



# Enzyme based cleavage strategy of *Bacillus lentus* BI377 in response to metabolism of azoic recalcitrant



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## HIGHLIGHTS

- ▶ Elucidation on enzyme dependant color removal strategy by *Bacillus lentus* BI377.
- ▶ Decolorization of diazo Reactive Red 141 was foremost than monoazo Reactive Red 2.
- ▶ Azoreductase cleaves azo bond in diazo RR141 whereas peroxidase mediated in RR2.
- ▶ Intermediate metabolites of RR2 and RR141 lead to mineralization via TCA cycle.

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## ABSTRACT

*Bacillus lentus* BI377 (*B. lentus* BI377) an alkaliphilic strain has accomplished the discriminate color removal strategy for Reactive Red sulfonated azoic recalcitrant irrespective of their molecular structure. During the decolorization experiment, it was observed that the diazo dye first followed chromophoric cleavage by azoreductase via typical azoreduction whereas, in case of monoazo dye, cleavage took place by peroxidase via successive electron transfers to oxide surface resulting in the asymmetric cleavage of the azo bond. Dismutation of oxidative stress by reactive metabolites has confirmed by superoxide dismutase activity. Carbon monoxide (CO) binding spectra, the content of cytochrome P450 and spectroscopy analysis by GCMS, FTIR and <sup>1</sup>H NMR of intermediate metabolites indicated the differentiate pattern of diazo and monoazo dye decolorization fuse to central metabolic pathway. Declined percentage of TOC and the cytotoxicity (MTT) study confirmed that environmentally benign intermediates may lead to mineralization.

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## 1. Introduction

Azo dyes are the largest chemical class of synthetic dyes widely used as colorants in textile dyeing, leather, plastics, food, cosmetics and paper printing (Bhatt et al., 2005; Pandey et al., 2007). These azo dyes are electron deficient xenobiotics and thus are capable to be degraded via azo reduction (Xu et al., 2007; Pandey et al., 2007). However, diverse structures present in the synthetic dyes and change in the chemical structures would significantly affect its decolorization capability. Additionally, many recent reports strongly believe that the specificity of azoreductase towards azo linkage is conditional on the electron-withdrawing capability of substituent in the proximity of azo linkage(s) and their chemical

structure thus determine susceptibility of dye decolorization (Chen, 2002; Hsueh and Chen, 2008; Suzuki et al., 2001). However, recent report of Mutambanengwe et al. (2007) has stated that bacterial xenobiotic metabolism is controlled by a nonspecific enzymatic catalysis to certain degree. A multistep conversion process of monooxygenase is employed for biodegradation of xenobiotics under aerobic condition is more beneficial over anaerobic ones due to their oxygen involving mechanism in which conversion of saturated toxic aromatic amines into environmental benign metabolites (Stern, 1999; Kodam et al., 2005; Pandey et al., 2007). In some aerobic bacteria, active manganese or lignin peroxidases are involved not only in decolorization but also further degradation process via successive cleavage of azo bond (Clarke et al., 2010; Kalyani et al., 2009).

Different existed mechanisms of the sulfonated dye degradation (Chen, 2002; Suzuki et al., 2001) have proposed that the efficient strains are capable due to their enzyme inducing ability according to the chemical structure of dye (Hsueh and Chen, 2008; Hsueh

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