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Urea Decompotion during Phytoplankton Growth in Enriched Water from Lake Biwa

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Abstract

Decomposition rate of urea and assimilation rate of urea nitrogen by phytoplankton, after enrichment of nitrogen and phosphorus in surface water collected from eutrophic area of Lake Biwa, were investigated. The rate of urea carbon incorporation into the particulate matter and urea decomposition (as sum of carbon incorporation rate and CO_2 liberation rate) corresponded to chlorophyll-a amount and photosynthetic rate. This suggests that urea in water is decomposed by phytoplankton associated with photosynthesis. A close relationship between the decompositon rate of urea carbon and the assimilation rate of urea nitrogen was observed. The decompositon of urea seems to be equivalent to the utilization of urea nitrogen by phytoplankton.

Introduction

In recent studies, some informations indicate that urea is utilized as a nitrogen source for phytoplankton, as well as ammonia and nitrate (MCCARTHY, 1972[1] ; MCCARTHY and EPPLEY, 1972[2] ; EPPLEY *et al.*, 1973[3] ; MCCARTHY *et al.*, 1977[4] ; MITAMURA and MATSUMOTO, 1981[5]) , and is decomposed by phytoplankton rater than bacteria (CARPENTER *et al.*, 1972a[6] ; MITAMURA and SAIJO, 1975[7] , 1976[8] ; WEBB and HAAS, 1976[9]) in the sea and freshwater lakes. Urea is a changeable compound in dissolved organic nitrogen compound in relation to the biological activity, as indicated by MITAMURA and SAIJO (1981)[10] from the seasonal observation of dissolved organic matters in Lake Biwa and by ISHIDA and MITAMURA (1986)[11] in a small eutrophic pond. They reported that urea seemed to play a important role in nitrogen cycle in natural waters.

MITAMURA and SAIJO (1986)[12] measured the decomposition of urea and the assimilation of urea nitrogen by natural phytoplankton in Lake Biwa. They suggested that the urea was mainly decomposed with the urea nitrogen assimilation by phytoplankton. ISHIDA and MITAMURA (1986)[13] reported the utilization of nitrogenous nutrients by phytoplankton in enriched lake waters collected from three different trophic areas in Lake Biwa. They show that the urea is a important nitrogen source for the phytoplankton growth in culture experiments.

To extend the knowedge regarding the behavior of the urea in narural waters, the decomposition of urea carbon and the assimilation of urea nitrogen during phytoplankton growth in enriched water collected from eutrophic area in Lake Biwa were investigated.

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Methods

The sample water was taken from surface layer at a very eutrophic narrow bay located in the southern basin of Lake Biwa in October 1975. The collected water was immediately enriched with urea, ammonia, nitrate and phosphate. Each nutrient was added to give the following concentration of added nutrient, respectively; 5-7 μg at. N $\cdot l^{-1}$ with urea, ammonia and nitrate, and 3 μg at. P $\cdot l^{-1}$ with phosphate. The enriched water sample was incubated in a transparent plastic container (20 l) under continuous illumination (5,000 lux) using fluorescent lamps at 19-20 ℃, continuously bubbling with air pump (*ca.* 1 l $\cdot$ min.$^{-1}$).

During the incubation, 200-500 ml of subsample water for the determination of nutrients, chlorophyll-a, particulate carbon (PC) and nitrogen (PN) were successively collected at 6-12 hr. intervals from the enriched culture. The collected subsample water was immediately filtered through a Whatman GF/C glass-fiber filter which was free of organic matter by ignition at 450℃, then the filter and filtrate were frozen and stored at -20 ℃.

Urea was determined by the method of NEWELL *et al.* (1967)[14], ammonia after SAGI (1966)[15] and nitrate by the method of WOOD *et al.* (1967)[16]. PC and PN were determined with a CHN Corder (Yanagimoto, MT-2 type). Chlorophyll-a was determined by the method of SCOR/Unesco (1964)[17].

The assimilation rate of nitrogenous nutrient by phytoplankton was measured at approximately 12 hr. intervals using ^{15}N-labelled compounds. The subsample water was poured into glass bottles, and small volumes of ^{15}N-labelled urea, ammonia and nitrate were added to each bottle. Then, the bottles were incubated for a few hours under 30,000 lux at 20 ℃ in a water tank. After the incubation, a formaldehyde solution was immediately added to each bottle to stop the biological activity. Sample water in each bottle was filtered through a Whatman GF/C glass-fiber filter. The ^{15}N: ^{14}N ratio in filter sample was determined by the technique of optical emission spectrometry with a ^{15}N Analyzer (Nihonbunko NIA-1 type) after combusting each sample by micro-DUMAS' method. The nitrogen assimilation rates of urea, ammonia and nitrate were calculated using a linear transformation of the MICHAELS-MENTEN equation, assuming that the rates were related to the ambient nitrogen concentrations.

The subsample water was poured into two series of bottles for the measurement of urea decomposition rate. After adding ^{14}C-labelled urea solution to each bottle, these bottles were incubated for a few hours under 30,000 lux at 20 ℃. A formaldehyde solution was added to each bottle to stop the biological activity, then sample water was filtered through a Milipore HA-type filter. The filter was put in a scintillation vial, and Bray scintillation fluid was added. The radioactivity was measured with Aloka Model LSC-651 liquid scintillation spectrometer to measure the rate of urea carbon incorporation into the organic matter. The filtrate of each sample was poured into a separate Erlenmeyer flask and a CO_2 absorption tube containing n-ethanolamine was placed in each flask to absorb the $^{14}CO_2$ liberated from the sample sloution by acidification. After adding dilute sulfuric acid solution to each filtrate, the flasks were sealed tightly and left for four days at room temperature. The radioactivity of $^{14}CO_2$ absorbed into n-ethanolamine solution was determined as the measurement of urea carbon incorporation into

the organic matter.

Photosynthetic carbon assimilation rate was determined by the ^{14}C technique of STEEMANN NIELSEN (1952) [18], simultaneously with the measurements of nitrogen assimilation rate and urea decomposition rate. The total CO_2 in the sample water was determined with an infra-red CO_2 analyzer, described by SATAKE *et al.* (1972) [19]

Results and Discussion

The changes of urea, ammonia, nitrate, PC, PN and chlorophyll-a concentration during the incubation are shown in Table 1. The concentrations of PC, PN and chlorophyll-a increased with time. Total nitrogenous nutrient (TNN; urea + ammonia + nitrate) concentration decreased with the increase of chlorophyll-a concentration. The cholorophyll-a concentration decreased, after the TNN concentration became low. The decrease of TNN concentration well corresponded to the increase of PN concentration. These nitrogenous nutrients seem to be utilized by phytoplankton. The nitrate concentration decreased after the ammonia was almost an undetectable value and urea became low. It seems that phytoplankton preferentially utilize ammonia, urea and nitrate in that order, from the changes of these nitrogenous nutrients concentrations.

The results of the photosynthetic carbon assimilation, the assimilation rate of urea nitrogen and the total nitrogen assimilation (as sum of urea, ammonia and nitrate assimilation rate measured using ^{15}N-labelled nitrogen compounds) are shown in Table 2. The photosynthetic rate was low values untill 60 hr.. A maximum photosynthetic rate was observed at 79 hr. and then the rate decreased. The photosynthetic rate by unit amount of chlorophyll-a showed a similar change to the photosynthetic rate, showing the low values from 30 hr. to 60 hr.. The assimilation of ammonia played the greater part in the total nitrogen assimilation untill 60 hr., whereas nitrate contributed in the total nitrogen assimilation after 79 hr.. The highest assimilation rate of urea was obtained at 60 hr.. The contribution of urea assimilation in the total nitrogen assimilation showed an appreciable change with time. Urea assimilation accounted for 18 to 27 % in the total nitrogen assimilation in the presence of ammonia, but it showed 95% at 60 hr. when the ammonia concentration became extremely low. The ratio of photosynthetic carbon assimilation rate to the total nitrogen assimilation rate (C/N assimilation ratio) was 0.2 to 10.2 (by weight) . The ratio of carbon to nitrogen in particulate matter was 6.5 to 8.9 (by weight) , and showed no appreciable change during the incubation period. MITAMURA and MATSUMOTO (1981) [5] reported that the C/N assimilaion ratio was 10.6 to 16.5 (by weight) in surface water of Lake Biwa. The ratios in the latter period of incubation of the present study are similar to those obtained by them.

The results of the decomposition rate of urea carbon (as sum of incorporation rate of urea carbon into the particulate matter and CO_2 liberation rate into the water from urea) during incubation are shown in Table 3. The urea decomposition rate ranged from 132 to 1139 $\mu gC \cdot m^{-3} \cdot hr.^{-1}$, and the highest rate was obtained at 79 hr.. The change of the urea decomposition rate resembled those of chlorophyll-a concentration and photosynthetic carbon assimilation rate. The contribution of urea carbon incorporation in the urea decomposition revealed more than 70% after 60 hr., while this was much low in the earlier period of the incubation. The percen-

Table 1 Changes of urea, ammonia and nitrate nitrogen, particulate carbon (PC), particulate nitrogen (PN), and chlorophyll-a (Chl.a) concentration during the incubation of enriched lake water.

Time (hr.)	Urea	Ammonia (μg at.N·1^{-1})	Nitrate	PC (μg C·1^{-1})	PN (μg N·1^{-1})	Chl.a (μg·1^{-1})
0	10.0	13.0	46.1	850	115	1.5
6	9.8	12.9	47.0			2.5
12	9.7	11.9	46.9	800	98	3.5
18	9.5	11.9	47.3			4.6
24	9.6	10.4	48.5	1220	157	4.6
30	9.5	10.2	49.8			8.1
36	9.4	8.7	49.2	1580	188	8.5
42	9.6	7.1	48.6			15.6
48	9.2	5.2	49.9	2300	339	20.7
54	8.4	2.1	49.9			27.7
60	6.7	0.0	48.5	3100	408	35.4
79	1.6	0.0	28.1	4480	690	31.9
89	1.4	0.0	12.5	5530	798	57.2
101	1.4	0.0	3.6	5900	856	57.4
112	1.2	0.0	3.1	6710	924	43.3
128	1.0	0.0	2.9	7850	1006	36.3
138	1.0	0.0	2.3	8330	996	30.5
152	1.1	0.0	1.9	9700	1090	26.4

Table 2 Rates of photosynthetic carbon assimilation, urea nitrogen assimilation and total nitrogen assimilation (as sum of urea, ammonia and nitrate nitrogen assimilation), and the ratio of urea nitrogen assimilation and photosynthetic carbon assimilation to total nitrogen assimilation, and PC/PN ratio in the particulate organic matter.

Time (hr.)	Photosyn. (μg C·1^{-1}·$hr.^{-1}$)	Urea assim. (μg N·1^{-1}·$hr.^{-1}$)	TNN assim.	Urea assim./TNN assim. (%)	Photosyn./TNN assim. (Wt./Wt.)	PC/PN
0	8.0	0.52	1.92	27	4.2	7.4
6	6.9					
12	5.5	0.46	1.78	26	3.1	8.2
18	4.2					
24	4.1	0.48	2.70	18	1.5	7.8
30	4.1					
36	3.6	0.99	4.35	23	0.8	8.4
42	2.9					
48	2.1	1.54	7.60	20	0.3	6.8
54	1.3					
60	0.9	3.67	3.85	95	0.2	7.6
79	118		11.56		10.2	6.5
89	109		11.68		9.3	6.9
101	48					6.9
112	25					7.7
128	8.1					7.8
138	3.7					8.4
152	1.6					8.9

Table 3 Rates of decomposition of urea carbon, incorporation of urea carbon into the particulate organic matter, CO_2 liberation into the water from urea, and the ratio of carbon incorporation and CO_2 liberation to decomposition of urea carbon.

Time (hr.)	Decomp.	C-incorp.	CO_2 lib.	C-incorp. / Decomp.	CO_2 lib. / Decomp.
	($\mu g\ C \cdot m^{-3} \cdot hr.^{-1}$)				
0	142	18	124	0.13	0.87
6	164	19	145	0.12	0.88
12	146	22	124	0.15	0.85
18	132	18	114	0.14	0.86
24	146	25	121	0.17	0.83
30	155	30	125	0.19	0.81
36	166	43	123	0.26	0.74
42	171	54	117	0.32	0.68
48	184	69	115	0.38	0.62
54	205	105	100	0.51	0.49
60	397	289	108	0.73	0.27
79	1139	812	327	0.71	0.29
89	777	681	96	0.88	0.12
101	1063	770	293	0.72	0.28
112	582	568	14	0.98	0.02
128	357	337	20	0.94	0.06
138	287	275	12	0.96	0.04
152	238	196	42	0.82	0.18

tage of carbon incorporation in urea decomposition increased with the phytoplankton growth. These results suggest that the rate of urea decomposition and urea carbon incorporation have relation to the phytoplankton growth and the photosynthetic activity. MITAMURA and SAIJO (1886) [12] reported that 11 to 36% of the urea decomposition was incorporated into the particulate phase in the light, but this was much lower in the dark in Lake Biwa. On the other hand, CARPENTER *et al.* (1972b) [20] indicated that the carbon incorporation from urea was negligible, using a culture diatom, *Stephanopyxis costata* (*Skeletonema costatum*) , under continuous light. In the present study, the percentages of the urea carbon incorporation in the urea decomposition during the phytoplankton growth showed much higher values than those obtained by MITAMURA and SAIJO (1986) [12] . It seems to be indicate that the contribution of carbon incorporation and CO_2 liberation in the urea decomposition might be partly affected by the physiological state of phytoplankton.

The relationship between urea carbon decomposition and chlorophyll-a or photosynthetic carbon assimilation is shown in Figs. 1 and 2. The urea decomposition rate and the carbon incorporation rate appear to be related to the chlorophyll-a concentration. The correlation coefficients for urea decomposition rate and carbon incorporation rate against chlorophyll-a were high values (r=.78 and r=.87) , as the results of regression analysis (Table 4) . This suggests that the urea decomposes by phytoplankton, and urea carbon is simulteniously incorporated into phytoplankton cell. The correlation coefficients between the urea decompotisiton and carbon in corporation against photosynthesis also showed high values (r=.86 and r=.82) . This indicates that the urea decomposition and the carbon incorporation are associated with photosynthesis of

Table 4 Regression equation of urea decomposition (U; μ g $C \cdot m^{-3} \cdot hr.^{-1}$) against chlorophyll-a (C; μ g $chl.a \cdot l^{-1}$) or photosynthetic carbon assimilation (P; $\mu g\ C \cdot l^{-1} \cdot hr.^{-1}$) from the linear regression analysis.

Urea Decomposition	U=13.6× C +45	(r =.78)
Carbon Incorporation	U=13.3× C −67	(r =.87)
CO_2 Liberation	U= 0.3× C +112	(r =.06)
Urea Decomposition	U= 7.7× P +207	(r =.86)
Carbon Incorporation	U= 6.4× P +113	(r =.82)
CO_2 Liberation	U= 1.2× P +93	(r =.54)

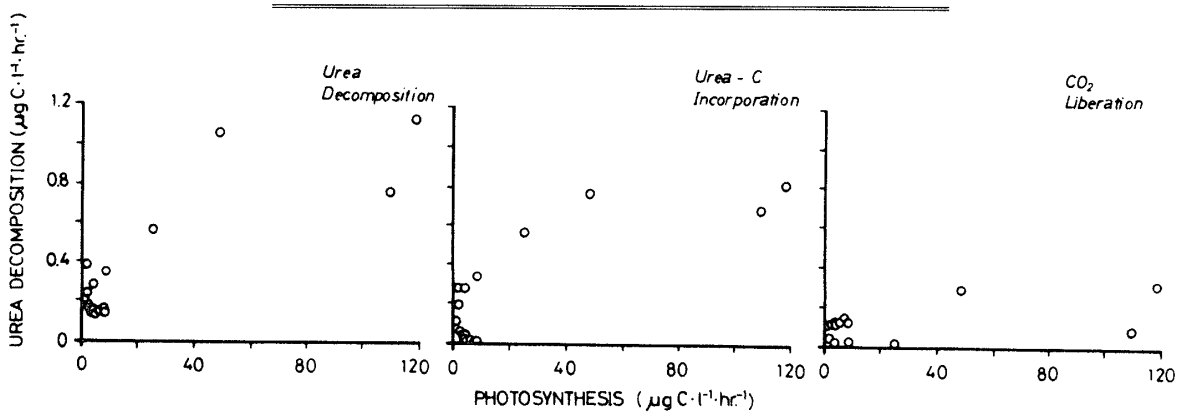

Figure 1. Ishida and Mitamura
Relationship between urea decomposition rate and chlorophyll-a

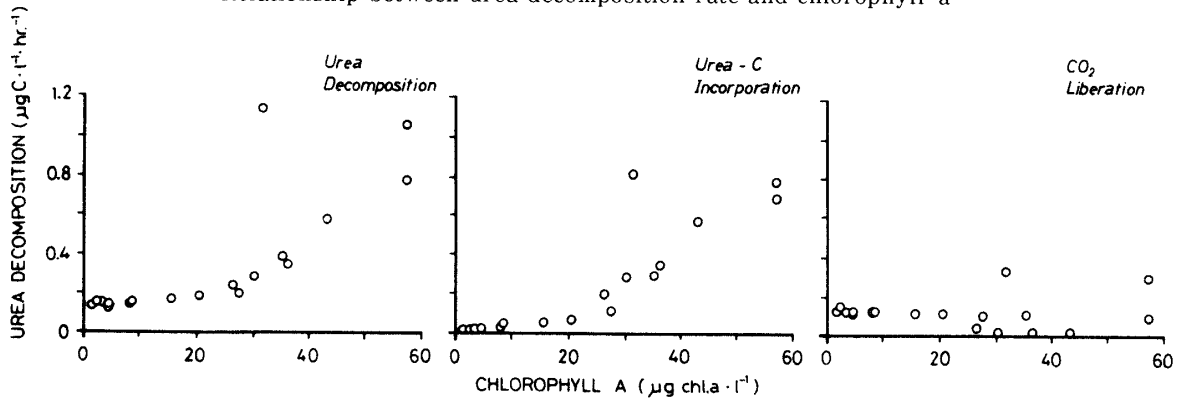

Figure 2 Ishida and Mitamura
Relationship between urea decomposition rate and photosynthetic carbon assimilation rate.

phytoplankton. MITAMURA and SAIJO (1986)[12] reported similar results. No appreciable correlation coefficient was found between CO_2 liberation and chlorophyll-a (r=.06) or photosynthesis (r=.54).

The results of the decomposition rate of urea carbon measured with ^{14}C-labelled urea and

the assimilation rate of urea nitrogen with ^{15}N-labelled urea are plotted in Fig. 3. The urea carbon decomposition had a close relationship with the assimilation of urea nitrogen by phytoplankton, showing high correlation coefficient (r=.98) . MITAMURA and MATSUMOTO (1981)[5] found that the ratio of urea carbon decomposition to urea nitrogen assimilation averaged 1.09 (by unit mole urea) in the light. MITAMURA and SAIJO (1986)[12] reported that the urea carbon decomposition rate corresponded to the urea nitrogen assimilation rate and the regression coefficient was calculated as 0.98 (by unit mole urea) from the regression analysis. In this study the ratio ranged from 0.25 to 0.75 (average 0.5) , expressed as unit mole urea, showing low values during low photosynthetic rate. Further information regarding the influence of physiological state of phytoplankton on the activity of urea utilization and urea decomposition will be required.

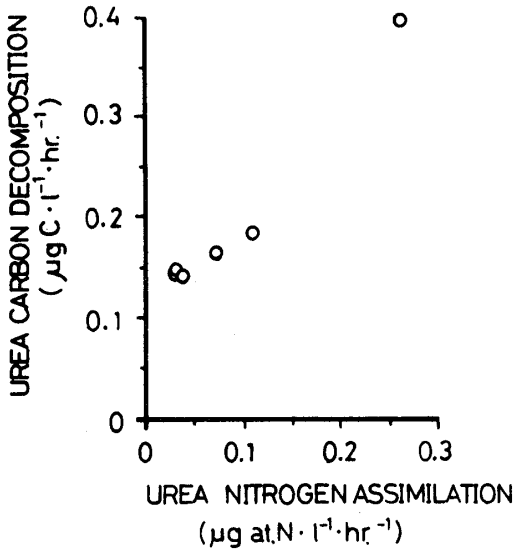

Figure 3 Ishida and Mitamura
Relationship between urea decomposition rate of urea carbon measured with ^{14}C-labelled urea and assimilation rate of urea nitrogen with ^{15}N-labelled urea.

PRICE and HARRISON (1988)[21] measured urea uptake by axenic cultures of *Thalassiosira pseudonana*, using ^{14}C-labelled urea, ^{15}N-labelled urea, and disappearance of dissolved urea from the medium. They reported that in nitrate-sufficient cells the average uptake rate of urea using ^{14}C-labelled and ^{15}N-labelled urea was lower compared with the disappearance rate of urea, since these differences depended on the nitrogen status of this species. In the present study, the disappearence rate of urea was comparable to the assimilation rate of urea, measured with ^{15}N-labelled urea, during the period of decreasing urea concentration. The average decomposition rate of urea carbon was lower compared with above values. The discrepancy among those values seems to be caused by the difference of the physiological processes for the urea contribution as the carbon and nitrogen source for phytoplankton.

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摘　　要

琵琶湖の富栄養化の進んだ水域から採取した表面水に栄養塩を添加して植物プランクトンを増殖させた系について，植物プランクトンによる尿素の分解と尿素態窒素の利用を調べた．尿素態炭素の有機物への取り込みと尿素分解の増加は，クロロフィルaの増加および光合成の増加と対応し，水中の尿素は植物プランクトンにより光合成と関係して分解されると考えられる．尿素分解と尿素態窒素の取り込みの関係は尿素の分解が尿素態窒素の取り込みと対応して生じることを示した．

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