MAGNETIC IRON OXIDE NANOPARTICLES AS A PLATFORM FOR ENZYME IMMOBILIZATION: ENHANCING THE BIOCATALYTIC ACTIVITY OF EVERSA® TRANSFORM 2.0 LIPASE FOR BIODIESEL PRODUCTION

Kaiany M. dos Santos[1*], Patrick S. Sousa[2], Juliana de F. Serpa[1], Viviane de C. Bizerra[1], Paulo G. de Sousa Junior[1], Valdilane S. Alexandre[1], Francisco S. Neto[2], Maria Cristiane M. de Souza[1] & José Cleiton Sousa dos Santos[1]

[1] Chemistry/Auroras Campus/University of International Integration of Afro-Brazilian Lusopohny - UNILAB, Institute of Engineering and Sustainable Development - IEDS, Redenção-CE, Brazil.
[2] Department of Chemical Engineering, Federal University of Ceara, Fortaleza – CE, Brazil.
** Corresponding author's email address: patricksilva@alu.ufc.br*

ABSTRACT

The research centers on synthesizing ethyl oleate through the esterification of oleic acid with ethanol. Magnetic nanoparticles were created via co-precipitation, followed by activation with glutaraldehyde and functionalization using γ-aminopropyltriethoxysilane (APTES). Optimal conditions for immobilizing the enzyme ET 2.0 were found to be a pH of 10, a 25 mM sodium carbonate buffer, an enzyme loading of 200 U/g, and a contact time of 1 hour. These conditions resulted in a yield of 78% and an enzymatic activity of 205.9 U/g. post-immobilization assessments indicated that the immobilized enzyme outperformed its free-form counterpart. Kinetic experiments were conducted under these optimized conditions (ranging from 2 to 96 hours at 150 rpm and 37°C). The biocatalyst was then tested for ethyl oleate synthesis using oleic acid and ethanol, achieving a conversion rate of 88.1%. Subsequent cycles maintained approximately 80% conversion up to the fourth cycle, demonstrating the sustainability of ester production. Molecular docking studies showed a binding affinity of -5.8 kcal/mol for the oil-enzyme complex, indicating that the substrate-lipase combination was stable and suitable for esterification.

Keywords: Magnetic nanoparticles. Immobilization. Eversa® Transform 2.0. Biodiesel.

1 INTRODUCTION

Biodiesel, a renewable biofuel mainly consisting of fatty acid esters, has become a promising alternative to traditional fossil-based diesel. Its production relies on two main chemical processes: transesterification and esterification[1,2]. These processes involve the catalytic reaction of vegetable oils or animal fats with primary alcohols to produce biodiesel and glycerin. Among these primary alcohols, methanol is preferred over ethanol in industrial applications

This preference is largely due to methanol's cost-effectiveness and its inherently higher reactivity, which often results in greater biodiesel yields. Within this transformative framework, enzymes have gained prominence as eco-efficient catalysts, owing to their exceptional specificity and reduced byproduct formation. This high specificity offers two main advantages: it boosts overall yield and eliminates the need for complex post-reaction separation processes. Both aspects significantly enhance the economic viability and environmental sustainability of biodiesel production[3].

Among the many commercially available lipases, Eversa® Transform 2.0 (ET 2.0) stands out as a particularly promising catalyst for biodiesel production. Developed by Novozymes, ET 2.0 is a liquid lipase derived from genetically modified Thermomyces lanuginosus. It offers several advantages, including high substrate specificity, robust catalytic activity under moderate conditions, and an extended shelf life[4]. Despite their compelling advantages, enzymes in their native, soluble state face several operational challenges, such as thermal instability and complex recovery processes from reaction mixtures. The production of ethyl oleate holds significant industrial importance due to its diverse applications across various sectors. Ethyl oleate, a fatty acid ester derived from oleic acid and ethanol, is widely used as a green solvent, emulsifier, and plasticizer in the polymer industry.

The current investigation has a multifaceted goal. Firstly, it seeks to synthesize and comprehensively characterize magnetite (Fe_3O_4) nanoparticles using an efficient and streamlined method. Secondly, the study aims to assess the suitability of these synthesized Fe_3O_4 nanoparticles as a solid support for immobilizing ET 2.0. To optimize the immobilization process, various operational parameters will be explored, including pH conditions, enzyme loading rates, and duration of enzyme-substrate contact. Furthermore, the study examines the inherent properties and operational stability of the magnetite material, all with the objective of enhancing its applicability in ethyl oleate synthesis.

2 MATERIAL & METHODS

The main materials used in the study included ET 2.0 supplied by Novozyme Latin America Ltda. located in Araucária, Paraná, Brazil. Gamma-aminopropyltriethoxysilane (APTES), a 25% (w/v) solution of grade II purity glutaraldehyde, p-nitrophenyl butyrate (pNPB), and p-nitrophenol (pNP) were purchased from Sigma-Aldrich, São Paulo, Brazil. All other reagents of analytical grade were obtained from Distribuidora Cequímica, Fortaleza, Ceará, Brazil. Additionally, the following analyses were conducted:

- Synthesis of magnetic nanoparticles (NPM);
- Treatment with gamma-aminopropyltriethoxysilane (APTES);
- Support for Activation with Glutaraldehyde (GLU);
- Immobilization of enzymes;
- Measurement of enzyme activity;
- Determination of enzymatic activity;
- Comprehensive characterization of supports and biocatalysts;
- Vibrating sample magnetometry (VSM);
- Scanning electron microscopy (SEM) and X-ray fluorescence (XRF);
- Fourier transform infrared spectroscopy (FTIR);
- Effect of pH on enzymatic activity;
- Effect of enzymatic load;
- Impact of contact time;
- Synthesis of ethyl oleate;
- Stability and operational kinetics;
- Characterization of esters;
- Homology modeling;
- Preparation of proteins;
- Obtaining the ligand;
- Molecular docking and calculation visualization.

3 RESULTS & DISCUSSION

Here is a summary of some of the results obtained from the aforementioned analyses: The role of glutaraldehyde in enzyme immobilization showed that activation resulted in a higher immobilization yield of 92% and an enzyme activity of 195.36 U/g. In contrast, without activation, the yield was 83.1% with an enzyme activity of 101.25 U/g. This highlights the significant improvement in immobilization yield and enzyme activity achieved through glutaraldehyde activation[5].About influence of nanoparticle concentration and steric hindrance, the author[5] it was noted that higher nanoparticle concentrations led to increased lipase loading, although it never reached 100%, potentially due to inaccuracies in the Bradford quantification method. In our study, the improved yield and activity with glutaraldehyde activation could also be attributed to a more effective interaction between the enzyme and nanoparticles.

The characterization of magnetic nanoparticles and biocatalysts included an investigation into the influence of magnetic loading on fabricated supports. The observed saturation magnetization (M_s) values were approximately 75, 70, 55, and 50 emu/g for the respective samples. These values reflect a noticeable decrease upon the functionalization of the naked magnetic particles (NPM) with APTES (γ-aminopropyltriethoxysilane) and glutaraldehyde. This trend is consistent with findings reported by Costa et al.[6] who also reported a similar decrease in M_s values upon APTES and glutaraldehyde coating of Fe_3O_4 nanoparticles[6]. However, the resulting nanosystem retains sufficient magnetization for effective magnetic separation, as demonstrated by Freire et al.[7] confirmed the successful functionalization and utility of Fe_3O_4/APTES_GLU_ET 2.0 as a biocatalyst support [7].

The Integration of scanning electron microscopy (SEM) and X-ray fluorescence (XRF) for material characterization SEM images and X-ray fluorescence (XRF) spectra of the nanomaterials—NPM, Fe3O4/APTES, Fe3O4/APTES_GLU, and Fe3O4/APTES_GLU_ET 2.0— are shown together in figure 2 (panels a, b, c, and d). SEM micrographs reveal nuanced differences in the morphological characteristics and porosity of the different engineered supports. A striking change in material porosity is indicative of rapid compositional changes. XRF spectral analysis supports minor elemental shifts, in particular a marginal decrease in iron (Fe) content associated with the appearance of sulfur (S) and other elements.

The operational stability and performance of the biocatalyst over 14 cycles of 16 hours each exhibit promising initial results, starting with an 88.1% conversion of oleic acid to ethyl oleate in the first cycle. Stability is maintained until the fourth cycle, with conversions hovering around 80%. However, a gradual decline is observed from the fifth cycle onwards, dropping to 39.9% in the final cycle. The modification of magnetic nanoparticles (MNPs) with APTES and glutaraldehyde proves advantageous in maintaining enzyme activity across successive cycles. ET 2.0-NPM demonstrates impressive efficiency in ethyl oleate production. The initial high conversion rates, coupled with sustained performance over multiple cycles, suggest that this biocatalyst is a strong contender for replacing more environmentally harmful chemical catalysts. Additionally, its ease of separation and recovery using a magnetic field further enhances its industrial appeal.

4 CONCLUSION

The study concluded that the ET 2.0-NPM biocatalyst exhibited exceptional efficiency in synthesizing ethyl oleate, achieving a high conversion rate of 88.1% under optimized conditions. This substantial conversion rate underscores the biocatalyst's performance, indicating that the enzyme's structural integrity and catalytic potential are well-maintained on the magnetic

nanoparticle (MNP) support. These findings strongly support the use of APTES and glutaraldehyde-modified MNPs as a superior approach for immobilizing enzymes in biodiesel production.

REFERENCES

[1] A. MODENEZ, I.; SASTRE, D.; C. MORAES, F.; MARQUES NETTO, C. Influence of Glutaraldehyde Cross-Linking Modes on the Recyclability of Immobilized Lipase B from Candida Antarctica for Transesterification of Soy Bean Oil. Molecules 2018, 23 (9), 2230.

[2] FREIRE, T. M.; DUTRA, L. M. U.; QUEIROZ, D. C.; RICARDO, N. M. P. S.; BARRETO, K.; DENARDIN, J. C.; WURM, F. R.; SOUSA, C. P.; CORREIA, A. N.; DE LIMA-NETO, P.; FECHINE, P. B. A. Fast Ultrasound Assisted Synthesis of Chitosan-Based Magnetite Nanocomposites as a Modified Electrode Sensor. Carbohydr Polym 2016, 151, 760–769.

[3] COSTA, V. M.; SOUZA, M. C. M. DE; FECHINE, P. B. A.; MACEDO, A. C.; GONÇALVES, L. R. B. Nanobiocatalytic Systems Based On Lipase-Fe3o4 And Conventional Systems For Isoniazid Synthesis: A Comparative Study. Brazilian Journal of Chemical Engineering **2016**, 33 (3), 661–673.

[4] RAVINDRA B. MALABADI; SADIYA MR; KIRAN P. KOLKAR; RAJU K. CHALANNAVAR. Biodiesel Production: An Updated Review of Evidence. International Journal of Biological and Pharmaceutical Sciences Archive 2023, 6 (2), 110–133.

[5] MALLA, F. A.; BANDH, S. A.; WANI, S. A.; HOANG, A. T.; SOFI, N. A. Biofuels: Potential Alternatives to Fossil Fuels. In Biofuels in Circular Economy; Springer Nature Singapore: Singapore, 2022; pp 1–15.

[6] SOUZA, M. C. M. de. Imobilização de Lipase de Candida Antarctica Do Tipo B Em Nanopartículas Magnéticas Visando a Aplicação Na Síntese de Ésteres., Universidade Federal do Ceará, Fortaleza, 2013.

[7] CARBALLARES, D.; ABELLANAS-PEREZ, P.; DE ANDRADES, D.; POLIZELI, M. DE L. T. DE M.; ROCHA-MARTIN, J.; FERNANDEZ-LAFUENTE, R. Reutilization of the Most Stable Coimmobilized Enzyme Using Glutaraldehyde Chemistry to Produce a New Combi-Biocatalyst When the Coimmobilized Enzyme with a Lower Stability Is Inactivated. ACS Sustain Chem Eng 2024, 12 (17), 6564–6572

ACKNOWLEDGEMENTS

This study is supported in part by the financial support of the following Brazilian agencies for scientific and technological development: Fundação Cearense de Apoio ao Desenvolvimento Científico e Tecnológico (FUNCAP) (PS1-0186-00216.01.00/21), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (311062/2019-9), and Coordenação de Aperfeiçoamento de Ensino Superior (CAPES) (finance code 001).