**Activity of extracellular enzymes from *Pleurotus ostreatus* fungi.**

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**Highlights**

* Cultivation of *P. ostreatus* on the waste plant biomass for enzymes production.
* The highest enzyme activities were detected after 8th day of fungi cultivation.
* Stabilization of extracellular enzymes from *P. ostreatus* was done using CLEA method.

**1. Introduction**

Forest and agricultural waste can be a major development and ecological opportunity and can serve as a substrate for medicinal mushrooms cultivation such as *Pleurotus ostreatus*, which can be easily cultivated by everyone. It can successfully grow on a wide range of waste lignocellulosic material [1,2]. *P. ostreatus* is a woody fungus that acts as a saprophyte. It is involved in a process of degradation of hemicellulose, cellulose and lignin. The enzymes laccase and cellulase, which are part of the lignocellulolytic enzyme system, contribute to this degradation. Various substrates affect the activity of the thus obtained enzymes from *P. ostreatus*. The utilization of the waste biomass from forestry and agriculture for the cultivation of *P. ostreatus* mushrooms in order to obtain different enzymes can be integrated to waste management and the development of the bio-economy. Produced enzymes from *P. ostreatus* could be used as nutritional supplements or they can be immobilized as an enzyme cocktail, e.g. laccase and cellulase in the form of cross-linked enzyme aggregates (CLEA) (without a carrier) to improve the enzyme stability. Immobilization of enzymes allows the recycling of these biocatalysts, which is otherwise almost impossible and can improve their stability. In addition, the cost of the biocatalyst could be reduced due to easily separation of biocatalyst from the reaction mixture. Immobilization in the form of CLEA allows the use of so called crude enzymes and therefore, it is not necessary to use a pre-purified enzyme, which reduces processing costs and is interesting for industrial use [3]. The cultivation of *P. ostreatus* fungi on a solid medium (wheat bran) was performed. The activity of laccase and cellulase from fungi extracts after different cultivation time was studied. The optimal time for *P. ostreatus* cultivation to obtain the highest specific activity of laccase and cellulase was determined. The immobilization of extracellular enzymes produced by *P. ostreatus* in the form of CLEA was done and their activity was also studied.

**2. Methods**

Cultivation of *P. ostreatus*

*P. ostreatus* was cultivated on wheat bran at 27 °C and at different period of time.

Enzyme extraction from *P. ostreatus*

After defined growing time of *P. ostreatus,* extracellular enzyme extraction with 0.05 M sodium citrate buffer was performed.

Preparation of CLEA

Preparation of CLEA laccase was performed in two steps: precipitation with different solvents (acetone, ethanol etc.) and cross-linking using glutaraldehyde (GA) at different concentrations.

Determination of laccase and cellulase activity

Laccase activity was determined at 420 nm using 2,2-azino-bis(3-etilbenz-tiazolin-6-sulfonat) as a substrate and cellulase activity was defined at 340 nm with a UV-Vis spectrophotometer using the method with Sigmacell solution as a substrate.

**3. Results and discussion**

After 8th and 14th day of fungi cultivation, enzyme extraction was carried out to determine the activity of the selected enzymes. The optimal incubation time was 8 days, since the activities at that time were the highest. Specific activity of cellulase was 6.3166 U/mgprotein and of laccase was 6.7280 U/mgprotein (Figure 1). The obtained enzymes extract was used for CLEA preparation. The optimal precipitation reagent was found to be acetone, since highest specific activity of laccase and cellulase was obtained after precipitation in that solvent. With the increase in GA concentration from 1% (v/v) to 8% (v/v) an increase in immobilization efficiency and in specific activity of cellulase and laccase were determined.



**Figure 1.** Influence of cultivation time of *P. ostreatus* on laccase and cellulase specific activity.

**4. Conclusions**

Cultivation of *P. ostreatus* was successfully performed on solid medium using wheat bran as a substrate. The activities of extracellular laccase and cellulase were the highest after 8th day of *P. ostreatus* cultivation. Extracted extracellular enzymes from *P. ostreatus* were immobilized in the form of CLEA for possible application in industrial cascade bioprocesses.

**References**

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