THE ROLE OF FUNGI IN BIOREMEDIATION

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Fungi represent a major portion of metabolically active microbial biomass in soil and other compartments of the biosphere. Thanks to their ecological interactions and to their biocatalytic potential, they are able to degrade organic pollutants and to contribute to the removal of other environmental threats, such as the accumulation of heavy metals. The fungal mycelial growth lifestyle is thought to be conducive to enhanced bacterially-mediated bioremediation activities. However, the best understood and exploited feature of fungi is their possession of catabolic enzymes, particularly oxidoreductases, whose low substrate specificity renders them ideal biocatalysts for environmental cleanup of diffuse and concentrated pollution. Together with many other fungal groups, the diverse ecophysiological group known as white-rot fungi (WRF) includes organisms that act as key regulators of the global C-cycle and have become a subject of intense study in recent years. The WRF lignin-modifying enzymes, i.e., manganese peroxidases (MnP), lignin peroxidases (LiP), and laccases (Lac) are involved not only in the degradation of lignin in their natural lignocellulosic substrates but also in the degradation of various xenobiotic compounds including aliphatic and (poly)aromatic hydrocarbons, chloro-, nitro- and amino-aromatics, polychlorinated buphenyls, azo and anthraquinonic dyes and, last but not least, a variety of emerging micropollutants, endocrine disruptor compounds EDC), pharmaceuticals and personal care products. Oxidoreductases like laccases can oxidize various compounds of phenol-like structure plus several non-phenolic substrates indirectly via the oxidized form of mediator molecules.

This prodigious bioremediation potential is illustrated with a number of studies from our laboratory. In the area of highly recalcitrant explosives, no bacterial strain has so far been shown to mineralize trinitrotoluene (TNT) whether in aerobic or anaerobic conditions, the partial mineralization of the carbon skeleton of TNT by WRF under ligninolytic conditions occurs via its initial reduction to aminonitrotoluenes, which are substrates for subsequent oxidative transformation by extracellular ligninolytic enzymes.

In the field of synthetic dyes, raw mixed-dye wastewater from a textile dye-producing plant was found to be decolorized by an agaric WRF, *Clitocybula dusenii*, which displayed higher Mn peroxidase (MnP) and laccase activities when grown with dye effluent than in control cultures. In a similar context, the effectiveness of ozonation, of a treatment using WRF and their enzymes and of a combined ozonation/fungal process were compared in terms of residual color and (geno)toxicity. The combined treatment (ozone/WRF) caused not only decolorization but an adequate abatement of the toxicity.

Finally WRF laccases have been successfully produced and immobilize or insolubilized in the form of cross-linked enzyme aggregates (CLEA) and applied for the treatment of EDC in adapted reactor systems. Furthermore, we have identified key factors for the production of CLEAs of laccases alone or of combined oxidoreductases, and we have improved these novel biocatalysts by applying rational experimental design and optimization methodologies. An illustration of the powerful possibilities afforded by multi-enzyme aggregates involves our co-aggregation of laccases from *Coriolopsis polyzona*, with an acidic pH-optimum, and from *Coprinopsis cinerea*, with a neutral pH-optimum, in a single biocatalyst. The combi-CLEAs obtained showed activity over a broad pH-range implying their suitability for the treatment of real wastewaters with varying pHs.

The creative fusion of fungal ecology and physiology, biocatalysis and chemical engineering fundamentals opens up particularly attractive horizons towards a new green solutions for environmental stewardship and sustainability.

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