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EFFECT OF MAGNETIC FIELD ON LIPASE STRUCTURE AND PERFORMANCE

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ABSTRACT

Lipases are widely used in several reactions at large scale, being used in the free and immobilized forms. Owing to this, many strategies have already been developed trying to improve their performance, such as magnetic field (MF) application. This review aims to explore and discuss the progress concerning the application of magnetic field on lipase structure and performance. It was verified, based on a bibliometric keyword analysis of 30 articles, the studies in this field can be categorized in three main groups, where the first is focused on MF application during lipase immobilization, the second category studies magnetic fluidized bed reactors to different reactions, mainly transesterification. The last category is depicted as a connection between the two others, but more focused on alternating magnetic field. Considering a comprehensive and rigorous analysis, it is possible to conclude that magnetic field can not only affect lipase structure, but also shows great results related to improve lipase performance.

Keywords: Enzyme immobilization, magnetic nanoparticles, magnetically bed reactors, alternating magnetic field.

1 INTRODUCTION

Lipases (triacylglycerol acylhydrolases, EC 3.1.1.3) are α/β hydrolases that have a catalytic triad consisting of catalytic residue (aspartate or glutamate), histidine, and a nucleophilic residue (serine). Furthermore, lipases are interfacial enzymes, which means their active site may be covered by a lid (protein structure), potentially interfering with the contact between substrate and active site in aqueous medium.[1] Despite the particularity of lipases concerning interfacial activation, they are among the most used enzymes due to their ability to catalyze reactions such as hydrolysis, esterification, transesterification, and alcoholysis[2].

Given the significance and distinctive characteristics of lipases, several strategies have been developed to improve lipase activity, structure, and stability, for example immobilization, protein engineering, and the use physical approaches, such as high-pressure treatment, ohmic heating, and magnetic field (MF). According to Want et al.[3], MF has the advantages of having convenient operation, simplified equipment, no secondary pollution, and low cost. Besides, it can be applied either on immobilized lipases or enzymatic reaction systems [4]. Based on these factors, this review aims to explore and discuss the progress concerning application of magnetic field on lipase structure and performance.

2 MATERIAL & METHODS

To better understand the research in the field, we conducted a bibliometric analysis on the Web of Science© Core Collection. This database has been widely used for bibliometric analysis in different fields because it contains many indexed journals with high scientific relevance. For this analysis, the keywords "lipase*" and "magnetic field" were used associated with Boolean operator AND. Following this search, a total of 118 documents were found, being 116 experimental articles. Next, we conducted a screening based on the titles and abstracts of the articles, selecting only those related to the application of magnetic fields on lipase or in bioprocesses involving lipases (either free or immobilized). Studies solely focused on lipase immobilization on magnetic nanoparticles were excluded, since immobilization strategies have been extensively explored in this field. After this screening process, 30 articles were selected for further analysis, and a bibliometric analysis to analyze the keywords was performed using the software VOSviewer.

3 RESULTS & DISCUSSION

Based on the collected data an analysis of keywords was carried out using the author keywords containing at least 2 co-occurrences. The result is shown in Figure 1.

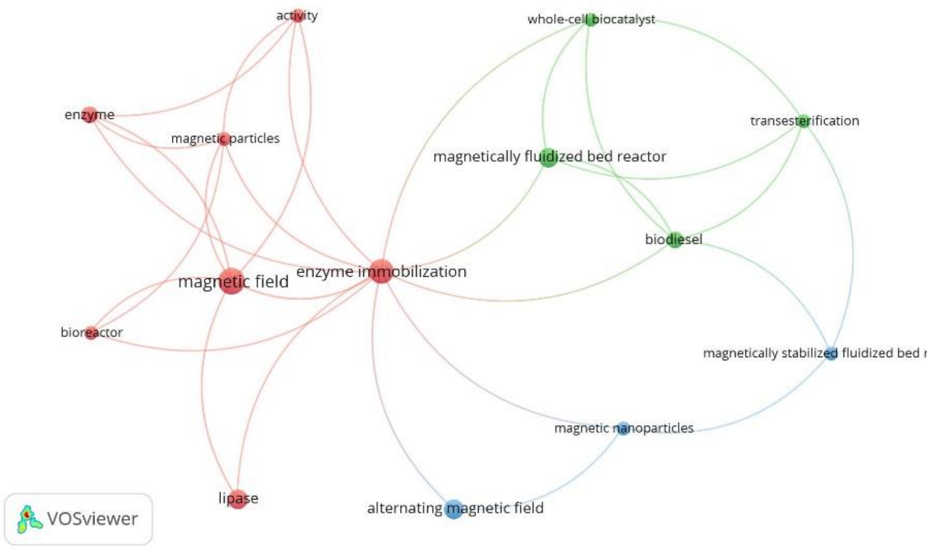

Figure 1 - Keyword analysis of scientific publications on lipase and magnetic field on Web of Science Core Collection database

As depicted in the Figure 1, three main clusters are observed, being the first related to the use of magnetic field on enzyme immobilization, characterized mainly by magnetic nanoparticles (red), second concerning the use of magnetic fluidized bed reactors in oil transesterification using whole-cell biocatalyst (green) and the last about magnetic stabilized fluidized bed reactors and alternating magnetic field related to magnetic nanoparticles and enzyme immobilization (blue). It is important to note that, although we have expression such as magnetic particles in the figure, we are not focusing on lipase immobilization process on magnetic nanoparticles in this review since it is a subject already widely explored, so in these studies the authors applied magnetic field in a process containing immobilized lipases on magnetic particles [4–6]

Proceeding with a more thorough investigation into the first cluster, Wang et al.[3]studied the application of magnetic field to improve the activity of immobilized *Candida antarctica* lipase B. Authors reported activity of treated lipases at 0.175 T was 2-fold the original enzyme, also an improvement concerning catalytic efficiency, substrate affinity, thermal stability, and reusability. In relation to enzyme structure, they found changes in enzyme structure after the magnetic field treatment, accessing it by fluorescence spectroscopy and Fourier transform infrared spectroscopy. In alignment with this outcome, Fraga et al.[7] also reported changes in Eversa® Transform 2.0 structure and activity after being treated by magnetic field of 0.7 T and 1.34 T. The behavior in both studies is similar, Wang et al.[3] reports that fluorescence intensity of treated samples was much higher than the original samples, and Fraga et al. [7] as well, correlating it with changes in spatial structure of the enzyme making Tryptophan more exposed.

Prado et al.[8] report activity increment and stability of treatment effect throughout the time, but also highlight that these changes are reversible, being necessary to understand the phenomena. In their study, Wang et al.[3] suggest a protein energy landscape model based on the theory of protein folding. In this model, the increase of energy may lead protein from a stable stage to an active stage and then to inactivation, where they have more polypeptide chain than β-strand and α-helix structure.

Advancing to the second cluster, which is focused on the application of magnetic field in reactors, Silva et al. (2023)[9] conducted an esterification process to produce 2-ethylhexyl oleate in a continuously operated magnetically stabilized fluidized bed reactor (MSFBR) using lipase immobilized on magnetic poly(styrene-co-divinylbenzene). Authors reported that under magnetic fields of 5, 10, and 15 mT productivity was 0.752, 0.800 and 0.850 mmol g^{-1} h^{-1}, respectively. A noteworthy point, they believe that MSFBR provided better agitation and mixing, which lead to increase in reaction rate. An additional research inquiry Chen et al.[10] observed magnetic whole-cell biocatalysts (MWCBs) could be used to biodiesel production in a magnetically fluidized bed reactor achieving yields higher than 80% by applying magnetic field intensity from 6 to 20 mT. Nonetheless, they noted that field intensity over 14 mT causes MWCBs agglomerates due to the presence of strong interparticle attractive forces. Furthermore, at 22 mT a fully compressed form of MWCBs was observed and a yield of less than 60% was achieved. In this context, it is evident that the field intensity plays a crucial role in the process.

Following this, a subsequent and more recent study, drawing upon Chen´s et al.[10] framework verified four variables to optimize conditions of MWCBs-catalyzed transesterification. They reported biodiesel yield of 89.0 under the following best conditions: 10 wt%, 17 mL^{-1} min^{-1}, 13.2 mT and 35 ˚C. Moreover, after 10 cycles a yield of 82.5% was found, have elucidated that in the presence of an appropriate magnetic field, the MWCBs disperse effectively within the MFBR, thereby mitigating the risk of microsphere rupture and cell leakage resulting from particle attrition and collisions with the reactor wall[11].

Reaching the third cluster, the subject concerning alternating magnetic field (AMF), which is a result of both Néel and Brown relaxation, stands out. According to some studies, AMF may activate magnetic carriers, make magnetic nanoparticles rotate and collide creating magnetic hyperthermia, disturb enzymatic kinetic improving collision rate between biocatalyst and substrate, change crosslinking enzyme aggregate aggregation, etc. [12,13]. AMF has been employed to induce alterations in enzyme properties and optimize reactor processes. Dai et al.[14] showed an increase in acetylation of 1-methyl-3-phenylpropylamine using Fe_3O_4-APTES-CS_2-lipase magnetic nanoparticle when magnetic field intensity increased in the range of 4 to 12 Gs. Beyond 12 Gs, a decrease in conversion was observed, which happens because there is a positive correlation between the intensity of an applied magnetic field and the resulting heat generated by the magnetic particles, the changes in the enzyme's internal structure were likely explained by this relationship and resulted in the lower activity of the immobilized lipase. Tang et al.[15] also showed benefic results in the application of AMF on CALB immobilized onto Fe_3O_4-pTAP nanoparticles, concluding that the rate of the reaction catalyzed by immobilized CALB was enhanced as the magnetic field frequency (25–500 Hz) and strength (2.8-162.3 Gs) were increased.

4 CONCLUSION

Considering the points previously shown, it is possible to verify that the application of magnetic field, either in the immobilization process or in the reaction can significantly affect lipase structure, which can lead to an improvement in their performance, but also to denaturation/aggregation depending on the intensity of magnetic field. In this context, it is important to note that more studies aiming to understand the effects of magnetic fields on different lipases and processes are still necessary.

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