ENZYMATIC SYNTHESIS OF ALKYL LEVULINATES USING LIPASE FROM *Rhizomucor miehei*

Everton M. Feitosa[1,2], Luan C. Silva[1,2], Maria Fernanda S. Mota[1], Denise M. G. Freire[1] & Erika C. G. Aguieiras[1,2]*

[1] *Laboratório de Biotecnologia Microbiana, Instituto de Química, Centro de Tecnologia, Universidade Federal do Rio de Janeiro, Ilha do Fundão, Rio de Janeiro, RJ, Brazil.*

[2] *Universidade Federal do Rio de Janeiro, Campus UFRJ Duque de Caxias, Duque de Caxias, RJ, Brazil.* Corresponding author's email address: erika@xerem.ufrj.br*

ABSTRACT

Alkyl levulinates have applications in several areas, such as fuel additives and flavoring and fragrance. The enzymatic synthesis of alkyl levulinates through the esterification reactions between levulinic acid and a monohydroxylated alcohol offers advantages over conventional chemical esterification such as mild reaction conditions, lower side reactions, and minimal waste generation. This study investigated the synthesis of alkyl levulinates catalyzed by a commercial lipase from *Rhizomucor miehei*, immobilized in macroporous acrylic resin. The effect of the alcohol chain size (C4, C6, C8, and C12), molar ratio of reagents, and type of solvent were studied. Higher conversions (> 85%) were attained for octanol (C8) and dodecanol (C12). Moreover, it was possible to conduct the reaction in a solvent-free system using dodecanol as an acyl acceptor.

Keywords: Levulinic acid. Alkyl levulinates. Lipase. Esterification. Biocatalysis.

1 INTRODUCTION

Due to the growing energy demand, the climate problems associated with the burning of fossil fuels, and concerns about its finite nature, there is pressure in the world to produce sustainable and renewable energy[1]. In recent years, the production of chemical platforms derived from biomass processing has gained attention. The most important source of biomass is from lignocellulosic composition, due to its largely renewable nature and its composition that can yield a variety of chemical products through different pathways, in addition to not competing with food products. The reserves of lignocellulosic materials generate simple sugars, which are of great importance due to their potential for use and transformation into important commodities or chemical platforms[2].

Among the products generated through the processing of lignocellulosic biomass, levulinic acid is classified as one of the 12 promissory building blocks and an intermediate organic promissory block for the synthesis of several molecules with chemical applications[3]. Levulinic acid is a 5-carbon acid composed of a ketone group and a carboxylic group, making it an important chemical platform molecule due to its high reactivity. Esters derived from levulinic acid have several industrial applications such as solvents, flavorings, and plasticizers[4].

The use of lipases as biocatalysts for alkyl levulinates synthesis can bring advantages such as high selectivity, specificity and purity of products generated under milder conditions[5]. Microbial lipases have great value due to the variety of catalytic activities, higher yield, stability, and higher growth rates in low-cost media[6].

While free enzymes exhibit higher catalytic activity, their low stability poses challenges for their application, particularly in terms of reusability. The immobilization of these enzymes is an alternative way to improve operational stability and favor their use, increasing the competitiveness of the biocatalytic process[6].

The general objective of this project is the enzymatic synthesis of alkyl levulinates from esterification reactions between levulinic acid and different alcohols using a commercial immobilized biocatalyst (NS40086).

2 MATERIAL & METHODS

NS 40086 (immobilized lipase from *Rhizomucor miehei*) was used as a biocatalyst. The reactions were carried out for 24 hours in a closed batch reactor with a capacity of 15 mL, equipped with magnetic stirring, with a controlled temperature at 50°C, in duplicate. The reaction medium consisted of a mixture of different alcohols (n-butanol (C4), hexanol (C6), octanol (C8), or dodecanol (C12)) and levulinic acid in different alcohol: acid molar ratios (3:1, 5:1 or 7:1) with 5% (w/w) of enzyme, and 10% (v/v) of the solvents methyl tert-butyl ether (MTBE) or hexane. The solvent-free system was also evaluated.

For analysis, 600 µL of each reaction sample were collected at 0 and 24 hours of reaction. The samples were centrifuged at 10,000 rpm for 5 minutes to remove the biocatalyst and the supernatant was stored at 4°C. A water bath at 100°C for 20 minutes was used for the removal of the solvent by evaporation. The production of esters was evaluated by neutralization titration to determine the levulinic acid content in the medium. 100 µL from each sample were dissolved in 40 mL of acetone/ethanol 1:1 (v/v) and titrated against 0.04 M NaOH solution until pH 11.0 using the Mettler model DG20 automatic titrator.

3 RESULTS & DISCUSSION

The reaction was initially carried out using levulinic acid and different alcohols (hexanol, octanol, n-butanol or dodecanol) in the alcohol: acid molar ratio 7:1 in the presence of two solvents (MTBE or hexane) for 24 hours. Higher conversions (> 70%) were obtained with both solvents for hexanol, octanol, and dodecanol. For the medium with butanol, there was no observed practical conversion (below 5%) (Figures 1a and 1b). This difference can be related to the preference of lipases to catalyze reactions with long-chain substrates since hexanol, octanol, and dodecanol have 6, 8, and 12 carbons, respectively[4]. Furthermore, the smaller carbon chain of butanol gives this alcohol a polar characteristic and can lead to lipase inactivation. This effect is enhanced by the action of the levulinic acid on the enzyme [4,5].

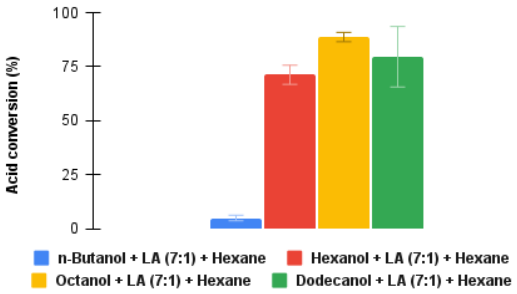

Figure 1a: Influence of the size of the alcohol chain in the conversion of levulinic acid. Reactions were conducted in alcohol: acid molar ratio 7:1, 5% (w/w) of NS 40086 at 50°C in a medium with hexane.

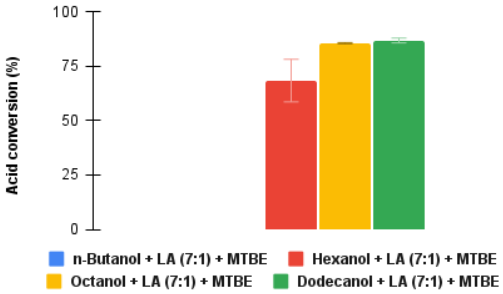

Figure 1b: Influence of the size of the alcohol chain in the conversion of levulinic acid. Reactions were conducted in alcohol: acid molar ratio 7:1, 5% (w/w) of NS 40086 at 50°C in a medium with MTBE.

The use of a suitable solvent can improve the solubility, number of effective collisions, and mass transfer of the reactants, increasing the conversion[8]. However, solvent-free systems offer the advantages of employing larger reaction volumes with fewer resources required to produce levulinate esters, besides to the environmental aspect. The results of the reactions carried out without solvent are presented in Figure 2. The absence of solvents did not have negative effects on the enzyme activity, for the reactions with dodecanol (approximately 95% conversion). For octanol and hexanol there was a significant decrease in the conversion from (85% to about 50% and 82% to about 56%, respectively). This result can be explained by the fact that levulinic acid also exerts an inhibitory effect on the enzyme and the solvent works to protect the enzyme from the effect of the acid.

Subsequently, reactions were carried out with different molar ratios between dodecanol and levulinic acid without solvent (3:1 and 5:1) for 24 hours. Higher conversions were obtained using the alcohol: acid molar ratio of 5:1 (91.9%) compared to 3:1 (6.5%). Furthermore, the conversions at the 5:1 alcohol: acid molar ratio were similar to the results previously observed in the 7:1 molar ratio (Fig. 2).

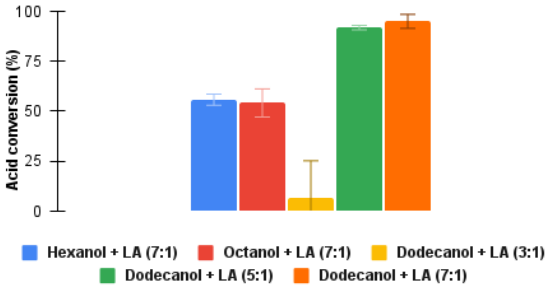

Figure 2: Conversion of levulinic acid in the reactions conducted without solvent using different alcohols and 5% (w/w) of NS 40086 at 50°C.

The effect of the molar ratio was also evaluated for hexanol and octanol in a medium with MTBE as solvent. The use of the 3:1 molar ratio of octanol and levulinic acid (Figure 3-a) was more promising than the use of hexanol (Figure 3-b) where a conversion of 12.4% was observed. This conversion with hexanol in a 3:1 ratio may be related to the higher concentration of levulinic acid in the reaction medium. Another probable reason already presented in the literature is the influence on the size of the alcohol carbon chain[4]. The reactions carried out in the 5:1 molar ratio showed similar conversions to those previously analyzed in the 7:1 molar ratio with octanol and hexanol.

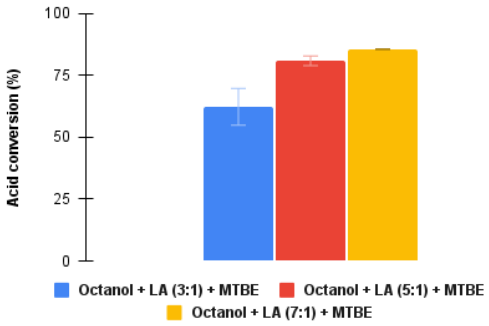

Figure 3a: Effect of molar ratio on levulinic acid conversion in the reactions conducted with octanol, 5% (w/w) of NS 40086, 10% MTBE at 50°C.

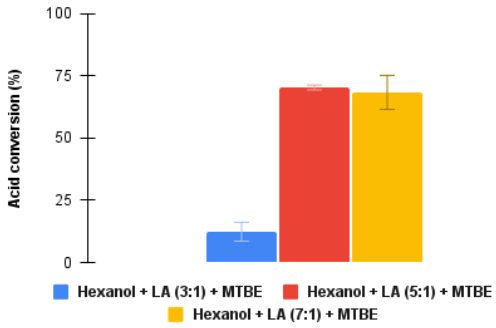

Figure 3b: Effect of molar ratio on levulinic acid conversion in the reactions conducted with hexanol, 5% (w/w) of NS 40086, 10% MTBE at 50°C.

CONCLUSION

The synthesis of alkyl levulinates using commercial lipases offers a promising route to produce these high-value compounds. Compared to chemical catalysis, where the production of harmful residues to the environment occurs, lipases are effective biocatalysts for this reaction, thus ensuring a more sustainable process. In addition, no studies using immobilized lipase of *R. miehei* NS 40086 for the esterification reaction between levulinic acid and alcohols were found in the literature.

REFERENCES

[1] BOULAL, A., ATABANI, A.E., MOHAMMED, M.N., KHELAFI, M., UGUZ, G., SHOBANA, S., BOKHARI, A., KUMAR, G., 2019. Biocatal. Agric. Biotechnol. 20, 101234.
[2] DÉMOLIS, A., ESSAYEM, N., RATABOUL, F., 2014. ACS Sustainable Chem. Eng. 2, 1338–1352.
[3] PILEIDIS, F.D., TITIRICI, M.-M., 2016. Levulinic Acid Biorefineries: New Challenges for Efficient Utilization of Biomass. ChemSusChem 9, 562–582.
[4] ZHOU, L., HE, Y., MA, L., JIANG, Y., HUANG, Z., YIN, L., GAO, J., 2018. Bioresour. Technol. 247, 568–575.
[5] AGUIEIRAS, E.C.G., CAVALCANTI-OLIVEIRA, E.D., DE CASTRO, A.M., LANGONE, M.A.P., FREIRE, D.M.G., 2014. Fuel 135, 315–321.
[6] CHANDRA, P., ENESPA, SINGH, R., ARORA, P.K., 2020. Microb Cell Fact 19, 169.
[7] JIA, B., LIU, C., QI, X., 2020. Fuel Process. Technol. 210, 106578.
[8] BADGUJAR, K.C., BADGUJAR, V.C., BHANAGE, B.M., 2022. Mater. Sci. Energy Technol. 5, 232–242.

ACKNOWLEDGEMENTS

The authors are grateful to the funding agencies Conselho Nacional de Desenvolvimento Científico (CNPq PIBIC UFRJ), and Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ).