Ulva lactuca for nutrients biofiltration in a recirculating shrimp effluent treatment system

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Abstract

An outdoor tank scale shrimp cultivation system was developed with integration of green macroalgae species, *Ulva lactuca*. Intensive shrimp farming produces large amount of waste nutrients, due to excess uneaten feed and excretion. Prior harvesting, shrimp effluent discharged without proper treatment and alleviates water quality of receiving water bodies. Coastal water degradation consequently causes negative environmental impact. This integrated system with *U. lactuca*, greatly reduced the nutrient loads and incorporated into its biomass. Phosphate removal was 90%, followed by ammonium and nitrate with removal efficiencies of 70% and 42%, respectively. The mean specific growth rate of *U. lactuca* was 2.25 ± 0.9 % day^{-1} and shrimp was 0.76 ± 0.09 % day^{-1}. Shrimp growth rate in control tank was significantly lower compared to the treatment tank ($P<0.001$) and survival rate declined throughout the experimental period. Integration of *U. lactuca* significantly enhanced the dissolved oxygen with mean value of 5.49±0.06 mg/L and creates a stable pH values compared to control treatment tank. Thus, *U. lactuca* is a prominent biofilter with rapid growth rate and greatly reduces the overall environmental impact of aquaculture water degradation and stabilize the culture environment and this study highly recommended utilization of *U. lactuca* as a biofilter component in an integrated multi-trophic aquaculture treatment system.

Keywords

Growth rate; nutrient biofiltration; macroalgae; recirculation; shrimp; *Ulva lactuca*

1. INTRODUCTION

Aquaculture industry steadily increases with an average annual growth rate of 6.9% (Troell, *et al.*, 2009). Approximately 75% of global shrimp production dominated by countries in Asia where the three leading nations are China, Thailand and Vietnam (Anh, Kroeze, Bush and Moi, 2010). Furthermore, Malaysia is one of the top ten shrimp producer in the world with production capacity of 2.8% in total world production of shrimp (FAO, 2012). At present, the world production data alone is 7 million tons and it could reach 18 million tons by the year 2030. Massive shrimp aquaculture industry progressively increased to meet the demand of food production for human consumption. Despite the existing shrimp farms, many new farms are also developed with the current cultivation technology. However, the increased number of farms with high shrimp production rates also produces high amount of effluent discharge which ultimately causes environmental degradation of the receiving waterways. Shrimp effluent has high load of excess nutrients and organic matter, in the form of excretory waste products and excess leftover feed materials. Thakur and Lin (2003) had revealed on nutrient budget in a closed shrimp culture system that shrimp could assimilate only 23-31% nitrogen and 10-13% phosphorus of the total inputs and the remaining entrained in the water. Consequently, the high amount of nutrient and organic loads will cause eutrophication and occurrence of red tide which affects marine organism and degrades the sustainability of coastal environment (Buschmann, Troell, Kautsky and Kautsky, 1996; Fei, 2004; Thakur and Lin, 2003; Xu, *et al.*, 2011). Large amount of nitrogen discharge into rivers are notable from intensive shrimp farming without concern on the impact to the environment.

Thus, implication of sustainable shrimp cultivation is at immense need for amelioration of effluent water quality prior to discharge into waterways. The potential solution as treatment approach is by biological means, using macroalgae as a biofilter integrated with shrimp cultivation, which has considerable capacity for nutrients reduction from the effluent water and also safe for the cultured species. There are several studies on integration of macroalgae with fish culture have been reported with various macroalgae species (Hayashi, *et al.*, 2008; Msuya and Neori, 2002; Amir Neori, Shpigel and Ben-Ezra, 2000; Rodrigueza and Montaño, 2007; Zhou, *et al.*, 2006). However, there are less studies focused on shrimp effluent treatment using macroalgae (Jones, Preston and Dennison, 2002; Marinho-Soriano, Morales and Moreira, 2002; Marinho-Soriano, Panucci, Carneiro and Pereira, 2009). The selected macroalgae is also considered as potential candidate to be part of component of IMTA system- integrated culture of fed species (e.g: shrimp), extractive (e.g shellfish) and inorganic extractive species (macroalgae). The macroalgae should possess superior characteristics and ability as biofilter, to produce a highly efficient treatment system. A suitable macroalgae species that is tolerant to hypereutrophic conditions of effluent, with ecophysiological characteristics that is suitable for local environment and culture conditions, naturally grown, valuable resource for commercialization and easily available is selected. A species that could meet these requirement is *Ulva lactuca* (cholorophyta), recognized as green macroalgae. This species has a rapid growth rate, highly resistant to infection, and successfully cultivated in an integrated system. The aim of the present study was to assess the biofiltration capacity of *U. lactuca* in a recirculating shrimp effluent treatment system with the enhancement of shrimp productivity.

2. METHODOLOGY

2.1 Preparation of algal sample

Macroalgae were collected from Danga Estuary, Johor Bahru, Malaysia, during low tide. Healthy thalli of the macroalgae were and transported to the laboratory in a polystyrene box filled with water sample. In the laboratory the macroalgae were washed under running water and cleaned of epiphytes. The macroalgae were pre-cultured in a polyethylene tank under natural light and temperature condition with constant aeration for a week, without addition of nutrients. Prior to experiment, a portion of healthy fragments removed to be used for the nutrient study experiment.

2.2 Experimental design

The nutrients removal during shrimp cultivation was investigated in tank treatment system built outdoor, with continuous recirculation of shrimp cultivation water to the macroalgae and control tanks (without macroalgae). Triplicate treatments were set up using glass aquarium (45cm x 32cm x 32cm), which were stocked with three prawns. Macroalgae were placed in an inert plastic aquarium with volume of 30 L above the shrimp aquaria to allow continuous vertical flow of shrimp cultivation water in the recirculating system. The inflow to each macroalgae aquarium was controlled by registers and out flow was gravity fed to the shrimp aquariums with water exhange of 1 volume d^{-1}. Shrimps of size 10.0 ± 0.2 g were stocked and observed its effluent water quality. Forty-five day-old post larvae of *Panaeus monodon* (PL_{45}) were purchased from a hatchery with an average weight of about 2.0 g for acclimatization in the brackish water (22-28 ppt) with light intensity of 100µmol m^{-2} s^{-1}, before the outdoor experiment and reared until it reached the required size for experiment.

Shrimp were fed 6% of its body weight per day with commercial shrimp feed (dry weight) composed of 40% crude protein, 21.2% carbohydrate, 6% crude fat, 6% crude fiber, 11% moisture, 15% ash and 0.8% phosphorus. The stocking density (fresh weight) of *U. lactuca* was 3.0g/L. The treatment was observed for 4 weeks and the nutrient removal rate by macroalgae was measured.

2.3 Measurements of physico-chemical parameters.

Temperature, salinity, dissolved oxygen (DO) concentration, and pH were measured. The pH of the water was monitored using YSI 1200 laboratory pH meter while other parameters were measured using YSI Proplus 55. Water samples were taken weekly from cultivation tank at early morning to analyze the dissolved nutrients such as ammonium (NH_4^+), nitrate (NO_3^-), phosphate (PO_4^{3-}). All of the measurement were performed in triplicate replications. For ammonium determination, was conducted according to Nessler method. The samples was analysed colorimetrically with HACH DR 5000 spectrophotometer, by using Nessler reagent (method No. 8038). In addition, for nitrate quantification, cadmium reduction method (No. 8171) and for phosphate quantification, ascorbic acid method (No. 8048) were used and measured with HACH DR 5000 spectrophotometer.

2.4 Analytical procedures

Fresh weights of shrimp and macroalgae were recorded fortnightly. The macroalgae were blotted on paper towel to remove excess water, weighed and restored back to original aquarium for determining specific growth rates (SGR, % d^{-1}) using Eq (1) (Lobban and Harrison, 1994) as follows:

$$SGR = \left[(\frac{w_t}{w_0})^{1/t} - 1 \right] * 100\% \qquad (1)$$

where W_0 is the initial fresh weight (g), W_t is fresh weight at time t (g), and t is time interval (days).

Nutrient removal efficiency (NRE %) achieved by macroalgae are generally based on the concentration reduction of a given nutrient in the culture medium and estimated as in Eq. (2).

$$NRE = 100 * (C_{initial} - C_{final}) / C_{initial} \qquad (2)$$

2.5 Statistical analysis

For all the experiments done, the replication data were expressed in terms of mean ± standard deviation. Data analyses were performed using the software SPSS 16.0 version. For physico-chemical parameters between the two periods, student's t-test was applied to determine the significant differences in mean between two groups. In addition, significant of nutrient removal rate by the macroalgae and SGR of macroalgae and shrimp were determined by using one way analysis of variance (ANOVA). The SGR of macroalgae analysed with ANOVA test and Tukey's multiple comparison test (α=0.05) was applied for weekly variation in growth. In all the cases, null hypothesis was rejected at the 5% significance level, which means that the significant differences among the treatment was at $P<0.05$.

3. RESULTS AND DISCUSSIONS

3.1 Physico-chemical analysis

Data for physical parameters for the treatment system is presented in Figure 1. It includes temperature, pH, dissolved oxygen (DO) and salinity that measured daily throughout the experimental period. Water temperature in all the aquariums were almost constant throughout the course of study and ranged between 26.5 ºC to 29.0ºC. The variations in temperature was less than 4 ºC and no significant differences between both aquariums ($P>0.05$). In contrast, pH and dissolved oxygen in aquariums integrated with *U. lactuca* had demonstrated significantly higher value compared to control aquariums. The mean of pH in macroalgae and control aquariums were 7.92 ± 0.4 and 7.60± 0.2, respectively. Dissolved oxygen (DO) content was about 12% higher in macroalgae aquariums compared to control that has the minimum DO of 4.78 mg/L. The mean concentration of DO in macroalgae aquarium was 5.50 mg/L and significantly varied ($P<0.01$) from shrimp and control aquarium. Salinity was in the range of 24 to 26 ppt, in macroalgae aquarium and increased about 5% in control aquarium. The rise was associated with loss of water vapour during day time by evaporation. As a consequence, left with concentrated salt in the control aquariums, since without macroalgae thalli and exposed to high irradiance from the sunlight that influenced the rate of evaporation.

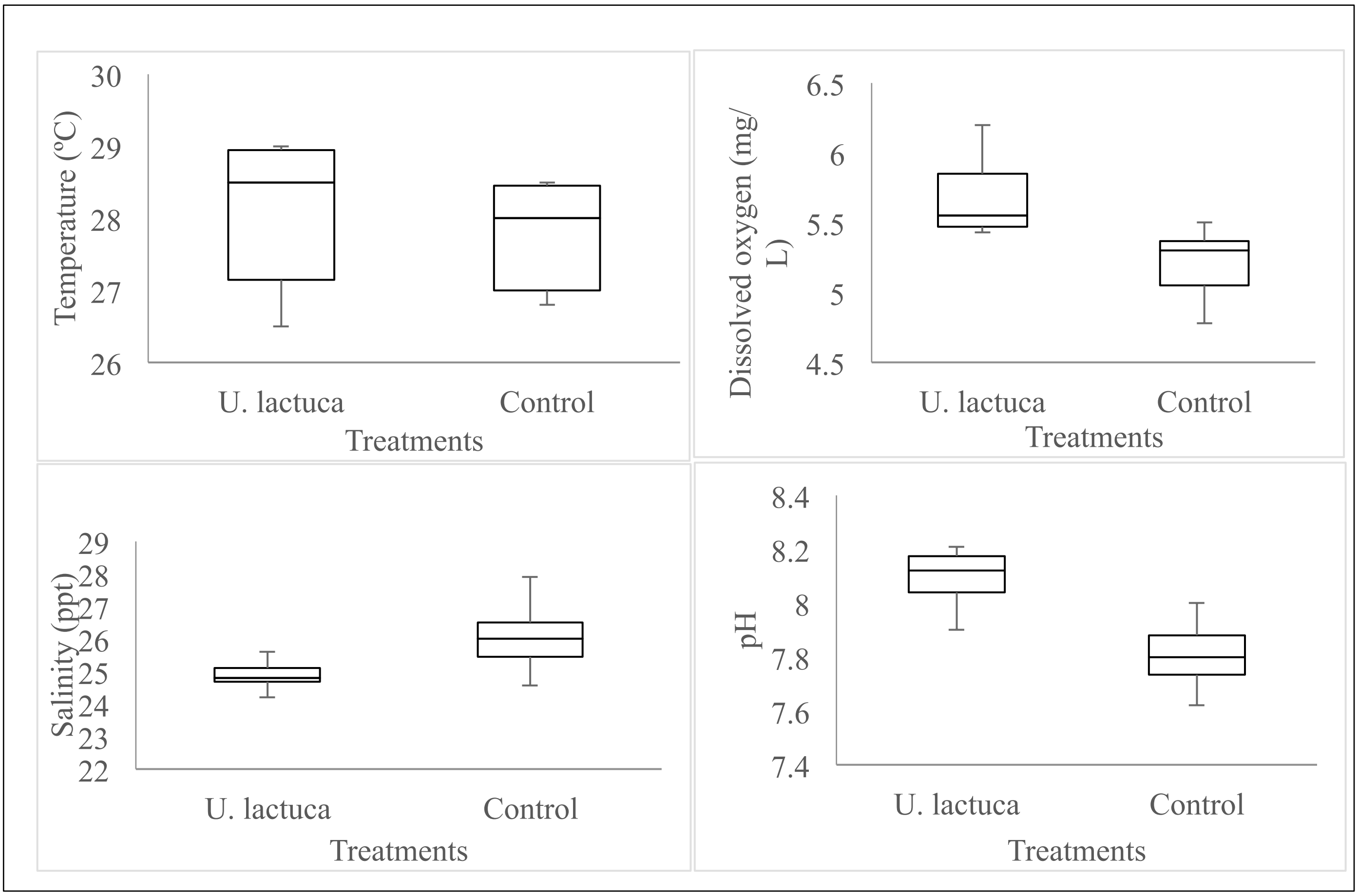

Figure 1: Water physico-chemical parameters in *U. lactuca* and control aquariums in the outdoor recirculating shrimp effluent water during cultivation period.

3.2 Nutrient removal rate

U. lactuca showed high potential for removal of nutrients such as ammonium, nitrate and phosphates (NH_4^+, NO_3^- and PO_4^{3-}) from the shrimp effluent and subsequently improved its water quality. Nutrient removal efficiency (%) in the treatments were calculated based on the difference in nutrient concentration between inflow and outflow. Initial NH_4^+ concentration was in the range of 6.0 to 6.4 mg/L as presented in Figure 2 (a). At the end of fourth week, a significant declined in NH_4^+ concentrations were observed in treatment with

U. lactuca. There were significant differences in ammonium concentration (P<0.05) between treatment and control aquariums. *U. lactuca* exhibited 70% nutrient removal efficiency compared to control, without macroalgae, that has shown only 15% of nutrient removal. However, this value had not shown significant (P>0.05) removal compared to the aquarium with *U. lactuca*. Control aquarium has shown ammonium removal as much as 15%, compared to removal of nitrate (-10%) and phosphate (-20%) which has increased in the final concentration and presented negative value for nutrient removal efficiency. The reduction in ammonium at control aquariums probably driven by microbial and phytoplankton activity that assimilates the ammonium for their cell metabolism and growth, and volatilization of some amount of ammonia to the external environment.

Higher initial phosphate concentration was illustrated in Figure 2 (b), in the range of 10.5 to 11.5 mg/L. Size of shrimp at 10.0 ± 1.2 g weight, generated high phosphate concentration in water. *U. lactuca* recorded remarkable phosphate removal efficiency of 90% compared to control treatment which showed a negative value (P<0.05). Control treatment without macroalgae has shown rapid increased in phosphate since shrimp excretion increases in the water and no macroalgae in this tank to serve as biofilter. Thereby, this aquarium has the lowest phosphate removal of -26%. In addition, Figure 2 (c) exhibits nitrate removal by *U. lactuca* with initial concentration ranged was 3.5 ± 0.5 mg/L in all the treatments. High removal efficiency achieved by macrolagae aquariums presented 42% removal in the continuous shrimp effluent recirculating system. There was a significant variation in nitrate removal between both treatments (P<0.05). Final concentration of nitrate in control treatment was higher than the initial concentration with value of 3.95 mg/L at -11% removal efficiency.

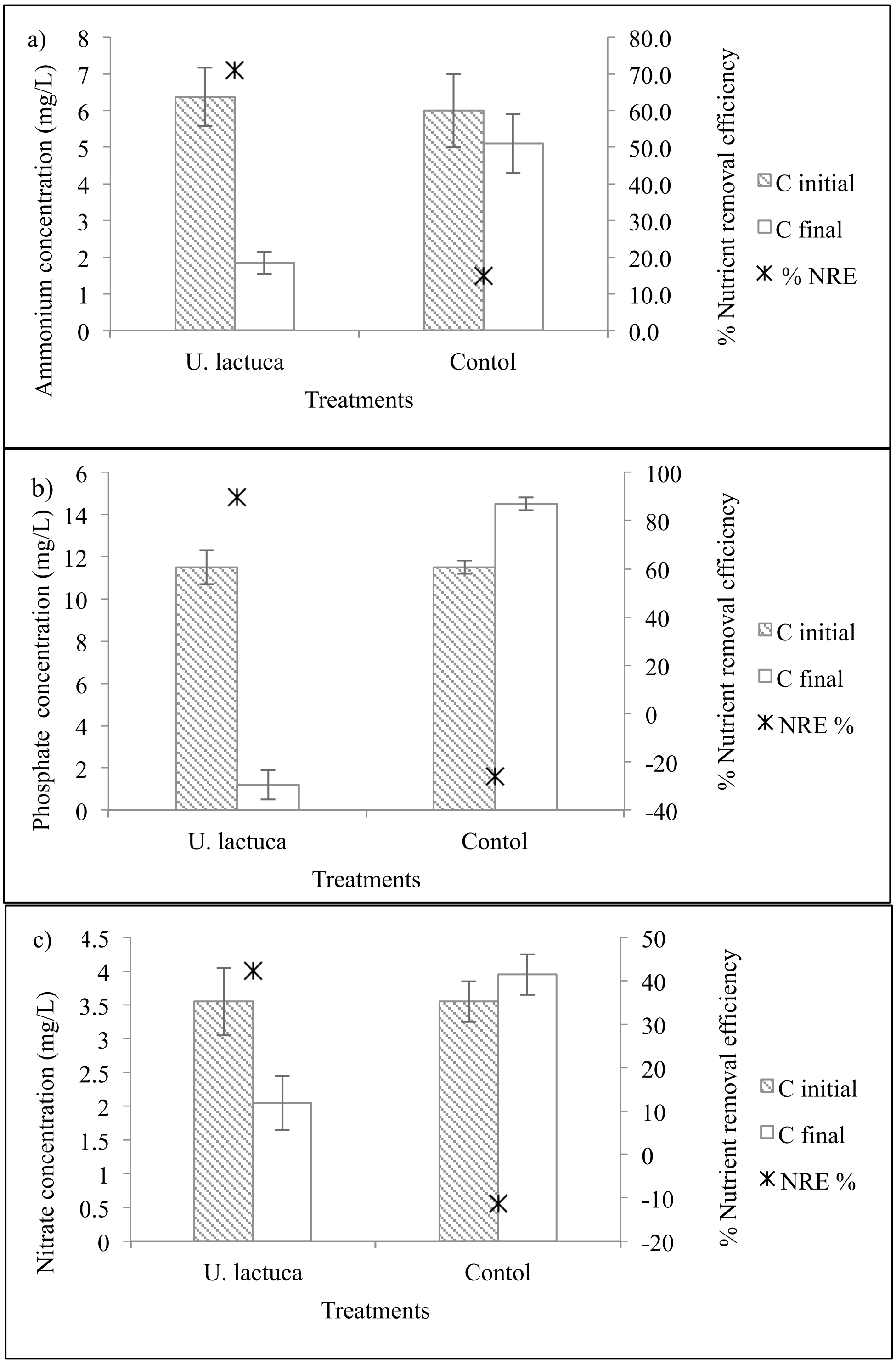

Figure 2: The initial and final nutrient concentrations during treatment period shown as bars. The '*' mark indicates the nutrient removal efficiency in *U. lactuca* and control treatments. (a) Ammonium concentration, (b) phosphates concentration and (c) nitrate concentration.

3.3 Specific growth rate and tissue content

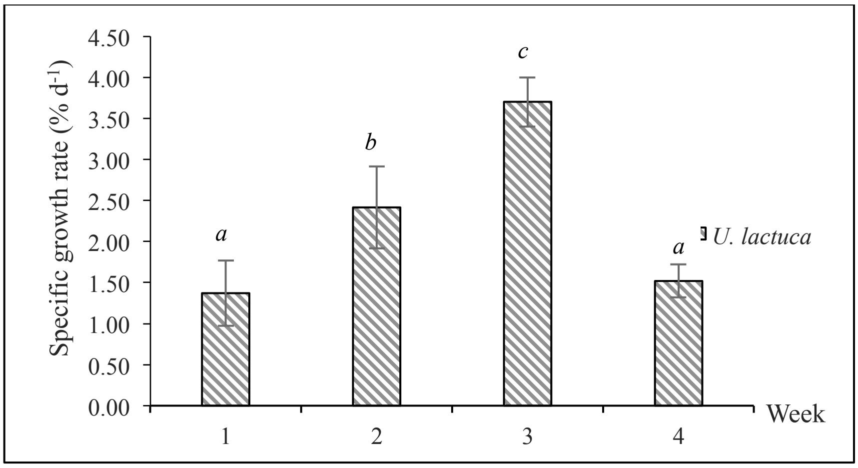

Figure 3: Specific growth rate of *U. lactuca* during the cultivation period integrated with shrimp cultivation. Each data point is the mean of three replicates (means ± SD). Bar marked with the same letters are not significantly different according to Tukey's multiple comparison test (P=0.05).

The mean specific growth rate (SGR) of *U. lactuca* in this experiment was 2.25 ± 0.9 % day^{-1}. The SGR of *U. lactuca* elevated considerably and a peak on the third week with SGR of 3.7% day^{-1} was recorded (Figure 3). Subsequently, the growth rate dropped with almost similar value as that of the first week (1.52% day^{-1}). In addition, SGR of shrimp that was cultivated in the aquariums were also determined. The initial biomass of shrimp used were larger in size with mean weight of 10.0 ± 1.2 g and mean length of 11.0 ± 0.5 cm. Shrimp biomass in macroalgae aquariums had gained about 25% more weight during the completion of experiment compared to shrimp in control aquariums that had a net gained of only 11%. Furthermore, at individual aquariums with integration of *U. lactuca,* the SGR of shrimp was 0.76 ± 0.09 % day^{-1} and 0.32 ± 0.04 % day^{-1} in control. There was no large variation was observed in the SGR of shrimp between aquariums with macroalgae (F=4.7, P>0.05). While, shrimp in control aquariums, without macroalgae had significantly differed in shrimp growth to that of integrated with macroalgae (One way-ANOVA:F=89.4, P<0.001).

4. DISCUSSION

4.1 Water quality and nutrient removal rate efficiency

The tropical climate temperature is favourable for the growth of *U. lactuca* and the range recorded in this study is suitable for growth with year round production. A stable DO and pH achieved in macroalgae aquariums, supported by the photosynthesis reaction that occurred during day time by sequestering the sunlight energy and utilizes the ambient carbon dioxide, significantly affects and maintain these values. It is recommended to maintain an optimum DO level of more than 4 mg/L for *P. monodon* cultivation, otherwise it will be lethal for shrimps. DO amount below this value will drastically effect shrimp growth and survival. Boyd (2003) discussed that in intensive pond, sufficient mechanical aeration is provided to prevent critical level of DO concentration and also to promote nitrification and other aerobic process for water purification purpose. Environmental parameters are crucial criteria to maintain the water quality for higher production of shrimps and survival rate.

Nutrient removal efficiency (NRE, %) is defined as the percentage reduction in a particular nutrient from the initial concentration that is found in the water medium. It was explicitly shown that *U. lactuca* had high preferences for phosphate and ammonium compared to nitrate. Of the two N sources, ammonium absorption occurred more rapidly as a result of immediate incorporation into the amino acid pool of the tissues and the ammonium supply was ample for the growth of macroalgae compared to nitrate. The preference of macroalgae for ammonium rather than to nitrate is common and takes place as it is in reduced form and readily available without the expenditure of energy (Naldi and Wheeler, 2002; Runcie, Ritchie and Larkumb, 2003). However, there was a significant reduction for nitrate also and indicated that both ammonium and nitrate are assimilated by the macrolagae simultaneously, not creating a competitive environment for both N source nor produce an inhibitory effect. *U. lactuca* has demonstrated good biofiltration efficiency for all the nutrients in this study with significant nutrient uptake rates, which presents its characteristics for assimilation for N and P in enhancing the growth rate and for cell metabolism. The N and P sources are known as important limiting nutrients that affects the algal growth.

This study also produced a remarkable findings for high phosphate reduction of 90% by *U. lactuc*a in a tank scale of continuous shrimp effluent recirculating system. There are several studies on phosphate removal, such as exemplified in a laboratory study for phosphate assimilation using fish effluent with high removal efficiency in *U. rotundata* (99.6%) after 7 hour of incubation. This study has shown that net uptake of phosphate is affected by high flow rate with 2 volumes d^{-1} turnover rate (Martínez-Aragón, Hernández, Pérez-Lloréns, Vázquez and Vergara, 2002). *U. lactuca* has applied in a continuous-flow system as a tertiary treatment of a sewage treatment plant to reduce risk of eutrophication in which the phosphate was reduced by 50% (Tsagkamilis, Danielidis, Dring and Katsaros, 2010).. The green pigment, chlorophyll-a, of this species possibly require a high phosphate concentration as vital energy source used in the form of ATP (adenosine triphosphosphate) during photosynthesis reaction by the sunlight energy entrapped during this reaction to synthesis organic compounds.

In addition, the higher nutrient removal rate by *U. lactuca* was associated with high growth rate. *Ulva* spp. is an ubiquitous species that thrives naturally with substantial affinity to high nitrogen sources, for instance, this genus possesses good assimilation capacity for ammonium and phosphate as proven in several research reports (Hernandez, Fernandez-Engo, Perez-Llorens and Vergara, 2005; Msuya, Kyewalyanga and Salum, 2006; Amir Neori*, et al.*, 2003). *U. lactuca* appears to grow rapidly by assimilating N and P sources form shrimp effluent. *U. lactuca* can be repeatedly harvested from the cultivation system when it has reached its maximum capacity for biofiltration.

4.2 Potential of *Ulva lactuca* as biofilter in IMTA system

The selection of *U. lactuca* as a potential biofilter candidate has been accompanied with several superior characteristics. *U. lactuca* is an ubiquitous species that grows rapidly, assimilates and metabolizes nitrogen quickly, resistant to environmental stresses, and possess anti-epiphytes properties (Gil, Torres and Esteves, 2005; A. Neori, Cohen and Gordin, 1991; Tsagkamilis*, et al.*, 2010; Vandermeulen and Gordin, 1990). *Ulva* species has been tested for assimilation of nutrients from nutrient-rich water and reduces algae bloom phenomena (Fei, 2004; Tsagkamilis*, et al.*, 2010; Yang*, et al.*, 2006; Zhou*, et al.*, 2006). Microalgae has a high growth rate and multiplies rapidly compared to macroalgae. However, rapid blooming of microalgae results in harmful algae bloom (HABs) that eventually causes dissolved oxygen depletion and affect living aquatic life. Furthermore, macraolgae biomass are easy to control and harvest could be performed with less cumbersome, labour save and produces valuable by-products. Integrated multi-trophic aquaculture (IMTA) allows a system to virtually use much of the nutrients reducing the production of waste. Thus, *U. lactuca* is the most promising species as biofilter and integration in shrimp effluent is considered as an efficient and sustainable treatment solution. There has been less research efforts on physiology and ecology study for *Ulva* species and a profound study provides information about the potential application of this species as biofilter. With respect to the aims of present study, this has been fulfilled and the potential use is confirmed in IMTA system.

5. CONCLUSIONS

The present study demonstrated that ammonium, phosphates and nitrates in effluent released by shrimp significantly reduced by *U. lactuca*. This confirmed that this species has remarkable abilities for removing nutrients from shrimp effluent and effectively function as a biofilter. This macroalgae also has considerable economic potential and it would be advantageous to use it in IMTA systems. The scaling up of shrimp effluent biofiltration efficiency in IMTA system with *U. lactuca* produces an efficient treatment system with higher biomass production accompanied with improved water quality that can be reused and safe for shrimp cultivation in the recirculating system. Massive production of *U. lactuca* in the biofiltration system that has high protein content serves as natural feed materials and greatly contributes to economic development. This ensures long-term sustainability of the industry, besides enhancing shrimp and *U. lactuca* productivity.

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