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RESEARCH ARTICLE

Evaluation of Magnetic Spent Tea as a Sustainable Carrier for Laccase Immobilization and Polycyclic Aromatic Hydrocarbon Biodegradation

Dylan Nathaneal Jeory[a], Mohd Syahlan Mohd Syukri[b*], Mohd Afit Rustan[b], Mohamad Firdaus Hamid[b], Cahyo Budiman[a], Ismail Ware[a]

[a]Biotechnology Research Institute, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia; [b]Faculty of Engineering, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia

Abstract Due to their persistence, hydrophobicity, and carcinogenic nature, polycyclic aromatic hydrocarbons (PAHs) produced from the petroleum-based sector give rise to various threats to the environment and human health. Laccase exhibits significant potential for enzymatic bioremediation; however, its practical application is constrained by its low stability and limited reusability. In this study, three synthesis parameters to develop magnetic spent tea (MST) as a sustainable carrier for laccase immobilization were studied, namely precipitation pH (9 to 11), alkaline additive type (NaOH vs NH_4OH), and temperature (40 to 80°C). To ensure the formation of Fe_3O_4 magnetite, the theoretical stoichiometric ratio Fe^{2+}:Fe^{3+} of 1:2 was applied. Immobilization of laccase with MST was performed via a crosslinking method with glutaraldehyde, and thereafter, its catalytic activity, recovery, stability, and PAH degradation ability were evaluated. The highest immobilized laccase activity of 5.32 U and recovery of 26.41% were obtained with NH_4OH at pH 9 and 80°C. FTIR spectra showed characteristic peaks corresponding to Fe–O, O–H, and C–N stretching vibrations, denoting that magnetization and enzyme immobilization were successfully achieved. Meanwhile, SEM images confirmed the deposition of Fe_3O_4 nanoparticles on the fibrous structure of spent tea. HPLC analysis revealed that after 24 hours, 22.6% of naphthalene and 6.6% of anthracene were successfully degraded. After five cycles of reuse, the immobilized system retained approximately 20% of its initial activity, indicating promising recyclability. This study demonstrates that magnetic spent tea (MST) is a promising, affordable, and sustainable carrier for laccase immobilization with potential applications for the biodegradation of PAHs.

Keywords: Magnetic spent tea, laccase immobilization, naphthalene and anthracene biodegradation.

Introduction

***For correspondence:**
msyahlan.syukri@ums.edu.my

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Human activities such as industrial operations, oil spills, and biomass burning have led to the accumulation of polycyclic aromatic hydrocarbons (PAHs). PAHs belong to a major class of persistent organic pollutants (POPs), consisting of two or more fused benzene rings, and they pose harmful biological and carcinogenic effects on the environment and human health [1]. The dense π-electron cloud on the aromatic ring structure of PAHs such as naphthalene and anthracene confers chemical stability and high resistance to biodegradation, thereby enabling their accumulation in the environment [2]. As Malaysia's ongoing industrial and urban sectors continue expanding, PAH contamination is also increasing, emphasizing the urgency for effective degradation treatment.

For the time being, conventional physical and chemical methods have been exploited to address PAH removal. These methods include adsorption, advanced oxidation, and thermal treatment [3]. Nevertheless, these methods are often very expensive, energy-intensive, and may generate secondary pollutants as by-products. On the other hand, biological approaches, particularly enzyme systems, could be an alternative to tackle PAH degradation, operating under milder conditions and at minimal cost.

Laccase is one of the most well-known oxidative enzymes and has attracted considerable interest among researchers due to its ability to oxidize a wide range of phenolic and non-phenolic compounds using molecular oxygen as the electron acceptor [4,5]. This catalytic activity can aid PAH degradation, highlighting its promising potential for wastewater treatment.

However, laccase application in industrial sectors faces limitations, such as poor stability and reusability across a broad pH and temperature range. It can only operate under moderate conditions whilst unable to remain stable under harsh industrial conditions. This will consequently lead to an increase in the operational costs [6]. Enzyme immobilization techniques can help in minimizing these limitations [7]. Immobilization of enzymes is a method where enzymes are attached to or wrapped in a carrier or solid support, consequently increasing the reuse of the catalyst and its shelf life [8]. The success of enzyme immobilization techniques thus depends heavily on the type of carriers used, as they contribute to the stability of the enzyme's structure, thus enhancing its activity. To date, there are various types of support used for laccase immobilization, such as synthetic polymers and numerous nanoparticles (silica, nanotubes, iron oxide, etc.). Studies have shown that these supports often achieve high degradation rates within relatively short treatment times. However, these materials can be costly and may include complex synthesis procedures [2,9].

Conversely, natural and waste materials have been extensively studied in recent years as a favourable alternative for solid supports in enzyme immobilization, since they are eco- and cost-friendly. Among them are spent tea leaves, an abundant lignocellulosic agricultural biomass, widely available in Malaysia [10]. They are rich in cellulose, hemicellulose, and lignin, and contain abundant hydroxyl, tannin, and phenolic groups, which facilitate better interaction with laccase and crosslinkers [11]. Spent tea is a promising alternative immobilization carrier because it is renewable, eco-friendly, and inexpensive.

Integrating magnetic properties into the carrier support will enable easy separation and recovery using external magnetic fields [12]. A recent study has documented that spent tea could be magnetized through co-precipitation with iron salts under alkaline conditions, while retaining its functional structure [11]. A stoichiometric Fe^{2+}:Fe^{3+} ratio of 1:2 was used in the co-precipitation process to coat the spent tea with Fe_3O_4. The synthesis parameters, which are pH, alkaline additive type, and temperature, greatly influence the performance of MST as a carrier for laccase immobilization.

Although numerous studies have investigated engineered natural carriers, limited research has focused on evaluating the synthesis parameters of waste-derived MST for laccase immobilization. Moreover, comparative studies correlating MST synthesis conditions with laccase activity, reusability, and PAH degradation performance remain limited. Therefore, an investigation of MST synthesis parameters is necessary to identify suitable conditions for improving its performance as a laccase immobilization carrier. In this study, three synthesis parameters of MST were investigated, namely precipitation pH (9–11), alkaline additive type (NaOH and NH_4OH), and reaction temperature (40–80°C).

MST prepared under the most suitable synthesis condition was subsequently immobilized with *Trametes versicolor* laccase via glutaraldehyde crosslinking. The resulting immobilized laccase system was further evaluated in terms of enzymatic activity, operational stability, and degradation capability toward PAH compounds, namely naphthalene and anthracene.

Materials and Methods

Spent tea was collected from local restaurants, then boiled, rinsed, and dried overnight. For the synthesis of MST, anhydrous ethanol and sodium hydroxide were purchased from Sigma-Aldrich, iron (II) sulphate heptahydrate ($FeSO_4.7H_2O$) was obtained from Merck, iron (III) chloride hexahydrate ($FeCl_3.6H_2O$) from VWR International, and ammonium hydroxide solution (25%) from Acros Organics. For laccase immobilization, laccase *Trametes versicolor* and sodium acetate buffer were obtained from Sigma-Aldrich, and glutaraldehyde from Alfa Aesar. Meanwhile, for the characterization and biodegradation analysis, 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) was purchased from Bio-Rad Laboratories, and naphthalene and anthracene from Sigma-Aldrich. All chemicals used in this study were of analytical grade. Distilled water was used to prepare all chemicals, including buffers.

Synthesis of MST

Magnetic spent tea was synthesized via the co-precipitation method. Ferrous sulphate heptahydrate ($FeSO_4 \cdot 7H_2O$) and ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$) were added to 160 mL of distilled water and stirred vigorously. The mixture was then heated to the desired temperature, and alkaline additives were added to induce the formation of Fe_3O_4 nanoparticles [13]. The effects of the synthesis parameters

were evaluated sequentially. Temperature was first evaluated while keeping the pH at 9 and NH_4OH as the alkaline additive. The most suitable temperature was selected based on the laccase activity assay. Next, pH evaluation was conducted by fixing the selected temperature. Finally, evaluation of alkaline additives between NH_4OH and NaOH was performed using the selected temperature and pH conditions.

Immobilization of Laccase onto MST (IMST)

Laccase was immobilized onto magnetic spent tea (MST) by using glutaraldehyde as a crosslinker. MST was added to 3 mL of laccase solution, and the mixture was shaken at room temperature for 6 hours. After 6 hours of binding, 25% glutaraldehyde was added to the suspension and shaken for an additional 30 minutes for covalent crosslinking. The unbound laccase solution was discarded, and the IMST was filtered and soaked in 3 mL of Tris buffer (pH 7.0) for 10 minutes to quench any active aldehyde groups in the glutaraldehyde. IMST was then rinsed with acetate buffer (pH 4.5) to remove any excess chemicals [11].

Enzyme Activity Determination

Free laccase and IMST activities were determined using a UV-Vis spectrophotometer by measuring the oxidation of ABTS at 420 nm. Both were evaluated separately in a cuvette filled with 1.35 mL of ABTS solution (1 mM) and 1.5 mL of 0.1 M sodium acetate buffer (pH 4). Absorbance value was recorded after 3 minutes. Laccase activity (U) was calculated using Eq. (1):

$$U = \frac{\Delta A \times V \times 10^6}{\varepsilon \times L \times t} \quad (1)$$

where, ΔA is the change in absorbance after reaction at 420 nm, V is the reaction volume, ε is the molar extinction coefficient measured in M^{-1} cm^{-1}, L is the optical path, and t is the reaction time. Enzyme activity (U) is the quantity of enzyme required to oxidize 1 nmol of ABTS per minute. Meanwhile, the activity recovery was calculated using the following Eq. (2):

$$Activity\ Recovery\ (\%) = \frac{A_{il}}{U_I} \times 100\% \quad (2)$$

where, A_{il} is the activity of immobilized laccase, while U_I is the activity of free laccase. Finally, to determine the relative activity for IMST, Eq. (3) given below was used:

$$Relative\ Activity\ (\%) = \frac{U_C}{U_{max}} \times 100\% \quad (3)$$

where U_c is the specific activity of free or immobilized laccase, while U_{max} is the highest specific activity under the tested conditions.

Characterization of MST and IMST

Detection of surface functional groups and confirmation of magnetization were performed using Fourier transform infrared spectroscopy (FTIR) in the range of 950-4000 cm^{-1}. Meanwhile, the surface morphology of spent tea, MST, and IMST was examined using scanning electron microscopy (SEM).

PAHs Biodegradation

Naphthalene and anthracene were used as model polycyclic aromatic hydrocarbons (PAHs). IMST was incubated at 20°C under controlled agitation in individual aqueous solutions of naphthalene or anthracene. After 24 hours, samples were collected and analyzed using high-performance liquid chromatography (HPLC) to determine residual PAH concentrations. All the samples were prepared in triplicate, and the degradation rate was calculated using the equation below:

$$Degradation\ Rate, \% = \frac{(Initial\ Concentration - Final\ Concentration)}{Initial\ Concentration}\ x\ 100$$

Reusability Test

The operational stability of IMST was evaluated through ABTS oxidation over ten consecutive cycles. IMST was magnetically recovered and washed with buffer after each cycle, then reused in the subsequent cycle. Laccase activity in each cycle was determined using UV–Vis spectrophotometry at 420 nm. The reusability of IMST was then determined based on the relative activity calculated using Eq. (3).

Results and Discussion

Effect of MST Synthesis Temperature on IMST Activity

Physicochemical properties such as the crystalline phase and magnetite nanoparticle size deposited on MST are highly influenced by the synthesis temperature, which in turn affects the laccase immobilization performance. To determine the suitability of MST as a carrier for laccase immobilization, three synthesis temperatures (40, 60 and 80°C) were investigated.

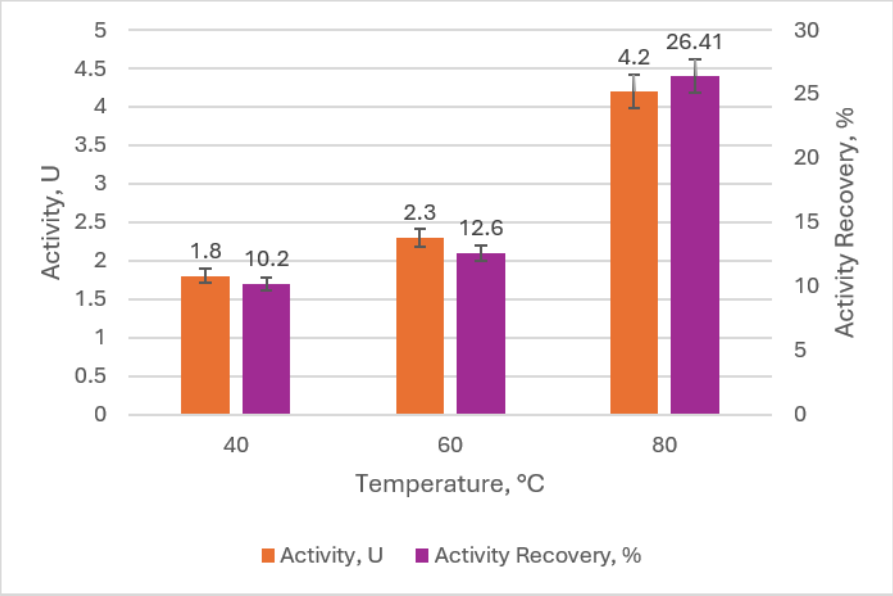

Figure 1. Activity and recovery activity at different temperature

In Figure 1, improvement in laccase activity and recovery was observed as the synthesis temperature increased. 80°C was observed to yield the highest activity of 4.2 U and activity recovery of 26.41%. At a synthesis temperature of 80°C and above, the system is prone to the formation of crystalline Fe_3O_4 with improved magnetic properties [14]. Proper formation of crystalline Fe_3O_4 on MST at the right temperature will then enhance the distribution of magnetic nanoparticles on the surface of spent tea, subsequently increasing the surface area for enzyme loading and reducing mass transfer resistance [15]. This results in higher activity and recovery. Moreover, the right temperature will affect nanoparticle size and surface charge, thereby inducing changes in electrostatic interactions between MST and laccase [16,17]. As the temperature increased to 80°C, improved crystallinity and formation of Fe_3O_4 nanoparticles enhanced the interaction between the carrier surface and laccase molecules, resulting in higher catalytic performance.

At lower synthesis temperatures, such as 40°C and 60°C, incomplete magnetite crystallization may occur, resulting in fewer Fe_3O_4 nanoparticles deposited on the surface of spent tea. This might be due to the conversion of amorphous hydrated iron oxyhydroxides to Fe_2O_3, rather than the formation of proper Fe_3O_4 [14]. This may limit the available surface area and active sites for laccase immobilization, subsequently leading to lower activity and activity recovery.

It should be noted that immobilization efficiency and enzyme activity are two distinct parameters. Activity recovery represents the catalytic activity retained after immobilization, whereas immobilization efficiency reflects the amount of enzyme immobilized onto the carrier. A higher immobilization efficiency does not

necessarily result in higher catalytic activity, as factors such as conformational changes, enzyme orientation, steric hindrance, and mass transfer limitations may influence enzyme performance following immobilization [5].

Effect of Precipitation pH of MST on IMST Activity

Precipitation pH is one of the key factors that determine the size and surface charges of magnetic nanoparticles deposited on spent tea, which then influence the activity and activity recovery in the laccase immobilization system with MST. In this study, the highest immobilized laccase activity and recovery were found when MST was synthesized at pH 9 (4.24 U, 14.62%), whereas a decreasing pattern was observed as the pH increased to 10 and 11, as shown in Figure 2.

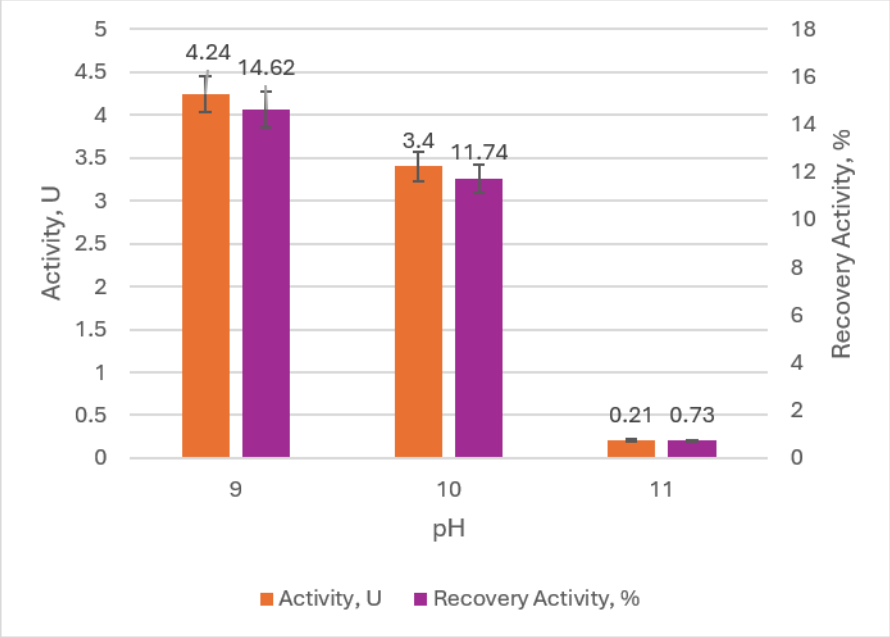

Figure 2. Activity and recovery activity at different pH

This trend could be explained by the size dependence of magnetic nanoparticles on changes in pH. According to the study by Jolivet et al. [16], increasing the synthesis pH leads to changes in nanoparticle size. This also affects the surface chemistry and subsequently influences the electrostatic interactions between MST and laccase. Thus, at synthesis pH 9, pure magnetite is likely formed, inducing the formation of well-crystallized Fe_3O_4 nanoparticles, resulting in higher activity and activity recovery.

However, excessively high pH is more prone to partial oxidation of Fe^{2+} and the production of secondary iron hydroxide particles such as goethite [19,20]. These less crystalline phases can compromise the surface properties of MST, hence reducing the amount of active immobilized enzyme. Other than that, nucleation and uncontrolled particle growth could easily take place as the pH increases, leading to particle agglomeration. This may eventually induce steric hindrance, thereby limiting enzyme accessibility and reducing the activity and activity recovery, as observed in the trend from pH 10 to 11 [18].

Effect of Alkaline Additives During MST Synthesis on IMST Activity

The type of alkaline additive used during MST synthesis indeed plays an important role in determining the activity of immobilized laccase. In this study, two types of alkaline additives were used, namely sodium hydroxide (NaOH) and ammonium hydroxide (NH_4OH). As shown in Figure 3, MST synthesized with 25% ammonium hydroxide (NH_4OH) yielded higher laccase activity of 5.32 U and higher activity recovery of 18.35%. Meanwhile, NaOH achieved only 0.89 U of activity and 3.09% activity recovery.

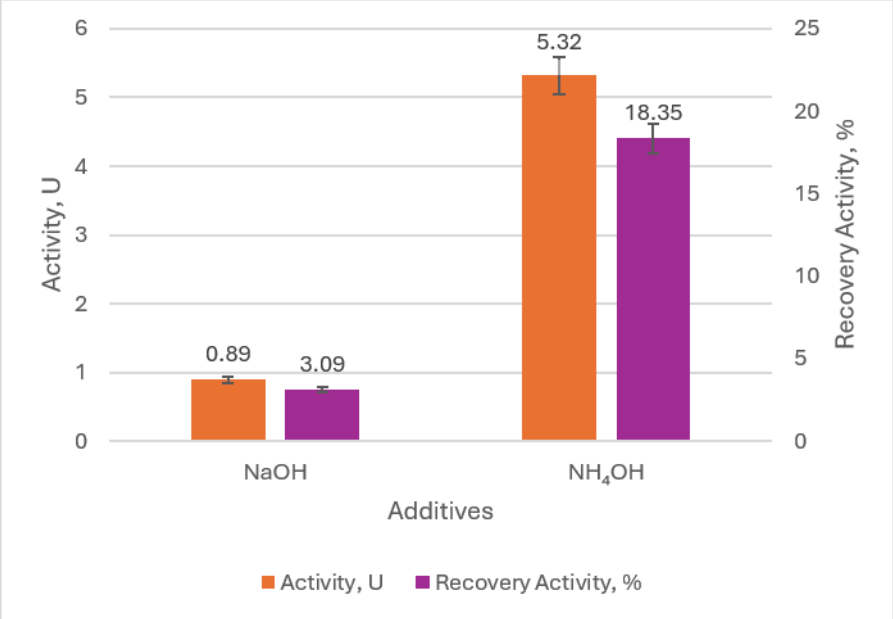

Figure 3. Activity and recovery activity at different alkali additives

This trend could be explained by the strength of the alkaline medium used during MST synthesis. The formation of Fe_3O_4 during precipitation is strongly influenced by the alkaline environment. NaOH is a strong alkaline medium. Therefore, adding NaOH as a hydrolyzing agent during MST synthesis can lead to the production of non-magnetic iron components [19,20]. As a result, crystalline Fe_3O_4 on MST is not properly formed, which affects enzyme loading and mass transfer resistance, yielding lower immobilization activity and activity recovery [14,15].

In contrast, NH_4OH acts as a milder alkaline additive and releases a controlled amount of hydroxide ions, which promotes the proper nucleation and growth of Fe_3O_4 nanoparticles [19]. Based on the results, it is deduced that NH_4OH increases the formation of magnetic nanoparticles on MST.

Structural Characterization by FTIR Spectroscopy

The FTIR spectra validate the magnetization of spent tea and the successful immobilization of laccase. In Figure 4, the broad peak around 3330 cm^{-1} indicates the presence of O–H stretching due to the lignocellulosic components such as polyphenols and cellulose. This peak became broader in MST and IMST due to enhanced hydrogen bonding interactions. The C=C stretching of lignin near 1608 cm^{-1} in ST was observed, whereas in IMST, the peak is shifted to 1634 cm^{-1}, which suggests the amide I band of laccase, confirming successful enzyme attachment to MST. C–O stretching was observed around 1020–1030 cm^{-1}, and after immobilization, it shifted, which indicates covalent bonding via glutaraldehyde crosslinking.

The most essential part to differentiate between ST, MST, and IMST was the peak around 545–547 cm^{-1}, indicating the presence of the Fe–O bond, thereby proving the successful incorporation of magnetite particles onto spent tea. There is a decrease in the Fe–O peak intensity in IMST, suggesting that more magnetite particles are covered by the immobilized laccase after enzyme attachment. All these ranges of bands are supported by other authors [10,21-23].

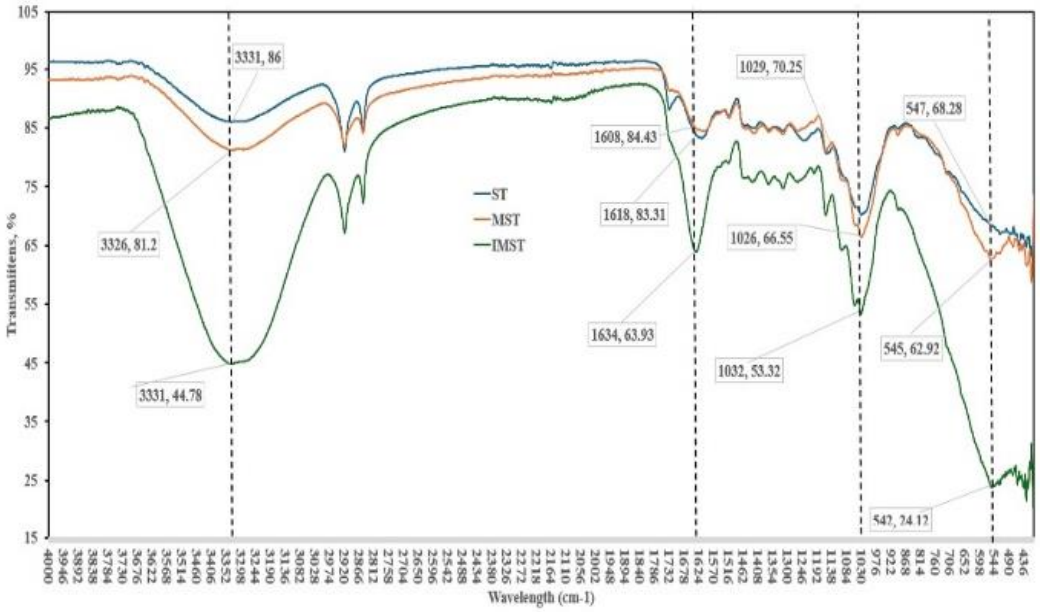

Figure 4. FTIR analysis for Spent Tea (ST), Magnetic Spent Tea (MST) and Immobilized laccase (IMST)

Surface Morphology Analysis Using SEM

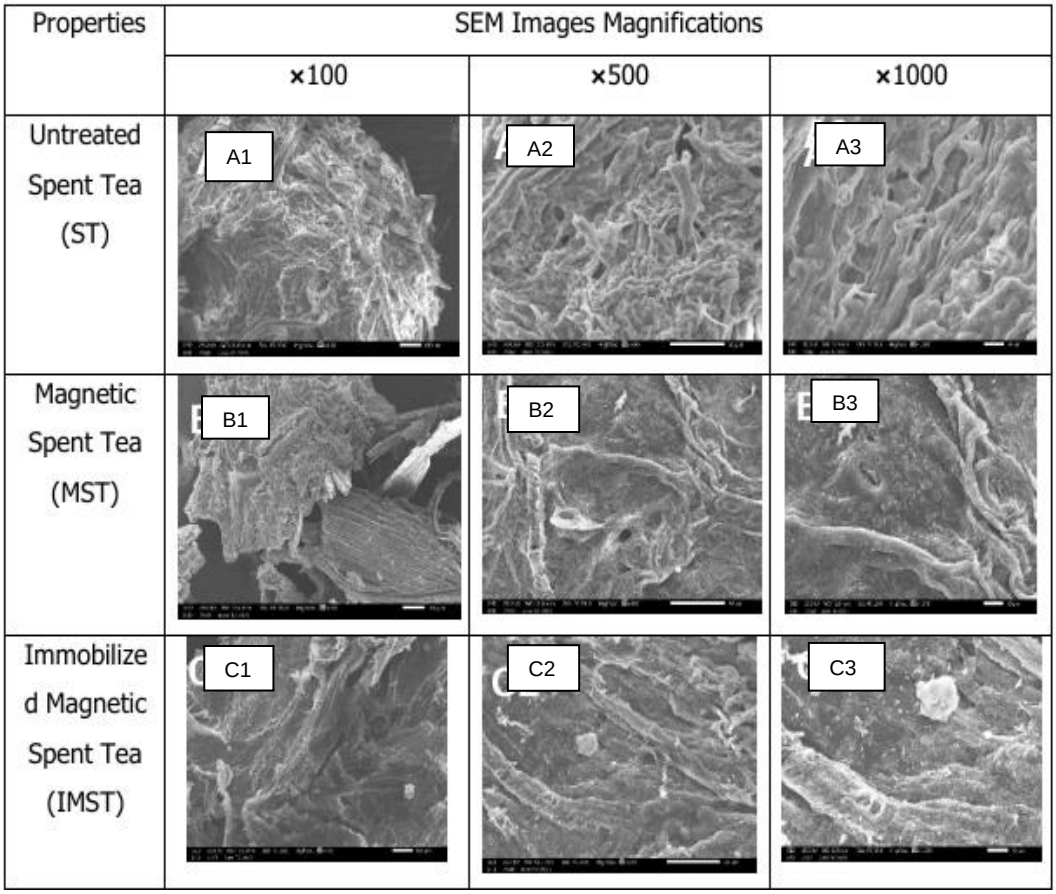

Figure 5. SEM images for ST, MST and IMST under different magnifications

SEM images from Figure 5 reveal clear morphological changes across the samples of ST, MST, and IMST. SEM images of ST (A1–A3) can be seen to have a fibrous structure and tiny holes, which is very common for plants such as tea leaves. After the magnetization of spent tea, small, nearly round particles are observed to appear and cover the ST surface. These particles are suspected to be magnetite, as also supported by the FTIR analysis. The deposition of magnetite particles indicates successful coating on the surface of the spent tea. After immobilization, a significant change in surface morphology is observed, where IMST has a rougher texture and noticeable irregularities compared to ST. This alteration indicates the successful attachment of laccase molecules onto the surface of MST, forming an additional layer that modifies the surface texture. Several studies reported similar observations after magnetization and immobilization through SEM analysis [10,24].

PAHs Biodegradation

Naphthalene and anthracene were used as model polycyclic aromatic hydrocarbons (PAHs). Figure 6 presents the biodegradation efficiencies of immobilized laccase on magnetic spent tea (IMST) in comparison with MST alone without immobilized laccase as the control. Biodegradation efficiencies of 22.61% for naphthalene and 6.64% for anthracene were achieved within 24 hours after IMST was incubated at 20°C under controlled agitation in individual aqueous solutions of naphthalene or anthracene, whereas the MST control exhibited only 4.26% and 0.47% degradation for naphthalene and anthracene, respectively. These results suggest that the contribution of the carrier matrix alone was minimal, whereas the catalytic activity of the immobilized laccase played a major role in the enhanced PAH degradation.

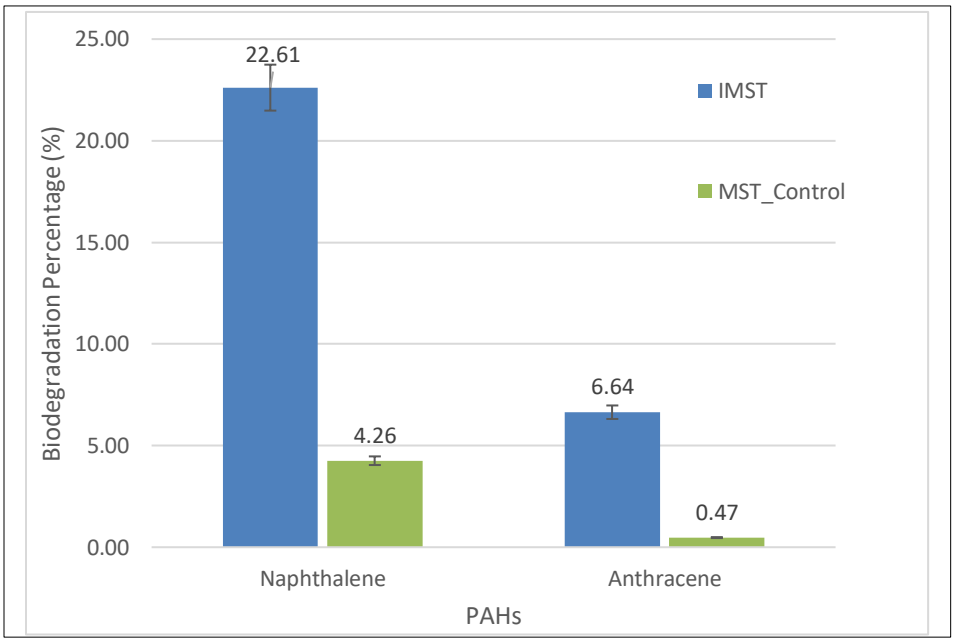

Figure 2. Degradation rate of anthracene and naphthalene by IMST and MST alone

The difference in the degradation of naphthalene and anthracene can be accounted for by the fact that naphthalene has simpler double rings and smaller molecules compared to anthracene [25,26]. Naphthalene is composed of lower molecular weight and lower structural complexity, which contribute to enzyme accessibility and its oxidation by IMST. A similar trend of results was observed where Okoro et al. [26] reported that naphthalene underwent greater degradation than anthracene using zinc- and copper-terephthalate metal–organic frameworks.

In comparison, based on a study conducted by Ramsay et al. [27], a microbial consortium provided with oxygen as terminal electron acceptors showed that 37.5% of naphthalene and 8% of anthracene were completely degraded in 30 and 160 days, respectively. A similar trend of results was also seen in the present study (22% for naphthalene and 6% for anthracene), albeit slightly lower in value and with differences in the period. Nevertheless, the microbial consortium required weeks to reach maximum mineralization of PAHs, whereas the IMST system used in the current study managed to reach almost the same result in a shorter reaction time, thus demonstrating its potential for rapid degradation of PAHs,

provided that certain improvements and optimizations are undertaken.

Reusability

The IMST system was evaluated for its reusability over eight consecutive cycles. Cycle 1 was recorded as 100% relative activity, and a rapid decrease to approximately 64% was observed in cycle 2, followed by 24% in cycle 3 (Figure 7). After the third cycle, relative enzyme activity remained stagnant until cycle 8, notably at low activity levels. The drastic decrease in IMST activity might be due to enzyme leaching during repeated washing, damage or distortion of the support, and conformational changes in the IMST structure, thereby reducing catalytic efficiency [28,29].

In addition, prior studies have reported that inorganic supports generally exhibit better operational stability compared to organic-type supports like spent tea due to their mechanical and chemical strength [30]. Liu et al. [31] also mentioned that approximately 36% of the initial activity was retained after five cycles when laccase was immobilized on an Fe_3O_4@chitin nanofiber composite, which shows a slightly similar trend of gradual loss to IMST.

Nonetheless, IMST still retained activity even after multiple consecutive cycles and early-stage activity reduction, suggesting that a fraction of the immobilized enzyme still remained operationally stable. Overall, the IMST system still shows the potential and advantages of MST as a carrier that can be recoverable and reusable, thus highlighting it as an alternative for wastewater treatment processes, provided that further optimization of the immobilization conditions is required to improve long-term reusability.

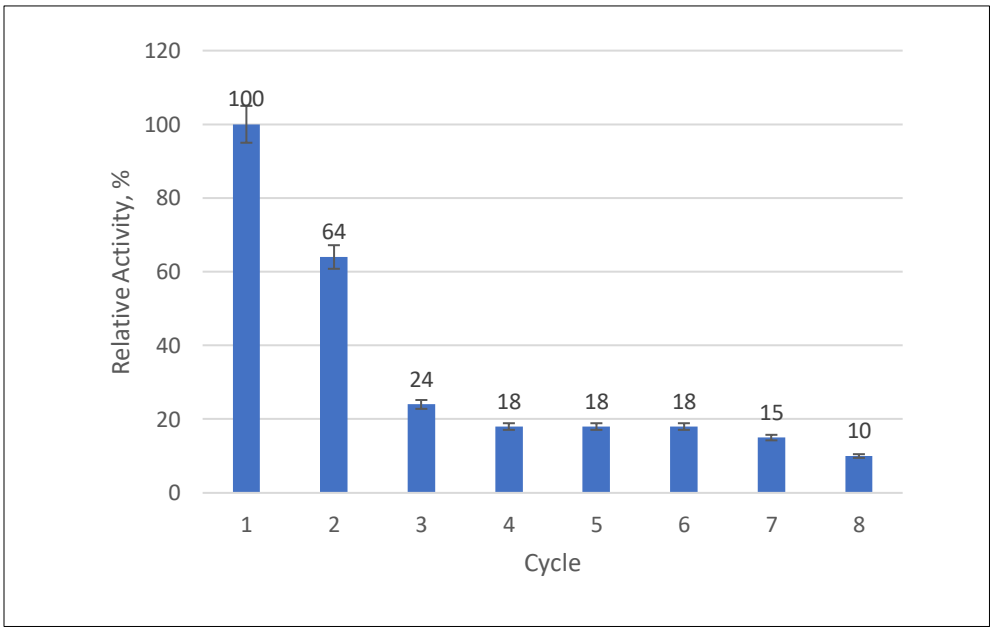

Figure 3. Relative activity of immobilized laccase over 8 reuse cycles

Comparative Study

The comparison in Table 1 shows several immobilization results obtained from other studies alongside the results obtained in the current study. It can be observed that some inorganic and synthetic supports exhibit higher operational stability, better enzyme activity, and greater degradation capability compared to organic carriers. Nevertheless, organic carriers also demonstrate several advantages, including biodegradability, renewability, and environmental friendliness. The MST developed in this study uses waste-derived spent tea incorporated with Fe_3O_4 nanoparticles, offering advantages in sustainability, cost-effectiveness, and recoverability via an external magnetic field. Therefore, MST, as an immobilization carrier, is a promising alternative for laccase immobilization and PAH biodegradation applications.

Table 1. Comparison of the performance of inorganic and organic carrier materials for laccase immobilization

Carrier	Enzyme Activity	Reusabilty	Degradation	Reference
		Inorganic Carrier		
CPC nano-porous silica beads	-	85% of initial activity at 30th cycle	(2,4-dinitrophenol), 91%, 12 h	[32]
Titania nanoparticle	0.085 ± 0.011U/mg	-	(ABTS), Remaining activity of 0.12 U/mg	[33]
Concanavalin A-activated Fe_3O_4 nanoparticles	849 U/g	82.8% of initial activity at 10th cycle	(Sulfonamide antibiotics), 95%, 30 min	[34]
Epoxy-functionalized silica	1.5 U/mg	61% of initial activity at 5th cycles	(Catechol), 95%, 2 h	[35]
Diatomaceous earth	0.8 U/g	-	(Nonylphenol), 60%, 20 min	[36]
Bentonite	800 U/g	46% of initial activity at 5th cycles	(Tetracycline), 60%, 3 h	[37]
Goethite-adsorbed laccase	200 U/g	-	-	[38]
Activated carbon fibers	97.1 U/g	40% of initial activity at 6th cycle	-	[39]
CNTs- hydrophilic polyvinylidene fluoride membrane	0.45 U/cm^2	90% of initial activity at 4th cycle	(Bisphenol-A), 90%, 48 h	[40]
Light expanded clay aggregate	6.67 U/g	95.4% of initial activity at 4th cycle	-	[41]
		Organic Carrier		
Chitosan beads	-	22.07% of initial activity at 14th cycle	(Acid Black 172), 68.84%, 48 h	[42]
Agar-Agar	38% (recovery activity)	24.8% of initial activity at 8th cycle	-	[43]
Modified Cellulose/CF beads	25% (recovery activity)	60% % of initial activity at 7th cycle	(Chlorinated Biphenyl), 85%, 8h	[44]
Solid lignocellulosic residues	5 U/g	Maintained removal efficiency after 1 reuse	(17α-Ethinylestradiol), 97%, 24 h	[45]
Eggshell membrane	1.3 U/g	30% of initial activity at 6th cycle	(Syringic acid), 57%, 24 h	[46]
Digested spent grain	0.24 U/g	26% of initial activity at 7th cycle	(Methyl Orange), 66%, 180 min	[47]
Rice-husk ash nano-silica/chitosan/alginate composite	3.8 U/g	50% of initial activity at 6th cycle	(Syringic acid), 100%, 24 h	[48]
Green coconut fiber	1 U/g, 4% (recovery activity)	55% of the initial activity at 13th cycle	(Reactive Red 239), 30%, 3.5 h	[49]
Alginate-gelatin mixed gel	52 U/mg	44% of initial activity at 2nd cycle	(Congo red dye), 80%, 24 h	[50]
Present study: Magnetic spent tea	106 U/g	24% of initial activity at 3rd cycle	(Naphthalene), 22%, 24 h	

Conclusions

In summary, MST was successfully prepared as a sustainable carrier for laccase immobilization using NH_4OH at pH 9 and 80°C, yielding the highest immobilized laccase activity of 5.32 U and an activity recovery of 26.41% among the tested synthesis conditions. FTIR and SEM analyses confirmed successful Fe_3O_4 coating and enzyme attachment onto MST. The IMST system preserved about 24% of its activity after the third cycle, indicating moderate operational stability and recoverability. Naphthalene and anthracene were degraded by 22.6% and 6.6%, respectively, within 24 hours, demonstrating its potential for PAH degradation. Overall, MST is a promising low-cost and environmentally friendly carrier for laccase immobilization and enzymatic biodegradation of PAHs. Further studies are necessary to improve the immobilization conditions and enhance the efficiency and long-term stability of the IMST system.

Conflicts of Interest

The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper.

Acknowledgment

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