Phytoremediation of Methylene Blue and Methyl Orange Using *Eichhornia crassipes*

Kah Aik Tan, Norhashimah Morad, and Jie Qi Ooi

***Abstract*—Phytoremediation is defined as the use of plants and their associated microorganisms to remove harmless pollutants from contaminated sites. It is a promising approach in the treatment of dye wastewater due its cost-effectiveness. The aim of this study was to determine the effectiveness of water hyacinth (*Eichhornia crassipes*) in removing color. Water hyacinth was used to treat 50 mg/L of methylene blue (MB) and 50 mg/L of methyl orange (MO) for 20 days under ambient temperature (30±1 °C). The pH of the synthetic dye wastewater was observed throughout 20 days. Results obtained showed that percentage of color removal was higher for MB compared to MO which were 98.42% and 66.80% respectively. The relative growth of *Eichhornia crassipes* in MB and MO were also being determined. The cell structure of *Eichhornia crassipes* (leaf, shoot and root) before and after the plants were exposed to dye wastewater was analysed using light microscope.**

***Index Terms*—*Eichhonia crassipes*, methylene blue, methyl orange, phytoremediation**

I. Introduction

Dyes can give color to water bodies even when they exist in small amount. They are widely used in various industries such as textile, plastic, paper and rubber industries [1]. Among these industries, textile industry ranks first in the usage of dyes for coloration of fiber. The textile wastewater is well known to contain strong color, large amount of suspended solids, high fluctuation in pH, high temperature, high COD concentration and other organic contents [2]. Due to the usage of dye and pigments during the dyeing process, the strong color and turbidity of the textile wastewater effluents caused many problems because of its negative visual impact [3].

Some of the dyes are toxic and carcinogenic in nature. Methylene blue (MB) is a basic dye which is used extensively in the dyeing and printing of cotton, silk etc [4]. MB can have various harmful effects. The high concentration of this dye in contact with the eye can cause corneal injury in human beings. Doses in the range 500 mg can lead to anemia, dizziness, headache, abdominal pain, nausea, profuse sweating and mental confusion [5]. On the other hand, methyl orange (MO) is an example of azo dye. Azo dyes are the most versatile and the largest group of dyes. They are considered to be toxic and mutagenic to living organisms [6]. Their discharge into a water environment can cause serious health problems as well as acute and chronic effects on aquatic life [7]. Thus, the impact and treatment of the dye to the environment has been studied by many researchers in the past decades. Many treatment methods have been developed for the removal of dyes from wastewater, including adsorption, oxidation processes, microbiological or enzymatic decomposition, and etcetera [8]. However, phytoremediation proves to be quite an efficient method in comparison to these methods [8].

Phytoremediation is the use of plants for environmental cleanup. It is popular because it has a lot of advantages such as cost-effectiveness, aesthetic advantages, long-term applicability and it can be directly applied at the polluted sites where other treatment methods are too expensive [9]. In addition, plants offer protection against wind and water erosion, preventing contaminants from spreading. It is easier to manage because of its autotrophic system that has large biomass and require little nutrient input [9]. Plants that are fast growing, with deep and fibrous roots, such as grasses, are very useful in phytoremediation [9], [10]. There are five types of phytoremediation techniques, namely phytoextraction, phytostabilization, phototransformation, phytodegradation, and rhizofiltration [11].

Water hyacinth (*Eichhornia crassipes*) is a free floating aquatic plant that originated from tropical South America and is now widespread in all tropical climates. It is listed as one of the most productive plants and also one of the world's worst aquatic plants. Due to its fast growth rate, large biomass production, high tolerance to pollution and its heavy metal and nutrient absorption capacities, *Eichhornia crassipes* has a potential to cleanup various types of wastewaters [12].

II. Methodology

A. Preliminary Study

Eichhornia crassipes were collected from Tasik Harapan, Universiti Sains Malaysia. All the plants were washed thoroughly with tap water to remove any dirt and soil particles adhered to the plants. The plants were then grown in basins using tap water for one week to allow the plants to adapt to the new environmental conditions. After one week, the plants were introduced to 6 L of MB and 6 L of MO with different concentrations (50 mg/L, 100 mg/L, 150 mg/L, 200 mg/L) for 7 days, to determine the most effective concentration for color removal. The conditions of the plants were observed throughout the experimental works.

B. Experimental Study

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Based on the preliminary study, concentrations of 50 mg/L of MB and 50 mg/L MO were chosen and prepared to grow the plants. The plants were wiped dry to remove excess water. Plants of almost the same size, which weighted between 210 g to 240 g and height within 15 cm to 20 cm were put into the basins which contained 6 L of 50 mg/L MB and 6 L of 50 mg/L MO dye aqueous solution. Experiments were carried out in triplicates with one control set each. The plants were left to grow for 20 days in open air and exposed to sufficient sunlight. Any decrease in volume of solution in each basin was added with deionized water to the mark to ensure their volume were maintained at 6 L to counter water loss due to evapotranspiration. 90 mL of samples were taken every 2 days.

The absorbance of MB and MO were measured by using HACH, DR 2400/2500 spectrophotometer with λ_{max} 665 nm and 465 nm respectively. Equation 1 was used to calculate the percentage of dye removal.

$$\text{percentage of dye removal (\%)} = [(C_o - C_f) / C_f] \times 100 \quad (1)$$

where, C_o is the initial dye concentration (mg/L) and C_f is the final dye concentration (mg/L).

The plant growth assessment was done by monitoring the wet weight and length of the plants before and after the experiment. The relative growth rate of plant was calculated as in (2) [13],

$$\text{relative growth rate (day}^{-1}\text{)} = (\ln w_2 - \ln w_1) / t \quad (2)$$

where, w_1 is the wet weight of Eichhornia crassipes before exposure to dye contaminant, w_2 is the final wet weight of Eichhornia crassipes after exposure to dye contaminant, and t is the duration (day).

C. Plant Cells Analysis

The light microscope (Nikon Eclipse E20) was used to observe the plant cells (leaf, shoot, and root) before and after exposure to the dyes. The plants were cut into three parts, which were leaves, shoot, and root. Samples were prepared by cutting a very thin slice of the cell and put on a microscope slide. The plant cells of each part of the plant were examined under the light microscope with 40x magnification. The images of the cells were captured.

III. Results and Discussion

A. Variation of pH

The pH can affect the efficiency of color removal. It has been reported that *Eichhornia crassipes* can grow within a pH range of 4.4 to 10.0 in different water stream [14]. Therefore, the pH value of MB and MO must be well-monitored. Fig. 1(a) and 1(b) show the pH readings of MB and MO for 20 days. The pH value of MB and its control set were within the range of pH 4 to pH 6 throughout the experiment works. However, the MO and its control set fluctuated between pH 5 and pH 8. The fluctuation of the pH value is due to the free diffusion of the carbon dioxide with the atmosphere and the absorption of nutrient by the plants [15].

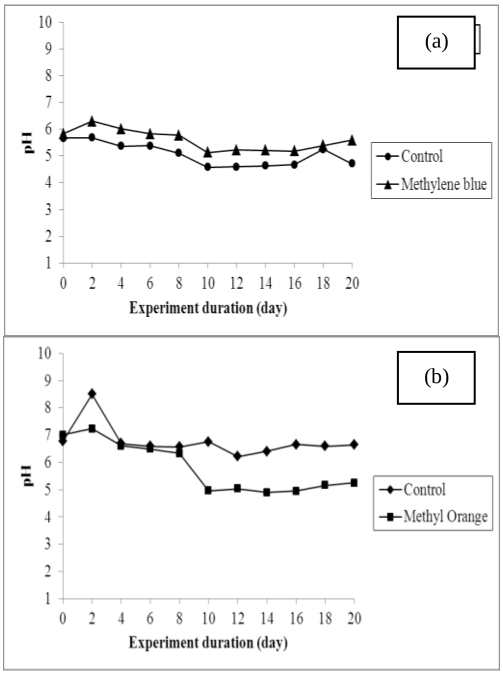

Fig. 1. pH reading of (a) MB and (b) MO for 20 days.

B. Color Removal of MB and MO

The percentages of color removal were calculated for MB and MO remediated by *Eichhornia crassipes*. Fig. 2(a) and 2(b) show the percentages of the color removal of MB and MO by *Eichhornia crassipes* after 20 days. From Fig. 1(a) and 1(b), the percentage of color removal of MB is higher than the percentage of color removal of MO which is 98.42 % and 66.80% respectively. This is because MO has a higher molecular weight and the structure of MO contains azo bond (-N=N-) as compared to MB which has a lower molecular weight. Many researchers have claimed that the rate of color removal is also dependent on the dye class rather than the molecular features [16]. The color removal of the control set is due to the photodegradation of dye molecules as the basins were exposed to sunlight. Photodegradation of dye occurs even in the presence of ultraviolet light only [17].

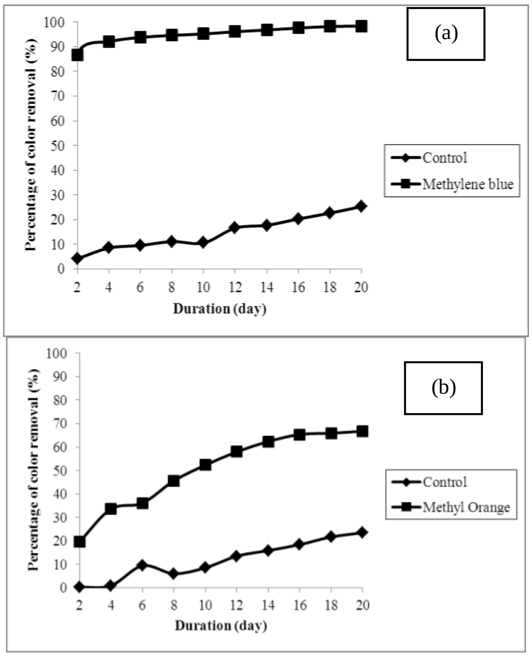

Fig. 2. Color removal of (a) MB and (b) MO for 20 days.

C. Plant Cells Analysis

A light microscope was used to observe changes in the plant cells before and after exposure to MB and MO dye wastewater. Fig. 3(a), 3(b) and 3(c) show the leaf cell structure of *Eichhornia crassipes* before and after exposure to MB and MO dye aqueous solution respectively. MB and MO are not visible in the leaves of the plants. This indicates that the leaf cell do not absorb MB and MO because the plants do not transfer MB and MO dye aqueous solution to the leaves within 20 days.

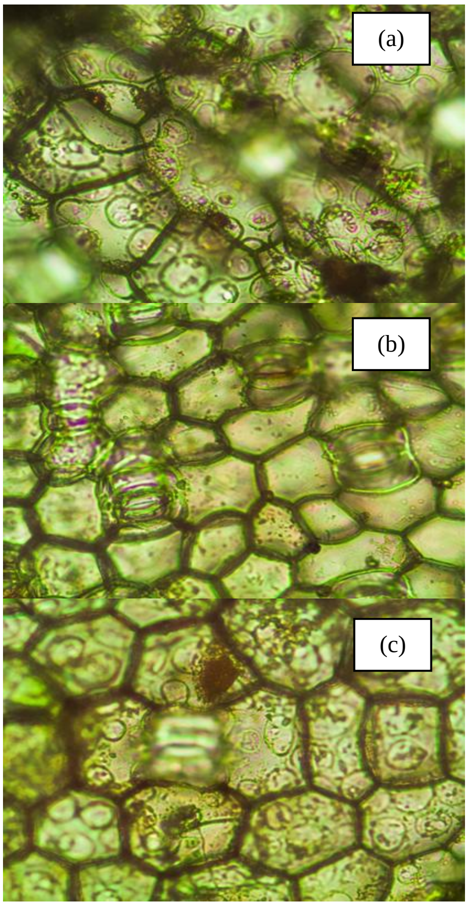

Fig. 3. The leaf cell structure of *Eichhornia crassipes* (a) before, (b) after exposure to MB and (c) after exposure to MO for 20 days.

However, Fig. 4(a), 4(b) and 4(c) show the shoot cell structure of *Eichhornia crassipes* before and after exposure to MB and MO dye aqueous solution. MB is not visible in the shoot of the plant. But, there is an orange spot found in Fig. 4(c). This indicates that the shoots had absorbed the MO and the plants transferred MO to their shoot faster than MB. Many researchers have reported that plants required longer time for the contaminants to reach the shoots as compared to root [12]. Thus, this explains the absence of MB in the shoot cell.

Fig. 5(a), 5(b), and 5(c) show the root cell structure of *Eichhornia crassipes* before and after exposure to MB and MO for 20 days. From Fig. 5(b) and 5(c), blue spot and orange spot can be observed. This indicates that the dye contaminations have been absorbed by the roots of *Eichhornia crassipes*. It is often observed that dye contaminations are accumulated more in the roots than in the shoots due to the difference of the two tissues for the biochemistry uptake. Similar case also been published in the color removal of synthetic reactive dye wastewater by narrow-leaved cattails [18].

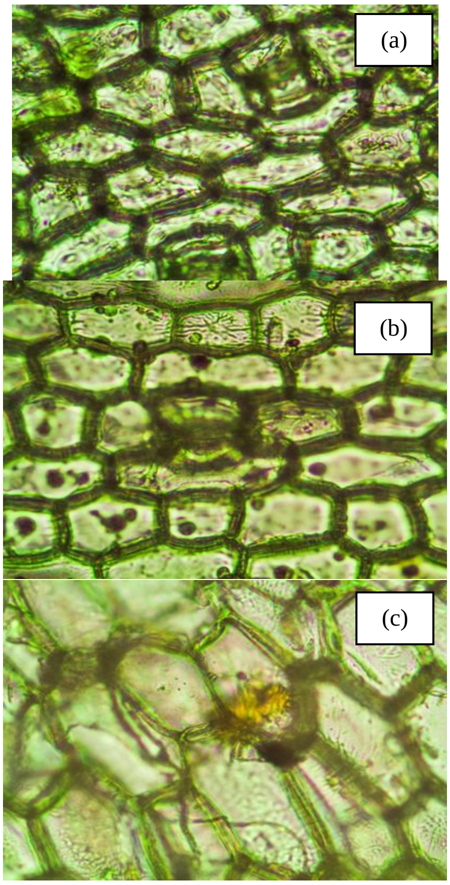

Fig. 4. The shoot cell structure of *Eichhornia crassipes* (a) before, (b) after exposure to MB and (c) after exposure to MO for 20 days.

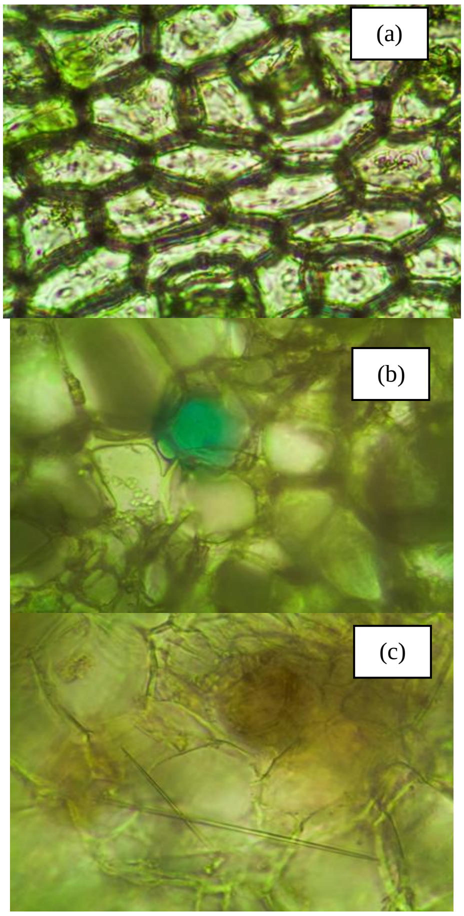

Fig. 5. The root cell structure of *Eichhornia crassipes* (a) before, (b) after exposure to MB and (c) after exposure to MO for 20 days.

D. Relative Growth Rate of Eichhornia crassipes

Relative growth rate of the plant was investigated to determine the ability of the plants to grow and survive in the contaminated wastewater. Table I shows the relative growth rate of *Eichhornia crassipes* in MB and MO dye aqueous solution. The results show that all of the plants had increased in wet weight after exposure to the dye contaminants. The wet weight increases from 213.1 g to 316.7 g and from 237.9 g to 397.6 g in MB and MO dye aqueous solution respectively. The relative growth rate of Eichhornia crassipes in MO dye aqueous solution is higher than it in MB which is 0.03 day^{-1} and 0.02 day^{-1} respectively. This indicates that plants grew better in MO dye aqueous solution than in MB dye aqueous solution.

TABLE I: THE RELATIVE GROWTH OF *EICHHORNIA CRASSIPES* IN MB AND MO DYE AQUEOUS SOLUTION

Dye	Wet weight of plant (g)		Relative Growth (day^{-1})
	Before	After	
MB	213.1	316.7	0.02
MO	237.9	397.6	0.03

E. Average Length of Eichhornia crassipes after 20 Days

Table II shows the average length of the plant before and after 20 days in MB and MO dye aqueous solution. The results showed that all the plants had an increase in their length after 20 days. The average increase are 1.6 cm and 3.1 cm when the plants exposed to MB and MO dyes aqueous solution respectively. However, the plants in MB and MO dye aqueous solution started to show wilting symptoms after 18 days of the experimental works. Wilting is a biochemical changes that prevent the healthy tissues of the plants from damage by surrounding the damaged area with a high concentration of phenolic compounds [18]. The overall results indicated that *Eichhornia crassipes* has a potential to be used in phytoremediation since it is highly tolerant to the pollution and can be used in wastewater treatment in ponds [8].

TABLE II: THE AVERAGE LENGTH OF *EICHHORNIA CRASSIPES* IN MB AND MO DYE AQUEOUS SOLUTION BEFORE AND AFTER 20 DAYS

Time (day)	Average length of plants (cm)	
	MB	MO
0 day	16.8	19.7
20 days	18.4	22.8

IV. CONCLUSION

This study has indicated that *Eichhornia crassipes* has a potential to decolorize different synthetic dyes. Based on the results obtained, the percentage of color removal of MB is 98.42%, while MO is 66.80%. The pH of the MB and MO dye aqueous solutions fluctuated throughout the experiment. This was due to the free diffusion of the carbon dioxide with the atmosphere and the uptake of nutrients by the plant.

The relative growth rate of *Eichhornia crassipes* in MB dye aqueous solution and the MO dye aqueous solution was 0.02 days^{-1} and 0.03 day^{-1} respectively. New growth was observed and the plants started to bloom during the experiment. This shows that the plant can survive and grow in contaminated dye solution. However, the plants in MB dye aqueous solution and methyl orange dye aqueous solution showed wilting symptoms after 18 days of exposure. The observation of the plant cells using light microscope analysis proved that the roots of *Eichhornia crassipes* can absorb the dyes. Thus, it can be concluded that *Eichhornia crassipes* is a suitable plant to be used in the phytoremediation of dye wastewater and has a potential to be used in water bodies contaminated with dyes.

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