REMOVAL OF SYNTHETIC DYE FROM WATER BY *CHLORELLA VULGARIS* MICROALGAE

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A project report submitted in partial fulfilment of the requirements for the award of Bachelor of Engineering (Hons.) Petrochemical Engineering

Faculty of Engineering and Green Technology
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DECLARATION

I hereby declare that this project report is based on my original work except for citations and quotations which have been duly acknowledge. I also declared that it has not been previously and concurrently submitted for any other degree or award at UTAR or other institutions.

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APPROVAL FOR SUBMISSION

I certify that this project entitled **REMOVAL OF SYNTHETIC DYE FROM WATER BY *CHLORELLA VULGARIS* MICROALGAE** was prepared by **CHIN JIAN YEAN** has met the required standard for submission in partial fulfilment of the requirements for the award of Bachelor of Engineering (Hons) Petrochemical Engineering at Universiti Tunku Abdul Rahman.

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ABSTRACT

Textile dyeing is in the ranking number 2 of the polluter of fresh water. To prevent the coloured waste water to be discharged which polluted the environment, the coloured waste water commonly will be treated to the acceptance level which was comply with the environmental protection laws before discharged to the environment. Microalgae is one of the potential method that can remove the textile dyes from the wastewater. It is a promising alternative solution to remove the textile dyes because it is clean, environmental friendly and reduce the greenhouse gases. Moreover, the biomass of microalgae can be produce as biofuels and contain many nutrients that benefits to human. However, the properties of synthetic dyes will affect the potential of bioadsorption, biodegradation or bioconversion of dye by microalgae. Therefore, the study onto different synthetic dye with different property is important to investigate the dye removal mechanism used by microalgae. In this study, *Chlorella vulgaris* microalgae is used to remove two synthetic dyes with different surface charge that are methylene blue which is positive surface charge and acid orange 7 which is negative surface charge. Results show that the microalgae able to remove the methylene blue by electrostatic interaction through adsorption while the acid orange 7 dye is not able to be removed. The highest removal efficiency of methylene blue was at initial concentration of 100mg/L which was 83.04 ± 2.94% at day 3. The removal mechanism used by the microalgae was more favour towards Langmuir isotherm with 128.21mg/g maximum adsorption capacity (q_{max}), 0.0096L/mg adsorption equilibrium constant (K_L) and 0.8216 R^2 value. The incubation period was prolonged to 4 weeks for initial concentration of 50mg/L and 100mg/L methylene blue was done and found out that dye removal efficiency had reached to stationary state after incubated for 1

week. The cell density in this both concentration were found decrease during the incubation period. Biochemical composition of the microalgae were studies after the treatment of the synthetic dye. Result showed that the total protein, total carbohydrates and total chlorophyll were the highest at week 1 which were 46.65 ± 2.6 mg/L, 73.33 ± 40.55 mg/L and 247.31 ± 17.88 mg/L at 100mg/L initial concentration of methylene blue.

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Currently, textile dyeing is in the ranking number 2 of the polluter of fresh water. World Bank predicted about 17 to 20 % of industrial water pollution problem are come from the process of textile dyeing and finishing treatment given to fabric. A total 200,000 tons of dye stuff is estimated lost from the processes of textile industry was drained into the global environment every year and the concentration of azo dyes can reach up to 500 parts per million which also equivalent to 500 mg/L in the textile effluent. In Indonesia, hundreds of textile factories along the line of the Citarum River are discharging the waste water dye into the fresh water river. A pound of chemical will be illegally drained into the Citarum River for one pound of production of textile. However, a total 35 million of people are still using Citarum River as their drinking water source. This scenario caused increase in disease rate such as cancer, skin disease and slow mental development in children due to the high concentration of dyes according to the Daily Mail (LaRose, 2017).

In textile industry, dyes are the tools that have been used for producing the textile art. Dye can be classified by many classes which depending on the classify ways. For example, they can classified by according to chemical constitution, manufacturer, application and colour. There are several types of dyes which are direct dyes, vat dyes, sulphur dyes, azoic dyes, reactive dyes, mordant dye, acid dyes, disperse dyes and acid dyes which are classified according by their chemical properties. These dyes are synthetic

organic dyes that made from the cracking of crude oil which are petrochemicals products from petrochemical industry (Tripathy et al., 2017).

To prevent the coloured waste water to be discharged which polluted the environment, the coloured waste water commonly will be treated to the acceptance level which was comply with the environmental protection laws before discharged to the environment. There are 3 techniques to remove the textile dye waste water before it is discharged into the environment. These 3 methods can be classified as chemical technique, physical technique and biological technique. Absorption by activated carbon, membrane filtration and electro-kinetic coagulation are the physical method. Activated carbon can remove wide variety of dyes but is very expensive. Membrane filtration method can removes all types of dyes but produce concentrated sludge after filtration. Electro-kinetic coagulation method is very economical but high sludge production. The chemical treatment consist of photochemical, electrochemical destruction and Fenton reagent. The advantage of photochemical technique is no sludge production and foul odours are produced but the disadvantage is formation of by-products. Electrochemical destruction process do not consume chemical and without sludge build up but the disadvantage in this process is high flow rate tend to cause a direct decrease in dye removal performance. Fenton reagent is cheap reagent and effective process in removal of dye but sludge is created and has problem on disposal (Dawood and Sen, 2014). The biological techniques are decolourization of dye by bacteria, decolourization by white rot fungi and adsorption by living or dead microalgae biomass. The advantage of decolourization of dye by bacteria is can removed in efficient and low operational cost but needs to provide suitable growth condition for the growth of bacteria. The advantage of white rot-fungi are using the enzyme to degrade the dye but the enzyme production are unreliable. The advantage of the adsorption by living or dead microalgae biomass is certain dyes can bind with microalgae species but not effective to all dyes (Doble and Kumar, 2005; Adegoke and Bello, 2015).

Microalgae which are also known as microphytes are unicellular species. They will commonly found in fresh water and aquatic system which exists individually, in

chains or in groups. They absorb light energy from the sun and carbon dioxide and then convert into sugar, proteins and expel oxygen through photosynthesis. Over this 2.7 billion years, microalgae are the one who drives, balances and maintains global ecosystem by creating half the world oxygen. Nowadays, microalgae not only just maintain ecosystem, they also help to manage the waste by absorbing such as chemicals, heavy metals and pesticides (Abdel-Raouf et al., 2012). Furthermore, microalgae can absorb carbon dioxide to reduce the greenhouse gases (Sayre, 2010). Moreover, they also can be as biomass to produce as biofuels which are more efficient than other feedstock (Arenas et al., 2016). This is because a small amount of microalgae can produce more biofuels than other feedstock.

Biofuel can be classified by several generations. The first generation of biofuels are came from food crops that can be consumed by human such as sugar, starch or grains. The disadvantages of the first generation of biofuels are they have caused the world price of food and animals feed increases and requires lots of land to produce biofuels (Advanced Hardwood Biofuels Northwest, 2014). Due to this advantages of first generation of biofuels, here came the second generation of biofuels which also known as advanced biofuels that are made from non-food type crops such as wheat and corn wastes, lignin polymers and woody cellulose (Advanced Hardwood Biofuels Northwest, 2014). These feedstocks will not compete with the food crops. The third generation of biofuels are derived from microalgae. Previously, microalgae were stick with the second generation of biofuels. Since microalgae inputs to produce biofuels is much lower than the other resources and produce a higher yield than other feedstock (Gao et al., 2012), it has been suggested promote into their own category which is third generation of biofuels (Alam et al., 2015).

There are several applications for biofuels nowadays. The first application of biofuels are in transportation. The biofuels which are biodiesel and bio-ethanol can replace the diesel and gasoline. They can be used by the engine vehicles with none or with little modification of cars. They can replaced the diesel due to the lower combustion emissions than diesel per equivalent power output and biodiesel are renewable resources (Mata et

al., 2010). The second application of biofuels will be as fuels in heating energy generation sector and in the electrical energy generation sector. Biofuels as fuels in the heating generation sector can reduce 12 % of carbon dioxide emission while reduce 30% carbon dioxide emission in the electrical energy generation sector (Adeniyi et al., 2018). The third application is biofuel can clean up the oil spillage. Biodiesel will be commonly as the cleaning agent to clean the oil spillage by spraying on to the crude oil and the crude oil will dissolves into the biodiesel. Then the mixture of biodiesel and crude oil can be remove by water to separate out the mixture (Pereira and Mudge, 2004).

The application of microalgae to treat the coloured waste water is found novel and outstanding if it can treat the waste water and at the same time able to produce biomass for biodiesel production purpose. Therefore, this project will study the performance of microalgae in coloured waste water treatment purpose and also for biomass production purpose.

1.2 Problem Statement

From literature review, one of the weakness by employing microalgae to treat the coloured waste water is this method is not effective to all dyes (Doble and Kumar, 2005; Adegoke and Bello, 2015). The properties of synthetic dyes will affect the potential of bioadsorption, biodegradation or bioconversion of dye by microalgae (Ghazal et al., 2018). Therefore, the study onto different synthetic dye with different property is important to investigate the dye removal mechanism used by microalgae. In this study, two synthetic dyes with different surface charge which are cationic methylene blue and anionic acid orange 7 are chosen for this study.

From the study of Hamadi et al. (2017), they proved that the *Spirullina platensis* microalgae had removed the synthetic dyes, which are Acid Black 210 and Acid Blue 7, up to 95.35 % and 92.56 % respectively. This finding had proven the method of using

microalgae to treat the coloured waste water is reliable. From the study of El-Kassas and Mohamed (2014), they found that *Chlorella vulgaris* microalgae able to treat the textile waste effluent and had removed the dye up to 75.68 % by using the waste water with moderate concentration as the cultivation medium. Moreover, they had demonstrated the capability of biomass production too. From literature review, *Chlorella vulgaris* is a potential biofuel producer due to it contain 28 to 33 % oil in cells (Abdullah et al., 2017). In medical research, *Chlorella vulgaris* contains high quality protein, chlorophyll and vitamins that are beneficial to human body which are improve the immune system, detoxify and heal, slow down ageing process and protects against degenerative and chronic health problems (Kantilal, 2003). Therefore, *Chlorella vulgaris* is being selected as study model in this research. The ability of *Chlorella vulgaris* to remove the selected synthetic dyes from water should be studied. Moreover, the growth of microalgae and also the characteristics of the biomass in function of wastewater treatment duration should be investigated. The study on the effect of synthetic dye toward the quality of biomass is important to ensure the usage of *Chlorella vulgaris* biomass produced.

1.3 Objectives

1. To characterize the microalgae.
2. To study the mechanism of synthetic dye removal by *Chlorella vulgaris* microalgae.
3. To study the growth and biochemical composition of the microalgae after dye removal.

1.4 Outline of Study

In chapter one, background of current textile dyeing and microalgae are introduced. Problem statement are about the problem faced by microalgae when treat coloured wastewater. The objectives are listed out in this study.

In chapter two, the types of synthetic dyes are reviewed. The physical, chemical and biological method to remove dyes from wastewater are also reviewed. These methods efficiency of removing the synthetic dyes are explained in details. The characteristics and potential of microalgae are also reviewed.

In chapter three, a flow diagram is drawn to explain the flow of experiment which was cultivation of the *chlorella vulgaris* microalgae until the biomass investigation. In this research, the materials and equipment used are listed out. Moreover, the methodology are explained in details.

In chapter four, the characteristics of synthetic dye and microalgae are studied in this research. The ability, efficiency of the microalgae removing the synthetic dye and mechanism of removing the dye by microalgae are also discussed. Furthermore, the biochemical composition of microalgae are also studied and discussed too.

In chapter five, the results and discussion are concluded. Furthermore, there are some recommendations listed down in this research for further improvement.

CHAPTER 2

LITERATURE REVIEW

2.1 Wastewater in Textile Industry

Nowadays, textile industry will be the most important industry in India due to textile industry is stand about 14 % of the total industrial production and it also stand about 3 % of total Gross Domestic Product in the country. Large amount of water will be consumed in textile industry to produce the textile which is about 200 m^3 of water per ton of product. Volume of wastewater produced from the production will be about 90 % of the consumed of water which is about 180 m^3 of water per ton of product. Dye can be classified as a component that very difficult to treat in the textile waste water. Commonly there will be dye washed off during the dyeing process due to the inefficiency of dyeing process. So, the residual dye present in the dye bath effluent will be highest about 50 % of the initial dye usage (Mondal et al., 2017). From the study of Shyam Sundar et al. (2018), the dye concentration in the waste water textile will be 800 mg/L. A total 5 to 10 mg/L of dye concentration can be found in the river on 50 days each year while the worst case is 1200 to 1500 mg/L of dye concentration found in the river on 2 days each year from some scenario analysis (Carmen and Daniela, 2012).

2.2 Synthetic Organic Dyes

Synthetic organics dye which are made from petrochemicals are mainly used by the textile industries. Mauve dye, a fuchsia colour dye is a first synthesis dye which was discovered by W.H. Perkin in 1856. The synthetic dye are produced by chemical synthesis. Synthetic dye are aromatic compounds which contains aromatic rings in the structure of the synthetic dye. The delocalized electrons and different functional groups can be found in the aromatic rings. Chromogen-chromophore structure represent the colour of the synthetic dye and it is also an acceptor of electrons. Auxochrome group which are also ionizable groups are the donor of electrons and also represent the dyeing capability. Dyeing capability refers to the binding capacity of the dyes onto the textile materials. Benzene, naphthaline or antrancene are the aromatic structure that are establish as the chromogen. Azo group (-N=N-), carbon-nitrogen (=C=NH; -CH=N-), carbon-sulphur (=C=S; ≡C-S-S-C≡), carbonyl group (=C=O), ethylene group (=C=C=), methine group (-CH=), nitro (-$N0_2$; -NO-OH) or nitrozo (-N=O; =N-OH) can be represent as the chromophore structure. Amino (-NH_2), carboxyl (-COOH), sulphonate (-SO_3H) and hydroxyl (-OH) are the example of auxochrome groups (Suteu et al., 2011). The chromogen will be binding with chromophore as shown below in the Figure 2.1. Table 2.1 shows the some chromophore groups presents in organic dyes.

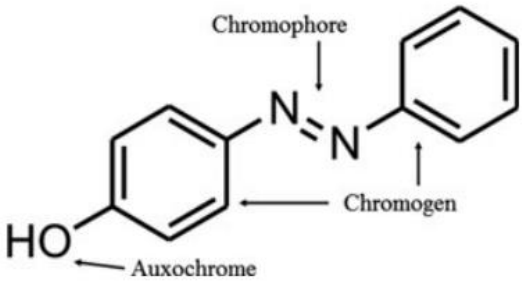
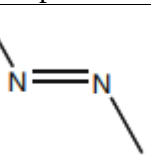
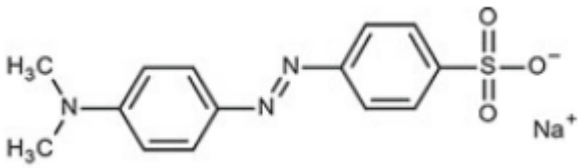
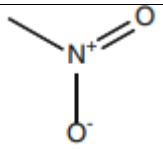
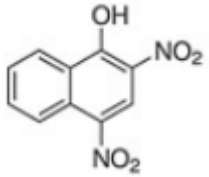
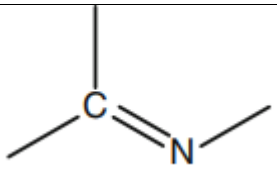
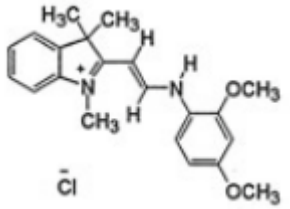
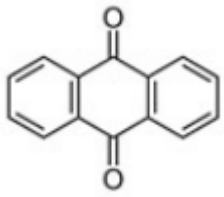
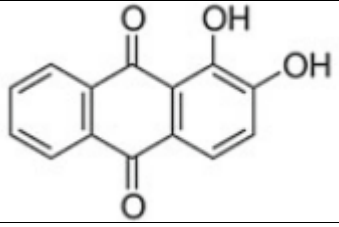
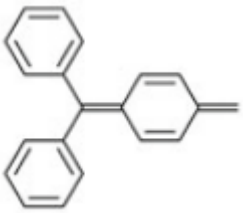
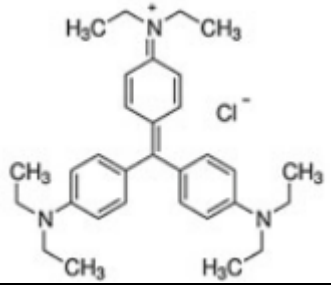
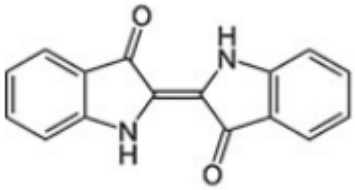
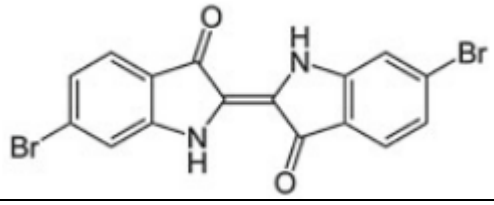
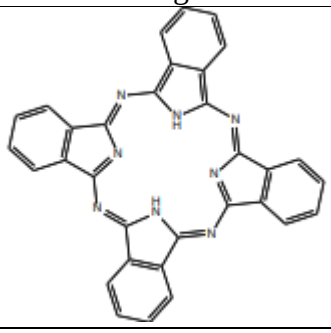
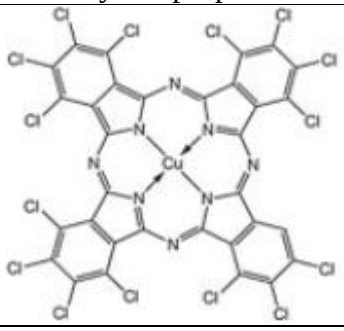


Figure 2.1: The chromogen-chromophore structure of 4-Hydroxyazobenzene (Gürses et al., 2016).

Table 2.1: Chromophore groups presents in organic dyes (Gürses et al., 2016).

Chromophore Groups	Sample Dye
	
Azo	Methyl Orange
	
Nitro	Martius Yellow
	
Methine	Basic Yellow 11

	
Anthraquinone	Alizarin
	
Triarylmethane	Ethyl Violet
	
Indigo	Tyrian purple
	
Phtalocyanine	Pigment green 7

Dyes also have certain uses such as used as indicators and used in colour photography, but most of the dyes are as a tools in colouring the fibres in textile industries. Dyes can be classified by many ways. According to Carmen and Daniela (2012), they can be classified by their chemical structure or application method. Dye that classified by according to their chemical structure will be Azo dyes, nitro dyes, indigoid dyes, anthraquinone dyes, phthalein dyes, triphenyl methyl dyes and nitroso dyes. Direct dye,

vat dyes, sulphur dyes, azoic dyes, reactive dyes, acid dyes, basic dyes and disperse dyes are classified according to their application method. The dye also can be separated into three category which are cationic dye, anionic dye and non-ionic dye. Direct dye, acid dye and reactive dyes are anionic dyes while the basic dye will be cationic dye. Disperse dyes are non-ionic dye which does not ionise in the aqueous solution.

2.2.1 Direct Dyes

Direct dyes are an anionic dye which contain an azo group, stilbene, phthalocyanine, dioxazine, formazan, anthraquinone, quinolone or thiazole as the main chromophore. According to M. Clark (2011), parameters such as chromophore, fastness properties or application characteristics can be classified by the direct dyes. Direct dyes are water soluble dyes and they are commonly applied on cellulosic fibres. Wash-fastness performance of the direct dye is only moderate has led to the replacement by the reactive dyes which has higher washing fastness properties on cellulosic substrate. Washing-fastness performance is a test that to determine the ability of fabrics retain on dyes that used to colour on them. The direct dye also can be used as pH indicator, biological stains, paper and leather.

2.2.2 Vat Dyes

Vat dyes are water insoluble dyes. They can be change to water soluble dyes which also known as leuco compound by adding reducing agent under alkaline condition (M. Clark, 2011). Vat dyes can be converted to water soluble dye due to the presence of carbonyl groups (C=O) in their structure. According to (Klaus, 2004), the reducing agent can be sodium dithionite in the presence of sodium hydroxide. This water soluble dye can be

absorbed by the cellulose. After that, regenerates of the parent form which are the insoluble vat dyes occurs by the oxidation of the leuco compound within cellulose fibres. Vats dyes generally will be used on cellulose fibres and cotton.

2.2.3 Sulphur Dyes

Sulphur dyes can be divided to four groups according to the Colour Index (M. Clark, 2011). Colour Index is a name of the particular dye has been given to describe a commercial product by its recognised usage class in term of hue and a serial number. The four groups will be C.I. Sulphur dyes, C.I. Leuco Sulphur dyes, C.I. Solubilised Sulphur dyes and C.I. Condenese Sulphur dyes. C.I. Sulphur dyes are insoluble in water, C.I. Leuco Sulphur dyes are soluble in water, C.I. Solubilised Sulphur dyes are highly soluble in water. C.I. Condenese Sulphur dyes are not made in the market nowadays. Among all the classes of dyes, sulphur dyes can be classified has the dullest range of colours and inexpensive (Benkhaya et al., 2017). Sulphur dyes will be mostly used for cotton and linen.

2.2.4 Azo Dyes

Azo dyes are water insoluble dyes. At least one azo group (-N=N-) can be found in the azo dyes which attached to one or two aromatics rings such as benzene and naphthalene. Aromatic heterocyclic units also can attach with the azo group. Monoazo dyes contains one azo linkage, diazo dyes contain two azo linkage while triazo contain three azo linkage (Benkhaya et al., 2017). Azo dyes are produced by the interaction of a diazonium compound with a coupling component and directly dye on to the textile fibres. The particular combination of diazo and coupling components used will determined the hue produced (M. Clark, 2011). The highest dyestuff production volume nowadays will be azo

dyes due to the simplicity of coupling reaction. These dyes used in cosmetic, textile and pharmaceutical.

2.2.5 Reactive Dyes

Reactive dyes are anionic dyes. According to Klaus et al. (2004), reactive dyes contains specific functional groups such as OH, SH, and NH_2 groups. This functional groups will form covalent bond with the substrate such as cellulose fibres that to be coloured on it. According to Ahmed (1995), and Nkeonye (1989), covalent bond that had been formed with the dye and the substrate would produce a high washing fastness properties. The energy to break the covalent bonds will be same as breaking the fibres itself. Reactive dyes commonly contains a reactive group, a chromophore group, a bridging group and a solubilizing group. Reactive group in the reactive dye is the ability of the dye molecule to form a covalent bond. Reactive group can be monofunctional or bifunctional reactive system (Nkeonye, 1989). Monofunctional reactive system can only react once with the nucleophilic groups in the fibres. Bifunctional reactive system are contains two separate reactive centres. This two separate reactive centres are for the reaction with the groups that contain in the fibres. Chromophore groups in the reactive dyes can be monoazo, diazo, metallized monoazo, metallized diazo, formazan, anthraquinone, triphenodioxazine, and phthalocyanine (Waring D.R., 1990). The responsibility of linking the chromophore and the reactive system will be the bridging groups. These groups are very important in the reactive dyes because they can affect the reactivity, stability of the reactive dyeing and other characteristics such as substantivity. Water solubility, substantivity and wash off are characteristics that under solubilizing groups.

2.2.6 Acid Dyes

Acid dyes are soluble in water and produce coloured anions in solution. Acid dyes usually contain sulphonic acid in their structure. Water solubility of the acid dye are due to the presence of this group structure. According to Vashi et al., (2014), the chromophore group of the acid dye can be azo, anthraquinone, azine, pyrazalone, nitro and nitroso compounds. Acid dye can be apply to the dyeing of polyamide, leather, paper, cosmetic and plastic.

2.2.7 Basic Dyes

Basic dyes are cationic dyes and are water soluble dyes. They are attracted to the substrate with a negative charge by electrostatic forces. The salt linkage will be formed between the substrate and cationic dyes. According to Benkhaya et al. (2017), basic dyes have a poor migration properties. Normally, basic dyes will applied with retarder agent to overcome the migration properties. High substantivity of the dye for the substrate is the major issue that produce the poor migration properties. Basic dye can be used on dyeing acrylic fibres, wool and silk.

2.2.8 Disperse Dyes

Disperse dyes are non-ionic dyes and also scarcely soluble in water. According to Becerir and Iskender (2003), the disperse dyes can be azo and anthraquinone dyes. The disperse dyes will normally contain auxiliaries such as the dispersing agent. The dispersing agent purpose is to soluble and disperse the dye in the dye bath which can help for a uniform dyeing of the substrate. The disperse dye also dissolve in water at a higher dyeing temperature. The disperse dyes will be normally dyes on the polyester fibres.

2.3 Physical, Chemical and Biological Method to Remove Dyes

There are several methods to remove dyes from the textile wastewater. The methods can be classified to physical, chemical and biological methods. Physical methods will be absorption by activated carbon, membrane filtration and electro-kinetic coagulation. Chemical method will be photochemical, electrochemical destruction and Fenton reagent. The biological method are decolourization of dye by bacteria, decolourization by white rot fungi and adsorption by living or dead microalgae biomass.

2.3.1 Activated Carbon Method

Activated carbon can remove dye from the textile water. They are highly porous materials which lead them to have a high surface area and high adsorption capacity. According to Baysal et al. (2018) experimental result, sodium hydroxide activated carbon by using sunflower pith based had removed 527.9 mg/g of methylene blue dyes in a 530 mg/L of initial methylene blue dye concentration. In 1000 mg/L of methylene blue dye solution, the activated carbon had removed 958.9 mg/g of dyes. The time taken for the adsorption rate are 180 minutes. Sago waste had been converted to activated carbon with aid of the sulfuric acid and ammonium per sulphate according to Kadirvelu et al. (2005). A total 6 mg/g of rhodamine-B dye has been adsorb by the sago activated carbon in 10 mg/L of concentration of rhodamine-B dye with equilibrium time of 180 minutes. In 40 mg/L concentration of rhodamine-B dye, the adsorption was 14 mg/L with equilibrium time of 210 minutes. The adsorption rate is decreased as the dye concentration increase in this case. The duration for the adsorption rate will be 240 minutes. The factor that can affect the adsorption rate are particle size of adsorbent and the pH condition. The advantage of the activated carbon is can remove many types of dyes. From my point of view, although some activated carbon can removed the dyes in very high efficiencies, but there are many steps to done through to produce the activated carbon. For example according to Mahamad et al. (2015) the raw materials need to be immersed in the chemical activation agent for

24hours. Then it was dried at the oven at 110 ^{o}C for 1 days and then followed by carbonization at 500 ^{o}C for 1 hour. This needed large amount of time to synthesis the activated carbon and the cost of the activated carbon to be very expensive.

2.3.2 Membrane Filtration Method

Membrane filtration is the physical method that can remove the dyes by using reverse osmosis or nanofiltration. According to Abid et al. (2012), reverse osmosis membrane has removed 48 mg/L which is 96 % removal of acid red dye in initial concentration of 50 mg/L of acid red dye while the nanofiltration membrane has removed 93.77 % of acid dyes which are 46.885 mg/L in initial concentration of 50 mg/L of acid red dye. The duration for carrying this experiment is by using 2 hours. A removal of 97.7 mg/L of methylene blue in wastewater which initially contain 100 mg/L of methylene blue by using nanofiltration membrane system at 15 bar (Cebeci and Torun, 2011). For this experiment, the duration usage for the filtration is 9 hours. The factors that can affect the membrane filtration are the feed temperature, operating pressure on permeate flux, dye concentration and the pH condition. Membrane filtration is highly of removing the synthetic dyes, but it has several disadvantages. Sludge will be produced after the process of membrane filtration and membrane fouling may occur which the accumulation of feed stream within the pores of the membrane.

2.3.3 Electro-Kinetic Coagulation Method

Another physical method to remove the dyes will be electro-kinetic coagulation. Electro-kinetic coagulation is a process that forming the coagulants in situ by electrolytic dissolution of anode using the principles of electrochemistry. The anode will be usually iron or aluminium sheet. The coagulant will neutralize the charges of the particulates and

the particulates will stick together to form agglomerates (Butler et al., 2011). According to Malinović et al. (2017), the dye removal efficiency is 96.01 % which is 48.005 mg/L in the initial dye concentration of 50 mg/L and dye removal efficiency of 80.64 % which is 161.28 mg/L in the initial dye concentration of 200 mg/L. The dye that used in that experiment is Ostalan Black SR. According to Ghosh et al. (2008), the removal dye efficiency was 99.75 % which is 99.75 mg/L when the initial dye concentration is 500 mg /L. The dye that removing in this experiment is crystal violet dyes. The duration for the removing of the dye is set for 60 minutes. The factor that can affect the removal of dyes are initial dye concentration, pH value of the solution and inter electrode distance. Electro-kinetic coagulation can remove dyes with very high percentages but sludge will be generates after the treatment process.

2.3.4 Photochemical Method

Photochemical is an advanced oxidation chemical method that can remove the dyes. The dyes will be photodegraded under ultraviolet light using a catalyst such as TiO_2 or H_2O_2. They can degrade the dyes molecules to carbon dioxide and water. The molecule that responsible degraded the dye will be the hydroxyl radical. According to the result of Jafari et al. (2012), 94.8 % of dye removal is achieved which is 47.4 mg/L in the initial concentration of 50 mg/L within 80 minutes. But for the dye removal in the 100, 200, 300 and 500 mg/L, the result get were 72.3 % (72.3 mg/L), 48.6 % (97.2 mg/L), 33.5 % (67 mg/L) and 19.2 % (96 mg/L) within 90 minutes. According to the result of Chatzisymeon et al. (2013), the remazol black B dye removal is 53 % which is 84.27 mg/L in initial dye concentration of 159 mg/L in the presence of 1 g/L of TiO_2. When increasing the TiO_2 catalyst to 2 mg/L, the removal dye concentration is 77 % (122.43 mg/L) while 4 mg/L of TiO_2 catalyst remove 84 % (133.56 mg/L) of the initial dye concentration which is 159 mg/L. The duration that carried out this experiment are 300 minutes. The factor that can affect the result are the catalyst concentration and radiation source. Photochemical process

can remove dye in high percentage, no sludge production and foul odours are produced but there is still have a limitation and causes this process is still in the stage of experiment. Photocatalytic process requires ultraviolet irradiation for the activation of the catalyst, thus giving in an inefficiency of utilizing the sunlight (Dong et al., 2015). This is because UV in sun light just stand a small fraction which is 5 % while the UV in visible light are 45 %.

2.3.5 Electrochemical Destructive

Electrochemical is a technique that can treat the dye in the textile effluent. Two electrode will be insert in this process to remove the dyes. The selection of anode plate material will be more important due to the anode plate will be responsible on creating of oxidants in the wastewater (Radha and Sirisha, 2018). In the study of Yunus et al. (2009), the initial concentration of rhodamine 6G dye is 800 mg/L and the removal of the dye can be greater than 99.5 % which is only left less than 0.5 mg/L of dye in the wastewater within 8 minutes and conducted at pH 2, presence of 0.2 M NaCl with current of 1.9 A. According to Singh et al. (2013) , the Basic Green 4 dye with initial solution of 125 mg/L has been removed about 98.6 % (123.35 mg/L) while Basic Green 4 dye with initial solution of 325 mg/L has been removed about 78 % (253.5 mg/L). Both result that get are within 50 minutes. The factor that can affect the result are the electrolyte concentration, pH value and the distance between the electrodes. When increase of dye concentration, the dye removal rate decreases due to the insufficient of oxidants produced to interact with the high number of dye molecules or the fouling of the electrode which block the active site of the electrode. The disadvantages of the electrochemical technique is that the high flow rate can cause the removal of dye efficiencies decrease and high cost in electricity.

2.3.6 Fenton Reagent Method

Fenton reagent is an advanced oxidation process to treat wastewater that containing toxic and persistent pollutants. It is a homogeneous catalytic oxidation process that can produce of hydroxyl radical by induce a complex redox using the mixture of hydrogen peroxide iron II ions in acidic medium. The hydroxyl radical will react with the organics compound to form organics radical which can react with oxygen under oxidation process to form water and carbon dioxide (El Haddad et al., 2014). In the study of Bahmani et al. (2013), 97 % (242.5 mg/L) of reactive black 5 dye is remove by using initial concentration of 250 mg/L reactive black dye under optimum pH and temperature which is pH 3, 40 ^{o}C and using 50 mg/L of $FeSO_4$ and 300 mg/L of H_2O_2. According to Ertugay and Acar (2017), 94 % of direct blue 71 azo dye is removed under optimum pH, Fe^{2+} and H_2O_2 which are pH 3.0, 3 mg/L of Fe^{2+} , and 125 mg/L of H_2O_2. Both of the duration for the experiment of the dye to be treated in this study is set as 20 minutes. The factor that can affect the dye removal this process was the pH value, H_2O_2 dosage, Fe^{2+} dosage, initial dye concentration and temperature. Although this process is high efficiency remove the dye but there is a disadvantages which are formation of ferrous iron sludge (Hansson et al., 2012).

2.3.7 Decolourization of Dye by White Rot Fungi

Decolourization of dye by white rot fungi is a biological method. White rot fungi normally grow on lignin of the wood to degrade them. They are many kind of white root fungi that can degrade the dye such as *Trametes* sp, *Pleurotus ostrearus* and *Pleurotus calyptrarus*. White rot fungi can produce the extracellular oxidative enzymes which are Manganese Peroxidase, Laccase and Lignin Peroxidase (Sumandono et al., 2015). These enzymes that produce from the white rot fungi can help in the decolourization of the synthetic dye. According to Sumandono et al. (2015), three kind of white root fungi which are *Phanerochaete chrysosporium* ATCC 34541, *Ceriporiopsis subvermispora* ATCC 90467, and new isolated white fungus (KRUS-G) have been used to test to remove the Remazol

Brilliant Blue R (RBBR) dyes. The prepared method will be putting three mycelia plug with respectively white rot fungi with liquid nutrients in respective erlenmeyer flasks and are incubated. The result that get was more than 79 % (79 mg/L) decolourized of RBBR for KRUS-G while *C.subvermispora* decolourized 63 to 68 % (63 to 68 mg/L) and *P.crysosporium* decolourized 21 to 23 % (21 to 23 mg/L) which containing initial 100 mg/L of RBBR , operate at different pH which are 4, 5, and 6 while incubation of 6 days at 28 ^{o}C. In this experiment, the dye concentration and pH value will be factor that can affect the removal of dye and growth of fungal mycelia and also suggest that the responsibility of degrading the dyes was laccase enzymes. In the study of Chakraborty et al. (2013), novel white rot fungus *Alternaria alternate* CMERI F6 can decolourize initial concentration 600 mg/L of congo red dye with efficiencies of 99.99 % (599.94 mg/L) and decolourize efficiency of 78 % (624mg/L) in initial of 800 mg/L of congo red dye within 48 hours. The factors that can affect the removal of dye have studied in this experiment are the pH value, supply of carbon and nitrogen sources, dye concentration and heavy metals. In laboratory skill, commonly no problems will occurred for white rot fungi but in industrial scale, they have several disadvantages such as formation of mycelia aggregates, long growth cycle (Arenas et al., 2016).

2.3.8 Decolourization of Dye by Bacteria

Synthetic dye can be removed by bacteria which is a biological method by biodegradation. Using the bacteria to remove the dyes is an environmental friendly method. According to Bose and Anitha (2016), they have remove the dye by using bacterial consortium. Bacteria species of *Klebsiella* sp1, *Klebsiel* sp2, *Staphylcoccus aureus*, *Bacillus cereus* and *Pseudomonas fluorescence* have been consortium in this experiment. Yellow dye, green dye, blue dye, black dye and orange dye has been removed by 0.63 mg/L, 0.92 mg/L, 0.4 mg/L, 0.65 mg/L, 0.96 mg/L at the dye concentration of 1 mg/L by 48 hours. In the study of Saleh and Hazaa (2017), three types of bacteria are used which are

Achromobacter xylosoxidans MAM-29, *B. cerues* MAM-B22, *B. cerues* MAM-B11 and *Ochrobactrum* sp. MAM-C9. The bacteria *B. cerues* MAM-B22 removed the highest dye concentration of congo red between the other bacteria which was 24.23 mg/L, 46.67 mg/L, 89.82 mg/L, 123.765 mg/L, 151.14 mg/L, 207.54 mg/L and 266.92 mg/L of 25, 50, 100, 150, 200, 300 and 400 mg/L respectively by 3 days of incubation at 37 ^{o}C. In 100 mg/L of congo red dye, 90.21 mg/L, 90.03 mg/L, 89.62 mg/L, 85.84 mg/L was the maximum removal of dye by *Ochrobacterum* sp. MAM-C9, *B. cereus* MAM-B22, *A. xylosoxidans* MAM-29 and *B. cereus* MAM-B11 respectively at pH 7.0. According to Lalnunhlimi and Veenagayathri (2016), Direct Blue 151 and Direct Red 31 has been used to study for the removal of dye by the bacterial consortium from soil samples. In 200 mg/L of two types of dyes, 195.14 mg/L of Direct Blue 151 and 190.5 mg/L of Direct Red 131 have been removed by the bacterial consortium within 5 days. The factor may affect the removal of dyes by bacteria can be pH value and temperature. Although the dyes can be removed in efficient and by low operational cost, but the process duration was taking too long for the removal of the synthetic dyes and provide the suitable condition for the growth of the bacteria.

2.3.9 Decolourization of Dye by Microalgae

Microalgae can remove the synthetic dye by the adsorption and bioconversion. It is an economical way and eco-friendly technology on the removing of synthetic dyes of textile waters. In the study of Xie et al. (2016), live microalgae *Chorella sorokiniana* XJK was used to remove the synthetic dye of Disperse blue 2BLN. From the result they have got is removal of 83 % of the Disperse blue 2BLN which is 48.8 mg/L in the initial concentration of 60 mg/L in 6 days cultivation. In this experiment, they also study for the biomass content and the lipid content. A total 43 % of lipid content and 570 mg/L of biomass are obtained from the experiment. According to Hamadi et al. (2017), dead microalgae had been used as the study for the removal of the synthetic dye. Dead *Spirulina Plantensis* will

be the dead microalgae while the synthetic dye will be the Acid Black 210 and Acid Blue 7. Dead *Spirulina Plantensis* has removed 98.55 mg/L of Acid Black 210 and 97.05 mg/L of Acid Blue 7 in both initial concentration of 100 mg/L. Both result are using 0.5 mg/L of biomass and the removal time was 60 minutes for Acid Black 210 and 75 minutes for Acid Blue 7. In this experiment, optimum adsorption rate condition have been determined which are pH of 2.0, temperature of 60 °C, initial dye concentration of 125 mg/L and *Spirulina Plantensis* concentration of 0.5 g/L. In the study of Al-Fawwaz and Abdullah (2016), the live microalgae that used to treat the dye is *Desmodesmus* sp. The dye that use are methylene blue and malachite green to be treat by the microalgae. The microalgae will be in the form of immobilization or free state. In immobilization state, the *Desmodesmus* sp. has removed 95.7 % (4.75 mg/L) of methylene blue in initial concentration of 5 mg/L of methylene blue while removed 71.6% (14.32 mg/L) of methylene blue in initial dye concentration of 20 mg/L. In free state, the *Desmodesmus* sp. has decolourized 4.75 mg/L at 5 mg/L of methylene blue while decolourized 11.36 mg/L at 20 mg/L of methylene blue. In immobilization state and free state of *Desmodesmus* sp., both of the result removal are same which are 17.82 mg/L (89.1 %) at 20 mg/L of malachite green while 3.16 mg/L (63.2 %) at 5 mg/L of malachite green. Incubation that have used in this experiment was 6 days. The factor that can affect the alive microalgae removal of the synthetic dye are the initial dye concentration, contact time and the state of the microalgae which are free state and immobilization state while the dead microalgae can be affected by the pH value and microalgae concentration. Although microalgae still has some disadvantages which are need to use more time to decolourize the synthetic dyes and only decolourize some specific of dyes, but the advantages of microalgae are still more than the disadvantages. Microalgae are an economical way to remove the dye which can save the cost of the removing of synthetic dyes and eco-friendly to environment. Moreover, after the treatment of microalgae, biomass microalgae can be used to produce as biofuels. Therefore, microalgae is the best method on removing the synthetic dyes. In this research paper, *Chlorella vulgaris* green microalgae will be used to treat the synthetic dyes which are methylene blue and acid orange 7.

2.4 Characteristics of Microalgae

Microalgae are unicellular species and also known as microphytes. They are consists of two groups of microalgae which are eukaryotic species and prokaryotic species. They are photoautotrophic microorganisms which can undergoes photosynthesis due to their contained of chloroplast. *Glaucophyta*, *Chlorarachniophyta*, *Chlorophyta*, *Cryptophyta*, *Dinophyta*, *Euglenophyta*, *Haptophyta*, *Heterokontophyta* and *Rhodophyta* are the types of microalgae that under the eukaryotic species group while cyanobacteria which is a blue-green algae is under prokaryotic microalgae group (Duong et al., 2012). The size of the microorganisms can be ranged from 0.2 to 2 μm. They also can in the size which are higher than 100 μm. Normally, microalgae will be grow in the fresh water and the seawater but some of the species of microalgae can survive and grow in extremely saline environment such as Dead Sea in Israel (Singh and Saxena, 2015). Microalgae will using sunlight and carbon dioxide and converts into sugar, protein and oxygen by photosynthesis (Baehr, 2014). Over this 2.7 billion years, microalgae are the one who drives, balances and maintains global ecosystem by creating half the world oxygen (Abdel-Raouf et al., 2012). Microalgae can grow rapidly and has large amount of lipid in their body content in the form of triacylglycerides (Duong et al., 2012).

2.5 Potential of Microalgae

Microalgae has been classified as the third generation of biofuels. They can be classified as their own category due to the microalgae inputs to produce biofuels is much lower than the other resources and produce a higher yield than other feedstock (Gao et al., 2012; Alam et al., 2015). For example, algae oil can be produced 30 times higher than the crops oils which are between 20,000-80,000 L algae oil per acre (Ravindran et al., 2016). Microalgae lipids can be converted to biofuels by the method of biochemical and thermochemical (Ravindran et al., 2016).

There are several advantages of microalgae to produce as biofuels as the alternative energy. The land usage of the microalgae to growth is smaller than the other biofuels crops. The oil content of the microalgae is higher than the other biofuels crops. Wide range of environments can be grown by algae such as fresh water, seawater, saline water, municipal and wastewater. Greenhouse gases such as carbon dioxide can be remove by the microalgae and reduce the global warming. Biodiesel, ethane and methane can be produce by microalgae and which can perform more efficiency than the fossil fuels (Duong et al., 2012). Therefore, the microalgae can be the potential as raw materials to produce as biofuels due to the above.

CHAPTER 3

RESEARCH METHODOLOGY

3.1 The Flow of Experiment

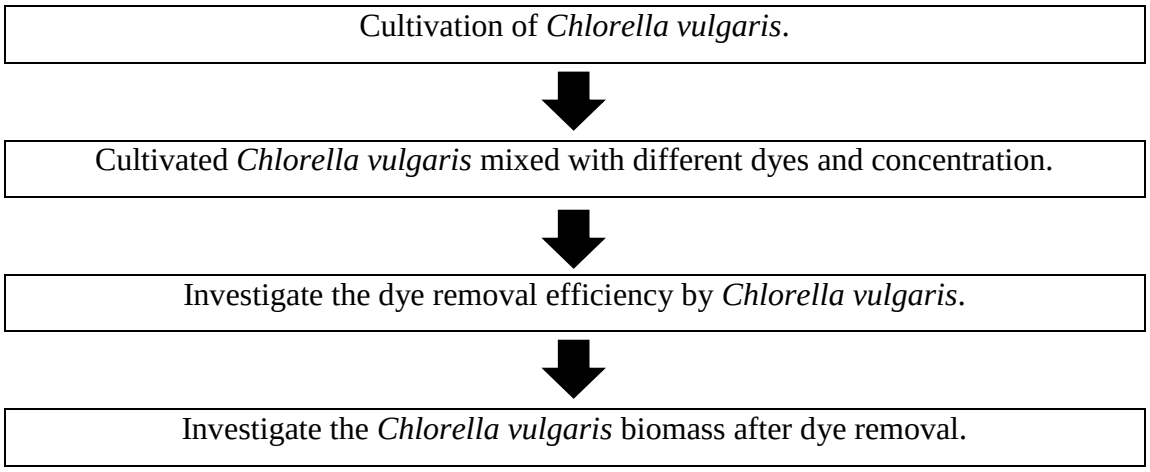


Figure 3.1: Schematic Diagram of Flow of the Experiment.

3.2 Materials and Chemicals

Material/Chemical	Supplier/Source
Methylene Blue	MERCK
Acid Orange 7	MERCK
Bold's Basal Medium (BBM)	USM
Microalgae Strain	USM

Equipment	Supplier/Source
Centrifuge	Ara Gemilang Saintifik Sdn.Bhd.
Autoclave	Himayama
Electronic Balance	BEC Enginee
Air Pump	Big Boy
Oven	Memmert
DR 6000 UV-Vis Spectrophotometer	Hach
Malvern zetasizer	Malvern Panalytical
Freeze Dryer	Bench Top

3.3 Cultivation of Microalgae

The *Chlorella vulgaris* strain are obtained from UMP, Pahang. A total of 10 L Bold's Basal Medium (BBM) are used to cultivate the stock of *Chlorella vulgaris* microalgae for 14 days. Air bubbling system and fluorescent lamp are installed to produce continuous light at 25 ºC (Toh et al., 2016).

3.4 Dye Removal by *Chlorella Vulgaris*

Before cell cultivation, temperature 121 ºC are set to autoclaved the medium and two blue cap bottle of 10 L for 15 min. A total of 250 mL of pre-cultivated medium at 14th day are taken and mix with the methylene blue and put into 500 mL conical flask. Experiment was carried out on the concentration of methylene blue dye of 50, 100, 300, 500, and 800 mg/L. After that, the dye removal efficiency by using *Chlorella vulgaris* microalgae will be measured by using UV spectrometer at 597 nm for everyday in 5 days. A calibration curve graph of concentration of methylene blue dye vs absorbance of solution are generated by series of dilution. The efficiency of dye removal was calculated by using the formula below:

$$Efficiency\ of\ dyes\ removed = \frac{C_c - C_o}{C_o} \times 100\ \% \quad (3.1)$$

where

C_0 = Initial dye concentration

C_c = Current dye concentration

The charge of the *Chlorella vulgaris* microalgae are measured by the Malvern Zetasizer. The biochemical composition of the biomass will be measured every week for 5 weeks. The experiment will be repeated by replacing methylene blue to acid orange 7 and measured by using UV spectrometer at 512 nm.

3.5 Measurement of Chlorophyll Content

A total of 2 ml of 90 % ethanol are used to extract the chlorophyll. Time to centrifuge are set for 2 minutes and at the speed of 10,000 g. After centrifugation, absorbance of the solvent extract are measured against a solvent blank. The below equation are used to estimate the chlorophyll content:

$$Total\ chlorophyll\ {}^{mg}/_{L} = (4\ \times A_{665}) + (25.5A_{650}) \quad (3.2)$$

where

A_{665} = Absorbance at wavelength of 665 nm

A_{650} = Absorbance at wavelength of 660 nm

3.6 Measurement of Carbohydrates

Sulfuric Acid-UV is a method to measure the carbohydrate content. A total 1 ml of supernatant was pipetted into a test tube and a total 3 mL of concentrated sulphuric acid solution is added into the test tube. Then, it is carefully vortexed for 30 s. The addition of sulphuric acid will rapidly increase the temperature in a few seconds. After that, the test tube with the mixture is dipped in the ice for 2 minutes to rapidly cool down the mixture to room temperature. Then, the absorbance of the solution is read by UV spectrometer at 315 nm. The amount of carbohydrate is determined by using the calibration curve that has constructed (Albalasmeh et al., 2013). The carbohydrate yield is defined as:

$$Carbohydrate\ yields(wt\ \%) = \frac{Carbohydrates\ concentration\ (g/L)}{Biomass\ concentration\ (g/mL) \times 1000} \times 100\ \% \quad (3.3)$$

3.7 Measurement of Protein Content

Water extraction method is employed to extract protein from the biomass. Dry biomass are mixed with distilled water (3 g/L). After that, the mixture of water and biomass are sonicate for 5 minutes. Then, centrifuge speed is set at 4000 g for the separation of the supernatant and cell debris for 5 minutes before testing of protein. BCA protein assay are used to measure the protein content by 96-well plate procedure. The mixture of 0.1 μL of

supernatant are placed in a well of the 96-well plate. Then 200 μL of working reagent are mixed well with the supernatant in a well of the 96-well plate. Next, the 96-well plate placed in oven for 30 min at 37 ºC. After that, the 96-well plate are cooled down to room temperature. Next, the absorbance of the solution are measured by UV spectrometer at 562 nm (Toh et al., 2016). Calibration curve of amount of protein (μg/L) against wavelength of absorbance of solution is constructed by series of dilution. The protein yield is defined as:

$$\text{Protein yield (wt \%)} = \frac{\text{Protein concentration ion (μg/mL)}}{\text{Biomass concentration (g/mL)} \times 10^6} \times 100\ \% \qquad (3.4)$$

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Characterize of the Microalgae.

Table 4.1: Charge of *Chlorella vulgaris* microalgae.

Surface	Zeta-potential (mV)
Chlorella vulgaris microalgae	-33.33 ± 1.60

From the Table 4.1 above, the result showed that the *Chlorella vulgaris* microalgae was negatively charged which was measured by Malvern zetasizer. The microalgae cell membrane will carried a net negatively charged due to the present of functional groups of –SH, -OH and –COOH which were the lipids, protein and sugars of the microalgae (Chan et al., 2012). The measurement of the net negatively charged result on the cell surface was due to the deprotonation of those ligands at natural pH water. In Figure 4.1 showed the FTIR spectrum of the *Chlorella vulgaris* microalgae before adsorption of the synthetic dye. As in the graph, there are two peaks which were the functional groups of –OH and –COOH which were peaks at 3422 cm^{-1} and 1410 cm^{-1}.

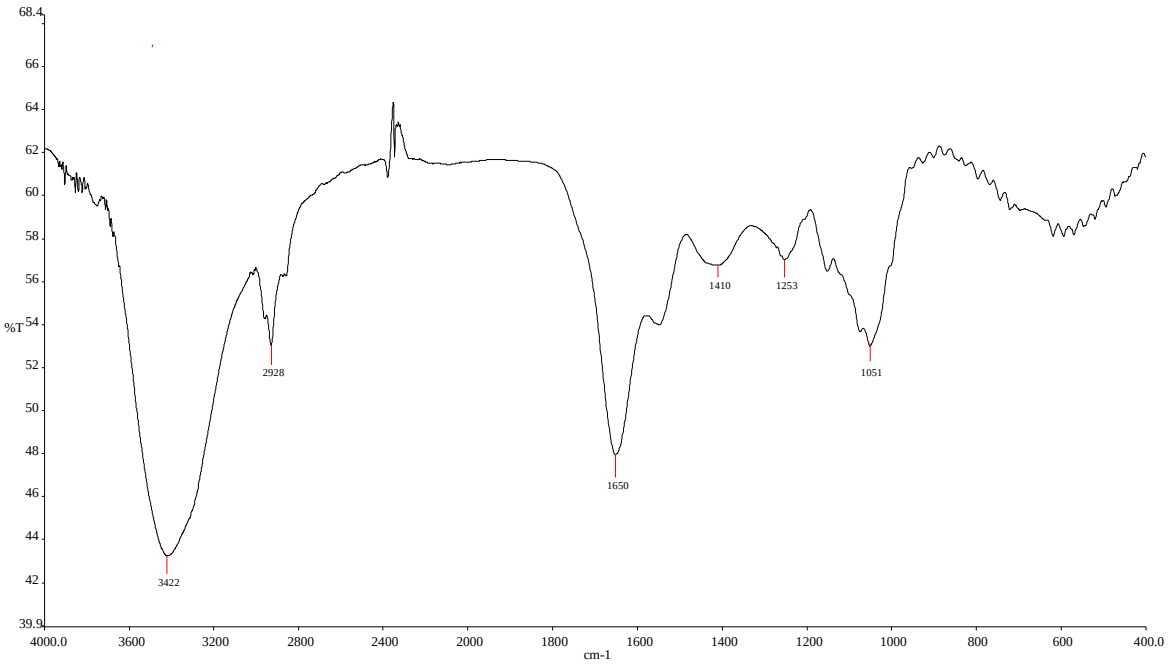


Figure 4.1: FTIR Spectrum of the *Chlorella vulgaris* Microalgae.

4.2 The Ability of Microalgae Remove Synthetic Dye

4.2.1 The Efficiency of Microalgae Remove Methylene Blue Dye

Figure 4.2 represent the efficiency of removing methylene blue dye at different initial dye concentrations by *Chlorella vulgaris* microalgae in function of day. The dye removal efficiency at day 0 that was right after the dye and microalgae mix together was significant. The dye removal efficiency almost maintained the same as day 0 even after 4 days of incubation. There were slight increase in dye removal efficiency from day 0 to day 1 when at initial dye concentration of 300 mg/L and above, but no change again for the following days.

Initial dye concentration of 100 mg/L removal was the highest, which was at 83.04 ± 2.94 % at day 3. The dye removal efficiency of methylene blue dye by *Chlorella vulgaris* microalgae was increased when initial dye concentration increase from 50 mg/L to 100 mg/L. However, the dye removal efficiency of methylene blue started decreased from 100 mg/L to 800 mg/L.

The methylene blue dye exhibits a positive charge while the *Chlorella vulgaris* microalgae exhibits a net negative charge on surface. The negative charge was due the present of functional groups such as hydroxyl group and carboxyl group which were the binding sites and cellulosic composition of the microalgae cell wall. These functional groups will acts as the adsorbing agent and attracted with the positive charge molecule by electrostatic force of attraction and leads to the adsorption of methylene blue dye (Al-fawwaz and Jacob, 2011). Hence, dye removal was achieved. Result showed that dye removal was effective on day 0 while not significant for the following days, which indicated that the main mechanism of dye removal by microalgal cells was through surface adsorption by electrostatic force. After surface adsorption, there was no significant uptake of dye by cells indicated that the microalgae would not consume methylene blue dye as their organic carbon source for growth (Lin and Wu, 2015).Therefore, the microalgae serve as a natural adsorbent for methylene blue dye through surface adsorption.

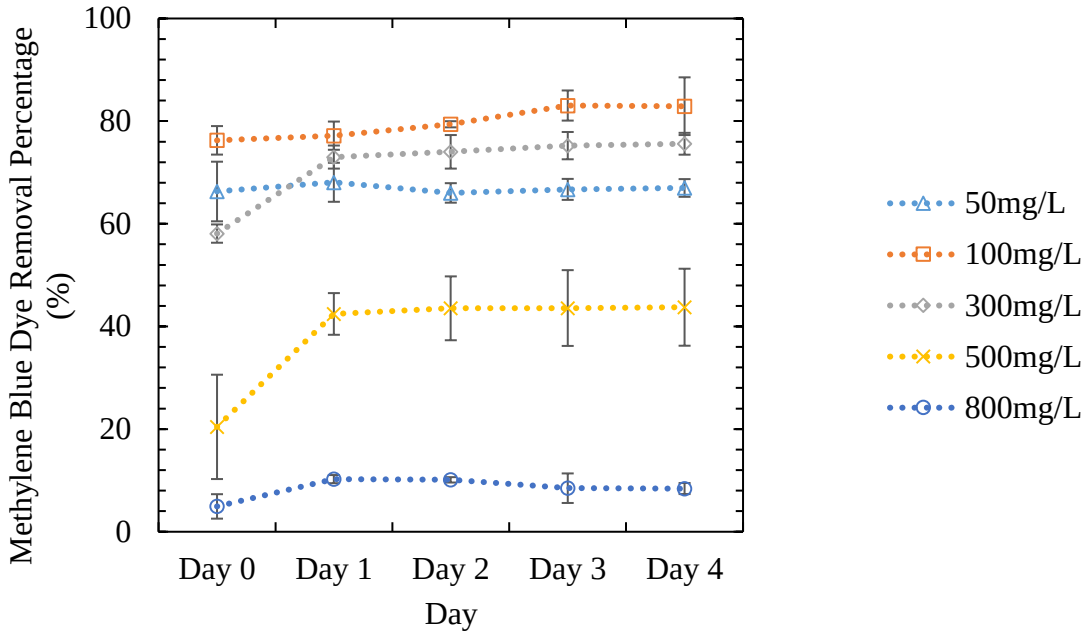


Figure 4.2: Methylene Blue Dye Removal Efficiency When at Different Initial Dye Concentration in Function of Time.

The capacity of methylene blue uptake by *Chlorella vulgaris* microalgae was shown in Figure 4.3 which was used to compare the performance of microalgae on dye removal when at different dosage of dye. The dye removal capacity by *Chlorella vulgaris* was increased from 50 mg/L initial dye concentration at 116.71 ± 10.22 mg/g to 100 mg/L of initial dye concentration at 134.26 ± 4.89 mg/g, which was the optimal dye removal capacity. The dye removal capacity was then decrease to 8.68 ± 4.19 mg/g when the initial dye concentration increase to 800 mg/L.

When the initial concentration of dye increased from 50 mg/L to 100 mg/L, there was more dye molecule found in the water medium when at 100 mg/L dye concentration compared to that of 50 mg/L dye concentration. The collision frequency between the dye molecule and microalgal cell was higher when at 100 mg/L dye concentration than that in 50 mg/L dye concentration (Mitrogiannis et al., 2015). Hence, more dye had adsorbed

onto cell surface successfully when at 100 mg/L dye concentration and so achieved higher dye removal efficiency.

When initial dye concentration further increase from 100 mg/L to 800 mg/L, the decreasing in dye removal efficiency showed that the microalgal cells had reached to an optimal for dye adsorption. The increment of initial dye concentration lead to the fullness occupy of *Chlorella vulgaris* microalgae surface functional groups. Moreover, the cells that had fully covered by a layer of dye tend to form repulsive forces with the free methylene blue dye molecules since both exhibited similar surface charge (Saeidi et al., 2017). Hence, dye removal capacity decreased when initial dye concentration more than 100 mg/L.

Therefore, the Chlorella vulgaris microalgae tend to remove methylene blue dye successfully through electrostatic attraction when at initial dye concentration of 100mg/L at day 0.

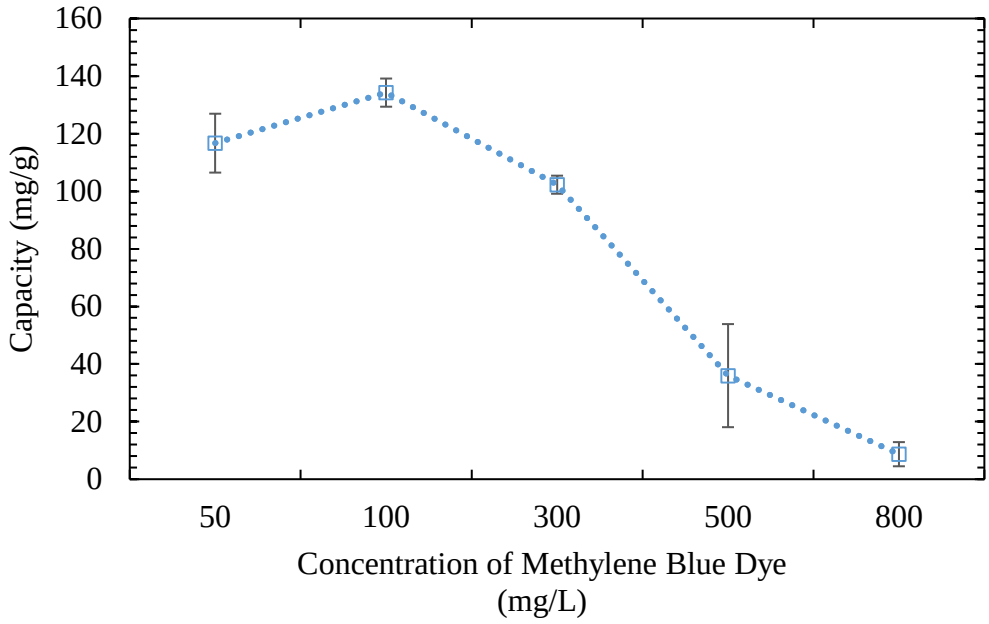


Figure 4.3: Capacity of Methylene Blue Dye Uptake in Function of Concentration of Methylene Blue Dye at Day 0.

4.2.2 The Efficiency of Microalgae Remove Acid Orange 7 Dye

Figure 4.4 showed the efficiency of removing acid orange 7 dye at different initial dye concentrations by *Chlorella vulgaris* microalgae in function of day. The dye removal efficiency almost maintained the same after the day 2 of incubation when at initial concentration of 50 mg/L, 100 mg/L and 300 mg/L. There were only slight increased in dye removal efficiency from day 2 to day 3 when at initial concentration of 800 mg/L but no change again for the following days.

The highest efficiency of removal acid orange 7 dye was 11.36 ± 1.53 % at day 4 which was at initial concentration of 500 mg/L. The lowest dye removal efficiency were 0 % when at initial concentration of 50 mg/L, 100 mg/L and 300 mg/L at day 2 and maintain for the following days.

The acid orange 7 dye and the *Chlorella vulgaris* microalgae exhibit a negative charge on surface. The acid orange 7 not able to adsorb onto the microalgal surface through electrostatic forces since both are same charge. Hence, dye removal was not able to achieve. There were small percentage of dye removal efficiency at day 0 was due to the weak Van der Waals forces between the acid orange 7 dye molecule and the microalgal surface (Hadjittofis et al., 2017).

In 500 mg/L and 800 mg/L concentration, there was suspected growth of fungus as seen through microscope in Figure 4.5. The decolourization of acid orange 7 dye when at initial concentration of 500 mg/L and 800 mg/L at day 4 can be contributed by fungus since there was no growth of any fungus at concentration of 50 mg/L, 100 mg/L and 300 mg/L and there were no removal of dye in these concentrations. Moreover, in the study of Saraf and Vaidya (2015), dead biomass fungal of *A. niger, A. oryzae, R. arrhizus* have removed acid orange 7 dye with efficiency of 43.97 %, 44.71 % and 45.93 % at pH 2 with same amount of dried biomass which was 50 mg and initial concentration of 50 mg/L of acid orange 7.

Therefore, the *Chlorella vulgaris* microalgae unable to remove acid orange 7 dye successfully through electrostatic attraction at all initial dye concentrations of acid orange 7.

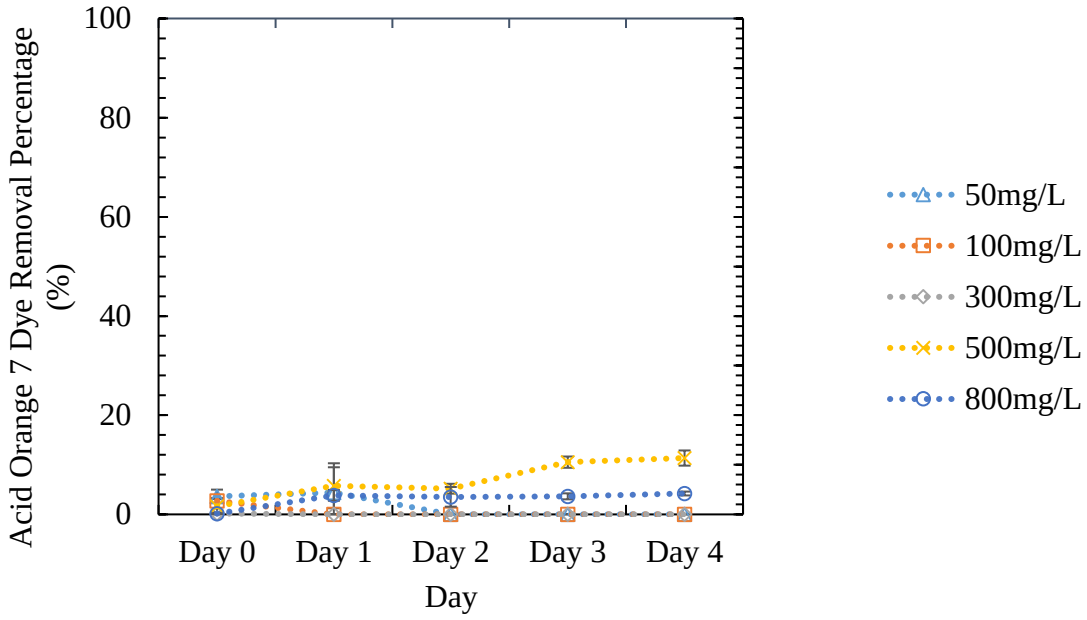


Figure 4.4: Acid Orange 7 Dye Removal Efficiency When at Different Initial Dye Concentration in Function of Time.

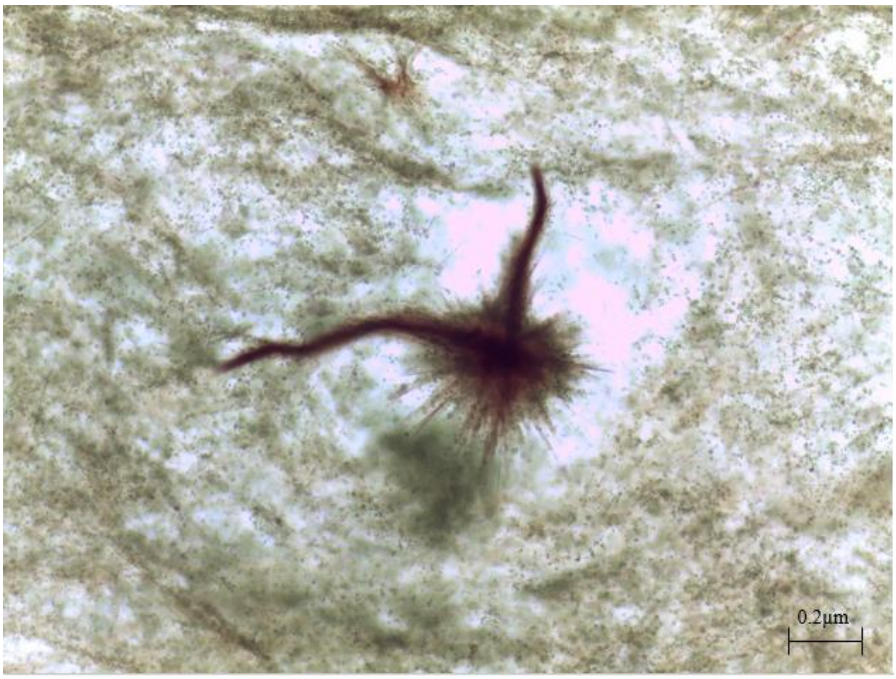


Figure 4.5: Growth of Fungus.

4.2.3 Methylene Blue Dye Removal by Adsorption

In theory of Langmuir isotherm had stated that the adsorption of the adsorbate was monolayer on the surface of the adsorbent which containing identical sites and without interact with the adjacent site (Langmuir, 1918; Sarwa and Verma, 2013). Langmuir isotherm of linear form equation can be represent by Equation 4.1:

$$\frac{C_e}{q_e} = \frac{1}{q_{max} K_L} + \frac{C_e}{q_{max}} \quad (4.1)$$

where C_e was represent as the equilibrium dye concentration in the solution (mg/L), equilibrium dye adsorb by the adsorbent was represent as q_e (mg/g), the maximum adsorption capacity of the adsorbent represent as q_{max} (mg/g) and K_L was referred as adsorption equilibrium constant (L/mg). R_L which was equilibrium parameter was added that to indicate the essential characteristics of Langmuir isotherm. The equation can be represent by Equation 4.2:

$$R_L = \frac{1}{1+K_L C_o} \quad (4.2)$$

where C_o was the highest initial concentration of adsorbate (mg/L) used in this study and K_L will be Langmuir constant (L/mg). The type of Langmuir isotherm to be either favourable, unfavourable, linear or irreversible was depending on the value of R_L. R_L greater than 1 was unfavourable ($R_L > 1$), R_L equal to 1 was linear ($R_L = 1$), R_L is between 1 and 0 was favourable ($0 < R_L < 1$) while R_L equal to 0 was irreversible ($R_L = 0$) (Che et al., 2014).

Table 4.2: Isotherm Result.

Langmuir isotherm coefficient				**Freundlich isotherm coefficient**		
Q(mg/g)	K_L(L/mg)	R^2	R_L	K_F (mg/g(mg/L)-1/n)	1/n	R^2
128.21	0.0096	0.8216	Favour	114.034	0.1374	0.0525

The Figure 4.6 shows the plotting of C_e/q_e versus C_e. This graph was described the relationship of amount of dye adsorb by *Chlorella vulgaris* microalgae biomass and the residual dye concentration. From the graph, the data that obtained showed that the maximum adsorption capacity (q_{max}) was 128.21 mg/g and R^2 value was 0.8216. The K_L value calculated was 0.0096 L/mg. The R_L value that calculated was 0.115 which was under favourable of Langmuir isotherm. These result proved that the methylene blue dye adsorption by *Chlorella vulgaris* microalgae was fit well to the Langmuir model. Therefore, the methylene blue dye molecules were monolayer adsorption on the *Chlorella vulgaris* microalgae surface and was not dependent on adjacent sites.

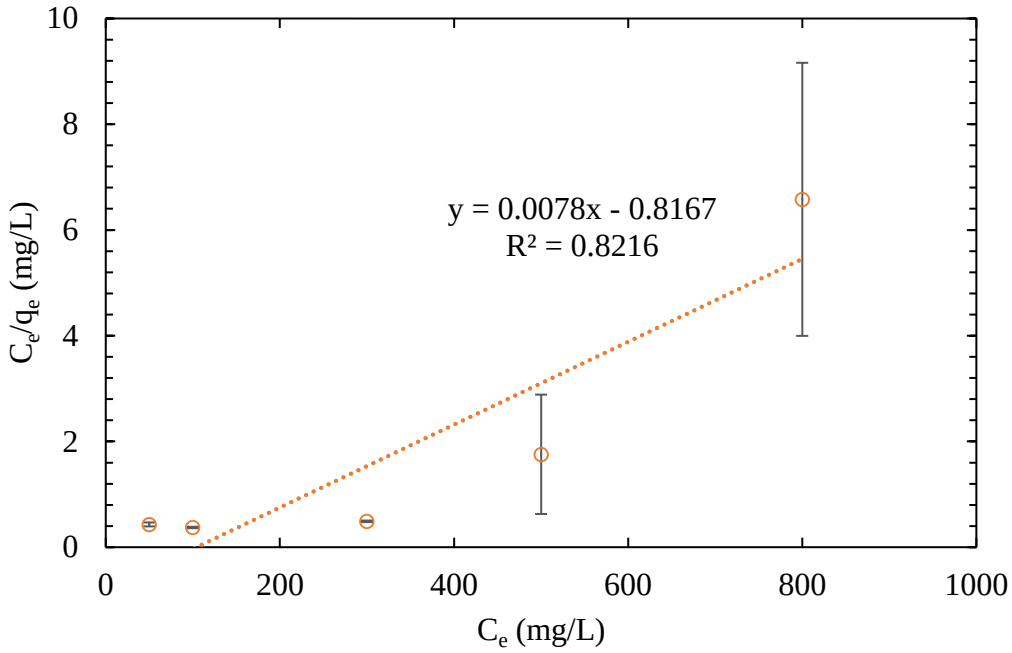


Figure 4.6: Langmuir Isotherm for Adsorption of Methylene Blue Dye by *Chlorella vulgaris* Microalgae.

In theory of Freundlich isotherm, the adsorption of the adsorbate was multilayer at heterogeneous surface between the liquid and solid phase capacity. There was an assumption made which was the adsorption sites were distributed exponentially with respect to the heat of adsorption. The equation was represent by Equation 4.3:

$$q_e = K_F C_e^{\frac{1}{n}} \quad (4.3)$$

Freundlich model in linear form was represent as Equation 4 after linearization of Equation 4.4:

$$\ln(q_e) = \frac{1}{n}\ln(C_e) + \ln(K_F) \quad (4.4)$$

where K_F (mg/g(mg/L)$^{-1/n}$) was represent as the multilayer adsorption capacity of adsorbent while n was indicate as an empirical parameter which was related to the intensity of adsorption and contrasts with the heterogeneity of adsorbent. The 1/n value which lies between 0.1 and 1 ($0.1 < 1/n < 1$) was represent as favourable Freundlich adsorption condition. Both K_F and n were Freundlich constant (Che et al., 2014; Lim et al., 2010).

From Figure 4.7, the R^2 value that calculated was 0.0525. The n value that got from the graph was 7.278 and the K_F value that obtained from graph was 114.034. The 1/n value was 0.1374 which was in between 0.1 and 1. Although the 1/n value was between the range of the favourable Freundlich adsorption condition, but the R^2 value was very low. These result obtained proved that was not fit well to Freundlich isotherm. Therefore, these data had been proved that the *Chlorella vulgaris* microalgae more favour in monolayer layer adsorption which was Langmuir isotherm than the multilayer adsorption which was Freundlich isotherm for dye removal.

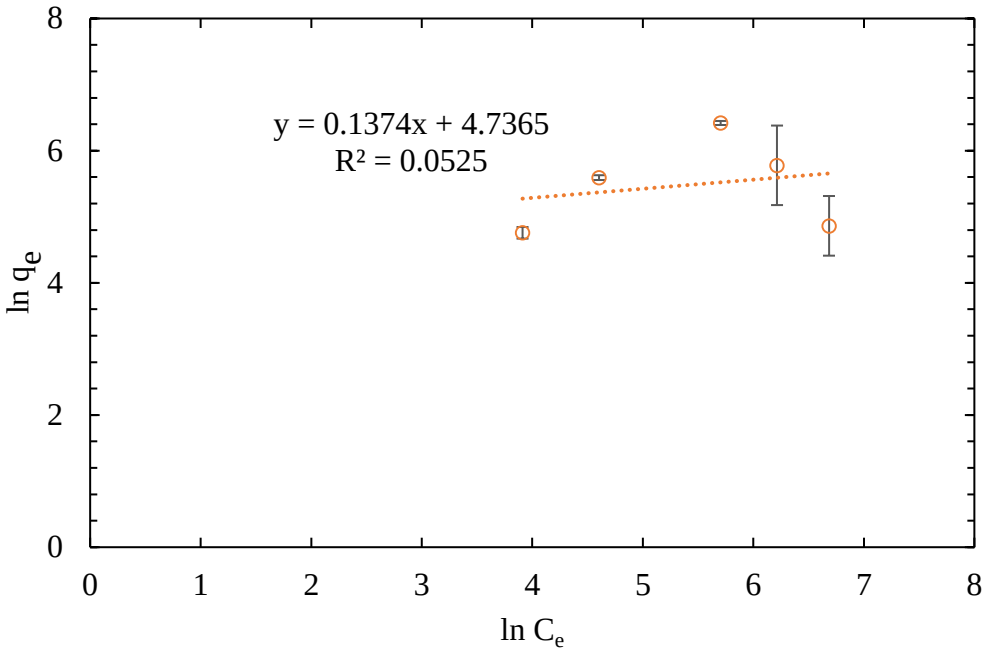


Figure 4.7: Freundlich Isotherm for Adsorption of Methylene Blue Dye by *Chlorella vulgaris* Microalgae.

4.2.4 Fourier-transform infrared spectroscopy (FTIR) Analysis

The FTIR spectrum of *Chlorella vulgaris* microalgae before and after adsorption of methylene blue were examined to determine the involvement of different functional groups of *Chlorella vulgaris* microalgae in dye adsorption. The responsibility of the adsorption process of adsorbate is the adsorbent that present with the chemical bands and molecular groups (Kumar et al., 2019). From Figure 4.8, there were total 6 peaks present before the adsorption of methylene blue dye by *Chlorella vulgaris* microalgae. After the adsorption of the methylene blue dye of microalgae, there were total 8 peaks found from Figure 4.9. There were 2 new peaks found after adsorption which were 1340 cm^{-1} and 1145 cm^{-1} which were the structure of C-N. The 1340 cm^{-1} peak was aromatic tertiary

amine CN stretch while the 1145 cm^{-1} peak was aromatic amine C-N stretch (Coates, 2006). These two structure were contained in the methylene blue dye as shown in Figure 4.10. Moreover, there were some peaks have been shifted after the adsorption of dye. The peaks of 3422 cm^{-1} and 1410 cm^{-1} were shifted to 3398 cm^{-1} and 1388 cm^{-1} after methylene blue dye adsorption. These have proved that there were involvement of methylene blue dye adsorption by hydroxyl group of surface of *Chlorella vulgaris* microalgae (Sarwa and Verma, 2013).

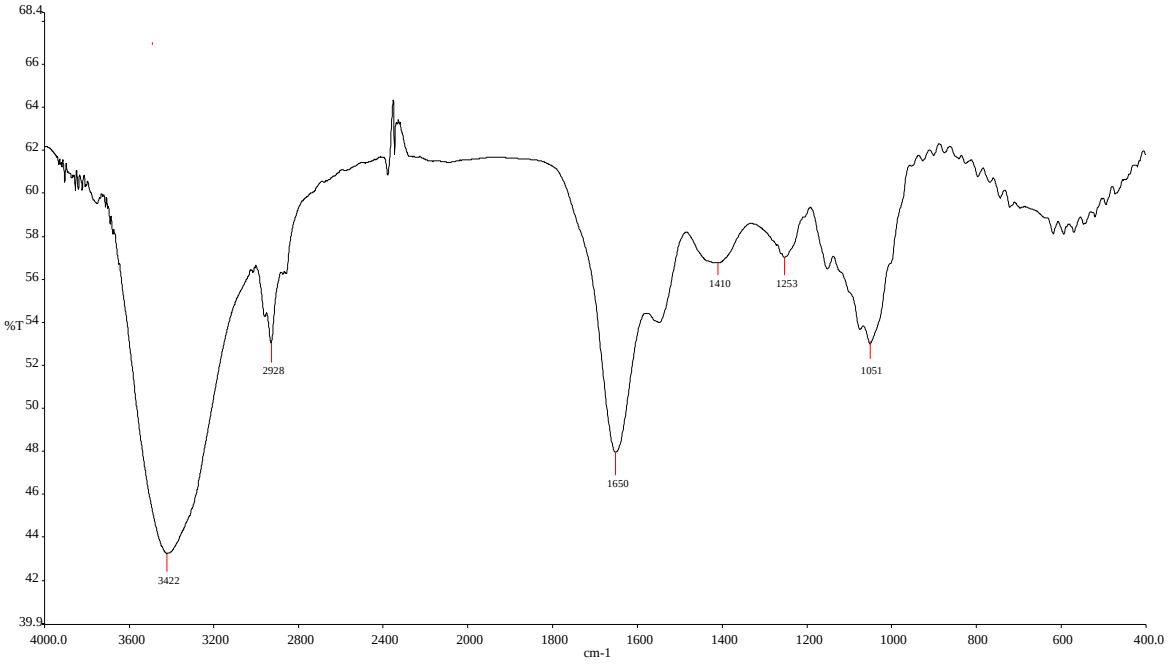


Figure 4.8: Before Adsorption of Methylene Blue Dye by *Chlorella vulgaris* Microalgae.

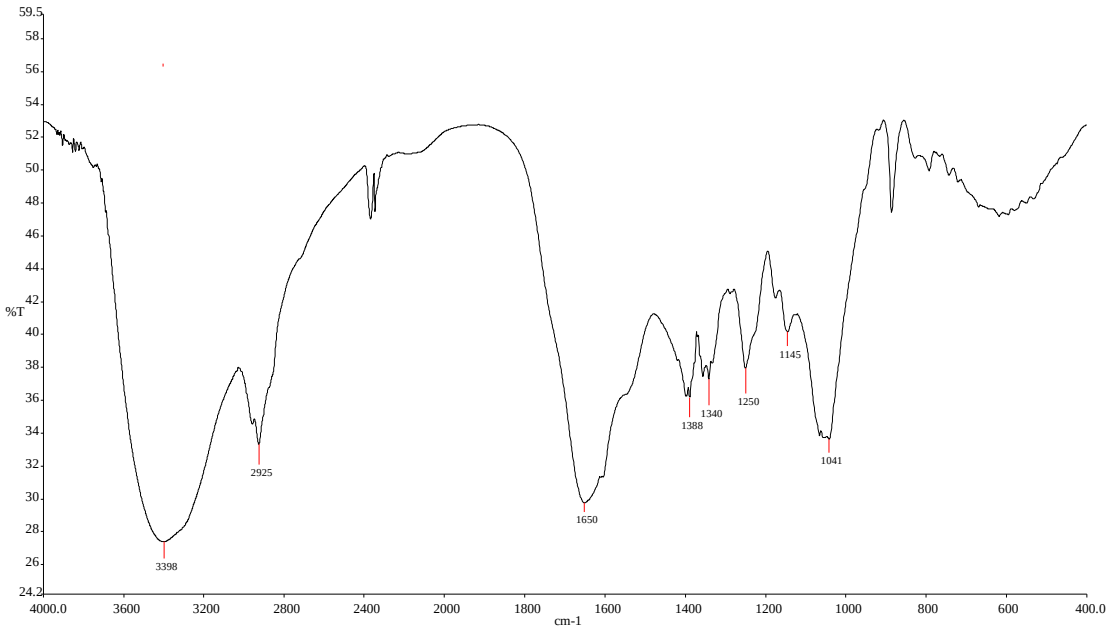


Figure 4.9: After Adsorption of Methylene Blue Dye by *Chlorella vulgaris* Microalgae.

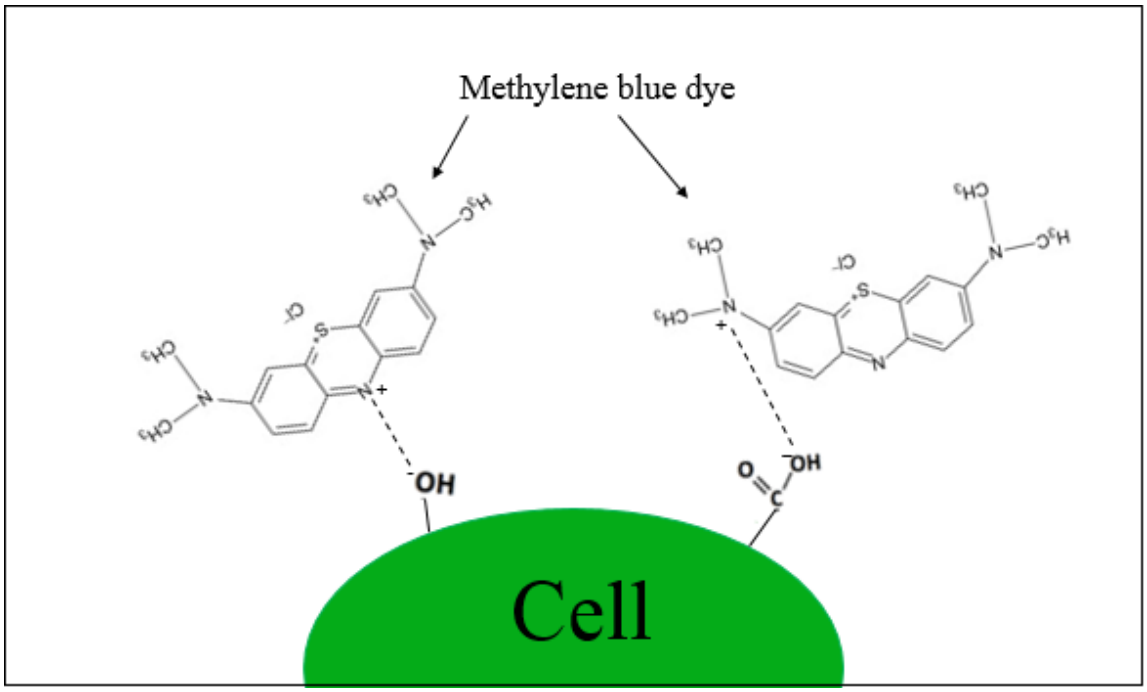


Figure 4.10: Electrostatic Interaction between the Microalgae and Methylene Blue.

4.3 The Growth and Biochemical Composition of Microalgae After Incubated with Synthetic Dye

4.3.1 Methylene Blue Dye Removal in a Month

In Figure 4.11, the duration of incubation for methylene blue dye at initial dye concentration of 50 mg/L and 100 mg/L was prolonged to 4 weeks since the dye removal efficiency of both concentration of dye had not reach to the stationary state after 4 days of incubation as shown in Figure 4.2. Figure 4.13 shown that the dye removal efficiency had reached to stationary state after incubated for 1 week. This result confirmed the mechanism of dye removal by microalgae was mainly through the electrostatic attraction interaction.

The slight fluctuation on the methylene blue dye removal between week 1 to week 4 was due to the molecule of methylene blue dye rapid diffusion from the microalgae surface to the free space of the medium of the adsorbate to attained equilibrium (Das and Swain, 2013). Furthermore, the ANOVA analysis confirmed there was changes on the dye removal from week 0 to week 4 in 50 mg/L concentration. However, the ANOVA analysis confirmed there was no changes on the dye removal from week 1 to week 4 in 100 mg/L concentration. At early stage, the time for the adsorption of methylene blue by microalgae would only affect the removal percentage of methylene blue but after the early stage the time would not be a factor that affecting the removal of the methylene blue dye. This was because at the early stage the microalgae surface functional group contain free availability for the rapid adsorption of the methylene blue dye molecules by electrostatic force and after the early stage the microalgae surface of the functional groups had been fully occupied by the methylene blue dye molecules(de Castro et al., 2017).

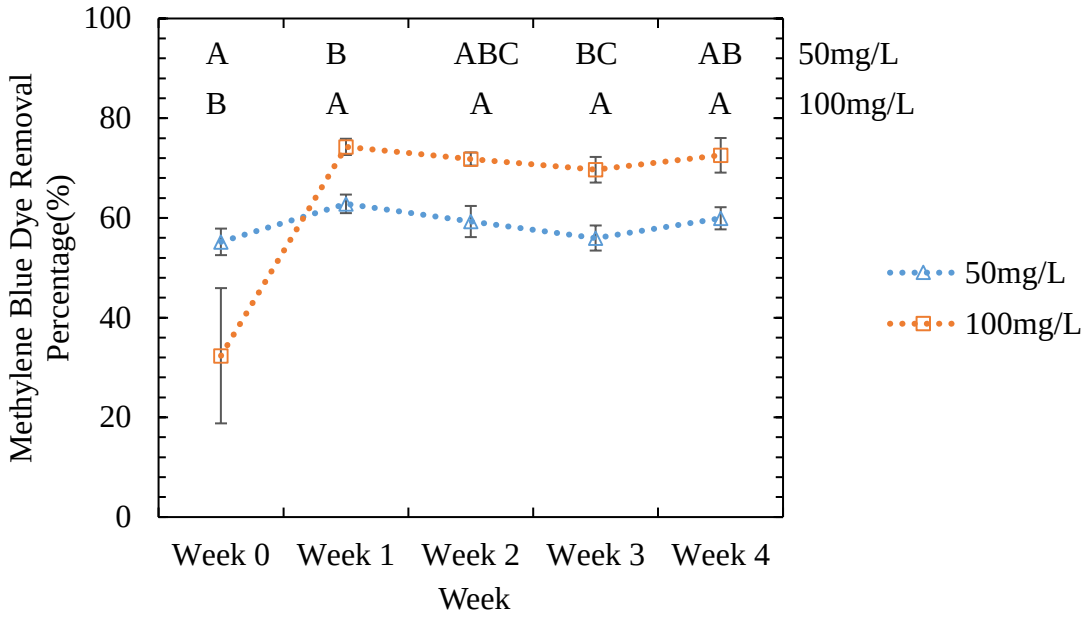


Figure 4.11: Methylene Blue Dye Removal Percentage in Different Concentrations in Function of Time. Statistically significant was evaluated based on one-way analysis of variance (ANOVA) followed by LSD all-pairwise comparison test at $p < 0.05$.

4.3.2 Cell Density of the *Chlorella Vulgaris* Microalgae in a Month

The cell density of the microalgae was being measured in function of week as shown in Figure 4.12. When at 50 mg/L of initial dye concentration, the cell density maintained from week 0 to week 2 and then decreased from week 2 to week 4. Result showed that the microalgae was started to die from week 2. When at 100 mg/L of initial dye concentration the cell density of the microalgae are gradually decreased from week 0 to week 4. Result showed that the microalgae was dying as well after incubated with 100 mg/L of dye.

The decreasing in cell density indicated that the microalgae was due to lack of the nutrients for the growth of the microalgae and also the presence of the dyes on the surface

of cells tend to block the light reach the cells for photosynthesis and hence prevent the normal growth of the microalgae (Carbajo Arteaga et al., 2018; Yang et al., 2018). From this study, result proved that the methylene blue dye was not the source of nutrient for the microalgae growth and it was only adsorption between the microalgae and methylene blue dye through the electrostatic force but the dye was not being uptake by microalgae for further growth.

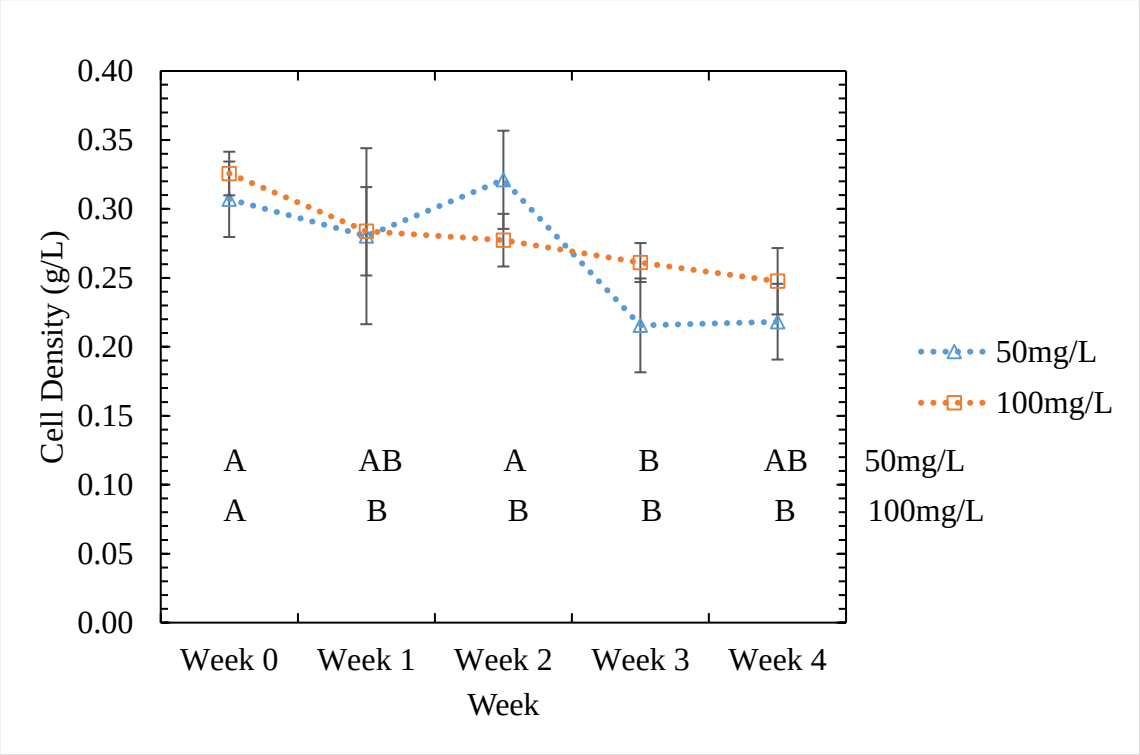


Figure 4.12: Cell Density of *Chlorella Vulgaris* Microalgae in Function of Time. Statistically significant was evaluated based on one-way analysis of variance (ANOVA) followed by LSD all-pairwise comparison test at $p < 0.05$.

After the water treatment of the synthetic dye by microalgae, the biochemical composition of the biomass of microalgae was being study to know whether the microalgae biomass had any other further usage or value. For collection of the microalgae biomass after water treatment, the microalgae can be undergo centrifugation for the

separation of microalgae biomass. But in industrial scale, the microalgae will undergo sedimentation process or flocculation process for the collection of microalgae biomass to reduce the cost. Since from the study the optimal was at 100 mg/L of initial methylene blue dye concentration and at week 1, so the following part just show the result for 100 mg/L.

4.3.3 Total Carbohydrate of *Chlorella Vulgaris* Microalgae

According to the Figure 4.13, the highest carbohydrate content was in week 1 which was 2.93 ± 0.79 wt %. From week 1 onwards, the carbohydrates content of microalgae decrease and remain constant.

The testing of the carbohydrate content was done because the carbohydrate content of the microalgae can be as the feedstock of the bioethanol production. The carbohydrate will undergoes fermentation process to produce bioethanol. The bioethanol production derived from microalgae can be from yeast fermentation or dark fermentation (El-Dalatony et al., 2017). Thus, the bioethanol production can replaced the limited fossil fuels. The high total content of carbohydrates can maximize the production of bioethanol. The microalgae can be harvested after the water treatment at week 1 for bioethanol production since the carbohydrate content was the highest at that moment.

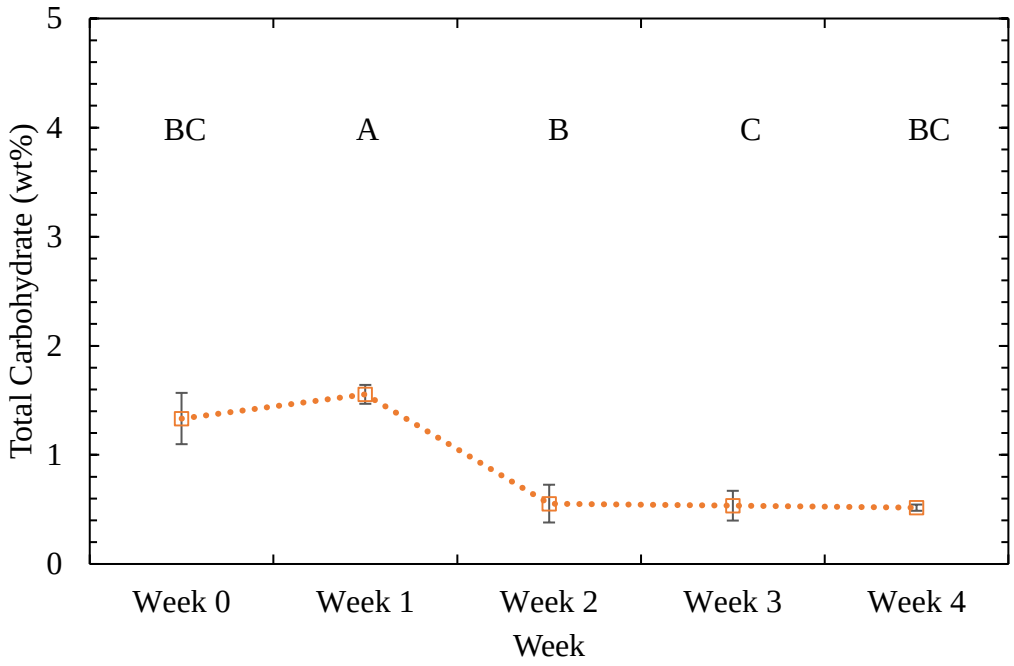


Figure 4.13: Total Carbohydrate of Microalgae in Function of Time. Statistically significant was evaluated based on one-way analysis of variance (ANOVA) followed by LSD all-pairwise comparison test at $p < 0.05$.

4.3.4 Total Protein of *Chlorella Vulgaris* Microalgae

The total protein content of microalgae was the highest at week 1 which was 2.44 ± 1.35 wt % according to the Figure 4.14. The total protein content was gradually decreasing from week 1 to week 4.

Microalgae contained protein nutrients which can be consume by humans as food. The derived protein from microalgae had complete Essential Amino Acid profiles. Moreover, the protein content in microalgae was higher than meat and dairy products. Thus, their protein nutrient was more benefits than the other conventional sources

(Koyande et al., 2019). Therefore, the microalgae can be harvested on week 1 and extracted the protein nutrient since the protein content was the highest at week 1.

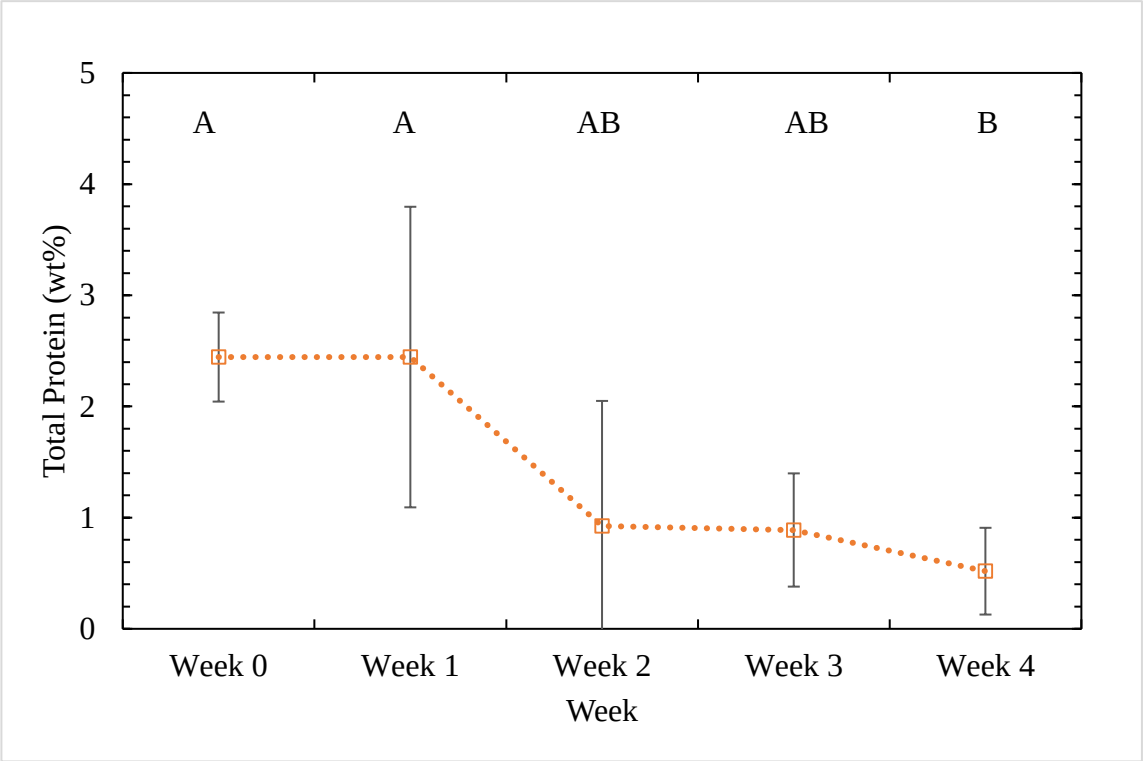


Figure 4.14: Total Protein of Microalgae in Function of Time. Statistically significant was evaluated based on one-way analysis of variance (ANOVA) followed by LSD all-pairwise comparison test at $p < 0.05$.

4.3.5 Total Chlorophyll of *Chlorella Vulgaris* Microalgae

According to the Figure 4.15, the highest total chlorophyll content of microalgae was at week 1 which was 247.31 ± 17.88 mg/L.

Chlorophyll was a green pigment which was important for the photosynthesis in algae by absorbing the sunlight and convert into chemical energy. Anti-inflammatory,

antimutagenic, antimicrobial and antioxidant properties will be obtain when chlorophyll was being consumed to the body (da Silva Ferreira and Sant'Anna, 2017). Moreover, the chlorophyll also can healing some of disease such as sores, ulcers and regulation of menstruation (Rani et al., 2018). Thus, the extraction of chlorophyll was very useful since there were many health benefits towards human.

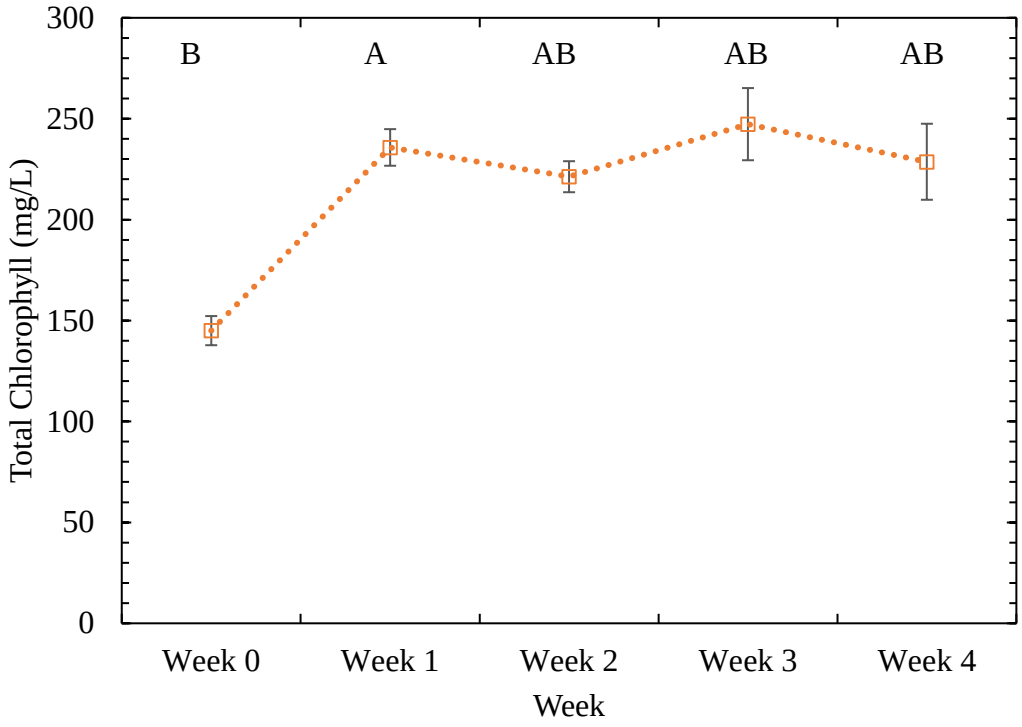


Figure 4.15: Total Chlorophyll of Microalgae in Function of Time. Statistically significant was evaluated based on one-way analysis of variance (ANOVA) followed by LSD all-pairwise comparison test at $p < 0.05$.

The removal of dye by using microalgae as natural adsorbent can perform in starting initial dye concentration of 100 mg/L and for 1 week duration. The biomass of microalgae after dye adsorption must be harvested right after 1 week of incubation in order to maintain the biomass quality for further processing such as dye desorption or

carbohydrate and protein extraction purpose. Carbohydrate can consume by yeast to undergo fermentation or dark fermentation process to produce bioethanol while protein and chlorophyll can be extracted for be as food since contained high nutrients which were benefits to human health.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The *Chlorella vulgaris* microalgae able to remove methylene blue dye by adsorption but not able to remove acid orange 7 dye by adsorption. This was because the *Chlorella vulgaris* microalgae surface was negative charge while the methylene blue dye was with positive charged were attracted by electrostatic forces while the acid orange 7 dye was similar charge with *Chlorella vulgaris* microalgae surface will repel with each other. The highest efficiency of the adsorption of methylene blue dye was at initial concentration of 100mg/L which was 83.04 ± 2.94 % at day 3. The lowest efficiency of removing methylene blue dyes was 8.41 % with initial concentration of 800 mg/L at day 4. When low concentrations of dyes, methylene blue dye molecule can rapidly occupy *Chlorella vulgaris* microalgae surface functional groups which thus increase the removal efficiency. When high concentrations of dyes, the saturations binding of *Chlorella vulgaris* microalgae surface functional groups with methylene blue dye molecules tend to repel the similar charge methylene blue dye molecules and thus inhibit further methylene blue dye adsorption. The acid orange 7 dye at day 4 showed dye removal occur at 500 mg/L and 800 mg/L were due to the growth of fungus and adsorption of the acid orange 7 dye.

In isotherm study, the methylene blue dye removal by *Chlorella vulgaris* microalgae was fixed to Langmuir isotherm in the range of favourable which was 0.115 and adsorption capacity of 128.21 mg/g.

Moreover, the mechanism of methylene blue dye removal by Chlorella vulgaris microalgae was confirmed is mainly through the electrostatic attraction interaction after prolong the duration of incubation to 4 weeks.

The cell density of microalgal decreased after 1 month of incubation was due to lack of the nutrients for the growth of the microalgae and also the presence of the dyes on the surface of cells tend to block the light reach the cells for photosynthesis and hence prevent the normal growth of the microalgae.

After the water treatment of the 100 mg/L of methylene blue dye by microalgae, the biochemical composition of the biomass of microalgae was being study .The total carbohydrates, total protein and total chlorophyll was the highest at week 1 which were 2.93 ± 0.79 wt %, 2.44 ± 1.35 wt % and 247.31 ± 17.88 mg/L at 100 mg/L respectively. These biochemical components provide many benefits to human such as production of bioethanol and acts as nutrients for health benefits. Therefore, the microalgae can be harvested at week 1 for the extraction of the nutrients and production of bioethanol since week 1 was the highest removal of methylene blue dye and highest biochemical composition of microalgal.

5.2 Recommendation

There were some recommendation to improve this research, such as:-

- The temperature surroundings of bioremediation of microalgae should be fixed to avoid the temperature surroundings affect the performance of microalgae.
- pH of the synthetic dye should be further researched to investigate the performance of removal of synthetic dye.

- Replaced chemical nutrients of cultivation of microalgae to lower cost substances for reducing cost.
- Study on the recovery of dye from microalgae which was desorption.
- Feasibility study onto real dye wastewater since the wastewater might contain lot impurities that we ignored in this fundamental study.

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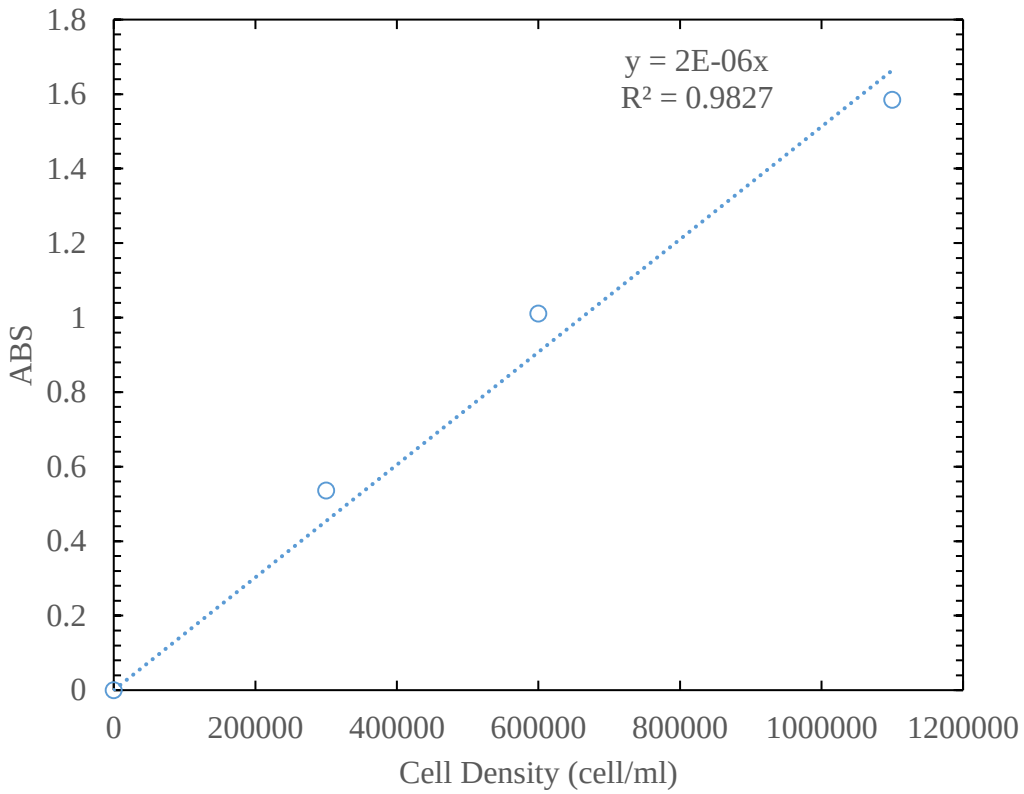
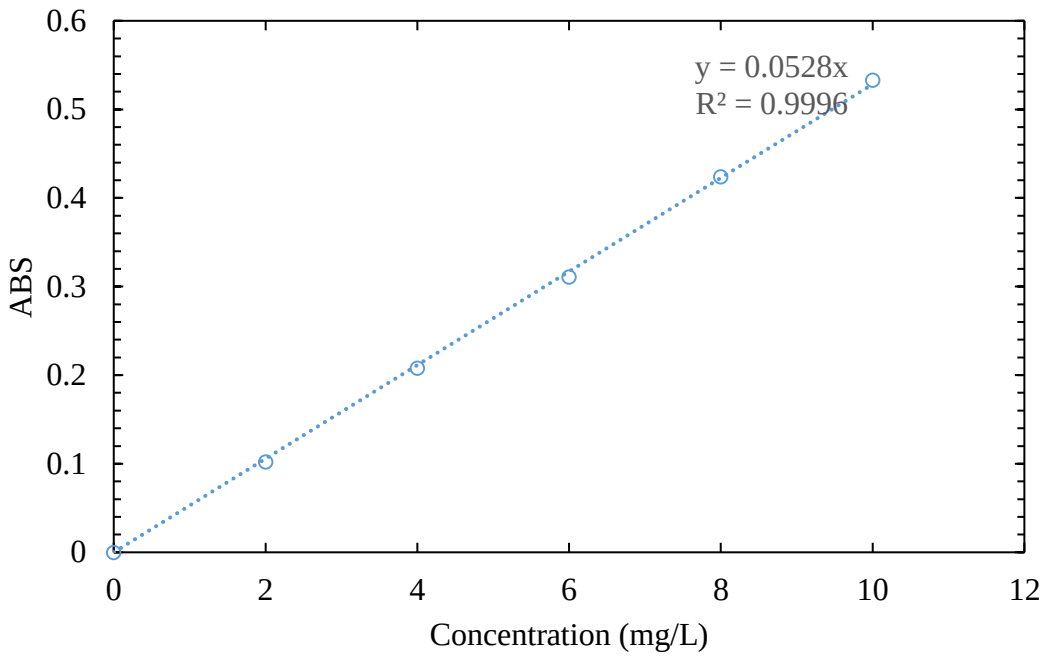
APPENDICES

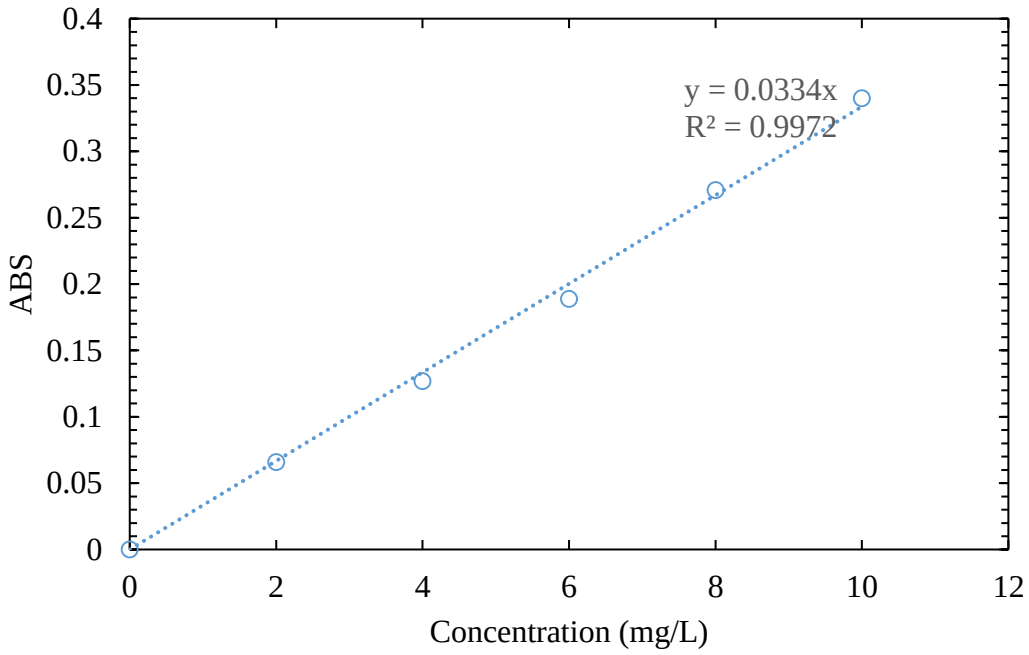
APPENDIX A: Calibration Curve of Microalgae Cell Density.



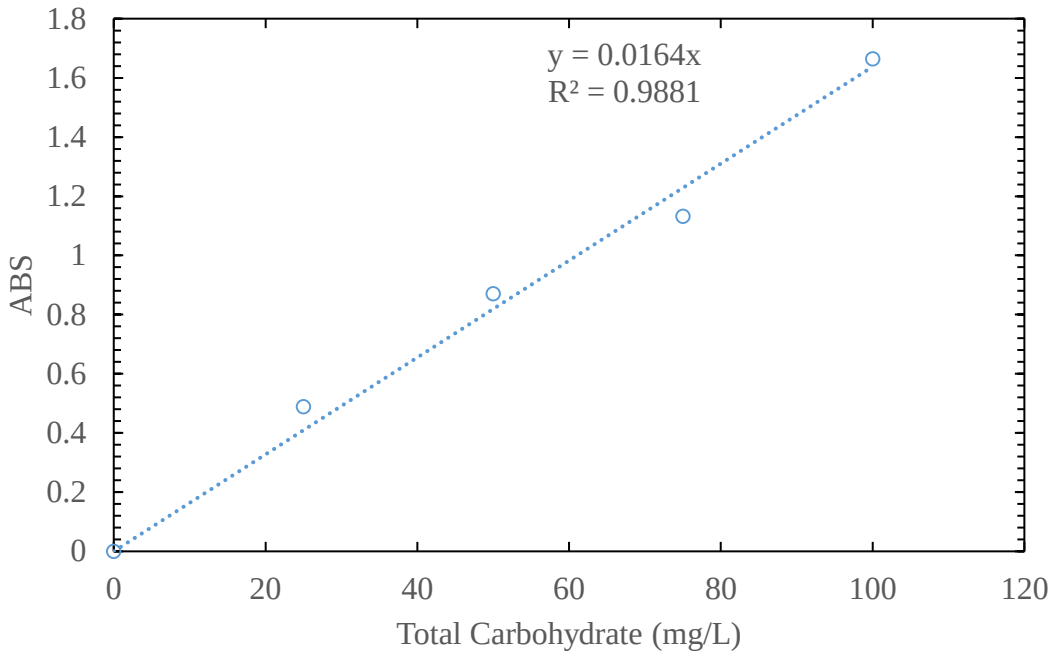
APPENDIX B: Calibration Curve of Acid Orange 7 Dye.



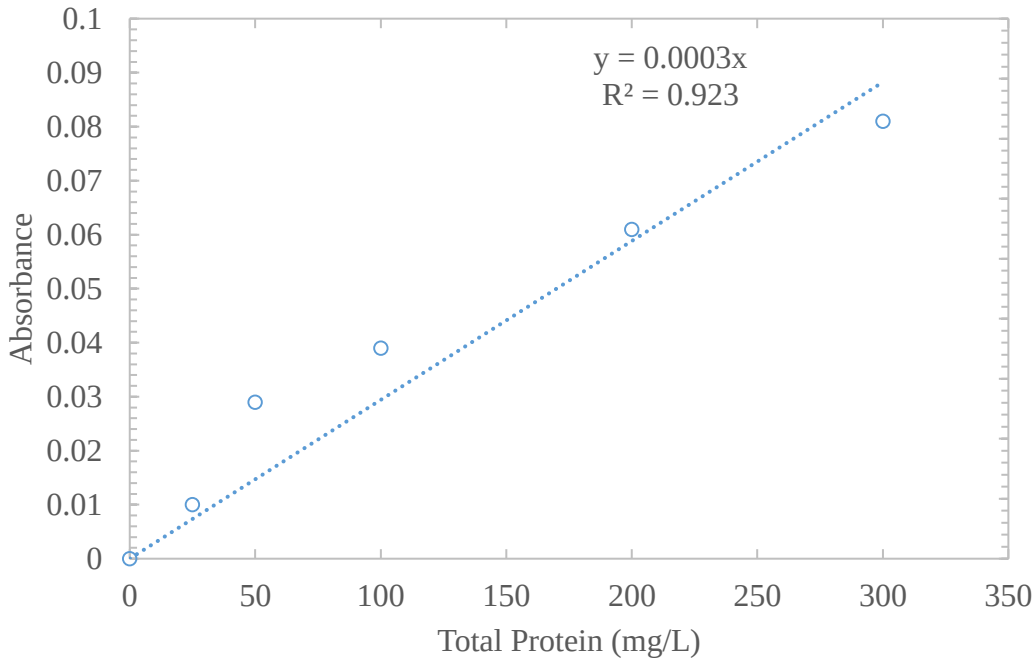
APPENDIX C: Calibration Curve of Methylene Blue Dye.



APPENDIX D: Calibration Curve of Total Carbohydrate.



APPENDIX E: Calibration Curve of Total Protein.



APPENDIX F: Removal Efficiency of Methylene Blue Dye and Acid Orange 7 Dye in Function of Day.

Day	Concentration (mg/L)	Acid Orange 7 Dye							
		Set 1		Set 2		Set 3		Average	SD
		ABS	Efficiency (%)	ABS	Efficiency (%)	ABS	Efficiency (%)		
0	50	0.212	3.64	0.215	2.27	0.209	5.00	3.64	1.364
	100	0.406	2.40	0.403	3.13	0.405	2.64	2.72	0.367
	300	1.24	0.00	1.187	0.50	1.222	0.00	0.17	0.290
	500	1.846	0.43	1.789	3.51	1.835	1.02	1.65	1.631
	800	2.332	0.13	2.332	0.13	2.33	0.21	0.16	0.049
1	50	0.198	10.00	0.222	0.00	0.213	3.18	4.39	5.109
	100	0.424	0.00	0.419	0.00	0.459	0.00	0.00	0.000
	300	1.248	0.00	1.203	0.00	1.236	0.00	0.00	0.000
	500	1.65	11.00	1.8	2.91	1.791	3.40	5.77	4.537
	800	2.247	3.77	2.27	2.78	2.219	4.97	3.84	1.094
2	50	0.23	0.00	0.256	0.00	0.232	0.00	0.00	0.000
	100	0.442	0.00	0.417	0.00	0.47	0.00	0.00	0.000
	300	1.284	0.00	1.235	0.00	1.268	0.00	0.00	0.000

	500	1.737	6.31	1.766	4.75	1.771	4.48	5.18	0.990
	800	2.212	5.27	2.303	1.37	2.242	3.98	3.54	1.986
3	50	0.228	0.00	0.243	0.00	0.236	0.00	0.00	0.000
	100	0.434	0.00	0.427	0.00	0.461	0.00	0.00	0.000
	300	1.288	0.00	1.224	0.00	1.259	0.00	0.00	0.000
	500	1.646	11.22	1.647	11.17	1.683	9.22	10.54	1.137
	800	2.248	3.73	2.265	3.00	2.237	4.20	3.64	0.604
4	50	0.228	0.00	0.229	0.00	0.241	0.00	0.00	0.000
	100	0.435	0.00	0.451	0.00	0.463	0.00	0.00	0.000
	300	1.267	0.00	1.223	0.00	1.289	0.00	0.00	0.000
	500	1.655	10.73	1.611	13.11	1.664	10.25	11.36	1.530
	800	2.227	4.63	2.238	4.15	2.245	3.85	4.21	0.389

Day	Concentration (mg/L)	Methylene Blue Dye							
		Set 1		Set 2		Set 3		Average	SD
		ABS	Efficiency (%)	ABS	Efficiency (%)	ABS	Efficiency (%)		
0	50	0.067	72.23	0.082	66.01	0.095	60.63	66.29	5.81
	100	0.062	75.74	0.067	73.78	0.053	79.26	76.26	2.78
	300	0.364	60.10	0.388	57.47	0.395	56.71	58.09	1.78
	500	1.128	9.31	0.88	29.25	0.961	22.73	20.43	10.17
	800	1.398	3.88	1.343	7.66	1.407	3.26	4.93	2.38
1	50	0.067	72.23	0.079	67.26	0.085	64.77	68.09	3.80

	100	0.051	80.04	0.065	74.56	0.085	76.91	77.17	2.75
	300	0.051	73.04	0.065	75.23	0.059	70.74	73.00	2.25
	500	0.717	42.35	0.665	46.53	0.766	38.41	42.43	4.06
	800	1.311	9.86	1.292	11.17	1.313	9.72	10.25	0.80
2	50	0.077	68.09	0.083	65.60	0.086	64.36	66.01	1.90
	100	0.051	80.04	0.054	78.87	0.053	79.26	79.39	0.60
	300	0.212	76.76	0.229	74.90	0.27	70.41	74.02	3.27
	500	0.745	40.10	0.613	50.71	0.749	39.78	43.53	6.22
	800	1.316	9.51	1.302	10.48	1.304	10.34	10.11	0.52
3	50	0.077	68.09	0.078	67.67	0.086	64.36	66.71	2.04
	100	0.043	83.17	0.051	80.04	0.036	85.91	83.04	2.94
	300	0.206	77.42	0.219	76.00	0.253	72.27	75.23	2.66
	500	0.762	38.73	0.596	52.08	0.747	39.94	43.59	7.38
	800	1.312	9.79	1.379	5.18	1.302	10.48	8.48	2.88
4	50	0.075	68.92	0.081	66.43	0.083	65.60	66.98	1.73
	100	0.038	85.13	0.033	87.09	0.06	76.52	82.91	5.62
	300	0.213	76.65	0.21	76.98	0.245	73.15	75.59	2.13
	500	0.751	39.62	0.592	52.40	0.756	39.22	43.75	7.50
	800	1.34	7.86	1.314	9.65	1.342	7.73	8.41	1.07

APPENDIX G: Isotherm Langmuir Study.

C_e	Mass of Microalgae (g)	Removal Efficiency (%)			C_i-C_f			q_e			C_e/q_e			Average	SD
		R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3		
50	0.071	72.23	66.01	60.63	36.115	33.005	30.315	127.165	116.215	106.743	0.393	0.430	0.468	0.431	0.038
100	0.071	75.74	73.78	79.26	75.74	73.78	79.26	266.690	259.789	279.085	0.375	0.385	0.358	0.373	0.013
300	0.071	60.10	57.47	56.71	180.3	172.41	170.13	634.859	607.077	599.049	0.473	0.494	0.501	0.489	0.015
500	0.071	9.31	29.25	22.73	46.55	146.25	113.65	163.908	514.965	400.176	3.050	0.971	1.249	1.757	1.129
800	0.071	3.88	7.66	3.26	31.04	61.28	26.08	109.296	215.775	91.831	7.320	3.708	8.712	6.580	2.583

APPENDIX H: Isotherm Freundlich Study.

lnC_e	lnq_e			Average	SD
	R1	R2	R3		
3.912	4.845	4.755	4.670	4.757	0.088
4.605	5.586	5.560	5.632	5.592	0.036
5.704	6.453	6.409	6.395	6.419	0.030
6.215	5.099	6.244	5.992	5.778	0.602
6.685	4.694	5.374	4.520	4.863	0.451

APPENDIX I: Removal Efficiency of Methylene Blue Dye in Function of Week.

Week	Concentration (mg/L)	Set 1		Set 2		Set 3		Average	SD
		ABS	Efficiency (%)	ABS	Efficiency (%)	ABS	Efficiency (%)		
0	50	0.268	52.48	0.238	57.80	0.252	55.32	55.20	2.66
	100	0.571	44.24	0.663	35.25	0.844	17.58	32.36	13.56
1	50	0.199	64.72	0.21	62.77	0.22	60.99	62.83	1.86
	100	0.277	72.95	0.245	76.07	0.269	73.73	74.25	1.63
2	50	0.232	58.87	0.211	62.59	0.246	56.38	59.28	3.12
	100	0.275	73.14	0.302	70.51	0.566	71.78	71.81	1.32
3	50	0.251	55.50	0.233	58.69	0.261	53.72	55.97	2.52
	100	0.341	66.70	0.297	71.00	0.294	71.29	69.66	2.57
4	50	0.214	62.06	0.225	60.11	0.239	57.62	59.93	2.22
	100	0.241	76.46	0.293	71.39	0.309	69.82	72.56	3.47

APPENDIX J: Cell Density of Microalgae in Function of Week.

Week	Concentration (mg/L)	Weight of empty centrifuge(g)			Weight of centrifuge with Microalgae (g)			Weight of Microalgae (g)			Cell Density (g/L)			Average	SD
		Set 1	Set 2	Set 3	Set 1	Set 2	Set 3	Set 1	Set 2	Set 3	Set 1	Set 2	Set 3		
0	50	1.0171	1.0061	1.0029	1.0733	1.0670	1.0700	0.0562	0.0609	0.0671	0.2810	0.3045	0.3355	0.307	0.027
	100	1.0149	1.0146	1.0053	1.0810	1.0762	1.0730	0.0661	0.0616	0.0677	0.3305	0.3080	0.3385	0.326	0.016
1	50	0.8154	0.7945	0.8498	0.8471	0.8451	0.8936	0.0317	0.0506	0.0438	0.2113	0.3373	0.2920	0.280	0.064
	100	0.7960	0.7946	0.7399	0.8441	0.8340	0.7801	0.0481	0.0394	0.0402	0.3207	0.2627	0.2680	0.284	0.032
2	50	0.8190	0.7403	0.7954	0.8715	0.7825	0.8452	0.0525	0.0422	0.0498	0.3500	0.2813	0.3320	0.321	0.036
	100	0.8212	0.8184	0.8108	0.8646	0.8567	0.8539	0.0434	0.0383	0.0431	0.2893	0.2553	0.2873	0.277	0.019
3	50	0.8227	0.7461	0.8424	0.8600	0.7732	0.8750	0.0373	0.0271	0.0326	0.2487	0.1807	0.2173	0.216	0.034
	100	0.8131	0.8071	0.7985	0.8500	0.8482	0.8380	0.0369	0.0411	0.0395	0.2460	0.2740	0.2633	0.261	0.014
4	50	0.8189	0.8160	0.7650	0.8544	0.8440	0.7997	0.0355	0.0280	0.0347	0.2367	0.1867	0.2313	0.218	0.027
	100	0.7422	0.8781	0.7965	0.7834	0.9140	0.8308	0.0412	0.0359	0.0343	0.2747	0.2393	0.2287	0.248	0.024

APPENDIX K: Total Chlorophyll of Microalgae in Function of Week.

Week	Concentration (mg/L)	Set 1			Set 2			Set 3			Average	SD
		ABS (665nm)	ABS (650nm)	Total Chlorophyll (mg/L)	ABS (665nm)	ABS (650nm)	Total Chlorophyll (mg/L)	ABS (665nm)	ABS (650nm)	Total Chlorophyll (mg/L)		
0	50	1.243	1.253	110.77	1.446	1.448	128.12	1.465	1.487	131.34	123.41	11.06
	100	1.582	1.587	140.16	1.721	1.734	153.30	1.583	1.602	141.55	145.00	7.22
1	50	1.561	1.591	140.44	1.316	1.323	234.00	1.466	1.458	258.26	210.90	62.21
	100	1.386	1.389	245.78	1.306	1.286	228.10	1.324	1.318	233.43	235.77	9.07
2	50	1.213	1.214	214.85	1.18	1.166	206.72	0.727	0.72	127.61	183.06	48.19
	100	1.847	1.796	228.96	1.297	1.293	213.59	1.205	1.207	221.27	221.27	7.68
3	50	0.777	0.742	132.17	0.969	0.929	165.39	0.906	0.878	156.08	151.22	17.14
	100	1.501	1.462	259.71	1.303	1.278	226.81	1.468	1.439	255.40	247.31	17.88
4	50	0.78	0.79	139.59	0.83	0.81	143.85	0.926	0.904	160.54	147.99	11.07
	100	1.216	1.186	210.64	1.306	1.28	227.18	1.43	1.398	248.21	228.68	18.83

APPENDIX L: Total Carbohydrates of Microalgae in Function of Week.

Week	Set 1		Set 2		Set 3		Average	SD
	ABS	wt%	ABS	wt%	ABS	wt%		
0	0.544	1.106	0.649	1.319	0.775	1.333	1.25	0.13
1	0.754	1.533	0.812	1.650	0.729	1.482	1.55	0.09
2	0.323	0.657	0.320	0.650	0.174	0.354	0.55	0.17
3	0.217	0.441	0.232	0.472	0.340	0.691	0.53	0.14
4	0.238	0.484	0.264	0.537	0.260	0.528	0.52	0.03

Appendix M: Total Protein of Microalgae in Function of Week.

Week	Set 1		Set 2		Set 3		Average	SD
	ABS	wt%	ABS	wt%	ABS	wt%		
0	0.064	2.780	0.062	0.560	0.057	2.440	1.93	1.20
1	0.055	1.780	0.053	1.560	0.075	4.000	2.45	1.35
2	0.042	0.330	0.041	0.220	0.059	0.930	0.49	0.38
3	0.042	0.330	0.048	1.000	0.051	1.330	0.89	0.51
4	0.044	0.560	0.047	0.890	0.040	0.520	0.66	0.20

Appendix N: One-Way Analysis Of Variance (ANOVA) Followed by LSD All-Pairwise Comparison Test.

Removal of Methylene Blue Efficiency in Function of Month at 50mg/L

Statistix 10.0 (30-day Trial) 06/04/2019, 18:08:23

One-Way AOV for: Week0 Week1 Week2 Week3 Week4

Source	DF	SS	MS	F	P
Between	4	115.693	28.9233	4.57	0.0234
Within	10	63.255	6.3255		
Total	14	178.949			

Grand Mean 58.641 CV 4.29

Homogeneity of Variances	F	P
Levene's Test	0.50	0.7344
O'Brien's Test	0.22	0.9190
Brown and Forsythe Test	0.13	0.9694

Welch's Test for Mean Differences

Source	DF	F	P
Between	4.0	4.15	0.0759
Within	5.0		

Component of variance for between groups 7.53258
Effective cell size 3.0

Variable	Mean
Week0	55.200
Week1	62.827
Week2	59.280
Week3	55.970
Week4	59.930

Observations per Mean 3
Standard Error of a Mean 1.4521
Std Error (Diff of 2 Means) 2.0535

Statistix 10.0 (30-day Trial) 06/04/2019, 18:10:50

LSD All-Pairwise Comparisons Test

Variable	**Mean**	**Homogeneous Groups**
Week1	62.827	A
Week4	59.930	AB
Week2	59.280	ABC
Week3	55.970	BC
Week0	55.200	C

Alpha	0.05	Standard Error for Comparison	2.0535
Critical T Value	2.228	Critical Value for Comparison	4.5756

There are 3 groups (A, B, etc.) in which the means
are not significantly different from one another.

Removal of Methylene Blue Efficiency in Function of Month at 100mg/L

Statistix 10.0 (30-day Trial) 06/04/2019, 21:56:22

One-Way AOV for: Week0 Week1 Week2 Week3 Week4

Source	DF	SS	MS	F	P
Between	4	3817.70	954.426	23.06	0.0000
Within	10	413.97	41.397		
Total	14	4231.67			

Grand Mean 64.127 CV 10.03

Homogeneity of Variances	F	P
Levene's Test	3.74	0.0412
O'Brien's Test	1.66	0.2341
Brown and Forsythe Test	1.85	0.1955

Welch's Test for Mean Differences

Source	DF	F	P
Between	4.0	5.97	0.0415
Within	4.8		

Component of variance for between groups 304.343
Effective cell size 3.0

Variable	Mean
Week0	32.357
Week1	74.250
Week2	71.810
Week3	69.663
Week4	72.557

Observations per Mean 3
Standard Error of a Mean 3.7147
Std Error (Diff of 2 Means) 5.2534

Statistix 10.0 (30-day Trial) 06/04/2019, 21:56:41

LSD All-Pairwise Comparisons Test

Variable	Mean	Homogeneous Groups
Week1	74.250	A
Week4	72.557	A
Week2	71.810	A
Week3	69.663	A
Week0	32.357	B

Alpha 0.05 Standard Error for Comparison 5.2534
Critical T Value 2.228 Critical Value for Comparison 11.705
There are 2 groups (A and B) in which the means
are not significantly different from one another.

Cell Density in Function of Month at 50mg/L

Statistix 10.0 (30-day Trial) 06/04/2019, 22:27:22

One-Way AOV for: Week0 Week1 Week2 Week3 Week4

Source	**DF**	**SS**	**MS**	**F**	**P**
Between	4	0.02914	7.286E-03	4.55	0.0237
Within	10	0.01600	1.600E-03		
Total	14	0.04515			

Grand Mean 0.2684 CV 14.90

Homogeneity of Variances	**F**	**P**
Levene's Test	1.90	0.1865
O'Brien's Test	0.85	0.5273
Brown and Forsythe Test	0.44	0.7788

Welch's Test for Mean Differences

Source	**DF**	**F**	**P**
Between	4.0	5.29	0.0492
Within	4.9		

Component of variance for between groups 1.895E-03
Effective cell size 3.0

Variable	**Mean**
Week0	0.3070
Week1	0.2802
Week2	0.3211
Week3	0.2156
Week4	0.2182

Observations per Mean 3
Standard Error of a Mean 0.0231
Std Error (Diff of 2 Means) 0.0327

Statistix 10.0 (30-day Trial) 06/04/2019, 22:28:45

LSD All-Pairwise Comparisons Test

Variable	**Mean**	**Homogeneous Groups**
Week2	0.3211	A
Week0	0.3070	A
Week1	0.2802	AB
Week4	0.2182	B
Week3	0.2156	B

Alpha 0.05 Standard Error for Comparison 0.0327
Critical T Value 2.228 Critical Value for Comparison 0.0728
There are 2 groups (A and B) in which the means
are not significantly different from one another.

Cell Density in Function of Month at 100mg/L

tatistix 10.0 (30-day Trial) 06/04/2019, 22:30:20

One-Way AOV for: Week0 Week1 Week2 Week3 Week4

Source	DF	SS	MS	F	P
Between	4	0.01054	2.634E-03	5.44	0.0137
Within	10	0.00484	4.844E-04		
Total	14	0.01538			

Grand Mean 0.2791 CV 7.89

Homogeneity of Variances	F	P
Levene's Test	1.40	0.3031
O'Brien's Test	0.62	0.6577
Brown and Forsythe Test	0.15	0.9569

Welch's Test for Mean Differences

Source	DF	F	P
Between	4.0	6.36	0.0348
Within	4.9		

Component of variance for between groups 7.166E-04
Effective cell size 3.0

Variable	Mean
Week0	0.3257
Week1	0.2838
Week2	0.2773
Week3	0.2611
Week4	0.2476

Observations per Mean 3
Standard Error of a Mean 0.0127
Std Error (Diff of 2 Means) 0.0180

Statistix 10.0 (30-day Trial) 06/04/2019, 22:30:59

LSD All-Pairwise Comparisons Test

Variable	Mean	Homogeneous Groups
Week0	0.3257	A
Week1	0.2838	B
Week2	0.2773	B
Week3	0.2611	B
Week4	0.2476	B

Alpha 0.05 Standard Error for Comparison 0.0180
Critical T Value 2.228 Critical Value for Comparison 0.0400
There are 2 groups (A and B) in which the means
are not significantly different from one another.

Total Carbohydrate in Function of Month at 100mg/L

Statistix 10.0 (30-day Trial) owcarboh100.sx, 06/04/2019, 22:17:47

One-Way AOV for: Week0 Week1 Week2 Week3 Week4

Source	DF	SS	MS	F	P
Between	4	2747.19	686.799	34.03	0.0000
Within	10	201.83	20.183		
Total	14	2949.03			

Grand Mean 26.955 CV 16.67

Homogeneity of Variances	F	P
Levene's Test	2.11	0.1539
O'Brien's Test	0.94	0.4802
Brown and Forsythe Test	0.61	0.6644

Welch's Test for Mean Differences

Source	DF	F	P
Between	4.0	71.58	0.0004
Within	4.3		

Component of variance for between groups 222.205
Effective cell size 3.0

Variable	Mean
Week0	40.000
Week1	46.646
Week2	16.606
Week3	16.037
Week4	15.488

Observations per Mean 3
Standard Error of a Mean 2.5938
Std Error (Diff of 2 Means) 3.6682

Statistix 10.0 (30-day Trial) owcarboh100.sx, 06/04/2019, 22:18:52

LSD All-Pairwise Comparisons Test

Variable	Mean	Homogeneous Groups
Week1	46.646	A
Week0	40.000	A
Week2	16.606	B
Week3	16.037	B
Week4	15.488	B

Alpha 0.05 Standard Error for Comparison 3.6682
Critical T Value 2.228 Critical Value for Comparison 8.1732
There are 2 groups (A and B) in which the means
are not significantly different from one another.

Total Protein in Function of Month at 100mg/L

Statistix 10.0 (30-day Trial) LSDprotein100.sx, 06/04/2019, 22:19:56

One-Way AOV for: Week0 Week1 Week2 Week3 Week4

Source	DF	SS	MS	F	P
Between	4	9274.0	2318.51	3.52	0.0486
Within	10	6592.6	659.26		
Total	14	15866.6			

Grand Mean 43.333 CV 59.25

Homogeneity of Variances	F	P
Levene's Test	2.34	0.1252
O'Brien's Test	1.04	0.4329
Brown and Forsythe Test	0.35	0.8403

Welch's Test for Mean Differences

Source	DF	F	P
Between	4.0	7.23	0.0279
Within	4.8		

Component of variance for between groups 553.083
Effective cell size 3.0

Variable	Mean
Week0	73.333
Week1	73.333
Week2	27.778
Week3	26.667
Week4	15.556

Observations per Mean 3
Standard Error of a Mean 14.824
Std Error (Diff of 2 Means) 20.964

Statistix 10.0 (30-day Trial) LSDprotein100.sx, 06/04/2019, 22:20:11

LSD All-Pairwise Comparisons Test

Variable	Mean	Homogeneous Groups
Week0	73.333	A
Week1	73.333	A
Week2	27.778	AB
Week3	26.667	AB
Week4	15.556	B

Alpha 0.05 Standard Error for Comparison 20.964
Critical T Value 2.228 Critical Value for Comparison 46.712
There are 2 groups (A and B) in which the means
are not significantly different from one another.

Total Chlorophyll in Function of Month at 100mg/L

Statistix 10.0 (30-day Trial) LSDchlorophyll100.sx, 06/04/2019, 22:21:01

One-Way AOV for: Week0 Week1 Week2 Week3 Week4

Source	DF	SS	MS	F	P
Between	4	19797.5	4949.36	28.52	0.0000
Within	10	1735.6	173.56		
Total	14	21533.1			

Grand Mean 215.61 CV 6.11

Homogeneity of Variances	F	P
Levene's Test	1.87	0.1915
O'Brien's Test	0.83	0.5340
Brown and Forsythe Test	0.45	0.7722

Welch's Test for Mean Differences

Source	DF	F	P
Between	4.0	48.16	0.0004
Within	4.9		

Component of variance for between groups 1591.93
Effective cell size 3.0

Variable	Mean
Week0	145.00
Week1	235.77
Week2	221.27
Week3	247.30
Week4	228.68

Observations per Mean 3
Standard Error of a Mean 7.6062
Std Error (Diff of 2 Means) 10.757

Statistix 10.0 (30-day Trial) LSDchlorophyll100.sx, 06/04/2019, 22:21:16

LSD All-Pairwise Comparisons Test

Variable	Mean	Homogeneous Groups
Week3	247.30	A
Week1	235.77	AB
Week4	228.68	AB
Week2	221.27	B
Week0	145.00	C

Alpha 0.05 Standard Error for Comparison 10.757
Critical T Value 2.228 Critical Value for Comparison 23.968
There are 3 groups (A, B, etc.) in which the means
are not significantly different from one another. L