

# LACCASE STABILIZATION IN THE PRESENCE OF COAL HUMIC ACIDS

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## I. INTRODUCTION

Extracellular enzymes in soil are assumed to be stabilized via formation of humic substances (HS) and enzyme complexes (Nannipieri et al., 1998). The formation of HS-enzyme complexes has been shown for most important enzymes presented in soil and dynamics of enzymatic activities for few of them have been studied. However there is no direct evidence for formation of laccase-HS complexes in spite of the fact that laccase activity has been detected in soil extracts (Masciandaro and Ceccanti, 1999). Therefore, the aim of this investigation was to study laccase interaction with humic acids (HA) and the effect of pH on the stability and activity of HA-laccase complex.

## II. MATERIAL AND METHODS

### *Laccase isolation and characterization*

The strain of *Coriolus hirsutus* producing extracellular laccase was kindly provided by Komarov Botanical Institute (St. Petersburg, Russia) and cultivated as described in (Koroleva et al., 2002). Extracellular laccase was isolated from the culture medium and purified in accordance with (Koroleva (Skorobogat'ko) et al., 1998).

Laccase activity was determined spectrophotometrically (Koroleva (Skorobogat'ko) et al., 1998) at 410 nm using 10 mM pyrocatechol as substrate in 0.1 M sodium acetate buffer (pH 4.5). One unit of activity is defined as change in A410 per min per mg of protein.

### *Humic acids*

Humic acids (HA) used in this study was a commercial preparation of leonardite HA Powhumus (Huminthech, Germany).

### *Stability of free laccase and laccase-HA with time*

The stability of free laccase and laccase-HA with time was monitored at pH 5.0 and 6.5, assaying the activity during 7 days (after 6h, 12h 24 h, 48 h, 72h, 96h, 120h, 144h and 168h). Experiments were conducted under the following conditions: concentrations of laccase and coal HA were 100 and 40 mg/L respectively; temperature 27°C. Phosphate buffer (50 mM, pH 5.0 or 6.5) was used as a background electrolyte.

UV-vis spectra of free laccase, HA and laccase-HA complex in 50 mM potassium phosphate buffer, pH 5.0 or 6.5, at 25 °C were recorded between 195-600 nm with a spectrometer PerkinElmer Lambda 25.

## III. RESULTS AND DISCUSSIONS

### *Inhibition of laccase in the presence and absence of coal HA*

The comparative study of free laccase and enzyme in presence of HA pH-stability has been carried out at pH 5.0 and 6.5. The enzymatic activity of free laccase as well as of HA-enzyme complex decreased faster at pH 6.5 than at pH 5.0. The significant stabilization of enzyme by HA has been observed at pH6.5 (Figure.1). Free laccase and laccase-HA complex completely lost enzymatic activity after 50 hours and 100 hours of incubation at pH 6.5 respectively. The curve of the enzyme inactivation with and without HA at pH 6.5 (Figure 1) demonstrates two stages of laccase

inactivation process with smooth region between 6 and 24 hours. This finding can be assigned to the conformational changes of both protein globule and active site assembly of four copper ions forming enzyme active center.

Laccase activity in the presence of HA was above zero value even after 150 hours, and the first stage of the enzyme inhibition lasted for about 50 hours. Therefore, laccase has been stabilized by HA at 6.5 probably due to formation HA-enzyme complex. The optical spectra of free laccase and enzyme-HA complex confirmed these suggestions.

At pH 5.0, which is laccase pH optimum in the buffer used the stabilization of enzyme has not been observed. Laccase stability at pH 5.0 was considerably higher than at pH 6.5. The residual enzyme activity comprised 66% and 69% without and with HA after 50 hours of incubation, respectively.

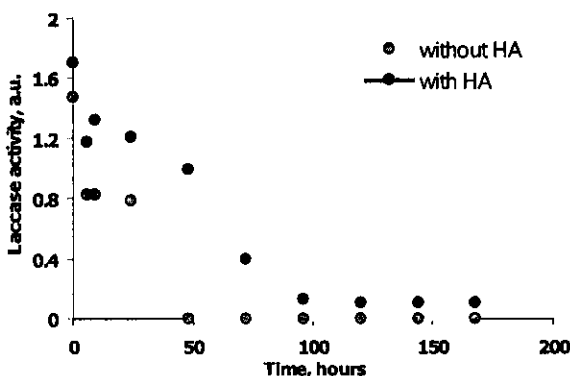


Figure 1 – Dynamic of laccase activity in the presence and absence of coal humic acids at pH 6.5.

The phenomenon observed can be seemingly explained by the formation of HA-enzyme complex. This resulted in stabilization laccase activity under the selected conditions that occurred mostly at pH 6.5.

#### IV. CONCLUSIONS

The results have shown that the laccase can form complexes with HA and this process is pH-dependent. The most significant effect in enzyme stabilization was been reached under unfavorable condition for expressing of laccase activity.

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