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SYNERGISTIC ACTION OF AZOREDUCTASE AND LACCASE LEADS TO MAXIMAL DECOLOURIZATION AND DETOXIFICATION OF MODEL DYE-CONTAINING WASTEWATERS

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Abstract

Many of the azo dyes used in the textile, paper, leather or plastic industries, and/or their breakdown products are toxic, potentially carcinogenic and can lead to the formation of bladder cancer in humans, tumours, allergies, nuclear anomalies in experimental animals, and chromosomal aberrations in mammalian cells. Among the few redox-active enzymes showing azo dyes degradative activity, azoreductases are particularly effective since they act on the reduction of the azo linkage, however, azoreductases require the addition of expensive cofactors such as NAD(P)H as electron donors for the reductive reaction and the products released are aromatic amines which are potentially toxic. In contrast, laccases are oxidoreductases that have a great potential for various biotechnological processes mainly due to their high non-specific oxidation, the lack of a requirement for cofactors, and the use of readily available oxygen as an electron acceptor capacity.

In the present study, the enzymatic degradation of an array of 18 azo dyes and of three model dye baths was tested with two enzymes with proven ability to degrade synthetic dyes, recombinant FMN-dependent NADPH azoreductase (PpAzoR) from *Pseudomonas putida* MET94 and recombinant CotA-laccase from the bacterium *Bacillus subtilis*. The PpAzoR showed a broader specificity for decolourisation of azo dyes than CotA-laccase. However, the final products of PpAzoR activity exhibited in most cases a 2 to 3-fold higher toxicity than intact dyes themselves. We showed that addition of CotA-laccase to PpAzoR treated reaction mixtures lead to a significant drop in the final toxicity. An *Escherichia coli* strain co-expressing *ppAzoR* and *cotA* genes was constructed where the sequential action of PpAzoR and CotA enzymes could be tuned by aeration conditions. Whole-cell assays of recombinant strain for the treatment of model dye wastewater resulted in decolourisation levels above 80% and detoxification levels up to 50%. The high attributes of this strain, make it a promising candidate for the biological treatment of industrial dye containing effluents.