DEVELOPMENT OF BIO-GREEN FLOATING AQUACULTURE SYSTEM AS BIOREMEDIATION FOR IMPROVEMENT OF WATER QUALITY AND GROWTH PERFORMANCE OF RED HYBRID TILAPIA

By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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Chairman : Professor Fatimah Md. Yusoff, PhD
Faculty : Agriculture

Aquaculture has been recognised as an important sector for income generation and economic development for many countries, especially in Asia where 89% of aquaculture products are generated. While aquaculture is the fastest-growing food sector worldwide, its production is still limited by feed quality, water quality deterioration, and diseases. In addition, untreated waste discharges from aquaculture activities can cause eutrophication in public waters. This study aimed to reduce nutrients, improve water quality and increase fish production in aquaculture systems by enhancing the nutrient uptake rate using plants and biofilms assembled as an efficient nutrient uptake system known as a bio-green floating aquaculture system (BFAS). The bio-green floating aquaculture system (BFAS) comprised an assemblage of biofilm-coated substrates and plants which were able to reduce nutrients, especially inorganic nitrogen and phosphorus from the aquaculture system.

In this study, three different plant species; water spinach (*Ipomoea aquatica*; WS), lemon basil (*Ocimum* × *africanum*; LB), and aromatic basil (*Ocimum basilicum*; AB), single and in combination (WS, LB, AB, WS+LB, WS+AB, LB+AB, WS+LB+AB) were tested to determine their nutrient uptake ability and plant growth performances. The highest ($P < 0.05$) nutrient uptake removal (39.08% of total nitrogen, 88.06% of total ammonia nitrogen, 72.61% of nitrite+nitrate nitrogen, 82.06% of total phosphorus, and 77.75% of soluble reactive phosphate) and plant growth performance was shown better in two combinations of water spinach and lemon basil (WS+LB).

A total of five different substrates (bamboo; BO, lava rock; LR, polyvinyl chloride; PVC, bio-ball; BA, bio-ring; BR) were used to investigate their periphyton abundance. Periphyton colonisation on lava rock and bamboo showed the highest ($P < 0.05$) biomass (2.18 ± 0.09 mg cm^{-2} and 2.05 ± 0.11 mg cm^{-2}, respectively) and periphyton abundance on the substrate amongst all the substrates used. A combination of individually selected plants (water spinach and lemon basil) and selected substrates (lava rock and bamboo) were tested to determine nutrient uptake efficiency and plant growth performance. It was found that nutrient removal efficiency in a single substrate (lava rock) combined with a single plant (water spinach) was significantly higher ($P < 0.05$) compared to other treatments with reduction of TAN (88%), NO_2-N (67%) and PO_4-P (30%). Water spinach yield showed the highest ($P < 0.05$) production compared to lemon basil. Results of this study showed that the combination of lava rock and water spinach efficiently controlled water quality with less accumulation of TAN, NO_2-N and PO_4-P. In addition, the attached biofilm, coupled with microbial nitrification (*Nitrospira*, *Nitrosomonas*, *Prosthecobacter*) and denitrification (*Acidovorax*, *Bosea*, *Dechloromonas*, *Flavobacterium*, *Thermomonas*, *Thiobacillus*), also played a significant role in the removal of nitrogen in the water spinach integrated with the lava rock system.

The development of BFAS using the selected plant (water spinach) integrated with the selected substrate (lava rock) with zero water exchange was used to investigate the efficiency of water quality improvement and production of monosex red tilapia (*Oreochromis* sp.) in 1000 L tanks. The growth performances of fish in BFAS were significantly higher ($P < 0.05$) compared to other treatments with the survival rate of 92.31% and specific growth rate of 3.58. The mean of nutrient concentrations in BFAS treatment were significantly lower ($P < 0.05$) compared to other treatments with the concentration of 0.01 mg L^{-1} in NH_3-N, 0.06 mg L^{-1} in NO_2-N and 0.75 mg L^{-1} in PO_4-P. Treatment with BFAS had the lowest FCR (1.16 ± 0.03) and also better fish health conditions compared to the treatments with non-BFAS with water exchange (1.50 ± 0.05) and without water exchange (2.15 ± 0.02). Furthermore, heavy metal concentrations in fish and plants of BFAS were safe for human consumption as the accumulation levels of the heavy metals were lower than the recommended safe limit. In addition, the most dominant bacteria in BFAS including Bacteroidota, Proteobacteria, and Nitrospirota were found, which are very important for nutrient cycling in the aquaculture system. This study illustrated that the newly developed BFAS containing substrates (lava rock) and plants (water spinach) could maintain the optimal water quality, providing better fish production, plant yields and lower production cost for tilapia aquaculture system.

Keywords: BFAS; monosex red tilapia; nutrient removal; periphyton (biofilm); plant; substrate

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PEMBANGUNAN SISTEM AKUAKULTUR TERAPUNG BIO-HIJAU SEBAGAI BIOREMEDIASI UNTUK PENINGKATAN KUALITI AIR DAN PRESTASI PERTUMBUHAN TILAPIA HIBRID MERAH

Oleh

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Akuakultur telah diiktiraf sebagai sektor penting untuk penjanaan pendapatan dan pembangunan ekonomi bagi banyak negara, terutamanya di Asia di mana 89% produk akuakultur dijana. Walaupun akuakultur adalah sektor makanan yang paling pesat berkembang di seluruh dunia, pengeluarannya masih terhad oleh kualiti makanan, kemerosotan kualiti air dan penyakit. Selain itu, pembuangan sisa yang tidak dirawat daripada aktiviti akuakultur boleh menyebabkan eutrofikasi di perairan awam. Kajian ini bertujuan untuk mengurangkan nutrien, meningkatkan kualiti air dan meningkatkan pengeluaran ikan dalam sistem akuakultur dengan meningkatkan kadar pengambilan nutrien menggunakan tumbuhan dan biofilm yang dipasang sebagai sistem penyerapan nutrien yang cekap dikenali sebagai sistem akuakultur terapung bio-hijau (BFAS). Sistem akuakultur terapung bio-hijau (BFAS) terdiri daripada himpunan substrat dan tumbuhan bersalut biofilm yang mampu mengurangkan nutrien, terutamanya nitrogen dan fosforus tak organik daripada sistem akuakultur.

Dalam kajian ini, tiga spesies tumbuhan berbeza; bayam air (*Ipomoea aquatica*; WS), selasih limau (*Ocimum* × *africanum*; LB), dan selasih aromatik (*Ocimum basilicum*; AB), tunggal dan gabungan (WS, LB, AB, WS+LB, WS+AB, LB+ AB, WS+LB+AB) telah diuji untuk menentukan keupayaan pengambilan nutrien dan prestasi pertumbuhan tumbuhan. Penyingkiran penyerapan nutrien tertinggi ($P < 0.05$) (39.08% daripada jumlah nitrogen, 88.06% daripada jumlah ammonia nitrogen, 72.61% daripada nitrit+ nitrat nitrogen, 82.06% daripada jumlah fosforus, dan 77.75% daripada fosfat reaktif larut) dan prestasi pertumbuhan tumbuhan ditunjukkan lebih baik dalam dua kombinasi bayam air dan selasih limau (WS+LB).

Sebanyak lima substrat berbeza (buluh; BO, batu lava; LR, polivinil klorida; PVC, bola-bio; BA, cincin-bio; BR) telah digunakan untuk mengkaji kelimpahan perifiton mereka. Pertumbuhan perifiton pada buluh dan batu lava menunjukkan biojisim tertinggi ($P < 0.05$) (2.18 ± 0.09 mg cm^{-2} and 2.05 ± 0.11 mg cm^{-2}) dan kelimpahan perifiton pada substrat di antara semua substrat yang digunakan. Gabungan tumbuhan terpilih secara individu (bayam dan selasih limau) dan substrat terpilih (batu lava dan buluh) telah diuji untuk menentukan kecekapan penyerapan nutrien dan prestasi pertumbuhan tumbuhan. Didapati bahawa kecekapan penyingkiran nutrien dalam substrat tunggal (batu lava) bersama dengan tumbuhan tunggal (bayam air) adalah lebih tinggi ($P < 0.05$) berbanding rawatan lain dengan pengurangan TAN (88%), NO_2-N (67%) dan PO_4-P (30%). Hasil bayam menunjukkan pengeluaran tertinggi ($P < 0.05$) berbanding selasih limau. Keputusan kajian ini menunjukkan gabungan batu lava dan bayam air dapat mengawal kualiti air dengan efisien dengan mengurangkan pengumpulan TAN, NO_2-N dan PO_4-P. Selain itu, biofilem yang melekat, ditambah dengan nitrifikasi mikrob (*Nitrospira*, *Nitrosomonas, Prosthecobacter*)/ denitrifikasi (*Acidovorax, Bosea, Dechloromonas, Flavobacterium, Thermomonas, Thiobacillus*), juga memainkan peranan penting dalam penyingkiran nitrogen dalam bayam air yang disepadukan dengan sistem batuan lava.

Pembangunan BFAS menggunakan tumbuhan terpilih (kangkung air) yang disepadukan dengan substrat terpilih (batu lava) dengan pertukaran air sifar digunakan untuk menyiasat kecekapan peningkatan kualiti air dan pengeluaran ikan tilapia merah monoseks (*Oreochromis* sp.) dalam tangki 1000 L. Prestasi pertumbuhan ikan dengan rawatan BFAS menunjukkan secara signifikan bilangan berat akhir ($P <0.05$) tertinggi (92.31 g.), kadar kemandirian (92.31%), dan kadar pertumbuhan spesifik (3.58). Purata kepekatan nutrien dalam rawatan BFAS adalah jauh lebih rendah ($P <0.05$) berbanding dengan rawatan lain dengan kepekatan 0.01 mg L^{-1} dalam NH_3-N, 0.06 mg L^{-1} dalam NO_2-N dan 0.75 mg L-1 dalam PO_4-P. Rawatan dengan BFAS mempunyai FCR terendah (1.16 ± 0.03) dan juga keadaan kesihatan ikan yang lebih baik berbanding rawatan dengan bukan BFAS dengan pertukaran air (1.50 ± 0.05) dan tanpa pertukaran air (2.15 ± 0.02). Tambahan pula, kepekatan logam berat dalam ikan dan tumbuhan BFAS adalah selamat untuk kegunaan manusia kerana tahap pengumpulan logam berat adalah lebih rendah daripada had selamat yang disyorkan. Di samping itu, bakteria yang paling dominan dalam BFAS termasuk Bacteroidota, Proteobacteria, dan Nitrospirot ditemui, yang sangat penting untuk kitaran nutrien dalam sistem akuakultur. Kajian ini menggambarkan bahawa BFAS yang baru dibangunkan yang mengandungi substrat (batu lava) dan tumbuhan (kangkung air) boleh mengekalkan kualiti air yang optimum, memberikan pengeluaran ikan yang lebih baik, hasil tumbuhan dan kos pengeluaran yang lebih rendah untuk sistem akuakultur tilapia.

Kata kunci: BFAS; penyingkiran nutrien; perifiton (biofilem); tilapia merah monoseks; tumbuhan; substrat

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µg	microgram
µL	microlitre
µm	micrometre or micron
µS	microSiemens
™	trademark
ºC	degree Celsius
AB	aromatic basil (*Ocimum basilicum*)
AFDM	ash free dry matter
AI	autotrophic index
anammox	anaerobic ammonia-oxidising
ANOVA	analysis of variance
BA	bio-ball
BFAS	bio-green floating aquaculture system
BO	bamboo stripes
BR	bio-ring
BW	body weight
CO_2	carbon dioxide
CHL	cholesterol
cm	centimetre
comammox	complete ammonia oxidiser
d	day
DM	dry matter
dL	decilitres

DNA	deoxyribonucleic acid
DO	dissolved oxygen
dS	deciSiemens
DWC	deep water culture/ floating raft
EC	electrical conductivity
EDX	energy dispersive x-ray
ELISA	enzyme-linked immunoassay
FCR	feed conversion ratio
FEP	fluorinated ethylene propylene
g	gram
Gluc	glucose
h	hour
H_2SO_4	sulphuric acid
HCl	hydrochloric acid
Hb	haemoglobin concentration
Ht	haematocrit
ICP-OES	Inductively Coupled Plasma Optical Emission Spectrometry
kg	kilogram
KNO_3	potassium nitrate
KH_2PO_4	potassium dihydrogen phosphate
L	litre
LB	lemon basil (*Ocimum* × *africanum*)
LR	lava rock
m	metre

MFD	media filled
min	minute
mg	milligram
mL	millilitre
nm	nanometre
mm	milimetre
mS	milliSiemens
N	normality
N	nitrogen
NaOH	sodium hydroxide
NED	N- (1-naphthyl)-ethylenediamine dihydrochloride
NFT	nutrient film technique
NFE	nitrogen free extract
ng	nanogram
NH_3	unionised ammonia
NH_3-N	unionised ammonia nitrogen
NH_4^+	ammonium
NH_4Cl	ammonium chloride
NH_4OH	ammonium hydroxide
NO_2-N	nitrite nitrogen
NO_3-N	nitrate nitrogen
NO_2+NO_3-N	nitrite and nitrate nitrogen
OD	optical density
OTU	operational taxonomic unit

PCR	polymerase chain reaction
PO_4-P	soluble reactive phosphate
ppm	part per million
psi	pounds per square inch
PVC	polyvinyl chloride
RBC	red blood cell
RBFAS	plant root samples of bio-green floating aquaculture system treatment
RGR	relative growth rate
RNA	ribonucleic acid
rRNA	ribosomal ribonucleic acid
RLRWS	plant root samples of water spinach with lava rock treatment
RM	Malaysian ringgit
s	second
SBFAS	substrate samples of bio-green floating aquaculture system treatment
SGR	specific growth rate
sp	species
spp	several species
SLRWS	substrate samples of water spinach with lava rock treatment
SR	Sedgewick-Rafter
T	temperature
TAN	total ammonia nitrogen
TDS	total dissolved solids
TN	total nitrogen

TP	total phosphorus
TRG	triglyceride
TSP	triple superphosphate
USD	United States dollar
v/v	volume per volume
WBC	white blood cell
WBFAS	Water samples of bio-green floating aquaculture system treatment
WG	weight gain
WLRWS	water samples of water spinach with lava rock treatment
WS	water spinach (*Ipomoea aquatica*)

CHAPTER 1

INTRODUCTION

1.1 Background of the study

Aquaculture has been recognised as an important sector for income generation and economic development for many countries, especially in Asia where 89% of aquaculture products are generated (FAO, 2018). While aquaculture is the fastest-growing food sector worldwide, its production is still limited by feed quality, deterioration of water quality, and diseases. Additionally, untreated waste discharges from aquaculture activities can cause eutrophication in public waters. Eutrophication is an environmental problem that many countries pay attention to solve the problem (Costa et al., 2018; Moal et al., 2019). Some countries such as the United States of America have invested more than 20 million USD in mitigating the eutrophication impact, especially in tourism areas (Lapointe & Bedford, 2007). Eutrophication can cause negative consequences for living organisms in the aquatic ecosystem. The main nutrient overload for eutrophication such as dissolved nitrogen and phosphorus mainly comes from household drainage, factories, agriculture and urban activities (Costa et al., 2018; Malone & Newton, 2020). It has been reported by the survey of the State of the World's Lakes project which eutrophication has been effected 54% of Asian lakes, 53% in Europe, 48% in North America, 41% in South America and 28% of those in Africa (Scannone, 2016). And by 2050, an average of 40 - 45% anthropogenic N input will be exported to the water ecosystem (Galloway et al., 2004; Malone & Newton, 2020). Environmental friendly criterion is a crucial concern for the development of remediation systems for water contaminants, and it tends to emphasise the need for simple maintenance, low cost and effective nutrient removal. Therefore, recycling and enhancement of nutrient utilisation by using aquatic plants or macrophytes and biofilm (periphyton) might be regarded as an environmental approach to alleviate nutrient excess in aquatic ecosystems. Integrated substrates with plant-based sustainable technology can produce foods and improve water quality as well as reduce operating costs in culture systems by recycling and converting nutrients (N and P) into high-value resources, eliminating (minimising) water exchange and increasing the profitability of fish and plant productions in the system (Li et al., 2019; Liu et al., 2021; Yep & Zheng, 2019). Wastewater can be removed from the water column which it has developed by using hydroponically grown plants. In addition, in order to increase the high yield of harvested production, the system has been attempted to combine with animal cultured mainly fish such as tilapia, and it subsequently shifts to aquaponics systems (Yep & Zheng, 2019).

Aquatic plant restoration (phytoremediation) is a subcategory of bioremediation, and it has been receiving increasing attention, as water plants have the ability to degrade or remove pollutants from their surrounding environment and retain huge amounts of nitrogen and phosphorus (Ansari et al., 2018; Herath & Vithanage, 2015; Moore et al., 2016; Wahab et al., 2014). Several studies on nutrient removal applying phytoremediation with aquatic plants have been performed to improve efficiencies of wastewater restoration (Abed et al., 2019; Su et al., 2019), aquaculture pond effluent (Ng

& Chan, 2021; Nizam et al., 2020), dairy effluent (Khan & Bano, 2018), and livestock wastewater (Hu et al., 2020). However, some factors such as light, nutrients and space can have an impact on plant development as well as nutrient removal efficiency (Estim et al., 2019; Saufie et al., 2020). In addition, integrated aquatic plants with the substrates can be alternative conventional practice to promote microhabitat and improve nutrient utilisation with symbiosis of plants and bacteria on substrates. However, using aquatic plants in aquaculture systems may have limitations such as the physiology of roots and seasonal variation. For example, some plants may have shorter roots or a slow growth rate at low temperatures (Bai et al., 2020; Liu et al., 2016). These characteristics may result in a reduction in nutrient uptake efficiency in the water column.

Recycling and enhancement of nutrient utilisation by integrated aquatic plants with biofilm (periphyton) can be an alternative way to their synergistic actions for water purification (Liu et al., 2021; Song et al., 2014). Therefore, enhancement of some organisms development along an aquatic food web such as immobilised substrate/biofilm integrated with aquatic plants as a floating raft system will possibly be an efficient way and environmentally friendly to improve nutrient utilisation (Cui et al., 2018; Li et al., 2019; Liu et al., 2021). Eco-technologies such as artificial floating bed systems have been applied worldwide for nutrient removal (Levy et al., 2017; Liu et al., 2016; Smith et al., 2019). Moreover, this integrated system is efficient in utilising nutrients, and it will be useful in the management of water bodies for various purposes such as aesthetics and recreational activities (Headley & Tanner, 2008; Pandey & Souza-alonso, 2019; Pollard et al., 2017). The efficiency of floating raft systems such as nutrient removal ability, heavy metal accumulation, biomass productions (fish and plants), fish health conditions as well as various microbial communities in floating raft systems should be investigated to achieve mass production and cost-effectiveness.

Therefore, the selection of available suitable substrates and plant species can achieve high nutrient removal and make effective use of floating rafts in aquaculture systems. The development of bio-green floating aquaculture system (BFAS) provides sustainable economically friendly technology for assessing the nutrient removal performances, improving fish production and generating alternative income for farmers with plant production, and reducing operation costs in fish culture system. Moreover, the development of bio-green floating aquaculture system (BFAS) might be helpful in developments for aquaponic farming to improve water quality and archive high yields of both fish and plant productions to meet food demand while also increasing nutrient use efficiency to reduce environmental problems and operating costs in aquaculture as well as for sustainable agricultural production.

1.2 Problem statement

In aquaculture, water is a precious resource and becoming a scarcity relative to human demand, and land resources also are decreasing and as demand for their utilisation to produce food increases their value will increase. Therefore, aquaponic systems and integrated farming practices will yield sustainable and environmentally sound farming methods. Although numerous studies have found that in aquaponics systems combined recirculating aquaculture system - RAS with hydroponics, poly-culture or integrated aquaculture (Estim et al., 2019; Goddek et al., 2015; Stathopoulou et al., 2021), but the application of this approach in aquaculture has several disadvantages such as in NFT and raft systems commonly use plastic or foam to cover water surface for not to allow primary and secondary production as well as high electricity cost due to water recirculation system. To overcome these issues, integrated substrates with plant-based sustainable technology should be developed to produce foods and improve water quality as well as reduce operating costs by zero water exchange culture system and converting nutrients (N and P) into high-value resources, eliminating (minimising) water exchange and increasing the profitability of fish and plant productions in the system. On the other hand, microorganisms in aquaculture systems are involved in multiple processes, including nitrification, organic matter decomposition, denitrification, phosphorus mineralisation, and iron cycling. Thus, there is still a need for the characterisation of aquaponics microbial communities related to fish and plants in the system.

1.3 Justification of the research

The bio-green floating aquaculture system (BFAS) is a floating raft system integrated between aquatic plants and substrates, which it functions and aims to benefit aquaculture and agriculture productions by increasing food availability, providing habitat for living organisms, improving water quality and increasing biomass both cultured animals and vegetation crops. Thus, improvement of water quality by using plants and substrates has been used for aquaponics, recirculation systems, lakes, and fish ponds. However, using the combinations of commercial plants integrated with substrates to remove contaminants from the aquatic environments and increase its aesthetic benefit should be more investigated. There is also limited information based on the aquaculture system integrated with substrates and economically plants in raft system. Although there are some invasive species such as water hyacinth and duckweed have been used for uptake of nutrient-rich in the water ecosystem. However, its negative impacts are more significant than the benefits such as costs of habitat restoration and native distribution areas. Using plants or macrophytes also has limited on its roots in deep water and their growth varies with seasonal changes, but not for substrates. Therefore, this study expects to reduce nutrient-rich in aquaculture systems by enhancing the nutrient uptake rate using plants and biofilms assembled as an efficient nutrient uptake system known as a bio-green floating aquaculture system (BFAS). The bio-green floating systems will be improved to be more efficient in nutrient removal of wastewater and increase fish production, plant biomass and nutrient uptake from biofilm communities. Thus, the research finding aims to develop a bio-green floating aquaculture system (BFAS) and increase cultured aquatic animal production as well as vegetation yield with a sustainable and environmentally friendly approach. Furthermore, BFAS can generate income as agro-tourism and aesthetic value for farmers and industry in the aquaculture system.

1.4 Objectives

The overall objective of this study is to examine the possible using a floating raft system which developed by integrated aquatic plants with substrates to enhance nutrient utilisation by recycling wasted nutrients by microorganisms and aquatic plants. Periphyton or biofilm coated around the substrates can be alternative food sources for the aquatic animals in the system as well as improving water quality and fish production in the aquaculture system.

Specific objectives

1. To examine the efficiency of nutrient removal of water spinach (*Ipomoea aquatica*), lemon basil (*Ocimum* × *africanum*; LB), and aromatic basil (*Ocimum basilicum*
2. To determine the efficiency of five different substrates (bamboo, lava rock, polyvinyl chloride bio-ball, and bio-ring) and their periphyton development
3. To assess the efficiency of the selected single plant, substrates, and their integrated preferred biofilm efficiency for nutrient uptake
4. To evaluate the efficiency of nutrient utilisation by the bio-green floating aquaculture system and its effects on water quality and monosex red tilapia production

1.5 Hypothesis

Hypothesis 1:

H_o: A single and in combination of selected plants do not improve water quality in raft systems

H_A: A single and in combination of selected plants improve water quality in raft systems

Hypothesis 2:

H_o: A selected substrate does not affect on periphyton growth and its development

H_A: A selected substrate affects on periphyton growth and its development

Hypothesis 3:

H_o: A selected plant combination with selected substrate does not improve water quality

H_A: A selected plant combination with selected substrate improves water quality

Hypothesis 4:

H_o: A bio-green floating system consisting of primary producers including plants, substrates, and periphyton does not improve water quality and fish production in an aquaculture system.

H_A: A bio-green floating system consisting of primary producers including plants, substrates, and periphyton improves water quality and fish production in an aquaculture system.

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