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# Application of Seaweed Cultivation to the Bioremediation of Nutrient-rich Effluent

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A seaweed biofilter/production system has been developed to reduce the environmental impact of marine fish farm effluent in coastal ecosystems as a part of the practice of integrated aquaculture. Several known seaweed taxa and their cultivars have been considered as biofilter organisms based on their species-specific physiological properties such as nutrient uptake kinetics and their economic value.

*Porphyra* is an excellent candidate and shows efficient nutrient extraction properties. Rates of ammonium uptake were maintained at around 3 $\mu$moles · g · dw$^{-1}$ · min$^{-1}$ at 150 $\mu$M inorganic nitrogen at 10°C. Ulva is another possible biofilter candidate with an uptake rate of 1.9 $\mu$moles · g · dw$^{-1}$ · min$^{-1}$ under same conditions.

A simple uptake/growth and harvest model was applied to estimate the efficiency of the biofilter/production system. The model was deterministic and used a compartment model structure based on difference equations. The efficiency of *Porpyra* filter was estimated over 17% of $NH_4^+$ removal from the continuous supply of 100 $\mu$mol · $l^{-1}$ $NH_4^+$ at 100 $l$ · sec$^{-1}$ flow rate.

**Key Words:** ammonium uptake, integrated aquaculture, *Porphyra*, seaweed, biofilter

## INTRODUCTION

Aquaculture has been supporting human demands for fish products for centuries and is an important industry worldwide (e.g., Chopin and Yarish 1998; Naylor *et al.* 2001). With the massive increase in world aquaculture production in 1990s, the current aquaculture industry is one of the fastest growing sectors in world food production (Anon 2000).

Fish aquaculture production on an intensive scale has caused many environmental problems (Wu 1995; Chopin and Yarish 1998). Modern intensive monoculture requires high inputs of water, feeds, fertilizers and chemicals and inevitably produces considerable wastes. Therefore, many aquaculture operations put enormous pressure on coastal habitats (Chopin and Yarish 1999; Black 2001). Waste products from fish farms consist mainly of nitrogen, phosphorus and carbon dioxide. In pen-based salmon aquaculture, the production of one ton of fish results in the discharge of about 56.4 kg of total nitrogen and 7.0 kg of phosphorus from the feed containing 45% protein and 1% phosphorus with the food conversion rate (FCR) of 1.2. These releases may vary with different FCR and feed composition (Stead and Laird 2002).

To reduce the nutrient burden of the fish farm effluents, the integration of seaweed cultivation with fish aquaculture has been proposed (e.g., Chopin *et al.* 2001). Integrated aquaculture system is not a new concept at al, although there have been significantly fewer examples of aquatic animals being polycultured with seaweeds than of aquatic animals with other aquatic animals (Stickney 2000). Studies on the methods for treating effluents from enclosed mariculture systems with macroalgae date to the work of Ryther and colleagues in the mid 1970s and this approach has recently gained new interest in 1990s (e.g., Troell *et al.* 1999). Various strategies for integrating seaweed cultivation with fish culture have been successful (Brzeski and Newkirk 1997). Several species of genera *Gracilaria* (Buschmann *et al.* 1994; Troell *et al.* 1997, 1999; Nelson *et al.* 2001; Jones *et al.* 2001, 2002), *Ulva*

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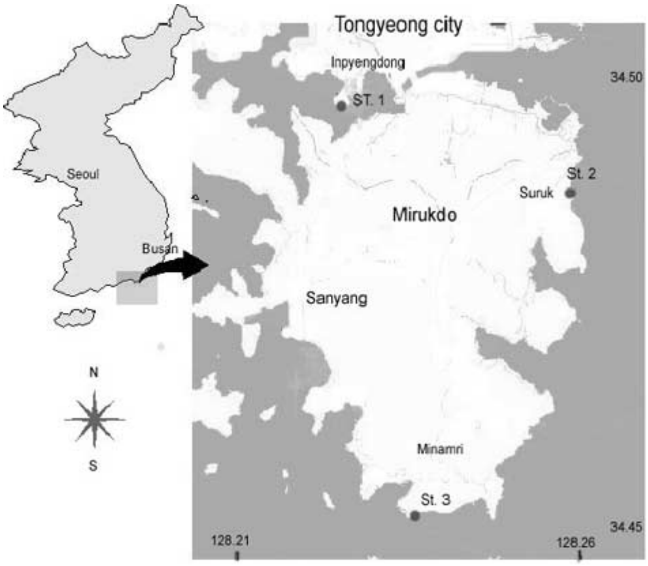


**Fig. 1.** Map of the study area in Tongyeong-Goeje area showing the three study sites.

(Krom *et al*. 1995; Neori *et al*. 1996, 1998, 2000; Dvir *et al*. 1999) and *Laminaria* and other macroalgae (Lüning 2002) have been considered in the integrated biofilter system and showed reasonably high efficiency in the removal of waste inorganic nutrients. Recently *Porphyra* (known as "Gim" in Korean) has been recommended as an attractive candidate for the integrated aquaculture with salmonoids (e.g., Chopin *et al*. 1999).

Korea has experience with polyculture. The National Fisheries Research Institute (NFRDI) of Korea has already set up guidelines of polyculture (Anon 1994). Those examples are *Porphyra tenera* and short necked clams (*Ruditapes philippinarum*) or surf clams (*Mactra veneriformis*); *Hizikia fusiforme* (actually now considered a species of *Sargassum*) or *Laminaria japonica* and sea squirt (*Halocynthia roretzi*); *Undaria pinnatifida* or *L. japonica* and abalone (*Haliotis discus hannai*); *H. fusiforme* and abalone. As finfish and shrimp aquaculture increased unexpectedly in 1990s, a changed concept and strategy of integrated culture to handle the effluent from these organisms is required. As a part of new project in 2002 - the development of an environmentally sound integrated systems has been in progress

The aim of this project was the development of a seaweed biofilter/production system to reduce the environmental impact of marine farmed finfish effluent in the coastal zone. Specifically, we sought to evaluate several species of seaweed with their performance for a biofilter/production system. Nutrient uptake kinetics, tissue nitrogen accumulation, and growth rate were considered here. We present preliminary results of some characteristics of these candidates for a seaweed biofilter/production system.

**Table 1.** The list of species studied for ammonium uptake experiment and their rates.

| Species | Uptake rates ($\mu$moles · g$^{-1}$ · dw · min$^{-1}$) | |
|---|---|---|
| | Average | Standard error |
| *Porphyra tenera* Kjellman | 3.44 | 0.856 |
| *Porphyra serriata* Kjellman | 2.89 | 0.708 |
| *Ulva pertusa* Kjellman | 1.91 | 0.690 |
| *Hypnea charoides* Lamouroux | 0.48 | 0.090 |
| *Gracilaria verrucosa* Papenfuss | 0.48 | 0.023 |
| *Gracilaria textorii* (Suringar) Hariot | 0.28 | 0.042 |

## MATERIALS AND METHODS

Tongyeong - Goeje area, as known as the 'Home of Korean Aquaculture' and located in the South Sea of Korea, was selected for the field observation. Three sites were chosen based on the different environmental characteristics. Site (St.) 1 is close to the dock area near the waste treatment plant of Tongyeong City, St. 2 is near the public beach area and St. 3 is facing to the open channel of the South Sea (Fig. 1).

The basic ecological aspects of seaweed community structure were surveyed monthly using the quadrat method (50 cm X 50 cm). The seawater nutrient contents were analyzed by standard methods (Parsons *et al*. 1984). The tissue elemental contents were analyzed by a CHNS/O elemental analyzer (Perkin Elmer 2400 Ser. II).

The rates of ammonium uptake were estimated from the 20-min, short-term ammonium-spiked batch incubations of several cultivated and field species (Table 1). Specimens were acclimated for 4 days at 10°C under 35 $\mu$mole · m$^{-2}$ · sec$^{-1}$ irradiance in filtered seawater. The seawater used for the experiment was collected from southwest part of the East Sea in October 2000. The average concentration of nitrate was less than 1 $\mu$M. After acclimation, specimens of ca. 0.5 g fresh weight were put into a 125 m*l* flask containing 50 m*l* of media, and incubated at 10°C under 150 $\mu$mole · m$^{-2}$ · sec$^{-1}$ for 20 min. The average rates were calculated as the difference between the initial ammonium concentration of 150 $\mu$M and final concentration from five to nine replicates.

Efficiencies of the seaweed biofilter/production system were estimated using a simple model. The model

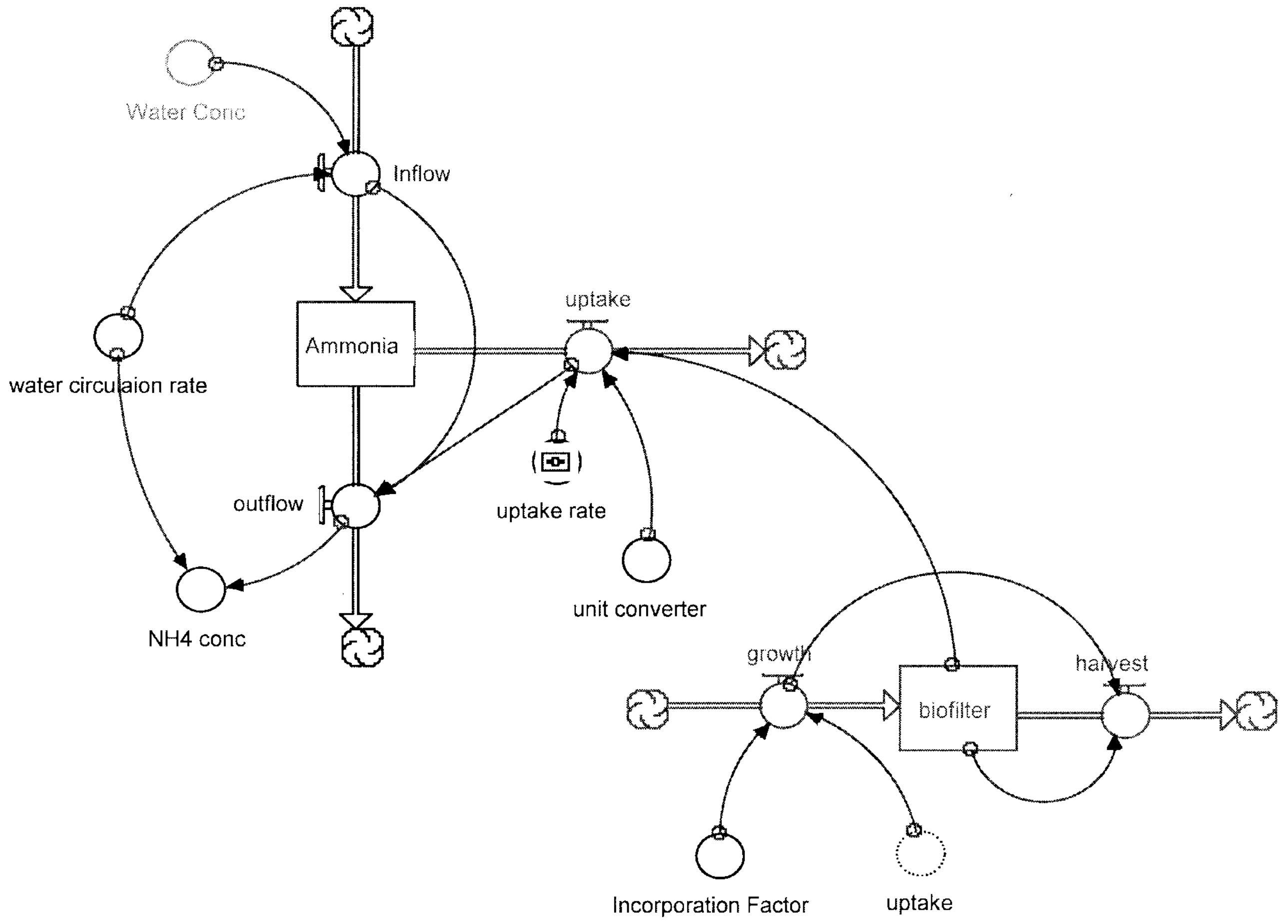


**Fig. 2.** Conceptual model representing the uptake/growth and harvest of a biofilter/production system.

included two submodels representing water column ammonium and the growth and harvest of seaweeds (Fig. 2). The water column ammonium submodel represented uptake process. The growth and harvest model represented growth and harvest as a result of ammonium uptake. The system was formulated as a deterministic compartment model based on difference equations programmed using Stella Research 7.0.2 High Performance System (2001) and runs on a personal computer. The initial values were defined by the user at the beginning of each simulation. Uptake rates of *Porphyra* sp. and growth and production information of *Porphyra* cultivation were applied to the model in estimating the efficiency from the continuous supply of 100 $\mu mol \cdot l^{-1}$ $NH_4^+$ at 100 $l \cdot sec^{-1}$ flow rate when the uptake rate of 2.5 $\mu mol \cdot g\ dw^{-1} \cdot min^{-1}$, with biomass of 15 nets (ca. 0.11 hectare in the field, about 0.5 $kg \cdot m^{-2}$), harvesting 15cm when frond grow to 20 cm for 5 months from October to March were applied.

## RESULTS

The changes in the fresh weight and dry weight of intertidal seaweed species in Tongyeong-Goeje area during the study period are shown in Fig. 3. The total biomass was highest at St. 3 and lowest at St. 1. Generally, there were decreasing trends in biomass during the study period. An unusually high value was recorded at St. 2 in June due to the contribution of *Ulva* sp. and *Codium* sp. *Ulva* sp. and *Pachymeniopsis* sp. showed coverage values of 22.5-95% and 10-27.5% respectively. *Undaria pinnatipida* and *Sargassum* sp. appeared with coverage of 5% to 46.7% during winter season and species of *Gelidium*, *Chondrus*, *Lomentaria*, *Hizikia* (now considered as a *Sargassum* sp.), and *Gigartina* were identified with 5-20% of coverage.

The concentration of total dissolved inorganic nitrogen (DIN; $NO_3^{-1}$ + $NO_2^{-1}$ + $NH_4^+$) and phosphate varied among sampling sites. The concentrations of DIN were

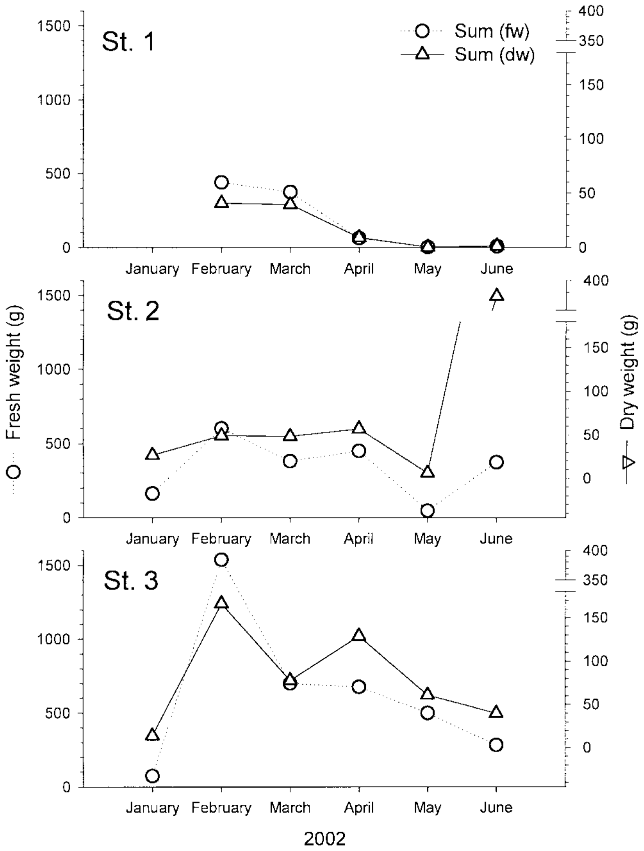


**Fig. 3.** The change of fresh and dry weight of intertidal seaweed species in Tongyeong-Goeje area.

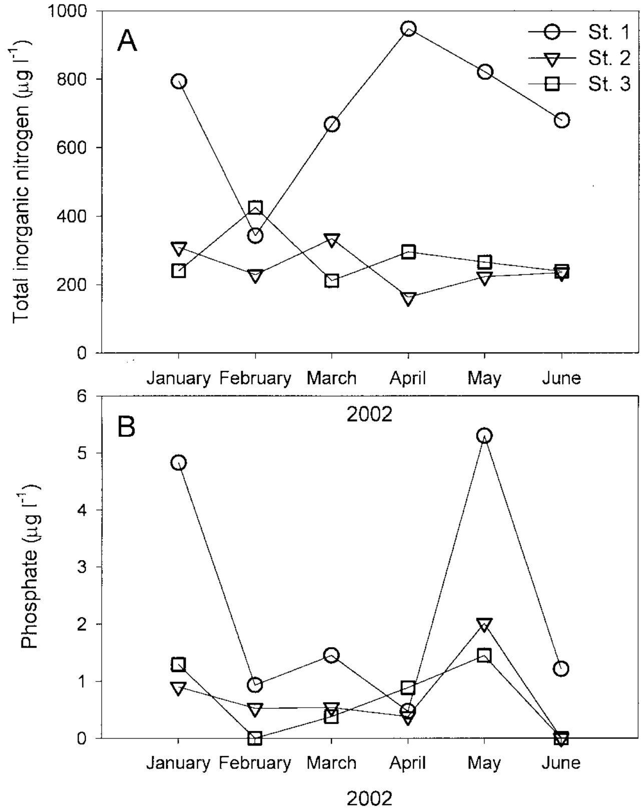


**Fig. 4.** The concentration of total dissolved inorganic nitrogen (A) and phosphate (B) in Tongyeong-goeje area.

high at St. 1 except in February (Fig. 4A). The phosphate seemed remain low around 1 $\mu g \cdot l^{-1}$ except in January and May (Fig. 4B).

The tissue nitrogen content of seaweeds was highest at St. 1 (average = 4.75%), where DIN was highest. Those of St. 2 and St. 3 averaged 3.13% and 2.65% respectively (Fig. 5A). The tissue nitrogen contents of *Porphyra* were up to 5.7% (5.4 ± 0.26%) of dry wt and showed highest value among seaweeds tested. *Sargassum* spp., *Enteromorpha* spp., and *Undaria* spp. showed 4.7%, 4.6%, and 4.2% of tissue nitrogen contents respectively in winter season. The nitrogen contents of green, brown and red algae were average of 3.18%, 2.91% and 3.42% respectively. In brown and red algae, the tissue nitrogen contents decreased during the period of study. However, the green algae, most of them were *Ulva* spp., showed large fluctuation (Fig. 5B).

There was a positive correlation between the tissue nitrogen content and seawater DIN. The correlation analysis showed the hyperbolic function seemed a better fit with higher correlation coefficient than those of the linear function; therefore, the rates of nitrogen accumulation in seaweed tissue seemed to reach maximum at higher DIN in seawater over 600 $\mu g \cdot l^{-1}$ (Fig. 6). This pattern was also found in Ulva spp. (Fig. 7).

*Porphyra* and *Ulva* showed four to six times higher rates of short-term $NH_4^+$ uptake than other red algal species tested in this study (Table 1). Based on the values of $NH_4^+$ uptake rates and growth, production and harvest information, the efficiency of $NH_4^+$ removal of Porphyra was estimated. The model predicted that more than 17% of ammonium would be removed from the continuous supply of 100 $\mu mol \cdot l^{-1}$ NH4+ at 100 $l \cdot sec^{-1}$ flow rate with conditions described in precious section.

## DISCUSSION

The algal coverage data showed that the green alga *Ulva* sp. could be regarded as a green tide especially in June. Recently the occurrence of green tides began to spread along the south coast of the Korean peninsula (Cuomo *et al.* 1993; Kim 2002). Inshore green tides and harmful algal blooms (HABs) in coastal waters are common in Korea, due mostly to the cultural eutrophication (Anon 2002). Fundamentally the growth of marine algae

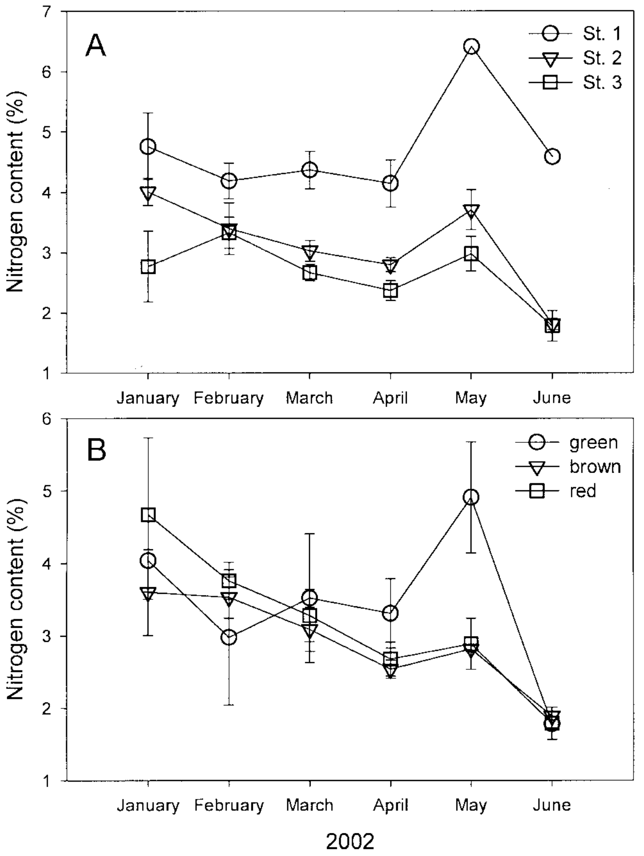


**Fig. 5.** The tissue nitrogen contents of seaweeds at different sites (A) and algal groups (B).

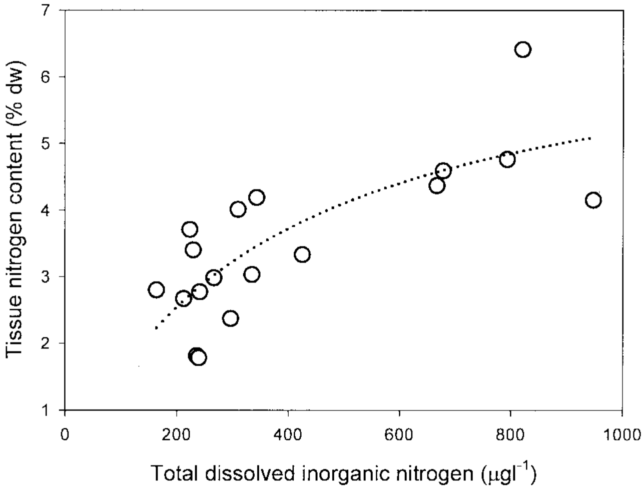


**Fig. 6.** A plot of total dissolved inorganic nitrogen vs. tissue nitrogen contents of seaweeds in Tongyeong-Goeju area.

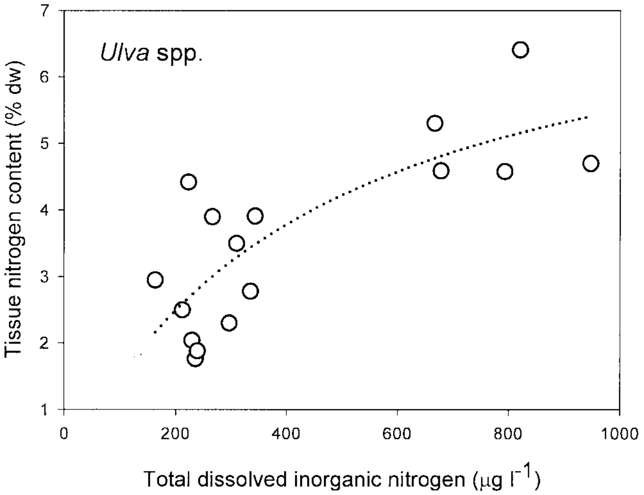


**Fig. 7.** A plot of total dissolved inorganic nitrogen vs. tissue nitrogen contents of *Ulva* sp. in Tongyeong-Goeje area.

in a given ecosystem should be proportional to the rate of supply of nutrients, temperature, light, and water motion (Schramm 1999; Smith *et al.* 1999). Therefore, the control of eutrophication should be accomplished by restricting the loading of key nutrients to the ecosystem. Deliberate nutrient loading reductions indeed have led to dramatic improvements in several areas in Japan (e.g., Smith *et al.* 1999).

The total nutrient load from aquaculture in an open system may be very small in comparison with other nutrient sources; however, its point source effects may have adverse effects on coastal ecosystems that can be significant (Chopin and Yarish 1999; Paez-Osuna *et al.* 1999). Seaweed cultivation can act as a nutrient scrubber system; integrated aquaculture of seaweeds with fish (or other forms of fed aquaculture) maybe is one of the most reasonable means for bioremediating coastal waters (McVey *et al.* 2002).

The tissue nitrogen contents of *Porphyra* and *Ulva* species showed ranges of values similar to those reported in other studies. The nitrogen contents of *Porphyra* were over 5% and *Ulva* was above 4%. For those species with cover greater than 10%, red algae had higher average tissue N than brown algae (though the difference was not significant). Red algae may have higher critical value due to their photosynthetic pigments (N-containing phycobiliproteins). Tissue nitrogen contents increased with increased concentration of dissolved nitrogen in seawater (Figs 6, 7). The internal tissue nitrogen contents are thought to be the complex results of the interactions among the external environments, uptake kinetics, assimilation, growth and reproductive processes (e.g. Hanisak 1983). The hyperbolic correlation between tissue nitrogen and DIN in seawater could be speculated as the result of hyperbolic pattern of $NH_4^+$ uptake as found in *Ulva lactuca* (Cohen and Neori 1991).

Tissue nitrogen contents of *Gracilaria* were also correlated with nutrient availability (Jones *et al.* 1996).

*Porphyra* tenera, which has been cultivated from the beginning of *Porphyra* cultivation in Korea, showed the highest rate of $NH_4^+$ uptake (3.4 $\mu$mol·g dw$^{-1}$·min$^{-1}$ at 150 $\mu$M) and nitrogen content (over 5%). The rate of uptake was a kind of surge uptake and may be higher than the accumulation uptake (Pedersen and Borum 1997). The values of *Pophyra serriata* and *Ulva pertusa* were slightly lower than that of *P. tenera* but four to five times higher rates than those of other species tested (Table 1). Although the present experiments were not designed to analyze the rate of $NH_4^+$ uptake according to the per surface area basis nor the ratio of surface area to volume, the values were similar to those reports of *Porphyra* and *Ulva* (Pedersen and Borum 1997; Taylor *et al.* 1998).

Several taxa such as *Ulva*, *Gracilaria*, *Laminaria*, and *Porphyra* have been investigated from different research groups (e.g., Chopin *et al.* 2001). The values of uptake rates in the present study were in the same range of other studies. Wallentinus (1984) found *Enteromorpha* sp. and *Cladophora* sp. showed about 2 $\mu$mol·g dw$^{-1}$·min$^{-1}$ at 60 $\mu$M $NH_4^+$ and Taylor *et al.* (1999) reported *Ulva* sp. exhibited about 2.0 $\mu$mol·g dw$^{-1}$·min$^{-1}$ and *Porphyra* sp. was about 4 $\mu$mol·g dw$^{-1}$·min$^{-1}$ at 100 $\mu$M $NH_4^+$.

Other studies on the rate of $NH_4^+$ uptake showed different values. Subandar et al. (1993) reported the rates of $NH_4^+$ uptake ranged between 0.1 and 0.4 $\mu$mol·g dw$^{-1}$·min$^{-1}$ in *Laminaria saccharina* using salmon farm effluent of 6.2-25.4 $\mu$mol $NH_4^+ \cdot l^{-1}$ in continuous flow culture.

An estimated 17% nitrogen removal efficiency could be easily achieved, because we applied a conservative uptake rate of 2.5 $\mu$mol·g dw$^{-1}$·min$^{-1}$ instead of 3.4 $\mu$mol·g dw$^{-1}$·min$^{-1}$ with conventional culture and harvesting methodigies. This conservative estimate is about half of the removal potential comparing with the estimates of Chopin *et al.* (1999). If higher uptake rates and stocking densities were applied, the efficiency could increase up to 50% in our model. However, the self-shading effects and circulation factors (such as flow and mixing rate) should be considered with increasing stocking densities (Cohen and Neori 1991; Quian *et al.* 1995; Nelson *et al.* 2001).

Previous studies showed the range of 10 to 90% of nitrogen removal efficiency in polyculture systems. *Ulva lactuca* removed 40-90% depending on $NH_4^+$ supply (Cohen and Neori 1991) and up to 85% in study of Vadermuelen and Gordin (1990). The efficiency of *Ulva rigida* in *Sparus aurata* waste water treatment was 76% in the daily input of 150 mmol $NH_4^+$·m$^{-2}$·day$^{-1}$ with 2.7g fw·$l^{-1}$ (Jimenez Del Rio *et al.* 1994). Troell *et al.* (1997) found slightly lower removal (5%) of dissolved inorganic nitrogen in a cage culture experiment with *Gracilaria*, but found much higher seasonal rates of NH4+ removal with 50% in winter and 90-95% in spring in a tank system (Troell *et al.* 1999). *Laminaria saccharina* removed 26-40% of incoming dissolved nitrogen from salmon farm effluent (Subandar *et al.* 1993).

The ultimate goal of this study is to develop a strategy for the sustainability of the coastal ecosystem based on the sound ecological and economic principles (Newkirk 1996). There are many conflicts among the users of coastal waters, and aquaculture should be conducted under the integrated coastal zone management with a balance between ‘extractive’ and ‘fed’ aquaculture (McVey *et al.* 2002; Rawson *et al.* 2002). The results of this preliminary study seem very promising, because the biology of the proposed species are well studied and the economic value of some are also high; nevertheless, more complete information should be obtained to expand integrated aquaculture systems (Troell *et al.* 1999; Harrison and Hurd 2001). Based on this study, a site-specific year-round system will be established depending on culture types, species, local hydrodynamics and other environmental factors. Moreover, monitoring and regulation of aquaculture systems need to be based upon carrying capacity models of each area, if there will be sustainable aquaculture along the Korean coastal zone (Fernandes *et al.* 2000, 2001).

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