Recovery of Laccase from Spent Mushroom Substrate

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Spent mushroom substrate (SMS) consists of composted organic material heavily colonized with *Agaricus bisporus* (J. Lge) Imbach mycelium. This mushroom species produces a variety of extracellular oxidative and hydrolytic enzymes, the most abundant of which is laccase, a polyphenoloxidase. Laccase exhibits broad substrate specificity and has received considerable research attention owing to its potential use in a variety of biotechnological applications. SMS is available cheaply and in large quantities as a by-product of the mushroom industry and is therefore being investigated as an alternative source of laccase for biotechnological application.

As part of an overall project aimed at optimization of the laccase extraction process, the present study examined the effects of surfactants on the extraction and stability of laccase from SMS. Extraction efficiencies were determined as a function of surfactant concentration for a variety of nonionic, anionic, and cationic surfactants relative to aqueous buffer alone. The effect of each surfactant on the catalytic properties of preextracted laccase was also evaluated to differentiate between surfactant effects on enzyme activity and those on enzyme release from the solid compost matrix.

When used at final concentrations ranging from 0.001% to 0.3% (w/v), nonionic surfactants and the anionic surfactant, sodium dodecyl sulfate, enhanced laccase extraction efficiencies by up to 10% relative to buffer alone. The cationic surfactant hexadecyltrimethylammonium bromide reduced extractable laccase activity by approximately 5%. However, these effects were highly variable and there was no clear relationship between surfactant concentration and extraction efficiency.

Addition of preextracted laccase to heat-inactivated compost followed by extraction into buffer alone or buffer with surfactant revealed that 40% of the added enzyme remained bound to the solid compost material for all buffers and surfactants examined. This was in contrast to similar studies performed with cellulase, where 100% recovery was achieved in one extraction step.

These results suggest that an appreciable amount of laccase remains bound to SMS following extraction via existing protocols. The adsorption of laccase to specific SMS components is currently being investigated to identify the nature of these binding interactions and to develop more efficient one-step extraction protocols for laccase recovery.

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