

Decolorization of Azo dyes contaminated water
using Native fungi from carpet industry
and fungal synthesized nanoparticle



Summary submitted for the award of degree of

Doctor of Philosophy

in

ENVIRONMENTAL SCIENCE

Supervisor

Prof S. K. Dwivedi

Department of
Environmental Science
School for Environmental Sciences

Submitted by

Garima Singh

Enrolment No. 683/17
Department of
Environmental Science
School for Environmental Sciences

Babasaheb Bhimrao Ambedkar University

(A central) University Lucknow, India

Year-2022

Summary

Azo dyes constitute the largest (60–70% of all textile dyes produced) and most important class of commercial dyes being used in textile industries. The bacterial cultures *Bacillus* sp. strain AK1, *Lysinibacillus* sp. strain AK2 and *Kerstersia* sp. strain VKY1 efficiently decolorized four structurally different azo dyes Methyl Orange, Ponceau 4R, Amido Black 10B and Fast Red E (individually and as well in mixture) under aerobic conditions.

Methyl Orange is a sulfonated azo dye having various industrial applications. It is also used as pH indicator in laboratories. This dye was decolorized by three bacteria *Bacillus* sp. strain AK1, *Lysinibacillus* sp. strain AK2 and *Kerstersia* sp. strain VKY1. The effect of various factors such as dye concentration, pH, temperature and NaCl concentration on decolorization was investigated. At 200 mg/L Methyl Orange concentration, the strains AK1, AK2 and VKY1 exhibited maximum decolorizing potential of 93%, 95% and 96% respectively at temperature 35 °C and pH 7.0 within 18 h of incubation. These strains decolorized the dye over a wide range of pH (5-10), temperature (15-55 °C), and NaCl concentration (5-20 g/L). Further, these strains decolorize up to 800 mg/L concentrations of Methyl Orange within 24 h. The dye decolorization efficiency was further increased by using different consortia of these three strains which could decolorize the dye completely within 12 h of incubation. The cell free extracts of the strains AK1, AK2 and VKY1 grown on

Methyl Orange exhibited the azoreductase activity of 0.4794, 1.56 and 1.01 $\mu\text{M}/\text{min}/\text{mg}$ protein respectively. HPLC and FTIR analysis of the dye decolorized sample indicated the formation of 4-aminobenzenesulfonic acid and *N,N*-dimethyl-*p*-phenylenediamine as breakdown products of azo bond.

The decolorization of toxic azo dye Ponceau 4R by three strains of bacteria *Bacillus* sp. strain AK1, *Lysinibacillus* sp. strain AK2 and *Kerstersia* sp. strain VKY1 individually and in consortia was studied. At optimal conditions, up to 95 %, 93 % and 87 % of the dye was decolorized by the strains AK1, AK2 and VKY1 respectively in 24 hours at 200 mg/L of the dye. Decolorization of the dye was optimized for different parameters such as the concentration of dye, pH, temperature and NaCl concentration. These strains were able to decolorize Ponceau 4R up to an initial concentration of 800 mg/L in the pH range of 5-10, temperature 25-55 °C and NaCl concentration up to 30 g/L. The dye decolorization efficiency of these strains was further enhanced by using different consortia of AK1, AK2 and VKY1 in various combinations. The complete decolorization of the dye by a consortium was achieved within 18 h at 200 mg/L. The cell free extract of these strains grown on this dye exhibited a remarkable activity of azoreductase involved in the breakage of the azo bond. The steady state kinetics of azoreductase validated the ping pong Bi-Bi mechanism of enzyme action. UV-Vis spectra, HPLC, FTIR and LC-MS

analysis of the dye decolorized sample showed the formation of 4-aminonaphthalene-1-sulfonic acid and 5-amino-6-hydroxynaphthalene-2, 4-disulfonic acid as the products of azo bond breakage. The phytotoxicity test of decolorized sample revealed a considerable reduction in the toxicity in comparison with the parent dye.

Similarly, a diazo dye Amido Black 10B and monoazo dye Fast Red E were also investigated for decolorization by bacterial cultures *Bacillus* sp. strain AK1, *Lysinibacillus* sp. strain AK2 and *Kerstersia* sp. strain VKY1. The effect of various parameters such as dye concentration, pH, temperature and NaCl concentration was investigated. The strains AK1, AK2 and VKY1 decolorized 78, 90 and 81% respectively in 36 h of incubation. Further, the consortium having all these three bacteria decolorized 71 % of dye in 12 h and 92 % of the dye in 24 h. While in case of the Fast Red E dye, the strain AK1 showed the highest decolorization of 95 %, AK2 decolorized 93% and VKY1 decolorized 87 % of the dye in 24 h. Consortia (AK1+AK2) and (AK1+AK2+VKY1) decolorized Fast Red E completely in 18 h.

Textile dye effluents contain a number of different types of dyes along with salts, chelating agents, precursors, by-products and surfactants. Thus decolourization of effluents requires an appropriate choice, perhaps a consortium of bacterial strains in conjunction with operative conditions.

Thus, in spite of high efficiency of dye decolorization by some selected strain, decolorization of real industrial effluents is quite a challenge. The mixed bacterial culture comprising of all the three strains efficiently decolorized four individual azo dyes tested (concentration of 200-1000 mg/L). However, with mixture of four structurally diverse azo dyes, decolorization was lesser in combination as compared to the decolorization of individual dyes. Among the three mixed bacterial cultures tested, (AK1+AK2+VKY1) and (AK1+AK2) proved to be the most efficient consortia.

The strains could decolorize dyes much above the reported dye concentration and therefore, is highly promising for applications in decolorization of azo dyes. The ability of the cultures to decolorize the dyes with high dye concentrations, varying pH and salinity conditions can have potential applications in the treatment of textile industry wastewaters.