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Assessment of Ammonium Uptake by Marine Macroalga *Gracilaria verrucosa* (Rhodophyta)

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Abstract: The present study explains the rate of ammonium uptake by *Gracilaria verrucosa* from two different concentrations (10 and 30 µM). The Mean uptake by *Gracilaria verrucosa* was estimated as 6.118 and 17.31 µM, when the ammonium conc. of 10 and 30 µM in the medium. Based on the rate of uptake, the process was divided into four phases. In the first phase (with in 10 min) the rate of uptake was minimal (0.24 in 10 µM initial conc. and 0.38 in 30 µM initial conc.). In the second phase (20 min to 1 h) maximum uptake was observed (3.3059 and 12.1796 µM). In the third phase (1-6 h), the uptake process was moderate (2.5284 and 4.221 µM). In the Last phase (7-8 h) uptake rate was very less (0.235 and 0.9051 µM). The rates of ammonium uptakes were positively correlated with the nutritional concentration in the environment and negatively correlated with time.

Key words: Ammonium uptake, macro algae, *Gracilaria verrucosa*

INTRODUCTION

Marine macro algae are ecologically very important and commercially valuable resource for food and other purposes. Animal mariculture and anthropogenic activities generates large quantity of inorganic wastes in the form of uneaten food and excretory products, out of which, more than 70% of them are released into the natural environment (Porter *et al.*, 1987). They lead to eutrophication to the environment (Neori *et al.,* 1991; Rathakrishnan, 2001) and acute toxicity to the animals (Troell *et al.*, 1999; Neori *et al.*, 2000). It is very necessary to minimize the organic load in the mariculture systems and reduce the ammonium rich effluent added to the natural environment. The chemical treatment and other processes to remove the excess ammonia from waste water and the culture ponds are very expensive and they may affect the environment (Troell *et al.*, 2003). So as an economicaly, feasible and eco-friendly proposal as the use of macro-algae to remove ammonium and nitrogen has been studied in recent years (Vandermeulen and Gordin, 1990; Cohen and Neori, 1991; Buschmann *et al.*, 1996; Rathakrishnan, 2001; Neori *et al.*, 2004; Seema and Reeta Jayasankar, 2005; Matos *et al.*, 2006). The Present study deals with the rate of ammonium uptake by the red seaweed *Gracilaria verrucosa* at different concentrations of ammonia.

MATERIALS AND METHODS

The rate of ammonium uptake experiments were conducted in CAS in Marine Biology Laboratory, Annamalai University using standardized multiple flask method (Hanisak and Harlin, 1978). For this experiment 10 L capacity glass jars were used as the incubation vessel. Glass Fiber Cellulose (GFC) filtered seawater was enriched with ammonium chloride and sodium nitrate for 10 and 30 µM initial concentrations of ammonia. *Gracilaria verrucosa* (10 g/L wet. weight) was added into the experimental jars. Replicates were maintained for each concentration. Jars without algae were kept as control. GFC filtered seawater without enrichment and algae were considered as blank.

Collection of algal materials: Healthy and epiphyte-free, seaweeds were collected from Vellar estuary (Lat. 11º29′ N; Long.79º 46′ E). They were washed with clean seawater and then placed into the polythene mesh bags. The bags were immersed in the jars containing GFC filtered sea water under the shadow of natural daylight with adequate aeration for 24 h.

Experimental setup and maintenance: Fifty milliliters of water samples were collected at every one hour intervals up to 8 h. From the experimental jars, additionally four water samples were collected at 10, 20, 30 min and 1 h intervals from the commencement of the experiment. Ammonium concentration was determined by Phenol-Hypochlorite method (Solörozona, 1969). Uptake rates were calculated directly from the depletion of ammonium conc. from the jars using modified method of Krom *et al.* (1985): $V = S_0 - S_1$.

Where, V = Uptake rate (μg^{-1}.wet.wt.h^{-1}), S_0 = Initial ammonium concentration and S_1 = Final ammonium concentration.

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RESULTS AND DISCUSSION

The ammonium concentration in the control jars showed no variation between the first and last measurements of the experiment. The possibilities to remove the ammonium from the jars by the process like nitrification, ammonium volatilization and absorption by seaweeds. Nitrification was not possible in the present study because there is no bacterial bio-filter system in the culture medium and nitrate conc. was always lower than the ammonium conc. This clearly indicates removal of ammonium from the culture medium mainly due to seaweeds only. Such kind of finding were earlier reported by Dy and Yap (2001) in *Kappaphycus alvarezii*

The mean ammonium uptake was 6.12 and 17.31 μM g^{-1}. wet.wt.h^{-1}. in low and higher concentrations respectively. Interestingly, the ammonium absorption process is divided into four phases. During the first phase (with in 10 min) seaweeds uptake only very minimum level of ammonium 0.24 μM (3.5%) in low conc. jars and 0.38 μM (2.24%) in high conc. jars (Fig. 1 and 2). Uptake process is controlled by the membrane transport and carrier protein particles present in the cell membrane (Sato *et al.*, 2006). In this phase less amount of ammonium is transported by the membrane cells that resulting the lesser assimilation of ammonium into soluble nitrogen components and the plant has taken some time acclimatize to sudden nutritional change in the environment (Fujita, 1985). Ammonium uptake was related to the adaptive responses to nitrogen limitation by the macro-algae (Fujita, 1985).

The second phase (20 min to 1 h) is termed as uptake phase. Surge uptake of ammonium occurs in this phase about 3.30 μM (58.64 %) and 12.18 μM (68.12%) in low and higher conc. jars respectively (Fig. 1 and 2). This is due to more ammonium transportation by the membrane cells and more assimilation of ammonium nitrogen into soluble nitrogen components and stored in algal cells (Dy and Yap, 2001; Chen, 2008).

The third phase (1 to 6 h) is called internally controlled phase. In this phase rate of ammonium uptake was constant 2.58 μM (34.12%) and 4.22 μM (24.42%) (Fig. 1). Because of the rate of membrane transport is equal to assimilation rate (Rosenberg *et al.*, 1984).

The fourth phase (6 to 8 h) is called externally controlled phase, in this phase the uptake rate was very less (Fig. 1 and 2). Because, the availability of ammonium for utilization and assimilation rate into the cells were decline, that resulting to reduce the uptake in later collections (Fig. 3). Similar kinds of the results were previously reported by D´ELia and Debore (1978), Chen (2008) and Fujita (1985). From this study we inferred that the rate of uptake is directly proportional to the nutrient concentration and inversely proportion to the time (Table 1).

The maximum percentage of removal occurs within an hour and subsequently the uptake process was

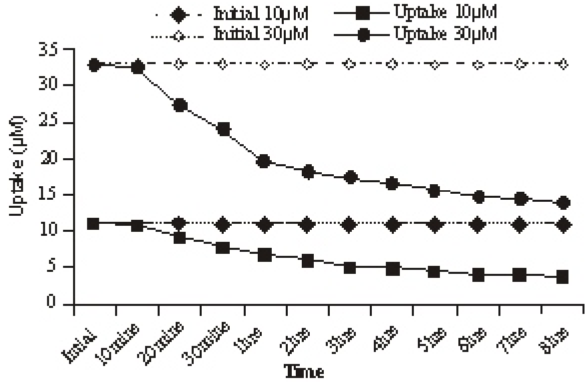

Fig. 1: Absorption of ammonium by *Gracilaria verrucosa* in different concentrations

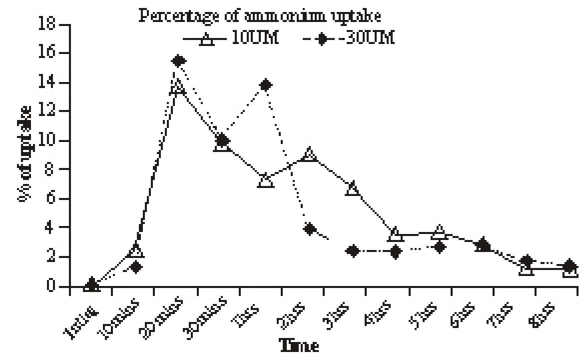

Fig. 2: Percentage of ammonium uptake between time and different concentrations

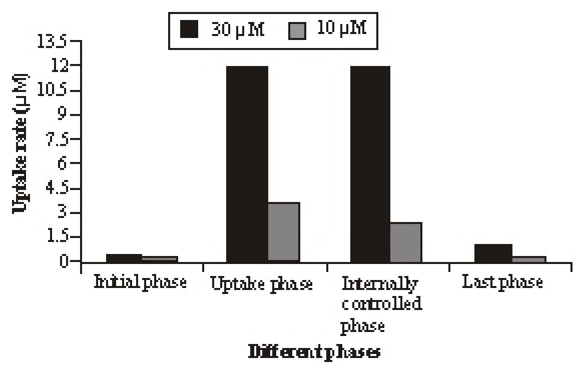

Fig. 3: Absorption of ammonium by *Gracilaria verrucosa* in different phases

considerably reduced (Fig. 3). Similar kinds of observations were earlier reported by Rosenberg *et al.* (1984), Pedersen (1994), DeBoer (1981) and Chen (2008).

Finfish and shellfish farms releasing effluents contain a wide range of ammonium conc. (5 to 30 μM). Due to the nutrient buffering capacity of seaweeds, they can act as a bio-filter to remove and transform the dissolved inorganic nutrients into soluble nitrogen components and these excess nitrogen components are utilized by the

Table 1: Multiple correlations between concentration, time and absorption in two different concentrations

	10 µm			30 µm		
	Conc	uptake	Time	Conc	uptake	Time
Conc	1			1		
uptake	0.522	1		0.410	1	
Time	-0.892**	-0.731*	1	-0.832**	-0.629*	1

*Correlation is significant at the 0.05 level (2-tailed)
** Correlation is significant at the 0.01 level (2-tailed)

seaweeds for their immediate growth (Littler, 1980; Lobban and Harrison, 1994; Choplin *et al*., 2001). From this study we inferred that *Gracilaria verrucosa* effectively reduce 60% ammonia from the medium. They also have more storage and the higher ammonium absorption capacity (Nagler *et al*., 2003; Hanisak, 1983; Rathakrishnan, 2001), so in any mariculture ponds, *Gracilaria verrucosa* can be used to reduce the ammonia load effectively. That will improve the quality of water, as well as the cultivable organisms also. The waste water of animal mariculture can be treated by the cultivation of *Gracilaria verrucosa*. This may to minimize the organic load and simultaneously give additional income by harvesting them.

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