9th Eastern European Young Water Professionals Conference

the international water association

BOOK of ABSTRACTS

Uniting Europe for Clean Water:

Cross-Border Cooperation of Old, New and Candidate Countries of EU, for identifying problems, finding causes and solutions

24-27 May 2017 Budapest, Hungary

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Budapest University of Technology and Economics,

Budapest 2017

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ISBN: 978-963-313-256-2

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Budapest 2017

Investigation of Acid Blue 193 and Crystal Violet Organic By-products Released Under Catalytic Reactions of the White-Rot Fungal Laccases

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INTRODUCTION

Wastewaters generated by textile dyeing processes represent an important pollution source of aquatic ecosystems mainly by intensive water colouration, dyes toxicity and/or persistence. Azo dye Acid Blue 193 and triphenylmethane dye Crystal Violet are identified among synthetic substances used in a large quantity in dyeing processes (Palma-Goyes et al., 2010; Ahmadi et al., 2015). Removal of the used compounds during dyeing processes is a necessity, several physical, chemical, and biological procedures being applied in this way. Taking into consideration the economic and ecologic impact, the biological degradation of dyes is considered the most appropriate (Kurade et al., 2017). However, direct exposure of the microorganisms (bacteria, microalgae, fungi) to the target pollutants could seriously affect wastewater treatment performance mainly due to the dyes' properties. In the mentioned above situation, pollutants' degradation with an extracellular enzymatic system is viewed as a more reliable method. However, it is important to identify intermediary and/or finally products released during degradation processes as they could show higher toxicity level than initial compound (Vandevivere et al., 1998). In this case, the study focused to determine the organic breakdown products of Acid Blue 193 and Crystal Violet resulted after enzymatic degradation with phenol oxidases (laccases) obtained from white-rot fungi.

MATERIALS AND METHODS

Laccases from white-rot fungus *Trametes versicolor* (0.53 U/mg) was purchased from Sygma Aldrich (St. Louis, MO, USA). The reaction mixture contained 1 L dye solution ($2.5 \cdot 10^{-2}$ mM Crystal Violet, pH 2.0 ± 0.1; $5 \cdot 10^{-2}$ mM Acid Blue 193, pH 4.0 ± 0.1) and laccases (200 U/L). Samples were kept at room temperature (23-26 ^{0}C) in dark for 24 h in steady state conditions. Identification of the organic breakdown products of the Acid Blue 193 and Crystal Violet was performed by gas chromatography – mass spectrometry (GC-MS) analytical method using gas chromatograph 7890A (Agilent, China) coupled with ion trapp mass spectrometer 240MS (Agilent, USA).

RESULTS

Taking into consideration the results of the GC-MS screening, two reaction pathways of the Acid Blue 193 dye degradation by laccases were assumed (Figure 1). One pathway (I) began with an asymmetrical cleavage of the azo dye keeping the azo bond (-N=N-) and releasing 1-diazenyl-naphthalen-2-ol and 2-naphtol products, the latter being easy biodegradable compounds. Another pathway (II) included dihydroxylation and oxidation reactions being released 1-naphtyl ether compounds which generated by hydrolysis 2-naphtols as one of the degradation compounds. Naphthalene products were not detected in the reaction mixture possible due to the ring cleavage by deoxygenation reactions as it was supposed by Hadibarata and collaborators (2013). It is worth pointing that cleavage of the other aromatic rings could be also occurred. However, these type products could not be detected by GC - MS method, further research being needed in this way.

In the case of triphenylmethane dye (Crystal Violet) several degradation pathways were also assumed. Thus, firstly it was supposed the bond cleavage between aliphatic carbon and carbon from phenyl group being released N,N-Dimethyl-4-methylaniline and N,N,N`,N`-tetramethyl-4,4`-methylendianiline products, the latter being subjected to demethylation reactions (I) with generation of 4,4`-Methylenebis(N-methylaniline) and oxidation reactions (II) resulting 4,4`-Bis(dimethylamino)benzophenone products which were turn by acid hydrolysis in 4-(dimethylamino)benzaldehyde (Figure 1) detected by GC-MS method.

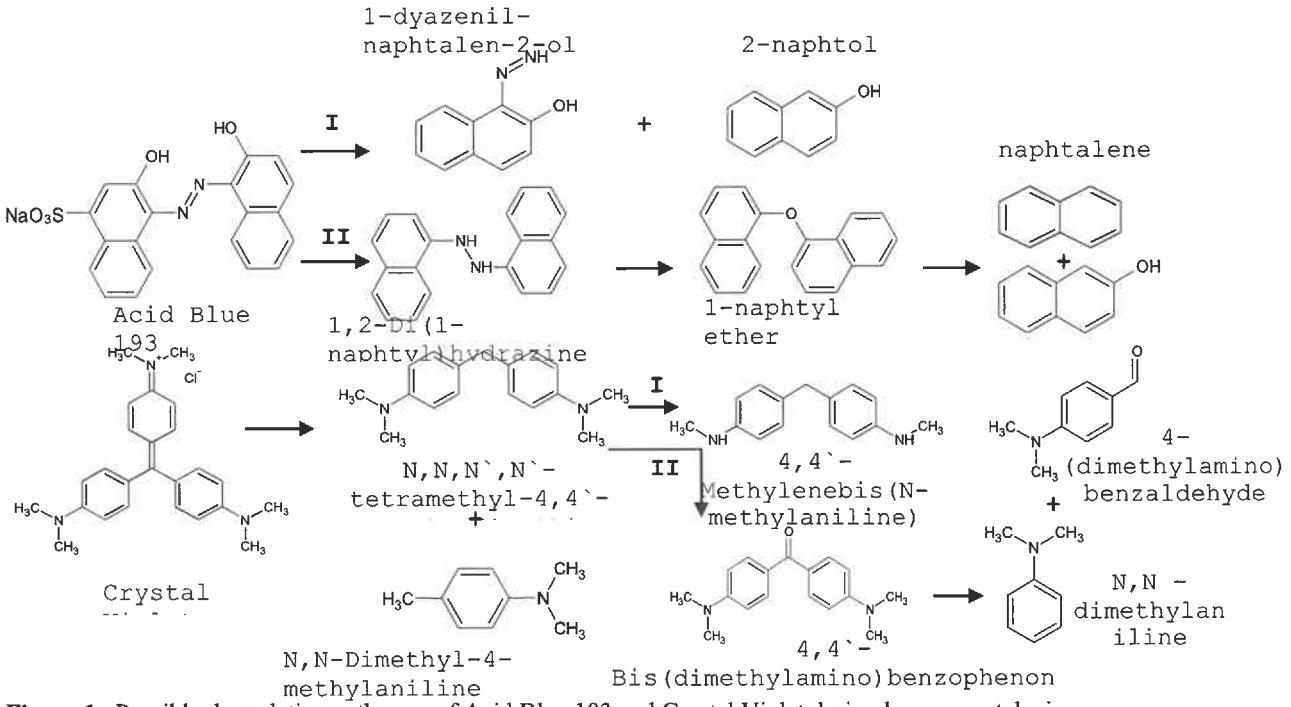

Figure 1. Possible degradation pathways of Acid Blue 193 and Crystal Violet during laccases catalysis.

CONCLUSIONS

The study investigated potential organic by-products released after Acid Blue 193 and Crystal Violet degradation by laccases obtained from white-rot fungi. Results showed that used laccases catalyzed different degradation reactions such as oxidation, demethylation, dihydroxylation, and hydrolysis conducting to the cleavage of the tested dyes in lower weight chemical structures. Moreover, taking into consideration the generation of easy biodegradable products, dyes degradation with laccases could be considered as a pretreatment step for increasing treatment performance of the dye contaminated wastewater in activated sludge process.

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