

# Use of Coral Rubble, Aquamat™ and Aquaponic Biofiltration in the Recirculating System of a Marine Fish Hatchery

---

A. Estim<sup>1\*</sup> and S. Mustafa<sup>1</sup>

<sup>1</sup>Borneo Marine Research Institute  
Universiti Malaysia Sabah  
Jalan UMS, 88400 Kota Kinabalu  
Sabah, Malaysia

\*Corresponding Author: bentin@ums.edu.my

Keywords: Aquaponic, biofilter, coral rubble, marine fish hatchery, water quality, *Eucheuma spp.*

## ABSTRACT

---

A preliminary study on the effect of combination biofilters, including coral rubble, geotextile Aquamat™ (Meridian Aquatic Technology, Silver Spring, MD, USA), and algal aquaponics in a marine fish recirculating system was investigated. Aquamat™ is an innovative product fabricated from highly specialized synthetic polymer substrates. Aquamat™ forms a complex three-dimensional structure that resembles seagrass in appearance, and has been used to support high stocking densities in fish culture ponds and enhance biological processes. In addition, coral rubble was used, and two seaweed species, *Eucheuma spinosum* and *E. cottonii*, were evaluated for their usefulness as aquaponic biofilters in a recirculating system. Results showed that the four different biofilters operating within the recirculating system were significantly different ( $P < 0.05$ ) in  $\text{NH}_3\text{-N}$  and  $\text{NO}_3\text{-N}$  concentrations. The lowest mean  $\text{NH}_3\text{-N}$  concentration was recorded in the recirculating tank using Aquamat™ + seaweed + coral rubble, while the highest mean  $\text{NO}_3\text{-N}$  concentration was recorded in the recirculating tank using Aquamat™ + coral rubble. Fish weight gain and survival rates were not significantly different ( $p < 0.05$ ) in the four recirculating systems. In the second experiment, three varieties

---

*International Journal of Recirculating Aquaculture 11 (2010) 19-36. All Rights Reserved, © Copyright 2010 by Virginia Tech, Blacksburg, VA USA*

of *Eucheuma* spp. grew poorly, and produced no noticeable effects on  $\text{NH}_3\text{-N}$ ,  $\text{NO}_2\text{-N}$  and  $\text{NO}_3\text{-N}$  concentrations. *Eucheuma cottonii* decayed in the early days, while the two varieties of *E. spinosum* decayed after 35 days. Once decayed, water quality impairment followed. This study concluded that *Eucheuma* species were not suitable as a method of biofiltration in a recirculating culture system. While these seaweeds do remediate water quality, they themselves require a good environment to perform this role. When conditions are not optimal for the stocked organisms, the co-culture system can produce negative results. Follow-up investigation is needed to determine the suitability of such integrated aquatic systems for a large-scale fish production in recirculation systems.

## INTRODUCTION

---

In recent years, there has been growing concern over the impact of aquaculture, especially the nutrient-rich wastewaters discharged from fish holding facilities into the environment. Scientific interest in nutrient pollution from aquaculture facilities has increased markedly since the 1980s (Camargo and Alonso 2006). It is estimated that 52-95% of the nitrogen, 85% of the phosphorus, 80-88% of the carbon and 60% of the total feed input in aquaculture ends up as particulate matter, dissolved chemicals or gasses (Wu 1995). Aquaculture has increasingly been viewed as environmentally detrimental (Naylor et al. 2000). Gutierrez-Wing and Malone (2006) explained that recirculating systems have been identified as one of the two main research areas in aquaculture that address this problem. These kinds of systems are gaining wider acceptance because of their ability to reduce waste discharge, improve water quality control and reduce cost of production.

The processes crucial to the treatment of water in recirculating systems are solids capture, biofiltration, aeration, degassification, and ion balance. There are many alternative technologies available for each of these processes. There is a great potential to realize significant cost reductions depending on the development of designs that integrate two or more of these processes (Losordo et al. 1999). The selection of a particular technology depends upon the species being reared, production site infrastructure, production management expertise, and other factors. In a recirculating system, the three most common types of water purification treatments include earthen ponds (sedimentation), a combination of

solids removal and nitrification, and a combination of solids removal and macrophyte-based nutrient removal (Van Rijn 1996). The combined culture of marine algae and animals has been tested in China and Taiwan (Qian et al. 1996), as well as Israel (Shpigel and Neori 2007). These systems are based on the concept that algae actively uptake CO<sub>2</sub>, release O<sub>2</sub> to the surrounding environment, and utilize the nutrients in metabolic waste originating from the stocked fish.

In this study, a combination of biofilters, including geotextile (Aquamat™), aquaponic algae, and coral rubble were incorporated into a marine fish recirculating system, and evaluated for their effectiveness (Estim et al. 2009, Estim and Mustafa 2010). Aquamat™ is a new and innovative product fabricated from highly-specialized synthetic polymer substrates. It forms a complex three-dimensional structure that resembles seagrass in appearance. This product has been principally used to support high stocking densities in fish culture ponds (Scott and McNeil 2001) and enhance biological processes that reduce ammonia concentrations (Bratvold and Browdy 2001, Estim et al. 2009). Additionally, two seaweed species, *Eucheuma spinosum* and *E. cottonii* (also known as *Kappaphycus alvarezii*) were tested as aquaponic biofilters in a recirculating system. These seaweed species are already cultured in the coastal areas of Sabah, Indonesia and the Philippines for their carrageenan contents, and were therefore easily available for integration with the fish aquaculture system. The objectives of this study were a) to compare dissolved inorganic nitrogen concentrations, fish weight gain, growth rates and survival rates in the four different recirculating systems and b) to measure the growth rate and biomass yield of three different seaweed varieties in a fish recirculating system.

Several studies have reported enhanced growth rates of seaweed and animals in integrated culture (Qian et al. 1996, Troell et al. 1999, Shpigel and Neori 2007). Schuenhoff et al. (2006) further elaborated that enhanced growth rates are achievable by integrated recirculating mariculture systems, which capture excess nutrients, making it possible to diversify the final products, provide a more efficient use of resources, and increase the income from the system while reducing operating costs.

## MATERIALS AND METHODS

### Aquamat™, Aquaponic Algae and Coral Rubble in Recirculating Systems

Twelve rectangular fiberglass tanks (0.5 x 0.55 x 0.5 m) were selected for the experiment. Each tank was equipped with a rectangular polyethylene bucket (0.2 x 0.15 x 0.1 m), which contained coral rubble (CR) in sizes ranging from 1.0 – 2.5 cm in diameter (Figure 1). Four combinations of recirculating biofilter systems were prepared in triplicate sets. The four types were as follows: CR + Aquamat™ (Aq), CR + Seaweed (Swd), CR + Aq + Swd, and CR alone (Control). Each of the recirculating systems was stocked with 55 juveniles of *Lates calcarifer*, (MW =  $1.06 \pm 0.41$  g) also known as barramundi. The water flow rate averaged  $0.05 \pm 0.01$  L/sec in each recirculating tank. A series of intensive samplings of dissolved inorganic nitrogen ( $\text{NH}_3\text{-N}$ ,  $\text{NO}_2\text{-N}$  and  $\text{NO}_3\text{-N}$ ) and *in situ* water quality (temperature, dissolved oxygen, pH, salinity, oxidation reduction potential (ORP) and conductivity) were carried out every four hours for 36 hours. After that, the sampling was repeated once daily (between 0900-1000 h) for one week.

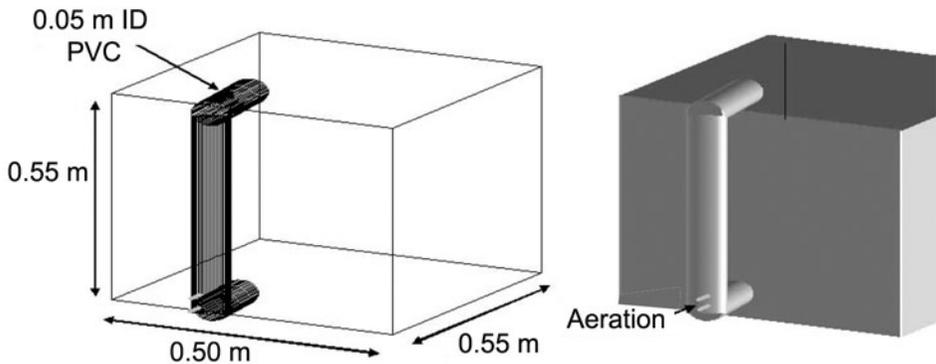


Figure 1. Layout of the recirculating tank in Experiment 1.

### Three Different Varieties of Seaweeds in Recirculating Systems

The second experiment was conducted over 56 days in duplicate recirculating systems with and without seaweed (Figure 2). Each recirculating system consisted of one circular tank (1000 L) and two rectangular fiberglass tanks (100 L). In the circular tank, Aquamat™ (with surface area of  $31.28 \text{ m}^2$ ) was installed and stocked with 150 *L. calcarifer* (mean weight =  $0.94 \pm 0.24$  g). In the first 100 L rectangular tank, eight kg CR was added. The other 100 L rectangular tank was

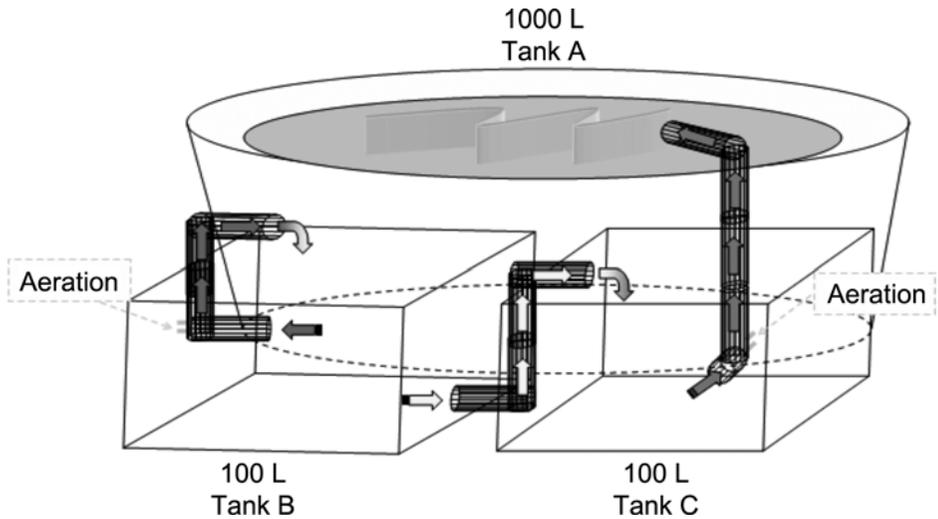


Figure 2. Layout of the recirculating systems of CR+Aquamat™ in Experiment 2.

planted with three varieties of seaweeds (Figure 3). The three different seaweeds were *Eucheuma cottonii* and two varieties of *Eucheuma spinosum* (brown and green varieties). Each seaweed cutting had an initial mean weight of  $20.13 \pm 6.55$  g for *E. cottonii*,  $18.07 \pm 2.60$  g for brown *E. spinosum* and  $18.52 \pm 2.96$  g for the green *E. spinosum*. A water flow rate of  $0.16 \pm 0.04$  L/sec was maintained in each recirculating system.

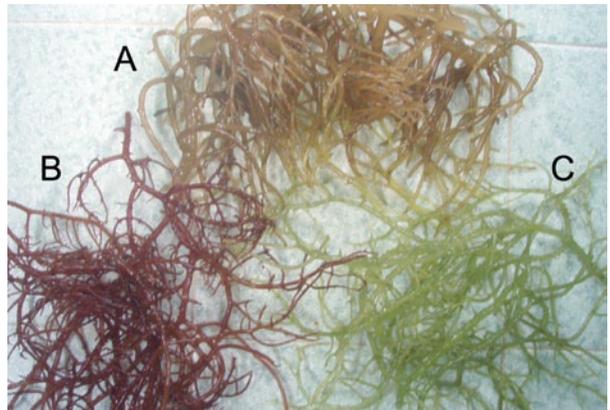


Figure 3. Three varieties of seaweeds. *E. cottonii* (A – light brown) *E. spinosum* (B – dark red) and *E. spinosum* (C – green).

The seaweed samples were collected from a seaweed farm in Bangi Island, North Borneo ( $7^{\circ}06'46.60''$  N;  $117^{\circ}05'57.17''$  E) and transported in a styrofoam box as described by Mysua and Neori (2002). In each treatment tank, a pre-weighed seaweed biomass was stocked to the initial density for the study. Seaweed was harvested every seven days, drained to eliminate the superficial water then weighed using a digital balance. Specific seaweed growth rates (SSGR) were calculated as

### Three Biofiltration Options in a Marine RAS

SSGR=[(Ln  $W_t$  – Ln  $W_o$ )/ $t$ ] x 100], where  $W_o$  is the initial weight or initial biomass, and  $W_t$  is the biomass at  $t$  culture days. The biomass yield (fresh weight) was calculated as the difference between the initial and the final weights and expressed in units of g/m<sup>2</sup>/day, based on the areas of the culture tanks. The seaweed weight gain (SWG) was determined as SWG=[[( $W_f$  –  $W_i$ )/ $W_i$ ] x 100], where  $W_i$  and  $W_f$  are the initial and the final weight or wet biomass, respectively.

### Water Quality

Dissolved inorganic nitrogen concentrations were analyzed using colorimetric methods as described by Parsons et al. (1984). The *in situ* water quality parameters [pH, temperature, oxidation reduction potential (ORP), conductivity and salinity] were monitored using a Cyberscan™ data logger (Eutech/Thermo Fisher Scientific, Ayer Rajah Crescent, Singapore). In the intensive experiment, seawater samples were collected every four hours initially, but later once a day between 0900-1000 for a week. Each time, after the seawater samples were collected from the recirculating tank, new seawater was added to maintain the volume and flow rate in each of the recirculating tanks. For the experiment involving the three varieties of seaweeds, water samples were collected from each tank every two days between 0900 and 1000 h. All seawater samples were filtered through GF/C Whatman filters (Whatman PLC, Maidstone, UK) with pore size of 0.45 µm. The light intensity in the culture set-up was measured with a digital light meter (TENMA® model 72-6693, Premier Farnell PLC, Bristol, UK) and was between 10.89 and 22.74 µmol/m<sup>2</sup>/sec on cloudy days; and 35.21 to 68.06 µmol/m<sup>2</sup>/sec on sunny days. Fish weight gain, specific growth rate and survival rate were determined.

### Data Analysis

All data were analyzed by ANOVA to determine the statistical significance of the different treatments. All the tests were conducted after the confirmation of homogeneity of variance (Levene's test). To satisfy the assumptions of normality and homogeneity of variance, data of dissolved inorganic nutrient concentrations were transformed by Ln ( $NH_3$ -N and  $NO_2$ -N), Cos ( $NO_3$ -N) and Log<sub>10</sub> for the DO concentrations prior to the statistical analysis. Multiple post-hoc comparisons among mean values were tested by Duncan test. In all cases, the null hypotheses were rejected at the five percent significance level.

## RESULTS

### Aquamat™, Aquaponic Algae, and Coral Rubble in Recirculating Systems

The four recirculating systems were not significantly different ( $P>0.05$ ) in seawater temperature, DO, pH, salinity, ORP, and conductivity levels. Water temperature ranged from  $25.99 \pm 0.82$  to  $26.05 \pm 0.82$  °C, DO ranged from  $5.64 \pm 0.37$  to  $5.95 \pm 0.24$  mg/L, pH ranged from  $8.06 \pm 0.09$  to  $8.11 \pm 0.05$ , salinity ranged from  $31.14 \pm 2.24$  to  $31.71 \pm 0.45$  ppt, ORP ranged from  $41.4 \pm 6.8$  to  $43.6 \pm 6.7$  mV, and conductivity ranged from  $48.57 \pm 0.55$  to  $48.63 \pm 0.60$   $\mu$ S/cm (Table 1).

Changes in  $\text{NH}_3\text{-N}$ ,  $\text{NO}_2\text{-N}$  and  $\text{NO}_3\text{-N}$  concentrations in the four recirculating tanks during the experiment are shown in Figure 4 and Figure 5. The variance analysis showed that the four recirculating tanks had significantly different ( $p<0.05$ ) values of  $\text{NH}_3\text{-N}$  and  $\text{NO}_3\text{-N}$  concentrations, but no significant difference in  $\text{NO}_2\text{-N}$  concentration (Table 1). The mean  $\text{NH}_3\text{-N}$  concentrations were  $0.85 \pm 0.76$  mg/L in the CR tank,  $0.72 \pm 0.71$  mg/L in the Swd + CR tank,  $0.35 \pm 0.23$  mg/L in the Aq + Swd + CR tank, and  $0.31 \pm 0.20$  mg/L in the Aq + CR tank. The mean  $\text{NO}_3\text{-N}$  concentrations were  $10.24 \pm 4.22$  mg/L in the Aq + CR tank,  $5.06 \pm 3.76$  mg/L in the Aq + Swd + CR tank,  $3.79 \pm 2.58$  mg/L in the CR tank and  $2.45 \pm 1.22$  mg/L in the Swd + CR tank. The mean  $\text{NO}_2\text{-N}$  concentrations ranged from  $0.20 \pm 0.04$  mg/L to  $0.80 \pm 0.21$  mg/L in the four recirculating tanks (Table 1).

*Table 1. Means ( $\pm$ SD) of in situ water quality,  $\text{NH}_3\text{-N}$ ,  $\text{NO}_2\text{-N}$  and  $\text{NO}_3\text{-N}$  concentrations in the four recirculating systems.*

	n	Control (CR)	Aq + CR	Swd + CR	Aq + Swd + CR
Temperature (°C)	39	$25.99 \pm 0.82$	$26.03 \pm 0.85$	$26.05 \pm 0.82$	$26.04 \pm 0.82$
DO (mg/L)	39	$5.95 \pm 0.24$	$5.64 \pm 0.37$	$5.66 \pm 0.24$	$5.71 \pm 0.29$
pH	39	$8.11 \pm 0.05$	$8.07 \pm 0.09$	$8.08 \pm 0.07$	$8.06 \pm 0.09$
Salinity (ppt)	39	$31.7 \pm 0.4$	$31.1 \pm 2.2$	$31.7 \pm 0.4$	$31.4 \pm 1.6$
ORP (mV)	39	$41.4 \pm 6.8$	$42.2 \pm 6.7$	$43.2 \pm 6.6$	$43.6 \pm 6.7$
Conductivity (uS/cm)	39	$48.63 \pm 0.60$	$48.58 \pm 0.57$	$48.57 \pm 0.55$	$48.59 \pm 0.56$
$\text{NH}_3\text{-N}$ (mg/L)	39	$0.85 \pm 0.76$ a	$0.31 \pm 0.20$ c	$0.72 \pm 0.71$ ab	$0.35 \pm 0.23$ bc
$\text{NO}_2\text{-N}$ (ug/L)	39	$0.80 \pm 0.21$	$0.55 \pm 0.15$	$0.20 \pm 0.04$	$0.32 \pm 0.10$
$\text{NO}_3\text{-N}$ (mg/L)	39	$3.79 \pm 2.58$ ab	$10.24 \pm 4.22$ c	$2.45 \pm 1.22$ a	$5.06 \pm 3.76$ b

Values with different superscripts within row are significantly different ( $P<0.05$ )

### Three Biofiltration Options in a Marine RAS

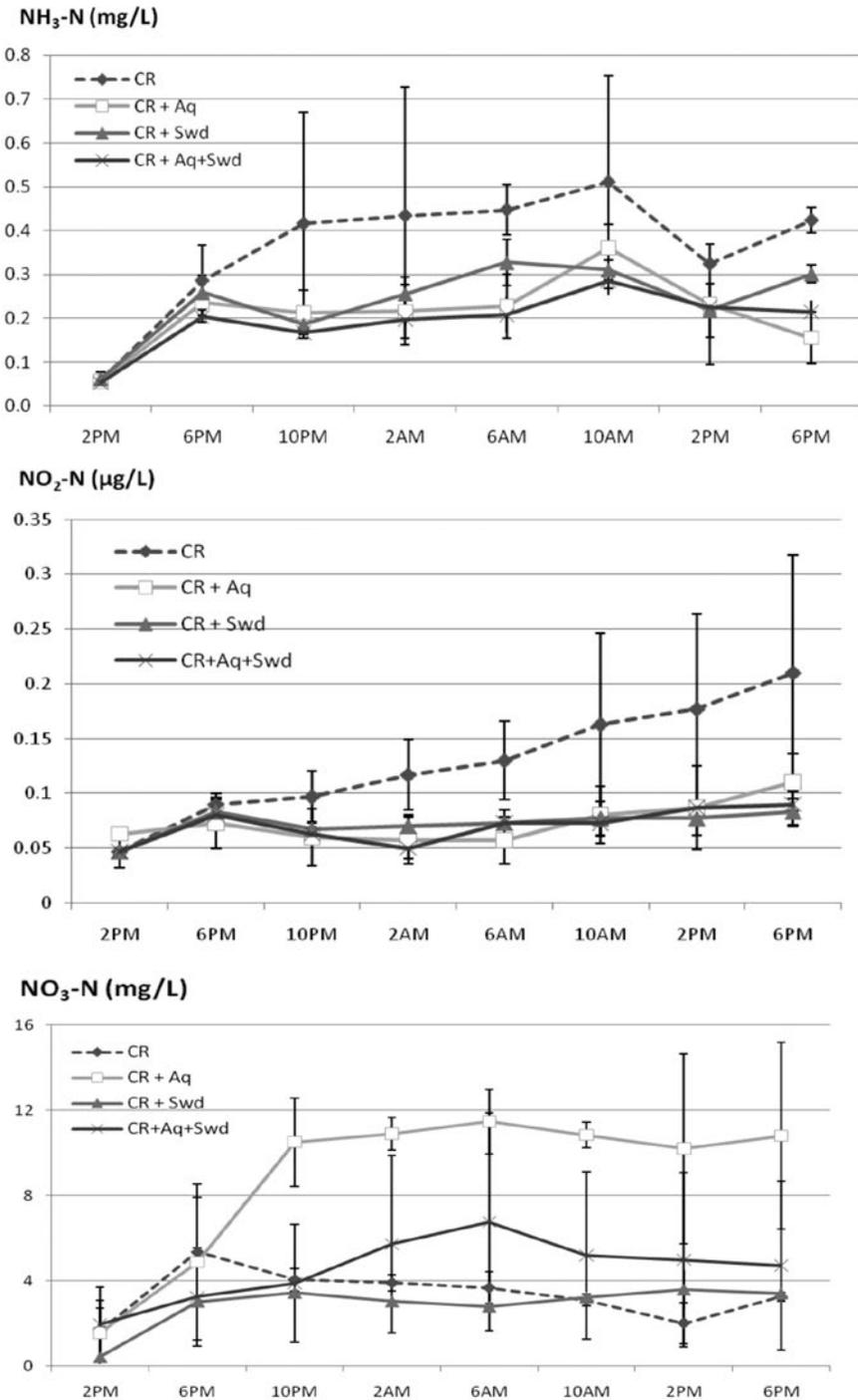


Figure 4. Changes (hours) in NH<sub>3</sub>-N, NO<sub>2</sub>-N and NO<sub>3</sub>-N concentrations (mean ± SD) in the four recirculating tanks.

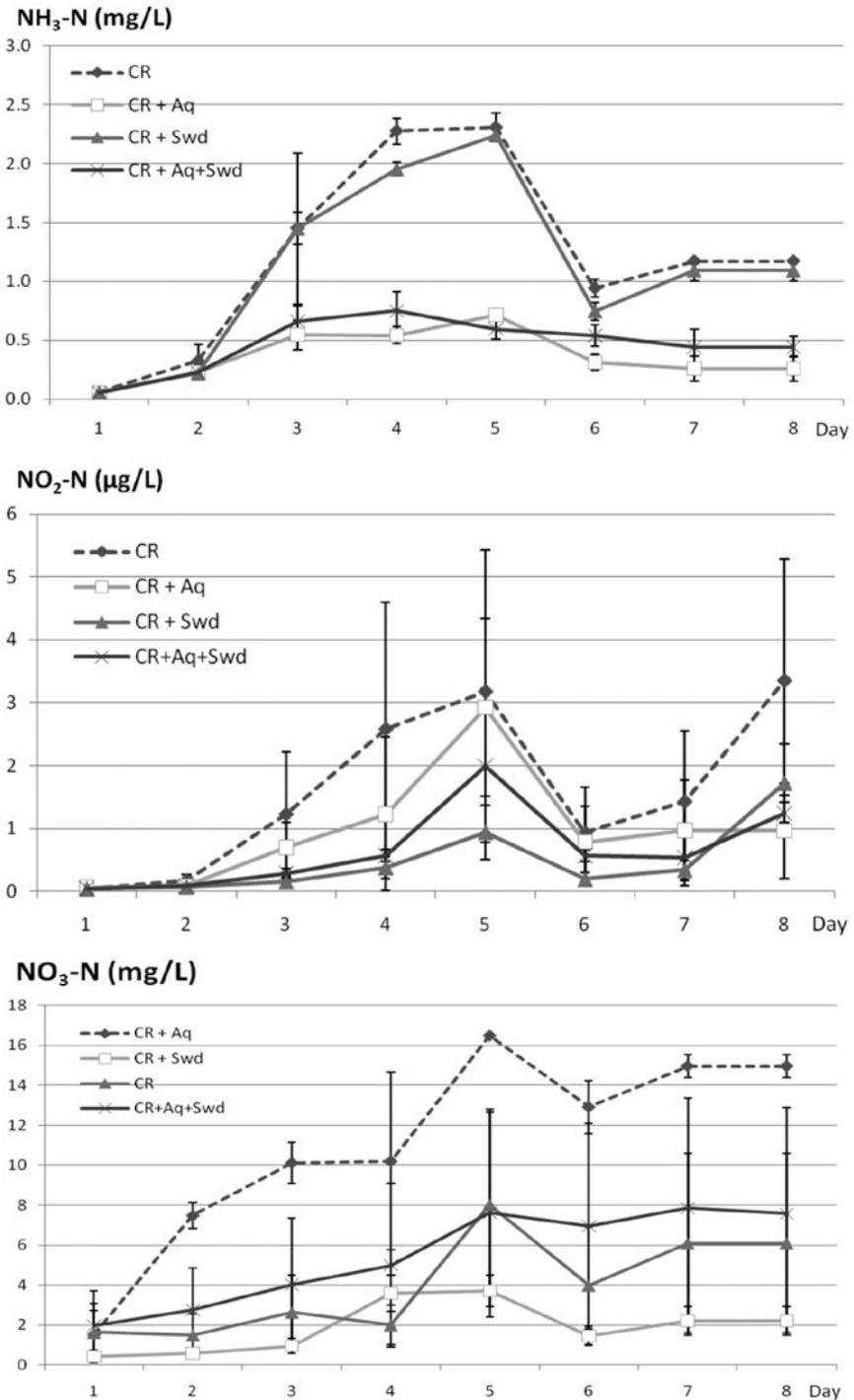


Figure 5. Changes (day) in NH<sub>3</sub>-N, NO<sub>2</sub>-N and NO<sub>3</sub>-N concentrations (mean ± SD) in the four recirculating tanks.

### Three Biofiltration Options in a Marine RAS

The mean fish weight gain and survival rate in the Aq + Swd + CR tank were  $96.4 \pm 53.4\%$  and  $96.4 \pm 4.8\%$ , respectively (Figure 6). The values for the Aq + CR tank were  $77.7 \pm 28.8\%$  and  $95.2 \pm 2.1\%$ , respectively; for the Swd + CR tank they were  $58.8 \pm 18.1\%$  and  $92.1 \pm 3.8\%$ , respectively; for the CR tank they were  $51.3 \pm 5.70\%$  and  $90.9 \pm 1.8\%$ , respectively (Table 1). It appeared that the fish weight gains and survival rates in the four treatment tanks were different (Figure 6). However,

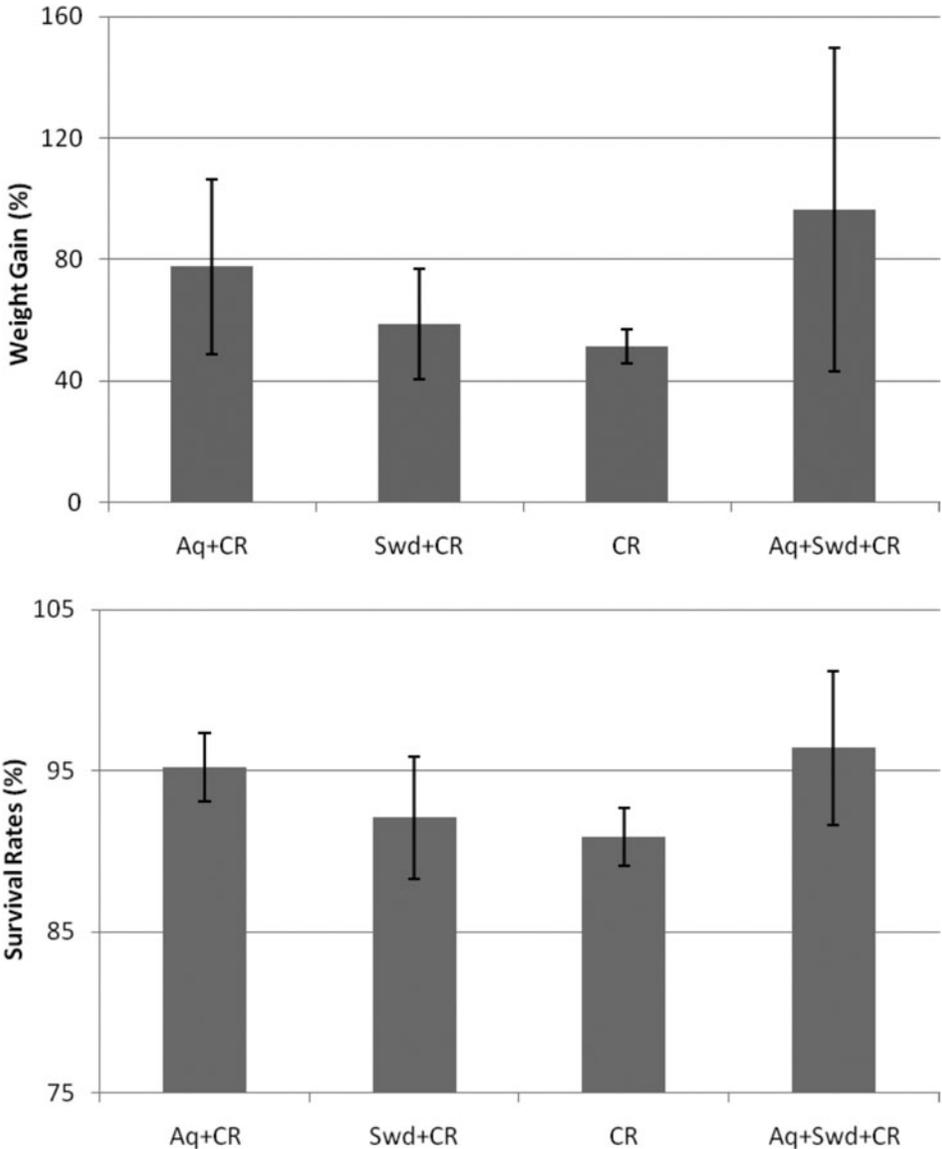


Figure 6. Means ( $\pm$  SD) of fish weight gain (%) and survival rate (%) in the four recirculating systems at the end of the experiment (7 days).

variance analysis showed that there was no significant difference ( $p < 0.05$ ) in fish weight gain or survival rate in the four tanks.

### Three Varieties of Seaweeds in Fish Recirculating System

Comparisons between the culture systems with and without seaweeds were not significantly different in temperature, pH, DO, and salinity levels. The seawater temperature averages in the culture tanks with and without seaweeds were  $26.75 \pm 0.51$  °C and  $26.77 \pm 0.50$  °C, respectively. The pH averaged  $8.06 \pm 0.40$  in the culture tank without seaweeds and  $8.22 \pm 1.84$  in the culture tank with seaweeds. The mean values of DO in the culture tanks with and without seaweeds were  $6.56 \pm 0.49$  mg/L and  $6.77 \pm 2.21$  mg/L, respectively. Salinities decreased from 31.1 to 23.4 ppt in both recirculating systems, due to the influence of rain after five, seven, 15, 21, and 26 days of the experiment. Once the salinity recorded dropped below 27 ppt in both recirculating systems, the water was exchanged with 75 % new seawater (Table 2).

The analysis of variance indicated no significant difference in  $\text{NH}_3\text{-N}$ ,  $\text{NO}_2\text{-N}$ , and  $\text{NO}_3\text{-N}$  concentrations in recirculating systems with and without seaweeds. The  $\text{NH}_3\text{-N}$  averaged  $0.44 \pm 0.24$  mg/L in the culture tank without seaweeds and  $0.44 \pm 0.25$  mg/L in the culture tank with seaweeds.  $\text{NO}_2\text{-N}$  and  $\text{NO}_3\text{-N}$  concentrations averaged  $0.0406 \pm 0.0066$  mg/L and  $109.0 \pm 113.9$  mg/L, respectively in the culture tank without seaweeds and  $0.0409 \pm 0.0060$  mg/L and  $112.1 \pm 112.4$  mg/L, respectively in the culture tank with seaweeds (Table 2).

*Table 2. Means ( $\pm$ SD) of in situ water quality in the recirculating systems with and without seaweed.*

Treatment Tanks	Without seaweed	With seaweed	$p < 0.05$
n	96	96	df=1; N=190
Temp.(°C)	$26.78 \pm 0.50$	$26.75 \pm 0.51$	F=0.754; MS=0.025
pH	$8.06 \pm 0.39$	$8.22 \pm 1.84$	F=0.701; MS=1.234
DO (mg/L)	$6.55 \pm 0.49$	$6.77 \pm 2.02$	F=0.962; MS=2.083
Salinity (ppt)	$27.42 \pm 2.50$	$27.52 \pm 2.41$	F=0.085; MS=0.510
$\text{NH}_3\text{-N}$ (mg/L)	$0.43 \pm 0.23$	$0.44 \pm 0.25$	F=0.082; MS=0.005
$\text{NO}_2\text{-N}$ (mg/L)	$0.0406 \pm 0.0066$	$0.0409 \pm 0.0060$	F=0.119; MS=0.000
$\text{NO}_3\text{-N}$ (mg/L)	$109.0 \pm 113.9$	$112.4 \pm 112.4$	F=0.035; MS=452.702

## Three Biofiltration Options in a Marine RAS

Two varieties of *E. spinosum* were grown in the recirculating system, however, after 35 days, both varieties showed signs of decay. The *E. cottonii* decayed in the first week of the experiment (Figure 7). The average specific growth rates of brown and green varieties of *E. spinosum* during the 35 days were  $0.329 \pm 0.129$  % per day and  $0.317 \pm 0.178$  % per day, respectively. Variance analysis proved that these two varieties did not differ significantly in terms of specific growth rates. The average yield per unit area of the brown and green varieties was  $1.555$  g/m<sup>2</sup>/day and  $1.476$  g/m<sup>2</sup>/day, respectively.

Table 2 shows that the fish growth rates and survival rates were not significantly different in both recirculating systems. The specific growth rates of *L. calcarifer* in the recirculating systems with and without seaweeds were  $1.96 \pm 0.90$  % per day and  $1.90 \pm 0.90$  % per day, respectively. *Lates calcarifer* survival rate was 94 % in the recirculating system with seaweeds and 86 % in the recirculating system without seaweeds.

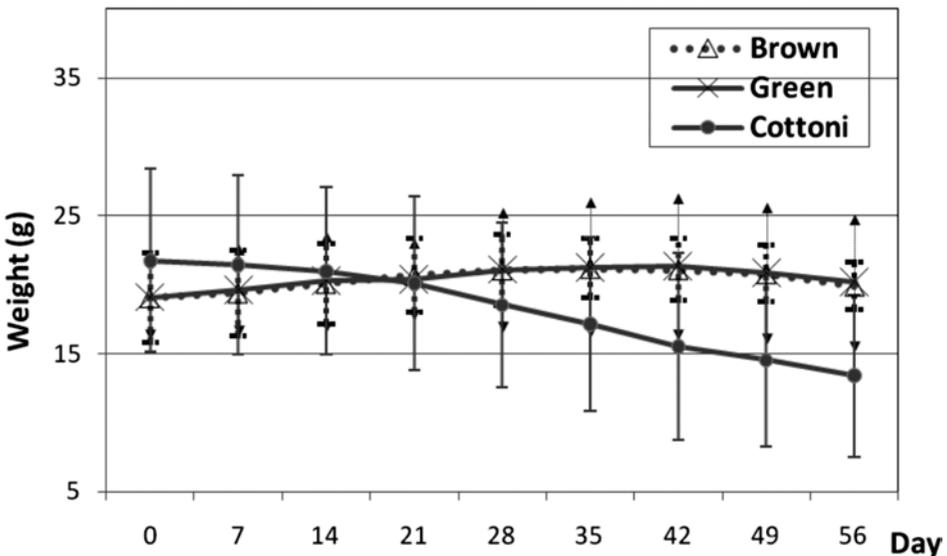


Figure 7. Weight (g) of three varieties of seaweeds (*Euclima cottonii*, brown *E. spinosum*, and green *E. spinosum*) in a fish recirculating system.

## DISCUSSION

The preliminary experiment showed that the nitrification process occurred in all recirculating tanks (Figure 4 and 5). This was evident from the observed increase in NO<sub>2</sub>-N and NO<sub>3</sub>-N concentrations in

the four recirculating tanks. The nitrification process is the biological oxidation of ammonia into nitrite, then into nitrate, which requires oxygen and bacteria. Nitrifying bacteria (*Nitrosomonas* and *Nitrobacter*) in the production system utilize ammonia-nitrogen as an energy source for growth and produce nitrite and nitrate as a by-product. Ammonia is a by-product of protein metabolism, which is excreted from the gills of fish as they assimilate feed, and is also produced when bacteria decompose organic waste solids within the system.

However, the rates of nitrification in the four recirculating systems were significantly different. Table 1 shows the four recirculating systems had significantly different ( $p < 0.05$ ) in  $\text{NH}_3\text{-N}$  and  $\text{NO}_3\text{-N}$  concentrations. The ammonia mean concentrations recorded in the Aq + CR and Aq + Swd + CR recirculating tanks were lower compared to the other two recirculating tanks (Swd + CR, and CR alone). This suggested that the nitrification process occurred faster inside the recirculating tanks of Aq + CR and Aq + Swd + CR compared to the other recirculating tanks. Aquamat™ and CR provided a substantial surface area for microbes to grow and enhance the nitrification process in recirculating systems. In biological filtration, a substrate with a large surface area is required for nitrifying bacteria to attach and grow (Stehr et al. 1995, Losordo et al. 1999, Estim et al. 2009). The rate of the nitrification reaction is highly dependent on a number of environmental factors. These include the substrate and oxygen concentration, temperature, pH, and the presence of toxic or inhibiting substances. Stehr et al. (1995) added that an increase in the surface area available in the oxygenated water column may also promote growth of specific bacterial groups such as nitrifiers, which are more likely to inhabit surfaces than to be free-floating. Previous studies showed that the bacteria colonies were, in fact, more numerous on the surface of Aquamat™ than in the water column in the culture system (Estim et al. 2009). Aquamat™ alone is still not sufficient to remove the dissolved inorganic nitrogen in a recirculating system (Figure 4 and 5), where the ammonia by-product, namely nitrate, also accumulates in the culture system. For aquatic animals, nitrate is the least toxic of the inorganic nitrogen compounds. However, if nitrate is released into the environment, it can stimulate harmful algal blooms (Estim et al. 2001). Some of the negative impacts attributed to aquaculture are due to the release of nitrogen and phosphorus into the surrounding environment; an excess of these nutrients can cause eutrophication and deteriorate the

environment (Camargo and Alonso 2006). Van Rijn (1996) explained that accumulation of other inorganic nutrients such as nitrate and phosphate have received little attention, but deserve increasing consideration.

The dissolved inorganic nitrogen concentration is lower in recirculating tanks with a combination biofilter using Aq + Swd + CR (Figures 4 and 5); this system also supported a marginally higher fish weight gain and survival rate over the other recirculating systems (Table 1). The inclusion of seaweed significantly reduced the load of dissolved nutrients that are returned to the environment (Neori et al. 1996, Msyua and Neori 2002, Shpigel and Neori 2007, Estim and Mustafa 2010, Troell et al. 1999). The methods for using seaweed to treat effluents from enclosed mariculture systems were initiated in the mid 1970s, and have recently garnered new interest, now that it has been shown that waste water from intensive and semi-intensive mariculture is suitable as a nutrient source for seaweed production.

In the second experiment, the three varieties of *Eucheuma sp.* were not seen to grow steadily and produced no noticeable effects on  $\text{NH}_3\text{-N}$ ,  $\text{NO}_2\text{-N}$ , and  $\text{NO}_3\text{-N}$  concentrations. It was noted that *E. cottonii* decayed in the early days, while the two varieties of *E. spinosum* decayed after 35 days. Qian et al. (1996) reported that *Kappaphycus alvarezii* in a co-culture system grows faster and removes nitrogenous waste released by pearl oysters. Besides, Msyua and Neori (2002) reported that *Eucheuma denticulatum* (also known as *E. spinosum*) did not survive after 10 days. They explained that the algae started to lighten in color, and then white lesions were observed at the tips, which is a typical sign of stress (peroxide formation). The specimens finally rotted and died. Those observations were also made on the *E. cottonii* in this experiment. Although Msyua and Neori (2002) reported that *E. denticulatum* died after 10 days, they also observed that pieces of *E. denticulatum* planted in the fishpond effluent channels survived until the fourth week. It was also observed that the new thallus of *E. spinosum* is slightly small and thin as reported before (Estim and Mustafa 2010).

During the study, fresh water (rain) influenced salinity inside the culture systems, which decreased from 31.1 to 23.4 ppt. This change most likely caused the early decay of *E. cottonii*. In addition, low temperatures during the experimental period may also have contributed to this process. Environmental conditions have to be optimal for stocked

species to give highest production (Qian et al. 1996). Therefore, when conditions are suboptimal, the co-culture system can produce negative results. Anggadirehja et al. (2002) explained that the suitable salinity for *Eucheuma spp.* was in the range of 28 to 34 ppt and that light played an important role in the photosynthetic activity and overall survival of the algae. The lower temperatures and decrease in light exposure may have resulted in setbacks to growth as well as biofiltration capacity (Schuenhoff et al. 2006). As detailed in Yan et al. (1998), the key elements in the successful management of this systemic photosynthesis are control over the respiration ratio and recycling of nutrients. Other factors affecting growth and survival are the concentration of dissolved oxygen, pH, temperature, and the concentration of ammonia and nitrite.

A concept and qualitative experimental results for integrated waste-recycling marine polyculture systems were described in the early 1970's (Yan et al. 1998, Shpigel and Neori 2007). In these studies, the source of nutrients was domestic effluents that were mixed with seawater to obtain brackish water for phytoplankton culture. In turn, the microalgae were fed to filter feeders (oysters and clams) as well as additional organisms that consumed the solid waste particles. Dissolved nutrients in the final effluent were biofiltered by seaweed. Replacement of the sewage water with effluents from fish culture and use of the seaweed for macroalgivore (abalone) culture were subsequently proposed (Shpigel and Neori 2007). In this study, it can be concluded that the *Eucheuma sp.* cannot survive for long under the conditions provided, and once dead, water quality impairment follows. While seaweeds carry out a degree of water quality remediation, they themselves require a good environment to perform the role. When the conditions are not optimal for the stocked organisms, the co-culture system can produce negative results. Follow-up investigations are necessary to determine the suitability of this type of integrated recirculating aquatic system for large-scale fish production. In fact, the variable costs of producing fish in recirculating systems (feed, fingerling, electricity, labor) are not much different than that of other production methods. The authors agree with conclusion of Yan *et al.* (1998) that although many forms of wastewater aquaculture are successful, they are not always universally applicable, and must be adapted to the local environmental, economic, social conditions. The integrated production of marine fish and seaweed has the potential to be ecologically, economically and socially more sustainable than current practices.

This will reduce environmental impact of fish farming, produce extra income for farmers and create additional jobs while helping to improve the public image of intensive aquaculture. Many developed countries have identified recirculating aquaculture as an area for research and development. Asia is lagging behind in this field. However, if as a result of intensive research, a feasible technology emerges, that technology will have a better chance of widespread application in the current climate, where environmental concerns are taking center stage in all industrial-scale operations, including seafood production.

## ACKNOWLEDGEMENTS

---

Special thanks to the Malaysia Ministry of Higher Education and the Malaysia Ministry of Science, Technology and Innovation for providing financial support under research grants of Fundamental FRG0041-ST-001 and Escience, SCF05-01-10-SF0053, respectively. I also would like to thank the staff of Borneo Marine Research Institute fish hatchery especially Prof. Dr. Shigeharu Senoo and Prof. Dr. Ridzwan A. Rahman.

## REFERENCES

---

- Anggadirehja, J.T., Zatnika, A., Purwoto, H. and Istini, S. **2002**. *Rumput Laut, Pembudidayaan, Pengolahan & Pemasaran Komoditas Perikanan Potensial*. Indonesia. Penebar Swadaya.
- Bratvold, D. and Browdy, C.L. Effects of Sand Sediment and Vertical Surfaces (Aquamat™) on Production, Water Quality and Microbial Ecology in an Intensive *Litopenaeus vannamei* Culture System. *Aquaculture* **2001**, 195:81-94.
- Camargo, J.A. and Alonso, A. Ecological and Toxicological Effects of Inorganic Nitrogen Pollution in Aquatic Ecosystems: A Global Assessment. *Environment International* **2006**, 32:831-849.
- Estim, A., Anton, A., Ridzwan, A.R. and Alexander, J. **2001**. NO<sub>3</sub>-N dan PO<sub>4</sub>-P Merangsang Peningkatan Populasi Alga Beracun (*Pyrodinium bahamense* var *compressum*) di Perairan Marin Sabah. *Proceedings Malaysian Science and Technology Congress 2001*. 24 – 26 September. Kota Kinabalu, Sabah.

- Estim, A., Mustafa, S. and Anton, A. **2009**. Dissolved Inorganic Nitrogen and Aquamat™ Application in Marine Fish Hatchery System. In Workshop on Protect Diversity of Bioresources in East and South-East Asia, 10 August 2009. JSPS Exchange Program for East Asian Young Researchers, Kagoshima University: Kagoshima, Japan.
- Estim, A. and Mustafa, S. Aquaponic Application in a Marine Hatchery System. *Aquaponics Journal* **2010**, 57:26-34.
- Gutierrez-Wing, M.T. and Malone, R.F. Biological Filters in Aquaculture: Trends and Research Directions for Freshwater and Marine Applications. *Aquacultural Engineering* **2006**, 34:163-171
- Losordo, T.M., Masser, M.P., and Rakocy, J. **1999**. Recirculating Aquaculture Tank Production Systems, A Review of Component Options. SRAC (Southern Regional Aquaculture Center) Publication No. 453.
- Msuya, F.E. and A. Neori. *Ulva reticulata* and *Gracilaria crassa*: Macroalgae that can Biofilter Effluent from Tidal Fishponds in Tanzania. *Western Indian Ocean Journal Marine Science* **2002**, 2:117-126.
- Naylor, R.L., Goldburg, R.J., Primavera, J.H., Kautsky, N., Beveridge, M.C.M., Clay, J., Folke, C., Lubchenco, J., Mooney, H. and Troell, M. Effect of Aquaculture on World Fish Supplies. *Nature* **2000**, 405:1017-1024.
- Neori, A., Krom, M.D., Ellner, S.P., Boyd, C.E., Popper, D., Rabinovitch, R., Davidson, P.J., Dvir, O., Zuber, D., Ucko, M., Angel, D. and Gordin, H. Seaweed Biofilters as Regulators of Water Quality in Integrated Fish-Seaweed Culture Units. *Aquaculture* **1996**, 141:183-199.
- Parsons, T.R., Maita, Y. and Lali, C.M. **1984**. *A Manual of Chemical and Biological Methods for Seawater Analysis*. Pergumon Press: Oxford, UK.
- Qian, P.Y., Wu, C.Y., Wu, M. and Xie, Y.K. Integrated Cultivation of the Red Algae *Kappaphycus alvarezii* and the Pearl Oyster *Pinctada martensi*. *Aquaculture* **1996**, 147:21-35.
- Schuenhoff, A., Mata, L. and Santos, R. The Tetrasporophyte of *Asparagopsis armata* as a Novel Seaweed Biofilter. *Aquaculture* **2006**, 252:3-11.

### *Three Biofiltration Options in a Marine RAS*

- Scott, S. and McNeil, R. Aquamats: How to Provide Habitat, Feed and Biofiltration for Hatchery and Nursery Tanks. *Hatchery International* **2001**, 2:19-21.
- Shpigel, M. and Neori, A. **2007**. Microalgae, Macroalgae and Bivalves as Biofilters in Land-Based Mariculture in Israel. In *Ecological and Genetic Implications of Aquaculture Activities*. Bert, T.M. (Ed.). Springer: New York, NY, USA, 433-446.
- Stehr, G., Böttcher, B., Dittberner, P. and Rath, G. The Ammonia-Oxidizing Nitrifying Population of the River Elbe Estuary. *FEMS Microbiology Ecology* **1995**, 17:177–186.
- Troell, M., Ronnback, P., Halling, C., Kautsky, N. and Buschmann, A. Ecological Engineering in Aquaculture: Use Seaweeds for Removing Nutrients from Intensive Mariculture. *Journal of Applied Phycology* **1999**, 11:89-97.
- Van Rijn, J. The Potential for Integrated Biological Treatment Systems in Recirculating Fish Culture - A Review. *Aquaculture* **1996**, 139:181-201.
- Wu, R.S.S. The Environmental Impact of Marine Fish Culture: Towards a Sustainable Future. *Marine Pollution Bulletin* **1995**, 31:159-166.
- Yan, J., Wang, R. and Wang, M. The Fundamental Principles and Ecotechniques of Wastewater Aquaculture. *Ecological Engineering* **1998**, 10:191-208.