



## Bioremediation Potential of Seaweed (*Kappaphycus alvarezii*) and Pacific Oyster (*Crassostrea gigas*) in a Tilapia (*Oreochromis Sp.*) Static Culture System

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### ABSTRACT

The possible role of integrating seaweed, *Kappaphycus alvarezii* and Pacific oyster, *Crassostrea gigas* in a tilapia static culture system was investigated. The effect of their bioremediating capacity on the growth performance of tilapia (iBEST strain) fingerlings were evaluated over a 28-day period. The study consists of four treatments T1 (control), T2 (seaweeds at 100g), T3 (oyster at 10pcs), and T4 (seaweeds at 100g + oyster at 10pcs) with three replicates arranged in a completely randomized design. Tilapia fingerlings with an average body weight of 7.5 grams were stocked at a density of 10 fish per aquarium. The experimental set-up consisted of a static culture system with 12 aquaria (15L capacity) continuously aerated. The fish were fed with commercial feeds with a daily feed ration equivalent to 10% of their average body weight. Results showed no significant differences in mean weight gain, specific growth rate, survival rate and feed conversion ratio of the tilapia fingerlings. This demonstrates that the presence of seaweed and oyster did not significantly affect fish growth. Additionally, there was also no significant variation observed in oyster growth. However, a negative weight gain, specific growth rate and survival rate of *K. alvarezii* were observed in *K. alvarezii* seaweed treatments, indicating an adverse effect on the seaweed. Regarding bioremediation potential, the control group had significantly higher levels of ammonia and nitrite compared to the treatments with oyster and seaweed. Nitrate concentrations remained relatively stable throughout the study, with no notable variations. A decreasing trend in phosphate levels was observed, indicating a reduction in nutrient concentrations, highlighting the significant impact of seaweed and oyster on water quality. In conclusion, integrating *Crassostrea gigas* oysters and seaweeds in the tilapia culture system is a promising approach. However, it is recommended to explore alternative seaweed species that can withstand a zero-water exchange system to enhance the overall sustainability of the integrated system.

**Keywords:** seaweed integration, bioremediation, phytoremediation, zooremediation, aquaculture sustainability, nutrient reduction, zero-water exchange

### 1. INTRODUCTION

Aquaculture plays a crucial role in meeting the global demand for food (Mishra et al, 2021), providing income for millions of people involved in seafood production (Martinez-Porchas & Martinez-Cordova, 2012). However, the intensification of aquaculture to meet this demand has led to negative environmental consequences, such as eutrophication and

disruptions in nitrification processes in ecosystems that receive aquaculture effluents (Martinez-Cordova et al., 2022). The excessive nutrients, including nitrogenous waste and phosphorous, present in aquaculture waste contribute to eutrophication, compromising water quality and potentially harming cultured fish (Crab et al., 2009).

Traditionally, water exchange has been used to mitigate these issues in aquaculture systems, but it is water-intensive and costly for farmers (Zablon et al., 2022). To achieve sustainable

aquaculture, there is a need for environmentally friendly technological innovations (Avnimelech, 2006). Bioremediation has emerged as a sustainable approach to mitigate the environmental impact of aquaculture waste, utilizing various organisms such as mollusks, echinoderms, plants, and bacteria (Martinez-Cordova et al., 2022).

Integrated aquaculture systems have shown promise in nutrient removal. Seaweeds, as photoautotrophic organisms, can counteract the environmental impact of heterotrophic-fed organisms and restore water quality (Neori, 2008). They efficiently assimilate nutrients and serve as an ecological and sustainable approach to wastewater remediation (Sivakumar et al., 2012). Bivalves, when stocked in integrated systems, filter organic particles and influence biogeochemical processes and nutrient fluxes through grazing, burrowing, and excretion (Betina et al., 2019).

The selection of macroalgae and bivalves for aquaculture wastewater bioremediation is crucial, as their nutrient removal efficiency depends on species and environmental conditions. The ideal choice should exhibit fast growth rates, high nutrient absorption capacity, adaptability to changing conditions, and should be sourced locally to prevent the introduction of non-endemic species (Nhat, et al., 2018).

*Kappaphycus alvarezii*, known locally as 'guso' or 'agal-agal,' constitutes a significant portion of seaweed exports in the Philippines (SEAFDEC, 2017). With its rapid growth rate and short harvest cycles (Vairappan, 2021), this species is highly suitable for filtering polluted water. There is tremendous potential to cultivate *K. alvarezii* in nutrient-impacted areas or within fish systems, utilizing its bioremediation capabilities (Hayashi et al., 2008).

Another aquatic organism renowned for its abundant aqua products is the Pacific oyster (*Crassostrea gigas*), it holds a prominent position in the global market. This species possesses the remarkable ability to significantly alter its environment, which can have both positive and negative implications for native species and ecosystems (Pira et al., 2022). Due to its profound impact on its surroundings, the Pacific oyster is often referred to as an ecosystem engineer (Dumbauld et al., 2009). *C. gigas* creates dense oyster reefs, forming thick mats that induce physical transformations in the surrounding habitat. This species is recognized for its capacity to convert soft substrates such as mud and silt into hardened substrates. Alongside this alteration of benthic habitats, Pacific oysters act as filter feeders, consuming suspended plankton and organic waste. Their metabolic ability to cleanse polluted environments makes them ideal candidates for bioremediation efforts.

In the Municipality of Kabasalan, Zamboanga Sibugay, the Pacific oyster *C. gigas*, locally known as 'talaba,' is cultivated, while the seaweed *Kappaphycus alvarezii* thrives in the coastal waters of Sibuguey Bay. Both of these species are abundant and persistent throughout the year in the Zamboanga Region, rendering it a suitable location for bioremediation initiatives. The combination of *K. alvarezii* and *C. gigas* offers a promising approach to mitigate environmental pollution. The efficient nutrient assimilation capabilities of seaweeds and the filtration and transformation abilities of Pacific oysters will make them valuable contributors to the bioremediation process. By harnessing the natural abilities of these organisms, it is possible to address the ecological challenges

posed by aquaculture and promote sustainable practices in the Zamboanga Region.

The main objective of this study is to investigate the bioremediation potential of seaweed (*Kappaphycus alvarezii*) and Pacific oyster (*Crassostrea gigas*) in a Tilapia (*Oreochromis sp.*) static culture system. Specifically, the study aims to: (1) assess the ability of *K. alvarezii* and *C. gigas* to remove or reduce pollutants, such as nitrogenous compounds (ammonia and nitrate) and phosphorous from the tilapia culture system; (2) investigate the growth performance and survival rate of tilapia reared in the presence of seaweed and oyster, compared to a control group without seaweed and oyster; and (3) evaluate the impact of seaweed and oyster presence on water quality parameters, such as dissolved oxygen levels, pH and nutrient concentrations.

## 2. MATERIALS AND METHODS

### 2.1 Experimental organisms

A total of 150 iBEST (Brackishwater Enhanced Selected Tilapia) fingerlings were sourced from the BFAR 9-Bagalupa Freshwater Hatchery in Pagadian City. Additionally, the Pacific oyster *Crassostrea gigas* was acquired from local growers in Kabasalan, Zamboanga Sibugay. Upon arrival at the experimental location, both the fingerlings and oysters underwent a gradual acclimation process to adapt to the culture conditions. This acclimation period lasted for 2 weeks, ensuring the proper adjustment of the aquatic organisms. Subsequently, the fingerlings and oysters were distributed into the aquariums, with a stocking density of 10 individuals per aquarium. This distribution allowed for appropriate observation and management of the tilapia and oyster populations throughout the study.

For the *K. alvarezii*, samples were collected from seaweed growers in Sibuguey Bay, specifically in RT Lim, Zamboanga Sibugay. Careful attention was given to the collection process to avoid cell breakage and minimize physiological stress to the thalli. Filaments were carefully gathered to achieve the desired weight without cutting them, ensuring the integrity and viability of the seaweed samples. The seaweeds were stocked at a rate of 100 grams (fresh weight) per aquaria.

### 2.2 Experimental Design

A total of twelve (12) aquaria with a 15L capacity were utilized in the study in which the water was continuously aerated. The biofilter set-up was done before the fish arrived. The oyster and seaweed served as biofilters. Before the experiment, seaweeds were kept in 15-liter aquariums of seawater that were gently aerated, without the addition of fertilizers, and under regulated environmental circumstances (natural photoperiod). The experiment consists of four (4) treatments, T1 (control), T2 (seaweed), T3 (oyster) and T4 (seaweed+oyster) as shown in Table 1 below. The treatments were replicated thrice and were laid in a completely randomized design (CRD). The investigation was carried out in the College of Fisheries Laboratory located in Kolambugan, RT Lim, Zamboanga Sibugay with a duration of 28 days.

Table 1. The different experimental treatments with the stocking densities

Treatment	Algal Density (g/aquaria)	Oyster Density (pcs/aquaria)	Fish Density (pcs/aquaria)
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1 (Control)	0	0	10
2	100	0	10
3	0	10	10
4	100	10	10

### 2.3 Feeding and Feed Ration

The feeding regime involved three feeding sessions per day, with the proportions distributed as follows: 30% in the morning at 8:00 am, 40% at noon (12:00 pm), and 30% in the afternoon at 4:00 pm. To determine the appropriate feed ration, the formula  $DFR = (ABW) \times (10\%)$  was utilized. Here, DFR represents the Daily Feed Ration, and ABW corresponds to the Average Body Weight of the fish. The feed ration was provided based on 10% of the fish's average body weight. To accommodate changes in weight and fish population, the feed ratio was adjusted every seven days.

For the experiment, commercial aquafeeds were used as the primary feed source. These aquafeeds were selected for their nutritional content and suitability for the tilapia and oyster species under study. Their formulation and composition were designed to meet the dietary requirements of the fish and promote healthy growth and development.

### Sampling Procedure

Prior to distributing the tilapia fingerlings, oysters, and seaweeds into the aquaria, the initial weight of the organisms was determined. This step ensured accurate baseline measurements for subsequent growth and biomass calculations. During the 28-day culture phase, sampling was conducted at consistent intervals of every seven days. Specifically, at 4:00 PM, the test organisms were carefully removed from the aquaria using a scoop net and transferred into a plastic basin. With utmost care to minimize stress, the organisms were weighed, taking into account any changes in their respective weights since the previous sampling. After the weighing process, the experimental organisms were promptly and gently returned to their respective aquaria to ensure minimal disruption to their natural habitat and overall well-being. This careful handling approach aimed to maintain the integrity of the experiment and prevent any unnecessary stress that could potentially impact the organisms' growth and behavior.

### 2.4 Nutrient Removal Efficiency Determination

Nutrient buildup in the water column primarily occurs due to various factors such as unconsumed feed, urine, fecal matter, and the decomposition of deceased organisms. These sources contribute to the accumulation of carbon, nitrogen, and phosphorus in both solid and dissolved forms. Monitoring and analyzing nutrient levels in the culture water are essential as excessive levels can have detrimental effects on the cultured species.

To assess the nutrient removal efficiency of seaweeds and oysters, key nutrients, including nitrates, ammonium, and phosphates, were determined using an API colorimetric water quality test kit. The procedure involved collecting a five-milliliter water sample from each tank using a test tube. Subsequently, a specific solution was added to the sample, inducing a color change in the water. After a five-minute period, the resulting color was compared to a standardized color chart to ascertain the nutrient concentration.

This method provided a convenient and reliable means of measuring the nutrient levels in the water column. By regularly monitoring these parameters, the researchers were able to gauge the effectiveness of the seaweeds and oysters in removing or reducing nitrates, ammonium, and phosphates, which are critical indicators of water quality in the tilapia static culture system.

### 2.5 Growth and Survival Determination

In order to assess the growth and survival of the fish, several parameters were calculated using the following formulas:

- Specific growth rate (SGR)% =  $100 \times \frac{(\ln W_t - \ln W_0)}{t}$  (%)
- Feed conversion ratio (FCR) =  $\frac{\text{Amount of feed consumed (g)}}{\text{Weight gain}}$
- Survival rate =  $\frac{(\text{number of remaining fish} / \text{initial number of fish}) \times 100}$

### 2.6 Monitoring of Water Quality Parameters

Regular monitoring of water quality parameters is crucial in fishponds and tanks to ensure optimal conditions for the fish and prevent stress and disease. These parameters play a significant role in the overall health and well-being of aquatic organisms. To accurately assess and monitor these parameters, a digital multi-parameter probe was employed. This specialized instrument allowed for the simultaneous measurement of various key water parameters, including temperature, dissolved oxygen (DO), salinity, and pH. By regularly checking these parameters before feeding the fish, the researchers gained valuable insights into the water quality and made any necessary adjustments to maintain an ideal environment for the fish.

### 2.7 Statistical Analysis

All statistical analyses for this study were conducted using the SPSS statistical package, a widely recognized and trusted software tool for data analysis. To assess significant differences among the treatments, a one-way analysis of variance (ANOVA) was employed. This statistical test allowed for the comparison of means across different treatment groups, providing insights into any significant variations. Furthermore, to identify specific treatment groups that exhibited significant differences, the Duncan Multiple Range Test was utilized.

## 3. RESULTS AND DISCUSSION

### 3.1 Bioremediating potential of seaweeds and oyster

The nutrient concentration of the tilapia culture water with seaweeds and oysters after a 28-day culture period is presented in Table 2. The data shows interesting trends and differences among the treatments in terms of ammonia, nitrite, nitrate, and phosphate levels (Figure 5).

Table 2. Concentration (ppm) of different nutrients in the culture water during the 28 days culture period.

Treatment	Ammonia		Nitrite		Nitrate		Phosphate	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final
T1 (Control)	0.83	1.67 <sup>a</sup>	0.67	1.00 <sup>a</sup>	8.00	8.17 <sup>a</sup>	0.83	0.50 <sup>a</sup>
T2 (Seaweeds)	0.83	0.42 <sup>b</sup>	0.42	0.42 <sup>b</sup>	7.67	7.83 <sup>a</sup>	1.00	0.25 <sup>a</sup>
T3 (Oysters)	1.00	0.67 <sup>b</sup>	0.50	0.42 <sup>b</sup>	7.50	7.67 <sup>a</sup>	0.83	0.50 <sup>a</sup>

T4(Seaweed+Oyster)	1.00	0.67 <sup>b</sup>	0.50	0.42 <sup>b</sup>	8.00	7.50 <sup>a</sup>	0.67	0.33 <sup>a</sup>
<b>Standard Values</b>	<b>0-0.5</b>	<b>&lt;1</b>	<b>&lt;10</b>	<b>&lt;0.4</b>				

Superscripts with different letter within the same column are significantly different from other treatments (p<0.05).

### Ammonia

The seaweed group (T2) had the lowest mean ammonia level of 0.42 ppm, followed by the oyster group (T3) and seaweed+oyster group (T4) with 0.67 ppm. In contrast, the control group (T1), had the highest mean ammonia level of 1.67 ppm, which was significantly higher than the other treatments. The results indicate that the presence of seaweed, oyster, or both in the culture system had a positive effect on reducing ammonia levels compared to the control group. Treatment 2, consisting of only seaweed, displayed the lowest mean ammonia level, suggesting that seaweed alone had a significant impact on ammonia reduction. This can be attributed to the ability of seaweed to absorb ammonia from the water column through its metabolic processes (Sato et al., 2006).

The oyster group (Treatment 3) and the seaweed+oyster group (Treatment 4) also exhibited lower mean ammonia levels compared to the control group, indicating the potential role of oysters in ammonia removal. Oysters are known for their filter-feeding behavior, where they actively remove particulate matter, excess nutrients, and organic materials from the water (Ayvazian, et al., 2021). This filtration process likely contributed to the reduction in ammonia levels observed in the oyster-containing treatments.

The control group (Treatment 1) had the highest mean ammonia level of 1.67 ppm, which was significantly higher than the other treatments. This result highlights the importance of incorporating seaweed and/or oysters in aquaculture systems to mitigate ammonia accumulation, as high levels of ammonia can be detrimental to the health and growth of cultured organisms.

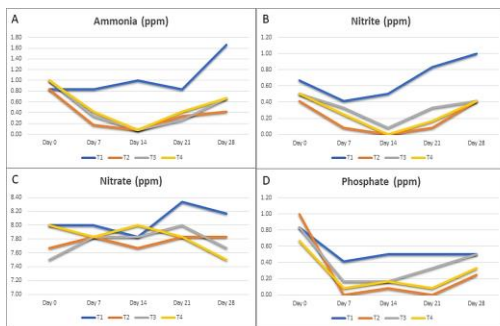


Figure 5. Concentration (ppm) of different nutrients in the culture water during the 28 days culture period.

Ammonia is a nitrogenous waste product excreted by fish and other aquatic organisms. In high concentrations, it can be toxic and negatively impact the overall water quality. Ammonia toxicity can lead to stress, impaired growth, and increased susceptibility to diseases in fish (Lin et al., 2023). Therefore, maintaining optimal ammonia levels is crucial for the well-being of cultured organisms and the success of aquaculture operations.

### Nitrite

For nitrite concentration, treatments 2, 3, and 4 displayed a mean level of 0.42 ppm, which was significantly lower than the mean concentration of 1.0 ppm observed in treatment 1. This suggests that the inclusion of seaweeds and oysters in the culture system contributed to the removal or transformation of nitrite in the culture water. Nitrite is a nitrogenous compound that can be toxic to aquatic organisms at high concentrations (Kim et al., 2019). Seaweeds can take up nitrite from the water column and utilize it as a nutrient source for their growth (Roleda & Hurd, 2019). Similarly, oysters are known filter feeders that can effectively remove suspended particles and organic matter, including nitrite, as they extract food from the water (Gallardi, 2014).

### Nitrate

In terms of nitrate concentration, treatment 4 exhibited the lowest mean value of 7.50 ppm, followed by treatment 3 with 7.67 ppm and treatment 2 with 7.83 ppm. Treatment 1 had the highest mean nitrate level of 8.17 ppm, but there were no marked variations among the treatments. Nitrate is a common form of nitrogen in aquatic systems, and its concentration is influenced by various factors, including nutrient inputs, biological processes, and water exchange rates. In aquaculture systems, nitrate can be derived from the breakdown of organic matter, such as uneaten feed and fish waste, as well as from the mineralization of ammonia and nitrite by nitrifying bacteria (Dauda et al., 2019). Overall, the nitrate concentrations remained within the standard values recommended by DAO (2016), indicating good water quality in the culture system.

### Phosphate

Regarding phosphate levels, treatment 2 had the lowest mean concentration of 0.25 ppm, followed by treatment 4 with 0.33 ppm. Treatment 1 and 3 had the highest mean phosphate levels of 0.50 ppm, but these values were not significantly different from the other treatments. Phosphate is an essential nutrient for aquatic organisms, including plants, algae, and aquatic animals. It plays a crucial role in various biological processes, such as energy transfer, DNA synthesis, and cellular metabolism (Lovio-Fragoso et al., 2021). However, excessive phosphate levels in aquatic ecosystems can lead to eutrophication, algal blooms, and poor water quality (Feng et al., 2023). The observed phosphate concentrations within the culture system remained relatively low and did not exceed the recommended standard values. This suggests that the presence of seaweed and oyster, as well as the culture management practices, helped maintain a favorable phosphate balance in the system.

It is noteworthy that the final values of the different nutrient concentrations in the culture water were lower than the initial values, indicating the nutrient removal capacity of the seaweed and oyster. The presence of seaweed and oyster in the culture system resulted in significantly lower levels of nitrate and ammonia compared to the control group, indicating their positive impact on water quality. Seaweed and shellfish culture have been recognized for their ability to improve ecosystems, as oysters, in particular, act as filter feeders, helping to clean the water by removing excess nutrients, organic materials, and algae (Theuerkauf et al., 2020).

The results demonstrated that the concentrations of nitrite, nitrate, and phosphate in the culture water remained within the standard

values recommended by DAO (2016). However, it is important to note that the ammonia levels were slightly higher than the standard values, suggesting the need for monitoring and management strategies to ensure optimal water quality for the cultured organisms.

### 3.2 Growth Performance of fish, seaweeds and oysters

#### Fish growth

The growth parameters of iBEST fingerlings after a 28-day culture period were evaluated in this study. The results, as presented in Table 3, provide insights into the performance of the different treatments. Treatment 4 exhibited the highest mean weight gain of 5.12 grams, followed by Treatment 1 with 4.55 grams, Treatment 2 with 4.12 grams, and Treatment 3 with the lowest mean weight gain of 4.08 grams. When considering specific growth rate (SGR), Treatment 4 again showed the highest value of 4.72, followed by Treatment 1 with 4.10, Treatment 3 with 3.57, and Treatment 2 with the lowest SGR of 3.51.

Survival rate, an important indicator of the health and well-being of the fish, was assessed across the treatments. Treatment 3 and 4 displayed the highest mean survival rate of 96.67%, followed by Treatment 2 with 93.33% and Treatment 1 with the lowest mean survival rate of 86.67%. It is worth noting that the control group, without the presence of seaweeds and oysters, exhibited the lowest survival rate. This could be attributed to the accumulation of toxic ammonia in the water, which is a potential issue when maintaining fish biomass without frequent water exchange, as highlighted by Crab et al., (2009). The poor water quality resulting from the accumulation of toxic substances may have also contributed to the stress experienced by the cultured organisms, ultimately leading to increased mortality.

Table 3. Growth parameters of Tilapia during the 28 days culture period

Treatment	Weight gain (g)	SGR (%)	Survival Rate (%)	FCR
T1 (Control)	4.55 <sup>a</sup>	4.10 <sup>a</sup>	86.67 <sup>a</sup>	2.16 <sup>a</sup>
T2 (Seaweeds)	4.11 <sup>a</sup>	3.51 <sup>a</sup>	93.33 <sup>a</sup>	2.54 <sup>a</sup>
T3 (Oysters)	4.08 <sup>a</sup>	3.57 <sup>a</sup>	96.67 <sup>a</sup>	2.49 <sup>a</sup>
T4 (Seaweed+Oyster)	5.12 <sup>a</sup>	4.72 <sup>a</sup>	96.67 <sup>a</sup>	2.40 <sup>a</sup>

Superscripts with different letters within the same column are significantly different from other treatments (p<0.05).

The feed conversion ratio (FCR), which reflects the efficiency of feed utilization by the fish, was also evaluated. Treatment 2 had the highest FCR with a mean value of 2.54, followed by Treatment 3 with 2.49, Treatment 4 with 2.40, and Treatment 1 with the lowest FCR of 2.16.

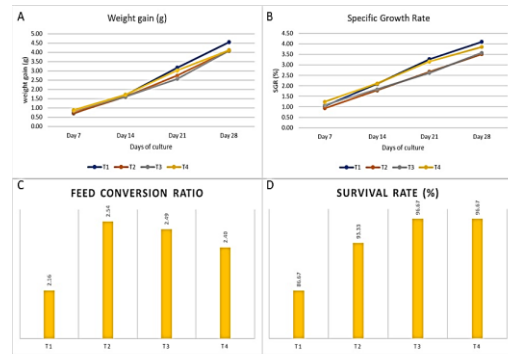


Figure 6. Growth parameters of Tilapia during the 28 days culture period. Statistical analysis was performed to determine if there were significant differences among the treatments for the measured growth parameters and survival rate. The results indicated that there were no significant differences (p>0.05) observed in the mean weight gain, specific growth rate (SGR), feed conversion ratio (FCR), and survival rate among the different treatments. These findings suggest that the presence of seaweeds and oysters, as well as the zero-exchange water system, did not have a negative impact on the growth and survival of iBEST fingerlings. The lack of significant differences in the growth parameters and survival rates among the treatments implies that the fish thrived well under all the experimental conditions. In fact, Figures 6A and 6B show an increasing trend in weight gain and specific growth rate, indicating favorable growth conditions.

#### Seaweed growth

The growth parameters of seaweed, *Kappaphycus alvarezii*, after a 28-day culture period are presented in Table 4. It is observed that there was a negative final value for weight gain, specific growth rate, and survival rate. A negative weight gain implies that the seaweed lost biomass during the culture period instead of gaining weight. Similarly, a negative SGR indicates a decrease in the growth rate of the seaweed. These observations suggest that the seaweed did not thrive or experience favorable growth conditions during the culture period. This decreasing trend is also evident in Figure 7.

Table 4. Growth parameters of seaweed during the 28 days culture period

Treatment	Weight (g)		Weight Gain (g)	SGR (%)	SR (%)
	Initial	Final			
T2 (Seaweeds)	100.00 <sup>a</sup>	48.70 <sup>a</sup>	-51.30 <sup>a</sup>	-2.57 <sup>a</sup>	48.7 <sup>a</sup>
T4 (Seaweed+Oyster)	100.00 <sup>a</sup>	50.30 <sup>a</sup>	-49.70 <sup>a</sup>	-2.45 <sup>a</sup>	50.3 <sup>a</sup>

Superscripts with different letter within the same column are significantly different from other treatments (p<0.05).



Figure 7. Growth parameters of seaweeds during the 28 days culture period. One possible explanation for this decline is the occurrence of the ice-ice disease, which resulted in the gradual decomposition of all seaweeds in Treatments 2 and 4 (100g seaweed). It appears that the seaweeds were unable to thrive in an environment with limited aeration. According to Yong et al., (2013), the optimal conditions

for seaweed cultivation include continuous aeration of 30.0 L per hour. Moreover, in the absence of sufficient water exchange, the seaweed tissues experienced discoloration, and the presence of pathogenic bacteria associated with the disease further contributed to the degradation of the seaweeds, ultimately leading to thalli disintegration (Largo et al., 1995). The accumulation of nutrients in the culture water also created a stressful environment for the seaweeds. It is evident that *K. alvarezii* is not able to withstand a closed system where there is no exchange of water. The lack of water exchange limited the availability of essential nutrients and disrupted the balance required for optimal seaweed growth.

**Growth of Oysters**

The growth parameters of Pacific oyster, *Crassostrea gigas*, after a 28-day culture period are presented in Table 5. Figure 8 demonstrates an increasing trend in weight gain and specific growth rate of the oysters, indicating that their growth was not adversely affected by the zero-exchange water system.

Table 5. Growth parameters of oyster during the 28 days culture period

Treatment	Weight (g)		Weight Gain (g)	SGR (%)	SR (%)
	Initial	Final			
T3 (Oyster)	62.69 <sup>a</sup>	67.35 <sup>a</sup>	4.66 <sup>a</sup>	0.26 <sup>a</sup>	93.33 <sup>a</sup>
T4 (Seaweeds+Oyster)	48.41 <sup>a</sup>	54.55 <sup>a</sup>	6.14 <sup>a</sup>	0.43 <sup>a</sup>	96.67 <sup>a</sup>

Superscripts with different letter within the same column are significantly different from other treatments (p<0.05).

Regarding survival rate, treatment 4 exhibited the highest mean at 96.67%, while treatment 3 had the lowest mean at 93.3%. However, statistical analysis indicated that these differences were not significant, suggesting that the survival rates among the treatments were relatively similar.

These findings suggest that the zero-exchange water system did not negatively impact the growth of Pacific oysters. The observed increase in weight gain and specific growth rate indicates that the oysters were able to thrive and achieve favorable growth under these cultural conditions. Additionally, the high survival rates across all treatments further support the notion that the zero-exchange water system was conducive to oyster culture.



Figure 7. Growth parameters of oysters during the 28 days culture period

It is important to note that while the differences in survival rates among the treatments were not statistically significant, further investigation or a larger sample size may be required to explore any potential variations in survival rates more comprehensively.

**3.3 Water Quality parameters of the culture water**

During the 28-day culture period, the physicochemical parameters of the water were monitored twice daily, and the initial and final values are presented in Table 6. The water temperature ranged from 27 to 28.4°C (Figure 8A), with no significant variation observed between the treatments. The dissolved oxygen concentration ranged from 12.6 to 12.9 mg/L (Figure 8B), and there were no

significant differences among the treatments. Similarly, the water pH ranged from 7.5 to 8.0 (Figure 8C), with no significant variation observed among the treatments. The salinity recorded ranged from 31.8 to 35.0 ppt (Figure 8D), and no marked variations were observed.

Table 6. Physico-chemical parameters of the culture water during the 28 days culture period

Treatment	Parameters							
	Temperature (°C)		Dissolve Oxygen (mg/L)		pH		Salinity (ppt)	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final
Control	27.40	27.57 <sup>a</sup>	12.80	12.57 <sup>a</sup>	7.97	7.67 <sup>a</sup>	32.47	34.87 <sup>a</sup>
Seaweed	27.33	27.57 <sup>a</sup>	12.87	12.57 <sup>a</sup>	8.01	7.67 <sup>a</sup>	32.03	34.97 <sup>a</sup>
Oyster	27.47	27.33 <sup>a</sup>	12.83	12.57 <sup>a</sup>	8.01	7.67 <sup>a</sup>	31.77	34.37 <sup>a</sup>
Seaweed+Oyster	27.67	27.67 <sup>a</sup>	12.87	12.63 <sup>a</sup>	8.02	7.50 <sup>a</sup>	32.10	34.97 <sup>a</sup>
<b>Standard Values</b>	<b>25-31</b>		<b>&gt;5.0</b>		<b>6.5-9.0</b>		<b>&gt;30</b>	

Superscripts with different letter within the same column are significantly different from other treatments (p<0.05).

The recorded values for water temperature, dissolved oxygen, pH level, and salinity were found to be within the standard values for the culture of the tilapia (DAO, 2016). These physico-chemical parameters play a significant role in the biology and physiology of fish. The observed stability and adherence to the standard values indicate that the culture system provided a suitable environment for the growth and survival of the tilapia.

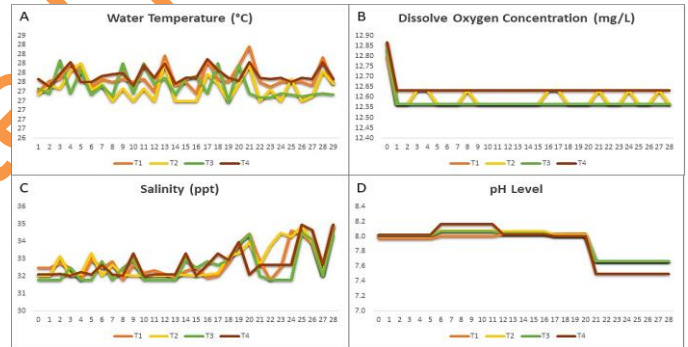


Figure 9. Physico-chemical parameters of the culture water during the 28 days culture period

Tilapia, including the iBEST strain, are known for their tolerance to various water conditions. They can thrive in low dissolved oxygen concentrations, such as 5 ppm or 6.5 ppm, and their optimal growth temperatures typically range from 22°C (72°F) to 29°C (84°F), as stated by Sallenave (2019). Additionally, tilapia can adapt to a wide range of salinity levels, with tolerances of up to 36 ppt. The ideal pH range for iBEST growth falls between 7.0 and 9.0. According to Mech (1996), the suitable pH range for fish culture generally falls between 6.7 and 9.5, with an ideal range of 7.5 to 8.5. Deviations from these ranges can induce stress in fish. In this study, the recorded physicochemical parameters remained within the suitable ranges for tilapia culture, ensuring optimal growth and survival. The stable and favorable water conditions provided by the zero-exchange water system contributed to the successful culture of iBEST fingerlings. These findings align with the established knowledge regarding the tolerance and adaptability of tilapia to a range of water conditions.

#### 4. CONCLUSIONS

In conclusion, the findings suggest that the integration of seaweeds and oysters in the Tilapia static culture system is a viable approach. The presence of oyster and seaweed in the system contributed to the bioremediation of nutrient concentrations, as evidenced by the decrease in nutrient levels by the end of the culture period.

The growth parameters and survival rate of the fish and oyster were not significantly impacted by the integration of seaweed and oyster, indicating that their presence did not have a negative effect on the growth of these organisms. However, it is important to note that the seaweed experienced negative growth during the culture period, highlighting the need to explore alternative seaweed species that are better suited to a zero-exchange water environment.

Further research and experimentation are recommended to identify seaweed species that can thrive in a zero-exchange water system and contribute positively to the overall performance of the integrated culture system. With careful selection and optimization, the integration of seaweeds and oyster holds potential for enhancing water quality and supporting the growth of cultured organisms in aquaculture systems.

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#### REFERENCES

Ayvazian, S., Mulvaney, K., Zarnoch, C., Palta, M., Reichert-Nguyen, J., McNally, S., Fulweiler, R. (2021). Beyond

Bioextraction: The Role of Oyster-Mediated Denitrification in Nutrient Management. *Environmental Science and Technology*, 14457-14465.

Betina, L., Zhao, L., Nicholaus, R., Yang, W., Zhu, J., & Zheng, Z. (2019). Bacterioplankton community in response to biological filters (clam, biofilm, and macrophytes) in an integrated aquaculture wastewater bioremediation system. *Environmental Pollution*, 254 (113035).

Boute, I., Tanguy, A., & Moraga, D. (2004). Response of the Pacific Oyster *Crassostrea gigas* to Hydrocarbon Contamination under Experimental Conditions. *Gene*, 147-157. doi:10.1016/j.gene.2003.12.027

Chavez-Crooker, P., & Obrique-Contreras, J. (2010). Bioremediation of aquaculture wastes. *Biotechnology*, 313-317. doi:DOI 10.1016/j.cobio.2010.04.001

Cicilia, S., Kambey, B., Calvyn, F., Sondak, A., & Chung, I.-K. (2020). Potential Growth and Nutrient Removal of *Kappaphycus alvarezii* in a fish floating-net cage system in Sekotong Bay, Lombok, Indonesia. *World Aquaculture Society*, 1-16.

Crab, R., Kochva, M., Vestræte, W., & Avnimelech, Y. (2009). Bioflocs technology application in over-wintering of tilapia. *Aquaculture Engineering*, 105-112.

DAO. (2016). Water Quality Guidelines and General Effluent Standards of 2016. *DENR Administrative Order No. 2016-08*.

Dauda, A., Ajadi, A., Tola-Fabunmi, A., & Akinwole, A. (2019). Waste production in aquaculture: Sources, components and management in different culture systems. *Aquaculture and Fisheries*, 81-88.

Dinesh, S., & Raja, K. (2014). Bioremediation by using microbes and algae with special reference to the coastline environment. *International Journal of Biosciences and Nanosciences*, 130-140.

Dominguez, B., Abreu, M. H., & Sousa-Pinto, I. (2015). On the bioremediation efficiency of *Mastocarpus stellatus* (Stackhouse) Guiry, in an integrated multi-trophic aquaculture system. *Journal of Applied Phycology*, 1289-1295.

Dumbauld, B. R., Ruesink, J. L., & Rumrill, S. J. (2009). The ecological role of bivalve shellfish aquaculture in the estuarine environment: a review with application to oyster and clam culture in West Coast (USA) estuaries. *Aquaculture*, 196-223.

Escapa, M., Isaach, J., Daleo, P., Alberti, J., Eribarne, O., & Borges, M. (2004). The Distribution and Ecological Effects of the Introduced Pacific Oyster *Crassostrea gigas* (Thunberg 1793) in Northern Patagonia. *Shellfish Research*.

Favas, P., Pratas, J., Rodrigues, N., D'Souza, R., Varun, M., & Paul, M. (2018). Metal (loid) accumulation in aquatic plants of a mining area: Potential for water quality biomonitoring and biogeochemical prospecting. *Chemosphere*, 194:158-170.

- Feng, W., Wang, T., Zhu, Y., Sun, F., Glesy, J., & Wu, F. (2023). Chemical composition, sources, and ecological effect of organic phosphorus in water ecosystems: a review. *Carbon Research*.
- Filippini, G., Dafforn, K. A., & Bugnot, A. B. (2023). Shellfish as a bioremediation tool: A review and meta-analysis. *Environmental Pollution*, Volume 316, Part 2. doi:<https://doi.org/10.1016/j.envpol.2022.120614>
- Gallardi, D. (2014). Effects of Bivalve Aquaculture on the Environment and Their Possible Mitigation: A Review. *Fisheries and Aquaculture Journal*.
- Ghaly, A. E., Kamal, M., & Mahmoud, N. S. (2005). Phytoremediation of aquaculture wastewater for water recycling and production of fish feed. *Environment International*, 1-13.
- Irfan, S., Modassar, M., Ranjha, A., Shafique, B., Ullah, M. I., Siddiqui, A., & Wang, L. (2022). Bioremediation of Soil: An Overview. *Springer*, 1. doi:[https://doi.org/10.1007/978-3-030-89984-4\\_1](https://doi.org/10.1007/978-3-030-89984-4_1)
- Jiang, Z., Wang, G., Fang, J., & Mao, Y. (2013). Growth and Food Sources of Pacific oyster *Crassostrea gigas* Integrated Culture with Sea Bass *Lateolabrax japonicus* in Ailian Bay, China. *Aquaculture International*, 45-52. doi:10.1007/s10499-012-9531-7
- Kim, J.-H., Kang, Y., Kim, K., Kim, S., & Kim, J.-H. (2019). Toxic effects of nitrogenous compounds (ammonia, nitrite, and nitrate) on acute toxicity and antioxidant responses of juvenile olive flounder, *Paralichthys olivaceus*. *Environmental Toxicology and Pharmacology*, 73-78.
- Labastida, A. V., Jumawan, C. Q., Abogado, A. A., Palma, R. B., & Sabillo, J. J. (2015). Growth performance of brackishwater enhanced selected tilapia (BEST) reared in brackishwater ponds. *SEAFDEC/AQD*. doi:<http://hdl.handle.net/10862/2810>
- Lefebvre, S., Barillé, L., & Clerc, M. (2000). Pacific oyster (*Crassostrea gigas*) feeding responses to a fish-farm effluent. *Aquaculture*, Volume 187, Issues 1-2. doi:[https://doi.org/10.1016/S0044-8486\(99\)00390-7](https://doi.org/10.1016/S0044-8486(99)00390-7)
- Li, M., Callier, M., Blancheton, J., & Gales, A. (2019). Bioremediation of fishpond effluent and production of microalgae for an oyster farm in an innovative recirculating integrated multi-trophic aquaculture system. *Aquaculture*, 314-325. doi:<https://doi.org/10.1016/j.aquaculture.2019.02.013>
- Lin, W., Luo, H., Wu, J., Hung, Tien-Chieh, Cao, B., Yang, P. (2023). A Review of the Emerging Risks of Acute Ammonia Nitrogen Toxicity to Aquatic Decapod Crustaceans. *Water*.
- Lovio-Fragoso, J., de Jesús-Campos, D., López-Eliás, J.A., Medina-Juárez, L.A., Fimbres-Olivarria, D., & Hayano-Kanashiro, C. (2021). Biochemical and Molecular Aspects of Phosphorus Limitation in Diatoms and their Relationship with Biomolecule Accumulation. *Biology*, 10(7): 565.
- Martinez-Cordova, L. R., Robles-Porchas, G. R., Vargas-Albores, F., Porchas-Cornejo, M. A., & Martinez-Porchas, M. (2022). Microbial bioremediation of aquaculture effluents. *Microbial Biodegradation and Bioremediation*, 409-417.
- Martinez-Porchas, M., & Martinez-Cordova, L. (2012). World Aquaculture: Environmental impacts and troubleshooting alternatives. *The Scientific World Journal*.
- Mishra, B., Tiwari, A., & Mahmoud, A. (2021). Microalgal potential for sustainable aquaculture applications: bioremediation, biocontrol, aquafeed. *Springer Nature*.
- Mithra, R., Sivaramaskrishnan, S., Santhanam, P., Dinesh Kumar, S., & Nanakumar, N. (2012). Investigation on Nutrients and heavy Metal Removal Efficacy of Seaweeds, *Caulerpa taxifolia* and *Kappaphycus alvarezii* for Wastewater Remediation. *Journal of Algal Biomass Utilization*, 21-27.
- Neori, A. (2008). Essential role of seaweed cultivation in integrated multi-trophic aquaculture farms for global expansion of mariculture: an analysis. *Journal of Applied Phycology*, 567-570.
- Nhat, P., Ngo, H., Guo, W., Chang, S., Nguyen, D., Nguyen, P., Guo, J. (2018). Can algae-based technologies be an affordable green process for biofuel production and wastewater remediation? *Bioresources Technology*, 491-501.
- Nayak, A., Bhushan, B., & Wilson, I. (2022). Current Soil Bioremediation Technologies: An Assessment. *Springer Nature Switzerland*, 17. doi:[https://doi.org/10.1007/978-3-030-89984-4\\_2](https://doi.org/10.1007/978-3-030-89984-4_2)
- Norita, S., Mohd Noordin, W., Ismail, N., & Hamzah, A. (2021). Red Hybrid (*Oreochromis* spp.) Broodstock Development Programme in Malaysia: Status, Challenges and Prospects for Future Development. *Asian Fisheries Society*, 73-81. doi:[doi.org/1033997/j.afs.2021.34.1.008](https://doi.org/10.1033997/j.afs.2021.34.1.008)
- Papenbrock, A. E., Turcois, A. E., & Papenbrock, J. (2014). Sustainable Treatment of Aquaculture Effluents—What Can We Learn from the Past for the Future? *Sustainability*, 836-856. doi:[10.3390/su6020836](https://doi.org/10.3390/su6020836)
- Pira, H., Risdian, C., Musken, M., Schupp, P. J., & Wink, J. (2022). *Winogradskyella luteola* sp.nov., *Erythrobacter ani* sp. nov., and *Erythrobacter crassostrea* sp.nov., isolated from the hemolymph of the Pacific Oyster *Crassostrea gigas*. *Archives of Microbiology*, 204(8):488.
- Qixing, Z., & Tao, H. (2004). Bioremediation: A review of applications and problems to be resolved. *PROGRESS IN NATURAL SCIENCE*, Vol. 14, No. 11.
- Rodriguez, M., & Montañó, M. (2007). Bioremediation potential of three carrageenophytes cultivated in tanks with seawater from fish farms. *Journal of Applied Phycology*, 755-762. doi:10.1007/s10811-007-9217-0
- Roleda, M., & Hurd, C. (2019). Seaweed nutrient physiology: application of concepts to aquaculture and bioremediation. *Phycologia*, 552-562.



Sato, K., Eksangsri, T., & Egashira, R. (2006). Ammonia-Nitrogen Uptake by Seaweed for Water Quality Control in Intensive Mariculture Ponds. *Journal of Chemical Engineering of Japan*, 247-255.

Seenivasagan, R., Karthika, A., Kalidoss, R., & Malik, J. (2022). Bioremediation of Polluted Aquatic Ecosystems Using Macrophytes. *Springer*, 57. doi:[https://doi.org/10.1007/978-3-030-89984-4\\_4](https://doi.org/10.1007/978-3-030-89984-4_4)

Sivakumar, G., Xu, J., Thompson, R. W., Yang, Y., Randol-Smith, P., & Weathers, P. J. (2012). Integrated green algal technology for bioremediation and biofuel. *Bioresource Technology*, 1-9.

Tanaka, Y., Ashaari, A., Mohamad, F. S., & Lamit, N. (2020). Bioremediation potential of tropical seaweeds in aquaculture: low-salinity tolerance, phosphorus content, and production of UV-absorbing compounds. *Aquaculture*, 518. doi:<https://doi.org/10.1016/j.aquaculture.2019.734853>

Theuerkauf, S., Barrett, L., Alleway, H., Costa-Pierce, B., St. Gelais, A., & Jones, R. (2020). Habitat value of bivalve shellfish

and seaweed aquaculture for fish and invertebrates: Pathways, synthesis and next steps. *Reviews in Aquaculture*, 54-72.

Vairappan, C. (2021). Probiotic fortified seaweed silage as feed supplement in marine hatcheries. *Advances in Probiotics*, 247-258.

Vidal, C., Oliveira, J., Silva, A., Ribeiro, C., & Farnese, F. (2019). Phytoremediation of arsenite contaminated environment. *Ecol Ind*, 104:794–801.

Yong, W., Ting, S., Yong, Y., Thien, V., Wong, S., Chin, W., & Rodriguez, K. (2013). Optimization of culture conditions for the direct regeneration of *Kappahycus alvarezii* (Rhodophyta, Solieriaceae). *Journal of Applied Phycology*, 1579-1606.

Zablon, W., Ogello, E., Getablu, A., & Omondi, R. (2022). Biofloc system improves protein utilization efficiency and growth performance of Nile tilapia, *Oreochromis niloticus* fry: Experimental evidence. *Aquaculture, Fish & Fisheries*, 1-10.

Zhou, Q., & Hua, T. (2004). Bioremediation: A review of applications and problems to be resolved. *Progress in Natural Science*.