Use of Agro-Industrial Waste for Production of Laccase and Manganese Peroxidase from White-Rot Basidiomycetes

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Ligninolytic enzymes have significant potential applications in various industries, including pulp and paper, bioremediation, textile, and laundry. Because the biotechnological applications require large amounts of low-cost enzymes, it is essential to search for powerful producers of enzymes as well as for abundant and inexpensive lignocelluloses wastes. Some of these wastes may contain significant concentrations of soluble carbohydrates and inducers of enzyme synthesis, enabling an efficient production of lignocellulolytic enzymes.

Various agroindustrial byproducts have been used for submerged fermentation of *Cerrena unicolor* (Bull.: Fr.) Murrill and *C. maxima* (Fr.) Ryvarden for ligninolytic enzyme production. Six agroindustrial byproducts were tested in shake flask trails. Wheat bran appeared to be the best growth substrate for fermentation of *C. unicolor,* enabling a very high accumulation of laccase (87.450 IU/L on day 7) in culture liquid. Kiwi, as a growth substrate, supported remarkable secretion of manganese peroxidase (2016 IU/L on day 7). Ethanol production wastes also provided a high yield of laccase, whereas other substrates (banana peels, peanut shells, and cotton stalks) appeared to be rather poor growth substrates for laccase and manganese peroxidase production.

Testing of *C. maxima* as a ligninolytic enzyme producer showed that this fungus is a weaker producer of laccase and peroxidase than *C. unicolor.* However, the supplementation of wheat bran, ethanol production wastes, and kiwi highly stimulated laccase production, which reached 6247 IU/L, 6141 IU/L, and 5569 IU/L, respectively. In contrast to *C. unicolor,* the second tested fungus, *C. maxima*, produced very low titres of manganese peroxidase (9.2–43.7 IU/L) in fermentation of five lignocellulosic substrates, whereas no manganese peroxidase was detected in submerged fermentation of kiwi. The data prove that the composition of lignocellulose substrates appear to determine the type and amount of enzyme produced by the wood-rotting Basidiomycetes.

Subsequently, *C. unicolor* cultivation was performed in a 10-liter fermentor using ethanol production wastes as a growth substrate. In order to maximize fungus growth and enzyme production, the pH was automatically controlled at 5.5 during the fermentation process. Laccase activity began on the second day and gradually increased, peaking on the 11th day (233.015 IU/L). Manganese peroxidase showed two peaks, on day 6 (107 IU/L) and on day 10 (142 IU/L).

Fermentation of *C. unicolor* on wheat bran supported lower laccase levels (185.640 IU/L, 12th day) but a much higher manganese peroxidase yield (6.300 IU/L, 11th day).

Similar to the shake flask trial, alcohol production waste supported quicker laccase production. On the 7th day, alcohol production waste and wheat bran reached 81% and 57% of their maximal laccase concentration, respectively.

We plan to elucidate the specific components in the substrates that affect the kinetics and regulate laccase synthesis. This aspect as well as enhancement of laccase synthesis is crucial for large-scale production. Continuation of yield increase will also include supplementation of inducers as well as further concentration of substrates via batch and fed-batch regimes.