

# Decolorisation, biodegradation and detoxification of benzidine based azo dye

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## Abstract

The present study deals with the decolorisation, biodegradation and detoxification of Direct Black-38, a benzidine based azo dye, by a mixed microbial culture isolated from an aerobic bioreactor treating textile wastewater. The studies revealed a biotransformation of Direct Black-38 into benzidine and 4-aminobiphenyl followed by complete decolorisation and biodegradation of these toxic intermediates. From cytotoxicity studies, it was concluded that detoxification of the dye took place after degradation of the toxic intermediates by the culture.

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## 1. Introduction

Azo dyes account for the majority of all textile dye stuffs produced and have been the most commonly used synthetic dyes in the textile, food, paper making, color paper printing, leather and cosmetic industries (Powell et al., 1979; Carliell et al., 1995; Chang et al., 2001). Synthesis of most azo dyes involves diazotization of primary aromatic amines followed by coupling with one or more nucleophiles (Zollinger, 1991). Benzidine (BZ)-based azo dyes have been found to be tumorigenic (Haley, 1975) and carcinogenic (Brown, 1977; National Institute for Occupational Safety and Health, 1980) due to their biotransformation to BZ (Heiss et al., 1992).

Azo dyes are recalcitrant xenobiotics and, therefore, conventional aerobic wastewater treatment processes usually cannot efficiently decolorise and degrade azo dye bearing effluents to the regulatory levels (Carliell et al., 1995). Electrochemical destruction (Jia et al., 1999), photocatalysis (Davis et al., 1994) and sorption (Vandevivere et al., 1998), though effective as tertiary treatments, are not economically viable. Primary, physical and chemical treatments of azo dye bearing wastewater (chemical coagulation and/or flocculation and/or precipitation), combined with conventional aerobic biological treatment, are effective but may generate significant amounts of chemical sludge categorized in India as hazardous waste (Ministry of Environment and Forests, 2003), whose disposal in a secure landfill increases process cost. Augmentation of biological treatment process without involving primary or tertiary treatment appears to be a techno-economically viable option. Biological degradation of disperse blue 79, an azo dye, has been reported recently (Melgoza et al., 2004). In the present

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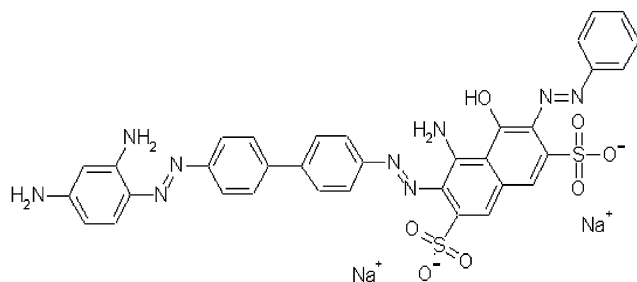


Fig. 1. Structure of Direct Black-38 dye.

study, Direct Black-38, a water soluble, benzidine based azo dye (Fig. 1), was selected for carrying out microbial decolorisation and biodegradation studies. Also, a comparative study of the toxicity potential of the dye before and after degradation treatment was carried out.

## 2. Methods

### 2.1. Dye solution preparation

Direct Black-38 dye was dissolved in double distilled water to prepare a stock solution (1000 mg/l). Solutions of the desired concentrations were obtained by successive dilution.

### 2.2. Screening and selection of microorganisms

Sludge was sourced from an aerobically maintained bioreactor treating textile wastewater. Around 10 g of sludge were added to 100 ml of minimal basal medium ( $\text{Na}_2\text{HPO}_4$ —6 g/l,  $\text{KH}_2\text{PO}_4$ —3 g/l,  $\text{NH}_4\text{Cl}$ —1 g/l,  $\text{NaCl}$ —0.5 g/l, 1 M  $\text{MgSO}_4$ —1 ml/l) and kept on an orbital shaker for 12 h at 150 rpm and 37 °C. Supernatant was collected after allowing the sludge to settle for 2 h. The microorganisms capable of degrading dye were screened by inoculating the Luria Broth Agar plates (casein enzymic hydrolysate—10 g/l, yeast extract—5 g/l,  $\text{NaCl}$ —5 g/l, agar—15 g/l; HiMedia Laboratories, India) containing 10 mg/l of Direct Black-38 with the supernatant. The colonies were selected on the basis of their ability to form clear zones on these plates. Such colonies were subsequently transferred to Luria Broth medium containing different concentrations of the dye.

### 2.3. Experimental setup for the decolorisation and biodegradation studies

Experiments were performed in flasks. Different concentrations of Direct Black-38 dye (25 mg/l, 50 mg/l and 100 mg/l) were added to Luria Broth medium inoculated

with 0.10 OD (600 nm) of culture. These flasks were incubated at 37 °C under static conditions till decolorisation and degradation were completed. The samples were withdrawn at different time intervals and analyzed for decolorisation and biodegradation. The biodegradation was monitored by ammonia release, HPLC and GC/MS analyses and cytotoxicity studies, while decolorisation was determined at 487 nm using an UV–Visible spectrophotometer.

### 2.4. Analytical methods

#### 2.4.1. Decolorisation study

The absorbance peak ( $\lambda_{\text{max}}$ ) of the Direct Black-38 dye was determined by UV scanning and found to be at 487 nm.

For the decolorisation study, 5 ml sample was taken and centrifuged at 12,000g for 10 min. The absorbance of the supernatant was spectrophotometrically determined at 487 nm (Perkin Elmer UV–Vis–NIR Lambda-900) at different time intervals (0, 4, 8, 12 and 24 h on the first day and thereafter every 24 h on subsequent days for a total period of 10 days).

#### 2.4.2. Biomass and biosorption study

The pellet from the centrifuged sample was suspended in 5 ml of distilled water. This was then vortexed and filtered using pre-weighed Whatman GF/C glass fibre filter paper circles. The biomass on GF/C was determined gravimetrically. The OD of the filtrate was measured at 487 nm for the biosorption study.

#### 2.4.3. Ammonia estimation

Ammonia was estimated by the Nesslerization method according to APHA, AWWA, WPCF (1991).

#### 2.4.4. Extraction and HPLC analysis

The dye degradation was monitored by HPLC (Waters, Model No. 501) as the decolorisation continued. Ten millilitres samples were taken at intervals (days 0, 4 and 10), centrifuged and filtered through a 0.45  $\mu\text{m}$  membrane filter (Millipore). The filtrate was then extracted three times with diethyl ether and flash evaporated in a rotary vacuum evaporator with a 45–50 °C water bath, after which the residue was dissolved in 2 ml methanol. Extracted samples were analyzed by HPLC using a mobile phase of 50:49.6:0.4% methanol:water:disodium hydrogen phosphate, a C-18 column (void volume 2.9 ml, length 250 mm and internal diameter 4.6 mm) with an eluant flow rate of 0.8 ml/min, chart speed of 1 cm/min and UV detector at 280 nm. Benzidine and 4-aminobiphenyl standards were procured from Sigma-Aldrich Chemicals, USA.

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