

**Title:** Production, purification and characterization of laccase from *Pleurotus ostreatus* grown on tomato pomace

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**Abstract:** A strain of *Pleurotus ostreatus* was grown in tomato pomace as sole carbon source for production of laccase. The culture of *P. ostreatus* revealed a peak of laccase activity (147 U/L of fermentation broth) on the 4th day of culture with a specific activity of 2.8 U/mg protein. Differential chromatographic behaviour of laccase was investigated on affinity chromatographic matrices containing either urea, acetamide, ethanolamine or IDA as affinity ligands. Laccase exhibited retention on such affinity matrices and it was purified on a Sepharose 6B-BDGE-urea column with final enzyme recoveries of about 60%, specific activity of 6.0 and 18.0 U/mg protein and purification factors in the range of 14-46. It was also possible to demonstrate that metal-free laccase did not adsorb to Sepharose 6B-BDGE-urea column which suggests that adsorption of native laccase on this affinity matrix was apparently due to the specific interaction of carbonyl groups available on the matrix with the active site Cu (II) ions of laccase. The kinetic parameters ( $V_{max}$ ,  $K_m$ ,  $K_{cat}$ , and  $K_{cat}/K_m$ ) of the purified enzyme for several substrates were determined as well as laccase stability and optimum pH and temperature of enzyme activity. This is the first report describing the production of laccase from *P. ostreatus* grown on tomato pomace and purification of this enzyme based on affinity matrix containing urea as affinity ligand.

**Author Keywords:** Laccase from *Pleurotus Ostreatus*; Lignocellulosic Enzymes; Affinity Chromatography; Reverse IMAC; Epoxy-Activated Sepharose 6B-urea; Tomato Pomace

**KeyWords Plus:** Chromatographic Behavior; Affinity-Chromatography; *Pseudomonas-Aeruginosa*; Versicolor; Xylanase; Adsorption; Industrial; Strains; Amidases; Lignin

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