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Degradation and detoxification of Navy Blue CBF dye by native bacterial communities: an environmental bioremedial approach

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ABSTRACT

Twenty four bacterial strains having potential capability to decolorize and degrade textile dye Navy Blue CBF, were isolated from activated sludge samples; amassed from azo dye contaminated sites of a local textile Industry of Faisalabad. Out of these, three potent strains were selected owing to their great decolorization potential. Various parameters like dye concentration, pH, temperature, and incubation time period were optimized to develop maximum dye decolorization. The most suitable pH and temperature for selected bacterial strain were 6.0–7.5 and 30–35°C, respectively, while dye concentration and incubation time were found to be 100 ppm and 144 h, respectively. Against Navy Blue CBF dye, maximum decolorization activity obtained in this study was around 90% under the optimized conditions, using the dye as sole source of carbon and nitrogen. Effects of additional carbon (2–10 mg/100 mL) and nitrogen sources (2–10 mg/100 mL) on color removal potential were also evaluated. A decrease in decolorization potential of the bacterial strains with all the carbon sources (23–27% decolorization) and nitrogen sources (23–31% decolorization) was observed. Water quality parameters like total organic carbon (TOC) and chemical oxygen demand (COD) were measured before and after the microbial treatment of dye. Under optimum conditions, significant reduction in TOC and COD by three bacterial strains was noticed as compared to untreated ones. UV–vis and FTIR analyses were carried out before and after decolorization process which indicated the biodecolorization and biodegradation of Navy Blue CBF dye into nontoxic metabolites.

Keywords: Dye; Decolorization; Amendments; COD; TOC; UV–visible spectral analysis; FTIR

1. Introduction

A serious problem arising in the modern world is the environmental pollution, caused by the discharge of a wide variety of dyes through industrial wastewater. Usage of synthetic dyes in textile industry is increasing day by day. It is becoming a universal dilemma that industrial dyeing processes are less effective in terms of dye usage as a considerable amount of dyes ranging from 10 to 15% is gone astray

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in the textile wastewaters [1,2]. Globally, an annual discharge of textile dyes into the textile wastewaters is nearly 280 kilo tons [3]. One of the chief challenges for environmentalists at present is the execution of water pollution [4,5]. About 70% of dyes used for various applications are azo ones which can neither be easily metabolized nor be eliminated from water through the application of previously used wastewater treatment methods. These dyes are mostly lethal and cancer causing [6–8]. To lessen the amount of impact of synthetic dyes onto the environment, a broad variety of physicochemical methods have been developed for their removal from the textile effluents. Some of these processes require sludge waste, whose disposal in a protected landfill enhances process cost [9]. Moreover, by reducing the light absorption, the dyes may considerably distress photosynthetic activity of aquatic life and become possibly toxic owing to the occurrence of aromatics or heavy metals in them [10,11]. These constraints have led to the consideration of advanced oxidation processes (AOP) and biological methods as attractive options for the treatment of dye-containing wastewaters [12–14]. Biodegradation is a substitute to many expensive physical and chemical technologies. It is more cost-effective, environment friendly, and does not produce huge amount of sludge [15,16].

A broad variety of micro-organisms that can capably degrade and destain various types of dyes include bacteria, fungi, yeast, and algae [17], playing a considerable role in degradation and subsequent complete mineralization of dyes [18,19]. Several biological degradation methods include the involvement of anaerobic bacteria for the metabolization of azo dyes [20–23]. Degradation of azo dyes through the usage of microbes in aerobic conditions is reported in a few studies [24–28]. It has been observed that under both conditions, either aerobic or anaerobic, the first step includes an extraordinary enzyme known as azoreductase which is responsible for the breakdown and reduction of the target azo bond; generating aromatic amines as a result of this breakdown. Afterward to the cleavage of azo bond, process of hydroxylation of the aromatic amines takes place (under aerobic conditions) which usually bring about ring opening of these intermediary metabolites [29,30].

The present study was aimed to isolate and identify new microbial strains with high potential to decolorize Navy Blue CBF dye. Experimental conditions were optimized to get maximum biodecolorization. Effect of co-metabolism on decolorization of selected reactive azo dye in presence of additional organic and inorganic carbon and nitrogen sources was also studied. COD and TOC values of Navy Blue CBF dye before and after decolorization were also compared.

2. Materials and Methods

The present research work was carried out in Environmental Sciences Laboratory, Institute of Soil and Environment Sciences, University of Agriculture, Faisalabad.

2.1. Dye and Chemicals

Navy Blue CBF dye used in this study was kindly provided by a local dyestuff Industry. It was pure and used for further study without any purification. All other chemicals were of analytical grade.

2.2. Collection of sludge samples and growth medium

Activated sludge samples were amassed from azo dye contaminated sites of a local textile industry Faisalabad, Pakistan. The bacterial strains were cultured using minimal salt media (MSM) containing NaCl (1.0 g L^{-1}), $MgSO_4{\cdot}7H_2O$ (0.5 g L^{-1}), $CaCl_2{\cdot}2H_2O$ (0.1 g L^{-1}), Na_2HPO_4 (1.0 g L^{-1}), KH_2PO_4 (1.0 g L^{-1}), yeast extract (4.0 g L^{-1}), and Agar (16.0 g L^{-1}).

2.3. Isolation and screening of efficient azo dye decolorizing bacterial strains

Collected activated sludge samples were used for subsequent isolation of potential bacterial strains [18]. Bacterial strains observed to be capable of growing on azo dyes from each inoculum's source were first augmented using MSM, with exclusive source of carbon and nitrogen being Navy Blue CBF dye. Dye was added to medium in equal amount to attain an absolute concentration of 100 mg/L. Bacterial cultures and Navy Blue CBF dye were added in flasks having 200 mL of MSM. Following the inoculation of 10 mL of activated sludge, flasks were incubated under shaking conditions at 25°C for the time period of 48 h. After that, cell suspensions were taken out from each flask and coated to the plates containing minimal salt agar media. Plates were incubated at 30°C for a time of 24 h. Visible microbial colonies in agar medium were gently washed using sterile H_2O. Next step was their resuspension into the flasks having fresh MSM broth with a mixture of various azo dyes. MSM agar plates having 0.1% yeast extract were reloaded with the cell suspensions. In these agar plates, 24 energetically emergent colonies having dissimilar properties of colony growth were observed which were then chosen and purified through double streaking technique. After purification, sample cultures were preserved in 15% (w/v) glycerol at a temperature of −20°C to be used for subsequent studies.

Decolorization capabilities of the individual bacterial strains isolated from enrichment cultures were tested, keeping the Navy Blue CBF dye as sole source of carbon and nitrogen. Samples without inoculation of bacterial cultures served as controls. Six different combinations of bacteria and dye were used in the experiments to allow intermediary measurements. From 24 bacterial isolates, 3 potential strains were screened out. These strains were then cultured in 0.1% yeast extract containing MSM (without dye) for 24 hours at 30˚C at 150 rpm.

2.4. In culture biodecolorization of Navy Blue CBF dye using selected strain

From the screening process, three efficient bacterial strains were chosen and subsequentially observed for their potential to degrade the CBF dye. Autoclaved flasks (having 0.4% yeast extract supplements) were filled with 90 mL sterilized MSM broth along with 100 mg/L CBF dye. Previous studies have reported the usage of yeast extract as a growth assistant of the azo dye degrading bacterial strains [31]. Keeping the azo dye as the only source of carbon and nitrogen, the addition of yeast extract was omitted in the present study. But yeast extract was added in the dye treatment study to measure the maximum decolorization potential of the selected bacterial strain. Selected bacterial cultures were then inoculated with inoculum having homogeneous cell suspension. Bacterial cultures were tightly sealed and incubated at 35˚C under shaking conditions. Controls were run in parallel to test samples. Six flasks were utilized for each strain. Aliquots were taken from time to time to measure the extent of dye decolorization (Eq. (1) given below). Results have been presented as average of triplicates:

$$\text{Decolorization}\,(\%) = \frac{I - F}{I} \times 100 \quad (1)$$

where I = Initial absorbance before decolorization and F = Absorbance after decolorization.

2.5. Measurement of decolorization potential via UV–vis Spectroscopy

To find out maximum absorbance (λ_{max}) for the Navy Blue CBF dye, dye solution (of 100 ppm concentration) was examined spectrophotometrically using UV–vis spectrophotometer (Model Hewlett Packard 8452A). λ_{max} for Navy Blue CBF dye was found to be 614 nm. After centrifugation of the bacteria-treated dye samples at 1,000 rpm for 15 min, supernatant was collected. In culture biodecolorization, assay was done by spectrophotometrical measurement of the absorbance of dye present in supernatants at λ_{max} of dye under study. Blank was kept to be the medium without the dye inoculation. Uninoculated cultures media devoid of dyes served as negative controls.

2.6. Biodegradation analysis via FT-IR Spectroscopy

Degradation analysis was monitored by FTIR. For this purpose, 100 mL of sample (after decolorization) was taken, centrifuged at 10,000 rpm for 20 minute, and extraction of metabolites was carried out from supernatant using equal volume of ethyl acetate. The extracts were dried over anhydrous Na_2SO_4 and evaporated to dryness in rotary evaporator. The crystals obtained were dissolved in small volume of analytical grade methanol and used for analysis. During UV–vis spectral analysis, changes in absorption spectrum in the decolorized medium (400–800 nm) were recorded in comparison with the results from the untreated samples (without decolorization). The FTIR analysis (Model no. Tensor 27, Bruker Optics, Germany) was done in the mid IR region of (1,000–4,000 cm^{-1}) with 16 scan speeds. The analysis was performed after the mixing of the samples with spectroscopically pure KBr in a ratio of 5:95 while the pellets fixed in a sample holder [32].

2.7. Optimization of culture conditions for efficient dye decolorization

Certain factors limit the growth and decolorization potential of newly isolated bacterial strains. To obtain maximum decolorization, optimization of culture conditions like dye concentration (20–120 ppm), pH (6–9), temperature (25–40˚C), and incubation period (48–240 h) was done by varying one factor at a time while keeping the others constant [33]. Subsequent to the addition of dye, flasks were kept on rotatory shaker at 150 rpm for optimal time of incubation. Percent decolorization was screened by UV–vis spectroscopy as described earlier. All assays were performed in triplicate. Unionculated controls were run parallel in all experiments.

2.8. Effect of additional carbon and nitrogen sources

The enriched dye-degrading bacterial strains were capable of growing with dye as the sole source of carbon and nitrogen at pH 7.2 and temperature 35˚C. After optimization of experimental parameters, various carbon and nitrogen sources were examined for

having an effect on bacterial growth and amount of dye decolorization [19]. Under previously optimized conditions, different additional carbon sources (glucose, starch, sucrose, and maltose) at 2–10 mg/100 mL and nitrogen sources (urea, thiourea, ammonium nitrate, and potassium nitrate) at 2–10 mg/100 mL were added for having their effect on the rate of dye decolorization. Control flasks were kept deprived of inoculum. All the samples were run in triplicate.

2.9. Measurements of water quality parameters

With the intention to examine organic load of water, the water quality parameters like total organic carbon (TOC) and chemical oxygen demand (COD) were measured [34].

2.10. Identification of selected microbial strain

Out of 24 bacterial strains, three bacteria isolates (14D, 3D, and BD) showing efficient decolorization potential were selected for degradation of dyes by inoculating onto the agar plates. These strains were then cultured in 0.1% yeast extract containing MSM (without dye) for 24 h at 30˚C under shaking condition at 150 rpm. Finally, one bacterial isolate having the best color removal activity was selected. For identification, the final strain has been submitted to NIBGE, Faisalabad, Pakistan.

3. Results

The present study was conducted in the Environmental Sciences Laboratory, Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad, Pakistan. It focused on batch scale study of decolorization of reactive azo dye.

3.1. Isolation and screening of dye-decolorizing bacterial strains

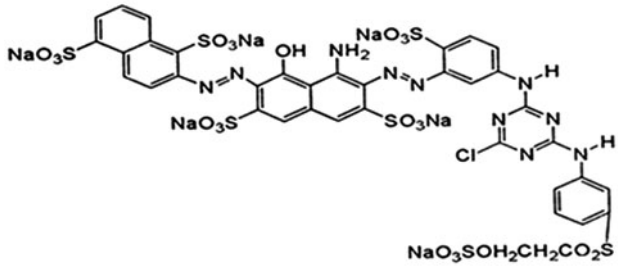
Activated sludge from contaminated sites gave out 24 different bacterial strains which were then evaluated for their potential to decolorize Navy Blue CBF under controlled laboratory conditions. Results declared that all the 24 bacterial cultures, isolated from activated sludge possess decolorizing activity in liquid medium with varying efficiency. It was also observed that azo dye did not affect the growth of bacterial strains. These strains were screened and their decolorizing capabilities were compared by measuring color intensity in liquid medium. Three most potential strains (BD, 3D, and 14D) showing 75–90% decolorization in 48 h were further screened through in culture biodecolorization of dye. Out of these three strains, BD was the most efficient strain with 89% decolorization followed by 3D and 14D showing 85 and 80% decolorization, respectively. These three most potent bacterial isolates (BD, 3D, and 14D) having highest decolorization potential were selected for further study.

3.2. Factors affecting biodecolorization of Navy Blue CBF

Potential of selected strains (BD, 3D, and 14D) was further investigated by optimization of various environmental conditions for decolorizing azo dye in liquid medium. Effects of various parameters, including dye concentration (20–120 ppm), incubation time (48, 72, 144, and 296 h), pH (6–9), temperature (30–40˚C), carbon sources (glucose, starch, sucrose, and maltose) and nitrogen sources (urea, thiourea, ammonium nitrate, and potassium nitrate) were investigated. The effects of dye concentration, temperature, pH, and incubation time on dye decolorization by newly isolated bacterial strains are shown in Figs. 1–4.

3.3. Effect of concentration of dye on decolorization (%)

It is evident from Fig. 1(a) that the decolorization of Navy Blue CBF azo dye sharply increased up to 100 ppm of dye concentration. After that, there was a decrease in % decolorization at 120 ppm concentration of Navy Blue CBF dye with all three strains (BD, 3D, and 14D). Maximum decolorization observed at 100 ppm was 80% (BD), 78% (3D), and 77% (14D).



3.4. Effect of incubation time on decolorization (%)

Time of incubation for maximum decolorization was also optimized. Decolorization increased gradually up to 144 h, whereas after that slight decrease in decolorization was observed. After incubation for 144 h, 88% color removal was achieved through BD while 3D and 14D showed 89 and 86% color removal, respectively (Fig. 1(b))

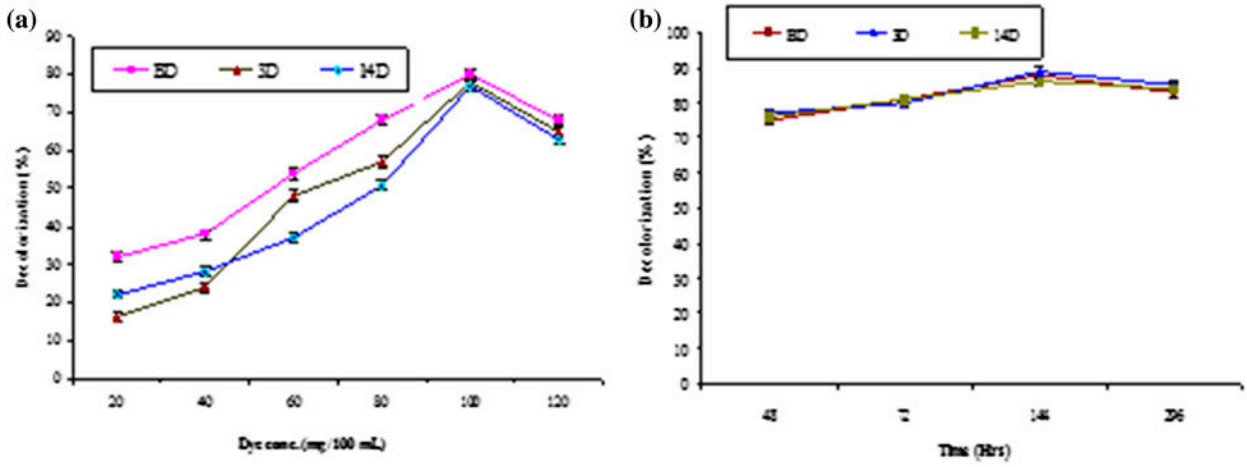


Fig. 1. Effect of conc. of dye: (a) incubation time and (b) on decolorization (%) of Navy Blue CBF by bacterial isolates.

3.5. Effect of pH on decolorization (%)

Experiments were conducted at different initial pH values between 6 and 9, keeping other factors constant (temperature; 35˚C, incubation period; 144 hrs, and dye concentration; 100 ppm) with azo dye being the sole source of carbon and nitrogen. It was observed that the optimal pH for decolorization ranged from 6 to 7.5 (Fig. 2(a)). Initially with the increase in pH from 6.0 to 7.5, decolorization increased with maximum color removal at pH 7.5. More than 82% color removal was obtained between this pH ranges with all three strains. Increase in pH from 7.5 to 9.0, resulted in decrease in decolorization process.

3.6. Effect of Temperature on decolorization (%)

Three levels (30, 35 and 40˚C) of temperature were assessed to find out optimal temperature for biodecolorization through selected bacterial strains. It is evident from Fig. 2(b) as temperature raised from 30 to 35˚C, decolorization extent went on well by all three bacterial isolates. Maximum decolorization potential was shown at 35˚C (90% by 3D, 87% by BD, and 85% by 14D); further increase in the temperature to 40˚C showed an adverse effect on the decolorization. Decolorization was found to be temperature sensitive as abiotic decolorization increased with the rise in temperature.

3.7. Effect of additional carbon sources on decolorization (%)

A variety of carbon sources (starch, glucose, maltose, and sucrose) were evaluated for their effect on Navy Blue CBF dye decolorization by bacterial strains. By adding 2 mg/100 mL of glucose, there was slight

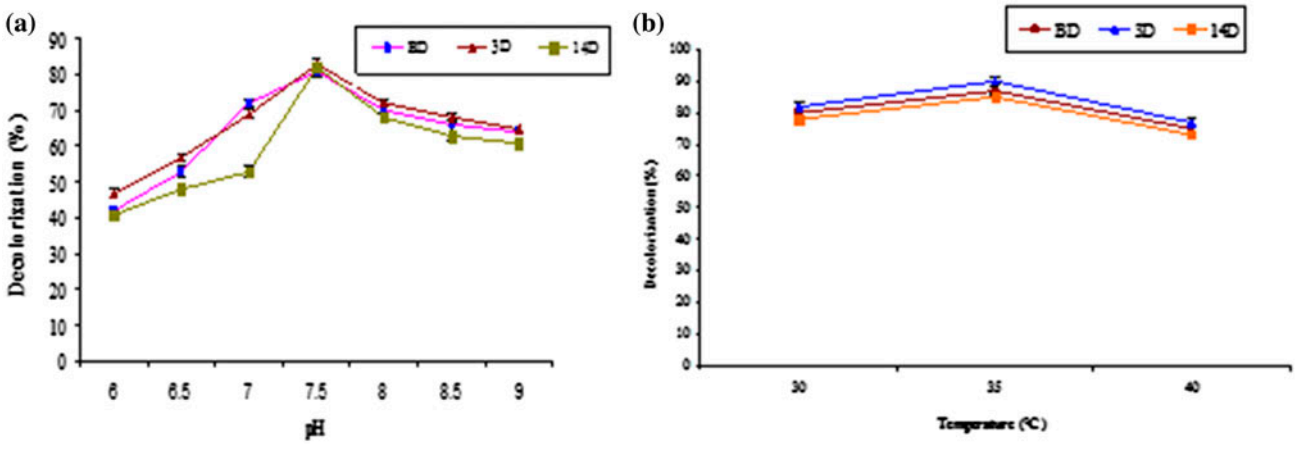


Fig. 2. Effect of pH: (a) temperature and (b) on on decolorization (%) of Navy Blue CBF by bacterial.

increase in decolorization of Navy Blue CBF dye for BD, 81–81.5%; for 3D, 71–71.6%; and 77.2–77.7% for 14D. Further increase in glucose resulted in decrease in decolorization (%). Minimum decolorization was observed at 10 mg/100 mL ranging from 25 to 30%. Similarly, using starch as an additional carbon source (2–10 mg/100 mL) continuous decrease in rate of decolorization was observed. Minimum decolorization was achieved at 10 mg/100 mL only from 26 to 32%. When sucrose was assessed at 2 mg/100 mL for having an effect on color removal activity, the rate of decolorization remained same i.e. 81% for BD, 75% for 3D and 77% for 14D (decolorization rate achieved with dye as sole source of carbon). But, the addition of further sucrose lead to a decrease in decolorization potential of the bacterial strain. Least decolorization was obtained at 10 mmg/100 mL i.e. 24–32%. Similar trend was followed when maltose was added as carbon source i.e. decrease in rate of decolorization by increasing the amount of maltose. Overall, with all the carbon sources bacterial strain showed least decolorization potential i.e. 23–27% (Fig. 3(a)–(d)).

3.8. Effect of additional nitrogen sources on decolorization (%)

Different nitrogen sources (urea, ammonium nitrate, potassium nitrate, and thiourea) were assessed for their effects on Navy Blue CBF dye decolorization by three bacterial strains. The results obtained on decolorization of Navy Blue CBF dye with addition of extra nitrogen sources have been presented in Fig. 4(a)–(d). No significant increasing effect of all the additional nitrogen sources (2–10 mg/100 mL) on decolorization was observed. However, an inhibitory effect was recorded at 10 mg/100 mL addition where only 26–32% decolorization was observed with all the nitrogen sources. By adding urea as nitrogen source,

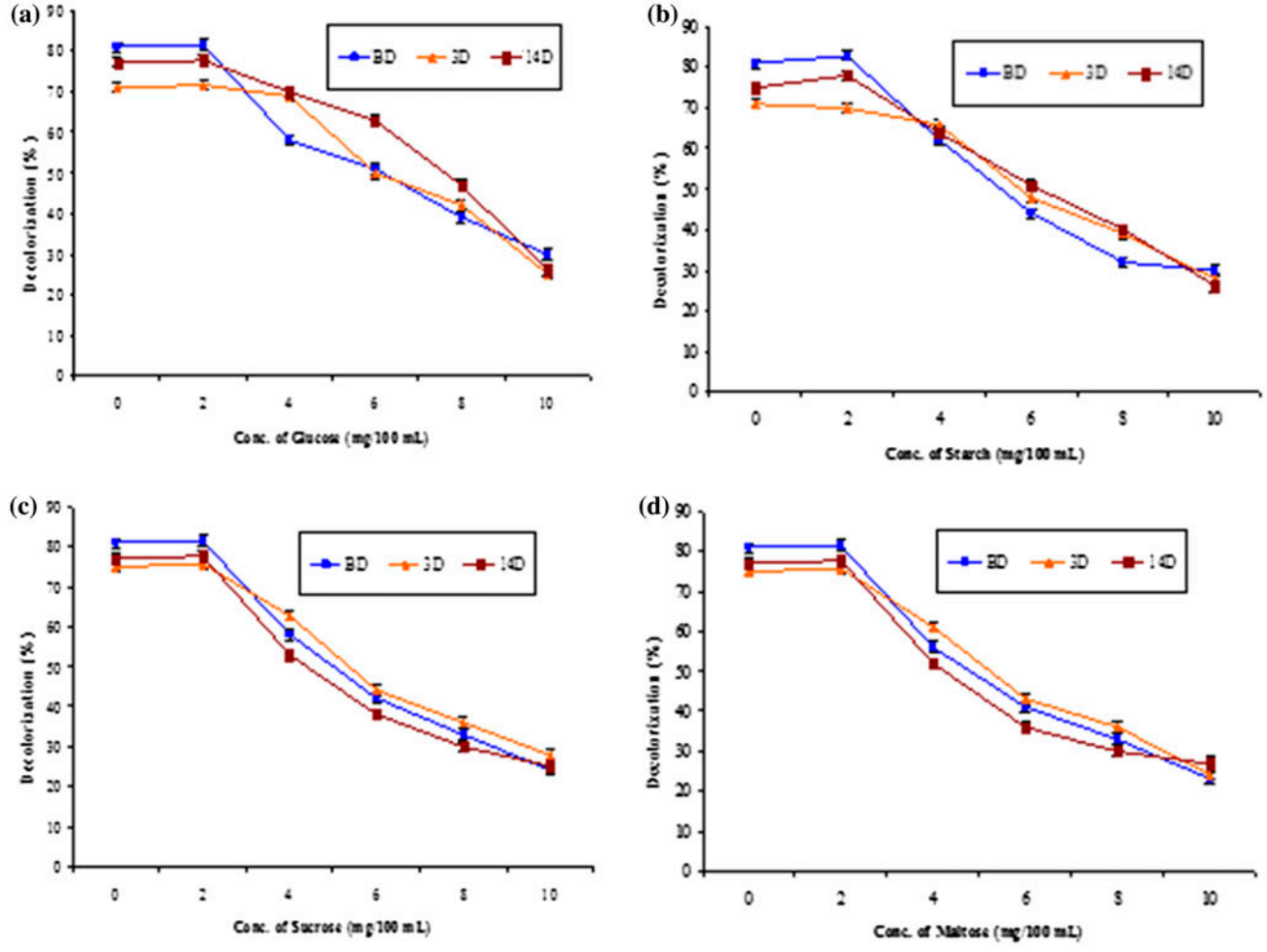


Fig. 3. Effect of glucose (a), starch (b), sucrose (c), and maltose (d) on decolorization (%) of Navy Blue CBF by bacterial isolates.

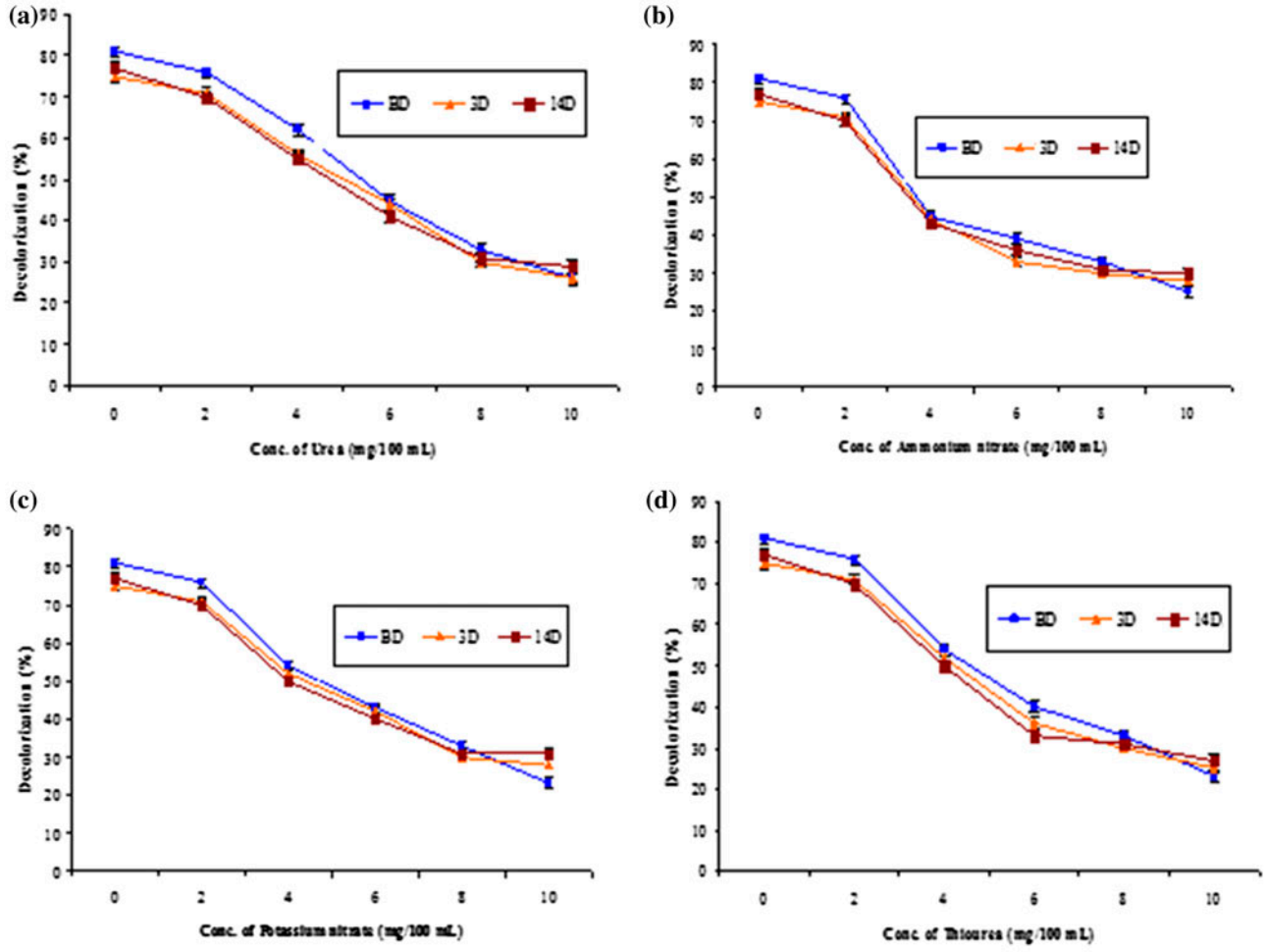


Fig. 4. Effect of urea (a), ammonium nitrate (b), potassium nitrate (c), and thiourea (d) on decolorization (%) of Navy Blue CBF by bacterial isolates.

minimum decolorization was observed at 10 mg/100 mL ranging 26–32%. Similar trends were noticed with ammonium nitrate (25–30%), potassium nitrate (23–31%), and thiourea (23–27%).

3.9. Water quality parameters

The intensity of biodecolorization and biodegradation of the Navy Blue CBF azo dye via microbial invasion can be evaluated through the determination of % mineralization. The % mineralization could be assessed through water quality parameters like TOC, COD, etc. TOC removal can be determined through the measurements of organic content removal at initial and final stage of process. Under optimum conditions, significant reduction in TOC ratio: 69% (3D), 52% (BD), and 47% (14D) was observed during decolorization (Fig. 5(a)), whereas the initial COD level in the wastewater medium was 1,560 mg/L which decreased to 613 mg/L (61%) under the optimum conditions by BD. Reductions in COD with other strains were 52% by 14D and 48% by 3D (Fig. 5(b)).

3.10. UV–visible analysis of Navy Blue CBF

The biodecolorization of Navy Blue CBF dye was also monitored by UV–vis analysis. Navy Blue CBF has maximum absorbance at 614 nm. Spectral line "A" showed dye before treatment and spectral line "B" showed decolorization of dye after 144 hours of bacterial treatments. At 309, 391, and 400 nm, low absorbance extra peaks were observed. When Navy Blue CBF was treated with bacterial strains, peaks in visible region disappeared. The results are shown in the Fig. 6(a)–(c). Biodegradation of the Navy Blue CBF azo dye with BD bacterial strain using aerobic treated

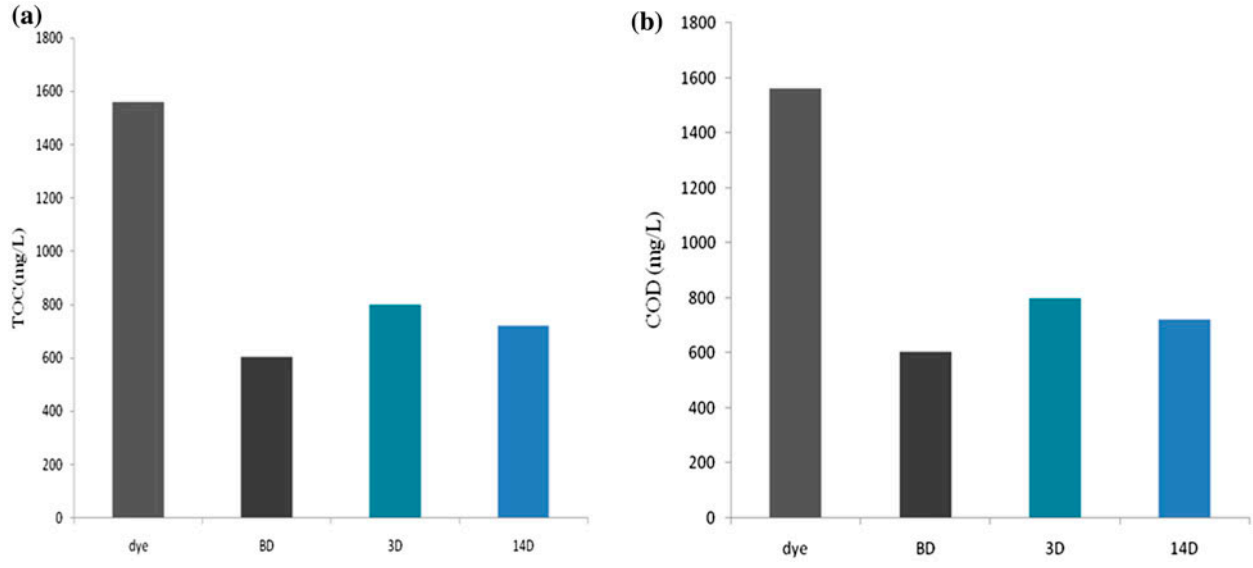


Fig. 5. Comparison of TOC (a) and COD (b) among various treatments.

solutions resulted in the disappearance of visible region absorbance peaks, which is an indicative of absolute color removal. The disappearance of the peaks previously formed at 391 and 400 nm within the UV spectrum and their replacement with the new peak in 350–360 nm range was observed. A small peak at 245 nm was also appeared. After treatment with 3D bacterial strain under optimized conditions all the peaks in the visible region vanished. In UV spectra, peaks at 224, 220, and 210 nm appeared with low absorbance. When Navy Blue CBF was treated with 14D bacterial strain the peaks in visible region disappeared. A new peak at 309 nm in the UV region appeared with low absorbance. Smaller peaks at 220, 244, and 260 nm were also observed.

3.11. FTIR analysis of Navy Blue CBF dye

FTIR analysis had been done for verification of the biodegradation process. Comparison of FTIR spectrum of control dye with bacterial treated samples are shown in Fig. 7(a)–(d).

Through the FTIR spectra, it was revealed that the samples without dye treatment form a number of peaks in the 3,300–3,500 cm^{-1} region. Stretching of H–N and H–O is generally observed in this region (3,300–3,500 cm^{-1}). These peaks are due to different attachments of O–H and N–H to aromatic carbons. These indicate the phenols and aromatic amines. The peaks in the region 2,800–3,000 cm^{-1} indicate C–H sp^3 bond starching. Peaks at 1,770 and 1,607 cm^{-1} are due to ketonic group and C=C (aromatic) starching, respectively. The peak at 1,373 cm^{-1} may be due to C–O–H and at 1,241 cm^{-1} due to C–O stretching. Presence of sulfone group could be observed by the peaks at 1,160 and 1,301 cm^{-1}. Peaks in the region of 1,000–1,100 cm^{-1} may be due to stretching of S–O group (of different types). After treatment with bacterial strains BD, 3D, and 14D under aerobic conditions, the peaks that were due to sulfones at 1,301 and 1,160 cm^{-1} got vanished. Disappearance of these peaks was due to the degradation of sulfones and formation of SO_2 gas. While peaks in the region of 3,300–3,500 cm^{-1} were still present but with very low intensity as compared to the parent dye sample. The decrease in intensity may be due to degradation. Peak at 1,738 cm^{-1} are indicative of five-member ketonic group which showed that there is disturbance in aromaticity after degradation.

4. Discussion

The key objective of our research work was to investigate the isolation, purification, and screening of bacterial strains having the potent ability to degrade and decolorize the azo dye Navy Blue CBF and secondly, to apply this treatment in textile wastewater treatment. For this purpose, 24 bacterial strains were isolated from azo dye-contaminated sites of a local textile Industry of Faisalabad. Out of these, three bacterial strains were selected on the basis of best color removal activity using dye as the sole source of carbon and nitrogen. They were further investigated for optimizing the culture conditions to remove dye color maximally.

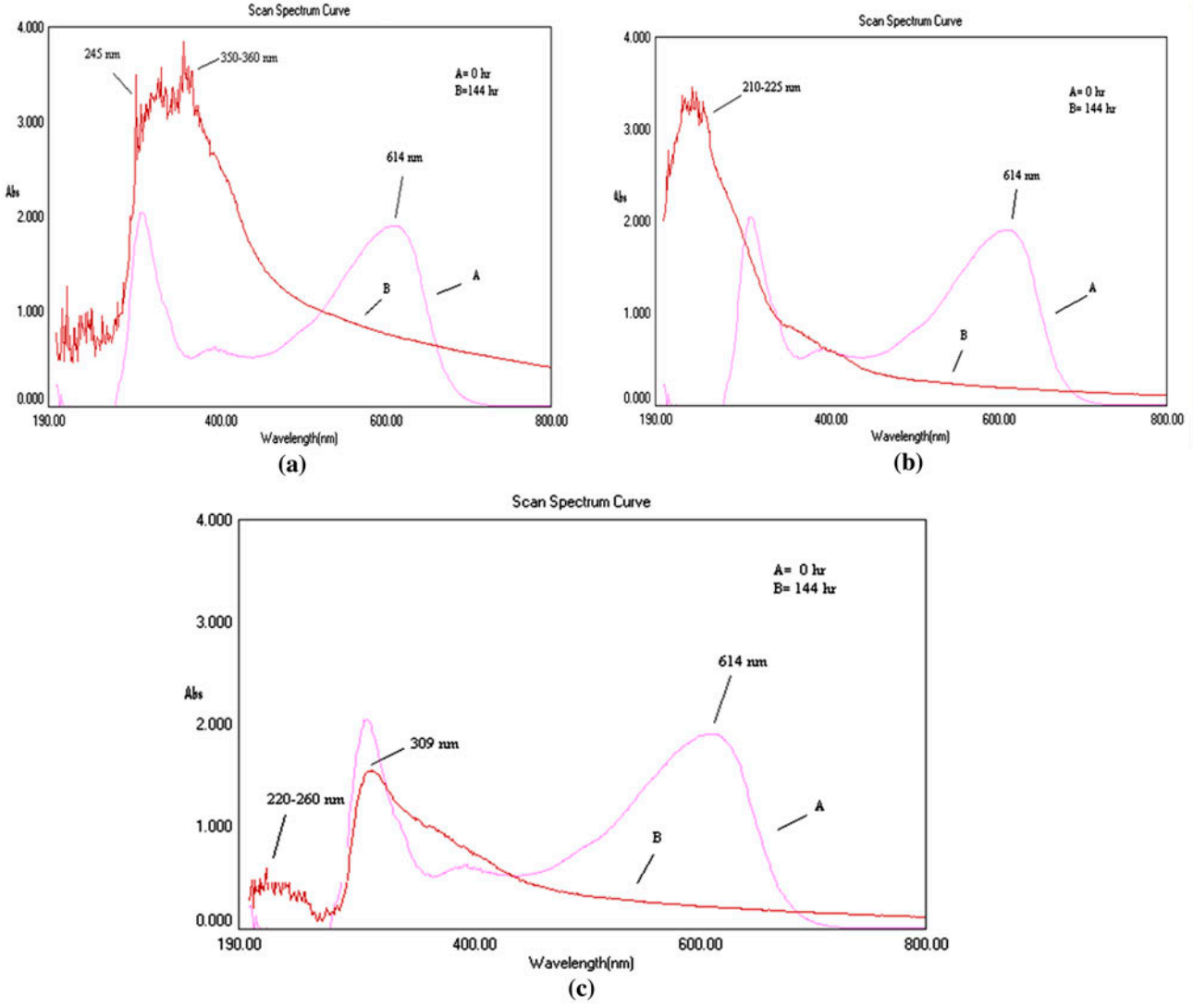


Fig. 6. UV–vis Spectrum before and after treatment with bacterial isolate BD (a), 3D (b), and 14D (c).

The results presented above illustrate the ability of all the three newly isolated microbial strains to use Navy blue CBF dye at a low concentration of 100 ppm and maximum biodecolorization in most selected strains was observed at 100 ppm dye in liquid medium. Whereas, increase in dye concentration showed a negative effect on decolorization. Similar trend was reported in the previous studies that dye decolorization can be strongly inhibited when a high concentration dyestuff was used to examine the poisonous effect of the dye on the degrading micro-organisms [33,35]. Elevated decolorization potential could be obtained at lesser dye concentrations [36–40]. Reduction in decolorization at high concentration of dye might be due to the toxicity of the dye and co-contaminants. On aromatic rings, one or more sulfonic-acid groups are generally present in azo dyes, which might inhibit the growth of micro-organisms by acting as detergents [41]. The existence of heavy metals (metal-complex dyes) might be another cause of the toxicity at higher concentration also the non-hydrolyzed reactive groups present in reactive dyes might be responsible for the toxic nature of the dye [31]. In the same way, reduction in decolorization potential with lower dye concentration could be due to the reduction in enzyme ability to identify the substrate effectively.

In the present study, the optimal decolorization pH was 7.5 using the dye as sole source of carbon and nitrogen. It might be concluded from this study that the ability of bacteria to decolorize Navy Blue CBF in liquid medium containing azo dye is supported by the neutral pH. This trend of decolorization being pH

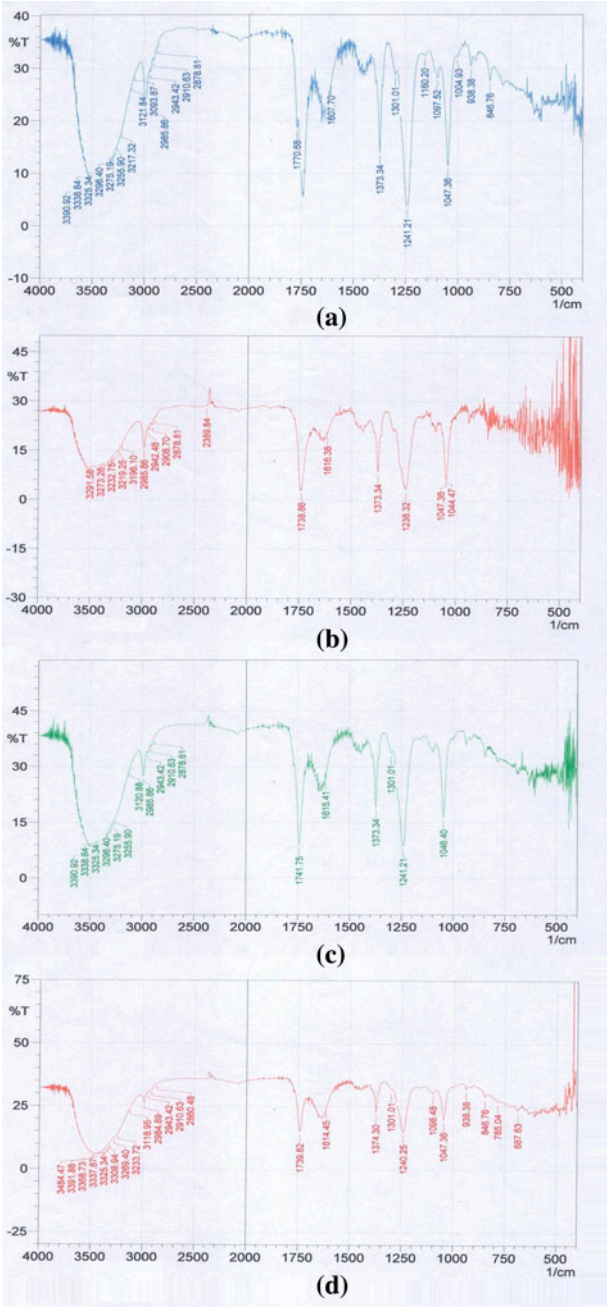


Fig. 7. FTIR Spectrum of Navy Blue CBF dye before treatment (a) after treatment with bacterial isolate BD, (b) with 3D and (c) with 14D (d).

dependent was comparable to that observed in previous studies [42,43]. Similarly, optimal temperature was found to be 35℃, further increase in temperature cause decrease in decolorization activity. Reduction in color removal at elevated temperature may be owing to the fact that enzyme which is responsible for decolorization is thermally deactivated at higher temprature. Consequently, all chosen isolates were mesophilic bacteria since they all exhibited enhanced decolorization activity in the temperature range of 25–35℃. These results are similar to those obtained by researchers [16] who reported the optimal temperature range being 28–35℃. At 30℃, the percentage decolorization attained the highest value i.e. 90%. Conventionally, the mesophilic range is used as it is a general assumption that the maintenance of high temperature would be too expensive, whereas degradation rate within the psychrophilic range is too time-consuming.

It is apparent from the above results presented that all the three newly isolated microbial strains were able to use the respective dye as sole source of carbon and nitrogen. Although, additional carbon and nitrogen sources were evaluated and compared for having any effects on decolorization potential. Different carbon sources like glucose, starch, sucrose, and maltose in liquid medium were tested in this context. All carbon sources tested resulted in a reduction in decolorizing ability of the all three microbial strains. Similarity with the results was also found in the previously conducted studies [16]. In this study, yeast extract or peptone present in the media enabled better growth of bacterial strains as well as complete decolorization of K-2BP. However, sucrose, lactose, glucose, starch, and glycerol consequently lessen the growth rates of bacteria and dye decolorization. Texts from other sources have also highlighted the maximal color removal mainly of azo dyes through further bacterial strains using yeast extract [27,44]. Similarly, different additional nitrogen sources (Urea, Thiourea, ammonium nitrate and Potassium nitrate) did not exhibit a remarkably positive effect on decolorization activity. Previous studies reported similar findings that inorganic nutrients like nitrogen does not enhance degradation of organic compounds always, since there are several other factors responsible to reduce microbial activity [45]. Other studies stated that when urea was dissolved in liquid culture, it shifted the pH further towards acidic side, which reduced the decolorization, enzyme activity of strains as well as growth. The process of decolorization might be slowed down by the presence of nitrate in culture media [22,46] as it might hinder the first step in dye decolorization by acting as electron acceptor.

Under optimum conditions, significant reduction in COD and TOC ratio was observed. The results showed that in order to achieve the complete mineralization of the dye the dye or its intermediary metabolites produced during degradation of dye may be used as carbon source by the bacterial strains. A similar method was implemented [46] for the sake of color elimination and measurement of the ratio of TOC

removal of Navy blue HER dye (azo dye) facilitated by *T. beigelii*. A noteworthy reduction in TOC (95%) was also observed. Reduction in COD level might be correlated to preceding study which stated that COD reduction might be due to the aromatic amines which are anaerobic recalcitrant in nature and inhibit the reduction of the COD level [38]. Another study presented that the alteration in the COD value might be due to the recalcitrant nature of 4BS dye to oxidative degradation [47]. By the microbial consortium, 4BS dye biodegraded to simple metabolites by the breakdown of azo bonds and the oxidation of phenyl and naphthyl rings, leading to the great enhancement in the COD value.

Several reaction intermediary metabolites for instance aromatic amines are usually formed predominantly in case of biodecolorization and biodegradation process of azo dyes. These metabolites might have more half-life and greater toxic effects as compared to those restrained by the original dye. In the UV–vis spectra, the absorption peak characteristic to the hydrogenated azo linkage (ArdNHdNHdAr0) is present at 245 nm which is likely pointing to incomplete disruption of azo linkage subsequent to the biological degradation of Navy Blue CBF dye with bacterial strains. The absolute color removal is pointed out by the absorbance peaks' disappearance in the visible region. Similar results were found in the earlier literature where benzene and naphthalene rings are corresponded by the declining absorbance peaks observed at 220 and 320 nm in the UV spectra, and the development of a new peak at 260 nm is suggestive of the reductive cleavage of the conjugated structure of azo compound [48,49].

Degradation was further confirmed by the FTIR spectra of control and bacterial strain treated dye samples. The disappearance of old major peaks which represents the bonding interactions in the original dye and their replacement with new small peaks supported biodegradation of the dye. The reduction in these peaks was observed throughout the treatment process and in a few studies these peaks were found to absolutely vanish in the spectra of bacterial strains treated dyes, verifying earlier results obtained by UV–vis spectroscopy concerning the azo bond disruption [50,51]. Throughout the azo dye treatment by anaerobic or aerobic processes, prediction of the destiny of the aromatic amines is very hard as their true elimination process (biodegradation, chemical reactions, or adsorption) is not clearly understood [52].

5. Conclusions

The study concluded that experimental parameters like pH, temperature, carbon, and nitrogen source have an important role on dye removal efficiency by isolated bacterial consortium from sludge, which showed enormous potential to degrade the textile dyes and resolve the problem of unnecessary dyes present in the effluents of textile industries. Further pilot-scale studies are required with these strains for actual industrial applications, and detailed study is needed to explore the mechanism involved. The UV–visible and FTIR spectral analysis showed decolorizing activity through a degradation mechanism rather than adsorption. This observation has established that the bacteria are adaptive in nature and can degrade contaminants. Application of traditional wastewater treatment requires enormous cost and continuous input of chemicals which becomes uneconomical and causes further environmental damage. Hence, economical and eco-friendly techniques using bacteria can be applied for fine tuning of wastewater treatment.

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