WASTEWATER TREATMENT USING MICROALGAE

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Abstract

Three species of selected microalgae isolated from Tasik Taman Jaya, *Oscillatoria limnosa*, S*cenedesmus quadricauda* and *Pediastrum duplex*, were cultured in *Chu no. 10* defined medium. After the microalgae reached their optimum growth they were grown in wastewater to observe their capability to remove inorganic nutrients in wastewater. The microalgae showed steady increase in cell density for the duration of laboratory observation of eighteen days. As the cell density of the microalgae increased, both nitrate and phosphate concentration in wastewater decreased. This study showed that all the three species of microalgae have the ability to reduce the nutrients. Among them, *Pediastrum duplex* was most efficient in reducing inorganic nutrients in wastewater. It reduced 60% and 75% of the total nutrients of wastewater from Station A and Station B respectively. By using this biological wastewater treatment, less chemical and physical treatment is needed to improve wastewater quality. The biologically treated wastewater can then be discharged into the reservoirs, rivers or streams.

Abstrak

Tiga spesies mikroalga yang diasingkan dari Tasik Taman Jaya iaitu *Oscillatoria limnosa*, *Scenedesmus quadricauda* dan *Pediastrum duplex* telah dikulturkan di dalam media pertumbuhan *Chu no. 10* di dalam makmal. Setelah mikroalga tersebut mencapai pertumbuhan yang optima, ia telah membiak di dalam air kumbahan, seterusnya menunjukkan keupayaannya untuk menyingkirkan nutrien tak organik dalam air sisa. Selepas 18 hari pemerhatian makmal dilakukan, ia menunjukkan peningkatan yang agak stabil dari sudut kepadatanya. Ketika kepadatan sel mengalami peningkatan, kandungan nitrat dan fosfat di dalam air sisa menunjukkan bacaan yang menurun. Dapatan dari kajian menunjukkan bahawa *Pediastrum duplex* ialah sel yang paling berkesan untuk mengurangkan kandungan nutrien tak organik di antara ketiga-tiga spesies mikroalga ini. Hasil dari bacaan makmal ke atas Stesen A dan B, ia menunjukkan bahawa sebanyak 60% dan 75% jumlah kandungan nutrien air sisa telah dikurangkan kepekatannya. Sehubungan itu, dengan mengaplikasikan kaedah rawatan secara biologi ke atas air sisa ia juga mampu mengurangkan kebergantungan ke atas kaedah rawatan secara kimia dan fizikal untuk memastikan kualiti air terjamin. Seterusnya, apabila proses rawatan secara biologi dilaksanakan maka penyaliran air sisa ke dalam kawasan reserviour dan sungai dapat disalirkan.

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List of Contents

Chapter 3

Chapter 4

List of Figures

List of Tables

List of Plate

List of Abbreviation

BOD = Biological Oxygen Demand

TSS = Total Suspended Solid

Cd = Cadmium

Hg = Mercury

Co = Cobalt

Pb = Plumbum

Cu = Cuprum

Cr = Chromium

Fe = Ferum

N = Nitrogen

P = Phosphorous

sp. = species

mg/L = milligram per liter

g/L = gram per liter

µm = micrometer

µg = microgram

List of Symbols

β	= beta
°C	= degree
±	= plus minus
%	= percent
pH	= power of Hydrogen
m^2	= meter square
m^3	= meter cube
Mg^{2+}	= Magnesium ion
Na^+	= Natrium ion
K^+	= Kalium ion
Ca^+	= Calcium ion

List of Appendices

Chapter 1

Introduction

Water that is released after being used by the domestic and industrial sector into the environment is called wastewater (Mohapatra, 2006). Wastewater is the major contributor to the aquatic ecosystem pollution. The waste contained high inorganic nutrients, mainly ammonia, nitrate, carbon and phosphate. Besides the inorganic nutrients, in the wastewater also contain heavy metals. These heavy metals and the inorganic nutrients may cause harm to the aquatic organisms, including humans, as water is the main fundamental source to human's daily life. The factors that contribute to the water pollutant are domestic wastewater, agricultural runoff, and landfill, industrial effluent and animal waste.

Thus, the physical and chemical characteristic of a water body has changed due to overloading of unwanted organic or inorganic substances. Consequently, it may reduce the quality of water and finally threatening an aquatic organism' life. In some situations, it may cause to the formation of eutrophication.

Eutrophication occurs when a lake is rich in phosphate and nitrate. This is indicated by existence of aquatic plants and algal biomass. Eutrophication changes the biodiversity cycle and biological communities mostly at all levels in the food web as well as entire communities (Hall and Smol, 1999). Phosphate and nitrate are the major nutrients that cause eutrophication in water bodies were treated sewage effluents are discharged into the lake (Shushu and Chipeta, 2002). These nutrients might produce an algal bloom as the nutrients overload the water bodies. Hutchinson (1967) noted that water clarity often indicates the overall lake's water quality and species of algae inhabiting it.

Nowadays, there are many ways to treat wastewater whether by chemical or biological methods. To be environmental friendly, a biological treatment has been chosen as an alternative way to treat wastewater. In biological treatments organisms are used. The organisms that are often used in this method are bacteria, fungi and microalgae. In some biological treatments, combinations of organisms are used in order to be more effective and give a good outcome. Besides using the microorganisms, aquatic plants are also useful in treating aquatic problems.

According to Tchobanoglous and Burton (1991), chemical and physical based technologies are more convenient to remove nutrients and metals from wastewater. However, these technologies are very costly and consumed a high magnitude of energy as well as chemicals. Therefore, through chemical treatment, it may cause other major problems such as an increasing of elements in the wastewater and high cost for operation (Hoffmann, 1998).

The purpose of this study is to investigate the effectiveness of microalgae in reducing the inorganic matter in the wastewater as well as to create an environmental friendly to the ecosystem without involve any chemicals in the treatment process.

Algae are aquatic organisms that are photosynthetic, oxygenic autotroph that are typically smaller and have less complex of structure compared to terrestrial plants (Graham and Wilcox, 2000). Algae can be divided into microalgae and macroalgae. Microalgae are also called phytoplankton, microphytes or planktonic algae, while macroalgae are commonly known as seaweed. Phytoplanktons are those plants that float aimless or swim freely to maintain a constant position against the water current (Graham and Wilcox, 2000). Freshwater phytoplankton forms the base of the aquatic food chain, and without it, the freshwater fisheries could not exist.

There are various types of shapes and sizes of the planktonic algae. Some of them occur as a single cell or unicell and some of them from a colony. A colony is a group of individual cells in which they may be either a variable number of cells that remain constants throughout the life of the individual. Some colony is referring to as coenobium (Graham and Wilcox, 2000). Another form of planktonic algae is the filament. It may be branched or un-branched, and it may form a single series of the cells (uniseriate) or multi series (pluriseriate). Although the planktonic algae have varied in structure forms, it can be either motile or non-motile. Mortality conferred in various ways; from swimming or creeping, pushing or by floating device. However, many non-motile planktonic algae are reproduced by the motile cells (Canter-Lund and Lund, 1995).

Becker (1994) noted that under natural condition, most of the algae grow as mixed communities, which include various species and genera. Thus, the successful growth of microalgae in a culture is depending of the environment factors. It may be either physical; such as temperature and light, or chemical; which provides all the raw materials use for their development of the algal cell (Becker, 1994). Wastewater treatment with microalgae offered a simpler solution than culture intended for the production of clean biomass and highly valuable material (De la Noüe *et.al*., 1992).

Microalgae were selected as the wastewater treated owing to their effectiveness in reducing the nutrient in the water bodies by utilizing the inorganic nutrients as for survival. Laliberté *et. al* (1994) found that the use of microalgae for wastewater treatment is affected by environmental condition including pH, temperature, light, inorganic carbon, effluent composition and retention time.

The wastewater treatment using microalgae, had been done about 40 years ago, which has been described by Oswald and Gotaas (1957). The focused was suspended microalgae growing in shallow, artificial ponds containing sewage (Hoffmann, 1998). According to Hoffmann (1998), non-suspended algae was used either as unialgal cultures immobilized in a polymeric matrix or as attached algal communities, which are grown in shallow, artificial streams or on the surface of rotating biological contractors (RBC/biodiscs).

There are several advantages of applying microalgae in the biological treatment compared to conservative treatments. The microalgae are very effective in consuming the inorganic nitrogen and phosphorus for their growth developments. In addition, they could purify waste by producing oxygen and removing heavy metals as well as

xenobiotic substance (Martínez *et.al*., 2000). Other than that, by using biological treatment, it also does not cause a secondary pollution if the biomass produce is re-used.

1.1 Objectives

Tasik Taman Jaya is a highly polluted lake. Therefore, a lot of effort must be taken to reduce the inorganic nutrients in the water bodies before the water can be discharged into rivers. Previous studies reported that this lake experienced an eutrophication process (Norhayati, 1995; Saravanamuthu, 1977). Having said that the researcher would like to extend this study by emphasizing the following objectives:

1. To study the composition and abundance of microalgae in the lake.
2. To identify algae species that are associated with the water pollution that can be used for wastewater treatment.
3. To examine the efficiency and the growth of the different selected microalgae in the wastewater and their ability to remove inorganic nutrients.

Chapter 2

Literature review

2.1 Water Pollution

Water plays a significant role in the development of the society due to adequate supply as well as safety for the humankind. Instead of man, animals and plants also need water for their survival. Approximately, 70 % of the component on the earth is water. However, only 3% is the freshwater and the rest is salty water. The freshwater can be finding either as the glacier (2.997%) or natural water at 0.003% (Abdul Aziz, 1999a). Thus it is important to maintain a very little quantity of natural water, because it most essential for daily uses. Recently, our water supply has become a problem. There are many polluted matters in the water bodies. This happens due to lack of awareness to protect the environment, especially aquatic environment.

According to The Malaysian Environmental Quality Act 1974 pollution is defined as" any direct or indirect alteration of the physical, thermal, chemical, biological or radioactive properties of any part of the environment by discharging, emitting or depositing waste to affect any beneficial use adversely, to cause a condition that is hazardous to public health, safety or welfare or to animals, birds, wildlife, fish or

aquatic life or plants to cause a contravention of any condition, limitation or restriction to which a license under the act is subject" (Abdul Salam, 1999).

Pollutions are usually classified into water pollution, air pollution, land pollution, thermal pollution as well as sound pollution. Pollution mostly results from human activities. Pollution problems become the worst year by year is due to the growing population as well as expanding per-capita use of materials and energy have increased the amount of by-product that comes into environment (Wright, 2005). Most of the materials used in home and industries are non-biodegradable; examples are aluminum cans, plastic packaging and synthetic organic chemicals. Thus, these materials cannot breakdown by the detritus feeder and decomposers. Due to this reason, the non-biodegradable is accumulated in the environment and causes the pollutions. Table 1 below shows type of pollutants that exits nowadays. These pollutants can be found in the air, water or soil, and it can be either metals or organic compounds that are not normally found in the nature (Scragg, 2005).

Table 2.1: Type of pollutants.

Type of pollutant	**Examples**
Inorganic 1) Metals 2) Radio nucleotides 3) Nitrate, nitrites, phosphates 4) Cyanide 5) Asbestos	Cd, Hg, Ag, Co, Pb, Cu, Cr, Fe.
Organic 1) Biodegradable 2) Petrochemicals 3) Synthetic	 -Sewage, domestic agricultural and process waste. -Oil, diesel, BTEX *. -Pesticides, organohalogens, PAH *.
Biology 1) Pathogens	 -Bacteria, viruses.
Gaseous 1) Gases 2) Volatile 3) Particulates	 -Sulphur dioxide, carbon dioxide, nitrous oxides, methane -CFCs *, VOCs *.

- BTEX = benzene, toluene, ethylbenzene, xylene
- PAH = polyaromatic hydrocarbon
- CFC = chloroflourocarbon
- VOC = volatile organic compound.

(Scragg, 2005).

Water pollution, air pollution and land pollution all inter-related. Water pollution occurs due to the development of lands as well as discharge of waste products that are not needed. There are several of land uses and human activities that contribute to the water pollution such as agro-based industries, intensive land clearing activities, animals' husbandry, especially pig rearing, logging activities, uncontrolled earthwork manufacturing activities, mining activities and maritime activities (Abdul Salam, 1999). All the pollution from the land seeps via the soil and goes through into groundwater as well as into the rivers system.

The air pollution occurs due to the releasing of gasses from factories, vehicles and open burning activities. The gasses releases to the air contain hazardous gasses, mainly from; carbon monoxide, chlorofluorocarbon (CFC), carbon dioxide, which is not good for human health. These gasses are removing by rain and flow into water bodies. Thus, the water bodies are the last destination for all types of pollution that exists.

There are two main source of water pollution: point source and non-point source (diffused source). The point source, are the effluent that are directly discharged into the receiving water bodies from stationary location. These included wastewater discharge from industries and urban areas, aquaculture pond, thermal discharge from power plants and also waste from boats and ships in harbour and port (Abdul Salam, 1999). The point-source discharges are easily to identify and easy to monitor compared to the non-point source (Wright, 2005).

The non-point sources are typically generated from human's activities that cannot be traced and difficult to monitor and control. The bases to the non-point source polluted are sediment, nutrient, fecal, bacteria, pesticides and herbicides. According to Abdul Salam (1999), all of these causes are come from a number of sources that include runoff from urban area, agricultural land and waste disposal site (landfills). However, the wastewaters contribute more to the water pollution nowadays.

Wastewater is defined as liquid waste from the residential, institutions, public areas, commercials and industries. The liquid waste originated from the toilets, bathrooms, tabs, kitchens and others. According to Abdul Aziz (1999a), about 70 to 80 % of the water supply has become wastewater. Overall, 99.9% of wastewater is water and 0.1% is solid. The solid component consists of organic and inorganic matter such as soaps and soil solids, urines, food residues, feces, papers and miscellaneous substances (Metcalf and Eddy, 1991) as well as rich in microorganisms. Therefore, by good management, wastewater can be used as fertilizer as well as to feed the microorganisms.

In urban areas, wastewater is usually classified as municipal or industrial wastewater. This municipal sewage is an unbalanced medium and is overly rich in phosphorus that is the essential nutrient for the algae. The nutrient content of the industry's waste is much more variable than that is from the domestic waste. Some industries discharge waste of low nutrient's content, which may require the addition of nutrient to facilitate biological treatment (Rohlich and Littormark, 1972).

Wastewater can be characterized according to its chemical constituents (nitrogen, phosphorus, sulfur, chloride) and pH. The pH of wastewater is usually more than 7.0. However, it becomes more acidic if it experiences an anaerobic process. This is due to

the process that produces methane (CH_4), carbon dioxide (CO_2) and hydrogen sulfide (H_2S) which give the acidic environment to the sewage. The anaerobic process occurred when organic matter is degraded by bacteria in oxygen lacking environment to release acidic gasses.

Organic matter (In sewage)	+ bacteria (Without oxygen)	⟶	$CO_2 + CH_4 + H_2S$ + new cells

In wastewater, the main nutrient source is nitrogen. Usually in wastewater nitrogen will undergoes nitrification process converting ammonia to nitrate and nitrite. Besides, nitrogen, phosphate is another main nutrient that can be finding in the wastewater. Phosphate is the main nutrient for the oxidation process (Jackson *et. al.*, 1990). It is occurred at level 5-15 mg/L.

The sulfate comes from many sources, but mainly from detergent. It causes problems to the culvert system as it releases hydrogen sulfide (H_2S) in anaerobic condition. As the H_2S combine with the water, it may form sulphuric acid, and it may cause erosion to the culvert (Abdul Aziz, 1999b). Chloride usually occurs at the level 50-100 mg/L in the sewage (Nicoll, 1988). It originated from urea. Wastewater can also be analyzed by occurrence of organic matter and metals. The physical characteristic of the wastewater are influenced by the environmental surrounding. The physical characteristics of the wastewater are shown in the Table 2.2 below.

According to Wright (2005), there are two basic strategies to bring water pollution under control. The first strategy is to reduce or remove the source. The next strategy is to treat the water by removing pollutants or converting them to harmless forms. The best solution to solve the point-source water pollution is by water treatment.

Source reduction although can be applied for both point and non-point source pollution more suitable for non-point source pollution.

Table 2.2: The Physical Characteristics of Wastewater

Characteristic	Description
Temperature	Little bit higher than the natural water. The dissolve oxygen is depending on the temperature of water.
Colours	The fresh wastewater is brownish and it is become darker and darker as it takes a long time.
Turbidity	Shows the total suspended solid is higher in the water bodies.
Odour	Hydrogen sulfide (H_2S) gasses are release from the process that occurs in the wastewater. It gives a bad smell to the environment.
Solids	Consists of three main suspense; total suspended (TS), Volatile suspended solid (VSS) and suspended solid (SS).

(Abdul Aziz, 1999b)

2.2 Eutrophication

Certain water quality standard has to be met for safe domestic uses, aquatic flora and fauna, recreational and other commercial functions (Ismail, 2003). Overloading of phosphate and nitrate, either into the artificial ecosystem or into the natural water, bodies will influence the formation of eutrophication. Recently, the increasing amount of nutrient reaching into the lakes, have brought big changes to the ecosystem, mainly in the development countries. Attention has been the focus on how to solve the problem of the nutrient enrichment in the lakes. Many works have been done to bring the lake to the normal environment, where the nutrients loading into the lakes are in a balance condition.

There are two categories of lakes; oligotrophic and eutrophic lakes. Low nutrient levels, low productivity and high species diversity characterize oligotrophic lakes (Graham and Wilcox, 2000). This category of lakes is good for a balance ecosystem,

especially for the aquatic life. While in the eutrophic lakes, its shows the characters are different with the oligotrophic lakes. The differences between oligotrophic and eutrophic lake are shown in the Table 3.

Table 2.3: The General Characteristic of Oligotrophic and Eutrophic Lake,

Characteristics	Oligotrophic	Eutrophic
Depth	Deeper	Shallower
Summer oxygen in hypolimnion	Present	Absent
Algae	Highly in species diversity, low density and productivity, often dominated by Chlorophyceae.	Low in species diversity, high density and productivity, often dominated by Cyanophyceae.
Blooms	Rare	Frequent
Plant nutrient flux	Low	High
Animal production	Low	High
Fish	Salmonids (e.g.; trout, char) and coregonids (e.g; white fish) often dominant	Coarse fish (e.g.; perch, roach, and carp) often dominant

(Manson, 1981).

Eutrophic lakes form either it can happen either naturally or artificially eutrophication. Natural eutrophication arises from nutrient increase by a non-human process that causes natural eutrophication are such as, a forest burning or other disaster (Ismail and Mohamad, 1992). Climatic shifts such as drought may also concentrate lake water nutrient or give rise to an increase contribution of nutrient-rich to the groundwater (Webster *et.al*, 1996).

Fogg *et. al*. (1973) has mentioned that lake eutrophication occurs when nutrient supplies, usually phosphorus and nitrogen, are elevated over rates that occur in the absence of any system perturbation and results in increase lake productivity. It is also considered that all lakes are originally oligotrophic until it is come to a point that it will turn to eutrophic by an evolution process. In a simple word, the lake experiences an 'aging process'. According to Edmondson (1974), a lake may become more productive

with time. This is due to the effect shallowing of the lake which affects the way it converts nutrients into the organism rather than an increase input of nutrient.

Artificial eutrophication is also known as cultural eutrophication. The cultural eutrophication occurs due to the loading of nutrients from humans' activities. Eutrophication may also happen by rapid development and density of human population, in a large urban settlement. Loading of additional nutrients such as food and modern detergent contribute to the changes in the catchments-lake (Reynolds, 1984). When outcomes of eutrophication are undesirable to human, it is considered as a form of pollution.

Eutrophication defined as nutrient or organic matter enrichment, or both that result in high biological productivity and a decrease volume within an ecosystem (Likens, 1972). It is occurred when the lakes are high in phosphorous concentration and exhibit increase plants and algal blooms as well as reduce biodiversity. The most important feature of a eutrophic lake is the concentration of oxygen in the hypolimnion become significantly reduces (Reynold, 1984) as well as the depth of the lake became shallower.

The lake ecosystem nutrient may exchange between three general compartments. Those are available nutrients, organic matter as well as primary and secondary minerals. According to Likens and Bormann, (1971), available nutrients are those dissolve in water or on exchange surface of pelagic particulate matter or bottom sediment. While the organic matter, are those nutrients that incorporated in living or dead organic matter. Both are in the pelagic region or in the sediment. The primary and secondary minerals

are those nutrients integrated in rock, which exists in the sediment or suspended in the water.

Eutrophication may also cause changes to biochemical cycles and biological communities. For instant, changes in the ratio of nitrogen and phosphate (N: P), often results in primary production shifting from primarily diatom and other smaller edible algae toward larger cyanobacteria that are better competitor for nitrogen (Tilman *et.al*., 1986).

There are several factors that contribute to eutrophication. They are mostly consequences of human activities. Opening of land for the development for residential or industries cause water run-off that brings soils into rivers or lakes. This causes lakes or rivers to become shallow. Industrial waste, such as from the electronic, chemicals, foods and textile and paper industries, where the wastes contain high in hazardous components also contribute to eutrophication. Eutrophication can also indirectly brought by air. For example, the oxidation nitrogen comes from the toxic gases of the vehicles, which bring along by the rain (Ismail and Mohamad, 1992)**.**

Dung from the animal husbandry such as chickens, ducks, goats or cows, contain highly in phosphate and nitrogen has been dispose thrown into the water body from farms. In Malaysia, the animal husbandry management system is not well organized. The owners simply throw the dung into the stream or lakes nearby. As the result, many lakes and streams experience eutrophication. According to Reynolds (1984), other factors that influence the changing of the lake are due to the uses of the modern inorganic fertilizers, forest clearance and the implementation of agricultural alter terrestrial nutrient.

Ismail and Mohamad (1992) said lakes with the high phosphate and nitrate content would trigger the development of the algae, mainly the blue-green algae. The rapid development of the algae may cause many problems. Some of the blue-green algae that can be finding are *Microcystis*, *Spirulina*, and *Nodularia*. These algae give an odour and smell to water. Besides, they also produce a toxic and release chemicals matter. Other effect causes by the eutrophication, such as the increasing of the turbidity; rate of the sedimentation will increase and shortening the life span of the lake; anoxic condition may develop as well as the diversity of the aquatic organisms decrease and the dominant of biota will change (Manson, 1981).

Eutrophication is a costly economic problem. When the massive algal bloom, it may increase water treatment cost, and sometimes it may cause treatment facilities to malfunction (Vaughn, 1961; Hayes and Greene, 1984). The polluted water may cause a disease such as the skin problem to human when they use or drink the water. The polluted lake is not suitable for recreational activity. The existence abundant with the algae may reduce the oxygen supply. A lack of oxygen, gives a negative impact to the aquatic life, particularly to the fish. Besides, due to this problem, many of the commercially important species may disappear and the amenity value of the water may decrease (Mason, 1981).

2.3 Phytoplankton

Sze (1998) pointed that lifestyles divide algae living submerged in either freshwater or marine environment into planktonic (floating) or benthic (bottom living). Benthic algae are associated with submerged substrate and water moves past them (Sze, 1998). Planktonic algae are also known as phytoplankton.

The first use of the term plankton is attributed to the German biologist, Viktor Hensen (Ruttner, 1953; Hutchinson, 1967). According to Hensen's (1887), plankton included all organic particles 'which float freely and involuntarily in open water, independent of shores and bottom' (Reynolds, 1984). However, the term of plankton by Hensen cannot be used nowadays due to many investigations shows that plankton do not float and most of them are dense than the water they inhabit.

According to Wright (2005), phytoplankton consists of numerous species of photosynthetic algae, protista and chlorophyll-*a* containing bacterium known as blue-green algae. They grow as microscopic single cells or small groups of cells. Phytoplankton lives suspended in water and are found wherever light and nutrient are available. They have to remain to the water surface to receive sufficient light for photosynthesis. They are mostly finding in few meters top of water bodies, and they tend to rise toward the surface in the early morning, depending on the light condition according to some species (Bowe, 2002).

Phytoplanktons have a variety of adaptations for floating. Some of them move with water flow, but some of them have flagella. The flagella allow them to swim for short distance but lack sufficient power to swim against the water (Sze, 1998). Although they do not exhibit the phenomenal size range of their marine relative, freshwater algae, nonetheless, display a wide diversity of form and function (Graham and Wilcox, 2000).

Phytoplanktons are classified into two groups; autotrophic and heterotrophic. Generally, the phytoplanktons are considered as autotrophic. The autotrophic algae requiring inorganic nutrient such as carbon dioxide, water, phosphate, inorganic nitrogen with light as the energy source (Sze, 1998). While the heterotrophic algae use

organic compound for their growth. Heterotrophic algae can be either phagocytotic or osmotrophic (Lee, 1999).

Table 2.4: Type of nutrition found in the algae.

Type of nutrient	**Principle source of energy for growth**	**Principle source of carbon for growth**
1) Autotrophic		
- photoautotrophic	-light	-carbon dioxide
- chemoautotrophic	-oxidation of organic compound	-carbon dioxide
2) Heterotrophic		
- photoheterotrophic	-light	-organic compound
- chemoheterotrophic	-oxidation of organic compound	-organic compound

(Lee, 1999).

Freshwater phytoplankton consists of Bacillariophyta, Chrysophyta, Chlorophyta, Cyanobacteria and Euglenophyta. The growths of this phytoplankton are depending primarily on five factors. The factors are; (i) rate of reproduction (ii) rate of removal of individual by death or grazing zooplankton (iii) photosynthesis; in relation to light intensity (iv) availability of nutrient and (v) temperature (Chapman and Chapman, 1973).

Cyanobacteria or blue-green algae were the first phytoplankton to evolve. They were the dominant forms of life on earth for more than 1.5 billion years (Graham and Wilcox, 2000). They are grouped under prokaryote. In the earlier studied, cyanobacteria were through as bacteria. However, an internal membrane called thylakoid, distinguished them from bacteria (Bagulia, 2008a). They are call blue-green algae because of the presence of principal bluish-green pigment (phycocyanin) along with chlorophyll-a, β-carotene and some quantity of myxoxanthin, myxoxanthophyll as well as small quantity of flavacin and phycoerythrin (Chapman, 1962). Besides, they also have glycogen as a storage product and their cell walls consisting of amino sugar and

amino acids (Lee, 1999). Their photosynthetic pigments are located in the thylakoid, which lie free in the cytoplasm. In thylakoids, it contains chlorophyll- *a* but absent from chlorophyll- *b* and chlorophyll- *c*. Their product from photosynthesis is starch.

Cyanobacteria is a photosynthetic producer in a wide range of freshwater and marine environment as well as common in terrestrial habitats and in the symbiotic system (Sze, 1998). They are frequently found in the phytoplankton of still or slowly flow through freshwater (Van de Hoek *et. al.,* 1995). They occur ubiquitously and have a wide range in their vegetative structure. They are unicellular, colonial members or as filamentous forms. The filamentous forms can be either branched or un-branched. While some of the colonies consist of the simple hollow sphere in which the cell arranged only in a single peripheral layer (Sharma, 1986). Sexual reproduction is not known for Cyanobacteria. They reproduce through simple division by non-motile endospores or spores as well as by vegetative fragmentation (Chapman and Chapman, 1973).

Part of the success of these blue-green algae is their ability to use low light intensity effectively. Thus, they can thrive below the surface, deep in the epilimnion. According to Prescott (1969), many of the Cyanobacteria are finding attached on stone that present in shallow water or down to depth of even up to 30.4 meter. Cyanobacteria has a special ability compare to another group of phytoplankton. They are able to fix nitrogen gaseous. Bold and Wynne (1978) had mentioned that there are three categories of Cyanobacteria that have the ability to fix the atmosphere nitrogen; (i) filamentous forms having heterocyst (ii) some unicellular non-heterocyst and (iii) some non-heterocyst with filamentous forms.

Oscillatoria, sp. is a filamentous blue-green alga, reproduce through the formation of hormogonia; the trichome is cylindrical and free with the mucilage sheath around them. The O*scillatoria* sp. belongs to order Oscillatoriales in the class of Cyanophyceae. They do not form a colony. The individual cells are disc-shaped and always contain ingrown cross-walls at different stages of development (Van de Hoek *et. al.,* 1995). This species is widely distributed in the sea freshwater, hot spring and areas affected by sewage effluent.

The Chlorophyta division consists of 500 genera and approximately 8000 species. Most of them live in freshwater, some are discovered in marine as well as in the terrestrial. Some of them are aerophytic, which are living on the tree trunks, soils and rock and even on snow and ice. Chlorophyta or are known as green algae, have chlorophyll- *a* and *b* as their major photosynthetic pigments. Their pigments are similar to the higher plants. They have a starch as the carbohydrate reserve and chloroplast envelope with two-associated membrane (Sze, 1998). Their morphologies are varied; many are unicellular and colonial; some are multicellular as well as filamentous. Their cells wall usually has cellulose as the main structural polysaccharides (Lee, 1999). Chlorophyta species can be produced either by sexual or asexual. From sexual production, it begins with isogamy to anisogamy and oogamy. While asexual production occurs from motile zoospores. Nevertheless, sometime it also forms from non-motile spores (Chapman and Chapman, 1973).

The division Chlorophyta, consist many classes as these green algae have a wide range of species. In Chlorophyta, there are macroalgae and microalgae. However, most of them are microalgae. Smith (1955) noted that only 10 % of them are living in the marine area, whereas 90 % are living in freshwater and lives as phytoplankton. Two

examples from this division are *Scenedesmus* sp. and *Pediastrum* sp. *Scenedesmus* sp. and *Pediastrum* sp. belong to the order Chlorococcales, in the class Chlorophyceae. Most of the species in this class occur in freshwater as phytoplankton, some in terrestrial forms and few of them found in brackish or marine habitat. *Pediastrum* sp. is a common species, which can be found in the freshwater rich in nutrient. They are mostly plankton, but some live on and between aquatic macrophytes (Van de Hoek *et. al.,* 1995). *Pediastrum* sp. usually forms colonies. Their structure is circular, flat and radially organized, usually one cell is thick. The cells around the colony margin generally bear horn-like projection; while those in the center do not have.

Species of *Scenedesmus* has a unique structure form. The individual cell forms are such elliptical to the spindle shaped and in many species, they bear spines (Van de Hoek *et. al.,* 1995). These individual cells attached together in a row about 4, 8 or 16 individual cells to form a colony. Sometimes, they lie in two rows of cells. The terminal cells of the species have spines, for example, *Scenedesmus quadricauda* (Richmond, 1986). Nevertheless, some of them have tufts of fine bristle, which have buoyancy. The cells are uninucleate and have a laminate chloroplast that contains pyrenoids (Richmond, 1986). Species of *Scenedesmus* is normally found in freshwater or brackish water in nutrient-rich condition. Their environment usually clears from pollution. In the laboratory work, they are often used to be cultured as they are easily grown in the culture condition.

2.3.1 Growth factors for phytoplankton

Several important environmental factors influence the growth of the phytoplankton. The factors are; (1) light (2) density stratification (3) nutrients (4)

floatation and sinking. However, the main factors that stimulate phytoplankton growth are light and nutrients.

Light are important for all groups of phytoplankton, as they use energy of light for their photosynthesis process. Photosynthesis in most algae occurs in the presence of light with wavelength at about 300 to 700 nm (Lee, 1999). A requirement for light by algae can be demonstrated by showing photosynthesis at different levels of light. Thus, the requirement for the light and the amount for absorbance are different to every species. For examples; the giant Kelp (brown algae), require brightness at least 70 E $m^{-2}year^{-1}$, in order to build up their thallus. However, more lights are needed as they were growing deepest in the ocean. While for phytoplankton, they occur on the surface of water to few meter depths, receive 1% of the surface irradiance (Lee, 1999).

The phytoplankton depending on species, have a compensation depth. It is said that, the amount of light allows only enough production to meet the requirement for cellular maintenance (respiration) without production of new biomass (Sze, 1998). According to Moss (1980), he defined the euphotic depth as the depth in the water column at which the energy absorbed in photosynthesis (gross photosynthesis), in an algal cell just balance the maintenance energy need of the cell for respiration. Thus, net for the photosynthesis is zero at the euphotic depth.

Light rapidly reduces as it penetrates the water column. The availability of light limits phytoplankton growth to a layer of water called the photic zone. Photic zone is the upper layer, where there is adequate light penetration. According to Sze, (1998), irradiance decreases exponentially with water depth because water as well as suspended particles absorb and scatter light. In water, light is attenuated by absorption and

scattering (Grossman *et. al.,* 1990). Trainor (1978) noted that, the chlorophyll pigment, absorb both red and blue light in the upper water, but as the red light is lost in the upper 10 meter, they can still function by absorption of the blue light down to 50 meters.

Near the surface, light is usually more than sufficient for photosynthesis, but nutrient availability may be limited. In some circumstance, the water of the lake is turbid and lack of sufficient light, thus it limits the phytoplankton growth (Trainor, 1978). It can be said that light can also be the limiting factor for the phytoplankton. Along with adequate light, the availability of nutrient as one of the most important factor regulating phytoplankton growth. If one of the essential elements is absence or the concentration is low, it will limit the phytoplankton growth.

Phytoplankton nutrients consist of basic elements; macroelements and microelements. Macroelements that needs by phytoplankton are carbon, hydrogen, sulfur, potassium, calcium, magnesium, phosphorous and nitrogen. Usually, the macroelements are taken in large quantities by phytoplankton. While, microelements were phytoplankton required usually taken in lower quantities and it is often acted as co-factors in the enzyme system (Sze, 1998). Examples of microelements are iron, manganese, copper, zinc and molybdenum. However, for diatoms they need another element, which is silicon.

Among the element that is commonly present in phytoplankton are carbon, nitrogen and phosphorous, which are regarded as critical one. This is due to the exogenous sources of the elements are among the most significant supply, that it can be limited phytoplankton metabolite rate and their growth. Reynolds (1984) also noted that, these three elements play an essential role in the enzymatic and energy transport.

Carbon is the most common nutrient utilized by the algae. They usually use the carbon that comes from either carbon dioxide (CO_2) or bicarbonate for photosynthesis. The total inorganic carbon (TIC) in-water body consists of carbon dioxide (CO_2), carbonic acid (H_2CO_3), bicarbonate ions (HCO^{-3}) and carbonate ions (CO_3^{2-}). The TIC derives from the atmosphere and from dissolution of carbonate rocks (Sze, 1998). However, in some condition, phytoplankton will release large quantities of organic carbon from their cells from photorespiration.

Nitrate is a common substance composed of nitrogen and oxygen. According to Faizah (2004), nitrate can stimulate the growth of microalgae in lake. The principal requirement for nitrogen to phytoplankton is in the synthesis of amino acid and protein. Several commonly occurring sources of nitrogen are potentially available to phytoplankton. There are different forms of nitrogen occurs in water include diatomic nitrogen (N_2), ammonium (NH^{+4}), nitrate (NO^{3-}), nitrite (NO^{2-}), other nitrogen oxides and nitrogen in organic compounds such as urea and free amino acids (Reynolds, 1984). However, occurrence of cyanobacteria as nitrogen-fixing algae are very important in the tropical and subtropical ocean with lower nutrient levels (Sze, 1998). When other forms of nitrogen are low, these cyanobacteria are often significant in converting nitrogen gas to forms useable by non-nitrogen fixing phytoplankton. Nitrogen fixing is inhibiting in the presence of nitrate or ammonium.

Nitrogen is an essential element in the synthesis of amino acids and protein of the phytoplankton. Its nitrogen content is about one-eighth to one-sixth by weight (Tang, 2001). It exists in aquatic environment as nitrate (NO_3), nitrite (NO_2) and ammonium ion (NH_4^-) as well as certain dissolved nitrogenous compounds such as urea and free amino acid peptides (Tang, 2001). Nitrogen often limits the phytoplankton in

the open water (Howarth, 1988), but is less commonly limiting in freshwater. In one tropical water body, concentration below 0.02 mg nitrate-N/liter might be limited the growth of plankton diatom (Prowse and Talling, 1958). However, elsewhere phytoplankton has been reported to utilize inorganic nitrogen at concentration below 0.1 mg nitrate-N/liter.

Phosphorus is essential to the function and growth of all plants including phytoplankton. This because it is a component of nucleic, acids and of adenosine triphosphate, which forms the basis of the enzyme synthesis as well as the intracellular energy transfer system (Tang, 2001). Reynolds (1984) mentioned that in water, phosphorous usually occurs in oxidized state either as inorganic orthophosphate ions (HPO2-4, $H_2PO_4^-$) or in organic forms, largely biogenic compound (Tang, 2001). Phytoplankton is able to produce its own phosphate in a special condition. When inorganic phosphorous is low but the organic forms of phosphorous are available, phytoplankton are able to excrete phosphate to break down a polyphosphate compound. However, when the amount of phosphate is overloaded, cells might take it up and store it as polyphosphate for later uses (Sze, 1998).

It is widely believed that in many freshwater bodies, growth of phytoplankton tends to be limit by the supply of inorganic phosphate. Concentration of phosphate-P, are often below of 10µg/L generally in range 0.1-1,000 μgPL^{-1} (Tang, 2001) and in some lakes, they might be reduced to 1 µg/liter or less by uptake during the growth of algae (Hutchinson, 1967; Gardiner, 1941).

Another element that important is silicon. It normally derived from rock weathering and dissolve in water as orthosilicic acid [Si $(OH)_4$] (Sze, 1998). Silicon is

present in most natural water as solid or colloidal silicate polymers, originate from catchment's soils or from biogenic source, for example, death diatom. Although all phytoplankton had a requirement for the small amount of silicon involve in protein and carbohydrate synthesis, the huge utilize for silicon is the Chrysophyta and among the diatoms. The silica is uses to strengthen their cell wall with unstructured silica polymer that the requirement becomes ecologically important (Reynolds, 1984). Hutchinson (1967) noted that phosphate, nitrate and silica are the most critical nutrients for the autotrophic production.

2.4 Microalgae and environment

The algae have two important roles that give balance to the main gasses in the atmosphere. They take part in the carbon cycle and as the equable level for dissolve oxygen in the water body. However, certain phenomena can disturb this cycle. Besides, they are producers of oxygen and organic materials, and they are responsible for 30 to 50% of the photosynthetic production on earth. Algae are important in producing nitrogen from nitrogen-fixing cyanobacteria. Cyanobacteria can convert nitrogen gas into forms of nitrogen than other organisms can use (Sze, 1998). Water quality also influences the development of the phytoplankton. This relationship gives two impacts; positive and negative. The positive impact can be seen by the occurrence of other aquatic organisms in the water bodies due to the abundant with phytoplankton. The phytoplankton is the main supplier for the food chain in the aquatic ecosystem as well as the producer of the oxygen.

However, the negative impact is the overloading nutrient into the water bodies happen may cause algal bloom. Besides it also polluted the aquatic system as well as they give an odour to the environment. In some cases, an abundant of it may reduce dissolve oxygen level, particularly during the night when there is no photosynthesis. This is because the phytoplankton utilizes oxygen for their respiration more than other aquatic organism. Consequently, these organisms are displaced (Ismail, 2003).

Oswald (1988a) said, that the presence of algae indicates tropic condition of a water-body. For examples, green algae and diatoms are frequent in relatively pure oligotrophic waters, while blooms of Cyanobacteria indicate a eutrophic state and water pollution. Effluents from urban, agricultural or industrial origin contain higher concentration of nitrogen and phosphate. These effluents have to be treated in primary and secondary treatment processes before they are discharged into natural water bodies. If the effluents loaded with too much of nitrogen and phosphate, which may cause eutrophication, heavy metal as well as toxic organic compounds, tertiary and quaternary treatment have been done. The cost of treating effluent increases as the stages number of treatment increase. Thus, an alternative way has to be introduced to the treatment system. One of the best ways is to treat wastewater with environment friendly microalgae.

Oswald and Gotaas (1957) were the first to propose large-scale production of algae biomass from waste. The microalgae biomass itself is also a potentially valuable (Lincoln and Earle, 1990) commodity because of its high-protein content. Removal of dissolved nitrogen and phosphate is the main aim of the wastewater treatment. Discharge of these nutrients in the water bodies may cause eutrophication and indirectly stimulate the algae bloom as well as unwanted plants like the aquatic macrophytes.

One of the principles reasons for removing the nutrients from wastewater effluent is to control eutrophication that is shows by the uncontrolled algae (Hammouda *et. al*., 1995). Microalgal cultures offer an elegant solution to tertiary and quaternary treatments due to the ability of microalgae to use inorganic nitrogen and phosphorus for their growth (Oswald, 1988a; 1988b; Richmond, 1986), and their capacity to remove heavy metals (Rai *et. al*., 1981), as well as some toxic organic compounds (Redalje *et. al*., 1989).

Under natural condition, most microalgae grow as mixed communities, which include various species and genera (Becker, 1994). In the treatment process, bacteria will break down remaining organic compounds and provide carbon dioxide for the algal growth. Algal will utilize phosphate, nitrogen and other elements as well as carbon dioxide. Then they will release the oxygen as their by-product. The advantageous from the treatment are removal of in organic elements and some organic compounds as well as water recovery for any uses, algal production and gas exchange (Trainor, 1978). Besides the water can be supersaturated with oxygen so that the animals can survive, and the algal biomass can be harvested for other uses such as animals feed.

A better technique of growing algae in laboratory has been giving a huge impact in studying algae. These improvements in the algal cultivation have made great advances in our knowledge of algal life histories, physiology, taxonomy, genetics, biochemistry and ultra-structure (Bold, 1974). Besides by culturing the algae, it has opened a new prospect in biotechnology mainly to the aquaculture industry.

Algae have tremendous ability to grow in water with variable load nutrients and to adapt quickly to the fluctuating nutrient and climate regime. They play a vital role in the rearing of mollusks; shrimps and fish as well as they have created a strategic interest for aquaculture industries. A majority of microalgae species that are cultured in aquaculture's ponds belongs to green algae, desmids and diatoms (Mohapatra, 2006). Cyanobacteria are less used in aquaculture due to the production of toxins. However, *Spirulina* sp., are cultured for its high biomass production and cellular protein contents (Mohapatra, 2006).

2.5 Application of microalgae in wastewater using various way of treatment.

Many studies have been conducted on wastewater treatment using microalgae. Oswald and Gotaas (1957) had reported that wastewater treatment using microalgae have already be attempt over 40 years ago. Studies using algal culture by many researchers previously, have demonstrated a success in removing nutrient compound in wastewater that are rich in nitrogen and phosphorous. The algae were grown in inorganic media. However, this approach is too expensive. Thus, to more economical, algae were grown in the wastewater. Waste contains all the macronutriens and micronutriens that required for the algal growth (Shelef *et.al*., 1978). In the earlier of the experimental work focus had been more to the suspended microalgae that grow in shallow, artificial pond containing sewage. However, the study of wastewater has been extended to non-suspended microalgae. This is due to the difficulties of harvesting the enormous suspended microalgae population developed in water after the treatment (de Bashan and Bashan, 2004).

Instead of cultivating microalgae in pond, another approach that had been attempt was to treat wastewater using immobilized microalgae. Chevalier and de la Noüe (1985) have used *Scenedesmus* sp. as the immobilized microalgae in *k*-carragenan beads to remove nutrients from wastewater. By employing immobilized microalgae, treatment of liquid is simplified, as the cells were trapped in the bead. Immobilized microalgae it may increase the retention time of cells in the reactor. Moreover, the cells may become well adapted to the substrate and therefore less risk of washout from the substrate (Travieso *et.al*., 1996). According to de-Bashan and Bashan (2004), algal cells that are trapped in the carrageenan and alginate bead grew much slower than suspended cells. However, these immobilized algae are able to remove over 95% of ammonium and 99% of phosphates from the wastewater in three days. Compared to suspended cells, the efficiency of removing the nutrients are only 50% of nitrogen and phosphate (Lau *et.al*., 1997).

The most used treatment in the whole world today is the High-Rate Algal Ponds (HRAP). These shallow oxidation ponds that have been used for many years have encouraged the growth of suspended microalgae (Hoffmann, 1998). It was the better treatment which can achieve a high level of treatment of both domestic and agricultural wastewater. These can be measured by reduction in BOD, TSS, nitrogen, phosphorous and metals (Shelef *et.al*., 1980; Fallowfield and Garrett, 1985; Picot *et.al*., 1991; Muttamara *et.al*., 1995).

Microalgae which are grown in the large pond to treat wastewater can be harvested. The harvest microalgae can be processed to become fertilizer or as animals feed. Thus, microalgae that have special characteristics should be grown in the pond.

Some of the characteristics are such as; they are able to grow in sewage, relatively high specific growth rate, ease to harvest and process (Azov *et al*., 1980).

Microalgae cultivation has been an alternative way for removing nitrogen and phosphorous from wastewater treatment. The microalgae may convert those nutrients to biomass. Through biotechnology, the biomass have become a useful product such as feed, food additive, source of value-added products or as supplements to biomass in an energy producing system (Mohapatra, 2006). Those products should be commercialized as an alternative to other products that are presently used.

The ability of microalgae in utilizing effluent for their growth, give a great potential on economic scale. The algal biomass could be used for animal feed (Shushu and Chipeta, 2002) as well as plant's fertilizer. A combination of algal protein production with wastewater, as an alternative for new protein sources has also been suggested (Tam and Wong, 1989). Jassby (1988), according to algae that grow on agro, industrial waste can be used for human nutrition. Thus, there are a lot of advantages from the cultivation of microalgae that are grown either in wastewater or culture media.

Chapter 3

Methodology

3.1 Tasik Taman Jaya and collection of water samples

Taman Tasik Jaya is situated at Petaling Jaya, Selangor. It is located between longitudes 03° 06’ north to 03° 07’ north and between latitude 101° 38’ east to 101° 40’ east. The surface area of the lake is 28,640 m^2 with capacity of 33,957 m^3. The lake is an ex-mining lake (Norhayati, 1995). This lake, which is situated near to the residential area, has been use as a recreational site. Besides, it is also surrounded by the main road and highway (Plate 3.1).

There are two major inlets and one outlet of the water into the lake**.** The effluents come from the residents surrounding the lake. These two inlets received water from the domestic waste, semi-treated waste from septic tank and water runoff from nearby housing area, main road and highway (Saravanamuthu, 1977). Thus, the composition of nutrient content in the water bodies is higher. The samples were taken at three different sites of the lakes to represent the whole area of the lake.

Water samples from Tasik Taman Jaya were collected from two stations (Stations A and B) between 9.30am to 10.30am in the year 2007. Water samples were collected twice and each time from the two stations. Water samples from the first collection were used to examine and identify microalgae that were present in the lake. Water samples from the second collection were used to carry out wastewater treatment experiments with microalgae. Station A was located at the first inlet of the lake (Plate 3.2) whereas station B was located between the second inlet (Plate 3.3) and outlet of the lake (Plate 3.4). Water samples from the first collection were used to identify microalgae from the two stations. Water samples were collected from or near the lake water surface using plankton net of with meshed size small enough to retain microalgae in the samples (Plate 3.5). Microalgae generally occurred near the water surface as they require sunlight for photosynthesis. The water samples were then keep in vial and were taken back to laboratory to examination.

Plate 3.1: Sampling location at Tasik Taman Jaya

Plate 3.2: Station A showing the first inlet of the lake

Plate 3.3: Station B showing the second inlet of the lake

Plate 3.4: Outlet of the lake.

Plate 3.5: The apparatus for sampling (plankton net, bottles and vials)

3.2 Microalgae identification.

One drop of water sample was placed on the slide and was observed using the microscope. Microalgae were identified by using the inverted microscope, Olympus BX51 (Plate 3.6). The observation of the microalgae was starting with lower magnification to the higher (x10 to x100). The identification of the microalgae found from the lake was done by referring to Salleh (1996).

Plate 3.6: The computer and microscope used for identification of microalgae

3.3 Water Quality

Water quality analysis and subsequent experiments was carried out using water collected from stations A and B during the second visit to the study site. Water samples were collected at or near water surface within arm-reach using plastic bottles. Water samples were kept in the plastic bottles and immediately brought back to the laboratory for analysis and subsequent experiments.

Water quality parameters that were analyzed were nitrate, phosphate and silica. Their initial concentrations and subsequent concentration during subsequent experiments were recorded and compared. These are explained further below.

a) Nitrate

Nitrate in the wastewater was measured using the Hach Spectrometer (Plate 3.7), with a selected program 353 N, Nitrate MR. The sample was added with Nitra Ver 5 Nitrate Reagent Powder Pillow. An amber colour will develop to shows the present of nitrate in the sample. The result will appear in mg/L NO_3^- . -N. The reading was done in triplicate to get averages.

b) Phosphate

Phosphate in wastewater was examined using the Hach Spectrometer, with 490 P React. PV. PhosVer 3 phosphate Powder Pillow was added into the sample vial. The reading was done in triplicate to get averages, and it is read in mg/L PO_4^{-3}.

c) Silica

Silica was examined using the Hach Spectrometer, 651 Silica LR. Two vials of sample were prepared. Fourteen drops of Molybdate 3 reagent were added into each of the sample cells and swirled to mix it. Then, Citric Acid Reagent Powder Pillow was added to each sample cell, and swirl to mix it. During this process, the destruction of possible phosphate interference occurred. One of the sample cells was added with Amino Acid F. Two-minute reactions began, and the present of silica was shown by the development of blue colour. The reading was in mg/L SiO_2. The reading was done in triplicate to get the average.

Plate 3.7: HACH Spectrometer for measuring water quality.

3.3.1 Water quality data analysis

Correlation between algae growth and nitrate, phosphate, and silica was analyzed using Microsoft Excel was used. An example of the calculation is shown in Appendix 4 to Appendix 9.

3.4 Culture Preparation

3.4.1 Media

Media were prepared from the premixed stock solutions. In this study *Chu no. 10* defined medium were used for the culture (Stein, 1973). Components of the medium were then added onto one liter of distilled water. The totals of the solution were combined and were adjusted to pH 6.5 – 7.0. The media were autoclave at 121 ˚C, 20 Ib/in^2 for 20 to 35 minutes. The liquid culture stock solutions were kept in the freezer before it was used. Following is the amount of the nutrient used for the stock culture solutions;

$Ca(NO_3)_2$	0.04 g/l
K_2HOP_4	0.01 g/l
$MgSO_4.7H_2O$	0.025g/l
$NaCO_3$	0.02 g/l
$NaSiO_3$	0.025g/l
$FeCl_3$	0.8 mg/l

3.4.2. Isolation

The isolation of a single algal unit into medium suitable for growth is required to establish a clonal, unialgal culture (Stein, 1973). The algae are isolated from the fresh collection from the field. The isolation process was done by using fine capillary pipette by taking aseptically a sterile Pasture-type pipette with a rubber bulb on the wide end. A forceps was used to hold the narrow end of pipette while it was heated up. As the pipette became softer, the narrow end of the pipette was pulled.

As the glass softened, the pippets were gently removed from the flame. The tip of the capillary pippet was then broken off. The bore of the pippet should be several times diameter size (75 to 150 µm) than the algal unit being isolated (Stein, 1973). The isolated algal were placed into a Petri disk and were looking through under the inverted microscope. The pippet was taken out of the liquid and positioned just above the algal unit. The pippet was slowly dipped into the liquid and by capillary action; the liquid with the desired cells should flow into the pippet. The flow of liquid can also be controlled by slight pressure of the rubber bulb.

The cells were placed into another disk, which contained distilled water. Algal cells were washed several times with the distilled water to remove dirt. The clean algal cells were placed in fresh water. After the cells showed rapid development they were transferred into a prepared medium (Chu 10).

3.4.3. Culture method

Liquid medium, that contained the algae were placed under florescence illumination so that the algae could grow without being damaged by direct sunlight. The room temperature was maintained at about 20 to 21 °C. 12 hours of light and 12 hours of darkness were done for the algae culture. Increases in algal cell number were observed after five days to two weeks depending to species. During the lag phase, the cells are adapted to the experimental environment. When the algae have entered the exponential phase, they were transfer into a larger flask to maintain their growth.

3.5 Nutrient removal

Fresh water sample collected from the Tasik Taman Jaya were filtered and placed into a 500 ml beaker. The water samples were filtered to remove the unwanted microalgae as well as suspended matter. Four beakers were set up and filled with three different microalgae species. One of the beakers was set up as a control and contains with filtered water sample.

The three different microalgae previously grown as the stock cultures were transferred into beaker that contained the wastewater. The stock cultures of the microalgae were *Oscillatoria limnosa*, *Scenedesmus quadricauda* and *Pediastrum duplex.* They were transferred into the water sample to examine their ability to remove

nutrient and reacted as water treatment. The nutrients that were removed from the water samples were nitrogen, phosphorous and silica. All the beakers were placed under florescence light with temperature maintained at 25°C to 26°C (room temperature). The measurements were done every 48 hours to monitor the reduction of the nutrient from the water samples with selected microalgae. During the experiment, stirring were done manually. Amount of nutrient removal (R) was calculated using the following formula.

$$R = T_0 - T_i$$

Where T_0 is the concentration at the start of the experiment and T_i is the consequences day the end of the experiment.

3.6 Growth

Growth measurement for the microalgae was done every 48 hours. One ml of water sample was put into sedimentation chamber. A few drops of Lugol's solution were added into water sample. It took about 30 to 60 minutes to allow the cells sink in the sedimentation chamber. Gently removed the chamber and put the cover slip on top of the slide. Then the cells counting were done by using inverted microscope. The growth was determined by the formula;

$$K = \frac{(\ln N_{t1} - \ln N_{t0})}{t_1 - t_0}$$

Where; $N_{t1}$is the number of the cells at sampling, and N_{t0} is the numbers of the cells at the beginning of the experiment (Oh-Hama and Miyachi, 1992).

Chapter 4

Result and discussion

4.1 Species of microalgae at Tasik Taman Jaya

A total of 22 species microalgae from Taman Jaya were identified in this study. Most of the species belongs to the division Chlorophyta and the division with the least number of species was Cyanophyta. All these are summarized in Table 4.1. Individuals belong to the division Euglenophyta were, however, the most commonly seen during the examination of microalgae of the water samples. Euglenophytes are generally able to adapt to and inhabit polluted water. They are able to utilize nutrients that pollute such water. According to Norhayati (1995), Tasik Taman Jaya was very polluted and eutrophic.

The effluents of the lake came from the residential area and surrounding road. The effluent bring along inorganic matter, detergents, kitchen wastes and others. With high constitution of the inorganic matter, it might trigger the development of the microalgae. Table 4.1 shows the existence of the microalgae in the Tasik Taman Jaya.

Table 4.1: Microalgae composition in Tasik Taman Jaya.

Division	**Species**	**Total number of species**
Euglenophyta (euglenoid)	*Phacus longicauda* *Phacus pleuronectus* *Phacus* sp. *Euglena sanguinnea* *Euglena* sp.	5
Chlorophyta (green algae)	*Chlamydomonas sp.* *Scenedesmus quadricauda* *Scenedesmus oblicuus* *Scenedesmus bijuga* *Scenedesmus dimorphus* *Scenedesmus acuminatus* *Pedistrum tetras* *Pediastrum duplex* *Tetraedron* sp. *Closterium* sp. *Crucigenia tetrapedia* *Chroococcus turgidus* *Chlorella sp.*	13
Cyanophyta (blue-green algae)	*Oscillatoria limnosa*	1
Bacillariophyta (diatom)	*Frustulia* sp. *Navicula* sp. *Cymbella* sp.	3

Initial trial in subsequent experiments indicated that *Scenedesmus quadricauda*, *Oscillatoria limnosa* and *Pediastrum duplex* grew well in culture under laboratory conditions. Hence they were selected for the wastewater treatment experiments of this study. The reason for choosing several species of was to compare their effectiveness in removing the nutrient from the wastewater as well as to stimulate their proliferation.

4.2 Water quality analysis

In the early stage of the experiment, the initial amount of nitrogen, phosphorous and silica in all wastewater samples from both stations were very high (Table 4.2 to Table 4.9). This indicated that Tasik Taman Jaya was polluted and experiencing eutrophication. Nutrients that were removed from wastewater effluent are to control eutrophication (Hammouda *et. al*., 1995).

Table 4.2: Nutrients concentration of water collected from station A and inoculated with *Oscillatoria limnosa*

Day	Nitrate (mg/L)	Phosphate (mg/L)	Silica (mg/L)
0	4.67 ±0.58	1.43±0.06	22.67±0.58
2	3.67±0.58	1.13±0.06	20.50±0.00
4	3.33±0.58	1.07±0.06	19.17±0.29
6	2.67±0.58	1.00±0.10	18.50±0.00
8	2.33±0.58	0.90±0.00	18.50±0.00
10	2.33±0.58	0.80±0.10	14.50±0.00
12	2.00±0.00	0.70±0.00	12.00±0.00
14	2.00±0.00	0.60±0.10	11.17±0.29
16	1.67±0.58	0.50±0.00	10.50±0.50
18	1.33±0.58	0.27±0.06	8.83±0.29

Table 4.3: Nutrients concentration of water collected from station A and inoculated with *Scenedesmus qaudricauda*

Day	Nitrate (mg/L)	Phosphate (mg/L)	Silica (mg/L)
0	6.33±0.58	1.03±0.06	22.83±0.76
2	5.67±0.58	1.00±0.10	21.00±1.00
4	5.33±0.58	0.83±0.03	19.17±0.29
6	5.00±0.00	0.80±0.00	16.67±0.29
8	4.67±0.58	0.77±0.12	16.33±0.29
10	4.33±0.58	0.67±0.06	15.83±0.76
12	4.00±0.00	0.60±0.10	14.83±0.29
14	2.33±0.58	0.47±0.06	13.83±0.76
16	2.00±0.00	0.37±0.06	13.00±0.50
18	1.33±0.58	0.23±0.12	12.33±0.29

Table 4.4: Nutrients concentration of water collected from station A and inoculated with *Pediastrum duplex.*

Day	Nitrate (mg/L)	Phosphate (mg/L)	Silica (mg/L)
0	5.00±1.00	1.13±0.06	21.67±0.58
2	4.33±0.58	0.83±0.06	18.83±0.29
4	4.00±0.00	0.77±0.06	18.33±0.29
6	3.33±0.58	0.70±0.10	18.00±0. 00
8	3.00±0.00	0.63±0.06	16.83±0.29
10	2.67±0.58	0.57±0.06	14.67±0.29
12	2.67±0.58	0.50±0.10	13.17±0.29
14	1.67±0.58	0.47±0.06	10.00±0.50
16	1.33±0.58	0.30±0.00	9.00±0.50
18	0.67±0.58	0.27±0.06	8.17±0.29

Table 4.5: Nutrients concentration of water collected from station A without inoculated

Day	Nitrate (mg/L)	Phosphate (mg/L)	Silica (mg/L)
0	6.00±1.00	1.33±0.06	24.00±0.00
2	5.67±0.58	1.23±0.06	23.83±0.29
4	5.33±0.58	1.17±0.12	23.17±0.29
6	4.67±0.58	1.10±0.10	19.67±0.58
8	4.00±0.00	0.90±0.10	19.50±0.00
10	4.00±0.00	0.87±0.15	19.17±0.29
12	2.67±0.58	0.83±0.15	18.50±0.00
14	2.00±0.00	0.63±0.06	16.17±0.29
16	1.67±0.58	0.53±0.06	15.00±0.00
18	1.67±0.58	0.43±0.06	12.00±0.00

Table 4.6: Nutrients concentration of water collected from station B and inoculated with *Oscillatoria limnosa*

Day	Nitrate (mg/L)	Phosphate (mg/L)	Silica (mg/L)
0	5.67±0.58	1.43±0.06	24.00±1.00
2	5.00±0.00	1.23±0.06	22.83±1.04
4	4.33±0.58	1.17±0.15	22.50±0.00
6	4.00±0.00	0.97±0.12	22.00±0.00
8	3.67±0.58	0.90±0.00	20.67±0.29
10	2.67±0.58	0.70±0.00	19.33±0.58
12	2.33±0.58	0.63±0.12	17.50±0.00
14	2.00±0.00	0.63±0.06	15.67±0.29
16	1.67±1.16	0.53±0.06	14.83±0.29
18	1.33±0.58	0.40±0.00	14.00±0.00

Table 4.7: Nutrients concentration of water collected from station B and inoculated with *Scenedesmus quadricauda*

Day	Nitrate (mg/L)	Phosphate (mg/L)	Silica (mg/L)
0	6.00±0.00	1.53±0.06	21.83±0.29
2	5.67±0.58	1.17±0.12	19.67±0.29
4	5.33±0.58	0.93±0.06	19.17±0.29
6	5.00±0.00	0.83±0.06	18.00±0.50
8	4.67±0.58	0.63±0.06	17.00±0.50
10	3.67±0.58	0.53±0.15	16.17±0.29
12	2.67±0.58	0.50±0.00	14.50±0.50
14	2.00±0.00	0.40±0.00	15.33±0.58
16	1.33±0.58	0.30±0.10	13.83±0.29
18	1.00±1.00	0.23±0.06	12.67±1.04

Table 4.8: Nutrient concentration in water collected from station B and inoculated with *Pediastrum duplex*

Day	**Nitrate (mg/L)**	**Phosphate (mg/L)**	**Silica (mg/L)**
0	6.67±0.58	0.93±0.06	22.33±0.29
2	6.00±0.00	0.87±0.06	20.83±0.29
4	6.00±0.00	0.80±0.10	20.17±0.29
6	5.67±0.58	0.70±0.10	19.67±0.58
8	4.67±0.58	0.63±0.06	18.67±0.76
10	3.67±0.58	0.60±0.00	17.50±0.87
12	2.33±0.58	0.57±0.06	16.67±0.76
14	2.00±0.00	0.50±0.10	14.17±0.29
16	1.33±0.58	0.37±0.06	13.83±0.76
18	1.33±0.58	0.23±0.06	13.00±0.00

Table 4.9: Nutrient concentration in water collected from station B without inoculation

Day	**Nitrate (mg/L)**	**Phosphate (mg/L)**	**Silica (mg/L)**
0	6.67±0.58	1.23±0.06	23.50±0.87
2	6.33±0.58	1.20±0.00	22.83±0.76
4	5.67±0.58	1.10±0.10	22.00±0.00
6	5.67±0.58	1.03±0.21	18.83±0.58
8	5.00±0.00	0.97±0.21	17.83±0.58
10	4.67±0.58	0.90±0.17	15.50±0.00
12	4.00±0.00	0.83±0.15	15.17±0.29
14	2.33±0.58	0.70±0.10	14.67±0.29
16	2.00±0.00	0.63±0.06	14.17±0.58
18	1.33±0.58	0.60±0.10	13.67±0.76

Table 4.10: Amount of nitrate reduction in water sample from Station A

Days	*Oscillatoria limnosa* (mg/L)	*Scenedesmus quadricauda* (mg/L)	*Pediastrum duplex* (mg/L)	Control (mg/L)
2	1.00	0.66	0.67	0.33
4	1.34	1.00	1.00	0.67
6	2.00	1.33	1.67	1.33
8	2.34	1.66	2.00	2.00
10	2.34	2.00	2.33	2.00
12	2.67	2.33	2.33	3.33
14	2.67	4.00	3.33	4.00
16	3.00	4.33	3.67	4.33
18	3.34	5.00	4.33	4.33

Table 4.11: Amount of phosphate reduction in water sample from Station A

Days	*Oscillatoria limnosa* (mg/L)	*Scenedesmus quadricauda* (mg/L)	*Pediastrum duplex* (mg/L)	Control (mg/L)
2	0.30	0.03	0.30	0.10
4	0.36	0.20	0.36	0.16
6	0.43	0.23	0.43	0.23
8	0.53	0.26	0.50	0.43
10	0.63	0.36	0.56	0.46
12	0.73	0.43	0.63	0.50
14	0.83	0.56	0.66	0.70
16	0.93	0.66	0.83	0.80
18	1.16	0.80	0.86	0.90

Table 4.12: Amount of silica reduction in water sample from Station A

Days	*Oscillatoria limnosa* (mg/L)	*Scenedesmus quadricauda* (mg/L)	*Pediastrum duplex* (mg/L)	Control (mg/L)
2	2.17	1.83	2.84	0.17
4	3.50	3.66	3.34	0.83
6	4.17	6.16	3.67	4.33
8	4.17	6.50	4.84	4.50
10	8.17	7.00	7.00	4.83
12	10.67	8.00	8.50	5.50
14	11.50	9.00	11.67	7.83
16	12.17	9.83	12.67	9.00
18	13.84	10.50	13.50	12.00

Table 4.13: Amount of nitrate reduction in water sample from Station B

Days	*Oscillatoria limnosa* (mg/L)	*Scenedesmus quadricauda* (mg/L)	*Pediastrum duplex* (mg/L)	Control (mg/L)
2	0.67	0.33	0.67	0.34
4	1.34	0.67	0.67	1.00
6	1.67	1.00	1.00	1.00
8	2.00	1.33	2.00	1.67
10	3.00	2.33	3.00	2.00
12	3.34	3.33	4.34	2.67
14	3.67	4.00	4.67	4.34
16	4.00	4.67	5.34	4.67
18	4.34	5.00	5.34	5.34

Table 4.14: Amount of phosphate reduction in water sample from Station B

Days	*Oscillatoria limnosa* (mg/L)	*Scenedesmus quadricauda* (mg/L)	*Pediastrum duplex* (mg/L)	Control (mg/L)
2	0.20	0.36	0.06	0.03
4	0.26	0.60	0.13	0.13
6	0.46	0.70	0.23	0.20
8	0.53	0.90	0.30	0.26
10	0.73	1.00	0.33	0.33
12	0.80	1.03	0.36	0.40
14	0.80	1.13	0.43	0.53
16	0.90	1.23	0.56	0.60
18	1.03	1.30	0.70	0.63

Table 4.15: Amount of silica reduction in water sample from Station B

Days	*Oscillatoria limnosa* (mg/L)	*Scenedesmus quadricauda* (mg/L)	*Pediastrum duplex* (mg/L)	Control (mg/L)
2	1.17	2.16	1.50	0.67
4	1.50	2.66	2.16	1.50
6	2.00	3.83	2.66	4.67
8	3.33	4.83	3.66	5.67
10	4.67	5.66	4.83	8.00
12	6.50	7.33	5.66	8.33
14	8.33	6.50	8.16	8.83
16	9.17	8.00	8.50	9.33
18	10.00	9.16	9.33	9.83

4.2.1 Nitrate removal

From the experiment, *Scenedesmus quadricauda* in water from station A, recorded highest nitrate reduction compared to the other microalgae. It reduces the nitrate content by 5.00 mg/L (Table 4.10) from the wastewater sample after eighteen days. The amounts of the nitrate decreased from 6.33 to 1.33 mg/L (Table 4.3). *Pediastrum duplex* reduced the nitrate from 5.00 to 0.67 mg/L (Table 4.4). Final nitrate concentration of the water inoculated with *Oscillatoria limnosa* was also 1.33 mg/L (Table 4.2). The final concentration control sample was 1.67 mg/L (Table 4.5).

For water from station B, the nitrate concentration at the last day of experiment inoculated with *Scenedesmus quadricauda* was 1.00 mg/L (Table 4.7), Those inoculated with *Oscillatoria limnosa, Pediastrum duplex* and control recorded the same final concentration value (1.33 mg/L) (Table 4.6, Table 4.8 and Table 4.9). Higher reduction of nitrate in water from station B inoculated with *Pediastrum duplex* was obtained as compared to that from station A. The reduction in nitrate content in the former was 5.34 mg/L (Table 4.13).

4.2.2 Phosphate removal

Initial phosphate concentration in water collected from station A ranged from 1.03 to 1.43 mg/L (Table 4.2, Table 4.3). Reduction in phosphate content during the 18 days experiment in water inoculated with the algae *Scenedsmus quadricauda*, *Pediastrum duplex*, *Oscillatoria limnosa* are shown Table 4.11. Water inoculated with *Oscillatoria limnosa* recorded the highest reduction of phosphate 1.16 mg/L. This showed that *Oscillatoria limnosa* was able to utilize phosphate more than *Pediastrum duplex* and *Scenedesmus quadricauda*. Meanwhile, in the control, the amount of phosphate reduction was about 0.90 mg/L (Table 4.11) with the final concentration of 0.43 mg/L (Table 4.5).

Reduction of phosphate in experiments with water sample collected from station B is shown in Tables 4.7 and Table 4.8). Final phosphate concentration in water sample inoculated with *Scenedesmus quadricauda* and *Pediastrum duplex* were both 0.23 mg/L The amount of phosphate reduced was in water samples inoculated with *Scenedesmus quadricauda* and *Pediastrum duplex* was 1.30 mg/L and 0.70 mg/L respectively. In

water sample inoculated with *Oscillatoria limnosa* phosphate reduction was 1.03 mg/L. There was a 0.63 mg/L reduction in phosphate concentration in the control (Table 4.9).

4.2.3 Silica removal

Table 4.12 shows the amount of silica reduction in water sample for station A. After eighteen days of the experiment, *Pediastrum duplex* reduced the silica at about 13.50 mg/L (Table 4.12). Silica concentration at the end of the experiment was 8.17 mg/L (Table 4.4). In water sample inoculated with *Oscillatoria limnosa* silica concentration decreased by 13.84 mg/L. In water sample inoculated with *Scenedesmus quadricauda* silica concentration decreased by 10.50 mg/L. Higher reduction of silica was obtained for the control which was by 12.00 mg/L. In water samples from station B inoculated with *Scenedesmus quadricauda*, there was a 9.16 mg/L reduction in silica. Reduction in concentration of silica in water sample from station B by all the algae are shown in Table 4.15. Water sample inoculated with *Scenedesmus quadricauda* showed the least reduction of silica concentration.

This study shows that unicellular and the filamentous algae were able to remove nutrient's concentration in the wastewater samples. A worked done by Shushu and Chipeta (2002) showed, *Oscillatoria* sp. is able to remove 100% of phosphate within six days of the experiment. Although in this experiment the stirring was done manually, almost 80% of the nitrate and phosphate as well as 50 % of silica has been removed from the wastewater by the microalgae. According to Martínez *et.al* (2000), stirring did not visibly affected this total elimination, except at 30° C. Stirring hardly affected adsorption, but at 30° C was the temperature at which stirring most influenced growth.

Backer (1994) state that, during the process of photosynthesis, organic carbon compounds are partially oxidizes to carbon dioxide which in turn is assimilated by the microalgae. In the same time, the microalgae utilized soluble nitrogen and phosphorous compounds from the medium and convert these inorganic matters, which are necessary for the cells' metabolism. Thus, it enhances the wastewater treatment. Figure 4.3 (a) and (b), figure 4.4 (a) and (b), and figure 4.5 (a) and (b), shows that *Oscillatoria limnosa*, *Scenedesmus quadricauda* and *Pediastrum duplex* are capable to removed nutrients. Both *Oscillatoria limnosa* and *Scenedesmus quadricauda* has been frequently used in wastewater treatment compared to *Pediastrum duplex*. However, from this study *Pediastrum duplex* also has the capability in removing the nutrients from wastewater.

4.3 Growth

The algal growths during the eighteen days experimental period are presented in Figure 4.1, Figure 4.2 and Figure 4.3. The higher levels of nutrient content of the wastewater sample support the proliferation of the microalgae. *Oscillatoria limnosa*, *Scenedesmus quadricauda* and *Pediastrum duplex* showed an increasing number of cells from the first day until the end of the experiment for both stations.

The initial growth for *Oscillatoria limnosa* at station A and station B was, 7.75 x 10^4 /ml and 8.00 x 10^4 /ml respectively (Appendix 3). Figure 4.1 shows the growth of *Oscillatoria limnosa* at station A and station B. For the first ten days, *Oscillatoria limnosa* at both stations showed a similar growth pattern. However, after ten days, the growth of cells increases more rapid at station B compared to *Oscillatoria limnosa* in station A. On the last day of the study, the number of cells at station B decreased as it

entered the stationary phase. The cells number at station A still show an increasing pattern after the eighteen day.

Hashimoto and Furukawa (1989) have pointed out various factors, which affect the growth of *Oscillatoria* sp. The factors that they have concluded are light intensity, nutrients concentration, inorganic carbon (IC) concentration and temperature. However, nitrogen concentration and IC concentration are the major factors controlling the growth of these filamentous microalgae in wastewater.

Scenedesmus quadricauda showed the similar growth pattern in both wastewater samples from the first day until day twelve (Figure 4.2). However, after two weeks in the wastewater sample, growth of *Scenedesmus quadricauda* at station B was higher than at station A. Thus, it does show a great development during the experiment was done. Generally, during the last day of experiment, those three microalgae have entered the stationary phase. In this phase, the development of the cells became slower or nearly stops.

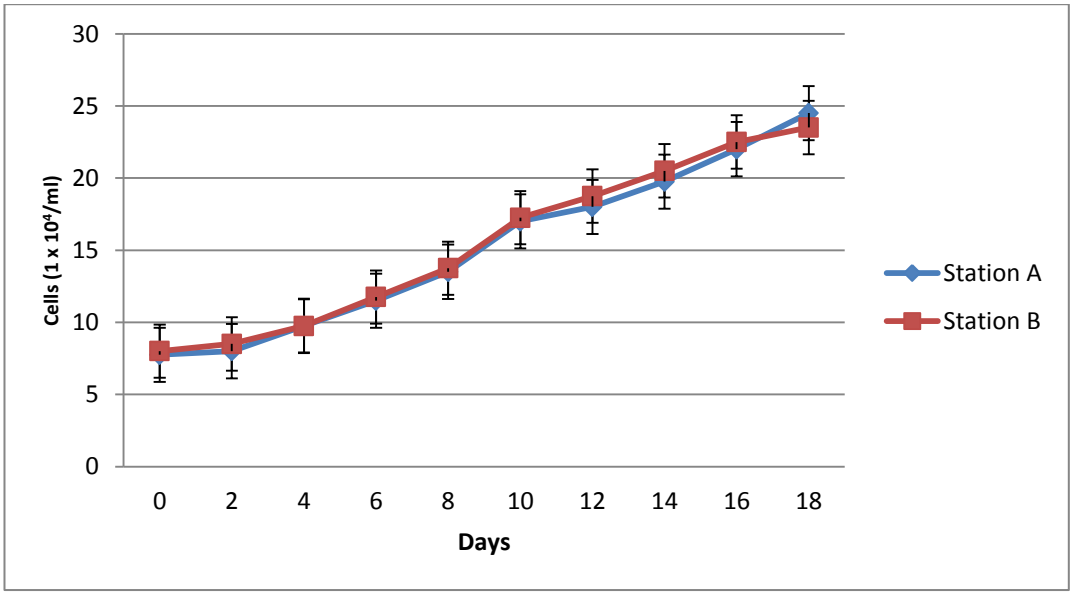

Figure 4.1: Growth of *Oscillatoria limnosa* in water sample from Station A and Station B during the experimental period

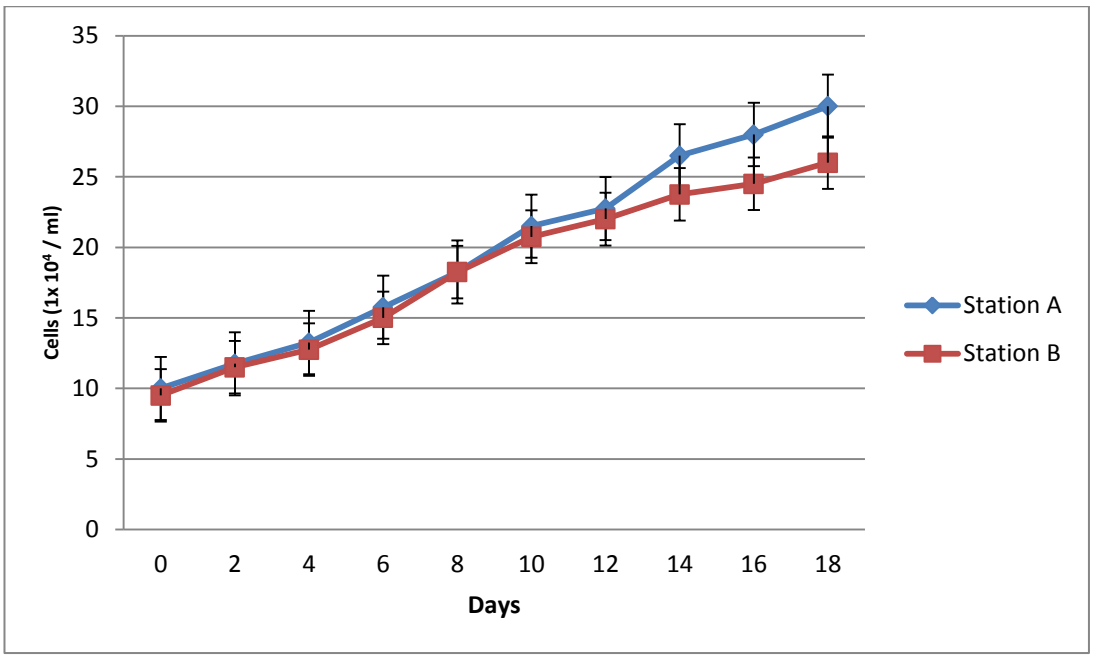

Figure 4.2: Growth of *Scenedesmus quadricauda* in water sample from Station A and Station B during the experimental period.

Besides *Oscillatoria limnosa*, *Pediastrum duplex* also shows a good development of growth. For the first week, the growth of *Pediastrum duplex* shows a slow development at station A and station B. This is shown in Figure 4.3. However, after eight days, the observation showed that the development of *Pediastrum duplex* has become more rapid, particularly at station B. This indicates that the species were tried to adapt to the environment before it grew well.

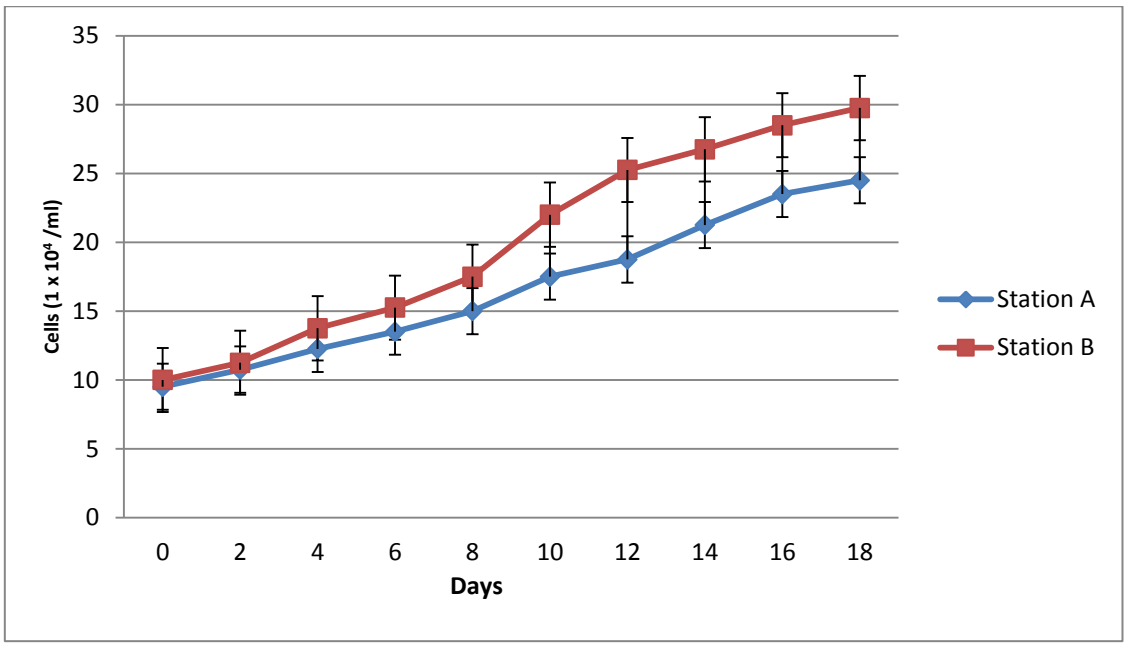

Figure 4.3: Growth of *Pediastrum duplex* in water sample from Station A and Station B during the experimental period

4.4 Relation between growth of microalgae and nutrients removal

Figure 4.4 indicates that the growth of *Oscillatoria limnosa* in wastewater sample from station A. Within a week, the cells' numbers of *Oscillatoria limnosa* increased in slower phase. Half of the nitrate was removed from the total amount. Compared to nitrate, the reductions of phosphate are slower in the beginning, but it decreases at the end days of the experiment. The rapid growth of *Oscillatoria limnosa* are increasing, did not affect the silica concentration so much. From the graph, it indicates that *Oscillatoria limnosa* is able to remove nitrate and phosphate during its growth but has not effect on silica content.

Figure 4.5 represent the growth of *Oscillatoria limnosa* together with the nutrient's removal during eighteen days of experiment on wastewater from station B. The results showed that, nitrate and phosphate have been reducing rapidly. These reductions are along with the development of the growth of *Oscillatoria limnosa.* From

the figure 4.5 it can be seen that *Oscillatoria limnosa* are able to remove silica in a small amount. This shows that silica is not an essential nutrient source for *Oscillatoria limnosa* proliferation.

From the observation, the growth of *Oscillatoria limnosa* was slower during the first four days of the experiment at both stations. During that period, *Oscillatoria limnosa* tried to adapt with the new environment. Meanwhile, the nutrients also showed slow reduction on those four days. This is shown in figure 4.4 and figure 4.5. After four days, *Oscillatoria limnosa* showed rapid development until the end day of the experiment. Observation shows that, *Oscillatoria limnosa* was able to utilize the nutrients for their growth and simultaneously removing nitrate, phosphate and silica from the wastewater samples.

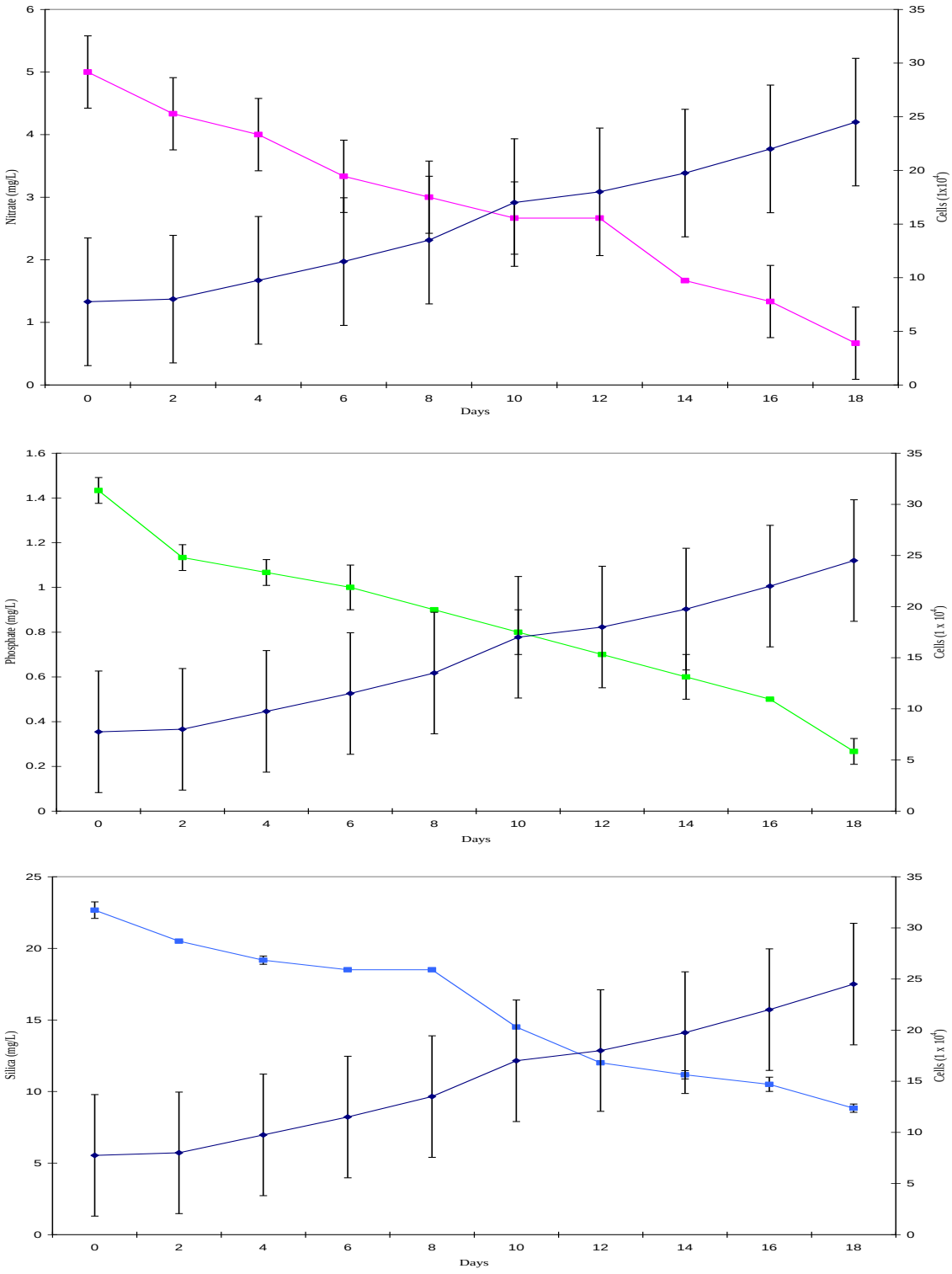

Figure 4.4: Nutrients reduction vs. growth of *Oscillatoria limnosa* in water sample from station A

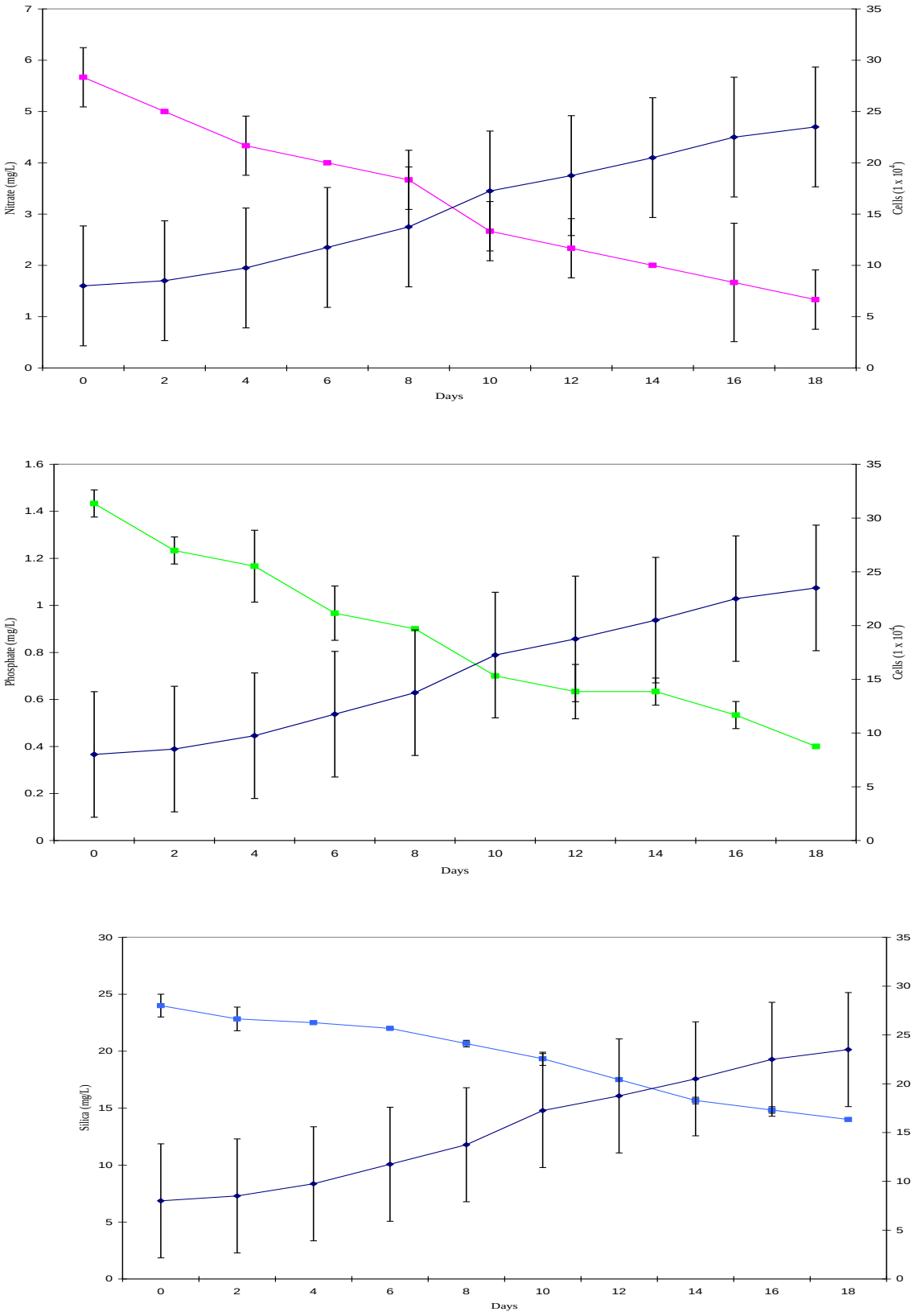

Figure 4.5: Nutrients reduction vs. growth of *Oscillatoria limnosa*. in water sample from station B

Figure 4.6 shows growth of *Pediastrum duplex* in wastewater from station A. Growth of the species increased rapidly with total number of cells 9.50 x 10^4 /ml (Appendix 3) from the first day of the experiment. At the end day of the experiment the numbers of cells become 24.50 x 10^4 /ml (Appendix 3). During the eighteen days of the study, nitrate and phosphate decreased rapidly along with the algae growth. This indicated that, *Pediastrum duplex* utilize the inorganic compound to increase their cells number. Silica also reduced along with the growth of *Pediastrum duplex*

At the beginning of the experiment for wastewater from station B, the developments of the *Pediastrum duplex* cells are slower with total numbers of cells 10.00 x 10^4 /ml (Appendix 3), but after a week they grew faster. At the same time, the nitrate, phosphate and silica were also decreased. The growth pattern and reduction of nutrients can be seen in figure 4.7. After a week, growth of *Pediastrum duplex* has accelerated and at the same time nitrate content there was a rapid drop. This also observed for phosphate. The increasing growth of *Pediastrum duplex* was due nitrate and phosphate assimilation. At the end of the experiment the total numbers of *Pediastrum duplex* was 29.75 x 10^4 /ml. The cells have utilized the nutrients for their proliferation but silica was not utilized. The same results were found in *Oscillatoria limnosa* where silica content did not reduce to much compare with nitrate and phosphate. On day 18, the silica reached to the level above of 10.00 mg/L. Thus, from the figure 4.6 and figure 4.7 show that *Pediastrum duplex* has an ability to reduce the nutrients in the wastewater.

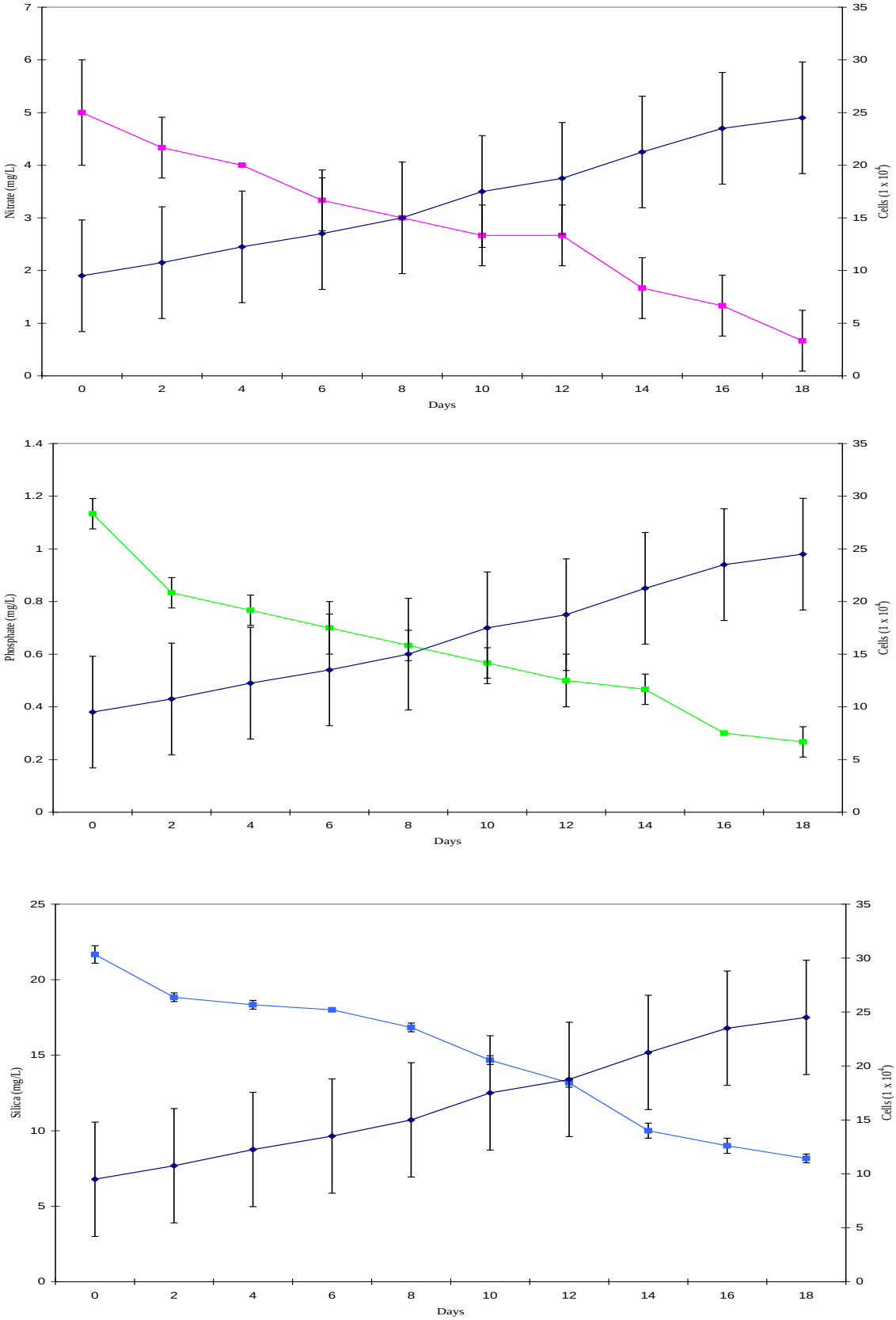

Figure 4.6: Nutrients reduction vs. growth of *Pediastrum duplex* in water sample from Station A

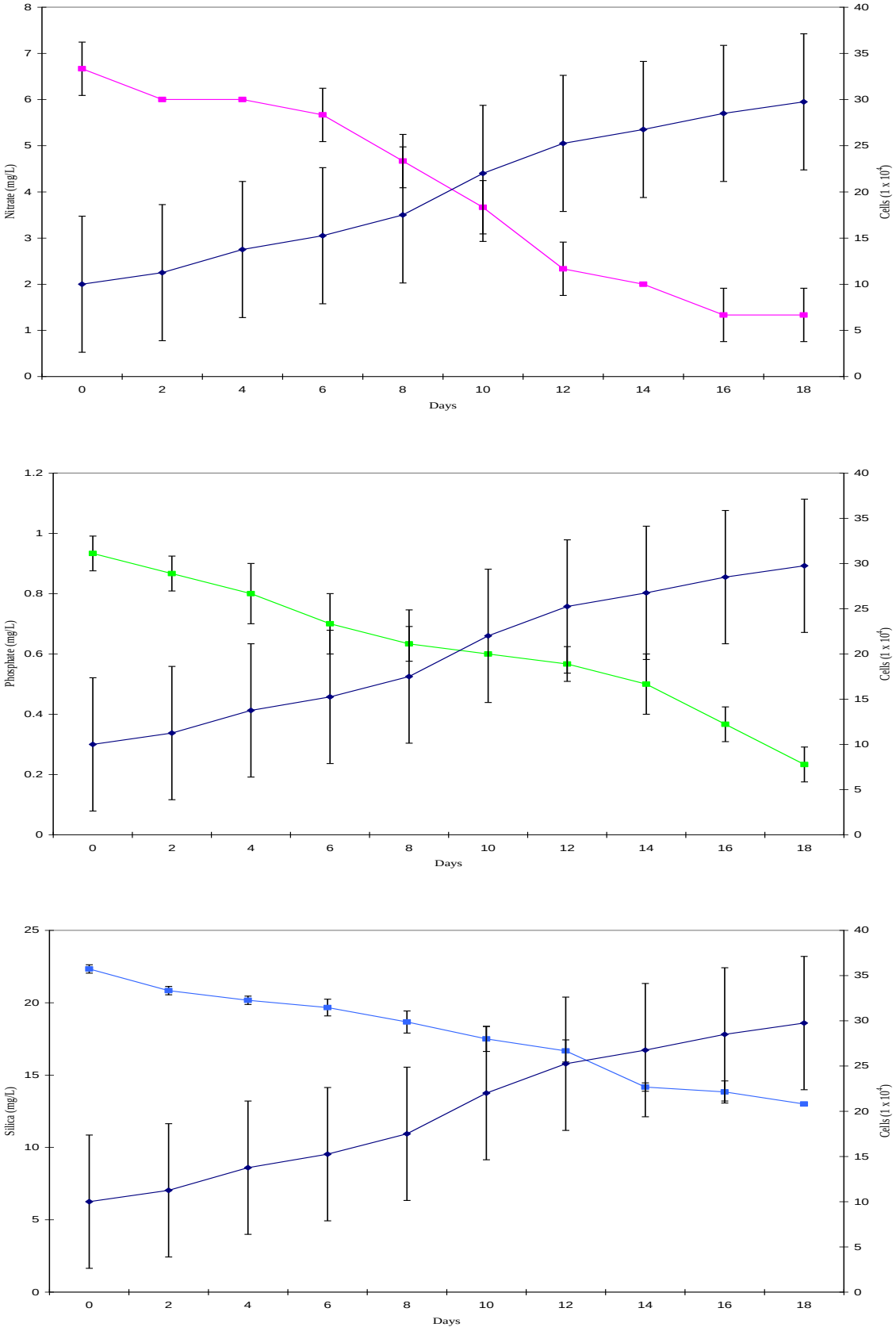

Figure 4.7: Nutrients reduction vs. growth of *Pediastrum duplex* in water sample from station B

Figure 4.8 indicates growth of *Scenedesmus quadricauda* by comparing with the nitrate, phosphate and silica during the eighteen days of experiment. *Scenedesmus quadricauda*. is able to grow on wastewater by utilizing the nutrients and at the same time removing it from the effluents. According to figure 4.8 nitrate and phosphate decrease rapidly together with the growth of *Scenedesmus quadricauda*. Hence, it can be said that the *Scenedesmus quadricauda* has utilized the nutrients as for their energy source. Similar with other's results, silica did not decrease accordingly with the growth. This is because, *Scenedesmus quadricauda*. itself do not utilize much the silica like nitrate and phosphate. Nitrate and phosphate are the most essential element of their proliferation but not silica. However, silica does show some reduction in the wastewater.

The growth of *Scenedesmus quadricauda* in station B is different from the growth in the station A. From the graph, the growth of *Scenedesmus quadricauda* is slower on first four days but enhance rapidly the day onwards. Figure 4.9 shows that the nitrate reduction is proportionally with the growth rate, but for the phosphate, it does shows rapid reduction on the first four day. After a week, the reduction of phosphate became slower and achieves to the lowest level. The results obtain for silica is still similar with *Oscillatoria limnosa* and *Pediastrum duplex* where, silica not removed much like nitrate and phosphate. Level of silica is still higher in the wastewater, along with the increases of *Scenedesmus quadricauda*. This shows that the silica is not the essential element for cells development of *Scenedesmus quadricauda*.

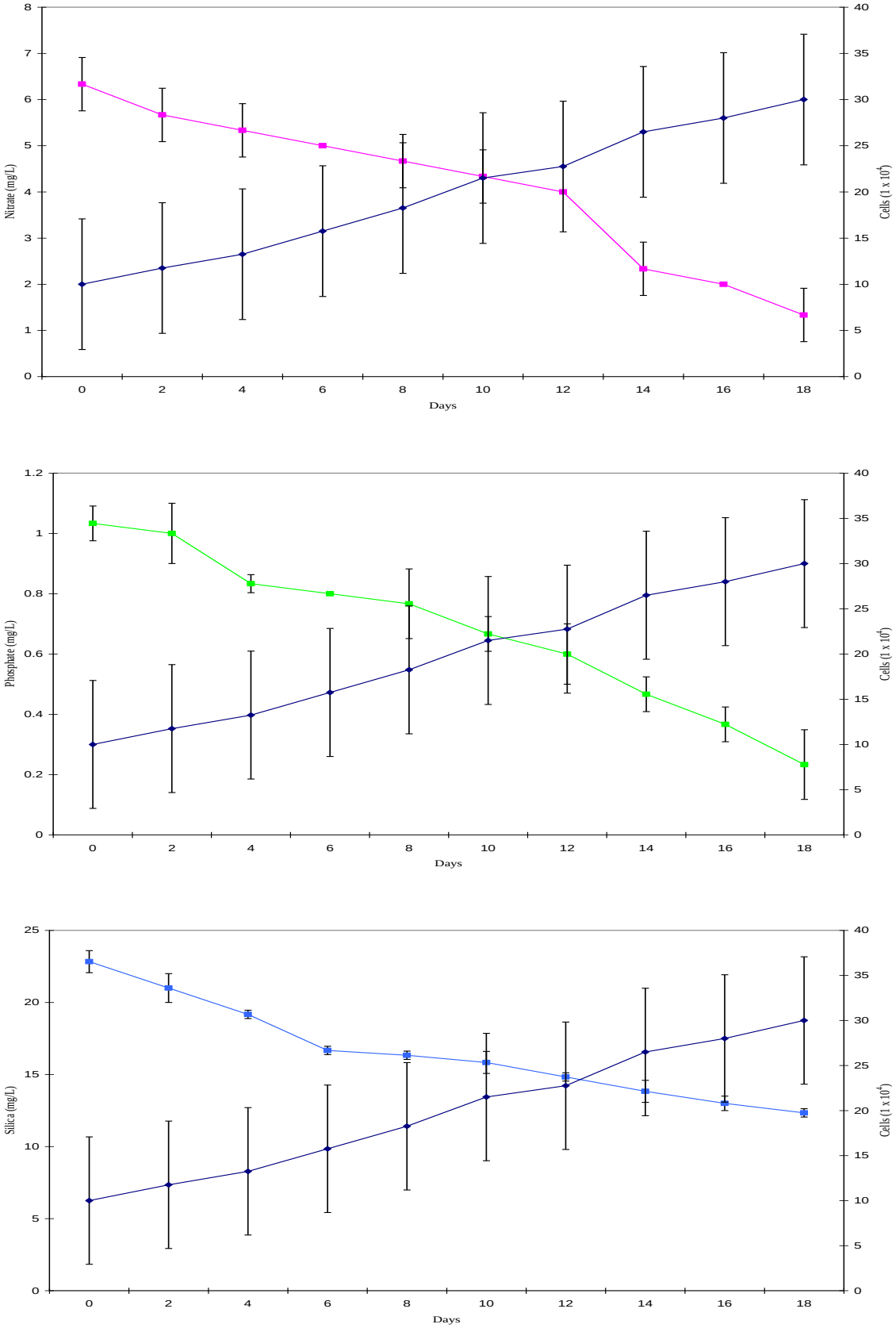

Figure 4.8: Nutrients reduction vs. growth of *Scenedesmus quadricauda* in water sample from station A

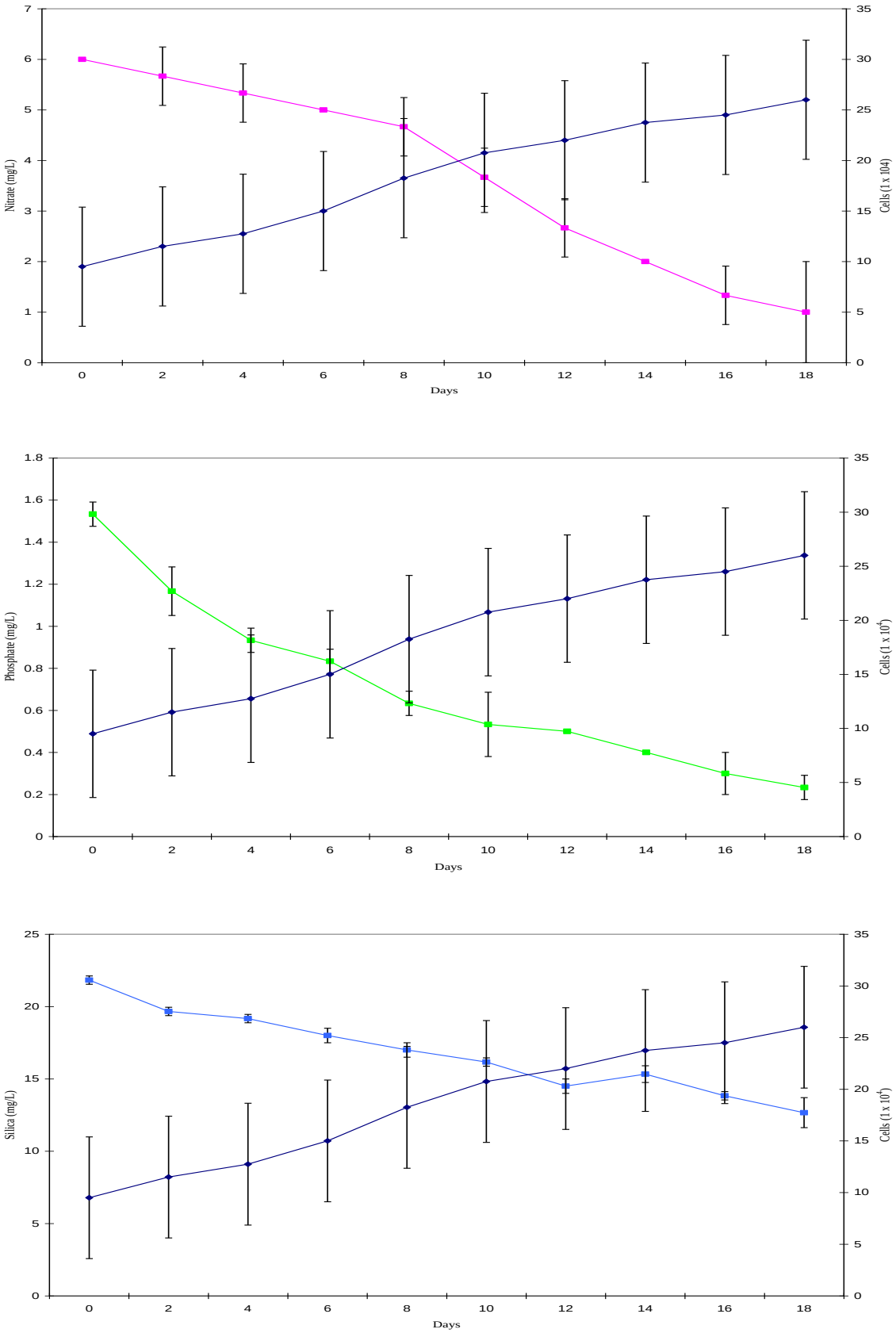

Figure 4.9: Nutrients reduction vs. growth of *Scenedesmus quadricauda* in water sample from station B

From the statistical analysis, it shows that there is a strong correlation between microalgae growth and the removal of the nutrients with the result is close to -1. Appendix 4 shows the correlation between *Oscillatoria limnosa* and nutrients in water sample from Station A. From the result, there was a negative correlation between growth and nitrate (-0.897), growth and phosphate (-0.980), growth and silica (-0.980). Similar result were obtain for *Oscillatoria limnosa* in Station B, where r = -0.987 for growth and nitrate, r = -0.976 for growth and phosphate and r = -0.989 for growth and silica (Appendix 5).

The correlation for *Scenedesmus quadricauda* and nutrients in water sample from both Station A and Station B are negatively correlated. All the results shows r = -1 (Appendix 6 and Appendix 7). Similar result was obtained for *Pediastrum duplex*, where r is close to - 1(Appendix 8 and Appendix 9). The growth is negatively correlated with the nutrients in water sample from Station A and Station B. The correlations are negative as the growths were increase and the nutrients are decrease during the experimental period.

According to Fay (1983), the blue-green algae have several characteristics, which made them well adapted in wastewater treatment. Some of the characteristics included the ability to accumulate inorganic phosphorous and nitrogen. Another one is that they have an ability to tolerate the highly variable condition that characterized polluted effluent.

Mohapatra (2006) noted that, green algae species prefer water bodies that are rich in nitrogen, with high N/P ratio. While water bodies with low N/P ratio are generally colonized by cyanobacteria. He also mentioned that, algal growth in

wastewater, not only remove the nutrients but also stabilize the pH and increase the dissolved oxygen content in wastewater, as well as it eliminating the requirement of artificial aeration.

Further study must on the use of *Pediastrum duplex* for wastewater treatment. *Pediastrum sp.* is bigger in size compared to *Scenedesmus* sp. and *Chlorella* sp. Fuhs *et.al.* (1972) have shown that rates of phosphorous uptake may be related to cell-surface area and maximum uptake rates are certainly greater in the larger such as *Pediastrum* sp. Besides this microalgae can grow well in culture medium as well as in the wastewater sample. Nevertheless, their growth development is influenced by many factors such as nutrients and light intensities.

According to Travieso *et.al*. (1996), microalgae are able to remove more nutrients under natural light as compared to under artificial light. In this study, after eighteen days of the experiment, these microlagae were able to grow under artificial light, with the nutrients are almost totally removed from the wastewater. This study also shows that artificial light can stimulate the microalgae growth. Light is critical for photosynthesis and nutrients are needed for the anabolic activities of the algal cells (Faizah, 2004). Kaplan *et.al*. (1986) stated that, at low light intensities, changes in the concentration of nitrogen and phosphate had not affected the growth rate of algae. When at medium light intensities, the growth rate was greater with higher concentration of nitrogen and phosphate. Under limited light condition, microalgae also can adapt their metabolism from autotrophy to heterotrophy. Hence, they are able to utilize both organic and inorganic compound from the medium (Becker, 1994).

Shelef *et. al*. (1978) concluded that microalgae are capable of taking nitrogen and phosphorous as their biomass. The increase in biomass yield proportionally enhances the removal of eutrophication causing nutrients from the effluent. Other studies revealed that the nutrient removal efficiency is related to (i) the content of nutrients in wastewater (ii) the degree of nutrient utilized by algal growth and (iii) the nutrient concentration in algal tissues (Matusiak *et. al*., 1976; Przytocka-Jusiak *et.al*., 1984). Tam and Wong (1989) mentioned that, the removal of total inorganic nitrogen from wastewater was more effective when algae were cultured in settled sewage. They stated that the removal of nitrogen, especially $NH_4^{+-}N$, from algal cultures is affected by two factors; direct utilization of nitrogen by algae and NH_3 stripping. They also added that the removal of total inorganic nitrogen from wastewater was, more effective when algae were cultivated in settle sewage than in activated sewage. Over 90% removal of total inorganic nitrogen was detected in treatments with settle sewage.

Chapter 5

Conclusion

An eighteen day laboratory experiment on waste water samples inoculated with different microalgae species shows:

1) 60 to 75% of the nutrients can be removed from wastewater.

2) Microalgae can grow and utilize nutrients in wastewater.

3) The growth rate of microalgae is negatively correlated with the nutrient.

4) *Pediastrum duplex* also has the potential to be used to remove nutrients from wastewater.

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Appendix 1

Nutrients removal in water sample from Station A

1) Nutrients remove by *Oscillatoria limnosa* in water sample from Station A

Day	Nitrate (mg/L)			Phosphate (mg/L)			Silica(mg/L)		
	Rep. 1	Rep. 2	Rep. 3	Rep. 1	Rep. 2	Rep. 3	Rep. 1	Rep. 2	Rep. 3
0	5.0	5.0	4.0	1.4	1.5	1.4	23.00	22.00	23.00
2	4.0	4.0	3.0	1.2	1.1	1.1	20.50	20.50	20.50
4	3.0	4.0	3.0	1.0	1.1	1.1	19.00	19.50	19.00
6	3.0	3.0	2.0	1.1	0.9	1.0	18.50	18.50	18.50
8	2.0	3.0	2.0	0.9	0.9	0.9	18.50	18.50	18.50
10	2.0	3.0	2.0	0.8	0.7	0.9	14.50	14.50	14.50
12	2.0	2.0	2.0	0.7	0.7	0.7	12.00	12.00	12.00
14	2.0	2.0	2.0	0.7	0.5	0.6	11.00	11.50	11.00
16	2.0	2.0	1.0	0.5	0.5	0.5	10.50	11.00	10.00
18	2.0	1.0	1.0	0.3	0.2	0.3	8.50	9.00	9.00

2) Nutrients remove by *Scenedesmus qaudricauda* in water sample from Station A

Day	Nitrate (mg/L)			Phosphate (mg/L)			Silica (mg/L)		
	Rep. 1	Rep. 2	Rep. 3	Rep. 1	Rep. 2	Rep. 3	Rep. 1	Rep. 2	Rep. 3
0	6.0	6.0	7.0	1.0	1.1	1.0	23.00	23.50	22.00
2	5.0	6.0	6.0	0.9	1.0	1.1	22.00	20.00	21.00
4	5.0	5.0	6.0	0.9	0.8	0.8	19.50	19.00	19.00
6	5.0	5.0	5.0	0.8	0.8	0.8	16.50	17.00	16.50
8	5.0	4.0	5.0	0.7	0.7	0.9	16.50	16.50	16.00
10	4.0	5.0	4.0	0.6	0.7	0.7	15.00	16.50	16.00
12	4.0	4.0	4.0	0.7	0.5	0.6	15.00	15.00	14.50
14	2.0	2.0	3.0	0.5	0.4	0.5	13.00	14.00	14.50
16	2.0	2.0	2.0	0.4	0.4	0.3	13.50	13.00	12.50
18	1.0	2.0	1.0	0.3	0.3	0.1	12.50	12.00	12.50

3) Nutrients remove by *Pediastrum duplex* in water sample from Station A

Day	Nitrate (mg/L)			Phosphate (mg/L)			Silica (mg/L)		
	Rep. 1	Rep. 2	Rep. 3	Rep. 1	Rep. 2	Rep. 3	Rep. 1	Rep. 2	Rep. 3
0	5.0	4.0	6.0	1.2	1.1	1.1	22.00	21.00	22.00
2	4.0	5.0	4.0	0.9	0.8	0.8	18.50	19.00	19.00
4	4.0	4.0	4.0	0.8	0.7	0.8	18.50	18.50	18.00
6	3.0	3.0	4.0	0.7	0.8	0.6	18.00	18.00	18.00
8	3.0	3.0	3.0	0.6	0.7	0.6	17.00	16.50	17.00
10	3.0	2.0	3.0	0.6	0.6	0.5	15.00	14.50	14.50
12	3.0	3.0	2.0	0.5	0.6	0.4	13.00	13.50	13.00
14	1.0	2.0	2.0	0.5	0.5	0.4	9.50	10.00	10.50
16	2.0	1.0	1.0	0.3	0.3	0.3	9.50	9.00	8.50
18	1.0	0.0	1.0	0.3	0.2	0.3	8.00	8.00	8.50

4) Nutrients removal for control in water sample from Station A

Day	Nitrate (mg/L)			Phosphate (mg/L)			Silica (mg/L)		
	Rep. 1	Rep. 2	Rep. 3	Rep. 1	Rep. 2	Rep. 3	Rep. 1	Rep. 2	Rep. 3
0	6.0	5.0	7.0	1.4	1.3	1.3	24.0	24.00	24.00
2	6.0	5.0	6.0	1.2	1.3	1.2	24.00	23.50	24.00
4	5.0	6.0	5.0	1.1	1.3	1.1	23.50	23.00	23.00
6	5.0	4.0	5.0	1.0	1.2	1.1	20.00	19.00	20.00
8	4.0	4.0	4.0	0.8	0.9	1.0	19.50	19.50	19.50
10	4.0	4.0	4.0	0.9	1.0	0.7	19.50	19.00	19.00
12	3.0	3.0	2.0	0.8	1.0	0.7	18.50	18.50	18.50
14	2.0	2.0	2.0	0.6	0.7	0.6	16.00	16.50	16.00
16	1.0	2.0	2.0	0.6	0.5	0.5	15.00	15.00	15.00
18	1.0	2.0	2.0	0.5	0.4	0.4	12.00	12.00	12.00

Appendix 2

Nutrients removal in water sample from Station B

1) Nutrients remove by *Oscillatoria limnosa* in water sample from Station B

Day	Nitrate (mg/L)			Phosphate (mg/L)			Silica (mg/L)		
	Rep. 1	Rep. 2	Rep. 3	Rep. 1	Rep. 2	Rep. 3	Rep. 1	Rep. 2	Rep. 3
0	5.0	6.0	6.0	1.5	1.4	1.4	23.00	24.00	25.00
2	5.0	5.0	5.0	1.2	1.3	1.2	22.00	24.00	22.50
4	4.0	5.0	4.0	1.0	1.2	1.3	22.50	22.50	22.50
6	4.0	4.0	4.0	1.1	0.9	0.9	22.00	22.00	22.00
8	3.0	4.0	4.0	0.9	0.9	0.9	20.50	21.00	20.50
10	2.0	3.0	3.0	0.7	0.7	0.7	19.00	19.00	20.00
12	2.0	3.0	2.0	0.7	0.5	0.7	17.50	17.50	17.50
14	2.0	2.0	2.0	0.7	0.6	0.6	15.50	15.50	16.00
16	1.0	3.0	1.0	0.5	0.5	0.6	15.00	14.50	15.00
18	2.0	1.0	1.0	0.4	0.4	0.4	14.00	14.00	14.00

2) Nutrients remove by *Scenedesmus quadricauda* in water sample from Station B

Day	Nitrate (mg/L)			Phosphate (mg/L)			Silica (mg/L)		
	Rep. 1	Rep. 2	Rep. 3	Rep. 1	Rep. 2	Rep. 3	Rep. 1	Rep. 2	Rep. 3
0	6.0	6.0	6.0	1.6	1.5	1.5	21.50	22.00	22.00
2	5.0	6.0	6.0	1.3	1.1	1.1	19.50	20.00	19.50
4	6.0	5.0	5.0	0.9	0.9	1.0	19.00	19.00	19.50
6	5.0	5.0	5.0	0.8	0.9	0.8	18.00	18.50	17.50
8	5.0	5.0	4.0	0.6	0.7	0.6	16.50	17.50	17.00
10	3.0	4.0	4.0	0.5	0.4	0.7	16.50	16.00	16.00
12	2.0	3.0	3.0	0.5	0.5	0.5	14.00	14.50	15.00
14	2.0	2.0	2.0	0.4	0.4	0.4	15.00	15.00	16.00
16	2.0	1.0	1.0	0.3	0.4	0.2	14.00	13.50	14.00
18	1.0	0.0	2.0	0.3	0.2	0.2	13.00	13.50	11.50

3) Nutrients remove by *Pediastrum duplex* in water sample from Station B

Day	Nitrate (mg/L)			Phosphate (mg/L)			Silica (mg/L)		
	Rep. 1	Rep. 2	Rep. 3	Rep. 1	Rep. 2	Rep. 3	Rep. 1	Rep. 2	Rep. 3
0	7.0	6.0	7.0	1.0	0.9	0.9	22.50	22.50	22.00
2	6.0	6.0	6.0	0.9	0.8	0.9	21.00	20.50	21.00
4	6.0	6.0	6.0	0.8	0.9	0.7	20.50	20.00	20.00
6	5.0	6.0	6.0	0.6	0.7	0.8	20.00	19.00	20.00
8	5.0	4.0	5.0	0.6	0.7	0.6	18.00	19.50	18.50
10	4.0	4.0	3.0	0.6	0.6	0.6	18.50	17.00	17.00
12	3.0	2.0	2.0	0.6	0.5	0.6	16.50	17.50	16.00
14	2.0	2.0	2.0	0.5	0.6	0.4	14.00	14.50	14.00
16	1.0	2.0	1.0	0.4	0.3	0.4	14.00	14.50	13.00
18	2.0	1.0	1.0	0.2	0.3	0.2	13.00	13.00	13.00

4) Nutrients removal for control in water sample from station B

Day	Nitrate (mg/L)			Phosphate (mg/L)			Silica (mg/L)		
	Rep. 1	Rep. 2	Rep. 3	Rep. 1	Rep. 2	Rep. 3	Rep. 1	Rep. 2	Rep. 3
0	7.0	6.0	7.0	1.3	1.2	1.2	24.50	23.00	23.00
2	7.0	6.0	6.0	1.2	1.2	1.2	22.00	23.00	23.50
4	5.0	6.0	6.0	1.1	1.2	1.0	22.00	22.00	22.00
6	6.0	6.0	5.0	0.8	1.1	1.2	18.50	19.50	18.50
8	5.0	5.0	5.0	0.8	0.9	1.2	18.50	17.50	17.50
10	5.0	4.0	5.0	0.8	0.8	1.1	15.50	15.50	15.50
12	4.0	4.0	4.0	1.0	0.8	0.7	15.50	15.00	15.00
14	2.0	3.0	2.0	0.7	0.6	0.8	14.50	15.00	14.50
16	2.0	2.0	2.0	0.6	0.6	0.7	14.50	13.50	14.50
18	1.0	2.0	1.0	0.7	0.6	0.5	14.50	13.50	13.00

Appendix 3

Growth of microalgae for eighteen days in the water sample (cell(1×10^4) /ml)

1) Station A

Days	*Oscillatoria limnosa*	*Scenedesmus quadricauda*	*Pediastrum duplex*
0	7.75	10.00	9.50
2	8.00	11.75	10.75
4	9.75	13.25	12.25
6	11.50	15.75	13.50
8	13.50	18.25	15.00
10	17.00	21.50	17.50
12	18.00	22.75	18.75
14	19.75	26.50	21.25
16	22.00	28.00	23.50
18	24.50	30.00	24.50

2) Station B

Days	*Oscillatoria limnosa*	*Scenedesmus quadricauda*	*Pediastrum duplex*
0	8.00	9.50	10.00
2	8.50	11.50	11.25
4	9.75	12.75	13.75
6	11.75	15.00	15.25
8	13.75	18.25	17.50
10	17.25	20.75	22.00
12	18.75	22.00	25.25
14	20.50	23.75	26.75
16	22.50	24.50	28.50
18	23.50	26.00	29.75

Appendix 4

Statistical Analysis for *Oscillatoria limnosa* from Station A

Station A

Days	Growth (cell (1×10^4) /ml)	Nitrate (mg/L)	Phosphate (mg/L)	Silica (mg/L)
0	7.75	4.67	1.43	22.67
2	8	3.67	1.33	20.5
4	9.75	3.33	1.07	19.17
6	11.5	2.67	1	18.5
8	13.5	2.33	0.9	18.5
10	17	2.33	0.8	14.5
12	18	2	0.7	12
14	19.75	2	0.6	11.17
16	22	1.67	0.5	10.5
18	24.5	1.67	0.27	8.83

Oscillatoria limnosa inoculated in water sample from Station A

	growth	*nitrate*
growth	1	
nitrate	-0.896579	1

The correlation for these two variable is = -0.897

	growth	*phosphate*
growth	1	
phosphate	-0.980123	1

The correlation for these two variable is = - 0.980

	growth	*silica*
growth	1	
silica	-0.980412	1

The correlation for these two variable is = -0.980

Appendix 5

Statistical Analysis for *Oscillatoria limnosa* from Station B

Station B

Days	Growth (cell (1×10^4) /ml)	Nitrate (mg/L)	Phosphate (mg/L)	Silica (mg/L)
0	8	5.67	1.43	24
2	8.5	5	1.23	22.83
4	9.75	4.33	1.17	22.5
6	11.75	4	0.97	22
8	13.75	3.67	0.9	20.67
10	17.25	2.67	0.7	19.33
12	18.75	2.33	0.63	17.5
14	20.5	2	0.63	15.67
16	22.5	1.67	0.53	14.83
18	23.5	1.33	0.4	14

Oscillatoria limnosa inoculated in water sample from Station B

	growth	*nitrate*
growth	1	
nitrate	-0.98656	1

The correlation for these two variable is = - 0.987

	growth	*phosphate*
growth	1	
phosphate	-0.97586	1

The correlation for these two variable is = -0.976

	growth	*silica*
growth	1	
silica	-0.98852	1

The correlation for these two variable is = -0.989

Appendix 6

Statistical Analysis for *Scenedesmus quadricauda* from Station A

Station A

Days	Growth (cell (1×10^4) /ml)	Nitrate (mg/L)	Phosphate (mg/L)	Silica (mg/L)
0	10	6.33	1.03	22.833
2	11.75	5.67	1	21
4	13.25	5.33	0.83	19.17
6	15.75	5	0.8	16.67
8	18.25	4.67	0.77	16.33
10	21.5	4.33	0.67	15.83
12	22.75	4	0.6	14.83
14	26.5	2.33	0.47	13.83
16	28	2	0.37	13
18	30	1.33	0.23	12.33

Scenedesmus quadricauda inoculated in water sample from Station A

	growth	*nitrate*
growth	1	
nitrate	-0.97607	1

The correlation for these two variable is = -0.976

	growth	*phosphate*
growth	1	
phosphate	-0.9827	1

The correlation for these two variable is = -0.983

	growth	*silica*
growth	1	
silica	-0.96078	1

The correlation for these two variable is = -0.961

Appendix 7

Statistical Analysis for *Scenedesmus quadricauda* from Station B

Station B

Days	Growth (cell (1×10^4) /ml)	Nitrate (mg/L)	Phosphate (mg/L)	Silica (mg/L)
0	9.5	6	1.53	21.83
2	11.5	5.67	1.17	19.67
4	12.75	5.33	0.93	19.17
6	15	5	0.83	18
8	18.25	4.67	0.63	17
10	20.75	3.67	0.53	16.17
12	22	2.67	0.5	14.5
14	23.75	2	0.4	15.33
16	24.5	1.33	0.3	13.83
18	26	1	0.23	12.67

Scenedesmus quadricauda inoculated in water sample from Station B

	growth	*nitrate*
growth	1	
nitrate	-0.96759	1

The correlation for these two variable is = - 0.968

	growth	*phosphate*
growth	1	
phosphate	-0.96689	1

The correlation for these two variable is = - 0.967

	growth	*silica*
growth	1	
silica	-0.97893	1

The correlation for these two variable is = -0.979

Appendix 8

Statistical Analysis for *Pediastrum duplex* from Station A

Station A

Days	Growth (cell (1×10^4) /ml)	Nitrate (mg/L)	Phosphate (mg/L)	Silica (mg/L)
0	9.5	5	1.13	21.67
2	10.75	4.33	0.83	18.83
4	12.25	4	0.77	18.33
6	13.5	3.33	0.7	18
8	15	3	0.63	16.83
10	17.5	2.67	0.57	14.67
12	18.75	2.67	0.5	13.17
14	21.25	1.67	0.47	10
16	23.5	1.33	0.3	9
18	24.5	0.67	0.27	8.17

Pediastrum duplex inoculated in water sample from Station A

	growth	*nitrate*
growth	1	
nitrate	-0.98528	1

The correlation for these two variable is =-0.985

	growth	*phosphate*
growth	1	
phosphate	-0.95519	1

The correlation for these two variable is = -0.955

	growth	*silica*
growth	1	
silica	-0.99129	1

The correlation for these two variable is = - 0.991

Appendix 9

Statistical Analysis for *Pediastrum duplex* from Station B

Station B

Days	Growth (cell (1×10^4) /ml)	Nitrate (mg/L)	Phosphate (mg/L)	Silica (mg/L)
0	10	6.67	0.93	22.33
2	11.25	6	0.87	20.83
4	13.75	6	0.8	20.17
6	15.25	5.67	0.7	19.67
8	17.5	4.67	0.63	18.67
10	22	3.67	0.6	17.5
12	25.25	2.33	0.57	16.67
14	26.75	2	0.5	14.17
16	28.5	1.33	0.37	13.83
18	29.75	1.33	0.23	13

Pediastrum duplex inoculated in water sample from Station B

	growth	*nitrate*
growth	1	
nitrate	-0.99266	1

The correlation for these two variable is = -0.993

	growth	*phosphate*
growth	1	
phosphate	-0.95643	1

The correlation for these two variable is = -0.956

	growth	*silica*
growth	1	
silica	-0.98256	1

The correlation for these two variable is = -0.983