Removal of Nitrate-Nitrogen in Seawater by Sterile *Ulva* sp.

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Introduction

Shrimp industry in developing countries is in a transition period to shift from the pond of open system to that of closed system in which little or no water is exchanged to minimize the risk of pathogen contamination from external water supply[1]. On the other hand, rapid accumulation of inorganic nitrogen, especially nitrate-nitrogen (nitrate-N) and ammonia-nitrogen (ammonia-N), is a serious problem in closed ponds. Ammonia-N generated by metabolism of shrimps is strongly toxic to them even in low concentrations[2]. While the toxicity of nitrate-N is much lower than that of ammonia-N[3], it is major pollutant for eutrophication. Sterile *Ulva* sp. was studied for water quality control in intensive shrimp mariculture ponds in developing countries as a simple and economical way to remove ammonia-nitrogen[4][5]. However, there are no study on relationship among uptake and content in alga cells of ammonia-nitrogen and nitrate-nitrogen, which is important for designing system of water quality control by macroalgae, and no studies on nitrate-N uptake under tropical conditions considering intensive shrimp mariculture. In this study, we studied nitrate-N uptake by sterile *Ulva* sp. in various conditions including tropical conditions considering application to intensive shrimp mariculture ponds.

1, Experimental

The preparation of Sterile *Ulva* sp. was carried out in the same way as the previous works[4][5]. The principal experimental conditions are summarized in **Table 1**. The "starved alga" prepared by the artificial seawater without any additives for several days was further cultured for 1 day with artificial seawater rich in nitrate-N and light of about 1800 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPF in apparatus as shown in **Fig. 1** to be converted to the ammonia-N "enriched alga". The portion of culture media was sampled and analyzed along time to obtain the time course of nitrate-N contents in culture media. In the case of the tropical condition, the temperature of 30 ± 2 °C was selected considering water temperature in intensive shrimp farms in Thailand[4][5]. In the existence of light, PPF (Photosynthetic Photon Flux) was approximately 1800 $\mu mol \cdot m^{-2} \cdot s^{-1}$ as the tropical condition according to the previous work[7]. An uptake run with addition of NH_4Cl at intervals to keep the concentration in the solution almost constant was also carried out under the tropical condition. The measurement of the amount of nitrate-N in alga cells was carried out as the previous work[8]. The concentrations of nitrate-N and ammonia-N in liquid solutions were determined by copperised-cadmium reduction method with Griess-Romijn reagent, and indophenol blue method, respectively [9].

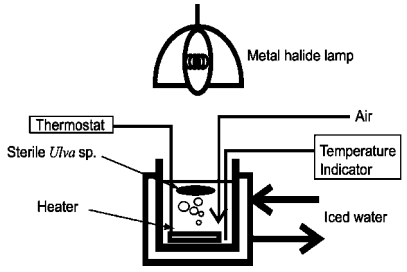

Fig. 1 Experimental apparatus (stainless steel tank)

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Table 1 Experimental condition

	Uptake runs Moderate condition	Uptake runs Tropical condition
Apparatus	glass beaker	stainless steel tank (Fig.1)
Light [$\mu mol \cdot m^{-2} \cdot s^{-1}$]	80	1800, 0
T [˚C]	28	30 ± 2
$C_{NN,0}$ [$\times 10^{-3}$ kg-N·m^{-3}]	0.20 - 10	8.4 ± 0.7
$C_{TAN,0}$ [$\times 10^{-3}$ kg-N·m^{-3}]	0	0, 1.5
$C_{P,0}$ [$\times 10^{-3}$ kg-P·m^{-3}]	0	0.10
$\pi_{NN,0}$[$\times 10^{-3}$kg-N· kgDM^{-1}·h^{-1}]	0.03 – 0.21	0.63 ± 0.3 (“starved”) 0.11 (“enriched”)
ρ [kgDM·m^{-3}]	0, 3.2	1.5
Feed of algae	“starved”	“starved” ,“enriched”
V [$\times 10^{-3}$ m^3]	0.50	5.0

2. Results and Discussion

In general, inorganic-nitrogen assimilation is shown as **Fig. 2**. The material balance of nitrate-N and ammonia-N in the culture medium phase for unit volume are given as **Eqs** (1) and (2), respectively.

$$-\frac{dC_{NN}}{dt} = \rho\frac{dC'_{NN}}{dt} + \rho\pi_{a,NN} = \rho\pi_{NN} \quad (1)$$

$$-\frac{dC_{TAN}}{dt} + \rho\pi_{a,NN} = \rho\frac{dC'_{TAN}}{dt} + \rho\pi_{a,TAN} \quad (2)$$

Table 2 shows the principal properties of sterile *Ulva* sp. with previous study[4][5][6]. These properties of sterile *Ulva* sp. in this work agreed well with the previous results and were reliable.

Table 2 Principal properties of sterile *Ulva* sp.[4][5] and *Ulva lactuca*[6]

	Sterile *Ulva* sp. (this study)	Sterile *Ulva* sp. (4)(5)	*Ulva lactuca*
F/D ratio [-]	4.2	4.3	3.7
Tissue H_2O [$m^3 \cdot kgDM^{-1}$]	3.2×10^{-3}	3.3×10^{-3}	2.7×10^{-3}
Specific surface area [$m^2 \cdot kgDM^{-1}$]	1.3×10^{-2}	9.1×10^{-3}	
C'_{TAN} [$\times 10^{-3}$ kg-N·kgDM^{-1}]	8.2×10^{-2} (“starved”)	3.3×10^{-2} (“starved”)	
C'_{NN} [$\times 10^{-3}$ kg-N·kgDM^{-1}]	8.0×10^{-2} (“starved”) 7.7×10^{-1} (“enriched”)		

Figure 3 shows the time courses of the nitrate-N and ammonia-N concentrations in the culture medium in the uptake runs under moderate conditions. **Figure 4** shows nitrate uptake under tropical conditions. The examples of this initial uptake rate were summarized in Table 1 with the experimental conditions. Algae took up both nitrate-N and ammonia-N under moderate condition. The rate of ammonia-N uptake was higher than that of nitrate-N. The initial uptake rates of both nitrate-N and ammonia-N were higher when the initial concentrations of those were higher. The rate of nitrate-N uptake was higher under the tropical condition than that under the moderate condition. The "starved alga" could take up nitrate-N under the tropical condition both with and without ambient ammonia-N in culture medium, whereas the "enriched alga" could hardly remove nitrate-N. This inhibition to uptake was observed by several researchers[6][10]. The amount of nitrate-N in alga cells was higher in "enriched alga" as shown in Table 2. In the

case of ammonia-N uptake, ammonia-N uptake was decreased when the amount of ammonia-N in alga cells was high[4][5]. In the case of nitrate-N uptake, the high amount of nitrate-N in alga cells was also deeply related to the uptake inhibition. The rate of nitrate-N uptake was higher under the tropical condition than that under the moderate condition. No significant difference in nitrate-N uptake was observed between with and without light. These properties are advantageous, because a lot of shrimp farms are located in tropical region, and nitrate-N must be removed without light at night there. With ambient ammonia-N in culture medium, the rate of nitrate-N uptake was lower than that without ammonia-N. On the other hands, we observed ammonia-N uptake was hardly inhibited by the existence of nitrate (data not shown). The averaged π_{uNN}, π_{uTAN}, π_{aNN} and π_{aTAN} in Fig.4 calculated by using Eqs. (1) and (2) were summarized in **Table 3** with the experimental conditions. The π_{aNN} was lower than π_{aTAN} in all the cases. The π_{aNN} and π_{aTAN} in "enriched alga" were lower. There were no significant differences in either π_{aNN} or π_{aTAN} between with and without light. There could be an explanation of the reason that nitrate-N uptake was inhibited by the existence of ambient ammonia-N in culture medium. The π_{aNN} was much lower than π_{uTAN} in the case with ambient ammonia-N, and was lower than π_{aTAN} in all the cases. When ammonia-N was taken up by alga, the amount of ammonia-N in alga cells became higher and the π_{aNN} was lower as shown in Table 3. This lower π_{aNN} would cause accumulation of nitrate-N in alga cell, which could inhibit nitrate-N uptake. **Figure 5** shows the results of the long-term experiment of the nitrate-N and ammonia-N concentrations in the culture medium in the uptake runs under the tropical conditions with addition of nutrients at interval, considering practical operation in intensive shrimp in developing countries. Ammonia-N added at interval was rapidly removed by algae. The algae took up nitrate-N almost completely in first 2 days, whereas hardly took up after that. Ammonia-N should be removed in shrimp ponds as soon as possible because ammonia-N is much more toxic to shrimps than nitrate. Therefore, this property is advantageous as biofilter in intensive mariculture.

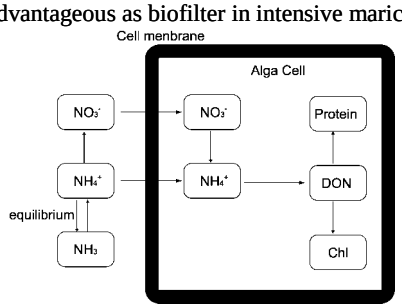
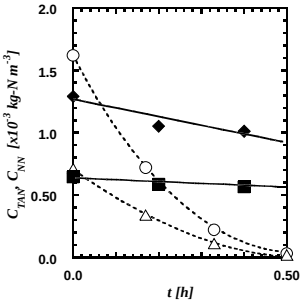


Fig.2 General concept of inorganic-nitrogen assimilation by macroalgae.



Keys	Inorganic Nitrogen	$C_{NN,0}$	$C_{TAN,0}$
◆	Nitrate-N	1.3	0
■		0.64	0
○	Ammonia-N	0	1.6
△		0	0.71

Fig.3 Time courses of the nitrate-N and ammonia-N concentrations in the culture medium in the uptake runs under the moderate condition.

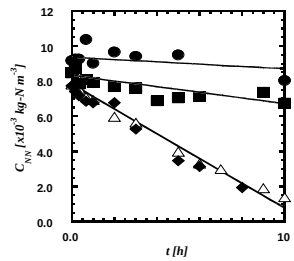


Fig.4 Time courses of the nitrate-N concentrations in the culture medium in the uptake runs under the tropical condition. Keys are shown in Table 3.

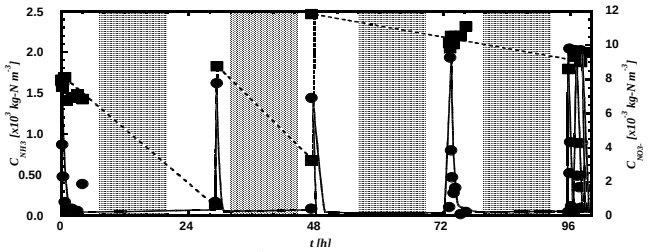


Fig.5 Long time courses of the nitrate-N and ammonia-N concentrations in the culture medium in the uptake runs under the tropical condition. (■: nitrate-N, ●: ammonia-N)

Table 3 Inorganic-N transport kinetics of alga under tropical conditions

Keys	C_{TAN}	$C'_{TAN,0}$	$C'_{NN,0}$	PPF	$\pi_{a,NN}$	$\pi_{a,TAN}$	$\pi_{u,NN}$	$\pi_{u,TAN}$
●	0	0.044	0.77	1800	0.080	0.35	0.054	0
◆	0	0.12	0.048	1800	0.68	0.82	0.50	0
△	0	0.049	0.19	0	0.92	1.1	0.48	0
■	1.5	0.13	0.076	1800	0.32	0.30	0.16	0.66

Conclusion

Sterile *Ulva* sp. could remove nitrate-nitrogen in the culture medium effectively, whereas the rate of nitrate-N uptake was lower than that of ammonia-N. The algae took up nitrate-nitrogen even without light. The nitrate-nitrogen uptake rate decreased with increasing nitrate-nitrogen content in alga cells. With ambient ammonia-N in culture medium, algae could take up nitrate-N, while the rate of uptake was lower. On the other hands, ammonia-N uptake was not inhibited by ambient nitrate-N. Consequently, nitrate-N and ammonia-N uptake by alga was proposed for controlling water quality in intensive shrimp aquaculture ponds in developing countries and the useful information for water quality control system design was provided.

Nomenclature

C_{NN} = concentration of nitrate-N [$\times 10^{-3}$ kg-N·m^{-3}], C_{TAN} = concentration of ammonia-N [$\times 10^{-3}$ kg-N·m^{-3}], C_P = concentration of phosphoric acid phosphorus [$\times 10^{-3}$ kg-P·m^{-3}], C'_{NN} = concentration of nitrate-N content in alga cells [$\times 10^{-3}$ kg-N·m^{-3}], C'_{TAN} = concentration of ammonia-N content in alga cells [$\times 10^{-3}$ kg-N·m^{-3}], $\pi_{a,NN}$ = specific assimilation rate of nitrate-N [$\times 10^{-3}$ kg-N·kgDM^{-1}·h^{-1}], $\pi_{a,TAN}$ = specific assimilation rate of ammonia-N [$\times 10^{-3}$ kg-N·kgDM^{-1}·h^{-1}], $\pi_{u,NN}$ = specific uptake rate of nitrate-N [$\times 10^{-3}$ kg-N·kgDM^{-1}·h^{-1}], $\pi_{u,TAN}$ = specific uptake rate of ammonia-N [$\times 10^{-3}$ kg-N·kgDM^{-1}·h^{-1}]

References

(1) Wang, J.K.; "Managing Shrimp Pond Water to Reduce Discharge Problems," *Aquaculture Engineering*, **9**, 61-73 (1990)

(2) Haywood, G. P.; "Ammonia Toxicity in Teleost Fishes: A Review," Canadian Technical Report of Fisheries and Aquatic Sciences, No. 1177. p. 35. Department of Fisheries and Oceans, Nanaimo, British Colombia, Canada (1983)

(3) J. Colt and G. Tchobanoglous; "Evaluation of the short-term toxicity of nitrogenous compounds to channel catfish, *Ictalurus punctatus*" : *Aquaculture,* **8**, 209-224 (1976)

(4) Sato, K. and R. Egashira; "Control of Ammonia Content Fluctuation in Closed Shrimp-culture Ponds with Using Seaweeds," The 10th APCChE Congress (APCChE 2004), October 21, 2004, Kitakyushu, Japan (2004)

(5) Sato, K., T. Eksangsri and R. Egashira; "Removal of Inorganic Nitrogen to Control Water Quality in Closed Shrimp-culture Ponds with Using Seaweeds," 7th World Congress of Chemical Engineering, July 2005, Glasgow, the U.K. (2005)

(6) Runcie, J. W., R. J. Ritchie and A. W. D. Larkum; "Uptake Kinetics and Assimilation of Inorganic Nitrogen by *Catenella nipae* and *Ulva lactuca*," *Aquatic Botany,* **76**, 155-174 (2003)

(7) Sakuratani, T., K. Watanabe, E. Nawata and C. Noichana; "Seasonal Change in Solar Radiation, Net Radiation and Photosynthetically Active Radiation in Khon Kaen, Northeast Thailand," *Environ. Control in Biol.* **40**, 395-401 (2002)

(8) McGlathey, K. J., M. F. Pedersen and J. Borum; "Change in Intracellular Nitrogen Pools and Feedback Controls on Nitrogen Uptake in *Chaetomorpha Linum* (Chlorophyta)," *J. Phycol*., **32**, 393-401 (1996)

(9) Kisho cho ed; Guide to Marine Observation (Kaiyo Kansoku shishin), pp. 182-184, the Oceanographic Society of Japan (1970)

(10) O'Brien, M.C. and P. A. Wheeler; "Short-term uptake of nutrients by *Enteromorpha prolifera* (Chlorophyceae)," *J.Phycol*. **23**, 547-556 (1987)