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LC-MS CHARACTERIZATION OF INTERMEDIATES AND PRODUCTS OF ACID ORANGE DYES AFTER LACCASE TREATMENT

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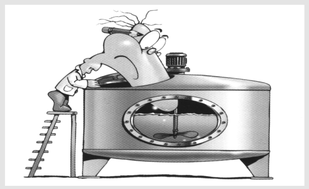


Introduction About 300.000 t/year azo dyes are used for textile dyeing operations, during which over 15% dyes are lost in the wastewater stream. The azo dyes are normally non-biodegradable under aerobic conditions. Under anaerobic conditions they are reduced to potentially toxic and mutagenic aromatic amines. The enzyme **Laccase** is a multicopper oxidase, which could decolorize azo dyes without cleavage of azo-bond and formation of aromatic amines. Degradation products of two **Acid Oranges dyes,** 3-(4-dimethylamino-1-phenylazo) benzene sulfonic acid and 3-(2-hydroxy-1-naphthylazo) benzene sulfonic acid by laccase ***Trametes villosa*** have been examined. Analyses of the products were performed using **ion pair chromatography coupled with a tandem mass spectrometer.**

Experimental

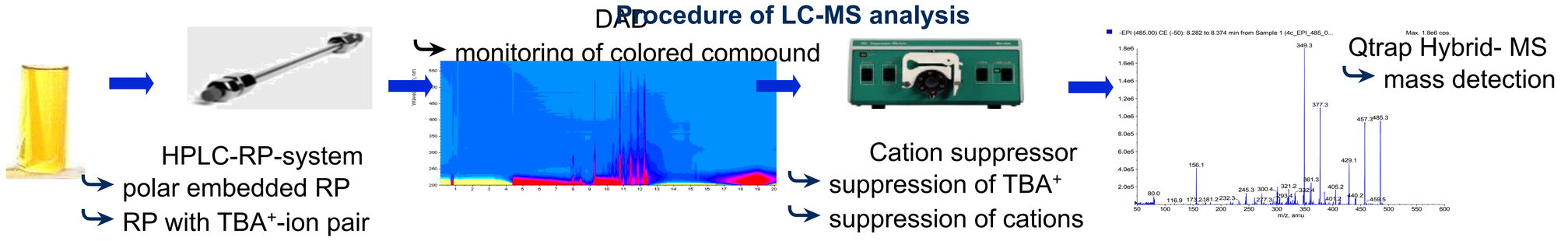
Conditions of Laccase degradation of dyes

The investigated dyes 3-(4-dimethylamino-1-phenylazo) benzene sulfonic acid (***dye I***) and 3-(2-hydroxy-1-naphthylazo) benzene sulfonic acid (***dye II***) have been prepared in a concentration of 0,001 M (in citrate buffer 0,1M at pH 5). Dye solutions were incubated at room temperature with laccase solution from *Trametes villosa* (5,3 mg protein/L, 600 U/mL).

Experimental details of LC-MS analyses:

Analyses were performed on an Agilent 1100 HPLC system (degasser, binary pump, column compartment, Diode Array Detector) coupled with a Qtrap ESI-MS/MS from Applied Biosystems (Canada). For chromatographic separation the following analytical columns were used: Synergi Hydro (Phenomenex, USA); ProntoSIL AQ (Bischoff; Germany); Nucleosil 100 - 3 C18 HD (Macherey-Nagel, Germany) with different gradient methods. A 753 suppressor module (Methrom, Swiss) installed between DAD and MS was used for cation suppression. The MS analyses were performed under negative ionisation in the Enhanced Mass Scan-, Enhanced Product Ion- , Precursor Scan Ion- and MS^3 Scan - mode.

Procedure of LC-MS analysis



Results

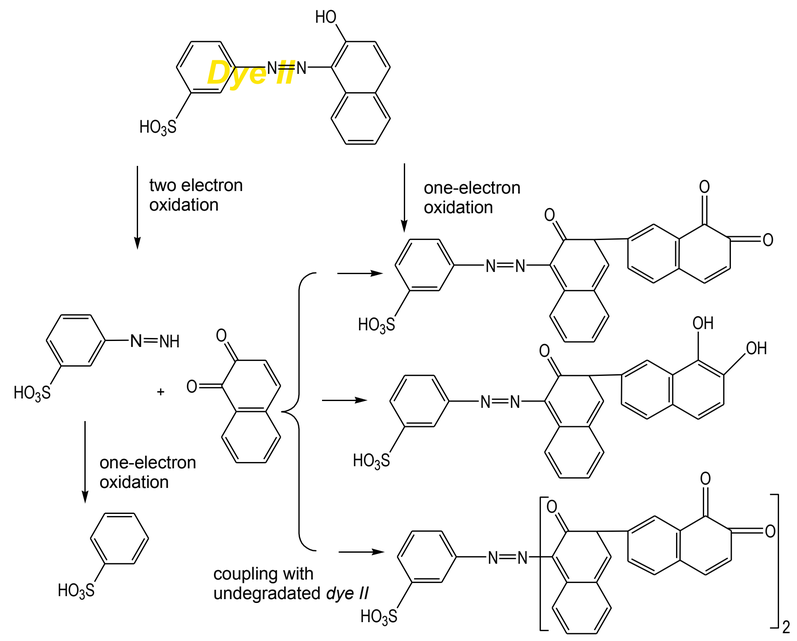
In the investigated samples **products of oxidation** by C-N bond cleavage have been observed and **oligomeric products of coupling of this oxidation products with undegradated molecules of *dye I* and *dye II***.

In both samples oxidation products, like benzenesulfonic acid, 3-hydroxy - benzenesulfonic acid and 3-diazenyl-benzenesulfonic and have been identified.

Degradation of ***dye I*** leads to the formation of dimeric products of coupling of *dye I* and one or two benzenesulfonic acid molecules.

Laccase treatment of ***dye II*** results in the formation of coupling products of *dye II* and one or two 1,2-naphthoquinone molecules and otherwise in the formation of coupling products of hydroxy-benzenesulfonic acid and one 1,2-naphthoquinone molecule. Additionall coupling of nitrogen molecules with degradation products has been observed.

Major products of laccase treatment of *dye II*



Conclusions

Several products of laccase treatment including several consecutive reaction products have been identified by an optimized LC-DAD-IC-MS technique. Attempts should be made to find conditions for complete degradation of the investigated azo dyes by laccase treatment without oligomer formation.