Evaluation of Dye Decolorization Potential of Laccase Producing *Trichodermaharzianum*DBS-1 and *Trichodermaviridae*DBS-2

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Abstract

Background: The presence of dye-containing effluents in water bodies and soil shows significant health risks to humans, plants, and animals. These adverse effects necessitate the search for safe, cost-effective, and environmentally friendly methods to remove dyes from these environments. This study aims to evaluate the dye decolourization potential of laccase-producing fungi, specifically *Trichoderma harzianum* DBS-1 and Trichoderma viridae DBS-2. Methods: Laccase-producing fungal isolates, *T. harzianum* DBS-1 and *T. viridae* DBS-2, were obtained from the laboratory at the Department of Microbiology, Ahmadu Bello University (ABU), Zaria, and stored on PDA slants. The isolates were screened for their ability to decolourize three dyes—Blue H3R, Yellow FG, and Red 3B—at concentrations of 50 ppm and 100 ppm. The fungi were inoculated into Malt Extract Broth containing each dye at the specified concentrations. Dye decolourization was assessed by measuring absorbance at 450 nm after 3, 6, and 9 days of incubation. Results: The results indicated that the fungal isolates could decolourize the dyes at varying rates. The highest decolourization was observed for Red 3B, with *T. viridae* DBS-2 achieving 71.32% decolourization at 50 ppm and 50.33% at 100 ppm after 9 days of incubation. Conversely, the lowest decolourization was recorded for Yellow FG by *T. harzianum* DBS-1, with 30.34% at 50 ppm and 20.87% at 100 ppm after 9 days. Blue H3R and Red 3B exhibited higher decolourization percentages compared to Yellow FG. Conclusion: The study demonstrates that laccase-producing isolates *T. harzianum* DBS-1 and *T. viridae* DBS-2 have significant potential for dye decolourization, particularly for Blue H3R and Red 3B dyes. These findings suggest that these fungal isolates could be employed as an effective, eco-friendly solution for the bioremediation of dye-contaminated environments.

Keywords: Dye, Laccase. Trichoderma, Decolourization, Environmental remediation.

Significance | Effective dye decolorization by laccase-producing fungi offers eco-friendly solutions to mitigate environmental pollution from industrial effluents.

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Introduction

Laccases are copper-containing polyphenol oxidases with the remarkable ability to degrade a wide variety of substrates, including lignin, phenolic compounds, and non-phenolic substances. These enzymes are found in various organisms, including bacteria, fungi, and insects, but fungi are the primary and most efficient producers of laccases (Perinbam, Bharath, Seeni, & Ravikumar, 2017). Over 60 fungal strains, particularly from the Ascomycetes, Deuteromycetes, and Basidiomycetes classes, have been identified as prolific laccase producers, making them crucial for biotechnological applications (Roseline & Joel, 2014; Shekher, Sehgal, Kamthania, & Kumar, 2011). Globally, the dyeing and printing industries rely on more than 10,000 different dyes and pigments, contributing to an estimated 800,000 tons of dye production annually. Unfortunately, a significant portion of these dyes, at least 10%, enters the environment through improper waste disposal practices, leading to

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substantial pollution concerns (Kowsalya, 2014). Synthetic dyes, commonly found in effluents from textile, paper, pulp, leather tanning, food processing, cosmetic, rubber, plastic, printing, and dyeing industries, pose a severe threat to aquatic ecosystems due to their high visibility, persistence, and toxicity (Camila et al., 2017). These dyes can persist in the environment for extended periods, particularly when not properly treated, further exacerbating environmental degradation (Zubbair, Ajao, Adeyemo, & Adeniyi, 2018).

The presence of synthetic dyes in water bodies, even in minimal concentrations, can significantly impact the aquatic environment. These dyes reduce the aesthetic quality of water, decrease transparency, and hinder gas solubility. Moreover, they block sunlight penetration, thereby impeding photosynthesis, which is vital for the growth and development of aquatic plants (Olumide, Aliu, Ayodeji, & Akinniyi, 2013). Beyond environmental concerns, these dyes pose serious health risks to humans and aquatic life. They are known to be carcinogenic, mutagenic, and teratogenic, leading to severe health issues such as cardiac failure, renal problems, skin irritation, allergies, and hepatic dysfunction (Camila et al., 2017; Zubbair et al., 2018).

Given the persistent nature of these pollutants and the limitations of conventional treatment methods, alternative approaches for their removal are essential. Laccases have gained significant attention for their ability to oxidize recalcitrant environmental pollutants, making them valuable in various biotechnological processes. They have been successfully employed in soil bioremediation, the biodegradation of phenolic pollutants, and the removal of endocrine disruptors (Adiveppa & Basappa, 2015). Fungi, particularly species of Trichoderma, are among the most efficient ligninolytic organisms. They have demonstrated the capability to degrade a wide range of dyes, including azo, heterocyclic, reactive, and polymeric dyes, making them ideal candidates for dye decolorization (Geethanjali, Gowtham, & Jayashankar, 2020).

The effectiveness of laccases in dye decolorization is due to their extracellular, non-specific, and non-stereo-selective enzyme system, which allows them to target a broad spectrum of dye molecules. Unlike traditional physicochemical methods of dye removal, such as coagulation, flocculation, adsorption, ion exchange, flotation, and membrane separation, enzymatic decolorization using laccase-producing fungi offers a cost-effective, eco-friendly, and efficient alternative (Abdulredha, 2013). Laccases not only degrade lignocellulosic biomass but also target biodegradation-resistant and toxic compounds, making them a promising solution for environmental remediation.

This study aims to evaluate the dye decolorization potential of laccase-producing *Trichoderma harzianum* and T. viridae, highlighting their potential as sustainable and effective agents in mitigating the environmental impact of synthetic dyes.

Materials and Methods

Collection and Reconfirmation of Fungal Isolates

The fungal isolates *Trichoderma harzianum* DBS-1 and Trichoderma viride DBS-2 used in this study were sourced from the Department of Microbiology, Faculty of Life Sciences, Ahmadu Bello University, Zaria, Nigeria. Upon collection, the isolates were stored on Potato Dextrose Agar (PDA) slants and maintained at room temperature to preserve their viability. To confirm the identity of these fungal isolates, both macroscopic and microscopic characteristics were assessed. The macroscopic examination involved observing colony morphology, color, texture, and growth pattern on PDA plates (Vantamuri & Kaliwal, 2015). Microscopic characteristics were further examined using light microscopy, focusing on the structure and arrangement of conidia, conidiophores, and hyphae (Forootanfar, Moezzi, Aghaie-Khozani, Mahmoudjanlou, Ameri, Niknejad, & Faramarzi, 2012). These observations were cross-referenced with a standard mycological atlas to ensure accurate identification and classification of the isolates (Adiveppa & Basappa, 2015).

Reconfirmation of Laccase Production Ability

The laccase-producing capability of the isolates *T. harzianum* DBS-1 and T. viride DBS-2 was reconfirmed using a tannic acid assay. For this purpose, mycelium from each fungal isolate was aseptically transferred onto PDA plates supplemented with 0.5% tannic acid. The plates were incubated at room temperature (approximately 25-28°C) for seven days. Laccase production was indicated by the formation of a reddish-brown halo around the fungal colonies, a result of the oxidation of tannic acid by the secreted laccase enzyme (Sahay, Yadav, & Yadav, 2009). The diameter of the halo was measured and recorded as a qualitative indicator of laccase activity (Camila, Johnatan, Eduardo, Roselei, Marli, & Aldo, 2017).

Determination of Percentage Dye Decolorization

The dye decolorization ability of the laccase-producing isolates was assessed using a liquid culture system. Malt Extract Broth (MEB) was prepared and supplemented with different synthetic dyes at concentrations of 100 ppm and 50 ppm. Each fungal isolate was inoculated into the MEB containing the dyes in duplicate to ensure reproducibility. The cultures were then incubated at room temperature under static conditions for up to nine days. Absorbance readings of the cultures were taken at 450 nm using a UV-Vis spectrophotometer at four time points: Day 0 (serving as the control), Day 3, Day 6, and Day 9. These readings were used to determine the extent of dye decolorization over time (Szabo, Csiszar, Toth, Szakacs, & Koczka, 2015).

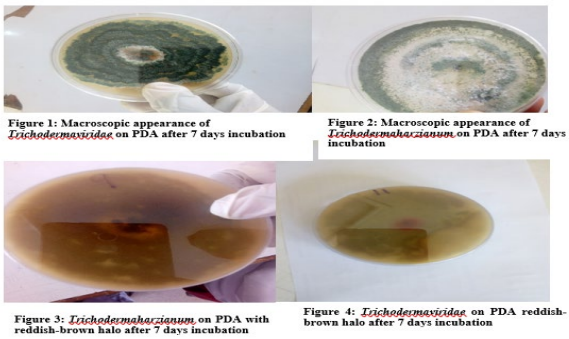

Figure 1: Macroscopic appearance of *Trichodermaviridae* on PDA after 7 days incubation

Figure 2: Macroscopic appearance of *Trichodermaharzianum* on PDA after 7 days incubation

Figure 3: *Trichodermaharzianum* on PDA with reddish-brown halo after 7 days incubation

Figure 4: *Trichodermaviridae* on PDA reddish-brown halo after 7 days incubation

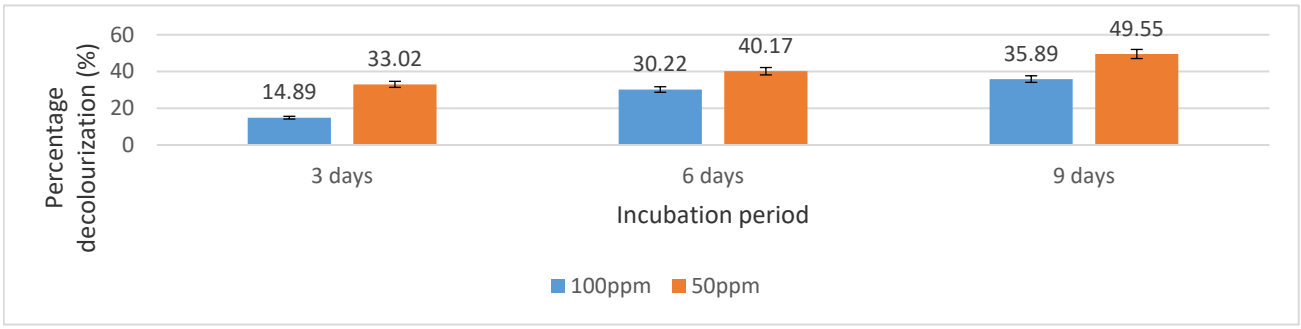

Figure 5. Percentages decolourization of Blue H3R dye by *Trichodermaharzianum*

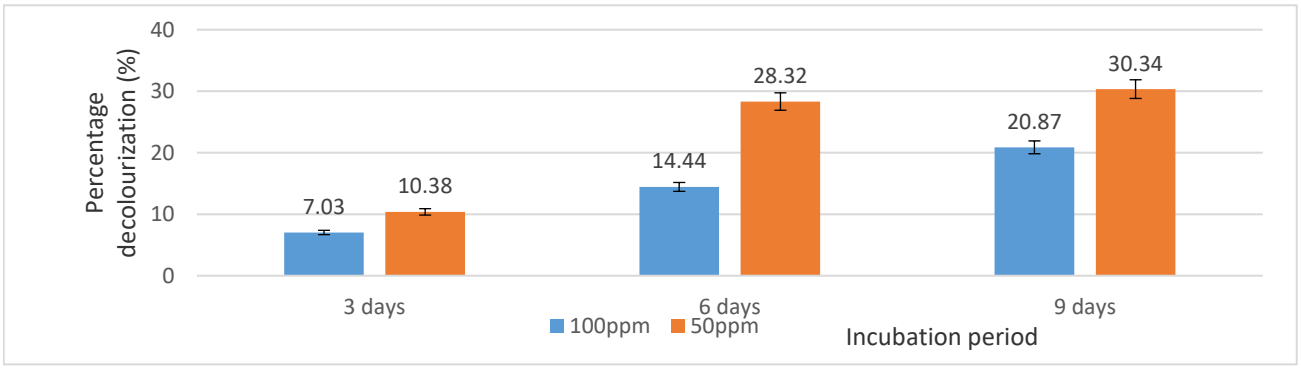

Figure 6. Percentages decolourization of yellow FG dye by *Trichodermaharzianum*

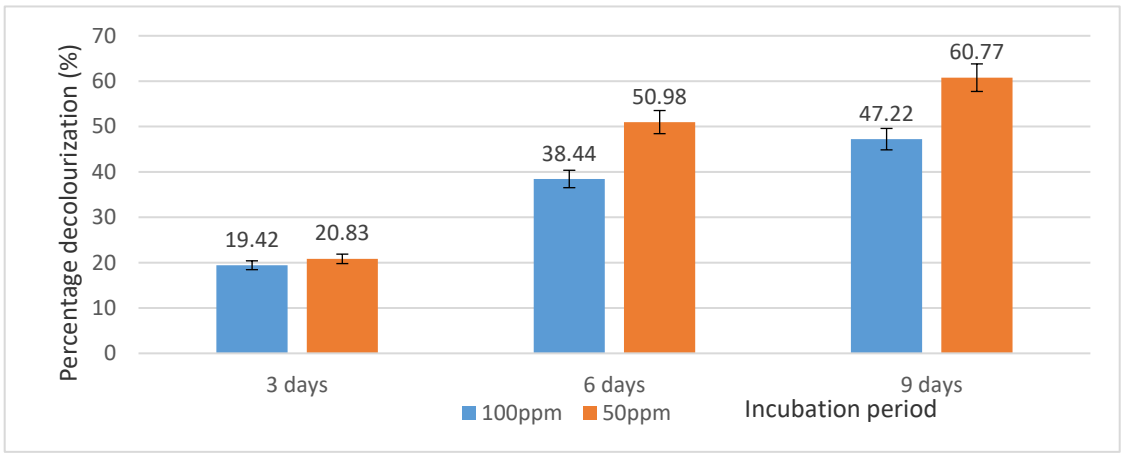

Figure 7. Percentages decolorization of Red 3B dye by *Trichodermaharzianum*

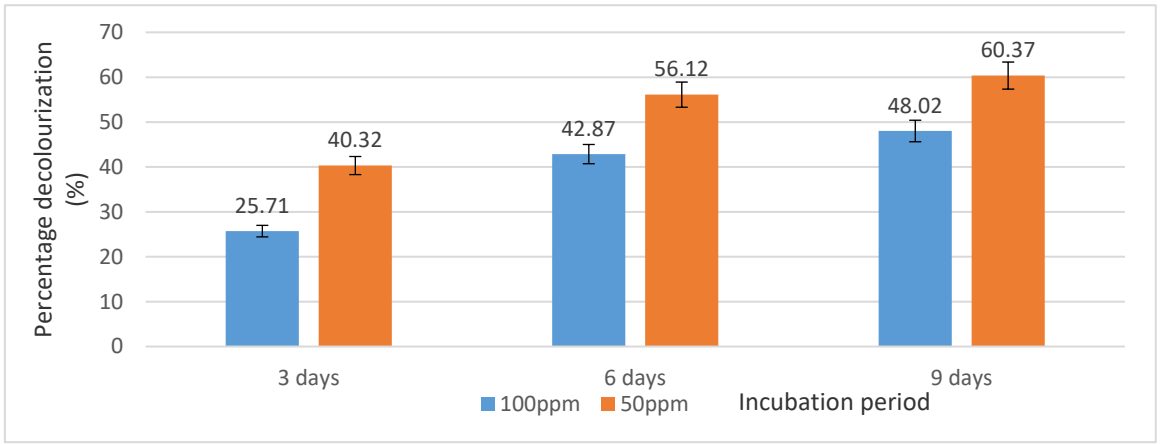

Figure 8. Percentages decolourization of Blue H3R dye by *Trichodermaviridae*

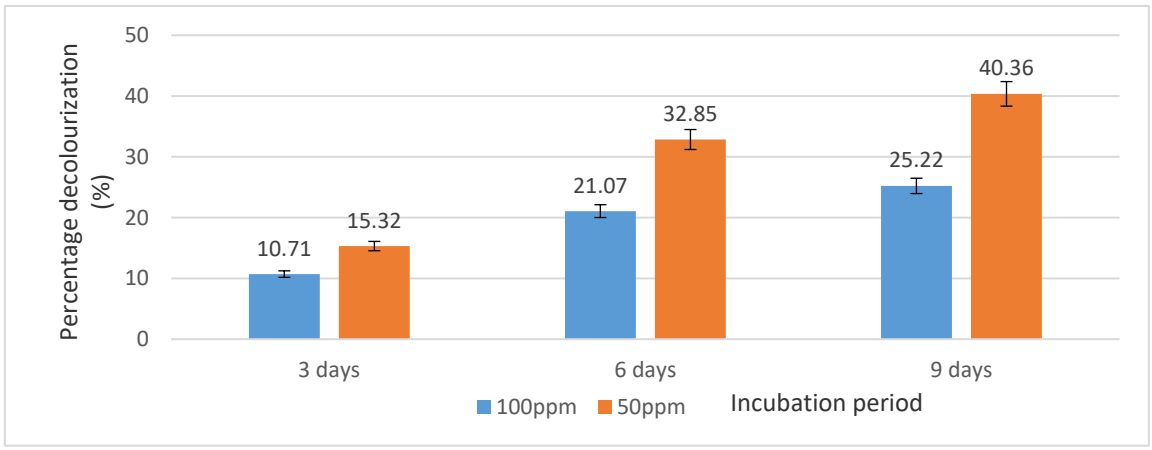

Figure 9. Percentages decolourization of yellow FG dye by *Trichodermaviridae*

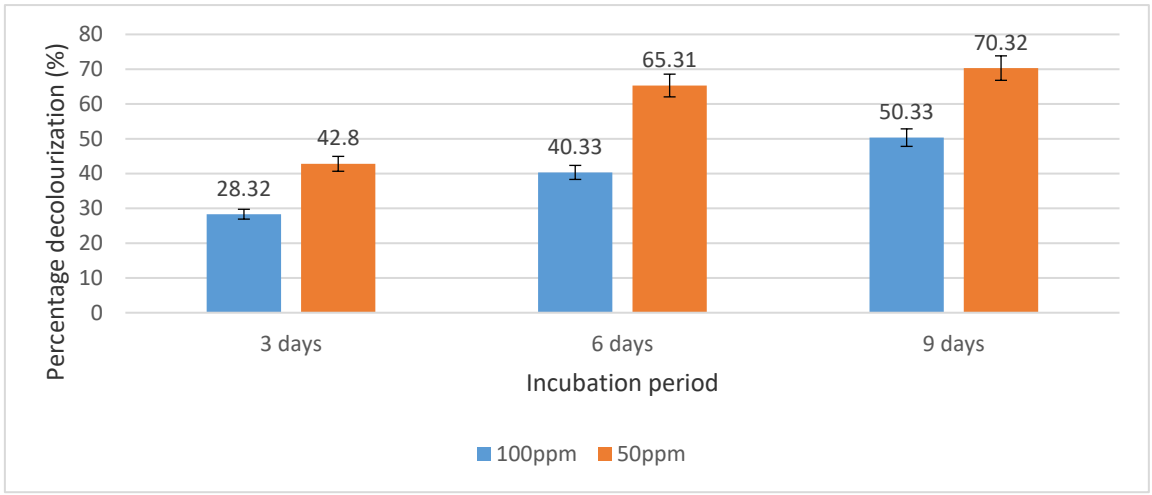

Figure 10. Percentages decolourization of Red 3B dye by *Trichoderma viridae*

This calculation provided a quantitative measure of the laccase-mediated dye degradation (Zhuo et al., 2011). To evaluate the significance of the combined effects of incubation time and dye concentration on the decolorization efficiency, the experimental data were subjected to a two-way Analysis of Variance (ANOVA). A significance level of 0.05 was applied to determine the statistical relevance of the results. All statistical analyses were performed using appropriate software tools, ensuring robust and reliable data interpretation (Wuyep et al., 2012).

Results and Discussion

The identity of the fungal isolates was confirmed, as was their laccase production ability (Risdianto, Sofianti, Suhardi, & Setiadi, 2012). Figures 1 and 2 show the macroscopic characteristics of the fungal isolates. The result of screening for laccase production is shown in Figures 3 and 4.

The findings of this study show that the three dyes (Blue H3R, Yellow FG, and Red 3B) were decolorized by the two laccase-producing fungal isolates used in this study—*Trichoderma harzianum* DSB-1 and Trichoderma viride DSB-2—at different rates (Vantamuri & Kaliwal, 2015). Mean decolorization percentages of Blue H3R dye, Yellow FG, and Red 3B by *Trichoderma harzianum* DSB-1 after 3 days, 6 days, and 9 days of incubation are presented in Figures 5, 6, and 7, respectively. An increase in percentage decolorization was observed with an increase in the incubation period for all the dyes. Higher decolorization percentages were observed at 50 ppm compared to 100 ppm across the three dyes (Sahay, Yadav, & Yadav, 2009). The least decolorized dye by *Trichoderma harzianum* DSB-1 was Yellow FG. The independent effects of dye concentration and incubation period on percentage decolorization were statistically significant ($p < 0.05$) for Blue H3R and Red 3B, but not statistically significant ($p > 0.05$) for Yellow FG (Szabo, Csiszar, Toth, Szakacs, & Koczka, 2015).

Figures 8, 9, and 10 show the mean decolorization percentages of Blue H3R dye, Yellow FG, and Red 3B by Trichoderma viride DSB-2, respectively, after 3 days, 6 days, and 9 days of incubation. The percentage decolorization of the dyes was found to increase with an increase in incubation period (Wakil, Eyiolawi, Salawu, & Onilude, 2019). Lower dye concentration (50 ppm) had higher decolorization percentages across the dyes. The least decolorized dye by Trichoderma viride DSB-2 was Yellow FG (Saqib et al., 2015). As with *Trichoderma harzianum* DSB-1, the independent effects of dye concentration and incubation period on percentage decolorization by Trichoderma viride DSB-2 were statistically significant ($p < 0.05$) for Blue H3R and Red 3B, while these effects were not statistically significant ($p > 0.05$) for Yellow FG (Toca-Herrera, Osma, & Couto, 2007).

The dye with the highest mean percentage decolorization was Red 3B, with 71.32% (for 50 ppm) and 50.33% (for 100 ppm) mean percentage decolorization by Trichoderma viride DSB-2 after 9 days of incubation. A lower mean percentage decolorization of Red 3B was observed by *Trichoderma harzianum* DSB-1 (60.77% for 50 ppm and 47.22% for 100 ppm) after 9 days of incubation. Yellow FG was the least decolorized dye, with a mean percentage decolorization of 40.36% and 25.22% by Trichoderma viride DSB-2 after 9 days of incubation. This indicates that Yellow FG is more resistant to decolorization by the laccase-producing fungal isolates and probably requires a longer time for decolorization (DeSouza-Ticlo, Tiwari, Sah, & Raghukumar, 2006). The difference observed could also be due to the structural differences of the dyes. This finding is in agreement with the findings of Eichlerova, Homolka, and Nerud (2006) and Pramanik and Chaudhuri (2018), who also reported varying decolorization rates of different dyes by laccase-producing fungi.

The higher mean percentage decolorization observed at 50 ppm concentration of the dyes indicates that the concentration of the dye in the culture medium affects the rate of decolorization. This result is in line with the report of Pramanik and Chaudhuri (2018). The higher dye decolorization ability expressed by Trichoderma viride DSB-2 in this study is due to differences in enzyme production ability among different fungal species and strains. Studies have shown that the decolorization potential of laccase-producing fungi and laccases varies and depends on the strain of the fungi (Forootanfar, Faramarzi, Shahverdi, & Tabatabaei-Yazdi, 2011; Zhuo et al., 2011; Forootanfar et al., 2012). An increase in percentage decolorization of the dyes was observed with an increase in incubation period. A similar trend in decolorization was reported by Pramanik and Chaudhuri (2018).

Conclusion

This study demonstrates that *Trichoderma harzianum* DBS-1 and Trichoderma viridae DBS-2 possess significant dye decolorization potential, particularly for Blue H3R and Red 3B dyes. *T. viridae* DBS-2 exhibited higher decolorization efficiency, achieving up to 71.32% for Red 3B at 50 ppm concentration after 9 days of incubation. The findings indicate that lower dye concentrations and extended incubation periods enhance decolorization rates. However, Yellow FG proved more resistant to degradation by both fungal isolates. These results highlight the potential of using laccase-producing fungi in the bioremediation of dye-contaminated environments, offering an eco-friendly and cost-effective alternative to conventional methods. Further research should explore optimizing conditions to improve decolorization of resistant dyes like Yellow FG.

Author contributions

A.M., conceptualized and developed the methodology, H.I.M., and G.S., prepared the original draft and collected, A.G., reviewed and edited the writing.

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Competing financial interests

The authors have no conflict of interest.

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