## Properties and Recycling of Covalently Immobilized Glycanases used for Aqueous Enzyme Assisted "Rosa Mosqueta" Oil Extraction

F. Dourado, P. Vasco, F. M. Gama and M.Mota Departamento de Engenharia Biológica, Universidade do Minho, Largo do Paço, IBFQ -Instituto de Biotecnologia e Química Fina4719 Braga Codex, Portugal

Keywords: Enzyme recycling, immobilization, oil extraction

In order to reuse enzymes after enzyme reaction and to enhance their stability, the immobilization of enzymes using an insoluble carrier has been widely studied. Cellulase was immobilized on Eudragit L, that is reversibly soluble depending on the pH of the medium, according to procedure described by Taniquchi et al [1]. It has been demonstrated that this method allows for improved stability, particularly at high temperatures, without major loss of specific activity. Also, it has been shown that the derivatised proteins have the adsorption equilibrium switched towards the liquid phase, thus making recycling possible. The enzyme assisted aqueous oil extraction may be useful to circumvent problems associated to the use of organic solvents. Commercial glycanases, used in this process to solubilize the cell wall polymers, are usually very stable enzymes, and, even if their price is lower and lower, its reutilization is still worth. As these enzymes adsorb on to the lignocellulosic materials where they perform their hydrolytic action, most of the protein remains attached to the solid phase after processing, and is therefore inaccessible for reusing. The experimental flow-sheet to be tested with the "Rosa Mosqueta" seeds included a) a milling step; b) addition of a pH 5,5 buffer plus immobilized enzymes (Celluclast) in a ratio of 1g protein / 100 g seeds, in order to hydrolyse the cell walls; c) water / azeotropic ethanol oil extraction; d) drying of the seeds before soxhlet hexane extraction, in order to quantify the residual oil. After the enzymatic treatment the enzyme is recovered by centrifugation and filtration, followed by acidification of the medium to precipitate the insoluble carrier of the enzyme.



Centre for Biological and Chemical Engineering Instituto Superior Técnico

International Conference on Protein Stabilisation

Biomolecule Stabilisation

From Molecular Interpretations to Bio-Industrial Applications