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BIOLOGICAL AND GEOCHEMICAL CHANGES IN LIGNOCELLULOSIC SUBSTRATES DURING THE SOLID STATE FERMENTATION OF *PLEUROTUS OSTREATUS*

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White-rot fungi are the most effective biotic degraders of lignin due to their suite of extracellular enzymes and mycelial growth habit. Their activity represents a key step in the global carbon cycle since it influences the forms in which carbon is released from lignocellulosic macrostructures such as tree trunks and grass stems as well as the pathways that the carbon will subsequently follow. This release of carbon is a biotic process that varies over time as the fungi grow, colonise substrates and express enzymes, suggesting that research will benefit from an approach that monitors multiple variables over time and that allows links to be made between the variables. Previous work in our laboratory (e.g. Vane et al. 2001a,b) has investigated time-dependent changes in lignin degradation products occurring during the breakdown of wheat straw lignin by white-rot fungi.

A model system with *Pleurotus ostreatus* growing on unamended wheat (*Triticum aestivum*) straw, common ash (*Fraxinus excelsior*) wood or Sitka spruce (*Picea sitchensis*) wood under solid-state fermentation conditions was used to investigate changes occurring during growth of this white-rot fungus. Sampling took place at regular intervals over 12 weeks. Lignin degradation was investigated using thermochemolysis in the presence of tetramethylammonium hydroxide (TMAH) which allowed identification of a suite of degradation products and changes in their relative abundances. Calculation of acid/aldehyde (e.g. 3,4-dimethoxybenzoic acid to 3,4-dimethoxybenzaldehyde) and side chain scission (e.g. 3,4-dimethoxybenzoic acid to 1,2-dimethoxy-4(1,2,3-trimethoxypropyl)benzene) ratios allowed lignin oxidation to be monitored. Changes in amounts of the fungal biomarker ergosterol were used as a proxy for fungal biomass. The activity levels of the extracellular enzymes were determined using targeted assays of the extracellular fluid removed from the solid state fermentation system. The ligninolytic enzymes manganese peroxidase and laccase were assayed, plus β-glucosidase which catalyses the breakdown of glucose oligomers.

Figure 1 a, b and c show the enzyme activity levels measured when *Pleurotus ostreatus* was grown on wheat straw; similar patterns were observed on ash and Sitka spruce. Of the two ligninolytic enzymes, laccase activity peaked one week earlier and subsequently declined more than manganese peroxidase activity. β-Glucosidase activity increased rapidly

in the first week but then remained relatively constant; the highest activity was measured in week 7. The pattern of enzyme activity contrasts with wheat straw lignin oxidation which displayed a time-lag of three weeks before the maximum rate of lignin oxidation (steepest gradient in plot) was observed (Figure 1d, in a separate experiment) and continued to increase throughout the incubation. Ergosterol concentration, and thus fungal biomass on wheat straw, also displayed a three-week time-lag before the maximum rate of increase was observed. These results indicate that enzyme production precedes fungal growth as well as lignin oxidation. Thus, the fungus probably devotes the highest proportion of its resources to producing ligninolytic enzymes when initiating lignin oxidation.

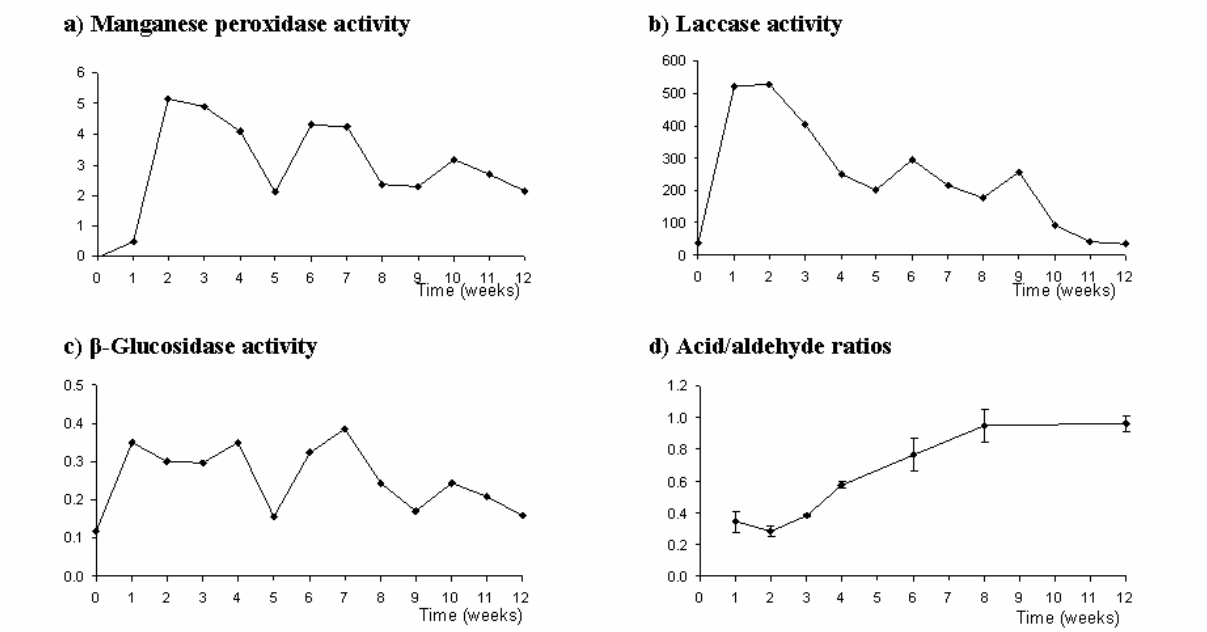

Figure 1:

a) Total extractable manganese peroxidase activity (manganese peroxidase assay units/g of dry straw) levels during 12 week solid state fermentation of wheat straw (*Triticum aestivum*) with *Pleurotus ostreatus*

b) Total extractable laccase activity (laccase assay units/g of dry straw) levels during 12 week solid state fermentation of wheat straw with *Pleurotus ostreatus*

c) Total extractable β-Glucosidase activity (β-Glucosidase assay units/g of dry straw) levels during 12 week solid state fermentation of wheat straw with *Pleurotus ostreatus*

d) Guaiacyl acid/aldehyde ratios (GCMS peak area ratios of 3,4-dimethoxybenzoic acid/3,4-dimethoxybenzaldehyde) indicating lignin oxidation during 12 week solid state fermentation of wheat straw with *Pleurotus ostreatus*. Data from a separate experiment to a), b) and c)

REFERENCES

Vane C.H., Abbott G.D. and Head I.M. (2001a) The effect of fungal decay (Agaricus bisporus) on wheat straw lignin using pyrolysis-GC-MS in the presence of tetramethylammonium hydroxide (TMAH). *Journal of Analytical and Applied Pyrolysis*, **60**, 69-78.

Vane C.H., Martin S.C., Snape C.E. and Abbott G.D. (2001b) Degradation of lignin in wheat straw during growth of the oyster mushroom (Pleurotus ostreatus) using off-line thermochemolysis with tetramethylammonium hydroxide and solid-state 13C NMR. *Journal of Agricultural and Food Chemistry*, **49**, 2709-2716.