PHYCOREMEDIATION OF WET MARKET WASTEWATER USING MICROALGAE *SCENEDESMUS* SP. FOR BIOMASS PRODUCTION

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DEDICATION

This thesis is specially dedicated to my beloved late father,

Allahyarham Haji Mohd Apandi Ahmad,

my mother,
Hajjah Hawa Haron,

my soulmate,
Mohd Ariff Rosli

I also would like to dedicate this piece of work to my parents in-law,
Haji Ab Aziz Wan Abdullah & Hajjah Saadah Ab Malek

all my siblings,
Farha, Affah and Nadeyah Mohd Apandi,

and not forgotten to all my family and friends, those who have encouraged and supported throughout my study. Last but not least, this novelty work is mostly dedicated to my lovely daughter,

Zeyra Sofea

I love you all….

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ABSTRACT

Rapid industrial development and escalating population growth are expected to contribute extremely to the world's environmental crisis due to excessive wastewater generation. Therefore, a technology by phycoremediation to reduce the wastewater contamination, coupled with biomass production of sustainable bioproduct has attracted interest worldwide. Thus, the aim of the study is to generate a potential bioproduct from microalgae *Scenedesmus* sp. combined with phycoremediation of wet market wastewater (WMW). The optimization study proved that the *Scenedesmus* sp. grew up well in the temperature between 24-32°C, light intensity of 245 µmol $m^{-2}s^{-1}$ and 18 hours of light exposure. In fact, this *Scenedesmus* sp. was much more tolerated in outdoor condition when integrated with wastewater phycoremediation in terms of biomass productivity and wastewater bioremediation. The best microalgae concentration was performed at 10^6cell/mL during phycoremediation process using WMW. The highest removal of nutrients (TP, TN and TOC) in WMW was up to 85%, 90% and 65% respectively under outdoor condition. Selected heavy metals (Cd, Cr, Fe, Pb and Zn) removed 87.24%, 85.55%, 90.35%, 88.5% and 87.1%, respectively. In microalgae cultivation, this study notably found the optimum of 64.26% of WMW concentration and 3.07 L/min of aeration rate were established by using statistical analysis by response surface design (RSM) in terms of biomass productivity with 6% error for the validation experiment with the value of biomass production of 68.32 mg/L/d. Besides that, the total protein and lipid content in the biomass yield produced in WMW (41.7% and 23.2%) which were more than the biomass produced in the BBM (37.4 and 19.2% respectively). The main functional of fatty acid detected 30 compounds in the biomass from WMW compared to 6 compounds in the BBM. This study notably found that WMW is a suitable and worthy media to be implemented in *Scenedesmus* sp. cultivation. Moreover, the quality composition of protein, lipid and fatty acid compound also have a bright perspective to be used as fish feed, agro feedstock, biodiesel and pharmaceutical potential and any related industry.

ABSTRAK

Pembangunan perindustrian pesat dan pertumbuhan penduduk menyumbang kepada krisis alam sekitar dunia disebabkan oleh air sisa yang berlebihan. Teknologi *phycoremediation* untuk mengurangkan pencemaran air sisa disamping berpontesi mengeluarkan biomas untuk bioproduk telah menarik minat di seluruh dunia. Oleh itu, matlamat kajian ini menjana potensi bioproduk dari microalgae *Scenedesmus* sp. digabungkan dengan *phycoremediation* air sisa pasar basah (WMW). *Scenedesmus* sp diperolehi daripada hutan tempatan. Kajian membuktikan bahawa *Scenedesmus* sp. membiak dengan baik pada suhu 24-32ºC, keamatan cahaya 245 µmol $m^{-2}s^{-1}$ dan 18 jam pendedahan cahaya. Malah, *Scenedesmus* sp. lebih banyak diterima dengan keadaan luar apabila disepadukan dengan *phycoremediation* air sisa dari segi produktiviti biomas dan bioremediasi air sisa. Kepekatan microalgae yang terbaik dilakukan pada 10^6 sel/mL semasa proses *phycoremediation* menggunakan WMW. Penyingkiran nutrien tertinggi jumlah (TP, TN dan TOC) dalam WMW sehingga 85%, 90% dan 65% masing-masing di bawah keadaan luar. Logam berat terpilih (Cd, Cr, Fe, Pb dan Zn) masing-masing dikeluarkan 87.24%, 85.55%, 90.35%, 88.5% dan 87.1%. Dalam penanaman mikroalga, kajian ini mendapati bahawa 64.26% kepekatan WMW dan 3.07 L/min menggunakan analisis RSM dari segi produktiviti biomas dengan ralat 6% untuk eksperimen pengesahan dengan nilai pengeluaran biomas sebanyak 68.32 mg/L/d. Selain itu, jumlah kandungan protein dan lipid dalam hasil biomas yang dihasilkan di WMW (41.7% dan 23.2%) adalah lebih tinggi daripada biomas yang dihasilkan dalam BBM (37.4 dan 19.2%). Fungsi utama asid lemak mengesan 30 sebatian dalam biomas dari WMW berbanding 6 sebatian dalam BBM. Kajian ini mendapati bahawa WMW adalah media yang sesuai untuk pembiakan *Scenedesmus* sp. Selain itu, komposisi kualiti protein, lipid dan sebatian asid lemak juga mempunyai perspektif yang cerah untuk digunakan sebagai makanan haiwan, biodiesel dan farmaseutikal dan industri yang berkaitan.

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LIST OF SYMBOLS AND ABBREVIATIONS

WMW	-	Wet market wastewater
CO_2	-	Carbon dioxide
O_2	-	Oxygen
RSM	-	Response surface methodology
FCCD		Face centered composite design
BOD	-	Biochemical oxygen demand
COD	-	Chemical oxygen demand
TSS	-	Total suspended solids
TN	-	Total nitrogen
TP	-	Total phosphorus
TOC	-	Total organic carbon
NO_3	-	Nitrate
NH_4	-	Ammonium
SO_4	-	Sulphate
CA	-	*Cicer Aretinium*
CC	-	*Cajanus Cajan*
H_2O	-	Water
HRAP	-	High rate algae pond
BBM	-	Bold's Basal Medium
APHA	-	American public health association
OD	-	Optical density
UTHM	-	Universiti Tun Hussein Onn Malaysia
MFA	-	Monounsaturated fatty acid
PFA	-	Polyunsaturated fatty acid
SFA	-	Saturated fatty acid
FKAAS	-	Faculty of Civil and Environmental Engineering

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LIST OF PUBLICATIONS

Najeeha Mohd Apandi, Radin Maya Saphira Radin Mohamed, Adel Ali Saeed Al-Gheethi, Paran Gani, Alfituri Ibrahim, Amir Hashim Mohd Kassim. 2018. *Scenedesmus* Biomass Productivity and Nutrient Removal from Wet Market Wastewater, A Bio-kinetic Study. *Waste and Biomass Valorization, Springer Nature.* (ISI, IF=1.8).

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CHAPTER 1

INTRODUCTION

1.1 Background

Rapid developments of industries together with escalating economic activities and increase of human population in developing countries have led to the increasing demand for traded goods and daily necessities. Therefore, authorities, governments and companies in these countries need to expand the diversity of sources associated with wet markets, supermarkets, shop retails and public markets. As one of the developing countries, Malaysia has a multi-racial population of 31.9 million people from 1950-2018 (10th April 2018) based on the latest United Nations, Department of Economic and Social Affairs, World Population Prospects (2018). Since its population is increasing steadily each year, the demands for fresh food are also escalating. A report by Agrofood Statistic, Ministry of Agriculture Malaysia (2016) showed that in the year of 2011 –2016, Malaysia's demands for local production of food such as poultry were (1.2–1.7 tan metric), meat (188.77 – 248.14 tan metric) and fishes (6.5 – 5.9 tan metric). Such amount of food demand is able to generate the production of wet market wastewater that is however could be harmful to public health and environment.

Wet market wastewater (WMW) is well known to have pollutants that can negatively impact on the environment if the pollutant load removal is not controlled and stabilized. WMW contains pollutants, nutrients and microbes in the forms of dissolved or suspended solids that could potentially risk the human health through exposure to pathogenic microorganism which is also hazardous to our environment. (Maizatul, Saphira & Mohamed, 2018). Furthermore, excessive nutrients such as nitrogen and phosphorus can negatively affect the environment especially on the

oceanic/marine living things (Sriram & Seenivasan, 2012; Jais *et al.*, 2017; Maizatul *et al.*, 2017). In addition, WMW may also contain poisonous heavy metals that appear in the food chain of aquatic and terrestrial ecosystem and pose hazard to the health (Jais *et al.*, 2017). Thus, discharging WMW into water bodies such as river, lake, sea and environment without any proper treatment may lead to pollutants loads and environment problems. Consequently, this can cause long-term problems that can affect access to safe and clean water for drinking and bathing. Apart from that, wastewater discharged from wet market may also contain sullage that requires a pre-treatment before being discharged into water bodies (Amneera *et al*., 2013). The quantity and quality of the wastewater are also varied depending on sources and wet market activities. In Malaysia, there are guidelines to treat wastewater, sewages, industrial effluents and water run offs. However, only a few guidelines have been established for the treatment of WMW. Considering all the information on WMW, this issue needs to be solved to ensure the pollutant loads into wastewater are in compliant with the local effluent standard before they are discharged into the environment.

The selection of wastewater treatment method either conventional, bioremediation or advanced technique has been debated among researchers (Abdel-Raouf, Al-Homaidan & Ibraheem, 2012; Rajasulochana & Preethy, 2016). Most of the current wastewater treatment practices have their own disadvantages as well as complicated maintenance requirements. The treatment practices also produce large volume of sludge which make them economically unviable (Capodaglio, 2017). The conventional wastewater treatment has extensive chemical usage which resulted in huge amount of sludge in the form of hazardous solid wastes together with accumulation of heavy metals and pathogens which are mostly harmful to the environment (Wei *et al.*, 2003; Rajasulochana & Preethy, 2016). Unlike other wastewater such as domestic wastewater, municipal wastewater and agricultural wastewater, there is no established or specific wastewater treatment for WMW. However, to overcome problems related to the physical and chemical treatment of WMW, bioremediation of wastewater using microalgae is applicable and offers a low energy requirement (Rawat *et al.*, 2011; Abdel-Raouf *et al.*, 2012). According to Abdel-Raouf *et al*., (2012) wastewater treatment using algae is an emerging technology which applies plants and associated rhizospheric microorganisms in removing and degrading the contaminants from soil, sediments, groundwater, surface water and atmosphere. Hence, using algae plant is a naturally good solution for

environmental problems such as earth's atmospheric temperature, depletion of ozone layer and climate change. This is because the microalgae produce more oxygen and glucose and at the same time consume carbon dioxide through photosynthesis (Mata, Martins & Caetano, 2010). Generally, wastewater treatment is highly expensive due to chemicals needed to treat the wastewater. However, the cost can be minimized by using microalgae as an alternative to reduce pollutants and at the same time biomass microalgae can be produced. Earlier studies have revealed dual advantages of this sustainable biological treatment method which are treatment of wastewater and creation of valuable biomass for generation of bioproduct (Zhang *et al.,* 2014; Gani *et al*., 2016a; Apandi *et al*., 2017).

The use of microalgae for nutrients and pollutants removal is known as phycoremediation. Phycoremediation is an effective technique that treats wastewater using algae in a very simple method unlike other conventional treatments (Abdel-Raouf *et al*., 2012). Phycoremediation can be classified as a sustainable method and cost saving. As such, it improves the economic perspective and environmental outline of the whole process since the phycoremediation process only requires nutrients like nitrogen and phosphorus and adequate sunlight to grow (Ajayan *et al*., 2015; Gani *et al*., 2016a). Remarkably, phycoremediation is not only able to remove nutrients, but also other organic pollutants and heavy metals depending on the selected microalgae, type of wastewater and culture condition (Rawat *et al.,* 2011; Chiu *et al.,* 2015). Additionally, growing in wastewater via phycoremediation process has more advantages over the conventional algae farms in terms of nutrient assimilation in wastewater. It does not require synthethic medium to support algae growth and henceforth improves the economical aspect and the whole production process of the environmental footprint as WMW is rich in nutrients. Hence, coupling the algae with WMW gives significant benefits by consuming the nutrients and turning it into biomass but at the same time cleaning the environment via carbon dioxide fixation (Liu *et al*., 2013; Jais *et al.*, 2017).

Scenedesmus sp. is a green microalgae that is widely found in freshwater bodies and has good adaptation ability in removing nutrients such as nitrogen and phosphorus. As such, *Scenedesmus* sp. is also very versatile as a raw material for bio product potential (Xin, Hong-Ying & Yu-Ping, 2011; Miranda, Passarinho & Gouveia, 2012). For example, the use of phycroremediation in treating domestic wastewater using *Scenedesmus* sp. could remove 92.9% of total nitrogen (TN) and 99.2% of total

phosphorus (TP) (Zhang *et al*, 2014). Meanwhile, Mohamed *et al.*, (2015) examined *Scenedesmus* sp. under outdoor condition in the phycoremediation of cafeteria wastewater. They found that the significant removal of TOC, TN and phosphate (PO_4^{3-}) was 73.36%, 90.78% and 35.9% respectively. Both studies have proven the fact that *Scenedesmus* sp. capability in the reduction of nutrients pollutants in various wastewater. WMW also contains high nutrients which can be essential for microalgae growth as claimed by researchers (Jais *et al*., 2017: Mohamed *et al*., 2017). This has led to the selection of *Scenedesmus* sp. as the best candidate in phycoremediation of WMW. Moreover, *Scenedesmus* sp. used in this study was locally collected and has been known to have high metabolic pathway and tolerant with local climate. Yet, there is no information concerning the removal of nutrients of WMW using microalgae *Scenedemus* sp for biomass production.

Based on the discussion above, there is a limited understanding and information on WMW towards bioremediation technologies. Thus, this research is aimed to construct a new resolution system in phycoremediation potential of microalgae *Scenedesmus* sp. in removing the nutrient pollutant loads of WMW. Furthermore, this thesis is also aimed to address some of the identified gap specifically to investigate the *Scenedemus* sp. production as a value added byproduct in biotechnology industry and for future guidelines on using *Scenedemus* sp. in treating WMW.

1.2 Problem Statement

According to the Environmental Quality Report of Department of Environment (DOE) (2013), a total of 1,475,444 water pollution were identified in Malaysia sourcing from 879 wet markets, 602 animal farms and 508 agro-based industries (DOE, 2013). Unfortunately, the WMW which consists of a variety of components such as nitrogen and phosphorus have been commonly released into the waterways freely (Amneera *et al*., 2013; Sakai *et al*., 2016; Jais *et al*., 2017). According to several preliminary site investigations conducted prior to this study and past researchers, WMW in several markets in Malaysia are directly discharged without going through any proper treatment. This has caused bad odor and drainage water pollution to surrounding areas, environment, and created uncomfortable scenes for the public. For an example, at one

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