SEMI-CLOSED CIRCULATION INTEGRATED MULTI-TROPHIC AQUACULTURE TREATMENT SYSTEM USING AQUATIC ORGANISMS AS BIOFILTERS TO IMPROVE SHRIMP EFFLUENT QUALITY

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Dedicated to my lovely

Parents, siblings and friends

thank you for your support, sacrifice and patience all this while

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ABSTRACT

Rapid development of aquaculture industry has contributed to the degradation of the coastal environment, and the waterways receiving effluent discharged. The wastewater contains high amount of excess nutrients and total suspended solids (TSS). Shrimp wastewater is rich in excretory waste products because the cultured shrimps could assimilate only 23-31% nitrogen and 10-13% phosphorus of the total inputs. This significantly causes water quality deterioration, outbreaks of shrimp diseases and affect shrimp production. This study focused on biological treatment option using macroalgae to balance the negative impact to the ecosystem. Thereby, Gracilaria edulis and Ulva *lactuca* were selected and potential as biofilters to improve shrimp water quality has been tested and verified in several laboratory and outdoor tank-scale experiments. Biofiltration potential of macroalgae in outdoor tank shrimp wastewater recirculation system had demonstrated considerably high nutrient removal efficiencies for ammonium, nitrate and phosphate concentrations such as 86%, 53% and 78% for G. edulis and 70%, 42% and 90% for U. lactuca, respectively. Furthermore, semi-closed circulation integrated multitrophic aquaculture treatment system fabricated at outdoor of laboratory with integration of tiger shrimp cultivation and treatment units such as sedimentation tank, green lipped mussel in a spray tank cultivation system and macroalgae tank. Sedimentation tank, reduced TSS, chlorophyll-a, and turbidity by 40%, 22% and 43%, respectively. Mussel system depicted reduction of 65%, 67% and 54.0%, respectively. Whereas, macroalgae had presented remarkable removal efficiencies for ammonium, nitrate and phosphate concentrations by 98%, 79% and 89% for G. edulis and 85%, 63% and 96% for U. lactuca. Besides that, G. edulis had exhibited removal for total nitrogen (TN) by 40%. On the other hand, U. lactuca had shown greater removal for total phosphorus (TP) with 80% removal efficiency, followed by G. edulis by 62.0%. Tissue analysis had demonstrated that the final nitrogen content almost doubled than the initial value in both macroalgae. The final phosphorus content of the tissue in G. edulis has doubled and U. lactuca has shown an increase of about 1.5 times. In addition, the mean growth rate for G. edulis and U. lactuca were about 4.4 % d⁻¹ and 3.7% d⁻¹ respectively. The mean growth rate of shrimp in treatment tank was 1.31 ± 0.76 % d⁻¹, doubled compared to control tank with 100% survival rate. Furthermore, maximum sustainable yield approach revealed the optimum macroalgae biomass for harvest to improve the performance of biofiltration capacity. Thus, G. edulis and U. lactuca are suitable as biofilters and potential applications of these findings include improvement of shrimp water quality to an acceptable level that ultimately enhance shrimp and macroalgae productivity besides produces an ecologically sustainable treatment and integrated system.

ABSTRAK

Pembangunan pesat industri akuakultur telah menyumbang kepada kemerosotan persekitaran pantai, dan laluan air penerimaan pelepasan. Air sisa ini mengandungi jumlah yang tinggi dalam lebihan nutrien dan pepejal terampai jumlah (TSS). Air sisa udang kaya dengan bahan buangan perkumuhan kerana udang yang diternak boleh mengasimilasikan hanya 23-31% nitrogen dan 10-13% fosforus daripada jumlah input. Oleh yang demikian, kualiti air udang bertambah teruk, penyebaran serangan penyakit dan menjejaskan Kajian ini memberi tumpuan kepada pilihan olahan biologi penghasilan udang. menggunakan makroalga untuk mengimbangi kesan negatif kepada ekosistem. Dengan itu, Gracilaria edulis dan Ulva lactuca dipilih dan potensi sebagai biofilter untuk meningkatkan kualiti air udang telah diuji dan disahkan melalui beberapa eksperimen di dalam dan di luar makmal berskala tangki. Potensi makroalga sebagai biofilter di dalam tangki air sisa udang sistem peredaran semula telah menunjukkan efisiensi penyingkiran nutrien yang tinggi untuk ammonium, nitrat dan fosfat seperti 86%, 53% dan 78% bagi G. edulis dan 70%, 42% dan 90% bagi U. lactuca, masing-masingnya. Tambahan pula, sistem olahan semi-peredaran tertutup 'integrated multi-trophic aquaculture' telah difabrikasi di luar makmal dengan integrasi penternakan udang harimau dan unit olahan seperti tangki pemendapan, kupang di dalam sistem 'spray tank cultivation' dan tangki makroalga. Tangki pemendapan, mengurangkan TSS, klorofil-a, dan kekeruhan sebanyak 40%, 22% dan 43%, masing-masing. Sistem kupang menggambarkan pengurangan 65%, 67% dan 54%, masing-masing. Manakala, makroalga telah menunjukkan potensi penyingkiran yang baik untuk ammonium, nitrat dan fosfat sebanyak 98%, 79% dan 89% bagi G. edulis dan 85%, 63% dan 96% untuk U. lactuca, masing-masingnya. Di samping itu, G. edulis telah mempamerkan penyingkiran untuk jumlah nitrogen (TN) sehingga 40%. Sebaliknya, U. lactuca telah menunjukkan penyingkiran lebih besar untuk julah fosforus (TP) dengan efisiensi penyingkiran sebanyak 80%, diikuti oleh G. edulis sebanyak 62%. Analisis tisu telah menunjukkan bahawa kandungan nitrogen akhir tisu hampir dua kali ganda daripada nilai awal dalam kedua-dua makroalga. Kandungan fosforus akhir tisu dalam G. edulis juga telah bertambah dua kali ganda, manakala U. *lactuca* telah menunjukkan peningkatan lebih kurang 1.5 kali dari nilai awal. Purata kadar pertumbuhan untuk G. edulis dan U. lactuca, masing-masing adalah 4.4 % d⁻¹ dan 3.7 % d^{-1} . Sebaliknya, udang telah mencatatkan kadar pertumbuhan dengan nilai purata 1.31 \pm 0.76 % d⁻¹, lebih dua kali ganda berbanding udang di tangki kontrol dengan 100% kadar hidup. Tambahan pula, pendekatan 'maximum sustainable yield' mendedahkan nilai biojisim macroalga optimum untuk tuaian bagi meningkatkan prestasi keupayaan biofiltrasi. Maka, G. edulis dan U. lactuca adalah sesuai sebagai biofilter dan aplikasi penemuan ini termasuk peningkatan kualiti air udang kepada tahap sesuai yang akhirnya juga meningkatkan produktiviti udang dan makroalga, selain meghasilkan sistem olahan dan integrasi yang seimbang dari segi ekologi.

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LIST OF ABBREVIATIONS

BOD	-	Biochemical oxygen demand
С	-	Carbon
Chl-a	-	Chlorophyll-a
C:N	-	Carbon to nitrogen
CO ₂	-	Carbon dioxide
COD	-	Chemical oxygen demand
DMRT	-	Duncan multiple range test
DO	-	Dissolved oxygen
D.wt	-	Dry weight
EPA	-	Environmental Protection Act
FAO	-	Food and Agriculture Organization
FCR	-	Food conversion ratio
G. edulis	-	Gracilaria edulis
HDPE	-	high density polyethylene
IMTA	-	Integrated Multi-trophic Aquaculture
L:D	-	Light and dark
MSY	-	Maximum sustainable yield
Ν	-	Nitrogen
NRE	-	Nutrient removal efficiency

NUR	-	Nutrient uptake rate
Р	-	Phosphate
PES	-	Provasoli Enriched Seawater (PES)
RAS	-	Recirculation aquaculture system
S	-	Substrate concentration
SGR	-	specific growth rate
SRP	-	Soluble reactive phosphorus
sp.	-	species
SSW	-	Sterilized seawater
TAN	-	Total ammoniacal nitrogen
TN	-	Total nitrogen
TP	-	Total phosphorus
TSS	-	Total suspended solid
U. lactuca	-	Ulva lactuca
UTM	-	Universiti Teknologi Malaysia
WQI	-	Water quality index
Wt.	-	Weight

LIST OF SYMBOLS

Co	-	Substrate concentration at initial period
Ct	-	Substrate concentration at final period
Ks	-	Half saturation constant
°C	-	degree celcius
t	-	time
V	-	Nutrient uptake rate
Wo	-	Initial weight of macroalgae
W _t	-	Final weight of macroalgae
SA	-	surface area
Y	-	yield

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CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Intensive shrimp production expands rapidly in most south-east Asian countries as it serves major protein source for human consumption (Naylor *et al.*, 2000; Anh *et al.*, 2010). In Malaysia, shrimp cultivation is supported actively due to its relatively clean and abundant supply of water with extensive coastline. Malaysia is one of the top ten shrimp producers in the world with production capacity of 2.8% in total world production of shrimp. In 2003, 70% of total aquaculture production in the country was generated by brackish water aquaculture species (FAO, 2012a) where shrimp production alone stood at 30,000 tonnes (Hashim and Kathamuthu, 2005) and in 2012 this figure has increased to 65,000 tonnes for meeting shrimp demand for local consumption and export targets (DoFM, 2012a). Furthermore, Asian countries are well known as shrimp producers and exporters since temperate regions have unfavourable environmental condition to culture similar shrimp species.

The rapid expansion of aquaculture industry has contributed to the degradation of coastal environment, due to the discharged of aquaculture wastewater directly to the sea or waterways prior to treatment. Thakur and Lin (2003) had revealed on nutrient budget in a closed shrimp culture system that shrimp could assimilate only 23-31% nitrogen and 10-13% phosphorus of the total inputs. Shrimp wastewater has high load of excess nutrient and organic matter, in the form of excretory waste products and

excess leftover feed materials. 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). Large amount of nitrogen discharge into rivers are notable in Cambodia, Malaysia, Thailand, and Vietnam with a value of 300,000 tonnes of nitrogen per year from various source (Todd *et al.*, 2010). This figure shows a large amount of nitrogen discharged without concern on the impact to the environment.

Integration of macroalgae cultivation in aquaculture wastewater is considered an ecologically sound aquaculture practice since macroalgae are known as potential nutrient strippers to assimilate excess nutrient from wastewater (Abreu *et al.*, 2011a; Kang *et al.*, 2011). Macroalgae biomass also contributes to the economic development with valuable by-products such as phycocolloid agar (Lüning and Pang, 2003; Neori *et al.*, 2003), human food (Norziah and Ching, 2000; Besada *et al.*, 2009), animal feed (Fleurence, 1999) and biofuel (Bastianoni *et al.*, 2008; Migliore *et al.*, 2012). The macroalgae genera most common in mariculture biofiltration are *Ulva* and *Gracilaria* which have been utilised in a few studies integrated with aquaculture animals in open sea, in ponds and as well as in tank culture systems (Buschmann *et al.*, 1996; Troell *et al.*, 1997; Fei, 2004; Hernandez *et al.*, 2005; Msuya *et al.*, 2006; Marinho-Soriano *et al.*, 2011).

Buschmann *et al.*, (1996) found that in an integrated salmon-*G. Chilensis* tank cultivation system, the macroalgae was capable of removing 95% of ammonium and 32% of orthophosphate. In addition, Troell *et al.*, (1997) stated that a considerable reduction in dissolved ammonium and phosphate can be achieved in an integrated culture of the *Gracilaria chilensis*-salmon, which ultimately reduce the risk of eutrophication. Fei (2004) reported that the *Gracilaria* can solve the eutrophication problem with 6.6% of growth rate. Tank cultivation of *G. caudata* and the filter feeder *Artemia franciscana* had biofiltration capacity for ammonium of 29.4% (Marinho-Soriano *et al.*, 2011). The *U. rotundata*, a type of *Ulva* genera, removes 54% of the total dissolved inorganic nitrogen in 600 L effluents of gilthead seabream in 70 days

(Hernandez *et al.*, 2005). Msuya *et al.*, (2006) found that *U. reticulata* can remove total ammonia nitrogen (TAN) of 6.5 g m⁻² day⁻¹ with 65% removal efficiency, which is a significant figure.

Based on the findings, this study proposed the integration of macroalgae for treatment of shrimp wastewater as a viable treatment approach to balance the negative impacts to the ecosystem. The hypothesis of this study is selected macroalgae, *U. lactuca* and *G. edulis* are potential biofilters in removing the excess nutrients from shrimp wastewater effectively and can result in higher biomass production of the shrimp and macroalgae. *G. edulis* and *U. lactuca* were selected as biofilters which have been found to grow naturally in canals around the shrimp cultivation pond and at Sg. Melayu estuaries of Johor Bahru, Malaysia. In order to assess biofiltration capacity of both macroalgae, several labroratory and outdoor experiments were conducted to confirm the hypothesis.

1.1.1 Preamble of Macroalgae

Macroalgae or also known as seaweed, are the most suitable candidate for biofiltration because of their high productivity of all plants and economically feasible to be cultured in large scale (Chopin *et al.*, 2001a; Neori *et al.*, 2004). Macroalgae biomass resource has contributes to large industrial products development such as food, soil conditioner, chemical and pharmaceutical products (Troell *et al.*, 1997; Capo *et al.*, 1999). Besides that, the major industrial use for macroalgae is as a source of phycocolloids, such as agar that mostly used as tissue culture media. The increasing demand for hydrocolloid extracts during the past 40 years has led to dramatic growth of the macroalgae industry in few countries (Briggs and Smith, 1993; Dawes, 1995b; Alveal *et al.*, 1997). In Eastern Asian countries, particularly in Japan, China and Korea, macroalgae cultivation has become an integral part of daily diet as protein source for human and animal feed (Bezerra and Marinho-Soriano, 2010). The society

is becoming aware of the benefits and potential of macroalgae and new algal products. An imminent in novel uses of macroalgae are stimulant to encourage more research and development in macroalgae cultivation (Lüning and Pang, 2003). Thus, cultivation of macroalgae has become a prominent industry where it generates income for the country and improved the socio-economic condition.

Besides that, this naturally growing species, has the potential to act as a biofiltration agent where it considerably reduces pollution load of aquaculture effluent and subsequently improve water quality (Buschmann *et al.*, 1996; Troell *et al.*, 1999; Martínez-Aragón *et al.*, 2002; Neori *et al.*, 2004). As a case study, Ann and his colleagues (2010) had discussed on water pollution occurred rapidly from intensive shrimp farming in south-east Vietnam, to meet the demand of food production for human consumption and impact to the environment. In this perspective, integration of macroalgae cultivation in aquaculture wastewater is a biological approach for mitigation of this problem and has been practiced by few researchers either in integrated manner or in an integrated multi-trophic aquaculture (IMTA) system (Jones *et al.*, 2001; Zhou *et al.*, 2006; Xu *et al.*, 2008a).

Macroalgae are important elements in many proposed integrated or multi trophic level mariculture systems as biofilters. Yet, there are only few successfully tested species integrated with shrimp cultivation out there and little information on economic viability as biofilters is available (Neori *et al.*, 2004; Troell *et al.*, 2003) and it should be economically interesting species to be cultivated. Macroalgae biomass relative growth rate increases by incorporating the excess nutrients of shrimp wastewater (particularly C, N and P) with the presence of solar energy. In integrated aquaculture, waste nutrients are considered not a burden but a resource that auxiliary macroalgae growth (Neori *et al.*, 2004).

1.2 Problem Statement

The study focused on the production of shrimp wastewater 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. The typical duration for shrimp cultivation cycle is 100 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. 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. Figure 1.1 exhibits the problems associated with shrimp effluents.

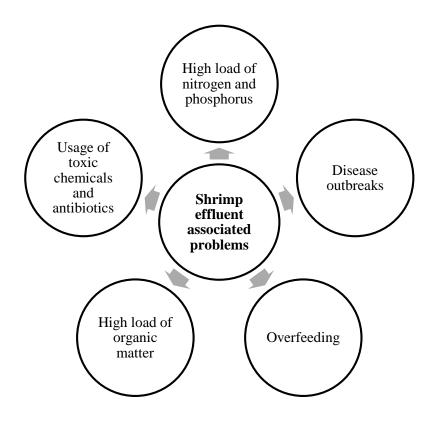


Figure 1.1: Associated problems of shrimp pond effluent.

Consequently, 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. 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 known as a more sustainable and ecologically sound aquaculture practices with biological treatment options by using macroalgae to improve the shrimp water quality.

However, another problem encountered when developing the integrated treatment system such as the choice of macroalgae used as biofilters that will significantly affect the shrimp production. 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, the hypothesis of this study suggests that U. lactuca and G. edulis 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. Typically, the integration involved between, waste produced species and macroalgae only and very little study is dedicated on combination of few species. Thereby, a second hypothesis was pointed out to develop integrated multi-trophic aquaculture (IMTA) treatment system that involved combination of organic extractive species such as green lipped mussel with the inorganic extractive, macroalgae species. Interestingly, the choices of species selected, the design consideration and semi-closed recirculation IMTA system is the breakthrough for this kind of integrated system. Furthermore, the macroalgae biomass improved by the approach of maximum sustainable yield (MSY) that suggests the optimum biomass for harvest to maintain the biofiltration efficiency of the treatment system.

1.3 Objectives of the Study

The aim of this study is to perform modification of an integrated macroalgae and aquaculture treatment system and assess potential of macroalgae as biofilters. 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:-

- 1. To investigate the dynamics of nutrients and organic matter that is produced during shrimp cultivation and characterization of its wastewater constituent for understanding the pollution load that is generated by shrimp cultivation.
- 2. To assess biofiltration capacity and kinetics of nutrient uptake by *G. edulis* and *U. lactuca* for nutrient removal from shrimp wastewater and the effect on macroalgae and shrimp biomass production.
- 3. To perform modification of a semi-closed circulation integrated multi-trophic aquaculture (IMTA) system that efficiently removes excess organic matter and nutrients from shrimp wastewater and reuse of the treated water for shrimp cultivation.
- 4. To perform process optimization of nutrient removal by a maximum sustainable yield (MSY) model approach that predicts the maximum biomass for harvest to maintain the optimum function of the biofiltration system and also biomass production of macroalgae.

1.4 Scope of the Study

This study was conducted using macroalgae species, *Gracilaria edulis* from the brackish water canal located at Fisheries Department of Brackish Water Culture of Gelang Patah, Johor, (PPTAP) and *Ulva lactuca* from the estuaries of Sg. Melayu, Johor, Malaysia. Both macroalgae species possesses different morphology, characteristics and physical properties that follows its taxonomic classification. However, both could act as potential biofilters in treatment of shrimp wastewater and significantly assimilates the dissolved inorganic nutrients.

The eco-physiological properties of the selected macroalgae could adapt and survive to brackish shrimp wastewater condition. In addition, the macroalgae species and green lipped mussels selected were found thrives naturally, for easy accessibility of culture materials. In addition, tiger shrimp, *Penaeus monodon*, of post larvae size was selected to observe its growth throughout shrimp production cycle and physico-chemical characteristics of the wastewater. The biofiltration capacity for nutrient removal and uptake rate investigated by using both macrolagae in several laboratories and outdoor tank-scale experiments. For outdoor tank-scale experiments, two treatment systems were designed- shrimp wastewater recirculation system and semiclosed circulation integrated multi trophic aquaculture (IMTA) system that was built outdoor of Environmental Engineering Laboratory, Universiti Teknologi Malaysia (UTM).

1.5 Significance of the Study

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., 2005b; 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. Application of ecological engineering tools such as IMTA for water pollution control by macroalgae integration is a viable approach for reducing the pollution load caused by shrimp cultivation. 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 IMTA system. In addition, once the biofilter is selected, harvesting rate could be the most important controlling factor in design of a biofiltration system. To date, research on MSY approach to develop a biofiltration system is not given much attention. Thus, the application of MSY concepts was highlighted in this study and contributes to the design of a high efficiency sustainable biofiltration system.

Typically, IMTA system is developed and practiced by a few countries with the aim to produce a more productive, economically profitable and environmentally sound aquaculture system. However, major modification was made where the combination of aquaculture species used were tiger shrimp, green lipped mussel and macroalgae species i.e. *G. edulis* and *U. lactuca*. This is a new configuration which have not been used by others and known as semi-closed circulation integrated multitrophic aquaculture (IMTA) system. In addition, the design of this system and execution in a semi-closed circulation approach, significantly improves the water quality and the combination of these species are safe for shrimp cultivation. Besides that, frequent water exchange that associated with the possibility of diseases outbreak during shrimp cultivation also improved in this closed circulation IMTA system. Thereby, this study claims novelty for the design of this integrated treatment system and has been patented. The importance of this study are as follows:-

- I. This study has revealed the amount of wastewater discharged that is produced from shrimp cultivation throughout its life cycle. The fate of nutrients that enter the shrimp tank in the form of feed were also identified.
- II. The study exhibited that the selected macroalgae species can be cultured in shrimp wastewater and produces extra biomass while utilizing nitrogen and phosphorus from the wastewater.
- III. This study provides a systematic treatment design which could be applicable for massive scale of shrimp cultivation and reuse of treated wastewater via the fabrication of a semi-closed circulation integrated multi-trophic aquaculture (IMTA) system.
- IV. This study also provides information to design an integrated treatment system with combination of several treatment units and species successfully with considerable reduction of nutrients and organic load, that results in better yield of macroalgae and shrimp production.
- V. The study provides information regarding MSY approach that can be applied for maximum harvest of macrolagae biomass for optimum biofiltration capacity.
- VI. This study also offers multi-product harvesting at the end of the treatment stage and conversion into useful products where macroalgae biomass could be processed into fertilizer or animal feed, with shrimp and mussel for seafood commercialization purposes.

1.6 Thesis Organization

The thesis consist of seven chapters. Chapter 1, introduction of the study was explained, which contains the underlying considerations made in embarking this study. Problem statement of the selected issues that concern of this study were highlighted. Followed by the objectives or motivation for the study to be carried-out, which reflects the solution proposed for the problems proclaimed previously. The scope of the study aimed to confine the study to meet the objectives and satisfies the significance of the study. Lastly, the output of the study would be required to conform the significance of study which is known as the driving force for the overall research that has been reported in this thesis.

Collection of literature reviews presented in Chapter 2. Many books, journals, literature collections, government reports, and proceedings were sought to form the foundation of this chapter. The prospect and challenges of shrimp and macroalgae industry, internationally and nationally were discussed. The significant integration of macroalgae with aquaculture wastewater was described and the efficiency of the existing IMTA system was discussed. The approach of maximum sustainable yield (MSY) is also pointed out.

Chapter 3 recorded the research materials and methodology applied to assist the research work to meet the objectives. Data analysis performed using Minitab software. Chapter 4 presents few laboratory experiments to assess on factors that affects macroalgae growth. Chapter 5 exhibits the potential of macroalgae as biofilters in an outdoor tank shrimp wastewater recirculation system integrated with macroalgae. Eventually, Chapter 6 represents the combined treatment efficiencies in a semi-closed circulation integrated multi-trophic aquaculture (IMTA) system. The conclusions were highlighted in Chapter 7. Moreover, this chapter also covers future research recommendation and exploration extended from this study.

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