

ABSTRACT

Widaad Afifah. 24020121120032. **Characterization of Recombinant Laccase Enzyme Protin From *Arthrobacter psychrolactophilus* Bacteria.** Thesis. Supervisor: Nurhayati and Fina Amreta Laksmi.

Laccase (EC. 1.10.3.2) is an enzyme capable of catalyzing the oxidation of various phenolic and non-phenolic substrates using molecular oxygen as the electron acceptor. This enzyme is widely applied in textile dye decolorization due to its environmentally benign reaction product, water (H₂O). In general, fungal laccase exhibit relatively low thermal stability, whereas laccases produced by thermophilic bacteria often show limited tolerance toward *organic solvents*. To address these limitations, a recombinant laccase from the extremophilic bacterium *Arthrobacter psychrolactophilus* was expressed in *Escherchia coli* BL21 Star DE3 to achieve high-level enzyme production while enhancing its stability toward temperature and organic solvents. The resulting Ap-Lac enzyme was characterized to determine its activity profile, stability, kinetic parameters, and application potential. Characterization included measurements of enzyme activity under varying pH, temperature, metal ions, organic solvents, and dye decolorization assays. All measurements were performed using a spectrophotometric method with 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) as the substrate. The results showed that Ap-Lac exhibits an optimal activity at pH 4 in 100 mM sodium acetate buffer and remains stable across pH 4 – 9 while retaining 92% of its activity. The optimal temperature was 60°C, with *half-life* of 462 minutes at 55°C, 85,5 minutes at 60°C, and 15,5 minutes at 65°C. Enzyme activity increased up to 1279% following the addition of 1 mM Cu²⁺, indicated by the appearance of a strong absorbance peak at 600 nm. The kinetic parameters of Ap-Lac showed K_m , K_{cat} , K_{cat}/k_m values of 0,55 mM, 1,13 S⁻¹, and 2 mM⁻¹S⁻¹, respectively. Ap-Lac demonstrated tolerance to organic solvents, retaining 70% activity in the presence of 10% methanol. Ap-Lac showed strong decolorization efficiency toward antraquinone dyes both with and without the mediator ABTS. The finidings indicated that Ap-Lac prossesses excellent stability and catalytic performance, highlighting its potential for application in industrial wastewater bioremediation.

Keywords: Multicopper, Oxidation, ABTS, Decolorization