UNIVERSITI PUTRA MALAYSIA

PRODUCTIVITY, PROXIMATE COMPOSITION AND TOXICITY OF NATURALLY OCCURRING MICROALGAE IN DIFFERENT STOCKING DENSITY OF CATFISH UNDER MALAYSIAN WEATHER CONDITIONS

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FS 2020 15

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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

December 2019

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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Presently global warming, increasing world population and coupled with food security are some of the issues that need to be addressed urgently. Naturally occurring microalgae found in fish culture is new possibility for cheap microalgal biomass production, potential food source and maintaining sustainable aquaculture under different weather conditions. Therefore, the objectives of this study are to determine the effect of different weather conditions and fish stocking densities on the dynamics of physico-chemical water parameter in catfish tanks, to evaluate the fish and microalgal productivity, biochemical composition and fatty acid profile of naturally occurring microalgae and catfish, to document the diversity and dynamics of those microalgae, to evaluate the toxicity of mixed microalgae in different fish stocking density and weather conditions. *Clarias gariepinus* with size 10±0.2 cm and 18.5±0.3 g was placed in polytank of 300 L under sheltered transparent roofing. The experimental set up comprising of control tank with 25 fishes and covered to prevent microalgal growth, and other exposed tanks with 10, 15, 20 and 25 fishes for treatment 1, 2, 3 and 4, respectively. Mean light intensity and temperature variation represent different weather conditions were logged every 30 minutes. Physico-chemical water parameter were measured using standard water quality techniques sampled every 2 days. Naturally occurring microalgae were sampled every 2 days for productivity (optical density, dry weight, total chlorophyll *a* & *b*, biomass, primary productivity, biochemical composition and fatty acid profile, diversity and species compositions) and toxicity of microalgae were determined. Catfish performances were determined for fish size (weight and length), survival rate, feed consumed record, and Feed Conversion Ratio (FCR). Correlation between fish stocking density and primary productivity, biomass productivity, diversity indices of mixed microalgae in different weather conditions was also evaluated. Mean±standard error and ranking score of all data were selected for data handling and interpretation. Weather conditions were represented by an average

daily light intensity and temperature with ranged from 184.02-575.07 µmol $m^{-2}s^{-1}$ and 29.29-36.90 ˚C. Physico-chemical water parameters were as follows; temperature (28.63 ±0.24 to 31.33 ±0.2 ˚C), pH (5.64±0.31 to 8.26±0.7), Total dissolve solids (TDS) (168.33±6.86 to 433.91±12.38 mg/L), Electrical conductivity (EC) (0.34±0.01 to 0.87±0.02 mS/cm), water transparency (2.13±0.31 to 28±0.00 cm), Nitrate (NO_3) (6.05±0.00 to 171.02±40.42 mg/L), Ammonium (NH_4) (7.02±3.11 to 105.25±18.65 mg/L), Phosphate (PO_4) (95.07±13.75 to 849.65±154.00 mg/L), Dissolved oxygen (DO) (2.62±0.05 to 6.49±0.10 mg/L), and Biological oxygen demand (BOD) (16.90±0.11 to 27.96±0.08 mg/L). Overall fish stocking density with water quality cumulative rank and score was the highest in treatment 3 (20 fish), 2(15 fish), 1 (10 fish) with 37 scores in dry weather conditions, treatment 1 (42 scores) in mix weather, and treatment 1(42 scores) in wet weather. Irrespective of the fish stocking density, the water quality in the mix weather conditions were the best, followed by wet weather conditions and the dry weather conditions with the worst water quality. Dry weight, optical density, total chlorophyll (*a* & *b*), biomass and primary productivity showed the highest value in treatment 4 (25 catfish) in dry weather conditions. There was positive correlation between different fish stocking density, primary productivity and microalgal biomass productivity. Proximate composition of naturally occurring microalgae contained 36% protein, followed by 29% total carbohydrate, 14% ash, 13% crude lipid, and 8% moisture. Fatty acid profile comprised of 58.82% saturated fatty acids with, followed by 23.53% monounsaturated fatty acid and 17.65% polyunsaturated fatty acid. Fish weight and length, Feed conversion ratio (FCR) of catfish was highest in control, followed by treatment 4. The survival rate ranged from 73.33-80% in all treatments. A total of 29 genera and 77 species of microalgae from 5 divisions were identified. The most abundant taxa were Chlorophyta (90%). Shannon diversity index (H'), Simpson index, evenness, and species richness indices ranged from 0.59-2.82, 0.21-0.97, 0.52-1.04 and 2.01-5.36, respectively. The highest stocking density of catfish (25 catfish; T4) in dry weather conditions produced highest microalgal diversity indices. Pearson's correlation analysis between fish stocking density and diversity indices showed strong positive values in all different weather conditions. Using probit analysis, the toxicity of naturally occurring microalgae have lethality concentration (LC_{50}) of 458.97-866.70 ppm for all weather conditions and all fish stocking density. This study concluded that the weather conditions and fish stocking density have profound effect on physico-chemical water quality, microalgae and fish productivity, proximate composition and fatty acid profile, species composition, diversity indices and toxicity of naturally occurring microalgae.

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

PRODUKTIVITI, KANDUNGAN PROKSIMAT DAN KETOKSIKAN MIKROALGA YANG WUJUD SECARA SEMULAJADI DALAM KETUMPATAN IKAN KELI BERBEZA DAN DALAM KEADAAN CUACA DIMALAYSIA

Oleh

MANTHAKA WEERAPHONG

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Pemanasan global dewasa ini, peningkatan penduduk dunia serta isu keselamatan makanan adalah diantara beberapa isu yang perlu ditangani dengan segera. Mikroalga semula jadi yang terdapat dalam kultur ikan adalah satu alternatif untuk pengeluaran biojisim yang murah, sumber makanan yang berpotensi dan mengekalkan akuakultur yang mampan dalam keadaan cuaca yang berbeza. Oleh itu, objektif kajian ini adalah untuk menentukan kesan keadaan cuaca yang berbeza dan ketumpatan stok ikan yang berbeza terhadap dinamik parameter fiziko-kimia air dalam tangki ikan keli, produktiviti mikroalga dan ikan, komposisi biokimia dan profil asid lemak mikroalga yang wujud secara semula jadi, kepelbagaian dan dinamik mikroalga tersebut, menilai ketoksikan mikroalga yang wujud secara semula jadi. *Clarias gariepinus* bersaiz 10 ± 0.2 sm dan berat 18.5 ± 0.3 g dikultur dalam polytank 300 L di bawah bumbung plastik lutsinar terlindung dari hujan. Eksperimen merangkumi tangki kawalan dengan 25 ikan yang ditutup untuk mencegah pertumbuhan mikroalga, dan tangki terdedah yang lain dengan 10, 15, 20 dan 25 ikan masing-masing untuk rawatan 1, 2, 3 dan 4. Keamatan cahaya dan variasi suhu untuk menunjukkan keadaan cuaca yang berbeza dicerap setiap 30 minit. Parameter fiziko-kimia air diukur menggunakan teknik kualiti air piawai yang diambil setiap 2 hari. Mikroalga yang wujud secara semula jadi diambil setiap 2 hari untuk produktiviti (ketumpatan optik, berat kering, jumlah klorofil *a* & *b*, biojisim, produktiviti primer, komposisi biokimia dan profil asid lemak, kepelbagaian dan komposisi spesies) dan ketoksikan mikroalga ditentukan. Tumbesaran ikan keli ditentukan berdasarkan saiz ikan (berat dan panjang), kadar kelangsungan hidup, rekod jumlah makanan digunakan, dan Nisbah Penukaran Makanan (FCR). Korelasi antara ketumpatan stok ikan dan produktiviti primer, produktiviti biojisim, indeks kepelbagaian mikroalga yang wujud secara semula jadi dalam keadaan cuaca yang berbeza juga dinilai. Purata ± sisihan piawai dan skor kedudukan untuk semua data telah dilaksanakan untuk pengendalian data dan

tafsiran. Keadaan cuaca diwakili oleh purata keamatan cahaya dan purata suhu setiap hari yang berjulat antara 184.02-575.07 µmol $m^{-2}s^{-1}$ dan 29.29-36.90 ºC. Julat parameter fizik-kimia air merangkumi suhu (28.63 ± 0.24 hingga 31.33 ± 0.2 ºC), pH (5.64 ± 0.31 hingga 8.26 ± 0.7), jumlah pepejal terlarut (TDS) (168.33 ± 6.86 hingga 433.91 ± 12.38 mg/L), kekonduksian elektrik (EC) (0.34 ± 0.01 hingga 0.87 ± 0.02 mS/sm), ketelusan air (2.13 ± 0.31 hingga 28 ± 0.00 sm), Nitrat (NO_3) (6.05 ± 0.00 hingga 171.02 ± 40.42 mg/L), Ammonium (NH_4) (7.02 ± 3.11 hingga 105.25 ± 18.65 mg/L), Fosfat (PO_4) (95.07 ± 13.75 hingga 849.65 ± 154.00 mg/L), Oksigen terlarut (DO) (2.62 ± 0.05 hingga 6.49 ± 0.10 mg/L), dan keperluan oksigen biologi (BOD) (16.90 ± 0.11 hingga 27.96 ± 0.08 mg/L). Ketumpatan stok ikan dengan kualiti air secara keseluruhan menunjukkan kedudukan dan skor kumulatif tertinggi untuk rawatan 3 (20 ikan), 2 (15 ikan), 1 (10 ikan) dengan 37 skor dalam keadaan cuaca kering, rawatan 1 (42 skor) dalam cuaca bercampur, dan rawatan 1 (42 skor) dalam cuaca basah. Tanpa mengambil kira ketumpatan stok ikan, kualiti air dalam keadaan cuaca campuran adalah yang terbaik, diikuti dengan keadaan cuaca basah dan keadaan cuaca kering yang mempunyai kualiti air paling teruk. Berat kering, ketumpatan optik, jumlah klorofil (*a* & *b*), biojisim dan produktiviti primer menunjukkan nilai tertinggi untuk rawatan 4 (25 ikan) dalam keadaan cuaca kering. Korelasi antara ketumpatan stok ikan yang berbeza, produktiviti utama dan produktiviti biojisim menunjukkan nilai positif. Komposisi mikroalga yang wujud secara semulajadi mempunyai protein kasar tertinggi sebanyak 36%, diikuti karbohidrat 29%, abu 14%, lipid kasar 13%, dan kelembapan 8%. Profil asid lemak terdiri daripada asid lemak tepu dengan (58.82%), diikuti oleh asid lemak mono tak tepu (23.53%) dan asid lemak tak tepu (17.65%). Berat dan panjang ikan, nisbah penukaran makanan (FCR) ikan keli menunjukkan nilai tertinggi dalam kawalan dan diikuti dengan rawatan 4. Kadar kelangsungan hidup untuk semua rawatan adalah dari 73.33-80%. Sejumlah 29 genera dan 77 spesies mikroalgae dari 5 bahagian telah dikenalpasti. Bahagian Klorofita merupakan kumpulan mikroalga paling dominan merangkumi 90% daripada jumlah mikroalga. Indeks kepelbagaian Shannon (H'), indeks Simpson, indeks kesamarataan, dan indeks kekayaan spesis berjulat antara 0.59-2.82, 0.21-0.97, 0.52-1.04 dan 2.01-5.36 masing-masing. Ketumpatan stok ikan keli (25 ikan; T4) dalam keadaan cuaca kering memberikan indeks kepelbagaian mikroalga tertinggi. Analisis korelasi Pearson diantara ketumpatan stok dan indeks kepelbagaian ikan menunjukkan nilai yang sangat positif untuk semua keadaan cuaca. Ketoksikan mikroalga yang wujud secara semulajadi menggunakan analisis probit menunjukkan kepekatan membawa maut (LC_{50}) berada dalam julat antara 458.97-866.70 ppm. Kajian ini menyimpulkan bahawa keadaan cuaca dan kepadatan stok ikan memberikan kesan yang ketara terhadap kualiti air fiziko-kimia, mikroalga dan produktiviti ikan, komposisi proksimat dan profil asid lemak, komposisi spesies, indeks kepelbagaian dan ketoksikan mikroalga yang wujud secara semulajadi.

ACKNOWLEDGEMENTS

Firstly, I really appreciate to be postgrad student from Universiti Putra Malaysia (UPM) where is the great one ranking university in Malaysia and world class.

Secondly, I would like to grateful thank you to my best supervisor, Dr. Hishamuddin Omar who always be great helpful since experimental design, support relevant tools, given some very useful comments, and believe and cheer me up to achieve goal. I would like also to really thank you my co-supervisors, Assoc. Prof. Dr. Mohammad Noor Amal Azmai and Dr. Syaizwan Zahmir Zulkifli who always kind supporting the equipment during experiment and given some value suggestions to fulfil my thesis.

Thirdly, I express my appreciation to thanks you all lecturers and staffs of Biology Department, Faculty of Science, UPM for serving the best knowledge and campaign to be the potential Ph.D. student.

Fourthly, I cannot miss to really thankful my lab mates in Plant physiology who are very helpful, including Chemistry laboratory where supported an experimental tool and complete my experiment.

Fifthly, I really deep thank you Nakhon Si Thammarat Rajabhat University (NSTRU) where support full-time scholarship for my graduation.
Finally, I express my deep thanks to my family "Weeraphong family" and my close friends who always activated, supported, cheered me up to achieve my graduation.

This thesis was submitted to the Senate of Universsiti Putra Malaysia and has been accepted as fulfilments of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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α	Alpha
°C	Degree celsius
%	Percentage
O_2	Oxygen
N_2O	Nitrous oxide
GHGs	Greenhouse gases
L	Litre
g	Gram
nm	Nanometer
µL	Microliter
µm	Micrometer
µmoles	Micromoles
$CaCl_2$	Calcium chloride
CO_2	Carbon dioxide
CH_4	Methane
Cu	Copper
BOD_5	Biochemical Oxygen Demand
DO	Dissolved Oxygen
FCR	Feed Conversion Ratio
GPP	Gross primary productivity
KCl	Potassium chloride
LC_{50}	Lethal concentration 50
mg	Miligram
$MgCl_2$	Magnesium chloride
MUFA	Monounsaturated Fatty acid
NaI	Sodium iodide
NaN_3	Sodium azide
NaOH	Sodium hydroxide
NH_4	Ammonium
NO_2	Nitrite
NO_3	Nitrate
NPP	Net primary productivity
PO4	Phosphate
ppm	Parts per million
PUFA	Polyunsaturated Fatty Acid
R	Community respiration
SFA	Saturated Fatty Acid
TN	Total nitrogen
TP	Total phosphorus

CHAPTER 1

INTRODUCTION

1.1 General background

Current issues

Presently, the world is experiencing major issues such as global warming, climate change, super storm, hurricane, drought, flooding, crop failures, diseases and others that have profound effect on all living organisms on earth. Fossil fuel combustion are the main cause of global warming resulting from human activities, rapid growing of human population, rapid economic growth in many developing countries and higher energy need of the developed industrial nation (Guldberg et al., 2018). These gases are carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O) and other gasses (Pires, 2017; Maenhout et al., 2019) also known as greenhouse gasses. CO_2 and CH_4 are the major greenhouse gasses that contribute to global warming because they act like blanket in the atmosphere, preventing heat from escaping and causing the earth to become warmer (Tsai et al., 2016).

Past and present problem to mitigate the issues

Many nations of the world were aware and making efforts to solve those problems by forming the campaign of climate change solution under the COP21; the conference of Parties, The United Nations Framework Convention on Climate Change (UNFCCC); an international environmental agreement and representative of a scenario on climate change which was held in November 30th-December 11th, 2015 at Paris, France. They agreed to avoid the worst impacts of global warming by managing the rising of average surface temperatures not exceeding 2°C. One of the primary objectives was to provide a detail plan within a short timeframe to reduce CO_2 emissions into the atmosphere annually and to encourage the investors around the world to invest more in infrastructures related clean energy within 2020 (COP21, 2015).

Among the efforts are the selection of crop and livestock, crop diversification, changing/improving cropping and grazing patterns, investing in irrigation technology with the ability to reduce the climate variability, changing/improving agricultural practices and soil and water conservation (Calzadilla et al., 2013). The alternative energy production such as the wind, water and sun, etc., bioenergy from organisms are some of the best alternative solutions in the future for sustainable energy and reduce CO_2 emissions to almost zero by 2100 (IPCC, 2014; Baicha et al., 2016).

Limitation to the present mitigation

Nevertheless, there are still major obstacles in implement the reduction of CO_2 emission due to serious economic implications that not many countries are willing to sacrifice. Rich countries do not want to lose their economic dominance and poor countries are struggling to adopt the CO_2 reduction initiative due to the lack of fund and limited technology. Therefore, it is very unlikely that the COP21 resolution will be realized in the near future soon. A cheaper, more practical and easier alternative that can be implemented by rich and poor nation must be sought.

How the proposed study can mitigate those issues

One of the possible solutions that fits above description is microalgae. Many studies have shown that microalgae have the potential of reducing atmospheric CO_2, produces O_2, producing protein, lipid and nutrient rich biomass and at the same time serve as effective bioremediation agent (Udaiyappan et al., 2017).

There are several cultivation technologies used for the production of microalgal biomass developed by researchers and adopted by commercial producers (Patil et al., 2008). The phototropic microalgae are the most commonly grown in open ponds and photobioreactors (Patil et al., 2005). The open pond cultures are economically more favorable, but there is some concern on land-based cost, water availability, and appropriate climatic conditions. Additionally, there is also concern about contamination by fungi, bacteria, protozoa, and competition by other microalgae (Lam et al., 2018).

Many publications favour photobioreactor because it offers a closed culture environment, which is protected from external influences, free from invading microorganisms, and stable culture environment where temperatures are controlled with an optimum CO_2 supply bubbled through culture medium (Mohsenpour & Willoughby, 2016). However, high initial capital investment and operational costs are the biggest challenge of close photobioreactor system for industrial reralization (Cho et al., 2016). Additionally, most of the studies are also done in small scale under laboratory conditions using single species with some exception using high valued species (Zalewska & Saniewski, 2011; Peters & Traunspurger, 2012).

Microalgae in fish culture system on the other hand, is the dynamic and the practical approach to achieve more sustainable aquaculture are by reducing waste water discharge, save water and minimizing contamination of the aquatic environment. Incidentally, aquaculture industry is a prominent industry for food supply, especially, fish is largest meat protein producer (FAO, 2017). Some quarters argued that although microalgae have the potential to sequester atmospheric CO_2, the production and harvesting cost can be prohibitive. The doubters need to observe the natural CO_2 sequester in the forest (tropical rainforest), mangrove and coral reef where multiple species co-exist with no or minimal human intervention. Microalgae is also the most efficient photosynthetic organisms on earth (Parmar et al., 2011; Singh & Ahluwalia, 2013).

Moreover, microalgae are shown to be improved the water quality as well as aquatic crop by utilizing wastes from uneaten feed and fish excrement (Brune et al., 2003), and preventing ammonia build up which can kill fish. The natural remediation by microalgae with other microorganisms enable the water to remain in the fish tank longer without replacing it. In conventional practice, once the microalgae reach certain density in the fish pond, the algae rich water is flushed out to the natural water way. This is such a waste because these microalgae can be harvested and convert to useful product such as fish and animal feed since microalgae are high protein content (50% on dry weight) which are potentially good substitute for fishmeal and enhance the survival and growth rates in other aquatic animals like prawn (Ju et al., 2012), biofuel and others. The only resources required are to harvest the microalgae periodically.

However, before these microalgal resource can be utilized, there are several information that are required such as the relationship of microalgal productivity to fish stocking density, weather conditions, water quality, microalgal species composition, species diversity, productivity of microalgae, proximate composition (protein, lipid and carbohydrate), fatty acid profile and toxicity test. Those details are placed in various chapters; chapter 3 deal with the effect of different stocking density of catfish and different weather conditions on water quality. Chapter 4 is about the productivities, proximate composition and fatty acids of naturally occurring microalgae, productivity of catfish under different weather conditions. Species composition and diversity of microalgae were reported in chapter 5. Toxicity study of mixed microalgae was illustrated on chapter 6.

1.2 Objectives of study

1. To determine the effect of different weather conditions and fish stocking densities on the dynamics of physico-chemical water parameter in catfish tanks.
2. To evaluate the productivity, biochemical composition and fatty acid profile of naturally occurring microalgae in different catfish stocking density and different weather conditions
3. To document the diversity and dynamics of naturally occurring mixed microalgae in different fish stocking density and weather conditions.
4. To evaluate the toxicity of mixed microalgae in different fish stocking density and weather conditions.

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LIST OF PUBLICATIONS

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