

Covalent Organic Frameworks as hydrophobic supports for *Candida rugosa* lipase immobilization

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ABSTRACT

Recent investigations have proven the advantages of enzyme adsorption within covalent organic frameworks (COFs). However, a comparative analysis of different COFs' interactions with these enzymes remains unexplored. In this study, we synthesized and characterized a library of 5 COFs. Through the immobilization of the commercial lipase *Candida rugosa* lipase B (CRL), we gained insights into the nano-scale interactions of an enzyme-COF system. First, we observed that neither COFs by itself altered the hydrolytic activities under the fluorescent model-substrate methylumbelliferyl heptanoate. Then, the immobilization presented enhanced adsorption of 99,4 to 99,9% of the CRL into all 5 COFs-CRL. The hydrolytic activity of the immobilized systems ranged from 12 to 353 U (nmoles/min.(ugCRL)), compared to 101.21 U for the non-immobilized form of CRL. The COF RIO-17-CRL system exhibited a 3.5-fold increase in activity compared to the free CRL. We also tested the transesterification activity of the complex COF-CRL under canola oil conversion on fatty acids and we found a conversion of 90,1% after 72 hours. Finally, we tested RIO-17-CRL in a plug flow reactor model. Altogether, these results indicate that COFs can be a plausible and highly effective platform for enzyme immobilization, enhancing both catalytic activity and operational stability for industrial biocatalysis applications.

Keywords: Covalent Organic Frameworks (COF). Lipase immobilization. Nanomaterials.

1 INTRODUCTION

Nanomaterials have catalyzed a transformative shift in the landscape of scientific inquiry and technological innovation. Among these materials, covalent organic frameworks (COFs) have emerged as a captivating class of nanoscale architectures, characterized by their precise structure and exceptional chemical tunability. (Gan et al 2021; Li et al 2020; Sun et al 2018).

The interest of COFs lies in their intricate design, wherein organic building blocks are meticulously assembled into periodic, crystalline networks. These structures offer a level of control and adaptability rarely seen in conventional materials. COFs exhibit an impressive surface area, robust chemical stability, and tailored porosity, rendering them ideal candidates for a myriad of applications. (Oliveira et al 2020).

Enzyme immobilization within COFs has opened exciting prospects in the realm of catalysis. These frameworks provide a solid substrate for enzyme attachment, enhancing their stability and recyclability. The synergy between COFs and enzymes has unlocked novel avenues for catalytic transformations, ranging from the synthesis of fine chemicals to the degradation of environmental pollutants. Such innovations hold immense promise for sustainable chemistry and industrial processes. (Gan et al 2021; Li et al 2020)

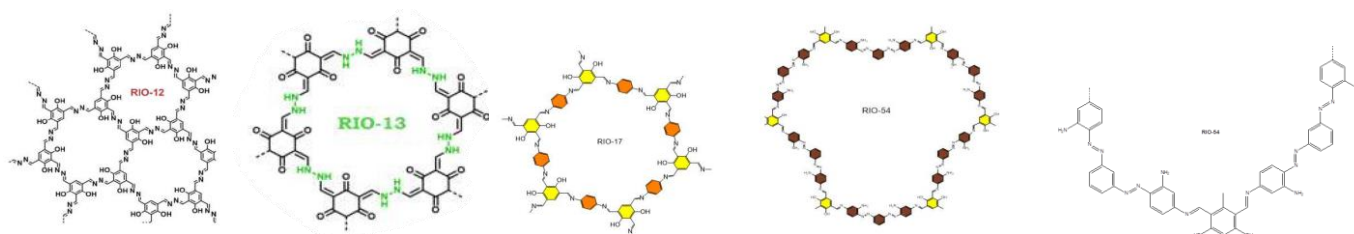


Figure 1 Nanomaterials base-structures of the Covalent Organic Framework synthesized at this work, to enzyme immobilization. COFs are namely RIO-nn.

At the same context, lipases, a class of enzymes (E.C.3.1.1.3.), have been used as catalysts in diverse industry fields due its versatility in catalyzing a diverse array of chemical reactions. The unique catalytic properties of lipases have been harnessed in various industrial processes, including the production of pharmaceuticals and the development of greener chemical technologies. When immobilized on COFs these enzymes exhibit enhanced catalytic efficiency and selectivity, marking a pivotal advancement in catalytic science (Feng et al 2024). As we envision the future, the integration of COFs, enzymes, and palladium can help the current challenges in chemistry (Oliveira et al 2020).

At this manuscript, we aim to elucidate the influence of distinct COFs on the immobilization of lipases. Our research endeavors to provide comprehensive insights into this dynamic interplay, with the goal of advancing the field of catalysis and contributing to the growing body of knowledge surrounding nanomaterials and enzyme-mediated reactions.

2 MATERIAL & METHODS

The COFs used in this project, named RIO-n (our acronym for Reticular Innovative Organic frameworks), included RIO-12 and RIO-13, whose synthesis has been previously described by our research group (Maia et al., 2018), RIO-17, previously reported as IISERP-COF15 (Chakraborty et al., 2019). RIO-54 production is first synthesized by our group and we did not published yet. And RIO-55, a CON (Covalent Organic Networks), was produced using dyes as building blocks (Freitas et al., 2020). All were obtained from solvothermal condensation reactions. Triformylresorcinol (TFR) was obtained through a previously described and adapted synthesis method (Maia et al., 2018). The synthesis of Triformylfluoroglucinol, was followed as described by Chong et al 2003.

3 RESULTS & DISCUSSION

The synthesized COFs, namely RIO-12, RIO-13, RIO-17, RIO-54, and RIO-55, exhibited high crystallinity, as confirmed by X-ray diffraction (Figure 2). Each COF structure was characterized by distinct pore sizes and surface areas, influencing their interaction with the immobilized *Candida rugosa* lipase B (CRL).

The 5 nanomaterials produced (Figure 1) presented high crystallinity as measured by X-ray Diffraction (Figure 2)

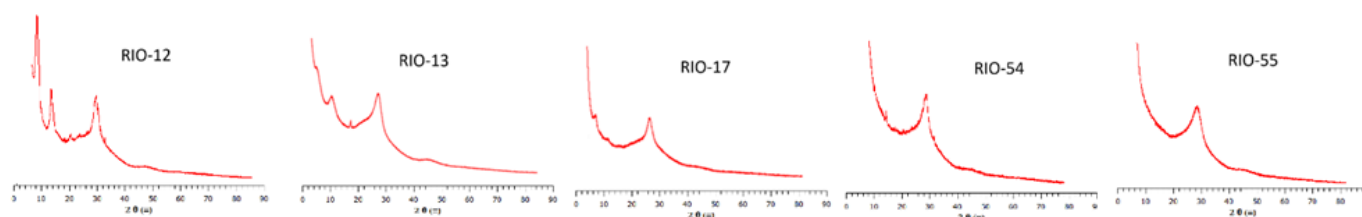


Figure 2 illustrates the X-ray diffraction patterns of the COFs synthesized in this study, demonstrating their crystalline nature and structural integrity.

The hydrolytic activities of the COF-CRL complexes were evaluated using the fluorescent model-substrate methylumbelliferyl heptanoate. The immobilization of CRL onto all five COFs resulted in enhanced enzyme loading, ranging from 99.4% to 99.9%, compared to the non-immobilized form. This immobilization strategy significantly affected the catalytic performance, with observed activities ranging from 12 to 353 U (nmoles/min.(μ gCRL)), compared to 101.21 U for free CRL. Notably, the COF RIO-17-CRL complex exhibited a remarkable 3.5-fold increase in activity compared to free CRL.

Furthermore, the transesterification activity of the COF-CRL complexes was assessed using canola oil, achieving a conversion rate of 90.1% after 72 hours, highlighting their potential in biodiesel production and other biotechnological applications. In a plug flow reactor model, the COF-CRL systems, particularly RIO-17-CRL, demonstrated sustained catalytic efficiency over extended periods, underscoring their stability and reusability in continuous-flow processes (data not shown).

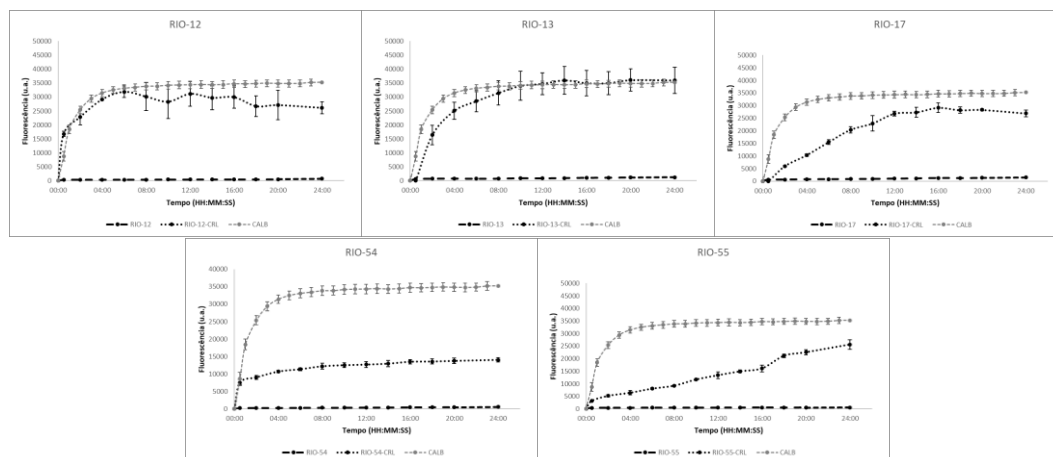


Figure 3 General profile of hydrolytic activities under the fluorescent model-substrate methylumbelliferyl heptanoate presented a Michaelis and Menten profile of behaviour. The systems represented are constituted by COFs (dashed lines), COFs with the *Candida rugosa* lipase (CRL) immobilized (dotted lines), forming the COF-CRL shell-core, and the free-form of CRL (grey lines).

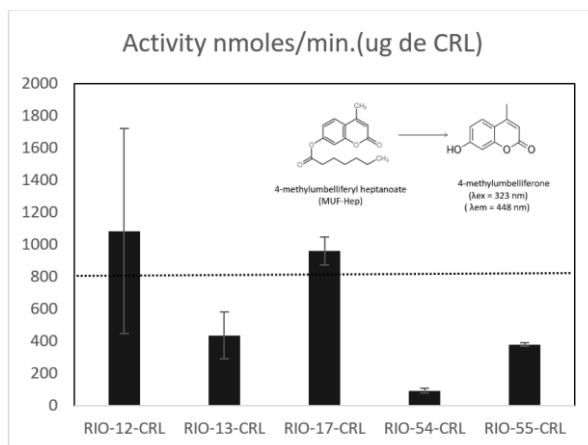


Figure 4 Hydrolytic activities under the fluorescent model-substrate methylumbelliferyl heptanoate are shown. The 5 systems represented are constituted by COFs and the *Candida rugosa* lipase (CRL) immobilized, forming the COF-CRL shell-core. COFs are namely RIO-*nn*. Dotted lines represent activity of the free form of the *Candida rugosa* lipase.

4 CONCLUSION

In this study, we have explored the synergistic potential of covalent organic frameworks (COFs), *Candida rugosa* lipase B (CRL) in the field of heterogeneous biocatalysis. Our research has focused on the immobilization of CRL onto COFs, aiming to enhance catalytic performance and expand the versatility of the system. We have observed promising results in terms of specific activity, highlighting the potential of this innovative catalytic platform.

This innovation has significant industrial and societal implications, aligning with green chemistry principles and sustainability goals. It offers potential applications in pharmaceuticals, fine chemicals, and sustainable energy production, enabling cleaner and more efficient catalytic processes. The COF + CRL contributes to reduced waste, energy consumption, and environmental impact, making it a valuable tool in the pursuit of greener and more sustainable chemical processes.

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