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Research Article

Sustainable and Economical Method for Malachite Green Removal via *Aspergillus versicolor* Biodegradation and Phytotoxicity Studies

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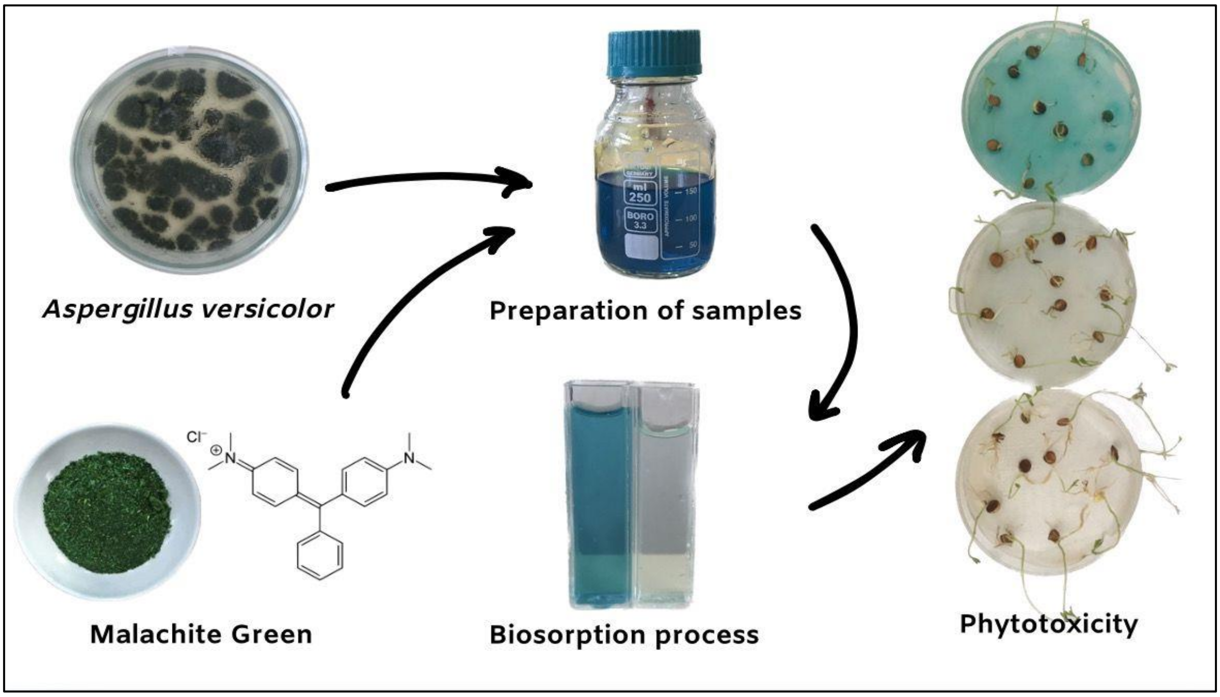
ABSTRACT: The rapid increase in population has accelerated industrial development, making wastewater purification difficult due to dyestuffs. High concentrations of dyes are widely used in the pharmaceutical, food, cosmetics, leather, and especially textile industries. Since dyes are designed to resist environmental factors such as sunlight and moisture, conventional treatment methods are generally inadequate. Among alternative treatment methods, biodegradation is cost-effective and environmentally friendly, and fungi are efficient agents for dye removal. Malachite Green, a trimethylolethane-based dye, is widely used in industry and discharged into wastewater, where it is difficult to remove. As a persistent pollutant, it poses risks to both human health and the ecosystem. In this study, Aspergillus versicolor was employed for the removal of Malachite Green dye. Experiments were conducted by varying temperature, dye concentration, and incubation time. Removal efficiencies of A. versicolor were evaluated daily at 25 °C, 30 °C, and 35 °C using different dye concentrations. The highest removal efficiency (93.12%) was achieved on the 2nd day at 35 °C with a concentration of 50 mg/L. This optimum condition was then used in the phytotoxicity phase of the study. Lens culinaris (green lentil) seeds were selected for the phytotoxicity test to evaluate potential ecological risks. Results indicated that the biosorption process did not show toxic effects, confirming the environmentally safe application of fungal biodegradation for dye removal.

Keywords: *Aspergillus versicolor, Biodegradation, Phytotoxicity, Malachite Green*



INTRODUCTION

The expanding sector and the growing human population today result in many problems, especially environmental pollution. Heavy metal industrial effluents, moreover, are highly hazardous to the biosphere and humans. As industrial manufacturing continues to be a key area, industry initiatives are set towards minimizing waste, decreasing its consequences, and recycling wastewater for reuse (Alkur et al., 2024). Dyes that are a major part of the textile sector are described as having chemical, photochemical, and biochemical degradation, which is impervious to nature. Although, this is the case with the majority, synthetics containing complex aromatic molecular structures are the main reason behind the stability and resistance of biological decomposition to substances. The aquatic systems are being sent out the dyes radiating out of the dam, thereby preventing and even stopping photosynthesis. Hence, the reduction of dissolved oxygen took place which further jeopardized the existence of aquatic creatures (Rezaei, 2014).

The textile industry uses a significant amount of water. Textile dyeing wastewater is one of the most highly polluting industrial wastewater types, accounting for more than 20% of total industrial wastewater (Zeng et al., 2021). Textile industry effluents are characterized by heavy metals, organic matter, turbidity, dissolved salts, and high pH levels. Even at deficient concentrations, the presence of dyes affects water quality and is considered undesirable (Oguntimein, 2015). Therefore, reducing the risks associated with using toxic dyes in the environment has become an important research topic (Yıldırım, 2020).

Other than these, there are many more techniques to depigmentate the colored wastewaters. Some of them are the methods of sedimentation, precipitation, filtration, adsorption, electrochemical processes, ion exchange, membrane separation, and oxidation, and their combinations (Özdoğan and Çelebi, 2018). As with everything good, there are pros and cons to each approach. Even though adsorption methods are focused on in the literature, these adsorbent materials (such as activated carbon) have been used successfully at dye removal from industrial wastewater because of their economic efficiency and effectiveness.

Sorour et al. (2024) centered their study on the adsorptive nature of date seeds, an agricultural waste, for the removal of Malachite Green dye from synthetic wastewater. The capability of the adsorbent to remove dye was found to decrease when operating at solution pH, adsorbent dose, temperature, and the initial dye concentration, with the best results of 98% at pH 5, adsorbent dose 0.1 g, temperature 25°C, and contact time of 30 minutes. Zhang et al. (2017) analyzed hydrochar generated from ABR and PE-ABR with Malachite Green to reveal how adsorption affects them. Among the four isotherm models, including Langmuir, Freundlich, Dubinin-

Radushkevich, and Temkin, the equilibrium was defined at ambient temperature, and one of the dye removal percentages was found to be 78.6% for ABR-hydrochar and 92.4% for PE-ABR after a contact time of 48 hours.

Sartape et al. (2012) tested palm kernel shells for their potential use as a low-cost, abundant, high-efficiency, and environmentally friendly adsorbent to remove Malachite Green dye as a less expensive alternative in the removal of colorants in aqueous solutions. The study investigated the effects of different variables such as dye concentration, pH, contact time, and temperature, amongst others, and the optimal conditions were found. The method was very effective for dye removal with a success rate of 98.87% after 3.30 hours at an initial concentration of 100 mg/L, pH 7-9, and 25°C with shaking at 150 rpm.

El-Bendary et al. (2023) performed a study about color changes and removal as well as degradation of the Malachite Green dye with the use of an isolated Pseudomonas plecoglossicide. Optimum adsorption conditions were determined by changing the parameters such as pH, contact time, and initial dye concentration. The maximum color removal of 91.5% was reached under optimal conditions of pH 6-7, contact time of 96 hours and incubation temperature of 35 degrees Celsius. Kul et al. (2022) reported a study regarding the adsorption process of Municipal Waste Pumice Stones from the Lake Van shores for the removal of Malachite Green. Considering the range of optimised parameters for the current study, one could say that it is possible to ensure an efficient removal of 86% under the following ideal conditions: adsorbent dosage of 0.1 g, contact time of 1 hour, 25 °C temperature, and use of the natural pH of the solution. Özdemir (2019) explored the adsorption of Malachite Green dye from aqueous solutions, employing peanut shells as an adsorbent. The study focused on the effects of dye concentration, temperature, adsorbent amount and equilibrium time. Although the experimental outcomes indicated that the ideal conditions consisted of 0.1 g of adsorbent, 1 hour of contact time, a temperature of 25°C, and the natural pH of the solution, this resulted in a removal efficiency of 86%.

Mat et al. (2018) aimed to remove the Malachite Green dye from aqueous solutions with the use of spent coffee grounds as a low-cost adsorbent. To compare the efficiency of dye removal various adsorption parameters, such as contact time, the initial concentration of a dye and the adsorbent dosage were varied. The findings indicated that as the initial dye concentration increased from 50 mg/L to 250 mg/L, the efficiency of removal diminished. This suggests a complex relationship; however, adsorbent doses ranging from 0.2 g to 1.0 g were found to be directly proportional to the percentage of dye removal. Notably, the maximum removal efficiency achieved was an impressive 99.63% at a temperature of 30°C after a duration of 90 minutes.

Ali et al. (2009) sought to eliminate Malachite Green dye through the utilization of Aspergillus flavus and Alternaria solani. The researchers prepared initial dye concentrations of 10, 20, 30, 40 and 50 µm, which were subsequently inoculated. After a period of six days, it was found that the removal efficiencies peaked at 96.91% for Aspergillus flavus and 97.43% for Alternaria solani when the initial dye concentration was set at 50 µm.

In this study, the researchers recognized the limitations (and high costs) associated with traditional methods of dye removal from wastewater. Consequently, they employed a biodegradation approach to eradicate Malachite Green (MG) dye using A. versicolor. A toxicity assessment was also conducted, because this alternative method may offer a more sustainable solution.

2. MATERIAL AND METHODS

2.1 Materials

In the investigation of biosorption utilizing live *A. versicolor*, the mold was inoculated onto Potato Dextrose Agar (PDA) medium, subsequently incubating at a temperature of 30°C for a duration of 7 days. During the biosorption process involving Malachite Green dye, solutions with various concentrations were meticulously prepared. These solutions were then analyzed through a UV spectrophotometer (Shimadzu UV-2600) at a wavelength of 617 nm, thereby facilitating precise measurements.

2.2 Experiments

The experimental workflow unfolds as follows

1- Four distinct dye concentrations (10 mg/L, 25 mg/L, 50 mg/L and 100 mg/L) were prepared in 50 mL flasks.

2- To ascertain the contact time, five aliquots of each dye concentration, supplemented with the biosorbent, were subjected to a shaking incubator operating at a stirring speed of 125 rpm. Daily measurements of the solutions were conducted to compute the percentage of dye removal efficiency.

3- For the temperature parameter, this process was replicated at temperatures of 25°C, 30°C and 35°C over one-week intervals.

4- Based on these parameters, a phytotoxicity study was executed to ascertain the optimum values derived.

The removal efficiency was calculated with the equation in **Eqn. 1**.

$$\% = \frac{(C_0 - C_e)}{C_0} \times 100 \qquad \textbf{Eqn. 1}$$

Where;

C_0, Initial concentration of dye (mg/L)

C_e, It is the dye concentration measured after biosorption (mg/L).

2.3 Toxicity tests

Toxicity tests were conducted using *Lens culinaris* (green lentil) to determine the effectiveness of biosorption treatments. *L. culinaris* was germinated in control water (tap water), a 50 mg/L solution of Malachite Green dye, and following the biosorption treatment with Malachite Green. The *Lens culinaris* seeds were first sterilized in bleach for one minute, then rinsed in sterile water, and then placed in sterile water for a total of ten minutes. Ten green lentil seeds were placed between filter papers in sterile Petri dishes and wetted with 10 mL of sterile water for planting. The seeds were watered with 5 mL of control water (tap water), a 50 mg/L concentration of Malachite Green dye (control), and 50 mg/L Malachite Green after the biosorption process (sample) for 7 days, after which seed germination was measured.

Germination percentage (**Eqn. 2)** was determined with the measurement of shoot and root lengths of the seeds (Doğaroğlu, 2018). **Eqn. 3** and **Eqn. 4**, on the other hand, give the formula for determining relative growth index (RGI), and growth index (GI) respectively (Sadıç, 2023).

$$(\%)\ \text{Germination} = \frac{\text{Number of seeds germinated}}{\text{Number of seeds planted}} \times 100 \qquad \textbf{Eqn. 2}$$

$$\textbf{RGI} = \textbf{RLS}/\textbf{RLC} \qquad \textbf{Eqn. 3}$$

Where;

RLS: root length of sample (positive and other concentration groups)

RLC: root length of control

$$\textbf{GI} = (\textbf{RLS}/\textbf{GSS})/(\textbf{RLCxGSC})\textbf{x100} \qquad \textbf{Eqn. 4}$$

GSS: is the number of germinated seeds in the standard solution.

GSC: is the number of germinated seeds in the control.

3. RESULTS AND DISCUSSION

In this study, the biosorption of Malachite Green dye, which is difficult to treat and costly to remove from wastewater, particularly in the textile industry, was investigated using *A. versicolor* fungus. The experimental process was designed by evaluating the parameters that affect biosorption efficiency, such as the initial dye concentration, temperature, and time.

3.1 Biodegradation results

To examine the effect of the initial dye concentration on biosorption, four different initial concentrations which are 10 mg/L, 25 mg/L, 50 mg/L, and 100 mg/L were tested. The study was conducted at temperatures of 25°C, 30°C, and 35°C. The comparison of removal efficiencies at different temperatures relative to concentration is shown in **Figure 1-3**.

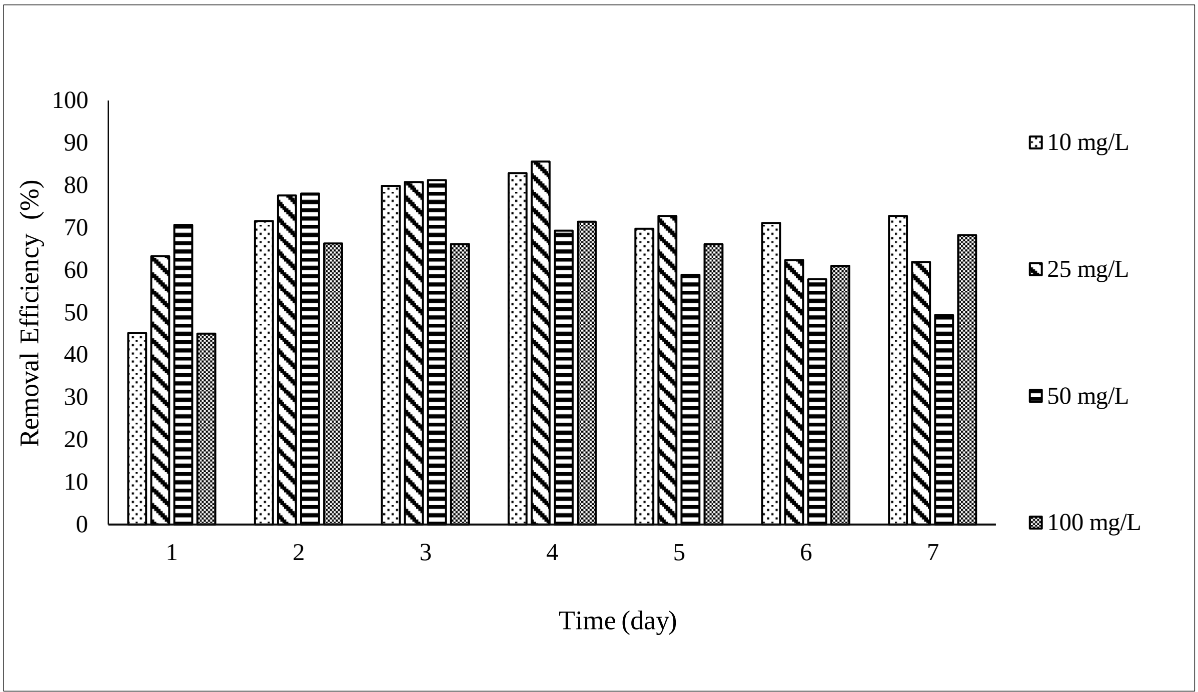


Figure 1. Removal efficiency in 7 days of biosorption at 25 °C

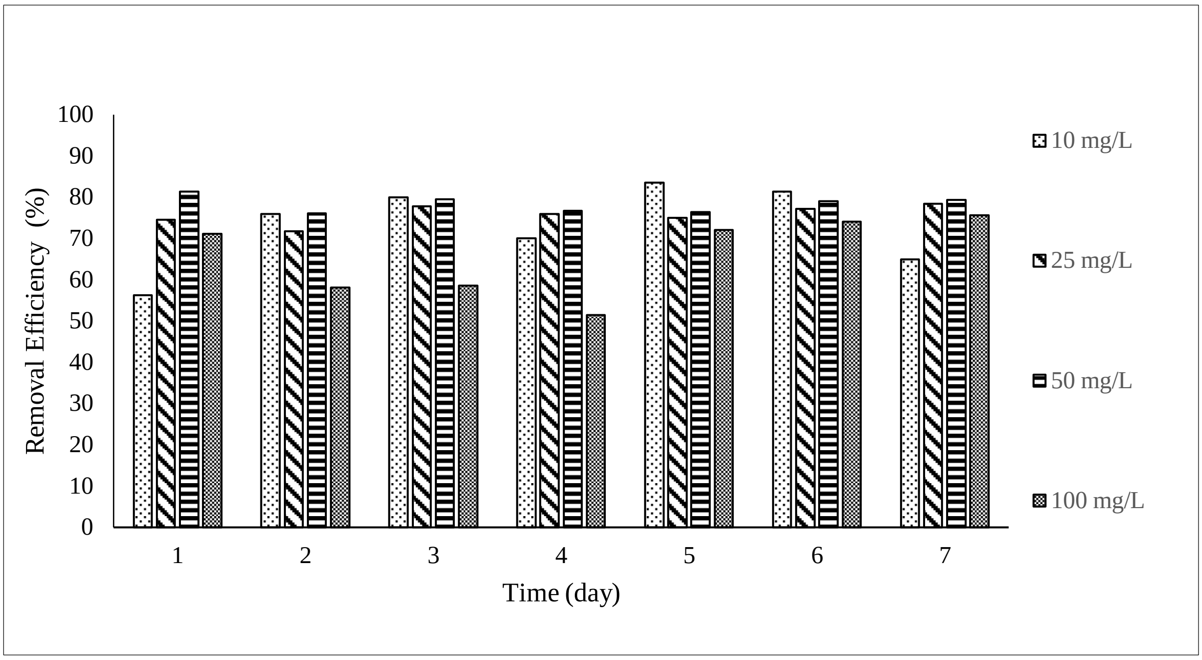


Figure 2. Removal efficiency in 7 days of biosorption at 30 °C

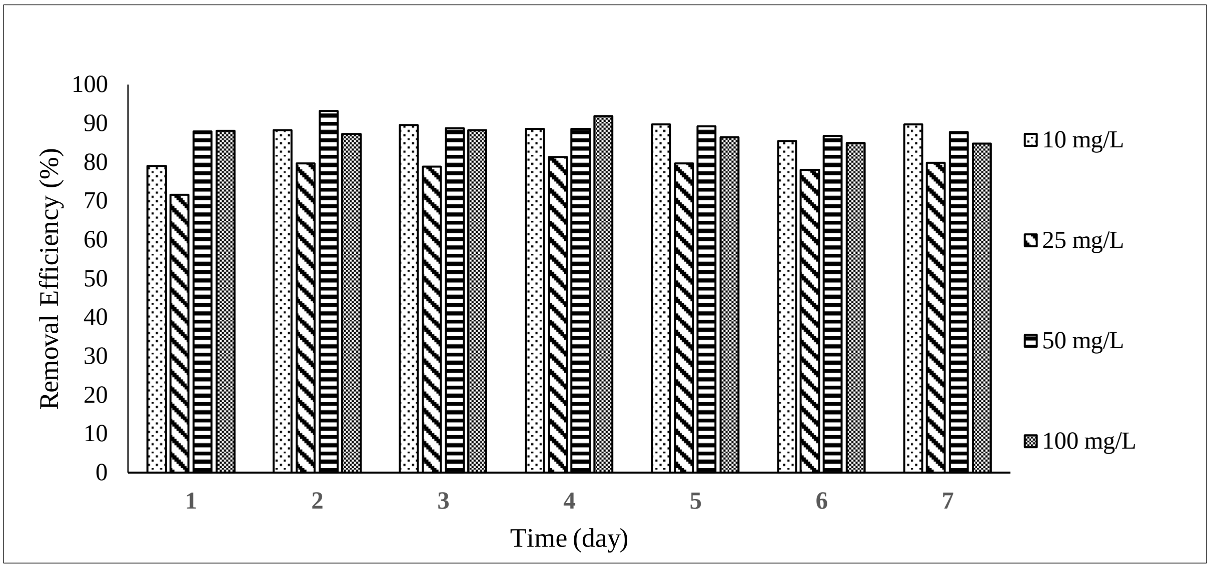


Figure 3. Removal efficiency in 7 days of biosorption at 35 °C

The comparison of removal efficiencies at different concentration values at 25°C, 30°C, and 35°C are shown in **Figure 4-7.**

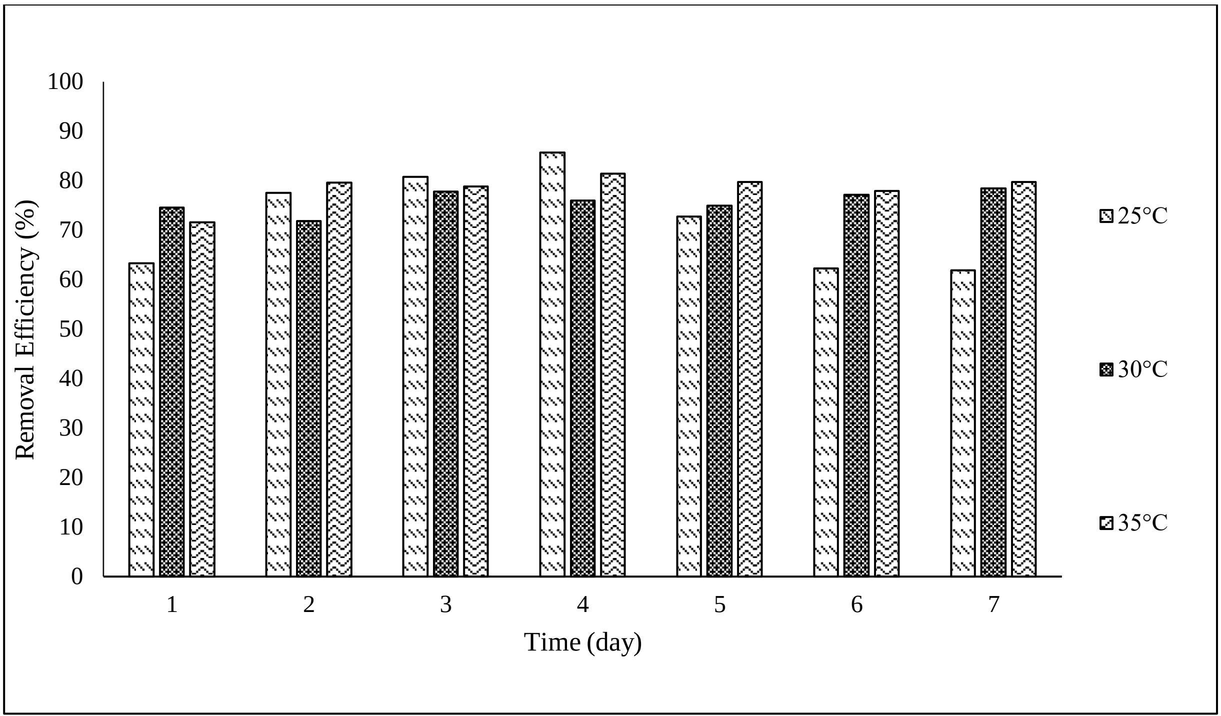


Figure 3. Biosorption process removal results at different temperatures for 25 mg/L

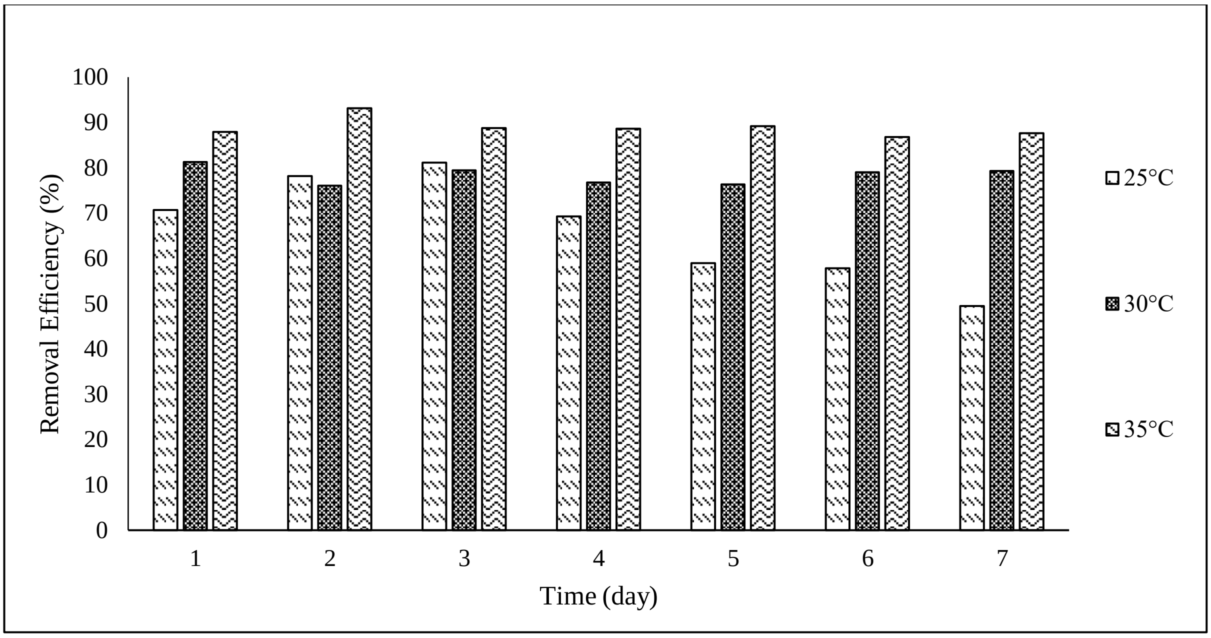


Figure 4. Biosorption process removal results at different temperatures for 50 mg/L

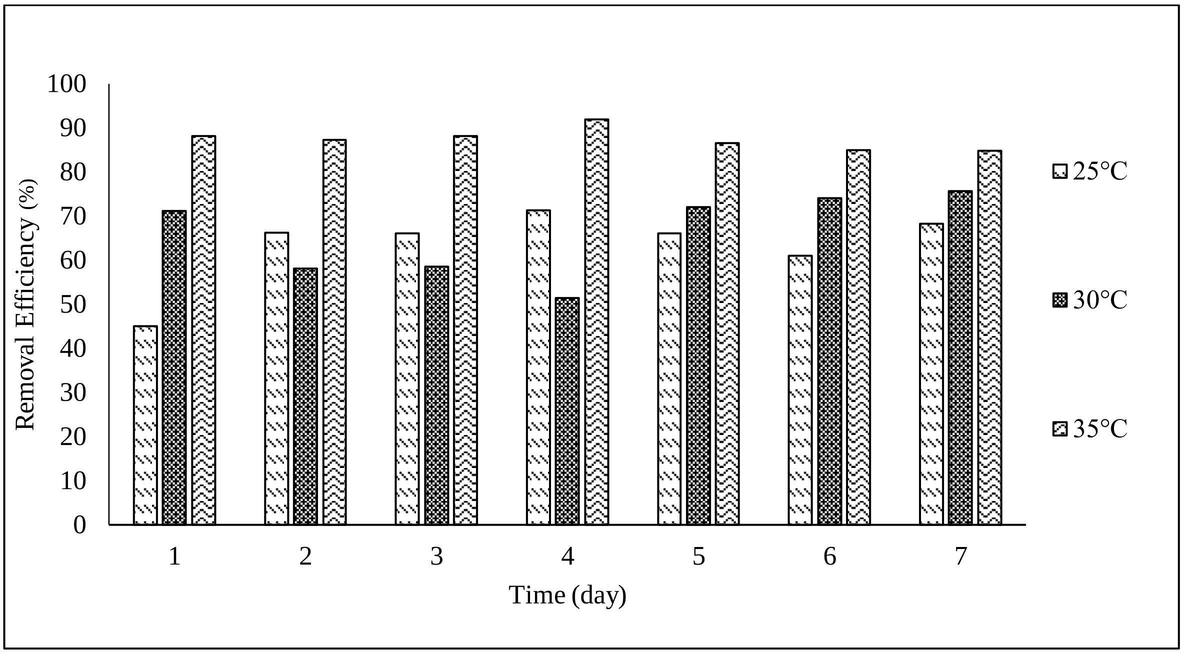


Figure 5. Biosorption process removal results at different temperatures for 100 mg/L

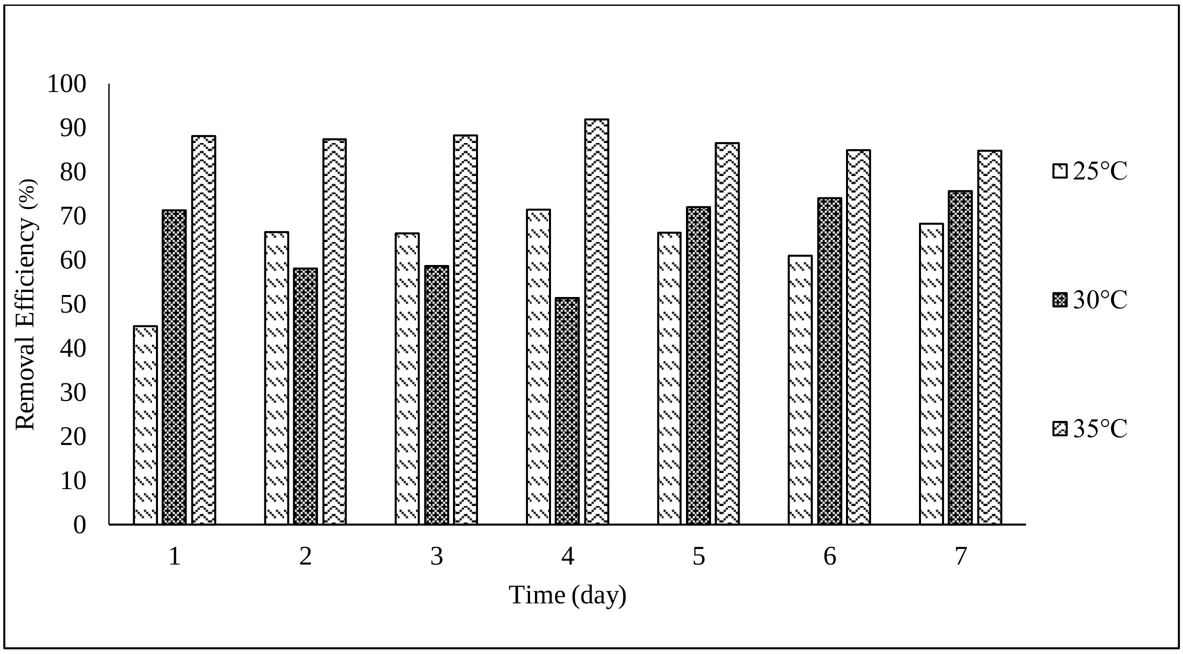


Figure 7. Biosorption process removal results at different temperatures for 100 mg/L

When the biosorption results were analyzed, the highest removal efficiency for the 10 mg/L dye concentration was found to be 89.71% at 35 °C on the 7th day. For the 25 mg/L dye concentration, the highest removal efficiency was 85.65% at 25 °C on the 4th day. For the 50 mg/L dye concentration, the highest removal efficiency was 93.12% at 35 °C on the 2nd day, and for the 100 mg/L dye concentration, the highest removal efficiency was 91.91% at 35 °C on the 4th day.

Measurements of the samples in which biosorbent was added were made in duplicate, and the findings were evaluated by determining the arithmetic mean. Consequent to the calculations, another approach to deal with dye pollution of wastewater was assessed. The maximum removal efficiency of treating Malachite Green by *A. versicolor* was achieved on the second day, namely, at 35 °C and the initial concentration of dye 50 mg/L, the removal efficiency reached 93.12% (**Table 1**).

Table 1. Removal efficiencies of adsorbent/biosorbent on MG at various temperatures and times

Adsorbent/Biosorbent	Temperature (°C)	Duration (minutes)	Removal Efficiency (%)	References
Palm kernel	25	30	98	(Sorour *et al.* 2024)
Hydrocar-ABR	25	2880	78.6	(Zhang *et al.* 2017)
Hydrocar- PE-ABR	25	2880	92.4	(Zhang *et al.* 2017)
Elephant Apple Peel	25	210	98.87	(Sartape *et al.* 2012)
P. plecoglossicide	35	5760	91.5	(El-Bendary *et al.* 2023)
Van Pumice	45	90	68.07	(Kul *et al.* 2022)
Peanut Shell	25	60	86	(Özdemir, 2019)
Coffee grouds	30	90	99.63	(Mat *et al.* 2018)
Aspergillus flavus	37.5	8640	97.43	(Ali *et al.* 2009)
Alternaria solani	37.5	8640	96.91	(Ali *et al.* 2009)
Aspergillus versicolor	35	2880	93.12	Our study

3.2 Phytotoxicity results

To evaluate the toxic properties of Malachite Green dye, *L. culinaris* (green lentil) seeds were used. *L. culinaris* seeds were germinated in three different conditions: 50 mg/L concentration of Malachite Green dye (blank), tap water (control), and Malachite Green after biosorption treatment (sample). The seeds were observed at the end of the 7th day, as shown in **Figure 8**.

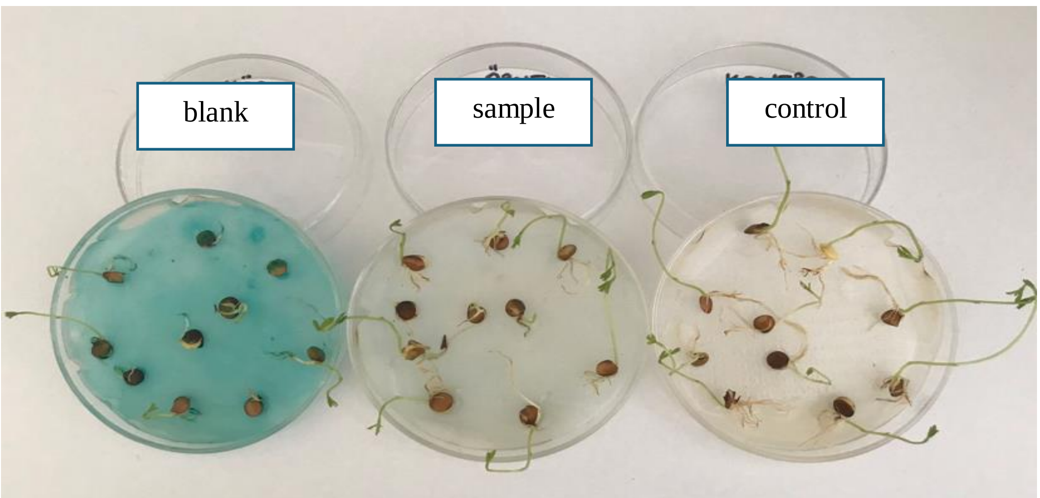


Figure 8. *Lens culinaris* (green lentil) phytotoxicity study

The germination rate, shoot, and root lengths obtained from *L. culinaris* (green lentil) seeds are presented in **Table 2**. The comparison of the root and shoot lengths of the seeds is shown in **Figure 9**.

Table 2. Toxicity results of Malachite Green on *Lens culinaris* (green lentil) seeds

	Tap water (control)	50 mg/L MG after biosorption (sample)	50 mg/L MG (blank)
Germination (%)	100%	100%	90%
Shoots (cm) ± SD	5.8 ± 1,31	4.8 ± 2.5	1.77 ± 1.66
Roots (cm) ± SD	1.47 ± 0.67	1.55 ± 0.8	0.72 ± 0.6

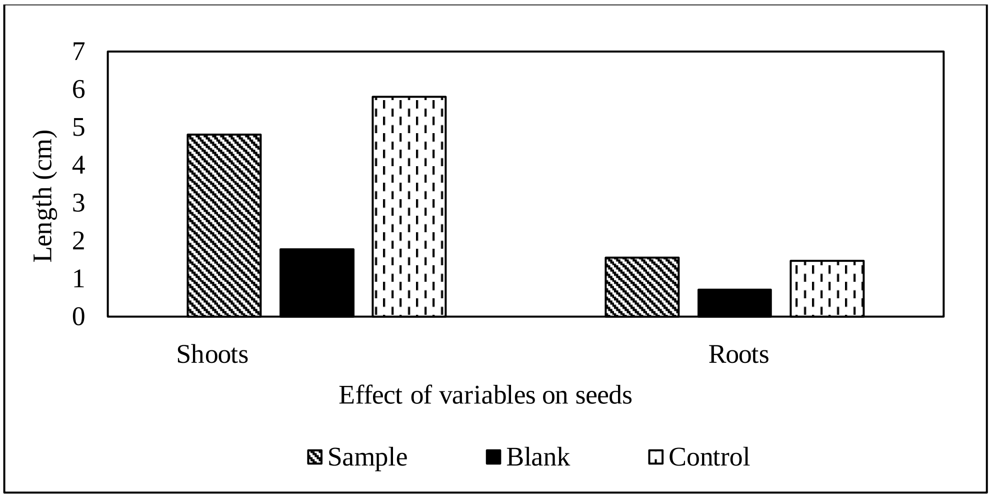


Figure 9. Root and shoot lengths of *Lens culinaris* (green lentil) seeds

At the end of the seventh day of germination, the following percentages of germination were observed: 90% for seeds treated with a concentration of 50 mg/L Malachite Green dye (blank), 100% for seeds irrigated with tap water (control), and 100% for seeds irrigated with the solution obtained from the biosorption of Malachite Green dye (sample).When the root and shoot lengths of germinated *L. culinaris* seeds were measured, the results were as follows: the shoot lengths of seeds irrigated with 50 mg/L concentration of Malachite Green, the solution obtained after the biosorption process, and tap water (control) were 1.77 cm, 4.8 cm, and 5.2 cm, respectively. The root lengths for the same groups were 0.72 cm, 1.55 cm, and 1.47 cm, respectively.

The relative growth index (RGI) of seeds irrigated with Malachite Green dye (control) was determined to be 0.46, while the RGI of seeds irrigated with Malachite Green (sample) was measured as 1.05.

The Growth Index (GI) calculations revealed that the GI value for seeds irrigated with Malachite Green dye (blank) was 0.52, while the GI value for seeds irrigated with Malachite

Green (sample) was 1.05. RGI values are categorized based on the observed toxicity effects as follows:

Inhibition of root growth (I): $0.0 < x \leq 0.8$
No significant effect (NSE): $0.8 < x \leq 1.2$
Stimulation of root growth (S): $x > 1.2$

At the end of the biosorption process, it was observed that the growth of the seeds irrigated with the resulting solution and with tap water (control) showed similar characteristics. In addition, it was observed that the seeds germinated with Malachite Green dye lagged in growth compared to the other seeds.

4. CONCLUSION

This study investigated the possibility of using *A. versicolor* as a biosorbent for the removal of the pollutant and carcinogenic dye Malachite Green from wastewater, as well as its biosorption in aqueous media. Finding the optimum conditions for the experiments to achieve the highest biosorption efficiency is the most critical point.

The parameters that were studied in this work are temperature, time, and dye concentration. The effect of temperature on biosorption was studied at 25 °C, 30 °C and 35 °C. In the case of living *A. versicolor*, the initial dye concentrations of 10 mg/L, 25 mg/L, 50 mg/L, and 100 mg/L were investigated. Each at three temperatures with five circles of biosorbent added to each sample. The samples were put in an agitating incubator with an agitation speed of 125 revolutions per minute and incubated for seven days, during which daily measurements were made to determine the percentage removal efficiency.

According to the findings of our study, the use of *A. versicolor* biosorbent applied to a 35°C adsorption process resulted in an above-average removal efficiency of 93.12% which is a strong performance. These results are consistent with other studies in literature, for example, the use of date seeds and hydrocar-PE-ABR compounds which reported almost similar or slightly higher efficiencies. Because of the clear environmental benefits, the use of biosorbents, particularly *A. versicolor*, can be noted to be effective even with colder temperatures and prolonged processing times. There may also be further enhancement in the removal efficiency with optimal conditions of temperature and time. To summarize, our biosorbent comes as a viable option capable of functioning in eco-friendly and sustainable contexts, outlining the overreliance on conventional adsorbents with minimal benefits.

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Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship Contribution Statement

Semra Malkoç: Conceptualization, Supervision, Data analysis, Draft preparation, Revision, Editing.

Aslı Çuhadaroğlu: Data curation and Investigation, Data analysis, Draft preparation.

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