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LACCASE IMMUNOLOGIZED ON PAN/O-MMT COMPOSITE
NANOFIBERS SUPPORTS FOR SUBSTRATE
BIOREMEDIATION: A DE NOVO ADSORPTION AND
BIOCATALYTIC SYNERGY

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Abstract: The engineering of supports for enzyme immobilization while retaining competent functionality is nontrivial. We attempted to enhance the removal efficiency of organics by adopting a relatively novel approach involving a synergy from the adsorption capabilities of a support incorporated with organically modified montmorillonite (O-MMT) and catalytic properties of immobilized laccase. Electrospun polyacrylonitrile (PAN)/O-MMT membranes after alkaline hydrolysis and carboxyl activation were chosen as the support system for laccase immobilization. Confocal laser scanning microscope confirmed a uniform enzyme distribution along with the fibers’ longitudinal surface. After enzyme immobilization, the optimum pH shifted from 3 to 3.5, while the optimum temperature remain unchanged at 50 °C. Its stability was preserved despite a large fluctuation in pH (50% of its initial activity retained over the pH range 2-6) and temperature (more than 80% of its initial activity retained over 30-70 °C). Compared to free laccase, the thermal stability of the immobilized laccase
was improved after being under 30 °C and 50 °C for 8 h. The operational stability was 68% of its initial after 10 times repeated usage, whiles also demonstrating 80% storage stability of initial activity even after two months. The immobilized laccase showed high removal efficiency of crystal violet at an optimum pH of 5 and temperature ~ 40 °C as well as an initial substrate concentration of 100 mg/L. Compared with that of individual support and the free laccase, the removal efficiency by immobilized enzyme is far higher to confirm a synergy in the immobilized enzyme and support system.

**Keywords:** Biocatalysis, Adsorption, Enzyme Immobilization, Electrospinning, Composite Nanofibers
**Introduction**

Triphenylmethane dyes (TPM) are synthetic colorants extensively used in paper, leather, and pharmaceuticals, but predominantly within the textile industries. Approximately thousands of tons of textiles dyes end up in receiving waters due to improper processing and dyeing technologies and thereby causing contamination. Among the many types of dyes, crystal violet (CV) results in 10-15% of unused dye released into the environment per annum which not only compromises water quality, but degrades the aesthetic value of water ecosystems. TPM dyes are recalcitrant to degradation due to their complex structure and aromatic rings while additionally being carcinogenic, toxic, and mutagenic. Crystal violet (CV) is also known to cause permanent injury to corneas, skin irritation, kidney failure, and permanent blindness after adventitious poisoning of drinking water systems.

Several physical and chemical methods, such as flocculation, coagulation, adsorption, membrane filtration and ozonation have been applied to treat wastewater containing colored effluents. However, the main drawbacks has been high cost, inability to totally remove recalcitrant dyes, and also an apparent production of large amounts of sludge which restrict their applications. Recently, the paradigm of enzyme-based bioremediation for contaminated waste water has been gaining ascendency over many other approaches because of superior physical and chemical operational parameters that include mild treatment conditions, high efficiency, low cost, and its competency to treat large effluent volumes. In one prototypical approach, laccase (polyphenoloxidase, EC 1.10.3.2), a multi-copper enzyme, has been shown to oxidize a variety of organic substrates. The process of laccase-based catalysis only requires the presence of the substrate and O\textsubscript{2} as the terminal oxidant to display
optimal behavior in decontamination schemes.  

Free laccase has certain limitations, such as low stability, recyclability, and reusability in industrial applications which has then led to the suggestion of a possible solution by enzyme immobilization, $^{10,11}$ which has been shown to significantly improve long-term enzymatic reactivity, stability, and efficiency even under harsh conditions, but the quest for appropriate supports is ongoing. $^{12-14}$ Zhang et al. $^{15}$ vividly identified bottlenecks for laccase-based catalysis in potential secondary pollution by-products. Thus, if the carrier can also work as an adsorbent for potential pollution by-products, it could effectively solve the issue mentioned herein.

Among immobilizing carriers, electrospun fibrous membranes possessing mesoporous structures have shown great potential because of their large surface area, inter-fiber porosity, fiber diameters from several micrometers to nanometers, and good mechanical strength. These properties ensure enhanced enzyme loading, recycling, and reuse. An additional benefit can be achieved through cross-linking for enzyme immobilization to ensure stabilization and prevention of leaching in larger mesoporous membranes. $^{16}$

In particular, electrospinning has proven useful to produce polymeric nanofibers membranes for absorbing contaminants. Among these polymeric nanofibers, PAN has been widely used for filtration and adsorption because of its good mechanical strength and stability. $^{17}$ Xu et al. $^{12}$ designed a mesoporous membrane (pore size 1.73-3.54 nm, pore volume 0.379 cm$^3$/g, and specific surface area = 542.91 m$^2$/g) by extracting hexadecyl trimethyl ammonium bromide (CTAB) from electrospun PAN nanofibers followed by laccase immobilization as a means to target Triclosan, a ubiquitous antibacterial/antifungal consumer
product. Remarkably, it was demonstrated that a combination of adsorption and degradation was indeed effective. In fact, the previous work in this lab demonstrated that an adsorption/enzyme catalysis-combined strategy \(^{13}\) demonstrates great value: PAN/organically-modified montmorillonite (PAN/O-MMT) electrospun composite nanofibrous membranes for laccase immobilization can be successfully prepared and demonstrated. However, that specific effort focused on system applications; thus, industrial-grade laccase and a rudimentary adsorption process were used for enzyme immobilization. Nevertheless, a mechanistic basis for its activity was not explored because the enzyme system was impure and displayed inherent instability, which is important in understanding the synergic mechanism; hence, making it possible to provide satisfactory results.

Thus, the current work examined an immobilized, highly pure laccase system covalently attached to the surface of electrospun PAN nanofibers within which O-MMT was incorporated as a versatile adsorbent and distributing agent. This study reveals that crystal violet (CV) can be effectively bio-remediated using a combined adsorption/biocatalysis process. The kinetic, stability, and physical properties of the immobilized laccase were studied and CV bio-remediation factors influenced by immobilized laccase were also investigated.

**Experimental**

**Materials**

Laccase from *Trametes versicolor* was purchased from Sigma-Aldrich.
2’-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), used as the substrate to determine enzyme activity, was obtained from Richu Biosciences Co. Ltd (Shanghai, China). 1-ethyl-3-(3-dimethylamino-propyl) carbodiimide (EDC) and N-hydroxysuccinimide (NHS) were purchased from Aladdin. Organically modified montmorillonite (O-MMT, modified by CTAB) was provided by Zhejiang Fenghong New Material Co., Ltd (Zhejiang, China). Polyacrylonitrile (PAN) ($M_w=50000$ g mol$^{-1}$) powders were purchased from Shangyu Wu & Yue Economic and Trade Co., Ltd (Zhejiang, China). All other reagents including crystal violet (CV), sodium hydroxide (NaOH), and N, N-dimethyl formamide (DMF) were purchased from Sinopharm Chemical Reagent Co., China. Deionized water was the solvent of choice in the study.

**Preparation of PAN/O-MMT composite nanofibers by electrospinning**

Electrospun PAN/O-MMT composite nanofibers were fabricated as follows: first, 0.15 g O-MMT powder was well dispersed in 20 mL DMF solution by sonication for 1 h followed by the addition of 3 g PAN powder into the slurry. It was magnetically stirred for 8 hrs until a light yellow transparent solution was obtained. The as-prepared electrospinning solution was poured into a 20 mL plastic syringe equipped with a stainless steel needle that was connected to a high-voltage generator. The applied voltage was set to 15 kV while a flow rate of 0.5 mL/h was maintained. The distance between the needle tip to the collector was 15 cm.

**Chemical modification of PAN/O-MMT composite nanofibers**

The as-electrospun nanofibrous membranes were chemically modified as follows: a relatively uniform area of the PAN/O-MMT composite nanofibrous membrane was cut into a circular shape at a diameter of 9 cm. A 200 mL blend solution of absolute ethanol and
deionized water with a volume ratio of 19:1 was prepared, into which 8 g NaOH was added.

The solution was transferred into a round-bottom flask after full dissolution of the NaOH. The membrane was placed into the flask and allowed to react at 85 °C for 20 min. The alkaline modified membrane was thoroughly washed until neutral pH was obtained. An alkaline-hydrolyzed PAN/O-MMT composite nanofibrous membrane was obtained.

EDC and NHS were used to activate the carboxyl group of the alkaline-modified PAN nanofibrous membranes using the protocol proposed in Kim’s work with slight modifications. A detailed process is briefly described as follows: first, the alkaline modified PAN membrane was immersed in a phosphate buffer saline (PBS) buffer solution (pH=6.5) for 20 min to remove impurities. The membrane was removed and immersed in an EDC/NHS buffer solution (the molar ratio of EDC·HCl/NHS=1:1) for 3 hrs at room temperature. The membrane was then removed and washed repeatedly.

Laccase immobilization

The activated PAN/O-MMT composite nanofibrous membrane was added into 100 mL of laccase solution (0.3 g/L, pH=4) and cross-linked for 3 h in a shaking bath at 25 °C. Afterward, the membranes were taken out and washed thoroughly with buffer solution until no enzyme was detected in the washing solution. The quantity of the immobilized laccase was calculated according to Bradford’s method. Later, the nanofibrous membrane with immobilized laccase was stored at 4 °C for further use.

Physicochemical characterization of the PAN/O-MMT nanofibrous membrane

Scanning electron microscope (SEM, Quanta 200, Holland FEI Company) was used to investigate the effect of alkali hydrolysis and carboxyl activation on the fibrous structure of
the PAN/O-MMT composite nanofibers. The samples were coated with a thin layer of gold by sputtering before the SEM imaging.

The immobilized enzyme on the surface of PAN/O-MMT composite nanofibrous membrane was labeled by an fluorescein isothiocyanate (FITC) aqueous solution and washed thoroughly by buffer solution before analysis. The membrane was carefully placed on a glass slide to which one drop of water was applied to fully unfold the membrane. A cover slip was applied to seal the membrane. Confocal laser scanning microscope (CLSM, TCS SP8) was used to characterize the immobilized enzyme at an excitation wavelength of 488 nm.

The functional groups of the PAN/O-MMT nanofibers before and after chemical treatment as well as the laccase immobilized nanofibers were obtained with Fourier Transform Infra-Red attenuated total reflectance (FTIR-ATR, Nicolet Nexus, Thermo Electron Corporation) equipped with a germanium crystal. The spectra were recorded after 16 scans at a resolution of 4 cm\(^{-1}\).

**Determination of enzyme activity**

The activity of the free and immobilized laccase was assayed at 30 °C using ABTS as the substrate. A detailed process was previously reported. Specifically, 15 mM of an ABTS solution was prepared by delivering 82.305 mg of ABTS to 10 ml of deionized water in a centrifuge tube. The activity of free and immobilized laccase was determined according to established assay protocols. In brief, the reaction assay consisted of 0.1 mM ABTS, 100 mM sodium acetate buffer (pH = 4.5) and a suitable amount of free and immobilized laccase. The assay was run by adding 0.1 mL ABTS (15 mM) into 2.9 mL sodium acetate buffer
containing a specific amount of free or immobilized laccase after which the absorbance was
monitored by UV-1700 spectrophotometry at a maximum absorbance wavelength of 420 nm.
For the immobilized laccase, the sample was centrifuged at 12,000 rpm for 40 sec, and the
supernatant was used for monitoring the laccase activity. All assays were conducted in
triplicate at minimum.

**Kinetic studies**

Kinetics studies were carried out at 30 °C in 100 mM sodium acetate (pH=4.5) buffer
using ABTS as the substrate having the substrate concentration varying from 0.1 to 1 mM.
The kinetic parameters of \( K_m \) and \( V_{max} \) were calculated according to the Lineweaver-Burk
double reciprocal models using Equation (1).

\[
\frac{1}{v} = \left(\frac{K_m}{V_{max}}\right)\left(\frac{1}{S}\right) + \frac{1}{V_{max}}
\]  

(1)

**Optimum pH and temperature**

To determine the optimum pH, the immobilized enzymes were incubated in buffers at pHs
ranging from 2 to 6 at 4 °C for 12 h and assayed for activity, while the optimum temperature
was determined by the activities of the immobilized enzymes incubated in buffers (pH 4.5) at
10 min over different temperatures ranging from 30 to 70 °C before adding ABTS.

**Stability of free and immobilized laccase**

The thermal stability of free and immobilized laccase was evaluated by the relative
activity at 30°C and 50 °C recorded at 2 h intervals. The experiments were conducted in 100
mM buffer solutions at pH 4.5.

The operational stability was studied by repeated usage (10 ×), while the relative enzyme
activity was recorded after each use. The experiments were carried out at 30 °C and pH 4.5.
All the control samples were made with the buffer solution at the same pH value as the assayed sample.

The storage stability of the immobilized enzyme was determined by the activity retention ratio during storage at 4 °C in 100 mM sodium acetate buffer solution (pH 4.5) at regular intervals up to 60 days.

**Removal of CV**

Effect of initial concentrations (10, 25, 50, 100, 150 mg/L), pH (2-6) and temperature (20-70 °C) on the removal efficiency of CV was determined using a UV-1700 spectrophotometer at a wavelength of 590 nm.

All assays were conducted in triplicate at minimum. Experimental data were reported as mean ± standard deviation. Statistical differences were analyzed using one-way ANOVA whiles statistical significance was represented as p < 0.05.

**Results & Discussion**

**Morphology and structural transformation after chemical modification and enzyme immobilization**

SEM images as well as their corresponding digital photographs are shown in Figure 1 (a-c) to evaluate the morphological and structural changes that were induced subsequent to alkaline hydrolysis and carboxyl activation.
Figure 1. SEM and digital photographs of PAN/O-MMT composite nanofibers before and after chemical modification: (a) PAN/O-MMT composite nanofibrous membrane; (b) PAN/O-MMT composite nanofibrous membrane after alkaline hydrolysis; (c) PAN/O-MMT composite nanofibrous membrane after carboxyl activation. (d) Confocal Laser Scanning Microscope (CLSM) photo-micrographs of PAN/O-MMT composite nanofibrous membranes after enzyme immobilization.

It was noted that the color of the PAN/O-MMT composite nanofibrous membranes ostensibly changed after chemical modification. Indeed, the alkaline hydrolysis produced a brownish yellow membrane that notably possessed much superior mechanical properties. The color change likely originated from intermediates that have poly-conjugated character generated from electrocyclization in the early hydrolysis stage. During this period of incubation, carboxyl and amide groups were formed that not only provide active sites for...
enzyme immobilization, but also gave rise to mechanical strength from a combination of hydrogen bonding effects. The ensuing EDC/NHS modification step for carboxyl activation changed the membrane color from brown-yellow to light yellow that is likely due to a chemical transformation/consumption of unstable intermediates.

It can be also noted from Figure 1 (a-c) that apart from the color changes, the fibrous microstructure also significantly changed. The original PAN/O-MMT composite nanofibers displayed good morphology with a diameter distribution over 200-300 nm (Fig. 1a). After alkaline hydrolysis, it was observed that the nanofibers assumed tortuous entanglements amongst one another in which adjacent nanofibers adhered to each other (Fig. 1b). This is likely induced by a chemical and physical “refining” arising from the hydrolytic action of OH⁻ at high temperatures. After EDC/NHS activation of the carboxyl group (Fig. 1c), it was observed that the fibers appeared to decouple as if they were relaxing to their original state. Specifically, the fibers generally repelled and redistributed individually.

Figure 1d illustrates a CLSM photo-image of PAN/O-MMT composite nanofibers containing the immobilized laccase. The FITC-labeled (green) laccase can be observed to be more or less uniformly distributed throughout the nanofiber network. This CLSM image also verifies that enzyme molecules have been successfully immobilized onto the surfaces of the solid support.
The chemical structure of PAN nanofibers was changed after alkaline hydrolysis and enzyme immobilization as confirmed by FT-IR presented in Figure 2. The original PAN nanofibers show a peak at 2241 cm$^{-1}$ for the nitrile group (–CN) and 1628 cm$^{-1}$ for the low level of vinyl groups (–C=CH–). The hydrolysis leads to the development of two new peaks at 1565 cm$^{-1}$ and 1668 cm$^{-1}$, respectively, indicating the presence of –COONa and –CONH$_2$ groups in the modified fiber. After enzyme immobilization, the FTIR spectrum of laccase-PAN nanofibers showed two peaks at 1564 and 1646 cm$^{-1}$, which can be attributed to amide I (the vibration of the C=O bonds) and amide II (a combination of C-N stretching and N-H vibration in protein backbone), respectively. Meanwhile, a new peak at 1073 cm$^{-1}$ appears, representing C-N bonds formed between the enzyme and PAN after laccase immobilization. This observation confirms the assertion that laccase is covalently immobilized onto the surface of PAN nanofibers.

Catalytic activity of free and immobilized laccase
Table 1. Kinetic parameters of the immobilized laccase.

<table>
<thead>
<tr>
<th></th>
<th>Enzyme loading (mg/g nanofiber)</th>
<th>Retention activity (%)</th>
<th>$K_m$ (µM)</th>
<th>$V_{max}$ (µmol/mg min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free Laccase</td>
<td>-</td>
<td>120.32</td>
<td>595.24</td>
<td></td>
</tr>
<tr>
<td>Immobilized Laccase</td>
<td>276</td>
<td>73.5</td>
<td>622.15</td>
<td>293.46</td>
</tr>
</tbody>
</table>

The amount of enzyme loading and kinetic parameters $K_m$ and $V_{max}$ are shown in Table 1. The enzyme loading is 276 mg per gram of support, a value that is higher than reported by Ran Xu (220 mg/g)\(^8\) whose work also used PAN nanofibrous membrane after chemical modification for laccase immobilization. The relative activity of the immobilized laccase was 73.5%, which is slightly higher than the laccase immobilized onto the amide-modified PAN nanofibers (72% of its specific activity).

The $K_m$ value of the immobilized laccase was much higher than that of free laccase while the $V_{max}$ value was significantly decreased. The difference between $K_m$ and $V_{max}$ after immobilization may be due to a lower accessibility between the substrate and active points of the immobilized enzyme because of higher diffusion limitations that arise from the space barriers of the support that result in a lower probability of forming an enzyme-substrate complex.\(^8\)

**Optimum pH and temperature**

Studies on the optimum pH and temperature for our system give information on suitable conditions for the enzymatic activity. Figure 4 shows the optimum pH and temperature of both free and immobilized laccase. The variation of pH has a pronounced effect on the enzyme stability likely from two reasons: first, a change in pH can denature the enzyme,
while secondly, pH can influence the dissociation of enzyme active sites; hence, restricting reaction with the substrate. 27 A pH range of 2-6 was therefore selected over which the enzyme activity could be evaluated.

The optimum pH for free laccase was 3, whereas that of immobilized laccase shifted to 3.5 (Fig. 3a). The same results were also observed in previous work with PAN/O-MMT in which it was used as a support for immobilized laccase via an adsorption method. 13 The basis for the observed results is that MMT intercalated within the PAN polymer matrix and was able to adsorb H\(^+\) from the buffer solution; thus, it was able to make the surface of the support more acidic than the buffer solution. Not surprisingly, the immobilized laccase demonstrated a higher activity over relatively higher pH. In addition, bound laccase could significantly broaden the operational pH range.

The optimum temperature for both free and immobilized laccase was 50 °C (Fig. 3b). In general, for free laccase, reduced or elevated temperature has a significant dampening effect on its activity in direct opposition to what we observed for the immobilized laccase. Over 30-70 °C, the variation of the enzyme activity was very small; even at 70 °C, it still retained more than 80% of its activity. Immobilization therefore can significantly improve enzyme activity despite fluctuations in temperature because of the presence of multi-linkages among nanofibers over which the enzyme molecules can maintain a stable conformation, thus preventing compromise of enzyme function.
Figure 3. Effects of pH (a) and temperature (b) on laccase activity.

Thermal stability

Figure 4. Thermal stability of free and immobilized laccase at (a) 30 °C and (b) 50 °C.

The thermal stability was studied at 30 °C and 50 °C and recorded every 2 h. The initial enzyme activity was set as 100%, while the relative enzyme activity at different time intervals was calculated; all results are shown in Figure 4.

At a lower temperature, both free and immobilized laccase showed better thermal stability than at higher temperatures. At 30 °C after 8 h, the free laccase retained 57 % of its initial activity, while the immobilized laccase maintained 85 % (Fig.4a). At 50 °C (8 h), the activity for both free and immobilized laccase were approximately 38 % and 66 %,
respectively (Fig. 4b). The results indicated a more preferable thermal stable laccase after immobilization. The improvement in resistance over temperature was probably due to a reduction in molecular mobility and conformational changes because of the immobilization on PAN/O-MMT composite nanofibers supports.  

Operational stability

Figure 5. Reusability of the immobilized laccase.

Reusability is one of the most important aspects for immobilized laccase. Figure 5 shows the operational stability at room temperature. After 5 cycles, it retained nearly 85% of its initial activity. Additionally, about 70% of the initial activity were retained after 10 cycles. The results shown indicate good reusability of the laccase immobilized on PAN/O-MMT composite nanofibers. This work showed better reusability that was able to maintain 70% of the initial activity after 10 cycles compared with other immobilization methods, whereas the reusability property of immobilized is essential for cost-effective use of the enzyme in biotechnological applications.
Storage stability

Figure 6. Storage stability of free and immobilized laccase at 4 °C.

Figure 6 illustrates the storage stabilities of free and immobilized laccase in aqueous solution. Free laccase showed continuous inactivation, while immobilized laccase exhibited negligible activity loss over two month. This result suggests that the multi-point covalent linkages for the laccase molecules effectively prevented the enzymes from being denatured and/or leaching.

The effect of initial concentration of CV on its removal

Figure 7. Effect of initial concentration on CV removal efficiency.

Figure 7 shows the effect of initial concentration of CV on removal efficiency. As the
initial concentration increased from 10 to 100 mg/L, the removal efficiency of CV by both free and immobilized laccase increased. However, when the concentration increased to 150 mg/L, the removal efficiency decreased. Due to diffusional limitations, immobilized laccase showed very low capacity in removing CV from aqueous solution when it was at low concentration.

**The effect of pH value and temperature on dye removal efficiency**

![Figure 8. The effect of pH value (a) and temperature (b) on dye removal efficiency.](image)

Variation of pH and temperature also affected dye degradation. The results of the removal efficiency of CV under different pH and temperature are shown in Figure 8.

When the pH increased from 2 to 5, CV removal efficiency increased; when pH further increased, the CV removal efficiency decreased (Figure 8a). As is well known, large quantities of H$^+$ in buffer solution compete with CV for adsorption sites on O-MMT. Thus, when the buffer solution becomes less acidic, the adsorption of CV is more efficient. However, when pH ~ 6, the laccase showed very low catalytic capacity, thus reducing the overall removal efficiency. This finding was consistent with that of Xu et al.$^{31}$

The optimum temperature for CV adsorption is 40 °C. It was revealed that higher
temperatures can assist CV molecules diffusion into the fiber matrix and improve adsorption; but when the temperature was further increased, laccase became partially denatured, thus decreasing the degradation efficiency. The results were similar to previous findings, possibly due to the common denaturing observed as a result of unfavorable conditions.\textsuperscript{31} 

The effect of time on dye removal efficiency

Figure 9. The effect of the free laccase (Lac), immobilized laccase, and the support on dye removal efficiency.

Figure 9 shows the removal efficiency of CV in a 3 h batch experiment. The total degradation of CV by free laccase was ~ 70 % over 3 h, while the adsorption ratio of CV by the support itself was 50 %. The removal efficiency of immobilized laccase is ~ 91 %, a value much higher than the degradation efficiency or adsorption effect itself, achieving equilibrium within 20 min, where the removal efficiency is higher than the total effect of both free laccase (45 %) and support (33 %). The result suggested a synergistic effect between the support itself and immobilized laccase. The synergistic removal efficiency was higher than the grapefruit peel,\textsuperscript{32} which adsorbed ~ 60% CV within 20 min and required 90 min to achieve 90% removal.
Conclusions

Electrospun PAN/O-MMT composite nanofibrous membranes after alkaline hydrolysis and carboxyl activation were successfully used as a support system to covalently immobilize laccase. CLSM confirmed that the surface of PAN/O-MMT composite nanofibrous membrane was uniformly coated with FITC-labeled laccase at a loading as high as 276 mg/g of nanofibrous membrane. Compared with free laccase, the stability of immobilized laccase was significantly improved despite significant fluxes in pH, elevated temperature, repeated usage, and extended storage. The removal of CV showed a synergistic effect between O-MMT and laccase, resulting in 91% of CV removal within 20 min. These results indicate that laccase immobilized on functional nanofibers demonstrates an attractive potential for remediating organic substrates in industrial waters, especially by the tandem adsorption and degradation phenomena that behaves in a remarkably efficient manner, and thus decrease the cost and encourage industrial applications.

Acknowledgments

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References


Graphical Abstract