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Prospective assessment of the *Enterobacter aerogenes* PP002 in decolorization and degradation of azo dyes DB 71 and DG 28

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ABSTRACT

The aim of the present study focused on the bacteriological decolourization and degradation of azo dyes Direct Blue 71 and Direct Green 28. The dye decolorizing and degrading proficient bacterial strain was isolated from the textile effluent tainted soil sample. The strain was identified as *Enterobacter aerogenes* (GenBank: KP770134.1) based on 16S rRNA sequencing. The influence of various culture conditions such as pH, temperature, agitation and concentration of dyes on the rate of decolorization were studied. The strain has shown the remarkably higher percentage of decolorization at 100 mg L^{-1} concentration, 37 °C, pH 7 and 168 h of incubation period for both dyes. Physicochemical properties of textile effluent were examined before and after treatments. The results emphasized that there was a remarkable reduction in BOD, COD, TS and TSS after degradation. The UV–vis, HPLC and FTIR analysis of metabolites after treatment confirmed that the decolorization was due to degradation. The adsorption kinetics was analyzed using zero-, first-, second-order and intra-particle diffusion models. It was found that the decolorization followed zero-order kinetic reaction with regard to the intra-particle diffusion. The phytotoxicity studies of control dye and degraded metabolites were tested. The phytotoxicity results divulge that the degraded metabolites have no adverse effects and it can be used for safe irrigation.

1. Introduction

In the state of elevating global development and industrialization, man has developed a numerous technologies in industrial sectors like textile, paper, printing, photography and pharmaceutical which consume huge quantity of water [1]. Due to the scarcity of fresh water and deterioration of water resources by industries as well as lack of awareness in effluent treatment methods leads to the transmission of waterborne diseases and pollution of ground water [2]. The dyes play a major role in all the above mentioned industrial sectors. Textile industry consumes largest quantity of dyes in their various stages of dying process. Among the different kinds of synthetic dyes used, the most prominent is azo dyes due to its brilliant color, ease of handling, usage and economical feasibility in synthesis when compared to other types of dyes. The azo dyes are toxic, carcinogenic and mutagenic in nature. They are characterized by the presence of one or more azo group (−N=N−). The azo group bonds with benzene or naphthalene groups, which contain several functional groups like hydroxyl (−OH), chloro (−Cl), amino ($-NH_2$), carboxyl (−COOH) and methyl ($-CH_3$) which lead to the formation of various kinds of azo dyes [3–5]. More than 3000 different variety of azo dyes are used in textile industries. Huge disposal of colored waste water by the textile industry leads to deleterious environmental pollution. The statistics states that worldwide annual discharge of textile effluent is around 2,80,000 tons [6,7]. The discharges of colored waste water causes a considerable environmental pollution in respect of decrease in water quality, dissolved oxygen concentration, limited photosynthesis as well as adverse effects towards aquatic flora and fauna. Moreover, azo dyes have a deleterious effects in terms of COD, BOD and TOC [8,9]. Numerous physical and chemical methods are employed to remove the azo dye residues from the industrial effluents. The progression of physical treatment methods like coagulation-flocculation, electrocoagulation, adsorption, ion exchange,

Abbreviations: DB 71, Direct Blue 71; DG 28, Direct Green 28; BOD, Biological Oxygen Demand; COD, Chemical Oxygen Demand; TS, Total Solids; TSS, Total Suspended Solids; MSB, Minimal Salt Basal; RSGI, Relative Seed Germination Index; UV–vis, Ultraviolet Visible; HPLC, High Performance Liquid Chromatography; FTIR, Fourier Transform Infra Red

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irradiation, ultrasound and membrane filtration are effective in dye decolourization but their efficiencies are limited due to the large quantity of sludge generation, deficient in removal of toxicity, low flexibility, high cost, low efficiency and incomplete color [10–12]. The chemical treatment methods which include ozonation, sodium hypochlorite, Redox-active metals, photochemical treatment and electrochemical destruction are employed to treat the dye waste water. Eventhough, these applications are efficient in dye removal, but these methods are commercially not feasible due to the short half-life, production of carcinogenic amines as a by product, generation of secondary pollutants and disposal crisis [13]. When compared to physical and chemical methods, bioremediation or biodegradation has takes place a prominent attention due to its eco-friendly, efficient removal of dye constituents, low cost and ease to do. A wide range of microorganisms has been reported to decolorize various kinds of dyes including bacteria, yeasts, fungi and microalgae. Among the microorganisms, fungi show higher decolourizing efficiency as well as production of diverse range of azo dye degrading enzymes. In spite of the application of fungi in dye waste water management is limited due to the ease of contamination by bacteria and unreliable usage in waste water treatment plants [14].

The prime focus on bacterial decolorization was due to its maximum rate of degradation, mineralization, detoxification and negligible amount of sludge formation [15–18]. A broad spectrum of aerobic and anaerobic bacteria have been extensively reported as degraders of various dyes such as Reactive Blue 13 by *Pseudomonas* sp., Crystal Violet, Brilliant Green, Acid Amaranth, Malachite Green, Great Red GR, Reactive Red KE-3B, Brilliant Blue K-GR and Reactive Red 198 by *Aeromonas hydrophila,* Gentian Violet, Basic Fuchsine, Methyl Red and Congo Red by *Citrobacter* sp. and Reactive Black 5, Direct Red 81, Acid Red 88, Disperse Orange by *Shewanella putrefaciens* [4,19,20]. Diverse ranges of bacterial strains have been reported as azo dye degraders with the aid of azoreductase enzyme which catalyze the cleavage of azo bonds both aerobically and anaerobically [13]. Bacteria consumes the azo dyes as their sole source of nutrient and there by producing the azoreductase enzyme. Azoreductase enzyme breaks down the azo dyes into non-toxic metabolites and subsequently mineralizes the compounds. The main drawback of azo dye decolourization is time consumption. This can be overcome by the usage of immobilized azoreductase enzyme with different type of matrix, and there by degradation of azo dyes can be carried out through continuous/column flow degradation. *Enterobacter* sp., a Gram-negative indigenous microflora effectively decolorize the dye Congo Red, but no literature available for bacteria decolourizing DB 71 and DG 28. Hence, this work has been focused on screening of efficient decolorizing indigenous bacteria *Enterobacter aerogenes* PP002 in decolorization and degradation of azo dyes DB 71 and DG 28 from the textile effluent. It is not only evaluates their decolorization efficacy on DB 71 and DG 28 at various concentrations but also optimizes the physiological condition (pH, temperature and agitation) for effective decolorization. Decolorization kinetics was studied in terms of reaction rate constant k_0, k_1, k_2 and k_i. Further, biodegradation of the dye was characterized by UV–vis, HPLC and FTIR. The toxicity of the dyes before and after treatment was examined by phytotoxicity analysis with millet *Sorghum vulgare* and a pulse *Arachis hypogoea.*

2. Materials and methods

2.1. Dye and chemicals

Azo dyes Direct Blue 71 (Molecular formula − $C_{40}H_{23}N_7Na_4O_{13}S_4$, λmax – 587 nm) and Direct Green 28 (Molecular formula − $C_{42}H_{27}N_{10}Na_3O_{11}S_2$, λmax −620 nm) was purchased from Merck India. Fig. 1 shows the chemical structure of DB 71 and DG 28. All the other chemicals for nutrient agar and minimal salt medium were analytical grade purchased from Himedia.

2.2. Physicochemical properties of textile effluent

Untreated effluent and dye contaminated soil were collected from textile industry at Tirupur, Tamilnadu, India. The effluent was subjected to physicochemical characterization and soil sample was used to isolate bacterial strains. Physicochemical characters of the effluent such as pH, color, COD, BOD, TS, TDS and TSS were evaluated using standard methods of examination of water and waste water [21]. Table 1 shows the methods followed during the physicochemical analysis of textile effluent. All the physicochemical properties of the effluent were evaluated before and after degradation.

2.3. Isolation, screening and identification of dye decolorizing microorganisms

Dye contaminated soil of about 0.1 g was serially diluted in distilled water upto 10^{-6} dilution. 100 µl of aliquots of each dilution were inoculated on the nutrient agar plates enriched with 100 mg L^{-1} of DB 71 and DG 28 by spread plate technique. The plates were incubated at 37 °C for 24 h. Morphologically distinct colonies with clear zone were selected and repeatedly streaked to get pure culture on nutrient agar plates. Further screening was carried out in nutrient agar plates supplemented with different concentration (100, 1000 and 2000 mg L^{-1}) of dyes. Potential decolorizing bacterial strains were selected based on the decolorization index calculated as:

Decolorization Index = zone diameter (cm)/colony size (cm)

The strain which shows the maximum decolorization was identified using 16S rRNA sequence analysis, the sequence was analyzed at BLAST in NCBI website (http://www.ncbi.nlm.nih.gov/BLAST). The nucleotide sequence obtained from 16S rRNA sequence analysis was submitted to the NCBI Gene Bank database. The Phylogenetic relationship of strain was assessed by the Maximum Likelihood method. The evolutionary distances were computed using the Kimura 2-parameter method. Evolutionary analyses were conducted in MEGA7 [22,23].

2.4. Decolorization assay

Decolorization experiments were carried out in minimal salt basal medium enriched with dye concentration of 100 mg L^{-1}. The medium pH was maintained at 7 with the aid of 0.1% NaOH or 0.1% HCL. The bacterial culture of 1% v/v (1.0%; OD = 0.1 at 595 nm) was inoculated and incubated at 37 °C under static conditions. The uninoculated MSB medium along with dye was kept as abiotic control. At regular intervals, 5 ml of sample was withdrawn and centrifuged at 10,000 rpm for 15 min to remove cell debris. The supernatent was analyzed for remaining dye content by the absorption of UV–vis spectrophotometer at 587 nm (λ_{max} of DB 71) and 624 nm (λ_{max} of DG 28). All the experiments were carried out in triplicate. Percentage of decolorization was calculated as follows

% of Decolorization = [(Initial Absorbance – Final Absorbance)/Initial Absorbance] ×100

Bacterial biomass was assessed by determining the difference between the weight of pellet before and after decolorizationa

Biomass = Initial Weight – Final Weight

2.5. Effect of operational parameters on decolorization

Influence of various physicochemical operational parameters like dye concentration (100 to 2000 mg L^{-1}), agitation (60 to 100 rpm), temperature (27 to 47 °C) and pH (5 to 9) on decolorization of DB 71 and DG 28 by *E. aerogenes* PP002 was examined.

In order to find out the effect of dye concentration on