DEVELOPMENT OF FORMULATED FISH FEED SUPPLEMENTED WITH MICROALGAE OF PHYCOREMEDIATION ON THE GROWTH PERFORMANCE AND FATTY ACIDS CONTENT OF *OREOCHROMIS NILOTICUS*

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DEDICATION

I would like to dedicate this achievement to my family especially to my beloved parents, Nur Hanisah Sarjit Binti Abdullah and Hairuddin Bin Haris. To my family members, Muhammad Ridhwan, Nurul Atikah, Nur Aisyah, Orked Nur Alesha, Mawar Nur Raisha and my beloved Kitty. Thank you for all your supports and always being there for me.

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ABSTRACT

The arising issues on the use of fishmeal which causes over exploitation of the wild caught fish and the increment cost of aquaculture is increasing day by day. This has led to find new alternatives to replace fishmeal such as microalgae. In this study, 2 % of *Scenedesmus* sp. cultivated in Bold Basal Medium and wet market wastewater was partially subtituted to replace fishmeal and tested to *Oreochromis niloticus* fingerlings for 12 weeks. The physicochemical properties of pelletized feed was analysed and compared between control formulation, formulation 1 and formulation 2. The results found that, addition of *Scenedesmus* sp. in the diet would not alter the hardness of the feed, yet the small increment of hardness in the diet formulation 1 and 2 might be due to the failure of maintaining weight of the feed before and after pelletizing process. Besides, the analysis of variance showed that moisture content of formulation 1 and 2 were significantly difference ($p<0.05$) and the mineral composition of the feed shows an improvement for formulation 2, however, it is still not meet the requirement due to the absence of complete diet ingredients in the formulations. Moreover, the growth performance of *Oreochromis niloticus* was analysed weekly using analytical balance and the results obtained showed an improvement in the final weight of the fish in formulation 1 and 2 up to 43.38g and 44.85 g respectively. The concentration of fatty acids obtained in this study was acceptable, though, the concentration of omega 6 is higher compared to omega 3. To conclude this finding, the addition of *Scenedesmus* sp. in the diet formulation for *Oreochromis niloticus* gives a small difference in physicochemical properties of the feed pellet, yet it facilitates in maintaining and improving the growth and the quality of muscle tissue of *Oreochromis niloticus.*

ABSTRAK

Isu berkaitan pengunaan makanan ikan asli yang menyebabkan eksploitasi ikan liar dan kenaikan kos akuakultur semakin meningkat dari hari ke hari. Hal demikian telah membawa kepada pencarian alternatif baru untuk menggantikan makanan ikan asli seperti mikroalga. Dalam kajian ini, 2 % daripada *Scenedesmus* sp. di ternak menggunakan media *Bold Basal* dan air pasar untuk menggantikan makanan ikan asli dan diuji kepada *Oreochromis niloticus* selama 12 minggu. Sifat fizikokimia pellet makanan dianalisis dan dibandingkan antara formulasi kawalan, formulasi 1 dan formulasi 2. Bedasarkan keputusan analisis varians, penambahan *Scenedesmus* sp. dalam diet tidak mengubah kekerasan pelet makanan, namun peningkatan kecil kekerasan pelet dalam formulasi 1 dan 2 mungkin disebabkan oleh kegagalan mengekalkan berat pelet sebelum dan selepas proses pembuatan pelet. Selain itu, analisis varians menunjukkan bahawa kandungan kelembapan formulasi 1 dan 2 mempunyai perbezaan yang ketara ($p<0.05$) dan kandungan mineral di dalam pelet makanan menunjukkan peningkatan untuk formulasi 2. Walau bagaimanapun, ia masih tidak memenuhi nilai keperluan yang dicadangkan kerana ketiadaan diet yang seimbang dalam formulasi. Selain itu, prestasi pertumbuhan ikan *Oreochromis niloticus* dianalisis setiap minggu menggunakan penimbang elektronik dan data yang diperolehi menunjukkan peningkatan berat akhir ikan dalam formulasi 1 dan 2 masing-masing sebanyak 43.38g dan 44.85 g. Kepekatan asid lemak yang diperolehi dalam kajian ini boleh diterima, walaupun, kepekatan omega 6 adalah lebih tinggi berbanding omega 3. Sebagai kesimpulan, penambahan *Scenedesmus* sp. dalam formulasi diet untuk *Oreochromis niloticus* memberikan perbezaan kecil dalam sifat fizikokimia pelet suapan, namun ia membantu dalam mengekalkan dan meningkatkan pertumbuhan dan kualiti tisu otot *Oreochromis niloticus*.

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LIST OF SYMBOLS AND ABBREVIATIONS

AAFCO	-	Association of American Feed Control Official
ANOVA	-	Analysis of variance
AOAC	-	Association of Official Analytical Chemist
B_1	-	Thiamine
B_2	-	Riboflavin
B_3	-	Niacin
B_5	-	Pantothenic Acid
BBM	-	Bold Basal Medium
Cell/mL	-	Cell per milliliter
DHA	-	Docosahexaenoic Acid
DO	-	Dissolved oxygen
DOF	-	Department of Fisheries
E	-	d-alpha-tocopherol
EPA	-	Eicosapentaenoic Acid
FAME	-	Fatty Acid Methyl Ester
FAO	-	Food and Agricultural Organization
FCR	-	Food Conversion Ratio
H_2SO_4	-	Sulphuric Acid
HCL	-	Hydrochloric acid
Hg	-	Mercury
HMSO	-	Highline Medical Services Organization
HNO_3	-	Nitric Acid
HPLC	-	High performance liquid chromatography
ICPMS	-	Inductive Coupled Plasma Mass Spectrometry
kg	-	Kilogram
KOH	-	Potassium Hydroxide
L	-	Liter

LC PUFA	-	Long Chain Polyunsaturated Fatty Acid
m	-	meter
MFAR	-	Malaysian Food Act and Regulation
mg/L	-	Milligram per liter
mL	-	Milliliter
mm	-	Millimeter
Mm Hg	-	Millimeter of Mercury
MUFA	-	Monounsaturated Fatty Acid
n3	-	Omega 3
n6	-	Omega 6
n9	-	Omega 9
NaOH	-	Sodium hydroxide
NH_3	-	Ammonia
nm	-	Nanometer
NO_3	-	Nitrate
NRC	-	National Research Council
NSP	-	Non-Starch Polysaccharide
PUFA	-	Polyunsaturated Fatty Acid
RPM	-	Revolution per minute
SFA	-	Saturated Fatty Acid
SGR	-	Specific Growth Rate
SPSS	-	Statistical Package for Social Science
SR	-	Survival Rate
USA	-	United States of America
USEPA	-	United States Environmental Protection Agency
Uv-vis	-	Ultraviolet-Visible Spectroscopy
WHO	-	World Health and Organization
β sheet	-	Beta Sheet
µL	-	Microliter
µm	-	Micrometer

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LIST OF PUBLICATIONS

Arifin, S. N. H., Mohamed, R. M. S. R., Al- Gheethi, A. A. S., Lai, C. W., Gopalakrishnan, Y., Hairuddin, N. D. H. & Vo, D. (2021). Photocatalytic degradation of triclocarban in aqueous solution using a modified zeolite/TiO_2 composite: kinetic, mechanism study and toxicity assessment. *Environmental Science and Pollution Research*. https://doi.org/10.1007/s11356-021-16732-y

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CHAPTER 1

INTRODUCTION

1.1 Research background

Aquaculture shows an increasing trend over the coming decades as it provides half of the fish for human consumption (Camacho-Rodriguez *et al*., 2017). According to FAO (2018), aquaculture remains as one of the major food sectors worldwide due to its efficiency in producing edible protein in response to the substantial rise in the demand for fish. However, the feed inputs in aquaculture practices contribute up to 75% of total aquaculture production and the price of fishmeal as a main traditional ingredient used in the aquafeed keep on increasing due to its high protein and fatty acid content (FAO, 2018).

Malaysia is encircled by sea, and have many rivers and lakes. These ecosystems provide natural sources of fish and other aquatic resources (Yusoff, 2015) for its residents. Therefore, fish has also been one of the important daily diets of Malaysians and a leading source of protein. Based on the consumption pattern by Yusoff (2015), an average one family spends about 20% of their food consumption on fish. In fact, the fish consumption index increased from 53.1 kg in 2011 and as in 2020, the consumption index has reached 61.1 kg. In Malaysia, the aquaculture industry contributes about 16% to the national seafood supply (Ng, 2009).

Moreover, the aquaculture industry contributes to national food security and bringing in foreign exchange for the country. Total fish production in 2012 contributed about 1.7 millions of tonnes cost at RM6 billion to the national fish supply (Yusoff, 2015). Development of the fisheries sector is expected to become one of the major contributors to the national economy, as Malaysia rely heavily on aquaculture as a source of income and animal protein. (Ibrahim *et al*., 2018). According to Yusoff

(2015), the demand for fish in Malaysia is expected to increase from 1.7 million tonnes in 2011 to 1.93 million tonnes by 2020. Besides, in producing a good diet formulation for fish, protein is considered as one of major food components. It is undeniable that fishmeal is a good protein for the diet of aquatic organisms. However, due to the high demand of aquaculture production with the increasing issues of using fishmeal as a main protein source in the diet formulation has led to the need to find new alternatives, such as animal and plant sources of proteins to replace fishmeal for the needs of aquaculture (Sirakov *et al*., 2015). Referring to Sirakov *et al*. (2015), the most accessible and inexpensive food component in aquaculture is microalgae.

Microalgae is photosynthetic organisms and it is the main source of cellular carbon and chemical energy for other organisms (Hattab *et al*, 2014). The popularity of microalgae in the aquaculture industry is increasing day by day due to their appropriate size for ingestion, high nutritional value, high growth rate, antioxidant property, and disease resistance. The nutritional value of microalgae is influenced by many factors such as size, shape, digestibility, and biochemical compositions (Guedes and Malcata, 2012). Hattab *et al*. (2014) has mentioned that, microalgae are cultivated for many purposes such as a bio filter to remove nutrients and other pollutants from wastewater and also as fish feed in aquaculture. Culturing of microalgae as feed in aquaculture develop since Allen and Nelson's triumph in 1910 by cultivating *Chlorella* sp. in Berlin, Germany (Preisig & Andersen, 2005). The common genus of microalgae used including, *Chlorella, Tetraselmis, Scenedesmus, Pavlova, Phaeodactylum, Chaetoceros, Nannochloropsis, Skeletonema* and *Thalassiosira*. As mentioned by Brown *et al*. (1997), analyses of microalgae in his study found that microalgae have similar contents of essential amino acids as in fishmeal. Many microalgae species have been used in fish feed formulations to assess their nutritional value, and its advantages in replacing the main protein source. For examples, *Chlorella* fed to Korean rockfish (Bai *et al*., 2002) and *Scenedesmus* fed to Tilapia (Tartiel *et al*., 2008).

Scenedesmus sp. is a green microalgae classified under family Chlorophyceae. The availability of essential amino acids required in fish growth and the high level of crude protein in this species make it possible to substitute fish meal (Bawdy *et al*., 2008). There are three major nutrients to be considered in microalgae to meet the needs of feed ingredients which are protein, carbohydrate and lipid. The nutritional value of protein component for microalgae is considered high if their essential amino acid composition is relatively close or similar to the requirements of the feeding animals (Misurcova *et al*., 2014). *Scenedesmus* is a source of single cell protein up to 40 to 70% by converting the solar energy, as claimed by Nasseri *et al*. (2011). Carbohydrates in microalgae are found in the form of starch, cellulose, sugars and other polysaccharides. In microalgae, carbohydrates serves two main functions which are, a structural components in the cell walls and as storage components inside the cell (Raven and Beardall, 2004). Lipids are highly significant at various growth stages of many aquaculture animals including fish larvae. In green algae, the average lipid content is 23% while in *Scenedesmus* sp. is 26% (Rakesh *et al*., 2016).

In addition, wastewater which is commonly discharged without treatment contained excess nutrients that are good for the growth of microalgae. Thus, due to the flexibility and adaptability of this microalgae towards cultivation in extreme conditions such as in wet market wastewater, has allowed it to undergo phycoremediation process which may results in microalgae biomass with a higher nutrient content that is ideal as a fish feed supplement and indirectly helped in reducing cost by lowering the use of fishmeal in the diet formulation (Mobin & Alam, 2014). Therefore, these strengthen that the phycoremediation process is good to improve the nutrient in microalgae to be used as fishmeal substitutes.

O. niloticus known as Nile tilapia, is one of the most cultured tropical fish worldwide (World Bank, 2013). *O. niloticus* possess five characteristics that make it as one of the selected fish species in the aquafarming industry which are high growth rate, versatility in environmental tolerance, stress and disease resistance, high reproductive rate and high acceptance for different diets (El- Sayed, 2006). The global production of this species was 4.5 million tonnes in 2013 and aimed to exceed 6.6 million tonnes by 2030 (FAO, 2014). Specific in Malaysia, a study by amal and Zamri-Saad (2011) has stated that, the production of *O. niloticus* reaches up to 20.8 metric tonnes. Moreover, this fish species is highly favoured in local and international market. Large scale production of *O. niloticus* through aquaculture practices would help to meet the demand of this species for both local supply and exportation. Overall, finding new alternative protein sources is important to minimize the problems of relying on fish meal and indirectly reduce cost in aquaculture practices. The phycoremediation process of *Scenedesmus* sp. in wetmarket wastewater would be one of the effective ways to improve the nutrient content in microalgae to replace fishmeal in fish feed diet formulations.

1.2 Problem statement

The ocean derived fishmeal and fish oil in aqua feeds has given rise to sustainability concerns as it will not meet the growing demand and will constraint aquaculture growth. As the aquaculture practices increased worldwide, the demand for fishmeal increases linearly, with prices rising by almost 300% in the past 10 years (Origin oil, 2014). It is inarguable that fishmeal is one of the best protein sources in the diet for fish, however, continuous exploitation of this natural resource will definitely lead to environmentally and economically unsustainable. Therefore, it is important to find alternative protein sources to replace fishmeal to fulfil the demand for fish in aquaculture production.

Currently, microalgae have received more attention because some of the species contain higher protein source that is almost similar to fishmeal. Comparing with animal by-product, microalgae is more preferable since animal by product requires the rendering process before using in the farm (Jedrejek *et al*., 2016). Moreover, according to Shah *et al*. (2017), the composition of commercial microalgae species such as proximate analysis, amino acids and fatty acids and their mineral content is comparable to the available feed ingredients in the aqua feed industry (Kent *et al*., 2015). Therefore, in this study, microalgae *Scenedesmus* sp. was cultivated in wet market wastewater as a protein replacer to partially replace fishmeal.

1.3 Significant of study

The State of World Fisheries and Aquaculture reported by FAO (2012) outlined that aquaculture is one of the fastest growing feed sectors, having almost 60 million tonnes globally for the past decades, and it is expected to grow up to 50% in the next years. According to FAO, a continuous expansion of the human population is predicted to pass nine billion by 2050, simultaneously the economic development gives a greater proportion with an income that allows them to choose their diet (Obach, 2012). Fishmeal has been used as a source of protein in the past decades in formulating artificial fish feed. About 36% of fisheries catch worldwide was processed into fishmeal to be incorporated into feeds for farmed fish every year (Ogello et *al*., 2014). Tacon and Metian (2008) stated that a global survey estimated aquaculture consumption of fishmeal is 3724 thousand tonnes in 2006. Aladetohun and Sogbesan (2013) added that, aquaculture practices are predicted to grow over the next 20 years indirectly would increase the demand for fishmeal and fish oil which indirectly may increase the pressure on the already threatened stocks of wild fish. Hence, the increasing demand of the production for aquatic animals must be supported by a corresponding increase in the production of formulated diets for culturing aquatic animals (Rahman et *al*., 2013). It is undeniable that fishmeal would fulfil the requirement of essential amino acids of the farmed animals to maximize growth. However, the use of fishmeal should be controlled to ensure food security and sustainability of the food chain.

In Asia, fish feed production increased from 40% in 2000 to 60% in 2008 while the usage of fishmeal for tilapia culture risen from 0.8 million tonnes to 1.7 million tonnes during the same period (Tacon & Metian, 2008). In addition, fish feed accounts for the highest operational cost in aquaculture with protein being the most expensive diet (Munguti et *al*., 2012) and fishmeal is the most expensive protein source in aquaculture feeds (Egerton *et al*., 2020). Moreover, the availability of fish meal and formulated fish feed in Malaysia is low and depending on importation which cause increasing of the operating cost (Chun, 2007).

The usage of microalgae in aquaculture as feed is uncommon. Microalgae are known as promising living cells which are at the base of the aquatic food chain due to their good nutritional value (Sathasivam *et al*., 2019). According to Allen and Nelson (1910), the first reports of the microalgae as feed in aquaculture practices were published since 1910, and the use of microalgae has developed widely (Duerr *et al*., 1998; Santos-Ballardo *et al*., 2015). The main application of microalgae in aquaculture could relate directly or indirectly to the nutrition impacts on many aquatic farmed organisms, for instance, rotifers and fish. Moreover, due to some characteristics such as lipids and carotenoids content, microalgae are known as the next generation of sustainable feedstock (Murray *et al*., 2013; Perez-Lopez *et al*., 2014).

In this study, freshwater microalgae from the genus *Scenedesmus* sp. was chosen to replace fishmeal in formulated fish feed. *Scenedesmus* sp. has a relatively similar essential amino acid profile to fishmeal. It is inarguable that additional cost will be used to produce formulated fish feed as Bold Basal's Medium (BBM) also will be used to cultivate *Scenedesmus* sp. However, the cultivation of *Scenedesmus* sp. using wet market wastewater will resulted in effluent with low nutrient and microalgae with higher nutrient composition. It is an economically fesible way to cultivate microalgae on a large scale. The ability of *Scenedesmus* sp. to perform phycoremediation would also ensure the nutrient recovery and indirectly prevent environmental pollution.

Therefore, the findings of this study are beneficial especially to the aquaculture and fish farming industry to enhance an increasing production of the eco- friendly formula of fish feed which consists of lower usage of fishmeal while maintaining the nutritional value for growth performance of *O. niloticus*.

1.4 Objective of study

The objectives of this study are:

I. To compare and formulate fish feed with 2% replacement of fishmeal with *Scenedesmus* sp. cultivated in Bold Basal Medium and wet market wastewater.

II. To study the physicochemical properties of the microalgae substitution cultivated in Bold's Basal Medium and wet market wastewater formulated feed.

III. To investigate the effect of microalgae supplemented products on fish growth performance, and fatty acids content in *O. niloticus* muscle tissue.

1.5 Scope of study

I. The study was focused on the formulation of feed with partial replacement of fishmeal with locally obtained *Scenedesmus* sp. that was cultivated in two different mediums which are standard medium and wet market wastewater.

II. In this study, the physicochemical properties of the formulated feed produced was analysed and compared with the control formulation and the feed prototype was tested to *O. niloticus* fingerlings.

III. The growth performance of *O. niloticus* and the water quality of the experimental tank was analysed weekly for 12 weeks. At the end of experimental period, the analysis on the fatty acids methyl ester in the muscle tissue of the fish was done to ensure a good quality of the fillet produced.

CHAPTER 2

LITERATURE REVIEW

2.1 Phycoremediation concept

The use of algae, either macroalgae or microalgae to treat wastewater has been discovered and practices for over 40 years (Ahmad *et al.*, 2013). Phycoremediation term was introduced by John (2000), which defines a bioremediation process performed by microorganisms such as microalgae (John, 2000; Gani *et* al., 2015). Besides, the use of microalgae culture to treat wastewater is firstly studied by Oswald *et al*. (1957). These photosynthetic microorganisms in wastewater aid in the bio transform the pollutant loads in their cell and clean the wastewater before being released to the environment (Rawat *et al*., 2011). According to Gani *et al*. (2016), there are some benefits of using microalgae in wastewater treatment which are effective in cost, environmentally friendly and not producing any toxic gases to the environment. Moreover, the algal biomass has the potential of high nutrient value, heavy metals removal and can be used as a live feed and formulated feed for both agriculture and aquaculture practices.

However, the removal efficiency of the pollutants by microalgae is depending on the species. According to Mohamed *et al*. (2017), phycoremediation of wastewater using microalgae occurs within 2 to 26 days to reduce pollutants to a minimum level. Only a few researchers have been done on phycoremediation of microalgae using various types of wastewater. Table 2. 1 below shows the study of using microalgae in wastewater treatment.

Based on Table 2.1, various microalgae species such as *Chlorella vulgaris*, *Nostoc commune*, *Scenedesmus* sp., *Botrycoccus* sp., and *Oscillatoria* sp. are used in wastewater for phycoremediation treatment. Latiffi *et al*. (2017) and Jais *et al*. (2015) studied the phycoremediation of meat processing wastewater and wet market wastewater by using the same type of microalgae which is *Scenedesmus* sp. while different studies on the physicochemical parameters' removal by *Botryococcus* sp. on two other type of wastewater which are dairy wastewater and Grey wastewater were conducted by Gani *et al*. (2015).

Table 2.1: Phycoremediation study using microalgae

Sources	**Phycoremediation summary**	**Reference**
Meat processing wastewater	Preliminary assessment of growth rates on different concentrations of microalgae *Scenedesmus* sp. in industrial meat food	Latiffi *et al*. (2017)
Wet market wastewater	Removal of nutrients and selected heavy metals in wet market wastewater by using microalgae *Scenedesmus* sp	Jais *et al*. (2015)
Dairy and Grey wastewater	Phycoremediation of greywater and dairy wastewater by *Botryococcus* sp. for physiochemical parameters removal	Gani *et al*. (2015)
Sewage treatment plant wastewater	Application of *Chlorella vulgaris* for reduction of organic and inorganic pollutant in sewage treatment plant wastewater	Sahu, (2014)
Industrial wastewater	Application of microalgal bacterial flocs for industrial wastewater treatment using sequencing batch reactors	Hende *et al*. (2014)
Municipal wastewater	Phycoremediation of municipal wastewater using *Oscillatoria* and *Nostoc commune* obtained from Mula Mutha river	Azarpira *et al*. (2014)
Domestic wastewater	Domestic wastewater bioremediation using microalgae *Chlorella vulgaris* and *Scenedesmus quadricauda* for physiochemical reduction	Kshirsagar, (2013)

The aforementioned study above, is almost similar to the research performed by Sahu (2014); Hende *et al*. (2014); Azarpira *et al*. (2014) and Kshirsagar (2013), which focus on the efficiency of the microalgae to remove the pollutants in the wastewater. It is common to study the wastewater treatment using microalgae. However, the applications of the by- product and its benefit should not be ignored. The illustration in Figure 2.1 shows the mechanism of wastewater treatment with the presence of microalgae, its biomass application, and the benefits of the wastewater after treatment.

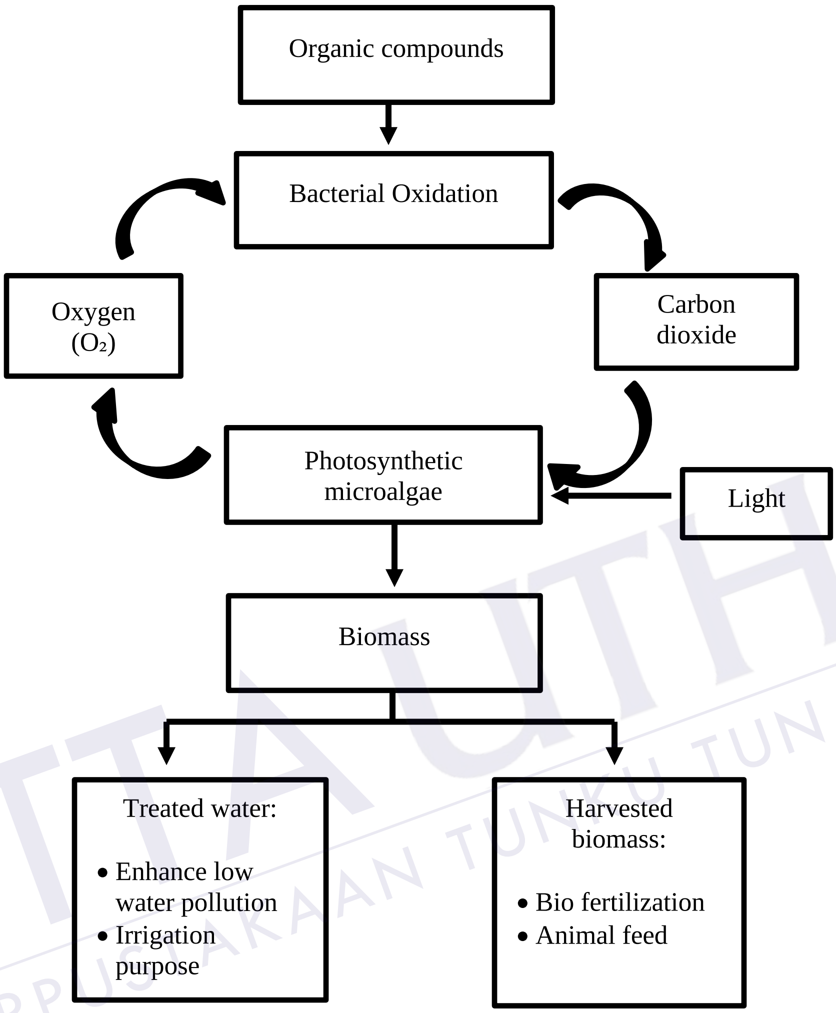

Figure 2.1: Mechanisms of wastewater treatment using microalgae

The phycoremediation of wastewater using microalgae starts with organic matter which enhance the growth of bacteria and carbon dioxide released. The carbon dioxide is then used by microalgae for photosynthesis with the aid of light from the surrounding. Moreover, this photosynthetic microalga will treat the wastewater by absorbing all the excess nutrients inside the medium and the oxygen released to the environment will be cycled for bacterial oxidation. Commonly, the microalgae by-product can be applied for many purposes such as for bio fertilization and as animal feed in aquaculture industry. Apart from that, the treated wastewater will ensure a discharge of wastewater into the aquatic ecosystem such as rivers and lakes with a low

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