *Hydrobiologia*, **151/152**, 431–441 (1987).
- 25) B. E. Lapointe: The effects of light and nitrogen on growth, pigment content and biochemical composition of *Gracilaria folifera* V. Angustissima (Gigartinales, Rhodophyta). *J. Phycol.*, **17**, 90–95 (1981).
- 26) J. A. DeBoer: Effects of nitrogen enrichment on growth rate and phycocolloid content in *Gracilaria folifera* and *Neoagardhiella baileyi* (Florideophycidae). *Proc. Int. Seaweed Symp.*, **9**, 263–271 (1979).
- 27) T. Waite and R. Mitchell: The effect of nutrient fertilization on the benthic alga *Ulva lactuca*. *Bot. Mar.*, **15**, 151–156 (1972).
- 28) H. S. Parker: Effects of simulated current on the growth rate and nitrogen metabolism of *Gracilaria tikvahiae* (Rhodophyta). *Mar. Biol.*, **69**, 137–147 (1982).
- 29) K. Schmitz: Translocation, in "The Biology of Seaweeds" (ed. by C. S. Lobban and M. J. Wynne), Blackwell Scientific Publication, Oxford, 1981, pp. 534–558.