THE PERFORMANCE OF SEAWEED *Kappaphycus alvarezii* AND *Kappaphycus striatum* IN TANK CULTURE SYSTEM

WAHIDATUL HUSNA ZULDIN

THESIS SUBMITTED IN PARTIAL FULFILLMENT FOR THE DEGREE OF MASTER OF SCIENCE

**BORNEO MARINE RESEARCH INSTITUTE
UNIVERSITI MALAYSIA SABAH**

2015

UNIVERSITI MALAYSIA SABAH

BORANG PENGESAHAN STATUS TESIS

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89157 Kota Belud,
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Tarikh: 15 Oktober 2015

(Tandatangan Pustakawan)

(Prof. Dr. Rossita Shapawi)
Penyelia

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15th October 2015

Wahidatul Husna Zuldin
MY1221006T

CERTIFICATION

NAME : **WAHIDATUL HUSNA ZULDIN**

MATRIC NO. : **MY1221006T**

TITLE : **THE PERFORMANCE OF SEAWEED *Kappaphycus alvarezii* AND *Kappaphycus striatum* IN TANK CULTURE SYSTEM**

DEGREE : **MASTER OF SCIENCE (AQUACULTURE)**

VIVA DATE : **17TH SEPTEMBER 2015**

CERTIFIED BY

1. **SUPERVISOR**
   Prof. Dr. Rossita Hj. Shapawi

Signatures

2. **CO-SUPERVISOR**
   Assoc. Prof. Dr. Suhaimi Md. Yassir

ACKNOWLEDGEMENTS

First and foremost, I would like to thank Allah the Almighty for all the opportunities and blessings given in my life. This research would have never been done without His blessings, wisdom, favour and protection. Through Him all things are possible. To my dearest supervisor, Prof. Dr. Rossita Hj. Shapawi, thank you for your amazing supervision, guidance, support, encouragements, constructive ideas, patience, kindness and enthusiasm contributed throughout my research and the writing processes. I would also like to express my gratitude to Assoc. Prof. Dr. Suhaimi Md. Yassir as my co-supervisor; I have benefited a lot from your constructive advices and views. All the kindnesses, encouragements and motivations made me matured as a student and future academician. Furthermore, I would also like to express my trillions appreciation to all the lecturers and staffs in Borneo Marine Research Institute (BMRI), who have helped and guided me in their own way to complete these research and thesis.

I would like to dedicate this work to my beloved parents and siblings for their love, hope, faith, endless encouragement, continued moral support as well as building up the values of hard work and respect in me. Also, my heartfelt thanks to all my fellow colleague and friends especially Syafiqah Saufie, Nik Nurhusna Nik Sin, Amirul Rashid Zaini and Chor Wei Kang for their continuous helps and supports throughout my research. Special thanks to all the staffs and management, Lusia Ransangan and the team in Shrimp Hatchery, IPMB, UMS and also Pulau Selakan Seaweed Farm, the cooperation is highly appreciated. Lastly, I am in great debt to those that I not mention here who have unselfishly given their time, talents and supports to assist me throughout my research. I would like to say thank you for all the supports. It would be impossible for me to complete this research without all the encouragements and supports.

Wahidatul Husna Zuldin

15th October 2015

ABSTRACT

Little is known on the performance of seaweed cultured in land-based facilities. The present study was conducted to determine the growth performance of *Kappaphycus* in tank and the effects of tank culture to the seaweed biochemical composition. Two tank culture systems were completed using circular and raceway tanks and three cultures of 40-days period were performed in each system. Red seaweeds, *Kappaphycus alvarezii* and *Kappaphycus striatum* were selected and cultivated in each tank culture system. Using circular tank, four different treatment tanks (T1 with only filtered seawater, T2 with fertilizer enrichment only, T3 with substrate addition only and T4 with the combination of fertilizer enrichment and substrate addition) were tested. In the circular tank culture system, both species were mutually cultivated via line culture method and the seaweed density was reduced accordingly in every culture (Culture 1: 2.40 g L^{-1}, 2: 1.92 g L^{-1} and 3: 0.96 g L^{-1}). Using Two-way ANOVA statistical analysis, there were no significant differences between the between the factors tested (fertilizer enrichment and substrate addition) and the daily growth rate of *Kappaphycus* ($p>0.05$) in every culture with different seaweed density. The maximum daily growth rate was recorded in T4 with lowest seaweed density of 0.96 g L^{-1} during Culture 3 on *K. striatum* (2.00±0.03% day^{-1}) and *K. alvarezii* (1.46±0.06% day^{-1}). In the second part of study, raceway tank was used and modified without any fertilizer enrichment and substrate addition needed. Both *Kappaphycus* were cultured using loose method in raceway tank. The results indicated that both *Kappaphycus* succeeded to grow with the highest average daily growth rate for *K. striatum* was 2.96±0.02% day^{-1} and for *K. alvarezii* was 2.13±0.03% day^{-1} with zero nutrient or substrate enrichment during Culture 2. *Kappaphycus striatum* was found to grow well in both system compared to *K. alvarezi.* The results also showed that the biochemical composition of both tank cultured *Kappaphycus* were comparable with the open sea culture where the average percentage of crude protein, crude fibre and carrageenan content of the *Kappaphycus* cultured in the tank were fall within the range mentioned in the previous literature. Thus, tank culture system did not significantly affect the biochemical composition of the *Kappaphycus.* In conclusion, the success in cultivating *Kappaphycus* using circular and raceway tank indicated that seaweeds are highly potential to be cultivated in land-based facilities comparable with the open sea culture seaweeds. These findings are significant to provide a baseline data and facilitate seaweed research area especially for high quality seaweed seedling production in the future.

ABSTRAK

PRESTASI RUMPAI LAUT Kappaphycus alvarezii DAN Kappaphycus striatum DALAM SISTEM PENGKULTURAN TANGKI

Sedikit yang diketahui mengenai prestasi pengkulturan rumpai laut di dalam kemudahan darat. Kajian ini telah dijalankan untuk menentukan prestasi pertumbuhan Kappaphycus dalam tangki dan kesan penggunaan tangki terhadap komposisi biokimia rumpai laut. Dua sistem pengkulturan tangki telah dilaksanakan menggunakan tangki bulat dan "Raceway" melibatkan tiga kali kultur dalam 40-hari tempoh pengkulturan bagi setiap tangki. Rumpai laut merah, Kappaphycus alvarezii dan Kappaphycus striatum telah dipilih dan dikultur di dalam setiap system pengkulturan tangki. Menggunakan tangki bulat, empat tangki rawatan (T1 hanya menggunakan air laut yang ditapis, T2 menggunakan aplikasi pertambahan baja sahaja, T3 menggunakan aplikasi pertambahan substrat sahaja, dan T4 menggunakan gabungan pertambahan baja dan substrat ke dalam tangki) telah diuji. Dalam tangki bulat, kedua-dua spesies ditanam melalui kaedah sistem kultur tali di mana ketumpatan rumpai dikurangkan pada setiap pengkulturan (Kultur1: 2.40 g L^{-1}, 2: 1.92 g L^{-1}dan 3: 0.96 g L^{-1}). Menggunakan ujian analisis statistik varian secara dua hala, tidak terdapat perbezaan yang signifikan ($p>0.05$) pada kedua faktor yang diuji iaitu pertambahan baja dan substrat dengan kadar pertumbuhan harian Kappaphycus pada setiap kultur yang mempunyai ketumpatan rumpai yang berbeza. Kadar pertumbuhan harian maksimum dicatatkan dalam tangki bulat dengan kepadatan stok terendah, 0.96 g L^{-1} ketika Kultur 3 pada K. striatum adalah 2.00±0.03% $hari^{-1}$dan K. alvarezii adalah 1.46±0.06% $hari^{-1}$. Dalam bahagian kedua kajian ini, tangki "Raceway" telah digunakan dan diubah tanpa perlu menggunakan penambahan baja dan substrat. Kedua-dua, Kappaphycus telah dikultur menggunakan kaedah pengampaian dalam tangki "Raceway". Hasil kajian menunjukkan bahawa kedua-dua Kappaphycus berjaya dikultur dengan kadar pertumbuhan purata harian tertinggi pada Kultur 2 sebanyak 2.96±0.02% $hari^{-1}$ untuk K. striatum dan 2.13±0.03 % $hari^{-1}$untuk K. alvarezii dengan sifar nutrient atau penambahan substrat. Kappaphycus striatum dipercayai berupaya untuk tumbuh dengan lebih baik di dalam setiap sistem berbanding K. alvarezii. Hasil kajian juga menunjukkan bahawa komposisi biokimia bagi Kappaphycus yang dikultur menggunakan tangki adalah setanding dengan rumpai laut yang dikultur di dalam laut, di mana peratusan purata kandungan protein, serat dan karrageenan Kapppahycus yang dikultur dalam tangki adalah di dalam julat yang dicadangkan dalam kajian lepas. Maka, sistem pengkulturan tangki tidak memberi impak kesan yang signifikan terhadap komposisi biokimia Kapppahycus. Kesimpulannya, kejayaan dalam mengkultur Kappaphycus menggunakan tangki bulat dan "Raceway" menunjukkan bahawa rumpai laut sangat berpotensi untuk ditanam di kemudahan darat setanding dengan rumpai laut yang dikultur di dalam laut. Penemuan ini adalah penting untuk menyediakan data asas dan memudahkan bidang penyelidikan rumpai laut terutamanya pembuatan benih rumpai berkualiti tinggi pada masa akan datang.

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LIST OF SYMBOLS AND ABBREVIATION

°	-	Degree
°C	-	Degrees Celcius
%day^{-1}	-	Percent per day
ug-atm/l	-	Microgram Atmosphere per liter
AMPEP	-	Acadian Marine Plant Extract Powder
ANOVA	-	Analysis of Variance
NSE	-	Natural Seaweed Extract
DO	-	Dissolved Oxygen
G	-	Gram
GF	-	GoFar60
gL^{-1}	-	Gram per liter
GNI	-	Gross National Income
HDPE	-	High Density PolyEthylene
H_2SO_4	-	Sulphuric Acid
HCl	-	Hydrochloric Acid
mg/L	-	Milligram per liter
NO^-_3-N	-	Nitrogen-Nitrite
NO^-_2-N	-	Nitrogen-Nitrate
NH_3-N	-	Nitrogen-Ammonia
Ppt	-	parts per trillion
T1	-	Treatment 1
T2	-	Treatment 2
T3	-	Treatment 3
T4	-	Treatment 4
UMS	-	Universiti Malaysia Sabah
µmol m^{-2}s^{-1}	-	Micro molar meter per second

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Seaweed farming is one of the top priorities set for development in Malaysia due to the increasing world demand for raw (biomass) and processed seaweed especially the commercial red seaweed, *Kappaphycus* that is important as the main source of carrageenan (Bindu and Levine, 2011). The seaweed farming activities has been identified as one of the entry point projects (ETPs) in Malaysia since the government introduced the Economic Transformation Programme, which aims to increase seaweed production up to 150,000 tons annually by 2020. Indeed, the reported global world demand of seaweed in 2012 was about 350,000 to 400,000 metric tonnes (Yassir, 2012).The seaweed farming industry was introduced to Malaysia in 1978 and the activity is rapidly on-going especially in Sabah as the only commercial seaweed producer in Malaysia (Sade and Ariff, 1987).

Most of the seaweed farming in Malaysia and several other countries such as Philippines, Indonesia, Vietnam and Myanmar involve open sea cultivation. The open sea cultivation of *Kappaphycus* (Rhodophytes) is adverse in Semporna waters especially for *K. alvarezii* and *K. striatum*. The common cultivation method practiced by local seaweed farmers in Semporna involves the repetitive vegetative propagation method using fixed long-line "tie-tie" culture system (Ali *et al.*, 2014). Vegetative propagation of *Kappaphycus* is easily done by breaking off and planting individual large pieces of the thalli (Doty, 1985). This is the most commonly used farming method currently used (Trono, 1974; Azanza-Corrales *et al.*, 1996). Normally, seaweeds are cultivated offshore using fixed long-line method and harvested after 30 to 60 days. Nevertheless, the offshore cultivation is quite risky with the ambiguous dependency on the seasonal growth variation, epiphytic infection and grazer's threats of turtles and fishes (Neish, 2003). Thus, the land-based tank cultured seaweed might lessen the risks on the threats in open sea cultivation.

However, the main concern on land-based seaweed cultivation is actually for research purposes; for instance, the production of high quality seaweed seedling in nursery through selective breeding, hybridization or grafting instead of using in-vitro micro-propagation technique by tissue culture. In 2007, Sabah government was looking to the possibility of applying seaweed tissue culture technology to improve the seaweed production efficiency by producing high-quality seaweed seedlings that feature high gel strength, faster growth rate and high resistance towards disease (Daily Express, 2007). Yong *et al.*, (2011) had performed a research on the in-vitro micropropagation of *Eucheuma* seaweeds in Universiti Malaysia Sabah and the result showed that the *Eucheuma* species had higher growth rate with the optimum condition including continuous aeration, suitable culture medium, temperature of 25°C to 30°C and salinity of 32 ppt. Nevertheless, the problem encountered during the field trial of the micropropagated *Kappaphycus alvarezii* (Doty) Doty ex Silva eventhough the seaweeds had gone through a successful acclimatization process in outdoor nursery system (Yoong *et al.*, 2013). Therefore, land-based seaweed cultivation might reduce the problem on the field trial of the micropropagated seaweed since the mock sea condition could be created in the tank culture system.

On the other hand, little is known for land-based seaweed cultivation using tank culture system especially in Malaysia. Recently, few researches were done on the *Kappaphycus* in terms of the nutritional content, micropropagation and efficiency of favourable cultivation method. Nevertheless, the cultivation of *Kappaphycus* in land-based nursery system such as in tank is very uncommon in Malaysia. Limited information is available for the seaweed cultivation in tank culture system especially in Malaysia. Therefore, the effort to culture seaweed in land-based facilities such as tank culture system deserved an investigation.

1.2 *Kappaphycus* as Farming Species

In this study, *Kappaphycus* (*K. alvarezii* and *K. striatum*) were selected as the experimental subject. These species were chosen due to the high commercial value as carrageenophytes producing marine biopolymer known as carrageenan. Carrageenophytes are the group that become the key source of *k*-carrageenan which is very significant in the phycocolloids industry. Carrageenan is the significant compound from seaweeds that can be applied commercially as gelling, thickening and stabilizing agents especially in food products, pharmaceutical formulation, cosmetics and also industrial usage such as mining (Naguit *et al.*, 2009). In fact, the commercial cultivation of the red seaweed, *Kappaphycus alvarezii* (Doty) Doty ex Silva has been satisfying the demand of the global carrageenan industry for more than 40 years (Bindu and Levine, 2011). In Malaysia, the seaweed farming industry is growing very fast nowadays with the increasing global demands for the dried seaweed supply. According to Dhargalkar and Pereira (2005), seaweeds are one of the important marine living resources that could be termed as futuristically promising plants with high market price value as these plants have been the source of food, feed and medicine in most of the Asian and Western countries worldwide.

Malaysian government has taken the initiative to further develop the seaweed farming industry with the opening of seaweed mini-estate system. The currently operating mini-estate is located in the Selakan Island, Semporna, Sabah and the most abundantly cultivated seaweed in the mini-estate are the *Kappaphycus* and *Eucheuma*. Indeed, the *Kappaphycus* is abundantly cultivated in the East Coast waters of Sabah, Malaysia. *Kappaphycus* was chosen in the present study due to the abundance, availability and good quality indicated by previous researchers in terms of the growth rate and the carrageenan content. *Kappaphycus alvarezii* is believed to have higher growth rate and carrageenan content (Neish, 2003). Meanwhile, *K. striatum* is believed to have high resistance towards diseases. One of the concerns in this study was to determine the performance of *Kappaphycus* in tank culture system focusing on the daily growth rate and biochemical composition. In terms of reproduction, *Kappaphycus* has the ability to reproduce through both asexual and sexual ways (Bulboa *et al.*, 2008). *Kappaphycus* is also believed to be able to undergo spores germination and vegetative propagation (Ask and Azanza, 2002). In fact, vegetative propagation is highly applicable for seaweed cultivation in tank using the hanging and suspension culture methods. Therefore, *Kappaphycus* might be able to survive and grow in tank through vegetative propagation with the presence of natural physical seawater quality parameters in the tank culture system.

1.3 Factors Affecting the Growth and Biochemical Composition of *Kappaphycus*

Among the limiting factors affecting the growth and biochemical composition of *Kappaphycus* are depth, light intensity, water motion or flow, salinity, dissolved oxygen level (DO), pH, temperature, water nutrient content, irradiance, nutrient enrichment, seaweed density and seasonal variation (Neish, 2003). *Kappaphycus* generally shows great variation in the nutrient content depending on the water temperature, salinity, light and nutrients availability for uptake (Kumar *et al.*, 2014). The present study was conducted to investigate the growth performance and biochemical composition of *Kappaphcyus* cultivated in the circular and raceway tank culture system. The growth performance was observed on the daily fresh weight increment of *Kappaphycus* and the biochemical composition was focused on the crude protein, crude fibre, ash, moisture and carrageenan content.

Using circular tank culture system, *Kappaphycus* was cultivated in three different culture time with improved seaweed density during every culture. The seaweed density referred to the total weight of *K. alvarezii* and *K. striatum* initially placed in the circular tank. The study hypothesized that reducing the seaweed density might increase the daily growth rate of the *Kappaphycus* in circular tank. The effect of fertilizer enrichment on the growth of *Kappaphycus* was also observed in every culture. The first hypothesis made was that the addition of Acadian Marine Plant Extract Powder, AMPEP fertilizer in the tank might increase the growth rate of *Kappaphycus* in the tank. Besides, the substrate addition was also tested on the growth of *Kappaphycus* through the addition of sands and corals in the tank in every culture. According to Preisig and Hans (2005), areas with coarse sandy to corally bottom substrate are suitable for seaweed cultivation. Therefore, the study hypothesized that the addition of sand and corals imitating the condition at real sea might aid in promoting the growth of *Kappaphycus* in the tank. In the second part of the study, after all the failures encountered with circular tank; the performance of seaweed was tested through an improved system using raceway tank to evaluate the growth and biochemical composition.

1.4 Tank Culture System

Circular and raceway tanks were used in the study to determine the performance of *Kappaphycus* in the tank culture system. The different types of tanks were implemented in two stand-alone experiments. Both systems were designed with the imitations of the sea condition suitable for *Kappaphycus* including depth, culture method and presence of substrate. Initially, the intention was to determine the possibility of *Kappaphycus* being hybridized by grafting using the circular tank culture system for research purpose. The aim was to improve the seaweed seed production by having hybridized *Kappaphycus* with selected criteria. Thus, both *Kappaphycus* were placed in the same circular culture tank and termed as mix-culture. Nevertheless, the grafting process failed after more than three trials and the research was proceed with determining the performance of *Kappaphycus* in the tank culture system for future research works. Still, the circular tank culture system failed to maintain the good growth of *Kappaphycus* in the tank due to several factors such as slow water movement in the tank. Therefore, new idea was generated to design a raceway tank with vigorous water movement and modified continuous aeration using the bubble-disc aeration stone with high air dispersion.

1.5 Significance of Study

In general, seaweed requires low capital investment and has a rapid annual rate of return. For instance, red seaweed (*Kappaphycus*) has high price especially for the carrageenan. Nowadays, offshore cultivation or also known as open sea cultivation is commonly practiced by most of the seaweed farmers in Asian countries such as Malaysia, Vietnam and Japan. However, threats were often faced by the farmers such as the predators (fishes and turtles) and also uncontrolled epiphytes in the open sea. Despite of that, the main problem faced by the industry is the lack of high quality seed stock production that might lead to increased production of high quality seaweed product.

Therefore, land-based cultivation might be an alternative way of cultivating seaweed in a controlled environment with fewer threats and become significant for good quality seed production facilitating experimental research proceduressuch as acclimatization of seedling produced from the laboratory tissue culture and genetics study.Other than that, food security can be promised with safe culture environment on land-based culture facilities. The methods used in this study are very practical and transferable to the local community especially the seaweed farming manpower. The present study might also help in leveraging the yield production of high quality seaweeds and act as one of the "blue-economy" strategy in sustaining the seaweed supply in the future. This experiment might be the first one conducted in Malaysia since there is not much study being done previously on seaweed culture in tank. Thus, this present study could provide a baseline data for the seaweed research and lead to the latest study on land-based seaweed cultivation in Malaysia especially for research purposes.

1.6 Objectives

The research focused on four main objectives:

a. To determine the growth and biochemical composition of *K. alvarezii* and *K. striatum* cultivated in circular tank.
b. To determine the suitable seaweed density of *K. alvarezii* and *K. striatum* in the circular tank.
c. To investigate the effect of fertilizer enrichment and substrate addition on the growth performance and biochemical composition of *K. alvarezii* and *K. striatum* in circular tank.
d. To determine the growth and biochemical composition of *K. alvarezii* and *K. striatum* in raceway tank.

CHAPTER 2

LITERATURE REVIEW

2.1 Seaweeds

Seaweeds are categorized as benthic marine macroalgae that generally distributed at the coastal area attached to the rock or hard substrate at the bottom of sea (Mine, 2008).Seaweeds are also known as marine plants due to the photosynthetic behaviour of converting sunlight into food sources and similar biochemical and physiological features with the higher plants (Dhargalkar and Pereira, 2005). In general, seaweeds are divided into three major groups; empirically distinguished by the Irish botanist William Henry Harvey (1811-1866) based on the colour of the thallus: red seaweed (Rhodophyta), brown seaweed (Phaeophyta), and green seaweed (Chlorophyta). The seaweed groups differ considerably in many ultra-structural and biochemical features including photosynthetic pigments, storage compounds, composition of cell walls, presence/absence of flagella, and ultra-structure of mitosis, connections between adjacent cells, and the fine structure of the chloroplasts (Hayes, 2011). Other than that, algae in general are classified into six different classes as shown in Figure 2.1.

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