UTILISATION OF *SARGASSUM POLYCYSTUM* FOR REMOVAL OF MALACHITE GREEN AND METHYLENE BLUE

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

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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 acknowledged. I also declare 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 report entitled **"UTILISATION OF *SARGASSUM POLYCYSTUM* FOR REMOVAL OF MALACHITE GREEN AND METHYLENE BLUE"** was prepared by **TANG PUEY HUA** has met the required standard for submission in partial fulfilment of the requirements for the award of Bachelor of Engineering (Hons.) Chemical Engineering at Universiti Tunku Abdul Rahman.

Approved by,

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Date : _____

Specially dedicated to
my beloved family and friends

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ABSTRACT

Dyes are one of the problematic contaminants. Dyes are toxic and even carcinogenic and they may affect aquatics life, human health and ecological system when dye wastewaters are discharged into water body. The most widely used methods for dye removal from wastewater systems include flocculation, coagulation, precipitation, adsorption, membrane filtration and electrochemical techniques. Adsorption process is superior to other removal techniques because it is more economical, simpler and due to the capability to treat dyes in a more concentrated form efficiently. A wide variety of well-known natural materials and waste materials from industries and agriculture can be employed as biological adsorbent in dye removal. Brown algae have been reported as promising biosorbents used in dye removal due to the presence of abundant functional groups. In the present study, the biosorption of malachite green (MG) and methylene blue (MB) in binary aqueous solution, using the brown algae, *Sargassum polycystum*, was investigated in batch mode of operation. In order to evaluate the biosorption capacity and characteristic, effect of initial dye concentration (50 to 100 ppm), sorbent dosage (0.2 to 1 g) and temperature (30 to 60℃) were studied. MB was found to be totally absorbed within 1 h while MG is also absorbed very fast and can reach equilibrium stage in 1 to 2 h for all studied concentration. With the increasing of initial dye concentration from 50 to 500 ppm, the sorption capacity of MG increased from 3.78 to 49.16 mg/g while sorption capacity of MB increased from 4.9 to 48.1 mg/g. Besides, the increasing of sorbent dosage also led to the increase of sorption capacity of MG (from 8.53 to 10.26 mg/g) and MB (from 9.45 to 10.25 mg/g). The results revealed that the biosorption process of MG and MB by *S. polycystum* is a thermo-independent process. The Langmuir and Freundlich models were used to describe the adsorption isotherm data.

Biosorption data fitted well to both of the models with high correlation coefficients and this implies that both monolayer sorption and heterogeneous surface conditions exist under the used experimental conditions. The best fit of the adsorption kinetic modelling was obtained using the pseudo-second-order kinetic model. In addition, thermodynamic parameters, include ΔG^{o}, ΔH^{o} and ΔS^{o}, were calculated, which indicated that the present system was spontaneous and exothermic physisorption process.

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LIST OF SYMBOLS / ABBREVIATIONS

C_0	initial solute concentrations in solution, mg/L
C_f	equilibrium solute concentrations in solution, mg/L
C_s	concentration of solute sorbed on surface of sorbent, mg/L
V	volume of the solution, L
M	amount of adsorbent used, g
q	sorption capacity, mg/g
q_e	amount of sorbate sorbed at equilibrium, mg/g
q_{max}	maximum sorption capacity, mg/g
q_t	amount of solute sorbed at time *t*, mg/g
b	Langmuir equilibrium constant, L/mg
R_L	separation factor (dimensionless)
K_f	Freundlich constant, $(mg/g)(L/mg)^{1/n}$
$1/n$	Freundlich exponent (dimensionless)
k_1	Pseudo-first-order equilibrium rate constant, 1/h
k_2	Pseudo-second-order equilibrium rate constant, g/mgh
R	gas constant, J/molK
t	contact time, h
T	absolute temperature, K
K_c	thermodynamic equilibrium constant at temperature *T*
U	agitation speed, rpm
ΔG^o	Gibbs free energy change, kJ/mol
ΔH^o	enthalpy change, kJ/mol
ΔS^o	entropy change, J/molK
K_c	distribution coefficient (dimensionless)
MG	malachite green
MB	methylene blue

CHAPTER 1

INTRODUCTION

1.1 Background

Dyes are used in many industries, such as food, pharmaceutical, cosmetic, dyestuffs, textile, paper and plastics. This results in coloured wastewater. It is found that colour is the first contaminant to be recognized in wastewater (Banat et al., 1996). There are more than 8000 chemical products associated with the dyeing process, while over 100000 commercially available dyes exist with over 7×10^5 metric tons of dye stuff produced annually (Banat et al., 1996). Dyes can be classified as follows (Mishra & Tripathy, 1993):

- Anionic : direct, acid, and reactive dyes;
- Cationic : basic dyes; and
- Non-ionic : disperse dyes.

Even a very small amounts of dyes present in water (less than 1 ppm for some dyes) is highly visible and undesirable (Banat et al., 1996; Robinson et al., 2001). Furthermore, many of the dyes are toxic and carcinogenic and this poses a serious hazard to aquatic living organisms (Vijayaraghavan & Yun, 2008). Dyes also interfere with the transmission of light and upset the biological metabolism processes which cause the destruction of aquatic communities present in ecosystem (Kuo, 1992; Walsh et al., 1980). In addition, the dyes have a tendency to sequester metal and may cause microtoxicity to fish and other organisms (Walsh et al., 1980). Therefore, it is necessary to develop an effective and appropriate treatment technique to remove the

dyes from the wastewater before discharging to natural water stream. There might have some difficulties in treating coloured wastewater due to the properties of dyes includes resistance to aerobic digestion, and stability to light, heat and oxidizing agents (Kumar et al., 1988; Sun & Yang, 2003). Conventional methods, such as adsorption, coagulation precipitation, filtration and oxidation are not so effective to those recalcitrant dyes and not economical or not so environmental friendly (Senthil et al., 2003).

Several physical, chemical and biological decolourization methods have been developed and reported in the past three decades and some of them have been accepted by the paper and textile industries (Ghoreishi & Haghighi, 2003). In addition, there are other methods including ozonation, irradiation, ion exchange and photo degradation. Some of these techniques may have some limitations, including excess amount of chemical usage, accumulation of concentrated sludge with disposal problems, expensive plant requirements and operational costs, lack of effective colour reduction and sensitivity to a variable wastewater input (Robinson et al., 2001).

Among these numerous techniques, adsorption is a desirable choice because it can be used to different types of colouring materials and can produce a high quality treated effluent (Derbyshire et al., 2001; Ho & McKay, 2003; Jain et al., 2003). Activated carbon is the most commonly used sorbent in commercial system to remove dyes because of its excellent adsorption ability (Crini, 2006). The activated carbon adsorption technique has been cited by the US Environmental Protection Agency as one of the best available control technologies (Derbyshire et al., 2001). However, the popularization of activated carbon is limited due to high cost. Therefore, cheaper and effective adsorbents such as natural materials, biosorbents, and waste materials from industry and agriculture have been studied. The examples of these materials are clay materials (bentonite and kaolinite), zeolites, siliceous material (silica beads, alunite, perlite), agricultural wastes (bagasse pith, maize cob, rice husk, coconut shell), industrial waste products (waste carbon slurries, metal hydroxide sludge), biosorbents (chitosan, peat, biomass) and others (starch, cyclodextrin, cotton) (Crini, 2006).

Application of biosorption for the removal of toxic pollutants or for the recovery of valuable resources from aqueous wastewaters is one of the most recent developments in environmental or bioresource technology. This non-conventional technology has high efficiency, minimum chemical or biological sludges, the ability to regenerate biosorbents, and the possibility of metal recovery following adsorption (Park et al., 2010). Biosorbents for the dye removal mainly classified to the following categories: bacteria, fungi, algae, industrial wastes, agricultural wastes and other polysaccharide materials. Marine algae, popularly known as seaweeds, are another important biosorbent which has gained momentum in recent years (Vijayaraghavan & Yun, 2008). They are biological resources and available in many parts of the world. The most important is the alginate gel presents in their cell walls offer a convenient basis for the production of biosorbent particles that are suitable for sorption process applications (Vieira & Volesky, 2000). Marine algae have been identified as potent metal biosorbents due to the presence of binding sites, such as carboxyl, sulfonate, amine and hydroxyl groups (Davis, Volesky & Mucci, 2003).

1.2 Aims and Objectives

In the present study, *Sargassum polycystum* was used as the biosorbent to remove basic dyes, which are malachite green and methylene blue, from a binary aqueous solution. Experiments have been conducted to collect the corresponding data and observe the efficiency of this technique. The objectives of the study are:

1. To investigate the sorption capacity of *Sargassum polycystum* in removal of malachite green and methylene blue from binary dye solution.
2. To investigate and study the effects of initial dye concentrations, sorbent dosage and temperature on the uptake of malachite green and methylene blue.
3. To model the sorption process by applying sorption isotherm, kinetic modelling and thermodynamic study.

1.3 Organization of Thesis

The thesis has been organized into several chapters:

Chapter 1: Presents the research background and research objectives.

Chapter 2 : Presents the literature review on the fundamental topics related to the research.

Chapter 3: A summary of the experimental procedures and methodologies used in the research.

Chapter 4: Presents the experimental results with discussion.

Chapter 5: Gives the conclusions and recommendations of the thesis.

CHAPTER 2

LITERATURE REVIEW

2.1 Contaminants in Water

Water contamination is one of the most important issues because water is essential to life. It is one of the principal elements to support the earth and all livings. Human activities and natural wastes are the common sources of water contaminants. Since a few decades ago, the existence of contaminants in water has been highly observed and studied. To remedy the environment and to protect human beings and any other livings from the impacts of water pollution, various techniques and methods have been developed to prevent water contamination and to remove contaminants that exist in water. The list of contaminants in water is lengthy so only the major categories of water contaminants have been done in the following sections.

2.1.1 Pathogens

Pathogens can cause serious diseases and even death. Their growth and multiplication replies on host (Master & Ela, 2008). The resulting growth of microorganism in a host causes infection. The pathogens are dispersed in the environment through the use of sewage sludge as fertilizer, agricultural practices, animal waste, and others (Master & Ela, 2008). The ability of pathogens to survive in surface water is variable. In general, survival is extended when water temperature is low. Other factors that influence survival include sunlight intensity, adsorption to

particles and the presence of aquatic microorganisms that may use the pathogens as food source or cause pathogen disintegration (Master & Ela, 2008). Pathogens may be removed during soil transfer by adsorption and inactivation (Dechesne & Soyeux, 2007).

2.1.2 Oxygen-demanding Wastes

Oxygen-demanding wastes are formed by the substances that oxidize in the receiving body of water. As bacteria decompose these wastes, they utilize oxygen dissolved in the water, which reduces the remaining amount of dissolved oxygen (DO) (Master & Ela, 2008). As DO drops, fish and other aquatic life are threatened and undesirable odors, tastes, and colours would appear. Oxygen-demanding wastes are usually biodegradable organic substances contained in municipal wastewaters, for example human waste and food residue or in effluents from certain industries, such as food processing and paper production. In addition, the oxidation of certain inorganic compounds and naturally occurring organic matter, such as leaves, crops residue and animal droppings contribute to the oxygen demand (Master & Ela, 2008).

2.1.3 Nutrients

Nutrients are principle requirement to support growth and reproduction. However, in terms of water quality, nutrients are considered as pollutants when their concentrations are sufficient to allow excessive growth of aquatic plants (Master & Ela, 2008). The typical example is the effect of excessive nutrients to algae. Nutrient enrichment leads to death and decomposition of algae (Master & Ela, 2008). Their decomposition removes oxygen from the water, potentially leading to levels of DO that are insufficient to sustain normal life forms (Master & Ela, 2008). Algae and decaying organic matter add colour, turbidity, odors, and objectionable tastes to water that are difficult to remove and that may greatly reduce its acceptability as a domestic water source (Master & Ela, 2008). Nutrients are chemicals, such as iron,

calcium and manganese while the three most important ones are carbon, nitrogen and phosphorus. Agricultural operations are a significant source of nutrient (Davis & Masten, 2009).

2.1.4 Salts

Water naturally accumulates a variety of dissolved solids, or salts, as it passes through soils and rocks on its way to the sea (Master & Ela, 2008). The components of the salts include cation such as sodium, calcium, magnesium, and potassium, and anions such as chloride, sulfate, and biocarbonate. The concentration of dissolved solids is an important indicator of the usefulness of water for various applications (Master & Ela, 2008). Water with high salinity is unsuitable for irrigation purpose because some crops have low tolerant to salt (Master & Ela, 2008). The high salinity of water is mainly caused by water evaporation. When water evaporates, the salts are left behind, and since there is less remaining fresh water to dilute them, their concentration increases (Master & Ela, 2008). This phenomenon always happens on the irrigated agriculture, especially in arid areas (Master & Ela, 2008). The salinity increases when irrigation water percolates through the soil and returns to the river. The salt concentration increases from the river head waters to the mouth as it is used and reused for irrigation as it flows downstream (Davis & Masten, 2009). In addition, high concentration of salts that discharged from industries and salt used to keep ice from forming on roads in the winter also cause relatively minor effect to high water salinity. Salt accumulation in soils is often controlled by flushing the salts away with additional amounts of irrigation water and construction of adequate drainage (Master & Ela, 2008).

2.1.5 Heavy Metals

Most metals are toxic. Some of the metals, such as chromium and iron, are essential nutrients in our diets, but they can cause a range of adverse impact on the body in

high doses (Master & Ela, 2008). These negative impacts include nervous system and kidney damage, creation of mutations, and induction of tumors (Master & Ela, 2008). Unlike other toxic substances, metals are totally nondegradable so they are virtually indestructible in the environment (Master & Ela, 2008). It is important to remediate metal pollution to minimize further environmental and human impacts. Various physicochemical processes, for examples adsorption, ion-exchange, microfiltration, chemical precipitation, and reverse osmosis and nanofiltration, have been developed to remove heavy metal from polluted water (Master & Ela, 2008). Furthermore, the application of biosorption by using food wastes or microorganisms as adsorbentents is studied and developed to remove heavy metal.

2.1.6 Pesticides

Pesticide covers a range of chemicals that kill undesired organism. Pesticides can be delineated as insecticides, herbicides, rodenticides, and fungicides (Master & Ela, 2008). Some of the pesticides last a long time in the environment before being broken down into other substances (Master & Ela, 2008). Pesticides are soluble in hydrocarbon solvents and so they easily accumulate in fatty tissue (Master & Ela, 2008). The accumulation in fatty tissue means that organisms at successively higher trophic levels in a food chain are consuming food that has successively higher concentration of pesticide. At the top of the food chain, body concentrations of these pesticides are the highest and toxicity is the most recognized (Master & Ela, 2008).

2.1.7 Volatile Organic Chemicals

Volatile organic chemicals (VOCs) are among the most commonly found contaminants in groundwater (Master & Ela, 2008). They are often used as solvents in industrial processes and a number of them are either known or suspected carcinogens or mutagens (Master & Ela, 2008). The most common method of

treatment to remove VOCs is to aerate the water to encourage them to vaporize and disperse in the atmosphere (Master & Ela, 2008).

2.1.8 Dyes

A dye or dyestuff is a coloured compound that can be applied on a substrate. A substrate is the material to which a colorant is applied by one of the various processes of dyeing, printing, surface coating, and so on. Generally, the substrate includes textile fibers, polymers, foodstuffs, oils, leather, and many other similar materials (Rangnekar & Singh, 1980). The preparation and application of dyestuffs is one of the oldest forms of human activities. Commercially, application of dyes in products and colour manipulation of products may add value to the products.

Dyes are complex compounds with a big complicated molecular structure and toxic properties. Thus, it can affect aquatics life, human health and ecological system when dye wastewaters are extremely discharged into water sources. It eventually makes changes of ecological system and other serious pollution problems (Molen, 2008).

Beside dyes, pigments are also one of the major types of colorants in the colouring industries. Normally, dyes are water-soluble or water dispersible organic compounds that are capable of being adsorbed into the substrate destroying the crystal structure of the substance. The dye molecules are usually chemically bonded to the surface and become a part of the material on which it is applied. On the other hand, pigments do not interact with the substrate and hence do not destroy the crystal structure of the substrate. Generally, pigments retain essentially their particulate or crystalline form during application (Molen, 2008).

Most of the synthetic dyes are aromatic organic compounds. A dye consists of a colour-producing structure, the chromogen (electron acceptor) and a part to regulate the solubility and dyeing properties, the auxochrome (electron donor). The chromogen is an aromatic body containing a colour-giving group commonly called

the chromophore (*"chroma"* means colour and "*phore"* means bearer). Chromophore groups cause colour by altering absorption bands in the visible spectrum. Some of the common chromophores are nitroso (-N=O), nitro (=NO-OH), azo (-N=N-), ethylene (=C=C=), carbonyl (=C=O), carbon-nitrogen (-CH=N-), and carbon-sulfur groups. The chromophore groups are the basis of one method of classifying dyes. Some molecules lose their colours when the chromophore groups are saturated. On the other hand, the auxochromes usually are: amino ($-NH_2$), hydroxyl (-OH), $-NR_2$, carboxylic acid (-COOH) and sulfonic acid ($-SO_3H$). These auxochromes (*"auxo"* means augment) are salt-forming which aids the solubility and adherence of the dye in acidic and basic medium. Auxochromes are the characteristic groups which intensify colour and improve the dye affinity to substrate (Rangnekar & Singh, 1980).

There are numerous types of dyes. Dyes may be classified according to a dual system devised by the Society of Dryers and Colourists and the American Association of Textile Chemists and Colorists. Published as the Colour Index(C.I.), dyes may be classified according to the chemical class, expressed an assigned number and the usage or application, expressed through its generic name (Verano, 2010). The classification of dyes by usage is summarized in Table 2.1. Aside from the dyes mentioned in Table 2.1, there are also azoic dyes, ingrain dyes, mordant dyes, and others. (Hunger, 2003; Rangnekar & Singh, 1980; Shenai, 1977)

Table 2.1: Usage classification of dyes

Class	Typical Applied	Method of application	Chemical Type
Acid	Nylon, wool, silk, paper ink, and leather	Usually from neutral to acidic dye–baths	Azo (including premetallized), anthraquinone, azine, triphenylmethane, xanthene, nitro and nitroso
Basic	Leather, wool, silk paper, modified nylon, polyacrylonitrile, polyester, and inks	Applied from acidic dye–baths	Cyanine, azo, azine, hemicyanine, diazahemicyanine, triarylmethane, xanthen, acridine, oxazine, and anthraquinone

Table 2.1: (Continued)

Class	Typical Applied	Method of application	Chemical Type
Direct	Cotton, paper, rayon, leather, and nylon	Applied from neutral or slightly alkaline baths containing additional electrolyte	Azo, phthalocyanine, stilbene, nitro, and benzodifuranone
Disperse	Polyester, polyamide, acetate, acrylic, and plastics	Fine aqueous dispersions often applied by high temperature/pressure or lower temperature carrier methods; dye may be padded on cloth and baked on or thermo fixed	Azo, anthraquinone, styryl, nitro, and benzodifuranone
Reactive	Cotton, wool, silk, and, nylon	Reactive site on dye reacts with functional group on fiber to bind dye covalently under influence of heat and alkaline condition	Azo, anthraquinone, phthalocyanine, formazan, oxazine, and basic
Solvent	Plastics, gasoline, varnishes, lacquers, stains inks, fats, oils, and waxes	Dissolution in the appropriate solvent or medium	Phthalocyanine, azo, anthraquinone and triphenylmethnae
Sulfur	Cotton and rayon	Dissolved in water (with the addition of sodium sulfide for the in soluble types); exhausted with glauber's salts	Indeterminate structures
Vat	Cotton, rayon, and wool	Water–soluble dyes solubilization by reducing with sodium hydrogen sulfite, then exhausted on fiber and reoxidized	Anthraquinone (including polycyclic quinones) and indigoids

(Sources**:** Shenai, 1977; Rangnekar & Singh, 1980; Hunger, 2003)

2.2 Basic Dye

Dyes are generally defined along the lines of being coloured, aromatic compounds that can ionise. They are thus able to interact with oppositely charged tissue constituents. Basic dyes are positively charged and are used to bind to negatively charged tissue components (StainsFile, 2005a).

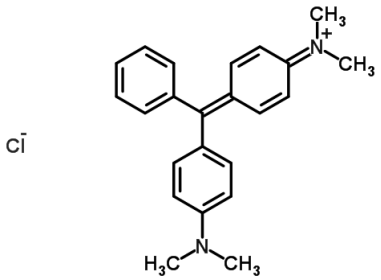
Basic dye is a class of dyes, usually synthetic, that act as bases, and which are mostly amino and substituted amino compounds (aniline dyes). Most are triarylmethane or xanthenes. Their colour base is not water soluble but can be made so by converting the base into a salt. The basic dyes, while possessing great tinctorial strength and brightness, are not generally light-fast. Therefore, their use in the dyeing of archival materials is largely restricted to those materials not requiring this characteristic. Basic dyes possess cationic functional groups such as $-NR^{3+}$ or $=NR^{2}+$. In an electric field the chromophore ion travels to the cathode or negative pole. Generally, basic dye forms salts with negatively charged (acidic) substances in tissue (chromatin, ergastoplasm, cartilage matrix, some granules). Some examples of basic dyes are crystal violet, safranin, basic fuchsin and methylene blue. It is applied to wool, silk, cotton and modified acrylic fibres. Usually acetic acid is added to the dyebath to help the take up of the dye onto the fibre (StainsFile, 2005a; Jagson Colorchem Limited, 2008).

2.2.1 Malachite Green

Malachite green (MG), a triarylmethane dye, is a dark green and crystalline solid prepared by condensing one part of benzaldehyde with two parts of diemethylaniline in the presence of concentrated sulphuric acid or zinc chloride (Srivastava, Sinha & Roy, 2004). Formally, MG refers to the chloride salt $[C_6H_5C(C_6H_4N(CH_3)_2)_2]Cl$, although the term malachite green is used loosely and often just refers to the coloured cation. MG can also be in oxalate salt form. However, the chloride and oxalate anions do not affect the colour of the dye. Table 2.2 summarises the physical-chemical properties of malachite green. Malachite green is most widely used for

colouring purpose, amongst all other dyes of its category (Jiang, Sun, Wang & Zhou, 2008). As malachite green is absorbed into the body, it is converted to carbinol form firstly, which is important because it spreads across cell membranes faster. When it is inside the cell, it is then metabolized into a form called leuco-malachite green (LMG). This form is toxic in addition and it is retained in the body for a longer period than the original form of malachite green (Webster's Online Dictionary).

Table 2.2: Properties of malachite green.

Property	Data
Common Name	Malachite Green
Other Names	Victoria Green B, Diamond Green B
C.I. Name	Basic Green 4
C.I. Number	42000
Class	Triarylmethane
Chemical Name	N-(4-{[4- (dimethylamino) phenyl] (phenyl) methylidene} cyclohexa-2,5-dien-1-ylidene) -N-methylmethanaminium chloride
Ionisation	Basic
Solubility Aqueous	Very
Solubility Ethanol	Very
Empirical Formula	$C_{23}H_{25}ClN_2$
Chemical Structure	
Molecular weight	364.91g/mol
Maximum Adsorption Wavelength	617-619nm
Colour	Green

(Source: StainsFile, 2010; ChemSpider, 2009)

Malachite green is used mainly industrially for leather, wool, cotton, silk, jute, paper and certain fibers. It is also used as a food colouring agent, food additive, a medical disinfectant and anthelminthic (Srivastava, Sinha & Roy, 2004). On the other hand, it is widely used in aquaculture as parasiticide, fungicide and antiprotozoan to control fungal attacks and protozoan infections (Srivastava, Sinha & Roy, 2004). In African aquaculture, it has been used against infection by bacteria, protozoans, cestodes, trematodes, nematodes, crustaceans, etc. (Hecht and Endemann, 1998). Traditionally, MG has been used to treat fungal infections on fish eggs. Malachite green is used as a biological stain for microscopic analysis of cell and tissue samples. In the Gimenez staining method, basic fuchsin stains bacteria red or magenta, and malachite green is used as a blue-green counterstain. Malachite green can also directly stain endospores within cells; here a safranin counterstain is often used. In addition, MG is rarely used as a saturable absorber in dye lasers, or as a pH indicator between pH 0.2 - 1.8. Leuco-malachite green, the primary metabolite of malachite, is used as a detection method for latent blood in criminalistics. Hemoglobin catalyzes the reaction between LMG and hydrogen peroxide, converting the colourless LMG to the chromatic form of malachite green. Therefore, the appearance of a green colour indicates the presence of blood (Webster's Online Dictionary, 2008).

MG discharged into receiving water bodies, even at low concentrations, will affect the aquatic life and cause detrimental effects in liver, gill, kidney, intestine and gonads. In humans, it may cause irritation to the gastrointestinal tract upon ingestion. Contact of MG with skin causes irritation, redness and pain (Daneshvar, Ayazloo, Khataee & Pourhassan, 2007). The toxicity of MG makes it a highly controversial compound. It has been reported to cause carcinogenesis, mutagenesis, chromosomal fractures, teratogenecity and respiratory toxicity (Srivastava, Sinha & Roy, 2004). Even the use of this dye has been banned in several countries and not approved by US Food and Drug Administration (Chang et al., 2001), it is still being used in many parts of the world due to its low cost, ready availability and efficacy (Schnick, 1988). The US Food and Drug Administration have nominated MG as a priority chemical for carcinogenicity testing (Culp & Beland, 1996). A considerable amount of research is being devoted to work out the wide spectrum of biological effects it exerts on different animals and mankind. There is concern about the fate of MG and

its reduced form, leuco-malachite green in aquatic and terrestrial ecosystems since they occur as contaminants and are potential human health hazards (Nelson & Hites, 1980; Burchmore & Wilkinson, 1993).

2.2.2 Methylene Blue

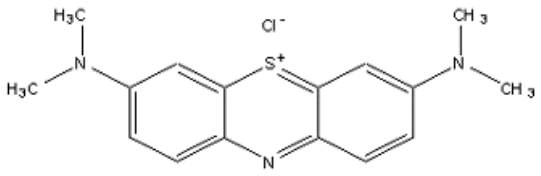
Methylene blue ([7-(dimethylamino)phenothiazin-3-ylidene] dimethylazanium chloride, molecular formula: $C_{16}H_{18}N_3SCl$), a heterocyclic aromatic chemical compound, has a molecular weight of 319.85. It is a cationic thiazine dye and a dark green powder that turn to deep blue colour when dissolved in water or alcohol. However, the reduced form of methylene blue, leukomethylene blue (LMB), is colourless (Cragan, 1999). Table 2.3 summaries the physical-chemical properties of methylene blue.

Methylene blue (MB) is an important dye widely used for printing calico, dyeing, printing cotton and tannin and dyeing leather, woal and coating for paper stock (Jiang, Sun & Wang, 2008; Aksu, Ertugrul & Donmez, 2010). MB is also used to test the adsorption capacity of various sorbents because it has a reasonably simple structure which allows examination of the adsorption mechanism. It also permits a quantitative comparison between the adsorption capacities of various sorbents (Cavas & Cengiz, 2008). It is used as a sensitizer in photo-oxidation of organic pollutants (Aksu, Ertugrul & Donmez, 2010). Besides, methylene blue is also widely used in biology, chemistry and medicine.

Methylene blue is a commonly used biological stain. Biologists often add a drop or two of methylene blue to bacteria on a glass slide before placing the slide under the microscope. The blue colour that stains the bacteria helps biologists see their shapes. Furthermore, methylene blue is used to bind to biological tissue as a result of chemical attractions. When in contact with acids, methylene blue is at its deepest shade of blue, indicating a strong attraction to acids. The location of cell nuclei, which contain the acid deoxyribonucleic acid, also known as DNA, are easily

identified under the microscope as the darkest areas of the cells stained with methylene blue (American Chemistry Council, 2010).

Table 2.3: Properties of methylene blue.

Property	Data
Common Name	Methylene Blue
Other Names	Swiss Blue
C.I. Name	Basic Blue 9, Solvent Blue 8
C.I. Number	52015
Class	Thiazin
Chemical Name	[7-(dimethylamino)phenothiazin-3-ylidene] dimethylazanium chloride
Ionisation	Basic
Solubility Aqueous	3.55%
Solubility Ethanol	1.48%
Empirical Formula	$C_{16}H_{18}N_3SCl$
Chemical Structure	
Molecular weight	319.85g/mol
Maximum Adsorption Wavelength	664nm
Colour	Blue

(Source: American Chemistry Council, 2011; StainsFile, 2005b)

Chemically, the colour of methylene blue indicates the presence or absence of oxygen or oxidizing agents because it is oxidized by them. Water containing the methylene blue indicator is blue when oxygen is present. A deep blue colour can be achieved by capping a solution of water and methylene blue and shaking it vigorously to mix oxygen from the air into the water. If oxygen is removed from the solution, the blue colour disappears. Methylene blue is added to pasteurized milk samples to be sure that milk was bacteria-free. If the blue colour of the sampled milk disappeared over the test period, it was likely that bacteria were consuming oxygen, and the milk had not been pasteurized properly. If the milk sample remained blue in

colour over the test period, bacteria were assumed not to be present (American Chemistry Council, 2010).

Methylene blue has been widely used in a variety of clinical settings to identify anatomic and pathologic structures. It has also been used to inactivate viruses in fresh frozen plasma, to treat chronic periodontitis, to aid in the diagnosis of and targeted therapy for cancer, and, experimentally, to treat septic shock (Cargan, 1999). Methylene blue is employed for the treatment of methemoglobinemia, which can arise from ingestion of certain pharmaceuticals or broad beans. Methylene blue is reduced to leucomethylene blue, which then acts to reduce the heme group from methemoglobin to haemoglobin (Brent, Burkhart & Wallace, 2005).

Although MB is not considered to be a very toxic dye, it can reveal very harmful effects on living things. MB can cause eye burns, which may be responsible for permanent injury to the eyes of human and animals, irritation to the gastrointestinal tract with symptoms of nausea, vomiting and diarrhea and also cause methemoglobinemia, cyanosis, convulsions, tachycardia, and dyspnea. Contact of MB with skin causes irritation (Jiang, Sun, Wang, & Zhou, 2008). After inhalation, symptoms such as difficulties in breathing, vomiting, diarrhea and nausea can occur in humans (Bhattacharyya & Sharma, 2005). According to Cragon (1999), intra-amniotic injection of MB during obstetric procedures is associated with serious adverse effects among newborns it was reported that the injection of MB during midtrimester amniocentesis will cause teratogenic effects.

2.3 *Sargassum polycystum*

Algae are a large and diverse assemblage of organisms that contain chlorophyll and carry out oxygenic photosynthesis. The algae can be microscopic or macroscopic in morphology and they are included in the plant kingdom, distinguished from other chlorophyllous plants on the basis of sexual reproduction. Several characteristics are used to classify algae, including the nature of the chlorophyll, the cell wall chemistry, flagellation and even phytopigments. Brown algae derive their characteristic colour

from the large amounts of the carotenoid fucoxanthin (which yields a brown colour) contained in their chloroplasts and the presence of various pheophycean tannins. Typical algal cell walls of Phaeophyta (brown algae) are comprised of at least two different layers. The innermost layer consists of a microfibrillar skeleton that imparts rigidity to the wall while the outer layer is an amorphous embedding matrix. The Phaeophyta algal embedding matrix is predominately alginic acid or alginate (the salt of alginic acid) with a smaller amount of sulphated polysaccharide (fucoidan) (Davis, Volesky & Mucci, 2003).

The carboxylic groups are generally the most abundant acidic functional group in the brown algae (Davis, Volesky & Mucci, 2003). The role of carboxylic groups in the adsorption process has been clearly demonstrated by a reduction in cadmium and lead uptake by dried *Sargassum* biomass following partial or complete esterification of the carboxylic sites (Fourest & Volesky, 1996). Finally, Fourier-transformed infrared (FTIR) spectral analyses have shown that cadmium biosorption to *Sargassum* arises from bridging or bidentate complex formation with the carboxylate groups of the alginate (Fourest & Volesky, 1996). The second most abundant acidic functional group in brown algae is the sulfonic acid of fucoidan. Sulfonic acid groups typically play a secondary role, except when metal binding takes place at low pH. Hydroxyl groups are also present in all polysaccharides but they are less abundant and only become negatively charged at pH>10, thereby, also playing a secondary role in metal binding at low pH (Davis, Volesky & Mucci, 2003).

The division Phaeophyta is subdivided into orders, which subsequently are divided into families, and then the familiar genus and specie are specified. From the point of view of biosorption, only two orders are of importance, namely the orders Laminariales and Fucales. Both of these orders are abundant in nature and include the most structurally complex algae. *Sargassum polycystum* (Figure 2.1) is belongs to the family named *Sargassaceae*, which is under Fucales (Davis, Volesky & Mucci, 2003).

S. polycystum is thalli with dark brown to yellowish brown colour and attached to rocks by a coarse branching holdfast. The stem of *S. polycystum* is short and cylindrical. The terete primary branches bear irregularly alternate secondary

branches with numerous simple and Y-shaped proliferations. Mature thalli have fewer and smaller oblanceolate leaves and the leaves is 7 to 15 mm long and 1.5 to 4 mm wide, with coarsely dentate or serrated margins. There are numerous stalked vesicles which in ovate or spherical shape, attach to the secondary, tertiary and terminal branches either singly or in clusters. *S. polycystum* is dioecious (Carpenter & Niem, 1998).

Figure 2.1: *Sargassum polycystum* (C. Agardh, 1824)
(Source: http://pkukmweb.ukm.my/ahmad/tesis/asmida/hurai.htm)

Usually, *S. polycystum* thrives in inner reef areas on coarse or sandy coralline substrate where not exposed to strong water turbulence. It is commonly found in tropical warm waters of the western Pacific, including the Philippines, China, Japan, Indonesia, Malaysia, and Guam (Carpenter & Niem, 1998).

Generally, seaweeds are the only source for the production of agar, alginate and carrageenan. *S. polycystum* is used as fertilizer, as human food, fodder and medicine. It contains auxin-like substance. Sometimes it used fresh or dried or burnt and the ash utilized as fertilizer on soils. It is also a source of alginate and may form dense stands and is therefore considered as a good biomass source for biogas production (Carpenter & Niem, 1998). Furthermore, *S. polycystum* is a suitable biosorbent with high sorption potential because of the high amounts of alginate

locked within its cellular structures, with abundant carboxylic groups capable of capturing cations present in solution (Diniz & Volesky, 2005).

2.4 Conventional Wastewater Treatment Methods

Dye is one of the important classes of the pollutants and once they enter the water it is no longer good and sometimes difficult to treat. It is because the dyes have a synthetic origin and a complex molecular structure which makes them more stable and difficult to be biodegraded (Forgacs, Cserhati & Oros, 2004; Rai et al., 2005). Overall at present there are more than 100,000 commercial dyes with a rough estimated production of 7×10^5 to 1×10^6 tons per year (Meyer, 1981; Zollinger, 1987; Hunger, 2003; Husain, 2006; Christie, 2007). The exact quantity of dyes discharged in environment is not available however, it is reported that 10 to 15% of the used dyes enter the environment through wastes (Husain, 2006; Hai, Yamamoto & Fukushi, 2007).

The technologies were reported to treat effluents which contained dye can be divided into four categories: (i) physical (ii) chemical (iii) biological and (iv) acoustical, radiation, and electrical processes. Table 2.4 shows major existing and emerging processes for dye removal. All of those technologies have advantages and drawbacks. Because of the high cost and disposal problems, many of these conventional methods for treating dye wastewater have not been widely applied at large scale in the textile and paper industries (Ghoreishi & Haghighi, 2003). There is no single process capable of adequate treatment, mainly due to the complex nature of the effluents (Pereira et al., 2003; Marco et al., 1997). In practice, a combination of different processes is often used to achieve the desired water quality in the most economical way. Some of the conventional methodologies used in dye removal are discussed in subsequent paragraphs.

Table 2.4: Principal existing and emerging processes for dye removal

	Technology	**Advantages**	**Disadvantages**
Conventional treatment process	Coagulation Flocculation	Simple, economically feasible	High sludge production, handling and disposal problems
	Biodegradation	Economically attractive, publicly acceptable treatment	Slow process, necessary to create an optimal favourable environment, maintenance and nutrition requirements
	Adsorption on activated carbons	The most effective adsorbent, great, capacity, produce a high-quality treated effluent	Ineffective against disperse and vat dyes, the regeneration is expensive and results in loss of the adsorbent, non-destructive process
Established recovery processes	Membrane separation	Removes all dye types, produce a high-quality treated effluent	High pressure, expensive, incapable of treating large volume
	Ion-exchange	No loss of sorbent on regeneration, effective	Economic constraints, not effective for disperse dyes
	Oxidation	Rapid and efficient process	High energy cost, chemicals required

Table 2.4: (Continued)

	Technology	Advantages	Disadvantages
Emerging removal processes	Advanced oxidation process	No sludge production, little or no consumption of chemicals, efficiency for recalcitrant dyes	Economically unfeasible, formation of by-products, technical constraints
	Selective bioadsorbents	Economically attractive, regeneration is not necessary, high selectivity	Requires chemical modification, non-destructive process
	Biomass	Low operating cost, good efficiency and selectivity, no toxic effect on microorganism	Slow process, performance depends on some external factors (ph, salts)

(Source: Crini, 2006)

2.4.1 Biological Treatments

Biological treatment is the most common and widespread technique used in dye wastewater treatment (Zhang, Knapp & Tapley, 1998; Bromley-Challenor et al., 2000; van der Zee & Villaverde, 2005; Frijters et al., 2006, Barragan et al., 2007; dos Santos et al., 2007). It is the most economical alternative when compared with other physical and chemical processes (Crini, 2006). A large number of species have been used for decolouration and mineralization of various dyes. The methodology offers advantages like being relatively inexpensive, having low running costs and the end products of complete mineralization not being toxic (Gupta & Suhas, 2009). However, biological treatment requires a large land area and is constrained by

sensitivity toward diurnal variation as well as toxicity of some chemicals, and less flexibility in design and operation (Bhattacharyya & Sarma, 2003). However, biological treatment is incapable of obtaining satisfactory colour elimination with current conventional biodegradation processes (Robinson et al., 2001). Moreover, although many organic molecules are degraded, many others are recalcitrant due to their complex chemical structure and synthetic organic origin. In particular, due to their xenobiotic nature, azo dyes are not totally degraded (Ravi et al., 1998).

The biological treatment process can be aerobic (in presence of oxygen), anaerobic (without oxygen) or combined aerobic–anaerobic (Gupta & Suhas, 2009). In aerobic treatment, bacteria and fungi are the two microorganism groups that have been most widely studied for their ability to treat dye wastewaters. In aerobic conditions, enzymes secreted by bacteria present in the wastewater break down the organic compounds. Ttriphenylmethane dyes have been found to be efficiently decolourized (92–100%) by the strain *Kurthia* sp. (Sani & Banerjee, 1999). Fungal strains capable of decolourizing azo and triphenylmethane dyes have been studied in detail by various workers (Bumpus & Brock, 1988; Vasdev, Kuhad & Saxena, 1995; Sani & Banerjee, 1999). Besides this, microorganisms including *Rhyzopus oryzae*, *Cyathus bulleri* and other microorganisms have also been tested for the deolorization of dyes (Zhang et al., 1999; Nigam et al., 2000; Salony et al., 2006). The decolourisation process is affected by several factors, which are concentration of pollutants, dyestuff concentration, initial pH and temperature of the effluent. The treatments are not suitable for all the dyes because some of them are recalcitrant to biological breakdown or are nontransformable under aerobic conditions (Pagga & Brown, 1986; Rai et al., 2005).

The potential of anaerobic treatment applications for the degradation of a wide variety of synthetic dyes has been well demonstrated and established (Delee et al., 1998; Forgacs et al., 2004; Rai et al., 2005). Azo dyes are nondegradable in conventional aerobic system yet were found to be decolourized under anoaerobic conditions (Rai et al., 2005). An anaerobic pretreatment (Delee et al., 1998) step could be a cheap alternative compared with aerobic systems as expensive aeration is omitted and problems with bulking sludge are avoided. It was suggested the advantages of anaerobic treatment to be that dyes can be reductively decolourised

with the efficient and cheap removal of BOD levels, heavy metals can be retained through sulfate reduction, no foaming problems with surfactants, high effluent temperatures can be favourable, high pH effluent can be acidified and degradation of refractory organics can be initiated (e.g., surfactants, chlorinated aromatics) (Delee et al., 1998). Nevertheless, Delee et al. (1998) suggested the drawbacks to be that BOD removal can be insufficient, dyes and other refractory organics are not mineralized, nutrients (N, P) are not removed and sulfates give rise to sulfide.

A combination of aerobic and anaerobic treatment is suggested in order to get better remediation of coloured compounds from the textile effluents. Generally, an anaerobic decolourization followed by aerobic post-treatment is recommended for treating dye wastewaters (Brown and Hamburger, 1987). These biological treatments are suitable for variety of dyes. The advantages of such system include the complete mineralization which is often achieved due to the synergistic action of different organisms, the reduction of the azo bond can be achieved under the reducing conditions in anaerobic bioreactors and the resulting colourless aromatic amines may be mineralized under aerobic conditions (Brown & Laboureur, 1983a; Brown & Laboureur, 1983b; Stolz, 2001). The factors like concentration of dyes, initial pH and temperature of the effluent, would affect the decolourisation process. The main drawbacks of the biological treatment is low biodegradability of the dyes, less flexibility in design and operation, larger land area requirement and longer times required for decolourisation–fermentation processes thereby making it incapable of removing dyes from effluent on a continuous basis in liquid state fermentations (Robinson et al., 2001; Bhattacharyya & Sarma, 2003; Crini, 2006).

2.4.2 Chemical Treatments

Chemical treatments include coagulation or flocculation combined, precipitation–flocculation with Fe(II)/$Ca(OH)_2$, electroflotation, electrokinetic coagulation, oxidation, irradiation or electrochemical processes. These chemical techniques are often expensive, and although the dyes are removed, accumulation of concentrated sludge creates a disposal problem. There is also the possibility that a secondary

pollution problem will arise because of excessive chemical use. Although these methods are efficient for the treatment of waters contaminated with pollutants, they are very costly and commercially unattractive. The high electrical energy demand and the consumption of chemical reagents are common problems (Crini, 2006).

Chemical treatment of dye wastewater with coagulants or flocculants (Wang et al., 2006; Shi et al., 2007; Zhou et al., 2008) is one of the robust ways to remove colour. The coagulants such as aluminum (Al^{3+}), calcium (Ca^{2+}) or ferric (Fe^{3+}) ions and flocculants are added in the water during the process (Gupta & Suhas, 2009). Sometimes combination of two may also be added to enhance the process (Wang et al., 2007). Generally, the process is economically feasible (but sometimes becomes expensive due to the cost of chemicals) with satisfactory removal of disperse, sulphur, and vat dyes (Gupta & Suhas, 2009). However, the main disadvantages of the process are that the final product is a concentrated sludge produced in large quantities and the removal is pH dependent (Kace & Linford, 1975; Lee et al., 2006). This process is not good for highly soluble dyes and the result with azo, reactive, acid and especially the basic dyes are generally not good (Raghavacharya, 1997; Hai et al., 2007).

Oxidation is another chemical treatment which using oxidizing agents to treat the wastewater. Commonly, two forms viz. chemical oxidation and UV assisted oxidation using chlorine, hydrogen peroxide, fenton's reagent, ozone, or potassium permanganate are used for treating the effluents, especially those obtained from primary treatment (sedimentation) (Gupta & Suhas, 2009). They only require low quantities and short reaction times and thus they are among the most commonly used methods for decolourisation processes. They are used to partially or completely degrade the dyes (generally to lower molecular weight species such as aldehydes, carboxylates, sulfates and nitrogen) (Gupta & Suhas, 2009). However, a complete oxidation of dye can theoretically reduce the complex molecules to carbon dioxide and water (Gupta & Suhas, 2009). pH and catalysts are important factors in oxidation process.

Electrochemical methodology is a tertiary treatment used to remove colour (Lin & Peng, 1994; Gupta et al., 2007). Decolourisation is achieved either by electro

oxidation with non-soluble anodes, such as iron, conducting polymer and boron doped diamond electrode etc. or by electro-coagulation using consumable materials (Gupta & Suhas, 2009). This technique is effective in decolourisation of soluble and insoluble dyes with reduction of COD. It is worthwhile pointing that among other variables the rate of colour and organic load removal depends, on the anode's material and the working potential. Nevertheless, the main inconveniences are high electricity cost, sludge production and pollution from chlorinated organics, heavy metals due to indirect oxidation (Gupta & Suhas, 2009).

2.4.3 Physical Treatments

Physical treatments include membrane-filtration processes and adsorption techniques. Filtration technologies include microfiltration, ultrafiltration, nanofiltration, and reverse osmosis. Each membrane process is best suited for a particular water treatment function (Cheremisinoff, 2002). Among them, microfiltration is seldom use for wastewater treatment because of its large pore size. Though ultrafiltration and nanofiltration techniques are effective for the removal of all classes of dyestuffs (Marmagne & Coste, 1996; Cheremisinoff, 2002), dye molecules cause frequent clogging of the membrane pores so their use in textile effluent treatment is limited too (Gupta & Suhas, 2009). The main drawbacks are high working pressures, significant energy consumption, high cost of membrane and a relatively short membrane life which makes their use limited for treating dye wastewater (Gupta & Suhas, 2009). Reverse osmosis forces water which through a membrane that is impermeable to most contaminants under pressure, is somewhat better at rejecting salts than it is at rejecting non-ionized weak acids and bases and smaller organic molecules generally molecular weight below 200 (Gupta & Suhas, 2009). Reverse osmosis is effective decolouring and desalting process against the most diverse range of dye wastes (Marcucci et al., 2001; Al-Bastaki, 2004; Sostar-Turk et al., 2005), and has been successfully employed for recycling (Gupta & Suhas, 2009).

Amongst the numerous techniques of dye removal, adsorption is the procedure of choice and gives the best results as it can be used to remove different

types of colouring materials (Derbyshire et al., 2001; Jain et al., 2003; Ho & McKay, 2003). The term adsorption refers to a process wherein a material is concentrated at a solid surface from its liquid or gaseous surroundings (Gupta & Suhas, 2009). It is now customary classified into physical adsorption and chemisorptions (Gupta & Suhas, 2009). The former occurs due to attraction between the solid surface and the adsorbed molecules are physical in nature while the latter's attraction forces are due to chemical bonding (Gupta & Suhas, 2009). Adsorption has been found to be superior to other techniques for water re-use in terms of initial cost, flexibility and simplicity of design, ease of operation and insensitivity to toxic pollutants. Adsorption also does not result in the formation of harmful substances.

The quantity of adsorbate that an adsorbent can accumulate is one of the most important characteristic of adsorbent. A good adsorbent should generally possess a porous structure (resulting in high surface area) and the time taken for adsorption equilibrium to be established should be as small as possible so that it can be used to remove dye wastes in lesser time (Linsen, 1970; Tien, 1994). Adsorbents which are often used for dye wastewater treatment are alumina, silica gel, zeolites, and activated carbon (Gupta & Suhas, 2009). Activated carbon is a popular sorbent to remove dyes in wastewater due to its excellent adsorption ability.

On the other side, liquid-phase adsorption is one of the most popular methods for the removal of pollutants from wastewater since proper design of the adsorption process will produce a high-quality treated effluent. This process provides an attractive alternative for the treatment of contaminated waters, especially if the sorbent is inexpensive and does not require an additional pre-treatment step before its application (Crini, 2006).

2.5 Biosorption

Biosorption is the passive uptake of pollutants from aqueous solutions by the use of non-growing or non-living microbial mass (Vijayaraghavan & Yun, 2008). Recovery and environmentally acceptable disposal of the pollutants can be achieved in

biosorption. The special surface properties of bacteria, yeasts, fungi and algae enable them to adsorb different kinds of pollutants from solutions (Aksu, 2005). During biosorption process, a number of metabolism-independent processes, such as physical adsorption, chemical adsorption, electrostatic interaction, ion exchange, omplexation, chelation, and microprecipitation, taking place essentially in the cell wall rather than oxidation though anaerobic or aerobic metabolism (biodegradation) (Aksu, 2005). Biosorption have advantages of high selectivity and efficiency, cost effectiveness and good removal performance. The mechanism of binding depends upon the type of biomass, chemical nature of the pollutant and the environmental conditions such as pH, temperature and ionic strength (Vijayaraghavan & Yun, 2008).

Bacteria, fungi, algae, wastes from other industrial operation (fermentation wastes, activated sludge process wastes), agricultural wastes and other polysaccharide materials can be used as biosorbents (Vijayaraghavan & Yun, 2008). Both living and dead (heat killed, dried, acid and/or otherwise chemically treated) biomass can be used to remove hazardous organics. However, it is difficult to maintain a viable biomass during adsorption because of the requirement of continuous supply of nutrients and avoidance of organic toxicity to the microorganisms (Aksu, 2005). Compare to living cells, dead microbial cells is more advantageous for the application of biosorption of water treatment as dead organisms are not affected by toxic wastes, do not require a continuous supply of nutrients and can be regenerated and reused for many cycles. Furthermore, dead cells may be stored or used for extended periods at room temperature without putrefaction occurring. It is also easy to prepare and regenerate dead cells. Moreover, dead cells have been shown to accumulate pollutants to the same or greater extent than growing or resting cells (Aksu, 2005). The mechanism of binding by inactivated biomass may depend on the chemical nature of pollutant (species, size, ionic charge), type of biomass, its preparation and its specific surface properties and environmental conditions (pH, temperature, ionic strength, existence of competing organic or inorganic ligands in solution). Biosorption is generally used for the treatment of heavy metal pollutants and organics in industrial waste streams and polluted natural waters (Aksu, 2005).

The biosorption process can be built in batch and continuous system. Numerous biosorption studies are performed in batch systems with single species of organics (Aksu, 2005). Suitable microbial biomass is contacted with aqueous solution containing organic pollutant molecules or ions for a sufficient time to allow the biomass to sequester these molecules and to reach equilibrium. Then the biomass is separated from the liquid phase and the pollutant-containing biomass is either regenerated or disposed in an environmentally acceptable manner. A major consideration with any biosorption scheme is the separation of liquid and solids after batch or counter current contacting (Aksu, 2005).

Continuous systems such as continuous stirred tank reactors, fluidized bed, moving bed and packed bed columns are generally practical in industrial processes. Packed column is the most convenient configuration for continuous operation and it ensures the highest possible concentration difference driving force (Aksu, 2005). Continuous packed bed biosorption has a number of process engineering advantages including high yield operations and relatively easy scaling up from a laboratory scale procedure (Aksu, 2005). A large volume of wastewater can be continuously treated using a defined quantity of biosorbent in the column. During the process, the pollutant may be concentrated in a small volume of solid material or desorbed into a small volume of eluant for recovery, disposal or containment (Aksu, 2005).

The dead biomass is reusable but it faces some problems to use the dead biomass in powdered form in the column. It is difficult to separate the biomass after biosorption, mass loss after regeneration, low strength and density and small particle size, which make it difficult to use in column applications (Aksu, 2005). These problems can be solved by immobilizing the dead biomass in a supporting material. Researchers have recognized that immobilizing nonliving biomass in a biopolymeric or polymeric matrix may improve biomass performance, biosorption capacity, increase mechanical strength and facilitate separation of biomass from pollutant containing solution (Aksu, 2005). Immobilization also allows higher biomass concentration, resistance to chemical environments and column operations and immobilized systems may be well suited for non-destructive recovery. In contrast, immobilized biomass has disadvantage of increasing the cost of biomass pre-treatment. Immobilization adversely affects the mass transfer kinetics of organics

uptake. When biomass is immobilized the number of binding sites easily accessible to organic molecules or ions in solution is greatly reduced since the majority of sites will lie within the bead (Aksu, 1998; Volesky, 2001; Aksu & Gönen, 2004).

There are many studies on the effect of pH, temperature, initial dye concentration, salts, heavy metal ions, other dyes, surfactants, shaking rate, and particle size on dye biosorption. pH is the most important parameter affecting not only biosorption capacity, but also colour of the dye solution and the solubility of some dyes. Dyed wastewater effluents are discharged at relatively high temperatures (50-60°C) and it contains salts, heavy metals, surfactants, and other dye species. These are the factors that influence biosorption capacity. Besides, larger particle size of biosorbent provides larger surface area for biosorption and the higher initial dye concentration could enhance the absorption process. In addition, there is a study show that an adequate stirring rate in a batch biosorption process contributes to overcome external mass transfer resistances.

CHAPTER 3

METHODOLOGY

3.1 Sorbent Preparation

3.1.1 Preparation of *Sargassum polycystum*

Sargassum polycystum was collected from Teluk Kemang, Port Dickson on 16 July 2010. Fresh seaweed was packed and stored in -4℃. *S. polycystum* was washed with tap water to remove salt, some epiphytes and other species of seaweed and then further rinsed with distilled water with 5 to 6 time until the water became clear. Then the material was dried by air in indoor for 2 to 3 days.

3.1.2 Chemical Modification

The cleaned and dried seaweed was subjected to chemical modifications with formaldehyde (0.2% v/v) to replace the natural mix of ionic species with protons. Seaweed (1.5 g) was put into 150 mL of 0.2% formaldehyde and shaked in an incubator shaker at 150 rpm and 30℃ for 24 hours. The mixture was subjected to undergo the cross-linking reaction. The modified seaweed was then separated from the solutions and washed by distilled water and then air dried. The dried mass was sealed well and stored with silica gel in room temperature.

3.2 Dye Solution Preparation

The dyes used in this study are malachite green and methylene blue. Before the experiment, stock solutions were prepared by dissolving accurately weighed dye powder in distilled water at a concentration of 1 g/L and left overnight to make the dye powder fully dissolved. The bottles were covered with aluminium foil in order the prevent decolourisation caused by light and stored in dark environment in room temperature.

Dye solutions with different concentrations were obtained by dissolving the stock solutions. The concentrations of dye solutions were determined by a UV-Vis spectrophotometer (Varian Cary 100) operating in the visible range on absorbance mode. Absorbance values were recorded at the corresponding maximum absorbance wavelength (λ_{max}). The λ_{max} of MG and MB are 617 and 664nm, respectively. Dye solution was initially calibrated for concentration in terms of absorbance units. The calibration curves of MG and MB binary solution were plotted respectively from the dye solution prepared in the concentration range from 10-500 mg/L. Figure 3.1 and 3.2 show the standard curve of MG and MB solution.

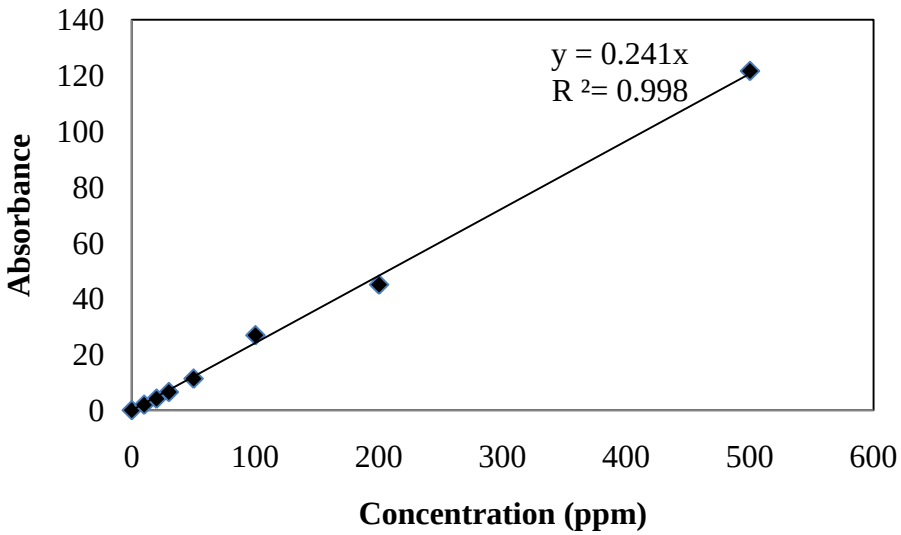


Figure 3.1: Standard curve of malachite green solution.

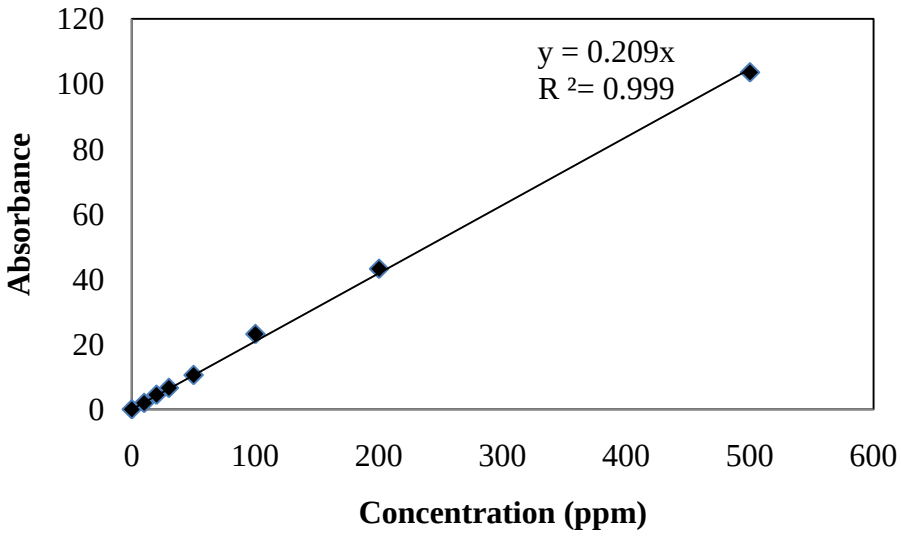


Figure 3.2: Standard curve of methylene blue solution.

3.3 Batch Adsorption Studies

The prepared adsorbent was tested for the removal of binary solution of malachite green and methylene blue using a batch mode process. In this study, three parameters, which are initial dye concentration, sorbent dosage and temperature, were investigated. The experiments were conducted in 250 mL flasks containing 100 mL of dye solution and 1 g of formaldehyde treated seaweed. The batch experiments were performed under shaking in an incubator shaker, with 150 rpm at 30℃. During the experiments, samples were taken to measure the dye removal at predetermined time intervals. The concentration of the dye solutions were determined by measuring their characteristic absorbance using double beam UV–vis spectrometer (Varian Cary 100) at maximum absorbance wavelength. All the experiments were conducted in triplicate and the average value were taken for analysis.

The amount of dye adsorbed on seaweed, also called sorption capacity, q(mg/g), was obtained as follows:

$$q = \frac{(C_0 - C_e)V}{W} \tag{3.1}$$

where,

C_0 = Initial liquid phase concentration (mg/L)

C_e = Equilibrium liquid phase concentration (mg/L)

V = Volume of the solution (L)

M = Amount of adsorbent used (g)

To express the percent of dye removal, the following equation was used:

$$\%\, removal = \frac{C_0 - C_e}{C_0} \times 100\% \quad (3.2)$$

where,

C_0 = Initial liquid phase concentration (mg/L)

C_e = Equilibrium liquid phase concentration (mg/L)

3.3.1 Preliminary Tests

Preliminary tests were carried out by taking sample every 1 hour until the solution concentration reached equilibrium. One gram of *S. polycystum* was put into a flask contained binary solution of MG and MB with a concentration of 100 mg/L and agitated at 150 rpm and 30℃.

3.3.2 Effect of Initial Dye Concentration

Binary dye solutions with initial concentration of 50, 100, 150, 200, 300 and 500 ppm were prepared. One gram of dried and treated biomass was put into the dye solution and agitated at 150 rpm and 30℃. Samples were taken to determine concentration after predetermined time intervals.

3.3.3 Effect of Sorbent Dosage

The effect of sorbent dosage on the sorption capacity was studied by changing the sorbent dosage to 0.2, 0.4, 0.6, 0.8 and 1 g. Sorbents with various weights were put into the dye solution with concentration of 100ppm and agitated at 150rpm and 30℃. Samples were taken every 15 minutes for 3 hours.

3.3.4 Effect of Temperature

The effect of temperature on the sorption capacity was studied at 30, 40, 45, 50 and 60◦C. One gram of sorbent was put into the dye solution with concentration of 100 ppm and agitated at 150 rpm and various temperatures. Samples were taken every 15 minutes for 3 hours.

3.4 Sorption Isoterms

Within this study, the Langmuir (Langmuir, 1918) and Freundlich (Freundlich, 1907) models, which are two-parameter models, have been used to describe sorption isotherm.

3.4.1 Langmuir Isotherm

Langmuir isotherm was used to model the biosorption process of MG and MB onto *Sargassum polycystum*. The Langmuir model is often represented as:

$$\frac{C_e}{q_e} = \frac{C_e}{q_{max}} + \frac{1}{bq_{max}} \tag{3.3}$$

This relation can be expressed in linear form as follows:

$$\frac{1}{q_e} = \frac{1}{q_{max}} + \frac{1}{b q_{max}\, C_e} \tag{3.4}$$

where,

q_e = Amount of sorbate sorbed at equilibrium (mg/g)

q_{max} = Maximum sorption capacity (mg/g)

b = Langmuir equilibrium constant (L/mg)

C_e = Equilibrium dye concentration in the solution (mg/L)

The Langmuir isotherm constants, b and q_{max}, were determined from the plot of $1/q_e$ versus $1/C_e$. The type of the isotherm can be indicated by the separation factor, R_L, which is dimensionless.

$$R_L = \frac{1}{1+bC_e} \tag{3.5}$$

3.4.2 Freundlich Isotherm

Freundlich isotherm is also studied to model the biosorption process and it can be represented as:

$$q_e = K_f C_e^{1/n} \tag{3.6}$$

The linearized equation can be written as

$$\ln q_e = \ln K_f + \frac{1}{n} \ln C_e \tag{3.7}$$

where,

q_e = Amount of sorbate sorbed at equilibrium (mg/g)

K_f = Freundlich constant ((mg/g)(L/mg)$^{1/n}$)

1/n = Freundlich exponent (dimensionless)

C_e = Equilibrium dye concentration in the solution (mg/L)

The Freundlich isotherm constants, K_f and 1/*n*, were determined from the plot of ln q_e versus ln C_e.

3.5 Sorption Kinetic Modelling

Pseudo-first and pseudo-second-order models have been used to describe biosorption kinetic data.

3.5.1 Pseudo-first-order Model

The pseudo-first-order model is described as:

$$\frac{dq_t}{dt} = K_1(q_e - q_t) \tag{3.8}$$

Equation 3.9 was integrated with initial condition that q_t = 0 at t = 0 and a linear form was formed.

$$\log(q_e - q_t) = logq_e - \frac{k_1}{2.303}t \tag{3.9}$$

where

q_e = Amount of solute sorbed at equilibrium (mg/g)

q_t = Amount of solute sorbed at time t (mg/g)

k_1 = Pseudo-first-order equilibrium rate constant (1/h)

t = Contact time (h)

The Pseudo-first-order constants, k_1 and $q_{e(cal)}$, were determined from the plot of (log ($q_{e(exp)}$-q_t) versus t).

3.5.2 Pseudo-second-order Model

The pseudo-second-order model is given as:

$$\frac{dq_t}{dt} = K_2(q_e - q_t)^2 \tag{3.10}$$

Equation 3.11 was integrated with boundary conditions t = 0 to t = t and q_t = 0 to q_t = q_t and a linear form was formed.

$$\frac{t}{q_t} = \frac{1}{k_2 {q_e}^2} + \frac{1}{q_e} t \tag{3.11}$$

where,

q_e = Amount of solute sorbed at equilibrium (mg/g)

q_t = Amount of solute sorbed at time t (mg/g)

k_2 = Pseudo-second-order equilibrium rate constant (g/mg/h)

t = Contact time (h)

The Pseudo-second-order constants, k_2 and $q_{e(cal)}$, were determined from the plot of (t/q_t versus t).

3.6 Thermodynamic Study

The thermodynamic study was conducted to study the thermodynamic parameters of the biosorption process. Gibbs free energy change, ΔG^o is estimated by applying thermodynamic equation as shown below

$$\Delta G^{o} = -RT \ln K_c \quad (3.12)$$

where,

R = Gas constant = 8.314 J/molK

T = Absolute temperature in K

K_c = Thermodynamic equilibrium constant at temperature T

K_c was estimated using the following equation:

$$K_c = \frac{C_s}{C_e} = \frac{C_0 - C_e}{C_e} \quad (3.13)$$

where,

C_s = Concentration of dye sorbed on surface of sorbent (mg/L)

C_e = Equilibrium dye concentration in the solution (mg/L)

C_0 = Initial dye concentration in the solution (mg/L)

Enthalpy change and standard entropy change of the biosorption process were calculated from van't Hoff equation with the following relation. ΔH^{o} and ΔS^{o} were calculated the slope and intercept of van't Hoff plots of ln K_c versus 1/T

$$lnK_c = \frac{\Delta S^{o}}{R} - \frac{\Delta H^{o}}{RT} \quad (3.14)$$

where,

ΔH^{o} = Enthalpy change

ΔS^{o} = Entropy change

3.7 Experiment Design

Figure 3.3 shows the general plan of the experimental design performed in the present study.

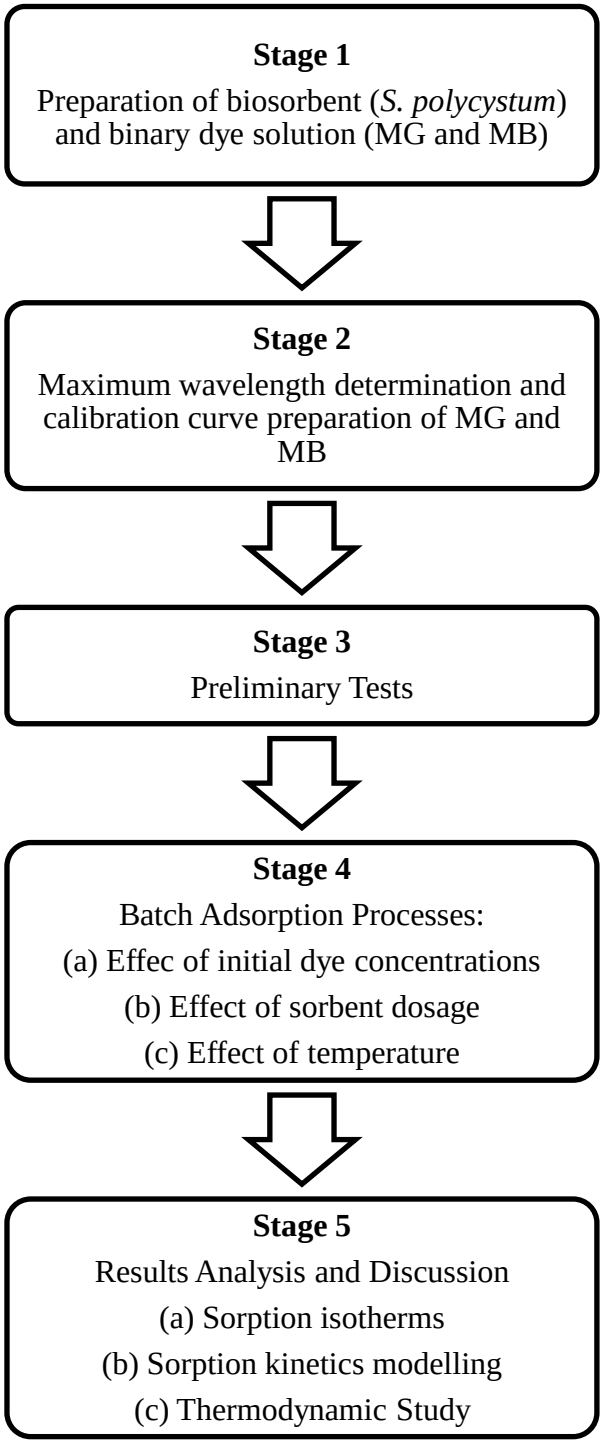


Figure 3.3: Proposed experimental works.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Preliminary Tests

Preliminary tests were run early in the experiment in order to learn about the efficiency of biosorption process and establish the baseline of configuration of experiments. One gram of *S. polycystum* was put into a flask contained binary solution of MG and MB with a concentration of 100 mg/L. Samples were taken every 1 h in the first 7 h and then do the last sampling after 24 h. The removal of each dye at different time was shown in Figure 4.1. Both MG and MB were removed very fast in the process and 100% MB removal was achieved after 1 h. The removal of MG is slightly lower than MB. Figure 4.2 has emphasized on the removal of MG started at 1 hour. Almost 98% of MG was removed within one hour and equilibrium was achieved after 2 h of agitation, with 98.3% of removal. The equilibrium sorption capacity of MG and MB is 9.9 mg/L and 9.83 mg/L respectively (Figure 4.3). The sorption capacity of the dyes considered no difference.

Both MG and MB are basic dyes. The dissociation of the dye molecules produce positively charged component. On the other hand, it was found that *Sargassum polycystum* contains three types of functional groups, include carboxyl, phosphonate and amine group (Yun, 2003; Gibert, Pablo, Cortina, & Ayora, 2004). These functional groups provide binding sites for positively-charged ions. As a result of electrostatic attraction, the dye molecules tend to be attracted by the negatively charged surface.

From the preliminary test, MG and MB were known that they can be adsorbented extremely fast by *S. polycystum* especially the latter. In the following experiments, samples would sometimes be taken every 15 minutes for 3 h for better observation.

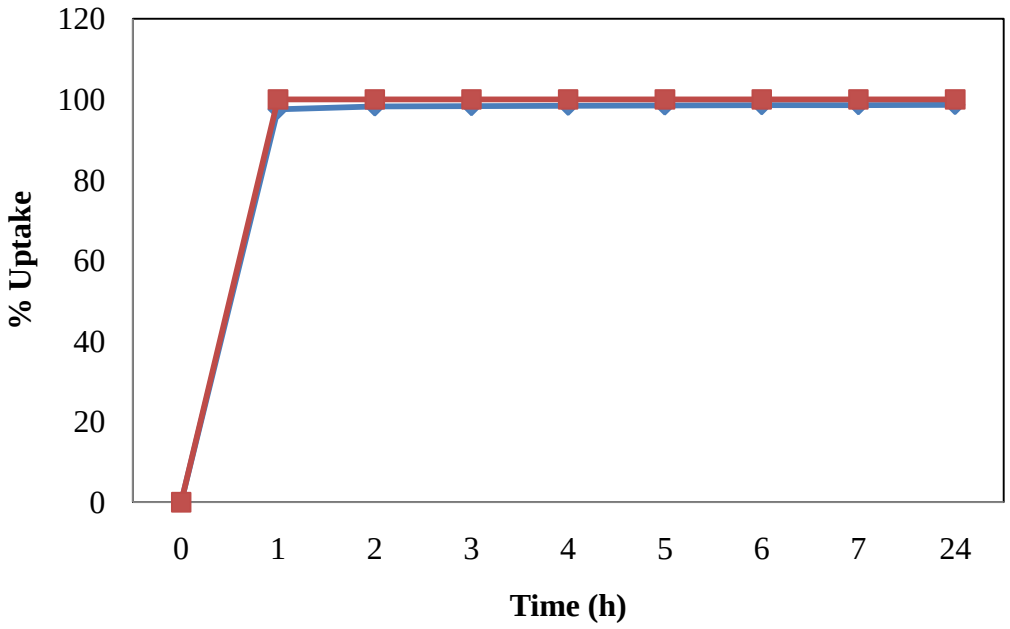


Figure 4.1: Removal of MG (◆) and MB (■) by *S. polycystum*. (*V*: 100 mL; C_i: 100 mg/L; *M*: 1.0 g; *T*: 30℃; *U*: 150 rpm)

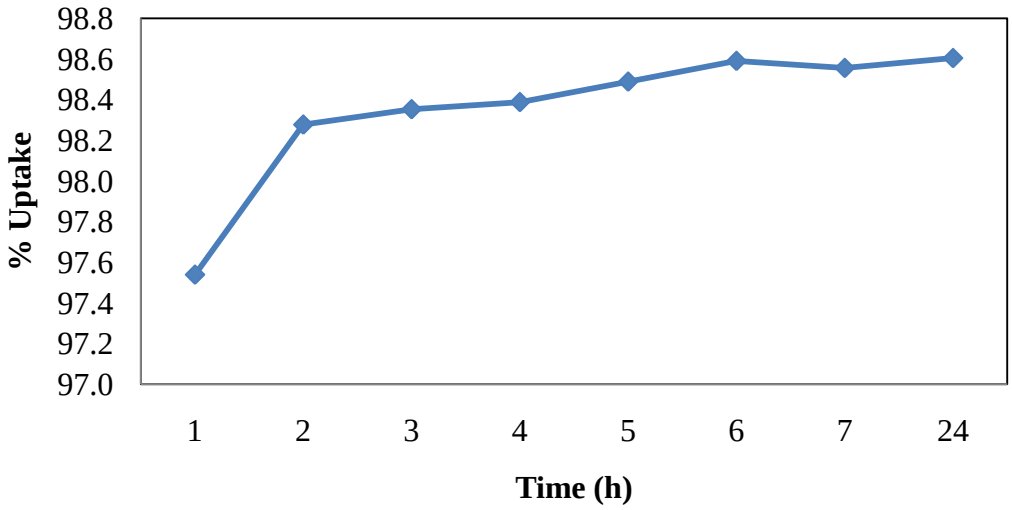


Figure 4.2: Removal of MG in binary solution with MB. (*V*: 100 mL; C_i: 100 mg/L; *M*: 1.0 g; *T*: 30℃; *U*: 150 rpm)

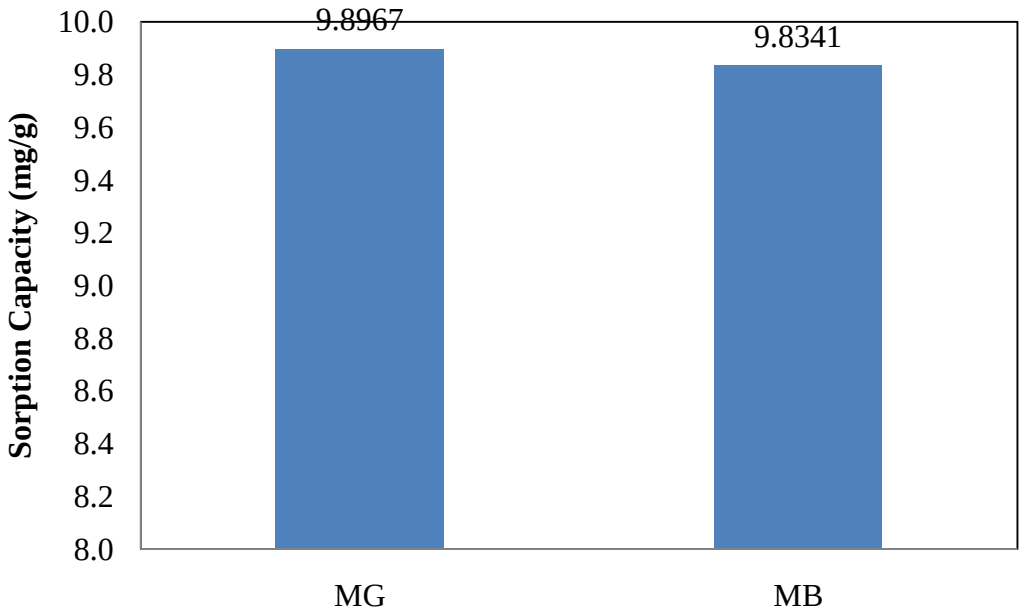


Figure 4.3: Sorption capacity of *S. polycystum* on removal of binary solution of MG and MB. (*V*: 100 mL; C_i: 100 mg/L; *M*: 1.0 g; *T*: 30℃; *U*: 150 rpm)

4.2 Effect of Initial Dye Concentration

The effect of initial dye concentration on sorption capacity of *S. polycystum* was investigated by changing concentration of both dyes from 50 to 500 mg/L. Figure 4.4 shows the equilibrium sorption capacity of MG and MB at various concentrations. From that figure, it can be observed that the amount of dye adsorbed varies with varying initial dye concentration and increases with increase in initial dye concentration. The effect of initial dye concentration on the sorption capacity has been found to be of considerable significance for the basic dye used. As shown in Figure 4.4, amount of MG adsorbed increased from 3.78 to 49.16 mg/g while that of MB increased from 4.9 to 48.1 mg/g for an increase in initial dye concentration from 50 to 500 mg/L. It was found that sorption capacity for MG and MB is very close but for most of the concentrations, sorption capacity of MB is slightly higher than MG because MB always achieved 100% removal in a very short time. An increase in initial dye concentration leads to an increase in the adsorption capacity of MG and MB on biomass.

A higher initial concentration provides an important driving force to overcome all resistances of the dye between the aqueous and solid phases, thus increasing the uptake. In addition, increasing the initial dye concentration increases the number of dye ions in the aqueous solution and hence enhance the number of collisions between dye ions and the seaweeds, which enhances the adsorption process (Aksu & Tezer, 2005).

As shown in figure 4.5, the removal of dye increases rapidly in the beginning and then became more gradual till equilibrium. No significant change in dye removal after about one to two hours. During the initial stage of sorption, a large number of vacant surface sites are available for adsorption. After laps of some time, the remaining vacant surface sites are difficult to be occupied due to repulsive forces between the solute molecules adsorbed on the solid surface and the bulk phase (Vijayaraghavan, Mao & Yun, 2008). This is the reach of equilibrium stage. Equilibrium time is one of the parameters for economical wastewater treatment plant applications. In binary system, MG at different initial concentration reaches dynamic equilibrium within one to two hours. Compare to solution with lower concentration, solution with higher concentration needs a longer time to reach equilibrium. On the other side, MB was completely adsorbed when the first sampling was carried out.

A study made by Hameed, Ahmad and Aziz (2006) observed that at the beginning of removal process, with the palm ash the adsorbent for acid green 25, the adsorption rate was fast. When the adsorption of the exterior surface reached saturation, the dye ions entered into the pores of palm ash and were adsorbed by the interior surface of the palm ash. This phenomenon takes relatively long contact time.

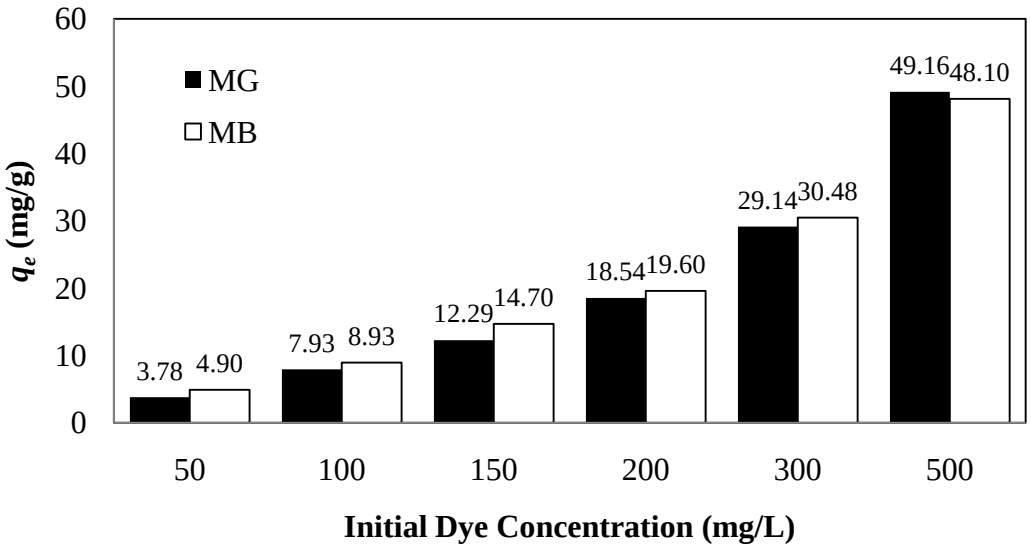


Figure 4.4: Sorption capacity of *S. polycystum* on removal of binary solution of MG and MB at different initial dye concentration. (*V*: 100 mL; C_i: 100 mg/L; *M*: 1.0 g; *T*: 30℃; *U*: 150 rpm)

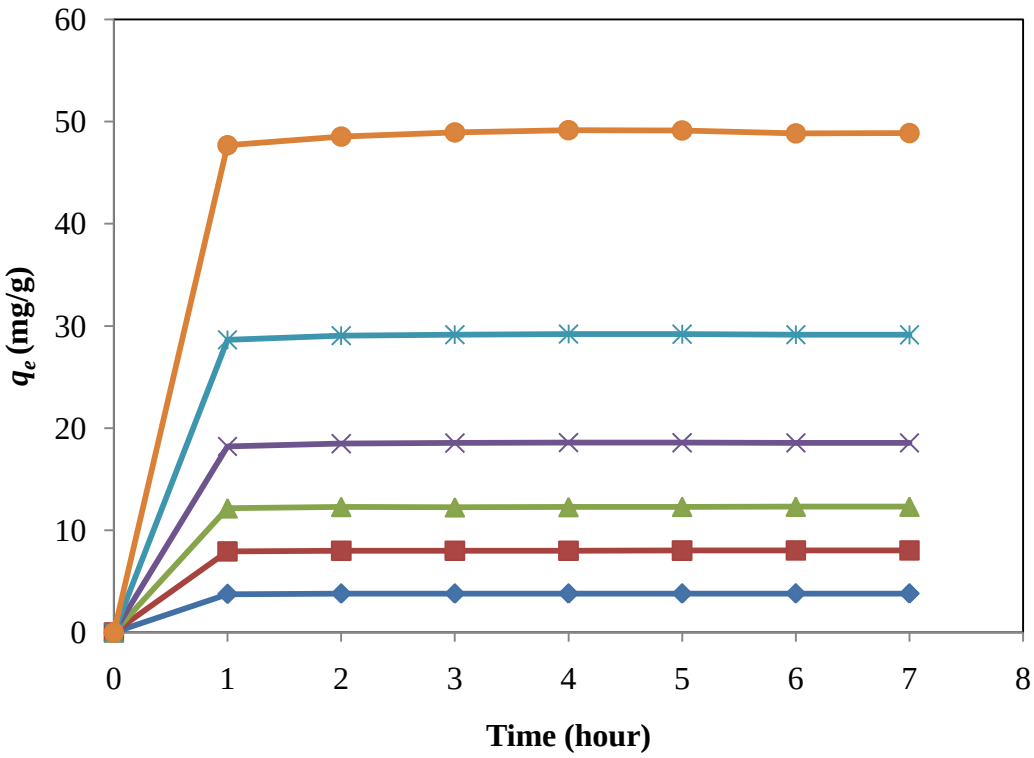


Figure 4.5: Effects of initial dye concentration on sorption of MG. Symbols: (◆) 50 mg/L; (■) 100 mg/L; (▲) 150 mg/L; (×) 200 mg/L; (✳) 300 mg/L; (●) 500 mg/L. (*V*: 100 mL; C_i: 100 mg/L; *M*: 1.0 g; *T*: 30℃; *U*: 150 rpm).

4.3 Effect of Sorbent Dosage

In order to investigate the effect of adsorbent dosage on the uptake of MG and MB onto *S. polycystum*, an initial dye concentration of 100 mg/L was selected and samples were taken every 15 minutes in 3 hours. The adsorbent dosages were in the range from 0.2 to 1.0 g. As can be seen from Figure 4.6, when the adsorbent dosage increases, sorption capacity of MG and MB also increases. Sorption capacity of MG and MB increased from 8.53 to 10.26 mg/g and from 9.45 to 10.25 mg/g, respectively as sorbent dosage was increased from 0.2 to 1.0 g. This indicates that with an increase of sorbent mass, more surface area is made available, and therefore an increase of the total number of sites. One gram is found that be the optimal sorbent dosage.

In addition, Figure 4.7 reveals that the time necessary to reach equilibrium stage decreased with the increasing of sorbent dosage. For the lowest seaweed dosage studied (0.2 g), equilibrium was reached after about 120 min, while for the highest sorbent dosage (1 g) equilibrium was attained after around 50 min. This is due to the higher availability of sorption sites for higher sorbent dosage.

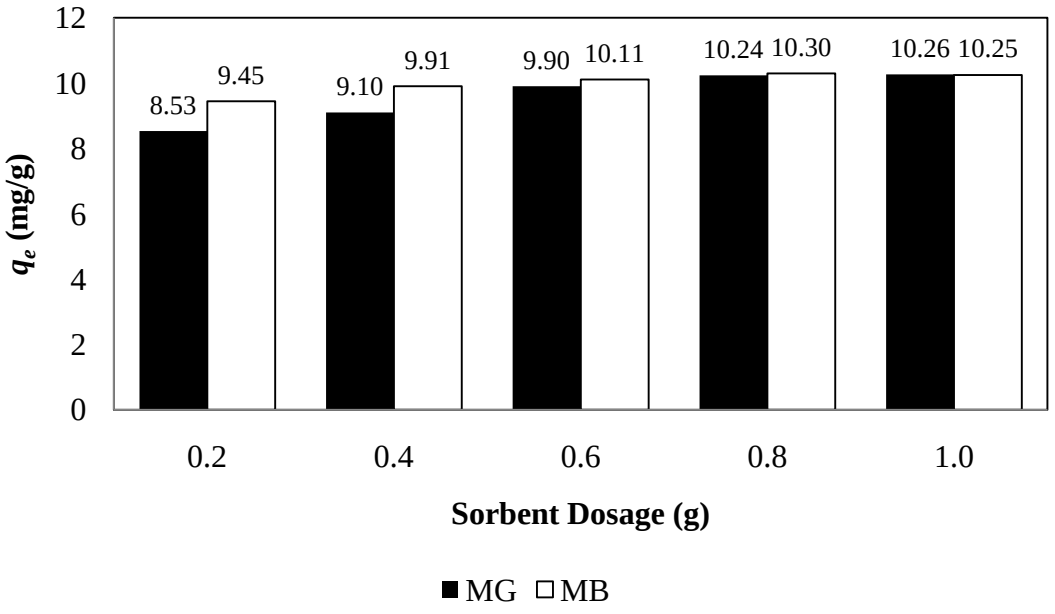


Figure 4.6: Sorption capacity of *S. polycystum* on removal of binary solution of MG and MB at different sorbent dosage. (*V*: 100 mL; C_i: 100 mg/L; *M*: 1.0 g; *T*: 30℃; *U*: 150 rpm)

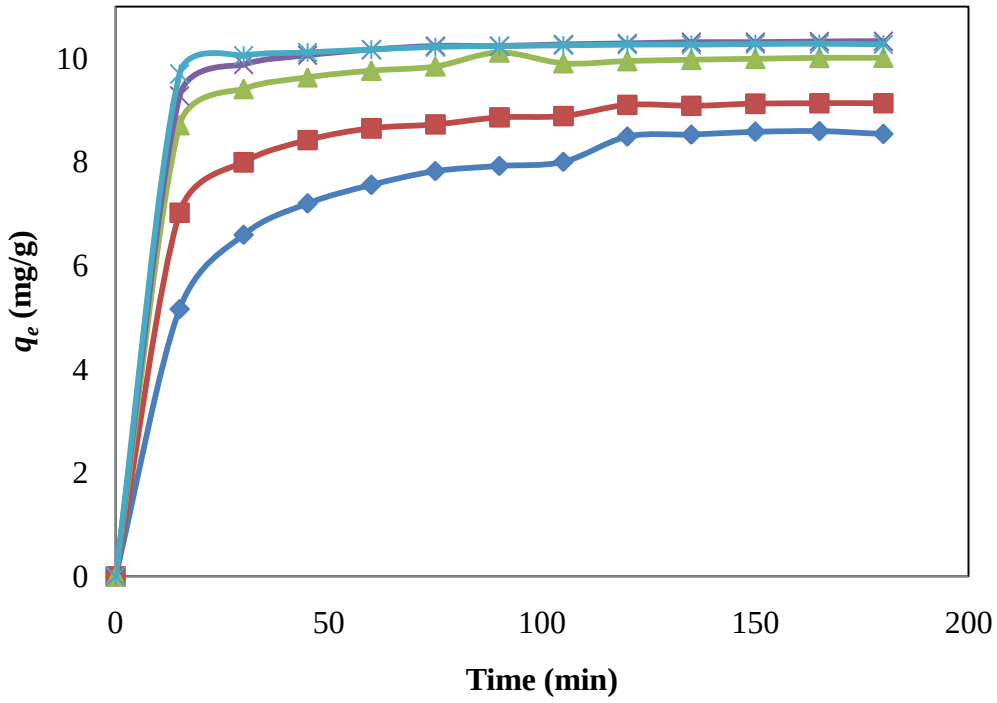


Figure 4.7: Effects of sorbent dosage on sorption of MG. Symbols: (◆) 0.2 g; (■) 0.4 g; (▲) 0.6 g; (×) 0.8 g; (✳) 1.0 g. (*V*: 100 mL; C_i: 100 mg/L; *M*: 1.0 g; *T*: 30℃; *U*: 150 rpm)

4.4 Effect of Temperature

The effect of temperature on the sorption capacity of MG and MB in binary solution was studied at 30, 40, 45, 50 and 60◦C. The results (Figure 4.8) show that the sorption capacity of MG decreased from 9.84 to 8.88 mg/g with temperature increased from 30 to 60◦C (Figure 4.8). The sorption capacity of MB also decreased from 9.83 to 9.13 mg/g. The decrease of sorption capacity with increasing temperature suggests that the adsorption process was exothermic and the mechanism was mainly physical adsorption, dominant at lower temperature (Deniz & Saygideger, 2010). This can be explained by the exothermic spontaneity of the sorption process and by the weakening of bonds between dye molecules and active sites of sorbents at high temperatures (Amin, 2009). However, in present study, the decrease of sorption

capacity of the MG and MB is not significant and this reveals that the adsorption process is independent of temperature.

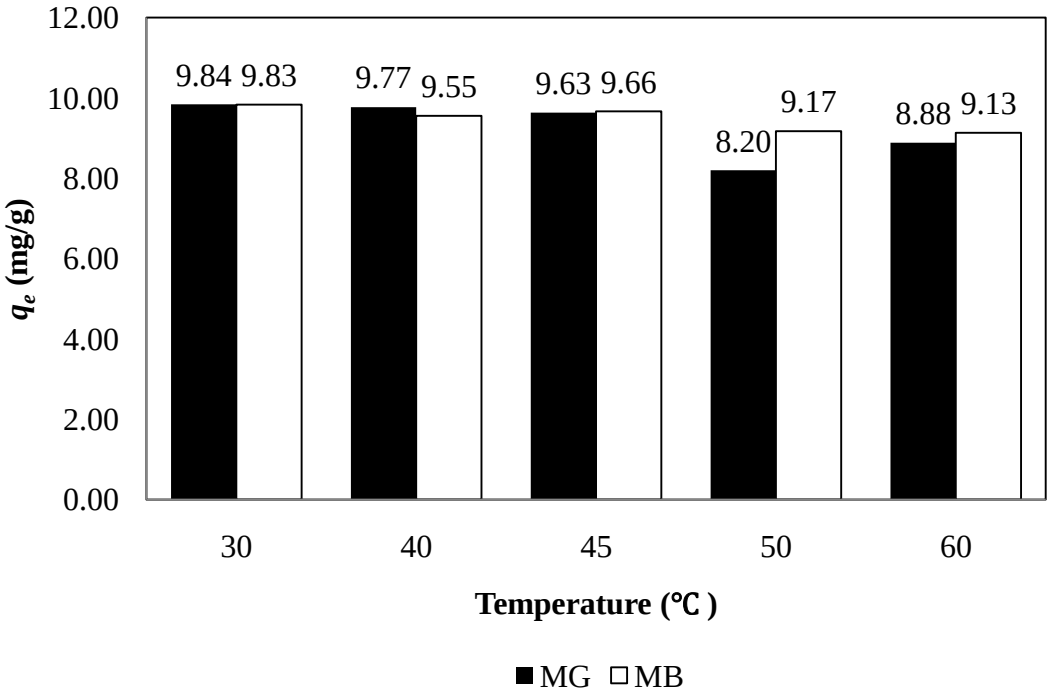


Figure 4.8: Sorption capacity of *S. polycystum* on removal of binary solution of MG and MB at different temperature. (*V*: 100 mL; C_i: 100 mg/L; *M*: 1.0 g; *T*: 30℃; *U*: 150 rpm)

4.5 Adsorption Isotherms

Models have an important role in technology transfer from a laboratory- to industrial-scale. Appropriate models can help in understanding process mechanisms, analyze experimental data, predict answers to operational conditions and optimize processes. As an effective quantitative means to compare binding strengths and design biosorption processes, employing mathematical models for the prediction of binding capacities can be useful (Volesky & Holan, 1995; Limousin et al., 2007). Within this study, the Langmuir (Langmuir, 1918) and (Freundlich, 1907) models,

which are two-parameter models, have been used to describe biosorption isotherm. The models are simple, well-established and have physical meaning and are easily interpretable, which are some of the important reasons for their frequent and extensive use.

MB was adsorbed too fast in the experiment and can be totally removed within 15 minutes. Consequently, it is inadequate to apply the experiment data of MB in the modelling parts. The following discussion only emphasize on MG. The biosorption isotherm of MG and MB removal by using *S. polycystum* at different initial dye concentration was studied using the Langmuir and Freundlich models. The model constants, along with correlation coefficients (R^2) obtained for the two isotherm models, are listed in Table 4.1.

The value of correlation coefficients, R^2 can describe how well a model fits a set of observations. Table 4.1 reveals that R^2 value of Langmuir and Freundlich isotherm are 0.9165 and 0.9303, respectively. Based on the correlation coefficients, the applicability of the isotherms was compared and it may assume that Langmuir model was slightly better fit to the adsorption data of MG than Freundlich model. In addition, from Table 4.1, Langmuir constant b and q_{max} are 0.0196 and 250, respectively while Freundlich constant K_f and $1/n$ were found to be 2.142 and 0.5428, respectively. Both two sets of the isotherms constants are valid and both support the applicability of the isotherms. These experimental results indicated that the sorption of MG (in solution with MB) onto *S. polycystum* followed both Freundlich and Langmuir models. Similar observation was reported for adsorption of malachite green on degreased coffee bean (Baek, Ijagbemi & Kim, 2010).

The Langmuir sorption isotherm suggests that when the sorbate occupies a site further sorption cannot take place at that site and it happens on homogeneous surface (Bekci, Cavas & Sekia, 2009). The constant b represents the affinity between the sorbent and sorbate. High b values are reflected in the steep initial slope of a sorption isotherm, indicating a desirable high affinity. Thus, for good general biosorbents, a high q_{max} and steep initial isotherm slope (high b) are desirable (Vijayaraghavan & Yun, 2008). The b value obtained in the experiment is 0.0196 and not considered as a high value. Another feature of Langmuir isotherm, R_L values

at different initial dye concentration (Table 4.3) are from 0.15 to 0.96. Further, R_L in the range from 0 to 1 indicates a favorable adsorption. Consequently, sorption of MG by *S. polycystum* conforms to Langmuir isotherm model.

In contrast with Langmuir isotherm, Freundlich equation suggests a multilayer adsorption and the sorption energy exponentially decreases on completion of the sorption centers of an adsorbent (Bekci, Cavas & Sekia, 2009). It is assumed that the stronger binding sites are initially occupied, with the binding strength decreasing with increasing degree of site occupation (Davis, Volesky & Mucci, 2003). Freundlich isotherms allowed the unlimited adsorption characteristic which could be an advantageous for describing the adsorption of low strength solution (Marungreueng & Pavasant, 2006). K_f is a constant indicative of the relative adsorption capacity of the adsorbent and *n* is a constant indicative of the intensity of the adsorption. High K_f and *n* values indicate that the binding capacity has reached its highest value and the affinity between the biomass and dye molecules was also higher. As seen in Table 4.2, Freundlich constant, the exponent 1/*n*, also known as heterogeneity factor, is smaller than 1 and *n* is 1.84. This reflects the favorable adsorption. Value of "*n*" between the ranges of 1 to 10 represented the efficient and beneficial adsorption (Kadirvelu & Namasivayam, 2000). The more heterogeneous the surface would bring the 1/*n* value closer to zero (Azira, Wong, Robiah et al., 2004). From the observation based of R^2 and Freundlich constant, the Freundlich isotherm is found that can be described the characteristic of biosorption of MG onto *S. polycystum* in a binary solution with MB.

The Langmuir model assumes monolayer coverage and constant adsorption energy while the Freundlich equation deals with heterogeneous surface adsorption. The applicability of both Langmuir and Freundlich isotherms to the studied system implies that both monolayer sorption and heterogeneous surface conditions exist under the used experimental conditions. This may be due to the different surface condition on the two sides of the leaves of seaweed.

Table 4.1: Langmuir and Freundlich constants for MB adsorption using linear regressive analysis.

Isotherm	Parameters		
Langmuir	b (L/mg)	q_{max} (mg/g)	R^2
	0.0196	250	0.9165
Freundlich	K_f (mg/g)(L/mg)$^{1/n}$	$1/n$	R^2
	2.142	0.5428	0.9303

Table 4.2: Shape of Langmuir isotherm.

Separation factor, R_L	Type of isotherm
$R_L > 1$	Unfavourable
$R_L = 1$	Linear
$0 < R_L < 1$	Favourable
$R_L = 0$	Irreversible

Table 4.3: Dimensionless separation factor, R_L.

Initial Dye Concentration (mg/L)	Separation factor, R_L
100	0.9623
150	0.9544
200	0.9470
300	0.8844
500	0.7926
1000	0.4681
2000	0.1495

4.6 Kinetic Modelling

The sorption kinetics in a wastewater treatment is significant, as it provides valuable insights into the reaction pathways and the mechanism of a sorption reaction (Ho &

McKay 1999). Also, the kinetics describes the solute uptake, which in turn controls the residence time of a sorbate at the solid-solution interface (Ho et al., 2000). The principle behind the adsorption kinetics involves the search for a best model that well represents the experimental data. Several kinetic models are available to understand the behavior of the adsorbent and also to examine the controlling mechanism of the adsorption process and to test the experimental data. In the present investigation, the adsorption data was analyzed using two simplest kinetic models, the pseudo-first-order and pseudo-second-order kinetic models.

From Table 4.4, it is very obvious to see that kinetics of adsorption of MG in binary solution with MB by *S. polycystum* are better described by pseudo-second-order kinetic model rather than pseudo-first-order. The linearity of the plot also shows the applicability of the pseudo-second-order kinetic model, which has regression coefficient of R^2 as 1 when the initial dye concentration increases from 100 mg/L to 500 mg/L and it is very close to 1 when at 1000 and 2000 mg/L while that of pseudo first order various from 0.36 to 0.91 randomly.

The calculated $q_{e(cal)}$ based on pseudo-first-order model increased from 0.12 to 500 mg/g while that of pseudo-second-order model increased from 8.02 to 158.73 mg/g when the initial dye concentration increases from 100 mg/L to 500 mg/L. Also, the theoretical values of calculated q_e for pseudo-second order kinetic model agreed very well with the experimental data. In contrast, $q_{e(cal)}$ values of pseudo-first-order kinetic model do not match the experimental values at all. This expresses the chemisorption behaviour of the biosorption process. This was consistent with the better results obtained with the pseudo-second-order model (Table 4.4). The values of predicted equilibrium sorption capacities showed reasonably good agreement with the experimental equilibrium uptake values (Senthilkumar, Vijayaraghavan, Thilakavathi et al, 2006).

Generally, in physical adsorption the attractive forces between adsorbed molecules and the solid surface is van der Waals forces and they being weak in nature result in reversible adsorption. In view of the higher strength of the bonding in chemisorption, it is difficult to remove chemisorbed species from the solid surface (Gupta & Suhas, 2009).

Table 4.4: Rate constant of kinetics models at various initial dye concentrations.

Initial dye concentration, C_i (mg/L)	$q_{e(exp)}$ (mg/g)	Pseudo-first-order rate constants			Pseudo-second-order rate constants		
		K_1 (1/h)	$q_{e(cal)}$ (mg/g)	R^2	K_2 (g/mg/h)	$q_{e(cal)}$ (mg/g)	R^2
100	8.0166	0.5843	0.1236	0.8018	15.5501	8.0192	1
150	12.3715	0.1854	0.1957	0.7422	2.7135	12.3916	1
200	18.6614	0.2151	0.3153	0.6022	1.9082	18.6916	1
300	29.3635	0.1504	0.4824	0.4386	0.9633	29.4118	1
500	49.5775	-0.1460	1.3128	0.3678	0.3367	49.7512	1
1000	79.1093	-0.4613	7.1730	0.5054	-2.7735	77.5194	0.9998
2000	151.0788	-1.0845	500.0565	0.9119	0.0074	158.7302	0.9943

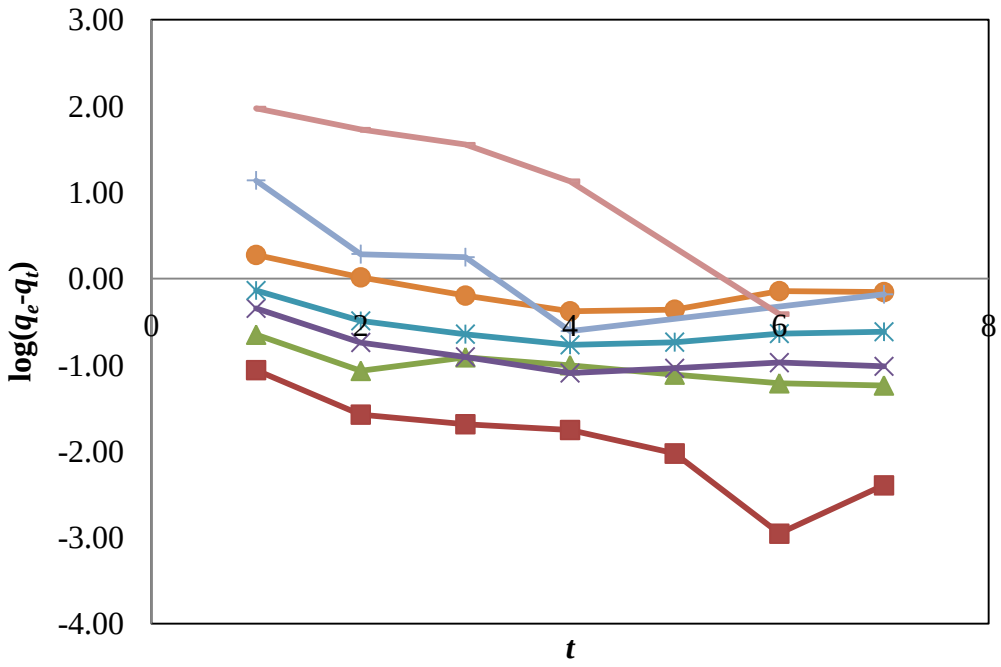


Figure 4.9: Pseudo-first-order model of kinetics curves. Symbols: (◆) 100 ppm; (■) 150 ppm; (▲) 200 ppm; (×) 300 ppm; (✳) 500 ppm; (+) 1000ppm; (-) 2000 ppm.

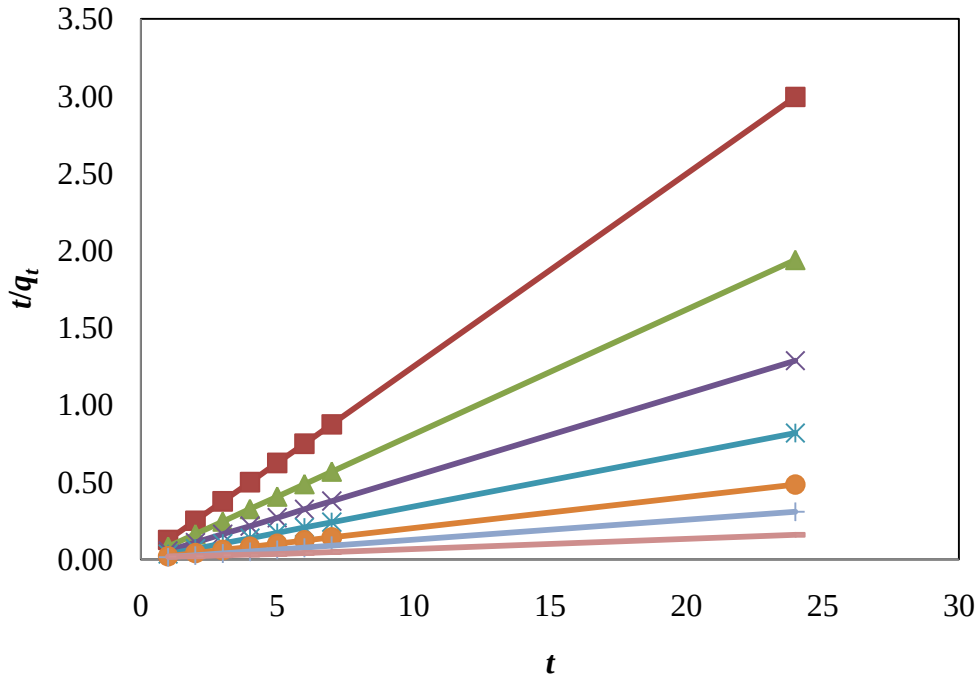


Figure 4.10: Pseudo-second-order model of kinetics curves. Symbols: (◆) 100 ppm; (■) 150 ppm; (▲) 200 ppm; (×) 300 ppm; (✳) 500 ppm; (+) 1000ppm; (-) 2000 ppm.

4.7 Thermodynamic Study

Based on fundamental thermodynamic concept, it is assumed that in an isolated system, energy cannot be gained or lost and the entropy change is the only driving force. In environmental engineering practice, both energy and entropy factors must be considered in order to determine which process would occur spontaneously.

Temperature dependence of the adsorption process is associated with several thermodynamic parameters. The Gibbs free energy change, ΔG^{o} is an indication of spontaneity of a chemical reaction and therefore is an important criterion for spontaneity. In addition, both energy and entropy factors must be considered in order to determine the Gibbs free energy of the process. The values of change in free energy (ΔG^{o}), change in enthalpy (ΔH^{o}) and change in entropy (ΔS^{o}) were shown in Table 4.5.

Distribution coefficient (K_c) indicates the capability of the seaweed to retain dye molecules and also the extent of its movement in a solution phase (Reddy & Dunn, 1986). According to Fontes and Gomes (2003), K_c is a useful parameter for comparing the adsorptive capacities of different adsorbent materials for any particular ion, when measured under same experimental conditions. Table 4.5 indicates that the K_c values at 303, 318 and 333 K are 64.64, 64.74 and 41.44 respectively, which shows a decrease with the increasing of temperature.

The absolute magnitude of ΔG^o may give an idea about the type of adsorption. Generally, the absolute magnitude of the change in free energy for physisorption is between −20 and 0 kJ/mol and chemisorption has a range of 80 to 400 kJ/mol (Yu, Zhuang & Wang, 2001). The values of ΔG^o for adsorption of dye onto *S. polycystum* are −10.5, −11.03 and −10.31 kJ/mol for 303,318 and 333 K, respectively, with an initial concentration of 100 mg/L. Hence, this process can be considered as physisorption. However, this result does not match with the result of the kinetic modelling analysis, which shows that the removal process of MG and MB by S. polycystum is chemisorptions.

The negative values of ΔG^o indicate that the adsorption of dye on the seaweed occurs spontaneously at a given temperature. It can also be noted that the change in free energy decreases with increase in temperature (Aravindhan, Rao & Nair, 2007). The negative free energy change value also suggests the feasibility of the MG biosorption process and confirms the affinity of the biosorbent towards the sorbate (Vijayaraghavan & Yun, 2008). The physical interaction between the seaweed surface and the MG molecules could be easily desorbed by physical means such as simply shaking or heating (Marungrueng & Pavasant, 2006).

The ΔH^o and ΔS^o in the biosorption of MG onto *S. polycystum* are -12.22 kJ/mol and -5.06 J/mol K, respectively. The negative value of change in enthalpy (ΔH^o) shows that the adsorption is exothermic in nature. Negative value of change in entropy (ΔS^o) suggests the decrease in adsorbate concentration in solid–liquid interface indicating thereby the increase in adsorbate concentration onto the solid phase (Amin, 2009). It also reflects the decreased randomness at the solid/solution interface during the adsorption of dye on seaweed. This is a direct consequence of: (i)

opening up of seaweed structure, (ii) enhancing the mobility and extent of penetration within the seaweed, and (iii) overcoming the activation energy barrier and enhancing the rate of intraparticle diffusion (Aravindhan, Rao & Nair, 2007). In addition, this is the normal consequence of the physical adsorption phenomenon, which takes place through electrostatic interactions (Amin, 2009).

Table 4.5: Thermodynamic parameters for the biosorption of MG onto *S. polycystum*.

Temperature (K)	K_c	ΔG^o (kJ/mol)	ΔH^o (kJ/mol)	ΔS^o (J/mol K)
303	64.6377	-10.5018	-12.2216	-5.0632
318	64.7393	-11.0258		
333	41.4411	-10.3109		

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

In present study, the competitive uptake of malachite green and methylene blue in binary solution by using *S. polycystum* has been investigated. The results showed that *S. polycystum* can efficiently remove both MG and MB from aqueous solution. MB was totally removed within 15 minutes. However, the % uptake of MG also up to 98% in 2 hours. The experiments were run in batch mode. It was found that the amount of dye sorbed increased with the increasing of initial dye concentration and the time taken to reach equilibrium decreased when the initial dye concentration increased. Besides, the sorption capacity also increased when higher amount of sorbent was used in the experiment. This biosorption process was found to be independent of temperature.

Two adsorption isotherms, Langmuir and Freundlich isotherm were applied to the equilibrium data and the results showed that both isotherms explained the biosorption of MG onto *S. polycystum* well, which mean both monolayer and heterogeneous sorption occurred in the process. In addition, biosorption kinetics of MG was best fitted to pseudo-second-order kinetic model, showing the rate limiting step may be chemical sorption. Thermodynamic paramaters indicate that it is a spontaneous and is exothermic in nature.

Considering the data and analysis presented in this study, it is suggested that *S. polycystum* would be well suited for the bioremediation of basic dye-bearing

industrial effluents. *S. polycystum* constitutes a promising material for the development of a low-cost biosorption technology for the removal of dyes from effluents. Furthermore, *S. polycystum* are abundant in many parts of Malaysia oceans and can serve as a cheap source for the production of biosorbent.

5.2 Recommendation and Future Research

As regards to the improvement on this part of thesis, the following future research and recommendations of application in industrial field are suggested:

(a) To determinate threshold point on various operating parameters are needed to further enhance the understanding the trend of the effect of initial concentration, sorbent dosage and temperature on the adsorption process.

(b) To investigate the effects of others operating parameters such as effect of pH, contact time, agitation speed, sorbent characteristic, and column studies.

(c) To investigate the sorption process by using various types of adsorption isotherms and kinetic models.

(d) To further study on removal of other hazardous materials present in dye effluent from textile and dyestuff industries such as heavy metal ions and other synthetic dyes by using *S. polycystum*.

(e) To carry out the experiment in water shaker in order to maintain the solution temperature throughout the experiment.

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