Environmental Engineering and Management Journal

March 2012, Vol.11, No. 3, Supplement, S86
http://omicron.ch.tuiasi.ro/EEMJ/

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P100

PRODUCTION OF OXIDATIVE ENZYMES BY *Trametes ochracea* ON THE HIGH-MOLECULAR WEIGHT FRACTION OF OLIVE-MILL WASTEWATER

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Abstract

Olive-mill wastewater (OMW), the incorrect disposal of which may cause serious and large-scale environmental impact, might be regarded as a possible resource due to its remarkable content in simple and complex sugars, lipids, residual oil, proteins, mineral elements and phenols with elevated biological activity (*e.g.*, oleuropein, ligstroside and verbascoside) that could be either directly recovered and purified or utilized for fermentative production processes.

The present work is part of a wider project aimed to assess the techno-economical feasibility of an OMW biorefinery that provides recovery of the phenolic substances by a membrane separation technology and the achievement of a permeate, containing a very low organic load, useful in the oil mechanical extraction process. The low-molecular weight phenols are tested as antioxidants in the animal feeding and/or in the preparation of various kinds of fresh foods while the residual high-molecular weight fraction (HMW) is used for microbial production of phenol oxidases.

A preliminary screening of several white-rot fungal species led to the selection of *Trametes ochracea* CBS 257.74 as the most promising strain for its ability to grow on the HMW-OMW and to release phenol oxidases. Therefore, the potential of this strain to produce laccase and Mn-dependent peroxidase (MnP) activities on HMW-OMW was tested using different single and combined supplements. Levels of dephenolization and detoxification of the final effluent were also detected.

The addition of carbon sources (*i.e.*, glycerol, glucose and fructose) did not significantly affect enzyme production, dephenolization and organic load removal. On the contrary, supplementation with nitrogen sources (yeast extract, casein peptone and diammonium tartrate) resulted in a significant improvement of both phenol oxidases activities and the highest laccase and MnP activities were reached with peptone (29.3 and 5.8 IU ml^{-1}, respectively). Among the tested putative inducers (*i.e.*, DMSO, ethanol, Tween 80, $MnSO_4$ and $CuSO_4$), $CuSO_4$ was the most effective one. In particular, laccase and MnP activity peaks reached 35.6 and 15.7 IU ml^{-1}, respectively, in the HMW-OMW medium supplemented with 0.75 mM $CuSO_4$ and peptone. These results confirm that the high-molecular weight fraction of OMW supplemented with peptone and $CuSO_4$ represents a good medium for phenol oxidases production by *T. ochracea*. A better definition of process parameters at bioreactor scale using pneumatic and mechanic systems are in progress to assess the real potential of the proposed fermentation.