Lignin Biodegradation by Selective White Rot Fungi as a Biotechnological Tool for Lignocellulosic Biorefinery

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Development of conversion systems from lignocellulosics into biofuels and chemicals has received much attention due to immense potentials for the utilization of renewable bioresources. In particular, production of bioethanol from lignocellulosics after saccharification with cellulolytic enzymes is a major concern. Since lignin makes the access of cellulolytic enzymes to cellulose difficult, it is necessary to decompose the network of lignin prior to the enzymatic hydrolysis. Biological pretreatment with white rot fungi in combination with thermochemical or physicochemical treatment is one possible approach for this purpose.

The lignin biodegradation by white rot fungi is an extracellular free radical event that proceeds in concert with the activation of molecular oxygen and redox cycling of transition metals. When wood is colonized by wood degrading fungi, their extracellular enzymes are not able to diffuse into the intact wood cell walls because the enzymes are too large to penetrate the pores of the wood cell walls. Hydroxyl radicals (•OH) are proposed as a principal low molecular mass oxidant that erodes wood cell walls to enhance the accessibility of the extracellular enzymes of wood rot fungi to wood cell wall components. In brown rot, hydroxyl radicals disrupt cellulose and hemicelluloses in wood cell walls, with concomitant modification of lignin substructures. Production of hydroxyl radicals are also reported for non-selective white rot fungi. In the Fenton system, catalysts for the reductive half cycle ($Fe^{3+} \rightarrow Fe^{2+}$) accelerate the hydroxyl radical formation. Wood rot fungi have versatile enzymatic and non-enzymatic systems to accelerate the reductive half cycle. In contrast to brown rot and non-selective white rot fungi, selective lignin-degrading fungi like *Ceriporiopsis subvermispora* are able to decompose lignin in wood cell walls without the intensive damage of cellulose. We first isolated a series of novel itaconic acid derivatives having a long alk(en)yl side chain at position C-3 of its core (ceriporic acids) from the cultures of *C. subvermisrpora*, and showed that ceriporic acids inhibited depolymerization of cellulose by the Fenton reaction, by suppressing a reductive half cycle from Fe^{3+} to Fe^{2+} [1-4]. Wood decay by *C. subvermispora* proceeds without the penetration of extracellular enzymes into the wood cell wall regions. As for the lignin degradation system at a site far from enzymes, lipid peroxidation has been proposed as a major pathway at an incipient stage of wood decay. We analyzed ligninolytic free radical species from hydroperoxides or peroxidizable compounds, and developed several ligninolytic free radical reactions.

The fungal and chemical pretreatments have been applied to various biomass conversion processes. A large scale solid state fermentation of a white rot fungus, *Phellinus* SKM2102 was applied to the pretreatment of Japanese cedar wood, in combination with microwave solvolysis. The pretreatments with white rot fungi were also applied to methane fermentation of wood biomass. The use of white rot fungi and biomimetic radical reactions is attractive not only for increasing saccharification yields but also for decreasing energy input for milling and transportation of the raw feedstock.

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