Effects of Inhibitory Factor on Uptake Rate of Ammonia-Nitrogen with Sterile *Ulva* sp. for Water Quality Control of Intensive Shrimp Culture Ponds

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Ammonia-nitrogen uptake by sterile *Ulva* sp. was studied for the control of culture pond water of intensive shrimp farming. The uptake rates were measured by batch and semi-continuous operations, and analyzed with the Michaelis-Menten model of uncompetitive inhibition. For the batch uptake operations, the Michaelis-Menten parameters were estimated, and the maximum rate and Michaelis constants were estimated as 3.4×10^{-2} kg kg^{-1} h^{-1} and 5.5×10^{-3} kg m^{-3}, respectively. The inhibitory factor increased with the uptake time and with the decrease of the seaweed density. In the cases of semi-continuous operations, the seaweed could continuously treat with the model farming culture solution. Although the ratio of the seaweed density relative to the rate of ammonia-nitrogen generation should be appropriately adjusted to keep lower inhibitory factor in the seaweed, the ammonia-nitrogen concentration could be maintained at a relatively low level during operation. Then the ammonia-nitrogen uptake by the alga water was roughly simulated and operation with moderate density of the algae in the pond could maintain the ammonia-nitrogen concentration at a sufficiently low level in the shrimp farming pond. The suggested treatment process might be attractive to control pond water quality for intensive shrimp farming.

Key words:
Ammonia-nitrogen uptake, sterile *Ulva* sp., water quality control of intensive shrimp culture ponds, Michaelis-Menten model, inhibitory factor

Introduction

Massive shrimp mortality caused by infectious diseases has been regarded as a serious issue for shrimp culture industries in developing countries. The white spot syndrome virus is one of the most serious pathogens and it has been causing frequent epidemics since the 1990s.[1–3] As a simple solution to this issue, shrimp farming in developing countries is shifting from open-culture systems to closed systems of intensive farming in order to avoid contamination.[4] This transition requires the technology to control the culture pond water quality. In closed-culture ponds the water quality is deteriorated mainly by the decomposition of the organic-rich sediment, derived from the uneaten feed of the cultured shrimp, as well as the metabolism of the cultured shrimp. In both cases, ammonia-nitrogen (ammonia-N) is a major contaminant and one of the most toxic compounds for most marine organisms, causing growth inhibition, and at worst, extinction of the farmed creatures.[5] The ammonia-N removal system is a key technology to control the quality of culture pond water for intensive shrimp farming. There were some reports on the treatment of seawater to control the water quality by macroalgae and microalgae. Most of them are related to the preservation of the ecosystem around shallows.[6] Only a few studies have been presented for marine cultivations purposes, while some proposals to remove the ammonia-N have been presented. Biofiltration was reported as one of the most promising technologies.[7–10] The system could successfully treat the culture pond water to maintain ammonia-N concentration at low levels for the intensive mariculture of *Sparus aurata*. Sterile *Ulva sp*., a kind of general seaweed, was studied to control the ammonia-N concentration in the culture pond water under tropical conditions.[11–13] The alga was characterized

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based on the ammonia-N uptake rate to show the characteristic feature of the Michaelis-Menten model with uncompetitive inhibition as well.[11] The ammonia-N uptake by sterile *Ulva* sp. was simulated experimentally and numerically, and it was found that the ammonia-N concentration could be kept low in the culture pond by using sterile *Ulva* sp. In many cases, the ammonia-N uptake rate was expressed by the Michaelis-Menten model, which has been used widely to express the ammonia-N uptake rates by some kinds of seaweeds. However, the inhibitor constant was not discussed well although the constant was influential on the uptake rates.[12] The distribution of ammonia-N between the culture solution and cell inside the algae was measured to analyze the ammonia-N permeation rate through the cell membrane of the algae.[13] However, the detailed mechanism of ammonia-N uptake is still unclear and possible later study is crucial.

This study aims to analyze the ammonia-N uptake rate in light of the inhibitor factor of the Michaelis-Menten model. The batch uptake measurements were conducted to overview the relationship between the uptake rate and inhibitory factor. In order to simulate the ammonia-N generation and uptake in the farming culture pond, the ammonia-N solution was continuously provided to the solution to model the generation and uptake of ammonia-N, and the ammonia-N uptake by the alga was measured. With these measurements, the inhibitory factors were measured and the uptake performances were evaluated.

Ammonia-nitrogen uptake by seaweed

The uptake rate of ammonia-N by sterile *Ulva* sp. was discussed in our previous works.[11,12] Fig. 1 shows the general concept of ammonia-N uptake by macroalgae. The ammonia-N uptake consists of two steps: ammonium ion permeation from culture solution into cytosol through the cell membrane by carrier-mediated transport and assimilation into dissolved organic nitrogen (DON). The material balance of ammonia-N around the seaweed could be described as:

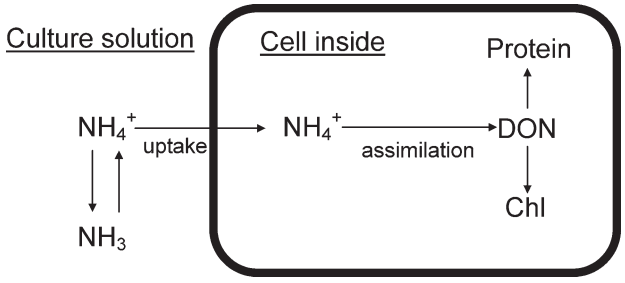


Fig 1 – *General concept of ammonia-nitrogen uptake by macroalga*

$$\frac{dC_{\mathrm{TAN}}}{dt} = r_{\mathrm{TAN}} - \rho_{\mathrm{u}} \cdot \pi_{\mathrm{u,TAN}} \tag{1}$$

where C_{TAN}, r_{TAN}, ρ_{u}, and $\pi_{\mathrm{u,TAN}}$ are the total concentration of ammonia-N in the culture solution, the rate of ammonia-N generation in a unit volume of the culture solution, the density of seaweed in the culture solution, and the rate of ammonia-N uptake by a unit dry mass of seaweed, respectively. In general, ammonia-N permeates as ammonium ions, NH_4^+, through the cell membrane and this overall uptake process could be written as:

$$A + C \rightleftarrows AC \tag{2}$$

$$AC \rightarrow A' + C \tag{3}$$

where A, C, AC and A' stand for NH_4^+ in the cultivation solution, a carrier in the membrane, a complex of the carrier and NH_4^+ in the membrane, and NH_4^+ in the cell, respectively. If the uncompetitive inhibition effect should be considered, the inhibition effect could be expressed as:

$$AC + I \rightleftarrows ACI \tag{4}$$

where I stands for the inhibitor of the uncompetitive inhibition. In our previous study,[11] it was discussed that sterile *Ulva* sp. should follow the model of the uncompetitive inhibition for the ammonia-N uptake. With these equations and the Michaelis-Menten model, $\pi_{\mathrm{u,TAN}}$ could be written as:

$$\pi_{\mathrm{u,TAN}} = \frac{V_{\max} \cdot C_{\mathrm{TAN}}}{K_{\mathrm{M}} + (1+\alpha) \cdot C_{\mathrm{TAN}}} \tag{5}$$

where $V_{\max}$ and K_{M} are the constants in the Michaelis-Menten model, and α stands for the inhibitory factor. When $\alpha = 0$, Eq. (5) is showing the Michaelis-Menten model without effects of inhibitor. Equation (1) can be rewritten with Eq. (5) to give:

$$\frac{dC_{\mathrm{TAN}}}{dt} = r_{\mathrm{TAN}} - \rho_{\mathrm{u}} \cdot \frac{V_{\max} \cdot C_{\mathrm{TAN}}}{K_{\mathrm{M}} + (1+\alpha) \cdot C_{\mathrm{TAN}}} \tag{6}$$

For the continuous operation of shrimp cultivation, the requirement for the ammonia-N concentration could be expressed as:

$$\frac{dC_{\mathrm{TAN}}}{dt} \leq 0 \tag{7}$$

that is;

$$r_{\mathrm{TAN}} \leq \rho_{\mathrm{u}} \cdot \frac{V_{\max} \cdot C_{\mathrm{TAN}}}{K_{\mathrm{M}} + (1+\alpha) \cdot C_{\mathrm{TAN}}} \tag{8}$$

As another restriction, the ammonia-N concentration in seaweed, C'_{TAN}, should not increase with

time at steady state because α increased with C'_{TAN},[11]

$$\frac{dC'_{\mathrm{TAN}}}{dt} \leq 0 \tag{9}$$

Under this condition, the assimilation rate, $\pi_{\mathrm{a,TAN}}$, should be equal to or larger than the uptake rate, $\pi_{\mathrm{u,TAN}}$, to give:

$$\pi_{\mathrm{u,TAN}} \leq \pi_{\mathrm{a,TAN}} \tag{10}$$

$$C_{\mathrm{TAN}} \leq \frac{\mathrm{K_M} \cdot \pi_{a,\mathrm{TAN}}}{V_{\max} - \pi_{\mathrm{a,TAN}} \cdot (1+\alpha)} \tag{11}$$

With these necessary conditions, the system of the shrimp culture pond would attain the steady state, and Eq.(1) could be rewritten as:

$$0 = r_{TAN} - \rho_{\mathrm{u}} \cdot \frac{V_{\max} \cdot C_{\mathrm{TAN,st}}}{K_{\mathrm{M}} + (1+\alpha) \cdot C_{\mathrm{TAN,st}}} \tag{12}$$

The ammonia-N concentration in the culture solution at steady state could be expressed as:

$$C_{\mathrm{TAN,st}} = \frac{V_{\max} \cdot r_{\mathrm{TAN}}}{\rho_{\mathrm{u}} \cdot K_{\mathrm{M}} - (1+\alpha) \cdot r_{\mathrm{TAN}}} \tag{13}$$

When α and C_{TAN} are small enough, and $(1+\alpha) \cdot C_{\mathrm{TAN}}$ is much smaller than K_{M}, the uptake rate could be rewritten as:

$$\pi_{\mathrm{u,TAN}} = \frac{V_{\max}}{K_{\mathrm{M}}} \cdot C_{\mathrm{TAN}} \tag{14}$$

The uptake rate should be proportional to C_{TAN} and $C_{\mathrm{TAN,st}}$ could be expressed as:

$$C_{\mathrm{TAN,st}} = \frac{K_{\mathrm{M}}}{\rho_{\mathrm{u}} \cdot V_{\max}} \cdot r_{\mathrm{TAN}} \tag{15}$$

When the value of α is large enough, and K_{M} is much smaller than $(1+\alpha) \cdot C_{\mathrm{TAN}}$, the Michaelis-Menten equation could be reduced as:

$$\pi_{\mathrm{u,TAN}} = \frac{V_{\max}}{\alpha} \tag{16}$$

Therefore, the uptake rate should be independent of C_{TAN}. In this situation, the operation to control the pond water quality for intensive shrimp farming would only depend on the seaweed density in the shrimp culture pond, ρ_{u}, and the essential condition for ρ_{u} is specified as:

$$\rho_{\mathrm{u}} \geq \frac{\alpha \cdot r_{\mathrm{TAN}}}{V_{\max}} \tag{17}$$

In this study, the effects of these parameters, especially for the inhibitory factor, on the ammonia-N uptake rates are discussed, which could be helpful for the development of the pond water quality control in intensive shrimp farming. The ammonia-N uptake measurements by batch operation were conducted to overview the ammonia-N uptake by seaweed, and to estimate the fundamental parameters of the Michaelis-Menten equation, such as $V_{\max}$ and K_{M} at initial conditions. The semi-continuous measurements were carried out for the estimation of α at steady state to clarify the contributions of the ammonia-N permeation and assimilation.

Experimental

Material

Sterile *Ulva* sp. was collected from Kanazawa Bay (Yokohama, Japan, 35°20′32N, 139°38′32E) and the principal properties of the used seaweed were listed in the previous papers.[12,13] The ratio of dry mass relative to fresh mass of the used algae, D/F, tissue H_2O, specific surface area were 4.2, 3.2×10^{-3} m^3 kg^{-1}, and 9.1×10^{-2} m^2 kg^{-1}, respectively. The artificial seawater containing commercial sea salt, Akuazarutsu purchased from Nisseisangyokabushikigasha, a joint-stock company, was used to prepare the culture solution. Ammonium chloride and sodium dihydrogen phosphate, special grade chemicals purchased from Wako Pure Chemical Industries, Ltd. (Japan), were used as sources of ammonia-N and phosphorous in the culture solution, respectively.

Preservation of seaweed

The principal procedure to preserve the collected seaweed was the same as the method shown in our previous work[12] and the preservation conditions are listed in Table 1. The collected seaweed was meticulously washed by artificial seawater to remove epiphytes and mud. To prepare "starved sea-

Table 1 – *Experimental conditions for preservation of seaweed*

V	[m^{-3}]	2.0×10^{-3}
$C_{\mathrm{TAN,0}}$	[kg m^{-3}]	0
$C_{\mathrm{P,0}}$	[kg m^{-3}]	0
Salinity of culture solution	[kg m^{-3}]	30
PPF	[μmol s^{-1} m^{-2}]	0 (nighttime), 1800 (daytime)
T	[K]	298 (nighttime), 303 (daytime)
Daytime/nighttime cycle	[h]	14 / 10

weed", the washed seaweed was cultivated for more than 24 hours in artificial seawater containing no additive agent. A glass container of $5.0 \cdot 10^{-3}$ m^3 was used as an aquarium, equipped with aeration agitation and metal halide lamp, EYE Clean-Ace M400DL/BUDP, purchased from Iwasaki Electric Co., Ltd. (Japan). The temperature and photosynthetic photon flux, PPF, were controlled at 295 K and 800 μmol m^{-2} s^{-1} as daytime conditions and at 295 K and 0 μmol m^{-2} s^{-1} as nighttime conditions, respectively. The daytime/nighttime cycle was fixed as 14 hours and 10 hours, respectively. After preservation, the dry mass of the seaweed, DM, was measured by the same method as in our previous work.[11–13] The "starved seaweed" was wiped with paper towels to remove solution on the surface, and kept in the desiccator with silica gel at room temperature for several days until the mass of dried seaweed became constant.

Batch uptake of ammonia-nitrogen by seaweed

The principal conditions of uptake measurement by batch operation are summarized in Table 2. Before each uptake measurement, the starved seaweed was acclimatized for more than 0.5 h at 295 K and 800 μmol m^{-2} s^{-1} in the culture solution without ammonia-N. When $t = 0$ h, the specified amount of ammonia-N solution was added to the culture solution and the uptake was allowed to start. The cultivation was assumed to be conducted in a tropical climate, and the temperature and PPF were fixed at 303K and 1800 μmol m^{-2} s^{-1}.

Table 2 – *Experimental conditions for uptake measurement by batch operation*

V	[m^{-3}]	5.0×10^{-4}
$C_{TAN,0}$	[kg m^{-3}]	$0.25 \times 10^{-3} - 2 \times 10^{-3}$
$C_{P,0}$	[kg m^{-3}]	0.1×10^{-3}
Salinity of culture solution	[kg m^{-3}]	30
$\rho_{u,0}$	[kg m^{-3}]	0.12 – 3.4
PPF	[μmol s^{-1} m^{-2}]	1800
T	[K]	303

The experimental apparatus of the batch uptake run is schematically shown in Fig. 2. A commercially available glass beaker of 5.0×10^{-4} m^3 was used as an aquarium. A metal halide lamp was used as a light source, and PPF was measured at several points of the solution surface by a quantum meter, same as in the preservation. A magnetic stirring tip was used to completely mix the culture solution. To avoid the seaweed from being torn apart by contact with the stirrer tip, a nylon net of 3360 μm line gap and 1000 μm diameter line was equipped at the bottom inside of the aquarium. The seaweed could not pass through the net and the solution in the vessel could be fully agitated. The artificial seawater and starved seaweed were agitated at a fixed stirring rate for more than 0.5 h before each uptake measurement. After this acclimatization, the uptake measurement was allowed to start by adding the specified amount and concentration of ammonia-N solution. The cultivation solution of 5 ml was taken at specified periods for observation of ammonia-N concentration changes.

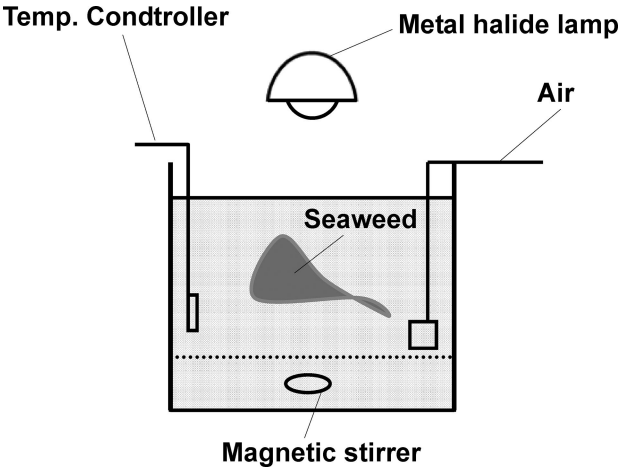


Fig. 2 – *Schematic diagram of experimental apparatus for batch uptake measurements*

Semi-continuous uptake of ammonia-nitrogen by seaweed

The uptake measurements by semi-continuous operation were conducted to analyze the uptake performance at steady state conditions. The principal conditions of uptake measurements by semi-continuous operation are listed in Table 3 and the apparatus is shown in Fig. 3. A glass beaker of 5.0×10^{-4} m^3 was used as an aquarium. Before starting uptake measurements, the starved seaweed was acclimatized in the culture solution without ammonia-N for at least 0.5 h, same as the batch uptake measure-

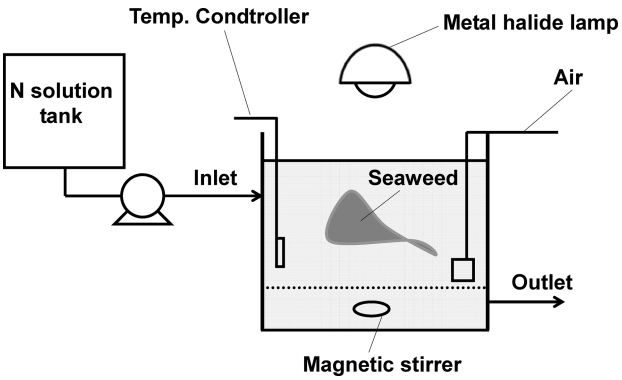


Fig. 3 – *Schematic diagram of experimental apparatus for semi-continuous uptake measurements*

Table 3 – *Experimental conditions for uptake measurements by semi-continuous operation*

V	[m^{-3}]	5.0×10^{-4}
$C_{TAN,0}$	[kg m^{-3}]	$0.25 \times 10^{-3} - 2 \times 10^{-3}$
$C_{P,0}$	[kg m^{-3}]	0.1×10^{-3}
Volume rate for ammonia-N supply	[m^3 h^{-1}]	1.2×10^{-4}
Salinity of culture solution	[kg m^{-3}]	30
$\rho_{u,0}$	[kg m^{-3}]	0.12 – 3.4
PPF	[μmol s^{-1} m^{-2}]	0 (nighttime), 1800 (daytime)
T	[K]	298 (nighttime), 303 (daytime)
Daytime/nighttime cycle	[h]	14 / 10

ments. At $t = 0$ started the supply of the ammonia-N solution for the cultivation solution with a plunger pump, and the ammonia-N solution was continuously provided to simulate generation of ammonia-N from shrimp, r_{TAN}. The overflowed solution was continuously taken from the aquarium and analyzed for ammonia-N concentration. The ammonia-N concentration in overflowed solution was assumed to be same as that in the aquarium. For short-time uptake measurements PPF was fixed at 1800 μmol m^{-2} s^{-1}. For long-time uptake measurements, a metal halide lamp was controlled to simulate the daytime and nighttime conditions and the cycle was fixed as 14 hours daytime and 10 hours nighttime.

Analysis

The concentrations of ammonia-N, NH_3 and NH_4^+, in the solutions were determined by the indophenol blue method.[14] PPF was measured at several points of water surface by a quantum meter, Model QMSS purchased from Apogee Instruments Inc. TM.

Results and discussion

Figs. 4 and 5 show the changes in ammonia-N concentrations in culture solutions, C_{TAN}, over the uptake time in the batch uptake measurements, and the fractional removal of ammonia-N, defined as the following equation;

$$E = \frac{C_{TAN} \cdot V}{C_{TAN,0} \cdot V_0} \tag{18}$$

In all cases, C_{TAN} and E decreased with time. The degree of the reduction in C_{TAN} increased with the initial concentration of ammonia-N in the culture solution. E was also affected by the initial ammonia-N concentration, and the removal efficiency improved with the decrease of initial concentration. The employed seaweed could treat more effectively with the culture solution of lower ammonia-N concentration. This property must be more favorable for the treatment of intensive culture pond water to keep the ammonia-N concentration at such a low level.

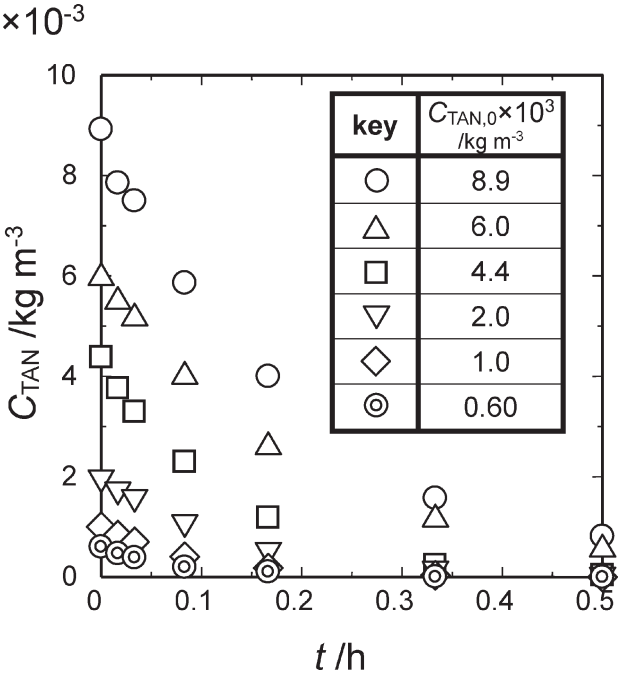


Fig. 4 – *Time courses of ammonia-nitrogen concentration in culture solution for batch uptake measurements. T: 303 K, PFP: 1800 μmol m^{-2} s^{-1}, $\rho_{u,0}$: 2.4 kg m^{-3}*

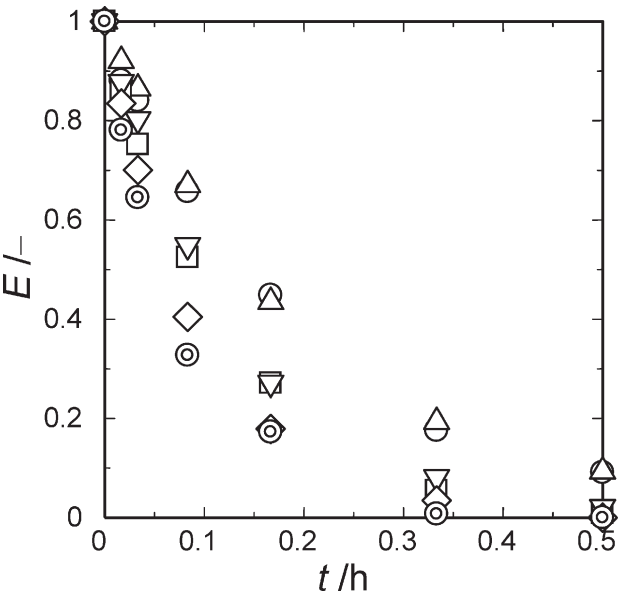


Fig. 5 – *Time courses of fraction removal for batch uptake measurements. T: 303 K, PFP: 1800 μmol m^{-2} s^{-1}, $\rho_{u,0}$: 2.4 kg m^{-3}, keys are same as Fig. 4.*

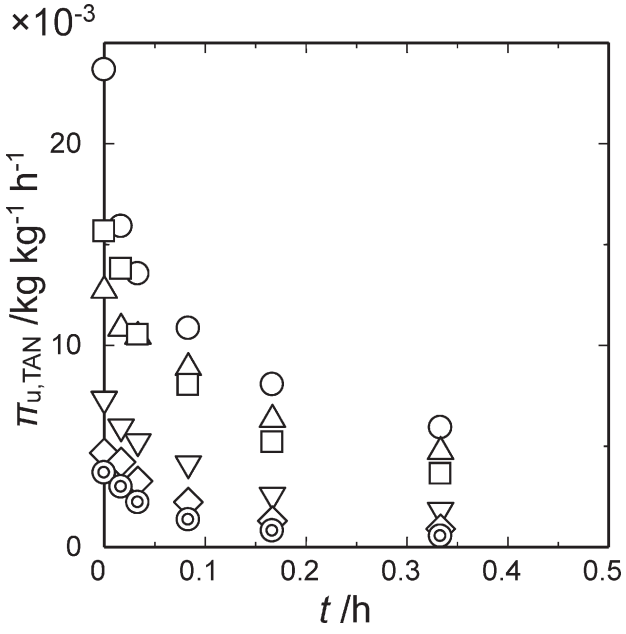
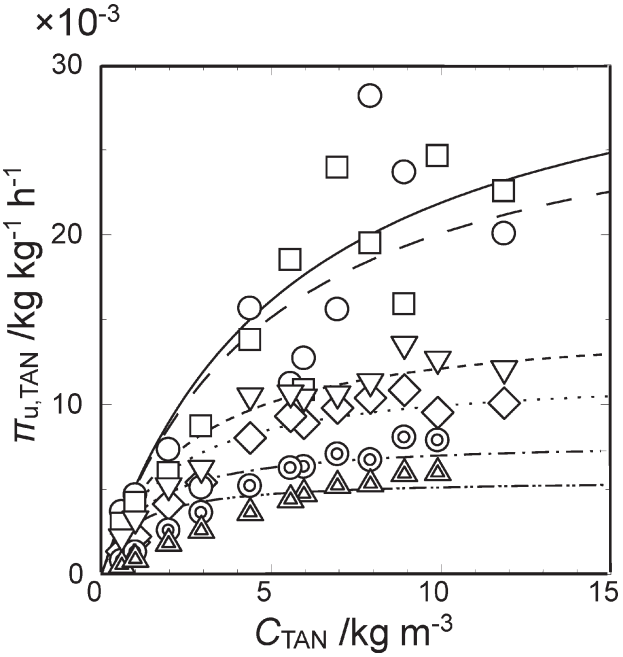


Fig. 6 – *Time courses of uptake rates for batch uptake measurements; T: 303 K, PFP: 1800 μmol m^{-2} s^{-1}, $\rho_{u,0}$: 2.4 kg m^{-3}, keys are same as Fig. 4.*

Fig. 6 shows the changes in uptake rate of ammonia-N by the seaweed, $\pi_{u,TAN}$, over time in the batch uptake measurements where the seaweed density ρ_u was fixed at 2.4 kg m^{-3}. The uptake rate decreased with uptake time or with an increase in the initial ammonia-N concentration. Based on the data of the uptake measurements, the Michaelis-Menten plots are shown at various points of uptake time in Fig. 7. The plots are classified according to uptake time to evaluate the inhibitory factor. The seaweed should ideally be in same conditions to compare the inhibitory factors and only seaweed at $t = 0$ h was actually in same conditions. The seaweed conditions at various times were not always identical even if the uptake periods were the same, because the ammonia-N concentration inside the cell body might be different from each other. In order to determine the approximate trend of the α change, the Michaelis-Menten plots were classified according to the specified uptake time, as shown in Fig. 7. The uptake rate at initial conditions increased with the initial ammonia-N concentration, reaching an asymptotic value as expressed by Eq.(5). From the Hanes-Woolf plots, the Michaelis-Menten constants, V_{max} and K_M were estimated at 3.4×10^{-2} kg kg^{-1} h^{-1} and 5.5×10^{-3} kg m^{-3}, respectively, where the value of α was assumed to be zero at $t = 0$. Table 4 summarizes the α values at various uptake times and the estimated uptake rates are shown in Fig. 7 as lines. The α values increased with time and attained 5.1 when $t = 0.33$ h. This increment might be caused by the ammonia-N accumulation inside the seaweed cell, as mentioned in the previous work.[11]



key	line	*t* [h]
○	———	0
□	– – – –	0.033
▽	- - - - - -	0.083
◇	.....	0.16
◎	—·—·—	0.33
△	—··—··	0.50

Fig. 7 – *Michaelis-Menten plotting for batch uptake measurements; T: 303 K, PFP: 1800 μmol m^{-2} s^{-1}, $\rho_{u,0}$: 2.4 kg m^{-3}*

The uptake process should consist of two main serial steps for ammonia-N metabolism; permeation through cell membrane and assimilation of permeated compounds. As the first step, the ammonia-N compound should permeate through the membrane and be stored in the cell inside. The stored ammonia-N is then assimilated by sytosol to synthesize amino acids and so on. The permeation and assimilation steps are closely linked to control the overall uptake rates[12, 13] and both steps are serial processes. For the case of the batch uptake measurements, the

Table 4 – *Measured inhibitory values in batch uptake measurements*

t [h]	α [–]
0	0
0.033	0.14
0.083	1.3
0.16	1.9
0.33	3.3
0.5	5.1

ammonia-N concentration in the solution should change along the uptake time and become smaller. Although the inhibitory factor increased with uptake time, the effects of the accumulation also became unclear. Meanwhile, for the semi-continuous uptake measurements, the generation rate of ammonia-N in the culture solution could be controlled, and the effects of the factor would become evident to clarify the contribution of the permeation and assimilation to the ammonia-N uptake. For steady state operation, when the assimilation rate is high enough relative to the permeation rate, and the permeation is the controlling step for the uptake, the ammonia-N should not be accumulated in the cell body to maintain the ammonia-N at low concentration in the cell body. In other words, the α value should not increase to be kept low. In a contrasting situation, the permeation rate is high enough relative to the assimilation rate, and the assimilation is the controlling step for the uptake. The resistance at the permeation step could be negligible and the ammonia-N should be accumulated in the cell inside. Consequently, the α value cannot be maintained at low level.

Fig. 8 shows the ammonia-N uptake results by the semi-continuous operation for short-time uptakes. The measurements were conducted for 10 hours under daytime conditions. The ammonia-N load relative to seaweed density, φ, was selected as an experimental parameter, defined as:

$$\phi = \frac{r_{\mathrm{TAN}}}{\rho_{\mathrm{u}}} \tag{19}$$

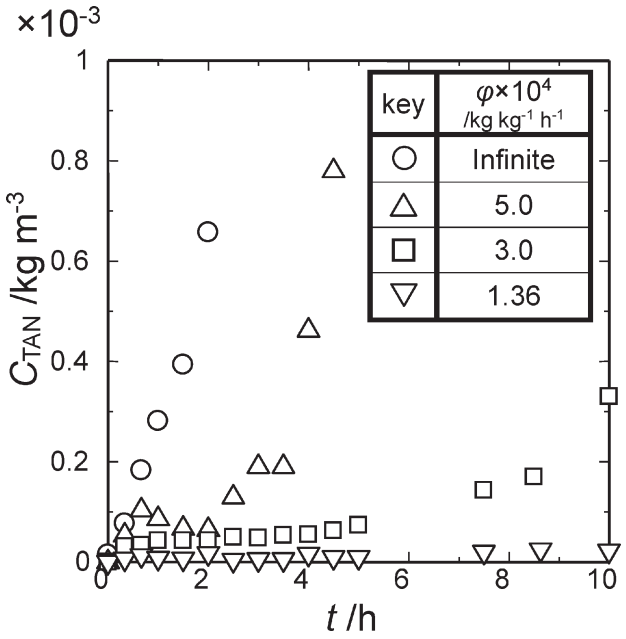


Fig. 8 – *Time courses of ammonia-nitrogen concentration in culture solution for semi-continuous uptake measurements; C_{in}: 1.0 × 10^{-3} kg m^{-3}, V_{in}: 0–6.0 × 10^{-4} m^3 h^{-1}, $\rho_{u,0}$: 2.4 kg m^{-3}, T: 303 K, PFP: 1800 μmol m^{-2} s^{-1}*

In the case of no seaweed, as a reference run, the ammonia-N concentration linearly increased. At initial ammonia-N load, $\varphi_0 = 5.0 \times 10^{-4}$ kg kg^{-1} h^{-1}, the ammonia-N concentration was kept low for the initial two hours but after that the concentration increased linearly in the same way as the reference run. When $\varphi_0 = 3.0 \times 10^{-4}$ kg kg^{-1} h^{-1} the ammonia-N concentration was low for the initial five hours and increased gradually. In this measurement, the uptake run of $\varphi_0 = 1.4 \times 10^{-4}$ kg kg^{-1} h^{-1} could maintain the ammonia-N concentration at low level, and the system could attain steady state. The values of α were estimated with Eq. (12) to be 34, 113 and 250 when φ_0 were 5.0×10^{-4}, 3.0×10^{-4} and 1.4×10^{-4}, kg kg^{-1} h^{-1}, respectively. The obtained αs were larger than those in the batch uptake measurements, and lower φ caused higher α. The second term of the denominator on the right side of Eq.(5) of Michaelis-Menten equation, $(1+\alpha) \cdot C_{\mathrm{TAN}}$, was much larger than K_{M}, and the uptake rate could be expressed as Eq. (16). Consequently, the ammonia-N uptake was controlled by the assimilation of the seaweed for the semi-continuous uptake measurements.

Fig. 9 shows the time course of ammonia-N concentration for the semi-continuous uptake measurements, in which the ammonia-N uptake run was conducted for more than 200 hours. The daytime/nighttime cycle was fixed as 14hr/10hr. The initial ammonia-N load, φ_0, was specified as 1.4×10^{-4} kg kg^{-1} h^{-1}, which was confirmed that the system could attain steady state in the former uptake measurement. The ammonia-N concentration increased with uptake time and the system should have attained steady state after 100 hours. The daily change of ammonia-N concentration at steady state is shown in Fig. 9(b). The ammonia-N concentration could be kept at low level during daytime, however at nighttime the concentration continued to increase until the end of the nighttime. Fig. 10 shows the time courses of ammonia-N uptake rate, estimated by the following equation;

$$\rho_u \cdot \pi_{\mathrm{u,TAN}} = V_{\mathrm{in}} \cdot C_{\mathrm{TAN,in}} - V_{\mathrm{out}} \cdot C_{\mathrm{TAN,out}} \tag{20}$$

where V_{in} and $C_{\mathrm{TAN,in}}$ stand for the volume rate of the solution containing ammonia-N supplied to the vessel and the ammonia-N concentration in the supplying solution, respectively. V_{out} and $C_{\mathrm{TAN,out}}$ mean the volume rate of overflowed solution from the vessel, and the ammonia-N concentration in the solution. Substantially $V_{\mathrm{in}} = V_{\mathrm{out}}$ and $C_{\mathrm{TAN,out}} = C_{\mathrm{TAN}}$. The uptake rate showed the maximum at initial conditions and decreased with time. After 100 hours of uptake run, the uptake rate became constant to attain steady state conditions. The behavior of ammonia-N uptake rate over a day at steady state is shown in Fig. 10(b). The uptake rate suddenly decreased after the nighttime condition started. It was reported that sunlight was necessary for the effective uptake of ammo-

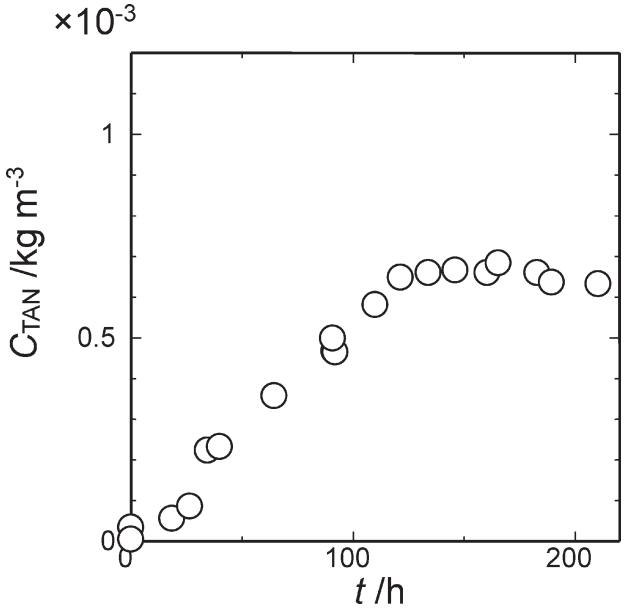


Fig. 9(a) – *Time courses of ammonia-nitrogen concentration in semi-continuous uptake measurements; C_{in}: 1.0 × 10^{-3} kg m^{-3}, V_{in}: 1.2 × 10^{-4} m^3 h^{-1}, $\rho_{u,0}$: 2.4 kg m^{-3} φ_0: 1.36×10^{-4} kg kg^{-1} m^{-1}, Daytime conditions: T: 303 K, PFP: 1800 μmol m^{-2} s^{-1}, t: 14 h, Nighttime conditions: T: 298 K, PFP: 0 μmol m^{-2} s^{-1}, t: 10 h*

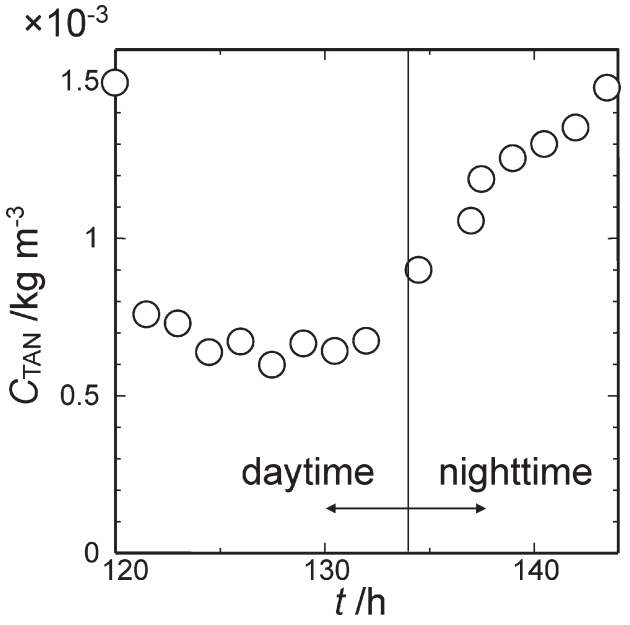


Fig. 9(b) – *Time courses of ammonia-nitrogen concentration in culture solution for semi-continuous uptake measurements; C_{in}: 1.0 × 10^{-3} kg m^{-3}, V_{in}: 1.2 × 10^{-4} m^3 h^{-1}, $\rho_{u,0}$: 2.4 kg m^{-3} φ_0: 1.36×10^{-4} kg kg^{-1} m^{-1}, Daytime conditions: T: 303 K, PFP: 1800 μmol m^{-2} s^{-1}, t: 14 h, Nighttime conditions: T: 298 K, PFP: 0 μmol m^{-2} s^{-1}, t: 10 h*

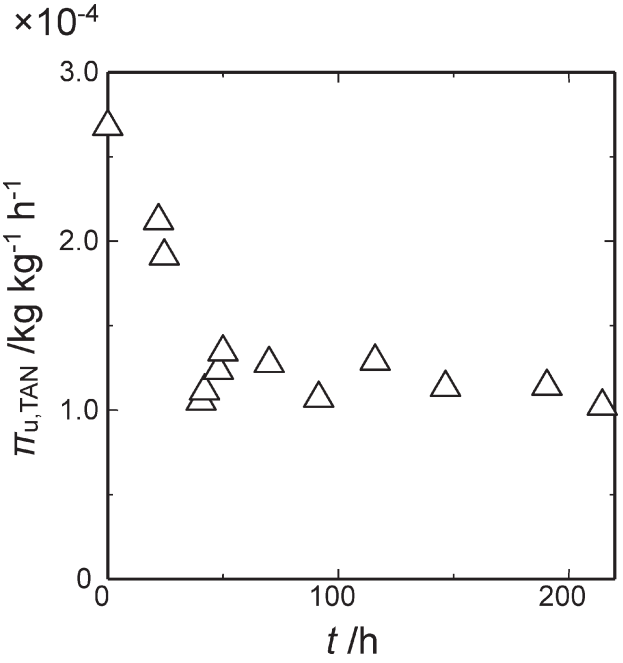


Fig. 10(a) – *Time courses of ammonia-nitrogen uptake rate in semi-continuous uptake measurements; C_{in}: 1.0 × 10^{-3} kg m^{-3}, V_{in}: 1.2 × 10^{-4} m^3 h^{-1}, $\rho_{u,0}$: 2.4 kg m^{-3} φ_0: 1.36×10^{-4} kg kg^{-1} m^{-1}, Daytime conditions: T: 303 K, PFP: 1800 μmol m^{-2} s^{-1}, t: 14 h, Nighttime conditions: T: 298 K, PFP: 0 μmol m^{-2} s^{-1}, t: 10 h*

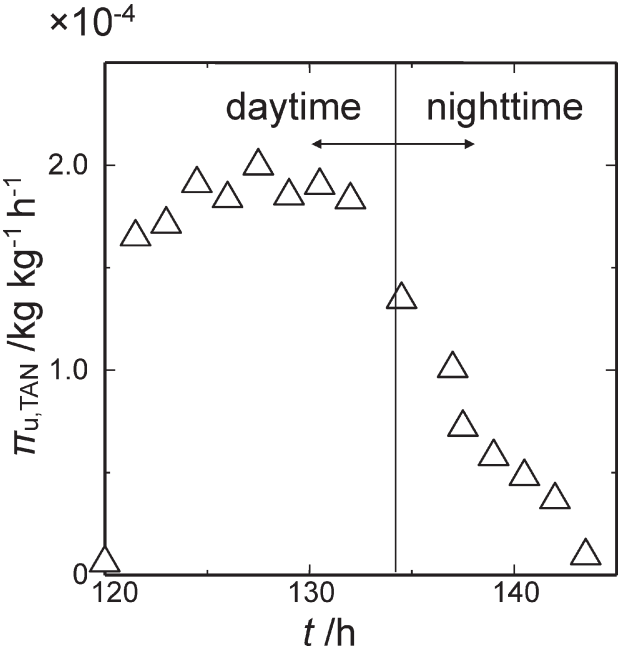


Fig. 10(b) – *Time courses of ammonia-nitrogen uptake rate in semi-continuous uptake measurements; C_{in}: 1.0 × 10^{-3} kg m^{-3}, V_{in}: 1.2 × 10^{-4} m^3 h^{-1}, $\rho_{u,0}$: 2.4 kg m^{-3} φ_0: 1.36×10^{-4} kg kg^{-1} m^{-1}, Daytime conditions: T: 303 K, PFP: 1800 μmol m^{-2} s^{-1}, t: 14 h, Nighttime conditions: T: 298 K, PFP: 0 μmol m^{-2} s^{-1}, t: 10 h*

nia-N.[11] The uptake rate was larger during daytime conditions than during nighttime conditions; however, the seaweed could uptake the ammonia-N even under nighttime conditions. The α value was estimated at approximately 220 under daytime conditions at steady state, similar to the value estimated in the short-time semi-continuous operation. The α value was relatively high and the uptake rate could be expressed by Eq. (16), independent of the ammonia-N concentration. Therefore, the ammonia-N uptake process must be controlled by the assimilation in this measurement. The range of r_{TAN} could be estimated from 1.0×10^{-6} to 8.0×10^{-5} kg m^{-3} h^{-1} in the ordinary

intensive shrimp culture pond for giant tiger prawns.[8] The required ρ_u could be estimated as 0.51 kg m^{-3} at most even if $\alpha = 220$. The estimated r_{TAN} values did not contain the effects of the ammonia-N generated from the decomposition of the sediment. The estimated required ρ_u was smaller than the experimental conditions and the suggested method can be regarded as a promising solution for pond water quality control for intensive shrimp farming.

Conclusions

The ammonia-nitrogen uptake by sterile *Ulva* sp. was measured and the uptake rate was analyzed based on the Michaelis-Menten model. The inhibitory factor of the model increased with the ammonia-nitrogen uptake and the factor exceeded more than 200. This increase might be attributed to the increment of the ammonia-nitrogen concentration in the cell body, and caused the reduction in uptake performance of ammonia-nitrogen. In the measurement range of this study, the controlling resistance of the ammonia-nitrogen uptake was laid at the assimilation step and the assimilation rate decreased with an increase in the ammonia-nitrogen concentration in the cell. By semi-continuous operation, the seaweed could continuously treat with the model farming culture solution to keep the ammonia-nitrogen concentration relatively low level when the ratio of the seaweed density relative to the rate of ammonia-nitrogen generation was appropriately adjusted. Despite high inhibitory factor, the pond water quality might be preferably controllable with moderate seaweed density. This seaweed does not require a long startup period and the treatment of the seaweed must be easily operated, same as other biofilter plants. The suggested configuration of the pond water control system was assessed to be preferable for ammonia-nitrogen content removal in intensive shrimp aquaculture ponds in developing countries. However, further study to evaluate other factors, such as effects of light and temperature, other chemicals, creatures and any other parameter, is necessary for actual application.

Nomenclature

C_{TAN} – concentration of ammonia-N in culture solution, kg m^{-3}

$C_{TAN,in}$ – concentration of ammonia-N in supply feed solution in semi-continuous uptake measurements, kg m^{-3}

$C_{TAN,out}$ – concentration of ammonia-N in solution overflowed from vessel in semi-continuous uptake measurements, kg m^{-3}

C'_{TAN} – mass fraction of ammonia-N in seaweed, kg kg^{-1}

C_P – concentration of phosphoric acid phosphorus, kg m^{-3}

E – fractional removal of ammonia-nitrogen, defined as Eq.(18), -

F/D – mass ratio of fresh relative to dried seaweed, -

K_M – Michaelis coefficient, kg m^{-3}

r_{TAN} – rate of ammonia-nitrogen generation in a unit volume of culture solution, kg m^{-3} h^{-1}

T – water temperature, K

t – time, h

V – volume of culture solution, m^3

V_{in} – supply rate of feed solution into vessel in semi-continuous uptake measurement, m^3 h^{-1}

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

V_{out} – overflowing rate of solution from vessel in semi-continuous uptake measurements, m^3 h^{-1}

α – inhibitory factor, -

$\pi_{a,TAN}$ – assimilation rate of ammonia-nitrogen by a unit dry mass of seaweed, kg kg^{-1} h^{-1}

$\pi_{u,TAN}$ – uptake rate of ammonia-nitrogen by a unit dry mass of seaweed, kg kg^{-1} h^{-1}

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

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

Subscript

0 – initial

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