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Research Article

Effective decolorization of Direct blue-1 dye by *Bacillus cereus* isolate for its possible application in environmental sustainability

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Abstract

Azo dyes which are found in the textile wastewater are one of the major environmental contaminants which pose threats for healthy ecosystem. Industrial developments resulted in the surge of dye contaminated wastewater, therefore, creating global environmental concern due to the intricate structure of dyes. Bacterial remediation of dyes from contaminated aquatic systems is an environmentally friendly approach. The aim of this study was isolation and identification of a bacterial strain with the potential of decolorizing an azo dye, Direct Blue-1, from dye-laden wastewater, and optimization of process parameters to achieve maximum level of dye decolorization. Total 9 bacterial cultures were isolated at 50 mg/l dye concentration from dye-contaminated wastewater. While single bacterial isolate was capable to decolorize Direct Blue-1 dye at 200 mg/l in minimal salt agar medium. Morphological, biochemical and 16S-rRNA gene sequence analysis showed that the isolated bacterium was *Bacillus cereus* strain MT-4. A Maximum of 97% decolorization was achieved at 5% v/v of inoculum size, under optimized cultural condition. The study highlights the potential of these bacterial strains in effective removal of Direct Blue-1, and their possible applications for clean and sustainable aquatic environment.

Keywords: direct blue dye; microbial decolorization; 16S-rRNA gene; sustainable environment; wastewater.

Highlights

- The study supports the eco-friendly and cost-effective potential of microbial remediation for dye-contaminated wastewater.
- *Bacillus cereus* MT-4 was capable of decolorizing Direct Blue-1 dye.
- Optimal conditions included pH 7.0, temperature 35°C, inoculum size 5% v/v, dye concentration 50 mg/L, and 48 h incubation.
- Static culture conditions were more effective (94.7%) than shaking conditions (83%).
- Dye concentrations above 200 mg/L inhibited bacterial growth and decolorization efficiency.

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1. Introduction

Ecosystem pollution continues to be a major global challenge with dye-laden effluent emerging as one of the major concerns. The wide-spread utilization of coloring agents driven by accelerated industrial expansion and increasing demand in multiple sectors has severely aggravated the issue [1]. Around 5-10% of dyes are discharged into industrial effluent, with azo-dyes representing 60% of the total dyes utilized [2-4]. Currently, azo-dyes and anthraquinone dyes are the two primary classes utilized for fabric dyeing. Under typical conditions, azo dyes are resistant to degradation and not efficiently removed by the traditional wastewater treatment processes due to their complex structure and anthropogenic nature of azo- dyes. Some of the dyes and their breakdown products facilitate the spread of toxic groundwater and surface water contamination from industrial textile efflux further affecting alternative water sources [5-7].

While different types of pollutants include azo dyes, heavy metals, microplastics, and other emerging pollutants are adversely affecting ecosystem [8-11]. Azo- dyes present pose significant environmental and health concern due to their tumorigenic, toxic and mutagenic properties [12]. Direct blue (DB) dye is used in textiles, and many researchers studied the microbial based remediation of DB dyes [13-15]. Azo-dyes and their degradation byproducts, including aromatic amines are recognized as mutagenic and carcinogenic compounds [16,17]. Researchers Hernández-Zamora et al. [18] discussed about the toxic impacts of dye on microalgae, cladocerans, and embryos of zebrafish.

Dye pollution also results in the undesirable water coloration, hinders the penetration of light and reduces the dissolved oxygen levels in the riverine habitat, threatening water- dwelling species. Therefore, proper treatment of dye- laden industrial effluent is crucial before their release into water bodies. Biological processes for the treatment of textile effluents are preferred over physicochemical methods due to their affordability, eco-friendly nature and

lower sludge production [19,20]. Microbe based remediation is suitably employed for remediation of environmental pollutants [21, 22]. Researchers discussed remediation and decolorization of azo dyes from contaminated environment [14, 23]. As a sustainable solution for the dye detoxification, microbe-mediated processes are being globally recognized.

Bacterial remediation approach has been developed into a key strategy for treating industrial effluent due to their multiple advantages. Particularly, they are able to persist and develop substantial biomass even under the nutrient limited conditions. Additionally, their potential in adapting harsh environmental conditions through various facultative anaerobic, anaerobic and aerobic pathways highlights their ecological effectiveness [24-26]. Researchers reported dye remediation by microbes like Di-Azo dye Direct Red 81 degraded by bacterial mixed culture [27], and Direct blue-1 dye decolorization and degradation by fungus *Aspergillus terreus* GS28 [14]. Optimization of different process factors need to study for achieving maximum level of dye decolorization. Moreover, application of single efficient bacterial isolate may be cost-effective approach for on-site dye decolorization. In this study, a potential Direct Blue-1 decolorizing bacteria was isolated from dye wastewater and identified by morphological and molecular methods. Effect of potent strain on degradation of Direct blue-1 was optimized at different temperature for identifying the maximum level of dye decolorization for its possible role in environmental bioremediation.

.2. Materials and Methods

2.1. Sampling

The dye contaminated colored wastewater samples in sterile bottles are collected nearby the discharge points of power looms, Tanda, Ambedkar Nager, Utter Pradesh, India, and stored (at 4^{o}C) in refrigerator for further experimentation.

2.2. Isolation and screening of potential Direct blue 1 dye decolorizing bacterial

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Pour plate technique was employed to isolate bacteria (at 35°C for 48 h), which were capable to decolorize Direct blue-1 on solid minimum salt medium (MSM) containing with 50 mg dye concentration. For the screening of the most potent Direct blue-1 dye decolorizing bacterium and its further application in decolorization studies, MSM agar plates were added with altered with dye concentration (50-300 mg/l), incubated and observed for further study.

2.3. Identification of potential Direct blue 1 dye decolorizing bacterium

Morphological and biochemical identification basis were used [28]. Additionally, molecular characterization was used to identify bacterium at species level by using 16S rRNA gene sequence application. The 16S rRNA gene sequence analysis was carried out at CytoGene Research & Development, Lucknow (India).

2.3. Culture conditions for dye decolorization

The dye decolorization trials were performed in the medium broth, and inoculated with selected potential bacterial isolate, and incubated at 35^0C for 72 h. There were three duplicates of the experiment. Periodically, the media samples were taken out and the extent of dye discoloration were examined at every 24 h, and analysed spectrophotometrically (at 593 nm).

2.4. Factors

Different process factors that effect on bacterial dye decolorization efficiency were studied for attainment of optimal values for the biodecolorization.

2.4.1. Effect of static and shaking of culture medium

In order to determine, the impact of shaking and static culture conditions, the extent of dye discoloration were measured at 48 h incubation.

2.4.2. Effect of dye concentration, incubation time, pH, temperature, and inoculum dose

The impact of different parameters like dye level (50-200 mg/l) pH (5.0-9.0), temperature (25^0C-40^0C), incubation time (24h -72h) and inoculum dose (1-4% v/v) on discoloration of

Direct blue 1 dye was investigated. The samples were processed as . per the above culture conditions.

2.5. Measurement for the extent of dye discoloration

In order to determine Direct blue-1 dye discoloration, the samples drawn at every 24 hours were centrifuged (at 10000 rpm for 10 min). Further, the supernatant was analysed to determine the percent discolourization by using UV-vis spectrophotometer (Labtronics) [14, 29]. The extent of dye discoloration was calculated using the following formula:

$$\text{Decolorization extent (\%)} = \frac{\text{Initial absorbance - Final absorbance}}{\text{Initial absorbance}} \times 100$$

Statistical analyses

The bacterial decolorization experiments were executed in triplicates and the statistical analysis (standard deviation) was done by using Microsoft Excel program.

3. Results and discussion

The improper release coloured wastewater to the aquatic environment is one of the significant concerns. The presence of azo dyes in aquatic ecosystem poses adverse impacts to humans, animals and plants. Microbe-based discoloration is one of viable option that helps in environmental sustainability by removing dyes pollution. In order to facilitate bacterial based bio-discoloration, optimization of process parameters was executed.

3.1. Isolation of potential bacteria for discoloration of Direct Blue-1 dye

In order to isolate dye decolorizing bacteria, the sample was inoculated on MSM agar medium. In this study, the dye was decolorized in the medium after 48 h incubation (Figure 1). Further, the bacterial colonies were isolated and purified on the same medium.

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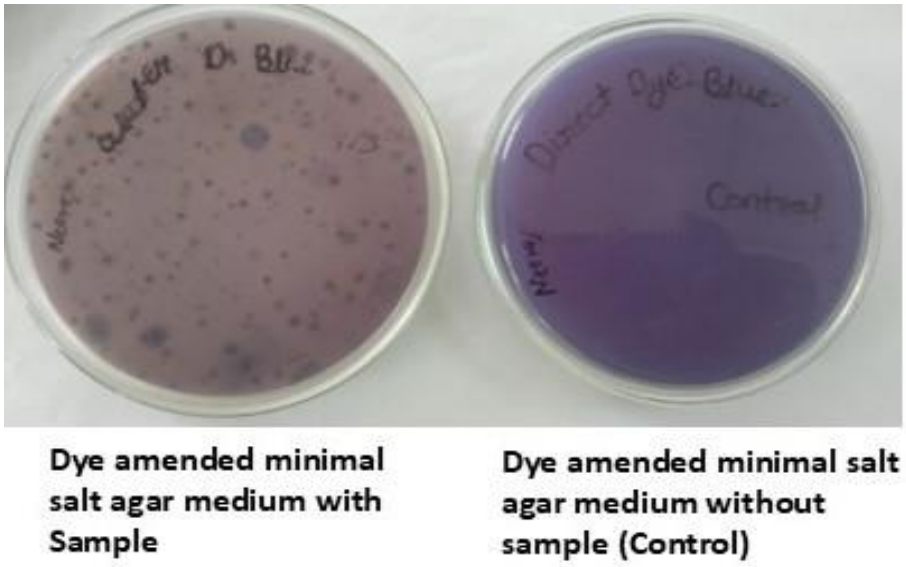

Figure 1: Isolation of Direct Blue-1 decolorizing bacteria

Total nine bacterial isolates were found to capable to decolorize Direct blue 1 dye at 50 mg/l concentration within 48 h incubation. While, single bacterial isolate was capable to grow on MSA medium amended with 200 mg/l dye concentration (Table 1). There was no growth of any bacterial isolates above 200 mg/l dye concentration. Higher concentration of dye (above 200 mg/l Direct Blue 1) was inhibitory for bacterial growth. Several researchers studied the effect of dye level on the extent of microbe-based decolorization [23,30-32]. In a research investigation, dye decolorization was assessed using mixed consortia of *Bacillus* sp., at different dye concentration from 200 to 1000 mg/l, and reported decreased extent of decolorization with increasing dye concentration [33]. The increasing concentration of dye might be toxic to bacterial isolates that results in lesser dye decolorization.

Table 1 Screening of the most potential Direct blue-1 decolorizing bacterium

Sr. No.	Dye concentration (mg/l)	Number of Bacterial isolates
1	50	9
2	100	5
3	150	2
4	200	1
5	250	-
6	300	-

3.2. Morphological, biochemical 16S rRNA gene sequence analyses for identification of potential bacterial isolate

In this study, a potential Direct blue-1 decolorizing bacterial isolate NSW-1 was isolated. Morphologically, the bacterial isolate was rod shaped, and biochemical characterization showed the isolate was Gram-positive, catalase positive and capable to utilize carbohydrates like glucose, sucrose, etc. Further, the isolate NSW-1 was identified as *Bacillus cereus* MT-4 using 16S rRNA gene sequence analysis. Figures 1S and 2S (supplementary) showed the gel picture of isolated genomic DNA and polymerase chain reaction (PCR) amplified products, and the experiments for extracting DNA through agarose gel electrophoresis and PCR were carried out at Cytogene Research Lab, Lucknow.

The accession number LC830210 was assigned after depositing the 16S rRNA gene sequence (1470 bp) to the DNA Data Bank of Japan database (https://www.ncbi.nlm.nih.gov/nuccore/LC830210.1/). The phylogenetic tree was constructed to show the relatedness with other related genera (Figure 2).

Figure 2: Phylogenetic tree of Direct blue-1 decolorizing bacterial isolate NSW-1

Optimization of factors affecting dye decolorization

Different biodecolorization conditions like dye concentration, temperature, incubation time, pH, culture medium shaking, and inoculum size were optimized. Biodecolorization of Direct blue-1 was better under static condition (94.7%) compared to shaking condition (83%) at 48 h incubation. This might be due better enzymatic activity with no shaking culture conditions. While the percent decolorization was lesser at 24 h, and insignificant change in percent decolorization was observed after 48 h incubation. Dye decolorization efficiency was attempted with varying dye concentration from 50 to 200 mg/l. It was observed maximum decolorization was achieved at 50 mg/l, further increase in dye concentration cause decrease in the extent of percent decolorization. Minimum 17% decolorization was observed at 200 mg/l concentration. In this study, the extent of dye decolorization was calculated using optical density (OD) measurement. Other researchers also used OD for measuring decolorization of dyes [6, 34]. In a research investigation, Afrin et al. [34] reported degradation of textile dyes

using bacterial consortium by measuring OD at optimized situations pH 7.0, 37 °C, at 100 mg/L dye concentration.

Further, Direct blue-1 (50 mg/l) amended medium for bacterial decolorization were exposed to a wide range of temperature of 25º-40ºC. It was observed that temperature alteration can significantly impact the bacterial decolorization of azo dyes. Approximately 95% of decolorization of Direct Blue-1 dye was observed by using 4% v/v inoculum dose at pH 7.0 and 35ºC. While there was decrease in decolorization at various other tested temperatures (Figure 3). Further decolorization studies were attempted at 48 h incubation and 35ºC.

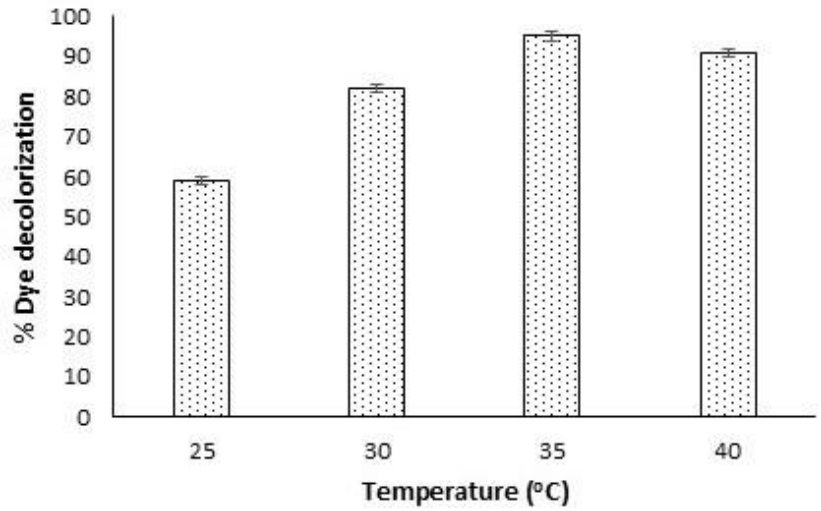

Figure 3: Decolorization of Direct blue-1 under the influence of temperature

Previous studies by multiple researchers such as, El Awady et al. [35] observed the optimal decolorization of different functional azo dyes by *Streptomyces albidoflavus* 3MGH around the temperature of 35ºC. Similarly, Barathi et al. [7], reported the similar trend of maximum dye decolorization at 35ºC of Reactive Blue 160 textile dye by *Bacillus subtilis*. In another investigation, Srivastava et al. [36] found efficient decolorization at 40ºC. These findings suggested that the temperature range of 35-40ºC, bacteria showed significant decolorization which might be due to better enzymatic activity. Several researchers studied on decolorization of dyes for environmental sustainability and pollutant remediation [6, 7, 13].

The extent of dye degradation was significantly influenced by the quantity of bacterial inoculum. The range of inoculum dose taken into account for the investigation of Direct Blue-1 dye decolorization was 1.0-6.0% v/v. At 5% of inoculum size, the decolorization was found to be maximum 97%, while decreased percent decolorization was reported with any deviation from optimal value of inoculum dose. In the present study, the results showed that with an increase in the size of the inoculum, the decolorization of dye rises which might be due to higher enzyme production. In a study, Srivastava et al. [36] studied biodecolorization of Reactive Black 5 dye by *B. albus* DD1 isolated from textile water effluent, and they found highest decolorization (74.7 ± 2.1%) with higher inoculum size (25% v/v), while lesser decolorization (54.9 ± 1.2%) with 5% inoculum size. However, in this study, no significant change in decolorization was observed by increasing inoculum size from 5% to 6%, it might be due to depletion of nutrients because of increased biomass which may results in lesser enzymatic function [36,37].

pH is one of the crucial factors for any bioprocess and significantly influences microbial functions. The pH range for the Direct Blue-1 dye decolorization taken was from 5 to 9. It was observed that at pH 7, maximum decolorization (97%) of dye was obtained. Further deviation in pH from optimal value results in decreased biodecolorization. Similarly, pH for the maximum decolorization by *Bacillus alba* was at pH 7.0 reported by Srivastava et al. [36] in their investigation. In a study, Neetha et al. [38] optimized decolorization of Direct Blue-14 dye by *B. fermus* isolate. In another investigation, El Bouraie & El Din [39] also observed the maximum result for dye decolorization at pH 7. The findings suggest that the bacterium *Bacillus* sp. prefer neutral pH for their maximum decolorization capability. Many researchers also investigated decolorization and detoxification of Direct blue dye by bacteria [40-42]. Wu et al. [43] studied enzymatic discoloration of methylene blue dye by *Bacillus thuringiensis*.

These studies indicate bacterial based decolorization may be an effective way for the remediation of dyes from contaminated aquatic environment.

Conclusions

Addressing dye contamination is a critical requirement in today's world. Different bioremediation strategies can be highly effective in the successful breakdown of synthetic dyes. In our study, the maximum decolorization 97% was obtained by using a single culture of bacterium *Bacillus cereus* MT-4 strain under optimized cultural conditions pH, temperature, inoculum size and dye concentration were 7.0, 35ºC, 4.0% and 50 mg/l dye, respectively. Further research investigations are required for possible large scale decolorization of Direct Blue-1 dye by isolated bacterium.

List of Abbreviations

DB: Direct Blue
MSM: Minimum Salt Medium
MSA: Minimal Salt Agar
OD: Optical Density
PCR: Polymerase Chain Reaction
16S rRNA: 16S ribosomal Ribonucleic Acid

Author Contributions

Manikant Tripathi: Conceptualization, writing—review and editing, supervision; **Neeraj Pathak:** Investigation, writing—original draft preparation; **Gaurav Yadav:** Investigation, writing—original draft preparation; **Sukriti Pathak:** Writing—original draft preparation; **Pankaj Singh:** Writing—review and editing; **Pradeep Kumar Singh:** Writing—review and editing; **Diksha Garg:** Writing—review and editing.

Availability of Data and Materials

The data supporting the findings of this study are available within the article.

Consent for Publication

Not Applicable

Conflicts of Interest

All author(s) declare no conflicts of interest regarding this manuscript.

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Supplementary Material

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