BACTERIAL DECOLORIZATION AND PHYSICOCHEMICAL CHARACTERIZATION OF TEXTILE INDUSTRY EFFLUENT

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Abstract

A potent bacterial strain (*Bacillus* spp.) was employed for the decolorization of effluent collected from textile industries. The present study includes biochemical characterization of the bacterium, physicochemical characterization of six distinct effluents (E1, E2, E3, E4, E5 and E6) and optimization of culture conditions for decolorization of effluent (E6) collected from the outlet of industrial area. The highest decolorization efficiency (74.8%) of bacterium was achieved within 90 hours of incubation. The effluent E6 collected from the main outlet of industrial area was highly polluted that have exhibited highest BOD, COD, DO, EC and TDS in comparison to other tested effluents. It was found that the pH 8, 35 °C temperature, 25% of bacterial inoculum and supplementation of glucose showed the optimal efficiency of effluent decolorization. Therefore, based on the result of the present study it can be explored that bacterial decolorization of effluent is very cost-effective and environment-friendly, which can be recommended for the decolorization of textile industry effluents containing carcinogenic dyes in large amounts, through industrial effluent treatment plant (ETP).

Keywords: Decolorization, Textile effluent, Bioremediation, *Bacillus* spp., Physicochemical.

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1. Introduction

The textile sector is considered to be backbone of the Indian economy, but it is steadily becoming more troublesome due to the untreated, extremely colored industrial effluents it releases into the environment, which contain a significant amount of dyestuffs. Several hazardous chemicals are used in the textile industries at different points in the process, including finishing agents, whitening, desizing, softening, and sizing [1]. The textile effluent contains a wide range of compounds, including dioxin, hydrogen peroxide, formaldehyde, surfactants, reducing agents, alkalis, complex dyes, alkalis, salts, humectants, binders, and dispersants [2]. Approximately 10-15% of the colors used in dyeing processes are discharged into wastewater due to inefficiency [3]. These highly pigmented synthetic dyestuffs are exceedingly carcinogenic to natural organisms in ecosystems, particularly in water bodies. Usually, the waste water is discharged straight into the river [4]. Furthermore, the release of untreated textile dye waste into water habitats also results in decreased sunlight penetration and has a detrimental impact on the levels of dissolved oxygen, photosynthetic activity, and several important water characteristics [5]. The discharge of effluent from textile industries contains a high concentration of coloring dyes, resulting in a very dark color. These dyes persist in water and soil for extended periods, leading to significant health hazards for living organisms. Additionally, they reduce soil fertility and hinder the photosynthetic activity of aquatic plants. Consequently, this creates anoxic environments for aquatic fauna and flora, posing serious hazards to the environment and affecting the natural ecosystem [5, 6]. The removal of colors from effluents generated during textile dyeing is today a significant ecological issue [7]. While, the government has established a policy covering the discharge of effluents from the textile sector after they have undergone both biological and non-biological treatments, it is not acceptable to directly release these effluents. The ongoing production of untreated wastewater holding textile dye poses a significant environmental hazard, disrupting the overall integrity of the ecosystem and leading to unanticipated worldwide health issues [8].

The decolorization of textile dye effluents is mostly performed using physical and chemical methods, which are not environmentally friendly. Recently, numerous bioremediation methods utilizing microbial organisms have been utilized to remediate textile effluents in an environmentally acceptable and cost-effective manner. In recent decades, many bioremediation methods utilizing microbial cells, such as bacteria, fungi, and yeast, along with their enzymes, have demonstrated significant potential for effectively decolorizing dyes in textile effluents [9,10]. Enzymes have been studied for their potential in treating industrial effluents containing complex dyes. The microbiological decolorization of dyes has drawn significant interest due to its cost-effective nature in decolorizing colors [11].

Currently, there has been a significant focus on utilizing microbial agents for cleaning dye effluents in order to develop more sustainable and cost-effective treatment approaches [3, 12]. In consequence of the decolorization process, the metabolites produced were shown to be less hazardous than the untreated effluents [13]. Therefore, the resulting effluent can be safely released into the environment without causing any harm. Efforts have been made to decolorize textile effluent using various microorganisms such as bacteria, fungus, actinomycetes, algae, etc. [14, 15, 16, 17,18]. However, bacteria and fungi stand out among them due to their superior ability to breakdown colors using various mechanisms for color removal. From the perspective of a large-scale treatments, bacteria have received greater attention due to their shorter growth period and their ability to breakdown and mineralize dyes [10]. Additionally, bacteria are more easily applicable compared to other organisms.

Hence the present study was aimed to investigate the physicochemical characteristics of distinct textile effluents, evaluation of decolorization efficiency of bacterium and optimization of the culture conditions for optimal decolorization of the textile effluents bearing heavy load of recalcitrant textile dyes and other pollutants.

2. Materials and Methods

2.1. Sampling

The sampling of effluents from the outlets of diverse textile industries located in Bhadoi, Uttar Pradesh (Latitude: 25º23’ 35.70” 12:00N; Longitude: 82º33’ 56.84” 12:00E), India. The samples were collected in aseptic plastic containers and carried to the laboratory in a refrigerated container. All samples were processed within one hour and stored at a temperature of 4 ^{0}C for subsequent investigations.

2.2. Screening of effluent degrading bacteria

A total of over 120 different bacterial isolates with varied morphological characteristics were examined for their capacity to decolorize the textile effluent. The bacterial strains were screened on nutrient agar medium supplemented with 10 ml of effluent. The bacterial isolates were inoculated into separate Petri plates containing nutrient agar. The plates were then incubated at a temperature of 37°C for a duration of 50 hours. Effluent decolorization was observed through regular monitoring during the incubation period. The isolate, which has changed the color of the effluent, was selected and transferred to an agar slant for storage at a temperature of 4°C for further studies.

2.4. Biochemical characterization of bacteria

The biochemical characterization of the potent isolate was carried out through multiple biochemical tests including; Vogus-proskaur, oxidase, indole production, methyl red, catalase, citrate utilization, gelatin liquefaction, nitrate reduction, H_2S production, urease hydrolysis, sucrose, mannitol, glucose and lactose fermentation as per Bergey's Manual of Determinative Bacteriology [19].

2.5. Identification of bacteria

Presumptive identification of the potent bacterial isolate was performed on the basis of the results of biochemical tests following the descriptions of the Bergey's Manual of Determinative Bacteriology.

2.6. Bacterial strain

The *Bacillus* spp. used in the investigation was isolated from the textile effluent and kept in the laboratory at Veer Bahadur Singh Purvanchal Purvanchal University, Jaunpur, Uttar Pradesh, India (25.7464° N, 82.6837° E). The culture was regularly cultured in nutrient broth at a temperature of 30ºC. Decolorization was performed in a medium holding glucose (5.0 g L^{-1}), NH_4Cl (1.5 g L^{-1}), K_2HPO_4 (0.5 g L^{-1}), $MgSO_4$ (0.1 g L^{-1}), $CaCl_2$ (0.1 g L^{-1}), $FeSO_4$ (0.07 g L^{-1}), and with a pH of 7.0.

2.7. Maintenance of bacteria

Bacterial strain was maintained into nutrient agar (Peptone 0.5%, beef extract 0.3%, NaCl 0.5%, Agar 1.5%, pH 7) slants. The culture was preserved in slants and stored in refrigerator at 5^0C and it was revived every fortnightly**.**

2.8. Physicochemical analysis of textile effluents

The effluents were subjected to physicochemical characterization through analysis of various parameters, including color, pH, total dissolved solids (TDS), biochemical oxygen demand (BOD), electrical conductivity (EC), dissolved oxygen (DO), and chemical oxygen demand (COD). These parameters were analyzed using the standard methods for examining water and sewage, as explained in APHA [20]. However, the pH was measured by digital pH meter (Cyberscan).

2.9. Decolorization of effluent

Decolorization of effluent was conducted under submerged conditions employing bacterial isolate. The aliquots of effluent were inoculated by the inoculums of bacterial culture. All experimental sets were conducted in 200 ml sized Erlenmeyer conical flasks containing 100 ml effluent in at least triplicates (n=3). The inoculated sets were incubated at 37^0C in BOD incubator to perform effluent decolorization and consistently monitored. The samples from each set were taken after regular intervals of 10, 20, 30, 40, 50, 60, 70, 80 and 90 hours.

2.10. Determination of decolorization

The percent (%) decolorization of effluent was calculated as follows:

$$\text{Decolouration (\%)} = \frac{100(Abs_{t0} - Abs_{tf})}{Abs_{t0}}$$

Where,

Abs_{t0} = Absorbance at initial time of culture.

Abs_{tf} = Absorbance at the final time of culture.

2.11. Statistical analysis

This study was carried out three times (n=3) and the standard deviation (SD) was computed using Microsoft Excel. The results have been presented as the mean ± SD value.

3. Result and Discussion

3.1. Physicochemical characteristics of textile effluents

The results of the physicochemical properties are shown in Table 1. All of the tested samples exhibited sedimentary coloration. E1, E2, E4, and E6 had a strong and unpleasant fragrance, while E3 had a distinct fishy odor and S5 produced an offensive smell. All the samples, except for S3 and S4, exhibited levels greater than the national environmental quality standard (N.E.Q.S) value of TDS (3500 mg/L). The textile effluents had higher TDS values, ranging from 1738 to 6040 mg/l. This was followed by the textile effluents with TDS values of 1738 and 5200 mg/l, and E1 with a TDS value of 3684 mg/l. The pH values of all effluent samples exhibited variability, ranging from 2.5 to 10.7.The E4 had the maximum pH of 10.7, while the lowest pH recorded was 3.7. Therefore, the E4 exhibited higher pH value when compared to standard value of pH. As in case of other parameters the COD and BOD values for all the samples were higher than the standard values. The highest COD values were observed for the textile effluents (3451 mg/l) of E6 followed by 1234 mg/l for E2, which was much higher in comparison of standard. The highest BOD values were obtained for textile effluents E6 (650 mg/l), while the lowest values were observed for E4 (85 mg/l), which is quite close to the standard value range of 80-250 mg/l. In addition, the TDS values were higher than the standard value, except for E3 and E4, which had TDS values lower than the standard.

Table 1. Physicochemical characteristics of six different textile industry effluents.

Parameter	E1	E2	E3	E4	E5	Main outlet effluent (E6)	N.E.Q.S.
Color	Brown	Black	Yellow	Green	Brown	Black	Colourless
Appearance	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid
Odour	Pungent	Pungent	Fishy	Pungent	Offensive	Pungent	No odour
Turbidity	89.6	83.3	69.4	21.7	45.9	100.78	-
TDS (mg/l)	3684	3532	1738	2179	4851	5200	3500
EC (μm hr/cm)	9429	2887	9635	8423	84914	98000	-
pH	6.8	8.1	7.7	10.7	3.7	2.5	6-9
DO (mg/l)	3.58	1.8	1.6	3.4	3.15	6.4	-
BOD (mg/l)	142	420	152	85	150	650	80-250
COD (mg/l)	2342	1234	1653	1456	1869	3451	150-400
Bacterial count (CFU/ml)	3.2×10^6	1.4×10^5	4.8×10^7	5.9×10^6	6.8×10^7	9.8×10^5	-

N.E.Q.S.- National Environmental Quality Standard (2000)
Sample = E_1, E_2, E_3, E_4, E_5 and E_6
TDS = Total dissolved solids, EC= Electrical conductivity, DO = Dissolved oxygen,
BOD = Biological oxygen demand, COD = Chemical oxygen demand

3.2. Effect of pH on effluent (E6) decolorization

The pH level is a crucial environmental component that impacts both bacterial growth and the functioning of intracellular enzymes involved in decolorization process. The pH level also affects the transportation of dye molecules inside bacterial cells for the decolorization. The study was conducted to examine the impact of pH on bacteria, with pH levels in between 4 to 9. The pH level of 7 resulted in the most effective decolorization of textile effluent, with a total decolorization percentage of 74.8% achieved within 90 hours of submerged fermentation. However, the pH 8 demonstrated the second greatest level of decolorization for textile effluent, with a recorded decolorization rate of 68.25% within a 90 hours of incubation period. Only 19% of dye decolorization was observed in the sets of pH 4 after 90 hours of incubation, indicating the lowest level of effectiveness. In this regard, it was noted that a pH of 7 significantly facilitated the decolorization of textile dye in a relatively short duration of 90 hours. The study observed a decrease in textile effluent decolorization in the following order: 74.8, 68.25, 60.56, 57.8, 38, and 19% corresponding to pH levels of 7, 8, 6, 5, 9 and 4 (Figure 1). Costa [21] found that a significant level of decolourization was achieved at a low pH of 4.0 for both natural textile dye effluent (Remazol Turquoise Blue G) and synthetic dye effluent (Lanaset Blue 2R). The decolourization reached 59% for the synthetic dye effluent using *Enterobacter asburiae*, a bacterium, which had the ability to remove color from dye effluent across a broad pH range, making it suitable for treating effluents from dye industries [22].

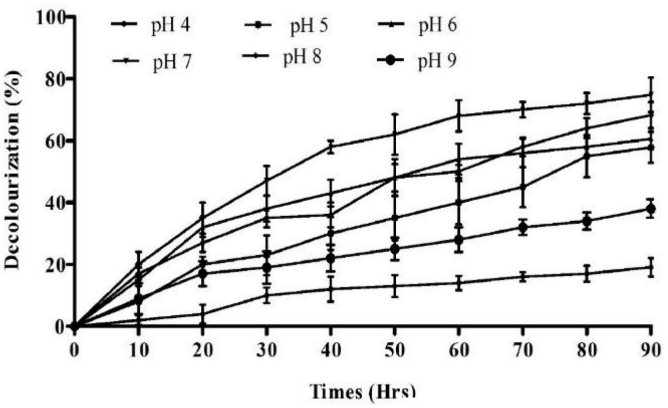

Figure 1: Influence of pH (4, 5, 6, 7, 8 & 9) on bacterial decolorization of textile effluent.

3.3. Effect of temperature on effluent (E6) decolorization

Temperature is a most effective factor that affects the activity of bacteria during dye decolorization. The ideal temperature for bacterial decolorization of textile effluent was found to be 35 °C. The highest textile effluent decolorization was achieved at 35°C, which was 74.8 % within 90 hours of submerged fermentation. The textile effluent decolorization achieved a maximum percentage of 68% and a minimum percentage of 36% for effluent decolorization at a temperature of 20°C, during a period of 90 hours. As a result, it was noted that a temperature of 35°C significantly boosted the removal of color from the textile effluent, achieving decolorization within a short period of 90 hours. The order of textile effluent decolorization, from highest to lowest, was as: 74.8 > 68 > 62.58 > 50.22 > 36.14, corresponding to temperatures of 35°C, 30°C, 25°C, 40°C, and 20°C (Figure 2). Thus, the most favorable temperature for removing color from the effluent using bacteria was found to be 35°C, resulting in a decolorization rate of 74.8% (Figure 2). Vishnoi et al., [23] reported a similar observation, where all the bacteria exhibited optimal growth at a temperature of 35°C, however their growth declined when the temperature exceeded this threshold of temperature.

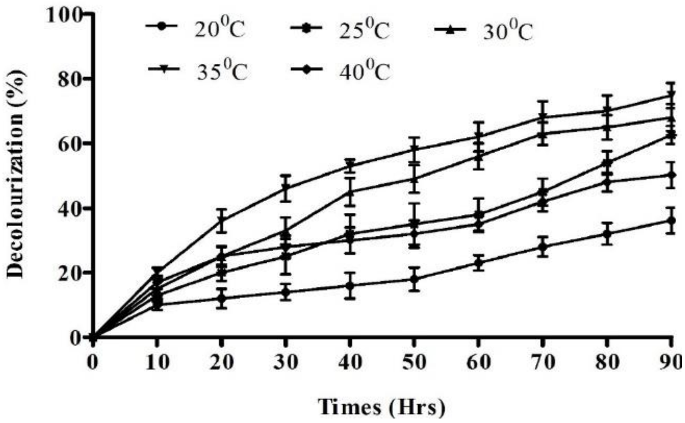

Figure 2: Influence of temperature (20, 25, 30, 35 & 40 ºC) on bacterial decolorization of textile effluent.

3.4. Effect of inoculum size on effluent (E6) decolorization

The decolorization of dye was primarily dependent on the quantity of bacterial inoculum. An increase in the bacterial inoculum was shown to correspond with an increase in the percentage of effluent decolorization. The textile effluent achieved the highest level of decolorization (74.8%) when 25% inoculum was used. This was followed by a decolorization of 72% with a 20% inoculum, 68% with a 15% inoculum, 60% with a 10% inoculum, 56.72% with a 5% inoculum, and 43.2% with a 1% inoculum, all within a period of 90 hours (Figure 3). Increasing the size of the inoculum resulted in an increase in both the growth of microorganisms and the synthesis of biomass. This is because when the inoculum size is less, it takes longer for the cells to divide into a sufficient number to produce enzymes and utilize the substrate [24, 25].

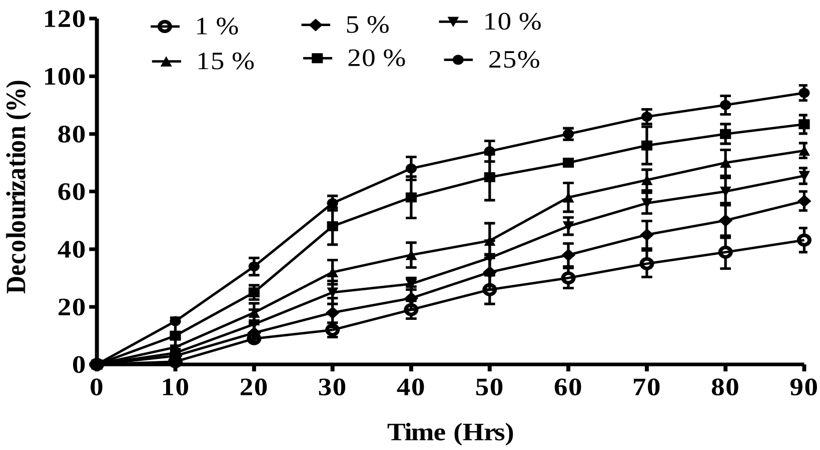

Figure 3: Effect of different inoculum sizes (1, 5, 10, 15, 20 & 25% (v/v)) on the bacterial decolorization of textile effluent.

3.5. Effect of carbon sources on effluent (E6) decolorization

Textile wastewater is typically regarded as carbon-deficient [26]. The decolorization process was adjusted to achieve efficient decolorization in the presence of a diverse variety of carbon sources, including glucose, maltose, starch, sucrose, and fructose. The dye achieved a maximum decolorization of 74.8% when the concentration was 0.1 g/L. This occurred after 90 hours of static incubation at a pH of 7 and an incubation temperature of 35°C. Subsequently, sucrose had the second highest decolorization percentage (70%), followed by fructose (64%), maltose (55%), and starch (40%) (Figure 4). In their study, Guadie et al. (2017) [27] described that *Bacillus* sp. strain CH12 exhibited efficient degradation of textile effluent when provided with various carbon sources. The bacterial strain demonstrated a remarkable degradation efficiency of 95-100%, significantly higher than the carbon-free culture, which only achieved an efficiency of 27-51%.

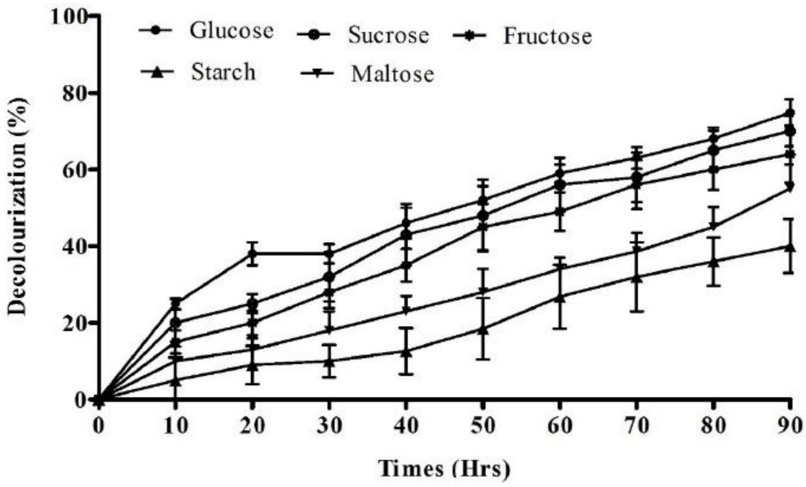

Figure 4: Effect of different carbon sources (Glucose, maltose, starch, sucrose & fructose) on the bacterial decolorization of textile effluent.

3.6. Biochemical characterization of dye decolorizing bacteria

The isolate that showed potential in removing color from textile effluent was analyzed using several types of biochemical and physiological assays in order to categorize it according to Bergey's Manual [19]. The results are displayed in Table 2 below:

Table 2: Morphological and biochemical characteristics of the bacterial isolate

Morphological & Biochemical traits	Results
Gram's reaction	+ve
Shape	cocci
Motility	-ve
Endospore	-ve
Oxidase test	+ve
Catalase test	+ve
Indole test	-ve
Methyl red	+ve
Vogesproskauer	-ve
Citrate utilization	+ve
Nitrate reduction	+ve
Urease test	-ve
Glucose fermentation	+ve
Lactose fermentation	-ve
Sucrose fermentation	+ve
Casein test	-ve
Gelatin hydrolysis test	-ve

+ ve = Positive; - ve = Negative

n= 3 (All experiment performed in triplicates)

3.7. Identification of bacteria

The interpretation of the biochemical test results pertaining to the potent effluent decolorizing bacterial isolate was conducted in accordance with the guidelines provided in Bergey's Manual of Determinative Bacteriology. The identification of the isolate as *Bacillus* spp. was facilitated by the presumptive observation that it belonged to the *Bacillus* genus.

4. Conclusion

The remediation of widely utilized carcinogenic textile dyes poses a significant issue in terms of environmental sustainability and protection. Several conventional physicochemical technologies have shown to be inadequate because of their high cost. The present research has discovered a new bacterium that has the capacity to remove color from textile waste water. This bacterial strain was able to remove up to 74.25% of the color within a period of 90 hours using a submerged fermentation process. The study additionally improved the treatment parameters in order to achieve the maximum rate of decolorization of textile effluent. Hence, taking into account the effectiveness of the newly identified bacterium, the optimum conditions for decolorizing textile effluent can be applied on a broad scale for treating both dyes and effluents in the industry. The utilization of the bacteria and its optimum process conditions can be a highly cost-effective technique, promoting environmental sustainability.

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