Uptake Rate of Ammonia-nitrogen With Sterile *Ulva* sp. for Water Quality Control of Intensive Shrimp Culture Ponds in Developing Countries

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Ammonia-nitrogen uptake by seaweed was modeled based on the concept of ammonia-nitrogen permeation through cell membrane, and the derived model of uptake rate was experimentally verified. In this study, sterile *Ulva* sp. was employed as seaweed to treat model culture solution, and the distribution equilibrium of the ammonia-nitrogen between the culture solution and cell inside was measured to obtain the equilibrium. For this measurement, the seaweed was pretreated before the uptake runs to inhibit the assimilation by methionine sulfoximine for removal of the assimilation effects on the uptake rate. The parameters of the distribution equilibrium and permeation rate of ammonia-nitrogen were measured. The pretreated seaweed could uptake ammonia-nitrogen and the ammonia-nitrogen permeated through the cell membrane from the culture solution into the cell according to the concentration gradient. The seaweed saturated with ammonia-nitrogen was immersed in the culture solution without ammonia-nitrogen and it could excrete ammonia-nitrogen once taken in. In both cases of the uptake and excretion, the systems attained equilibrium after around 6 hours. The ammonia-nitrogen concentration in the cell increased with the concentration in the culture solution at equilibrium. The flux of ammonia-nitrogen was almost proportional to the concentration difference, defined as that between the ammonia-nitrogen concentration in the cell and the hypothetical concentration of ammonia-nitrogen in the cell which is in equilibrium with the culture solution. The overall permeation coefficient was measured as $9.1 \cdot 10^{-3}$ m h^{-1} for both cases of uptake and excretion, and this relationship was valid when the concentration difference was large enough relative to the flux.

Key words:
Ammonia-nitrogen uptake, sterile *Ulva* sp., water quality control of intensive shrimp culture ponds, uptake mechanism

Introduction

The shrimp industries in the developing countries have been devoting their efforts in risk reduction of massive shrimp mortality due to serious infectious diseases or in-pond water quality deterioration. The white spot syndrome virus has been causing considerable mortality in shrimps since 1990s[1–4] because it is highly contagious for almost all kinds of crustaceans, including crustacean planktons. Thus, the shrimp industry has been faced with the option to employ closed-system aquafarming in order to avoid this infection. In closed systems, control of the pond culture water quality is crucial and some harmful compounds must be continuously removed during cultivation, especially ammonia-nitrogen compounds (ammonia-N). Ammonia-N, excreted by the metabolism of fish and crustaceans, is toxic to them, inducing growth inhibition and at worst extinction,[5] and some proposals to remove ammonia-N have been presented. One of them is biofiltration, which could successfully treat the pond water and maintain a low level of ammonia-N concentration in an intensive mariculture of *Sparus* aurata.[6–8] Authors have reported that sterile *Ulva sp*. could effectively take in ammonia-N under tropical conditions[9] and the algae was characterized based on the ammonia-N uptake rate to evaluate the control of intensive mariculture with the algae.[10] The Michaelis-Menten has been generally used expressing the ammonia-N uptake rate and the uptake rate was organized only under the specified situation that the inhibitory factor was negligible.[10] The nitrogen uptake rate followed the Michaelis-Menten model with the algae pretreated to be starved, and the effects of the uptake conditions on the inhibitory factor were unclear. The inhibitory factor increased as the seaweed took in the ammonia-N and the increment could not be analyzed. Therefore it is necessary to evaluate the ammonia-N uptake by the

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seaweed in general conditions, not sticking to the inhibitory factor, and the alternative uptake model was tried in this case.

This study aims to arrange the uptake model of ammonia-N by sterile *Ulva sp*. Firstly, the uptake model is discussed with regard to ammonia-N transfer through the cell membrane. Secondly, the uptake rates were experimentally measured under the conditions that the assimilation was inhibited using inhibitor methionine sulfoximine. This method was expected to extract only the effect of the ammonia-N transfer rate through the cell membrane due to removal of the assimilation effects, and the ammonia-N distribution equilibrium around the cell was also measured. With these data the performance of ammonia-N uptake was discussed for clarification of the ammonia-N uptake mechanism.

Ammonia-nitrogen uptake by seaweed

The Michaelis-Menten model has been generally used to represent uptake rates of ammonia-N or nitrate-N by some kinds of seaweed, such as *Catenella nipae, Polysiphonia decipiens*, *Ulva lactuca*, *Ulva* sp. and sterile *Ulva* sp. The uptake rates are affected by many environmental and cell/metabolic conditions, and the mechanism of uptake and assimilation of nitrogen compounds are much too complicated for clear analysis. The Michaelis-Menten model was employed to simply express the overall uptake rate of nitrogen compounds, and the physical meaning of the Michaelis-Menten parameters is still being elucidated. The uptake rate is expressed based on the following Michaelis-Menten concept. The material balance for the unit volume of the culture medium is given by

$$\frac{\mathrm{d}\gamma_{\mathrm{TAN}}}{\mathrm{d}t} = \rho_{\mathrm{u}} \cdot \frac{\mathrm{d}\gamma'_{\mathrm{TAN}}}{\mathrm{d}t} + \rho_{\mathrm{u}} \cdot \pi_{\mathrm{a,TAN}} \qquad (1)$$

$$\frac{\mathrm{d}\gamma_{\mathrm{TAN}}}{\mathrm{d}t} = \rho_{\mathrm{u}} \cdot \pi_{\mathrm{u,TAN}} \qquad (2)$$

where γ_{TAN} is the concentration of ammonia-N in the culture medium, γ'_{TAN} is that in seaweed cells, $\pi_{\mathrm{a,TAN}}$ is the specific assimilation rate, $\pi_{\mathrm{u,TAN}}$ is the specific rate of ammonia-N uptake by seaweed, and ρ_{u} is the density of seaweed in the solution. In general, ammonia-N uptake by seaweed is governed by the permeation of ammonium ions, NH_4^+, through the cell membrane. This ammonia-N transfer was facilitated by carrier in the membrane and this overall uptake process can be written as:

$$\mathrm{A} + \mathrm{C} \rightleftarrows \mathrm{AC} \qquad (3)$$

$$\mathrm{AC} \rightarrow \mathrm{A}' + \mathrm{C} \qquad (4)$$

where A, C, AC and A′ stand for NH_4^+ in the cultivation solution, carrier in the membrane, complex of the carrier and NH_4^+ in the membrane and NH_4^+ in the cell, respectively. If the effect of inhibitor, I, is considered, the process can be expressed as;

$$\mathrm{AC} + \mathrm{I} \rightleftarrows \mathrm{ACI} \qquad (5)$$

With these equations and the Michaelis-Menten model, $\pi_{\mathrm{u,TAN}}$ can be written as:

$$\pi_{\mathrm{u,TAN}} = -\frac{V_{\max} \cdot \gamma_{\mathrm{TAN}}}{K_{\mathrm{M}} + (1+\alpha) \cdot \gamma_{\mathrm{TAN}}} \qquad (6)$$

This model employs several assumptions, e.g.; 1. The dissociation of eq. (3) is a rapid reaction; 2. The reaction of eq. (4) is irreversible; and 3. The reaction of eq. (4) is a controlling step in the process. It is generally said that seaweed releases ammonia-N as nighttime and is once taken into the cell body during daytime. In other words, the reaction of eq. (4) should be reversible and rewritten as;

$$\mathrm{AC} \rightleftarrows \mathrm{A}' + \mathrm{C} \qquad (7)$$

This reaction surely does not change the molecule of ammonia-N and can be considered as simple dissociation. Accordingly, this can be regarded as a rapid reaction, same as reaction (3). In relation to these assumptions, the mass transfer resistance should be allocated, not in the reactions, but in the mass transfer steps. Moreover, the following assumptions were used to develop the simple permeation model: 1. The permeation resistance is uniform relative to the effective specific surface area; 2. The amount of carrier in membrane is sufficient in relation to the amount of ammonia-N; 3. The swelling and growth of the cell are ignored; 4. The permeated ammonia-N is homogeneously distributed in the cell. The concentration profiles in the rate process are conceptually shown in Fig. 1 as dashed lines. The ammonia-N molecule diffuses from the bulk of the culture solution to the surface of the seaweed and reacts with carrier at the surface to generate the complex form of AC. The complex form diffuses to another surface contiguous to the cell inside and dissociates again into A′ and C, diffusing into the cell inside. Accordingly, if the ammonia-N concentrations are high enough in the culture solution and low enough in the cell, the ammonia-N should permeate through the cell membrane into the cell inside, and vice versa.

If the assimilation is inactive, the system should attain equilibrium condition in infinite time. The concentration profiles in equilibrium condition are shown in Fig. 1 as solid lines. The dissociation coefficients at both sides of the membrane can be expressed as;

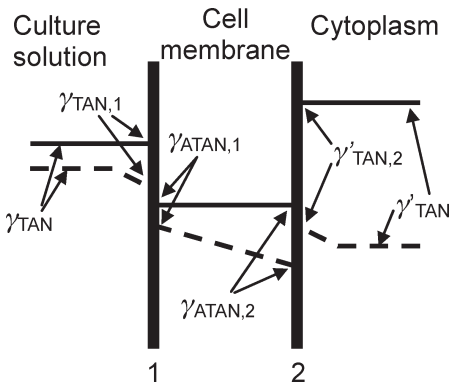


Fig. 1 – *Ammonia-N concentration profiles around cell membrane solid lines: ammonia-N concentrations at equilibrium dashed lines: ammonia-N concentrations during uptake*

$$K_1 = \frac{\overline{\gamma_{\mathrm{ATAN},1}}}{\overline{\gamma_{\mathrm{A}}} \cdot \gamma_{\mathrm{TAN},1}} \tag{8}$$

$$K_2 = \frac{\overline{\gamma_{\mathrm{ATAN},2}}}{\overline{\gamma_{\mathrm{A}}} \cdot \gamma'_{\mathrm{TAN},2}} \tag{9}$$

here $\overline{\gamma_{\mathrm{A}}}$, $\overline{\gamma_{\mathrm{ATAN},1}}$, $\gamma_{\mathrm{TAN},1}$, $\overline{\gamma_{\mathrm{ATAN},2}}$ and $\gamma'_{\mathrm{TAN},2}$ stand for the total carrier concentration in membrane, the concentration of complex form of carrier and ammonia-N in membrane at interface 1, the concentration of ammonia-N in culture solution at interface 1, the concentration of complex form of carrier and ammonia-N in membrane at interface 2, and the concentration of ammonia-N in culture solution at interface 2, respectively. At equilibrium,

$$\gamma_{\mathrm{TAN}} = \gamma_{\mathrm{TAN},1} \tag{10}$$

$$\overline{\gamma_{\mathrm{ATAN},1}} = \overline{\gamma_{\mathrm{ATAN},2}} \tag{11}$$

$$\gamma'_{\mathrm{TAN},2} = \gamma'_{\mathrm{TAN}} \tag{12}$$

therefore γ'_{TAN} can be expressed as;

$$\gamma'_{\mathrm{TAN}} = \frac{K_1}{K_2} \cdot c_{\mathrm{TAN}} \tag{13}$$

The local mass transfer rates can be expressed as;

$$J_{\mathrm{e}} = k_{\mathrm{e}} \cdot (\gamma_{\mathrm{TAN}} - \gamma_{\mathrm{TAN},1}) \tag{14}$$

$$J_{\mathrm{m}} = k_{\mathrm{m}} \cdot (\overline{\gamma_{\mathrm{ATAN},1}} - \overline{\gamma_{\mathrm{ATAN},2}}) \tag{15}$$

$$J_{\mathrm{c}} = k_{\mathrm{c}} \cdot (\gamma'_{\mathrm{TAN},2} - \gamma'_{\mathrm{TAN}}) \tag{16}$$

Eqs. (14), (15) and (16) stand for the ammonia-N transfer rates in the culture solution, in the membrane and inside the cell. The overall permeation coefficient, *P*, can be defined as

$$J = P \cdot (\gamma'^{*}_{\mathrm{TAN}} - \gamma'_{\mathrm{TAN}}) \tag{17}$$

here the driving force of the permeation is expressed as the concentration difference of the hypothetical concentration of ammonia-N inside the cell in equilibrium to the ammonia-N concentration in the culture solution, $\gamma'^{*}_{\mathrm{TAN}}$, and the ammonia-N concentration inside the cell, γ'_{TAN}. The overall permeation coefficient, *P*, can be written as

$$\frac{1}{P} = \frac{K_2}{K_1} \cdot \frac{1}{k_{\mathrm{e}}} + \frac{1}{\overline{\gamma_{\mathrm{A}}} \cdot k_{\mathrm{m}}} + \frac{1}{k_{\mathrm{c}}} \tag{18}$$

To obtain the overall permeation coefficient, the equilibrium information is essential for estimation of $\gamma'^{*}_{\mathrm{TAN}}$ values and the ammonia-N distribution should be measured under the conditions that the assimilation would be inhibited.

To measure the uptake rate under conditions without assimilation, methionine sulfoximine[11] was used as an inhibition agent. This agent is known as a general metabolic inhibitor, and the effects on other parts of the cell are not discussed. Therefore this agent might have direct influence on the cell membrane and consequently it possibly affects the distribution and permeation of ammonia-N compounds through the cell membrane. Here, the effects of methionine sulfoximine on the cell membrane were not being experimentally confirmed, and therefore it was assumed that it would work only as an inhibition agent. The seaweed was pretreated with methionine sulfoximine solution to inhibit assimilation and the treated seaweed was utilized for ammonia-N uptake runs. The change of ammonia-N concentration in the culture solution was measured to obtain the ammonia-N permeation rate through the cell membrane. After enough time, the system attained equilibrium, and the distribution of the ammonia-N concentrations inside and outside of the cell could be obtained. The detailed procedures are given in Experimental chapter.

Experimental

Material

Sterile *Ulva* sp. was collected in July in 2009 from Kanazawa Bay (Yokohama, Japan, 35°20′32N, 139°38′32E). The principal properties of this seaweed were reported in previous papers.[10] Commercial sea salt, purchased from Akuazarutsu, Nisseisangyokabushikigasha, a joint-stock company, was used to prepare the culture solution for the artificial seawater. Ammonium chloride, sodium dihydrogen phosphate and methionine sulfoximine, special grade chemicals purchased from Wako Pure Chemical Industries, Ltd. (Japan), were used as

sources of ammonia-N and phosphorous in the culture solution and as an assimilation inhibitor, respectively.

Preservation of seaweed

The principal procedure to preserve the collected seaweed was the same as the method shown in our previous work.[10] The collected seaweed was thoroughly washed with artificial seawater to remove epiphytes and mud. To prepare "starved seaweed", the washed seaweed was cultivated for more than 24 hours in artificial seawater containing no additives. A glass container of 0.3×0.2×0.2 m (W×D×H) was used as an aquarium and equipped with aeration agitation and metal halide lamp, EYE Clean-Ace M400DL/BUDP, purchased from Iwasaki Electric Co., Ltd. (Japan). The lamp was situated just above the center of the container and the level of the container was adjustable with lab-jack to control the photosynthetic photon flux on the surface of the solution. The temperature and photosynthetic photon flux, PPF, were controlled at 295 K and 1800 μmol m^{-2} s^{-1}, respectively. Before the uptake runs, the starved seaweed was pretreated for 2 hours in an aqueous solution of 1 mmol L^{-1} methionine sulfoximine to inhibit assimilation. After preservation, the dry mass of the seaweed, DM, was measured by the same method as described in our previous work.[10] The "starved" seaweed was wiped with paper towels and was kept in the desiccator with silica gel at room temperature for several days until the mass of dried seaweed was constant. The mass of the dried seaweed was defined as DM. The surface area, a, of the used seaweed was measured by spreading the fresh seaweed on a sheet of graph paper. DM and a were utilized to estimate γ'_{TAN}, γ'^{*}_{TAN} and $\pi_{u,TAN}$.

Uptake run

After the starved seaweed was pretreated in an aqueous solution of 1 mmol L^{-1} methionine sulfoximine for 2 hours, it was washed with artificial seawater and wiped with paper towels, Kimtowel wipers, purchased from VRECIA Corp., to roughly remove the seawater left on the seaweed surface.

The principal conditions of uptake runs are summarized in Table 1. The cultivation was assumed to be conducted in the city of Rayong in Thailand and these conditions were based on information from NNDC Climate Data Online, which were observed from October 2007 to October 2008. The temperature and PPF were fixed at 303 K and 1800 μmol m^{-2} s^{-1} for daytime conditions, and at 298 K and less than 5 μmol m^{-2} s^{-1} for nighttime conditions, respectively.

Table 1 – *Experimental conditions for uptake and excretion measurements*

V	[m^3]	$2.0 \cdot 10^{-3}$
$\gamma_{TAN,0}$	[kg m^{-3}]	$0.25 \cdot 10^{-3}$ – $2 \cdot 10^{-3}$
$\gamma_{P,0}$	[kg m^{-3}]	$0.1 \cdot 10^{-3}$
salinity of culture solution	[kg m^{-3}]	30
ρ_u	[kg DM m^{-3}]	$0.064 \cdot 10^{-3}$ – $0.61 \cdot 10^{-3}$
PPF	[μmol s^{-1} m^{-2}]	0 (nighttime), 1800 (daytime)
T	[K]	298 (nighttime), 303 (daytime)
F/D	[-]	3.8 – 6.77

The apparatus for the uptake experiment is schematically shown in Fig. 2. A commercially available glass beaker of $2.0 \cdot 10^{-3}$ m^3 was used as a main aquarium for uptake run, equipped with magnetic stirrer. To avoid the seaweed to be torn apart by contact with the stirrer tip, the nylon net of 3360 μm line gap and 1000 μm line diameter was equipped with the bottom inside of the aquarium. The seaweed could not pass through the net and the solution in the vessel could be fully agitated. A metal halide lamp was also provided for a light source to situate just above the center of the glass beaker. The diameters of the illumination part of PPF generator and the shade were 0.12 m and 0.42 m, respectively. The level of the surface of the solution could be adjusted for PPF value to be controlled. PPF was measured at several points of the solution surface by a quantum meter and there was no distribution of PPF on the solution surface. After the artificial seawater and the starved seaweed were stirred at a fixed agitation rate and the temperature became stable, the uptake measurement started by addition of the ammonia-N solution of the specified concentrations. The cultivation solution of 5 mL

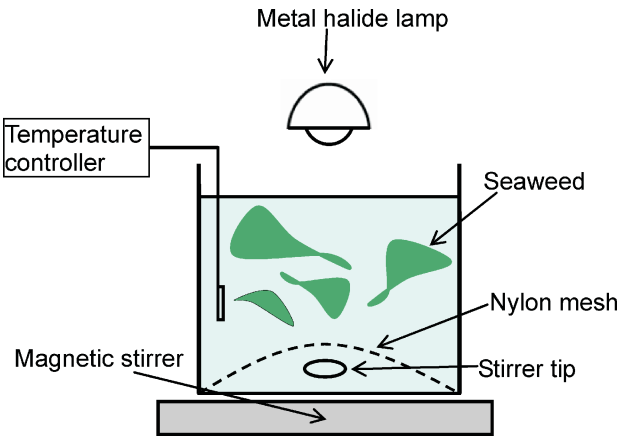


Fig. 2 – *Schematic diagram of experimental apparatus*

was taken at specified periods for the observation of ammonia-N concentration changes. For some cases, excretion measurements were conducted in which after the specified uptake period the seaweed was taken and put into fresh seawater containing no additives. By this measurement, the excretion rates and the distribution equilibrium were measured to compare the effects of the ammonia-N transfer direction.

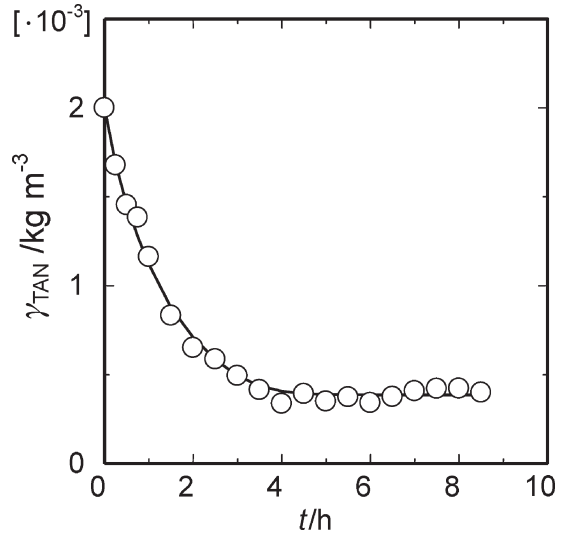
Analysis

The concentrations of ammonia-N, NH_3 and NH_4^+, in solutions were determined by the indophenol blue method.[12] PPF was measured at several points of water surface by a quantum meter, Model QMSS purchased from Apogee Instruments Inc. TM.

Results and discussion

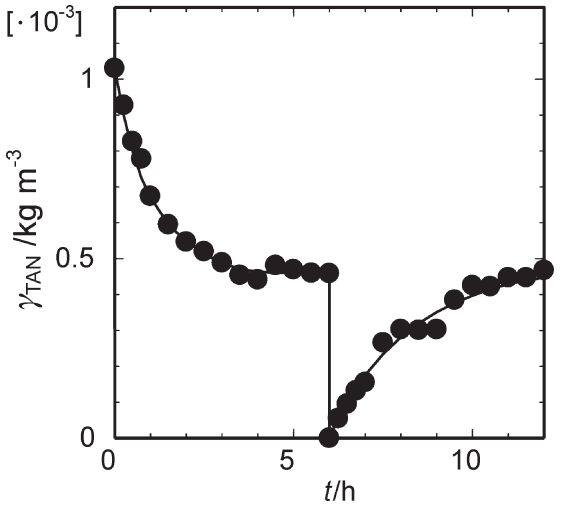
Before the uptake runs, the specific surface area, *a*, and the mass ratio of fresh and dried seaweed, *F/D*, were measured. The average values were 146 $m^2\ kg^{-1}$ DM and 4.8, which were slightly lower than those of our previous work.[9]

Fig. 3 shows one example of the ammonia-N concentration change in the culture medium over the uptake time under the conditions of daytime. The ammonia-N concentration in the culture medium decreased with time, and after 6 hours the concentration became constant for all cases to attain the equilibrium conditions. At equilibrium, the ammonium-N concentration in the seaweed, γ'_{TAN}, increased with γ_{TAN} and the detailed results about the ammonia-N distribution will be discussed later.



F i g . 3 – *Example of time course of ammonia-N concentration in culture solution in uptake measurement in daytime conditions; D/M: 4.6,* $\gamma_{TAN,0}$*=2.0 · 10⁻³ kg m⁻³,* ρ_u *= 0.28 · 10⁻³ kg DM m⁻³*

Fig. 4 shows an example of the results of excretion measurements. Some uptake measurements were continued for the excretion measurements. At t = 6 hrs, attaining the equilibrium condition, the culture solution was changed to the fresh artificial seawater containing no additives. The excretion measurements were conducted to confirm whether the ammonia-N once taken in would be excreted into the culture solution and to compare the uptake and excretion behaviors. As shown in Fig. 4, the γ_{TAN} was changed in the same way as the result shown in Fig. 3 in the first 6 hrs, and the system attained the equilibrium conditions at 6 hours. After changing the culture solution to the fresh artificial seawater, the seaweed started to excrete the ammonia-N to the culture solution and the concentration of ammonia-N began to increase. The ammonia-N concentration became constant until reaching another equilibrium condition after 6 hours from solution replacement. γ'_{TAN} also increased with γ_{TAN} at equilibrium for the excretion measurement, which will be discussed later.



F i g . 4 – *Example of time course of ammonia-N concentration in culture solution in uptake and excretion measurements in daytime conditions; D/M: 4.3,* $\gamma_{TAN,0}$ *= 1.0 · 10⁻³ kg m⁻³,* ρ_u *= 0.20 · 10⁻³ kg DM m⁻³*

The results of the uptake runs conducted under nighttime conditions are shown in Fig. 5. The ammonia-N concentration in the culture solution decreased with time at the initial stage; however, the concentration increased in most cases. In other words, the seaweed could once take in the ammonia-N at the initial stage and the seaweed started to excrete the ammonia-N within several hours. The reason causing this behavior is unclear at this moment. Originally, the performance of ammonia-N uptake was degraded in nighttime conditions. Sunlight might work not only on the assimilation but also on the active transport of ammonia-N through

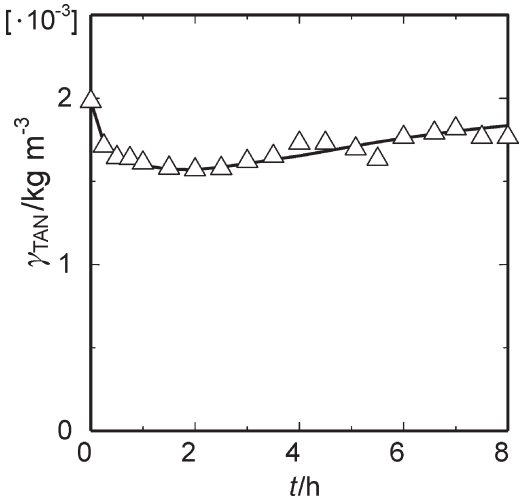


Fig. 5 – *Example of time course of ammonia-N concentration in culture solution in uptake measurement in night-time conditions; D/M: 4.9,* $\gamma_{TAN,0} = 2.0 \cdot 10^{-3}$ *kg m*$^{-3}$, $\rho_u = 0.35 \cdot 10^{-3}$ *kg m*$^{-3}$

the cell membrane. The assimilation of ammonia-N by seaweed can be basically facilitated under daytime conditions. In the early stage of the uptake run, the seaweed could once uptake the ammonia-N compound because it was just after the change of the culture solution and the ammonia-N compound might permeate due to the large concentration difference between inside and outside. However, the seaweed could not afford to keep the ammonia-N inside of the cell because the assimilation was limited and the seaweed might excrete the excessive ammonia-N compound out of the cell.

Fig. 6 shows the relationships between the ammonia-N concentrations in the seaweed, γ'_{TAN}, and the ammonia-N concentration in the culture medium, γ_{TAN}. Black and white keys stand for the results measured in the uptake and excretion measurements, respectively. Here γ'_{TAN} and γ_{TAN} were defined as the values measured at 6 hours in the uptake rate measurements, and at 6 hours after change of the culture solution in the excretion measurements, respectively and γ'_{TAN}s were estimated from the mass balance eq. (1) when the assimilation rate, π_a, was fixed at zero. The relationships were assumed to be equilibrium between the inside and outside of the cell. In all cases, γ'_{TAN}s of uptake measurement were larger than those of excretion measurement. γ'_{TAN} increased with an increase in γ_{TAN} and γ'_{TAN} sharply increased in the low range of γ_{TAN} for both cases. γ'_{TAN} gently increased in the range of $\gamma_{TAN} > 0.5 \cdot 10^{-3}$ kg N m^{-3} for the cases of uptake measurements and in the range of $\gamma_{TAN} > 0.1 \cdot 10^{-3}$ kg N m^{-3} for the cases of excretion measurements, respectively. Based on this relationship, the hypothetical ammonia-N concentration equilibrium to the culture medium, γ'^{*}_{TAN} in eq. (17), could be estimated.

Fig. 7 shows the effects of the ammonia-N concentration, γ_{TAN}, on the ratio of the inside and outside concentrations at equilibrium, φ, and black and white keys represent the results of uptake and excretion measurements, respectively. φ was estimated by the following equation.

$$\varphi = \frac{\gamma'_{TAN} \cdot \rho_u}{\gamma_{TAN}} \tag{19}$$

where ρ_u stands for the density of the dry-mass based seaweed used in the measurement relative to the culture solution. The ratios were larger in case of the uptake measurements than that in case of the excretion measurements, as shown in Fig. 5. The ratio increased with the decrease of γ_{TAN} and the up-

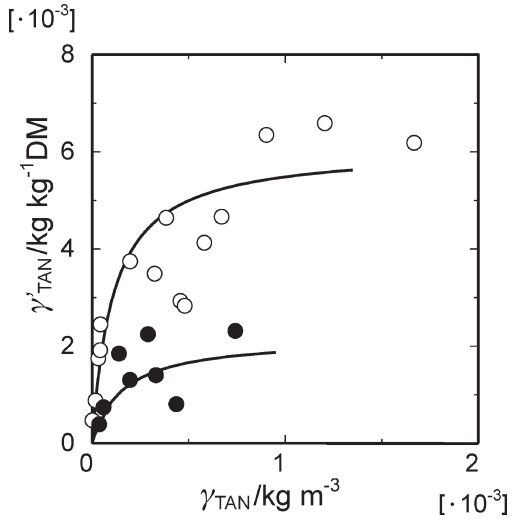


Fig. 6 – *Ammonia-N distribution equilibrium between culture solution and cell inside; ○: uptake measurements, ●: excretion measurements*

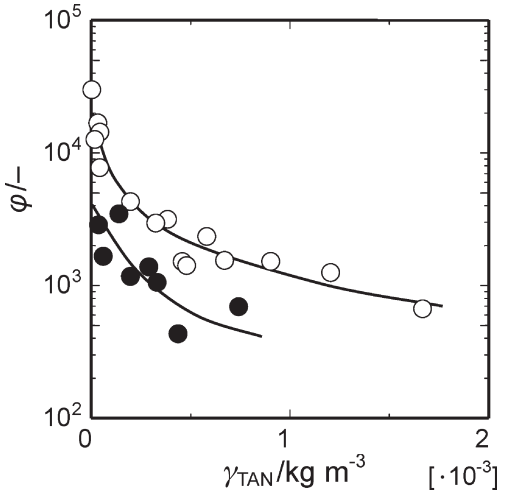


Fig. 7 – *Effects of ammonia-N concentration ratio in seaweed inside and culture solution at equilibrium, defined as eq. (19); ○: uptake measurements, ●: excretion measurements*

take efficiency was enhanced in the low concentration region, especially for the results in uptake measurements. This behavior is so preferable for the treatment of seawater in case that the ammonia-N concentration should be kept so low, like the case of shrimp farming.

Fig. 8 shows the Michaelis-Menten plots in daytime conditions. The white and black keys stand for the uptake rates measured at $t = 0$ and $t = 1$ h, respectively and the solid line represents the initial uptake rate modeled by the Michaelis-Menten equation, measured in the previous work.[10] The model can fully represent the uptake rate at the initial conditions and the uptake rates measured at $t = 1$ h were smaller than the estimated values. As mentioned in our previous work,[10] the conditions of the seaweed inside could be consistent only by the pretreatment. It was so difficult to compare the uptake rates measured in the course of the runs because the conditions of other than the ammonia-N concentration might be influential and could not be made consistent, and the Michaelis-Menten model must be sensitive to the change in conditions. The inhibition factor, α, or another model of uptake rate is therefore required to express the rates for any given condition. As discussed above, it was confirmed that the ammonia-N compound which permeated through the cell membrane into the inside of the cell should permeate back to the culture medium. This should require the Michaelis-Menten model to be modified and the permeation model might be more reasonable for this case. Then the permeation model was used for the expression of the uptake rate.

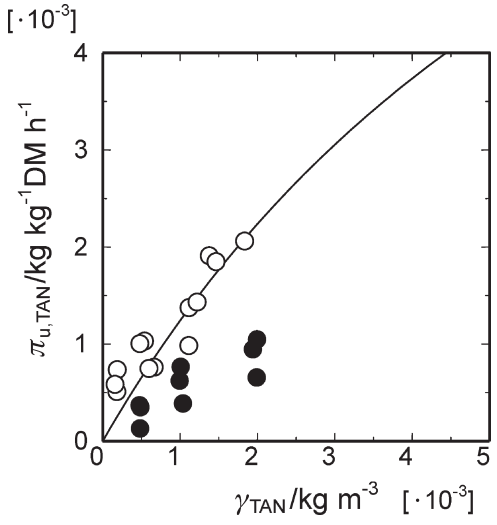
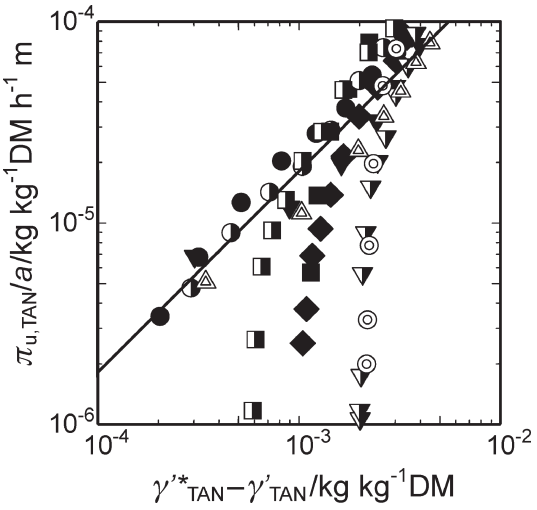


Fig. 8 – *Michaelis-Menten plotting; ○: values of $\pi_{u,TAN}$ at initial, ●: values of $\pi_{u,TAN}$ at t =1 h*

Figs. 9 and 10 show the effects of the concentration difference between γ'_{TAN} and the hypothetical concentration of ammonia-N equilibrium to



Key	$\gamma_{TAN,0}\cdot10^3$	$\rho_u\cdot10^3$
●	2.0	0.28
▲	1.0	0.20
■	0.5	0.18
▼	1.0	0.20
◆	0.5	0.23
◑	2.0	0.35
◭	2.0	0.18
◨	1.0	0.23
◬	0.5	0.26
◎	1.0	0.23
◬	2.0	0.13

Fig. 9 – *Effects of driving forces on ammonia-N flux in uptake measurements; Solid line: $\pi_{u,TAN}/a = 1.81 \cdot 10^{-2}$ $(\gamma'^{*}_{TAN} - \gamma'_{TAN})$*

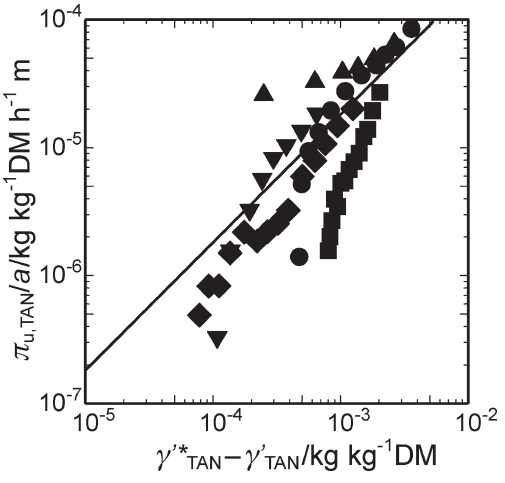


Fig. 10 – *Effects of driving forces on ammonia-N flux in excretion measurements; Solid line: $\pi_{u,TAN}/a = 1.81 \cdot 10^{-2}$ $(\gamma'^{*}_{TAN} - \gamma'_{TAN})$, keys are same as in Fig. 9*

γ_{TAN}, γ'^{*}_{TAN}, on the overall flux of the ammonia-N through the specific surface area of seaweed, J, expressed by eq. (17) at the uptake and excretion measurements, respectively. The uptake and excretion rates were estimated from the results as exemplified in Figs. 3 and 4. The relationship between the uptake/excretion rate and overall flux is expressed as follows,

$$J = \pi_{u,TAN}/a = P \cdot (\gamma'^{*}_{TAN} - \gamma'_{TAN}) \quad (20)$$

Here the value of a was measured before each run and the average value was 143 $m^2\ kg^{-1}$ DM. The overall flux was approximately proportional to the driving force for both cases of the uptake and excretion runs. The overall permeation coefficient was estimated to be $9.1 \cdot 10^{-3}$ m h^{-1} and the fluxes for both uptake and excretion cases could be expressed by the same line. However, in the ranges of small driving forces the measured values were deviated to be lower than the estimated line. These points were so close to the equilibrium conditions and the error of the driving forces essentially became large in this range. In other words, when the permeations were measured in the low initial ammonia-N concentration range or the driving force was so small, the deviations from the estimated line became large. The sufficient driving force could be maintained in the ranges of higher $c_{TAN,0}$ and in the initial periods of uptake runs. Consequently, the overall permeation coefficient was decided from the data in the regions of larger driving forces. In the cases of excretion, similar trends to the uptake runs could be observed and the flux followed the same line as that of uptake rate. The tendency of deviation was also the same and in the range of smaller driving forces the error became large. The relationships in cases of the uptake and excretion, valid in the specified conditions of γ'_{TAN}, could be expressing the permeation flux of ammonia-nitrogen. As mentioned in previous work (9), the effects of the inhibitory factor became larger as the seaweed took in the ammonia-N and the factor could be specified only when the starved alga was used. The suggested model, within only limited situation, could express the permeation rate not only in case of starved conditions. However, this model was applied only to the ammonia-N permeation through the cell membrane and should be developed to incorporate the following steps until the assimilation step. The method then would be possibly effectual for the design and operation of the shrimp farming in the closed pond.

Conclusion

The ammonia-nitrogen uptake performance by sterile *Ulva* sp. was studied for the development of intensive closed-system shrimp farming to control the pond water quality. Based on the concept of ammonia-nitrogen distribution around the culture solution and cell, the equilibrium and uptake behaviors were in advance discussed to develop the uptake model and the permeation model was suggested to analyze the uptake performance. The ammonia-nitrogen distribution equilibriums were measured in the cases of both uptake and excretion using the seaweed pretreated with methionine sulfoximine to inhibit the assimilation. At equilibrium, the ammonia-nitrogen concentration in the cell increased with that in the culture solution and the ratios of the inside and outside concentrations were larger for the uptake runs than those for the excretion runs. The suggested model, under only limited conditions, could express the ammonia-nitrogen permeation rate and the overall permeation coefficient was estimated as $9.1 \cdot 10^{-3}$ m h^{-1}. The model was valid only in case that the driving force of the ammonia-nitrogen flux was large enough and both inward and outward transfers of ammonia-nitrogen through the cell membrane were observed to follow the permeation model. The measured information about distribution equilibrium and permeation rate should be helpful in evaluating a system of water quality control for intensive shrimp pond culture with sterile *Ulva* sp.

Nomenclature

- a – specific surface area of seaweed in culture solution, m^{-1}
- $\overline{\gamma_i}$ – concentration of component i in membrane, kg m^{-3}
- γ_{TAN} – concentration of ammonia-N in culture solution, kg m^{-3}
- γ'_{TAN} – concentration of ammonia-N in seaweed, kg kg^{-1}
- γ'^{*}_{TAN} – hypothetical concentration of ammonia-N in seaweed equilibrium to γ_{TAN}, kg kg^{-1} DM
- γ_P – concentration of phosphoric acid phosphorus, kg m^{-3}
- F/D – mass ratio of fresh relative to dried seaweed, –
- J – overall permeation flux of ammonia-N, kg kg^{-1} DM h^{-1} m^{-2}
- J_c – flux of ammonia-N of ammonia-N transfer in cell, kg kg^{-1} DM h^{-1} m^{-2}
- J_e – flux of ammonia-N of ammonia-N transfer in culture solution, kg kg^{-1} h^{-1} m^{-2}
- J_m – flux of ammonia-N of ammonia-N transfer in membrane, kg kg^{-1} DM h^{-1} m^{-2}
- K_1 – dissociation coefficient, in eq. (8), kg^{-1} m^3
- K_2 – dissociation coefficient, in eq. (9), kg^{-1} kg DM
- K_M – Michaelis coefficient, kg m^{-3}
- k_e – mass transfer coefficient of ammonia-N in culture solution, m h^{-1}
- k_m – mass transfer coefficient of ammonia-N-carrier complex in membrane, m h^{-1}
- k_c – mass transfer coefficient of ammonia-N in alga cell, m h^{-1}
- m – dry mass of algae, kg DM
- T – water temperature, K
- t – time, h
- V – volume of culture solution, m^3

V_{max} – saturated uptake rate of ammonia-N in the Michaelis-Menten equation, kg kg^{-1} DM h^{-1}

α – inhibitory factor, –

φ – ammonia-N concentration ratio of the inside and outside concentrations at equilibrium, defined as eq. (19), –

$\pi_{a,TAN}$ – specific assimilation rate of ammonia-N, kg kg^{-1} DM h^{-1}

$\pi_{u,TAN}$ – specific uptake rate of ammonia-N, kg kg^{-1} DM h^{-1}

ρ_u – culture density of seaweed, kg DM m^{-3}

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