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Exploring docking and aerobic-microaerophilic biodegradation of textile azo dye by bacterial systems

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ABSTRACT

Aeromonas hydrophila MTCC 1739 and *Lysinibacillus sphaericus* MTCC 9523 non-adapted bacteria were investigated for decolorization of 100 mg L^{-1} of a textile azo dye Drimaren Red CL-5B in Nutrient Broth at 37 °C, pH 8 under sequential aerobic-microaerophilic conditions for 72 h. Docking by Biosolve IT software was used to calculate docking score, identify active site residues and molecular interactions between laccase and azoreductase of both bacteria with dye. Docking score and decolorization percentage were assessed comparatively. UV–vis spectroscopy, HPLC, FTIR and GC–MS were performed for biodegradation analysis. Laccase, veratryl alcohol oxidase and azoreductase enzymes were assayed. Docking score of −31.9921, −18.1289 and −27.2792, −2.5185 kJ/mol for laccase and azoreductase while 91.96 and 88.35 % decolorization were achieved by *A. hydrophila* MTCC 1739 and *L. sphaericus* MTCC 9523 respectively. Docking studies analyzing interactions between dye and dye-degrading enzymes were in agreement with decolorization percentage. Biodegradation analysis supported biotransformation of dye into simpler products. Enzyme studies revealed high expression of dye degrading enzymes in both non-acclimatized bacteria on exposure to azo dyes compared to controls. It was suggested that *A. hydrophila* MTCC 1739 and *L. sphaericus* MTCC 9523 can be used for efficient decolorization and biodegradation of azo dye containing textile wastewater. Thus, an advanced approach consisting of an *in silico* preliminary screening and subsequent bacterial dye degradation is validated for an ecofriendly, economical and time-efficient bioremediation to reuse treated water.

1. Introduction

Azo dyes contain one or more azo bonds (—N═N—) along with aromatic groups and can be classified as acidic, basic, direct, disperse, mordant, reactive and solvent dyes. They are widely used in textile, cosmetics, food and paper printing industries [1]. Textile industry is one of the oldest and largest employers in India. Commercially azo dyes are majorly used in these textile industries as they are cost-effective, versatile with broad range of colours, easy to manufacture and resistant to damage. These dyes are stable at different pH range, high temperature and light conditions [2]. But they harm aquatic plants, animals, crops and humans as they are highly toxic, mutagenic and recalcitrant [3]. Colour removal and treatment of harmful by-products is significant for reuse of treated water. Bioremediation techniques intend to use natural processes to recycle water, a precious resource and prevent pollution by destroying the contaminants of anthropogenic activities permanently [4].

Biological decolorization and degradation of azo dyes is advantageous as it is an eco-friendly, efficient and cheaper option with lower sludge and higher efficiency for a wide range of azo dyes [5]. Use of bacterial systems for degradation is of particular interest due to their ease of synthesis, maintenance and an inherent capacity to survive in stressful conditions and break down the dyes into non-toxic products attributed to the presence of various oxidative and reductive enzymes for biotransformation. *A. hydrophila* and *L. sphaericus* have been isolated from the soil and water exposed to textile industry wastewaters in various studies [6,7]. Bacteria-mediated degradation of azo dyes is generally non-specific and faster [8]. The recalcitrant textile dyes which remain stable in the environment can be decolorized by strains with high degradation and tolerance capacity [9]. The use of pure cultures ensures reproducibility and efficient interpretation of experimental data compared to consortium. Cell growth takes place under higher aeration due to shaking while bacterial decolorization is reported to occur in microaerophilic or static conditions often initiated by enzymatic reduction of azo bonds [10]. Microaerophilic state provides the benefits of both aerobic and anaerobic process and aromatic amines obtained by dye decolorization are further degraded and mineralized in microaerophilic phase [11]. Remazole Brilliant Violet 5R degraded in microaerophilic conditions due to bacterial respiration linked to

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Table 1
Structure and other details of Drimaren Red CL-5B dye.

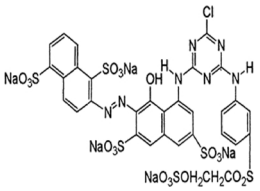
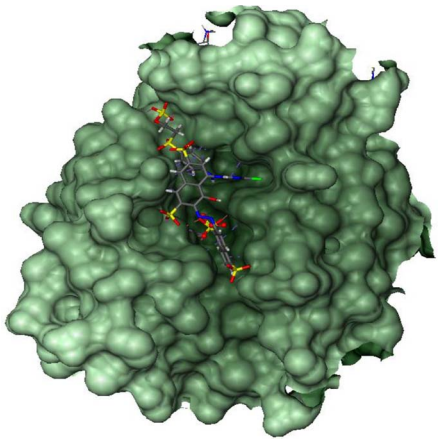
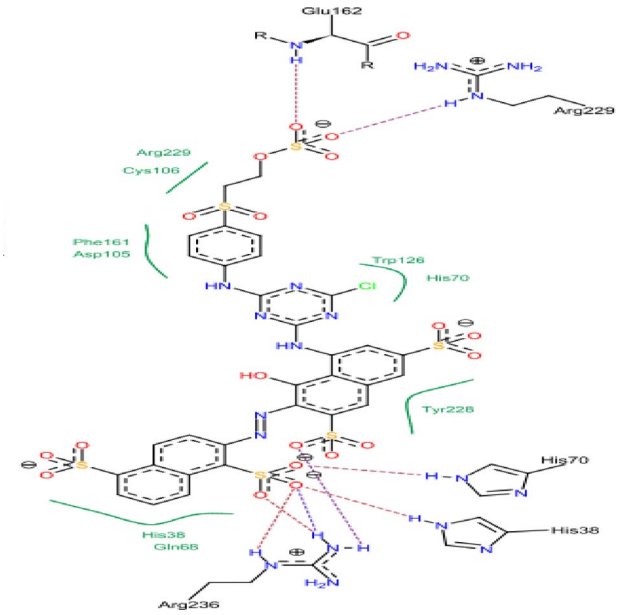
Structure	Features	Molecular class	λ_{max}
	C.I. Reactive Red 195 Reactive Red 3BS, Reactive Red SP-3B, Reactive Red M-3BE, Reactive brilliant Red ME-3BS $C_{31}H_{19}ClN_7Na_5O_{19}S_6$ M.W: 1136.32 g/mol CAS 93050-79-4	Single azo	520 nm

Table 2
Docked complex and interactions of Drimaren Red CL-5B with *A. hydrophila* MTCC 1739 (* – bonded interactions; others – non-bonded interactions).

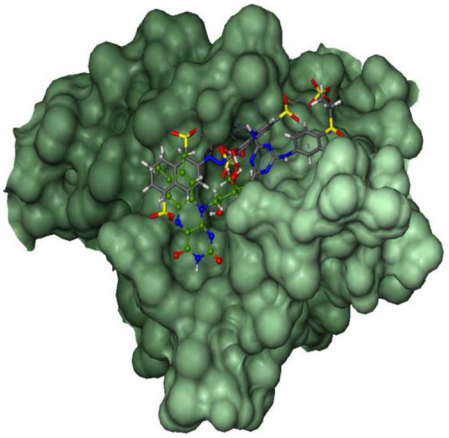
Laccase

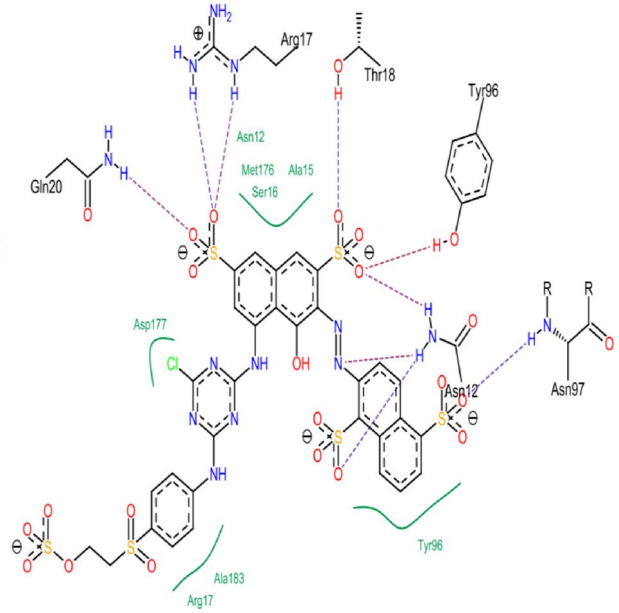




Dock score: −31.9921 kJ/mol
Active site residues: His38*, His70*, Glu162*, Arg229*, Arg236*, His38, Gln68, His70, Asp105, Cys106, Trp126, Phe161, Tyr228, Arg229

Azoreductase





Dock score: −18.1289 kJ/mol
Active site residues: Asn12*, Arg17*, Thr18*, Gln20*, Tyr96*, Asn97*, Asn12, Ala15, Ser16, Arg17, Tyr96, Met176, Asp177, Ala183