# BIOREMEDIATION OF SHRIMP POND EFFLUENT USING OUTDOOR SEAWEED RECIRCULATING CULTURE SYSTEM

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## ABSTRACT

In Malaysia, the uses of water for domestic, commercial and industrial purposes is increasing considerably, resulting in a rapid increase in the wastewater volume and pollutants. The mariculture industries are also facing the same problems. This research focuses on the impact of shrimp farming activities on the environment. The continuing discharge of contaminants from the pond obviously shows that no concern to conform with environmental regulations and good farming practices. Therefore, an efficient treatment system must be developed to improve the water quality at low operational cost and environmentally viable. In the present study, the performance of *Gracilaria changii* and *Gracilaria edulis* as biofilters for nutrients removal from shrimp pond in a laboratory scale and outdoor environment were assessed. The outdoor experiments were conducted in an Outdoor Recirculating Culture System (ORCS). The water flow rate was 200 L/hr. In the laboratory batch culture experiments, both species demonstrated considerably high nutrient removal efficiencies for ammonium, nitrate and phosphate concentrations. The removal efficiencies were 72.5%, 58.8% and 45.9% for G. *edulis,* and 71%, 56.8% and 43.5% for G. *changii,* respectively. The mean specific growth rate (SGR) for G. *edulis* was 3.5 ± 1.0 % $day^{-1}$, while G. *changii* was 3.3 ± 0.9 % $day^{-1}$. As for experiments conducted in ORCS, the nutrient removal efficiencies for ammonium, nitrate and phosphate were 86.2%, 59.3% and 52.0% for G. *edulis,* and 78.1%, 55.5% and 65.9% for G. *changii,* respectively. The mean SGR for G. *edulis* was 3.91 ± 1.0 % $day^{-1}$ and G. *changii* was 3.69 ± 0.6 % $day^{-1}$. The removal efficiency and SGR of G. *edulis and G. changii* in ORCS were higher compared to the laboratory batch culture experiments. The efficiency of biosand filters (BSF) to reduce total suspended solid, turbidity and chlorophyll-a of shrimp pond water was also assessed. The results showed that total suspended solid, turbidity and chlorophyll-a concentrations decreased significantly. The BSF depicted 70.6%, 70.0% and 60.0% efficiencies in the reduction of TSS, turbidity and chlorophyll-a, respectively. The integration of shrimp culture with G. *edulis* in ORCS was conducted as well, and the study proved to be successful. Results showed that shrimp and seaweed grew well in the system. The mean SGR for shrimp and G. *edulis* were 1.31 ± 0.76 % $d^{-1}$ and 4.4 % $d^{-1}$, respectively. High survival rate of shrimp (91%) was observed in the treatment unit. The design considerations, the combination of cultured species and application of G. *edulis* as biofilter in this study provides useful information for aquaculture field. The findings include improvement of shrimp water quality to an acceptable level that ultimately enhances shrimp and seaweeds productivity and produces an ecologically sustainable treatment and cultivation system.

## ABSTRAK

Di Malaysia, peningkatan penggunaan air secara berlebihan untuk kegunaan domestik, komersial dan industri telah meningkatkan isipadu air buangan dan pencemaran. Industri marikultur juga menghadapi masalah yang sama. Kajian ini memberi fokus terhadap kesan aktiviti penternakan udang ke atas alam sekitar. Pembuangan enapcemar secara berterusan dari kolam-kolam ternakan menunjukkan kecenderungan untuk tidak mematuhi peraturan-peraturan alam sekitar dan amalan baik akuakultur. Justeru itu, satu kaedah yang berkesan perlu dibangunkan untuk memperbaiki kualiti air buangan dengan kos yang rendah serta mesra alam. Dalam kajian ini, kecekapan *Gracilaria changii* dan *Gracilaria edulis* sebagai penapis bio bagi mengurangkan nutrient dalam air kolam ternakan udang pada skala makmal dan lapangan telah dinilai. Kajian di lapangan telah dijalankan dalam sistem ternakan secara pusingan. Kadar aliran air adalah sebanyak 200 L/jam. Kajian di makmal menunjukkan kedua-dua spesies ini berjaya meningkatkan kadar kecekapan penyingkiran nutrien seperti ammonia, nitrat dan fosfat. Kadar kecekapan penyingkiran adalah 72.5%, 58.8% dan 45.9% bagi *G. edulis* dan 71%, 56.8% dan 43.5% bagi *G. changii*. Kadar tumbesaran khusus bagi *G. edulis* adalah 3.5 ± 1.0 % hari$^{-1}$ manakala *G. changii* adalah 3.3 ± 0.9 % hari$^{-1}$. Bagi kajian yang dijalankan dalam sistem air pusingan pula, kadar kecekapan penyingkiran untuk ammonia, nitrat dan fosfat adalah 86.2%, 59.3% dan 52.0% bagi *G. edulis* dan 78.1%, 55.5% dan 65.9% bagi *G. changii*. Purata kadar tumbesaran khusus untuk *G. edulis* adalah 3.91 ± 1.0 % hari$^{-1}$ dan *G. changii* adalah 3.69 ± 0.6 % hari$^{-1}$. Kadar kecekapan penyingkiran dan kadar tumbesaran khusus dalam sistem ternakan secara pusingan ini adalah lebih tinggi berbanding dengan kajian di makmal. Keberkesanan penapis pasir untuk mengurangkan jumlah pepejal terampai, kekeruhan dan klorofil-a juga telah dinilai. Keputusan menunjukkan kepekatan jumlah pepejal terampai, kekeruhan dan klorofil-a berjaya diturunkan secara ketara iaitu sebanyak 70.6%, 70.0 % dan 60.0% masing-masingnya. Kajian integrasi udang dengan *G. edulis* dalam sistem air pusingan juga Berjaya dijalankan.. Keputusan menunjukkan udang dan rumpai laut tumbuh dengan baik. Kadar tumbesaran bagi udang dan *G. edulis* adalah masing-masing 1.31 ± 0.76 % hari$^{-1}$ dan 4.4 % hari$^{-1}$. Kadar hidup udang yang tinggi (91%) telah diperhatikan dalam sistem rawatan. Pertimbangan daripada segi reka bentuk, kombinasi spesies ternakan dan penggunaan *G. edulis* sebagai penapis bio dalam kajian ini memberi maklumat berguna kepada bidang akuakultur. Hasil penemuan meliputi peningkatan kualiti air ternakan ke paras yang sesuai untuk meningkatkan pengeluaran udang dan rumpai laut dan seterusnya menghasilkan sistem rawatan yang lestari secara ekologi.

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| | | |
|---|---|---|
| ANOVA | - | Analysis of Variance |
| APHA | - | America Public Health Association |
| BOD | - | Biochemical Oxygen Demand |
| C | - | Carbon |
| CAS | - | Control Aquaculture System |
| Chl-a | - | Chlorophyll-a |
| C:N | - | Carbon to Nitrogen |
| DIN | - | Dissolved Inorganic Nitrogen |
| DMRT | - | Duncan Multiple Range Test |
| DO | - | Dissolved Oxygen |
| DOF | - | Deparment of Fisheries |
| FAO | - | Food and Agriculture Organization |
| FCR | - | Food Conversion Ratio |
| HDPE | - | High Density Polyethylene |
| IMTA | - | Integrated Multitrophic Aquaculture |
| JAS | - | Jabatan Alam Sekitar |
| LSD | - | Least Signicant Difference |
| N | - | Nitrogen |
| NRE | - | Nutrient Removal Efficiency |
| NUR | - | Nutrient Uptake Rate |
| P | - | Phosphate |
| PL | | Post Larvae |
| RAS | - | Recirculation Aquaculture System |
| RGR | - | Relative Growth Rate |
| SA | - | Surface Area |
| SD | - | Standard Deviation |
| SGR | - | Specific Growth Rate |
| SRP | - | Soluble Reactive Phosphorus |
| sp. | - | species |
| TAN | - | Total Ammoniacal Nitrogen |

| | | |
|---|---|---|
| TN | - | Total Nitrogen |
| TP | - | Total Phosphorus |
| TSP | - | Triple Super Phosphate |
| TSS | - | Total Suspended Solid |
| UTM | - | Universiti Teknologi Malaysia |
| UV | - | Ultra Violet |
| WQI | - | Water quality index |
| Wt. | - | Weight |

## LIST OF SYMBOLS

| | | |
|---|---|---|
| $C_o$ | - | Substrate concentration at initial period |
| $C_t$ | - | Substrate concentration at final period |
| $^oC$ | - | Degree celcius |
| t | - | Time |
| *V* | - | Nutrient uptake rate |
| $W_o$ | - | Initial weight of macroalgae |
| $W_t$ | - | Final weight of macroalgae |
| Y | - | Yield |

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# CHAPTER 1

# INTRODUCTION

## 1.1 Background of the Study

In Malaysia, aquaculture plays an important role in food-fish supply. Aquaculture also provides employment and business opportunity to the people of the country. In 2015, there were 23,832 aquaculture farmers (DOF, 2016). Aquaculture is supported by its relatively clean and abundant supply of water with extensive coastline brackish water species contributed to about seventy percent of the btotal aquaculture production.

Shrimp aquaculture is a rapidly growing and is expected to continue this pattern through 2030 due to the increasing global demand and high value of cultured shrimp. Poorly managed coastal shrimp farming has been cited for degrading nearshore water quality through nutrient enrichment (Boyd and Clay, 1998). Shrimp pond effluent is generally greatly enriched in nutrients due to animal feces, added fertilizers and uneaten feed (Primavera, 1993). Nutrient enrichment from shrimp aquaculture is of particular concern due to the projected rapid growth of shrimp industry, potential ecological impacts on adjacent nearshore ecosystems and the possible implications for farm productivity. Consequently, the high amount of nutrient and organic loads will cause eutrophication and occurrence of red tide which affects marine organism and degrades the sustainability of coastal environment (Buschmann *et al.*, 1996; Thakur and Lin, 2003; Fei, 2004)

Most shrimp farms are located near estuaries and mangrove swamps, and this has contributed to increase eutrophication and ecological imbalance of coastal areas. Many studies have shown tropical marine ecosystems to be highly susceptible to the impacts of excess nutrients. For example, nutrient enrichment has been associated with worldwide declines in seagrass and coral reef ecosystems (McGlathery, 2001; Bellwood et al., 2004). Nutrient enrichment could also affect the shrimp farms themselves through self-pollution (intake of their own or neighboring farms' waste effluents). Poor water quality may reduce farm productivity by diminishing shrimp growth and promoting shrimp disease outbreaks (Hargreaves, 1998; Lin, 1989).

Integration of seaweed culture in aquaculture wastewater is considered an ecologically sound practice. Seaweeds are known as potential nutrient bioremediators of excess nutrient from wastewater (Abreu et al., 2011a). Besides, seaweed valuble by-products such as phycocolloid agar, animal feed and biofuel contributes extra income to the farmers. The seaweed genera most common in mariculture biofiltration are *Gracilaria. Gracilaria* have been utilised in many studies such as integration with aquaculture animals in open sea, ponds and as well as in tank culture systems (Buschmann et al., 2007; Marinho-Soriano et al., 2011). In tank cultivation system with salmon, *G. Chilensis* was capable of removing 95% of ammonium and 32% of orthophosphate (Buschmann et al., 1996). A considerable reduction in dissolved ammonium and phosphate will ultimately reduce the risk of eutrophication. It is report tha *G. edulis* removes 54% of the total dissolved inorganic nitrogen from gilthead seabream effluent within 70 days (Hernandez et al., 2005).

In addition to the environmental issues, the discharge of untreated pond effluent represents an economic loss of costly nutrients, thereby reducing farm profitability. One of the major challenges facing the shrimp farming industry is to overcome environmental concerns and to improve economic efficiency by developing and implementing an integrated approach to reducing nutrient waste. Besides improvements in feed formulations and feed management, this approach must include improved design and management of effluent treatment systems at reasonably low cost. This study proposed an integration shrimp culture with seaweed as a viable

treatment approach to balance the negative impacts to the ecosystem. *G. edulis* and *G. changii* were selected as biofilters which have been found to grow naturally in canals around the shrimp cultivation pond and estuaries of Peninsular Malaysia. In order to assess biofiltration capacity of both seaweed, several labroratory and outdoor experiments were conducted.

## 1.2 Problem Statement

The study focused on the production of shrimp and the effect of its effluent to the environment prior to discharge without any proper treatment. The increased number of farms with high shrimp production rates and high effluent discharge has significantly causes environmental degradation in the shrimp ponds and the adjacent waterways (Thakur and Lin, 2003; Anh et al., 2010). The typical duration for shrimp cultivation cycle is 90 to 120 days and harvested after this period. After harvesting, shrimp effluent in ponds will be discharged to the adjacent canals, or waterways without any treatment (Anh et al., 2010) The effluent is highly polluted and causes deleterious effect. Shrimp wastewater produces large amount of excess nutrient, such as ammonia that is harmful for shrimps and organic matter during the cultivation cycle (Marinho-Soriano et al., 2009b)

Reducing the negative environmental impacts associated with aquaculture activities is a key to ensuring the long-term sustainability of the industry. One potential avenue is the increased use of land-based recirculating aquaculture systems (RAS). Such systems allow more effective control of culture conditions, and permit many negative impacts on the surrounding environment to be minimised (Piedrahita, 2003). Waste products can be contained within the facility, habitat degradation is decreased or eliminated and decreased interaction between culture organisms and wild organisms

prevents spread of disease or escape of exotic species. However, the accumulation of nitrogenous wastes is a major drawback of RAS (Wright, 1995).

The amount of nutrients and organic load from the wastewater has to be reduced to ensure better survival and productivity of the shrimp. Typically, shrimp wastewater will be discharged and replenished with fresh seawater as a method of treatment (Anh et al., 2010). However, this method of treatment is not environmentally friendly. Thus, the present study proposed on integration of macroalgae, where it could function as biofilters by removing excess nutrients load in the wastewater. This method is more sustainable and ecologically sound treatment options by using macroalgae to improve the shrimp water quality.

However, when developing the integrated treatment system the choice of macroalgae as biofilters is very impotant. The selection of the potential macroalgae species must take into consideration few aspects such as its tolerance to the hypereutrophic condition of the wastewater, high yield and growth rate, has economic values, abundantly found and thrives naturally, and safe for integration with shrimp. Thus, this study suggests that *G. edulis and G. changii* are assume to meet these requirements. In addition, this study has also solved the bottleneck problem of design consideration for a sustainable treatment system that is affordable by farmers. Interestingly, the choices of species selected, the design consideration of recirculation system is new for this kind of integrated system.

## 1.3 Objectives of the Study

The aim of this study is to develop a low-cost method of effluent treatment system that can improve the water quality of shrimp ponds and to introduce a zero discharge effluent shrimp culture system. Special attention is dedicated on the physicochemical effect and removal performances specifically on the nutrient removal. In addition, organic and nutrient loads were also observed for the enhancement of the treatment system. This can be achieved by the following specific objectives:-

i. To investigate the potential of locally available *Gracilaria* species (*Gracilaria edulis* and *Gracilaria changii*) for bioremediation of nutrients from shrimp pond wastewater in a laboratory scale.

ii. To assess the efficiency of biosand filter (BSF) in reducing total suspended solid (TSS), turbidity and chlorophyll-a.

iii. To investigate the potential of locally available *Gracilaria* species for bioremediation of nutrients from shrimp pond wastewater in an outdoor recirculating culture system model (ORCS).

iv. To perform outdoor recirculating culture system model (ORCS) which integrate seaweeds as biofilters to removes excess nutrients for a period of 90 days.

## 1.4 Scope of the Study

The research work was conducted at Fisheries Research Institute (FRI), Gelang Patah. The main aim of the study is to treat the shrimp pond effluent to maintain optimum water quality for shrimp culture. The study was conducted into three phases.

The first phase of the experiment is to evaluate potential of locally available *Gracilaria sp*. i.e *G. edulis and G. changii* for bioremediation of nutrient from shrimp pond in a laboratory scale batch culture. Batch culture experiment was conduted to determine the optimum biofiltration rate that can be supported by the seaweeds at minimun amount of phytoplankton.

The second phase is to set up an outdoor recirculating culture system model (ORCS) at Fisheries Resarch Institute (FRI), Gelang Patah and to conduct a bioremediation studies using *Graciliaria edulis* and *Gracilaria changii* in ORCS. The recirculating water system consists of fibreglass tanks, seaweeds and biosand filter (BSF). BSF is a biological filter using natural and cheap filtering materials such as gravels and sand. Prior to that, an assessement on the efficiency of biosand filter (BSF) in reducing suspended solid and turbidity will be carried out.

The last phase is to conduct the shrimp culture integrated with seaweeds in an experiment in an outdoor recirculating culture system model (ORCS) for a period of 90 days. The growth of shrimp, growth of seaweed and water quality parameters were monitored.

## 1.5 Significance of the Study

This research focuses on the impact of shrimp farming activities and its impacts to the environment. The continuing discharge of contaminants from this pond (e.g. nutrient and organic wastes) pollutes obviously shows that no concern to conform on environmental regulations and good farming procedures. Therefore, an efficient treatment system must be developed to improve the water quality at low cost operation and environmentally viable. To date, very limited study has been done to develop a systematic local farming discharge system in order to control the effluents.

Aquaculture wastewater treatment have been extensively reported, particularly, for wastewater derived from varieties of fish cultivation integrated with macroalgae cultivation (Hernandez et al., 2005; Yang et al., 2005; Zhou et al., 2006; Hayashi et al., 2008; Abreu et al., 2011b; Huo et al., 2012). However, very few research have been conducted on treatment of shrimp wastewater and improvement of its water quality. Thus, in this study water quality deterioration of shrimp wastewater has been addressed and as a mitigation, application of macroalgae biomass which act as a biofilter has been proposed as a biological treatment options. Besides its potential as biofilters, these macroalgae have considerable economic value and advantageous to be used in integrated system

This study proposed an outdoor recirculating system which integrates shrimp culture with seaweed. The design of this system and execution recirculation approach, significantly improves the water quality and the combination of these species are safe for shrimp culture. In conclusion, the significant of this study is to introduce an integrated shrimp culture with seaweed in an outdoor recirculating culture system model and thus to introduce a zero discharge of wastewater of shrimp culture system.

## 1.6 Thesis Organization

The thesis consist of seven chapters. Chapter 1 consist of an introduction which contains the underlying considerations made in embarking this study. Then, followed the problem statement which highlighted the issues that concern of this study. Next, the objectives of the study to be carried out and achieved. Lastly, the scope of the study which aimed to confine the study to meet the objectives and satisfies the significance of the study.

Chapter 2 presented the collection of literature reviews. Books, journals, literature collections, government reports, and proceedings were sought to form the foundation of this chapter. The prospect and challenges of shrimp and seaweed industry, internationally and nationally were discussed. The significant integration of seaweed with shrimp was described and the efficiency of the existing recirculating aquaculture system was discussed.

Chapter 3 recorded the research materials and methodology applied to assist the research work to meet the objectives. Data analysis was performed using SPSS software. Chapter 4 presents nutrients removal by *Gracilaria* at laboratory scale experiments. Chapter 5 exhibits the potential of seaweed as biofilters in an outdoor tank shrimp wastewater recirculation system. The efficiency of of biosand filter in reducing suspended solid, turbidity and chlorophyll-a were also elaborated in this chapter. Chapter 6 represents the treatment efficiencies of Gracilaria in removing nutrients and maintaining suitable water quality in integrated shrimp culture in a recirculation shrimp culture system. The discussions and conclusions were highlighted in Chapter 7. This chapter also covers the future research recommendation.

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