Bioremediation efficiency and biochemical composition of *Ulva reticulata* Forsskål (Chlorophyta) cultivated in shrimp (*Penaeus monodon*) hatchery effluent

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Abstract

The rapid growth of aquaculture is accompanied by increased discharge of nutrient-rich wastewaters into rivers and coastal waters; leading to eutrophication and deterioration of water quality. Seaweeds are suitable candidates to reduce dissolved inorganic nutrient concentration discharged through aquaculture effluent, and can improve water quality and allow for sustainable aquaculture. In this study the de-eutrophication ability of *Ulva reticulata* was investigated in a shrimp hatchery; in Kuala Selangor, Malaysia; by evaluating its ability to remove nutrients from shrimp brood stock effluent (SBE) in a batch culture system. The biofiltration ability of *U. reticulata* was confirmed by the significant reduction in nutrient concentrations during a 12 day period. The concentration of ammonical-nitrogen (NH_3-N) was reduced by 100 % (after 12 h), nitrite (NO_2-N) by 100 % (after 18 h), orthophosphate (PO_4-P) by 89 % (after 12 days) and nitrate (NO_3-N) by 33 % (after 12 days). An 18.5 % increase in biomass of the seaweed over the experimental period was also observed. The mean relative growth rate (RGR) of *U. reticulata* reached 1.6±0.1 % d^{-1}.The *U. reticulata* grew well in SBE, producing protein (6.1 ±1.1 %) and carbohydrate (39.9 ±4.5 %). Carbohydrate ($p<0.05$) and protein ($p>0.05$) content in seaweed growing in SBE were higher than seawater. The results of this study indicate that *U. reticulata* can be used directly as an effective biofilter for nutrient removal from shrimp hatchery effluent.

Keywords: *Ulva reticulata*, Chlorophyta, Bioremediation, Water quality, Seaweed, Nutrient removal, Shrimp hatchery effluent

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Introduction

Aquaculture systems release large amounts of nutrients into the marine ecosystem, in the form of excretory products and excess feed (Zhou *et al*., 2006; Rodrigueza and Montaño, 2007; Marinho-Soriano *et al*., 2009). Eutrophication is a general phenomenon in coastal waters, commonly attributed to the increase of shrimp, fish and shellfish aquaculture (Liu *et al*., 2010; Mao *et al*., 2005), resulting in an increase in harmful algal blooms and deterioration of water quality (Rodrigueza and Montaño, 2007; Marinho-Soriano *et al*., 2009; Huo*et al*., 2011). The major challenge in aquaculture industries has been to minimize the negative effects of nutrients in pond water and aquaculture effluents. A number of chemical, physical and biological methods applied to conventional waste water treatment have been used in aquaculture systems. Physical and chemical remediation technologies are very expensive systems to create and control (Ruenglertpanyakul*et al*., 2004). Bioremediation processes are more economical and are based on anaerobic/aerobic fermentation, microbial denitrification and biological removal of nitrogen and phosphorus through primary producers like microalgae, seaweeds and seagrasses. In developing tropical countries, microalgae have been used for treating aquaculture effluent, but seaweeds which are easier to harvest, also have the ability of nutrient uptake (Tsagkamilis*et al*., 2010). Seaweeds may play an important role in the control of eutrophication, improving water quality and enhancing sustainable aquaculture at low costs (Neori, 2008; Copertino*et al*., 2009; Huo*et al*., 2011). Seaweeds can remove up to 90% of the nutrients discharged from an intensive fish farm (Shpigel and Neori, 2007). Integrated multi-trophic aquaculture (IMTA) has developed over the last decade. In IMTA systems, seaweeds can have many economic values (Copertino *et al*., 2009; Figueroa *et al*., 2009).

The green algae *Ulva* spp., due to their morphological characteristics (high ratio of surface area to volume) exhibit high tolerance and affinity for ammonium uptake from shrimp pond effluent (Copertino *et al*., 2009). Species of are considered as ideal biofiltering candidates because of their high rates of nutrient removal, high photosynthesis and high specific growth rate (Hernández, *et al*., 2002; Martínez-Aragón *et al*., 2002; Neori *et al*., 2003; Copertino *et al*., 2009; Tsagkamilis *et al*., 2010). *Ulva* species grow well in high nitrogen concentrations and produce large biomass, thus removing large amount of nutrients (Bolton *et al*., 2009). They were studied for production of feedstock for biofuels, food ingredients and fertilizers (Tsagkamilis *et al*., 2010; Ale *et al*., 2011).*Ulva* species can produce allelopathic substances and inhibit growth of epiphytes and phytoplankton; and are therefore effective in competing for nutrients and light with phytoplankton (Wang *et al*., 2001; Copertino*et al*., 2009) and against pathogens (Lu *et al*., 2008). *U. lactuca* has been shown to be a good source of vitamins (A, B_2, B_{12} and C); in addition to their antioxidant, antimicrobial and antiviral properties (Orttiz*et al*., 2006; Abd El-Baky*et al*., 2008; Ale *et al*., 2011). Species of*Ulva*,such as *U. clathrata*, have

high yields and growth rates under wide ranges of salinity, temperatureand pH; and with low water exchange and without inorganic carbon supplementation (Copertino*et al*., 2009). *U. pertusa* can uptake dissolved organic nitrogen as urea and alanine directly without bacterial breakdown to inorganic nitrogen (Tarutani*et al*., 2004).

In South African abalone farms, *Ulva* is cultivated for abalone feed, while simultaneously reducing ammonia and bacterial levels in the effluent (Flodin, 2005; Bolton *et al*., 2009). The removal efficiency of *U. reticulata* and *U. pertusa* for the uptake of dissolved inorganic nitrogen were reported to be higher than that of *Gracilaria crassa*, *Chaetomorpha crassa, Eucheuma denticulatum, G.lemaneiformis, Underia pinnatifida* and *Hizikia fusiforme* (Msuya and Neori, 2002; Liu *et al*., 2010).

In Malaysia, shrimp farming is the largest contributor of revenue generated by the aquaculture industry. However, the expanding aquaculture industry may result in the degradation of the coastal ecosystem. The cultivation of seaweeds in shrimp pond effluent may provide a potentially efficient system for bioremediation, while producing useful seaweed biomass for valuable products.

In this paper, we evaluated the nutrient removal ability of *U. reticulata,* cultured in a shrimp (*Penaeus monodon*) hatchery effluent, using a batch culture system. The biochemical composition of the *U. reticulate* was also determined to assess the potential utilization of this seaweed biomass.

Materials and methods

Source of Ulva and maintenance of stocks

The study was conducted in a shrimp hatchery using a batch culture system in an outdoor setting. The *U. reticulata* used in this study was collected from the seashore of Pinang, Malaysia, and transferred to a *P.monodon* (Tiger prawn) hatchery in Kuala Selangor (03º17'38" N, 101º 17' 8" E). For the acclimatization stage, *U. reticulata* stock was kept in several plastic tanks (200 L) containing filtered natural seawater, with continuous aeration and water exchange (30 %) every other day for three weeks until the start of the bioremediation experiments. During this time, the seaweeds were maintained free of epiphytes and acclimatized to shrimp hatchery conditions. Necrotic portions of the seaweeds were removed. After acclimatization, the healthiest thali of *U. reticulata* were selected and transferred to the treatment tanks with 150 L capacity (70 cm ×105 cm ×25 cm; W×L×D) and placed under a sheltered place, open on three sides, and roofed with transparent polycarbonate sheets for the bioremediation experiment.

Culture of U. reticulata in shrimp brood stock effluent (SBE)

In the hatchery, *P.monodon* brood stocks were cultured in a 3000 L cement pond for larvae production. About 70 shrimps, with an average weight of 150 g were maintained in the cement pond with a daily water change of 30 %. This discharge water was used for the experiments.

The experimental design for seaweed cultivation consisted of four treatments in triplicates. The treatments were: (i) tanks

containing seawater (SW); (ii) tanks containing *U. reticulata* and seawater (SW+U); (iii) tanks containing shrimp brood stock effluent (SBE); (iv) and tanks containing *U. reticulata* and shrimp brood stock effluent (SBE+U). Twelve plastic tanks (4treatments×3replicates=12) with 150 L capacity were used. For treatments with seaweed, 450 g ($3gL^{-1}$) of fresh weight (FW) *U. reticulata* were placed in each tank. The tanks' contents were continuously aerated with air-pumps and air stones submerged in the water. This batch culture system was continued for 12 days.

Biomass and relative growth rate (RGR)

The biomass (gL^{-1} FW) and relative growth rate ($\%d^{-1}$) of *U. reticulata* were measured at six-day intervals for 12 days. The seaweed was completely removed from each tank, it was then spread and shaken in a plastic basket and allowed to drain the excess water for 15 min. The seaweeds were weighed using the Sartorius BL610 (±0.01g) balance. Relative growth rate (RGR) with reference to day 0 (RGR_i) and previous samples (RGR_{t-1}) were calculated using the following equations (Phang *et al*., 1996):

RGR_i (% d^{-1}) = $[(W_t-W_i)/ (W_i*\Delta t)]*100$

Where W_i is the initial fresh weight, W_t is the fresh weight on day t, Δt is the time interval

RGR_{t-1} (% d^{-1}) = $[(W_t-W_{t-1}) (W_{t-1}*\Delta t)]*100$

Where W_t is the fresh weight on day t and W_{t-1} is the fresh weight on the previous sampling day, Δt is the time interval

Environmental and water physico-chemical parameters

During the trial experiment, water physico-chemical parameters and environmental variables were monitored every 48 h. The water physico-chemical parameters such as temperature (ºC), conductivity (ms), salinity (‰), percentage dissolved oxygen (DO %) and pH were measured *in situ* in the morning (11 am) by a YSI (Model 85) multi-parameter probe. Irradiance was measured using the LI-COR (LI - 250A) light meter. The irradiance throughout the experiment was in the range of 50 to 109 µmol photons m^{-2} s^{-1} with an average of 91.5±21.0 µmol photons $m^{-2}s^{-1}$. Water samples for the determination of nutrient concentration were taken at 0, 2, 4, 6, 12, 18, 24, 48(2^{nd} day), 72(3^{th} day), 144(6^{th} day), 216(9^{th} day), 288(12^{th} day) hours after the start of the experiment. Water samples were stored in polyethylene bottles and transported in the ice chest (5-10ºC) to the laboratory for analysis. The concentration of ammonical-nitrogen (NH_3-N) was measured by the salicylate method, nitrite (NO_2-N) by diazotization, nitrate (NO_3-N) by cadmium reduction, orthophosphate (PO_4-P) by the ascorbic acid method, and chemical oxygen demand (COD) by the reactor digestion method using the HACH Spectrophotometer (Model DR/ 2500) (Clesceri *et al*., 1998). The C:N:P ratio of the SBE was calculated according to Phang and Ong, 1988. The other parameters; such as total suspended solids (TSS), total solids (TS), total volatile solids (TVS)and total dissolved solids (TDS); were measured in the laboratory using various analytical

methods according to the standard methods for examination of water and wastewater (Clescer*iet al*., 1998)

Removal efficiency (RE %) of nutrients

Percentage removal efficiency (RE %) of nutrients in each tank was calculated by subtracting the initial (inflow) and final (outflow) concentration of nutrients during sampling times as:

RE % = [(*Inflow* - *Outflow*)/ *Inflow*] ×100

Where: *Inflow* = nutrient concentration in each tank at the start of experiment (mg L^{-1})

Outflow = nutrient concentration in each tank water at the time of sampling (mg L^{-1})

The nutrient removal of *U. reticulata* in the treatments containing seaweed (SBE+*U* and SW+*U*) was estimated by subtracting the removal efficiency of the nutrients in its control tanks (SBE and SW) at the same times.

Biochemical composition of U. reticulata

For the biochemical compositions assay, seaweed samples were taken from the treatments and control tanks, cleaned by washing several times in fresh water, blot-dried and weighed. The fresh samples were used for biochemical analyses.

Protein (Bradford, 1976), carbohydrate (Dubois, 1956), chlorophyll *a*, carotenoids and phycobiliproteins (Lim *et al*., 2010) were determined on the first day (day 0) and the last day (day 12) of the experiment. The carbohydrate and protein content of *U. reticulata* were analyzed and expressed in percentage dry weight (% DW). Ash content was calculated from the weight remaining after the incineration of dry matter for 6h at 550 °C in the muffle furnace (Phang*et al*., 1996).

Statistical analyses

Normal distribution of the data was tested using the Kolmogorov-Smirnov test. A factorial analysis of variance (ANOVA) was used to analyze the influence of time (hour) and treatment on the concentration and removal efficiency of nutrients by *U. reticulata*. Tukey's-HSD comparisons were applied to determine statistically significant differences (p<0.05) among time and treatment following ANOVA.

One-way ANOVA was used for the analysis of physico-chemical parameters and solid contents in different treatments. Independent sample t-tests were utilized to determine the effect of treatment on the biochemical composition of *U. reticulata* at the end of the experiment. A significance level of 95 % (p<0.05) was set for all the tests. The statistical analyses were carried out using SPSS software, version 15 (SPSS Inc., USA).

Results

Biomass and relative growth rate of U. reticulata

The biomass (gL^{-1} FW) of *U. reticulata* and percentage increase of biomass (% ΔW) in the treatments of SBE+U and SW+U over time is shown in Fig1. ANOVA analysis showed a significant increase in the biomass of *U. reticulata* (p<0.05) in SBE+U and SW+U treatments on day 12. After 12 days the average biomass in treatment SBE+U (3.6±0.1 g L^{-}

^{1}FW) was higher than SW+U (3.3 ±0.1 g L^{-1} FW) but it was not statistically significant (p>0.05).

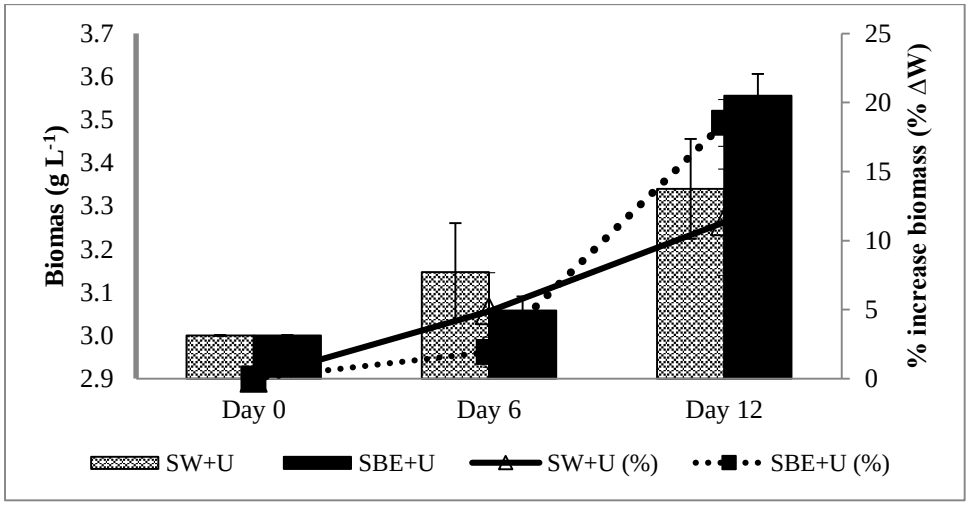

Figure 1:Biomass of *U. reticulata* (g L^{-1} FW) (histogram), and percentage increase of biomass (% ΔW)(Line graph) vs. day during 12 days in shrimp brood stock effluent (SBE) and seawater (SW); Error bars are standard deviation.

The relative growth rate of *U. reticulata*, calculated as RGR_i and RGR_{t-1},are shown in Fig 2. After 12 days the average RGR_iin treatment SBE+U (1.5±0.1%d^{-1}) was significantly (p<0.05) higher than SW+U (0.9±0.3% d^{-1}). The average $RGR_{t-1}$from day 6 to 12 in treatment SBE+U (2.7±0.1 %d^{-1}) was significantly (p<0.05) higher than treatment SW+U (1.0±0.1%d^{-1}) (Fig.2).

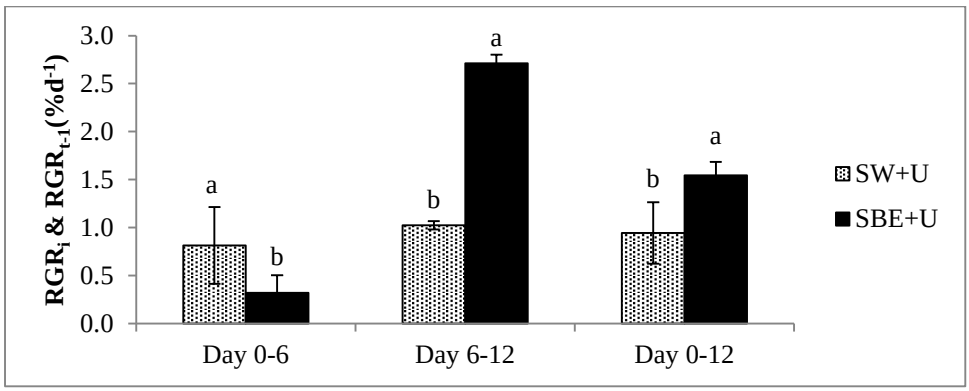

Figure 2:Mean relative growth rate of *U. reticulata* in shrimp brood stock effluent (SBE) and seawater (SW) with reference to the initial day (RGR_i,Day 0-6 & Day 0-12) and with reference to the previous sampling day (RGR_{t-1}, Day 6-12); Error bars represent standard deviation.

Water quality

The characteristics of the shrimp brood stock effluent (SBE) in the experiment are shown in Table 1. The C:N:P ratio of the SBE was 300:4:1.

Physico-chemical characteristics of water from different treatments on day 0 and 12 are shown in Table2. Water temperature in tanks without seaweed (SW and SBE) were significantly (p<0.05)

higher than tanks with seaweed (Table 2).On average, pH values in tanks with *Ulva* were significantly (p<0.05) higher than the values found in tanks without *Ulva*.

Table 1: Some parametersof the shrimp brood stock effluent measured at the start of experiment.

Parameter	Concentration
NH_3-N (mg L^{-1})	1.00 ± 0.01
NO_3-N (mg L^{-1})	0.40 ± 0.01
NO_2-N (mg L^{-1})	0.13 ± 0.01
PO_4-P (mg L^{-1})	0.40 ± 0.01
C.O.D. (mg L^{-1})	320 ± 28
Salinity (‰)	26 ± 0.2
pH	8.0 ± 0.1
TS (mg L^{-1})	29046 ± 3146
TSS (mg L^{-1})	142 ± 4
TVS (mg L^{-1})	5974 ± 65
TDS (mg L^{-1})	28904 ± 3104
Total carbon (mg L^{-1}) =(COD × 12/32)	120
Total nitrogen (mg L^{-1}) =(NH_3-N + NO_3-N + NO_2-N)	1.53
C: N: P	300:4:1

TS, Total Solids; TSS, Total Suspended Solids; TVS, Total Volatile Solids; TDS, Total Dissolved Solids; COD, Chemical Oxygen Demand.

Table 2: Physico-Chemical characteristics (mean ± SD) of the water in tanks with *Ulva*and without *Ulva* (Control) on day 0 and 12.

Treatment	Temperature (°C)		pH		DO (%)		Conductivity (ms)		Salinity(‰)	
	Day 0	Day 12	Day 0	Day 12	Day 0	Day 12	Day 0	Day 12	Day 0	Day 12
SBE+U	25.5±0.1	24.0±0.0	7.8±0.1	8.7±0.3	74.4±1.7	83.2±5.9	38.9±0.1	41.1±0.2	27.5±0.1	27.9±0.2
SBE	26.7±1.0	25.4±1.0	8.0±0.0	8.3±0.0	69.2±0.1	91.8±0.1	39.3±0.1	39.9±0.1	25.8±0.0	27.1±0.0
SW+U	25.4±0.2	24.1±0.3	8.2±0.0	8.8±0.1	76.6±3.5	85.7±5.4	38.9±0.1	41.5±0.1	25.8±0.2	27.3±0.4
SW	26.5±1.0	25.2±1.0	8.1±0.0	8.4±0.0	71±0.1	76±0.1	41.8±0.1	42.6±0.1	27.6±0.0	27.9±0.0

SBE+U, Shrimp brood stock effluent with *Ulva*; SBE, Shrimp brood stock effluent; SW+U, Seawater with *Ulva*; SW, Seawater .

Nutrient removal efficiency

Table 3 gives the nutrients' (NH_3-N, NO_3-N, NO_2-N, PO_4-P) concentration (mgL^{-1}, mean±SD) in the treatments of SBE+U, SBE, SW+U and SW during the experiment period and the nutrient removal efficiency (RE %) of *U. reticulata* on the 12th day. In general the presence of *U. reticulata* in SBE+U and SW+U, resulted in a significant (p<0.01) decrease in all nutrients' concentration on the 12th day compared to the control tanks (Table 3). The Tukey-HSD test (p<0.01) indicated that treatment, time (hour) and the interaction between treatment and time, had a significant (p<0.01) effect on concentration of NH_3-N, NO_3-N, NO_2-N and PO_4-P during the culture period.

Table 3: Concentration (mean ± SD) of nutrients (mgL^{-1}) during the culture period and removal efficiency (%RE) between the start of experiment and day 12 in different treatments.

Treatment	Time (hour)											
	0	2	4	6	12	18	24	48	72	144	216	288
SBE+U	1.0 ±0.0	0.4 ±0.1	0.2 ±0.1	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0±0.0	0.0 ±0.0
SBE	1.0 ±0.0	1.0 ±0.0	0.8 ±0.0	0.7 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	0.9 ±0.0	0.8 ±0.0	0.8 ±0.0	0.8±0.0	0.7 ±0.1
SW+U	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
SW	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
SBE+U	0.5 ±0.0	0.4±0.1	0.4 ±0.1	0.4 ±0.0	0.4 ±0.0	0.4 ±0.1	0.4 ±0.1	0.4±0.05	0.4±0.1	0.5±0.1	0.3±0.0	0.3 ±0.0
SBE	0.4 ±0.0	0.6 ±0.0	0.6 ±0.0	0.6 ±0.0	0.6 ±0.0	0.7 ±0.0	0.7 ±0.0	0.8 ±0.0	0.8 ±0.0	1.0 ±0.0	1.6 ±0.0	1.9 ±0.0
SW+U	0.4 ±0.0	0.3 ±0.1	0.3 ±0.1	0.3 ±0.0	0.3 ±0.1	0.3 ±0.1	0.3 ±0.1	0.3 ±0.0	0.3±0.0	0.3±0.0	0.3 ±0.1	0.4±0.0
SW	0.5 ±0.0	0.3 ±0.0	0.3 ±0.0	0.4 ±0.0	0.5 ±0.0	0.5 ±0.0	0.6 ±0.0	0.6 ±0.0	0.5 ±0.0	0.4 ±0.0	0.5 ±0.0	0.8 ±0.0
SBE+U	0.0 97 ±0.0	0.015 ±0.0	0.012 ±0.0	0.005 ±0.0	0.001±0.0	0.003 ±0.0	0.000±0.0	0.001±0.0	0.001±0.0	0.001 ±0.0	0.001 ±0.0	0.001 ±0.0
SBE	0.130 ±0.0	0.134 ±0.0	0.135 ±0.0	0.137 ±0.0	0.140 ±0.0	0.140 ±0.0	0.140 ±0.0	0.170±0.0	0.190 ±0.0	0.260 ±0.00	0.640 ±0.0	0.63 0.0
SW+U	0.020 ±0.0	0.005 ±0.0	0.004 ±0.0	0.003 ±0.0	0.002 ±0.0	0.001±0.0	0.000 ±0.0	0.001±0.0	0.001±0.0	0.001 ±0.0	0.001 ±0.0	0.001 ±0.0
SW	0.050 ±0.0	0.048 ±0.0	0.049 ±0.0	0.053 ±0.0	0.056 ±0.0	0.060 ±0.0	0.062 ±0.0	0.065 ±0.0	0.070 ±0.0	0.076±0.0	0.080 ±0.0	0.090±0.0
SBE+U	0.5 ±0.0	0.4 ±0.1	0.3 ±0.1	0.1 ±0 .0	0.1±0.0	0.1 ±0.0	0.1 ±0.0	0.1 ±0.0	0.0±0.0	0.0±0.0	0.0 ±0.0	0.0 ±0.2
SBE	0.4 ±0.0	0.3 ±0.0	0.3 ±0.0	0.2 ±0.0	0.4±0.0	0.4 ±0.0	0.5 ±0.0	0.3 ±0.0	0.3±0.0	0.3 ±0.0	0.3 ±0.0	0.6 ±0.0
SW+U	0.1 ±0.0	0.1 ±0.0	0.1 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0±0.0	0.03 ±0.0	0.1 ±0.0	0.1 ±0.0	0.1 ±0.1
SW	0.1 ±0.0	0.1 ±0.0	0.1 ±0.0	0.1 ±0.0	0.1 ±0.0	0.1±0.0	0.1 ±0.0	0.1 ±0.0	0.08 ±0.0	0.10 ±0.0	0.1 ±0.0	0.1 ±0.0

Values in any one row not followed by the same superscript letters are significantly different at P<0.05; nd, not detectable; Time of sampling based on hour after start of experiment; SBE+U, Shrimp brood stock effluent with *Ulva*;SBE, Shrimp brood stock effluent without *Ulva*; SW+U, Seawater with *Ulva*; SW, Seawater without *Ulva*.

The ammonical-nitrogen concentration was non-detectable in all treatments except for SBE after day 12(Table3). The variation in NH_3-N concentration (mgL^{-1}) and removal efficiency (RE %) in treatments with seaweeds (SBE+U, SW+E) over time is presented in Figs.3a, 3b.

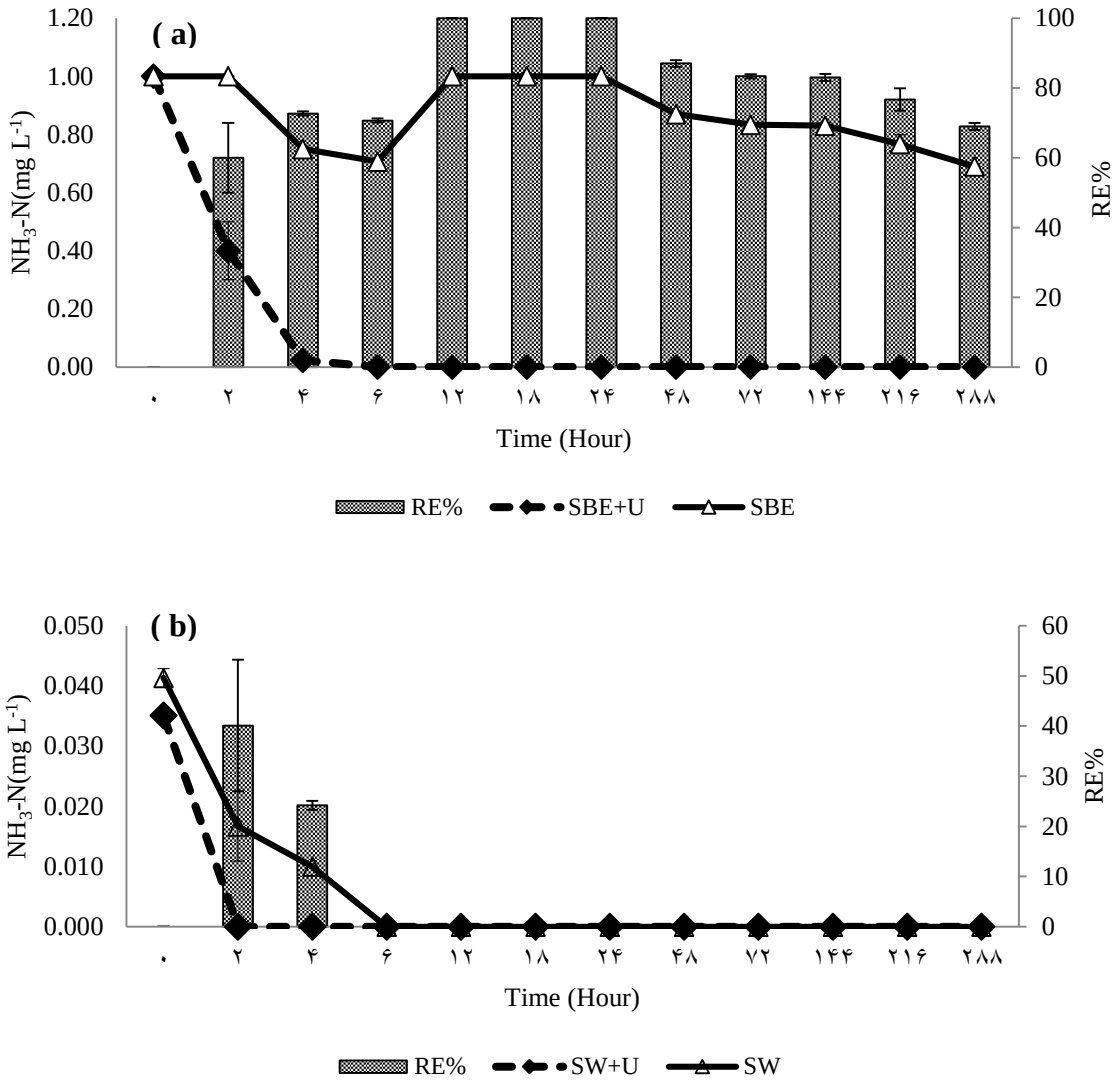

Figure 3: Ammonical-nitrogen concentration (line graph; mg L^{-1}) and the removal efficiency (histogram; RE%) of *U. reticulata* in different treatments during 288 hours; a) Shrimp brood stock effluent without *Ulva* (SBE) and Shrimp brood stock effluent with *Ulva*(SBE+U); b) Seawater without *Ulva* (SW) and Seawater with *Ulva* (SW+U); Values presented as average (n=3); Error bars represent standard deviation.

NH_3-N removal by *Ulva* in the treatment of SBE+U increased by 60 % after two hours and by 100 % after 12 hours which was significantly higher (p<0.01) than the removal in the SBE control (Fig. 3a). In seawater tanks containing *Ulva*(SW+U), the NH_3-N concentration was very low and reduced by 40 % after two hours, and was undetectable in both SW and SW+U after 12 hours (Fig. 3b).

Nitrate (NO_3-N) concentration and removal efficiency (RE %) fluctuated under various treatments and time of experiments (Figs. 4a, 4b). Nitrate regeneration in treatments of SBE, SW and sometimes in SBE+U observed during the culture period which were significantly (p<0.01) different under various treatments and time of experiments (Table 3, Figs. 4a, 4b). At the final day, significant (p<0.05)

maximal decrease in the concentration of nitrate were determined in the tanks containing seaweed (SBE+U, SW+U). Nitrate concentration in SW+U treatment was measured less than treatment without seaweed (SW =control). Nitrate removal increased over the culture period in both SBE+U and SW+U, indicating the adaptation of the seaweed. The maximum nitrate removal in SBE+U was determined to be 33 % (12th day) and 33.3 % (2nd day) in SW+U (Figs.4a, 4b).

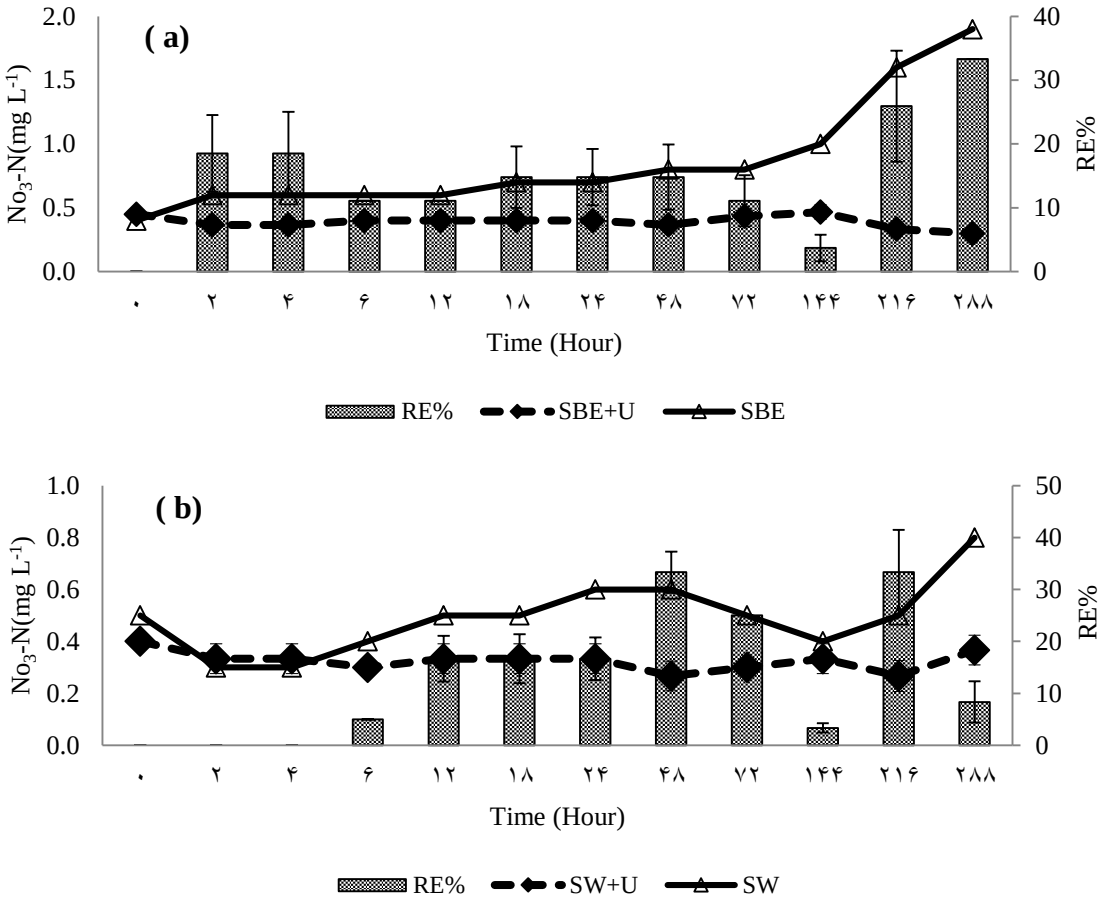

Figure 4: Nitrate concentration (line graph; mg L^{-1}) and removal efficiency (histogram; RE%) of *U. reticulata* in different treatments during 288 hours; a) Shrimp brood stock effluent without *Ulva* (SBE) and Shrimp brood stock effluent with *Ulva*(SBE+U); b) Seawater without *Ulva* (SW) and Seawater with *Ulva* (SW+U); Values presented as average (n=3); Error bars represent standard deviation.

Nitrite concentration in treatment with *Ulva* (SBE+U, SW+U) significantly (p<0.01) decreased with time over the period of the study. In fact, nitrite concentration in control tanks increased for both SBE and SW on the 12th day. The maximum nitrite removal was 100 % in SBE+U and 99 % for SW+U after 18 and 24 hours, respectively (Table 3, Figs. 5a, 5b).

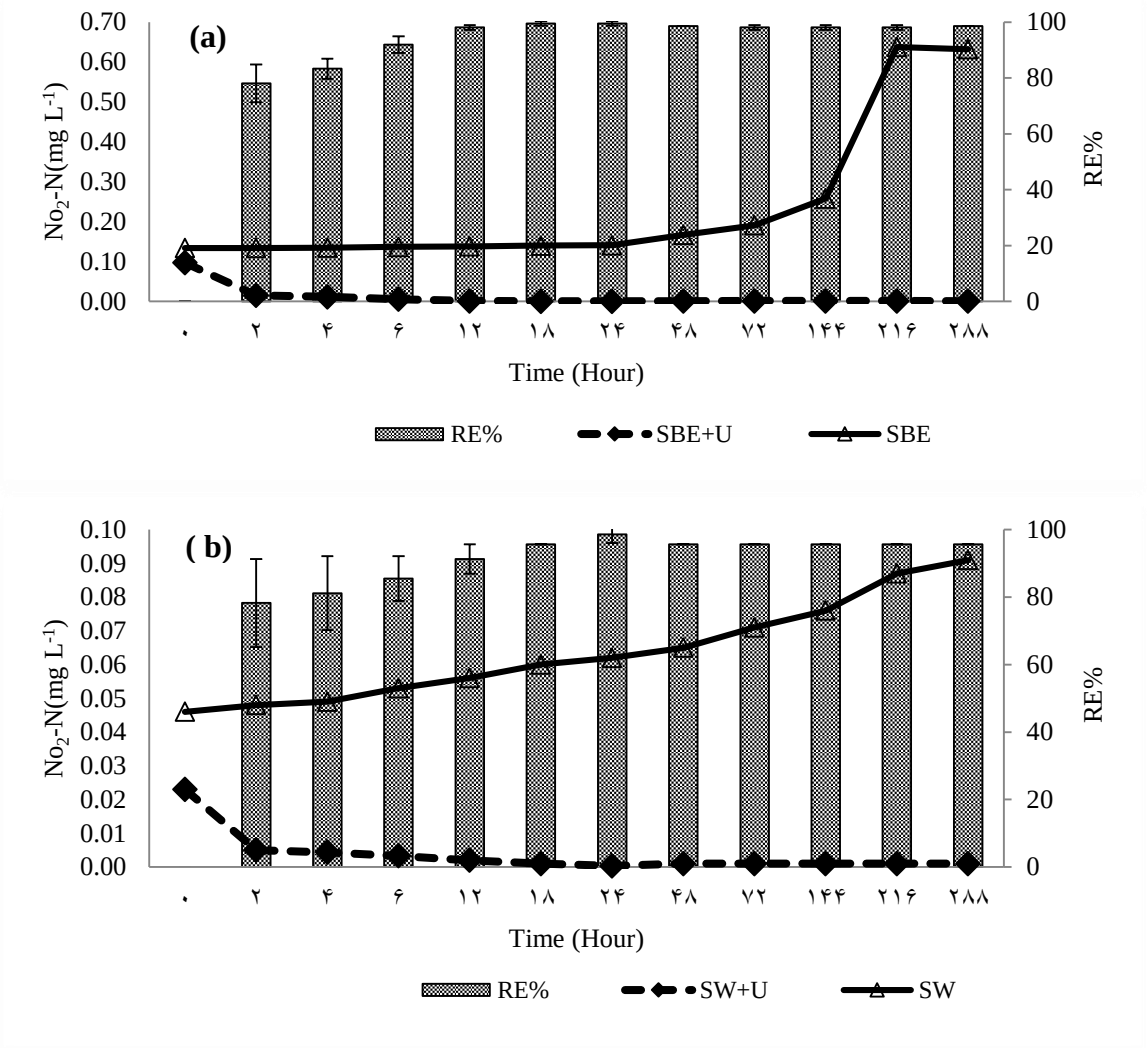

Figure 5: Nitrite concentration (line graph; mg L^{-1}) and removal efficiency (histogram; RE%) of *U. reticulata* in different treatments during 288 hours; a) Shrimp brood stock effluent without *Ulva* (SBE) and Shrimp brood stock effluent with *Ulva*(SBE+U); b) Seawater without *Ulva* (SW) and Seawater with *Ulva* (SW+U); Values presented as average (n=3); Error bars represent standard deviation.

Orthophosphate concentration in SBE+U, SBE, SW+U and SW treatments changed from 0.50 to 0.05, 0.39 to 0.62, 0.09 to 0.11 and 0.12 to 0.10 mg L^{-1}after 12 days, respectively. Tukey-HSD analysis showed that orthophosphate concentration in tanks with seaweed were significantly (p<0.01) lower than tanks without seaweed (Table 3). *U. reticulata* in SBE+U treatment showed greater removal efficiency (89 %) for orthophosphate compared to SW+U (35 %) (Table 3 and Figs. 6a, 6b).

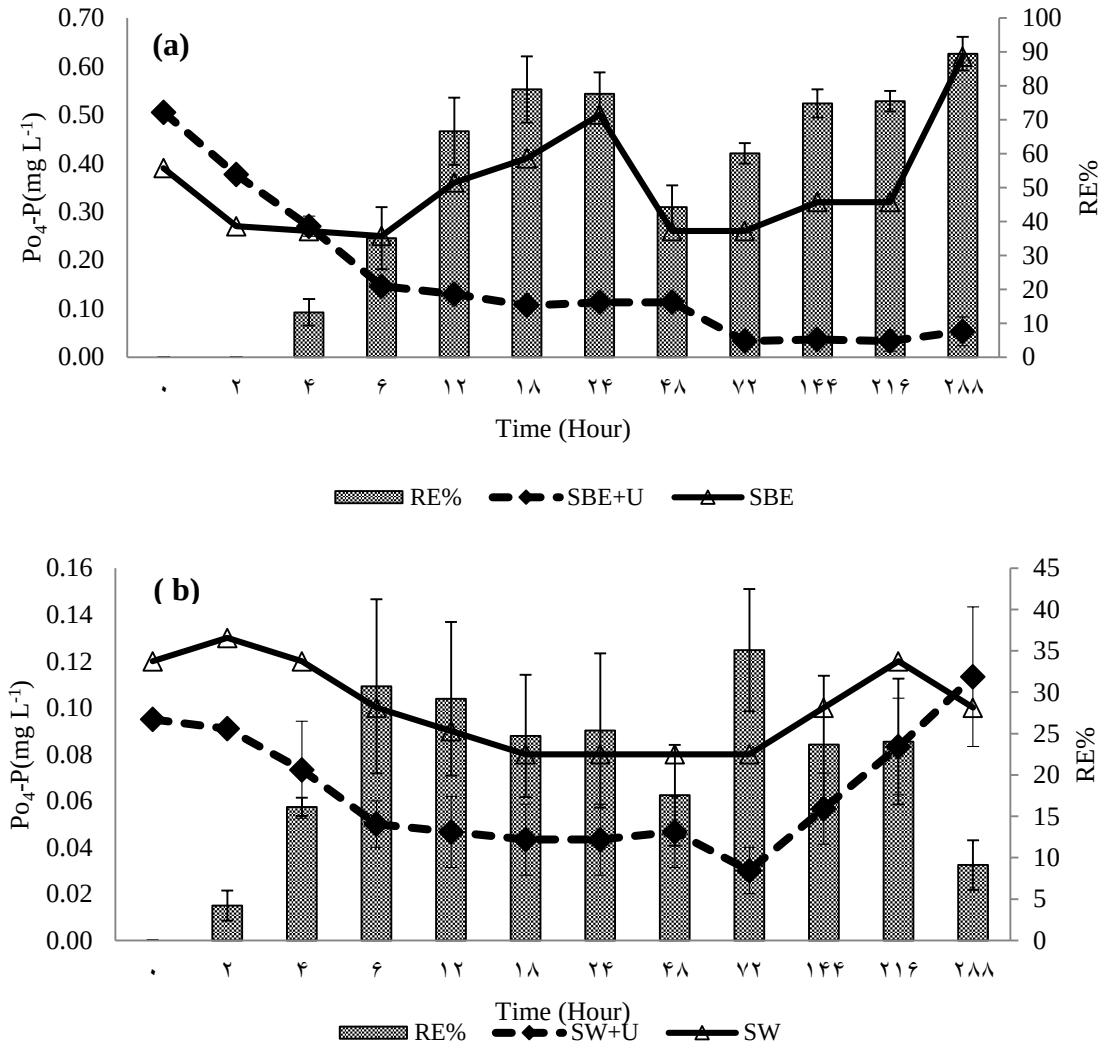

Figure 6: Phosphate concentration (line graph; mg L^{-1}) and removal efficiency (histogram; RE%) of *U. reticulata* in different treatments during 288 hours; a) Shrimp brood stock effluent without *Ulva* (SBE) and Shrimp brood stock effluent with *Ulva*(SBE+U); b) Seawater without *Ulva* (SW) and Seawater with *Ulva* (SW+U); Values presented as average (n=3); Error bars represent standard deviation.

Biochemical Composition

The protein content (%DW) of *U. reticulata* had decreased in both SBE+U (from 8.3 ±0.3 to 6.1 ±1.1) and SW+U (from 8.2 ±0.3 to 4.2 ±0.4) treatments. The carbohydrate content also showed a decreasing trend in SBE+U (from 42.3 ±3.2 to 39.9±4.5) and SW+U (42.1 ±3.0 to 39.4 ±4.6 % DW) after 12 days (Table 4).

The chlorophyll *a* and carotenoid content of *U. reticulata* both increased in SBE+U from 0.23 ±0.05 to 0.25 ±0.05 mg g^{-1} and 122 ±42 to 136 ±14 µg g^{-1} at the end of experiment, respectively. While in treatment SW+U, the chlorophyll *a* and carotenoid content decreased from 0.23 ±0.10 to 0.14±0.02 mg g^{-1} and 121 ±42 to 100±9 µg g^{-1}, respectively. At day 12, the chlorophyll *a* and carotenoid content of *U.*

reticulata in SBE+U were significantly (p<0.05) higher than SW+U (Table 4).

Table 4: Biomass (FW) and chemical composition of *U. reticulate*in two treatments (SBE+U, SW+U) and day (0 & 12).

Source	Day	Treatment		T
		SBE+U	SW+U	
Biomass (g FW tank^{-1})	0	450±0	450±0	0.0 ns
	12	523±17	511±34	0.6 ns
Protein (% DW)	0	8.3±0.3	8.2±0.3	0.0 ns
	12	6.1±1.1	4.2 ±0.4	2.7 *
Carbohydrate (% DW)	0	42.3±3.2	42.1±3.0	0.0 ns
	12	39.9±4.5	39.4±4.6	0.1 ns
Ash (%)	0	24.9±0.8	24.9±0.8	0.0 ns
	12	23.8±1.2	23.2±0.2	0.8 ns
Chlorophyll a (mg g^{-1})	0	0.23±0.05	0.23±0.10	0.0 ns
	12	0.25±0.05	0.14±0.02	3.3 *
Carotenoid (µg g^{-1})	0	122±42	121±42	0.0 ns
	12	136±14	100±9	3.5*

* significant difference at p<0.05; ns, not significant; SBE+U, Shrimp brood stock effluent with *Ulva*; SW+U, Seawater with *Ulva*; T, Independent sample t-test.

Mean ash content of *U. reticulata* growing in SBE (23.8 ±1.2 % DW) did not show a significant (p>0.05) difference to SW+U (23.2 ±0.2 % DW) (Table 4).

Discussion

Growth of Ulvareticulata

The mean relative growth rate (RGR_i =1.6 ±0.1, RGR_{t-1}=2.7±0.1%d^{-1}) of *U. reticulata* in SBE tanks are comparable to the values previously reported for *U. reticulata*(1.2%d^{-1})in fishpond effluent (Msuya and Neori, 2002),*U. clathrata*(1–3%d^{-1}) under static and flow regime in tank cultivation (Copertino*et al*., 2009), *U. reticulata* (5–8 % d^{-1}) in finfish effluent (Mwandya*et al*., 2001). Studies have shown that the daily growth rate of *Ulva*

species can be different based on species, growth conditions and culture duration (Neori*et al*., 2004).

In this experiment, the *U. reticulata* was found to be able to uptake nutrients from SBE which resulted in an 18.5% increase in biomass, which was higher than that of the control tanks (11.3 %). Figueroa *et al*. (2009) reported the same biomass for *U. lactuca* in high and low nutrient concentration, but *G.tenuistipitata* in co-culture with rainbow trout (*Oncorhynchusmykiss*) showed better growth than mono-culture (Zhou *et al*., 2006).

The low growth rate of *U. reticulata* in seawater in the present study could be attributed to culture conditions such as low nutrient concentration. The depigmentation of *U. reticulata* in control tanks (seawater) can be another reason for the low growth rate. In tropical waters nitrogen and phosphorus levels enhancement and water movement can prevent seaweed bleaching by reducing the light intensity and temperature effects (Santelices, 1987). Figueroa *et al*. (2009) showed that a higher

nutrient level can increase pigment content and photosynthesis in *U. lactuca*.This is probably why the *U. reticulata* had reduced pigment content in the seawater compared with the SBE treatment.

Removal efficiencies of nutrients

Seaweeds have been proposed as ideal biofilters for nitrogen sources in aquaculture effluents (Marinho-Soriano *et al*., 2009). In general, few studies have focused on phosphate uptake by seaweeds (Jones *et al*., 2001; Kang *et al*., 2011). In this study, we evaluated the biofiltering efficiency of *U. reticulata* for nitrogen and phosphate sources. Nutrient reduction in *U. reticulata* tanks were mainly related to biofiltering effects of seaweed and to a lower degree due to the uptake of nutrients by microphytes, especially cyanobacteria (Mwandya*et al*., 2001).In this study, *U. reticulata* in SBE tanks exhibited the highest rate of NH_3-N uptake (100 %); followed by Nitrite (98.6 %), phosphate (89.4 %) and nitrate (33.3 %). The slight decrease in NH_3-N concentration in the control tanks (without *U. reticulata*) may be related to a diurnal cycle of nutrient loss.

The Results of our study are similar to Seema and Jayashankar, (2005) who reported 94 % NH_3-N removal efficiency for *U. reticulata* in shrimp pond effluent and 97.7 % for *U. rotundata*n seabass tanks (Martinez-Aragón *et al*., 2002). Chung *et al*., (2002) reported, 40–90 % of NH_4^+ removal efficiency for *U. lactuca* and 76 % for *U. rigida* from fish waste water. *U. lactuca*c ultivated in effluents of fish pond could efficiently remove 85–90 % of total ammonia-nitrogen (Neori*et al*., 2003). Similar results have been reported for *U. pertosa*and *Gracilaria*, which exhibited higher NH_4^+uptake than NO_3 and NO_2 (Kang *et al*., 2011). Mwandya*et al*., (2001) reported 63 % removal efficiency for ammonia, 58 % for phosphate and 54 % for nitrate by *U. reticulata* cultivated in finfish effluent.

Seaweeds are noted to have the greatest preference for NH_3-N uptake compared to other nutrients (Buschmann and Varela, 2008). Most seaweed species showed that uptake rates of NH_4^+exceeded those of NO_3 under natural environmental conditions because NH_4^+can be directly incorporated into the composition of amino acids (Ahn*et al*., 1998; Shpigel and Neori, 2007).

In this experiment the average removal efficiencies of *U. reticulata* for nitrite, phosphate and nitrate in shrimp effluent and batch culture system were 98.6 %, 89.4 % and 33.3 %, respectively. Seema and Jayashankar, (2005) reported removal efficiencies of 22% for nitrite, 5% for nitrate and 45% for total nitrogen by *U. reticulata* grown in shrimp effluent. *U. pertusa* showed a 54% removal efficiency for dissolved inorganic nitrogen, 22% for NO_3^-+NO_2 and 36.6 % for phosphate in fish effluent (Kang *et al*., 2011). *U. clatrata* showed a higher removal efficiency for total ammonical-nitrogen (70–82%) than for phosphate (50%) (Copertino *et al*., 2009).

In the present study *U. reticulata* showed faster and more efficient removal (RE%) for NH_3-N (100%) after 12 hours, followed by NO_2 (100%) after 18 h and NO_3 (33%) after 12 days. The ability to

remove NO_2 and NO_3 increased with time, showing an adaptation to the presence of these nitrogen sources.

The high affinity of *U. reticulata* for NH_3-N and NO_2-N uptake can be a useful strategy for gaining large amounts of nitrogen. NH_4^+ is the most reduced form of inorganic nitrogen, therefore, from an energetic point of view it is the most useful nitrogen source for seaweed growth (Marinho-soriano *et al*., 2009). In general, seaweed has shown a high affinity for the uptake of nitrogen sources, as well as storing them for growth during nitrogen starvation periods (Abreu *et al*., 2011).

In this study, a removal efficiency of 89.4% for PO_4-P was measured in SBE +U after 12 days. Studies have shown that phosphate can be taken up efficiently in integrated fish-*Ulva* cultivation systems. Kang *et al*. (2011) reported 38.1%, 30.6 % and 20.2% phosphate uptake for *Gracilariopsis chorda, U. pertusa* and *Saccharina japonica*, respectively. Tsagkamilis *et al*., (2010) found *U. lactuca* reduced phosphate content by about 50 % in sewage effluent. Martinez-Aragón *et al*., (2002) found that *U. rotundata* removed nearly 99.6% of phosphate at low flow rates in fish tanks.

Biochemical composition

The results of this study showed that at the end of the experiment the protein content in *Ulva* cultured in SBE (6.09±1.1% DW) was significantly (p<0.05) higher than samples grown in seawater (4.20±0.37% DW). Manivannan *et al*. (2008) reported 3.25% (DW) of protein content for *U. lactuca*. High protein content in *U. reticulata* cultured in SBE showed that the seaweed can uptake and store excess nitrogen in protein form. Msuya and Neori (2008) reported that the protein content of *U. lactuca* significantly increased with increasing nutrient loading levels. The protein content of *U. reticulata* cultivated in fish effluent could increase up to 26 % (Msuya and Neori, 2002).

In this study, on the final day the carbohydrate content in *U. reticulata* cultured in SBE (39.9±4.5% DW) was higher than SW+U (39.4±4.6% DW) but was not significant (p>0.05). Carbohydrate content varies with the nutritional state of the cells (Msuya and Neori, 2002). Shanmugam and Palpandi (2008) reported 50.2% DW carbohydrate in *U. reticulata*. Manivannan*et al*., (2008) reported a mean carbohydrate of 23 % for *U. lactuca*.

U. reticulata cultivated in SBE tanks became darker in colour than the ones in seawater tanks. The darker colour of thalli in SBE may be due to the high nutrient concentration in SBE tanks. Chlorophyll *a* in *U. lactuca* grown in high nutrient supply was reported to be higher than those in low nutrient supply (Fiquaroa *et al*., 2009). The nitrogen supply in medium culture can influence pigment content, protein and carbon uptake in many species of seaweeds (Cómez-Pinchetti*et al*., 1998; Fiquaroa *et al*., 2009).

Ulva reticulata was shown to grow well in shrimp effluent without dilution. The seaweed grown in SBE had a higher relative growth rate than that of the seawater treatment. The biomass had relatively high protein (6.1% DW) and carbohydrate (39.9% DW) content. Our study showed that *U. reticulata* is a good

biofilter for removing nutrients in shrimp brood stock effluent, especially for ammonical-nitrogen, nitrite and phosphate after one day of culture.

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