APPLICATION OF PINEAPPLE AGRO-INDUSTRIAL WASTE AS A NEW SUPPORT PROPOSAL FOR OBTAINING SUSTAINABLE BIOCATALYSTS

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ABSTRACT

Although there are a number of catalysts used in industry, there is a growing demand for technologies that enable the application of green chemistry in processes such as hydrolysis, esterification and biodiesel production. In this context, the immobilization of enzymes has emerged as an advantageous and environmentally friendly technique. This study aimed to immobilize different lipases. The lipase immobilization process was carried out using physical adsorption techniques. After obtaining the immobilized derivatives, the best temperature and pH conditions were evaluated. Preliminary results showed that the Burkholderia cepacia lipase immobilized via physical adsorption on pineapple crown treated with NaOH had the highest hydrolytic activity, with a value of 1763.22 U g^{-1}. The pH and temperature variance tests for the fresh pineapple crown support showed that the best results were obtained at pH 8.0 and a temperature of 60°C, respectively. Compared to the treated crown, there was better hydrolytic activity at pH 8.0 and a temperature of 45°C. The thermal stability test revealed a half-life of approximately 6.8 hours for the raw support and 21.53 hours for the treated biomass.

Keywords: Pineapple Crown. Support. Biocatalyst. Lipase. Immobilization.

1 INTRODUCTION

The use of enzymes as biocatalysts has been widely pursued by industry. In addition to their high catalytic performance, this class of catalysts also provides more ecological and sustainable industrial processes [1]. In the last twenty years, there has been considerable progress in biocatalysis, driven by significant advances in areas such as metagenomics, protein engineering and bioinformatics. At the same time, the use of enzymes has become more economically viable, thanks to advances in immobilization technologies and the application of immobilized enzymes in continuous operations in packed bed reactors [2].

Among biocatalysts, lipases (triacylglycerolacylhydrolases; EC 3.1.1.3) stand out, forming part of a class of hydrolytic enzymes with diverse origins and properties [3]. However, there are several factors that influence the stability of enzymes in their free form, including variations in pH, temperature and the possibility of losing catalytic activity. Immobilization of enzymes is therefore a viable solution, as it increases the thermal and operational stability of the biocatalyst [4]. Immobilized biocatalysts have a wide application in industry, especially in esterification and transesterification reactions. A prominent example is in the synthesis of biodiesel, a sustainable and renewable fuel produced from vegetable and animal fats [5].

In view of the growing search for the reuse of agro-industrial waste, the use of the crown and peel of the pineapple has proved attractive, especially considering that Brazil is the world's third largest producer of this fruit [6]. Given this context, the aim of this work is to make it feasible to use pineapple agro-industrial waste to produce a sustainable support for immobilizing lipases, with a view to obtaining sustainable catalysts that can be used in synthesis reactions.

2 MATERIAL & METHODS

The leaves of the pineapple crown were washed under running water and cut into small pieces by hand. After this stage, they were taken to the oven. After drying, they were ground [7].

The experiments were carried out using commercial lipase preparations of microbial origin from *Burkholderia cepacia* (LBC). The chemical characterization of the support was based on previous studies [8], such as the alkaline method with NaOH.

The method used to immobilize the lipases was physical adsorption. 1g of the support was soaked in hexane under agitation (150 rpm) for 2 hours. The excess hexane was then separated from the support and 100 µL of polyethylene glycol solution (5 mg/ml) and 0.25 g of lipase or 250 µL of lipase in its liquid form were added. The support was fixed under agitation for 2 hours at room temperature, followed by a further 18 hours at 4°C. The derivative was filtered and rinsed with hexane [9].

The enzymatic activities of the immobilized lipases were determined using the olive oil hydrolysis method [9]. The fatty acids released were quantified by titration with a solution of KOH (0.04 mol L^{-1}), using phenolphthalein as an indicator. Activities were expressed in μmol g^{-1} min^{-1} (U g^{-1}). Experiments were carried out in triplicate and activities were calculated according to equation 1:

$$Activity\ (U\ g^{-1}) = \frac{(Va-Vb).N.10^3}{t.m} \quad (1)$$

Where: Va= volume of KOH spent titrating the sample, Vb= volume of KOH spent titrating the blank (time 0 min), N= concentration of KOH solution (mol L^{-1}) t= reaction time (min), m= dry mass of biocatalyst (g).

In order to analyze the influence of the pH and temperature variables on the hydrolytic activity of LBC immobilized in pineapple crown, experiments were carried out using the methodology for the hydrolysis of olive oil [9], in different pH and temperature ranges in order to establish the optimum conditions. After selecting the best conditions for the biocatalyst, based on the hydrolytic activity values, the thermal stability of the *Burkholderia cepacia* lipase was checked. The effect of temperature on the stability of the immobilized lipase was determined by incubating samples of the immobilized lipase (0.05g) at the optimum temperature of each support situation in organic solvent at a ratio of 1:10 (v/v) for different incubation periods.

3 RESULTS & DISCUSSION

The chemical characterization of the pineapple crown was previously carried out, with the aim of determining the content of components such as lignin, cellulose and hemicellulose, as described in Table 1. This information will be of great relevance to the study of the crown, with a view to improving the interaction between the support and the lipase [8].

Table 1 Approximate lignin, cellulose and hemicellulose content in fresh and treated pineapple crown [8].

fractions (%)	Fresh pineapole crown	Pineapple crown treated with NaOH 4%
Lignin	24.3	10.9
Cellulose	17.4	53.3
Hemicellulose	19.1	20.1

The lignin content in the alkaline-treated crown is reduced by more than half. In addition, it has been observed that the pineapple crown represents around 30% of the fruit's mass and is composed mainly of lignin, cellulose and hemicellulose, as well as other components in smaller percentages, such as waxes and water [8].

The hydrolytic activity values for lipase in its immobilized form are lower compared to the enzyme in its free form [9]. The best hydrolytic activity value was the lipase from *Burkholderia cepacia* treated immobilized by physical adsorption (1763.22 U/g). with the lowest value, there is the activity of the fresh crown (1301.82 U/g).

The amount of lignin and hemicellulose in the bleached fiber decreased significantly compared to the raw crown, indicating the effectiveness of NaOH in separating hemicellulose and lignin from the raw crown. This pretreatment efficiency may be correlated with an improvement in the catalytic activity of the process [8].

Figure 1: Residual activity of the immobilized enzyme in treated and fresh pineapple crown according to heat treatment time.

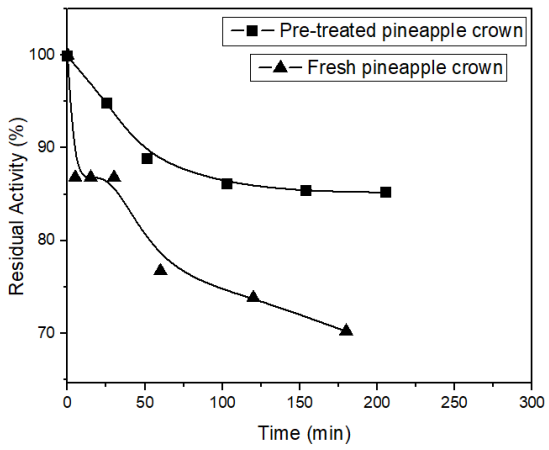

Figure 1 shows that during the first hour of testing on the raw crown, the immobilized derivative recorded a loss of just over 20% of hydrolytic activity. After 3 hours of testing, a yield of 70% of enzymatic activity was achieved. In comparison with studies conducted on the treated crown, an improvement in stability was revealed, with a loss of around 13% after 4 hours of testing. In other studies [9], which assessed the thermal stability of the enzyme immobilized on different supports at the same temperature, a loss of activity of around 25 and 40% was observed in the second hour of testing. This confirms that the thermal stability conferred by immobilization makes the pineapple crown biomass support a good option for certain reactions.

Table 2 Biochemical parameters and half-life time of lipased and *Burkholderia cepacia* immobilized in fresh and NaOH-treated pineapple crown biomass.

Parameters	Fresh pineapple crown-immobilized LBC	Pre-treated pineapple crown-immobilized LBC
Optimal pH	8.0	8.0
Optimal Temperature (ºC)	60	45
Half-life time ($t_{1/2}$) at 45ºC (h)	6.79	21.53

The tests carried out to determine the best enzymatic activity at different pH values show that, for both treated and raw corona, *Burkholderia cepacia lipase* (LBC) proved to be most effective at a pH of 8.0. As for the optimum temperature, the treated crown showed greater hydrolytic activity at 45°C, while fresh pineapple crown, the best performance was observed at 60°C. Comparative data on the deactivation constant [10], shows that the lower the value, the greater the stability of the biocatalyst. The immobilized derivative of the treated pineapple crown showed the best half-life value (h), being approximately 3.45 times longer compared to that immobilized in Nb_2O_5, 3.17 times longer than the raw crown and 7.77 times longer than that immobilized in SiO_2-PVA [9], This shows that the pineapple crown is a promising support for immobilizing lipases, suggesting its viability as a matrix for biotechnological applications.

4 CONCLUSION

In the search for alternatives to boost production, reduce costs and contribute to sustainable development, the research project explored the immobilization of enzymes in lignocellulosic biomass matrices, such as the pineapple crown. In this phase, the enzymatic activities of *Burkholderia cepacia* lipase in the raw and treated pineapple crown were partially evaluated. The aim was to find the best result and move forward with the tests. As a result, the treated pineapple crown represented higher hydrolytic activity values than those immobilized on the raw support, and a better half-life was demonstrated in the thermal stability tests, proving that the treatment is effective for immobilizing enzymes.

Preliminary results indicated that the pineapple crown is a promising lignocellulosic support for enzymes, showing catalytic activities and biochemical properties comparable to those found in previous studies, such as studies who used hybrid supports [9], and a example who used cellulose-based supports [11].

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