Mechanism of ammonia nitrogen uptake by sterile *Ulva* sp. and its application to water quality control for intensive shrimp culture pond in developing country

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不稔性アオサによるアンモニア窒素摂取の機構と

開発途上国型エビ養殖池の水質制御に対する適用

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まず、アオサのキャラクタリゼーション（炭素、水素、窒素、クロロフィル組成、等の測定）、様々な条件における培養液ー藻体内部間のアンモニア窒素の分配平衡、アオサ表面の膜におけるアンモニア窒素の透過速度、およびアオサ内部におけるアンモニア窒素同化速度の実測、等を行った。昼間を想定した条件において透過速度は内部濃度基準の総括濃度差に比例し、昼間の条件の場合に比較して夜間の条件における透過速度は著しく低かった。アンモニア窒素同化速度と摂取速度はほぼ同等であった。これらの実験結果および既往のエビ養殖池における窒素循環モデルを用いてアオサによる水質制御計算を行った。養殖水中のアンモニア窒素濃度を低く抑えることができ、本法を簡便な水質制御法として提案した。

1 Introduction

Since the 1990s the shrimp industry, which has given high profit and foreign exchange to developing countries, has suffered many disease outbreaks[1]. Some shrimp farmers introduced none or quite small water exchange culture system to avoiding the infection of disease. However, employing the system began to cause the serious water deterioration and the water quality control became more important. Ammonia-nitrogen (ammonia-N,AN) excreted by shrimp respiration and from decomposition of bottom sediment is main cause of water deterioration. Ammonia-N is toxic to fishes and crustaceans[2] and even if the concentration is low, the growth of shrimp is inhibited[3]. Most of nitrogen component in the shrimp feed supplied into pond is finally discharged into the pond as the form of ammonia-N through excretion by shrimp and decomposition of accumulated sediment.

In this study, we investigated mechanism of ammonia nitrogen uptake by sterile *Ulva* sp. and simulated water quality control in basis of nitrogen balance and ammonia-N uptake mechanism of alga.

2 Mechanism of ammonia nitrogen uptake by sterile *Ulva* sp.

2.1 Materials

Sterile *Ulva* sp. collected from April to November 2008 at Kanazawa Bay (Yokohama, Japan 35°20’32N, 139°38’32E). Commercial sea salt was used to prepare artificial seawater (salinity: 30‰).

2.2 Experimental

2.2.1 Characterization of alga

A vessel made of glass of which capacity $5\times10^{-2}m^3$ and used to store algae. Light source was 400W metal halide lamp.

Elemental analysis was carried out by CHN corder MT-6. Chlorophyll concentration was determined by Scor/Unesco method.

2.2.2 Distribution of ammonia-nitrogen between culture medium and nitrogen pool in alga and permeation through surface film of algae

Algae were cultivated for 2h in artificial seawater containing 1mM methionine sulfoximine, which inhibits the assimilation of ammonia-N, before uptake runs. Concentrated ammonia-N solution added to culture medium at the beginning of uptake run and measured ammonia-N concentration of culture medium.

Table 2.2.2-1 shows the principal conditions of uptake runs. Some conditions were assumed daytime, others were nighttime. In all runs, the algae without ammonia-N assimilation ability were used to know the distribution equilibrium of ammonia-N between the culture medium and the ammonia-N pool in alga. After equilibration, ammonia-N excretions were also conducted by exchanging the medium with ammonia-N free seawater.

2.2.3 Assimilation of ammonia-nitrogen in alga

Concentrated ammonia-N solution added to culture medium at the beginning of uptake run and measured ammonia-N concentration of culture medium. The ammonia-N concentration in alga was measured by adding carbonyl cyanide m-chlorophenilhydrazone into the medium.

2.3 Results and discussion

2.3.1 Characterization of alga

Table 2.3.1-1 shows of mass of Carbon, Hydrogen, and Nitrogen per unit dry biomass. Wt% of CHN decreased with increase of stock cultivation time. This is considered those nutrients, which were pooled in the sea, were exhausted for growth.

Table2.3.1-2 shows amount of chlorophyll-a,b,c per unit dry biomass. There is not great differences of chlorophyll concentrations in each sample of alga.

Table 2.3 1-1 Results of elemental analysis

Stock cultivation time [day]	Biomass $\times10^3$ [kgDM]	H [wt-%]	C [wt-%]	N [wt-%]
0	0.0581	5.30	28.29	3.26
0	0.0281	5.34	29.39	3.50
0	0.0646	5.25	27.80	3.13
5	0.0589	4.31	22.09	2.35
5	0.0206	4.78	24.97	2.55
5	0.0229	4.66	24.42	2.39

Sampling date 2008, Oct, 17th

Table2.3.1-2 Chlorophyll concentration of alga

Biomass × 10^3 [kgDM]	$C_{Chl\text{-}a} \times 10^3$ [kg-Chl kgDM^{-1}]	$C_{Chl\text{-}b} \times 10^3$ [kg-Chl kgDM^{-1}]	$C_{Chl\text{-}c} \times 10^3$ [kg-Chl kgDM^{-1}]
0.064	1.5	0.6	0.6
0.053	2.1	1.0	1.0
0.044	2.7	1.2	1.1
0.048	2.2	0.9	0.7
0.033	2.3	1.0	0.8
0.043	2.0	0.9	0.8

2.3.2 Distribution of ammonia-nitrogen between culture medium and nitrogen pool in alga and permeation through surface film of algae

Figure 2.3.2-1 shows the equilibrium relation between ammonia-N concentrations in culture medium and in the ammonia-N pool of alga. In condition assumed daytime, equilibrium ammonia-N concentrations in alga after uptake were higher than those after excretion. Equilibrium ammonia-N concentrations of internal algae in condition assumed daytime were higher than that concentration in condition assumed nighttime. These differences of equilibrium relation were considered to be chemical potential deference between external and internal algae.

Figure 2.3.2-2 shows the relation between ammonia-N uptake rate and concentration difference $C'^{*}_{N}-C'_{N}$. C'^{*}_{N} was estimated by the equilibrium relationship after uptake given in Fig.2.3.2-1. Mean gradient of collinear approximations not through origin was 0.73 h-[1]

Table 2.2.2-1 Experimental condition

	Daytime	Nighttime
Initial concentration of culture medium $C_{TAN,0}$ [kg-N m^{-3}]	0.25~2.00	
Culture density of alga ρ_U [kgDM m^{-3}]	0.07~0.66	
Cultivation time [h]	7~12	
Light intensity, PPF [μmol s^{-1} m^{-2}]	1800	5>
Temperature [K]	303	298

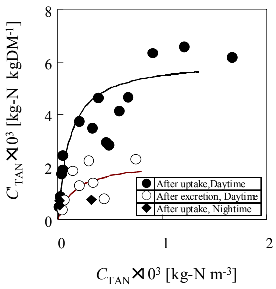

Fig.2.3.2-1 Equilibrium between AN concentrations in culture medium and in alga

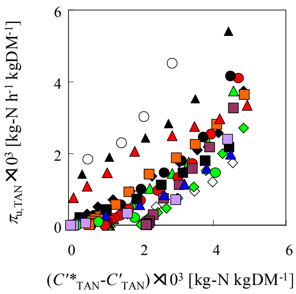

Fig.2.3.2-2 Relation between AN uptake rate and overall concentration difference, $C'^{*}_{TAN}-C'_{TAN}$

2.3.3 Assimilation of ammonia-nitrogen in alga

The material balance in a unit volume of the culture medium is given by,

$$\rho_U \pi_{u,TAN} = -\frac{dC_{TAN}}{dt} = \rho \frac{dC'_{TAN}}{dt} + \rho_U \pi_{a,TAN} \qquad \textbf{(1,2)}$$

where $\pi_{u,TAN}$ is ammonia-N uptake rate by alga, C'_{TAN} is the ammonia-N concentration in alga, and $\pi_{a,TAN}$ is the assimilation rate. Integration of this equation from time t_1 to t_2 leads to,

$$C_{TAN,1} + \rho C'_{TAN,1} = C_{TAN,2} + \rho C'_{TAN,2} + \rho \overline{\pi_{a,TAN}}(t_2 - t_1) \qquad \textbf{(3)}$$

where $\pi_{a,TAN}$ is mean assimilation rate between t_1 and t_2. **Figures 2.3.3-1** and **2.3.3-2** show the time courses of ammonia-N concentration in medium and those in alga with assimilation, respectively. **Figure 2.3.3-3** shows relation between mean ammonia-N concentration in alga and ammonia-N assimilation rate. Assimilation rate increased with increase of ammonia-N concentration in alga at first 1 hour. However, assimilation rates rapidly decreased with time. This decrease is provably due to exhaustion of other nutrients such as carbon.

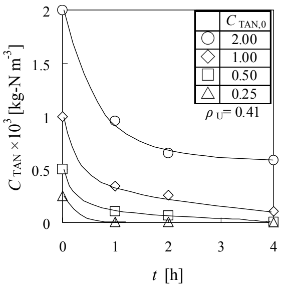

Fig.2.3.3-1 Time courses of AN concentration in culture medium

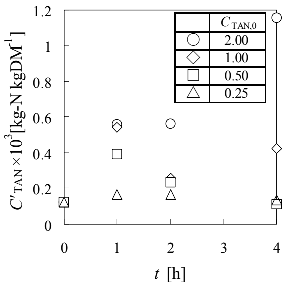

Fig.2.3.3-2 Time courses of AN concentration in alga

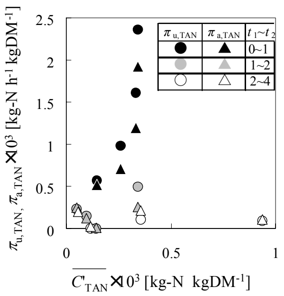

Fig.2.3.3-3 Relation between mean AN concentration in alga and AN assimilation rate

Table 2.3.3-1 Experimental conditi

$C_{TAN,0}$ [kg-N m^{-3}]	0.25~2.00
ρ_U [kgDM m^{-3}]	0.41
PPF [μmol s^{-1} m^{-2}]	1800
Temperature [K]	303

3 Water quality control for shrimp pond

3.1 Nitrogen balance in shrimp pond

Figure3.1-1 shows a concept of nitrogen dynamics model.

Nitrogen as the form of the shrimp feed are supplied into pond. Much of feed is eaten by shrimp(1), and a portion of feed is uneaten(2). Shrimp excretes ammonia-N through respiration (4) and other nitrogen metabolite(feces, shed shell etc.)(3). Ammonia-N also generate from sediment decomposition(7). Ammonia-N in water column was removed by algal uptake(5), water exchange(8), and other removal process(9). Algae are removed from pond(6) as one of final form of nitrogen.

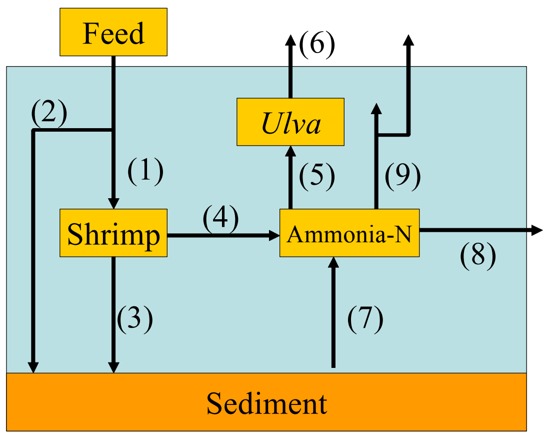

Fig.3.1-1 Concept of nitrogen dynamics model

3.2 Basic equation and calculation

Material balance;

The material balance in a unit volume of pond water is given by,

$$\frac{\mathrm{d}C_{\mathrm{TAN}}}{\mathrm{d}t} = r_{\mathrm{total,TAN}} - r_{\mathrm{total,remove}} \tag{4}$$

where $r_{\mathrm{total,TAN}}$ is generation rate of ammonia-N, $r_{\mathrm{total,remove}}$ is removal rate of ammonia-N.

Ammonia-N generation

Generation rate of ammonia-N is given by,

$$r_{\mathrm{total,TAN}} = r_{\mathrm{e,TAN}} + r_{\mathrm{sed,TAN}} \tag{5}$$

where $r_{\mathrm{e,TAN}}$ is rate of ammonia-N excreted by shrimp respiration, $r_{\mathrm{sed,TAN}}$ is rate of ammonia-N generated by sediment decomposition.

$r_{\mathrm{e,TAN}}$ is given by,

$$r_{\mathrm{e,TAN}} = q_{\mathrm{TAN}} r_{\mathrm{mtb,N}} \tag{6}$$

where q_{TAN} is ratio of ammonia-N from shrimp respiration to total nitrogen metabolite. $r_{\mathrm{mtb,N}}$ is excretion rate of nitrogen metabolite from shrimp per unit volume of pond water and given by,

$$r_{\mathrm{mtb,N}} = a_{\mathrm{mtb,N}} \rho_{\mathrm{s}} M_{\mathrm{s}}^{\gamma} \tag{7}$$

where $a_{\mathrm{mtb,N}}$ is nitrogen metabolite from shrimp per unit shrimp mass. ρ_{s} is culture density of shrimp. M_{s} is average shrimp mass and estimated from growth model. γ is allometric scaling of shrimp metabolism.

On the other hand, $r_{\mathrm{sed,TAN}}$ is given by,

$$r_{\mathrm{sed,TAN}} = r_{\mathrm{r}} M_{\mathrm{sed,N}} \tag{8}$$

where r_{r} is rate constant of decomposition. $M_{\mathrm{sed,N}}$ is amount of nitrogen contained in sediment per unit volume of pond water. $M_{\mathrm{sed,N}}$ include the sediment derived from uneaten feed and nitrogen metabolite from shrimp.

Ammonia-N removal

Removal rate of ammonia-N is given by,

$$r_{\mathrm{total,remove}} = \rho_{\mathrm{U}} \overline{\pi_{\mathrm{u,TAN}}} + f C_{\mathrm{TAN}} + o C_{\mathrm{TAN}} \tag{9}$$

Where $\overline{\pi_{\mathrm{u,TAN}}}$ is average uptake rate, f and o is rate constants of water exchange and other removal process, respectively. In the case that saturation ratio of photosynthesis affect uptake performance, $\overline{\pi_{\mathrm{u,TAN}}}$ is given by,

$$\overline{\pi_{\mathrm{u,TAN}}} = \overline{q_{\mathrm{light}}} \pi_{\mathrm{u,TAN}} \tag{10}$$

where $\overline{q_{\mathrm{light}}}$ is average saturation ratio of photosynthesis ($\overline{q_{\mathrm{light}}}$ equaled 1 in case of not taking the effect of saturation ratio of photosynthesis on uptake into account.) Relation between saturation ratio of photosynthesis q_{light} and light intensity is approximated following equation,

$$q_{\mathrm{light}} \left(= \frac{p}{p_{\mathrm{MAX}}} \right) = \frac{I}{I + K_{\mathrm{light}}} \tag{11}$$

where I is light intensity at specific depth from pond surface. K_{light} is constant depending on temperature. I can be described by Beer-Lambert law as,

$$I = I_0 \, exp(-kz) \tag{12}$$

where I_0 is light intensity of pond surface, k is overall light extinction coefficient, z is distance from pond surface. k is given by,

$$k = k_{\mathrm{U}} + k_{\mathrm{other}} \tag{13}$$

where k_{U} and k_{other} is light extinction coefficient of alga and pond water, respectively. k_{U} is determined by chlorophyll concentration in alga and ρ_{U} and given by,

$$k_{\mathrm{U}} = k_{\mathrm{Chl}} \rho_{\mathrm{U}} C_{\mathrm{Chl}} \tag{14}$$

where k_{Chl} is light extinction coefficient per unit mass of chlorophyll-a, C_{Chl} is chlorophyll-a amount per unit dry biomass of alga. $\pi_{\mathrm{u,TAN}}$ is, from relation between $\pi_{\mathrm{u,TAN}}$ and overall concentration difference, given by

$$\pi_{\mathrm{u,TAN}} = P_{\mathrm{u}} \left(C'^{*}_{\mathrm{TAN}} - C'_{\mathrm{TAN}} \right) \tag{15}$$

where P_{u} is overall mass transfer coefficient. Change rate of ammonia-N concentration in alga can be obtain from material balance and given by below equation including growth dilution of ammonia-N in alga pool,

$$\frac{\mathrm{d}C'_{\mathrm{TAN}}}{\mathrm{d}t} = -C'_{\mathrm{TAN}} \overline{\pi_{\mathrm{a,TAN}}} R_{\mathrm{Biomass/N}} + \left(\overline{\pi_{\mathrm{u,TAN}}} - \overline{\pi_{\mathrm{a,TAN}}} \right) \tag{16}$$

where $R_{\mathrm{Biomass/N}}$ is ratio of dry biomass to nitrogen in dry biomass. As with $\overline{\pi_{\mathrm{u,TAN}}}$, $\overline{\pi_{\mathrm{a,TAN}}}$ is given by

$$\overline{\pi_{\mathrm{a,TAN}}} = \overline{q_{\mathrm{light}}} \pi_{\mathrm{a,TAN}} \tag{17}$$

From Fig. 4-3, assimilation rate is almost the same as uptake rate and that can be written by,

$$\pi_{\mathrm{a,TAN}} = \pi_{\mathrm{u,TAN}} \quad (\text{when} \quad \pi_{\mathrm{a,TAN,MAX}} \geq \pi_{\mathrm{u,TAN}}) \tag{18}$$

$$\pi_{\mathrm{a,TAN}} = \pi_{\mathrm{a,TAN,MAX}} \ (\text{when} \quad \pi_{\mathrm{a,TAN,MAX}} \leq \pi_{\mathrm{u,TAN}}) \tag{19}$$

where $\pi_{\mathrm{a,TAN,MAX}}$ is maximum assimilation rate of ammonia-N.

Calculation

Parameters of equations in chapter3.2 were estimated from chapter2 and cited previous study[4][5].

Table3.2-1 shows the parameters of fixed value in all calculation. *Penaeus* monodon was selected as culture target. Cultivation time was 120days. It was assumed nitrogen input was only feed and was assumed 20% of total nitrogen input was assimilated by shrimp and 10% of that was uneaten by shrimp.

Table3.2-2 shows the parameters that were varied in each calculation run. (1) was calculated as standard condition, (2) as prediction of effect of culture density of shrimp, (3) effect of saturation ratio of photosynthesis, (4) effect of water exchange and other removal process, (5) effect of ratio of nitrogen metabolite through respiration to total nitrogen metabolite, (6) effect of water turbidity, and (7)effect of algal harvest.

Table3.2-1 Parameters of fixed value

$a_{\mathrm{mtb,N}}$ [kg-N h^{-1} kg-shrimp]	0.0000274
C_{Chl} [kg-chl kgDM^{-1}]	0.002131
P_{U} [h^{-1}]	0.7307
$\pi_{\mathrm{a,TAN,MAX}}$ [kg-N h^{-1} kgDM^{-1}]	0.002042
$R_{\mathrm{Biomass/N}}$ [kgDM kg-N^{-1}]	30.40
h [m]	1.0
k_{Chl} [kg-Chl^{-1} m^{-1}]	11800
$I_{0,\mathrm{day}}$ [μmol s^{-1} m^{-2}]	1800
$I_{0,\mathrm{night}}$ [μmol s^{-1} m^{-2}]	0
T_{day} [K]	303.15
T_{night} [K]	298.15
γ [-]	0.75
r_{r} [h^{-1}]	0.0025

Table3.2-2 Calculatiuon conditions

	$\rho_{u,0}$ [kgDM m^{-3}]	$\rho_{s,0}$ [m^{-3}]	$\overline{q}_{light}$ [-]	f [h^{-1}]	$o\times10^3$ [h-1]	q_{TAN} [-]	k_{other} [m^{-1}]	H_u [kgDM h^{-1}m^{-3}]
(1)	0~2.0	40	1	0	0	1	2.5	※3
(2)	0~2.0	40~120	1	0	0	1	2.5	※3
(3)	0~2.0	40~120	※1	0	0	1	2.5	※3
(4)	0~2.0	40~120	1	※2	8.33	1	2.5	※3
(5)	0~2.0	40~120	1	0	0	0~1	2.5	※3
(6)	0~2.0	40	※1	0	0	1	1.0~4.0	※3
(7)	0~2.0	40	1	0	0	1	2.5	0

※1, q_{light} was calculated by equation(10)~(14)

※2,Water exchange was carried out at night; Change rate is 0.8%, 8%, 12%, and 16% day^{-1} in 0~30, 31~60, 61~90 and 91~120 day, respectively

※3, Algae were harvested to keep culture density of alga

3.3 Results and Discussion

Figure3.3-1 shows effect of culture density of shrimp(calculation run(1)(2)**).** Ammonia-N concentration increase with time, also increase with increase of culture density of shrimp.

Figure3.3-2 shows relation between initial culture density of algae and limit culture density of shrimp (calculation run(2)(3)). Limit culture density of shrimp, which was defined that maximum value of average ammonia-N concentration in nighttime does not exceed 0.001kg-N m^{-3}, slightly decreased with degree of light limitation $\overline{q}_{light}$.

increased by water exchange and decreased by light limitation.

Figure3.3-3 shows relation between ratio of nitrogen metabolite through respiration to total nitrogen metabolite q_{TAN} and limit of culture density of shrimp. Limit culture density of shrimp was increase with increase of q_{TAN}.

Figure3.3-4 shows relation between light extinction coefficient of pond water and limit of culture density of shrimp.

In the case that harvest of algae was not conducted, final culture density of algae exceeded 2.0 kgDM m^{-3}. This

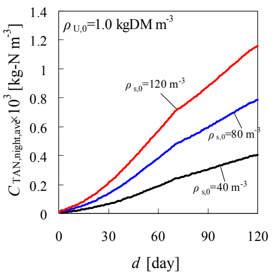

Fig.3.3-1 Time courses of average ammonia-N concentration at nighttime on each day

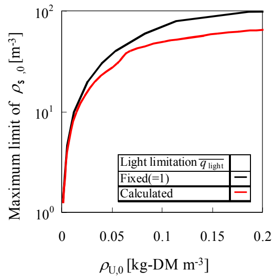

Fig.3.3-2 Relation between initial culture density of alga and limit of culture density of shrimp

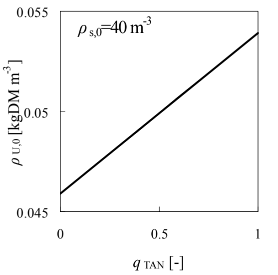

Fig.3.3-3 Relation between ratio of nitrogen metabolite through respiration to total nitrogen metabolite and minimum limit of

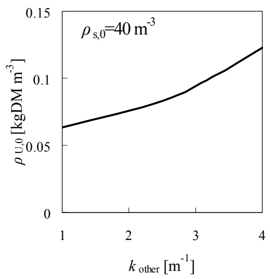

Fig.3.3-4 Relation water turbidity and minimum limit of culture density of alga

range of culture density may inhibit the shrimp culture management.

4 Conclusion

We measured the ammonia-N distribution equilibrium relation between culture medium and in alga, ammonia-N permeation rate, and assimilation rate. Secondly, we carried out calculation of water quality based on these data.

The water quality control method by using sterile ulva can remove ammonia-N in shrimp pond more effectively

Nomenclature

$a_{mtb,N}$ Nitrogen metabolite excretion rate [kg-N h^{-1} kg-shrimp^{-1}]
γ Allometric scaling of metabolism [-]
C_{TAN} Ammonia-N concentration [kg-N·m^{-3}]
$C_{TAN,night,ave}$ Average ammonia-N concentration in nighttime [kg-N·m^{-3}]
$C_{TAN,night,MAX}$ Maximum value of average ammonia-N concentration in nighttime [kg-N·m^{-3}]
C'_{TAN} Ammonia-N concentration in alga[kg-N·kgDM^{-1}]
C'^{*}_{TAN} Equilibrium ammonia-N concentration in alga [kg-N·kgDM^{-1}]
$C_{Chl\text{-}a}$ Chlorophyll concentration in alga [kg-Chl-a kgDM^{-1}]
d Cultivation time[day]
f water exchange rate [h^{-1}]
H_U Harvest rate of algae[kgDM h^{-1} m^{-3}]
h Pond depth[m]
I Light intensity [μmol s^{-1} m^{-2}]
k Overall light extinction coefficient [m^{-1}]
k_{Chl} Light extinction coefficient of chlorophyll [m^2 kg-Chl-a]
k_{other} Light extinction coefficient of pond water [m^{-1}]
k_U Light extinction coefficient of alga [m^{-1}]
K_{light} Constant of apporoximation [μmol s^{-1} m^{-2}]
M_s Shrimp mass [kg-shrimp]
$M_{sed,N}$ Nitrogen in sediment per unit volume of pond water[kg-N m^{-3}]
o Overall Rate constant of other ammonia-N removal process [h^{-1}]
p Photosynthesis rate [μl-O_2 cm^{-2} h^{-1}]
p_{MAX} Saturated photosynthesis rate [μl-O_2 cm^{-2} h^{-1}]
P_U Overall mass transfer coefficient [h^{-1}]
q_{light} Saturation ratio of photosynthesis [-]
q_{TAN} Ratio of nitrogen metabolite through respiration to total nitrogen metabolite [-]
$R_{Biomass/N}$ Ratio of dry biomass to nitrogen in alga
$r_{e,TAN}$ Excretion rate of ammonia-N by shrimp respiration[kg-N h^{-1} m^{-3}]
$r_{mtb,N}$ Nitrogen metabolite excretion rate by shrimp [kg-N h^{-1} m^{-3}]
r_r Rate constant of sediment decomposition [h^{-1}]
$r_{sed,TAN}$ Ammonia-N generation rate from sediment [kg-N h^{-1} m^{-3}]
$r_{total,TAN}$ Total rate of ammonia-N generation [kg-N h^{-1} m^{-3}]
$r_{total,remove}$ Removal rate of ammonia-N [kg-N h^{-1} m^{-3}]
t Cultivation time [h]
T_{day} Temperature at daytime [K]
T_{night} Temperature at nighttime [K]
$\pi_{a,TAN}$ Assimilation rate of ammonia-N [kg-N·kgDM^{-1}·h^{-1}]
$\pi_{a,TAN,MAX}$ Maximum assimilation rate of ammonia-N [kg-N·kgDM^{-1}·h^{-1}]
$\pi_{u,TAN}$ Uptake/permeation rate of ammonia-N [kg-N·kgDM^{-1}·h^{-1}]
ρ_s Culture density of shrimp [m^{-3}]
ρ_U Culture density of algae[kgDM·m^{-3}]

<Subscript>

a = assimilation ,Chl = chlorophyll, f = feed, mtb = metabolism, N = nitrogen, s = shrimp, TAN = Total Ammonia-Nitrogen, u = uptake, U = *Ulva*, 0 = initial or pond surface, ¯ = average

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