

BIOSORPTION STUDY  
OF METHYLENE BLUES  
DYES USING E.SPINOSUM:  
INTERACTION STUDY  
BETWEEN pH,  
INITIAL CONCENTRATION AND DOSAGE

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STUDY BETWEEN pH, INITIAL CONCENTRATION  
AND DOSAGE

NOR HASYIMAH BINTI SUDIN

Thesis submitted in fulfillment of the requirements  
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## ABSTRAK

Penyingkiran pewarna dari industri telah menimbulkan minat yang mendalam di kalangan penyelidik dalam membangunkan teknologi kos rendah dan keberkesanan yang tinggi. Penyerapan yang menggunakan alga laut telah menjadi alternatif yang sesuai untuk mengeluarkan pewarna dalam larutan cecair. Kajian ini bertujuan untuk mengkaji interaksi parameter dan keadaan optimum. Parameter operasi yang terlibat ialah dos biomas (0.1-3g / L), kepekatan awal (5-150mg / L) dan pH (2-12). Prestasi penyerapan ditentukan dengan menjalankan eksperimen penyerapan berkumpulan. Hasilnya menunjukkan dimana dos biomas meningkat di samping itu peratusan penyingkiran juga turut meningkat tetapi pada had tertentu. Pelbagai pH tidak memberikan kesan yang besar kepada proses penyerapan. Dari data ujikaji, menunjukkan bahawa kepekatan awal berada pada 5mg / L dan dos optimum adalah pada pH 6. Maksimum peratusan penyingkiran pewarna di interaksi antara dos biomas dan kepekatan awal adalah 95% dah berada pada kepekatan 150mg/L. Dalam pada itu, untuk interaksi antara pH dan dos biomas, peratusan penyingkiran pewarna ialah 86% iaitu pada pH 6.

## **ABSTRACT**

Removal of dyes from the industries has growing interest among the researchers in developing low cost and high efficiency technologies. Biosorption that using marine algae has become the ideal alternative for removing the dyes in aqueous solution. The study is aimed to the interaction of parameters and optimum conditions. The operating parameters that involved are biomass dosage (0.1-3g/L), initial concentration (5-150mg/L) and pH (2-12). The biosorption performance was determined by conducting the batch biosorption experiments. The results indicate as the biomass dosage increasing the percentage of removal also increasing. The variety of pH do not give a large effect to the biosorption process. From the experiment data, its show that the initial concentration is at 5mg/L and the optimum dosage is at pH 6. The maximum removal percentage for interaction between biomass dosage and initial concentration is 95% at concentration 150mg/L. Meanwhile, for the interaction between pH and biomass dosage, the maximum removal percentage is 86% at pH 6.



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## LIST OF SYMBOLS

$q_e$ (mg/g)	Equilibrium biosorption capacity
$C_i$	Initial concentration
$C_e$	Equilibrium concentration
$M$	Mass of seaweed biomass
$V$	Volume of MB dye
$R, \%$	Percentage removal

## LIST OF ABBREVIATIONS

H+	Hydrogen ion
OH-	Hydroxyl ion
DOE	Department Of Environment
BOD	Biochemical Oxygen Demand
COD	Chemical Oxygen Demand
MB	Methylene Blue
NaOH	Sodium hydroxide
HCl	Hydrochloric acid
USA	United States of America



## **CHAPTER 1**

### **INTRODUCTION**

#### **1.1 Introduction**

Recent years, an environment issue has become the main topic among the society in order to protect the environment for future. Many types of pollution had occurred such as air, water and soil because of the presence of chemical contamination. Every year, a huge amount of wastewater has been produced by many types of industries such as textile, food, paper, pharmaceutical and cosmetic that contain various organic and inorganic pollutants such as dye, that cause coloured waste water. The coloured wastewater discharged to the water resources and polluted the water resources. Figure 1.1 shows the number of river for each level of quality from 2005 until 2014. This environmental quality report was obtained from the Department of Environment. From this report, its show that the number of rivers that is clean had decreased from 2007 to 2014.

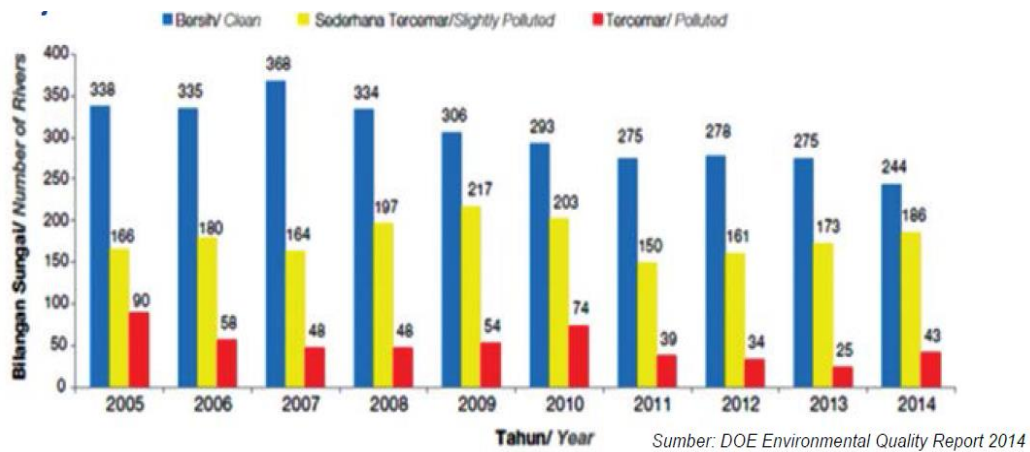


Figure 1.1: The Number of River for Each Level of Quality (DOE Environmental Quality Report, 2014)

Nowadays, about ten thousands of different dyes and pigments are used industrially and over 0.7 million tons of synthetic dyes are produced worldwide (Ehsan et al., 2012,). The industries that usually used synthetic dyes are textile, leather, cosmetics, food and also pharmaceuticals. Most of synthetic dyes are used in textile industry which is used to paint various fabrics. Dyeing effluents that comes from the dyes are the most hazardous component because the dyes are carcinogenic, toxic and mutagenic for humans and also other organisms. The Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) of water will increase because of the dyes in the wastewater. The toxicity of aquatic environment will also rise due to that dye is a toxic. These will cause severe health effect to human and also the animal that used the water such as central nervous system, disfunction of kidney and also reproductive system (Ehsan et al., 2012).

Some of these synthetic dyes are directly discharged into environment without any proper treatment by the industries. This is due to limitation of cost for the operation that has been applied by the industry to prevent the industry from loss. So, to cut the operation cost, the industry just discharged the wastewater that containing the dyes directly into river. Furthermore, the current cost for the treatment of the wastewater is high and some of the treatments are not an eco-friendly. If the treatment of the wastewater is conducted, the industry will need to increase the cost of the operation. This could make the industry loss due to the increase on the operation cost. Even some of the industry conducted the treatment

for the wastewater, the dyes are still presence in the wastewater. This is because the method that the industry used has low efficiency and just wasted the money. Therefore, a suitable low cost, eco-friendly and also has high efficiency method need to be suggested to treat the wastewater that containing dyes.



Figure 1.2: The River Polluted (Environmental Department, 2017)



Figure 1.3: The Waste Water Discharged into River (Environmental Department, 2017)

## 1.2 Problem Statement

Nowadays, there are a lot of issues regarding pollution has been discussed by the community and government especially about water pollution. This problem has attracted a lot of attention due to water is one of the most important resource for humans and also others organisms. The percentage of water uses in daily life can be categorized with 4% for drinking, 8% for washing up, 7% for outdoor, 30% for toilet flushing, 21% for baths and taps, 12% for showers, 13% for clothes washing and 5% for others.

Water is polluted due to the presence of chemical substances such as dyes. The colour of water is caused by the dyeing effluent that comes from the dyes. Dyes can cause serious environmental problem because they are carcinogenic, toxic and mutagenic for human and others organisms. In addition, the dyes also affect photosynthesis activity of aquatic environment and also reduce light penetration that will give a high impact in environment quality.



Figure 1.4: The Polluted River (DOE Environmental Quality Report, 2014)

Dye that is a toxic will increase the toxicity of aquatic environment. The water will become more toxic that dangerous for humans and animals if used it. By using the water that already polluted, it can cause severe health affect such as disfunction of kidney, liver, brain and reproductive system.

This problem can be solved by using several methods such as chemical oxidation, membrane separation, and reverse osmosis but those methods have low

efficiency and also high cost. Biosorption has shown good potential for decolourization of dyes in wastewater. Biosorption can be done by using seaweed. This is because the seaweed has a large surface area and also eco-friendly.

### **1.3 Aims and Objectives**

- 1) To study the interaction between operating parameter
- 2) To determine the optimization condition of biosorption

### **1.4 Limitations and Scope of Study**

1. The study used marine macro algae as the absorbent of dye and the type of algae used is red seaweed.
2. The type of dye used in this study is Methylene Blue only.
3. The method used for this study is biosorption experiment and conducted in environment lab.
4. The experiment conducted based on three operating parameters; pH, initial concentration and biosorbent dosage and the interaction between these operating parameter.
5. The experiment conducted to determine the optimization condition of biosorption.

### **1.5 Significant of Study**

The study will be conducted to solve the problem of dyes in wastewater. Comparing with other method, the biosorption of dyes using seaweed is cheaper. When using seaweed that has larger surface area, the sorption capacity will be increase. In other meaning, the biosorption efficiency is higher so that shown this method is effective. Seaweed is also one of renewable resources and biological. So, this method is an eco-friendly and suitable to use in way to protect the environment.



Figure 1.5: The Polluted River Caused by the Dyes (India, 2017)

Moreover, this study also will help the industry to treat the wastewater with low cost. This will save the industry budget for the cost of operation. By that, there will no issue the industries discharged the wastewater that polluted directly into the environment since there is a new method that the industry can apply.

Furthermore, the community also will be secure to use the water because the wastewater that discharged from the industry has been treated. So, the water is safe to be used. There no more health diseases that caused by polluted water.

Besides that, the government also will have less complaint regarding the water pollution. Less action that needs to be taken since these industries has obeyed the law regarding this matter.

Therefore, wastewater that polluted with dyes will become no more issue since that problem has been solved with this method.

## **CHAPTER 2**

### **LITERATURE REVIEW**

#### **2.1 Introduction**

This chapter consists of the literature review from the journal. This chapter had been divided to five categories such as wastewater, dyes, type of biomass, removal method and operating parameters.

#### **2.2 Wastewater**

Wastewater is water that physical, chemical or biological properties have been changed as a result of the introduction of certain substances which render it unsafe for some purposes such as drinking (Peace Amoatey & Richard Bani, 2011). Every human daily activity is mainly water dependent and then discharge the waste into water. The substances such as laundry powder, body wastes, detergent, food scraps, and household cleaners can affect the people health and also damage the environment. It is known that much of water supplied ends up as wastewater which makes its treatment very important.

The wastewater can be described as in the figure below.

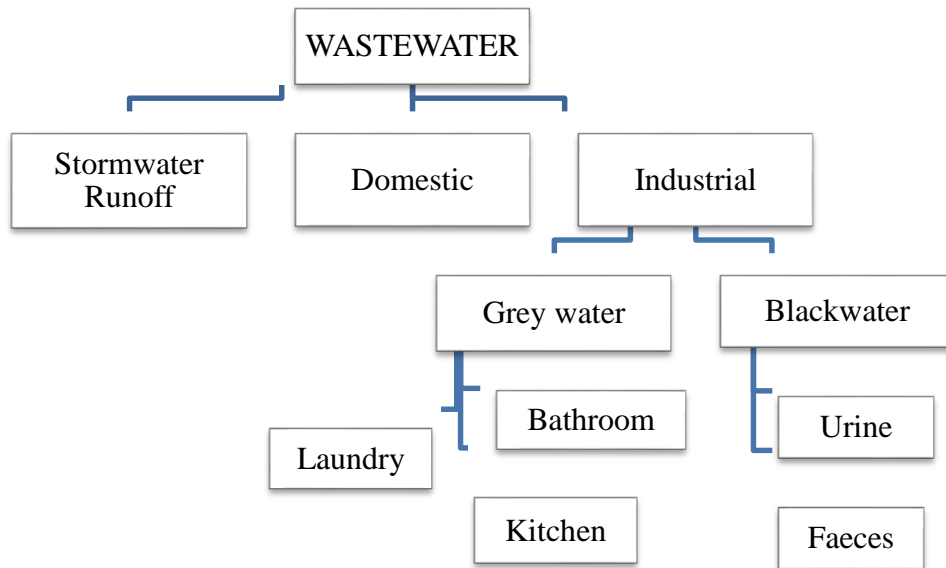


Figure 2.1: Types of Wastewater. (Peace Amoatey & Richard Bani, 2011)

### 2.2.1 Stormwater Runoff

Storm water runoff means the water from the streets and open yard after a rainfall event which is run through drains or sewers. When raining, the water will fall onto the ground and run through the drains.



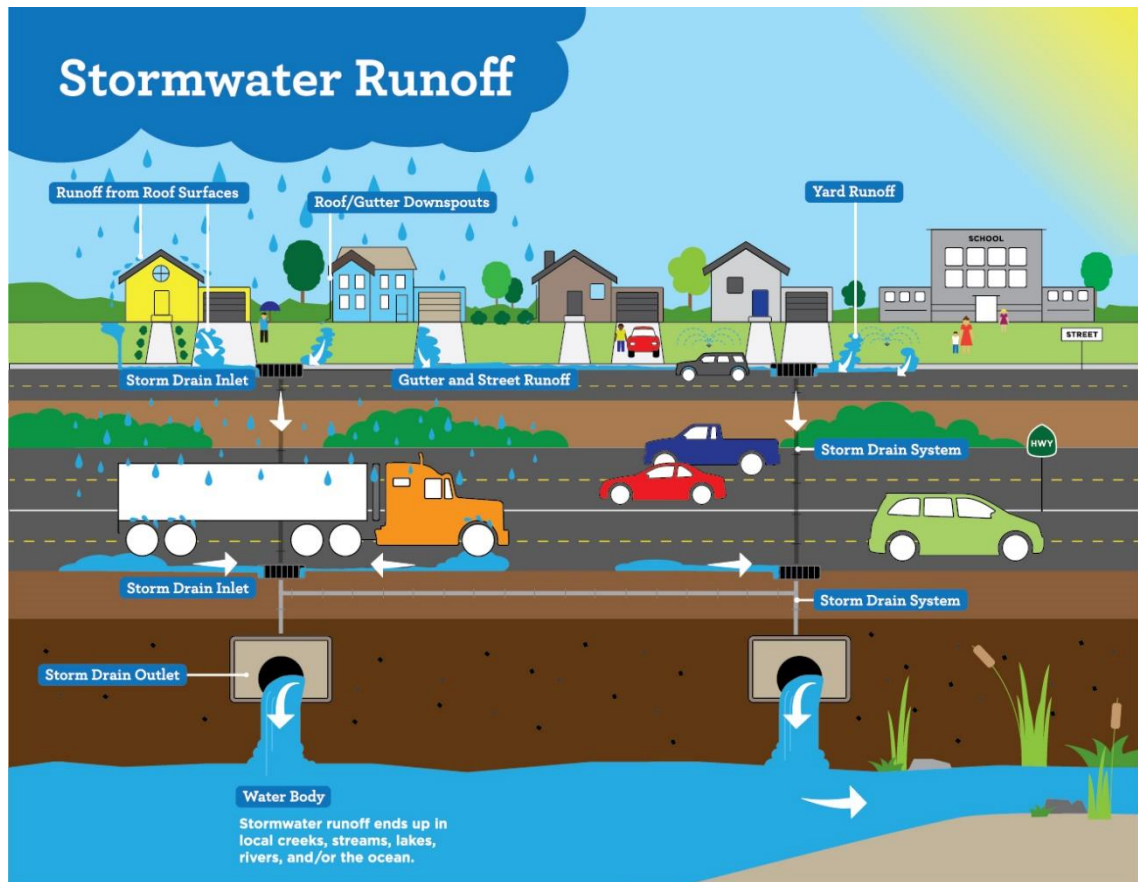


Figure 2.2 The Stormwater Runoff (City of Columbia,2018)

### 2.2.2 Domestic Wastewater

Domestic wastewater also known as municipal wastewater. It is basically wastewater that comes from the residence, business buildings and also institutions. Domestic wastewater usually contain waste that not very harmful to human or other organism. Domestic wastewater may be contained waste from the kitchen, bathroom or toilet.

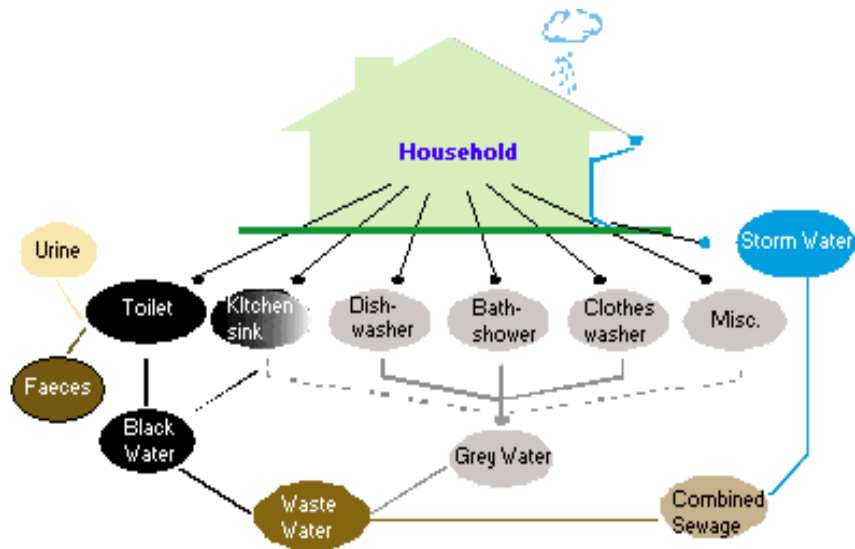


Figure 2.3: The Range of Possible Sources of Household Wastewater Showing Wastewater from Toilet, Kitchen, Bathroom, Laundry and Others (Nandita Singh, 2017)

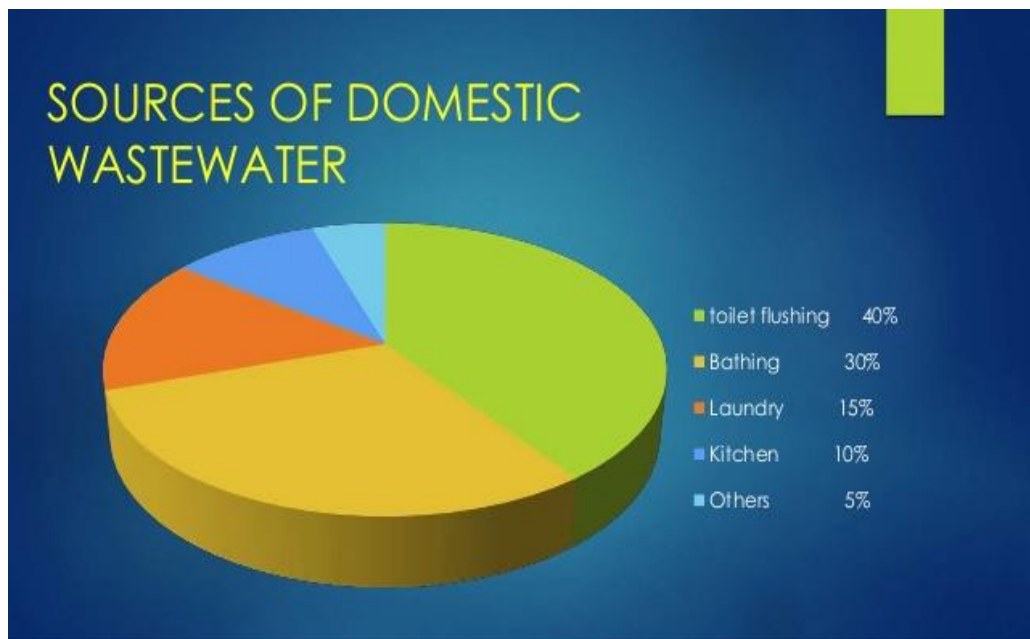


Figure 2.4: The Sources of Domestic Wastewater (Akruthi Enviromental Solution, 2017)

### 2.2.3 Industrial Wastewater

For industrial wastewater is liquid waste that discharge from the industrial such as factories and production unit. Industrial wastewater divided to two categories which are grey water and black water. For grey water, its waste from the laundry, kitchen and bathroom. Meanwhile for the black water, it contains the faeces and urine only.



Figure 2.5: The Industry Sectors (University of Stuttgart, 2015)

### 2.2.4 Different between Domestic Wastewater and Industrial Wastewater

According to Suriani (2017), there is inherent different between the domestic wastewater and the industrial wastewater. The difference can be seen from the number of contaminants in the wastewater. For the industrial wastewater, the number of the contaminants can run from zero to 100,000 ppm (parts per million). Meanwhile, the number of contaminants for the domestic wastewater can only run from 100 to 1,000 ppm. This shown that the industrial wastewater carried more degradable organic matter compared to the domestic wastewater. Untreated industrial wastewater can be dangerous matter to human and also others organisms.

### 2.3 Dyes

Recent years, synthetic dyes substances had been widely used in various industrial activities such as textile, pharmaceutical, food, cosmetics, plastics, leather, carpet, photographic and paper industries. The textile dyeing process becomes as the largest generator of the colored effluent among these industries. At the same time, the effluent from the industries not only colored, but also contains toxic organics. The colored wastewater that discharged into water sources had damaged the environment as it disturbs the human health and also toxic to flora and fauna. This had caused the photosynthetic activity of autotrophic organism reduce.

According to E. Daneshvar et al (2017), the dyes can be categorized as; anionic-direct, acid and reactive dyes;cationic-basic dyes;non-ionic-disperse dyes. There are also azo-dyes. Due to its simplicity in production, cost effectiveness, ease of application, durability and available in various colors had make the azo-dyes been selected to be used more than half of the global industries. As the azo-dye are toxic substances, an exposure to the dyes may leads to allergic, carcinogenic and mutagenic for human and also other organisms.

The common dye that used in textile industries is Methylene Blue (MB) and it is categorized under azo-dye group cationic which carries a positive charge in its molecules (E. Daneshvar,2012). MB has categorized as not strongly hazardous but still had several report about the acute exposures to living form. The effect could be varies such as kidney problem, gastrointestinal irritation or reproduction problems. The effect of dyes also can be very harmful such as from gastrointestinal become a cancer.



Figure 2.6: The Synthetic Dyes used in Industry (Environmental Department, 2017)

Chemical structure

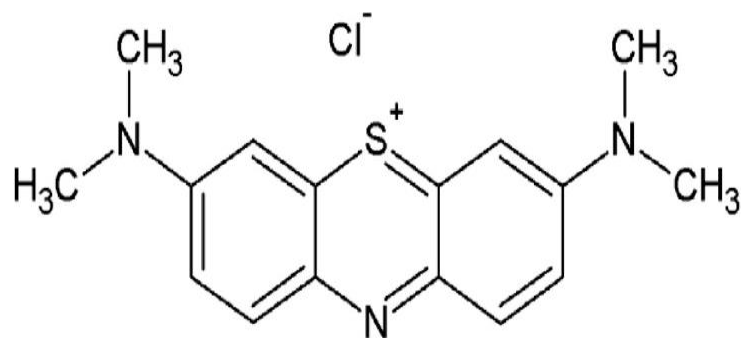


Figure 2.7: Chemical Structure of Methylene Blue. (E.Daneshvar et al, 2017)

## 2.4 Type of Biomass

Nowadays, the marine macro alga had attracted a wide attention as a viable option among the researchers to treat waters containing dyes. These marine algae become a good biosorbent by having some of important characteristics such as the presence of unique chemical composition, high surface area and also high binding affinity. Moreover, algae are fast and easy growth in simple medium. The marine algae also cheap and available around the world. In the sorption system, the biosorbent can be used either in living or non-living form but non-living is more practical and favourable. This is because the algae are not affected by the toxicity of dyes molecule. Therefore, many researchers suggest the marine algae as the new option for the biosorbent.

According to S.Thanigaivel et al (2016), the marine algae had been classified into several set. These set are based on the environment that the marine algae found and what the potential that the marine algae had. The seaweed production had increased mainly in the tropical and subtropical marine species. The seaweed species had been categorized according to the various properties that based on the seaweed's fundamental structural and functional characteristics.

Table 2.1 The Type of Algae

Type	Genus sp.
Red algae ( <i>Rhodophyta</i> )	<i>K.alvarezii</i> <i>E. spinosum</i> <i>K. striatum</i>
Green algae ( <i>Chlorophyta</i> )	<i>C.lentillifera</i>
Brown algae ( <i>Phaeophyta</i> )	<i>S.polycystum</i>



Figure 2.8: *E.spinosum* (American Carrageenan Technology , 2012)

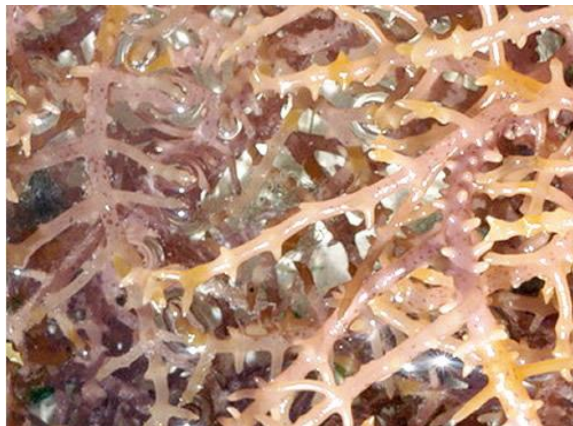


Figure 2.9: *K.striatum* (SEAWEED UNIDIP, 2013)



Figure 2.10: *S. polycystum* (Niemeyer, 2012)



Figure 2.11: *C. lentillifera* (M. K. Ab Ismail, 2016)

Meanwhile, the algae also can be categorized based on color and colloid content which are brown (*Phaeophyta*), red (*Rhodophyta*) and green (*Chlorophyta*). Alginate and sulphate are the predominant active group that found in the brown algae. The red algae are characterized by the presence of the sulphated polysaccharides such as carrageenan. For green algae, it has mainly cellulose and protein bonded to polysaccharides in the cell wall.

Many types of algae have been used in previous research specifically for heavy metal removal. Red algae as biosorbents have been used by several researches for the removing various organics and inorganics pollutants. So, the red algae had been selected to be used in this study for removing the MB dyes. This is due to the red algae has the highest biosorption capacity among these three algae and can be observed in the figure below.



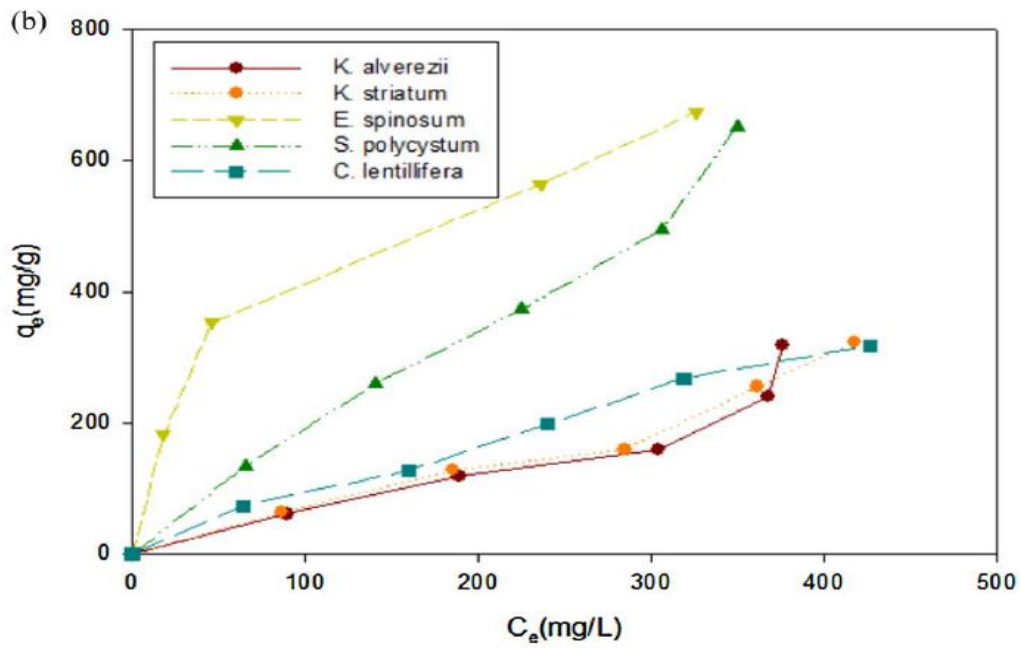


Figure 2.12: Equilibrium Biosorption Isotherms by Different Algae Biosorbent. (N.Mokhtar et al, 2017)

The figure above shown that at MB concentration lower than 1000 mg/L, *E.spiniosum* (red) has outperformed others followed by *S. polycystum* and the followed by *C. lentillifera*, *K.striatum* and *K. alvarezii*. This has proved that *E.spiniosum* has the greatest performance in binding the MB onto the seaweed surface in terms of  $q_{max}$  and biosorbent affinity (N.Mokhtar et al, 2017).

## 2.5 Removal Method

As the dyes are resistant to microbial degradation due to their complex molecular structure and are stable to light, heat and oxidizing agents had make the removal color dye be difficult. Several treatments have proposed to overcome that problem such as coagulation and flocculation, biological oxidation, chemical precipitation, activated carbon adsorption, ion exchange, photo-catalys, photo-oxidation and membrane filtration. There are a lot of disadvantages of these methods such as not environmentally friendly, technical limitation, high sludge production and usually dependent on the concentration of the waste. Among these techniques, the effective technique for the treatment of dye-containing wastewater is adsorption. However, its need high capital and operational costs.

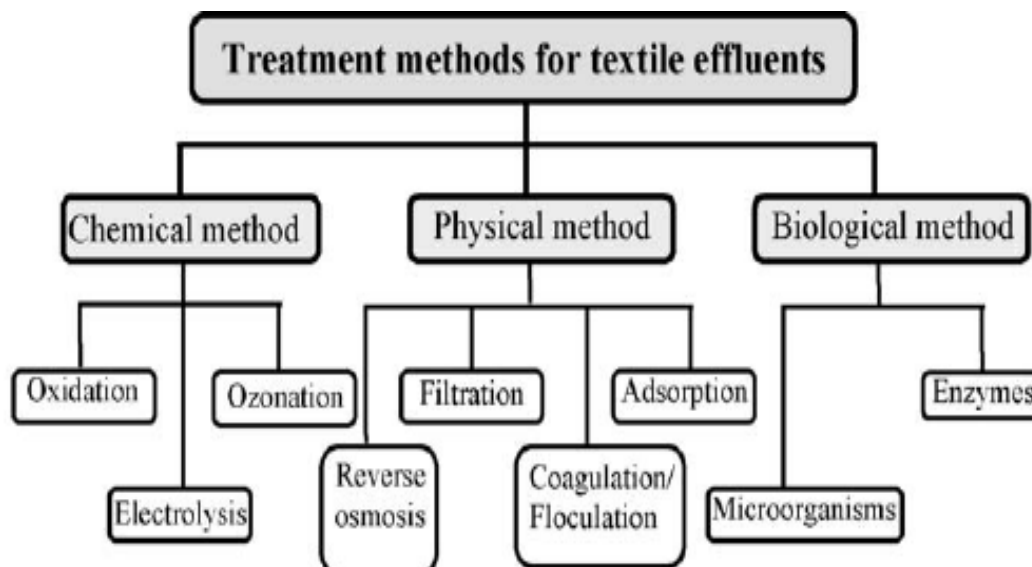


Figure 2.13: Treatment Methods for Textile Effluents (India, 2017)

The interest among the researcher had developed in finding new method for the wastewater treatment that is low cost and high efficiency. Biosorption method had shown a good potential to replace conventional treatment methods for removal of dyes. This is because it less investment in terms of initial cost, immense effectiveness under favourable conditions, versatility which is means it is practically to remove organic and nonorganic pollutants simultaneously, minimal volume of disposable sludge and simple regeneration.

According to Vijayaraghavan and Yun (2015), biosorption is a technique that can be used for removing the pollutants from waters, especially those that are not easily biodegradable such as metals and dyes. Biosorption also can be defined as an action removing of substances from solution by biological material. Biosorption using low cost biosorbent offers an attractive alternative for dye remediation that involving physic-chemical mechanism of removal.

Conception of biosorption is multidimensional that had been evolving over a few decades ago. Biosorption term is related to the existence of many mechanisms, the biosorbents used, environmental factors and for living organism's case, the presence or absence of metallic processes. There are diverse range of processes for the biosorption term that had been used by the authors including bioabsorption, bioadsorption, biosorption and also bioaccumulation.

According to M.Fomina et al. (2014), sorption is a physico-chemical process that means one substances attached to another substance. The term of 'bio' shows that the involvement of a biological entity. So, biosorption can be simply defined as physico-chemical process which are the removal of substance from solution by biological material. Both adsorption and absorption had used the sorption terms. Absorption can be defined as the incorporation of the substances in one state to another of a different state. For example, the gas being absorbed by water or liquid being absorbed by solid. Meanwhile, adsorption is the bonding of ions or molecules onto surface of another molecules

Based on previous research, the biosorption had been considered as subcategory of adsorption. The most common sorption is adsorption that involved in traditional clean-up technologies. Sorption also can be used to describe any system that a sorbate interacts with a sorbents and resulting the accumulation at the sorbate-sorbent interface.

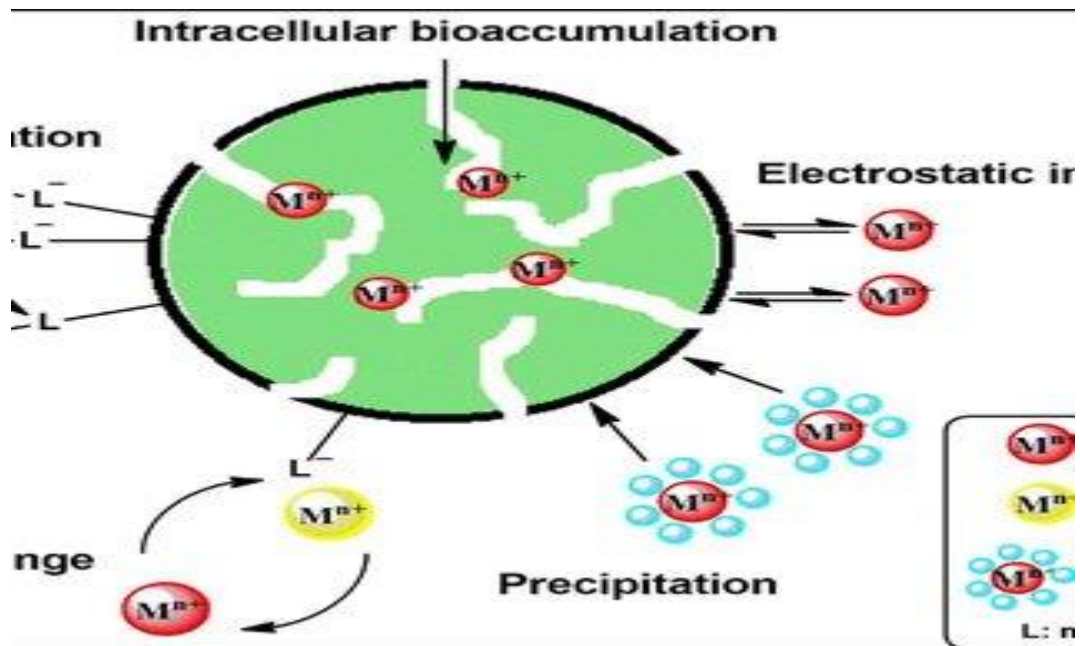


Figure 2.14: Biosorption simulation (Researchgate,2017)

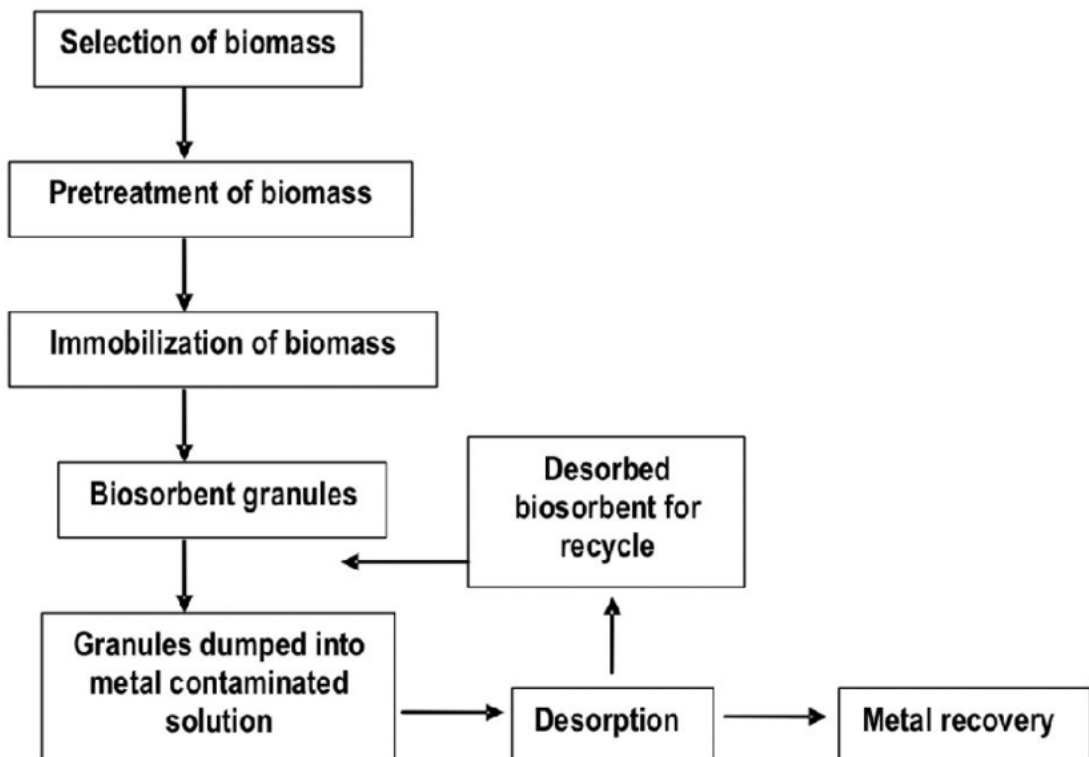


Figure 2.15: Schematic Diagram of a Biosorption Process

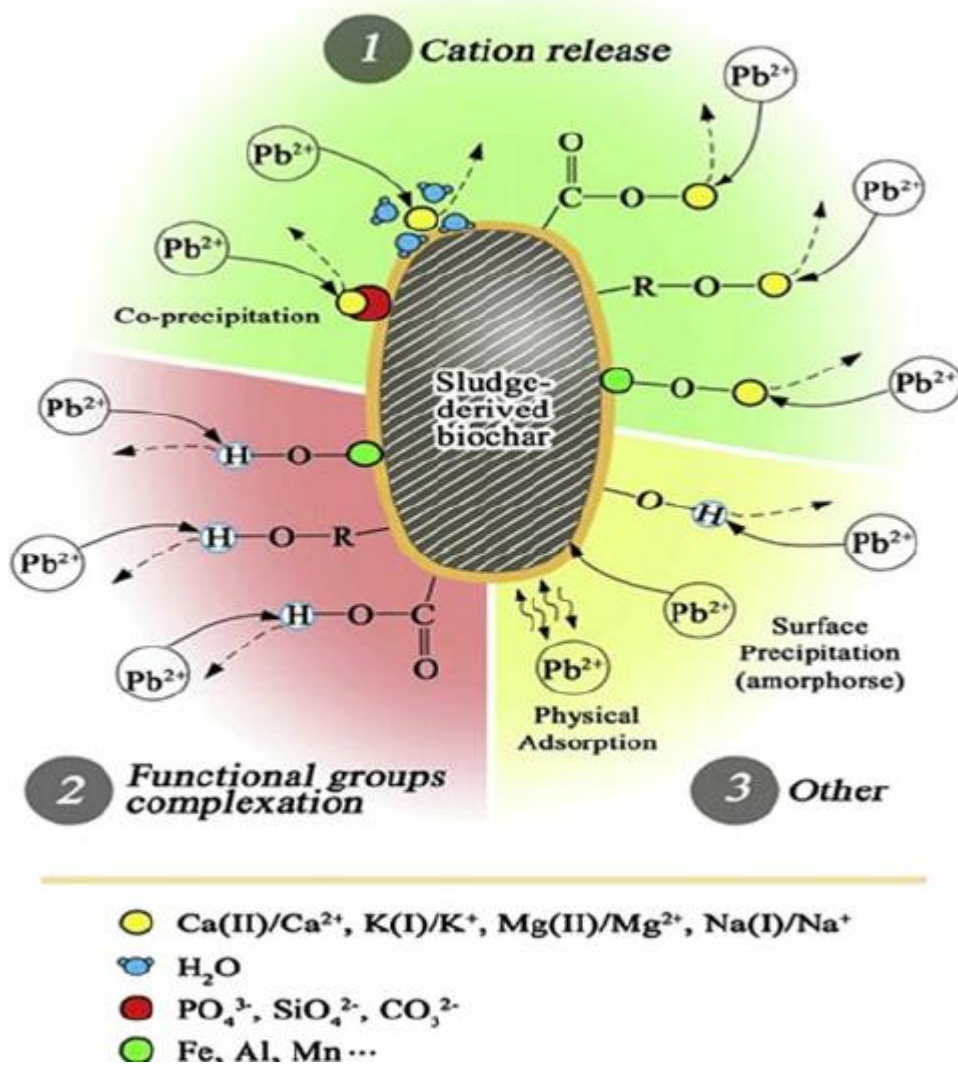


Figure 2.16: Conceptual of Biosorption

## 2.6 Operating Parameters

Parameter is a limit. In term of mathematic the parameter can be describe as a constant in an equation. Now, any system will have own parameter that define it operation. The operating parameter means that the parameter or limit that involved in this researcher. There are three parameters that involved in this research which are pH, initial concentration of MB and biomass dosage.

### 2.6.1 pH

E. Daneshvar et al. (2012) reported in the adsorption of any solute the solution pH is an important parameter because of the strong influence on its solubility as well as the adsorption capacity. Therefore, its effect on biosorption of the MB onto red seaweed was studied including its interaction between the initial concentration and biomass dosage.

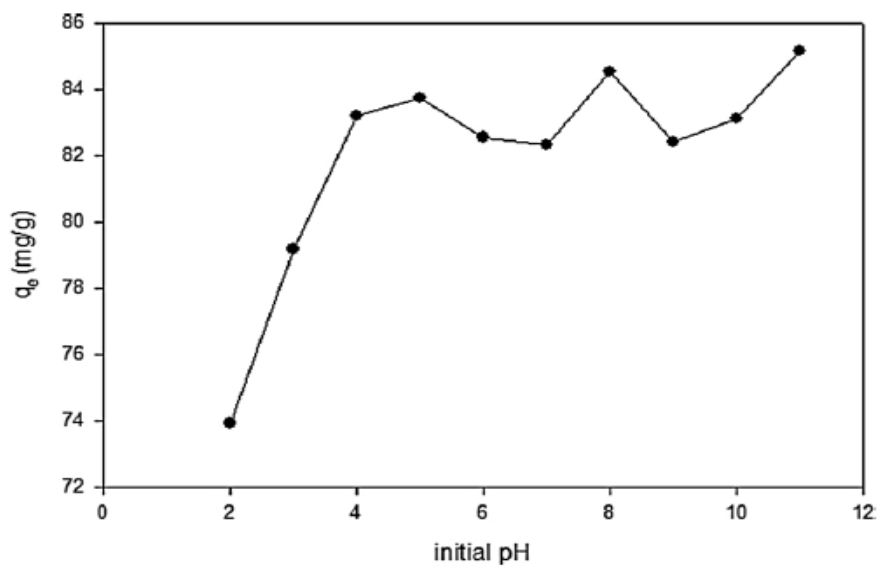


Figure 2.17: The Effect of pH on Biosorption of MB Dye. (N.Mokhtar et al,2017)

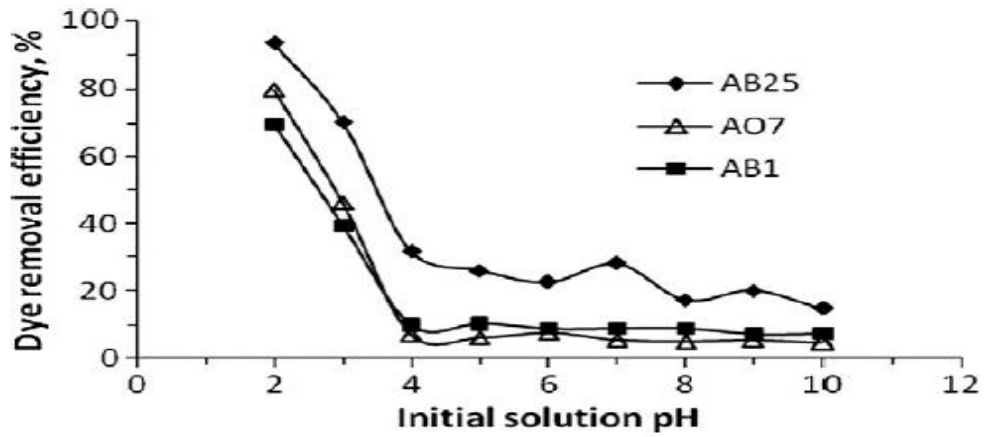


Figure 2.18: The Effect of Initial pH on Acidic Dyes Removal Efficiency of *S. marginatum* (E.Daneshvar et al, 2012)

The figure above shows the result that obtains from E.Daneshvar et al. (2012) study. The study is about the effect of PH on biosorption of the three acid dyes AB25, A07 and AB1 onto *S. marginatum* biomass in batch experiments carried out a 27 °C using 30 mg/L dye concentration and 1.0 g/L biomass level, where the initial pH was varied in the range 2-10.

### 2.6.2 Biomass Dosage

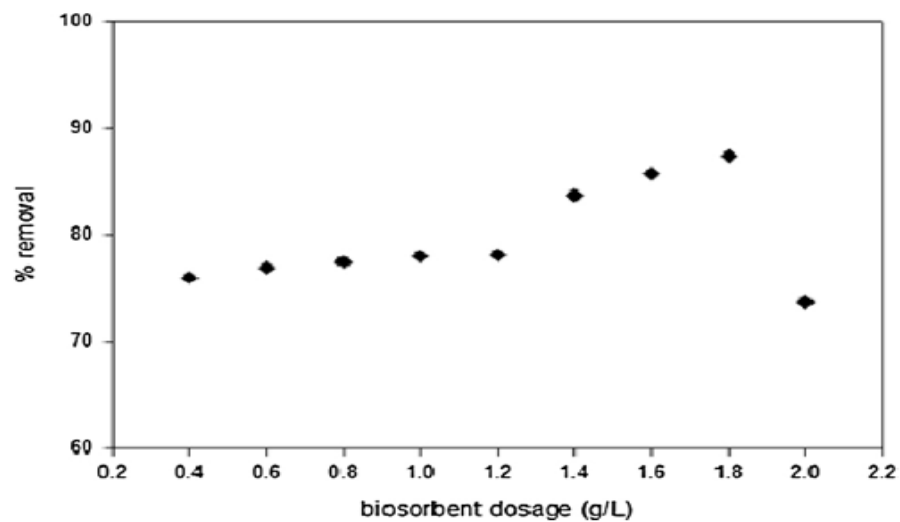


Figure 2.19: The Effect of Biosorbent Dosage to the Removal of MB Dye in Aqueous Solution (N.Mokhtar et al, 2017)

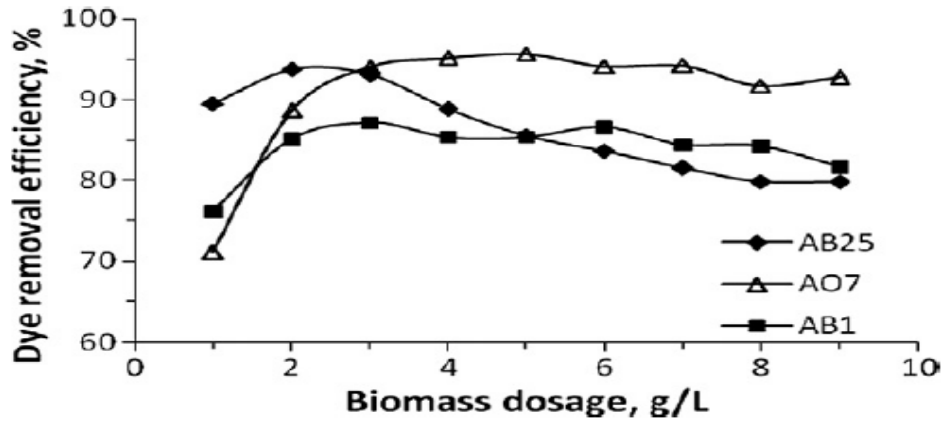


Figure 2.20: The effect of *S. marginatum* biomass level on acidic dyes removal efficiency. (E.Daneshvar et al, 2012)

According to E.Daneshvar et al (2012), there are nine level different values of biosorbent level in the range 1-9 g/L, while the initial pH, dye concentration, temperature and contact time were kept constant at 2, 30 mg/L, 27 °C and 60 min, respectively for all three dyes. The sorption efficiency of these three acidic dyes are progressively increased with the biosorbent and reached the maximum values.

### 2.6.3 Initial Concentration

The main function of the initial sorbate concentration is as driving force that overcome the mass transfer resistance of all molecules between sorbent and sorbate.



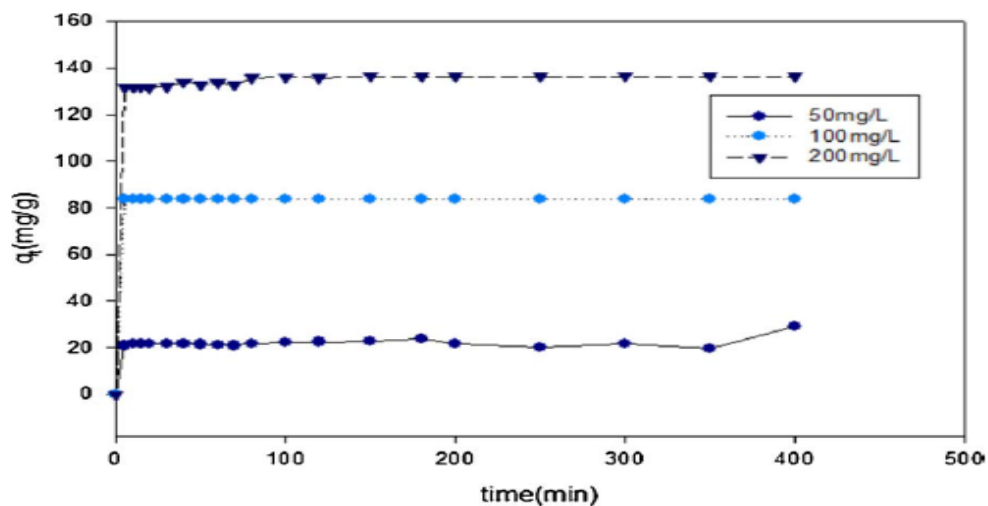


Figure 2.21: The Effect of Initial Concentration on the Biosorption of MB Dye. (N.Mokhtar et al,2017)

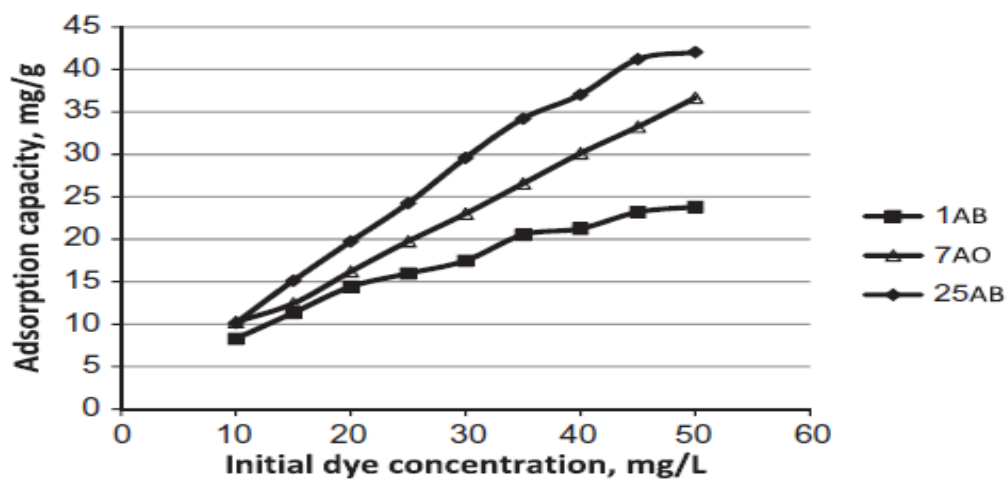


Figure 2.22: The Effect of Initial Dye Concentration on Acidic Dyes Removal Capacity of *S. marginatum* biomass. (E.Daneshvar et al, 2012)

Figure 2.22 shows that the higher the concentration of sorbates, the higher the adsorption capacity (E.Daneshvar et al, 2012)

#### **2.6.4 Interaction of Parameters**

The interaction of the operating parameter such as pH with initial concentration and biomass dosage will be studied in this research. According to the previous research, when the pH level and the initial concentration or biomass dosage increase the removal efficiency also increases. This shows that there is an interaction between these parameters that will affect the biosorption process.

## CHAPTER 3

### METHODOLOGY

#### 3.1 Introduction

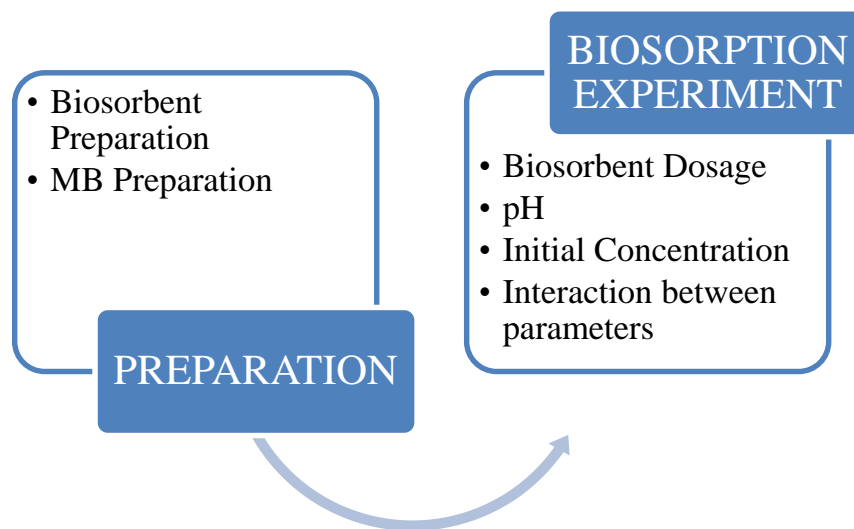


Figure 3.1 Methodology of Research

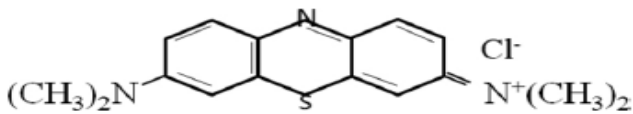
##### 3.1.1 Biosorbent Preparation

The red macroalgae was used as biosorbent for the biosorption of MB dye. The samples of the adsorbent were obtained from an algae factory in Kunak, Sabah. The adsorbents were cleaned by washing repeatedly with tap water to eliminate dirt and surface-adhered particles. Then followed with distilled water several times to remove extraneous and salt. They were then dried in an oven (UFB500, Memmert) at 60 °C for 24 h. Dried biomass was grounded, sieved and grouped into particles size ranging between 0.6 and 1.18 mm. Then, stored in tight plastic bottles.

### 3.1.2 MB Preparation

The dye that used in this study was Methylene Blue (MB). 1 g of Methylene Blue was dissolved in 1 L of distilled water to prepare an aqueous MB solution of 1000 mg/L. Initial pH was adjusted by addition of 0.1M of sodium hydroxide (NaOH) or 0.1M of hydrochloric acid (HCL). All chemicals were purchased from HmbG with analytical grade. Table 3.1 lists the properties of MB dye (N. Mokhtar et al, 2017).

Table 3.1 Properties of Methylene Blue

Item	Genus sp.
Commercial name	Methylene Blue
Abbreviation	MB
Application class	Basic Blue 9
CI No:	52015
Molecule structure	
Chemical formula	$C_{16}H_{18}ClN_3S$
Molecular weight	319.86 g/mol
Maximum wavelength	640 nm
Chromosphere	Thiazine

### 3.2 Batch Biosorption Procedure

A series of batch experiment must be undergone by biosorbent to study the interaction of operating parameters. The factors of sorbate such as dosage (1-2 g/L), pH (pH 2-12) and initial concentration (100-500 mg/L) of MB were studied in an attempt to understand the interaction. The pH was adjusted by adding 0.1 M HCL or 0.1 M NaOH solution. The interactions that are studied are the interaction between biomass dosages with pH, interaction between biomass dosages with initial concentration and also interaction between pH with initial concentration.

The tests were conducted by placing various biomass dosages (1-2 g/L) in the 25 mL of dye at various concentrations from 100 to 500 mg/L. Then the samples were placed on an orbital shaker for an agitation at 130 rpm and taken at predetermined time intervals. All experiments were carried out at room temperature of 27 ° C. The MB solution was centrifuged at 4000 rpm for 3 s and the analysed. (N. Mokhtar et al, 2017).

The equilibrium biosorption capacities,  $q_e$  (mg/g) and the percentage removal (R, %) of MB dye was calculated based on:

$$q_e = \frac{(C_i - C_e)M}{V} \quad 3.1$$

$$R(\%) = \frac{(C_i - C_e)}{C_i} \times 100 \quad 3.2$$

Where  $C_i$  and  $C_e$  (mg/L) represent the initial and equilibrium concentration of MB dye respectively,  $M$  (g) is the mass of seaweed biomass and  $V$  (L) is the volume of MB dye (N. Mokhtar et al, 2017).

### 3.3 Biosorption Performance

By using UV-vis spectrophotometer (UV-vis spectrophotometer, DR5000, Arachem), the concentration of the MB in the solution before and after biosorption was measured at 640 nm wavelength, corresponding to the maximum absorbance for MB. All the equipment was properly calibrated in accordance to manufacturer instructions. The pH values were measured by using pH meter (Metler Toledo). The agitation speed was set at 130 rpm using orbital shaker (Excelsa E1, Edison, New Jersey, USA). By using centrifuge (Centrifuge 2420, Japan), the solutions were centrifuged at 4000 rpm for 3 s. All the data represented the mean of triplicates with average and standard deviation.

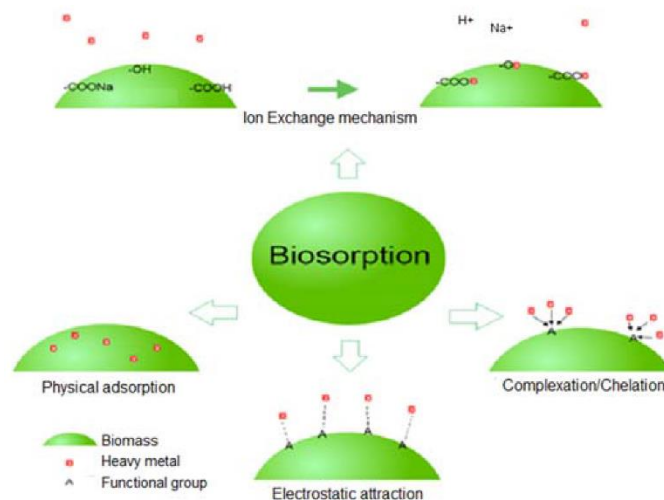


Figure 3.2: Biosorption Mechanism

## CHAPTER 4

### RESULTS AND DISCUSSION

#### 4.1 Introduction

This chapter consist of results obtained from biosorption study of preliminary analysis and interaction of three effect parameters such as pH, biomass dosage and initial concentration of Methylene Blue. A detailed explanation and discussion were discussed in this chapter.

#### 4.2 Methylene blue wavelength

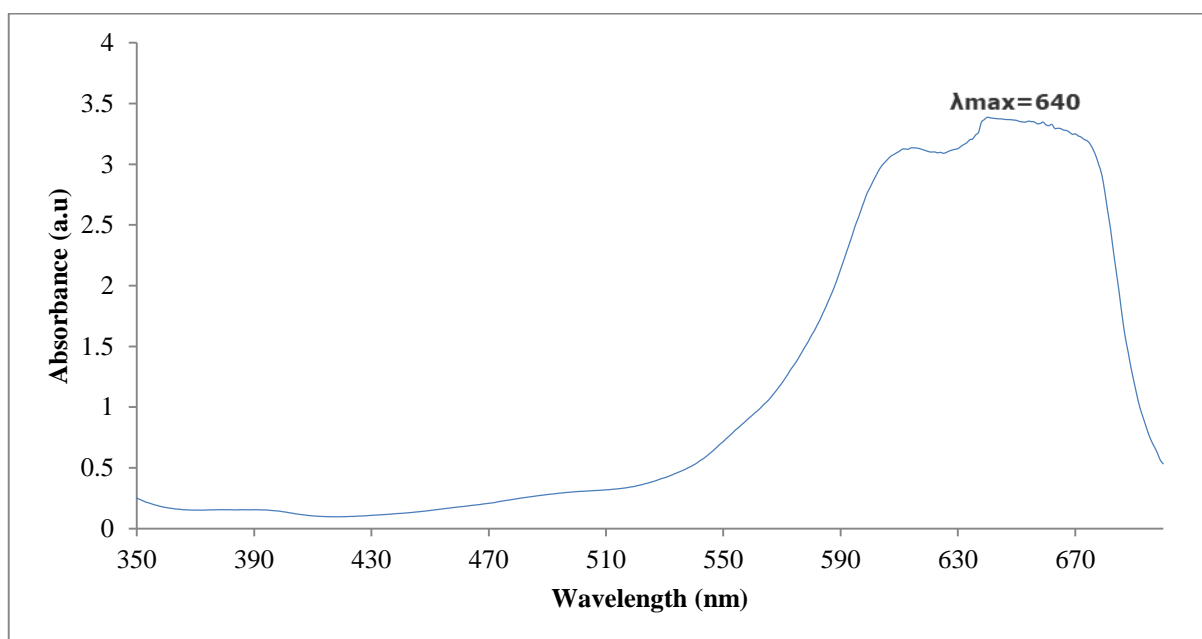


Figure 4.1 Wavelength of MB from UV-VIS Spectra

From the figure 4.1 its show the wavelength of the Methylene Blue that obtained from the UV spectra. The optimum wavelength can be obtained from the graph at 640nm.

### 4.3 Interactive Effect

#### 4.3.1 Effect of Dosage and Initial Concentration

Table 4.1 Data Analysis

concVSdosage	5mg/L	25mg/L	50mg/L	100mg/L	150mg/L
<b>0.1</b>	10	84	77	81	69
<b>0.5</b>	23	85	83	91	95
<b>1</b>	50	86	82	89	90
<b>1.5</b>	58	90	81	87	89
<b>2</b>	68	86	77	78	86
<b>2.5</b>	52	85	77	78	83
<b>3</b>	7	83	75	77	82

From the table, it shows the percentage of removal for each of concentration for dosage from 0.1 until 3g/L. For concentration 5mg/L, the percentage of removal were 10%, 23%, 50%, 58%, 68%, 52% and 7%. For concentration 25mg/L, the percentage of removal were 84%, 85%, 86%, 90%, 86%, 85% and 83% meanwhile for 50mg/L are 77%, 83%, 82%, 81%, 77%, 77% and 75%. The percentage of removal for concentration 100mg/L were 81%, 91%, 89%, 87%, 78%, 78% and 77% meanwhile for concentration 150mg/L were 69%, 95%, 90%, 89%, 86%, 83% and 82%. The highest percentage of removal were at concentration 150mg/L which is 95% and biomass dosage was at 0.5g/L.

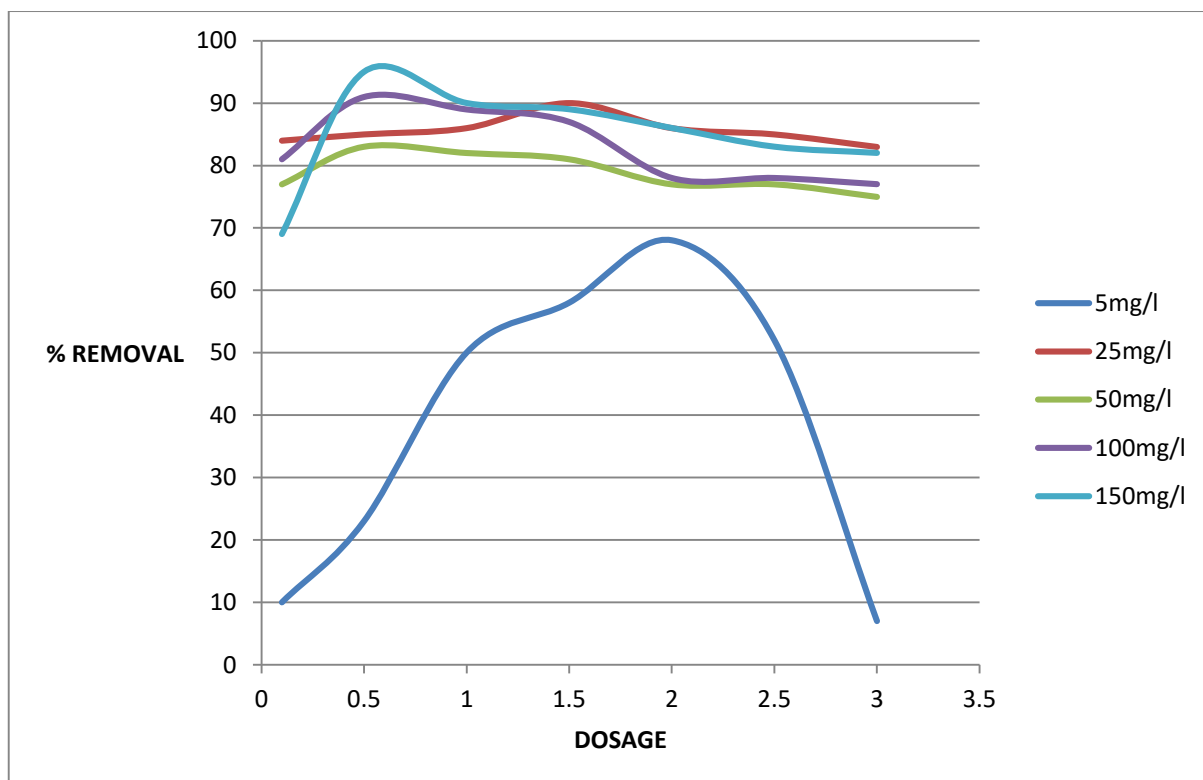


Figure 4.2: Interactions between Initial Concentration and Biomass Dosage

Figure 4.2 shows the interaction between initial concentration and biomass dosage. The initial concentration is within the range from 5mg/L to 150mg/L which are 5mg/L, 25mg/L, 50mg/L, 100mg/L and 150mg/L. Meanwhile for the biomass dosage range is between 0.1g/L, 0.5g/L, 1.0g/L, 1.5g/L, 2.0g/L, 2.5g/L and 3.0g/L. The experiment was prepared with variety initial concentration and biomass dosage but at fixed pH which is at 7 and also fixed room temperature, 27°C.

As can be seen the result that plotted in Figure 4.2, it found that, the percentage of MB removal were increased with the increasing biomass dosage even in different initial concentration. This is due to the facts that the higher biomass dosage contributes to higher active sites in the process. The similar effects based on the usage of different biomass dosage were reported in the literatures (N. Mokhtar et al. 2017).

However, by further increase of biomass dosage, the percentage of removal decreases as shown in Figure 4.2. This result due to the increased turbidity of the suspension, which reduces light penetration. This also could probably happen due to aggregation of biomass at higher dosage (N. Mokhtar et al. 2017). The initial



concentration was obtained from this result which is 5mg/L as the curve of the graph looks like a bell. This is because the concentration is the most significant compared to others concentration for the interaction between concentration and dosage. This is also due to variety removal percentage by different biomass dosage. Therefore, further experiment of biosorption process by using 5mg/L for initial concentration.

#### 4.3.2 Effect of Dosage and Ph

Table 4.2 Data Analysis

<b>PHVSdosage</b>	<b>2</b>	<b>4</b>	<b>6</b>	<b>8</b>	<b>10</b>	<b>12</b>
<b>0.1</b>	69	65	53	61	80	38
<b>0.5</b>	71	72	86	80	81	64
<b>1</b>	78	81	77	75	85	59
<b>1.5</b>	84	69	72	72	78	43
<b>2</b>	75	63	65	70	77	37
<b>2.5</b>	65	54	57	69	73	35
<b>3</b>	52	53	52	67	72	33

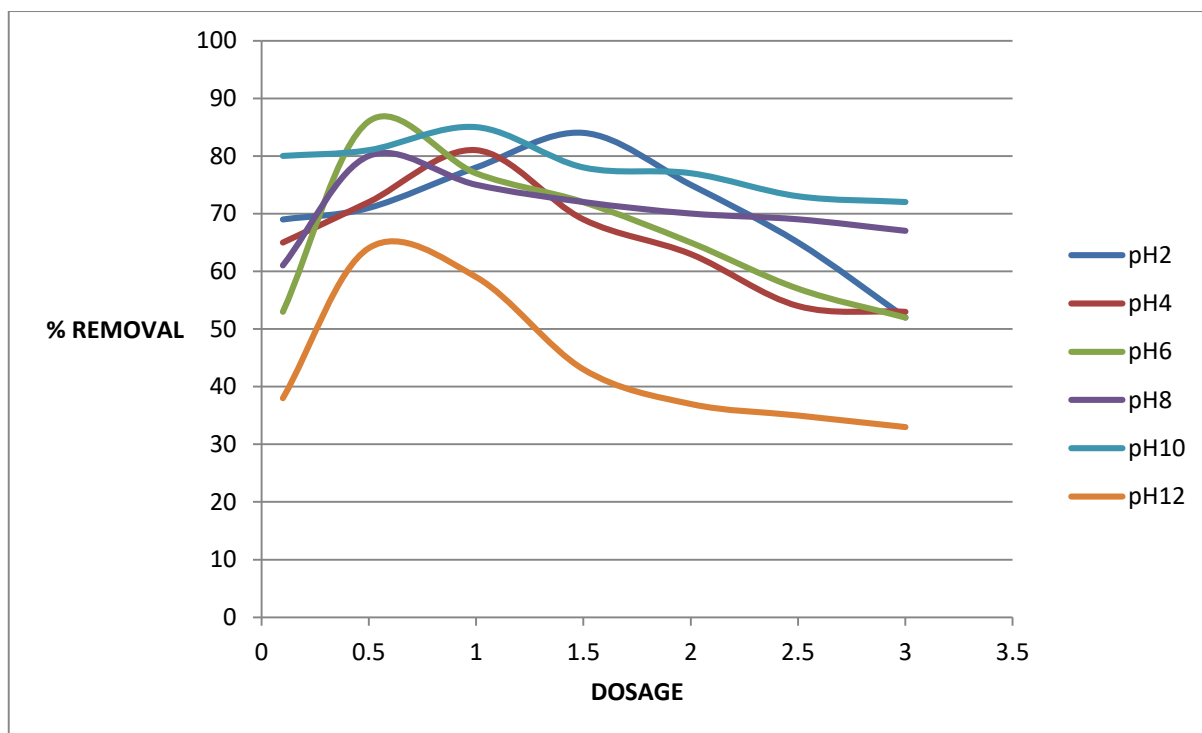


Figure 4.3 Interactions between Dosage and pH

Experiment further demonstrates in order to study second interaction which is tested by different of pH reading. The pH solution is the one of the important parameter that influences the percentage of MB removal. In this study, the effect of pH on biosorption of MB was investigated by varying the pH values (2, 4, 6, 8, 10, 12) and the result are presented in Figure 4.3. The ranges of dosage still same because used the optimum dosage. From the result in Figure 4.3, it showed that, at different pH, the percentage of removal is increased as the biomass dosage increased.

Surely, pH variation changes charge on surface of MB particles and varies potential of catalyst reactions. It full with  $H^+$  ions when pH lower than 7, while it full with  $OH^-$  ions when pH greater than 7. The dye binding was led by electrostatic attraction between the cationic species of  $MB^+$  and negatively charged functional group such as hydroxyl. In certain conditions such as acidic, the algae binding sites can be protonated hence reducing the electrostatic attraction between ions which is causing MB uptake is lower (E. Daneshvar et. al. 2012, N.Mokhtar et.al 2017).

### **4.3.3 Optimization Condition**

Optimization can be achieved when choosing 5mg/L with dosage 0.5g/L and pH 6. Optimization condition can be obtained from the max point of the bell curve. Optimization condition means the molecules of methylene blues were fully saturated at the available active site located at the biosorbent surface.

## **CHAPTER 5**

### **CONCLUSION**

#### **5.1 Conclusion**

Methylene Blue had successfully adsorbed onto algae. There are many types of algae that can be used for biosorption such as green algae, brown algae, and red algae. However, red algae were chosen for these experiments due to low purchasing price, effective absorbent and eco-friendly. Batch adsorption experiments were conducted to study the interaction of operating parameters and optimum conditions. The parameters studied were biomass dosage, initial concentration and pH.

For the interaction between the biomass dosage and initial concentration, the maximum percentage of removal is 95% for concentration 150 mg/L. Meanwhile for interaction between pH and biomass dosage, the maximum removal percentage is 86% for pH at 6.

Further investigation demonstrated at pH 6, the optimum dosage is at 0.5g/L. The initial concentration was obtained during experiments which are at 5mg/L. Methylene Blue molecules were fully saturated at the available active site that located at the biosorbent surface during the optimum conditions.

## **5.2 Recommendation**

Based on the result obtain in this work, a few recommendations that need to be considered for future establishment of data in order to get better data and higher efficiency of Methylene Blue dye removal. The perspective that need to be considered is it will be effective if there are more experiment on various biosorbent could be done continuously. And in term of experiment, maybe after this, biosorption using macroalgae could be applied in real industry wastewater with optimum condition of biosorption.

The real industry could apply this method in order to treat the wastewater before discharged into the water resources. This method of removal which is biosorption of dye by using the *E. Spinosum* must be apply with the optimum condition for an effective result.

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## APPENDIX A



Figure A-1: E.Spinosum



Figure A-2: Methylene blue stock solution



Figure A-3: Sieved E.Spinosum

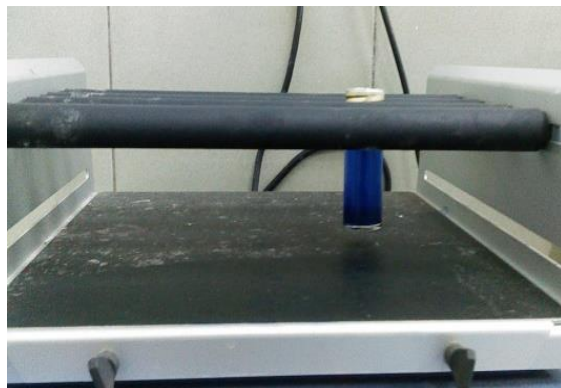


Figure A-4: Shaker



Figure A-5: Biosorption



Figure A-6: Crushed E. Spinosum



Figure A-7: Biosorption Process

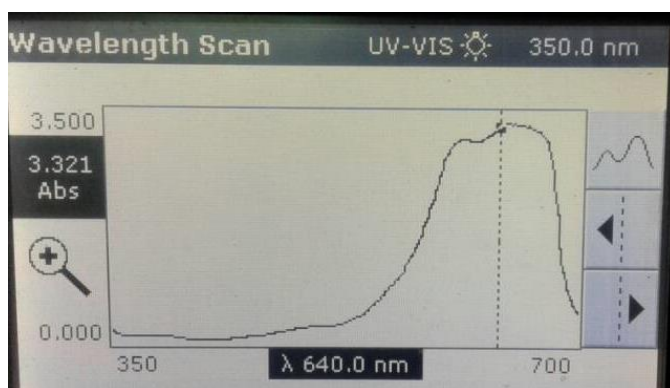


Figure A-8: Wavelength Scan from UV-VIS Spectra



Figure A-9: Oven Dry E.Spinosum

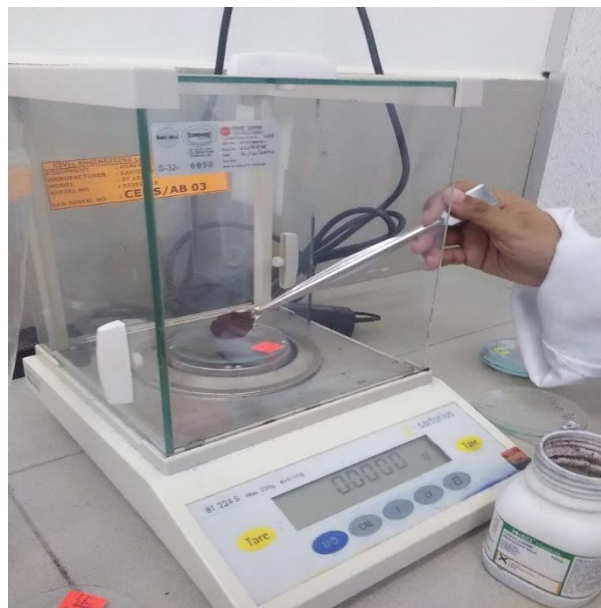


Figure A-10: Methylene Blue Dyes

pH:2			pH:8		
y(ppm)	ml	5 mg/l	y(ppm)	ml	5 mg/l
1	5	0.217	1	5	0.131
2.5	12.5	0.567	2.5	12.5	0.462
pH:4			pH:10		
y(ppm)	ml	5 mg/l	y(ppm)	ml	5 mg/l
1	5	0.261	1	5	0.125
2.5	12.5	0.563	2.5	12.5	0.453
pH:6			pH:12		
y(ppm)	ml	5 mg/l	y(ppm)	ml	5 mg/l
1	5	0.183	1	5	0.144
2.5	12.5	0.602	2.5	12.5	0.445



Figure A-11: abs Reading for pH

SUMMARY OUTPUT								
<i>Regression Statistics</i>								
Multiple F	1							
R Square	1							
Adjusted	65535							
Standard E	0							
Observati	2							
<i>ANOVA</i>								
	<i>df</i>	<i>SS</i>	<i>MS</i>	<i>F</i>	<i>gnificance F</i>			
Regressio	1	1.125	1.125	#NUM!	#NUM!			
Residual	0	0	65535					
Total	1	1.125						
	<i>Coefficients</i>	<i>Standard Err</i>	<i>t Stat</i>	<i>P-value</i>	<i>Lower 95%</i>	<i>Upper 95%</i>	<i>Lower 95.0%</i>	<i>Upper 95.0%</i>
Intercept	0.07	0	65535	#NUM!	0.07	0.07	0.07	0.07
5 mg/l	4.285714	0	65535	#NUM!	4.285714	4.285714	4.285714	4.285714
y=4.29x+0.07								

Figure A-12: Regression Analysis for pH 2

y=4.29x+0.07											
V/L	INITIALCCPH	DOSAGE,g	DOSAGE,g	reading1	reading2	average	Cf	Ce	qe	R(%)	
0.025	5	2	0.1	0.0025	0.032	0.037	0.0345	0.345	1.55005	34.4995	69
0.025	5	2	0.5	0.0125	0.036	0.029	0.0325	0.325	1.46425	7.0715	71
0.025	5	2	1	0.025	0.039	0.008	0.0235	0.235	1.07815	3.92185	78
0.025	5	2	1.5	0.0375	0.021	0.014	0.0175	0.175	0.82075	2.786167	84
0.025	5	2	2	0.05	0.036	0.018	0.027	0.27	1.2283	1.88585	75
0.025	5	2	2.5	0.0625	0.044	0.034	0.039	0.39	1.7431	1.30276	65
0.025	5	2	3	0.075	0.061	0.047	0.054	0.54	2.3866	0.871133	52

Figure

A-13: Example Data Analysis for pH 2



SUMMARY OUTPUT								
<i>Regression Statistics</i>								
Multiple R	0.999601							
R Square	0.999202							
Adjusted R	0.998804							
Standard Er	0.111635							
Observatio	4							
<i>ANOVA</i>								
	<i>df</i>	<i>SS</i>	<i>MS</i>	<i>F</i>	<i>Significance F</i>			
Regression	1	31.22507509	31.22508	2505.532	0.000398878			
Residual	2	0.024924907	0.012462					
Total	3	31.25						
	<i>Coefficient</i>	<i>Standard Error</i>	<i>t Stat</i>	<i>P-value</i>	<i>Lower 95%</i>	<i>Upper 95%</i>	<i>Lower 95.0%</i>	<i>Upper 95.0%</i>
Intercept	0.095143	0.135037266	0.704568	0.554072	-0.48587547	0.6761615	-0.48587547	0.676161457
50mg/L	4.193396	0.083775283	50.05529	0.000399	3.832940065	4.553852	3.832940065	4.553851969

Figure A-14: Regression Analysis for Concentration 50mg/L

		abs									
	y=4.19x-0.095										
V/L	INITIAL CONC. PH	DOSAGE,g/L	DOSAGE,g	reading 1	reading 2	average	Cf	Ce	qe	R(%)	
0.025	50	7	0.1	0.0025	0.262	0.281	0.2715	2.715	11.28085	387.1915	77
0.025	50	7	0.5	0.0125	0.195	0.207	0.201	2.01	8.3269	83.3462	83
0.025	50	7	1.1	0.0275	0.218	0.224	0.221	2.21	9.1649	37.12282	82
0.025	50	7	1.3	0.0325	0.206	0.24	0.223	2.23	9.2487	31.34715	82
0.025	50	7	1.5	0.0375	0.228	0.221	0.2245	2.245	9.31155	27.12563	81
0.025	50	7	1.7	0.0425	0.215	0.246	0.2305	2.305	9.56295	23.7865	81
0.025	50	7	1.9	0.0475	0.244	0.218	0.231	2.31	9.5839	21.27163	81
0.025	50	7	2.1	0.0525	0.273	0.277	0.275	2.75	11.4275	18.36786	77
0.025	50	7	3	0.075	0.282	0.308	0.295	2.95	12.2655	12.57817	75

Figure A-15: Data Analysis for Concentration 50mg/L

y	ml	50mg/L	ml	100mg/L	ml	150mg/L	ml	300mg/L	ml	25mg/L
2.5	1.25	0.558	0.63	0.493	0.41667	0.358	0.208333	0.75	2.5	0.628
5	2.5	1.201	1.25	1.045	0.833333	0.842	0.416667	1.233	5	1.258
7.5	3.75	1.752	1.88	1.63	1.25	1.552	0.625	2.197	7.5	1.646
10	5	2.36	2.5	1.937	1.66667	2.131	0.833333	2.641	10	2.39

Figure A-1: abs Reading for Initial Concentration