Thesis for the Degree of Master of Fisheries Science

Performance of Seaweed Biofiltration in A Small-Scale Recirculating System

by

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The Graduate School

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August 2008

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소규모 순환여과 시스템에서 해조류를 이용한 생물여과의 성능

Advisor: Jae-Yoon Jo

by
Lilik Teguh Pambudi

A thesis submitted in partial fulfillment of the requirement for the degree of

Master of Fisheries Science

in the Department of Fisheries Biology, Graduate School, Pukyong National University

August 2008

릴릭 터구흐 팜부디의 수산학석사 학위논문을 인준함

2008 년 8 월

주 심 이 학 박 사 김 종 명 (인)

위 원 영 양 학 박 사 배 승 철 (인)

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August, 2008

Contents

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Abstract

A small-scale recirculating system with three seaweeds (*Enteromorpha compresa*, *Ulva pertusa*, *Sargassum piluliferum*) as biofilters was designed to measure biofiltration performance. The system used 9 small aquaria (23 L) for seaweed biofilter reactor (triplicates) and 1 small aquarium (23 L) as a control. Two reservoirs (288 L and 500 L) and submersible pump also were used to maintain water recirculating system which also maintain flow rate on the aquaria at 1.095 L min^{-1}. Artificial wastewater was used as nutrients for seaweed and 1 peristaltic pump was controlled to supply it into system. Ammonia loading rate on the system was 20 and 50 g TAN m^{-3} d^{-1}. The photoperiod was 12 h light:12 h dark and irradiance was 100 µmol photons m^{-2} s^{-1}. The water temperature regimes on the system were 10°C and 15°C. Ammonium, nitrate, nitrite and phosphate were determined between inflow and outflow on each aquarium daily. The experimental period was 15 days for each treatment (two different water temperatures and two different ammonia loading rate).

The results showed that nutrients concentration of effluents between the control and three seaweeds reactor are significantly different ($p \leq 0.05$), except on nitrite. Nutrient uptake rates and biofiltration efficiencies of ammonium by *E. compresa*, *U. pertusa*, and *S. piluliferum* on this research are 55.85-68.07 μM NH_4^+ g^{-1} FW d^{-1} (26.65-89.66%), 42.14-50.98 μM NH_4^+ g^{-1} FW d^{-1} (20.68-67.98%) and 27.47-34.32 μM NH_4^+ g^{-1} FW d^{-1} (13.18-46.24%), respectively. Nutrient uptake rates and biofiltration efficiencies of nitrate-nitrogen by *E. compresa*, *U. pertusa*, *S. piluliferum* in this experiment are 10.73-22.99 μM NO_3-N g^{-1} FW d^{-1} (17.77-52.26%), 13.57-21.29 μM NO_3-N g^{-1} FW d^{-1} (17-50.48%) and 12.24-19.30 μM NO_3-N g^{-1} FW d^{-1} (14.88-46.62%), respectively. In this research, nutrient uptake rates and biofiltration efficiencies of nitrite-nitrogen by *E. compresa*, *U. pertusa*, *S. piluliferum* are 0.19-0.22 μM NO_2-N g^{-1} FW d^{-1} (5.36-12.26%), 0.18-0.28 μM NO_2-N g^{-1} FW d^{-1} (4.78-11.32%) and 0.17-0.27 μM NO_2-N g^{-1} FW d^{-1} (4.60-11.63%), respectively. Thus, nutrient uptake rates and biofiltration efficiencies of orthophosphate by *E. compresa*, *U. pertusa*, and *S. piluliferum* are 1.16-2.41 μM PO_4^{3-} g^{-1} FW d^{-1} (29.62-81.74%), 0.69-1.82 μM PO_4^{3-} g^{-1} FW d^{-1} (16.70-65.15%) and 0.75-1.22 μM PO_4^{3-} g^{-1} FW d^{-1} (14.72-45.23%), respectively.

Furthermore, the volumetric ammonia removal rates by these seaweeds also higher than biofilm and RBC biofilter but on nitrate is lower than activated sludge reactor due to another research.

According to these results, it can be stated that *E. compresa* shows the better biofiltration performance than *U. pertusa* and *S. piluliferum* and also has a chance to be prime candidate as biofilter to maintain mariculture ponds effluent or will be used in seawater recirculating system at 10-15^{o}C of water temperatures..

Keywords: seaweed, biofiltration, recirculation, wastewater

List of Tables

List of Figures

I. INTRODUCTION

Both land-based and open-water cultures confirm that nutrients were released from fish, shrimps and bivalves and these are suitable for seaweed growth. The nitrogen (NH_3) released from such organisms is the preferred nitrogen source for seaweeds (Lobban and Harrison, 1994; Carmona et al., 2006). The phosphorus released increases phosphate (PO_4^{3-}) concentrations in the water, which is the most suitable form of phosphorus for seaweed growth (Lobban and Harrison, 1994; Neori, 1996; Chopin and Wagey, 1999). In addition, some seaweed species in integrated cultures take up nutrients beyond their requirements for growth (Troell et al., 1997).

Most studies confirmed that nutrients from land-based and open-water marine aquaculture operations are suitable as biofilter (Troell et al., 2003). A main issue in the effective implementation and optimal scale-up of biofiltering is a detailed understanding of algal ecophysiology. The optimization of the overall biofiltration efficiency necessitates a compromise between apparently conflicting aims: water flow, biomass production, nutrient uptake or reduction efficiency (Chopin et al., 2001). However, a reliable synthesis is still lacking on the many factors that can determine seaweed biofilter design and functioning in commercial integrated marine aquaculture especially only very few studies focused on closed recirculation systems. Thus, further research to identify species and technology that could also collect the nutrients in the water column, not only in the surface waters is required.

A conceptual framework of environmental problems associated with traditional monocultures indicates that short-term productivity gains at the cost of longer term sustainability. For this reason, the challenge for modern aquaculture is to make use of ecosystem process and functions, minimizing in this way the environmental effects. Most works on the use of seaweeds to treat

effluent water from land-based marine aquaculture has used integrated systems with seaweeds of *Ulva*, *Gracilaria* with finfish such as gilthead seabream. Vandermeulen and Gordin (1990) reported some of the first marine aquaculture experiments with *Ulva lactuta* in an integrated system of finfish, *Sparus aurata*. They found that *Ulva* removed total ammonia nitrogen (TAN) efficiently from fishpond effluent water. The author also described the use of *Ulva* as a viable cost-effective way of removing nutrients. High growth rates, high yields and reduced C/N ratios were subsequently reported by Neori et al. (1991) who cultivated *Ulva lactuta* using fishpond effluent water in outdoor tanks. Jimenez del Rio et al. (1996) reported removal rate of dissolved inorganic nitrogen by *Ulva rigida* as biofilter during summer and winter season.

Buschmann et al. (1996) presented that a case study for integrating salmon and *Gracilaria* in an intensive tank cultivation system and he founded *Gracilaria* is an efficient species for reducing nitrogen loads in fish effluents.

Enteromorpha compresa, *Ulva pertusa* and *Sargassum piluliferum* are common seaweeds in Korea and very abundant in a eutrophic coastal area. These seaweeds have different morphology and surface area:volume (SA:V) ratio and according to Lobban and Harrison (1994), *Enteromorpha compresa* has higher SA:V ratio, following by *Ulva pertusa* and *Sargassum piluliferum*. The seaweed which has higher SA:V ratio is also has higher uptake rates of nutrients in the water than the lower one. Present study was testing the hypotheses of uptake rates, biofiltration efficiency and also specific growth rate which are related to SA:V ratio among of *Enteromorpha compresa*, *Ulva pertusa* and *Sargassum piluliferum*.

Present study measured nutrient uptake and biofiltration efficiency of ammonia, nitrate, nitrite and phosphate using *Enteromorpha compresa*, *Ulva pertusa* and *Sargassum piluliferum* as a bioflter and specific growth rate also were calculated in a recirculating bioreactor system under laboratory conditions by two different ammonia loading rate and two water temperature regimes.

Furthermore, the volumetric nutrient uptake rates and biofiltration efficiency of these seaweeds were also compared to other type of biofilter (i.e. sand, RBC) in nitrification and denitrification.

II. MATERIALS AND METHODS

1. System design

The experimental system was designed to allow an evaluation of the biofiltration capacity of seaweeds in recirculating system (Fig. 1). The system consists of 10 aquaria (25 x 40 x 24 cm) which contained 23 L of seawater, 500 L plastic tank as upper reservoir (head tank), a submersible pump, a wooden tank as bottom reservoir (90 cm x 80 cm x 60 cm) which contained 288 L of seawater, and a cartilage filter (Figure 1). Nine aquaria were used for 4 treatments, 3 seaweeds (*Enteromorpha compresa*, *Ulva pertusa*, *Sargassum piluliferum*) with 3 replications and 1 aquarium for control. On the top of aquaria, 3 fluorescent light sources (3 x 40 watt) were installed as the light intensity of 100 μmol photons m^{-2} s^{-1} for supporting photosynthesis process to each aquarium. Aeration was supplied on each aquarium to supply air and circulation of seaweed in water column. Plastic-plate screen was provided in each aquarium (distance is 5 cm of in front) to prevent seaweeds flow out through the outflow pipe (S-shape pipe). Thermostatic heating and cooling system were used to maintain water temperature. One peristaltic pump was used for input the synthetic wastewater to the reservoir. The direction of water flow also can be shown on Figure 1 and the water flowed to each aquarium was maintained at 1.095 mL min^{-1}. This all experimental process was operated at December, 2007 until March, 2008.

2. Seaweeds tested

Three seaweeds, *Enteromorpha compresa*, *Ulva pertusa* and *Sargassum piluliferum* were placed in each of triplicated aquarium as biofilters. All of the seaweeds were collected from the lower intertidal zone in Songjeong beach area.

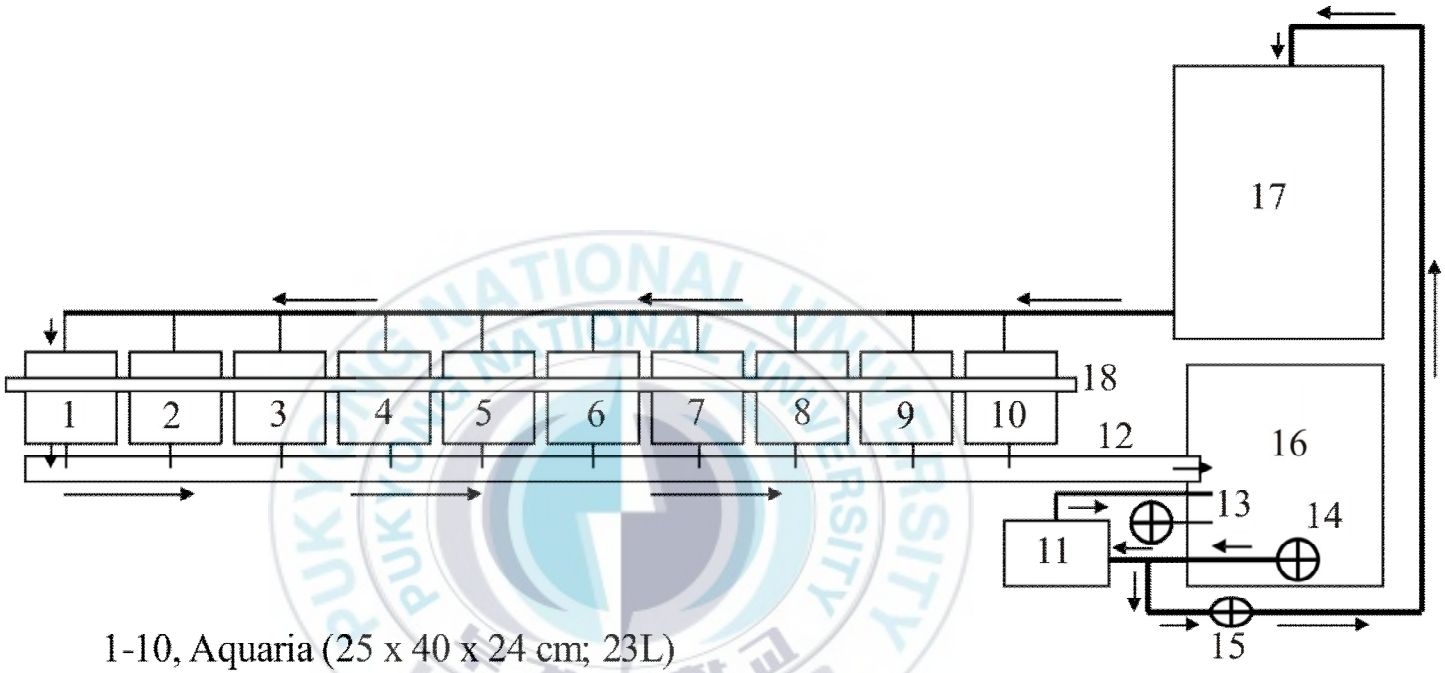

Fig. 1. Recirculating system for testing of seaweeds (*Enteromorpha compresa*, *Ulva pertusa*, *Sargassum piluliferum*)

The seaweeds were moved into the laboratory, and treated by 5 ppm solution of GeO_2 to remove sediments and epiphytes. Then the seaweeds were acclimated for 2 days in aquaria (90 L) under 10-13^{o}C and light condition of 30 μmol photons m^{-2} s^{-1}. From these stocks, fresh thalli (FW) were selected and stocked at the rate of 5 g FW L^{-1} in each aquarium according to Aragon et al. (2002).

3. Experimental procedure

Three seaweeds (*Enteromorpha compresa*, *Ulva pertusa*, *Sargassum piluliferum*) were conditioned by supplying of synthetic wastewater. Total ammonia nitrogen (TAN) uptake rate were evaluated at 20 g m^{-3} d^{-1} and 50 g m^{-3} d^{-1} of TAN loading rates. These TAN loading rate were based on the ammonia excretion rate of Korean rockfish *Sebastes schlegeli* (Lei Peng, 2003) and TAN loading rate of fishpond effluents on mariculture operation (Myusa, et al., 2006). The formulation of synthetic wastewater (Table 1) was based on Rogers and Klemetson (1985).

The 20 L synthetic wastewater tank was installed, the synthetic wastewater feeding was supplied by a peristaltic pump (Cheon Sei, Korea) and water flow was adjusted at 14 mL/min to reservoir tank. The peristaltic pump was controlled to ensure that the reservoir tank received the fixed hydraulic loading rate of synthetic wastewater.

Water flow rate of each aquarium was set at 1.095 L/min to represent the experimental system by Myusa, et al. (2006). Water temperature was maintained at 10^{o}C and 15^{o}C by a thermostatic heating system and cooler equipment in winter season (Kang et al., 2007). This study used 12L:12D of photoperiod according to Aragon, et al. (2002).

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Table 1. Composition of synthetic wastewater (20 L) (Rogers and Klemetson, 1985)

Composition	Ammonia Loading Rate 20 g m^{-3} d^{-1}	Ammonia Loading Rate 50 g m^{-3} d^{-1}
$(NH_4)_2SO4$	20.777	51.942
$NaHCO_3$	26.657	66.642
Na_2HPO4	6.867	17.167
Dextrose	5.378	13.445
$MnSO_4$	0.032	0.08

4. Water sampling

Water was sampled daily at daylight period (15:00 pm) from inflow (control) and outflows (control and treatment) of aquaria with 11 samples daily. Other parameters (water temperature, dissolved oxygen, salinity and pH) were measured daily in each aquarium. Since the synthetic wastewater (20 L d^{-1}) was added into the system for 24 hours by a peristaltic pump, approximately 20 L of excess water in the system was over flowed continuously.

5. Method of water quality measurements

Nutrients (NH_4^+, NO_3-N, NO_2-N, PO_4^{3-}) were determined by HACH DR/2000 Spectrophotometer. Ammonium was determined by Nessler Method, nitrate-nitrogen by Cadmium Reduction Method, nitrite-nitrogen by Diazotization Method and orthophosphate by Ascorbic Acid Method (Standard Method for the Examination of Water and Wastewater, 1994).

Dissolved oxygen and water temperature were measured by Oxyguard®, salinity by refractometer (ATAGO Inc.) and pH by Pin Point pH Meter (American Marine Inc.).

5.1. Nutrient uptake rates

Nutrient uptake rates (NUR, μM g^{-1} FW d^{-1}) were determined by following equation (Aragon et al., 2002):

$$((C_{in}-C_{Out}) \times Q \times \Delta t)/(B \times \Delta t))$$

C_{in}-C_{out} , mean inflow and outflow of nutrient concentration (μM)
Q, flow rate (L d^{-1})
B, biomass of seaweed (g fresh weight)
Δt, time interval (d)

5.2. Biofiltration efficiency

Biofiltration efficiency (B_e, %) was calculated by following equation (Jimenez Del Rio et al., 1994):

$$B_e = ((C_{in}-C_{out})/C_{out}) \times 100$$

C_{in}, concentration of nutrient in inflow (μM)
C_{out}, concentration of nutrient in outflow (μM)

5.3. Volumetric nutrient removal rates

Volumetric nutrient removal rates (VNR, g $m^{-3}d^{-1}$) were calculated by following equation (modified from Oh, 2001):

$$VNR = (C_{in}-C_{out}) \times Q \times V^{-1}$$

C_{in}, concentration of nutrient in inflow (mg/L)

C_{out}, concentration of nutrient in outflow (mg/L)

Q, flow rate (m^3/day)

V, volume of bioreactor (m^3)

6. Biological measurements

Fresh weight of seaweed were determined by removing waters with soft towel and weighed on electric balance (EEA Inc.) and dry weight to fresh weight ratio was determined after drying 10 g of seaweed (n=9) at 80°C for 48 h (Phillips and Hurd, 2004). This ratio was needed if the determining of nutrients uptake rate based on dry weight of seaweed.

6.1. Specific growth rate

Specific growth rate (SGR, %) rates were calculated as (Rosenberg et al., 1984):

$$SGR = 100 \times [\ln(w_t/w_o)]/t$$

w_o, initial biomass (g)

w_t, biomass at given times (g)

t, culture days (days)

7. Statistical analysis

Data were analysed by analysis of variance (ANOVA) to determine whether there were significant differences in the results of the measured parameters among the treatments and between treatment and control. This processed analysis was using Minitab 15 statistical software version (Minitab

Inc, 2007). The differences between the means of treatment were analysed using Tukey's HSD Test ($p \leq 0.05$).

III. RESULTS

1. Treatment I: Ammonia loading rate of 20 g m^{-3} d^{-1} at 10°C

1.1. Condition during experiment

In Treatment I, water temperatures were ranged 9.9-10.2°C and pH maintained 7.64-7.93 in inflow and outflow of *E. compresa* were 7.78-7.98, *U. pertusa* were 7.77-7.96 and *S. piluliferum* were 7.72-7.94. The pH was slightly increased from inflow to outflow because of photosynthetic activities by seaweeds. The dissolved oxygen level was 11-11.3 ppm in all bioreactor (aquaria). Water salinity during this treatment was 35 psu. Furthermore, water temperatures, pH level, D.O. level and water salinity in the system during Treatment I were in optimal range for uptaking nutrient by seaweeds.

1.2. Nutrient concentrations

On the 1st day of the experiment after ammonia loading rate was maintained at 20 g m^{-3} d^{-1} for 15 days of Treatment I period, ammonium (NH_4^+) concentration of all outflows were ranged 19.11-93.17 µM and the outflow of *E. compresa* was lowest (19.11 µM) and that of the control was highest (93.17 µM). At the same time, ammonium concentration in the outflow of *U. pertusa* was 28.67 µM and that of *S. piluliferum* was 38.22 µM. The highest concentration of ammonium in the outflow of control reached to 210.70 µM, and in the outflow of *E. compresa*, *U. pertusa*, *S. piluliferum* reached to 119.44 µM, 134.97 µM, and 160.05 µM, respectively (Fig. 2).

At the end of experiment, the ammonium concentration in the outflow of control, *E. compresa*, *U. pertusa*, and *S. piluliferum* were 116.10 µM, 34.64

μM, 77.64 μM and 104.39 μM, respectively. Statistically significant differences ($p \leq 0.05$) were found in ammonium concentration among the treatments (Fig. 2).

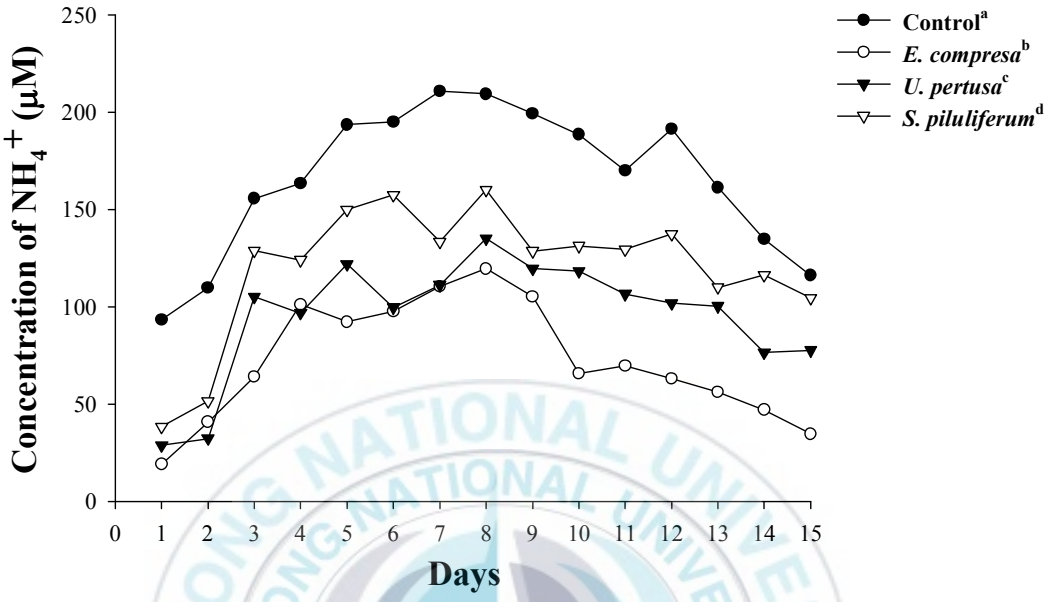

Fig.2. Changes of average concentration of ammonium in the outflow of control, *Enteromorpha compresa*, *Ulva pertusa*, and *Sargassum piluliferum* under the ammonia loading rate of 20 g m^{-3} d^{-1} at 10°C. The different superscript letter of treatments in the legend shows statistically different in the means.

In the Treatment I, concentration of nitrate-nitrogen (NO_3-N) in the outflow of the control, *E. compresa*, *U. pertusa* and *S. piluliferum* at the 1st day were 50 μM, 9.28 μM, 12.14 μM and 26.42 μM, respectively. The highest concentration of nitrate-nitrogen in the outflow of control reached to 100 μM, and thos of seaweeds, *E. compresa U. pertusa* and *S. piluliferum* reached to 64.28 μM, 61.90, and 66.42 μM, respectively. At the end of experiment, the

nitrate-nitrogen concentration in the outflow of the control, *E. compresa*, *U. pertusa*, and *S. piluliferum* were 50 μM, 42.85 μM, 35.71 and 42.85 μM, respectively. Nitrate-nitrogen concentration at the outflow of the three seaweeds were significantly lower than that of the control ($p \leq 0.05$), but those among the seaweeds were not significantly different ($p > 0.05$) (Fig. 3).

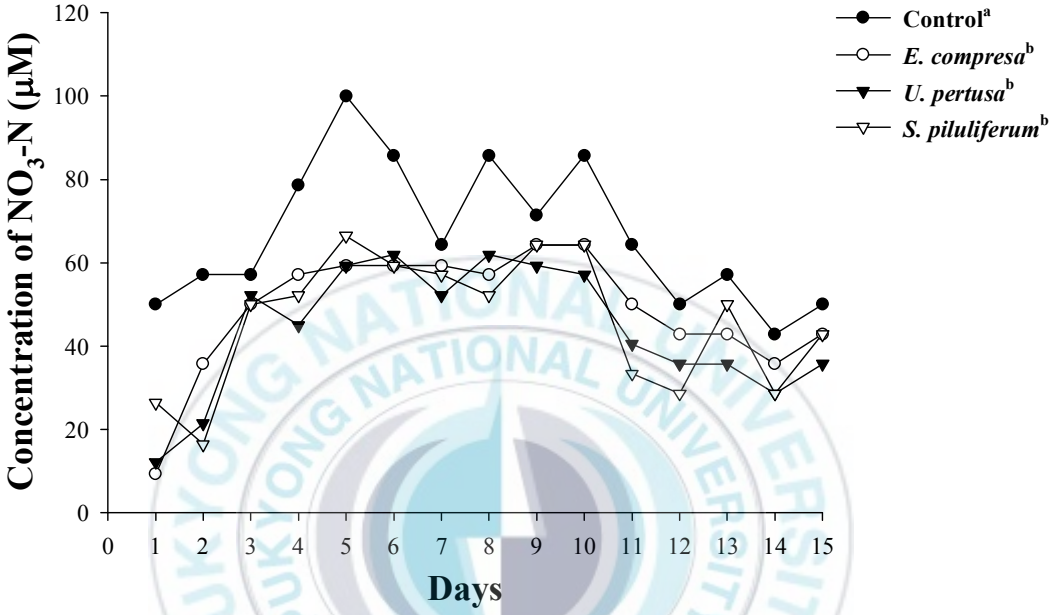

Fig.3. Changes of average concentration of nitrate-nitrogen in the outflow of the control, *Enteromorpha compresa*, *Ulva pertusa*, and *Sargassum piluliferum* under the ammonia loading rate of 20 g m^{-3} d^{-1} at 10°C. The different superscript letter of treatments in the legend shows statistically different in the means.

Concentration of nitrite-nitrogen (NO_2-N) in the outflow of control, *E. compresa*, *U. pertusa* and *S. piluliferum* in the Treatment I at 1st day were 2.50 μM, 2.35 μM, 2.43 μM and 2.21 μM, respectively. The highest concentration

of nitrite-nitrogen in the outflow of control reached to 6.93 μM, and those of seaweeds, *E. compresa*, *U. pertusa* and *S. piluliferum* reached to 6.50 μM, 6.64 μM, and 6.64 μM, respectively. At the end of experiment, concentration of nitrite nitrogen in the outflow of control, *E. compresa*, *U. pertusa* and *S. piluliferum* were 5.85 μM, 5.71 μM, 5.78 μM and 5.71 μM, respectively. No significant differences ($p>0.05$) were found in nitrite-nitrogen concentration among the treatments (Fig. 4).

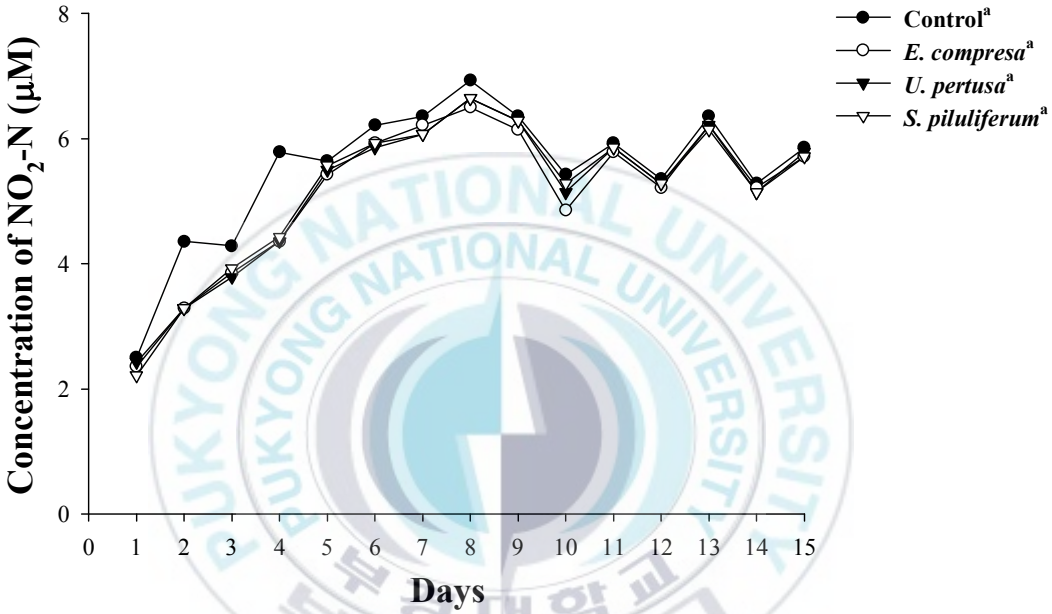

Fig.4. Changes of average concentration of nitrite-nitrogen in the outflow of the control, *Enteromorpha compresa*, *Ulva pertusa*, and *Sargassum piluliferum* under the ammonia loading rate of 20 g m^{-3} d^{-1} at 10°C. The different superscript letter of treatments in the legend shows statistically different in the means.

On the 1st day of experiment, concentration of orthophosphate (PO_4^{3-}) in the outflow of the control, *E. compresa*, *U. pertusa*, and *S. piluliferum* were 0.70 μM, 0.62 μM, 0.10 μM and 0.40 μM, respectively. The highest concentration of orthophosphate in the outflow of control reached to 2.72 μM, and those of seaweeds, *E. compresa*, *U. pertusa* and *S. piluliferum* reached to 0.86 μM, 1.73 μM, and 1.54 μM, respectively. No significant differences in orthophosphate concentration ($p>0.05$) were found only between in the outflow of *U. pertusa* and *S. piluliferum* (Fig. 5).

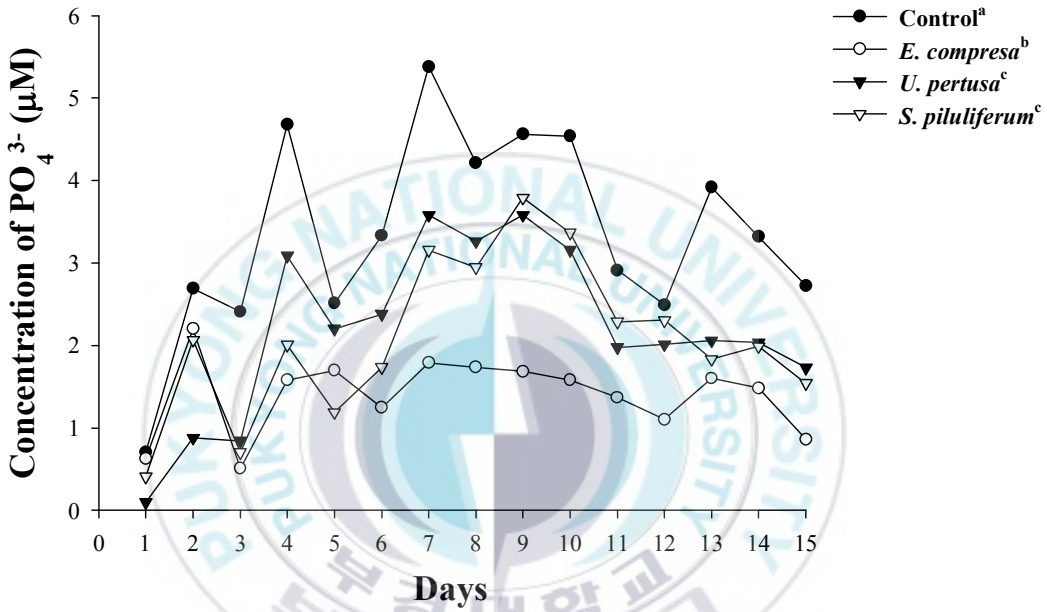

Fig.5. Changes of average concentration of orthophosphate in the outflow of control, *Enteromorpha compresa*, *Ulva pertusa*, and *Sargassum piluliferum* under the ammonia loading rate of 20 g m^{-3} d^{-1} at 10°C. The different superscript letter of treatments in the legend shows statistically different in the means.

1.3. Nutrient uptake rates and biofiltration efficiencies

Three seaweeds showed nutrients uptake activities for ammonium, nitrate-nitrogen, nitrite-nitrogen and orthophosphate. *E. compresa* showed highest uptake ability on ammonium, nitrate-nitrogen, nitrite-nitrogen uptake rate followed by *U. pertusa* and *S. piluliferum*. Furthermore, in the orthophosphate uptake rate, *E. compresa* showed the highest uptake rate followed by *S. piluliferum* and *U. pertusa*. Thus, *E. compresa* had highest biofiltration efficiencies on ammonium, nitrite-nitrogen, orthophosphate and *U. pertusa* as well on nitrate-nitrogen as shown in Table 2.

Table 2. Nutrient uptake rate (μM g^{-1} FW d^{-1}) and biofiltration efficiency (%) of *Enteromorpha compresa, Ulva pertusa* and *Sargassum piluliferum* under ammonia loading rate of 20 g m^{-3} d^{-1} at 10°C (Treatment I)

Nutrients	*E. compresa*	*U. pertusa*	*S. piluliferum*
Ammonium (NH_4^+)	55.85 (58.07%)[a]	42.14 (43.82%)[b]	27.47 (28.60%)[c]
Nitrate (NO_3-N)	10.73 (26.59%)[a]	13.57 (34.68%)[a]	12.24 (31.02%)[a]
Nitrite (NO_2-N)	0.22 (7.12%)[a]	0.20 (6.39%)[a]	0.19 (6.47%)[a]
Orthophosphate (PO_4^{3-})	1.16 (54.33%)[a]	0.69 (38.36%)[b]	0.75 (38.21%)[b]

*Number with the different letter in the same row shows significant difference ($p \leq 0.05$).

1.4. Volumetric nutrient removal rates

For volumetric nutrient removal rate, *E. compresa* showed the highest rate for ammonia and *U. pertusa* for nitrate-nitrogen (Table 3).

Table 3. Volumetric nutrient removal rates (g m^{-3} d^{-1}) of *Enteromorpha compresa, Ulva pertusa* and *Sargassum piluliferum* under ammonia loading rate of 20 g m^{-3} d^{-1} at 10°C (Treatment I)

Nutrients	*E. compresa*	*U. pertusa*	*S. piluliferum*
TAN	179.25±34.42[a]	135.24±31.29[b]	88.03±34.48[c]
NO_3-N	17.28±11.57[a]	21.85±10.36[a]	19.71±11.10[a]

*Number with the different letter in the same row shows significant difference ($p \leq 0.05$).

2. Treatment II: Ammonia loading rate of 20 g m^{-3} d^{-1} at 15°C

2.1. Condition during experiment

During Treatment II, water temperatures were maintained between 14.9-15.1°C and pH in inflow of the treatments were ranged 7.81-7.88 and that in outflows of *E. compresa*, *U. pertusa* and *S. piluliferum* were 7.91-7.98, 7.88-7.92, and 7.87-7.92, respectively. The prone of slightly increasing pH from inflow to outflow of all treatments was because of photosynthetic activities by seaweeds. The dissolved oxygen levels were ranged 11-11.3 ppm in all bioreactor (aquaria). Water salinity during this treatment was remained 35 psu. According to the data mentioned above, water temperatures, pH level, D.O. level and water salinity in the system of Treatment II were in optimal range for uptaking nutrient by seaweeds.

2.2. Nutrient concentrations

After ammonia loading rate was maintained at 20 g m^{-3} d^{-1} for 15 days in Treatment II period, the ammonium (NH_4^+) concentration in the outflow of the

control, *E. compresa*, *U. pertusa*, and *S. piluliferum* at the 1st day were 43 μM, 7.17 μM, 7.17 μM and 28.67 μM, respectively. The highest ammonium concentration in the outflow of control reached to 139.75 μM (at 6th and 9th day), and those of seaweeds, E. compresa, U, pertusa and S. piluliferum reached to 35.83 μM (at 8th day), 46.58 μM (at 5th and 9th day), and 78.83 μM (at 12th day), respectively. At the end of experiment, ammonium concentration in the outflow of the control, *E. compresa*, *U. pertusa*, and *S. piluliferum* were 107.50 μM, 7.17 μM, 35.83 μM and 53.75 μM, respectively. Statistically significant differences ($p \leq 0.05$) were found in ammonium concentration among the treatments (Fig. 6).

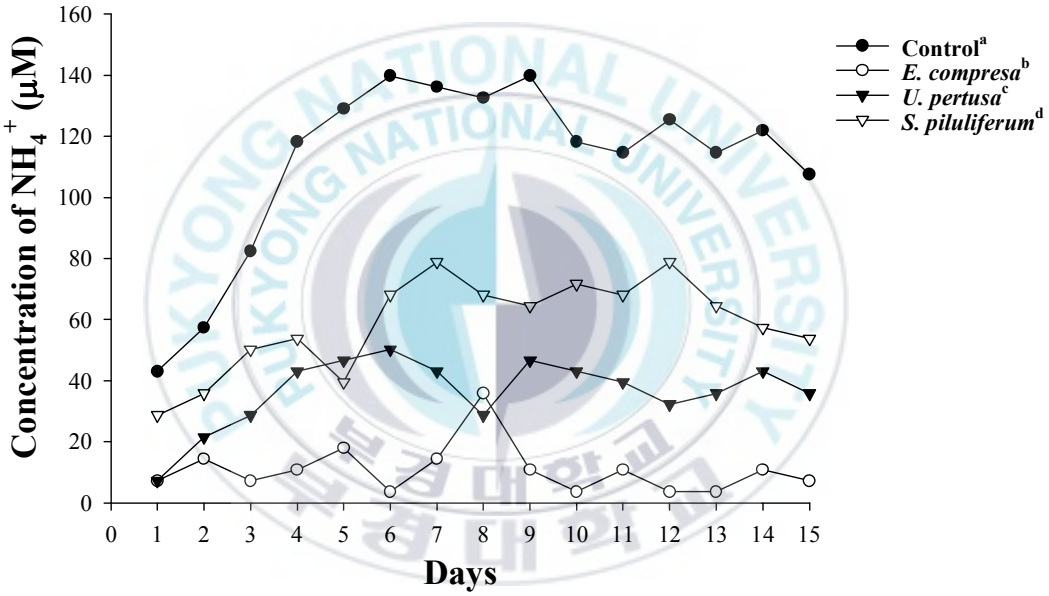

Fig.6. Changes of average concentration of ammonium in the outflow of the control, *Enteromorpha compresa, Ulva pertusa* and *Sargassum piluliferum* under the ammonia loading rate of 20 g m^{-3} d^{-1} at 15°C. The different superscript letter of treatments in the legend shows statistically different in the means.

In the Treatment II, concentration of nitrate-nitrogen (NO_3-N) in the outflow of the control, *E. compresa*, *U. pertusa* and *S. piluliferum* at the 1st day were 35.71 μM, 7.14 μM, 7.14 μM and 14.28 μM, respectively. The highest nitrate-nitrogen concentration in the outflow of the control reached to 78.57 μM (at 10th and 14th day), and those of seaweeds, *E. compresa*, *U. pertusa* and *S. piluliferum* reached to 50 μM (at 14th day), 50 μM (at 14th day), and 50 μM (at 3rd day), respectively. At the end of experiment, the nitrate-nitrogen concentration in the outflow of the control, *E. compresa*, *U. pertusa*, and *S. piluliferum* were 42.85 μM, 28.57 μM, 21.42 and 28.57 μM, respectively. Significant differences in nitrate-nitrogen concentrations were found between the outflow of the control and the three seaweeds ($p \leq 0.05$), but there was no differences among of seaweeds ($p > 0.05$) (Fig.7).

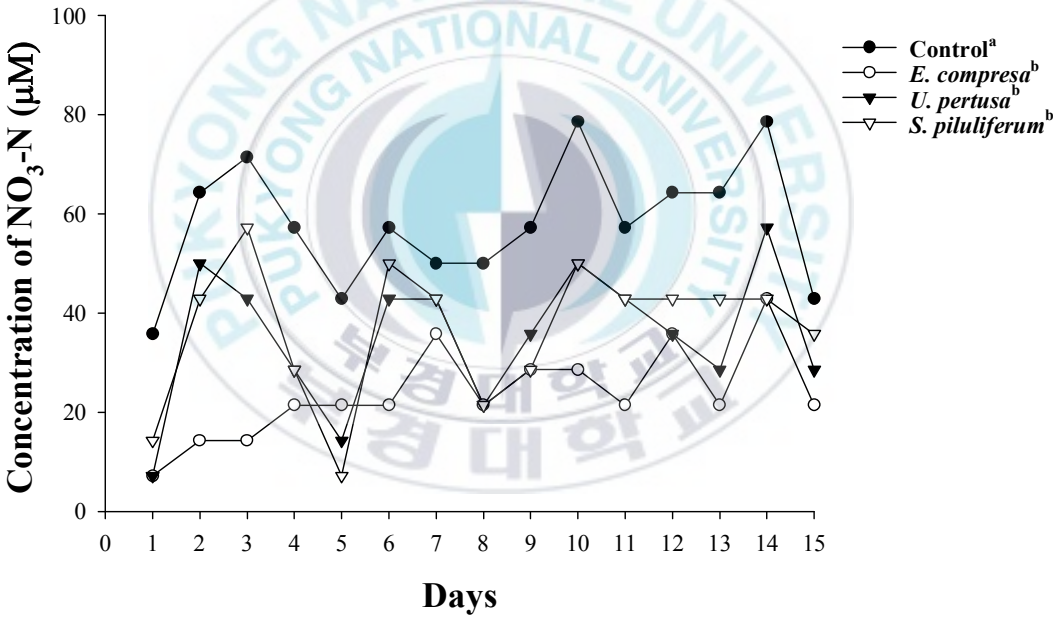

Fig.7. Changes of average concentration of nitrate-nitrogen in the outflow of the control, *Enteromorpha compresa, Ulva pertusa* and *Sargassum piluliferum* under the ammonia loading rate of 20 g m^{-3} d^{-1} at 15°C. The

different superscript letter of treatments in the legend shows statistically different in the means.

Concentrations of nitrite-nitrogen (NO_2-N) in the outflow of the control, *E. compresa*, *U. pertusa* and *S. piluliferum* in the Treatment II at 1st day were 1.57 μM, 1.28 μM, 1.35 μM and 1.14 μM, respectively. The highest concentration of nitrite-nitrogen in the outflow of the control reached to 5.42 μM, and three seaweeds, *E. compresa*, *U. pertusa* and *S. piluliferum* reached 5.42 μM, 5.57 μM, and 5.57 μM, respectively. At the end of experiment, concentration of nitrite nitrogen in the outflow of the control, *E. compresa*, *U. pertusa* and *S. piluliferum* were 4.92 μM, 4.64 μM, 4.71 μM and 4.64 μM, respectively. No statistically difference ($p \geq 0.05$) was found in nitrite-nitrogen concentration in the treatment (Fig. 8).

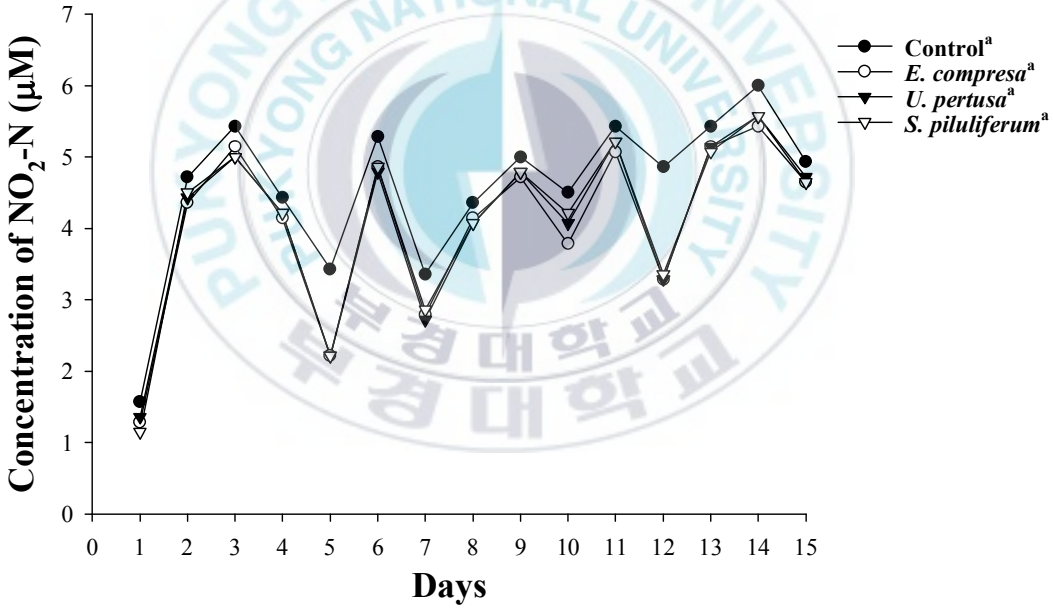

Fig.8. Changes of average concentration of nitrite-nitrogen in the outflow of the control, *Enteromorpha compresa, Ulva pertusa* and *Sargassum*

piluliferum under the ammonia loading rate of 20 g m^{-3} d^{-1} at 15°C. The different superscript letter of treatments in the legend shows statistically different in the means.

Concentrations of orthophosphate (PO_4^{3-}) in the outflow of the control, *E. compresa*, *U. pertusa*, and *S. piluliferum* at the 1st day were 0.52 μM, 0.31 μM, 0.10 μM and 0.21 μM, respectively. The highest concentration of orthophosphate in the outflow of the control reached to 4.31 μM, and three seaweeds, *E. compresa*, *U. pertusa*, and *S. piluliferum* reached to 0.94 μM, 1.68 μM, and 2.42 μM, respectively. Statistically differences ($p \leq 0.05$) were found in orthophosphate concentration among the treatments (Fig. 9).

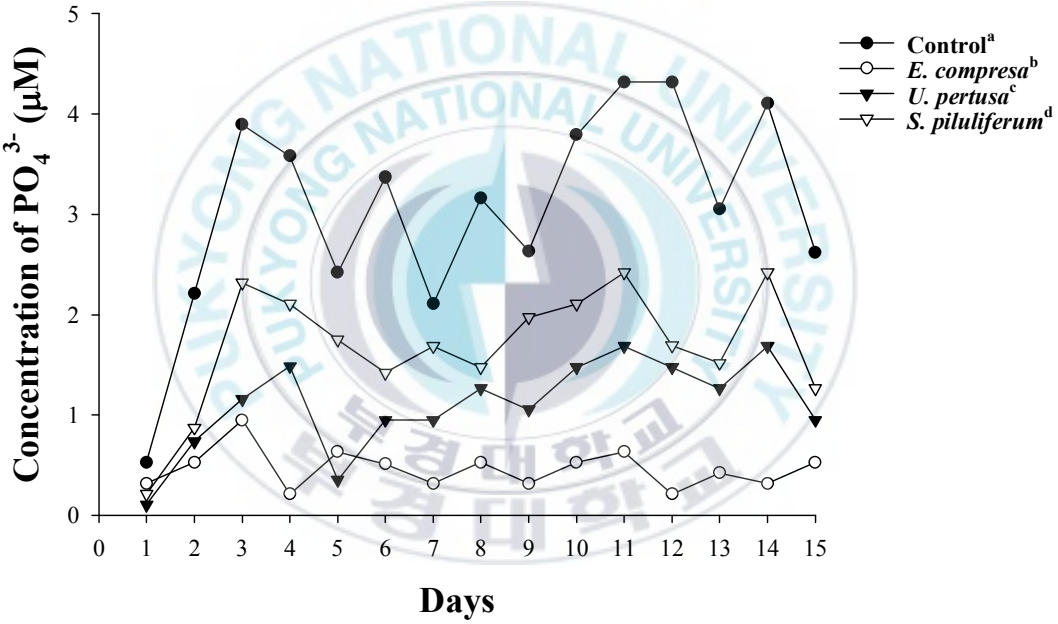

Fig.9. Changes of average concentration of orthophosphate in the outflow of the control, *Enteromorpha compresa, Ulva pertusa* and *Sargassum piluliferum* ammonia loading rate of 20 g m^{-3} d^{-1} at 15°C. The different superscript letter of treatments in the legend shows statistically

different in the means.

2.3. Nutrient uptake rates and biofiltration efficiencies

In the experiment of Treatment II, seaweeds actively uptakes ammonium, nitrate-nitrogen, nitrite-nitrogen and orthophosphate. *E. compresa* showed highest ammonium, nitrate-nitrogen, nitrite-nitrogen and orthophosphate uptake rate followed by *U. pertusa* and *S. piluliferum*. Furthermore, *E. compresa* also had highest biofiltration efficiencies on ammonium, nitrate-nitrogen, nitrite-nitrogen, orthophosphate than other seaweeds as shown in Table 4.

Table 4. Nutrient uptake rate (μM g^{-1} FW d^{-1}) and biofiltration efficiency (%) of *Enteromorpha compresa, Ulva pertusa* and *Sargassum piluliferum* under ammonia loading rate of 20 g m^{-3} d^{-1} at 15°C (Treatment II)

Nutrients	*E. compresa*	*U. pertusa*	*S. piluliferum*
Ammonium (NH_4^+)	60.38 (89.66%)[a]	45.14 (67.98%)[b]	31.76 (46.24%)[c]
Nitrate (NO_3-N)	17.88 (52.26%)[a]	17.03 (50.48%)[a]	15.89 (46.62%)[a]
Nitrite (NO_2-N)	0.30 (12.26%)[a]	0.28 (11.32%)[a]	0.27 (11.63%)[a]
Orthophosphate (PO_4^{3-})	1.55 (81.74%)[a]	1.17 (65.15%)[b]	0.82 (45.23%)[c]

*Number with the different letter in the same row shows significant difference ($p \leq 0.05$).

2.4. Volumetric nutrient removal rates

For volumetric nutrient removal rate, *E. compresa* showed the highest rate for ammonia and nitrate-nitrogen following by *U. pertusa* and *S. piluliferum* as shown in Table 5.

Table 5. Volumetric nutrient removal rates (g m^{-3} d^{-1}) of *Enteromorpha compresa, Ulva pertusa* and *Sargassum piluliferum* under ammonia loading rate of 20 g m^{-3} d^{-1} at 15°C (Treatment II)

Nutrients	*E. compresa*	*U. pertusa*	*S. piluliferum*
TAN	193.79±55.36[a]	144.88±38.42[b]	101.92±38.42[c]
NO_3-N	28.79±9.05[a]	27.42±7.32[a]	25.59±8.38[a]

*Number with the different letter in the same row shows significant difference ($p \leq 0.05$).

3. Treatment III: Ammonia loading rate of 50 g m^{-3} d^{-1} at 10°C

3.1. Condition during experiment

In Treatment III, water temperatures were ranged 9.9-10.1°C and pH maintained 7.62-7.91 in inflow and outflow of *E. compresa* were 7.76-7.98, *U. pertusa* were 7.75-7.96 and *S. piluliferum* were 7.68-7.98. The pH was slightly increased from inflow to outflow because of photosynthetic activities by seaweeds. The dissolved oxygen level was 11-11.3 ppm in all bioreactor (aquaria). Water salinity during this treatment was 35 psu. According to those mentioned data, water temperatures, pH level, D.O. level and water salinity in the system in Treatment III were in optimal range for uptaking nutrient by seaweeds.

3.2. Nutrient concentrations

At the 1st day of experiment after ammonia loading rate was maintained at 50 g m^{-3} d^{-1} for 15 days of Treatment III period, ammonium concentration in the outflow of the control, *E. compresa*, *U. pertusa* and *S. piluliferum* were

315.33 μM, 207.83 μM, 200.67 μM and 229.33 μM, respectively. The highest ammonium concentration in the outflow of the control reached to 404.92 μM, and three seaweeds, *E. compresa*, *U. pertusa*, and *S. piluliferum* reached to 318.92 μM, 336.83 μM and 358.33 μM, respectively. At the end of experiment, ammonium concentration in the outflow of the control, *E. compresa*, *U. pertusa*, and *S. piluliferum* outflow were 333.25 μM, 261.58 μM, 275.92 μM and 301 μM, respectively. Statistically significant differences ($p \leq 0.05$) were found in ammonium concentration among the treatments (Fig. 10).

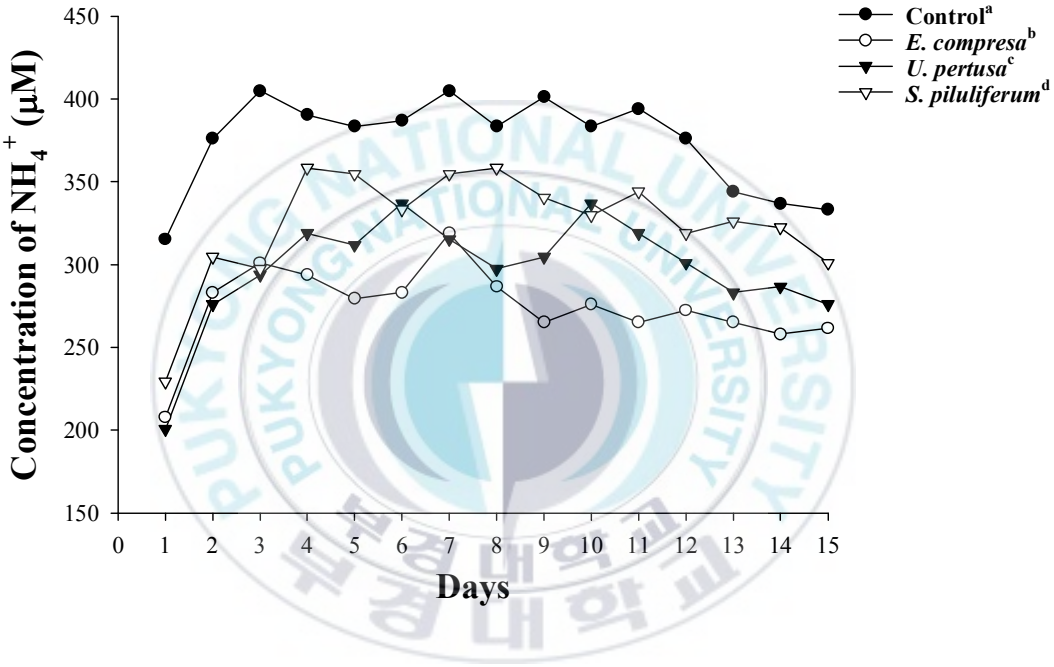

Fig.10. Changes of average concentration of ammonium in the outflow of the control, *Enteromorpha compresa, Ulva pertusa* and *Sargassum piluliferum* under the ammonia loading rate of 50 g m^{-3} d^{-1} at 10°C. The different superscript letter of treatments in the legend shows statistically different in the means.

In the Treatment III, concentration of nitrate-nitrogen (NO_3-N) in the outflow of the thecontrol, *E. compresa*, *U. pertusa* and *S. piluliferum* day at the 1st were 142.86 μM, 64.29 μM, 57.14 μM and 85.71 μM, respectively. The highest concentration of nitrate-nitrogen in the outflow of the control reached to 242.86 μM, and three seaweeds, *E. compresa*, *U. pertusa*, and *S. piluliferum* reached to 200 μM, 207.14 μM, and 214.29 μM, respectively. At the end of experiment, the nitrate-nitrogen concentration in the outflow of the control, *E. compresa*, *U. pertusa*, and *S. piluliferum* were 135.71 μM, 128.57 μM, 121.43 and 121.43 μM, respectively. Statistically significant differences in nitrate-nitrogen concentration were found between the outflow of control and the seaweeds ($p \leq 0.05$), but among of seaweeds were not significant differences ($p > 0.05$) (Fig.11).

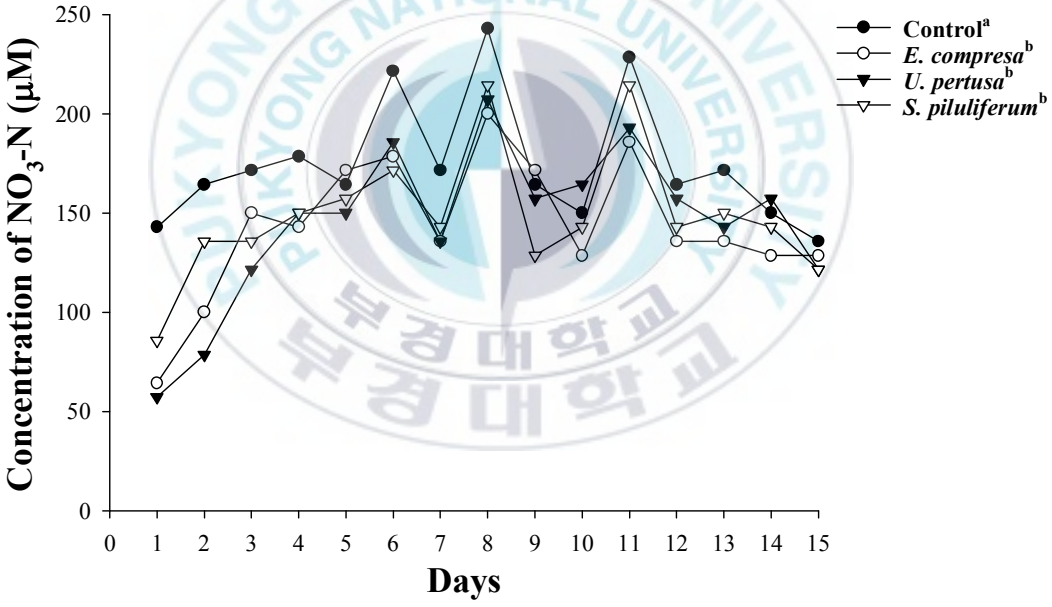

Fig.11. Changes of average concentration of nitrate-nitrogen in the outflow of the control, *Enteromorpha compresa, Ulva pertusa* and *Sargassum*

piluliferum under the ammonia loading rate of 50 g m^{-3} d^{-1} at 10°C. The different superscript letter of treatments in the legend shows statistically different in the means.

Concentrations of nitrite-nitrogen (NO_2-N) in the outflow of the control, *E. compresa*, *U. pertusa* and *S. piluliferum* at 1st day were 5.43 μM, 4.64 μM, 5.07 μM and 5.14 μM, respectively. The highest concentration of nitrite-nitrogen in the outflow of the control reached to 8.14 μM, and those of seaweeds, *E. compresa*, *U. pertusa* and *S. piluliferum* reached to 7.86 μM, 8 μM, and 8.07 μM, respectively. At the end of experiment, concentration of nitrite-nitrogen in the outflow of the control reached to 7.79 μM, and those of seaweeds, *E. compresa*, *U. pertusa* and *S. piluliferum* were 7.29 μM, 7.43 μM and 7.14 μM, respectively. No significant differences ($p \geq 0.05$) were found of nitrite-nitrogen concentration in the treatment (Fig. 12).

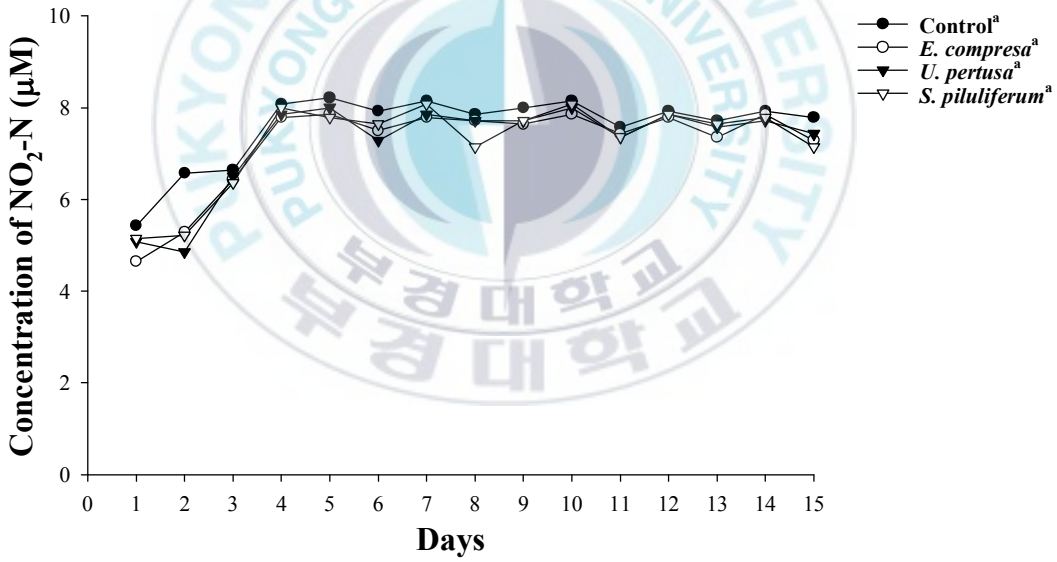

Fig.12. Changes of average concentration of nitrite-nitrogen in the outflow of the control, *Enteromorpha compresa, Ulva pertusa* and *Sargassum*

piluliferum under the ammonia loading rate of 50 g m^{-3} d^{-1} at 10°C. The different superscript letter of treatments in the legend shows statistically different in the means.

In the Treatment III, concentration of orthophosphate (PO_4^{3-}) in the outflow of the control, *E. compresa*, *U. pertusa*, and *S. piluliferum* at the 1st day were 7.05 μM, 3.79 μM, 5.58 μM and 5.68 μM, respectively. The highest concentration of orthophosphate in the outflow of the control reached to 12.42 μM, and three seaweeds, *E. compresa*, *U. pertusa*, and *S. piluliferum* reached to 8.74 μM, 10.42 μM, and 10.32 μM, respectively. At the end of experiment, orthophosphate concentration in the outflow of the control, *E. compresa*, *U. pertusa*, and *S. piluliferum* were 11.05 μM, 6 μM, 9.68 μM and 8.53 μM, respectively. No significant differences in orthophosphate concentration ($p>0.05$) were found only between the outflow of *U. pertusa* and *S. piluliferum* as shown in Fig. 13.

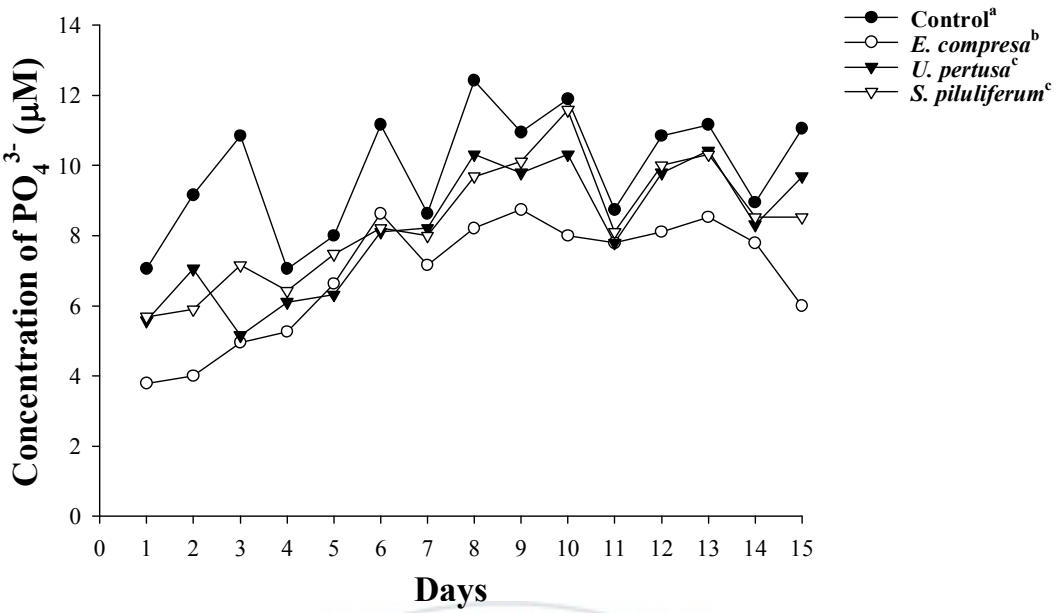

Fig.13. Changes of average concentration of orthophosphate in the outflow of the control, *Enteromorpha compresa, Ulva pertusa* and *Sargassum piluliferum* under the ammonia loading rate of 50 g m^{-3} d^{-1} at 10°C. The different superscript letter of treatments in the legend shows statistically different in the means.

3.3. Nutrient uptake rates and biofiltration efficiencies

Three seaweeds actively uptakes ammonium, nitrate-nitrogen, nitrite-nitrogen and orthophosphate. *E. compresa* showed highest ammonium, nitrate-nitrogen, nitrite-nitrogen and orthophosphate uptake rate followed by *U. pertusa* and *S. piluliferum*. Furthermore, *E. compresa* also had highest biofiltration efficiencies on ammonium, nitrate-nitrogen, nitrite-nitrogen, orthophosphate than other seaweeds as shown in Table 6.

Table 6. Nutrient uptake rate (μM g^{-1} FW d^{-1}) and biofiltration efficiency (%) of *Enteromorpha compresa, Ulva pertusa* and *Sargassum piluliferum* under ammonia loading rate of 50 g m^{-3} d^{-1} at 10°C (Treatment III)

Nutrients	*E. compresa*	*U. pertusa*	*S. piluliferum*
Ammonium (NH_4^+)	59.53 (26.65%)[a]	46.00 (20.68%)[b]	15.33 (14.88%)[c]
Nitrate (NO_3-N)	18.45 (17.77%)[a]	17.60 (17.00%)[a]	15.89 (46.62%)[a]
Nitrite (NO_2-N)	0.22 (5.36%)[a]	0.20 (4.78%)[a]	0.19 (4.60%)[a]
Orthophosphate (PO_4^{3-})	1.76 (29.62%)[a]	0.99 (16.70%)[b]	0.88 (14.72%)[b]

*Number with the different letter in the same row shows significant difference ($p \leq 0.05$).

3.4. Volumetric nutrient removal rates

For volumetric nutrient removal rate, *E. compresa* showed the highest rate for ammonia and nitrate-nitrogen following by *U. pertusa* and *S. piluliferum* as shown in Table 7.

Table 7. Volumetric nutrient removal rates (g m^{-3} d^{-1}) of *Enteromorpha compresa, Ulva pertusa* and *Sargassum piluliferum* under ammonia loading rate of 50 g m^{-3} d^{-1} at 10°C (Treatment III)

Nutrients	*E. compresa*	*U. pertusa*	*S. piluliferum*
TAN	200.19±41.48[a]	156.77±46.49[b]	103.75±47.91[c]
NO_3-N	29.71±22.39[a]	28.34±27.65[a]	24.68±14.38[a]

*Number with the different letter in the same row shows significant difference ($p \leq 0.05$).

4. Treatment IV: Ammonia loading rate of 50 g m^{-3} d^{-1} at 15°C

4.1. Condition during experiment

In the Treatment IV, water temperatures were ranged 14.9-15.1°C and pH maintained 7.71-7.89 in inflow and outflow of *E. compresa* were 7.82-7.99, *U. pertusa* were 7.85-7.95 and *S. piluliferum* were 7.82-7.91. The pH was slightly increased from inflow to outflow because of photosynthetic activities by seaweeds. The dissolved oxygen level was 11-11.3 ppm in all bioreactor (aquaria). Water salinity during this treatment was 35 psu. According to those mentioned data above, water temperatures, pH level, D.O. level and water salinity in the system during Treatment IV were in optimal range for uptaking nutrient by seaweeds.

4.2. Nutrient concentrations

In Treatment IV, ammonium concentrations in the outflow of the control, *E. compresa*, *U. pertusa* and *S. piluliferum* at the 1st day were 232.92 µM, 136.17 µM, 150.50 µM and 182.75 µM, respectively. The highest ammonium concentration in the outflow of the control reached to 326.08 µM, and those of seaweeds, *E. compresa*, *U. pertusa* and *S. piluliferum* reached to 204.25 µM, 240.08 µM, and 258 µM, respectively. At the end of experiment, ammonium concentration in the outflow of the control, *E. compresa*, *U. pertusa*, and *S. piluliferum* were 308.17 µM, 200.67 µM, 218.58 µM and 247.25 µM, respectively. Statistically significant differences ($p \leq 0.05$) were found in ammonium concentration among the treatments (Fig. 10).

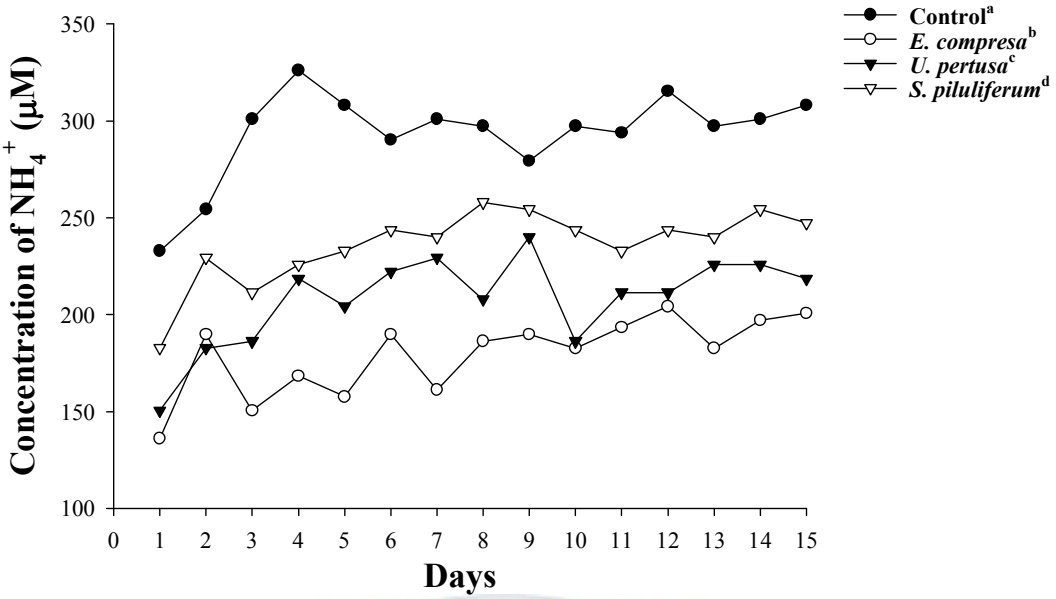

Fig.14. Changes of average concentration of ammonium in the outflow of the control, *Enteromorpha compresa, Ulva pertusa* and *Sargassum piluliferum* under the ammonia loading rate of 50 g m^{-3} d^{-1} at 15°C. The different superscript letter of treatments in the legend shows statistically different in the means.

In the Treatment IV, concentration of nitrate-nitrogen (NO_3-N) in the outflow of the control, *E. compresa*, *U. pertusa* and *S. piluliferum* at the 1st day were 150 μM, 71.43 μM, 78.57 μM and 92.86 μM, respectively. The highest concentration of nitrate-nitrogen in the outflow of the control reached to157.14 μM, and three seaweeds, *E. compresa*, *U. pertusa*, and *S. piluliferum* reached to 135.71 μM, 135.71 μM, and 128.57 μM, respectively. At the end of experiment, the nitrate-nitrogen concentration in the outflow of the control, *E. compresa*, *U. pertusa*, and *S. piluliferum* were 121.43 μM, 107.14 μM, 100 μM and 92.86 μM, respectively. Significant differences in nitrate-nitrogen concentrations were found between the outflow of control and all the three

seaweeds ($p \leq 0.05$), but there was no differences among of seaweeds ($p > 0.05$) as shown in Fig.15.

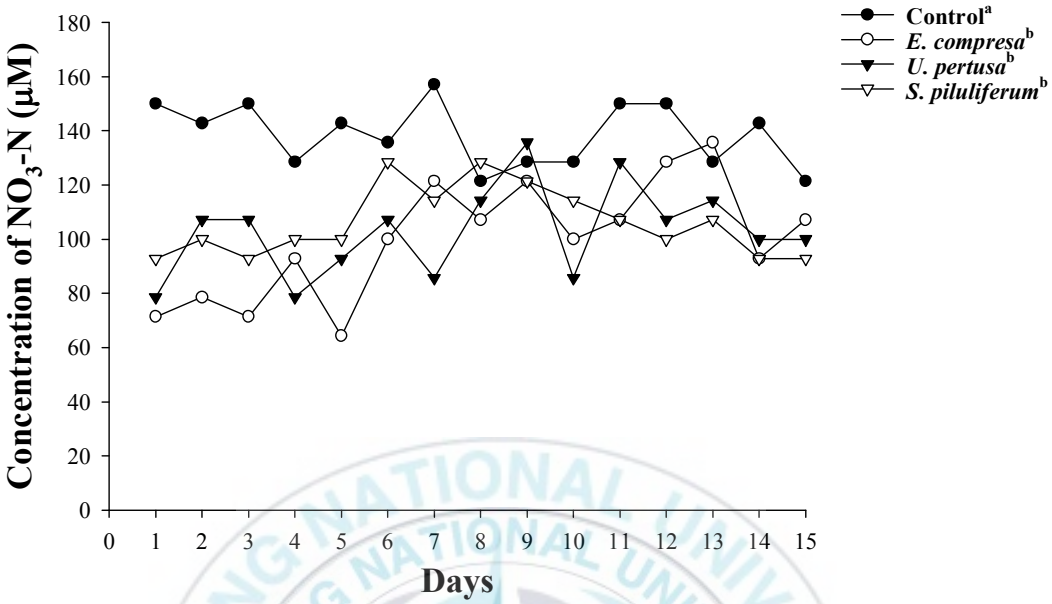

Fig.15. Changes of average concentration of nitrate-nitrogen in the outflow of the control, *Enteromorpha compresa, Ulva pertusa* and *Sargassum piluliferum* under the ammonia loading rate of 50 g m^{-3} d^{-1} at 15°C. The different superscript letter of treatments in the legend shows statistically different in the means.

In the Treatment IV, concentrations of nitrite-nitrogen (NO_2-N) in the outflow of the control, *E. compresa*, *U. pertusa* and *S. piluliferum* at 1st day were 4 μM, 4 μM, 3.64 μM and 3.71 μM, respectively. The highest concentration of nitrite-nitrogen in the outflow of the control reached to 5.93 μM, and three seaweeds, *E. compresa*, *U. pertusa*, and *S. piluliferum* reached to 5.57 μM, 5.50 μM, and 5.57 μM, respectively. At the end of experiment, concentration of nitrite-nitrogen in the outflow of the control, *E. compresa*, *U.*

pertusa and *S. piluliferum* were 5.14 μM, 5.07 μM, 5.14 μM and 5.14 μM, respectively. No statistically differences ($p \geq 0.05$) were found in nitrite-nitrogen concentration among the treatments as shown in Fig. 16.

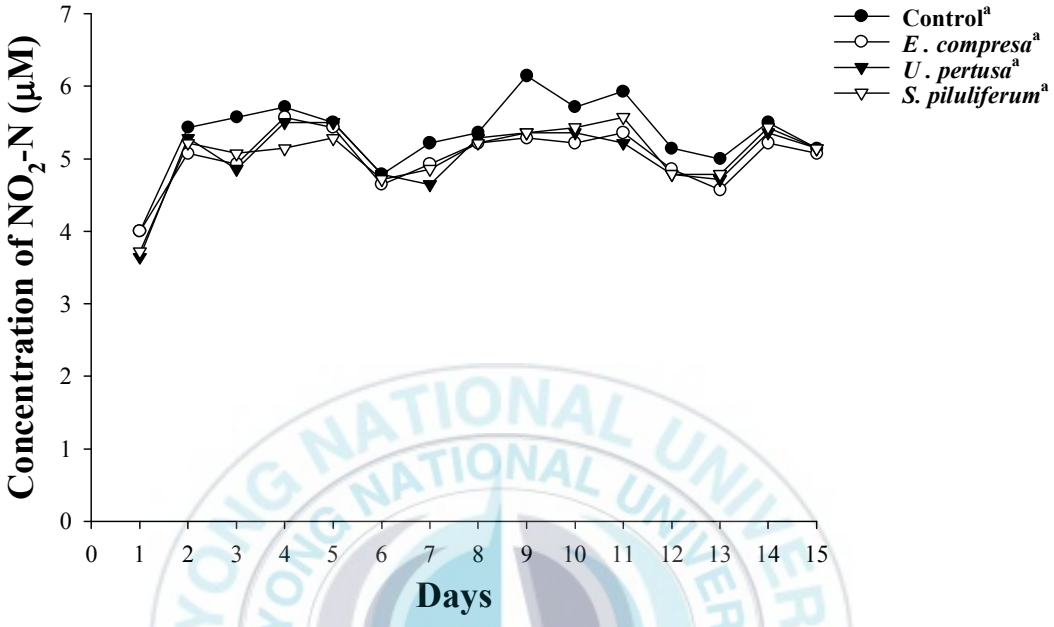

Fig.16. Changes of average concentration of nitrite-nitrogen in the outflow of the control, *Enteromorpha compresa, Ulva pertusa* and *Sargassum piluliferum* under the ammonia loading rate of 50 g m^{-3} d^{-1} at 15°C. The different superscript letter of treatments in the legend shows statistically different in the means.

In the Treatment IV, concentration of orthophosphate (PO_4^{3-}) in the outflow of the control, *E. compresa*, *U. pertusa*, and *S. piluliferum* at the 1st day were 6.63 μM, 3.47 μM, 3.58 μM and 4.32 μM, respectively. The highest concentration of orthophosphate in the outflow of control reached 10.74 μM, and three seaweeds, *E. compresa*, *U. pertusa*, and *S. piluliferum* reached to

7.58 μM, 8 μM, 9.26 μM, respectively. At the end of experiment, orthophosphate concentration in control, *E. compresa*, *U. pertusa*, and *S. piluliferum* were 9.47 μM, 4.74 μM, 5.89 μM and 6.95 μM, respectively. Statistically differences ($p \leq 0.05$) were found in orthophosphate concentration among the treatments (Fig. 17).

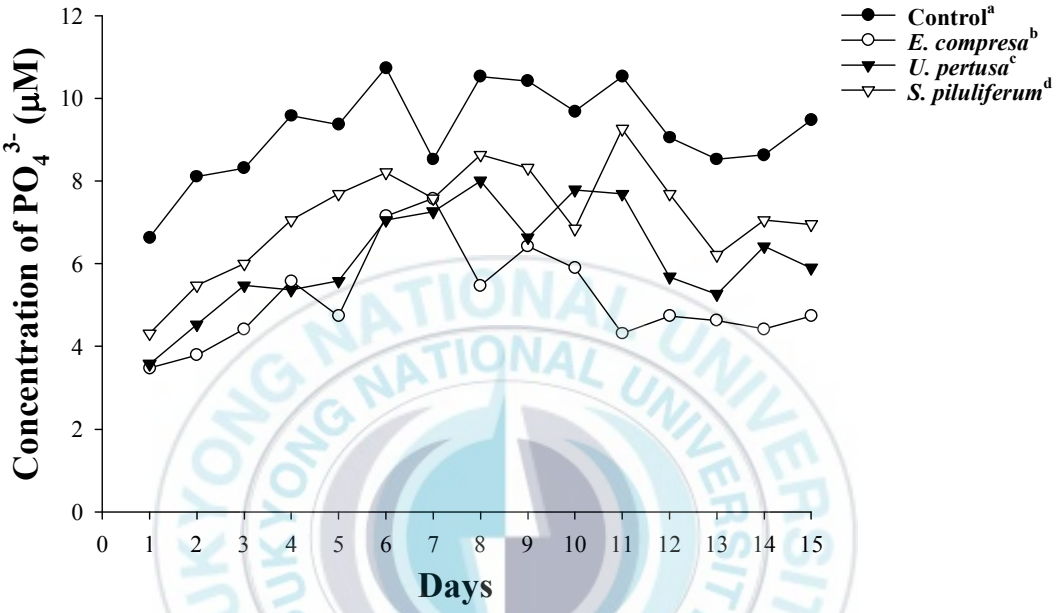

Fig.17. Changes of average concentration of orthophosphate in the outflow of the control, *Enteromorpha compresa, Ulva pertusa* and *Sargassum piluliferum* under the ammonia loading rate of 50 g m^{-3} d^{-1} at 15°C. The different superscript letter of treatments in the legend shows statistically different in the means.

4.3. Nutrient uptake rates and biofiltration efficiencies

In the Treatment IV, seaweeds actively uptake ammonium, nitrate-nitrogen, nitrite-nitrogen and orthophosphate. *E. compresa* showed highest ammonium, nitrate-nitrogen, nitrite-nitrogen and orthophosphate uptake rate followed by *U. pertusa* and *S. piluliferum*. Furthermore, *E. compresa* also had highest biofiltration efficiencies on ammonium, nitrate-nitrogen, nitrite-nitrogen, orthophosphate than other seaweeds as shown in Table 8.

Table 8. Nutrient uptake rate (μM g^{-1} FW d^{-1}) and biofiltration efficiency (%) of *Enteromorpha compresa, Ulva pertusa* and *Sargassum piluliferum* under ammonia loading rate of 50 g m^{-3} d^{-1} at 15°C (Treatment IV)

Nutrients	*E. compresa*	*U. pertusa*	*S. piluliferum*
Ammonium (NH_4^+)	68.07 (38.69%)[a]	50.98 (29.09%)[b]	34.32 (19.39%)[c]
Nitrate (NO_3-N)	22.99 (27.01%)[a]	21.29 (25.12%)[a]	19.30 (22.59%)[a]
Nitrite (NO_2-N)	0.19 (5.74%)[a]	0.18 (5.78%)[a]	0.17 (5.42%)[a]
Orthophosphate (PO_4^{3-})	2.41 (43.99%)[a]	1.82 (33.58%)[b]	1.22 (31.08%)[c]

*Number with the different letter in the same row shows significant difference ($p \leq 0.05$).

4.4. Volumetric nutrient removal rates

For volumetric nutrient removal rate, in the Treatment IV *E. compresa* showed the highest rate for ammonia and nitrate-nitrogen following by *U. pertusa* and *S. piluliferum* as shown in Table 9.

Table 9. Volumetric nutrient removal rates (g m^{-3} d^{-1}) of *Enteromorpha compresa, Ulva pertusa* and *Sargassum piluliferum* under ammonia loading rate of 50 g m^{-3} d^{-1} at 15°C (Treatment IV)

Nutrients	*E. compresa*	*U. pertusa*	*S. piluliferum*
TAN	218.47±48.81[a]	163.62±39.03[b]	110.15±40.10[c]
NO_3-N	37.02±26.02[a]	34.28±21.05[a]	31.08±19.19[a]

*Number with the different letter in the same row shows significant difference ($p \leq 0.05$).

5. Specific growth rates

During Treatment I (ammonia loading rate of 20 g m^{-3} d^{-1} at 10°C), *E. compresa* had the highest specific growth rate (SGR) followed by *U. pertusa*, and *S. piluliferum* and the SGR levels were 1.61 % day^{-1}, 1.33 % day^{-1} and 0.66 % day^{-1}, respectively (Fig.18).

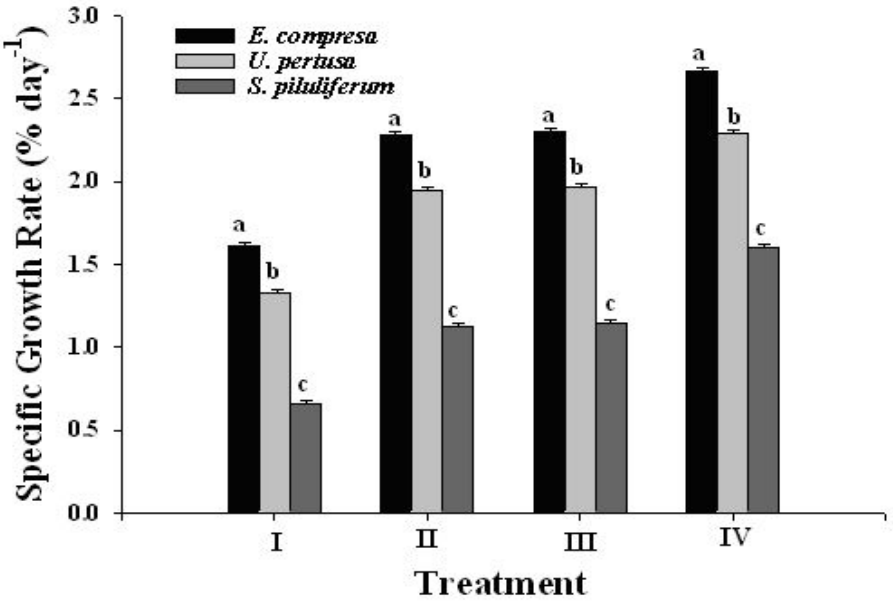

Fig.18. Integrated specific growth rates of *Enteromorpha compresa, Ulva pertusa* and *Sargassum piluliferum* under different combinations of ammonia loading rates 20 and 50 g m^{-3} d^{-1} and temperatures (10°C and 15°C).

In the Treatment II (ammonia loading rate of 20 g m^{-3} d^{-1} at 15°C), the highest SGR level was achieved by *E. compresa* (2.28 % day^{-1}) followed by *U. pertusa* (1.95 % day^{-1}) and *S. piluliferum* (1.12 % day^{-1}). In the Treatment III (ammonia loading rate of 50 g m^{-3} d^{-1} at 10°C) , *E. compresa* showed the highest SGR followed by *U. pertusa* and *S. piluliferum* and the SGR levels were 2.30 % day^{-1}, 1.97 % day^{-1} and 1.14 % day^{-1}, respectively. Furthermore, the highest SGR in the Treatment IV (ammonia loading rate of 50 g m^{-3} d^{-1} at 15°C) was found in the *E. compresa* (2.66 % day^{-1}) followed by *U. pertusa* (2.29 % day^{-1}) and *S. piluliferum* (1.60 % day^{-1}). Significant differences ($p \leq 0.05$) were found in the specific growth rates among the three seaweeds in all treatment (Fig.18).

IV. DISCUSSION

1. Condition during experiments

Water temperatures in all of treatments were ranged 9.9-15.1 ^{o}C which was safe and suitable range for the seaweeds, *E. compresa, U. pertusa and S. piluliferum* (Lobban and Harrison, 1994; Neori et al., 2000; O'Brien and Wheeler, 1997). Dissolved oxygen levels in all treatments were ranged 11-11.3 mg/L. Salinity level was remained 35 psu in all of treatments (Lobban and Harrison, 1994). Furthermore, the given light irradiance and dark-light period also provide appropriate condition for seaweeds on photosynthetic process (Kang et al., 2007). The abundance of nutrients also gave appropriate condition for uptake process by seaweed because the limitation of nutrients concentration affect to seaweed physiological process. For instance, the lack of nutrient (i.e. ammonia, phosphate) gave direct effect to seaweed growth. Generally, conditions during experiment were suitable for nutrient uptake process by seaweeds according to Lobban and Harrison (1994).

2. Nutrient concentrations

During Treatment I and II (ammonia loading rate of 20 g m^{-3} d^{-1} at 10^{o}C and 15^{o}C), the average ammonium (NH_4^+) concentrations in the outflow of seaweeds were ranged 10.75-120 μM and these are within the similar range of Neori et al. (1996). Ammonium concentrations of their fish tanks were generally lower than 100 μM with maximum of 180 μM. However, in the Treatment III and IV (ammonia loading rate of 50 g m^{-3} d^{-1} at 10^{o}C and 15^{o}C) average ammonium concentrations in the outflow of control and seaweeds were higher than the results of Neori et al. (1996). The differences seem to be

due to higher ammonia loading rate in the present study.

Comparisons of ammonium concentrations in the outflow of control with all seaweeds treatments, the concentration of control was significantly higher than those of seaweeds. It can be stated that seaweeds has a good ability to uptake the ammonium.

Generally, ammonium concentrations in this study were higher (10.75-324.89 μM) than those in the integrated culture of oyster and *Gracilaria edulis* by Jones et al. (1996). The ammonium concentrations of them in the oyster treatment with and without *G. edulis* were 51-1.3 μM and 18-51 μM, respectively. Furthermore, Neori et al. (2000) reported that the ammonium concentration in effluent water from Japanese abalone farm with *Ulva* and *Gracilaria* were 15-17 μM and 1-5 μM, respectively. These differences seems to be due to the species differences and ammonia loading in this experiment.

Concentrations of nitrate-nitrogen (NO_3-N) in this study were ranged from 28.10-143.81 μM. According to Neori et al. (1996) the NO_3-N concentration during the integrated cultivation of gilthead seabream, *Sparus auratus* and *Ulva lactuta*, were ranged 0-180 μM. It can be stated that concentrations of nitrate-nitrogen (NO_3-N) during present study are on the safe range for aquaculture activities. However, concentrations of NO_3-N in the outflow of control were always significantly higher than that in the outflow of seaweed treatments in this experiment. It means that seaweeds can reduce NO_3-N concentration from wastewater. Carmona et al. (2006) also mentioned this point when they tested several species of *Porphyra* to remove nitrate and ammonium from the water.

Concentrations of nitrite-nitrogen (NO_2-N) in the present study was about 3-8 μM and this was lower than that mentioned by Neori et al. (1996). They measured NO_2-N concentration in the integrated cultivation of gilthead seabream and *Ulva lactuta* and the NO_2-N concentration was increased up to 230 μM. This means that even though the NO_2-N concentrations in the

outflow of control and seaweeds treatments were not significantly different, those concentrations are in the safe range for aquaculture activities.

Concentrations of orthophosphate (PO_4^{3-}) in the present study were lower than that of the Neori et al. (1996). The orthophosphate concentrations in this study were about 2-12 µM while those in Neori et al. (1996) were about 20-50 µM. However, the concentrations of PO_4^{3-} in the outflow of control of this study were always significantly higher than those in outflow of seaweed treatments. It means that seaweeds used in this experiment can reduced orthophosphate from wastewater.

3. Nutrient uptake rates and biofiltration efficiencies

Ammonium (NH_4^+) uptake rates by *E. compresa* in this experiment were about 445.8-543.3 µmol NH_4^+ g^{-1} DW h^{-1} (or 55.85-68.07 µM NH_4^+ g^{-1} FW d^{-1}) and these are much higher than those reported by O'Brien and Wheeler (1987) which was about 39-188 µmol NH_4^+ g^{-1} DW h^{-1} .

Ammonium uptake rates by *U. pertusa* in this study were ranged 240-290.34 µmol NH_4^+ g^{-1} DW h^{-1} (or 42.14-50.98 µM NH_4^+ g^{-1} FW d^{-1}) and these numbers are much lower than that of *E. compresa*. But these results are similar of the Pedersen and Borum (1997) that noted 240±61 µmol NH_4^+ g^{-1} DW h^{-1} but higher than those reported by Hernandez et al. (2002) whose result with *Ulva* was about 1.33 µmol NH_4^+ g^{-1} DW h^{-1} because they used lower ammonia concentration.

Ammonium uptake rates by *S. piluliferum* in this study were 185-231.13 µmol NH_4^+ g^{-1} DW h^{-1} (or 27.47-34.32 µM NH_4^+ g^{-1} FW d^{-1}) and these results were higher than reported by Schaffelke and Klumpp (1998) whose data were about 111.1 µmol NH_4^+ g^{-1} DW h^{-1}.

Biofiltration efficiencies of *E. compresa* on ammonium removal in this study were ranged between 26.65%-89.66%. This means that *E. compresa*

removed ammonium efficiently from waste waters. However, the highest biofiltration efficiency in ammonium removal by *E. compresa* in this study was lower than that reported by Hernandez et al. (2002) who achieved biofiltration efficiency up to 95.5% with *Enteromorpha*. It is assumed that the different efficiencies were due to the use of lower ammonia concentration media (34-62 μM) and higher water temperature (18 ^{o}C) in their experiment.

In this study, biofiltration efficiencies of ammonium by *U. pertusa* were ranged 20.68%-67.98%. This level of biofiltration efficiencies is lower than thad using *Ulva* by Hernandez et al. (2002). Their ammonium removal efficiency increased upto 88.2%. However, biofiltration efficiencies of ammonium by Msuya et al. (2006) with *Ulva* was 65% and this is lower than the results of present study.

Nitrate-nitrogen (NO_3-N) uptake rates by *E. compresa* in this study were 85.69-183.59 μmol NO_3-N g^{-1} DW h^{-1} (or 10.73-22.99 μM NO_3-N g^{-1} FW d^{-1}) and these results are slightly higher than that of O'Brien and Wheeler (1987) (75-169 μmol NO_3-N g^{-1} DW h^{-1}) by *Enteromorpha*.

Thus, nitrate-nitrogen uptake rates by *U. pertusa* in this study were 240-376.53 μmol NO_3-N g^{-1} DW h^{-1} (or 13.57-21.29 μM NO_3-N g^{-1} FW d^{-1}) and these results are higher than that reported rate (20 μmol NO_3-N g^{-1} DW h^{-1}) by Pedersen and Borum (1997).

Furthermore, biofiltration efficiencies of nitrate-nitrogen by *E. compresa* were ranged 17.77%-52.26%. This result showed that this seaweed can remove NO_3-N efficiently from wastewaters. While the biofilteration efficiencies of nitrate-nitroge by *U. pertusa* and *S. piluliferum* were ranged about 17.0%-50.48% and 14.88%-46.62%, respectively. According to above results in this study, these seaweeds show higher biofiltration efficiencies than that reported by Wang et al. (2007) in the integrated culture system with with juvenile sea cucumber (*Apostichopus japonicus*) and *Ulva*.

Uptake rates and biofiltration efficiencies of nitrite-nitrogen (NO_2-N) by

E. compresa, *U. pertusa* and *S. piluliferum* were ranged 3.56%-12.26%, 4.78%-11.32%, and 4.6%-11.63%, respectively. While biofiltration efficiencies of nitrite-nitrogen by *U. pertusa* in the results of Wang et al. (2007) showed 40.11% and much higher efficiencies than the present study.

Orthophosphate (PO_4^{3-}) uptake rates by *E. compresa* in this study were ranged 13.21-27.46 μmol PO_4^{3-} g^{-1} DW h^{-1} (or 1.16-2.41 μM PO_4^{3-} g^{-1} FW d^{-1}) and these results are higher than those of Aragon et al. (2002) and Hernandez et al. (2006). Uptake rates of orthophosphate of Aragon et al. (2002) were 5.8 μmol PO_4^{3-} g^{-1} DW h^{-1} by integrated culture of *Enteromorpha*, *Ulva*, and *Grasilaria* with sea bass (*Dicentrarchus labrax*). Uptake rates of orthophosphate of Hernandez et al. (2006) was 0.3 μmol PO_4^{3-} g^{-1} DW h^{-1} by *Grasilaria*

Meanwhile, orthophosphate uptake rates by *U. pertusa* in this experiment were ranged 3.97-6.17 μmol PO_4^{3-} g^{-1} DW h^{-1} (or 1.17-1.82 μM PO_4^{3-} g^{-1} FW d^{-1}) while that of Aragon et al. (2002) using *Ulva* was 2.8 μmol PO_4^{3-} g^{-1} DW h^{-1} and the later result was slightly higher than the former.

Orthophosphate uptake of *S. piluliferum* in this experiment were ranged 0.75-1.22 μM PO_4^{3-} g^{-1} FW d^{-1} (or 5.11-8.31 μmol PO_4^{3-} g^{-1} DW h^{-1}) which is higher than the result of 0.43 μmol PO_4^{3-} g^{-1} DW h^{-1} by Schaffelke and Klumpp (1998) with *Sargassum*. This difference of efficiency between two studies seems to be due to the concentrations of phosphate medium that was 10 μM orthophosphate in the former and 0.3 μM in the later.

Therefore, biofiltration efficiencies of orthophosphate in this experiment with *E. compresa*, *U. pertusa* and *S. piluliferum* were 29.62%-81.74%, 16.70%-65.15% and 14.72%-45.23%, respectively. These efficiencies were lower than the results of Aragon et al. (2002) that range was 71.4-91.5%.

According to those results mentioned above, *E. compresa* showed better performance on nutrients uptake rate and biofiltration efficiencies followed by *U. pertusa* and *S. piluliferum* in this study. It is assumed that these differencies

of efficiencies are due to the surface area:volume ratio (SA:V ratio) among the seaweeds. According Rees (2003), the SA:V ratio of *Enteromorpha* was 529, and that of *Ulva* was 400. There was no reference of SA:V ratio of *Sargassum* but the SA:V ratio of *Fucus* that is similar of *Sargassum* was 30-34. Also, Wallentinus (1984) measured higher uptake rates for nitrate, ammonium and phosphate in short-lived, opportunistic, and filamentous, such as *Enteromorpha* that had high SA:V ratios. He also got the lowest uptake rates of *Fucus* that is long-lived, with low SA:V ratios.

4. Comparison of volumetric nutrients removal rates and efficiencies

In this experiment, volumetric total ammonia nitrogen (TAN) removal rates of three seaweeds *E. compresa, U. pertusa and S. piluliferum* were ranged 88-218 g TAN m^{-3} d^{-1} (Table 10). According to Kim et al. (2000), volumetric TAN removal rates were ranged 2.8-82 g TAN m^{-3} d^{-1} by polyvinyl alcohol bead filter. Chen et al. (2006) reported that volumetric TAN removal rate of a biofilm was 20 g TAN m^{-3} d^{-1}. Wheaton et al. (1994) reported that volumetric TAN removal rate with RBC was 76 g TAN m^{-3} d^{-1}. Using sand as a biofilter medium, Summerfelt (2006) reported volumetric TAN removal rate was 146 g TAN m^{-3} d^{-1}) while that by Tsukuda et al. (1997) was 1500 g TAN m^{-3} d^{-1}. Except the result of Tsukuda et al. (1997), all other results are within the ranges of present study.

Table 10. Comparison of volumetric total ammonia nitrogen removal rates (g TAN m^{-3} d^{-1}) of present study to other biofilter in nitrification process

Biofilter	Volumetric nutrients removal rate	References
Polyvinyl alcohol bead filter	2.8-82	Kim et al. (2000)
Biofilm	20	Chen et al. (2006)
RBC	76	Wheaton et al. (1994)
E. compresa, *U. pertusa*, *S. piluliferum*	88-218	**Present study (2008)**
Sand	146	Summerfelt (2006)
Sand	1500	Tsukuda et al. (1997)

Results of removal efficiencies of TAN by three seaweeds *E. compresa, U. pertusa* and *S. piluliferum* and other biofilters materials are shown in Table 11. The removal efficiencies of TAN by three seaweeds in the present study were ranged 13-89%. Removal efficiency of TAN by Chen et al. (2000) was 15% while that by Kim et al. (2006) was 58-90%. Xiaojing et al. (2006) used oyster shell and plastic balls as media and TAN removal efficiencies were ranged 63-97%. Summerfelt (2006) used sand as a biofilter medium and TAN removal efficiency was 82%. Tsukuda et al. (1997) also used sand as biofilter medium and the TAN removal efficiency was 89%. By using fluidized bead filter, TAN removal efficiencies were ranged 50-90% (Summerfelt et al., 2001). The TAN removal efficiencies of present study was quite similar with other results. However, direct comparisons among the TAN removal efficiencies are impossible because it must concern with the other data such as flow rates, hydraulic retention time, and ammonia loading rate, etc.

According to the information above, those seaweeds have similar

removal efficiency with other biofilters. Generally, in the nitrification process, those seaweeds have great potential to apply the nitrification process. Even though, the volumetric TAN removal efficiency of sand biofilter was about 7.5 times higher than those of seaweeds, sand biofilter has disadvantage to remove TAN because it requires high energy costs for lifting up the sand in biofilter (Summerfelt, 2006). Due to this information, those seaweeds can be another option to remove TAN in marine recirculating aquaculture systems.

Table 11. Comparison of removal efficiency (%) of present study to other biofilter in nitrification process

Biofilter	**Removal efficiency (%)**	**References**
Biofilm	15	Chen et al. (2000)
Polyvinyl alcohol bead filter	58-90	Kim et al. (2006)
E. compresa, *U. pertusa*, *S. piluliferum*	13.18-89.66	**Present study (2008)**
Oyster shell and plastic balls	63.6-97.5	Xiaojing et al. (2006)
Fluidized bead biofilter	50-90	Summerfelt et al. (2001)
Sand	89	Tsukuda et al. (1997)
Sand	82	Summerfelt (2006)

In the denitrification process, removal of NO_3-N, volumetric removal rate of those seaweeds were ranged about 17-37 g NO_3-N m^{-3} d^{-1} and comparisons were made to other biofilers (Table 12). Park et al. (2001) reported that volumetric removal rate of polyvinyl alcohol bead filter was ranged 8-18 g NO_3-N m^{-3} d^{-1}. Except this, all other reports showed the volumetric removal rates of NO_3-N m^{-3} d^{-1} were ranged from 40 to 2400 g NO_3-N m^{-3} d^{-1} and were significantly higher than present study. Removal efficiencies of NO_3-N of

other researchers were ranged 9-98% and were mostly higher than present study (Table 13).

Table 12. Comparison of volumetric nitrate removal rates (g NO_3-N m^{-3} d^{-1}) of present study to other biofilter in denitrification process

Biofilter	**Volumetric removal rate**	**References**
Polyvinyl alcohol bead filter	8-18	Park et al. (2001)
E. compresa, *U. pertusa*, *S. piluliferum*	17.28-37.02	**Presenst study (2008)**
Porous medium	40.8	Honda et al. (1993)
Freeze dried alginate beads	62.4	Tal et al. (2003)
Plastic balls	158.4	Menasveta et al. (2001)
Porous medium	201.6	Grguric et al. (2000)
Plastic medium	576	Tal and Schreier (2004)
Single sludge reactor	80-600	Klas et al. (2006)
Moving bead biofilm reactor	100-1700	Labelle et al. (2005)
Sand	1742.4	Gelfand et al. (2003)
Brick granules	2400	Sauthier et al. (1998)

Table 13. Comparison of removal efficiency (%) of present study to other biofilter in denitrification process

Biofilter	Removal efficiency (%)	References
E. compresa, *U. pertusa*, *S. piluliferum*	14.88-52.26	**Present study (2008)**
Single sludge reactor	60-70	Klas et al. (2006)
Porous medium	70	Grguric et al. (2000)
Moving bead biofilm reactor	9-88	Labelle et al. (2005)
Polyvinyl alcohol bead filter	40-98.4	Park et al. (2001)

According to the information above, nitrate removal efficiencies of the seaweeds showed lower ability than other biofilters. It means that those seaweeds tested in this experiment showed lower denitrification ability. However, one of the big advantages of denitrification by seaweeds is no need anaerobic conditions in the denitrification process. Other methods using biofilter media must have anaerobic conditions to activate anaerobic denitrification bacteria. In this process, oxygenation process is necessary to increase dissolved oxygen level in the water from denitrification biofilter before return to rearing system. Due to this reason, those seaweeds can be another options for denitrification biofilter in marine recirculating aquaculture systems.

5. Specific growth rates

Specific growth rate (SGR) of *E. compresa* showed the highest among the tested seaweeds followed by *U. pertusa* and *S. piluliferum*. The reason of the best SCR of *E. compresa* might be due to higher nutrient uptake rates than

two other seaweeds. The SGRs of *E. compresa* and *U. pertusa* and *S. piluliferum* during experimental period in this study were 1.61-2.66% day^{-1}, 1.33-2.29% day^{-1}, and 0.66-1.60% day^{-1}, respectively. But all of SGRs of these seaweeds were significantly lower than that of Aragon et al. (2002) which was 3-8.5% day^{-1}. The reason of the different SGR between present study and Aragon et al. was due to the higher temperature. The temperature of Aragon et al. (2002) was 18^{o}C and present study was 10 and 15^{o}C.

The SGR of *U. pertusa* in this study was 0.66-1.60% day^{-1} that was much lower than that by Wang et al., (2007), 3.3% day^{-1}. The reason of the different SGR was due to the size of culture facility which was 24 L aquarium in this study and a 70 m^3 tank of Wang et al. (2007). The SGR of *S. piluliferum* was the lowest among seaweeds in this study. That was because of this algae has the lowest SA:V ratio and lowest nutrient uptake rate among the seaweeds.

During this study, all SGRs were increased with water temperature and ammonia loading rate increase. Lobban and Harrison (1994) also mentioned that nutrients uptake rate is increasing with temperature.

V. CONCLUSION

1. Three seaweeds, *Enteromorpha compresa, Ulva pertusa* and *Sargassum piluliferum* were tested as a biofilter under the conditions of two ammonia loading rates (20 and 50 g m^{-3} d^{-1}) and two different water temperature regimes (10°C and 15°C). Concentrations of nutrients (NH_4^+, NO_3-N, PO_4^{3-}) were significantly reduced through the all seaweed aquaria.
2. *Enteromorpha compresa* showed the highest nutrients uptake rate followed by *Ulva pertusa* and *Sargassum piluliferum* on ammonium (68.07 μM NH_4^+ g^{-1} FW d^{-1}), nitrate-nitrogen (22.99 μM NO_3-N g^{-1} FW d^{-1}), nitrite-nitrogen (0.19 μM NO2-N g^{-1} FW d^{-1}) and orthophosphate (2.41 μM PO_4^{3-} g^{-1} FW d^{-1}).
3. Comparisons of volumetric ammonium removal rate among these seaweeds and other biofilters i.e. biofilm and RBC showed that seaweeds have higher TAN removal rates and can be considered as a biofilter for nitrifying process. However, volumetric nitrate-nitrogen removal rate by seaweeds were lower than those of denitrifying biofilters. But they can still be applicable to use for denitrification process because of very simple methods of application and need not have anaerobic conditions like other denitrification biofilters.
4. *Enteromorpha compresa* can be considered as prime candidate for nitrification and denitrification biofilter in th recirculating aquaculture systems and for the treatment of pond effluents.

ACKNOWLEDGEMENTS

I would like to thank Professor Jae-Yoon Jo for his guidance, advice, and support during my graduate study. Also special thanks to Dr. In-Bae Kim and Dr. Seong-Yoon Hong, for the advices, discussion and support.

I would like to give thanks to Myung-Seok Han for his kindness for information and identification of the seaweeds, and Dicky Harwanto, Sang-Joon Choi, Dr. Sung-Yong Oh, Ho-June Min, Gwang-Chol Nam, and all members of Aquaculture Engineering Laboratory, Pukyong National University, for their supports during my study in the laboratory.

Finally, I want to express a special appreciation to my family and my parents for their patience in love and invaluable support.

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