## Characterization and stability of free and immobilized Laccase in the Dyestar dyeing effluent

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## Summary

In this study the stability (in terms of half-life time) and decolourisation efficiency of free and immobilised laccase was determined in four industrial dyeing effluents. The dyeing liquors composition and the chemical structure of the dyes influence significantly the stability and the decolourisation ability of the enzyme. The dyeing in enzymatically decolourised Reactive Black 5 effluent provided consistency of the colour with both bright and dark dyes. Additionally the number of washing cycles necessary to remove the hydrolyzed dyestuff from dyed textiles was reduced using an enzymatic pre-treatment. In decolourisation experiments with immobilised laccase, two phenomenons were observed - initial decolourisation due to adsorption on the support and dye degradation due to the enzyme action.

This research focuses on the stability and decolourisation ability of free and immobilised laccase in industrial dyeing effluents and their reuse for dyeing. In our experiments the stability of the immobilised laccase in dyeing liquors was unexpectedly lower than the stability of the free enzyme. The stability of the laccase in dyeing effluents is a result of the additive effect of all components presented. The decrease of enzymatic activity could be associated with the potential protein – dyeing effluent components interactions. Azoaromatic sulfonate dye anions could provide enzyme stabilisation. However, the structure of the immobilised laccase possibly became less accessible than the free enzyme for interaction with the anionic dyes, and thereby the stabilisation was not efficient. Non-ionic surfactants present in the dyeing liquors are able to stabilise the enzyme in solution by formation of a surrounding shell. The surfactant stabilisation was effective only on free enzyme. The salts in the dyebaths also displayed enzyme stabilising properties. The presence of CrVI and CrIII in the mordant dyeing effluent could cause significant loss of activity. The colour removal with immobilized laccase is due to the additive effects of dye-support adsorption, dyeprotein adsorption and enzymatic dye degradation. In the decolourisation experiments carried out in pure dyes, the higher the hydrophilicity of the dye, the lower was the decolourisation. The Reactive Blue 158 adsorbed on the alumina support could act as mediator in laccase-assisted reactions, explaining the higher decolourisation of Reactive Black 5 with immobilized enzyme. The decolourisation efficiency in dyeing effluents was affected by the stabilization/destabilisation interactions in the dyeing formulation. Acceptable colour difference was achieved in dyeing with dyebaths prepared with laccase decolourised Reactive Black 5 effluent. Enzymatic treatment of dyed textiles provided water savings, reducing the number of the after-dyeing washing cycles.