# *Original Research Article*

## Dye Decolorization of Industrial Soil by using Dye Degrading Bacteria

**ABSTRACT:**Today's world needs to control the industrial pollution through smarter ways. As a significant class of synthetic organic compounds, textile dyes are frequently used in industrial processes and cause pollution. They are mass-produced and have the potential to pollute the environment both during manufacture and during the dying process of the fiber. Therefore, it is necessary to create treatment techniques that are more successful in removing dyes from the source, which is the soil used for textile waste. Out of 11 isolates, Staphylococcus saprophyticus was shown to be the most active dye degrader. It was isolated from the soil of a textile plant. Investigations were conducted on the significant factors that affect crystal violet decolorization, such as temperature, pH, carbon, and nitrogen supply. With sucrose and beef extract serving as the energy sources; dye decolorization (92.35%) was effectively accomplished under ideal conditions in 120 hours at $30^0$C and pH 8.

**Key Words:** *Staphylococcus saprophyticus*, crystal violet, textile waste, industrial soil, temperature, pH, carbon and nitrogen source.

**INTRODUCTION:**Since the advent of the industrial revolution in the 19th century, the environmental pollution has grown with time and it badly affected the air, water, soil and related ecosystems. Pollution results from different agricultural or urban/industrial sources in the form of fertilizers, pesticides, dyes, etc. The textile and paint industries release large quantities of polluted water containing artificial dyes in the surrounding ecosystem (Bibi *et al*., 2018).Dyes are aromatic xenobiotic compounds and are used extensively in the textile industry, to dye nylon, wool, silk, and paper, cotton, and leather industries. Dyes are colorant that becomes molecularly dispersed at some points during application to fiber and exhibit some degree of permanence. There are many application classes of dyes, including acid dyes, natural dyes and synthetic dyes, some of the used as a biological stains and in veterinary medicine. The total world textile dye production is estimated to be in the range of 700000 tons per year. During the textile dyeing process, up to 40% of dyes may remain unfixed to the fiber and could contaminate the industrial waste water, which in many cases are flushed directly into water ways. This represents a world dye release ranging from 30000 to150000 tons per year. These organic colored substances are very stable and difficult to degrade. The textile factory daily discharges millions of liter so fun treated effluents in the form of waste water into public rains that eventually empty into rivers. This alters the pH, increases the biochemical oxygen demand (BOD) and chemical oxygen demand (COD), and gives the rivers intense colorizations Ajayi and Osibanjo (1980). The use of these water resources is limited and the ecosystem isaffected. About 10-15% of all dyes are directly lost to waste-water in the dyeing process. Thus, the waste- water must be treated before releasing into the natural environment. Several dye degrading microorganisms have been reported and characterized such as *Bacillus* sp, *Staphylococcus* sp., *Streptomyces* sp., *Arthobacter*sp., *Micrococcus* sp., *Vibreo*sp., *Pseudomonas* sp., *Mycoplasma* sp., *Sarcina*sp. and *E. coli.* Banat *et al.* (1996) & Azmi *et al.* (1998). Ozonation can degrade such molecules. But the initial investment and subsequent operation costs are high. Therefore, developing a bioremediation process seems essential. Bio-treatment offers a cheaper and eco-friendly alternative for colour removal in textile effluent. The ubiquitous nature of bacteria makes them invaluable tools in effluents bio-treatment. The aim of present study was to isolate and identify potential dye decolorizing bacterial strains from industrial soil samples and to optimize variousculturepara metersto enhancedye decolorization.

### Materials and Methods

**Isolation of bacteria:** Bacteria were isolated from industrial soil using serial dilution method described by (Parshetti*et al*., 2006).

**Screening of isolated bacteria for dye decolorization:** Soils containing industrial effluents were collected from different industries like saw mill, textile mill, bangle mill and cotton mill. After collecting a soil sample serial dilution of each sample up to $10^{-6}$ to $10^{-8}$ level were made. These dilutions were used in pour plate method. After plating, the isolates were inoculated in nutrient broth at $37^0$C for 24h. To check the dye decolorizing activity 1ml of inoculated nutrient broth was added to 98ml sterilized nutrient broth and with 1ml of dye (crystal violet) the volume was made up to 100ml and it was left on shaker for 24-168h at room temperature. Then the dye decolorization activity was detected by spectrophotometer at 590nm. This method was used for all the isolates (Parshetti*et al.*, 2006).

**Determination of decolorization activity:**Colour was measured at the dye's optimum wavelength (590nm). For this purpose, samples were centrifuged at 3000rpm for 15min and absorbance of supernatants was determined. The decolorization efficiency was expressed as a following equation described by Chong *et al*. (2006).

Decolorization (%)= (I-F)/I*100

Where I= initial absorbance and F= absorbance of decolorized medium

Selection of dye decolorizing bacteria: The strain, which was showing highest decolorizing percentage on Nutrient Broth, was selected for further study.

**Effect of temperature:**Arrange of temperature (20-60°C) was used to study the effect of the temperature on dye decolorization. In this medium 10ml inoculums (10%v/v) was added with 1ml dye (0.50mg/1000 ml) along with 89 ml sterilized nutrient broth at pH7 to make a volume of 100ml then it was incubated for different time intervals (24-120h), respectively. After every time interval i.e. 24, 48, 72, 96 and 120h, the dye decolorization activity was detected by centrifuging the supernatant at 3000rpm for 15min in spectrophotometer at 590nm. This method was used for all the parameters.

**Effect of pH:** Effects of pH for decolorization of industrial soil containing dyes (0.50mg/1000ml) were studied over a pH range 5 to 10 by adjusting pH with HCL and Na0H. In this process 10ml inoculums (10%v/v) was used with 1ml dye (0.50mg/1000ml) along with 89ml sterilized nutrient broth at $30^0$C to make a volume of 100ml then it was incubated over a period of 24-120h.

**Effect of different carbon sources on decolorization:** Effect of various carbon sources on decolorization of industrial soil was assessed by growing the isolate in the sterilized 79ml nutrient broth with 10ml inoculums and 1ml dye at $30^0$C and pH8 along with different carbon source such as glucose (1%), mannitol (1%), sucrose (1%), maltose (1%) and lactose (1%), respectively then incubated for different time intervals i.e. 24-120h.

**Effect of different nitrogen sources on decolorization:** Effect of various nitrogen sources on decolorization was assessed by growing the isolate in the 69ml sterilized nutrient broth with 10ml inoculums, 1ml dye and 10ml carbon source i.e. sucrose (1g/100ml) at $30^0$C, pH 8 and different organic nitrogen source such as peptone (1%), beef extract (1%), malt extract (1%), yeast extract (1%) to make a volume of 100ml. Same procedure was used for different inorganic nitrogen sources such as $NaNO_3$ (1%), $NH_4NO_3$ (1%),$KNO_3$ (1%), $NH_4Cl$ (1%) and $(NH_4)_2SO_4$ (1%), respectively.

**Statistical analysis:** The data recorded during the course of investigation was statistically analyzed using analysis of variance ANOVA (two way classification), correlation and t-test at 5% significance level.

**Results and Discussion**

**Incidence of dye decolorizing bacteria in industrial soil samples:** In the present study total 12 bacteria were obtained from the soil sample of saw mill among them three (25%) isolates showedpositive dye decolorization activity.

Table 1 Sample collection sites

| Sample No. | Sample site | Total No. of isolates | Total No.ofpositiveisolates |
|---|---|---|---|
| 1 | Sawmill(Naini) | 12 | 3(25%) |
| 2 | Textilemill(Naini) | 15 | 3(20%) |
| 3 | Bangle mill(Ferozabad) | 10 | 3(30%) |
| 4 | Cottonmill(Prayagaraj) | 09 | 2(22.22%) |

Significant $t_{cal}$ (6.52)>$t_{tab}$ (5%) (2.44);dof=6

Almost same observation was obtained from textile mill, where total 15 isolates were obtained in which 3 (20%) showed positive result. From bangle mill,number of isolated bacteria was 10 in which 3 (30%) isolates have dye decolorizing capacity. From cotton mill 9 bacteria were isolated among them 2 (22.22%) isolated showed positive test i.e. showing dye decolorization potential. The result was found to be statistically significant ($p<0.05$) there was a significant difference in the incidence of dye decolorizing bacteria from different industrial soil samples (Table-1).

These observations were similar to the findings of Meehan et al. (2001) who found an azo dye reducing, endospore forming bacterium isolated from textile industry. *Staphylococcus saprophyticus* was isolated from textile soil and selected as the most active azodye degrader of 19 isolates by Seesuriyachan*et al*., (2007). Most of the results were found in textile industries, the reason was that textile industry contains azo compounds which were very toxic in nature and very hard to degrade but there was an enzyme called laccase was present not in every bacteria but most of them. This enzyme had ability to degrading dyes, Joseph *et al*., (2007).

**Screening of potential isolate for dye decolorization**: The isolate showed random increased decolorization % between 24-120h, after that the increasing percentage was nearly steady. Total 12 isolates from saw mill soil samples were obtained out of which 3 isolates i.e. SM1, SM2 and SM3 showed dye decolorizing ability.

When the isolates were studied at different time intervals, it was observed that SM1, SM2 and SM3 showed maximum dye decolorizing activity after 120h i.e. 48.48, 47.45 and 73.39%, respectively. Similar percentage activity was showed by three isolates of textile mill namely TM1, TM2 and TM3. Maximum was at 120h with 29.16% (TM1), 40.19% (TM2) and 84.17% (TM3) activity. Almost similar observations were seen in the soil samples collected from bangle mill. Three isolates were obtained that possess dye decolorization ability and were designated as BM1, BM2 and BM3. On comparing BM1, BM2 and BM3 isolates, maximum activity was observed with BM1 after 120h. of incubation. In the case of cotton mill isolates namely CM1 and CM2, the maximum decolorization was observed at 120h i.e. 43.66% (CM1) and 42.99% (CM2).

From all the data obtained it was clearly observed that TM3 was showing most decolorizing percentage at 120h after that time is seems to be constant. It was observed that the rate of removal of acidic dyes increases with increase in contact time to some extent. Further increase in contact time does not increase the uptake due to deposition of dyes on the available adsorption site on adsorbent material, Sumanjit *et al*., (2008). So all the parameters were optimized at 120h and this strain was selected for further studies. The results were found to be significant ($p< 0.05$) means the decolorization was supported by the time duration (Table-2). These observations were similar to the findings of Chong et al., (2006) who found 53.60% decolorization of directblue 15 at 120h.

**Table-2:**Percentage dye decolorization by isolated bacteria.

| Sl. | Sample no. | Isolate no. | Decolorization% 24h. | 48h. | 72h. | 96h. | 120h. | 144h. | 168h. |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Saw mill | SM1 | 21.44 | 25.12 | 34.01 | 42.24 | 48.48 | 48.64 | 48.00 |
| | | SM2 | 17.63 | 22.72 | 24.36 | 39.27 | 47.45 | 47.63 | 47.81 |
| | | SM3 | 42.59 | 62.06 | 66.95 | 69.12 | 73.39 | 73.46 | 73.54 |
| 2 | Textile mill | TM1 | 18.16 | 24.18 | 27.65 | 28.72 | 29.16 | 30.44 | 32.17 |
| | | TM2 | 18.70 | 28.32 | 37.23 | 39.56 | 40.19 | 40.64 | 40.73 |
| | | TM3 | 30.97 | 40.17 | 55.57 | 76.93 | **84.17** | 84.42 | 84.59 |
| 3 | Bangle mill | BM1 | 32.54 | 41.38 | 57.58 | 59.35 | 60.53 | 60.67 | 60.82 |
| | | BM2 | 19.17 | 28.02 | 37.46 | 44.24 | 51.90 | 52.21 | 52.50 |
| | | BM3 | 14.35 | 23.58 | 30.51 | 35.89 | 42.82 | 43.33 | 44.10 |
| 4 | Cotton mill | CM1 | 17.09 | 21.60 | 28.70 | 36.64 | 43.66 | 44.27 | 44.73 |
| | | CM2 | 20.42 | 26.84 | 36.34 | 40.61 | 42.99 | 43.46 | 44.18 |

Significant for hour:
$F_{cal}$ (55.45)>$F_{tab}$ (5%) (2.17)

S.E. = 2.72
C.D. = 5.9024

Significant for isolates:
$F_{cal}$(46.6)>$F_{tab}$(5%) (1.91)

S.E. = 2.17
C.D. = 4.1447

**Table-3:** Effect of temperature on decolorization of crystal violet.

| **Temperature** | **Decolorization%** | **Variation%** |
|---|---|---|
| $20^0$C | 54.83 | –29.34 |
| $30^0$C | 80.36 | –3.81 |
| $40^0$C | 52.80 | –31.37 |
| $50^0$C | 39.44 | –44.73 |
| $60^0$C | 27.76 | –56.41 |

Non-significant $t_{cal}$(3.15)< $t_{tab}$ (5%) (3.18); r =-0.7628;dof=3

**Table-4:** Effect of pH on decolorization of crystal violet at $30^0$C.

| **pH** | **Decolourization%** | **Variation%** | **Temperature** |
|---|---|---|---|
| 5 | 40.58 | –43.59 | 30°C |
| 6 | 42.33 | –41.84 | |
| 7 | 66.77 | –17.4 | |
| 8 | 82.28 | –1.89 | |
| 9 | 33.22 | –50.95 | |
| 10 | 28.01 | –56.16 | |

Non-significant $t_{cal}$(0.3875) < $t_{tab}$ (5%) (2.776); r=-0.1902; dof = 4

**Table-5:** Effect of carbon source on decolorization of crystal violet at $30^0$C and pH8.

| Carbonsource (1%) | Decolorization % | Variation % | Temperature | pH |
|---|---|---|---|---|
| Mannitol | 58.77 | –25.4 | 30°C | 8 |
| Maltose | 52.81 | –31.36 | | |
| Glucose | 47.19 | –36.98 | | |
| Sucrose | 89.66 | +5.49 | | |
| Lactose | 45.39 | –38.78 | | |

Significant $t_{cal}(7.28) > t_{tab}(5\%)$ (2.13); dof = 4

Microbial decolorization of acid black 24 (dye conc. 0.1g/l) a water soluble, benzidine based azo dye was studied by Ozdemir *et al*., (2005). They found decolorization was 74% under shaking conditions within 336h, although dye reduction was stable between 144h and 336h. In our study the dye decolorization was stable between 120-168h. In shaking conditions the dye decolorization was due to adsorption of dye by viableor died cells.

**Identification of bacterial strain:** Based on cultural, morphological and biochemical characteristics tests the TM3 was identified as Staphylococcus saprophyticus (Holt *et al*., 1986).

**Effect of temperature:** The decolorization of crystal violet was most efficient at $30^0$C, with80.36% of colour removal, followed by 20, 40, 50 and $60^0$C. The decline of microbial activity on decolorization could be due to the loss of cell viability and denaturation of enzyme Pearce (2003). The results were found to be non-significant ($p>0.05$). Our findings were similar to the findings of Ilhan *et al*., (2004) who discovered a bacterial strain Staphylococcus saprophyticus which has ability to degrade or remove chromium, lead and copper ions from industrial waste water at $30^0$C with 66, 91 and 48% degradation at 120h, respectively.

**Effect of pH:** The optimum pH for decolorization in this study was pH8, in which decolorization efficiency was 82.28%. Amongst pH,pH8was usedfor subsequence experiments as it was slightly basic and suitable for industrial application. The results were found to be non-significant ($p>0.05$) (Table-4). Similar findings are reported by Sumanjit *et al*.(2007) who tested the dye decolorizing activity was maximum at $30^0$C and pH8 which is slightly basic against Staphylococcus saprophyticus with 78% decolorization of acid red 119. These results indicated that dye decolorization was due to the degradation or conversion of complex substances into smaller substances by particular strain.

**Effect of carbon source:** In an attempt to enhance decolorization performance with extra supplements of carbon source in a medium 89.66% decolorization with sucrose (1%) was found in 120h with pH8 and temperature$30^0$C. Results were found to be statistically significant ($p<0.05$) (Table-5).

The decolorization process requires carbon substrates as an energy source. Sucrose (1%) was the best energy source tested with Methyl Orange. This particular dye was degraded to 39% of the normal level in the presence of sucrose by Lactobacillus caseias described by Seesuriyachan et al. (2007).

**Effect of organic nitrogen source:** In an attempt to enhance decolorization performance with extra supplements of organic source in a medium we found 92.35% decolorization with beef extract (1%), in 120h with carbon source sucrose, pH8 and temperature 30°C. The results were statistically significant ($p<0.05$) (Table-6).

**Table-6:** Effect of organic nitrogen source on decolorization of crystal violet at $30^0$C and pH8 with carbon source sucrose.

| Organic nitrogen Source (1%) | Decolorization % | Variation % | Temperature | pH | Carbon source |
|---|---|---|---|---|---|
| Peptone | 78.28 | –5.35 | $30^0$C | 8 | Sucrose |
| Beef extract | 92.35 | +8.18 | | | |
| Malt extract | 55.09 | –29.08 | | | |
| Yeast extract | 83.46 | –0.71 | | | |

Significant $t_{cal}$ (9.72) > $t_{tab}$ (5%)(2.35); dof=3

**Table-7:** Effect of inorganic nitrogen source on decolorization of crystal violet at 30°C and pH 8 with carbon sourcesucrose

| Inorganic nitrogen Source (1%) | Decolorization % | Variation % | Temperature | pH | Carbon source |
|---|---|---|---|---|---|
| $NaNO_3$ | 70.00 | –14.17 | $30^0$C | 8 | Sucrose |
| $NH_4NO_3$ | 80.97 | –3.2 | | | |
| $KNO_3$ | 88.95 | +4.78 | | | |
| $NH_4Cl$ | 14.28 | –69.89 | | | |
| $(NH_4)_2SO_4$ | 20.11 | –64.06 | | | |

Significant $t_{cal}$(3.39) > $t_{tab}$ (5%) (2.13); dof=4

**Effect of inorganic nitrogen source:** In an attempt to enhance decolorization performance with extra supplements of inorganic nitrogen source in a medium we found 88.95% decolorization with potassium nitrate (1%), in 120h with carbon source sucrose, pH8 and temperature 30°C. The results were found to be statistically significant ($p<0.05$) (Table-7).

Wong and Yuen (1998) studied the decolorization of n,n-dimethyl-p-phenylene diamine by *Klebsiella pneumoniae* and *Acetobacter liquefaciens* with adding nitrogen source as ammonium sulphate (1%) they found 80 and 45% decolorization by both the strains.

The study revealed that maximum decolorization was obtained against crystal violet (0.50mg/l) at $30^0$C, pH8; sucrose (1%) and beef extract (1%). Using all these modified parameters the decolorizing activity was increased up to 92.35% otherwise it is 84.17%.



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