REMOVAL OF NUTRIENTS AND HEAVY METALS FROM DOMESTIC AND INDUSTRY USING BOTRYOCOCCUS SP.

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DEDICATION

Dedicated to my beloved family, friend and my supervisor

Thank you for your support and being the reason for me to complete my study.

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ABSTRACT

Microphytes or microalgae are the most basic food source of many types of organisms on earth and blooms during the presence of dissolved inorganic phosphorus. Wastewater is a body of water that is dangerous to organic life forms when consumed or used. It contains many pollutants that can cause health problems and also affect the ecosystem of an environment. This study aims to improve the water quality of wastewaters using phycoremediation process. The objectives of this study are to determine the growth of *Botryococcus sp.* in different types of wastewater in terms of resistance and survival of *Botryococcus sp*. in phycoremediation performance, to measure the environmental factor effecting the growth of *Botryococcus sp.* of phycoremediation process, to optimize the physiochemical and heavy metal removal in different types of wastewaters and to evaluate the effectiveness of *Botryococcus* sp. to remove the pollutants in wastewaters. Phycoremediation or bioremediation process is using macroalgae or microalgae for removing pollutants, nutrients, xenobiotics and heavy metals from wastewater. This research was done by collecting microalgae sample, isolating and culturing the required *Botryococcus* sp. Growth optimization and followed by phycoremediation process is done to remove unwanted elements from wastewaters. The optimum growth rate of algae is achieved when salinity is at 0M, temperature at 33^0C, photoperiod at 12:12 and light intensity of 18000 Lux. Result shows that the highest nitrate removal percentage occurs in semiconductor (100%), followed by palm oil mill effluent (97.29%), textile wastewater (98.04%) and domestic wastewater (85.43%). Total Phosphorus removal indicates the highest percentage for domestic wastewater (100%), palm oil effluent (99.2%), textile wastewater (98.44%) and semiconductor (50.39%). From this research, it is found that the best overall removal of physiochemical and heavy metal content occurs in palm oil mill effluent followed by domestic wastewater, semiconductor wastewater and textile wastewater.

ABSTRAK

Microphytes atau mikroalga adalah sumber makanan asas pelbagai jenis organisma di bumi dan ia tumbuh dengan kehadiran fosforus bukan organik terlarut. Air sisa ialah jenis air yang berbahaya kepada semua bentuk kehidupan apabila dimakan, diminum atau digunakan. Ia mengandungi banyak bahan pencemar yang boleh menyebabkan masalah kesihatan dan memberi kesan kepada ekosistem persekitaran. Kajian ini bermatlamat untuk membaiki kualiti air sisa menggunakan proses "phycoremediation". Objektif-objektif kajian ini adalah untuk mengkaji pertumbuhan *Botryococcus sp.* di pelbagai jenis air sisa terutamanya dari segi faktor ketahanan dan kebolehidupan *Botryococcus sp.* dalam prestasi proses bioremediasi, untuk mengkaji faktor persekitaran yang memberi kesan kepada pertumbuhan *Botryococcus sp.* sebelum proses bioremediasi, untuk mengoptimumkan fizikokimia dan logam berat dalam pelbagai jenis air sisa dan untuk menilai tahap efektif *Botryococcus sp.* untuk membuang bahan pencemar dari air sisa. "Phycoremediation" atau bioremediasi menggunakan mikroalga untuk menyingkirkan bahan pencemar, nutrisi, xenobiotik dan logam berat daripada air sisa. Kajian ini dijalankan dengan mengumpul sampel mikroalga, mengasingkan dan mengkultur *Botryococccus* sp. yang diperlukan. Seterusnya pengoptimuman dan proses 'phycoremediation' dijalankan untuk menyingkir unsur - unsur dari air sisa. Kadar pertumbuhan optimum bagi alga dicapai apabila kemasinan 0M, suhu 33^0C, kadar foto pada 12:12 dan keamatan cahaya 18000 Lux. Keputusan menunjukkan bahawa penyingkiran tertinggi peratusan nitrat berlaku dalam air sisa semikonduktor (100%), diikuti oleh air sisa kilang kelapa sawit (97.29%), air sisa tekstil (98.04%) dan air sisa domestik (85.43%). Jumlah penyingkiran Fosforus menunjukkan peratusan tertinggi bagi air sisa domestik (100%), air sisa kelapa sawit (99.2%), air sisa tekstil (98.44%) dan air sisa semikonduktor (50.39%). Menurut kajian ini, didapati bahawa penyingkiran terbaik keseluruhan kandungan logam fisiokimia dan berat berlaku dalam air sisa kilang kelapa sawit diikuti oleh air sisa domestik, air sisa semikonduktor dan air sisa tekstil.

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LIST OF SYMBOL AND ABBREVIATIONS

%	Percentage
^0C	Degree Celsius
µm	Micrometer
AAS	Atomic Absorption Spectrometer
APHA	American Public Health Association
As	Arsenic
Ba	Barium
BBM	Bold Basal Medium
BOD	Biochemical Oxygen Demand
Ca	Calcium
Cd	Cadmium
COD	Chemical Oxygen Demand
CO_2	Carbon Dioxide
Cr	Chromium
Cu	Copper
DO	Dissolved Oxygen
DOE	Department of Environment
Fe	Iron
FKAAS	Faculty of Civil and Environmental Engineering
Hg	Mercury
IC	Inorganic Carbon
ICP	Inductively Coupled Plasma
L	Liter
Lx	Lux
Mg	Magnesium
mL	Milliliter
MS	Mass Spectrometry
Ni	Nickel

NTU	Nephlometric Turbidity Unit
Pb	Lead
POME	Palm Oil Mill Effluent
Sr	Strontium
TC	Total Carbon
TN	Total Nitrate
TOC	Total Organic Carbon
TP	Total Phosphorus
TS	Total Solid
TSS	Total Suspended Solid
UTHM	Universiti Tun Hussein Onn Malaysia
Zn	Zinc

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Wastewater contains pollutants load that can negatively impact the environment if not controlled in terms of removal. This is because wastewater containing nitrogen and phosphorus that can affect the natural ecosystem. Therefore, this research used microalgae to treat wastewater biologically, commonly known as phycoremediation. The phycoremediation technology is useful to remove excess nutrients such as nitrogen, phosphorus from wastewater and also have ability to remove various pollutants.

In addition, phycoremediation technology also can produce sustainable product such as biodiesel. According to Mata, Martins, & Caetano (2010) biodiesel can be produced from the vegetable oils (edible or non-edible) and animal fats while Amaro, Guedes, & Malcata (2011) found that the microalgae from *Botryococcus sp.* can produce 75% of oil content which have a high potential as new renewable energy. As the demand for energy continues to increase globally, fossil fuel usage will likewise continue to rise. There is still a plentiful supply of fossil fuels at reasonably low cost, although this is likely to change in the future, but more critically a rising use of fossil fuels is unlikely to be sustainable in the longer term principally due to the attributed increase in greenhouse gases emission from using these fuels and the environmental impact of these emission on global warming (Hill, Nelson, Tilman, Polasky, & Tiffany, 2006). With these types of problem that are around, the need for a solution seem dire.

The presences of conventional methods, bioremediation methods or any other different methods seem to be a matter of growing interest in researchers. This research is using the bioremediation method or more specifically, phycoremediation where microalgae *Botryococcus sp.* is used to treat the wastewaters that have been selected. *Botryococcus sp.* remediates the pollutants that are contained in the wastewaters by consuming them and using them to grow itself in numbers. At its best conditions, it provides the optimum growth of cells and pollutant removal percentages. The microalgae enhance the removal of nutrients, heavy metals and pathogens and furnish oxygen to heterotrophic aerobic bacteria to mineralize organic pollutants, using in turn the carbon dioxide released from bacterial respiration (Kotteswari, Murugesan & Ranjith, 2012). Therefore, the application of microalgae is very important as another method of pollutant minimization.

1.2 Problem Statement

Wastewater is a common water body that can be found anywhere nowadays. It can be found in the textile industry, electronic production factories, palm oil mills, residential houses and even in a restaurant. The contents of the wastewater are long known to affect the health of humans and also aquatic animals (Gani *et al.*, 2015). The common problems that rise are unwanted algae bloom, eutrophication and contamination of water sources due to improper treatment of wastewater.

The current problem with wastewater treatment plants is the high-energy consumption where 50% - 60% of plant power usage is dedicated to treating wastewater (Votano *et al.*, 2004). Other than that, highly trained operators are required to handle the treatment plant as the responsibility of running the plant differs according to seasonal changes. Sludge on the other hand means the residue generated during physical, chemical and biological treatment of wastewater. These sludges are toxic and need to be disposed safely to avoid any environmental problems. Finally, treatment plants occupy large land area and mainly processes such as sedimentation, settling and aeration are involved in this. Even the wastewater contents play a significant role as a problem to the users.

One of the main contaminants of wastewater is heavy metals. The most common heavy metals that could be found as stated by Barakat (2011) that due to the discharge of large amounts of metal-contaminated wastewater, industries bearing heavy metals, such as Cadmium (Cd), Chromium (Cr), Copper (Cu), Nikel (Ni), Arsenic (As), Lead (Pb) and Zinc (Zn) are most hazardous among the chemical-intensive industries.

Other than that, nutrients such as phosphorus and nitrogen have proved to be dangerous when they are in high amounts in the waters (Olguín & Sánchez-Galván, 2011). The water becomes not safe for consumption due to the excessive contents, contaminated by fertilizers from agricultural activities and causes eutrophication

Four common wastewaters that are found in Malaysia are domestic wastewater, textile wastewater, semiconductor wastewater and palm oil mill effluent. Domestic wastewater is found in residential areas and is treated at conventional treatment plants/ Domestic wastewater have high levels of phosphorus and nitrate which need to be treated. Textile and semiconductor wastewaters are common in industrial areas but the treatment of these wastewaters differ from conventional methods. Textile wastewater contain high levels of COD, BOD, nitrogen, heavy metal and dyes whereas semiconductor wastewater have been produced in large amounts as the ultra pure water is largely used in rinsing of components and purification after chemical-mechanical polishing. Palm oil mill effluent is very common in plantation estates that produce oil from the fruits. These factories discharge high amounts of effluents after treatment and there are high levels of biomass in them even after treatment.

Therefore, the National Water Quality Standards for Malaysia (2011) is a very important guide to make sure that the water or effluent that is being released into the environment is complying with the standards and is safe to its suitable classes. This is important to humans as water is used daily for drinking, cooking, washing and other works. Microalgae are the solution to this problem and *Botryococcus sp.* shows to be promising in becoming the best answer. The wastewaters which are filled with pollutants are cleaned biologically and instead of just cleaner water, it also provides more oxygen to the environment while bio remediating it (Gani *et al.*, 2016).

1.3 Objectives of Study

Based on the above problem statement, these objectives have been selected to find a solution: -

i. To determine the growth rate of *Botryococcus* sp. in different types of wastewater in terms of resistance and survival of *Botryococcus* sp. in phycoremedition performance.
ii. To measure the environmental factors (photoperiod, temperature, light intensity, salinity) effecting the growth of *Botryococcus* sp. for cultivation.
iii. To evaluate the effectiveness of *Botryococcus* sp. to remove the nutrients and heavy metal pollutants in wastewaters.

1.4 Scope of Study

Based on the objectives stated above, these scopes of studies have been selected to guide the research into achieving the results: -

i. Culture and harvest the required *Botryococcus sp.* using Bold Basal Medium (BBM) stock culture
ii. The optimum condition is determined for *Botryococcus sp.* growth concerning the environmental factor of photo period, temperature, light intensity and salinity
iii. The phycoremediation process is conducted at laboratory culture scale and outside culture scale using domestic, palm oil, textile and semi-conductor wastewater
iv. The changes in wastewater is measured as pH, BOD, COD, Total Phosphorus, Total Nitrogen and heavy metal before and after running the phycoremediation process

1.5 Significance of the Study

This study here is to save the ecosystem from the pollution that is happening. The pollution causes an unstable growth of aquatic plants which at the end, affects the other living organisms in the habitat or ecosystem. Other than that, the presence of heavy metals causes aquatic life forms to be affected and indirectly causes the accumulation of heavy metals in the human body. Therefore, the ecosystem needs to be cleansed from foreign contaminants.

Other than that, this study is also done to apply a natural process that can be a platform for wastewater treatment to extract the nutrients and heavy metals before it reaches the main water body. With this, it can also lower the cost or even abolish the cost of treating an ecosystem that was polluted by the nutrients or heavy metals by applying the use of the microalgae in the aquatic environment itself. Nowadays, these 4 wastewaters can be treated via phycoremediation.

Phycoremediation has many advantages such as reducing sludge formations, increasing dissolved oxygen through photosynthesis and lowering cost for wastewater treatments. This creates a treatment of wastewaters which is done biologically without any foreign chemicals where the addition of the chemicals may cause side effects.

The success of this study is able to change the conventional ways of treating wastewater of many types while being able to produce a useful byproduct that can be used in different industries. It also reduces costs in terms of maintenance and plant running expenditures.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Wastewater is the type of water that can cause many health and environmental problems because of its dangerous and untreated contents. With this in account, a proper management system is to be provided so that the wastes of domestic, municipal and industry waters will be treated as required to avoid the adverse effects. A water body can be labeled as polluted when it is unsafe for human and animal consumption, normally caused by wastes that are thrown into the water.

The main contributors to this problem are from industrial activities and agricultural activities. Malaysia, as a country with an abundant water resource should not have a problem with water pollution as it has to supply the citizens with clean water daily without any disruptions. But, with the increment of human advancement and technological developments, it has gone the other way around where the water resources are getting polluted by the advancements itself.

Organic and inorganic substances which were released into the environment as a result of domestic, agricultural and industrial water activities lead to organic and inorganic pollution (Abdel-Raouf, Al-Homaidan, & Ibraheem, 2012). At the end, it may also lead to the abundant clean water to turn into a water crisis. The attitude of certain humans at many communities, industries and agricultural sectors, the lack of law enforcement and monitoring also leads this to a higher problem level to be dealt with.

2.2 Types of Wastewaters and Characteristics

There are numerous types of wastewaters that are formed through our daily activities. These wastewaters can be categorized based on their origins of formation and also their chemical and physical contents that vary from each other. Wastewater originating from municipal, agricultural and industrial waste supply a good area of algae growth where the treatment of wastewater using algae in nutrient removal while producing biofuels (Pittman, Dean, & Osundeko, 2011).

2.2.1 Domestic Wastewater

This wastewater is the water which was used and mixed with contaminants. Examples of buildings that produce this wastewater are residential, office and institutions where they are originated from toilets, sinks and bathrooms. The contents of the wastewater comprise of mainly: -

i. Floating solids and suspended solids
ii. Small solid materials
iii. Fine solids in suspension
iv. Pollutants

Rawat *et al.,* (2011) has stated that untreated wastewater generally contains high levels of organic material, pathogenic microorganisms, as well as nutrients and toxic compounds. The contents of wastewater when left untreated are hazardous as they contain many viruses and bacteria's. The tropical temperature and the amount of heat and sunshine that is received are high therefore, the amount of water involved in evaporation is high, leaving the pollutants and turning septic. The treatment of wastewaters using algae has many advantages such as no secondary pollution, environmental friendly, biomass reusability and effective nutrient removal. (Raj *et al.*, 2015). Fiber was the biggest amount of organic waste that is found in organic wastewater (Huang, Li, & Gu, 2010).

The Table 2.1 shows the composition of this wastewater. Based on the table, the common pH range for domestic wastewater is slightly acidic to alkaline. BOD and

COD levels are high whereas dissolved solids are low. Other than that, amounts of suspended solids are high in this type of wastewater due to exposure to many environmental factors.

Table 2.1: Domestic Wastewater Composition (Kiran and Buyar, 2013; Malaysian Environment Quality Act 1974)

No	**Parameter**	**Kiran and Buyar (2013)**	**Malaysian Environment Quality Act (1974)**
		Concentration	Standard of Effluent
1	pH	6.7 – 9.5	5.5 – 9.0
2	Biochemical Oxygen Demand (mg/l)	916 – 4070	50
3	Chemical Oxygen Demand (mg/l)	1500 – 5500	< 100
4	Total Solid (mg/l)	1790 – 3730	-
5	Dissolved solid (mg/l)	1020 – 1760	< 5000
6	Suspended solid (mg/l)	990 – 1810	< 100
7	Alkalinity (mgCaCo3/l)	664 – 2610	-
8	Temperature (C)	-	40

Kasmirin (2014) has also stated that sewage from residential sources contain contaminants that could potentially cause a variety of disease which brings an impact on humans and also the environment. Domestic wastewater contains organic matter and human wastes such as urine, garbage and other solid materials. It also has dangerous bacteria. Metcalf and Eddy (2003) have stated that these bacteria will use organic matter to reduce new cells. Therefore, many methods such as physical, biological and chemical methods are being used to remove the bacteria from domestic wastewater that are being treated. Eutrophication happens in domestic wastewater as it is filled with high phosphorus levels and water environments are polluted by it (Thongtha *et al.,* 2014).

2.2.2 Palm Oil Mill Wastewater

Palm oil is the top export good of Malaysia to the world. The industry is one of the best contributors to our Malaysian economy. Based on the Malaysian Palm Oil Board (2004), Malaysia is producing 549.5% of the world's palm oil stocks. Palm oil mill effluent (POME) is one of the main sources of water pollution with regards to inland water pollution. With the growing rate of the natural oil production industry at the international level, there is a high demand in the palm oil sector as Malaysia is one of the main producers of the oil. As the demand increases, the production also increases and as a result, the effluent from these industries is also at a rise. Malaysian rivers are polluted highly because of palm oil mill industry waste generations (Wu *et al.*, 2010; Wong, Kadir, & Teng, 2009). Rupani & Singh, (2010) states that among the waste generated, palm oil mill effluent (POME) is considered the most harmful waste for the environment if discharged untreated. The environment is harmed via water pollution if the palm oil mill wastewater is discharged without legitimate treatment besides having emanation of a foul smell the neighborhood of a factory. Proper wastewater treatment strategies shall be utilized to avoid this (Chotwattanasak & Puetpaiboon, 2013).

Palm oil and rubber industry production was recorded highest total industrial pollution with 80% figure in total (Lorestani & Zinatizadeh, 2006). Palm oil mill effluent is a thick brownish liquid that contains high solids, oil and grease, COD and BOD values. Direct discharge of POME into the environment is not encouraged due to the high values of COD and BOD (Hadiyanto, 2013; Poh & Chong, 2009). The reason as to its importance to be treated before released to the environment is to make it survivable for marine life. Table 2.2 shows the characteristic of POME wastewater.

Table 2.2 : The Characteristic of POME (Khalek, 2007)

Parameter	**Unit**	**Concentration**			**Discharge limit**
		Rupani et al (2010)	Lorestani et al (2006)	Madaki & Seng et al (2013)	
Biochemical Oxygen Demand	mg/l	25000	25000	25000	100
Chemical Oxygen Demand	mg/l	50000	50000	51000	-
pH	-	4.7	4.7	4.2	5 – 9
Temperature	°C	80 – 90	-	-	45
Total Suspended Solid	mg/l	18000	18000	18000	400
Total Solid	mg/l	40500	40500	40000	-
Total Volatile Solid	mg/l	34000	34000	34000	-
Total Nitrogen	mg/l	750	750	750	200
Ammonical Nitrogen	mg/l	35	35	35	-
Oil and Grease	mg/l	4000	4000	4000	50

Basically a higher BOD means it has a poor quality of water, and the inverse hold true as well (Lorestani & Zinatizadeh, 2006). Raw POME has the BOD amount of 100 times higher than domestic wastewater. This is because POME which is oily requires high amount of oxygen to decompose in the water stream. Although after treatment, POME still holds high levels of organic matter and this is a factor which is impending to the environment.

POME is initially at high temperatures, acidic, possessing brownish suspensions and the pH value is low as inorganic acids in the POME are produced in the fermentation process. Rupani & Singh, (2010); Lorestani & Akbar Zinatizadeh, (2006); Madaki & Seng (2013) has stated that untreated POME is categorized with the same value of several characteristics by high amounts of total solids, oil and grease, COD and BOD. It is also identified as one of the major sources in aquatic pollution in Malaysia.

Kamyab *et al.*, (2013) showed that POME is a colloidal suspension consisting of 95 – 96% water, 0.6 – 0.7% oil and 4 – 5% total solids. POME is considered non-toxic as no dangerous chemicals are added during processing and raw POME has high amount of degradable matter.

The contents of POME can sustain the growth of microalgae, *Botryococcus sp.* as the growth of these algae can be sustained by the oxygen amounts and nutrients in it. There are also presence of fungi and bacteria in this wastewater such as *Aspergillus niger, Aspergillus flavus, Aspergillus fumigates, Fusarium, Penicillium, Mucor,* and *Candida* are examples of fungi which are in these wastewaters. But as the POME is released at its final stages, these fungi die as its effluent is at very high temperatures that the fungi could not sustain it.

2.2.3 Textile Industry Wastewater

The textile industry is one of the most rapidly growing industries in the world. As fashions are introduced, the need to produce the clothing rises by the day and as a side effect, the production of its wastewater also increases. The textile industry uses vegetable fibers such as cotton, animal fibers such as wool and silk, and a wide range of synthetic materials such as nylon, polyester, and acrylics. The production of natural fibers is approximately equal to the amount of production of synthetic materials of which polyester accounts for about half (Commission, 2002; Tüfekci, Sivri, & Toroz, 2007). During the dyeing process, high amounts of sodium sulphate are used and for the neutralization process, sulphuric acid is used. Untreated textile wastewater may be a wellspring of substantial metal contamination over oceanic biological communities (Sekomo *et al.*, 2012).

But even after the neutralization process, the presence of sodium sulphate is imminent in the effluent from the industry. The disposal of textile effluent in treatment plants which are not specifically designed to treat textile wastewater may cause no difference in its treatment as the pollutants may pass through all the stages of treatment and yet, stay unchanged or treated. Releasing these untreated waters will surely harm aquatic life when it is released into water sources. Various industries such as textile, pigments, plastics, mining, electroplate-ing, metallurgical processes, etc releases heavy metals into the environment (Bulgariu & Bulgariu, 2012). The Table 2.4 shows the characteristics of this wastewater.

Table 2.3: Characteristics of Textile Wastewater (Kdasi *et al.,* 2004)

Parameters	**Values**
pH	7.0 – 9.0
Biochemical Oxygen Demand (mg/l)	80 – 6000
Chemical Oxygen Demand (mg/l)	150 – 12000
Total suspended solids (mg/l)	15 – 8000
Total dissolved solids (mg/l)	2900 – 3100
Chloride (mg/l)	70 – 80
Total nitrogen (mg/l)	70 – 80
Color (Pt-Co)	50 – 2500

The pollutants that are present in the released waters will affect the environment is many ways such as dissolved oxygen depletion in water, lower photosynthesis functions and harm life forms in rivers to acids.

2.2.4 Semiconductor Industry Wastewater

The electric and electronic industry is blooming rapidly in many countries and Malaysia is not left out of it. Omar *et al.*, (2007) states that the semiconductor is used in computers and their peripherals, communication devices, electronic products, scientific and medical test equipment's.

The semiconductor industry discharges high levels of metal contaminated effluents after the production of its parts. Cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), strontium (Sr) and zinc (Zc) are the common heavy metals that can be found in the effluents from this industry. When the heavy metal filled effluents meet natural waters, the high solubility characteristics of the water environment makes the heavy metals easier to be adsorbed into the aquatic animals and thus, entering the food chain of the human population. In order to adapt to the toxicity level of the heavy metals, micro-algae have developed an extensive spectrum of mechanisms which are known as extracellular and intracellular (Singh, Tiwari, & Das, 2016). The Table 2.5 shows the characteristics of this wastewater.

These heavy metals affected aquatic life forms are consumed by human, the accumulation of the heavy metals in the human body may occur. When the level of heavy metals accumulation is reached in one's body, diseases such as in Table 2.6 may occur.

Table 2.4: Characteristic of semiconductor wastewater (Huang *et al.*, 2011)

No	**Parameter**	**Unit**	**Concentration**
1	pH	-	7.4
2	Conductivity	µs/cm	1845
3	Total bacterial count	CFU/ml	$2.38 \text{ X } 10^{-6}$
4	Suspended solid	mg/l	24.4
5	Turbidity	mg/l	25
6	Total organic carbon	mg/l	9.56
7	Chemical oxygen demand	mg/l	50.4
8	Ammonium	mg/l	10.6
9	Total phosphorus	mg/l	1.0
10	Phosphate	mg/l	0.4
11	Silicate	mg/l	9.0
12	Sulfate	mg/l	262.3
13	Chloride	mg/l	288.3
14	Total dissolved solid	mg/l	1103.6
15	Alkalinity	mg/l	177
16	Ca Hardness	mg/l	198.6
17	Total hardness	mg/l	281.1

Table 2.5: Heavy metals and their effects on human health (Singh *et al.*, 2011)

Pollutants	**Major sources**	**Permissible (mg/l)**
Arsenic	Pesticides, fungicides, metal smelters	0.02
Effects on human health	Bronchitis, dermatitis, poisoning	
Cadmium	Welding, electroplating, pesticides, fertilizers, Cd and Ni batteries, nuclear fission plant	0.06
Effects on human health	Renal dysfunction, lung disease, lung cancer, bone defects (Osteomalacia, Osteoporosis), increase blood pressure, kidney damage, bronchitis, gastrointestinal disorder, bone marrow, cancer	
Lead	Paint, pesticide, smoking, automobiles emission, mining, burning of coal	0.1
Effects on human health	Mental retardation in children, development delay, fatal infant, encephalopathy, congenital paralysis, sensor neural deafness, acute	

	or chronic damage to the nervous system, epilepticus, liver, kidney, gastrointestinal damage	
Manganese	Welding, fuel addition, ferromanganese production	0.26
Effects on human health	Inhalation or contact causes damage to central nervous system	
Mercury	Pesticides, batteries, paper industries	0.01
Effects on human health	Tremors, gingivitis, minor psychological changes, acrodynia characterized by pink hands and feet, spontaneous abortion, damage to nervous system, protoplasm poisoning	
Zinc	Refineries, brass manufacture, metal plating, plumbing	15
Effects on human health	Zink fumes have corrosive effect on skin, cause damage to nervous membrane	
Chromium	Mines, mineral sources	0.05
Effects on human health	Damage to the nervous system, fatigue, irritability	
Copper	Mining, pesticide production, chemical industry, metal piping	0.1
Effects on human health	Anemia, liver and kidney damage, stomach and intestinal irritation	

Wastewater formed in this industry may contain non – halogenated solvents, acids, salts and other organic contaminants. The contaminants are present into the water by process of slurry, wafer, and linearization, post polishing and cleaning process of the parts in the semiconductor itself. Lien & Lie, (2006) stated that in Taiwan, coagulation – flocculation treatment by polyaluminium chloride and polymer mainly treats the wastewater in most of the semiconductor manufacturers.

2.3 Impact of Wastewater on Environment

Wastewater has many points of insertion into the environment such as human waste or known as black water, cesspit leakage, septic tank discharge, sewage treatment plant discharge, highway drainage and industrial wastes, leaking in sewerage system and many more (Popa *et al.*, 2012). With these entry points, the quality of the water in that environment and its surrounding will be damaged. Other than that, if this is not controlled, the amount of water that is safe for human consumption will be scarce.

Wastewater also contains pathogens like bacteria, viruses and non-pathogenic bacteria. Organic soluble and particles such as hairs, food, paper fibers, humus, urea, drug and pharmaceuticals. Inorganic particles also can be found in wastewater of many types. Examples are sand, grit, metal and ceramic particles. Living life forms like protozoa, insects, arthropods and even small fish may also be present in the water body. Gases such as hydrogen, carbon monoxide, methane and others also will be present in the wastewater. Finally, wastewater also contains nutrients such as phosphorus, nitrogen and etc (Benit & Roslin, 2015).

When phosphorus is collected at the water bodies, it will cause an unexpected problem to the ecosystem in the water which is eutrophication (Singh *et al.,* 2016). The phosphorus that is in the wastewater will cause the growth of algae and other aquatic plants in the water body. Eutrophication is a state where the rapid increase of aquatic plant growth will cause oxygen levels in the water body to drop as the aquatic plant die. This caused heavy competition to the organisms in the water body to use oxygen for themselves.

Inclusion of wastewater to the water sources also disrupts fish population growth. Over blooming of algae causes fish to suffocate and reproduce very less and this affects the fish species wholly. It is also seen that involvement of wastewater into the environment causes deformation and poisoning to aquatic animals and plants, thus minimalizing the sustainability of a healthy species growth in the ecosystem.

The Environment Quality Act (DOE, 2000) states that pollution is the condition of the environment when a pollutant is present whereas pollutant is a contaminant or a mixture of several contaminants present in the environment in a concentration or quality greater than the permissible level determined by the regulation of the Government, or whose presence in the environment is prohibited by the regulation of the Government.

Heavy metals in the wastewater which is from industrial and domestic sources also causes major problems to the environment, especially to the aquatic ecosystem, resulting in the loss of biological diversity and the magnification and bioaccumulation of toxic agents in the food chain (Souza *et al.,* 2012). Serious harm will be caused to the nature’s domain as well as to the health of human beings if the wastewater is discharged into the nearby water bodies (Sriram & Seenivasan, 2012).

2.4 Algae

Simple plants which have a range of being the size of microscopic and to being as large as seaweeds. Algae perform approximately half of the photosynthesis on this planet and subsequently are instrumental in supporting the biosphere (Sankaran & Thiruneelagandan, 2015; Guschina & Harwood, 2006). Microscopic algae are known as microalgae whereas large algae are known as macroalgae. An example of macroalgae can be seen in the seas which is the giant kelp. Algae grow by using the process that is common to all plants which is photosynthesis, Photosynthesis works by converting the readily available sunlight, carbon dioxide, nitrogen, phosphorus and a few nutrients into a material known as biomass.

Photosynthesis only happens in the presence of daylight and does not occur in the darkness of the night. This is called as autotrophic growth. But, there are other algae that can grow in the dark. They use starch as energy and is called heterotrophic growth and in some species, the can use photosynthesis in the day and starch in the dark which is known as mixotrophic growth.

Algae are very diverse and can be found anywhere in the world. Their existence plays an important role in many environments and ecosystems, as well as producing 70% of the air that is breath. In case of many algae, maximal growth rate is observed at optimum temperatures between 28˚C and 35˚C (Soeder *et al.,* 1985). Algae are considered as the only alternative to current biofuel crop such as corn and soybean as they do not require arable land (Singh *et al.*, 2010). Moreover, a considerable lot of the algal species in wastewater treatment forms large colonies (50–200 lm), and cell aggregation may be accomplished through nutrient restriction or CO_2 addition which will bring down the expense of algae harvesting (Zhou *et al.*, 2012). The nitrogen and phosphorus removal efficiency is controlled directly and indirectly by the algae growth rate (Olguı´n, 2003).

There is a big chance where algae wastewater treatment that use high rate algal ponds produce algae from its by-product (Park *et al.*, 2011). Metal has been removed by dead biomass through passive sorption or by living cells via the alga (Mahdavi *et al.,* 2012).

2.4.1 Macroalgae

Macroalgae is widely known as seaweed. Macro brings the meaning of big so it is a big plant that lives in the water. It is a multicellular organism which causes them to have many cells that let the macroalgae function. They are composed of a thallus, a stem and a foot. Seaweed can be found in many colours such as red, brown and green.

Macroalgae has been used for many functions. Most of it are for food, herbalism and fertilizers. But the most prominent function can be seen when it is used as a filter. When a seaweed does photosynthesis, it uses nutrients that are in the water that they grow. Common nutrients used by the seaweed are ammonia, nitrite, phosphate, copper and carbon dioxide. This process has been running in the environment as natural filters for lakes and reefs.

As the seaweed or macroalgae filters the water by using up its excess nutrients, it continues to grow in size and quantity. If there are too much nutrients, it will grow exponentially and may become a threat to the ecosystem in the water body. These can be thwarted by removing excessive seaweed that grew in the water body and monitor the continued progress.

There is a problem in this method which is the occupation area that the seaweed uses when it grows. When used in a filtration pond or tank, the growth of the seaweed itself will lower the optimum capacity for water treatment. In this case, microalgae are seen as the solution to optimize treatment capacity.

2.4.2 Microalgae

Microalgae are also known as phytoplankton (Votano *et al.,* 2004). Microalgae are the largest autotrophic microorganisms of plant life taxa in the world (Rawat *et al.,* 2013). 2-200mm is the size of microalgae which are unicellular (Odjadjare *et al.,* 2015; Pereira *et al.,* 2013). Having simple structure, Microalgae are prokaryotic or eukaryotic photosynthetic microorganisms in which, due to their unicellular or simple multicellular structure, can grow rapidly and live under harsh conditions (Surendhiran & Vijay, 2012). Micro brings the meaning of very small so it is a very small plant that lives in the water. Many species of microalgae grow effectively in wastewater (Oswald *et al.,* 1957). Their size is very small that it is unobservable with normal eyes. They can be viewed under a microscope and are typically green in colour for the presence of chlorophyll in their cells. 60% of earths oxygen is produced by microalgae (Hernández et al., 2013; Ugoala *et al.,* 2012). Microalgae could reduce greenhouse gas emission by fixing the carbon dioxide from atmosphere as in photosynthesis (Boonchai *et al.,* 2012). As microalgae is capable of fixing several-fold more CO_2 per unit area when compared to trees or crops, it is the most promising production facilities (Salih, 2011).

Microalgae store more neutral lipids as compared to macroalgae (Krishna *et al.*, 2012). Microalgae are one of the most dependable sources of renewable energy but high land requirement to build raceway pond for microalgae mass culture was one of the major setback which limits the exploitation of microalgae (Janarthanan 2012).

Many types of bio-fuels can be produced from microalgae (Sivasubramanian, 2009). With higher fuel yield potential and lower water demand than traditional energy crops, Microalgae are novel aquatic biomass (Pragya *et al.*, 2013; Abou-Shanab *et al.*, 2013; Brennan & Owende, 2010; Griffiths & Harrison, 2009). Microalgae have been suggested as potential candidates for fuel production because of a number of advantages including higher growth rate compared to other energy-crops ((Kothari *et al.*, 2013; Halim *et al.*, 2012; Huang *et al.*, 2010; Huang 2009). For further conversion to biodiesel, extraction efficiency of total lipids from microalgae can be increased through cell disruption (Florentino de Souza Silva *et al.*, 2014). Microalgae cultures offer an elegant solution to tertiary and quandary treatments due to the ability of microalgae to use inorganic nitrogen and phosphorus for their growth (Abdel-Raouf *et al.*, 2012).

According to Ahmad *et al.*, (2013) municipal wastewater, industrial effluents and solid wastes can be treated aerobically as well as anaerobically using microalgae (Fouilland *et al.*, 2014). It is reasoned that immobilization of *C. vulgaris* and *S. rubescens* on twin-layers is a viable intends to lessen nitrogen and phosphorus levels in wastewater (Shi *et al.*, 2007). As long as the biomass produced is reused and permits efficient nutrient recycling, wastewater remediation by microalgae is an eco-accommodating process with no secondary contamination (Fathi *et al.*, 2013; Lee *et al.*, 2010).

2.4.3 *Botryococcus* sp.

Characterized by unusually high hydrocarbon contents, *Botryococcus braunii* is a chlorophycean, colonial, slow- growing fresh water microalga (Shimamura *et al.*, 2012; Dayananda *et al.*, 2005; Zhang & Kojima, 1998). Microalga *Botryococcus braunii* is unicellular and is of chlorophyceae (chlorophyta) (Banerjee *et al.*, 2002). It is a green microalga where its colonies are held together with lipid biofilm that is found in lakes. It is also the species of algae that can be found in freshwaters, lakes, dams and ponds (Dayananda & Kumudha, 2010). Due to its high sorption capacity and its availability in almost unlimited amounts, new biosorbent materials has focused especially on algae (Romera *et al.*, 2007). Microalgae have greater photosynthetic efficiency than terrestrial plants and require very little simple nutrients supply for growth (Rawat *et al.,* 2013). The pollutants of wastewater are bio-transformed via phycoremediation of *Botryococcus* sp. into microalgae cell (Gani *et al.,* 2015; Wu *et al.,* 2014; Fouilland, 2012).

It is a species of green algae where its cells form an irregularly shaped aggregate with thin filaments that connect the cells. The cell is a void with 6 -10 micron long and 3 – 6 micron wide. Classification for *Botryococcus sp.* is from kingdom *Plantae*, Division *Chlorophyto*, Class *Trebouxiophyceae*, Family *Botryococcaceae* and Genus *Botryococcus*. *Botryococcus braunii* is known for its ability to produce hydrocarbons even though it is a green colonial fresh water microalga (Watanabe *et al.,* 2014., Rao *et al.,* 2007).

When there is a significant increase of inorganic phosphorus that is dissolved a water body, these microalgae will bloom at an increased rate. Efficient growth and effective uptake of nitrogen have been observed in green alga like *Botryococcus braunii* growing in the piggery wastewater (An *et al.,* 2003). Moreover, both organic matter and nutrients in municipal and piggery wastewater can be essentially diminished by microalgae-based systems (Abou-Shanab *et al.,* 2013; Zhou *et al.,* 2012). This alga is important as it has an advantage where it is known to be able to remove heavy metals from waters. It can also be grown and breed in laboratory conditions.

The bioremediation of polluted waters mainly, heavy metal removal is a good advantage that these algae possess within it. This microalga is also known to produce high amounts of hydrocarbon, or also known as oil (Alam *et al.,* 2012). The amount is around 30% of its own dry weight and it can be harvested in an easy method too. Microalgae have faster growth rates than plants and are capable of growth in highly saline waters which are unsuitable for agriculture (Rawat *et al.,* 2013). Due to its ability to fix atmospheric CO_2 photosynthetically and produce long- chain hydrocarbons, the colonial green alga *Botryococcus braunii* has been proposed as a promising renewable petroleum substitute (Sakamoto *et al.,* 2012; Hu *et al.,* 2011) .

Botryococcus braunii is also vital as it is well known as a source of Triterpenes or known as hydrocarbons. This can be used to produce gasoline, kerosene and diesel (Metzger & Largeau, 2005). It can also be used in bioremediation of polluted waters to remove heavy metals. *Botryococcus braunii* is special in creating hydrocarbons (Emaka *et al.,* 2012). Secondary treated wastewater from municipal waste is suitable as a medium to grow *Botryococcus braunii* and removing nitrogen and phosphorus (Órpez *et al.*, 2009).

2.5 Phycoremediation

Phycoremediation is the use of macroalgae or microalgae for removal or biotransformation of pollutants, including nutrients and xenobiotics, from wastewater and carbon dioxide from waste air (Biosci *et al.,* 2014; Ahmad *et al.,* 2013). Phytoremediation is clearing polluted soil and aquatic system incorporating plant, fungi or algae to remediate heavy metals (Chekroun & Baghour, 2013). There are in existence of many methods to treat waters that are polluted with contaminants and nutrients. Mostly by bioremediation and by chemical treatment. Using algae to enhance water quality, remediate carbon dioxide levels via photosynthesis and removing excess nutrients at low cost is done by employing phycoremediation (Biosci *et al.*, 2014; Dominic *et al.*, 2009).

Microalgae assimilate a significant amount of nutrients because they require high amounts of nitrogen and phosphorus for the synthesis of proteins (45-60% of microalgae dry weight), nucleic acids and phospholipids. Nutrient removal can also be further increased by NH^3 stripping or Phosphate precipitation due to the rise in the pH associated with photosynthesis (Oswald, 2003; Nurdogan and Oswald, 1995; Laliberté et al., 1994).

In municipal wastewaters, high levels of heavy metals and toxic pollutants can be found. Therefore, a system to treat wastewater and its toxicity is a requirement nowadays. Through phycoremediation process, microalgae can become good heavy metal removers from wastewater and at the same time, able to sustain themselves by using the nutrients that are present in the wastewater itself. *Botryococcus* sp. removes 60% of nitrogen and 36% of total phosphorus from domestic wastewater (Gani *et al.*, 2015). Trapping the solar energy in its chloroplast cell and absorbing CO^2 along with nutrients from water to synthesis their biomass and produces oxygen are the main advantages of using micro algal species (Kotteswari *et al.*, 2012).

2.5.1 Applications of Phycoremediation

Microalgae has been used in many bioremediation processes as it is a reliable and environment friendly treatment for wastewater. This process does not cost as much as the conventional treatment available because phycoremediation employs non-pathogenic photosynthetic organisms which do not produce toxic byproducts (Dwivedi, 2012). Oxygen levels increase as photosynthesis occurs and carbon dioxide from the air is used which reduces greenhouse gasses (Gani *et al.*, 2015). Physiochemical and heavy metal elements are consumed by the microalgae, thus decreasing amounts of total dissolved solid and sludge formation in the wastewater (Azmi & Yunos, 2014).

During treatment process minimal odor is released as conventional treatment produces high odor densities (Dwivedi, 2012). Conventional treatment requires several stages of treatment which are complex and required high maintenance whereas phycoremediation is simpler (Lorestani & Akbar Zinatizadeh, 2006). Gani (2016) states that phycoremediation can be also applied in treating the food processing wastewater. Besides that, microalgae can adapt in any environment condition as mention by Latiffi *et al.*, (2016). Azarpira, Dhumal, & Pondhe, (2014) also stated that Gloeocapsa gelatinosa, Euglena viridis and Synedra affinis are prominent sewage algae that is found to reduce BOD, COD, NH, -N, Org, -N and hardness in wastewater.

2.5.2 Advantages of Phycoremediation

As an alternative to the conventional treatment methods, microalgae are suggested to be used to remove nutrients from wastewaters. According to Umamaheswari & Shanthakumar, (2016) the removal or biotransformation of pollutants, nutrients and xenobiotics from the wastewater can be done by using microalgae which is known as phycoremediation. Microalgae wastewater treatment is an eco-friendly solution and it also offers the advantage of a cost-effective way of nutrient removal and biomass production (Lee *et al.*, 2015; Mulbry *et al.*, 2008). The major advantages of wastewater treatment by microalgae cultures are such that there are no additional pollution when the biomass is harvested and it allows efficient recycling of nutrients (Biosci et al., 2014).

With that, Hanumantha (2010) has provided the advantages of applying the process of phycoremediation in the bioremediation of wastewater and heavy metal pollution of the ecosystem. The main advantage is the ability of microalgae to tackle more than one problem simultaneously, a solution not possible by conventional chemical processes. Other than that, microalgae work by case-specific as the process can be operated batch-wise, semi-continuously or in a continuous manner. Microalgae also has commercial benefits derived from the biomass and other extracted biochemical. It is also highly compatible with existing operations at treatment plants. Using microalgae for treatment is also cost-effective as it saves power and reduces the usage of a lot of chemicals. Carbon dioxide sequestration – a solution for the threat of global warming as it provides a viable option to mitigate it. Microalgae is also proven to have the flexibility to handle fluctuations in quality and quantity of effluent feed. It also has the ability to provide oxygenation for the environment and provide co-production of biofuels and biofertilizers. It is also robust to minimize automation, maintenance and the need for skilled operators while possessing the selective ability to remove only the contaminants under consideration while preserving its sustainability and eco-friendliness from an ecological perspective.

2.6 Bioremediation of Heavy Metal by Microalgae

The heavy metals in the water can be present via many sources. It could enter the water system by effluent from semi-conductor industry, contamination of water system by illegal waste dumping and other sources too. Industrial processes and agricultural practices also bring the side effect of heavy metal releases into the ecosystem. Heavy metals are a stable and stubborn contaminant as it cannot degrade or be destroyed. To adapt to the toxicity level of the heavy metals, microalgae have developed an extensive spectrum of mechanisms (extracellular and intercellular). Their suitability in practical applications of waste-water bioremediation is ascertained by their ability to grow and concentrate heavy metals along with their wide-spread occurrence (Kumar *et al.*, 2015).

Therefore, bioremediation by the microalgae on the heavy metals can be an alternative solution to this problem. A process of transforming hazardous contaminations in water to nonhazardous waste products using specific microorganisms is Bioremediation (Sunar *et al.*, 2015; Dwivedi, 2012). According to Rhodes, (2013) there is two approaches for bioremediation. It's the in situ and ex situ method. Contaminated samples that were treated at site is known as in situ whereas ex situ is when the sample is physically removed to be treated elsewhere. The functional groups present on the cell surface are able to bind to metal ions which makes microalgae excellent heavy metals scavengers (Krishna *et al.*, 2012).

The use of living cells is most efficient for removal of metal ions from large water bodies containing low concentrations (ppb range) of metal ions. Resistant microalgae species isolated from metal-contaminated sites have a higher capacity for accumulating heavy metals compared with species isolated from non-contaminated sites (Wong *et al.*, 2000). During algal growth, metals are removed from the surrounding environment and accumulated in the cells by both non-metabolic dependent processes (adsorption) and metabolic dependent ones (absorption).

Provided that adequate environmental conditions for supporting microalgae growth, such as light, temperature and pH are present, the use of living microalgae biomass offers an efficient, simple and cost-effective method. The pH value was taken into consideration in the study because it can be a basic parameter to indicate the photosynthetic activity of microalgae and the light is one of the important factors of photosynthesis process (Janarthanan 2012).

In such ambience, microalgae can grow effectively accumulating nutrients and metals, making them sustainable and suitable for low cost wastewater treatment (de-Bashan & Bashan, 2010). Nitrogen and phosphorus is utilized by the microalgae for their growth (Boonchai *et al.*, 2012). Satisfying the growth prerequisites of microalgae, they are as of now being used to decrease the high nutrient load (particularly N and P) from wastewaters, making it a reasonable cultivation medium for biomass production (Renuka *et al.*, 2015).

Biosorption of heavy metals by microalgae is generally a bi-phasic process. The first phase is adsorption by extracellular cell-associated materials, e.g. polysaccharides, mucilage and cell wall components, e.g. carboxy and hydroxy groups, as well as sulfate. This is a non-metabolic, rapid process, which occurs in both living and non-living cells. It is dependent on a few parameters: pH, heavy metal, type of algae, and biomass concentration. With concomitant carbon dioxide sequestration, microalgae could serve as a dual role of bioremediation of wastewater as well as generating biomass for high-protein feed supplements or biofuel production (Cheng *et al.*, 2013).

The second phase is absorption and accumulation inside the cell. This is a slow process involving active transport through the cell membrane into the interior and binding to proteins and other intracellular sites. It is a metabolic-dependent mechanism that is inhibited by low temperatures, absence of an energy source, metabolic inhibitors, and uncouplers, and occurs only in living cells. To clear the gap, application of *Botryococcus* sp was the reason to push on the research. Microalgae have excellent heavy metal scavenging property besides being cost effective and easy to handle (Krishna *et al.*, 2012). A summary of microalgae in heavy metal bioremediation can be seen in the Table 2.7.

Table 2.7: Schematic Review of Microalgae in Heavy Metal Bioremediation

No.	**Microalgae**	**Heavy metal**	**Removal efficiency (%)**	**References**
1	*Synechocystis salina*	Chromium, Cr	60	Worku & Sahu (2014)
		Iron, Fe	66	
		Nickel, Ni	70	
		Mercury, Hg	77	
		Calcium, Ca	65	
		Magnesium, Mg	63	
		Total Hardness	78	
2	*Chlorella marina*	Zinc, Zn (powder)	97	Kumar et al., (2013)
		Zinc, Zn (immobilized	55.3	
3	*Porphyridium cruentum*	Copper, Cu	92	Soeprobowati & Hariyati, (2013)
4	*Chlorella pyrenoidosa* (100% Conc.)	Chromium, Cr	52.8	Ajavan & Selvaraju, (2012)
		Copper, Cu	77.1	
		Lead, Pb	43.8	
		Zinc, Zn	68.9	
	Scenedesmus sp. (100% Conc.)	Chromium, Cr	52	
		Copper, Cu	79.2	
		Lead, Pb	47.8	
		Zinc, Zn	66	
5	Indigenous microalgae	Barium, Ba	91.2	Krustok et al., (2012)
		Iron, Fe	94.6	
6	*Botryococcus* sp.	Chromium, Cr	94	Onalo et al., (2014)
		Copper, Cu	45	
		Arsenic, As	9	
		Cadmium, Cd	2	

Onalo *et al.*, (2014) stated that heavy metals contained in industrial effluents are dangerous particularly in human health perspective due to their toxicity and bio-accumulating effects. The *Botryococcus* sp. has a promising future as it can absorb heavy metal contents from wastewater and also keeps itself prevented from contamination or being poisoned. Microalgae have shown high heavy metal binding affinity primarily due to short-chain amino acids they possess a large surface area-to-volume ratio that tends to be accessible for contact with the environment within as well as their accumulation ability which makes them potential remediating agents (Onalo *et al.*, 2014).

Botryococcus sp. has shown that it can remediate wastewater and absorb high levels of nutrients from the wastewater. In addition to that, it also increases oxygen levels and lowers carbon dioxide levels in the air. It grows great in optimum conditions and does not need to be regulated unless the water body that it is in has too much nutrient and pollutants which causes it to grow beyond control. These data show that *Botryococcus* sp. is great applicant for phycoremediation.

REFERENCES

Abdel-Raouf, N., Al-Homaidan, A. A., & Ibraheem, I. B. M. (2012). Microalgae and wastewater treatment. *Saudi Journal of Biological Sciences*, *19*(3), 257–275

Abou-Shanab, R. A I., Ji, M. K., Kim, H. C., Paeng, K. J., & Jeon, B. H. (2013). Microalgal species growing on piggery wastewater as a valuable candidate for nutrient removal and biodiesel production. *Journal of Environmental Management*, *115*, 257–264.

Ahmad, F., Khan, A. U., & Yasar, A. (2013). Comparative phycoremediation of sewage water by various species of algae. *Proceedings of the Pakistan Academy of Sciences*, *50*(2), 131–139.

Alam, F., Date, A., Rasjidin, R., Mobin, S., Moria, H., & Baqui, A. (2012). Biofuel from algae-Is it a viable alternative? *Procedia Engineering*, *49*, 221–227.

Amaro, H. M., Guedes, a. C., & Malcata, F. X. (2011). Advances and perspectives in using microalgae to produce biodiesel. *Applied Energy*, *88*(10), 3402–3410.

Apha, Awwa, & Wef. (2012). Standard Methods for the Examination of Water and Wastewater.

Azarpira, H., Dhumal, K., & Pondhe, G. (2014). Application of phycoremediation technology in the treatment of sewage water to reduce pollution load. *Advances in Environmental Biology*, *8*(7), 2419–2423.

Azmi, N. S., & Yunos, K. F. M. (2014). Wastewater Treatment of Palm Oil Mill Effluent (POME) by Ultrafiltration Membrane Separation Technique Coupled with Adsorption Treatment as Pre-treatment. *Agriculture and Agricultural Science Procedia*, *2*, 257–264.

Banerjee, A., Sharma, R., Chisti, Y., & Banerjee, U. C. (2002). Botryococcus braunii: A renewable source of hydrocarbons and other chemicals. *Critical Reviews in Biotechnology*, *22*(3), 245–279.

Barakat, M. a. (2011). New trends in removing heavy metals from industrial wastewater. *Arabian Journal of Chemistry*, *4*(4), 361–377.

Bastidas, O. (2014). Cell Counting with Neubauer Chamber (Basic Hemocytometer Usage), 1–6. Retrieved from http://www.celeromics.com/cell-counting

Benit, N., & Roslin, A. S. (2015). Physicochemical properties of wastewater collected from different sewage sources, *2*(11).

Biosci, I. J., Azarpira, H., Behdarvand, P., Dhumal, K., & Pondhe, G. (2014). Potential use of cyanobacteria species in phycoremediation of municipal wastewater, *6655*, 105–111.

Boonchai, R., Seo, G. T., Park, D. R., & Seong, C. Y. (2012). Microalgae Photobioreactor for Nitrogen and Phosphorus Removal from Wastewater of Sewage Treatment Plant. *International Journal of Bioscience, Biochemistry and Bioinformatics*, *2*(6), 407–410.

Brennan, L., & Owende, P. (2010). Biofuels from microalgae-A review of technologies for production, processing, and extractions of biofuels and co-products. *Renewable and Sustainable Energy Reviews*, *14*(2), 557–577.

Bulgariu, D., & Bulgariu, L. (2012). Equilibrium and kinetics studies of heavy metal ions biosorption on green algae waste biomass. *Bioresource Technology*, *103*(1), 489–493.

Chekroun, K. Ben, & Baghour, M. (2013). The role of algae in phytoremediation of heavy metals: A review. *Journal of Materials and Environmental Science*, *4*(6), 873–880.

Chen, C. Y., Yeh, K. L., Aisyah, R., Lee, D. J., & Chang, J. S. (2011). Cultivation, photobioreactor design and harvesting of microalgae for biodiesel production: A critical review. *Bioresource Technology*, *102*(1), 71–81.

Cheng, H., Tian, G., & Liu, J. (2013). Enhancement of biomass productivity and nutrients removal from pretreated piggery wastewater by mixotrophic cultivation of Desmodesmus sp. CHX1. *Desalination and Water Treatment*, *51*(37–39), 7004–7011.

Chinnasamy, S., Bhatnagar, A., Hunt, R. W., & Das, K. C. (2010). Microalgae cultivation in a wastewater dominated by carpet mill effluents for biofuel applications. *Bioresource Technology*, *101*(9), 3097–3105.

Chisti, Y. (2008). Biodiesel from microalgae beats bioethanol. *Trends in Biotechnology*, *26*(3), 126–131.

Chotwattanasak, J., & Puetpaiboon, U. (2013). Full Scale Anaerobic Digester for Treating Palm Oil Mill Wastewater. *Journal of Sustainable Energy & Environment*, *2*(3), 133–136.

Christenson, L., & Sims, R. (2011). Production and harvesting of microalgae for wastewater treatment, biofuels, and bioproducts. *Biotechnology Advances*, *29*(6), 686–702.

Dayananda, C., & Kumudha, A. (2010). Isolation, characterization and outdoor cultivation of green microalgae Botryococcus sp. *Scientific Research and Essays*, *5*(17), 2497–2505.

Dayananda, C., Sarada, R., Bhattacharya, S., & Ravishankar, G. A. (2005). Effect of media and culture conditions on growth and hydrocarbon production by Botryococcus braunii. *Process Biochemistry*, *40*(9), 3125–3131.

Dominic, V., Murali, S., & Nisha, M. (2009). Phycoremediation efficiency of three microalgae Chlorella vulgaris, Synechocytis salina and Gloeocapsa gelatinosa. *SB Academic Review*, *16*(1–2), 138–146.

Dwivedi, S. (2012). Bioremediation of heavy metal by algae: current and future perspective. *Journal of Advance Laboratory Research in Biology*, *III*(III), 195–199.

Elumalai, S., Ramganesh, S., & Sangeetha, T. (2014). International Journal of Current Phycoremediation for Leather Industrial Effluent - Treatment and Recycling Using Green Microalgae and its Consortia, *2*(10), 1–9.

Fathi, A. A., Azooz, M. M., & Al-Fredan, M. A. (2013). Phycoremediation and the potential of sustainable algal biofuel production using wastewater. *American Journal of Applied Sciences*, *10*(2), 189–194.

Florentino de Souza Silva, A. P., Costa, M. C., Colzi Lopes, A., Fares Abdala Neto, E., Carrh. C., Leit, O., R., Mota, C. R., & Bezerra dos Santos, A. (2014). Comparison of pretreatment methods for total lipids extraction from mixed microalgae. *Renewable Energy*, *63*, 762–766.

Fouilland, E. (2012). Biodiversity as a tool for waste phycoremediation and biomass production. *Reviews in Environmental Science and Biotechnology*, *11*(1), 1–4.

Fouilland, E., Vasseur, C., Leboulanger, C., Le Floc'h, E., Carrh, C., Marty, B., Sialve, B. (2014). Coupling algal biomass production and anaerobic digestion: Production assessment of some native temperate and tropical microalgae. *Biomass and Bioenergy*, *70*, 564–569.

Gani, P., Sunar, N. M., Latiff, A A, Joo, I. T. K., Parjo, U. K., Emparan, Q., & Er, C. M. (2015). Phycoremediation of Dairy Wastewater by Using Green Microlgae : Botryococcus sp . *Applied Mechanics and Materials*, *773–774*(2015), 1318–1323.

Gani, P., Sunar, N. M., Latiff, A A, Kamaludin, N. S., Parjo, U. K., Emparan, Q., & Er, C. M. (2015). Experimental Study for Phycoremediation of Botryococcus sp . On Greywater. *Applied Mechanics and Materials*, *773–774*(2015), 1312–1317.

Gani, P., Sunar, N. M., Matias-Peralta, H., & Abdul Latiff, A. A. (2016). Application of phycoremediation technology in the treatment of food processing wastewater by freshwater microalgae botryococcus SP. *ARPN Journal of Engineering and Applied Sciences*, *11*(11), 7288–7292.

Gani, P., Sunar, N. M., Matias-peralta, H., Aziz, A., & Latiff, A. (2015). Screening of Sustainable Hydrocarbon Extracted from Microalgae via Phycoremediation. *ARPN Journal of Engineering and Applied Sciences*, *In Press*(X), 1–6.

Gani, P., Sunar, N. M., Matias-peralta, H., Maya, R., Radin, S., Aziz, A. (2017). Accepted manuscript, 1–27.

Gokulan, R., Sathish, N., & Praveen Kumar, R. (2013). Treatment of grey water using hydrocarbon producing Botryococcus braunii. *International Journal of ChemTech Research*, *5*(3), 1390–1392.

Griffiths, M. J., & Harrison, S. T. L. (2009). Lipid productivity as a key characteristic for choosing algal species for biodiesel production. *Journal of Applied Phycology*, *21*(5), 493–507.

Guschina, I. A., & Harwood, J. L. (2006). Lipids and lipid metabolism in eukaryotic algae. *Progress in Lipid Research*, *45*(2), 160–186.

Hadiyanto 2013 - Phytoremediations of palm oil mill effluent (POME) by using aquatic plants and microalgae for biomass production.pdf.

Halim, R., Harun, R., Danquah, M. K., & Webley, P. A. (2012). Microalgal cell disruption for biofuel development. *Applied Energy*, *91*(1), 116–121.

Hanumantha Rao, P., Ranjith Kumar, R., Raghavan, B., Subramanian, V., & Sivasubramanian, V. (2011). Application of phycoremediation technology in the treatment of wastewater from a leather-processing chemical manufacturing facility. *Water SA*, *37*(1), 7–14.

Hernández, D., Riaño, B., Coca, M., & García-González, M. C. (2013). Treatment of agro-industrial wastewater using microalgae-bacteria consortium combined with anaerobic digestion of the produced biomass. *Bioresource Technology*, *135*, 598–603.

Hill, J., Nelson, E., Tilman, D., Polasky, S., & Tiffany, D. (2006). Environmental, economic, and energetic costs and benefits of biodiesel and ethanol biofuels. *Proceedings of the National Academy of Sciences of the United States of America*, *103*(30), 11206–11210.

Hu, H.-Y., Li, X., Yu, Y., Wu, Y.-H., Sagehashi, M., & Sakoda, A. (2011). Domestic Wastewater Reclamation Coupled with Biofuel/Biomass Production Based on Microalgae: a Novel Wastewater Treatment Process in The Future. *Journal of Water and Environment Technology*, *9*(2), 199–207.

Huang, G., Chen, F., Wei, D., Zhang, X., & Chen, G. (2010). Biodiesel production by microalgal biotechnology. *Applied Energy*, *87*(1), 38–46.

Huang, M., Li, Y., & Gu, G. (2010). Chemical composition of organic matters in domestic wastewater. *Desalination*, *262*(1–3), 36–42.

I, N. G., B, M. K., & I, A. R. (2012). Constraints to large scale algae biomass production and utilization. *J. Algal Biomass Utln*, *3*(2), 14–32.

Janarthanan P. , Kumaran P., S. N. (2012). Journal of Energy & Environment, 5–7.

Kamyab, H., Soltani, M., Ponraj, M., Din, M. F., & Putri, E. V. (2013). A review on microalgae as potential lipid container with wastewater treating functions, *1*(2), 76–80.

Kothari, R., Prasad, R., Kumar, V., & Singh, D. P. (2013). Production of biodiesel from microalgae Chlamydomonas polypyrenoideum grown on dairy industry wastewater. *Bioresource Technology*, *144*, 499–503.

Kotteswari, M., Murugesan, S., & R, R. K. (2012). Phycoremediation of Dairy Effluent by using the Microalgae Nostoc sp. *International Journal of Environmental Research and Development.*, *2*(1), 35–43.

Krishna, A. R., Dev, L., & Thankamani, V. (2012). An integrated process for Industrial effluent treatment and Biodiesel production using Microalgae. *Research in Biotechnology*, *3*(1), 47–60.

Latiffi, N. A. A., Mohamed, R. M. S. R., Shanmugan, V. A., Pahazri, N. F., Kassim, A. H. M., Matias-Peralta, H. M., & Tajuddin, R. M. (2016). Removal of nutrients from meat food processing industry wastewater by using microalgae botryococcus SP. *ARPN Journal of Engineering and Applied Sciences*, *11*(16), 9863–9867.

Latiffi, N. A., Maya, R., Mohamed, S., Apandi, N., & Kassim, H. (2015). Application of Phycoremediation using Microalgae Scenedesmus sp . as Wastewater Treatment in Removal of Heavy Metals from Food Stall Wastewater, *774*, 1168–1172.

Lee, J. Y., Yoo, C., Jun, S. Y., Ahn, C. Y., & Oh, H. M. (2010). Comparison of several methods for effective lipid extraction from microalgae. *Bioresource Technology*, *101*(1 SUPPL.), S75–S77.

Lee, K. Y., Ng, T. W., Li, G., An, T., Kwan, K. K., Chan, K. M., Wong, P. K. (2015). Simultaneous nutrient removal, optimised CO<inf>2</inf> mitigation and biofuel feedstock production by Chlorogonium sp. grown in secondary treated non-sterile saline sewage effluent. *Journal of Hazardous Materials*, *297*, 241–250.

Lokhande, R. S., Singare, P. U., & Pimple, D. S. (2011). Study on Physico-Chemical Parameters of Waste Water Effluents from Taloja Industrial Area of Mumbai, India. *International Journal of Ecosystem*, *1*(1), 1–9.

Lorestani, Akbar Zinatizadeh, A. (2006). Biological Treatment of Palm Oil Mill Effluent (Pome) Using an Up-Flow Anaerobic Sludge Fixed Film (Uasff) Bioreactor, (December), 53.

Mahdavi, H., Ulrich, A. C., & Liu, Y. (2012). Metal removal from oil sands tailings pond water by indigenous micro-alga. *Chemosphere*, *89*(3), 350–354.

Malla, F. A., Khan, S. A., Rashmi, Sharma, G. K., Gupta, N., & Abraham, G. (2015). Phycoremediation potential of Chlorella minutissima on primary and tertiary treated wastewater for nutrient removal and biodiesel production. *Ecological Engineering*, *75*, 343–349.

Mane, P. C., & Bhosle, A. B. (2012). Bioremoval of some metals by living algae spirogyra sp. and spirullina sp. from aqueous solution. *International Journal of Environmental Research*, *6*(2), 571–576.

Mata, T. M., Martins, A. A., & Caetano, N. S. (2010). Microalgae for biodiesel production and other applications: A review. *Renewable and Sustainable Energy Reviews*, *14*(1), 217–232.

Metzger, P., & Largeau, C. (2005). Botryococcus braunii: A rich source for hydrocarbons and related ether lipids. *Applied Microbiology and Biotechnology*, *66*(5), 486–496.

Odjadjare, E. C., Mutanda, T., & Olaniran, A. O. (2015). Potential biotechnological application of microalgae: a critical review. *Critical Reviews in Biotechnology*, *8551*(November), 1–16.

Olguin, E. J. (2003). Phycoremediation: Key issues for cost-effective nutrient removal processes. *Biotechnology Advances*, *22*(1–2), 81–91.

Olguín, E. J., & Sánchez-Galván, G. (2011). *Phycoremediation*. *Comprehensive Biotechnology* (Second Edi, Vol. 1). Elsevier B.V.

Onalo, J. I., Peralta, H. M. M., & Mohamed Sunar, N. (2014). Growth of Freshwater Microalga, Botryococcus sp. in Heavy Metal Contaminated Industrial Wastewater. *Journal of Science and Technology*, *6*(2).

Órpez, R., Martínez, M. E., Hodaifa, G., El Yousfi, F., Jbari, N., & Sánchez, S. (2009). Growth of the microalga Botryococcus braunii in secondarily treated sewage. *Desalination*, *246*(1–3), 625–630.

Paper, C. (2016). Application of Phycoremediation in Agro-based Industries – A Review Phycoremediation of Agro industrial wastewater Application of Phycoremediation in Agro-based Industries – A Review Phycoremediation of Agro industrial wastewater, (March).

Park, J. B. K., Craggs, R. J., & Shilton, A. N. (2011). Wastewater treatment high rate algal ponds for biofuel production. *Bioresource Technology*, *102*(1), 35–42.

Pereira, H., Amaro, H. M., Katkam, N. G., Barreira, L., Catarina Guedes, A., Varela, J., & Malcata, F. X. (2013). *Microalgal Biodiesel*. *Air Pollution Prevention and Control: Bioreactors and Bioenergy*.

Pittman, J. K., Dean, A. P., & Osundeko, O. (2011). The potential of sustainable algal biofuel production using wastewater resources. *Bioresource Technology*, *102*(1), 17–25.

Poh, P. E., & Chong, M. F. (2009). Development of anaerobic digestion methods for palm oil mill effluent (POME) treatment. *Bioresource Technology*, *100*(1), 1–9.

Popa, P., Timofti, M., Voiculescu, M., Dragan, S., Trif, C., & Georgescu, L. P. (2012). Study of physico-chemical characteristics of wastewater in an urban agglomeration in Romania. *The Scientific World Journal*, *2012*, 549028.

Pozza, C., Schmuck, S., & Mietzel, T. (2010). A novel photobioreactor with internal illumination using Plexiglas rods to spread the light and LED as a source of light for wastewater treatment using microalgae. *Qub.Ac.Uk*, 1–7.

Pragya, N., Pandey, K. K., & Sahoo, P. K. (2013). A review on harvesting, oil extraction and biofuels production technologies from microalgae. *Renewable and Sustainable Energy Reviews*, *24*, 159–171.

Qin, J. G., Qin, J. G., Li, Y., & Li, Y. (2006). Optimization of the growth environment of Botryococcus braunii Strain CHN 357. *Journal of Freshwater Ecology*, *21*(1), 169–176.

Raj GP, A. S. (2015). Botryococcus braunii as a Phycoremediation Tool for the Domestic Waste Water Recycling from Cooum River, Chennai, India. *Journal of Bioremediation & Biodegradation*, *6*(3).

Rao, A. R., Dayananda, C., Sarada, R., Shamala, T. R., & Ravishankar, G. a. (2007). Effect of salinity on growth of green alga Botryococcus braunii and its constituents. *Bioresource Technology*, *98*(3), 560–564.

Rawat, I., Ranjith Kumar, R., Mutanda, T., & Bux, F. (2011). Dual role of microalgae: Phycoremediation of domestic wastewater and biomass production for sustainable biofuels production. *Applied Energy*, *88*(10), 3411–3424.

Rawat, I., Ranjith Kumar, R., Mutanda, T., & Bux, F. (2013). Biodiesel from microalgae: A critical evaluation from laboratory to large scale production. *Applied Energy*, *103*, 444–467.

Renuka, N., Sood, A., Prasanna, R., & Ahluwalia, A. S. (2015). Phycoremediation of wastewaters: a synergistic approach using microalgae for bioremediation and biomass generation. *International Journal of Environmental Science and Technology*, *12*(4), 1443–1460.

Rhodes, C. J. (2013). Applications of bioremediation and phytoremediation. *Science Progress*, *96*(4), 417–427.

Romera, E., González, F., Ballester, a., Blázquez, M. L., & Muñoz, J. a. (2007). Comparative study of biosorption of heavy metals using different types of algae. *Bioresource Technology*, *98*(17), 3344–3353.

Ruangsomboon, S. (2012). Effect of light, nutrient, cultivation time and salinity on lipid production of newly isolated strain of the green microalga, Botryococcus braunii KMITL 2. *Bioresource Technology, 109*, 261–265.

Rupani, P., & Singh, R. (2010). Review of current palm oil mill effluent (POME) treatment methods: Vermicomposting as a sustainable practice. *World Applied Sciences*, *11*(1), 70–81.

Sakamoto, K., Baba, M., Suzuki, I., Watanabe, M. M., & Shiraiwa, Y. (2012). Optimization of light for growth, photosynthesis, and hydrocarbon production by the colonial microalga Botryococcus braunii BOT-22. *Bioresource Technology*, *110*, 474–479.

Salih, F. M. (2011). Microalgae Tolerance to High Concentrations of Carbon Dioxide: A Review. *Journal of Environmental Protection*, *2*(5), 648–654.

Sankaran, B., & Thiruneelagandan, E. (2015). Original Research Article Microalgal diversity of Parthasarathy temple tank , Chennai , India, *4*(4), 168–173.

Sankaran, K., Suriya Narayanan, G., & Premalatha, M. (2014). Enhancing Biogas Potential of Distillery Wastewater Through Phycoremediation. *Int. Conf. on Advances in Chemical Engineering and Technology ICACE TKMCE '14*, (April 2015), 220–223.

Sekomo, C. B., Rousseau, D. P. L., Saleh, S. A., & Lens, P. N. L. (2012). Heavy metal removal in duckweed and algae ponds as a polishing step for textile wastewater treatment. *Ecological Engineering*, *44*, 102–110.

Shi, J., Podola, B., & Melkonian, M. (2007). Removal of nitrogen and phosphorus from wastewater using microalgae immobilized on twin layers: An experimental study. *Journal of Applied Phycology*, *19*(5), 417–423.

Shimamura, R., Watanabe, S., Sakakura, Y., Shiho, M., Kaya, K., & Watanabe, M. M. (2012). Development of Botryococcus Seed Culture System for Future Mass Culture. *Procedia Environmental Sciences*, *15*, 80–89.

Singh, V., Tiwari, A., & Das, M. (2016). Phyco-remediation of industrial waste-water and flue gases with algal-diesel engenderment from micro-algae : A review. *Fuel*, (January).

Sivasubramanian, V. (2009). Current status of Research on algal bio-fuels in India, *1*(1), 1–4.

Souza, P. O., Ferreira, L. R., Pires, N. R. X., Filho, P. J. S., Duarte, F. A., Pereira, C. M. P., & Mesko, M. F. (2012). Algae of economic importance that accumulate cadmium and lead: A review. *Brazilian Journal of Pharmacognosy*, *22*(4), 825–837.

Sriram, S., & Seenivasan, R. (2012). Microalgae Cultivation in Wastewater for Nutrient Removal. *Journal of Algal Biomass Utilization*, *3*(2), 9–13.

Sunar, N. M., Matias-peralta, H., Aziz, A., Latiff, A., Parjo, K., Rafiq, A., & Razak, A. (2015). Phycoremediation of Wastewaters and Potential Hydrocarbon from Microalgae : A, *9*(20), 1–8.

Surendhiran, D., & Vijay, M. (2012). Microalgal Biodiesel - A Comprehensive Review on the Potential and Alternative Biofuel. *Journal of Chemical Sciences*, *2*(11), 71–82.

Suresh Kumar, K., Dahms, H.-U., Won, E.-J., Lee, J.-S., & Shin, K.-H. (2015). Microalgae – A promising tool for heavy metal remediation. *Ecotoxicology and Environmental Safety*, *113*, 329–352.

Thongtha, S., Teamkao, P., Boonapatcharoen, N., Tripetchkul, S., Techkarnjararuk, S., & Thiravetyan, P. (2014). Phosphorus removal from domestic wastewater by Nelumbo nucifera Gaertn. and Cyperus alternifolius L. *Journal of Environmental Management*, *137*, 54–60.

Tüfekci, N., Sivri, N., & Toroz, İ. (2007). Pollutants of Textile Industry Wastewater and Assessment of its Discharge Limits by Water Quality Standards. *Turkish Journal of Fisheries and Aquatic Sciences*, *103*, 97–103.

Votano, J. R., Parham, M., Hall, L. H., Kier, L. B., & Hall, L. M. (2004). Microalgae: the green gold of the future? *Chemistry & Biodiversity*, *1*(11), 1829–1841.

Wahidin, S., Idris, A., & Shaleh, S. R. M. (2013). The influence of light intensity and photoperiod on the growth and lipid content of microalgae Nannochloropsis sp. *Bioresource Technology*, *129*, 7–11.

Watanabe, H., Li, D., Nakagawa, Y., Tomishige, K., Kaya, K., & Watanabe, M. M. (2014). Characterization of oil-extracted residue biomass of Botryococcus braunii as a biofuel feedstock and its pyrolytic behavior. *Applied Energy*, *132*, 475–484.

Wong, Y. S., Kadir, M. O. a B., & Teng, T. T. (2009). Biological kinetics evaluation of anaerobic stabilization pond treatment of palm oil mill effluent. *Bioresource Technology*, *100*(21), 4969–4975.

Wu, T. Y., Mohammad, A. W., Jahim, J. M., & Anuar, N. (2010). Pollution control technologies for the treatment of palm oil mill effluent (POME) through end-of-pipe processes. *Journal of Environmental Management*, *91*(7), 1467–1490.

Wu, Y. H., Hu, H. Y., Yu, Y., Zhang, T. Y., Zhu, S. F., Zhuang, L. L., Lu, Y. (2014). Microalgal species for sustainable biomass/lipid production using wastewater as resource: A review. *Renewable and Sustainable Energy Reviews*, *33*, 675–688.

Yaakob, Z., Kamarudin, K. F., & Rajkumar, R. (2014). The Current Methods for the Biomass Production of the Microalgae from Wastewaters : An Overview, *31*(10), 1744–1758.

Yen, H. W., Hu, I. C., Chen, C. Y., & Chang, J. S. (2014). *Design of Photobioreactors for Algal Cultivation*. *Biofuels from Algae* (Vol. 23). Britian: Elsevier B.V.

Zhang, K., & Kojima, E. (1998). Effect of light intensity on colony size of microalga Botryococcus braunii in bubble column photobioreactors. *Journal of Fermentation and Bioengineering*, *86*(6), 573–576.

Zhou, W., Min, M., Li, Y., Hu, B., Ma, X., Cheng, Y., Ruan, R. (2012). A hetero-photoautotrophic two-stage cultivation process to improve wastewater nutrient removal and enhance algal lipid accumulation. *Bioresource Technology*, *110*, 448–455.