Upstream and downstream processing of fungal laccase

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Statement of the Problem: Laccase (EC 1.10.3.2, polyphenol oxidases) belongs to the group of oxidoreductases which is characterized by its specific catalytic properties and the ability to oxidize various organic compounds. Therefore the enzyme is very attractive for a wide range of industrial and environmental purposes. However, due to relatively low effectiveness and the possibility of gradual degradation of bioproducts in the reactor or during the separation and purification stages, there is a need for new approaches and research in this field. Therefore, the purpose of this research was to study and integrate the stages of up- and downstream processing (biosynthesis and purification) of laccase from *Cerrena unicolor* in order to obtain a highly active enzymatic product.

Methodology: The biosynthesis was performed in a 14 L bioreactor equipped with a set of sensors for process control. Modifications to the medium (addition of microparticles), MPEC, as well as various types of cultivation/growth strategies were examined. The supernatant was concentrated and purified by an aqueous two-phase system (ATPS) consisting of polyethylene glycol and phosphate buffer solutions and through foam fractionation (FF) at different pH values and with the addition of different detergents. Ultrafiltration and chromatography methods were also investigated. Molecular mass and isoelectric point was determined with the use of electrophoresis.

Findings: Laccase activity increased 3.5-fold after addition of microparticles to the culture media. The fed-batch mode resulted in high laccase activity (up to 4 U/mL) which remains stable during cultivation. The optimal conditions for laccase purification by FF and ATPS were determined with activity partitioning coefficients between foamate and retentate of almost 200 and 2000, respectively, and with yields reaching 50% and 90%, respectively.

Conclusions: Application of MPEC and fed-batch mode proved successful in increasing enzyme production. Hence, both ATPS and FF can be used for laccase purification.

Biography

Anna Antecka has received her PhD in Environmental Engineering in 2008 from the Lodz University of Technology in Currently, she is an Assistant Professor in the Department of Bioprocess Engineering at Lodz University of Technology. From 2004-2005, she has worked at the International Institute Zittau, Germany, in the Department of Environmental Biotechnology. In 2002, she studied at the University of Dortmund, Germany. Her main research interests are in microbial ecology and biotechnology of fungal enzymes especially laccase, including its production, purification and characterization, as well as enzyme applications for industrial and environmental purposes. Currently, she is working in the area of integrated continuous up- and downstream processes for the biosynthesis and purification of fungal laccases.

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