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LACCASE IMMOBILIZED 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 ℃ and 50 ℃ 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 ℃ 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. [1] 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 [2] 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. [3,4] 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. [5]

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.[6,7] Recently, the paradigm of enzyme-based bioremediation for contaminated waste water has been gaining ascendancy 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. [8] 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_2 as the terminal oxidant to display

optimal behavior in decontamination schemes.[9]

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,

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 [18] 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. [19] 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

149 the PAN/O-MMT composite nanofibers. The samples were coated with a thin layer of gold
150 by sputtering before the SEM imaging.

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

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

162

163 Determination of enzyme activity

164 The activity of the free and immobilized laccase was assayed at 30 °C using ABTS as the
165 substrate. A detailed process was previously reported.[20] Specifically, 15 mM of an ABTS
166 solution was prepared by delivering 82.305 mg of ABTS to 10 ml of deionized water in a
167 centrifuge tube. The activity of free and immobilized laccase was determined according to
168 established assay protocols. In brief, the reaction assay consisted of 0.1 mM ABTS, 100 mM
169 sodium acetate buffer (pH = 4.5) and a suitable amount of free and immobilized laccase. The
170 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). [21]

$$1/v=(K_m/V_{max})(1/S)+1/V_{max} \quad (1)$$

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.

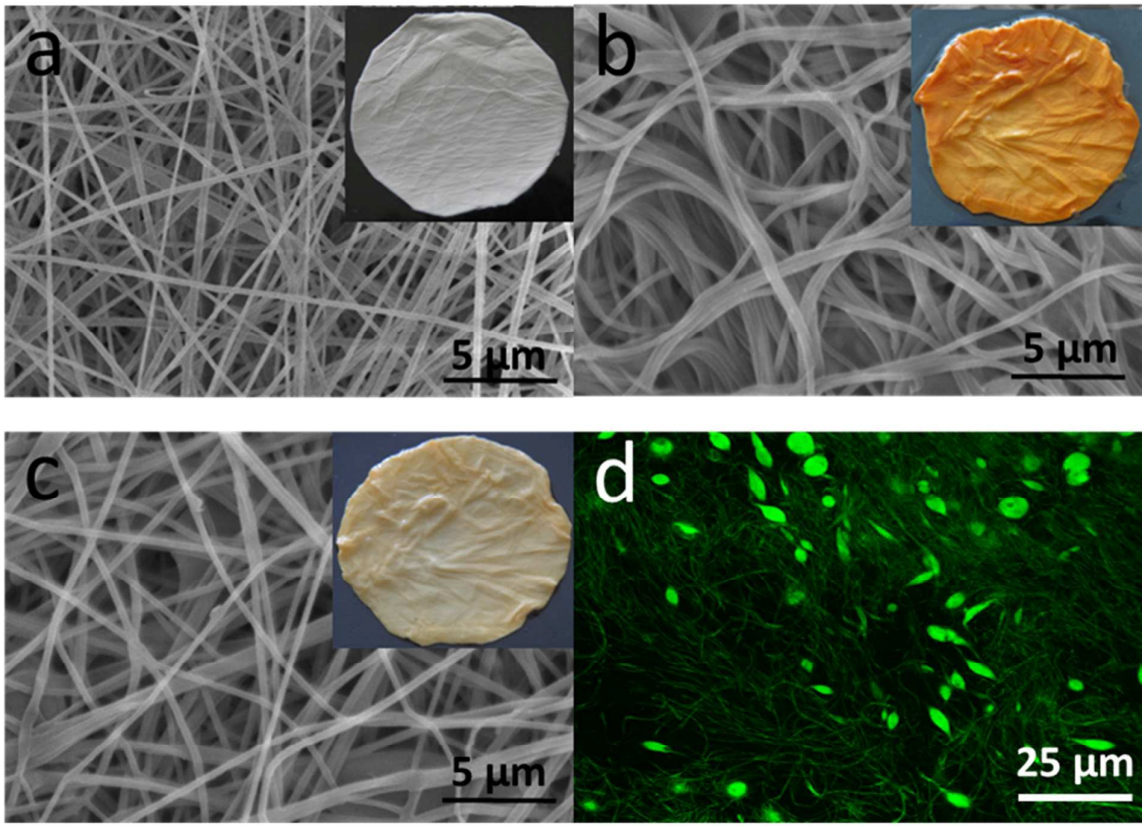


Figure 1. SEM and digital photographs of PAN/O-MMT composite nanofibers before and after chemical modification: (a) PAN/O-MMT composite nanofibrous membrane; (b) PAN/O-MMT composite nanofibrous membrane after alkaline hydrolysis; (c) PAN/O-MMT composite nanofibrous membrane after carboxyl activation. (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. [22-24] During this period of incubation, carboxyl and amide groups were formed that not only provide active sites for

225 enzyme immobilization, but also gave rise to mechanical strength from a combination of
226 hydrogen bonding effects. The ensuing EDC/NHS modification step for carboxyl activation
227 changed the membrane color from brown-yellow to light yellow that is likely due to a
228 chemical transformation/consumption of unstable intermediates.

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

238 Figure 1d illustrates a CLSM photo-image of PAN/O-MMT composite nanofibers
239 containing the immobilized laccase. The FITC-labeled (green) laccase can be observed to
240 be more or less uniformly distributed throughout the nanofiber network. This CLSM image
241 also verifies that enzyme molecules have been successfully immobilized onto the surfaces of
242 the solid support.

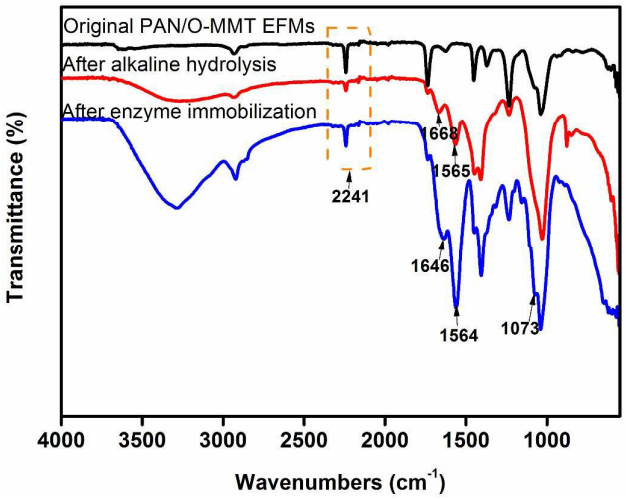


Figure 2. FTIR spectra of PAN/O-MMT composite nanofibers before and after alkaline hydrolysis and enzyme immobilization.

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-).[22] 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. [25] 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.[8, 26] Meanwhile, a new peak at 1073 cm^{-1} appears, representing C-N bonds formed between the enzyme and PAN after laccase immobilization.[8] 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.

	Enzyme loading (mg/g nanofiber)	Retention activity (%)	K_m (μM)	V_{max} (μmol/mg min)
Free Laccase	-		120.32	595.24
Immobilized Laccase	276	73.5	622.15	293.46

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.

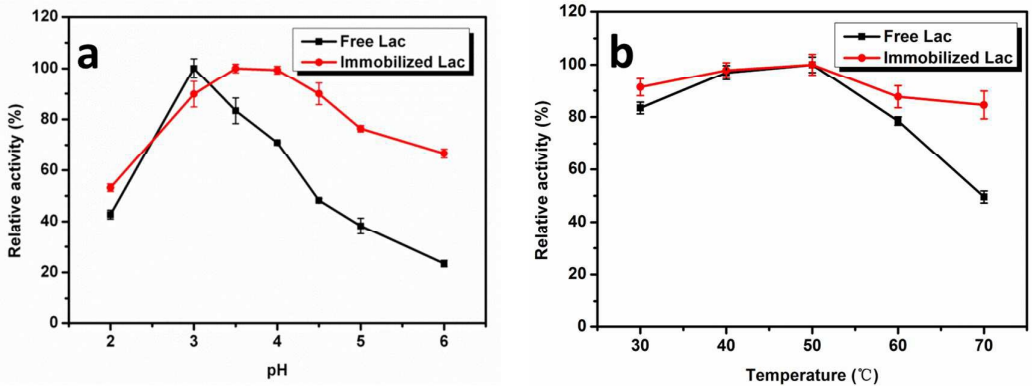


Figure 3. Effects of pH (a) and temperature (b) on laccase activity.

Thermal stability

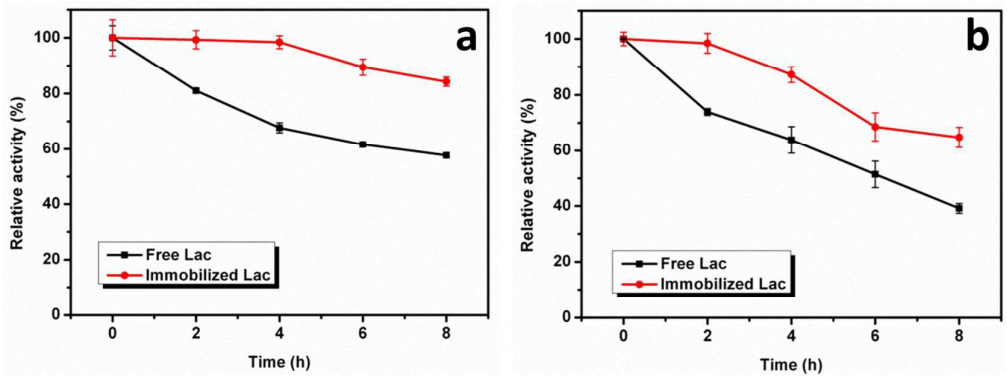


Figure 4. Thermal stability of free and immobilized laccase at (a) 30 °C and (b) 50 °C.

The thermal stability was studied at 30 ℃ and 50 ℃ 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.[28]

Operational stability

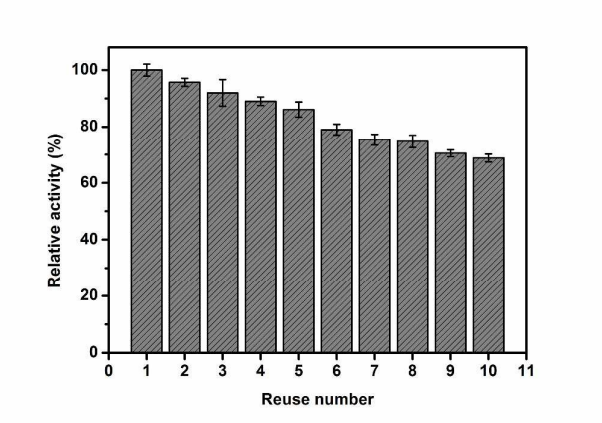


Figure 5. Reusability of the immobilized laccase.

Reusability is one of the most important aspects for immobilized laccase.[29] 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,[28,13] whereas the reusability property of immobilized is essential for cost-effective use of the enzyme in biotechnological applications.

Storage stability

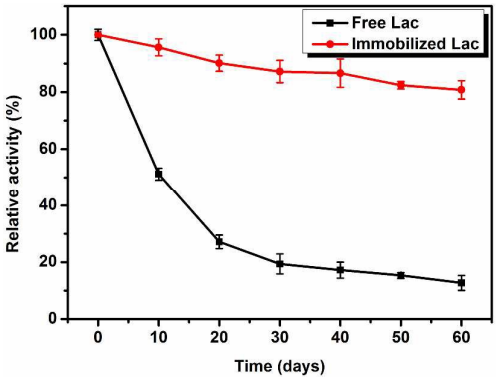


Figure 6. Storage stability of free and immobilized laccase at 4 °C.

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. [30]

The effect of initial concentration of CV on its removal

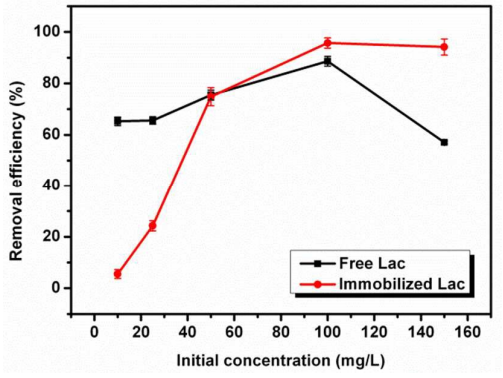


Figure 7. Effect of initial concentration on CV removal efficiency.

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

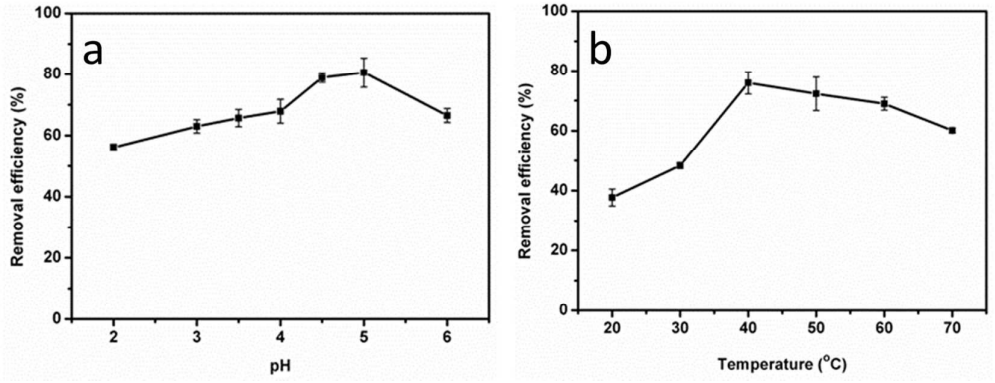


Figure 8. The effect of pH value (a) and temperature (b) on dye removal efficiency.

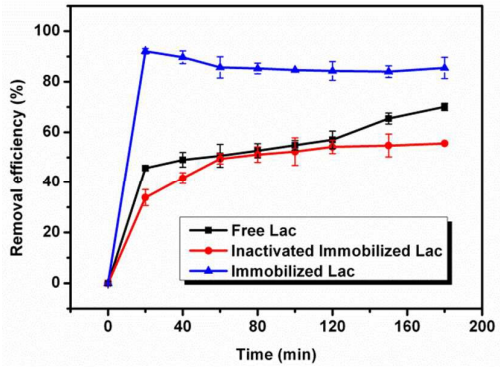
Variation of pH and temperature also affected dye degradation. The results of the removal efficiency of CV under different pH and temperature are shown in Figure 8.

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

364 temperatures can assist CV molecules diffusion into the fiber matrix and improve adsorption;
365 but when the temperature was further increased, laccase became partially denatured, thus
366 decreasing the degradation efficiency. The results were similar to previous findings, possibly
367 due to the common denaturing observed as a result of unfavorable conditions.[31]

368 The effect of time on dye removal efficiency



369

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

371

372 Figure 9 shows the removal efficiency of CV in a 3 h batch experiment. The total
373 degradation of CV by free laccase was ~ 70 % over 3 h, while the adsorption ratio of CV by
374 the support itself was 50 %. The removal efficiency of immobilized laccase is ~ 91 %, a
375 value much higher than the degradation efficiency or adsorption effect itself, achieving
376 equilibrium within 20 min, where the removal efficiency is higher than the total effect of both
377 free laccase (45 %) and support (33 %). The result suggested a synergistic effect between
378 the support itself and immobilized laccase. The synergistic removal efficiency was higher
379 than the grapefruit peel,[32] which adsorbed ~ 60% CV within 20 min and required 90 min to
380 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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Graphical Abstract