ELEPHANT GRASS AS A SUPPORT FOR LIPASE IMMOBILIZATION: STUDY OF BIOCHEMICAL, KINETIC PARAMETERS, AND THERMAL STABILITY OF THE BIOCATALYST

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ABSTRACT

The present study aimed to determine the biochemical parameters and thermal stability of *Burkholderia Cepacia* lipase (LBC) immobilized on elephant grass, both in its natural form and pre-treated by ultrasound, in different pH solutions. The chemical characterization of the natural support revealed a composition of 36.70% cellulose, 27.10% hemicellulose, 11.00% lignin, and 5.3% ash. The hydrolytic activity of LBC immobilized on the pre-treated support provided higher values than the biocatalyst immobilized on fresh elephant grass, reaching 850.2 U/g (pH 4), 1014.7 U/g (pH 7), 933.9 U/g (pH 10), and 647.6 U/g (fresh). The morphological change of the grass, due to ultrasonic treatment, was analyzed by scanning electron microscopy (SEM) and showed a decrease in particle size, consequently reducing pore diameter, providing the support with greater immobilization potential. Regarding the study of biochemical parameters, the optimal pH for both the biocatalyst immobilized on fresh and pre-treated support was in the range of 7 and 8. The optimal temperature was broader for the lipase immobilized on fresh support (45-60°C) compared to the pre-treated support (45°C). In the study of thermal stability, the lipase immobilized on treated grass showed a longer half-life time, on the order of 31.8 hours.

Keywords: Elephant grass. Support. Biocatalyst. Lipase. Immobilization.

1 INTRODUCTION

Enzymes play a fundamental role in various industries, such as cosmetics, pharmaceuticals, and food, facilitating the acceleration of chemical processes. However, challenges related to stability and recovery limit their widespread application [1]. To overcome this issue, immobilization on solid supports emerges as a viable alternative, allowing for the reuse of enzymes in subsequent batches [2].

The cost of enzymatic immobilization procedures often becomes burdensome due to the use of synthetic organic materials [3]. Therefore, the development of affordable and environmentally friendly alternatives becomes essential, ensuring a good interaction between the enzyme and the support. In this context, lignocellulosic biomass is gaining prominence as supports for lipase immobilization, considering a sustainable and environmentally favorable process [4].

Elephant grass (*Pennisetum purpureum,* Schum), on the other hand, is a lignocellulosic biomass with high productivity and ease of cultivation, containing high levels of cellulose and hemicellulose. However, due to its rapid growth, excess grass is often discarded through burning, resulting in adverse environmental impacts [5]. Given this context, the present study aimed to determine the biochemical parameters and thermal stability of *Burkholderia cepacia* lipase immobilized in elephant grass.

2 MATERIAL & METHODS

The elephant grass leaves were harvested in October and, after washing and drying at 60°C, the material was ground and sieved to the desired particle size (40-80 mesh). The fresh material was characterized by determining the contents of cellulose, hemicellulose, lignin, and ash, based on the methodologies described by Ferraz et al. (2000) [6] and Sluiter et al. (2008) [7]. Subsequently, the elephant grass was submerged in aqueous solutions with different pH values (4, 7, and 10) and subjected to an ultrasound bath to eliminate air bubbles present in the material. Later, the grass was subjected to ultrasound treatment with a probe for 30 minutes.

The lipases were immobilized on pre-treated and fresh elephant grass using the physical adsorption method, according to the methodology of Silva et al. (2023) [8]. The hydrolytic activity of the immobilized lipase was determined using the olive oil hydrolysis method, as described by Da Rós et al. (2010) [9]. The morphological structure of the surface of the fresh and pre-treated supports under the best pH condition was analyzed by scanning electron microscopy (SEM) using the TESCAN Mira 4th generation, located in the Materials Engineering Department (DEMAR) at EEL-USP.

The biochemical properties of *Burkholderia cepacia* lipase (LBC) immobilized on pre-treated and fresh elephant grass supports were analyzed by assessing the influence of temperature (40 to 60°C) and pH (6 to 8) variables on the hydrolytic activity of the biocatalyst, based on the methodology described by Da Rós et al. (2010) [9].

The thermal stability of the biocatalysts was evaluated by incubating samples of the biocatalyst (0.05 g) at 45°C in hexane, according to a methodology adapted from Soares et al. (1999) [10]. To assess the effect of substrate concentration on the initial rate of the hydrolytic reaction of immobilized LBC on pre-treated and fresh elephant grass, the Michaelis-Menten kinetic model was used. Reaction systems with variable concentrations of total fatty acids (20-80% w/v) obtained from oil-in-water emulsions were prepared. The kinetic constants Km and Vmax were determined using the Lineweaver-Burk method.

3 RESULTS & DISCUSSION

Elephant grass, used as a support for lipase immobilization, was characterized by measuring the contents of cellulose, hemicellulose, lignin, and ash. The results obtained were 36.7% cellulose, 27.10% hemicellulose, 11% total lignin, and 5.3% ash. Factors such as soil nutrients, humidity, temperature, and sunlight also influence the composition of this grass, directly impacting its growth and cellular composition [11].

Regarding the pre-treatment of the grass, the conducted experiments revealed different values of hydrolytic activity depending on the pH used: 850.2 U/g (pH 4), 1014.7 U/g (pH 7), and 933.9 U/g (pH 10). Thus, when compared to the hydrolytic activity of the lipase immobilized on the grass in its natural form (647.6 U/g), it is observed that the pre-treatment step was efficient in enhancing the catalytic activity of the biocatalyst, with the best result obtained at pH 7. Therefore, this condition was selected for the subsequent stages of the work. Figure 1 shows the morphology of the support (SEM) of the elephant grass, in its natural form (a), and in the best treatment condition (b).

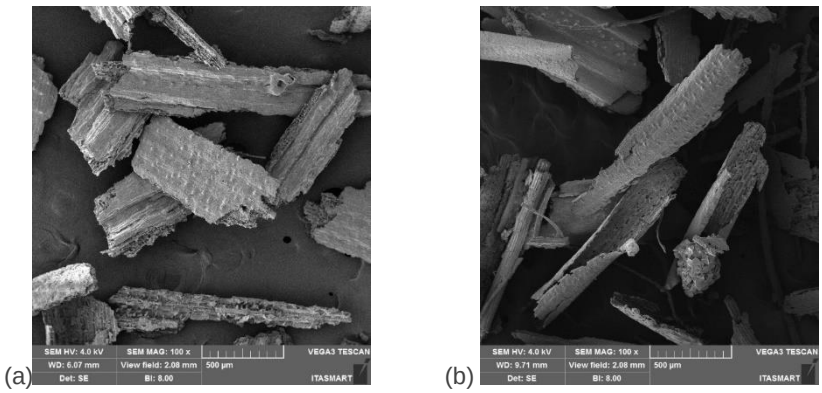

Figure 1 Images obtained by SEM with a magnification of 100x. (a) Fresh elephant grass. (b) Pre-treated elephant grass

Scanning electron microscopy analysis revealed significant transformations in the elephant grass post-ultrasonic treatment compared to its natural form (Fig. 1a). Initially, the particles were large and compact, and after the ultrasonic treatment, they underwent fragmentation, resulting in finer particles and smaller pore diameters (Fig. 1b). This change in morphology may explain a better interaction between the treated support and the lipase, suggesting a potential increase in enzyme immobilization efficiency.

The influence of temperature and pH, as well as the thermal stability and determination of the kinetic parameters Km and Vmax, were verified through the biochemical and kinetic characterization of *Burkholderia cepacia* lipase immobilized on pre-treated and fresh elephant grass. The results obtained are presented in Table 1.

Table 1 Biochemical and kinetic properties of *Burkholderia cepacia* lipase immobilized on fresh and pre-treated supports

Parameters	Fresh elephant grass-immobilized LBC	Pre-treated elephant grass-immobilized LBC
Optimal pH	7.0 – 8.0	7.0 – 8.0
Optimal Temperature (ºC)	45 – 60	45
Half-life time ($t_{1/2}$) at 45ºC (h)	27.4	31.8
Kinectic Parameters		
K_m (mM)	1839.5	1690.5
V_{max} (U/g)	5000	5000

The results show that the lipase immobilized on elephant grass, both in its natural form and after pre-treatment, exhibits a similar optimal pH range, while the lipase immobilized on fresh grass demonstrates a broader optimal temperature range. The half-life time of the lipase immobilized on pre-treated grass (31.8h) revealed slightly higher values at 45°C compared to the natural form (27.4h). Regarding the kinetic parameters, the lower Km values obtained for the lipase immobilized on pre-treated grass indicate a higher substrate affinity. Da Silva et al. (2020) [3] investigated the immobilization of *Burkholderia cepacia* lipase using a synthetic support of niobium oxide (Nb_2O_5) functionalized with γ-APTS and activated with glutaraldehyde, observing that the optimal activity was achieved at a temperature of 55°C and pH 6.5. The lipase's affinity for the substrate (olive oil emulsion) was higher when immobilized on elephant grass, whether in its natural or pre-treated form (K_m = 1838.5 and 1690.5 mM), compared to

immobilization on Nb_2O_5 (K_m = 2796 mmol/L mM) obtained by the authors. Similarly, Da Rós et al. (2010) [9] evaluated the immobilization of the same lipase on different synthetic supports: SiO_2-PVA and Nb_2O_5 activated with glutaraldehyde. When analyzing the thermal stability of these immobilized derivatives at 60°C, they observed a half-life time of 6.24h for the lipase immobilized on SiO_2-PVA and 2.77 hours for the lipase immobilized on Nb_2O_5, highlighting the better performance of immobilization on elephant grass, whether in its natural or pre-treated form, in terms of thermal stability.

4 CONCLUSION

Based on the presented data, the immobilization of *Burkholderia cepacia* lipase on elephant grass emerges as a promising and sustainable alternative for the production of heterogeneous biocatalysts. The chemical characterization of the support revealed favorable properties, such as a high content of cellulose and hemicellulose. The results show a similar optimal pH range but a broader optimal temperature range compared to fresh grass. Additionally, the lipase immobilized on pre-treated grass showed higher thermal stability and superior affinity with the substrate. Comparing with synthetic supports highlights the advantage of elephant grass in terms of ecological and kinetic performance. Thus, the immobilization of the lipase on this material emerges as a sustainable and efficient strategy in biocatalyst production, driving advancements in industrial biotechnology and the exploration of natural materials as supports for enzymatic immobilization, promoting ecologically conscious industrial practices.

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