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LACCASSES STABILIZATION BY COVALENT IMMOBILIZATION ONTO FUNCTIONALIZED MAGNETIC AND SEPABEADS SUPORTS

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Introduction

Lacases (benzendiol: oxygen reductases) belong to the group of copper protein enzymes, which catalyze the oxidation of substrates, generally phenolic, with the simultaneous reduction of molecular oxygen to water [1]. Laccases present advantages in food, pharmaceuticals textile and paper fields, in biodegradation of environmental pollution [2] but their use at an industrial level has been restricted due to: low stability, reusability issues, high sensitivity to denaturing agents, and high production costs [3]. Although the immobilization of laccases has been previously reported the development of efficient supports that can retain a higher enzymatic activity during immobilization remains an interesting goal [4].

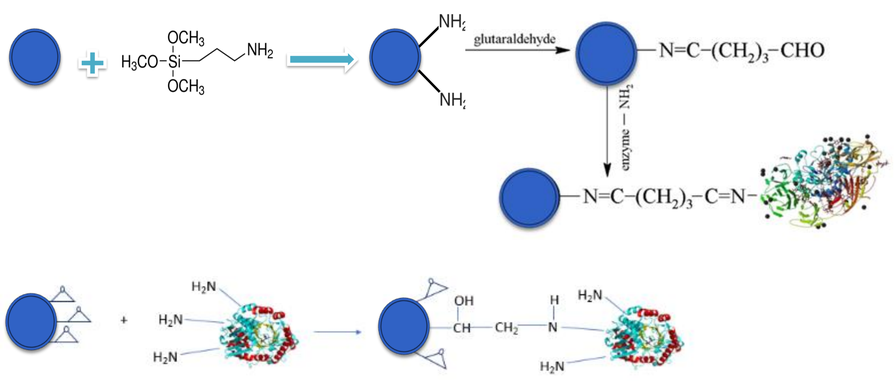
Aim of the study

In this work, two native commercially available laccases from *Aspergillus* sp. and *Trametes versicolor* were covalently immobilized onto six functionalized solid supports: three magnetically and three methacrylic polymers matrices (ReliZyme™). Compared to the well-known Fe_3O_4 magnetic particles, in this work, Ni-Zn or Ni-Zn-Co based magnetic particles spinel ferrites (MFe_2O_4) with different metallic cations (M: Zn, Mn, Co, Cr, Ni) were used. These particles have attracted interest due to their magnetic properties like superparamagnetism, spin glass behavior, with a range of applications in different fields such as magnetic recording, high frequency electronic cores, biomedical applications [5].

Results and discutions

Screening of supports for covalent immobilization

Six solid supports were selected: three magnetic supports containing different amounts of Co, Ni and Zn and three methacrylate resins bearing amino and oxirane functional groups. The immobilization was performed as presented in Scheme 1.



Characterization of magnetic supports

The magnetic particles were obtained by the co-precipitation method [5]. The size distribution of the particles was measured by laser particle size analyzer (Figure 1), showing that the particles average diameter was less than 10 µm. The magnetic parameters of the powders, measured by vibrating sample magnetometer (VSM), are presented in Table 1. The VSM values indicate that for the Co containing samples the magnetic parameter values are slightly higher.

Table 1 Magnetic parameters of the magnetic particles measured by vibrating sample magnetometer (VSM)

	Saturation magnetization, Ms emu/g	Remnant magnetization, Mr (emu/g)	Coericivity, Hc (Oe/g)
$Ni_{0,5}Zn_{0,5}Fe_2O_4$	53	1,44	54
$Ni_{0,4}Co_{0,2}Zn_{0,4}Fe_2O_4$	56	2,1	110

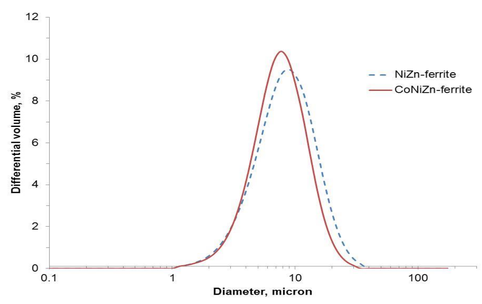
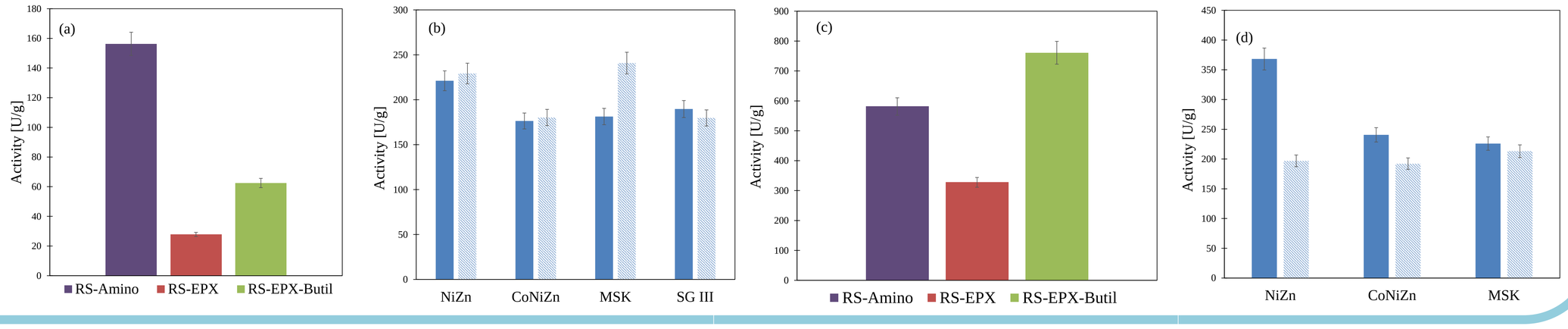


Fig. 1. The size distribution of the obtained magnetic particles

Enzymatic activity of immobilized laccases

The performances of the immobilized laccases were first evaluated using 2,6 dimethoxyphenol as substrate. The results are presented in Figure 1. a-d

Fig. 2. *Aspergillus sp*. and *Trametes versicolor* laccases activity immobilized on metacrylic resins functionalized with amino and epoxy groups (a), (c) and magnetic particles functionalized using two different silane precursors 3-aminopropyltrimethoxysilane (blue) 3-aminopropyltriethoxysilane (blue line) (b), (d)



Characterization of the immobilized laccases

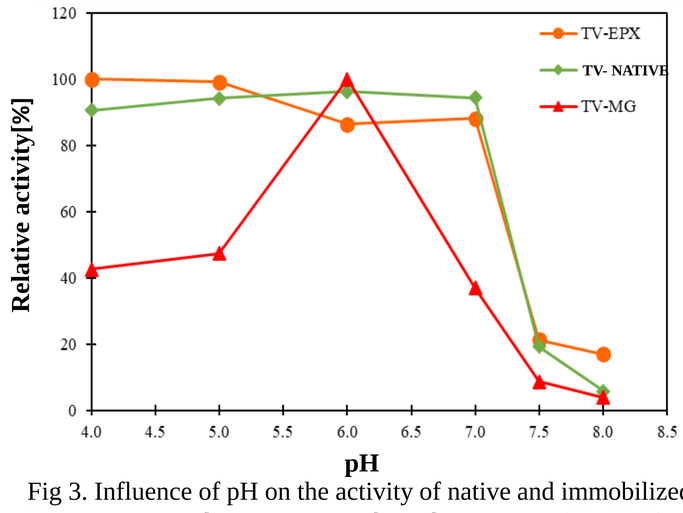


Fig 3. Influence of pH on the activity of native and immobilized Trametes versicolor on epoxymethacrylate support (TV-EPX) and on MSK magnetic support functionalized with 3-NH2-PrTMOS group (TV-MG)

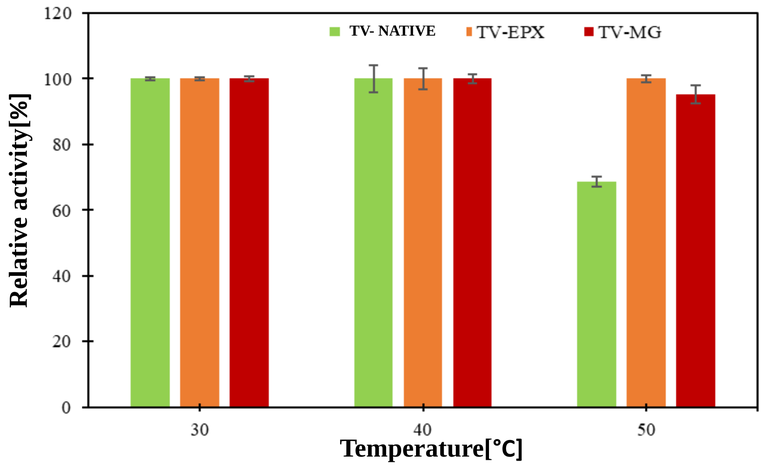


Fig 4.The effect of temperature on the activity of the immobilized laccase from *Trametes versicolor*

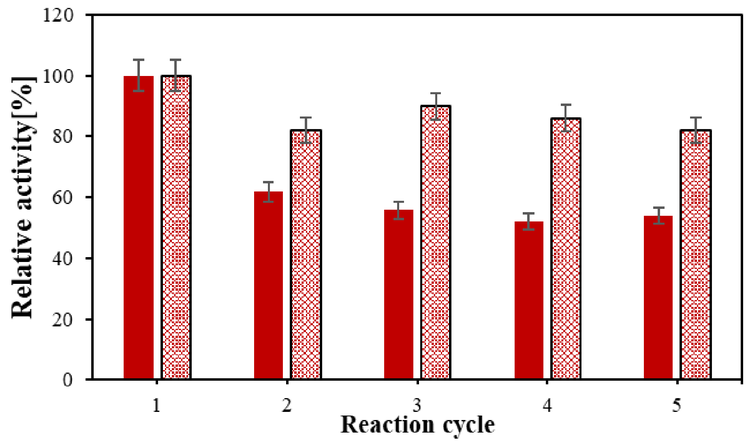


Fig. 5. Operational stability of *Trametes versicolor* laccases preparate immobilized on magnetic nanoparticles functionalizated with 3-NH_2-PrTMOS group without (red) or with the reducing action of $NaBH_4$ (dashed red)

Conclusions

- ✓ **The laccases from Trametes versicolor and Apergillus sp. were successfully immobilized. by covalent binding obtaining 24 enzymatic preparations. The highest values of enzymatic activity, for *Trametes versicolor* - 760 µmol / min / g biocatalyst, were obtained when epoxymethacrylic resin was used as support. The highest enzymatic activity using magnetic nanoparticles was obtained for the *Trametes versicolor* lacase 360 µmol / min / g biocatalyst, on NiZn support.**
- ✓ **The immobilized biocatalysts were successfully reused in fivereaction cycles, showing excellent operational stability.**
- ✓ **The reduction of the imine bound with NaBH4 resulted in an increase in operational stability of approximately 20%.**

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