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Extended Abstract

Optimization of Laccase Extraction from Spent Pleurotus Substrate †

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Spent mushroom substrate (SMS) is a significant source of enzymes and bioactive compounds that needs efficient extraction treatments in order to increase its economic value. Mushrooms are multifunctional components used in the human diet for their medicinal and nutritional value. Selenium (Se) is a microelement which assures the essential intake of antioxidant enzymes through Se aminoacids [1]. Laccase (EC 1.10.3.2. diphenol:oxygen oxidoreductase) is an enzyme from the oxido-reductase class that oxidizes a large variety of phenolic compounds using molecular oxygen as an oxidizing agent. The aim of this study was to optimize the extraction of laccase from spent Pleurotus substrate (SPS) by varying the pH, temperature, and liquid/solid ratio (L/S). We also wanted to see the influence of Se on Pleurotus growth, metabolism, and laccase expression and activity. Pleurotus mushrooms were grown in specific tents, in plastic bags, in the presence or absence of 50 and, respectively 100 µM Se, until formation of fruiting bodies. We used a respirometer equipment (Echo Instruments, Slovenske Konjce, Slovenia) to determine the amount of CO_2 produced and the O_2 consumed by mycelium growth in the presence and absence of Se [2]. The substrate was lyophilised, and then subjected to protein extraction in a water bath with agitation (100 rpm) for 4 h. The extraction of laccase was optimized by response surface methodology (RSM) using a face-centered design (FCD) with one nominal factor (the buffer system: acetate and phosphate) and three numeric factors, namely pH (6, 7, 8 for phosphate and 3.8, 4.7, 5.6 for acetate), temperature (25, 45 and 65 °C) and L/S ratio (10/1, 30/1 and 50/1). Three center points were added per buffer system to estimate the standard error. The response was the enzyme activity which was determined by measuring the absorbance of ABTS at 420 nm with a UV-VIS spectrophotometer [3]. The design was created with a randomized run order and analyzed using Design Expert® Version 11 software. Most protein extracts significantly oxidized ABTS, except the extracts obtained at 65 °C and at pH ≥ 7, parameters that inactivated the enzyme. The oxidation rate increased after concentrating the extract by ultrafiltration, indicating an enzymatic-induced process. The cultivated Pleurotus expressed significant amounts of active laccase, which was active at pH < 7, as expected. Preliminary data showed that Se could positively influence the expression of laccases, but the effect is highly dependent on the dose and optimizations are necessary for positive outcomes, as the induction of enzyme expression could be balanced by the toxicity effects of Se. Se did not significantly influence the CO_2 production nor the O_2 consumption by mycelium.

In conclusion, we used solid-state fermentation to obtain fruiting bodies from Pleurotus for dietary supplements or food industry use, and SPS as a sub-product for a cost-effective source of ligninolytic enzymes. The influence of Se on enzyme expression needs an in-depth investigation.

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