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Laccase Immobilization in Core-Shell Polymer Particles for Degradation of Anticancer Doxorubicin

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ABSTRACT

Doxorubicin (DOX) is an anti-cancer compound, which has been found in groundwater in quantities capable of negatively impacting the ecosystem. Efficient removal of DOX is necessary and can be achieved using laccase as a biocatalyst. The present work investigated the immobilization of laccase on core-shell polymeric support, the surface functionalization steps required for immobilization, and the effectiveness of the biocatalyst in the degradation of DOX in comparison to free laccase. The results revealed a laccase immobilization yield greater than 50%, with the amount of enzyme per mass of support being 1.138 to 1.230 mg of enzyme/g of support. The biocatalyst showed greater thermal resistance compared to the free enzyme, in tests carried out at temperatures from 30 to 70°C using ABTS as substrate. Furthermore, the immobilized laccase maintained its activity for more than five cycles of use. Regarding DOX degradation tests, degradation above 92% (comparable to the free enzyme) was obtained for all drug concentrations evaluated (250 μg L^{-1} and 500 μg L^{-1}) in a shorter time (180 to 240 min) compared to that achieved by the free enzyme (more than 540 min). This demonstrates the efficiency of the enzymatic derivative in the degradation process.

Keywords: Laccase. Immobilization. Core-shell polymer. Drug degradation. Doxorubicin.

1 INTRODUCTION

Antineoplastic drugs are widely used in the treatment of various types of cancer and are becoming more common as cases of the disease increase worldwide. Doxorubicin (DOX), for example, has been detected in wastewater from oncology hospitals and effluents from common treatment plants[1,3]. Given this, there is growing concern related to the negative impact of these compounds on the ecosystem. In this scenario, laccases are enzymes capable of catalyzing the oxidation of various substrates, such as phenols and aromatic or aliphatic amines. They are promising for the degradation of pharmaceutical compounds. However, there is a disadvantage in using free laccase due to its inactivation in wastewater and unavailability for reuse[6,7]. Immobilization of enzymes on a solid support is a proven strategy for biocatalyst reuse, product separation, recycling, and continuous process development. To achieve this, the enzyme support must present characteristics and immobilization conditions appropriate to sustain the activity of the enzyme, such as high chemical and mechanical stability, high specific area, hydrophilicity, and available functional groups. In this context, polymeric particles with a shell-core structure offer several advantages when applied as a support for enzyme immobilization, such as the ability to achieve specific properties that would not be achieved by individual materials[5]. Among the possible materials for the composition of the structure, polyacrylonitrile (PAN) has been investigated because it is a low-cost material that can be easily modified and functionalized to interact well with laccase[4,6]. The present work investigated the degradation of doxorubicin by laccase immobilized on polymeric particles with specific shell-core morphology composed of a polypropylene/polystyrene core and a polyacrylonitrile shell.

2 MATERIAL & METHODS

Poly(propylene-styrene)/polyacrylonitrile core-shell particles obtained as described by GURGEL *et al.,* (2024)[2] were evaluated as a support for laccase immobilization. Before immobilization, the particles were functionalized. The steps used to functionalize the polyacrylonitrile (PAN) surface were described by TAHERAN *et al.* (2017)[4] and VIEIRA *et al.* (2023)[6], consisting of four steps: (I) alkaline hydrolysis, (II) acid hydrolysis, (III) amination and (IV) treatment with glutaraldehyde. From this, the possibility of immobilizing the laccase without functionalizing the support or reducing the number of steps was investigated. Thus, five different supports were obtained concerning the functionalization steps used, aiming to reduce costs in the process, as described in Table 1.

Table 1 Nomenclature and description of prepared CS supports

Support	Description
CS-0	Core-shell particles without any functionalization step
CS-I-II	Core-shell particles functionalized with steps I and II
CS-I-II-III	Core-shell particles functionalized with steps I, II, and III
CS-I-II-III-IV	Core-shell particles functionalized with all the steps described
CS-I-IV	Core-shell particles functionalized with steps I and IV

After each functionalization step, samples were taken to perform FTIR analysis to evaluate the incorporation of specific functional groups on the surface of the material. For immobilization, each support was treated with laccase solution at a concentration of 0.25 mg mL^{-1}. For all tests, immobilization kinetics were monitored for 7 h, measuring the enzymatic activity of the solution in the oxidation of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS). After immobilization, the biocatalysts obtained were washed and the immobilization yield was determined from the decay of enzymatic activity in the supernatant. The support with the lowest number of functionalization steps and which maintained good immobilization performance was chosen for the characterizations. The enzyme derivative was then characterized for thermal stability (at temperatures of 30°, 50°, 60°, and 70° C), stability at different pHs (3, 5, 6, and 8), storage stability (at room temperature and refrigerator), the operational stability and kinetic parameters of the Michaelis-Menten equation were also determined. Doxorubicin degradation tests were carried out under conditions of temperature equal to 30 °C and pH 6, conditions close to those found in real effluents. For these tests, DOX concentrations of 250 and 500 μg L^{-1} were used. The operational stability of the enzyme derivative in DOX degradation was also monitored.

3 RESULTS & DISCUSSION

The results of FTIR analyses and immobilization kinetics for each support (treated with different functionalization steps) allowed us to observe that the supports treated with NaOH (alkaline hydrolysis) and glutaraldehyde offered a higher laccase immobilization yield, thus, the two supports with the highest immobilization performance were CS-I-II-III-IV and CS-I-IV, as can be seen in the results presented in Figure 1 and Figure 2.

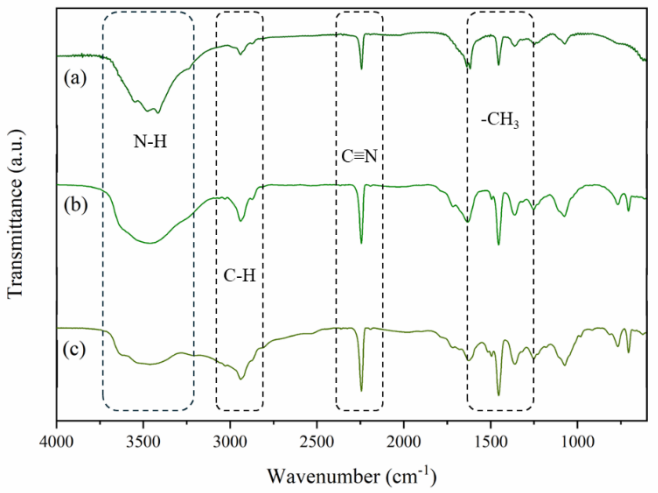

Figure 1 FTIR spectrum of surface samples of CS-I-II-III-IV (a), CS-I-II-III (b), and CS-I-IV (c)

Through FTIR results, the presence of the characteristic peak of polyacrylonitrile (C≡N) was noticed; there is also the presence of the -NH group, indicated by the wave numbers of 2245 cm^{-1} and 3460 cm^{-1}, respectively[8]. After treatment with glutaraldehyde, no new functional groups appear, but it is indicated that the polymer is cross-linked, which is desired in most enzyme immobilization processes. Despite the new groups added, what was observed was that there are still non-functionalized nitrile groups available.

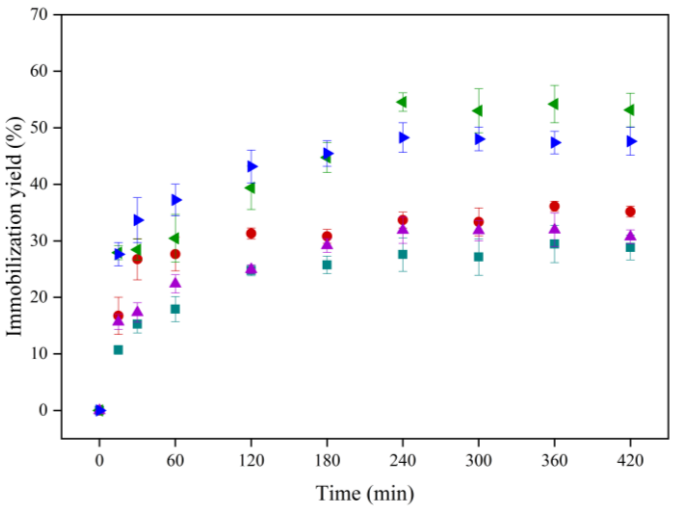

Figure 2 Immobilization yield as a function of time for supports (using 0.25 g L-1 initial concentration of laccase, 8 mL of volume, and 0.85 g of support), where (●) CS-0, (■) CS-I-II, (▲) CS-I-II-III, (◀) CS-I-II-III-IV and (▶) CS-I-IV

From Figure 2, it is observed that for all supports, the maximum immobilization yield is reached within 2 to 4 hours after the start of contact between the laccase solution and the CS particles. It is also possible to note that the supports functionalized with steps I and II (CS-I-II) or steps I, II, and III (CS-I-II-III), as shown previously, offered affinity with laccase similar to the support without any functionalization step (CS-0). These supports offered around 30% immobilization yield, while the supports that were treated

by stage I and stage IV of functionalization reached 50% immobilization yield (supports CS-I-II-III-IV and CS-I-IV). This behavior indicates a maximum capacity for laccase to bind to the supports in question. It may be related to several factors, including the availability of binding sites on the support (functional groups on the surface of the particles), the structure and spatial conformation of the laccase (which may limit accessibility to some binding sites), and even some kinetic factors. This maximum amount that can be immobilized on the support is equivalent to approximately 1.14 milligrams of laccase per gram of support for the CS-I-IV support and approximately 1.28 mg of laccase/g of support for the CS-I-II-III-IV support.

Based on these results, the support with the lowest number of steps (CS-I-IV) was selected as the enzymatic derivative of this study. In relation to the free laccase, the laccase immobilized on the CS-I-IV support demonstrated greater thermal stability, maintaining more than 80% of the activity at a temperature of 50° C. As for operational stability, the enzymatic derivative maintained 60% of the activity after five reuse cycles. The kinetic parameter values found (K_M and V_{max}) indicated high affinity between the derivative and the substrate (ABTS), which were 1.0299 mM and 61.713 U L^{-1}, respectively. The results obtained for DOX degradation are shown in Figure 3.

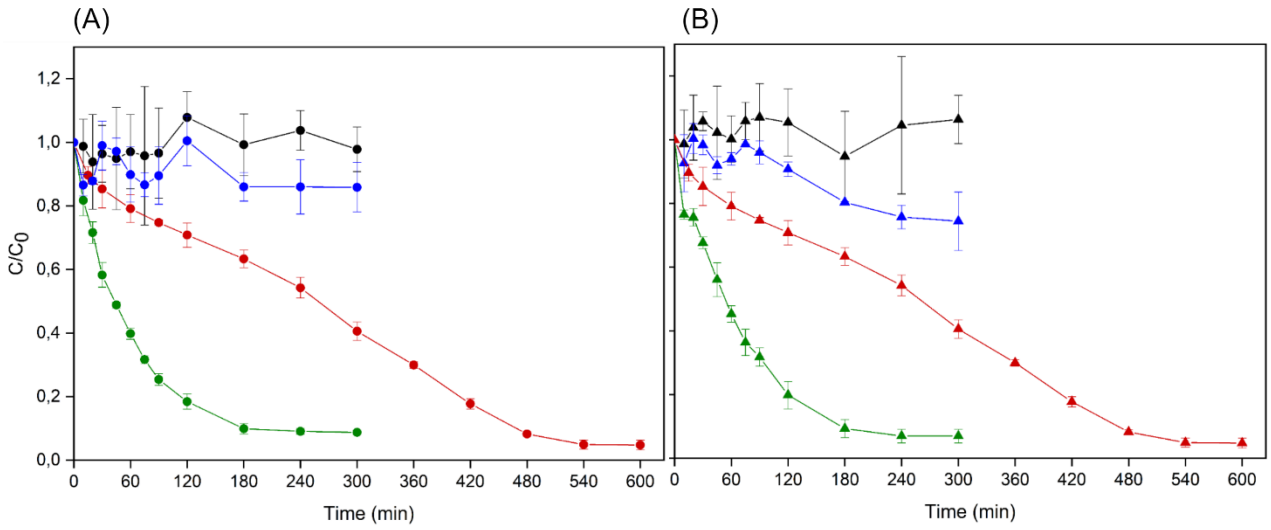

Figure 3 DOX removal kinetic curves by: (A) Hydrolysis (●), Adsorption (●), Degradation with immobilized (●) and free laccase (●) at DOX concentration of 250 µg L-1; and (B) Hydrolysis (▲), Adsorption (▲), Degradation with immobilized (▲) and free laccase (▲) at DOX concentration of 500 µg L-1 (using 1.5 g of enzyme derivative and for the free enzyme assay an amount of 1.7 mg of laccase)

For tests using a concentration of 250 µg L^{-1}, the immobilized laccase was able to degrade approximately 92% of DOX in 180 minutes. For tests using 500 µg L^{-1}, the immobilized laccase showed even more promising results. In 180 minutes, the degradation was 91%, reaching 93% in 240 minutes, while the free laccase showed degradation of 96% in around 480 minutes, indicating that the immobilization of the enzyme to the support was able to improve the degradation capacity of the laccase. DOX by laccase. Furthermore, the immobilized laccase could be reused at least five times, when it still maintained activity above 60%; After the sixth reuse cycle, the derivative promoted 29% DOX degradation.

4 CONCLUSION

The immobilization of laccase to the shell-core polymeric support was able to provide significant advantages, including increased DOX degradation capacity, greater stability, as well as ease of recovery and reuse, which can contribute to the economic viability of the process.

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