Performance of Ammonia-nitrogen Uptake by Sterile *Ulva* sp. under Tropical Condition

TAJIRI SHIN
Student ID : 02-1504-8
Supervisor: EGASHIRA RYUICHI
Department of International Development Engineering, School of Engineering,
Tokyo Institute of Technology, Tokyo 152-8552, Japan

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 farmer introduced none or quite small water exchange culture system that called "closed" or "semi-closed" system to avoiding the infection of disease. However, water deterioration is more serious in "closed" or "semi-closed" pond. Deterioration of rearing condition accompanies asymptomatic disease onsets and results in massive mortality.

Ammonia-nitrogen (ammonia-N) generated from bottom sediment and metabolism of fishes or crustaceans 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 prevented[3]. Much amount of nitrogen entered into the ponds as formulated feed is not retained by shrimp and becomes sediment accumulated on the bottom of pond and pond water [4].

In this study, we applied macroalgae to the removal of nitrogen in form of ammonia-N in shrimp aquaculture ponds in developing countries. We used sterile *Ulva* sp. as model macroalgae to measure the rates of ammonia-N uptake under tropical condition.

1. Experimental

1.1 Materials

Sterile *Ulva* sp. collected from September to November 2006 at Kanazawa Bay (Yokohama, Japan 35°20'32N, 139°38'32E). Commercial sea salt was used to prepare artificial seawater (salinity: 30‰). NH_4Cl and Na_3PO_3 were used as sources of ammonia-N and phosphorus in the seawater, respectively.

1.2 Cultivation apparatus

Three different types of apparatuses were used as cultivation apparatus. One was a vessel made of glass of which capacity was $5\times10^{-2}m^3$ and used to store algae (apparatus A). The second was a $5\times10^{-4}m^3$ glass beaker for batch cultivation (apparatus B; **Fig.1**). The other was a custom-made tank made of acrylic resin for long time semi-continuous cultivation (apparatus C; **Fig.2**). Light source was 400W metal halide lamp. The culture medium was agitated by magnetic stirrer (apparatus B and C) or aeration (apparatus A).

1.3 Procedure and conditions

The principal conditions of algae preparation for uptake runs are summarized in **Tables 1** and **2**. Collected algae were washed thoroughly and were stored in apparatus A for uptake runs. In the case of short time cultivation with apparatus B, concentrated ammonia-N solution added to culture medium at the beginning of uptake run and ammonia-N concentration of culture medium were measured. In the case of long time cultivation with apparatus C, rates of ammonia-N uptake were calculated from the time course of ammonia-N concentration of outflow.

1.4 Analysis

The concentrations of ammonia-N in liquid solution were determined by the indophenol blue method (Japan Meteorological Agency, 1970).

Table 1 Experimental condition

	Cultivation/storage	short time uptake
Apparatus	A	B
Light [μmol m^{-2} s^{-1}]	800	1800
T[**℃**]	22	30±1
Culture medium	Airtificial seawater	Airtificial seawater
$C_{N,0}$ [$\times10^{-3}$ kg-N m^{-3}]	0	0-12
$C_{P,0}$ [$\times10^{-3}$ kg-P m^{-3}]	0	0
$\rho_{u,0}$ [kgDM m^{-3}]		2.38
V [$\times10^{-3}$ m^3]	20	0.5

Table 2 Experimental conditions

	long time uptake
Apparatus	C
Light [μmol m^{-2} s^{-1}]	1800
Light : dark periods [hour]	14:10
Culture medium	Airtificial seawater
T_{light} [**℃**]	30±1
T_{dark} [**℃**]	22±1
$\rho_{u,0}$ [kgDM m^{-3}]	0.53
$C_{N,in}$ [$\times10^{-3}$ kg-N m^{-3}]	1.5, 2.5 ,3.5
$C_{N,in}$ /$C_{P,in}$ [-]	35
inflow rate [$\times10^{-3}$ m^3・h^{-1}]	0.125
outflow rate[$\times10^{-3}$ m^3・h^{-1}]	0.125
V [$\times10^{-3}$ m^3]	0.45

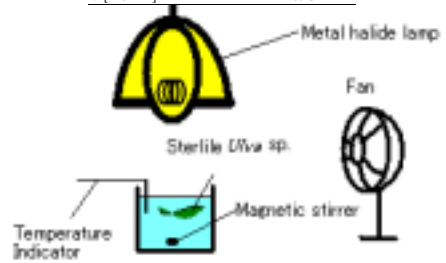

Fig.1 Experimental apparatus B

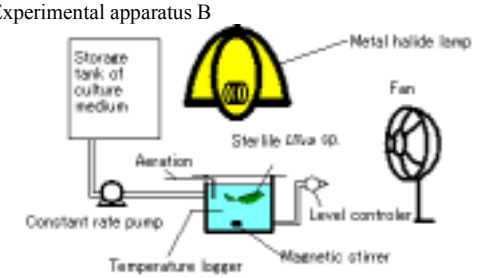

Fig.2 Experimental apparatus C

Received on February 16, 2007. Correspondence concerning this article should be addressed to Shin TAJIRI (E-mail address: stajiri@ide.titech.ac.jp).

2. Results and Discussion

2.1 Basic relationship in ammonia-N uptake

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

$$-\frac{dC_N}{dt} = \rho_u \frac{dC'_N}{dt} + \rho_u \pi_{a,N} \quad (1)$$

$$-\frac{dC_N}{dt} = \rho_u \pi_{u,N} \quad (2)$$

In this equation, $\pi_{u,N}$ is ammonia-N uptake rate by alga which can be represented by the Michaelis–Menten equation incorporating uncompetitive inhibition as,

$$\pi_{u,N} = \frac{V_{max} C_N}{K_M + (1+\alpha) C_N} \quad (3)$$

where α is the inhibitory factor.

2.2 Uptake performance in batch cultivation

Figure 3 shows examples of the time course of ammonia-N concentration in culture medium. In all cases, alga removes ammonia-N in culture medium.

Figure 4 shows the initial rates of ammonia-N uptake calculated from the time course of ammonia-N concentration in "tropical" (Light: 1800 μmol·m^{-2}·s^{-1}, Temperature: 30±1°C) and "moderate" (80 μmol·m^{-2}·s^{-1}, 25±2°C[5]) The Michaelis–Menten constants, V_{max} and K_M are summarized in **Table 3** with previous work. $\pi_{u,N}$ and V_{max} are higher under "tropical" condition than that under "moderate" condition.

Figure 5 shows a Michaelis–Menten plot for the ammonia-N uptakes by "Starved" alga which was cultivated under limited ammonia-N supply and "Enriched" alga which was cultivated in ammonia-N rich medium (2.0×10^{-3} kg-N·m^{-3}, 2.38 kgDM·m^{-3}) for 0.5 h. There is not remarkable difference of uptake rates between "Starved" alga and "Enriched" alga. This may resulted from insufficient enrichment in this study.

2.3 Uptake performance in semi-continuous cultivation

Figure 6 shows the time course of ammonia-N uptake rate. The rates of uptake were nearly constant when the cultivation time exceeds about 100 h. Uptake performance of semi-continuous cultivation were estimated from sterile *Ulva* sp. that was cultivated for more than 100 h and summarized in **Table 4**. Uptake rate of semi-continuous cultivation is quite smaller than batch cultivation.

Figure 7 shows the daily fluctuation of ammonia-N concentration in culture medium. While sterile *Ulva* sp. kept ammonia-N concentration in culture medium nearly constant in light periods, ammonia-N concentration gradually increased in dark periods. This decrease of ammonia-N uptake rate in dark periods may result from temperature decrease, nonphotosynthetic condition, or both.

Conclusion

Ammonia-N uptake rates of Sterile *Ulva* sp. under "tropical" condition were higher than under "moderate"[5] condition. Uptake rate in semi-continuous cultivation is quite smaller than batch cultivation.

Nomenclature

C_N	= concentration of ammonia-N	[kg-N·m^{-3}]
C'_N	= concentration of ammonia-N in alga cell	[kg-N·kgDM^{-1}]
C_P	= concentration of phosphoric acid phosphorous	[kg-P·m^{-3}]
C_{in}	= ammonia-N concentration of inflow to the cultivation tank	[kg-N·m^{-3}]
C_{out}	= ammonia-N concentration of outflow from the cultivation tank	[kg-N·m^{-3}]
K_M	= Michaelis coefficient	[kg-N·m^{-3}]
t	= time	[h]
V	= volume of artificial seawater	[m^{-3}]
V_{max}	= saturated uptake rate of ammonia-N in the Michaelis–Menten equation	[kg-N·kgDM^{-1}·h^{-1}]
α	= inhibitory factor	[–]
$\pi_{a,N}$	= specific assimilation rate of ammonia-N	[kg-N·kgDM^{-1}·h^{-1}]
$\pi_{u,N}$	= specific uptake rate of ammonia-N	[kg-N·kgDM^{-1}·h^{-1}]
ρ_u	= culture density of algae	[kgDM·m^{-3}]

Literature Cited

[1] Primavera, J.H.; "Tropical Shrimp Farming and Its Sustainability," Tropical Mariculture, S. S. De Silva ed., pp. 257-289, Academic Press, San Diego, U.S.A. (1998)

[2] Haywood, G.P.; "Ammonia Toxicity in Teleost Fishes: A Review," *Can. Tech. Rep. Fish Aquat. Sci.*, 1177, 1-35 (1983)

[3] Chen, J. C. and C. C. Tu; "Influence of Ammonia on Growth of *Penaeus monodon* Fabricius Post–Larvae," *Aquaculture and Fisheries Management*, 22, 457-462 (1991)

[4] Briggs M.P.R and Funge–Smith S.J.; "Anutrient budget of some intensive marine shrimp ponds in Thailand," Aquaculture and Fisheries Management, 25, 789-811 (1994)

[5] Sato, K., Eksangsri, T., Egashira, R.; "Ammonia-Nitrogen uptake by Seaweed for Water Quality Control in Intensive Mariculture Ponds," J. Chemical Engineering of Japan, 39, 247-255 (2006)

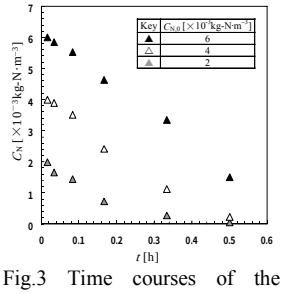

Fig.3 Time courses of the ammonia-N concentration in culture medium in the short time uptake run

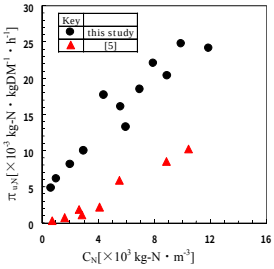

Fig.4 Michaelis–Menten plotting for the ammonia-N uptakes under "tropical" and "moderate"

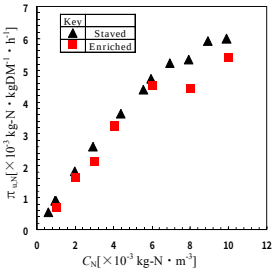

Fig.5 Michaelis–Menten plotting for the ammonia-N uptakes by "Starved" and "Enriched" sterile *Ulva* sp. $\pi_{u,N}$ is average rate of 0.5 h

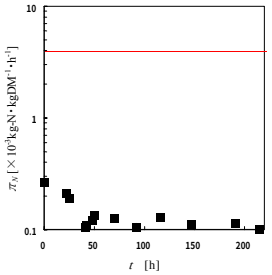

Fig.6 Time course of the ammonia-N uptake rate in semi-continuous cultivation (medium was only sampled in light periods), Red line: $\pi_{N,0}$=3.9×10^{-3} kg-N・kgDM^{-1}・h^{-1} (at $C_{N,0}$=0.76×10^{-3} kg-N・m^{-3})

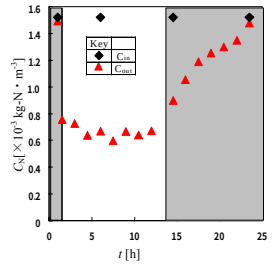

Fig.7 The daily fluctuation of ammonia-N concentration in culture medium (semi-continuous cultivation); shading and non shading area shows dark and light periods, respectively

Table 3 Comparison of Michaelis–Menten coefficients with previous study

	This study	[5]
V_{max}[×10^{-3} kg-N·kgDM^{-1}·h^{-1}]	34.6	16.4
K_M [×10^{-3} kg-N·m^{-3}]	5.65	6.91

Table 4 Examples of data obtain by semi-continuous cultivation

	run 1	run 2	run 3
C_{in} ×10^3	1.52	2.47	3.51
$C_{out,light}$ ×10^3	0.76	1.92	2.89
$C_{out,dark}$ ×10^3	**1.22**	**2.17**	**3.31**
$\pi_{u,N,light}$ ×10^3	0.157	0.111	0.104
$\pi_{u,N,dark}$ ×10^3	0.088	0.042	0.031
α_{light}	220	309	332
α_{dark}	391	822	1109