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Preliminary Studies of Methylene Blue Remotion from Aqueous Solutions by *Ocimum basilicum*

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Abstract: The continuous expansion in the textile industry results in high loads of coloured wastewaters that heavily pollute the limited freshwater sources. Therefore, a wide array of treatment methods has been used to remediate water/wastewater from dyes. One common practice is the use of plants to degrade, absorb, metabolise, and detoxify different types of pollutants, including dyes. This study employs sweet basil (*Ocimum basilicum*) as a phytoremediation model herb to remove different concentrations (5–25 mg/L) of methylene blue (MB) dye from synthetic water, taking into account the effects of the MB dye concentration (5–25 mg/L) and contact time (up to 10 days). The results showed that the ability of *Ocimum basilicum* to absorb MB dye decreased with the increase of the MB dye concentration and increased with the increase of the contact time. The best removal of the MB dye was 93% when the concentration of the MB dye was 25 mg/L and the contact time was 10 days. Additionally, it was noticed that the relative growth rate (RGR) of the herbs was adversely influenced by increasing MB dye concentrations and that the best RGR value was 2.2 g/day when the MB dye concentration was 5 mg/L.

Keywords: bioremoval; methylene blue; phytoremediation; sweet basil; *Ocimum basilicum*

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1. Introduction

Various industrial sectors are increasingly employing various dyes, resulting in the buildup of hazardous chemicals in the environment [1]. Dyes are used in a variety of sectors, including textiles, plastics, paper, concrete, pharmaceuticals, and rubber [2]. Furthermore, the textile sector is thought to be responsible for more than 50% of dye wastewater [3]. As a result, significant amounts of textile effluent must be treated before being released into receiving waterways. The production of dye metabolites and the presence of heavy metals within the dye structure are both responsible for toxicity in textile effluents [4]. Dyes are considered dangerous because of their toxic characteristics, including carcinogenic, allergic, and dermal impacts. In addition to the toxicological considerations, the most serious environmental adverse impact with dyes is the restriction of sunlight penetration into surface waters, which inhibits the growth of microorganisms (phytoplankton or/and cyanobacteria) and submerged aquatic plants, resulting in oxygen depletion in the water [5].

The removal of dyes from wastewater is critical, especially when the concentration of the dye is relatively low (below 1 ppm) and due to the major impacts of the dyes on the aquatic environment. The main negative impacts of dye-contaminated wastewater on aquatic life are the severe decrease in dissolved oxygen (DO) and sunlight penetration and the increase in biochemical oxygen demand (BOD) [6].

Methylene blue (MB) is a basic aromatic heterocyclic cationic dye that is widely present in dye-based industrial effluent due to its widespread use in the textile industries for colouring leather, cotton, printing, and tanning [7,8]. When ingested by humans, MB dye can induce disorientation, shortness of breath, vomiting, elevated blood pressure, a

variety of allergic responses, and cancer [1]. It is classified as a hazardous pollutant because of its negative consequences for the environment [8].

To reduce the environmental effects of dyes, a variety of techniques for removing them from waterways and wastewaters have been developed, including adsorption [9], electrocoagulation [10,11], membrane filtration [12], and advanced oxidation [13,14]. All of these techniques are more expensive than biological treatment ones. However, these are generally utilised in series with biological treatment [7]. In comparison to these above techniques, however, phytoremediation appears to be a very successful technology [15]. The use of plants to clean up the environment is known as phytoremediation. It is popular because it offers a number of benefits, including cost-effectiveness, aesthetic benefits, long-term application, and the ability to be used directly on contaminated sites [16]. Growing aquatic plants like *Phragmites australis* and free-floating plants like *Lemna minor* are among the plants that researchers currently use for bioremediation [6]. Plant photosynthetic activity and growth rate are important factors in the economic viability of phytoremediation [15]. In the phytoremediation of MB dye by *Spirodela polyrrhiza*, Manghabati and Pazuki [17] found out that the exposure duration was the most critical element. MB dye has a maximum degradation efficiency of 90%, according to Al-Baldawi et al. [18], which may be attained by *Azolla pinnata* over five days at a 25 mg/L starting concentration. Physical observation revealed a clear sign of bio-decolourisation by duckweed (*Lemna minor*) following 24 h of dye exposure, according to Imron et al. [19]. The Ultraviolet-visible spectroscopy (UV/V) tests revealed a decrease in MB dye absorbance throughout the 24 h test period, with up to 80.56% decolourisation. The removal percentage of MB dye reached up to 96% within five days in research conducted by Ewadh [20], indicating that the aquatic plant Coontail (*Ceratophyllum demersum*) shows a high potential as a phytoremediation agent to remove MB dye from the wastewaters.

Identifying the appropriate plant to remove pollutants is a crucial step in phytoremediation. Aromatic and medicinal herbs can be utilised to clean up polluted locations since metals are less likely to be transferred from the soil to the essential oil or change its composition [21]. Sweet basil (*Ocimum basilicum*), a medicinal, aromatic herb, is high in minerals and Vitamin A. Basil's distinctive smell comes from the essential oil of the green leaves, which is high in aldehydes, terpenes, and phenols [22]. Basil is grown for use as a culinary herb, as a dried or frozen leaf condiment or spice, and as a source of fragrant essential oils for use in meals, flavours, and perfumes. Furthermore, fresh herbage is used in medical therapy [23].

This study aims to assess the bioremoval of MB by sweet basil (*Ocimum basilicum*) using the phytoremediation concept.

2. Materials and Methods

2.1. Sweet Basil (Ocimum basilicum) Herbs

In this study, sweet basil (*Ocimum basilicum*) was gathered from the researcher's own garden. All of the plants were fresh and carefully cleaned with tap water to eliminate any dirt or soil particles that were attached to them. The plants were then cultivated for one week in vessels with tap water and grown in a Hoagland nutritional solution to allow them to adjust to their new surroundings [17].

2.2. Preliminary Experiments

MB dye was prepared in a stock solution with a concentration of 100 mg/L by dissolving 0.1 g of MB dye in 1 L ultra-pure water and then diluting it with distilled water to the desired concentration [24]. In all experiments' vessels, the temperature was approximately 28 ± 2 °C, and the pH was adjusted using HCl (0.1 M) and NaOH (0.1 M) solutions.

2.3. Removal Experiments

After one week, the plants were exposed to different concentrations of MB aqueous solution (5, 10, 15, 20, and 25 mg/L) for ten days to identify the most effective concen-

tration for decolourisation. Throughout the trial, the characteristics of the plants were monitored [16]. Experiments were carried out in duplicate, each with one control set. The bioremoval of MB cationic dye was investigated using a UV–Vis spectrophotometer (Shimadzu 1201, Kyoto, Japan) and a photometrical measurement at the range of the MB dye-visible wavelength (660–665 nm) [8,18,20]. It is worth noting that a calibration curve was prepared using a reference water sample and coloured samples (known dye concentrations). This calibration curve was used later in the measurements of the residual dye concentrations.

The experiments on the bioremoval by *Ocimum basilicum* were carried out in a glass container containing 100 mL of dyed solutions that contained different MB dye concentrations (5, 10, 15, 20, and 25 mg/L). The herbs of *Ocimum basilicum* had been exposed to these varying MB dye concentrations for ten days. The herbs' weight used in the bioremoval experiments was 5 g per experiment. To analyse the absorbance value spectrophotometrically, samples were collected every 0, 2, 4, 6, 8, and 10 days. To eliminate undesirable particles before the spectrophotometric analysis, the sample was filtered with 40 µm Wattman filter paper [19,25].

Reference samples were freshly prepared at the same time as the spectroscopic test and disposed of immediately after the test. Reference samples were not kept to be used in the following tests because of the relatively long time between two successive tests (days) to avoid any changes in the properties of the reference samples.

2.4. Calculating the Percentage of Bioremoval

The bioremoval of MB dye from the aqueous solution as a percentage was estimated after application of phytoremediation experiments, using Equation (1):

$$R = \frac{C_1 - C_2}{C_1} \times 100 \quad (1)$$

where R is the percentage of MB dye bioremoval (%), and C_1 and C_2 are the initial and final concentrations of MB dye in the aqueous solution (mg/L), respectively [16,18].

2.5. Analysis of Herbs Growth Rate

The growth profile of *Ocimum basilicum* herbs was assessed by measuring the relative growth rate. The relative growth rate (RGR) was calculated using Equation (2) based on the increasing fresh biomass weight of herbs [16,19]:

$$\mathrm{RGR} = \frac{\ln W_2 - \ln W_1}{T} \quad (2)$$

where RGR is the relative growth rate of a plant (g/day), W_1 and W_2 are the initial and final biomass weights of used herbs, respectively (g), and T is the experiment time (day).

3. Results and Discussion

3.1. The Bioremoval of MB Dye by Ocimum basilicum

In contrast to a control flask, the physical bioremoval of MB dye from an aqueous solution at a concentration of 25 mg/L utilising *Ocimum basilicum* after a 10-day incubation interval is shown in Figure 1. The effectiveness of the bioremoval as a percentage of MB dye at various concentrations and incubation intervals of up to 10 days is shown in Figure 2a–e. After ten days of contact time, the bioremoval of MB dye was 93, 88, 84, 79, and 76% for an MB initial concentration of 5, 10, 15, 20, and 25 mg/L, respectively. These removal efficiencies are satisfactory compared to the results in the literature [19,26]. The effectiveness of the bioremoval of MB dye at a given concentration using sweet basil was related to the contact (experimental) duration between the herbs and the aqueous dye solution. These results were consistent with the findings of the previous studies [19,26]. This may be explained by the fact that extending the exposure period of the dye on herbs increases the likelihood of dye molecules coming in touch with the herb's surface, resulting in a larger

surface area for dye molecule sorption [27]. Figure 2 reveals the residual concentration of MB dye in an aqueous solution and its bioremoval as a percentage. The results shown in Figure 2 indicate that the residual concentration of MB dye in the aqueous solution decreases with an increasing contact time; that is, the residual concentration of MB dye and contact time are inversely proportional.

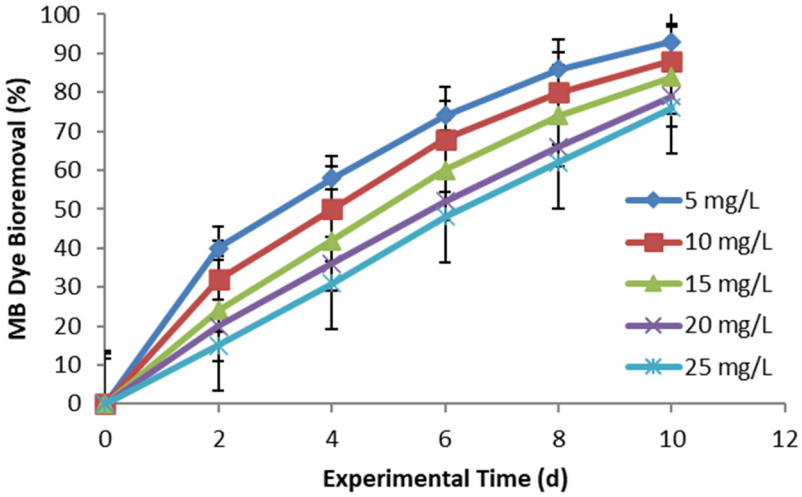

Figure 1. The bioremoval of MB dye as (%) at different concentrations.

The ability of plants, including *Ocimum basilicum*, to accumulate dyes' particles in their bodies could be attributed to the presence of biomolecules, such as carbohydrates, proteins, and coenzymes, in their bodies, which can convert dye molecules into forms that can be easily accumulated in the body of the living cells [28,29].

3.2. The Growth Profile of Ocimum basilicum

Figure 3 shows the relative growth rate (RGR) throughout the 10 day contact time. The RGR was influenced by increasing MB dye concentrations, as seen in Figure 3. The RGR value of sweet basil (*Ocimum basilicum*) in aqueous solutions containing 5, 10, 15, 20, and 25 mg/L MB dye was 2.2, 1.6, 1.1, 0.08, and 0.05 g/day, respectively. Thus, MB dye had a detrimental influence on the RGR of *Ocimum basilicum*, as revealed by the mentioned findings. The plant's growth rate was influenced by the MB dye. As a result, as the dye concentration increased, the RGR value decreased. A number of researchers also noticed the same result [26,27,30].

It is worth noting that the literature indicated that depleted plants and microalgae (after the treatment) could be disposed of in different ways, such as using them as a source of biofuel [31,32].

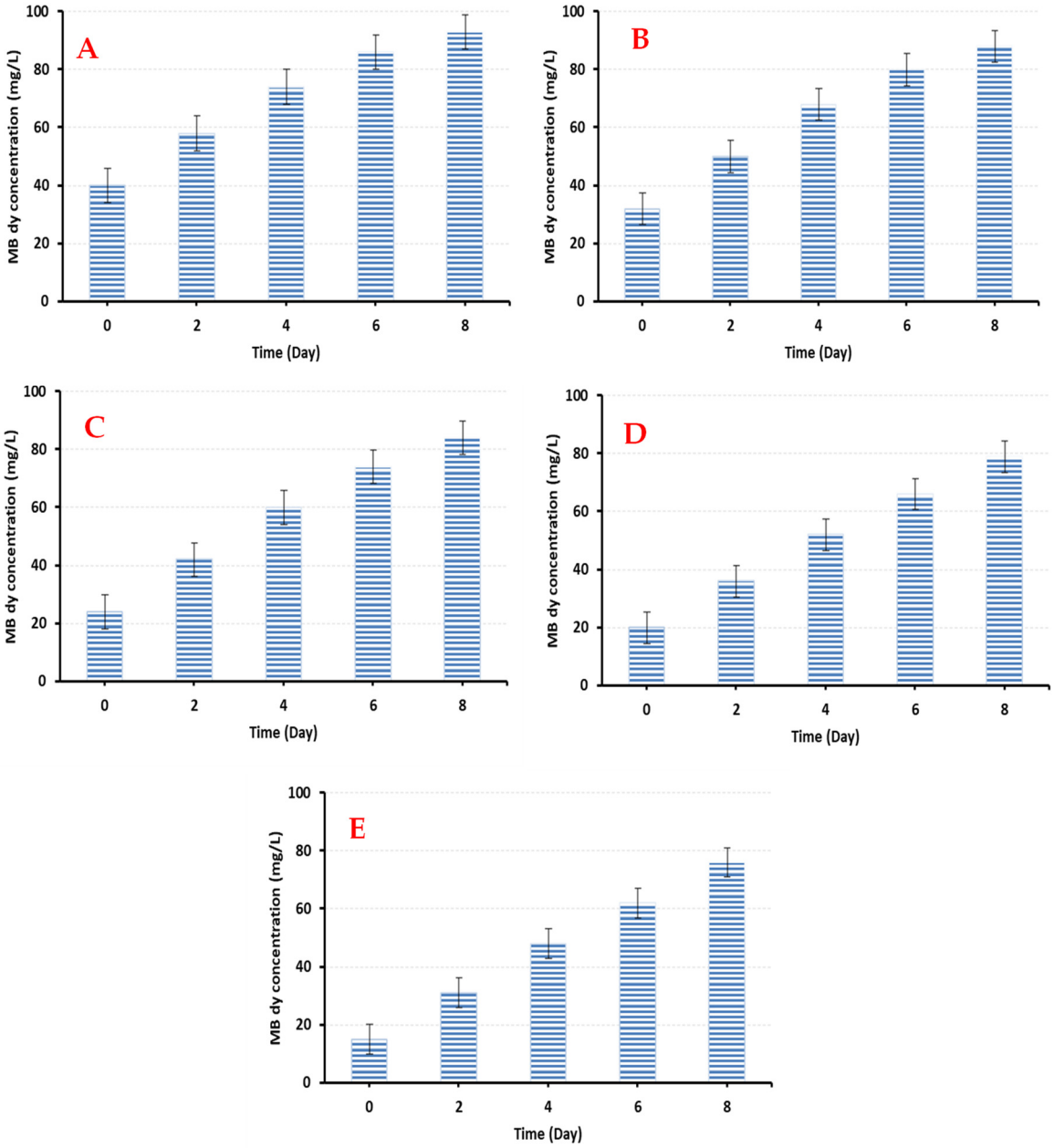

Figure 2. The bioremoval and the residual concentration for different concentrations of MB dye in the aqueous solution; (**A**) 5 mg/L; (**B**) 10 mg/L; (**C**) 15 mg/L; (**D**) 20 mg/L; and (**E**) 25 mg/L.

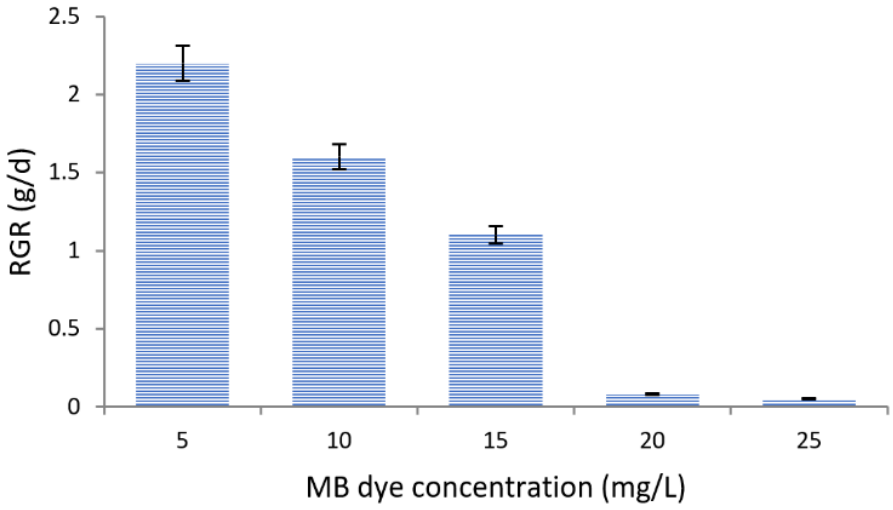

Figure 3. The relative growth rate (RGR) of *Ocimum basilicum* after 10 days of contact time at different concentrations of MB in the aqueous solution.

4. Conclusions

Plants are a cost-effective and environmentally friendly way to break down, absorb, metabolise, and detoxify various pollutants. Sweet basil (*Ocimum basilicum*) was used as a phytoremediation model plant in this study to evaluate the bioremoval of MB dye from an aqueous solution at concentrations of 5, 10, 15, 20, and 25 mg/L. The results revealed that *Ocimum basilicum* had the potential ability to absorb MB dye for a specified limit during a contact time extending to 10 days. The bioremoval of MB dye was 93, 88, 84, 79, and 76% for an MB dye initial concentration of 5, 10, 15, 20, and 25 mg/L, respectively. The MB dye adversely affected the growth rates of basil herbs, according to findings on the relative growth rate (RGR). The results showed that the relative growth rate values declined as the methyl blue dye concentration was increased in the aqueous solution. The RGR for *Ocimum basilicum* decreased from 2.2 to 0.05 g/day as the MB dye concentration increased from 5 to 25 mg/L in the aqueous solution.

For future studies, the authors recommend investigating the ability of *Ocimum basilicum* to remove very small concentrations of MB dye, such as $\leq$1 ppm.

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