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REMOVAL OF METHYLENE BLUE BY COONTAIL (*CERATOPHYLLUM DEMERSUM*) USING PHYTOREMEDIATION CONCEPT

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Abstract

Effluents containing dyes are discharged to the environment without treatment pose harm to humans' aquatic life by presenting to those contaminants. The goal of this research is to study the ability of the aquatic plant Coontail (*Ceratophyllum demersum*) to remove methylene blue (MB) dye. Coontail (25 g) was exposed to a concentration of 5 mg/L MB dye for 5 days. The absorbance values were measured at (0-5) days with a wavelength of 600-665 nm. The dye concentration was analysed through absorbance with a UV/Vis spectrophotometer and showed a reduction from 0.095 at day 0 and decreased to 0.0045 at 5 days. The dye removal percentage rate during exposure to MB was observed. The removal percentage was up to 96% for 5. The plants have strong potential as a phytoremediation agent to remove dyes from the wastewater treatment system.

Key words: Coontail, *Ceratophyllum Demersum*, Green environment, methylene blue, phytoremediation.

Introduction

Phytoremediation is the method of utilizing plants and their related microorganisms to expel toxins from the contaminated sites. It is a promising strategy in the treatment of dye wastewater due its cost-effectiveness. Synthetic dyes are considered as one of the most significant classes of environmental pollutants that adversely mark the human and aquatic life (Sinha *et al*., 2016; Taheri *et al*., 2014; Hasani Zonoozi *et al*., 2015). More than 7×10^5 metric tons of dyes are formed worldwide every year and 5-10% of them are discharged in wastewater (Yao *et al*., 2016; Hakimelahi *et al*., 2012). Wastewater containing dye is produced by different productions such as textile, paper, leather, pharmaceuticals and food processing (Modak *et al*., 2016; Mehrali *et al*., 2010; Vaigan *et al*., 2010; Gupta *et al*., 2013). Dyes have several negative effects on environmental systems such as: visibility even at very low concentrations, disturbing sunlight penetration, toxicity/mutagenicity/carcinogenicity of their degradation products, resisting physicochemical/ biological attack, and increasing COD/BOD levels of aquatic sources, the effluent containing dyes is released to the environment, thereby seriously affecting the environment by damaging ecosystems, causing water pollution and reducing light penetration for aquatic ecosystems (Robinson *et al*., 2001; Hosseini Koupaie *et al*., 2013a; Karimifard and Alavi Moghaddam, 2016a; Al Farraj *et al*., 2019). The aromatic molecular structure of most dyes makes them very stable and difficult to remove (Mehrali *et al*., 2012; Azizi *et al*., 2011b; Hasani Zonoozi *et al*., 2014; Hosseini Koupaie *et al*., 2012).

Phytoremediation has been effectively utilized for adsorption and removing dye from water such as *Glandularia pulchella* L. (Kabra *et al*., 2012), *Salvinia molesta* (Chandanshive *et al*., 2016) and *Typha angustifolia and Paspalum scrobiculatum* (Chandanshive *et al*., 2017).

Floating plant of *Salvinia molesta* was able to degrade azo dye Rubine GFL up to 97% at a concentration of 100 mg/L within 72 h., using 60 g of root biomass (Chandanshive *et al*., 2016). Plants have the potential to degrade textile dyes (Khataee *et al*., 2012). There are three categories of technology are commonly used to degrade dyes, namely, chemical, physical and biological (Al-Farraj *et al*., 2019; El-Hassani *et al*., 2019; Hadibarata *et al*., 2018; Imron and Titah, 2018). The chemical processes usually involve the use of chemicals through the oxidation process to degrade dyes (Hassaan *et al*., 2017), the by-products of the chemical process like sludge is require additional processing, because they also contain hazardous elements. In addition, the high

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costs of the chemical procedure. The same thing with the physical processes like coagulation-flocculation, adsorption, ion exchange and membrane filter by treating the sludge and the cost of treating, (Azimi *et al*., 2017). The biological process is more suitable for degrading dyes than chemical and physical processes because biological process produced nontoxic by-products, such as CO_2 and H_2O which are safe to the environment and ecofriendly and easy to operate in addition the biological process and releases nontoxic by-products to the environment (Al-Farraj *et al*., 2019; Imron *et al*., 2019a; Titah *et al*., 2019). The biological treatment based on living organisms, like microorganisms and plants by transforming the pollutants via the metabolic pathway (Imron *et al*., 2019b). The use of plants as bioremediation agents is very promising for degrading various dyes and organic and inorganic pollutants (Ali, 2010; Singh and Singh, 2017). Plants are very delicate to pollution, like organic and inorganic pollutants (Purwanti *et al*., 2018; Tangahu *et al*., 2019; Titah *et al*., 2018). Some aquatic plants, such as Azolla pinnata and Lemna minor, have the potential to degrade azo dyes (Al-Baldawi *et al*., 2018; Khataee *et al*., 2012; Reema *et al*., 2011). Based on Al-Baldawi *et al*. (2018), the maximum degradation efficiency of methylene blue (MB) is 90%, which can be achieved by A. pinnata for 5 days at 25 mg/L initial concentration. According to Khataee *et al*., (2012), L. minor can degrade Acid Blue 92 dyes up to 80% for 6 days. MB is a heterocyclic aromatic used in biological and chemical industries, textile industries and medicine (Al-Baldawi *et al*., 2018; Contreras *et al*., 2019). MB dye produces serious effects, such as headache, vomiting and high blood pressure when consumed by humans. However, studies on the phytoremediation of MB dye using coontail (*Ceratophyllum demersum*) are lacking in the scientific literature. To fill this research gap, this study aims to investigate the removal of MB dye using coontail (*Ceratophyllum demersum*) a species of *Ceratophyllum* which it is a submerged, free-floating aquatic plant. The results of this study will be useful for industries as alternative biotechnology by using plants that can be used to treat dye-contaminated water.

Materials and methods

Plant cultivation

Coontail (*Ceratophyllum demersum*) a species of *Ceratophyllum* which it is a submerged, free-floating aquatic plant. (about ½-12' long) that branches at right angles (90°). Coontail (*Ceratophyllum demersum*) plant were collected from different sites of Babylon archaeological area channel during January, 2019 to March, 2019. The plant was adapted for 7 days in fresh clean tap water before the main experiment. All used plants were healthy and in good conditions. In our study, the phytoremediation of *C. demersum* was conduct by exposing the plant to MB for 5 days, the rootless, submerged, free-floating macrophyte *Ceratophyllum demersum* and the removal was monitored.

Table 1: Characteristics of methylene blue.

Parameters	Properties
Molecular formula	$C_{16}H_{18}ClN_3S$
Molecular weight (g/mol)	319.851
Colour index number	52015
Density (g/mL)	1.0
Melting point (C)	190
λ max (nm)	665

Dye analysis

Methylene Blue ($C_{16}H_{18}ClN_3S$) (Merck, Germany) was used as the pollutant to be removed in this experiment. MB characteristics and chemical structure are presented in fig. 2 and table 1. The initial pH of the dye solution ranged from 7.1 to 7.9 and the initial temperature was the room temperature around 28°C in all reactors. The MB was measured with UV/Vis Microplate Spectrophotometer (Apel PD-303S, Japan) at maximum absorption wavelength, 660-665 nm (Al-Baldawi *et al*., 2018; Low *et al*., 2012).

Decolorization experiments

Decolorization experiments were conducted in a container containing 50 mL of MB as a stock concentration of 50 mg/L (Pego *et al*., 2017) and 5mg/L as working stock. 10 grams of coontail were exposed to MB-contaminated water at room temperature. The reactor was designed to replicate the floating treatment wetland under batch system reactor (Lucke *et al*., 2019). It was ensured that all plant roots reached the bottom of

Fig. 1: Coontail used in the study.

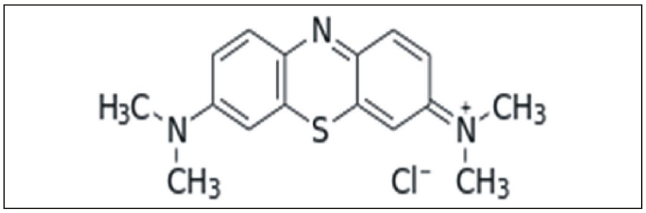

Fig. 2: Chemical structure of methylene blue.

the reactor during the test to accommodate the root sorption capability and the rhizosphere removal mechanisms (Almuktar *et al*., 2018). Samples (1 mL) were collected every single day to measure the absorbance value. The absorbance value was analysed using UV/Vis Microplate Spectrophotometer (Apel PD-303S, Japan) at maximum absorption wavelength 665 nm with distilled water used as blank (Al-Baldawi *et al*., 2018; Low *et al*., 2012). Before the absorbance was measured, the sample was filtered using 40 µm Wattman filter paper to remove unwanted particles. All measurements were conducted in three replicates. The removal of MB was approached by using absorbance analysis in terms of decolorization. The decolorization percentage was calculated based on Eq. 1, (Al Farraj *et al*., 2019; Khataee *et al*., 2012), as follows:

Decolorization % = A_{0-} At/ A_0

where,

A_0 the initial absorbance at 0 hour

At the final absorbance at t-hour

Results and Discussion

Coontail removal of MB was investigated for 5 days. The absorbance values were calculated everyday with replicate to ensure the accuracy of the results. The absorbance value decreased with the increasing of exposure duration. The physical observed reactor can be seen in fig. 3. The decrease of absorbance on the main reactors indicated that the MB dyes were decolorized by Coontail.

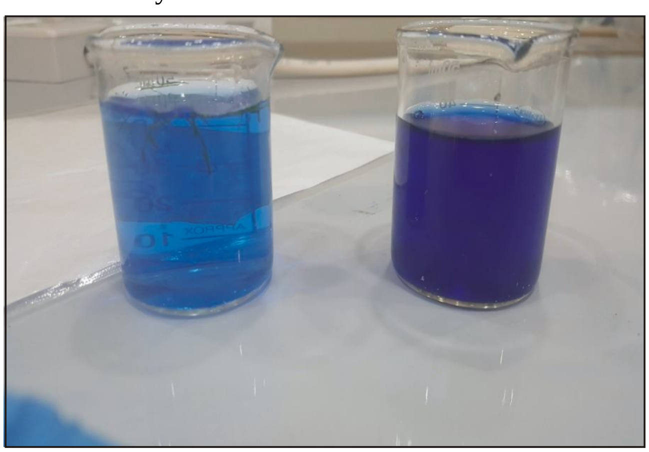

Fig. 3: Sampling day 3 comparing with control.

Reema *et al*., (2011) reported that the dye uptake capacity increased with increasing contact time. Based on the decreasing absorbance value, it was indicated that Coontail can adsorb and accumulate MB inside the plant. Similar to results obtained by Reema *et al*., (2011) and Khataee *et al*., (2012), Coontail removed the dye-contaminated water by up to 80% for 3 days. The removal of MB was increased with increasing exposure time (Pathania *et al*., 2017; Vieira *et al*., 2009). In fact, increasing the exposure time of the dye on plants increases the probability of contact between dye molecules and plant surface (Khataee *et al*., 2012, 2010; Tangahu *et al*., 2019). The surface area for sorption of the dye molecule is greater when the exposure time is increased. This result was in agreement with that obtained by Nasrullah *et al*., (2015) and Pathania *et al*., (2017), in which the increasing incubation time resulted in high decolorization efficiency. The observed pH decreased throughout the 5 days research period, the decreasing in pH occurred due to the secretion of some plant metabolites. The initial step of the degradation mechanism step of MB is the cleavage of the bonds of C-C into two benzene rings. Instead of producing a toxic aromatic compound, a hydroxylation process of an aromatic ring produced a phenolic compound was conducted (Pandey *et al*., 2007; Sarkar *et al*., 2017). The phenolic compound is important in biodegradation related to antioxidant and enzyme properties (Khataee *et al*., 2012) After that, the enzyme produced by plants oxidized the phenolic compounds to generate a phenoxy radical and carbonium ion (Singh *et al*., 2015; Sudha *et al*., 2014). The production and secretion of these ions increased the acidity of the living environment, thereby resulting in lower observed pH at the end of the test period. The physical observation of the MB removal after treatment by Coontail are shown in fig. 3 and 4.

From the figure above the decolorization process can be examined by using UV/Vis spectra based on Al Farraj *et al*., (2019) and as presented in fig. 3 and 4. It is clear that the peak at 665 nm shows a significant decrease.

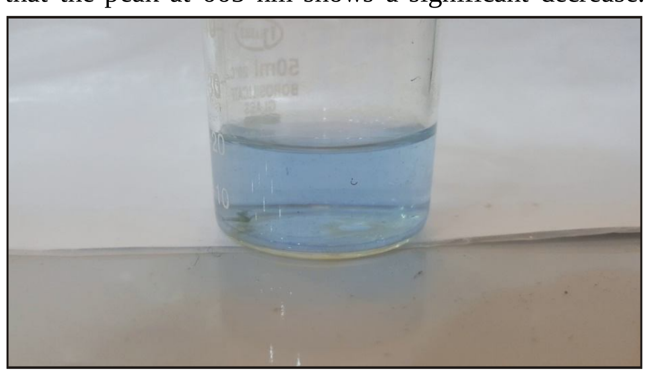

Fig. 4: Sampling day 5.

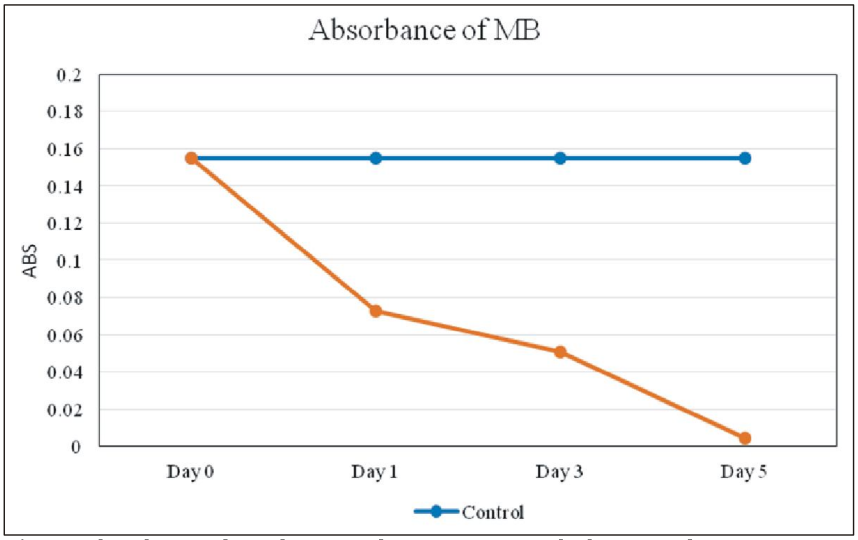

Fig. 5: Absorbent values during 5 days compare with the control.

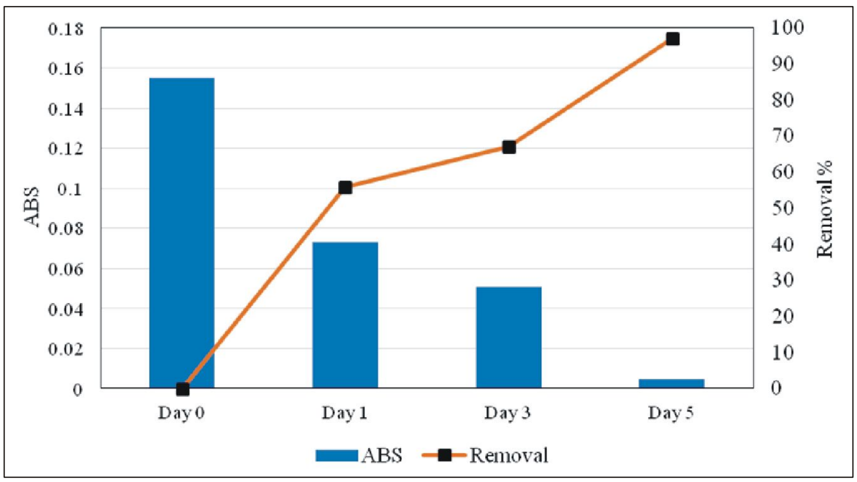

Fig. 6: Removal percentage during 5 days.

Geoffroy *et al*., (2004) reported that during the degradation of dye by plants and microorganism, different enzymes convert the pollutant into a less toxic compound. The absorbance according to UV-Spectrometer reading were 0.155, 0.0.73, 0.051 and 0.0045 at day 0 to 5 day for 5 mg/L MB-dye concentrations respectively as illustrated in fig. 5 and the removal percentage illustrate in fig. 6.

As shown in fig. 5 and 6, the removal was detected from the first day with a value of removal 56% and for the third and fifth day was 67% and 97%, which is a high percent of removal which give an indicator that this plant have a high ability to remove dyes from water stream and good to applied in phytoremediation process.

Conclusion

The removal of Methylene Blue (MB) by coontail is significantly affected by the contact time. Physical observation showed a clear indication of decolorization by coontail after 5 days of exposure to the dye. UV/Vis spectra analysis also illustrated the decrease of MB absorbance throughout the 5 days of the test period and showed up to 95% of decolorization. Our findings suggest that coontail has the potential to be used as phytoremediation agent in treating dye-contaminated wastewater. Further study related to different dyes types, combination of different aquatic plants and extended time exposure will greatly contribute to the knowledge on phytoremediation of dye-contaminated wastewater. The application of aquatic plants as phytoremediation agent of dyes is considered an environmentally friendly technology. Phytoremediation of dyes using aquatic plants has gained wide consideration as an alternative technology due to its low operational cost, nontoxic by products, eco-friendliness and low amount of sludge production. These findings were limited to one type of dye and one type of aquatic plant. Thus, further study on different wastewater, combination of different types of aquatic plants and the extended period of treatment is needed. These findings are very helpful for textile industries and by using an alternative biotechnology to treat dye-contaminated wastewater using plants (phytoremediation).

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