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MAXIMIZING SUSTAINABILITY: ENZYMATIC GLYCEROLYSIS IN THE PRESENCE OF LIGNIN, USE AND REUSE OF NOVOZYM 435

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ABSTRACT

This study investigates the potential of using and reusing the immobilized enzyme Novozym 435 in the transesterification reaction in the presence of lignin at different concentrations in the medium to develop lignopolyols for application in the development of polyurethanes. Lignin is the second largest natural polymer. For paper industries, the lignin is considered production waste. However, this material is rich in functional groups that add important values to polymeric materials, such as increased mechanical strength. Several authors have already studied the exploitation of chemical glycerolysis in the presence of lignin. However, enzymatic glycerolysis in the presence of lignin still needs to be studied, and the biocatalyst (enzyme) reuse is not found in the literature. Lignopolyols produced at values above 5 wt.% inhibited enzyme activity after the second cycle, with % drop-in enzyme activity of over 80%. Meanwhile, less than 60 % activity loss values were found for lignopolyols produced with 0 % and 5 %. This study is a pioneer in exploring the catalytic potential of enzymes as biocatalysts in the transesterification reaction in the presence of lignin and its reuse. As this is an innovative subject, new research related to the subject should be investigated.

Keywords: Transesterification. Lignopolyol. Novozym 435. Reusability.

1 INTRODUCTION

Lignin, found naturally in plant cell walls, is the second most common biopolymer, making up 15% to 35% of the total, just after cellulose. It is mainly produced as a by-product in biorefineries and the pulp and paper industries through various processes. However, due to its complex structure, lignin can negatively affect its mechanical properties when making different products. This challenge underscores the importance of maximizing lignin abundance while improving its properties for specific uses[1].

The transformation of lignin through transesterification, which derivatizes its hydroxyl (OH) groups, offers significant advantages regarding compatibility[2]. This method proves helpful in expanding the industrial applications of lignin. For instance, it can produce polyurethane foams from lignin, enhancing its properties[3]. Industries are increasingly turning to biotechnological processes for more sustainable solutions. Enzymatic glycerolysis of lignin is gaining attention as a promising method for efficiently converting this complex polymer into valuable chemical products, offering environmental advantages over traditional approaches that use inorganic acids as catalysts[4].

Using reusable enzymes in biotechnological processes reduces waste production[5]. Syntheses catalyzed by these enzymes offer sustainable alternatives to traditional industrial chemical methods, meeting the growing demand for environmentally friendly technologies. An excellent example of this approach is Novozym 435 (N435), an immobilized lipase developed by Novozymes that is widely accessible on the market. N435, created by immobilizing *Candida antarctica* lipase B on a specific resin made of a macroporous poly(methyl methacrylate) support crosslinked with divinylbenzene, stands out as one of the most utilized commercial biocatalysts in academia and industry[6,7]. Its widespread use underscores its importance and effectiveness [7,8,9].

The use of N435 not only means progress in catalytic efficiency but also aligns with the sustainability principles that drive biotechnology advances. Within this framework, our study sought to evaluate Novozym 435 effectiveness as a biocatalyst in the glycerolysis reaction of lignin to produce polyols. We carried out initial and subsequent cycle tests to investigate its performance.

2 MATERIAL & METHODS

In a solvent-free system, lignopolyols were obtained by enzymatic glycerolysis using castor oil, commercial glycerol, and lignin. The methodology was adapted[10]. The lignopolyols were synthesized in a jacketed glass reactor using castor oil, glycerol in a 1:6 molar ratio, lignin (0, 5, 10, and 15 wt.%) related to castor oil and glycerol, 16% Tween 80 related to castor oil, glycerol, and lignin and 9% of biocatalyst. The substrates and lipase were mixed and kept at 70 °C, 600 rpm for 2 h.

Enzyme activity was based on the methodology[8]. It assumed the principle of esterification reaction between lauric acid and propanol of molar ratio 1:1. For homogenization of the system, the mixture was kept in stirring at 250 rpm at 60 ºC for 40 min. Before adding the enzyme, a sample aliquot was collected for blank titration. After this collection, 5% enzyme was added by mass about the substrates. The esterification reaction occurred for 40 min; then a 150 µL aliquot was collected, diluted in 20 mL of acetone:ethanol solution (1:1), and submitted to titration with NaOH 0.04 N. Determining the enzymatic activity was

calculated as the amount of enzyme necessary to consume 1 µmol of lauric acid per minute. The calculation is described in equation (1):

$$\frac{U}{g} = \frac{[(V^0 NaOH) - (V^{40} NaOH)] \cdot N \cdot 10^3}{t \cdot w} \quad (1)$$

Where:
N = Molarity of NaOH solution;
V^0 = Volume de NaOH spent in mL to titrate the control (sample at time zero);
V^{40} = Volume de NaOH spent in mL to titrate the sample (sample at time 40);
t = reaction time in minutes;
w = weight of enzyme in grams used.

3 RESULTS & DISCUSSION

Enzymatic activity was analyzed before and after the cycles conducted in this study. Before the first cycle, a value of 174.0 U/g was observed. According to Figure 1, after the first cycle, there was a reduction of approximately 45% in the activity of the N435 enzyme. It is common for the biocatalyst to experience a decrease in activity after the initial contact with the substrate and the onset of the reaction process. This occurs due to potential variations in pH, viscosity, and the formation of new compounds in the medium during the reaction[4,11]. After the second cycle, this decrease in activity becomes more pronounced, reaching over 70 % for the enzymes used in media containing 10 % and 15 % lignin. In contrast, it did not exceed 50 % loss for the others.

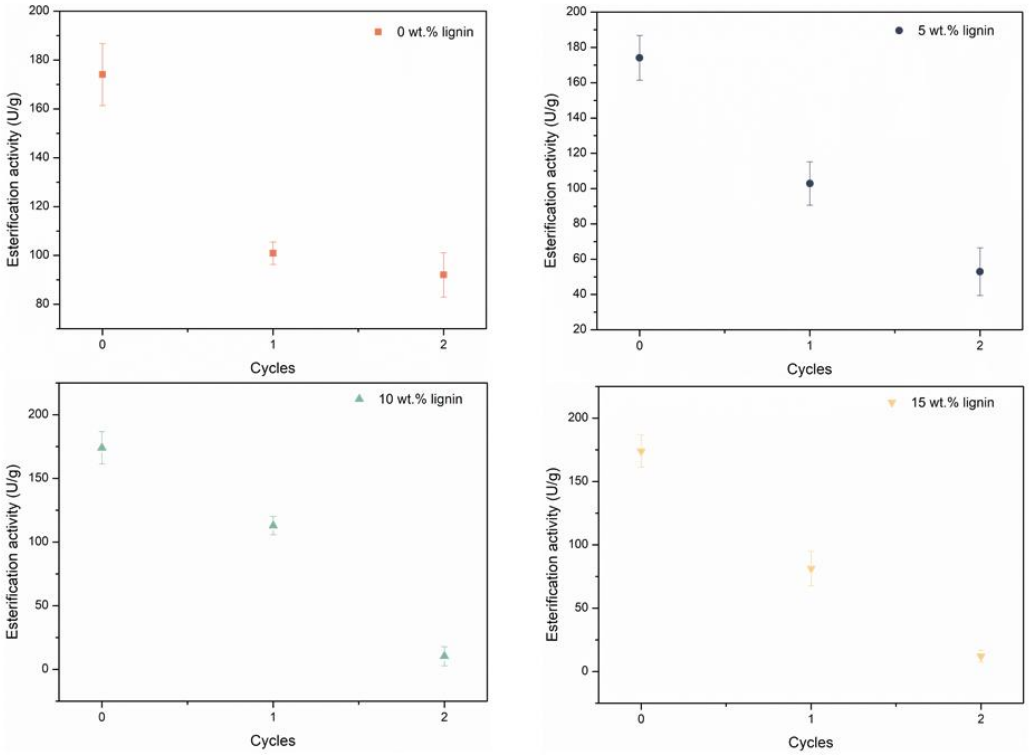

Figure 1 Reusability of Novozym 435 transesterification synthesis in the presence of lignin at different ratios.

Other studies have investigated the reuse of the N435 enzyme in esterification or transesterification reactions for producing various products. These studies have demonstrated a substrate-to-product conversion rate exceeding 90% in the initial reaction cycles. However, it is essential to highlight that extrinsic factors significantly impact the catalytic potential of the enzyme. Factors such as the substrate-enzyme ratio can influence the reaction efficiency, often leading to partial dissolution of the protein component during the chemical process. These considerations are crucial for optimizing the use of the N435 enzyme in industrial applications and ensuring consistent and satisfactory results[8,12,13,14].

Table 1 Novozym 435 activity before and after enzymatic glycerolysis cycles in the absence and presence of lignin.

Condition	Novozym esterification activity (U/g)				
	-	0 wt.%	5 wt.%	10 wt.%	15 wt.%
Before reaction	174.0±12.7	-	-	-	-
After the 1st cycle	-	100.9±4.6	102.9±12.2	112.9±7.1	81.3±13.6
After the 2nd cycle	-	92.0±9.0	52.9±13.4	10.3±7.5	12.1±4.7

4 CONCLUSION

This study showed that the N435 enzyme can produce lignopolyols through the transesterification of biopolymers in the presence of lignin. While promising for moderate concentrations of lignin, higher concentrations may compromise the feasibility of the process with immobilized enzymes. This fact highlights the importance of optimizing medium conditions to enhance biotechnological efficiency.

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