P39

Degradation of Azo Dyes by *Trametes villosa* Laccase under Long Time Oxidative Conditions

Andrea Zille^a, Barbara Górnacka^b, Astrid Rehorek^b Artur Cavaco-Paulo^a

^aUniversity of Minho, Department of Textile Engineering, 4800-058 Guimarães, Portugal; ^bUniversity of Applied Sciences Cologne, Institute of Chemical Engineering and Plant Design, Betzdorfer Str. 2, D-50679 Cologne, Germany *E-mail: <u>azille@det.uminho.pt</u>*

Trametes villosa laccase was used for direct azo dye degradation for which the reaction products were analyzed over long periods of time. Laccases have been extensively studied for the degradation of azo dyes [1-6]. These enzymes are multi-copper phenol oxidases that decolorize azo dyes through a highly non-specific free radical mechanism forming phenolic type compounds, thereby avoiding the formation of toxic aromatic amines [7,8].In the literature, there are a large number of papers reporting on decolorization of azo dyes however the fate of the products of azo dye laccase reactions is ignored [9-12]. Therefore, the purpose of this work is the study of the azo dye degradation products in the presence of laccase. Direct azo dye laccase degradation and amino-phenols polymerization was performed for several days. The formed soluble products were studied by LC-MS while the polymerized insoluble products were studied by ¹³C -NMR. LC-MS analysis shows the formation of phenolic compounds in the dye oxidation process as well as a large amount of polymerized products that retain the azo group integrity. The amino-phenols reactions were also investigated by 13 C-NMR and LC-MS analysis and the real polymerization character of laccase enzymes was shown. This study highlights the fact that laccases polymerize the reaction products obtained in long time batch decolorization processes of the azo dyes. These polymerized products provide unacceptable color levels in effluents limiting the application of laccases as bioremediation agents.

- [4] Novotny, C., K. Svobodova, A. Kasinath and P. Erbanova. 2004. Int. Biodeterior. Biodegrad. 54:215-223.
- [5] Peralta-Zamora, P., C. M. Pereira, E. R. L. Tiburtius, S. G. Moraes, M. A. Rosa, R. C. Minussi, and N. [1] [1]
- [6] Duran. 2003. Appl. Catal. B: Environ. 42:131-144.
- [7] Wesenberg, D., I. Kyriakides, and S. N. Agathos. 2003. Biotechnol. Adv. 22:161-187.
- [8] Wong, Y., and J. Yu. 1999. Wat. Res. 33:3512-3520.
- [9] Chivukula, M., and V. Renganathan. 1995. Appl. Environ. Microbiol. 61: 4347-4377.
- [10] Chagas, P. E., and R. L. Durrant. 2001. Enzyme Microb. Technol. 29:473-477.
- [11] Jarosz-Wilkolazka, A., J. Kochmanska, E. Malarczyk, W. Wardas, and A. Leonowicz. 2002. Enzyme [1] Microb. Technol. 30:566-572.
- [12] Robinson, T., B. Chandran, and P. Nigam. 2001. Enzyme Microb. Technol. 29:575-579.

^[1] Adosinda, M., M. Martins, N. Lima, A. J. D. Silvestre, and M. J. Queiroz. 2003. Chemosphere. 52:967-973.

^[2] Blanquez, P., N. Casas, X. Font, X. Gabarrell, M. Sarra, G. Caminal, and T. Vicent. 2004. Water Res. 38:2166-2172.

^[3] Maximo, C., and M. Costa-Ferreira. 2004. Proc. Biochem. 39:1475-1479.