NUTRIENT REMOVAL FROM SUNGAI PINANG BASIN WATER SAMPLES BY FREE AND IMMOBILISED CELLS OF Scenedesmus bijugatus Kützing

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2016

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by

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Thesis submitted in fulfillment of the requirements

for the degree of

Master of Science

September 2016

ACKNOWLEDGEMENT

First and foremost, my deepest appreciation goes to my supervisor, Professor Dr Wan Maznah Wan Omar, for her continuous guidance, patience and knowledge in supporting me throughout my postgraduate study.

I would like to thank all my fellow labmates: Ms. Lim, Ms. Zoya, Ms. Lia, Ms. Fatini, Ms. Mimi, Ms. Afiqah, Ms. Diyanah, Ms. Ayu, Mr. Basri and Mr. Azmir for being great companions to me and for sharing tears and joys together with me all these years.

Special thanks to Dean and other staffs of the School of Biological Sciences, USM, for their support and assistance during the course of this project. I also wish to acknowledge the Universiti Sains Malaysia Research University Grant (1001/PBIOLOGI/836008) for the financial support provided for the research conducted.

Last but not least, I would like to express my deepest gratitude to my parents and family members for their understanding and words of encouragement along the journey to accomplish my project.

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LIST OF SYMBOLS, ABBREVIATION OR NOMENCLATURE

DO	Dissolved oxygen
NO_2-N	Nitrite nitrogen
NO_3-N	Nitrate nitrogen
NH_4^+	Ammonium
PO_4-P	Phosphate
TDS	Total dissolved solids
°C	Degree Celsius
mL	Milliters
mg	Miligram
μg/mL/d	Microgram /Liter/Day
mg/L^{-1}	Microgram/Liter
mean ± s.e.	Mean ± Standard Error
BBM	Bold Basal Medium
T_g	Doubling time
μ	Growth rate

PENYINGKIRAN NUTRIEN DARIPADA SAMPEL AIR LEMBANGAN SUNGAI PINANG OLEH SEL TIDAK PEGUN DAN SEL PEGUN *Scenedesmus bijugatus* Kützing

ABSTRAK

Mikroalga hijau *Scenedesmus bijugatus* digunakan untuk menyingkirkan nutrien bukan organik (fosfat, ammonium, nitrit dan nitrat) dengan menginokulasi sel ke dalam sampel air dari lokasi terpilih, sekitar Lembangan Sungai Pinang dengan pelbagai tahap pencemaran. Kajian ini mengkaji kesan penyingkiran nutrien bukan organik oleh sel pegun dan tidak pegun *S. bijugatus* melalui pertumbuhan alga (kepekatan klorofil *a*) dan kecekapan sel pegun dan tidak pegun dalam pengambilan nutrien bukan organik (kadar pengambilan (*b*) dan penyingkiran (%)). Sampel air sungai tercemar dan sederhana tercemar mencatatkan kadar pertumbuhan *S.bijugatus* yang tinggi berbanding air sampel bersih. Antara enam stesen yang dikaji, kadar pertumbuhan yang tertinggi dicatat dalam sampel air surut muara Sungai Pinang, dengan sel tidak pegun mencatatkan kadar pertumbuhan yang tertinggi (0.285µg/mL/d), berbanding dengan sel pegun (0.062*µ*g/mL/d). Antara enam stesen sungai, penyingkiran fosfat tertinggi oleh *S. bijugatus* dicapai dengan sampel air sungai tercemar (hilir Air Itam), dengan penyingkiran sebanyak 90% untuk rawatan sel pegun manakala rawatan sel tidak pegun adalah sebanyak 40%. Semua sampel air sungai sederhana tercemar dan tercemar menunjukkan penyingkiran nitrogen yang tinggi, dalam lingkungan 80-100%, 80-90% dan sekurang-kurangnya 50% penyingkiran masing-masing, untuk ammonium, nitrit dan nitrat. Penyingkiran nutrien bukan organik dan pertumbuhan sel mikroalga antara rawatan sel tidak pegun dan sel pegun tidak berbeza dengan signifikan ($p>0.05$). Walaubagaimanapun,

terdapat perbezaan signifikan antara penyingkiran nutrient bukan organik dan pertumbuhan alga di antara stesen sungai air bersih, sederhana tercemar dan tercemar ($p<0.05$); ini menunjukkan sampel air tercemar menyokong pertumbuhan *S. bijugatus* dan penyingkiran nutrient dengan cekap berbanding dengan sampel air sungai bersih. Rawatan sel pegun dibuktikan dapat mempengaruhi penyingkiran fosfat dalam media melalui pemendapan kimia antara matriks manik dan ion fosfat. Sebaliknya, sel pegun (penyerapan matriks manik) hanya mempengaruhi penyingkiran nitrogen secara terhad, dan penyingkiran yang lain bergantung kepada assimilasi sel alga. Dengan penemuan ini, sel pegun boleh membantu mikroalga untuk menyingkir nutrient bukan organik secara cekap, terutamanya fosfat dalam sampel air tercemar secara organik dan merupakan calon terbaik dalam bioremediasi jika penambahbaikan dalam teknik sel pegun dapat dicapai.

NUTRIENT REMOVAL FROM SUNGAI PINANG BASIN WATER SAMPLES BY FREE AND IMMOBILISED CELLS OF *Scenedesmus bijugatus* Kützing

ABSTRACT

Green microalga *Scenedesmus bijugatus* was used to remove inorganic nutrients (phosphate, ammonium, nitrite and nitrate), by inoculating the cells into water samples collected from selected sampling sites along Sungai Pinang Basin with varying levels of pollution. This research studied the effects of inorganic nutrients removal by free and immobilised *S. bijugatus* cells through algal growth (chlorophyll *a* concentration) and the efficiency of free and immobilised cells in the uptake of inorganic nutrients (uptake rate (*b)* and removal (%)). Polluted and moderately polluted river water samples recorded a higher growth of *S. bijugatus* than clean river water samples. Among the six stations studied, the highest growth rate was recorded in Sungai Pinang estuary low tide water samples, with free cells treatment being the highest (0.285μg/mL/d) compared to immobilised cells (0.062μg/mL/d). Among the six river stations, the highest phosphate removal (%) by *S. bijugatus* was achieved by polluted river water samples; such as Air Itam downstream which recorded 90% removal for immobilised cells treatment while free cells treatment was about 40%. All moderately polluted and polluted river water samples showed high nitrogen removal, in the range of 80-100%, 80-90% and at least 50% removal for ammonium, nitrite and nitrate, respectively. Both inorganic nutrients removal and microalgal cells growth between free or immobilised *S. bijugatus* cells treatments were not significantly different ($p>0.05$). However, there were significant differences in the removals of inorganic nutrients and algal growth among the clean, moderately

polluted and polluted river water stations ($p<0.05$), showing that, polluted water supports *S. bijugatus* growth more efficiently than clean water samples and in removing nutrients. Immobilisation treatment has been found to greatly affect phosphate removal from the medium through the chemical precipitation of beads matrix and phosphate ions. On the other hand, immobilisation (beads matrix absorption) only affected nitrogen removal to a limited extend, and the rest depends on the algal assimilation pathway. With these findings, immobilisation can be used to aid microalgae in efficiently removing inorganic nutrients, especially phosphate from organically polluted waters and will be a good candidate for bioremediation if further enhancement in the immobilisation techniques can be achieved.

CHAPTER ONE

INTRODUCTION

1.0 Introduction

Water quality is the condition (physical, chemical and biological parameters) of a water body in its suitability in sustaining a certain function (e.g. recreational activities, drinking water and fishing) (Diersing, 2009).

According to Azhar (2000), river water provides about 98% of Malaysia's water requirement. Rivers play an important role in the development of Malaysian settlements since historical times. Major settlements started flourishing along the river banks, as it provides food and water for survivability, transportation, agricultural and economical values to the people who live there. However with rapid urbanization and industrialisation, the rivers become overstress and have deteriorated drastically. Besides deteriorating water quality, the rich biodiversity of aquatic organisms found in Malaysia is also badly affected (Chan *et al.*, 2003). Deforestation and building of hydro-electric power plant also affects the river landscape. Both activities change the landscape of rivers and will increase the sediment loading into the river systems. This leads to increase flash flood problems especially during monsoon season and further pollute the river (Chan *et al.*, 2003).

According to Yahya (2008), 17 rivers out of Malaysia's 186 river systems have become unsafe for human activities. Rivers all around Malaysia are affected due to rapid development. River provides Malaysians with the source for generating hydropower while the floodplains around the rivers provide fertile land for the development of agriculture sector, industrial area and even housing development. Uncontrolled human activities have caused the river water quality to deteriorate.

Rapid population growth, agriculture, urban and industrial activities in Selangor state has caused the river water quality to become polluted (Leong *et al.*, 2007). Besides that, Langat River Basin (Juahir *et al.,* 2011) in Selangor has undergone heavy development to such an extent that the pollution is becoming worse. In a study conducted by Leong *et al.* (2007), it has been found that Selangor River is highly contaminated with organochlorine and organophosphate pesticides.

Other than Langat River Basin, Inaman River estuary, Sabah (Mokhtar et al. 1994), Juru River Basin (Lim & Kiu, 1995), Linggi River Basin (Khan, 1991), Selangor River Basin (Santhi & Mustafa, 2013), Klang River Basin (Tan, 1995) and Sungai River Basin(DID, 2000; Kok, 2004) are all heavily affected by human activities along the rivers. According to a study conducted by Kok (2004), Sungai Pinang Basin water pollution is mainly caused by anthropogenic activities such as domestic sewage, agricultural and industrial wastes. If no proper measure is taken, water pollution in Malaysia will continue to deteriorate and will affect Malaysia economically as well as the overall wellbeing of living organisms (Jacky, 2010).

According to Dudgeon (1992), currently tropical Asian rivers are facing three threats. Firstly is degradation of drainage basins, caused by deforestation and overgrazing activities. Secondly, is a threat caused by river regulation and control. Although it is a planned development, damage to the environment is even greater. For example, the construction of dam blocks fish migration routes, alters the seasonal floodplain flooding pattern and land-water interactions. The third threat is river pollution due to untreated wastewater from household or industry areas.

Malaysia is badly affected by the three threats mentioned above. Being a developing country, Malaysia needs more resources and land for development and to

achieve that Malaysia need to clear and cut down the forest (deforestation) (DID, 2009). Malaysia is also depending on hydroelectric power to generate electricity by building dam (Shafie *et al.*, 2011) which contributes to the second threat. River regulation also leads to extensive flooding during the monsoon season as can be observed of the annual monsoon floods especially at the Northeast coast of Peninsular Malaysia (Chan, 1997). River pollution is a serious threat in Malaysia due to rapid development (Yahya, 2008).

1.1 Types of water pollutants

According to Hodges (1977), there are eight categories of water pollutants: 1) sewage and other oxygen-demanding wastes, consume oxygen during its degradation process by microorganisms and it affects the aquatic life devastatingly; 2) infectious agents, within waste water there are microorganisms that can bring diseases and sicknesses to mankind, animals and plants such as cholera and typhoid; 3) plant nutrients, such as phosphorus and nitrogen are essential for aquatic plant growth. However excessive plant nutrients lead to excessive plant growth. This will depletes dissolved oxygen one way or the other no matter the aquatic plant is growing or dying; 4) exotic organic chemicals, such as detergents, pesticides, industrious products can be toxic to aquatic life even in low concentration 5) inorganic minerals and chemical compounds, such as salts and acid mine drainage from industrial and municipal runoff can endanger aquatic life and pollute the drinking water; 6) sediments, such as soil and mineral particles are brought in through water erosion. Sediments can clog the waterways and cause problems; 7) radioactive substances, as a result of radioactive waste from nuclear power plants or industry can cause harmful effect to the water environment; and 8) heat, it affects the water density and its viscosity, causing both to drop. Heat also increases the settling of suspended solids

and evaporation rate. Besides that, the chemical reaction rate will increase too. Thus assimilation of waste will be faster and oxygen will depletes rapidly.

1.2 Sources of water pollution

According to Hodges (1977), major sources of water pollution can be classified into 1) domestic, mostly comes from household and commercial areas; 2) industrial, water mostly comes from a specific area where there are a large number of industrial factories; 3) agricultural, where water runoff from agriculture sectors such as fertilisers, insecticides and husbandry wastes; and 4) shipping waste waters, where shipping activities cause oil pollution and oil spillage besides human wastes resulted from human activities**.**

Nutrient inputs come from the surrounding area of an aquatic environment. The growth of aquatic biomass solely depends on the availability of nutrients at the start of the proliferation process. Eutrophication can be improved if the nutrients input into the water body can be controlled (Wainright, 1999). Human activities are usually the main cause of eutrophication (Chapman, 1996). Factors contributing to eutrophication can be divided into point and non-point sources. Point sources are sources that can be pin point such as urban wastewater and household detergents. Non-point sources are sources that cannot be determined exactly such as agriculture activities. Other factors that might offset eutrophication will be the climate: the thermocline effect at the lake of warm and sub-tropical region (Chapman, 1996).

Uptake of nutrients by aquatic life is fast but it requires time to grow. As a result, the eutrophication phenomenon has already manifested for some time by the time its effect is known. In many cases of eutrophication, its effect is only known

after a certain period of excess nutrients uploading into the affected water body. (Chapman, 1996)

According to Martinez *et al.* (2000), it has been found that ammonium, nitrate and phosphate are the three main substances that contributed to eutrophication. This is because nitrogen and phosphate are the main nutrient that promote growth of algae in the seas and lakes (Abe *et al.* 2000; Gao, *et al.* 2009)(Edited, ok?). As the promoting factors, nitrogen and phosphorus are also the limiting factors for growth when either one of them is depleted. Meybeck *et al.* (1989) pointed out that in an aquatic environment, if the N/P ratio is greater than 7, phosphorus will be the limiting factor and vice versa. (Chapman, 1996)

A known way of treating phosphate is through precipitation with the use of ferric chloride. However, some of the chemicals used are toxic and may harm the aquatic environment (Wainright, 1999). To reduce the side effects of chemicals, biological ways such as biosorption by microalgae has been tested widely by many researchers in the hope to treat remove polluting substances from the water body. Several researchers have conducted studies of algae growth on different types of water samples, such as municipal waste (Li *et al.*, 2011; Chi *et al.*, 2011) and industrial wastewater (Mulbry *et al.*, 2009).

1.3 Utilisation of algae for the biological treatment of polluted waters

In the old days, wastewaters from household or industry were released into the natural waters. When mankind activities grow, wastewater is treated at treatment plants. However treatment plants do not effectively remove polluting organic ions such as phosphate and the methods to fully remove these polluting compounds are expensive (Ruiz-Marin *et al.* 2010). In addition, agricultural runoff is another non-

point source of pollution to the natural waters which leads to the eutrophication phenomenon. (Cai, *et al.* 2013)

Algae provides oxygen, utilizes the minerals of its surroundings for growth and a primary producer in aquatic ecosystems (Shubert, 1984). Therefore, algae have been incorporated into the treatment plants to remove excess nutrients from the wastewaters before it is released into the natural environment (Graham, *et al.* 2009; Ruiz-Marin *et al.* 2010)

Algae are sensitive to the condition of its environment. By observing its reaction towards its surrounding, data such as the nutrients concentration can be collected to monitor the water quality (Shubert, 1984). Wastewater treatment ponds such as sewage treatment plants and algal ponds utilize algae in their treatments to absorb excess nutrients. There are a few types of design for algae pond such as the "high rate ponds" and the "algal turf systems" (Graham, *et al.* 2009; Hoffman, 1998; Pizarro, *et al.* 2006)

Wastewater system that utilizes algae, obtain the benefits to remove excess nutrients and to provide oxygen to the wastewater; does not produce odour, does not require energy and algae biomass can be easily removed from the wastewater. (Graham, *et al.* 2009)

1.4 Nutrient removal by green microalgae

Nutrient removal by green microalgae will be used in this experiment to treat water samples with four different water qualities: clean water, moderately polluted, most polluted and brackish water. Microalgae will be used in this nutrient removal experiment to treat water samples polluted with elements that leads to eutrophication such as ammonium, nitrogen and phosphate as microalgae depends on these three

nutrients for their growth. Besides the dependence of nutrients for their growth, microalgae can also clean the wastewater by releasing oxygen as a byproduct of photosynthesis (Martinez *et al.*, 2000).

Globally many researchers have done research on the process of nutrient removal using algae at their respective countries (Abe *et al.*, 2000; Martinez *et al.*, 2000; Romera *et al.*, 2006,). However, not much study of nutrient removal process using algae has been tested in rivers or used in wastewater treatment in Malaysia. Furthermore, Malaysia is experiencing the crisis of river pollution. Pinang River Basin, located in a northern part of Penang Island is one of the polluted rivers in Malaysia. So, this study was conducted to determine the potential of biosorption of nutrients by microalgae on Malaysian Rivers.

Most microalgae are usually suspended in water and can bloom easily under suitable conditions (de-Bashan & Bashan, 2010). Existing micro algal cultivation system are the open system, closed system and the hybrid system (Cai *et al.* 2013). All of them share the same common characteristics which are on land cultivation and uses suspended algae culture in their system. The main difference between the three systems is the system exposure towards their surrounding environment. (Cai *et al.* 2013). However, freely suspended cells in such microalgae culturing system always pose a problem for biomass removal after treatment (de-Bashan & Bashan, 2010; Jimenez-Perez *et al.* 2004; Mallick, 2002). The conventional harvesting methods are chemical, mechanical, electrical, and biological techniques. Chemical might leave residuals that are toxic to the environments while energy methods such as industrial filtration and centrifugation are expensive (Cai *et al.* 2013). Immobilization by natural or artificial means has been suggested and tested for the harvesting of micro

algae by many researchers such as (Cai *et al.* 2013; de-Bashan & Bashan, 2010; Hoffman, 1998; Mallick, 2002), and others

Besides using freely suspended cells in this study, studies of non-suspended cells, immobilization of algae in alginate beads will also be conducted. Entrapment method of the alginate beads will be used, as it is one of the natural gels besides carrageenan that has been widely used and is not made of toxic substances that might endanger the environment. Leenen *et al.* (1996) and Olguín (2003) pointed out that immobilization technique has been developed with the goal of low cost harvesting technique and high algae productivity in mind. Besides that, immobilized algae ensure the algae biomass can be removed efficiently after each treatment so that the discharged water will be ready to be reused for other functions (Hoffman, 1998).

According to Mallick (2002), besides fulfilling the requirements of a functional immobilized algal system, the characteristics of an ideal immobilization matrix are also very important. For example, conventional algal wastewater treatment only needs to consider about species selection, algal density and others. However the usage of immobilization techniques has shown that the characteristics of gel matrix are also to be taken into account in setting up the immobilized algal system (gel matrix and algal interaction). Table 1.1 outlines the comparison between a functional immobilized system and the characteristics of a "good" immobilization matrix.

Table 1.1: Basic requirements of an immobilized algal system and properties of an ideal matrix for immobilization (Mallick, 2002)

Requirements of a useful immobilized algal system	Properties of an ideal matrix for immobilization
Retention of viability	Non-toxicity
Ability to photosynthesize	Phototransparency
High density of cells	Stability in growth medium
Continued productivity	Retention of biomass
Low leakage of cells from matrix	Resistance to disruption by cell growth

1.5 The importance of the study

Immobilization of microalgae has emerged more than 20 years ago with the pioneering studies conducted by de la Noue and his collaborators (Chevalier and de la Noue, 1985 a, b; de la Noue and de Pauw. 1988; de la Noue et al., 1990). The main reason behind immobilization is to reduce the harvesting cost of algae biomass resulted from wastewater treatment. For this study, both types of experimental set up 1) freely suspended microalgae and 2) immobilization of algae within the gel matrix were used to test the efficiency of nutrient removal incubated in water river samples collected from the infamous polluted Sungai Pinang basins. Besides that, this study was conducted to provide a suitable biological process to reduce nutrients level in Malaysian waters and to study a way to effectively treat nutrients found in organically polluted rivers.

1.6 Hypothesis

1. Organically polluted river is better in sustaining green algae growth due to its high nutrient concentrations compared to clean river that have lower nutrient concentrations.
2. Different types of water samples have different effects on the growth of green algae and its nutrients uptake. For example, the micro algal cells treated in brackish water will have different growth rate compared to micro algal cells treated in other types of water sample.
3. Immobilised algae cells have higher uptake of nutrients compared to free cells.

1.7 Research objectives

1. To study the green algal growth collected from selected Sungai Pinang Basin water samples by referring to the algal density.
2. To determine the uptake of nutrients (ammonium, nitrite, nitrate and phosphate) by green micro algal incubated in water samples collected from selected Sungai Pinang Basin stations.
3. To determine the efficiency of immobilised micro algal cells in the uptake of nutrients as compared to free cells.

CHAPTER TWO

LITERATURE REVIEW

2.0 Literature Review

Algae have long been used in wastewater treatment system due to its capability to absorb inorganic nutrients especially phosphorus and nitrogen during photosynthesis. However after the treatment of algae, the algae biomass produced is difficult to be removed (Cordoba *et al.* 1995; Moreno-Garrido, 2008; de-Bashan & Bashan, 2010). To solve the difficulty of algae biomass separation, immobilization techniques have been studied and used. Several studies have been done to further investigate in the uptake of nutrients by microalgae.

A study conducted by Rai *et al.* (1992) to compare the removal of Cu and Fe between two different strains of algae and by using free and immobilized cells reported that immobilized algae strains have a higher rate of metal uptake compared to free cells. This is because the immobilization process has protected the algae strain from direct exposure towards the toxicity of metal (Rai *et al.*, 1992). In a study conducted by Mallick & Rai (1994), to compare the efficiency of metal and nutrients removal by different immobilization methods found that immobilized algae has higher turnout rate compared to free cells (alginate > chitosan > carrageenan)

Kaya *et al.* (1995) conducted a study on nutrient removal based on four types of system by using *Scenedesmus bicellularis*. The four systems being tested were non-immobilized cells with air bubbling (NCA); cells immobilized in alginate beads (CBW), cells immobilized on alginate screens (CSW) and cells immobilized on alginate screens but conditioned in air at 100% relative humidity (CSA). Among the

four systems, alginate screens are more efficient for N and P removal than alginate beads.

Mallick (2002) used immobilized algae for wastewater treatment to remove nitrogen, phosphorus and metal, and found out that immobilized microalgae improved the efficiency of nutrient uptake when it was used together with bioreactors. A study conducted by Al-Rub *et al.* (2004) compared the used of immobilized and free (suspended) cells. The study found out that immobilization improved biosorption capacity and shorten preparation time compared to free cells.

de-Bashan *et al.*(2002) studied the co-immobilize techniques of microalgae and the plant growth promoting bacterium(PGPB) to enhance the growth of microalgae and to increase the efficiency of nutrients absorption by microalgae during the wastewater treatment. The study found that the PGPB-microalgae combination works better compared to the nutrients absorption rate of microalgae alone. In a study conducted by Perez-Martinex *et al.* (2009), immobilised benthic algal species was used in the wastewater treatment. It has been found that benthic species show a high removal rate especially for phosphorus. Another advantage found for using benthic species in that study was that benthic species remained on the surface of the beads compared to other planktonic species.

2.1 Parameters measured *In situ*

The following parameters were measured *in situ* during water sample collection: total dissolved solid, dissolved oxygen, temperature, conductivity, salinity, and pH.

2.1.1 Total dissolved solid (TDS)

Total dissolved solid (TDS) is also known as filterable residue. TDS refers to elements found in natural waters that may remain as solid after its liquid form has dried up (Chapman, 1996).

2.1.2 Dissolved Oxygen

Dissolved oxygen (DO) refers to the amount of gaseous oxygen that is dissolved in an aqueous solution. Oxygen concentration is affected by the temperature, salinity, turbulence and the photosynthetic activity of algae and plants and atmospheric pressure. Solubility of oxygen will drop as value of temperature and salinity rise. The range of DO measured in freshwater at sea level is between 15 mg L^{-1} at 0 ºC to 8 mg L^{-1} at 25 ºC. Concentrations of DO measured in clean waters are between the ranges of 8 to 10 mg L^{-1}. If it drops below 5 mg L^{-1} aquatic organisms might not survive (Chapman, 1996).

2.1.3 Temperature

Surface water temperature is greatly affected by many environmental factors such as season, air circulation, cloud cover, depth of water and others. However temperature greatly affects the physical and biological activities within a water body. High temperature will decrease the solubility of gases in water such as oxygen that is vital for the survival of aquatic organisms (Chapman, 1996)

2.1.4 Conductivity

Conductivity (µS cm^{-1}) refers to the potential of a water body to transmit electricity. The range of conductivity for freshwater bodies is between 10 to 1000 µS cm^{-1}. However in polluted waters, the reading may exceed 1000 µS cm^{-1}.

Concentrations of total dissolved solids and major ions in a water body will affect conductivity (Chapman, 1996).

2.1.5 Salinity

The unit of measurement for salinity is practical salinity units (psu) or parts per thousand (ppt). It is estimated that for every 1 kg of seawater, there are about 35g of dissolved salts in it, but it may vary from place to place in between the range of 10%--70%. (Graham *et al.* 2009).

2.1.6 pH

pH refers to the acidity or alkalinity of a water solution. The range of pH scale is from 0 to 14. It starts with pH 0 being very acidic to pH 14 being very alkaline with pH 7 in between as the neutral condition. Natural water usually has a pH range of 6.0 to 8.5. Clean water pH is greatly influenced by the balance of natural compounds such as fulvic and humic acids and the bicarbonate ions (Chapman, 1996).

2.2 Pollutant sources and pathways

Discharge of pollutant into a water body differs in terms of time variation. Discharge of pollutant can vary from time to time. The time variations are categorized into permanent or continuous, periodic, occasional and accidental. Permanent or continuous waste refers to household and industrial wastes, while, periodic refers to periodic variation of some big event or activities taking place such as mass arrival of tourist in an area or during the celebration of a festive season. Occasional refers to random release of waste such as those from industrial area and accidental occasion refers to waste release due to accident such as leakage or truck

accident. As the discharge of pollutant vary from time to time, so does the effects it had on a water body. For example, permanent or continuous discharge from the city waste during rainy season may have sufficient amount of water to dilute the waste and the rate of waste degradation is enough to render the waste harmless to the water body. However, permanent or continuous discharge during the dry season will harm the water body directly. In conclusion, the lake volume and initial dilution play a role in the pervasiveness of the lake contaminant (Chapman, 1996; Hatt *et al.*, 2004; Kim & Kannan 2007).

2.3 Nutrients

2.3.1 Nitrite

Ideally, nitrite content within a water body should be between the range of 0.001mg L^{-1} NO_2-N and 1 mg L^{-1} NO_2-N. If nitrite is found in high concentrations, it means the water body contains industrial waste and poor microorganism's activity in the water (Chapman, 1996; Carpenter *et al.*, 1998; Smith *et al.*, 1999).

2.3.2 Nitrate

The World Health Organization (WHO) suggested that the concentration of NO_3^- shouldn't exceed 50 mg L^{-1} (or 11.3 mg L^{-1} as NO_3-N). NO_3-N concentration exceeding 0.2 mg L^{-1} NO_3-N will promote excess algae growth that will lead to the eutrophication phenomenon in lakes (Chapman, 1996; Carpenter, *et al.*, 1998; Burt *et al.*, 2011). DOE Interim Water Quality Standards for Malaysia has set a maximum standard of NO_3 to be maintained at less than 5 mg NO_3-N/L (WEPA, 2006).

Human and animal waste (Carpenter *et al.*, 1998; Wainright, 1999) or agricultural activities run-off (Carpenter *et al.*, 1998; Burow *et al.*, 2010) will cause the nitrate concentrations within a water body to increase as high as 5mg L^{-1} NO_3-N

(Chapman, 1996). Besides that, nitrate also comes from waste discharge and household detergents (O'Sullivan, 1971; Carpenter *et al.*, 1998; Wainright, 1999; Smith *et al.*, 1999; Hot, 2000).

Nitrate may cause a sickness called infant methemoglobinemia or blue baby syndrome in infant if drinking water with high nitrate content is used to prepare baby formula. Infant methemoglobinemia will cause the baby to be deprived of oxygen and may even cause death in infant if it is not treat early (Rittmann & McCarty, 2001).

2.3.3 Ammonia

The degradation of protein waste in water releases ammonia nitrogen. Ammonia being a base will form ammonium bicarbonate under the presence of carbon dioxide and water. Bicarbonate ion plays an important role as a natural pH buffer. With high concentration of protein waste, it also means a high ammonia content and high pH value. This usually leads to ammonia toxicity within a water body (Randall & Patricia, 1987; Rittmann & McCarty, 2001; Randall & Tsui, 2002).

Ammonia found in the aquatic environment originated from many different pathways. For example, ammonia may come from nitrogenous organic and inorganic material in soil and water and the byproduct of microorganisms' metabolism (Rittmann & McCarty, 2001). Ammonia is also released into the aquatic environment through municipal wastewater, industrial activities such as pulp and paper industry and agricultural activities such as husbandry ((Randall & Patricia, 1987; Randall & Tsui, 2002). Ammonia will be harmful to the aquatic organisms if it is found in large amount at a specific pH range (Randall & Patricia, 1987; Chapman, 1996; Randall & Tsui 2002).

Low concentrations of ammonia and its elements are found in clear water in less than 0.1mg L^{-1} as nitrogen. The ammonia content found on the upper part of the water layer is usually below 0.2 mg L^{-1} N but may go as high as 2-3 mg L^{-1} N. Household and industrial sewage are among the factors contributing to organic pollution in a water body. Ammonia being an organic substance is usually used as a sign for organic pollution. Periodic variations of ammonia concentration may happen due to the death and growth of organisms within the water body especially in eutrophic water. The base of the lake or pool undergoing anoxic condition may show signs of high ammonia concentrations (Chapman, 1996; Smith *et al.* 1999).

2.3.4 Phosphorus

Phosphorus occurs naturally in the aquatic environment in both dissolved and granular forms (Rittmann & McCarty, 2001; Selig *et al.*, 2002). Granular form includes living and dead plankton, precipitates of phosphorus, phosphorus adsorbed to particulates and amorphous phosphorus. The dissolved form includes inorganic phosphorus (orthophosphates and polyphosphates) and organic phosphorus (organically bound phosphates) (Chapman, 1996; Selig *et al.*, 2002). However, only orthophosphate will be absorbed for phytoplankton growth (Selig *et al.*, 2002). Interchange between the three forms of dissolved phosphates: orthophosphates, polyphosphates and organically bound phosphates happens from time to time as the process of decomposition, formation of organically bound forms and oxidation of the inorganic forms take place (Chapman, 1996).

The range of phosphorus in natural surface water, clean water, confined saline waters and groundwater levels are between 0.005 to 0.020 mg L^{-1} PO_4-P, 0.001mg L^{-1} PO_4-P and 200mg L^{-1} PO_4-P and 0.02 mg L^{-1} PO_4-P respectively. (Chapman, 1996)

Phosphorus is a vital macronutrient for the growth of autotrophic community such as algae (Rittmann & McCarty, 2001; Havens & Schelske, 2001; Selig *et al.*, 2002). Excess phosphorus leads to algae bloom, while limited phosphorus will restrict the growth of algae. Besides occurring naturally, animal husbandry and household detergents also contributes to the high concentration of phosphate found in water body (Carpenter *et al.*, 1998; Smith *et al.*, 1999; Wainright, 1999).

Detergents contain high concentration of sodium tripolyphosphate and it is one of the main sources in contributing to eutrophication. Although substitute for detergents phosphates such as zeolites with phosphate and polycarboxylic acids has been used, it has to be used in high amounts to see its effectiveness in cleaning. Detergent phosphates are no longer contributing to the source for phosphate pollution as its usage has been greatly reduced nowadays. However phosphate found in water body sediments can still contribute a huge amount of phosphate pollutants into the water body in the form of dissolved phosphate (Carpenter *et al.*, 1998; Wainright, 1999; Liu & Qiu, 2007).

Phosphorus is one of the important analyses performed when it comes to water quality surveys or monitoring as it is a growth-limiting factor for aquatic plants (Havens & Schelske, 2001; Selig *et al.*, 2002). Eutrophic lake usually has high phosphorus content within its water body. The knowledge in monitoring water quality such as phosphate content is essential to ensure a clean source of water supply and the balance of ecosystem for the survival of aquatic organisms (Chapman, 1996; Smith *et al.* 1999; Liu & Qiu, 2007).

Phosphorus can be removed from the water body through biological treatment. Phosphorus removal can either be done before, after or during the treatment. Certain

cations such as Ca^{2+}, Al^{3+}, or Fe^{3+} can be used to bind with phosphate anion (PO^{3-}_4) to form phosphate precipitations for phosphate removal. Rittmann and McCarty (2001) reported that three methods can be used to remove phosphorus: 1) normal phosphorus uptake into biomass where phosphorus content within an aquatic environment is usually taken up by plants such as algae (Chapman, 1996; Liu & Qiu, 2007); 2) precipitation by metal-salts addition to a microbiological process where under pH values suitable for the growth of microorganisms, aluminium and ferric cations will bind with orthophosphate anion to form precipitations. Generally, before wastewater enters or flows out of the bioreactor, metal salts such as Al^{3+} or Fe^{3+} are added and precipitate are removed together with the sewage sludge (de Haas *et al.*, 2000; Rittmann & McCarty, 2001); and 3) enhanced biological phosphorus uptake into biomass. In this method, some heterotrophic bacteria has the ability to absorb huge amount of phosphorus and store it as energy storage material, known as intracellular phosphate (poly P) is used. The final removal of phosphate from wastewater will be rather high (2 to 5 times the phosphate content compared to normal biomass) through the use of this bacteria species (de Haas *et al.*, 2000; Rittmann & McCarty, 2001). de Haas *et al.* (2000) found out in their study that enhanced biological phosphorus removal is highly regarded and will eventually leads to reduced implementation cost compared to the chemical precipitation removal methods although this method might incur higher initial implementation cost. (Rittmann & McCarty, 2001).

2.4 Algae

Algae are plants that are circular or filamentous in shape (Wainright, 1999), lack roots, stems and leaves but they have chlorophyll *a*, just like any plants in the plant kingdom (Lee, 1989). Algae are also known as thallophytes commonly found in water, be it freshwater, marine and even brackish water. Besides that, they are also found in almost every corner of the Earth, for example, the snow in American mountains, desert soils, hot springs, and even the north and South Pole of the Earth (Lee, 1989).

Algae play an important role in maintaining the wellbeing of a water body and in wastewater treatment. Inorganic minerals uptake from the aquatic environment is required for algae growth. Uptake of inorganic minerals causes chemicals such as pH, hardness and alkalinity in the aquatic environment to fluctuate (Rittmann & McCarty, 2001). Waste release into the water body will also promote the rapid growth of algae within an aquatic environment. Rapid growth of algae bring odour and taste problem to the water body, obstruct the filter at water treatment plant and even form buoyant mats that will interfere with water sports on the water body (Smith & Schindler, 2009). Certain algae especially cyanobacteria produce toxins that cause fish kills **(**Rittmann & McCarty, 2001; Paerl *et al.*, 2001; Smith & Schindler, 2009). However, the breakdown of algae will use up the oxygen within the water body and leads to eutrophication phenomenon. Thus, a stable community of algae plays an important role in sustaining a balanced ecosystem (Rittmann & McCarty, 2001).

2.4.1 Morphology

Algae can appear in many forms, for example, some algae are spherical in shape, some are rod-shaped, and spindle-shape or club-shaped. Some algae may grow as a membranous colony or as filaments clusters (Rittmann & McCarty, 2001).

All algae have chlorophyll *a* pigment. However one can differentiate the algae from one group to another through its chlorophylls pigment. For example, the chlorophyll of cyanobacteria is scattered all over the cell, this is a characteristic that is not shared by the eukaryotic algae. The chlorophyll of eukaryotic algae is enclosed within a membrane known as chloroplasts (Rittmann & McCarty, 2001).

Table 2.1**:** Four distinct evolutionary groups of algae (Lee, 1989)

Evolutionary Group	Description	Example
Group 1	Prokaryotic algae (characterized by their prokaryotic cell organization)	Cyanophyta(blue-green algae) Prochlorophyta(prochlorophytes)
Group 2	Eucaryotic algae with chloroplasts surrounded only by the two membranes of the chloroplast envelope	Glaucophyta Rhodophyta (red algae) Chlorophyta(green algae)
Group 3	Eucaryotic algae with chloroplasts surrounded by one membranne of chloroplast endoplasmic reticulum	Euglenophyta(euglenoids) Dinophyta(Dinoflagellates)
Group 4	Eucaryotic algae with chloroplast surrounded by two membranes of chloroplast endoplasmic reticulum	Cryptophyta(cryptophytes) Chrysophyta(golden-brown algae) Prymnesiophyta(haptophytes) Bacillariophyta(diatoms) Xanthophyta(yellow-green algae) Eustigmatophyta Raphidophyta(chloromonads) Phaeophyta(brown algae)

Chlorophyta

Chlorophyta, or commonly known as green algae are found in many places and are a versatile species. It is mostly used in wastewater treatment. Most green algae are unicellular, and some of it is motile due to the presence of flagella. Green algae have one chloroplast per cell. The commonly used algae for wastewater treatments are *Chlorella, Scenedesmus and Chlamydomonas* (Rittmann & McCarty, 2001).

Chrysophyta

Chrysophyta has a shell structure that is consisted of silica and has a yellow-brown pigment. One of the common species found in Chrysophyta is diatom. Siliceous shell left behind by the diatom species can be harvested as diatomaceous earth, and it is usually processed into a water filter aid (Rittmann & McCarty, 2001).

Euglenophyta

Euglenophyta are unicellular algae that are motile, due to the presence of flagella. The most widespread species of this group is Euglena. Euglena's red stain comes from its photoreceptor within its body. The photoreceptor helps the organism to move towards or away from the sunlight accordingly (Rittmann & McCarty, 2001).

Pyrrophyta

Certain species of the Pyrrophyta group have a hardened cellulose wall. The unfavorable red tide that has poisoned thousands of fish worldwide is caused by one of the Pyrrophyta species known as *Gonyaulax catanella*. This is due to a fatal toxin that is released by the algae. The occurrence of red tide is due to the unloading of high concentration of nutrients from the wastewater. Another reason that causes red

tide is the cold, nutrient rich water surging from the bottom of the ocean to the surface or the growth requirements for red tide algae has been met at certain period of the year (Rittmann & McCarty, 2001).

Cyanophyta

Cyanobacteria are one of the dominant species found in the group of Cyanophyta. It is also commonly known as blue-green algae. Bad taste, odours and unsightly massive floating mats are few of the many "disadvantages" caused by Cyanobacteria. Cyanophyta group has an advantage over the eukaryotic algae to grow in a water body that has limited nitrogen. It can fix nitrogen gas directly from the atmosphere and convert it to cellular protein and nucleic acids (Rittmann & McCarty, 2001).

2.4.2 Reproduction and Growth

Algae can propagate sexually or asexually. If the conditions to propagate is suitable, algae cells can divide very quickly. Nearly all algae are autotrophic, but some are heterotrophic and depends on complex organic substances such as acetate (Rittmann & McCarty, 2001).

The vital components for the survival of autotrophic organisms are carbon, hydrogen, oxygen, nitrogen, phosphorus, sulfur and iron. The compositions of algae are 50% carbon, 10% nitrogen, and 2% phosphorus. The components of nitrogen and phosphorus within an algal will drop to the range of 2 and 0.2% respectively, if the nitrogen and phosphorus content within the water body is lesser than the amount required by the algae (Rittmann & McCarty, 2001).

Despite the fact that certain minerals such as iron and carbon are found abundant in freshwater but very little in marine environment, nitrogen and phosphorus are often the restricting factor for the growth of algae in a water body. To fully utilize the growth of algae to our advantage in terms of promoting an equilibrium state or to slow down the process of eutrophication within a water body, the researchers must have some insights on the influence of inorganic nutrients towards the algae (Rittmann & McCarty, 2001).

Each individual alga has its own individual preferred optimum conditions for it to grow well. In terms of temperature, diatoms can be found in colder regions compared to cyanobacteria. Thus, temperature changes and along with other factors will determine the growth of the prevailing algal species in a water body (Rittmann & McCarty, 2001).

The optimum pH for the growth of algae is between near neutral to alkaline. This is due to the presence of bicarbonate ions acting as a pH buffer within a water body. Bicarbonate is formed in the presence of water and carbon dioxide and is released by the photosynthetic activity of aquatic plants and algae. So, as the algae and plant grows, so will fluctuations of water pH (Rittmann & McCarty, 2001).

Water pH in the range of 8.5 to 9.0 will inhibit algal growth. But, there are certain species of algae that can tolerate the high pH value and strive in the environment where no other algae species can survive (Rittmann & McCarty, 2001). It has been found that bloom forming or red-tide coastal species tolerate extremely high pH-levels in terms of seawater (above pH 9.0) (Hansen et al., 2007). One such species that commonly forms blooms (red tides) is *Heterocapsa triquetra* (Hansen *et al.*, 2007)