PRODUCTION OF XYLANASES BY *ASPERGILLUS TERRICOLA* USING WHEAT BRAN AS CARBON SOURCE: COMPARATIVE STUDIES BETWEEN DIFFERENT BIOREACTORS AND INFLUENCE OF AERATION AND INOCULUM CONDITIONS

Michele Michelin[1,2], Maria de Lourdes T. M. Polizeli[2], Daniel P. Silva[1], Denise S. Ruzene[1], António A. Vicente[1], João A. Jorge[2], Héctor F. Terenzi[2], José A. Teixeira[1]

[1] IBB - Institute for Biotechnology and Bioengineering, Centre of Biological Engineering, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal
email: silvadp@deb.uminho.pt / fax: +351 253 678986
[2] Department of Biology, Faculty of Philosophy, Sciences and Letters of Ribeirão Preto, University of São Paulo, 14040-901 Ribeirão Preto-SP, Brazil
email: mimichelin@yahoo.com.br

Keywords: Aspergillus terricola, xylanases, fermentation

Introduction. Hemicellulose is an important biomass reservoir in the plant cell wall and agricultural wastes containing hemicellulose are globally generated. With reference to biomass regeneration, hemicellulose degradation has been intensively studied in the last decade. Xylan, the principle component of hemicellulose, is comprised of β-1,4-linked D-xylopyranose residues with different substitute groups in the side chain. Xylanases that cleave the backbone and initiate the depolymerization of xylan have received most attention, mainly because of their potential industrial use. The successful application of xylanase requires its production in high amounts. (Davidov and Atev, 1996; Gawande and Kamat, 1999; Subramanyan and Prema, 2002).
In this work, the performance of different bioreactors (stirred-tank and air-lift), and the influence of aeration and inoculum conditions for xylanase production are evaluated.

Methods. *Aspergillus terricola* was used for the investigations conducted on a 2 L stirred-tank bioreactor (STB) (Bioengineering AG CH-8636 Wald) with 1 L working volume, under 300 rpm stirrer speed and 1 vvm aeration rate or without aeration. The spore concentration in the suspension was determined in a Neubauer counting chamber by microscope and around $2x10^8$ spores.mL^{-1} were inoculated directly into the bioreactor or on Erlenmeyer flasks (250 mL) containing 50 mL of culture medium. These were incubated on a rotary shaker (150 rpm) for 24 h, being two of them used as inoculum into the bioreactor. The air-lift bioreactor (ALB) was designed and constructed at the Department of Biological Engineering, University of Minho (Portugal), and made of Perspex (polymethylmethacrylate). The working volume was 6 L with a concentric draft tube. Filtered air was supplied at 0.4 or 1 L.min^{-1}. Sterile Vogel medium was used for cultivation with 0.5% wheat bran as carbon source and the cultivation conditions were 30ºC, pH 6.0. Samples were collected every 12 or 24 h, filtered and analysed. Xylanase was assayed by DNS using 1% birchwood xylan in citrate-phosphate buffer (pH 6.0) as substrate (Vilela et al., 1973; Miller, 1959). One unit of enzymatic activity was defined as the amount that liberated 1 μmol of product per minute on assay conditions.

Results and discussion. On STB, the maximum xylanase production (7.5 U.mL^{-1}) was obtained when the spores were inoculated directly into the bioreactor and the system was programed to 1 vvm aeration rate. Absence of aeration was limitant to xylanase production (0.75 U.mL^{-1}) on direct cultivation. However, when the pre-cultivation system was used to prepare the inoculum (without aeration), a good xylanase production (6.5 U.mL^{-1}) was obtained, but this production time (168 h) was higher that observed on optimum condition (36 h). The xylanase production on ALB was higher that observed on STB, being calculated as more than 9 U.mL^{-1} (144 h) with 0.4 L.min^{-1} aeration rate and around 13 U.mL^{-1} (96 h) when 1 L.min^{-1} of aeration was used.

Conclusions. In conclusion, the obtained results suggest that variables such as aeration, inoculum condition and bioreactor configuration are key variables on the definition of a strategy to optimize the biosynthesis and microbial production of the xylanase enzymes.

Acknowledgements. Biota-FAPESP/Brazil (Fundação de Amparo à Pesquisa do Estado de São Paulo); FCT/Portugal (Fundação para a Ciência e a Tecnologia).

References.

1. Davidov, E.R., Atev, A. (1996). Method for production of enzyme preparation. *Patent number Ru 2057179 C1.*
2. Gawande, P., Kamat, M. (1999). Production of *Aspergillus* xylanase by lignocellulosic waste fermentation and its application. *Journal of Applied Microbiology*, 87:511–519.
3. Subramanyan, S., Prema, P. (2002). Biotechnology of microbial xylanases: Enzymology, molecular biology and application. *Critical Reviews in Biotechnology*, 22:33–64.
4. Villela, G.G., Bacila, M., Tastaldi, H. (1973). *Técnica e experimentos de bioquímica.* Ed. Guanabara Koogan, p.523.
5. Miller, G.L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chem.*, 31:426-428.