

Assessment of Nutrients in Seaweed Tank from Land Based Integrated Multitrophic **Aquaculture Module**

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Abstract

Aquaculture industry is often generating waste that negatively impact to the environment. These wastes are rich in nutrients. Seaweed can utilize these waste nutrients. This experiment was conducted in a flow-through system (FTS) and a recirculation system (RS) in land-based integrated multi-trophic aquaculture module using seaweed Kappaphycus alvarezii as one of the components. The aim was to access the efficiency of the removal of nutrients in the integrated culture. Kappaphycus alvarezii was stocked at the density of 1 kg/tank in the rectangular plastic tank with 500 L of seawater. The waste generated from integrated multi-trophic aquaculture tanks was directed towards K. alvarezii culture tank. Water samples from inlet, outlet and inside of the culture tanks were drawn to determine the nutrients, namely, total nitrogen (mg/L) and total phosphorus (mg/L). Total nitrogen and total phosphorus in the seaweed sample and from the sediment of culture tanks were also analyzed. The total nitrogen amounting to 59.5% and 61.6% nitrogen was taken up by K. alvarezii in FTS and RS culture tank, respectively. The phosphorus showed the highest deposition of 61.1% and 31.6% in the sediment of in FTS and RS culture tanks respectively, whereas only 5.5% and 3.4% of phosphorus were taken up by K. alvarezii from FTS and RS culture tanks, respectively. The percentage of nitrogen remained in water was comparatively higher by 14.2% and 27.5% than phosphorus by 8.3% and 23.0% in water of both FTS and RS culture tanks, respectively. These results indicated that this species seaweed is efficient in the removal of nitrogen from both FTS and RS culture tank.

Keywords

Kappaphycus alvarezii, Nutrients Removal, Flow-Through System and **Recirculation System**

1. Introduction

The waste generated from aquaculture farm, although much smaller compare to that generated by the agricultural sector has contributed to environment degradation. In an intensive or semi-intensive system aquaculture species such as fish or shrimp assimilate only 23% - 31% of nitrogen and 10% - 13% of phosphorus from given feed, while the remaining 14% - 53% of nitrogen and 39% - 67% of phosphorus are execrated. These remain in water column or are deposited in sediment [1]. Both these nutrients are common effluents from aquaculture that occur as in particulate and dissolved forms. In aquaculture effluents, about 7% -32% of total nitrogen (TN) and 30% - 84% of total phosphorus (TP) and up to 27% of total carbon are bound in the particulate fraction and the remainder can be found in dissolved form [2]. The negative impact on the environment due to the release of effluents is related to eutrophication processes, especially in coastal and sheltered areas [3]. One of the best options to utilize these nutrients is to integrate the systems through cascading arrangements. By integrating fed aquaculture, the wastes generated from culture species become a resource for the other trophic level of species [4]. The generation of waste nutrient and their utilization by some stocked species is a sort of synergistic work. Balance of nutrients in culture tank proceeded various process. Nutrient introduced in each of integrated system must be utilized properly towards zero emission. Seaweed can be efficient in removal of dissolved inorganic and organic nutrients from effluent of intensive aquaculture system [4].

The use of seaweed integrated with fish culture has been studied in open water and land-based systems in Israel, Portugal, Brazil, and Indonesia [1] [5]. Seaweed performs better as a nitrogen absorber with ammonium than with nitrate which is excellent in the context of intensive fish aquaculture where most of the nitrogen is released as ammonium [6]. Seaweed (*Ulva lactuca*can) removes 30% of dissolved nutrient load from fishpond [7]. Shrimp (*Penaeus monodon*), mussel (*Mytilus edulis*) and seaweed *Gracillaria* is a good combination and recorded a reduction in the effluent of 72% of total nitrogen and 61% of total phosphorus in integrated system [3].

On the other hand, integrated multi-trophic aquaculture (IMTA) system has been proposed as a means of developing environmentally sound aquaculture practices and resource management through a balanced ecosystem approach. This system employs a combination of high nutrient uptake capacity seaweed and low trophic organisms which are herbivorous or omnivorous [4]. Most of the studies have focused on seaweeds integrated with fish culture [4] [8] [9] [10]. Studies have been conducted on the possibilities of IMTA systems of bivalves and seaweeds [11], but little information is available on the using spiny lobster as fed aquaculture species, sea cucumber as organic extractor and sea weed as the inorganic extractor in IMTA module. Thus this study was undertaken to assess the nutrient budget in the seaweed, *Kappaphycus alvarezii* culture tank in flowthrough and re-circulating integrated multi-trophic aquaculture systems.

2. Methodology

Three 500 L water capacity rectangular tanks were used in this experiment. One tank was stocked with spiny lobster (*Panulirus ornatus*) for fed aquaculture species. The waste from spiny lobster tank was diverted to sea cucumber (*Holothuria scabra*) unit and finally waste of dissolved organic and inorganic nutrients collected in seaweed (*Kappaphycus alvarezii*) culture tank. *Kappaphycus alvarezii* was obtained from the supplier at Kampung Baru, Tuaran, Sabah, Malaysia, and transported to University Malaysia Sabah Shrimp Hatchery. The seaweed was acclimatized in the culture tank before start of the experiment. Stocking density was 1 kg/400 L water. Pieces of 50 g of *Kappaphycus alvarezii* were arranged in a row and tied by using nylon cotton thread in the seaweed tank. Waste comprising of dissolved and particulate organic and inorganic nutrients from spiny lobster (*Panulirus ornatus*) and sea cucumber (*Holothuria scabra*) tanks were used as the source nutrients in seaweed cultivation tank.

The *insitu* water quality parameters were monitored every day morning and evening in *Kappaphycus alvarezii* tank by using digital YSI multi-probe equipment. The parameter includes, dissolved oxygen concentration (mg/L), pH, temperature (°C), water salinity (psu), and light intensity (lux). Water samples were collected at every two week interval from three locations, inflow of water to *Kappaphycus alvarezii*, water inside of culture tank and water sample outflow point of seaweed tank. Three beakers of 500 ml were placed at the bottom of seaweed experimental tank and sediments were collected at the end of experiment. Collected sediments were placed in an oven at 60°C temperature until complete dryness. Samples were powdered and stored in a fridge in sealed plastic bag until analysis. Sample of seaweed was taken before start of the experiment and at the end of the experiment and placed in oven at 60°C temperature for drying. The dried seaweed was grinded into fine powder and stored in a sealed plastic bag. The bags containing samples were stored into a fridge until analysis.

3. Analytical Parameters

Total nitrogen (mg/L) and total phosphorus (mg/L) from the samples of water and sediment were analyses according to standard methods [12] and dried *Kappaphycus alvarezii* were analyzed with standard methods [13]. In brief, total nitrogen in water was determined by persulfate oxidation method followed by the spectrophotometric measurement. The total phosphorus in water sample was converted into soluble phosphorus (Orthophosphate) by digestion with a mixture of sulfuric acid and ammonium per-sulfate. Soluble phosphorus was then measured by the ascorbic acid method. Total nitrogen in the sediment and *Kappaphycus alvarezii* dry samples were digested with strong sulphuric acid and followed by distillation in Kjeltech auto-analyzer. Total phosphorous in sediment and also *Kappaphycus alvarezii* samples were analyzed by acid mixture digestion and spectrophotometric method.

Removal efficiency (RE%) of nutrients was calculated according to formula

described as below [14]:

$$\text{NRE} = \left[\left(M_t - M_o \right) / M_o \right] \times 100$$

where: NRE refer as Nutrient Removal Efficiency, M_o as initial nutrient concentration and M_t as nutrient concentration at time *t*.

4. Results

The values of the *insitu* water quality parameters in the *Kappaphycus alvarezii* experimental tank were observed similar in both flow-through and recirculation systems as shown in **Table 1**. Thus, the influences of environmental parameters on the nutrients flux of this tank were negligible.

4.1. Total Nitrogen Utilization in Kappaphycus alvarezii Tank

Nutrient budget showed that the maximum percentage of total nitrogen was removed by *Kappaphycus alvarezii* in both FTS (59.55%) and RS (61.6%) systems. Total nitrogen remaining the inside tank water of RS (27.5%) system was comparatively higher than the percentage of total nitrogen (14.2%) obtained inside the tank of FTS system. On the other hand, the highest amount of 19.1% total nitrogen was obtained in sediment of FTS system, while only 6.35% of total nitrogen was recorded in sediment of RS system (**Figure 1** and **Figure 2**).

4.2. Total Phosphorus Utilization in Kappaphycus alvarezii Tank

Nutrient budget showed that the maximum of percentage of total phosphorus remained in the sediment and accounted 61.1% and 31.6% in FTS and RS systems,

Table 1. The concentration of dissolved oxygen (mg/l), pH, temperature (°C), salinity (psu) and light intensity (lux) recorded from *Kappaphycus alvarezii* tank in flow-through system (FTS) and recirculation tank system (RS) (Values express as Mean \pm S.D).

Systems	DO (mg/l)	pН	Temperature (°C)	Salinity (psu)	Light intensity (lux)
Flow through	65.6 ± 0.31	8.07 ± 0.04	29.6 ± 0.88	31.2 ± 0.09	16641 ± 47.1
Recirculation	66.1 ± 0.11	8.10 ± 0.04	25.9 ± 0.61	33.2 ± 0.18	16323 ± 48.8



Figure 1. Utilization of total nitrogen (%) in Kappaphycus *al-varezii* tank in a land based IMTA flow through system (FTS).

respectively. The highest percentage in sediment was followed by estimation of total phosphorus in the range of 16.6% and 29.05% outlet point of FTS and RS systems, respectively. The lowest percentage of 5.55% and 3.41% phosphorus were removed by the seaweed in FTS and RS systems, respectively. Certain percentage of total phosphorus remained unaccounted in both FTS and RS systems compared to the total nitrogen these two systems (**Figure 3** and **Figure 4**).



Figure 2. Utilization of total nitrogen (%) in *Kappaphycus alvarezii* tank in a land based IMTA recirculation system (RS).



Figure 3. Utilization of total phosphorus (%) in *Kappaphycus alvarezii* tank in a land based IMTA flow through system.



Figure 4. Utilization of total phosphorus (%) in *Kappaphycus alvarezii* tank in a land based IMTA flow through system.

5. Discussion

The efficacy of total nitrogen removal in FTS and RS system was 59.5% and 61.6%, respectively. The removal efficacy of total nitrogen was observed to be the highest compared to removal of total phosphorus (3.41% - 5.55%) by Kappaphycus alvarezii. Nutrients like nitrogen and phosphorus in aquatic system are directly taken up by macro-algae for their growth. Seaweeds have high rates of productivity and they grow well in water bodies with higher nitrogen and other nutrients. Seaweeds are integrators of bio-available nutrients over time [7]. Among the nutrients Nitrogen is the primary one that limits seaweed growth, thus nitrogen uptake from water can control the growth of macro-algae. Typically, green macro algae take up nutrients faster per unit of biomass than thick thalli, the brown algae [15]. This result shows a better removal efficacy in assimilation of nitrogen excretory products from lobster fed tank by seaweed, Kappaphycus alvarezii in both FTS and RS systems. An estimated 17% nitrogen removal efficiency could be easily achieved, but it will depend on the supply of dissolved inorganic nitrogen. Nitrogen removal efficiency was reported to vary widely (10% - 90%) in poly-culture systems [16]. The removal of total nitrogen from the aquaculture system was found to be 45% when treated with seaweed. Efficient nitrogen uptake even at low substrate concentrations has been reported for Ulva species [17]. Seaweeds show great species-specific variations in the nutrient uptake and their composition, which reflects the influence of environmental factors such as water temperature, salinity, light and availability of nutrients. Not only environmental factors, nutrient removal vary with seaweed species, habitat, maturity and as well as with the various phases of algal growth. Seaweed performs better as nitrogen absorber with ammonium than with nitrate which serves the purpose in intensive fish aquaculture where most of nitrogen is released as ammonium [6]. Tank cultivated *Gracillaria* could remove as much as 90% - 95% of the ammonium in effluent waters released from salmon tanks [18]. Such rapid accumulation by seaweed has been documented to function even in different culture systems [19]. In both FTS and RT systems in this experiment, total nitrogen from the fed-based lobster culture is rapidly taken up by Kappaphycus alvarezii. It has been observed earlier that effluent of abalone (Haliotis tuberculata) culture tank divert into seaweed (Ulva lactuca) culture tank contained nitrogenous waste of which 58% was removed by seaweed [20].

In this experiment efficiently of phosphorus utilization by seaweed *Kappa-phycus alvarezii* was observed lower than that of nitrogen by *Kappaphycus alvarezii*. harvesting accounted for 35.5% and 6.1% of the total nitrogen and total phosphorus input into the ponds in cage poly-culture of white shrimp with seaweed *Kappaphycus alvarezii* [5]. This experiment also confirmed that nutrient availability in culture tank was observed to be controlled by water management system. In water recirculation system nutrient flux was observed to increase of 27.5% and 23.08% in total nitrogen and total phosphorus, respectively compared to flow through system. Thus nitrogen uptake efficiency of seaweed in recircula-

tion tank is slightly higher (61.6%) in comparison with flow through system (59.5%). The effects of water flow and nutrient availability influences the uptake efficiency. By increasing the water flow, nutrient flux will increase as well, which allows a high biomass production of nutrient-sufficient seaweeds. Nitrogen can be efficiently removed from water when in lower concentration and higher flow rate. The high flow rates are required in order to keep the nitrogen concentration at a constant level [21]. If the water flow is low, nutrients become limiting and seaweed biomass production decreases, but the nutrient uptake efficiency is higher [22]. Only the algae tissue did not account for all nitrogen removal from the cultivation media. This phenomenon was also described researcher [20] where the protein content of the harvested algae did not fully account for the total nitrogen removed from the system, but this was not observed in this experiment. The specific role of alternative pathways for nitrogen removal was also not studied in this experiment. Higher concentrations of nitrogen and phosphorus will be deposited in the tissue [23] but the excess nutrients in water do not necessarily lead to their high concentrations in tissues, even luxuriant consumption phenomena observed in seaweed [24]. In addition to that, the nutrient uptake efficiency sometimes was observed low and nutrient concentration remains high in the effluents. Nutrient uptake or removal efficiency depends on various physical parameters such as light, temperature, pH and salinity. The water temperature in the flow-through system remained fairly constant during the day because of the high flow rate through the fish culture and the algal tanks [25]. The greatest production for macro algae was found in pH values of 8.0 - 8.2 [26]. The salinity of 30% - 33% was suitable to the highest specific growth rate of 31% [27]. However, nutrient availability was higher in the flow-through culture, resulting in greater total nutrient uptake and thereby more growth, as indicated by the greater final biomass in these cultures. The removal efficiency for total phosphorus 36% - 50% in the flow-through system which was comparable with other experiments in which Kappaphycus alvarezii (27%) and Ulva reticulate (33%) have been used as biofilters in fish effluents [28].

The concentrations of nutrients in water depend on the vertical distribution of the macro-algae in the water column. Most of the nutrients regenerated were accumulated in the sediments. In fact, macro algae grew by taking up nutrients from the water and simultaneously decayed at the surface sediments. The sinks for total nitrogen in sediments of FTS and RS in present experiment were recorded 19.1% and 6.35%, respectively. Negligible percentages of total nitrogen (0.05% to 2.25%) remained unaccounted. Approximately 30% of the nitrogen unaccounted in intensive shrimp culture is assumed to be the nitrogen to the atmosphere as nitrogen or ammonia [29]. The highest percentage of total phosphorus (61.1% in FTS and 31.62% in RS) was observed in sediment compared to total nitrogen. It can be assumed that this amount is lost through consumption by microbes or fixed in minerals in sediment, although this was not taken into consideration in this study. The nitrogen and phosphorus in the surface sediment were normally had higher values than other layers because some soluble or granular matter containing nitrogen and phosphorus could be deposited on the sediment through flocculation, adsorption, sedimentation and so on [30]. Decrease in the concentration of total nitrogen and total phosphorus in water indicated that the significant amounts of nitrogen and phosphorus may be released from the sediment to form a new equilibrium between water and the sediment based on specific concentration gradients. The released nutrients will be absorbed by macro-algae. In intensive marine shrimp culture pond total phosphorus of 84% was retained in the sediments [29]. Major sources of nutrients in sediment are detritus and excreted product of feed species. In addition, some bacteria degrade the organic detritus in seaweed tank and release dissolved inorganic nutrients to the water. In well manage condition, 21% of nitrogen and 53% of P into the culture system accumulate in the bottom sediments [31].

Total phosphorus of out let water in this experiment was accounted of 16.6% and 29.1% in FTS and RS, respectively. This was discharged to environment in FTS or recycled back in RS. The marked increase in net phosphorus concentration in the water environments is considered to be a result of macro algal decomposition. In this experiment it is unclear what might have caused this increase in phosphorus in both systems. It is surprising that phosphorus was not efficiently removed from the water and 25% of it remained in water for incorporation into phosphorus-demanding macro algae [30]. On the other hand 8.35% and 12.8% of TP remain unaccounted in FTS and RS respectively. In Intensive culture 42.8% of total phosphorus input for the monoculture and 43.4% for the poly-culture system were unaccounted [8]. Effluent water constituted 10% of TP loss in the budget and this is mainly bound in the suspended solid fraction. Thus, trapping of the suspended solid fraction is important to minimize its environmental impact [29].

6. Conclusion

Seaweed (*Kappaphycus alvarezii*) in land-based IMTA culture tank has potential to be used in order to improve nutrient utilization, especially total nitrogen and also to decrease nutrient discharge to the environment. It is necessary to study the other species combinations in IMTA to enhance the systems' nutrient assimilation performance.

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