# IMMOBILIZATION AND STABILIZATION OF ACETYL XYLAN ESTERASE OF *Aspergillus nidulans*

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## 1. INTRODUCTION

Acetyl xylan esterase (EC 3.1.1.72) are enzymes that cleave the ester bonds of the O-acetyl groups of the xylan backbone to create new sites for subsequent hydrolysis of other enzymes, such as endoxylanases (BIELY et al., 2014). D-xylose is one of the main constituents of xylan, the main class of hemicellulose. To degrade this structure, it is necessary that enzymatic hydrolysis occurs synergistically with xylanolytic enzymes, such as endo-beta-1,4-xylanases, β-xylosidases, α-L-arabinofuranosidases, α-glucuronidases, feruloyl esterase and acetyl xylan esterase (AXE) (POLIZELI et al., 2016). The use of enzymes in their free form is expensive and expensive, in addition to some problems such as low stability, sensitivity to process conditions, and difficulties in recovering the products and recycling the enzyme from the reaction medium (MATEO et al., 2007). To assist in these difficulties, the immobilization process becomes essential for applying enzymes in industrial processes, as it contributes to stabilization reuse of the enzyme in several cycles, reducing the cost of the biocatalyst (SANTOS et al., 2015). In the present work, we purify, characterize an acetyl xylan esterase (AxeCE3) from *Aspergillus nidulans* and successfully immobilized in an activated agarose matrix with different reactive groups: monoaminoethyl-N-ethyl (MANAE), Glyoxyl and Glyoxyl-Iminodiacetic acid (IDA).

## 2. RESULTS AND DISCUSSIONS

The *axeCE3* gene was cloned into the pEXPYR vector and transformed into *A. nidulans* A773 strain for protein expression. AxeCE3 was purified and characterized for its biochemical properties and showed activity over a wide range of pH (3.0-9.0) and temperature (30-70 °C), with maximum activity at pH 7.0, 55 °C using *p*-nitrophenyl acetate (pNPA) as a substrate. After that AxeCE3 was immobilized on agarose-activated supports (MANAE, Glyoxyl and Glyoxyl-IDA).

**Table 1.** Main parameters of the immobilization of AxeCE3 from *Aspergillus nidulans* on agarose-activated supports.

| Support | Immobilization Efficiency[a] (%), IE | Recovered Activity[b](%) |
|---|---|---|

| | | |
|---|---|---|
| **MANAE** | 99.7 | 144 |
| **Glyoxyl** | 31 | 37 |
| **Glyoxyl-IDA** | 26 | 5 |

[a]Efficiency (%) – calculated by the difference between the initial activity and the final activity in the supernatant after immobilization. [b]Recovered activity (%) - calculated by comparing the theoretical adsorbed activity, obtained through the mass balance expressed in activity (described in U $g^{-1}$ of support) and the real activity of the immobilized enzyme. One unit of enzymatic activity (U) was defined as the amount of enzyme capable of promoting the production of 1 µmol of p-nitrophenol per minute under the assay conditions, using pNPA as a substrate.

According to Table 1, the immobilization efficiency is calculated from the disappearance of the activity present in the supernatant, the value > 95% was obtained for the MANAE support after 1 h of immobilization, with a retention high value 100%. Indicating that the enzyme had an interaction ion exchange with the amino group ($-NH_2$) of the MANAE support. However, the same profile was not observed after 16 h of reaction for the other two supports used, which showed immobilization efficiency and activity retention lower than 50%. AxeCE3 has a greater number of negatively charged amino acid residues, which explains the low efficiency and retention of activity in the Glyoxyl-IDA support, due to the exposed carboxyl group ($-COO^-$), it tends to make ionic bonds with the enzyme residues with positive loads of the enzyme. Regarding the Glyoxyl support, the low number of lysine residues on the surface of the protein may explain the low value of immobilization efficiency observed in this support. Concerning the stability at temperature of the MANAE derivative, it maintained 70% of its activity after 90 min of incubation at 60 °C, while the residual activity of free AxeCE3 was only 10%. The results obtained in this work provide a basis for the development of applications of immobilized AxeCE3 for use in the formulation of enzymatic cocktails for the hydrolysis of lignocellulosic residues.

## 3. REFERENCES

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## 4. ACKNOWLEDGMENTS

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