IMMOBILIZATION AND STABILIZATION OF THE MAIN BETA-XYLOSIDASE FROM *Penicillium janczewskii*

Authors: Terrasan, C.R.F [1], Terrone, C.C. [2], Carmona, E.C. [2], Pesela, B.C. [3], Guisan, J.M. [1]

Affiliations: [1] Departamento de Biocatálisis, Instituto de Catálisis y Petroleoquimica (ICP), Consejo Superior de Investigaciones Científicas (CSIC). Campus Universidad Autónoma de Madrid (UAM), Cantoblanco, 28049, Madrid, Spain.

[2] Biochemistry and Microbiology Department, Biosciences Institute, Univ Estadual Paulista - UNESP. PO Box 199, Av. 24 A, no. 1515, Bela Vista, 13506-900, Rio Claro, SP, Brazil.

[3] Departamento de Biotecnología y Microbiología de Alimentos, Instituto de Investigación en Ciencias de los Alimentos (CIAL), Consejo Superior de Investigaciones Científicas (CSIC). Campus Universidad Autónoma de Madrid (UAM), Cantoblanco, 28049, Madrid, Spain.

Abstract:

Enzyme immobilization is a possibility to improve the characteristics of an enzyme in terms of stability, stabilization and catalysis, as well as for processes improvement since it allows the reuse of the biocatalyst for many operational cycles. The aims of this study were to immobilize the main extracellular beta-xylosidase produced by the fungus *Penicillium janczewskii* by covalent immobilization and also verify some physicochemical properties of the immobilized enzyme. The partially purified enzyme was successfully immobilized by multipoint covalent attachments on agarose gels highly activated with glyoxyl groups. Due to the low stability in pH 10, the enzyme was previously stabilized in this condition with polyethylene glycol. Immobilization was then carried out initially in an ice-cold bath for 0.5 h followed by gradual temperature increase up to room temperature for additional 1.5 h. Optimum activity was observed in pH 4.0 and in the range between 70-80 °C, that is similar to the free enzyme. Besides, the immobilized enzyme presented higher thermostability: it was stable up to 60 °C, and it was not possible to calculate the half-life value because the activity remained above 75% even after incubation for 30 days. At 70 °C after 4h, approximately 60% of activity was observed, and its half-life remained between 4 and 24 h. At 80 °C, the derivative completely lost the activity after 30 min-incubation. Stability was also observed in a wide pH range from 4.0 to 9.0. The increase in temperature during immobilization is essential to increase enzyme-support attachments rendering much more stabilization to the derivative. By following the proposed protocol, both subunits of this dimeric enzyme remain strongly attached to the support as verified by SDS-PAGE and the derivative present more stability than the free-enzyme and the CNBr derivative (unipunctual immobilization).

Keywords: Enzyme immobilization; enzyme characterization; β-xylosidase; *Penicillium janczewskii*

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