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Magnetic Spent Tea Leaves as a Sustainable Carrier for Laccase Immobilisation in the Brilliant Green Removal from Aqueous Solution

Nurmin Bolong[1], Mohd Syahlan Mohd Syukri[1*], Azhar Omrah[1], and Dylan Nathaneal Jeory[2]

[1] Faculty of Engineering, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia,
[2] Biotechnology Research Institute, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia,
*Correspondence: msyahlan.syukri@ums.edu.my

*ORCID ID: 0000-0001-7153-5043
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Abstract: Synthetic dyes released into waterways through industrial effluents remain a major environmental concern because of their persistence and potential ecological effects. This study evaluated magnetic spent tea leaves (MSTL) as a low-cost and sustainable carrier for laccase immobilisation and assessed the performance of the resulting material (MSTL-LI) for Brilliant Green removal from aqueous solution. Laccase was immobilised onto MSTL, achieving an activity of 106.4 U/g and demonstrating successful enzyme attachment to the support. The effects of contact time, initial dye concentration, and adsorbent dosage on dye removal were investigated. Dye removal increased with contact time and reached 86.30% at 48 h for 100 ppm Brilliant Green using 50 mg of MSTL-LI, with only marginal improvement at 60 h, indicating that equilibrium had been approached. At the optimized contact time, the highest removal efficiency was obtained at the lowest tested dye concentration (100 ppm), while removal efficiency decreased progressively as the initial concentration increased from 200 to 400 ppm. Increasing the adsorbent dosage from 50 to 200 mg improved removal efficiency from 87.98% to 95.31%, although only marginal additional improvement was observed beyond 150 mg. These findings suggest that MSTL-LI is a promising waste-derived support for dye removal. Nevertheless, further studies involving material characterisation, reusability assessment, and mechanistic investigation are required to further evaluate its practical applicability in wastewater treatment.

Keywords: magnetic spent tea leaves; laccase immobilisation; brilliant green dye removal, wastewater treatment.

1. Introduction

Rapid industrialization and urban expansion have led to the massive production of wastewater containing hazardous pollutants that endangers nature and human life around the world [1,2]. These pollutants have recently attracted considerable concern as emergent contaminants (ECs), such as pharmaceuticals, pesticides, synthetic dyes, and industrial additives, which comprise persistent and bioaccumulative substances with negative environmental implications even at trace concentration levels [3-5]. Most of these compounds are present at parts per billion (ppb) or parts per trillion (ppt) levels and are difficult to eliminate with conventional water treatment methods due to their low biodegradability and complex chemical composition [6].

Furthermore, many ECs are not newly introduced compounds, but rather known substances whose adverse effects and mechanisms have only recently been recognized [7,8]. Although advanced oxidation, membrane filtration, and adsorption have demonstrated high removal efficiency, their wider application remains constrained by several limitations, including toxic by-product formation, membrane fouling, adsorbent saturation, and high operational costs [9–11].

Enzymatic catalysis has emerged as an attractive alternative because it can operate under mild conditions, at low temperature, and has the capability of degrading diverse aromatic and non-aromatic compounds using oxygen as the final electron acceptor [12,13]. However, the practical application of free laccase is limited by its poor operational stability, short service life, and poor reusability [14]. These limitations have been partially resolved through the use of enzyme immobilisation techniques, such as improving catalytic stability, storage stability, and recovery [15,16].

Concurrently, low-cost lignocellulosic materials, such as spent tea leaves (STL), have gained considerable attention as sustainable adsorbents because of their abundance, renewability, and high surface reactivity. Chemically modified STL has been successfully applied to remove heavy metals and organic pollutants from water [17]. However, its large-scale application is constrained by the difficulty in separating the chemically modified STL from the aqueous media and the relatively high cost associated with chemical modification procedures [18]. Previously, the research team successfully functionalized STL with magnetic Fe_3O_4 nanoparticles through a co-precipitation method, thereby imparting magnetic properties to the material [19]. Magnetic spent tea leaves (MSTL) offer a potential solution as they can be easily separated, reused and serve as a dual-purpose carrier for immobilised enzymes [20,21].

In this study, magnetic spent tea leaves (MSTL) were employed as a carrier for laccase immobilisation, and the performance of the resulting composite (MSTL-LI) was evaluated for Brilliant Green removal from aqueous solution. The effects of contact time, initial dye concentration, and adsorbent dosage were investigated. This work highlights the potential of a waste-derived magnetic support for enzyme immobilisation, while highlighting the need for further mechanistic, reusability, and material characterisation studies before practical application can be established.

2. Materials and Methods

2.1. Chemicals and Reagents

Laccase from *Trametes versicolor* in powder form (enzyme activity ≥ 0.5 U/mg), 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 25% glutaraldehyde solution Grade II in water, and Brilliant Green dye (content ~90%) were purchased from Sigma-Aldrich. Iron (II) sulfate heptahydrate was purchased from Merck, and iron (III) chloride hexahydrate was purchased from Systerm; both were used as precursors for Fe_3O_4 synthesis during the preparation of magnetic spent tea leaves.

2.2. Preparation of Spent Tea Leaves

Spent tea leaves (STL) were collected after use and washed several times with distilled water to remove adhering impurities. To eliminate residual caffeine, tannins, and colorants, the STL was boiled in distilled water at 100 °C for 1 h, followed by repeated washing until the rinse water became clear. The cleaned and decolorized STL was then oven-dried at 105 °C for 24 h, crushed, sieved to obtain a more uniform particle size, and stored in airtight containers until use.

2.3. Preparation of Magnetic Spent Tea Leaves

Magnetic spent tea leaves (MSTL) were prepared using the co-precipitation method, following previous work [21], to produce Fe_3O_4 nanoparticles with uniform composition and narrow size distribution. A Fe^{2+}: Fe^{3+} molar ratio of 1:2 was maintained during magnetite formation. The resulting Fe_3O_4-functionalized spent tea leaves were washed and collected, and their magnetic response was confirmed qualitatively using a magnetic rod. The magnetization step was intended to improve post-treatment recovery of the support from aqueous media.

2.4. Preparation of Laccase-Immobilised MSTL (MSTL-LI)

Laccase was immobilised on magnetic spent tea leaves (MSTL) through a sequential adsorption and cross-linking process adapted from previous work [21]. Briefly, 50 mg of MSTL was added to 3 mL of laccase solution (1.0 mg/mL) prepared in acetate buffer at pH 4 and incubated for 6 h under mild agitation. Glutaraldehyde (0.05%, v/v) was then added to promote cross-linking, and the mixture was agitated for a further 30 min at 200 rpm. The immobilised material was filtered and treated with 3 mL of Tris-HCl buffer (pH 7.0) for 30 min to quench unreacted glutaraldehyde and stabilize the preparation. The material was then washed thoroughly with acetate buffer, air-dried, and stored for subsequent experiments.

2.5. Laccase Activity Assay

Laccase activity was determined spectrophotometrically using ABTS as the substrate in sodium acetate buffer at pH 4. The increase in absorbance at 420 nm was monitored for 3 min using a UV-Vis spectrophotometer (BK-UV1800/BK-UV1600). One unit of enzyme activity was defined as the amount of enzyme required to oxidize one μmol of ABTS per minute. Laccase activity was calculated using Equation 1.

$$Laccase\ Activity\ (U/g) = \frac{\Delta A \times V \times 10^6}{\varepsilon \times L \times t \times m_{ST}} \quad (1)$$

where ΔA is the change in absorbance at 420 nm, V is the reaction volume, ε is the molar extinction coefficient of ABTS (36,000 M^{-1} cm^{-1}), L is the optical path length (1 cm), t is the reaction time, and m_{ST} is the MSTL weight used for immobilisation.

2.6. Dye Removal Experiments

The dye removal performance of Brilliant Green (BG) by laccase-immobilised magnetic spent tea leaves (MSTL-LI) was evaluated under different operating conditions, including contact time, initial dye concentration, and adsorbent dosage. MSTL was used as the control in the experiments. Batch experiments were conducted according to the conditions summarized in

Table 1. After treatment, the residual BG concentration in the solution was determined using UV-Vis spectroscopy at 625 nm. The dye removal efficiency was calculated using Equation (4), where C_0 and C_e represent the initial and final dye concentrations, respectively.

Table 1. Experimental design for adsorption study of Brilliant Green onto MSTL-LI

Factor	Conditions/ range	Fixed parameters
Contact time	12, 24, 36, 48, 60 h	Initial BG concentration = 100 ppm; MSTL-LI dosage = 50 mg; working volume = 15 mL
Initial dye concentration	100, 200, 300, 400 ppm BG solution	MSTL-LI dosage = 50 mg; contact time = 48 h; working volume = 15 mL
Adsorbent dosage	50, 100, 150, 200 mg MSTL-LI	Initial BG concentration = 100 ppm; contact time = 48 h; working volume = 15 mL

$$Removal\ Percentage\ (\%) = \frac{C_o - C_e}{C_o} \times 100\% \quad (4)$$

3. Results and Discussion

3.1. Laccase Activity after Immobilisation

The immobilised laccase on magnetic spent tea leaves (MSTL-LI) exhibited an average activity of 106.4 U/g, confirming that laccase was successfully attached to the MSTL support. This result indicates that the immobilisation approach preserved a measurable fraction of enzyme activity and produced an active biocatalytic material suitable for subsequent dye removal experiments.

A previous study on immobilisation of laccase on magnetic nanoparticles reported that a laccase activity of 0.3 U/g was achieved when laccase was immobilised on superparamagnetic nanoparticles [23], while an activity of 18 U/g was reported for laccase immobilised on $Fe_3O_4@SiO_2$ core-shell magnetic nanoparticles [24]. The differences in activity may be attributed to variations in the carrier material, surface chemistry, enzyme-support interaction, and immobilisation conditions.

A reduction in enzyme activity after immobilisation is commonly observed and may result from partial enzyme deactivation caused by strong chemical interactions between the enzyme and the support surface, which can alter the native conformation of the enzyme [25]. In addition, steric hindrance may reduce accessibility to the enzyme active sites after immobilisation, thereby lowering the measured catalytic activity [26].

Despite the expected reduction, the MSTL as the laccase support showed promising performance while offering additional practical advantages such as easy magnetic recovery, potential reusability, low-cost carrier preparation, and the use of waste-derived biomass. These features make MSTL-LI a promising material for dye removal applications in wastewater treatment, where operational stability, recoverability, and material cost are important considerations.

3.2. Effect of Contact Time

The removal of Brilliant Green increased progressively with contact time. Figure 1 shows the effect of contact time on BG dye removal by MSTL-LI at an initial dye concentration of 100 ppm and an adsorbent dosage of 50 mg. The dye removal was 29% after 12 h, 55% after 24 h, and 62% after 36 h. A marked increase was observed at 48 h, where removal reached 86%. Only a negligible change was observed at 60 h (86%), indicating that the system had approached equilibrium by 48 h. While 48 h was identified as the optimum contact time under the studied conditions, this relatively long contact period may limit the practical applicability of the system in large-scale wastewater treatment. The increasing trend with time can be attributed to the greater interaction between dye molecules and the available active sites on the MSTL-LI system. The long equilibrium time suggests that although the material is effective, further process optimisation would be required to improve the practicality of the treatment process for real applications. These results suggest that MSTL-LI provides stable dye removal performance while offering the added benefits of enzyme immobilisation and magnetic recoverability [26]. Similarly, the control using MSTL to remove Brilliant Green also showed an increase in the dye removal percentage with increasing contact time; however, the highest removal recorded was only 28.58% at 60 h. The result shows that MSTL alone has the ability to remove Brilliant Green, and the presence of laccase enhances its performance.

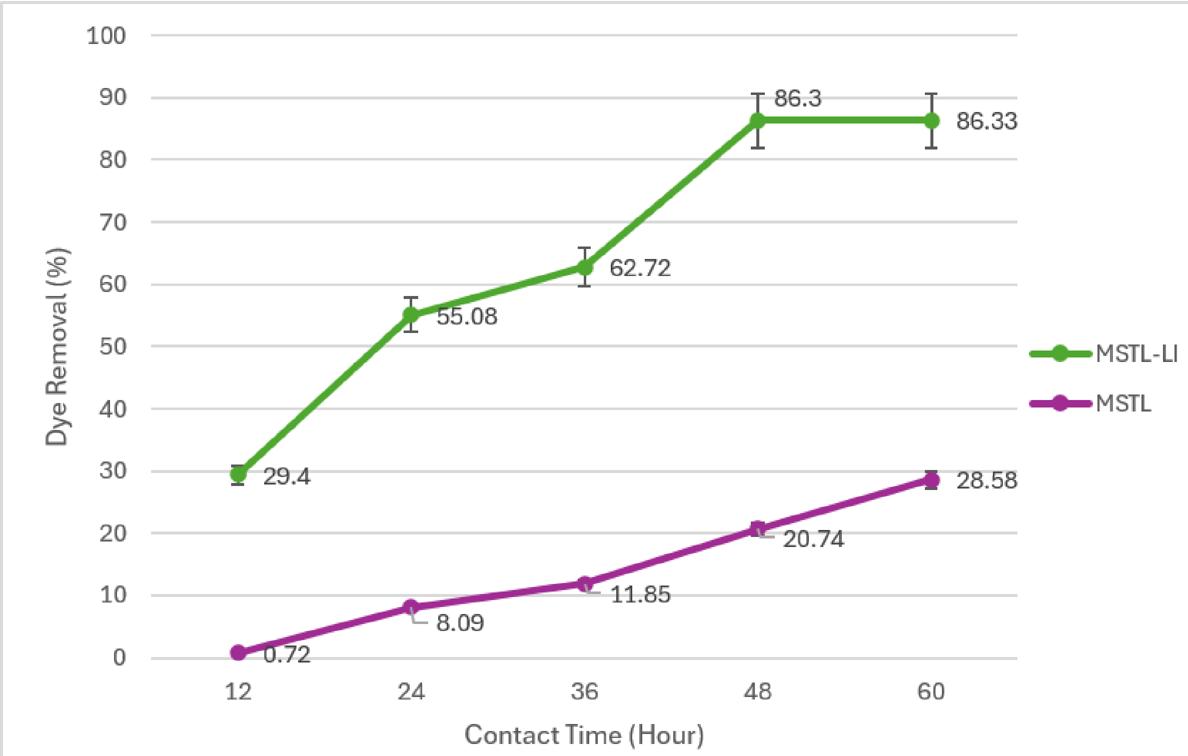

Figure 1. Effect of contact time on the removal of Brilliant Green by MSTL-LI and MSTL (control)

3.3. Effect of Initial Dye Concentration

The effect of initial BG concentration on dye removal by MSTL-LI was investigated at initial dye concentrations of 100, 200, 300, and 400 ppm using the optimized contact time of 48 h. As shown in Figure 2, the dye removal efficiency decreased with increasing initial dye concentration. The highest removal efficiency of 90% was achieved at 100 ppm, indicating that the available active sites on MSTL-LI were sufficient to effectively remove most dye molecules at lower concentration. A similar trend was reported when using MSTL as the adsorbent, although the removal efficiency was lower.

When the initial dye concentration increased to 200 ppm, the removal efficiency dropped to 56%, followed by 40% at 300 ppm and 19% at 400 ppm. This decline may be explained by the increasing number of dye molecules relative to the limited number of available active sites on the immobilised system. At higher concentrations, stronger competition among dye molecules for adsorption and catalytic interaction sites may occur, thereby reducing the overall removal efficiency. A similar concentration-dependent decrease in dye removal has been reported in previous studies [26]. These findings suggest that MSTL-LI performs more effectively at low to moderate dye concentrations, which may be relevant for practical wastewater polishing applications.

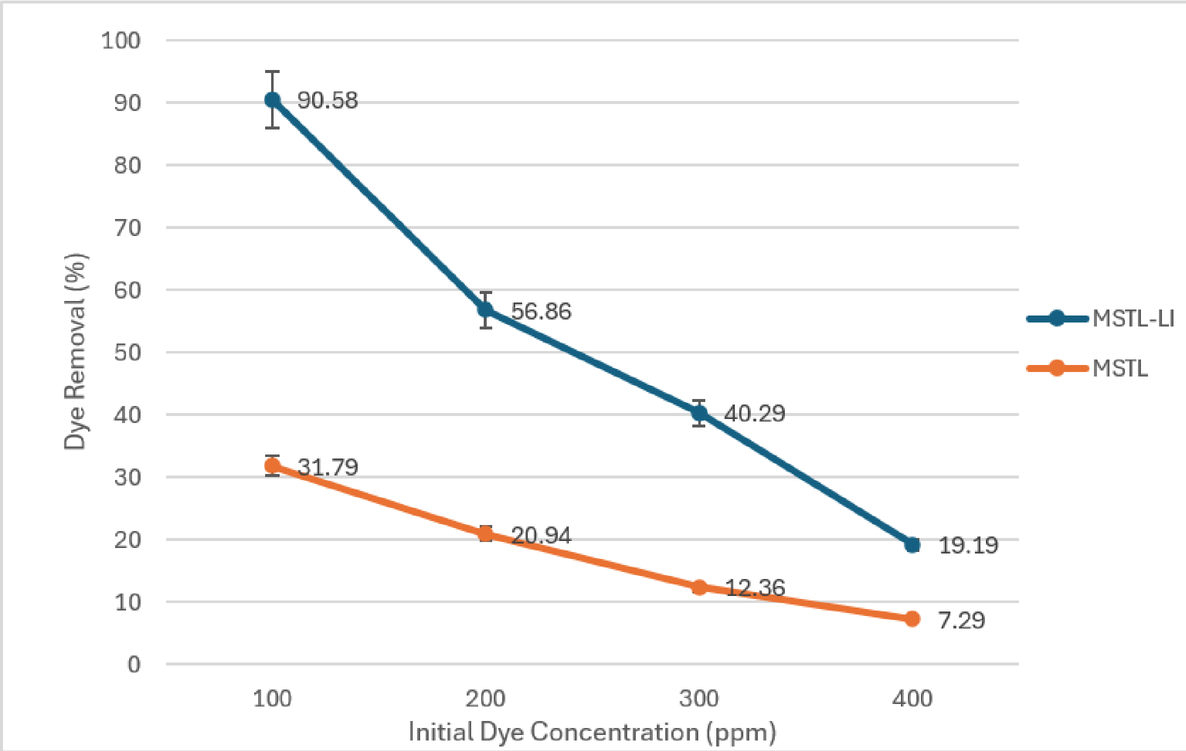

Figure 2. Effect of initial dye concentration on the removal of Brilliant Green by MSTL-LI and MSTL (control)

3.2.3. Effect of Adsorbent Dosage

The effect of adsorbent dosage on BG dye removal was examined by varying the amount of MSTL-LI from 50 to 200 mg, while maintaining the initial dye concentration at 100 ppm and the contact time at 48 h. As shown in Figure 3, the dye removal increased with increasing adsorbent dosage. The removal was 87% at 50 mg, and increased to 92%, 93%, and 95% at 100, 150, and 200 mg, respectively.

This behaviour is consistent with the increase in the number of available binding sites and accessible surface area as more material is added. However, the improvement beyond 150 mg was relatively small, indicating that the system approached saturation and that further addition of adsorbent provided only marginal benefit [26]. Although the highest removal was recorded at 200 mg, from a practical perspective 150 mg may already provide near-optimal performance with better material efficiency. Overall, these results confirm that adsorbent dosage is an important operating parameter in maximising dye removal by MSTL-LI.

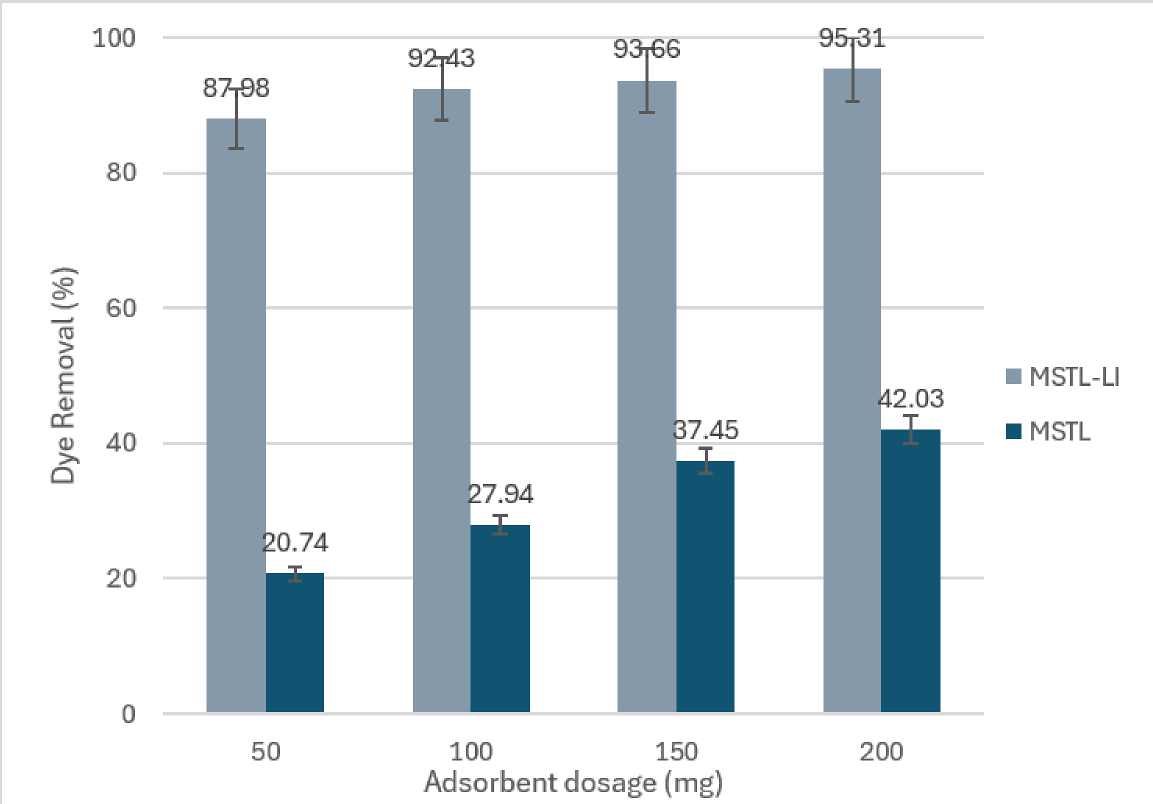

Figure 3. Effect of MSTL-LI dosage on the removal of Brilliant Green by MSTL-LI and MSTL (control)

4. Conclusions

Magnetic spent tea leaves were successfully used as a carrier for laccase immobilisation, and the resulting material showed promising performance for Brilliant Green removal from aqueous solution. The immobilised system exhibited an activity of 106 U/g. Dye removal increased with contact time and approached equilibrium at 48 h. Compared with some nano-based adsorbents or catalytic materials, this slower response highlights an area requiring further optimisation. The highest removal efficiency was observed at an initial dye concentration of 100 ppm, while efficiency decreased as the dye concentration increased. Increasing the dosage of MSTL-LI from 50 to 200 mg improved removal efficiency up to 95%, although the gain beyond 150 mg was limited. Overall, MSTL-LI represents a promising waste-derived and magnetically recoverable support for dye treatment. Nevertheless, further work is needed to verify material characterisation, reusability, adsorption kinetics, isotherm behaviour, and the specific contribution of enzymatic degradation before broader practical application can be concluded.

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Conflicts of Interest

The authors declare no conflict of interest.

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