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Decolorization of Reactive Dyes by Bacteria Isolated from Textile Dye-Contaminated Soil

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Abstract

Textile dye-contaminated soil samples were collected for the isolation of heterotrophic bacterial population. The isolates were screened primarily for dye decolorization using Luria Bertani broth containing 100 mgL^{-1} of selected reactive dyes namely Blue FNR (BF), Novacron Navy (NN), or Novacron Deep Cherry (NDC). Secondary screening of dye-decolorizing bacterial isolates was carried out in Bushnell Haas medium. The decolorization efficiency of the isolates was estimated spectrophotometrically. The selected isolates were identified by the analysis of their cultural, biochemical, and molecular characteristics. Among 38 bacterial isolates from the dye-contaminated soil, two bacterial genera *Klebsiella pneumoniae* strain OS-3 and *Bacillus tropicus* strain OS-8 were found to have strong ability in selected dye-decolorization. The optimum pH and temperature for dye decolorization in static conditions by both isolates were found to be 6-8 and 37°C within 72 hours, respectively. Efficient dye decolorization was observed under static condition in compared to shaking culture. Both isolates were able to induce decolorization in the presence of co-substrate. Starch, sucrose and glucose as a carbon source and yeast extract as an organic nitrogen source induced the efficiency of Blue FNR, Novacron Navy and NDC dyes decolorization, respectively by both isolates. On the other hand, it was observed that the percentage of dye degradation decreased in the presence of inorganic nitrogen source in the medium. Both isolates retained efficiency to decolorize selected reactive dyes by 70-80% at 5% salinity. The strong decolorizing ability in natural conditions indicates that both bacteria could be used in dye-polluted wastewater treatment.

Keywords: Decolorization, Reactive dyes, Bacteria, dye-contaminated soil

Introduction

Discharge of wastewater from different textile industries poses a hazardous risk to the environment. Reactive dyes are commonly used for dyeing textile fibers. These xenobiotic dyes cannot be degraded due to their complicated structures. Untreated textile wastewater discharge in aqueous habitats affects light penetration and dissolved oxygen in water bodies and depicts acute toxic effects on aquatic flora and fauna, causing severe environmental problems[1]. The degradation intermediates of synthetic reactive dyes are toxic, and in some cases, these compounds are carcinogenic and mutagenic to humans and other animals[2]. Some reactive dyes and their biodegradation products help the movement of toxic ground and surface waters that are contaminated by textile effluents, which may further contaminate the other water sources and soil. Hence, it is very important to process the textile dye wastewater before discharging it into water streams[3,4]. Several methodologies i.e. Filtration[5], chemical coagulation[6], ion exchange[7], and ozone treatment [8] are being developed for the removal of textile dyes. However, these methods possess many limitations such as high cost, the formation of sludge containing the dyes or their metabolites, etc.[9]. Due to genetic divergence and metabolic resources of microbes, bioremediation of textile dyes is a better alternative to physicochemical approaches[10]. Different groups of microbes can decolorize and mineralize dyes because they produce enzymes that catalyze dye removal and degradation reactions[11]. The present study

aimed to isolate dye-decolorizing bacteria from textile dye-contaminated soil and to evaluate their potentiality to decolorize commercially used synthetic reactive dyes.

Materials and Methods

Chemicals and dye

Chemicals used in the study were of analytical grade and procured from Merck (Germany), Sigma-Aldrich (USA) and Hi Media (India). The textile dyes used in the study were reactive dyes viz. Novacron Deep Cherry (NDC), Novacron Navy (NN), or Blue FNR (BF) was generously gifted by the K.D.S Textile Mills Ltd., Chattogram, Bangladesh.

Samples and sampling sites

Dye-contaminated soil samples were collected from nearby canals of the textile industries at Chattogram, Bangladesh. The collected samples were kept in the icebox and brought to the laboratory for microbiological analysis.

Isolation of dye-decolorizing bacteria

The isolation of heterotrophic dye-decolorizing bacteria in soil was analyzed by the streak plate technique employing enrichment agar media containing 1.0 gL^{-1} $(NH_4)_2SO_4$, 1.0 $gL^{-1}KH_2PO_4$, 0.5 gL^{-1} $MgSO_4.7H_2O$, 0.1 gL^{-1} $CaCl_2$, 15 gL^{-1} agar and 100 mgL^{-1} textile dye.

Screening and measurement of decolorization efficiency

The bacterial isolates were subjected to primary screening to select dye-decolorizing bacteria. Isolates were inoculated in Luria Bertani (LB) broth containing 100 mgL^{-1} dye, then incubated at 37°C for 72 hours. After incubation, the decolorization ability of the bacterial isolates was observed by the visual appearance of the color change compared with the uninoculated control. Potential dye-decolorizing bacterial isolates were selected for secondary screening.

Secondary screening of dye decolorizing bacterial isolates was carried out in Bushnell Haas (BH) broth medium with slight modification containing 1.0 gL^{-1} NaCl, 0.1 gL^{-1} $CaCl_2.2H_2O$, 0.5 gL^{-1} $MgSO_4.7H2O$, 1.0 gL^{-1} KH_2PO_4, 1.0 gL^{-1} Na_2HPO_4 and 100 mgL^{-1} textile dye. The medium was also supplemented with co-substrate 0.5% sucrose, 0.5% yeast extract, and 0.3% peptone[12]. After inoculation, tubes were incubated at 37°C for 72 hours under static condition. Then, 3 mL culture was taken in the Eppendorf tube and centrifuged at 5000 rpm for 20 minutes (Kubota 6930, Kubota Corporation, Japan). OD of the supernatants was measured using a spectrophotometer (Genesys 150, Thermo Scientific U.S) at the respective wavelength of each dye. The OD was converted in concentration using the standard curve of each dye. The decolorization efficiency (%) was measured by the following equation:

$$\text{Decolorization efficiency (\%)} = \frac{(\text{Initial dye concentration} - \text{final dye concentration} \times 100)}{\text{Initial dye concentration}}$$

Identification of dye-decolorizing bacteria

The isolates used in this study were identified based on cultural, biochemical, and 16S rRNA gene sequencing.

Statistical Analysis

The experiments were done in triplicates and the results were expressed as mean ± SD.

Optimization of various physicochemical parameters for maximum dye degradation

The effect of static and shaking condition (120 rpm), pH values (5-9), temperature (25-45°C), Salinity (0.1-5%), carbon sources (glucose, fructose, lactose, starch, mannitol, sucrose at 0.5 % concentration), and nitrogen sources (organic i.e. beef extract, yeast extract, peptone; inorganic: i.e., asparagine, NH_4Cl) were studied in terms of decolorization of dye using the spectrophotometer. The experiments were performed using actively growing cultures as inoculum and after incubation at selected physicochemical conditions, 3 ml culture from the dye medium was withdrawn and centrifuged at 5000 rpm for 20 minutes to obtain supernatant. After that, the OD of the supernatant was measured at λ_{max} of each dye. The same procedure was followed for control. Then, the absorbance data were

converted to concentration by using the standard curve. All the experiments were performed in triplicates.

Results and Discussion

Isolation, identification, and screening of potential dye-decolorizing bacteria

Streak plating of the dye-contaminated soil samples resulted in a collection of 38 bacterial isolates that were used in this study. Following the screening of 38 isolates for their decolorization ability, two potential isolates OS-3 and OS-8 were selected for further evaluation of the decolorization rate and effects of physicochemical parameters in BH minimal salt medium.

Identification of bacterial isolates OS-3 and OS-8 was done based on the cultural, biochemical and 16S rRNA gene sequence. The sequence of OS-3 and OS-8 isolate showed maximum similarity (99%) with *Klebsiella pneumoniae* and *Bacillus* sp. (99%), respectively during similarity search in the GenBank database of the National Center for Biotechnology Information, using the nucleotide BLAST suit optimized for highly similar sequences (Mega blast)[13], and identified as *Klebsiella pneumoniae* strain OS-3 (Accession number OQ845761) and *Bacillus tropicus* strain OS-8 (accession number OQ 845762), respectively. In phylogenetic analysis (Figure 1 and 2), OS-3 and OS-8 fall in the cluster of *Klebsiella pneumoniae* and *Bacillus* sp. respectively. The evolutionary history was inferred using the Neighbor-Joining method using MEGA 11.0 software[14].

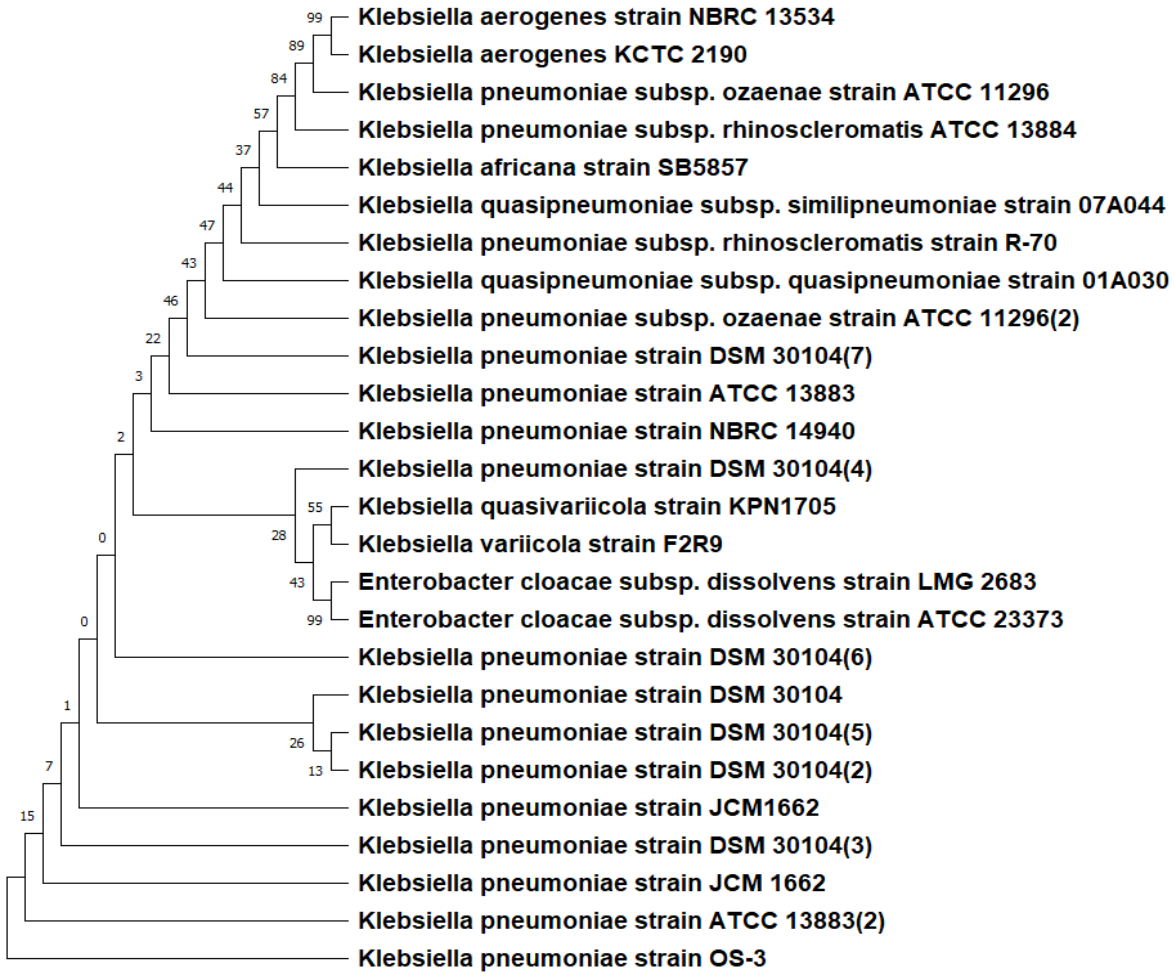

Figure 1. Phylogenetic tree of the *Klebsiella pneumoniae strain* OS-3 based on 16S rDNA partial sequences

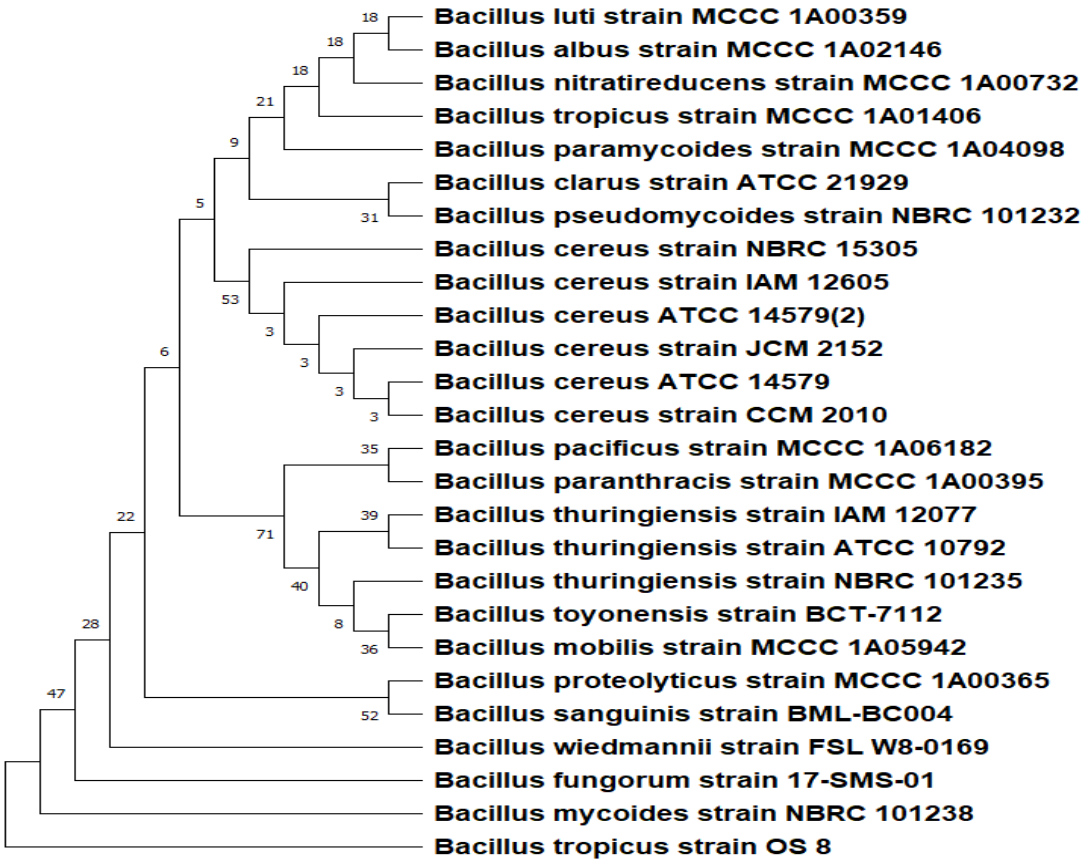

Figure 2. Phylogenetic tree of the *Bacillus tropicus* strain OS-8 based on 16S rDNA partial sequences

Optimization of various physicochemical parameters for maximum dye decolorization

Effect of static and shaking conditions

Microbial bioremediation of dyes in aerobic, anaerobic, or microaerobic systems has been extensively investigated throughout the last decade. Dye decolorization under static and shaking (aerobic) cultural conditions was reported by several researchers[2,15]. In the present study (Figure 3), *Klebsiella pneumoniae* strain OS-3 showed 89.86% decolorization of Blue FNR, 94.89% decolorization of Novacron Navy, and 95.40 % decolorization of Novacron Deep Cherry in static conditions whereas 76.33% decolorization of Blue FNR, 77.46% decolorization of Novacron Navy, and 62.1% decolorization of Novacron Deep Cherry in shaking conditions. On the other hand, *Bacillus tropicus* strain OS-8 showed 83.93% decolorization of Blue FNR, 96.53% decolorization of Novacron Navy, and 94.05% decolorization of Novacron Deep Cherry in static conditions, while 82.89% decolorization of Blue FNR, 82.04% decolorization of Novacron Navy, and 87.51% decolorization of Novacron Deep Cherry in shaking conditions. Singh *et al.* reported that *Staphylococcus hominis* RMLRT03 strain showed 85.52% decolorization of Acid Orange in static condition whereas in shaking condition only 32.47%[16]. The *Rhizobium radiobacter* showed 90% decolorization of Direct Black 38 in static condition while only 6% decolorization was observed in shaking conditions[17]. Tripathi and Srivastava also observed 90% decolorization of Acid Orange 10 under static condition by *Pseudomonas putida* strain whereas only 6% decolorization in shaking conditions [18].

Azoreductase-mediated microbial decolorization of azo dyes is normally inhibited by the presence of oxygen principally due to the competition in the oxidation of reduced electron carriers (e.g., NADH) with either oxygen or azo groups as the electron receptor[19]. In shaking conditions, the existence of oxygen deprives the azoreductase from receiving electrons required for azo bond cleavage, whereas under static conditions, these electrons are easily available to the enzyme from NADH to decolorize the azo dyes [20].

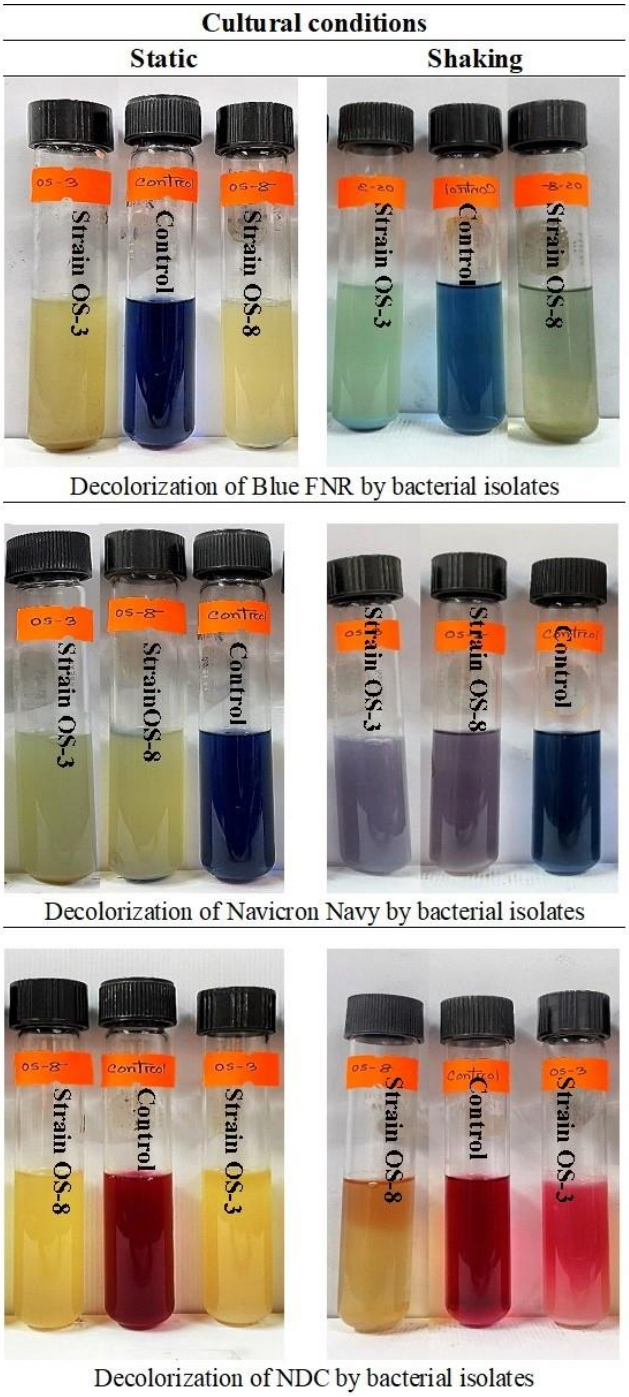

Figure 3. Effect of static and shaking culture on decolorization of reactive dyes

Effect of temperature

The decolorization of different reactive dyes by *Klebsiella pneumoniae* strain OS-3 and *Bacillus tropicus* strain OS-8 were increased with an increase in temperature from 25-37ºC (Figure 4,5). A further increase in temperature declines the decolorization activity of the bacterial strains. The optimum temperature for decolorization of different reactive dyes i.e. Blue FNR (89.87%), N. Navy (94.89%) and NDC (93.95%) by *Klebsiella pneumoniae* strain OS-3, and Blue FNR (94.46%), N. Navy (96.53%) and NDC (94.50%) by *Bacillus tropicus* strain OS-8 were found at 37ºC. Similar to our findings Modi *et al*. reported that the maximum decolorization of Reactive Red195 (RR195), Reactive Black5 (RB5), Reactive Yellow145 (RY145), and Reactive Black dye by *Bacillus cereus* M1 and M6 was observed at 37ºC [21]. Moosvi *et al.* also observed that bacterial consortium JW-2 showed maximum decolorization of Reactive Violet 5R at 37ºC [22]. It is known that in many bacterial systems, the decolorization rate of azo dyes increases with increasing temperature up to the optimal temperature, within a defined range. Then there is a marginal reduction in the decolorization activity. The decline in decolorization rate at higher temperature may be due to thermal deactivation of enzymes or loss of cell viability [23].

Effect of pH

The pH is an important parameter for microbial metabolism and decolorization of dyes. In the present investigation, the decolorization of different reactive dyes by *Klebsiella pneumoniae* strain OS-3 and *Bacillus tropicus* strain OS-8 were found in broad pH range of 4.0-9.0. The maximum decolorization of Novacron Navy (98.51%) was observed at pH 6.0; NDC (90.50%) and Blue FNR (94.38) at pH 7.0 by strain OS-3 (Figure 6). On the other hand, the maximum decolorization of Blue FNR (98.64%), Novacro Navy (96.52%) were recorded at pH 8.0 and NDC (93.53%) at pH 7.0 by the strain OS-8 (Figure 7). The findings are in accordance with most decolorizing bacteria that have a broad pH range in due decolorization[24,25,26]. It was reported that decolorization of Methyl Red by *Micrococcus* strain R3 was found in pH range of 6.0–8.0[27] and Remazol Black B by *Bacillus* sp. ETL-2012 in the pH range of 5.0–8.0[28].

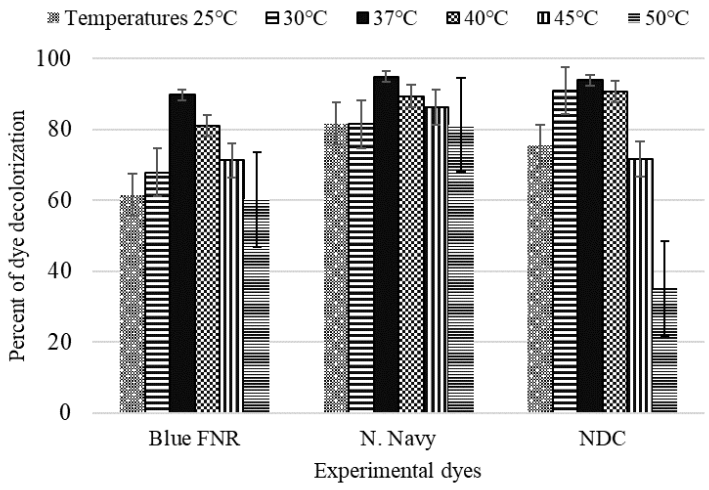

Figure 4. Effect of temperature on dyes decolorization by strain OS-3

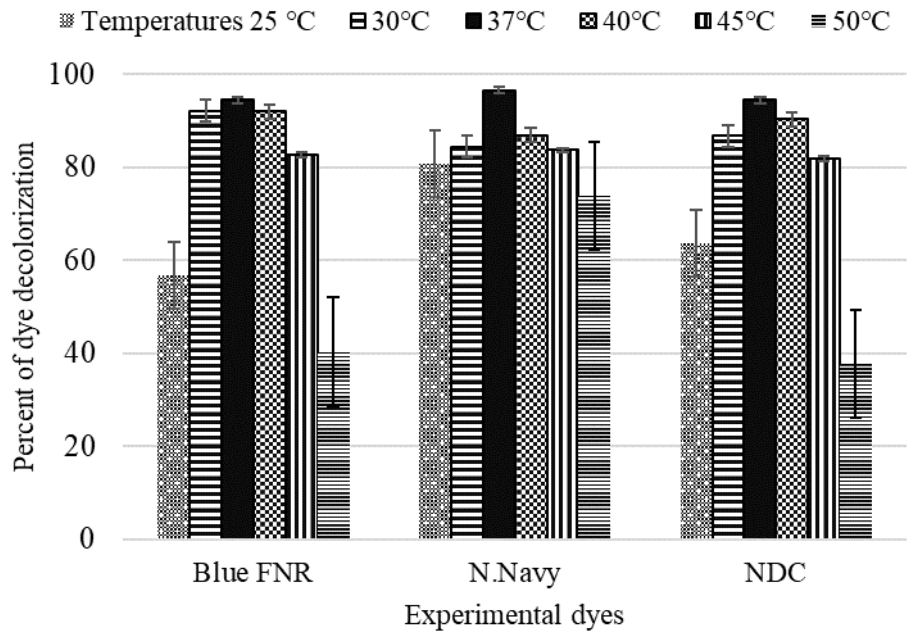

Figure 5. Effect of temperature on dyes decolorization by strain OS-8

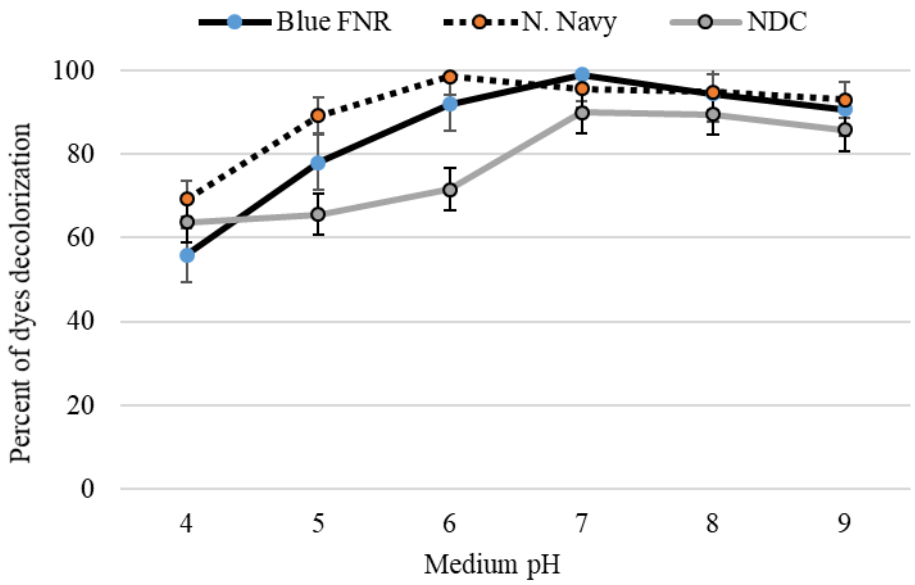

Figure 6. Effect of pH on dyes decolorization by strain OS-3

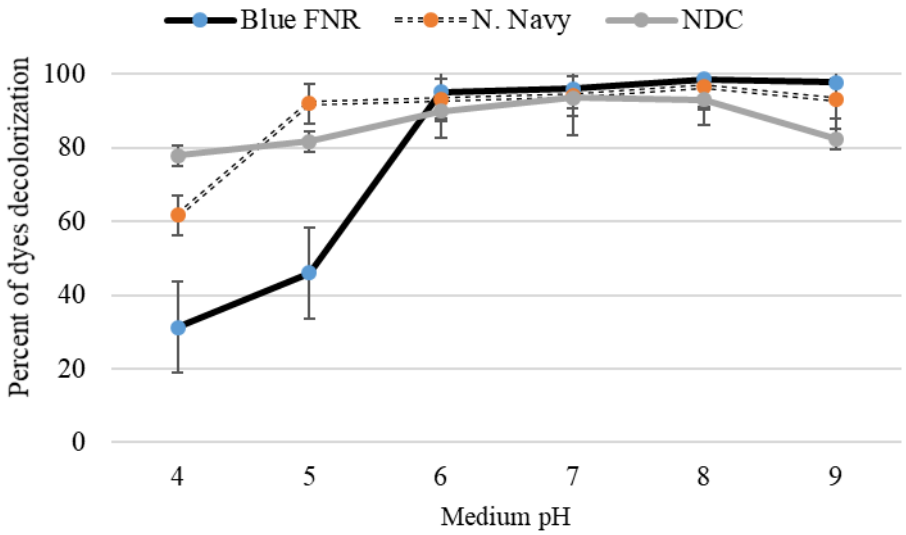

Figure 7. Effect of pH on dyes decolorization by strain OS-8

Effect of carbon and nitrogen sources

The effect of carbon sources on decolorization of reactive dyes was investigated. Among different carbon and nitrogen sources tested for efficient decolorization of Blue FNR, Novacron Navy and NDC by the strain OS-3, starch (94.86%), sucrose (95.42%) and glucose (94.1%) were found to be better carbon source respectively (Figure 8). On the contrary, the maximum decolorization of Blue FNR, Novacron Navy and NDC by the strain OS-8 was found in the presence of starch (94.34%), sucrose (97%) and fructose (94.88%) respectively (Figure 9). However, the suitable carbon source was varied from different bacterial strain. Because the metabolism of dye-decolorization pathway may be different [29]. Joe *et al*. observed that the presence of glucose in the medium induces the decolorization rate of Reactive Red 3B-A and Reactive Black 5 by *Clostridium bifermentans* strains[30]. Tan *et al*. also reported that sucrose as suitable carbon source showed maximum decolorization of Acid Scarlet 3R, Acid Red B, Acid Orange II, Acid Scarlet GR, Reactive Brilliant, Red K-2G dye by *Scheffersomyces spartinae* TLHSSF1[31]. Microorganisms used carbon sources for energy and as electron donors, which are necessary for the breakage of the azo bond [32]. These sources generate reducing equivalents which are transferred to the dye during decolorization process and work as an electron shuttle between a dye and an NADH-dependent azo reductase [33].

The decolorization efficiency of strain OS-3 and OS -8 in the presence of different nitrogen source (yeast extract, beef extract, peptone, Asparagine and ammonium chloride) was also investigated. Yeast extract was observed as suitable nitrogen source for maximum decolorization of Blue FNR, Novacron Navy and NDC by the strain OS-3 with decolorization 96.51, 97.89 and 93.61%, respectively and by the strain OS-8 with decolorization 96.45, 97.62 and 94.35% respectively (Figure 8, 9). Similar to our results were also reported by various researchers. Telke *et al*. observed that yeast extract was suitable co-substrates for decolorization of Reactive Red 141 by *Rhizobium radiobacter* which is similar to our findings[17]. Tan *et.al* also reported the enhanced decolorization of different dyes in the presence of yeast extract in the medium[31]. The metabolism of organic nitrogen sources regenerate NADH which acts as an electron donor for the reduction of azo dyes by bacterial system[32].

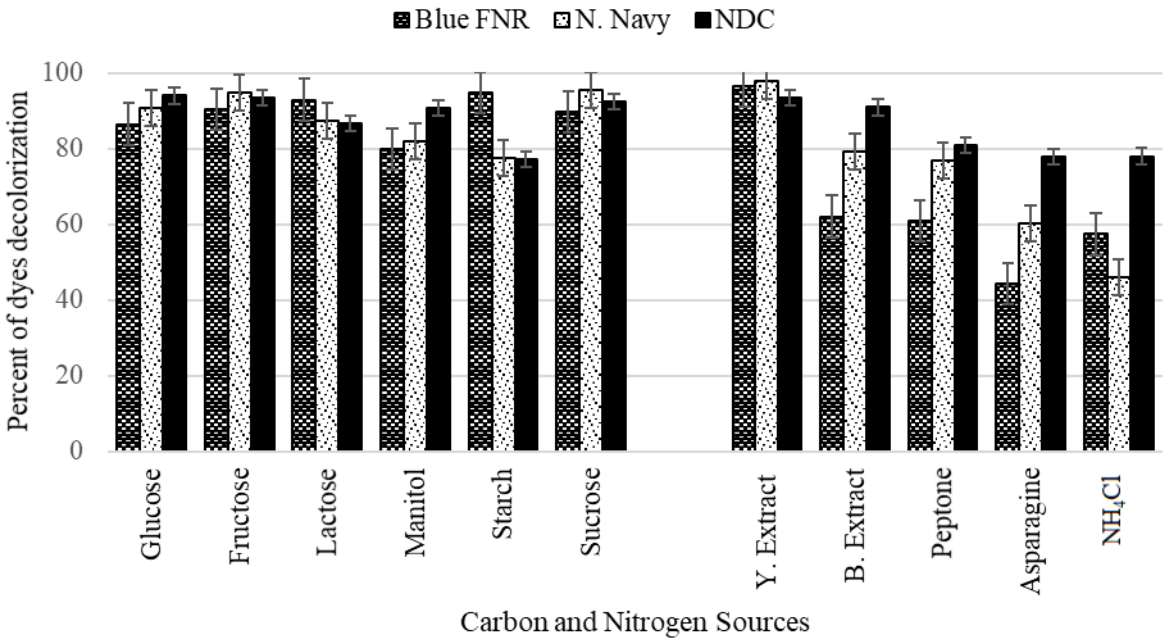

Figure 8. Effect of carbon and nitrogen sources on dyes decolorization by strain OS-3

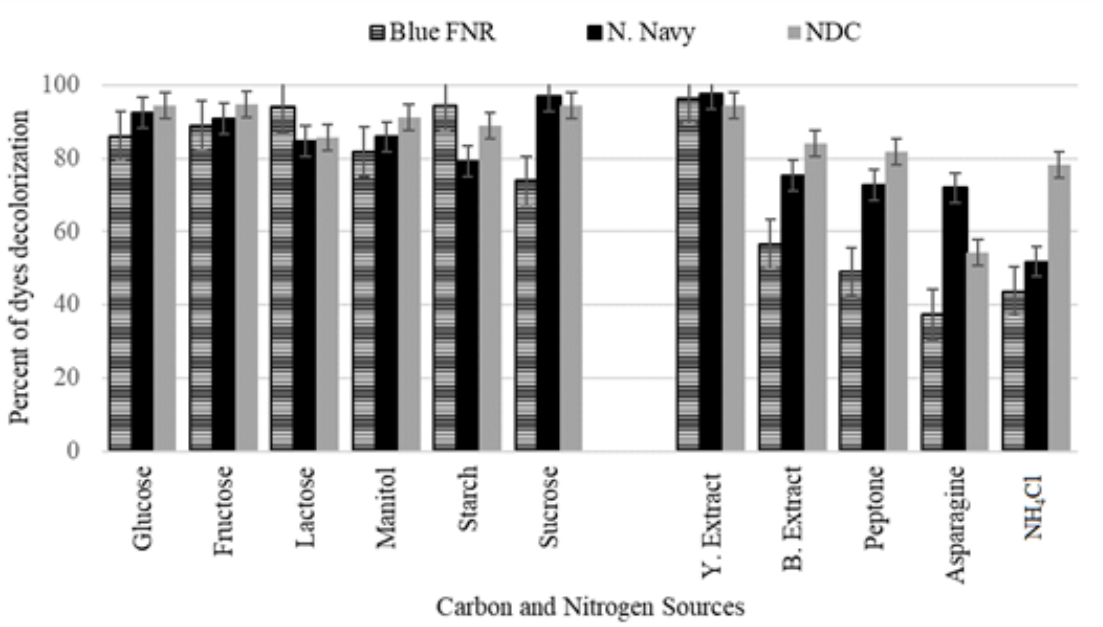

Figure 9. Effect of carbon and nitrogen sources on dyes decolorization by strain OS-8

The effect of salinity

The decolorization efficiency of *Klebsiella pneumoniae* strain OS-3 and *Bacillus tropicus* strain OS-8 at 0-5% salt concentration was also studied (Figure 10 and 11).

Although both strains exhibited maximum decolorization efficiency at 0-1% salt concentration but the decolorization of different reactive dyes by both strains were retained with an increase in salt concentration from 1.0-5.0%. Decolorization at increased salt conditions and dye degrading potentiality indicates the bacteria's ability to withstand the osmotic and ionic pressure, proving its ability to become a potential candidate in textile effluent treatment[34,35].

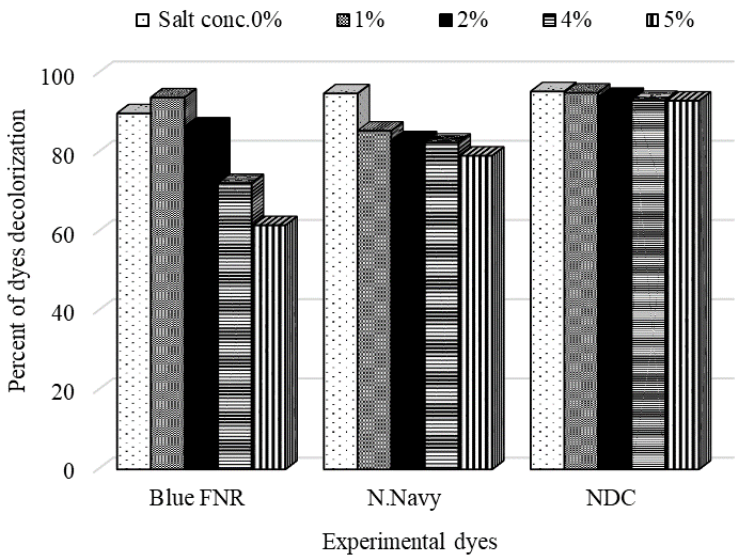

Figure 10. Effect of salinity on dyes decolorization by strain OS-3

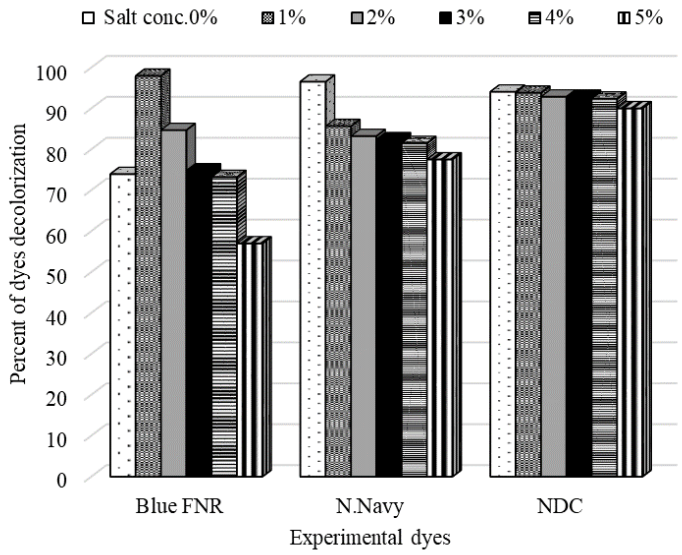

Figure 11. Effect of salinity on dyes decolorization by strain OS-8

Conclusion

Bacterial bioremediation is the most economical, efficient, and eco-friendly technique for removing dyes from textile wastewater. The degradation of complex organic pollutants by heterotrophic bacteria is crucial to clean up the contaminated environment. The dye-decolorization is the key step in textile wastewater treatment. So, it was necessary to explore potential bacterial strains to be used in dye polluted wastewater treatment. The isolated bacteria genera *Klebsiella pneumoniae* strain OS-3 and *Bacillus tropicus* strain OS-8 could decolorize reactive dyes Blue FNR, Novacron Navy, or Novacron Deep Cherry efficiently under optimized conditions. Hence, both bacterial strains may have practical application as an efficient biological tool for textile wastewater treatment.

Conflict of interest

The authors declared that have no conflict of interest.

Acknowledgments

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