

Isolation and Screening of Reactive Dye Decolorizing Bacterial Isolates from Textile Industry Effluent

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Abstract: Water pollution caused by industrial effluent discharges has become an alarming trend worldwide, while textile industries are considered as the most polluting among all others. In recent years, bio-treatment took attraction in removing the unwanted colour and toxicity of textile effluents than other conventional treatment processes. The present study objective is the isolation and the identification of indigenous bacteria from textile dye effluent and evaluation of their ability to decolorize commonly used dyes. The decolorizing activity was measured spectrophotometrically after incubation of the isolates for 24 h. in mineral salt medium modified with 100 mg/l of reactive blue 222 or reactive black 5. About 37 bacterial isolates were isolated from textile wastewater. Screening of dye decolorizing bacterial isolates showed that sixteen isolates appeared positive in response to decolorization of different dyes. Five bacterial strains were identified to be capable of decolorizing more than 60% of dyes up to species. The strains were *Pseudomonas* sp. (D4), *Salmonella* sp. (D7), *Aeromonas* sp. (D11), *Klebsiella* sp. (D22) and *Bacillus* sp. (D31). Isolate *Pseudomonas* sp. (D4) was the most efficient bacterial isolates to decolorize reactive black 5 and reactive blue 222 with 83% and 70% color removal efficiency, respectively, in 24 h. Furthermore, the isolates had the best performance at the dye concentration of 100 mg/L medium (pH 7) and at temperature 35°C. This study thus reveals that some bacteria inhabit in textile effluent whereby utilize the dyes as their source of energy and nutrition and imply their importance in the treatment of industrial effluents.

Key words: Water pollution • Industrial effluent • Bio-treatment • Decolorization • Textile effluent
• Bacterial isolate • Reactive dyes

INTRODUCTION

Surface and groundwater resources in Egypt suffer from many domestic and industrial contaminants including acids, bases, toxic organic and inorganic dissolved solids and colors. One of the most important environmental pollution problems is the color in water courses, although some of this color is normally present and of "natural" origins (e.g. the color originates from the activity of some microorganisms in ponds), a considerable proportion, especially in the lower reaches of rivers draining large industrial conurbations, originates from industrial effluents [1]. Synthetic dyestuffs are used extensively in textile, paper, printing industries and dye houses. The effluents of these industries are highly colored and the disposal of these wastes into receiving

waters causes damage to the environment [2]. The discharge of dyes into surface water obstructs light penetration and oxygen transfer into water bodies, hence affecting aquatic life [3]. The textile industry utilizes about 10000 different dyes and pigments. The worldwide annual production of dyes is over 7.105 tons [4, 5]. During textile dyeing, the amount of dye lost in effluent is dependent upon the class of dye used, varying from only 2% loss when using basic dyes to a 50% loss when reactive dyes are used. Approximately 20% of these losses enter the environment through effluents from wastewater treatment plants [6]. Therefore, textile effluents containing dyes must be treated before their discharge into the environment. Although, it is difficult to treat textile industry effluents due to their high Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), heat,

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color, pH and the presence of metal ions [7, 8, 9]. In recent years, numerous studies were carried out for the decolorization of textile effluent, including various physicochemical methods such as filtration, coagulation, chemical flocculation, use of activated carbon, advanced oxidation processes, ion exchange, electrochemical and membrane process. Few of them are effective but with high cost, low efficiency and lack of selectivity of the process [10, 11]. Biological treatment offers a cheaper and environment friendly alternative to dye decolorization and wastewater reutilization in industrial process [1, 6, 12]. The general approach for bioremediation of textile effluent is to improve the natural degradation capacity of the indigenous microorganism that allows degradation and mineralization of dyes with a low environmental impact and without using potentially toxic chemical substances, under mild pH and temperature conditions [13-16]. Approximately 75% of the dyes that are discharged by textile processing industries belong to the classes of reactive (36%), acid (25%) and direct (15%) dyes [9].

The objective of this study was focused on the isolation and the identification of efficient reactive dye decolorizing indigenous bacterial strains from the textile effluents. The bacterial isolates were originated from the dye contaminated textile wastewater from local industry and it is therefore expected that they could adapt to the prevailing local environmental conditions. Such bacteria can be used to develop an effective biological treatment system for the wastewaters contaminated with reactive dye.

MATERIALS AND METHODS

Dyes and Samples: Textile dyes (Table 1) and effluent samples were supplied by Al-Zaeton textile industry located at Cairo, Egypt. The dye was selected on the basis of structural diversity and frequent use in textile industries. The set of dyes selected based on their structural diversity and are commonly used in the textile industry in Egypt.

Culture Media: Nutrient agar media (Oxoid) was used for the enumeration and isolation of bacteria from the dye-effluent. The composition of the mineral salt media (MSM) [17] used in the present study was as follows (g/l): 3-glucose, 2-(NH₄)₂SO₄, 1-KH₂ PO₄, 10-K₂ HPO₄, 0.1-MgSO₄.7H₂O and 5-NaCl.

Physicochemical- Characterization of Textile Effluent: Effluent samples were collected in pre-sterilized polypropylene bottles from textile industry and

conventional parameters such as pH, total dissolved solids (TDS), temperature, electrical conductivity (EC), chemical oxygen demand (COD) and biological oxygen demand (BOD) were characterized as the procedure recommended by standard method for the examination of water and wastewater [18]. Samples were stored at 4°C for the isolation of microorganism [16].

Isolation of Bacteria from Wastewater Samples: Bacterial isolations were carried out by serially diluting textile effluent samples in sterile distilled water were subsequently plated onto nutrient agar plates [19]. The plates were incubated at 37°C for 24 h. and colonies with distinct morphology were picked up and purified by regular subculture. The strains were maintained on slants of nutrient agar.

Screening of Dye-Decolorizing Bacteria: The screening was carried out using selected dyes in mineral salt media (broth). Each selected strain was cultivated for 24 h. in nutrient broth. A 5% (v/v) of the inoculum with uniform cell density (OD: 0.8) was then transferred into 250 ml Erlenmeyer flasks containing 50 ml of mineral salt media. A final concentration of 100 mg of reactive dye/l of mineral salt medium was added into each flask and absorbance was taken at their absorbance maxima (λ_{max}) initially (t₀) after a period of 24 h (t₂₄) at 35°C. Based on the reduction in absorbance, the percentage of decolorization was estimated visually. Isolates that exhibited a high potential of decolorizing ability were chosen for further experimentation [16].

Dye Decolorization Assay: Decolorization activity expressed in terms of percentage was determined. The decrease in absorbance was monitored at λ_{max} for particular dye. Decolorized sample (5 ml) was withdrawn, centrifuged at 10000 rpm for 15 mins and its absorbance was measured at λ_{max} of the dye. The uninoculated dye free medium was used as blank. All assays were performed in triplicate and compared with uninoculated control. The color removal efficiency (Decolorization %) of bacterial isolates was expressed as the following equation [13, 20].

$$\text{Decolorization \%} = \frac{\text{Initial absorbance value} - \text{Final absorbance value}}{\text{Initial absorbance value}} \times 100$$

Identification of Selected Isolates: The selected isolates were examined for their morphological properties, such as size, shape, cell arrangement and staining properties. Cultural properties including form, colour, elevation, margin, surface of colonies on nutrient agar plate and

slant were also recorded. Physiological and biochemical characteristics of the isolates were evaluated by Voges–proskauer, methyl red, indole, catalase, oxidase, citrate utilization and H₂S production tests. The ability of the organisms in fermenting a number of sugars including glucose, fructose, sucrose, arabinose, mannose, rhamnose, galactose, maltose and lactose were also performed. The isolates were identified up to species based on comparative analysis of the observed characteristics with the standard description of bacterial strains in Bergey's Manual of Systematic Bacteriology [21].

Dye Decolorization Optimization: Factors like substrate concentration, temperature, Incubation time and pH were optimized during the experimentation for maximizing decolorizing efficiency of the isolates. Optimization studies included various concentration of dye (50, 100, 150, 200 and 250 mg/l), temperatures (25, 30, 35, 40, 45°C) and pH values adjusted initially using HCl or NaCl solutions (3, 5, 7, 9). Isolates that exhibited a high potential of decolorizing ability were tested to optimize their decolorization efficiency. While culture conditions were the same as used in decolorization experiment i.e., minimal salt medium was used along with the 100mg of reactive dye/l of mineral salt medium. Uninoculated blanks were run to check the abiotic decolorization during the experimentation [22].

Statistical Analysis: All analysis was conducted in triplicate and the results obtained from each set of data have been expressed in terms of mean (average) of triplicate and (\pm) standard deviation by using commercial spreadsheet package.

RESULTS AND DISCUSSION

Characterization of Textile Effluent: Textile effluent six samples (3 samples untreated and 3 treated) were collected from Al-Zaeton textile industry located at Cairo, Egypt. The color of raw effluent was reddish brown and changed finally after treatment to light brown. The pH of the untreated effluent was 9.9 which reduced after treatment to near neutral 7.6 (Table 2). The color absorbance of the treated sample was found to be higher than the permissible limit of Law 48 of 1982 which was due to the presence of dye content. The pollutant load of the effluent which had adverse effects on aquatic flora, fauna and even human beings, was found to be high. Hence decolorization and degradation of dye present in textile effluent were needed. The values of BOD and COD

were less in the treated sample in comparison to the very high values of BOD and COD in effluent.

Isolation, Screening and Identification of Dye Decolorizing Bacteria: A total of 37 bacterial strains were isolated from textile dye effluents based on their distinct colony characteristics on nutrient agar plate after incubation at 37°C for 24 hr. Screening of the bacterial isolates was performed to figure out the isolate capable of decolorization textile dyes (Reactive blue 222 and Reactive black 5) in mineral salt media containing 100 mg/L of respective dye. Screening of dye decolorizing bacterial isolates was shown that sixteen isolates from total Bacterial isolates (37 isolates) appeared positive in response to decolorization of different reactive dyes as shown in Table 3 and remained isolates (21 isolates) could not decolorize the tested reactive dyes. Only five bacterial strains were found to be capable of decolorizing more than 60% of dyes and could be considered as potential agents for decolorization textile dyes. Numerous researchers carried out similar works from the textile dye effluent [2, 20, 23, 24, 25], activated sludge [13, 26] and soil contaminated with dye [27, 17, 28], lake-mud and wastewater treatment plant which indicated the natural adaptation of these isolates to high dye concentration [29] and their survival in the presence of toxic dyes [30].

The most efficient bacterial isolate to decolorize reactive black 5 and reactive blue 222 was *Pseudomonas* sp. (D4) with 83% and 70% decolorization efficiency respectively in 24 h (Fig. 1). *Aeromonas* sp. (D11) was the second most efficient bacterial isolate and it decolorized the reactive black 5 and reactive blue 222 with 79% and 63% respectively in 24 h. The decolorization potential for reactive black 5 and reactive blue 222 with *Bacillus* sp. (D31) was 66 and 61% respectively followed by *Salmonella* sp. (D7) (64 and 60%) and *Klebsiella* sp. (D22) (61 and 60%). The five bacterial strains exhibiting strong decolorizing activity were investigated for their morphological, cultural, physiological and biochemical features as shown in Table 4. After scrutinizing the properties with that described in Bergey's Manual, the bacterial strains were identified as *Pseudomonas* sp. (D4), *Salmonella* sp. (D7), *Aeromonas* sp. (D11), *Klebsiella* sp. (D22) and *Bacillus* sp. (D31).

Dye Decolorization Optimization: Potential of selected isolates (*Pseudomonas* sp. (D4), *Salmonella* sp. (D7), *Aeromonas* sp. (D11), *Klebsiella* sp. (D22) and *Bacillus* sp. (D31)) were further investigated for the optimization of various environmental conditions for decolorizing the reactive dye in liquid medium.

Table 1: Textile dyes were used in this study

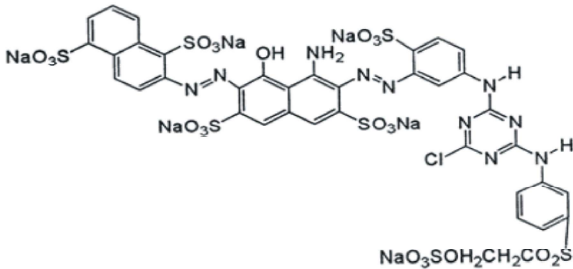
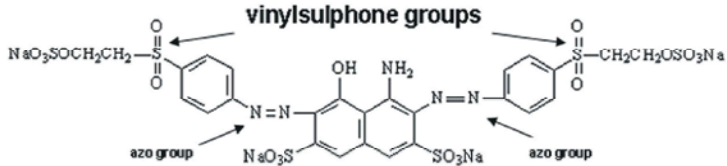
Dye	Type of dye	Maximum wavelength (nm)	Chemical structure
Reactive blue 222	Reactive dye	610	
Reactive black 5	Reactive dye	597	

Table 2: Physicochemical- characterization of textile effluent samples before and after treatment

Parameters	Untreated sample (average (±) standard deviation)	Treated sample (average (±) standard deviation)	Limits for disposing industrial wastes in surface water (Egyptian Law No.48 for 1982)
pH	10.5±0.5	7.5±0.5	6-9
Temperature	53±1.7	34±1.2	35°C
EC (µS/cm)	477±1	333±2	NA
TDS (mg/L)	282±2.2	197±1.3	Not more than 2000
COD (mg/L)	620±2	194±2	80
BOD (mg/L)	110±1	25±2.6	60
Color (Pt Co)	445±0.6	235±2	Free from coloured substances

(NA) Not Available; (EC) Electrical Conductivity; (TDS) Total Dissolved Solids; (COD) Chemical Oxygen Demand; (BOD) Biological Oxygen Demand; (±) means Standard Deviations.

Table 3: Screening of bacterial isolates for decolorization of different dyes in mineral salt medium

Name of dyes	Total bacterial isolates	Number of bacterial isolates decolorized the dyes	Codes of bacterial isolates
Reactive blue 222	37	8	D1, D3, D4, D7, D10, D11, D22 and D31
Reactive black 5		13	D4, D5, D7, D8 D11, D14, D17, D22, D26, D27 D31, D32 and D34

Table 4: Identification of the strongest dye decolorizing bacteria isolated from effluent

Tests to the five bacterial isolates	D4	D7	D11	D22	D31
Gram stain	-	+	-	-	-
Shape	Rod	Rod	Rod	Rod	Rod
Motility	Motile	Motile	Motile	Non-Motile	Motile
Spore formation	-	+	-	-	-
Glucose fermentation	-	+	+	+	+
Sucrose fermentation	-	+	+	+	-
Lactose fermentation	-	+	+	+	-
Indole production	-	-	+	-	-
Methyl red	-	-	-	-	+
Voges-Prausker	-	+	+	+	-
Citrate utilization	+	+	+	+	+
Catalase	+	+	+	+	+
Oxidase	+	-	+	-	-
H ₂ S production	-	-	+	-	+
Identity of the isolates	<i>Pseudomonas</i> sp.	<i>Bacillus</i> sp.	<i>Aeromonas</i> sp.	<i>Klebsiella</i> sp.	<i>Salmonella</i> sp.

(+) means positive growth/ present; (-) means no growth/absent

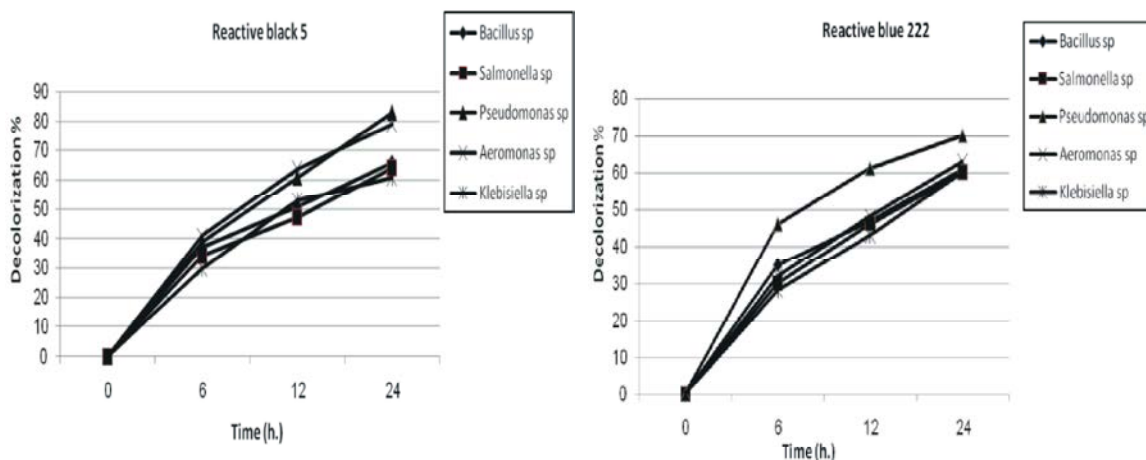


Fig. 1: Biodecolorization of reactive black 5 and reactive blue 222 in mineral salt medium by selected bacterial isolates

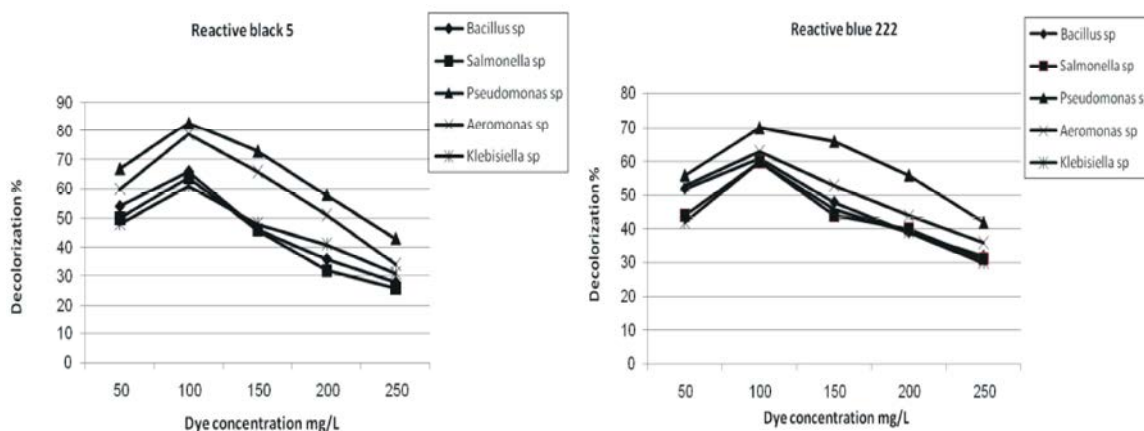


Fig. 2: Effect of different levels of dye concentration on biodecolorization of reactive black 5 and reactive blue 222 by bacterial isolates

Dye Concentration: It was evident (Fig. 2) that reactive black 5 and reactive blue 222 decolorization sharply increased up to 100 mg/l of dye concentration with the maximum decolorization efficiency observed at this concentration. Then, there was a gradual decrease in the dye decolorization for all bacterial strains. *Pseudomonas* sp. (D4) isolate was the most efficient dye decolorizing strain with 83% and 70% removal of the color at 100 mg/l respectively while after 100 mg/l concentration, again *Pseudomonas* sp. (D4) showed a decreasing trend. *Aeromonas* sp. (D11) isolate was the second at the rank with 79% and 63% decolorization at 100 mg/l. The same trend of decolorization was followed in case *Bacillus* sp. (D31), *Salmonella* sp. (D7) and *Klebsiella* sp. (D22) as observed in the case of *Pseudomonas* sp. (D4). Decrease in decolorization ability at high substrate concentration might be due to the toxicity of the dye. The dyes generally contain one or more sulphonic-acid groups on aromatic rings, which might act as detergents to inhibit the growth

of microorganisms [13]. Another reason of the toxicity at higher concentration may be due to the presence of heavy metals (metal-complex dyes) and/or the presence of non-hydrolyzed reactive groups which may retard the bacterial growth (reactive dyes) [31]. Similarly, reduction in decolorization at low concentration of the substrate might be due to the decrease in enzyme ability to recognize the substrate efficiently.

Effect of pH: pH is among the other most important factors for any microbial activity. For studying effect of pH value, different levels of pH ranging from 3 to 9 were used and incubation of all selected isolates was done at these levels (Fig. 3). Initially with the increase in pH value from 3 to 7 decolorization increased and maximum occurred at 7 pH. Similarly, further increase in pH from 7 to 9 had negative effect on decolorization capacity of various isolates. The maximum decolorization for reactive black 5 and reactive blue 222 was observed with *Pseudomonas* sp.

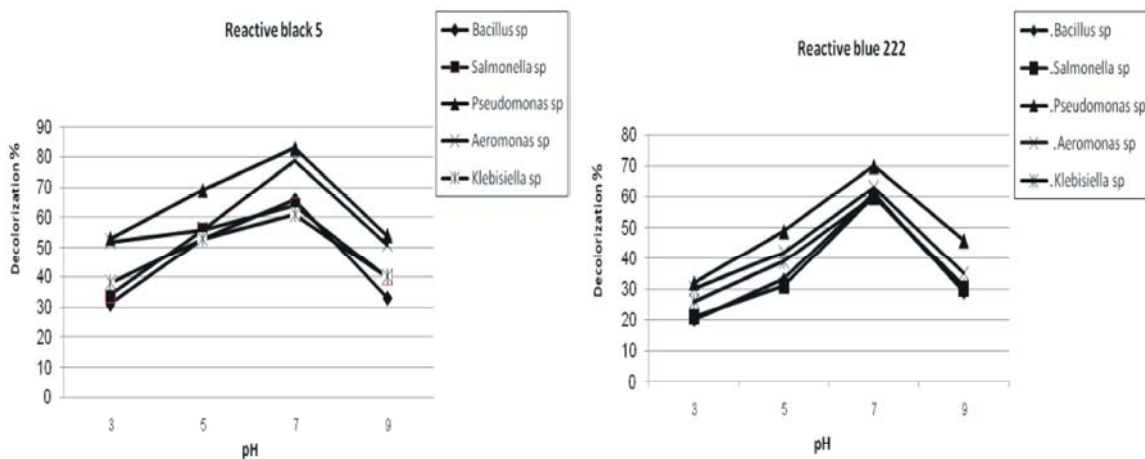


Fig. 3: Effect of different levels of pH on biodecolorization of reactive black 5 and reactive blue 222 by bacterial isolates

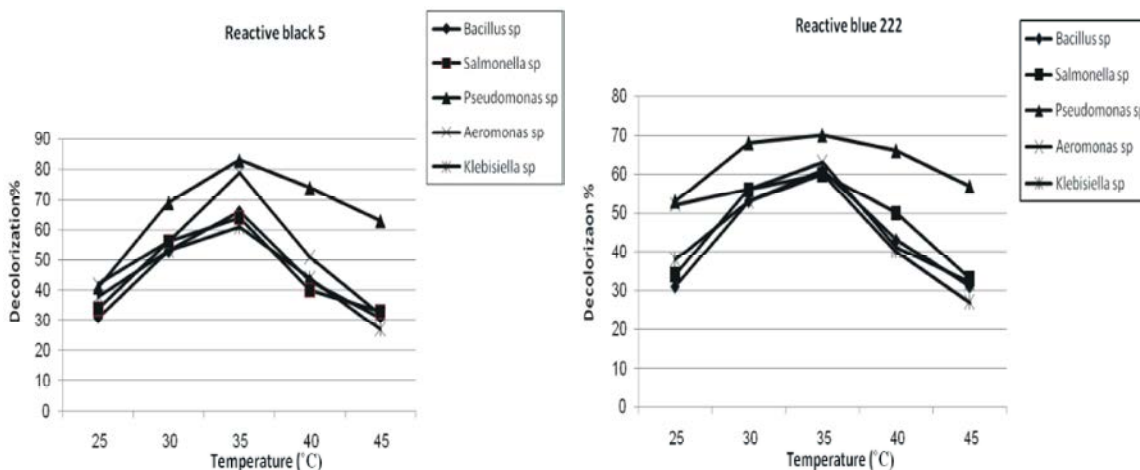


Fig. 4: Effect of different levels of temperature on biodecolorization of reactive black 5 and reactive blue 222 by bacterial isolates

(D4) isolate at pH 7 while minimum decolorization occurred at pH 9. Similar trends in remaining isolates *Bacillus sp.* (D31), *Salmonella sp.* (D7) and *Klebsiella sp.* (D22) were observed at pH 7. Overall, it was noted that all the bacterial isolates showed optimum decolorization from pH 5 to 7. The pH plays a critical role for the optimal physiological performance of microbial cells and the transport of various nutrient components across the cell membrane. Thus, the pH of the decolorization medium has a marked effect on the cell growth and enzyme production [1]. In the present study the optimum incubation pH for maximum color removal percentage for the two reactive dyes was neutral. Further increase in pH, dye decolorizing activity of the culture was decreased. This may be related to the transport of dye molecules across the membrane, which is considered a rate limiting step [32]. So, from this

study, it could be concluded that neutral pH supported bacterial activity to decolorize reactive dye in liquid medium [33].

Effect of Incubation Temperature: Five levels (25, 30, 35, 40 and 45°C) of temperature were used for assessing optimal biodecolorization of reactive black 5 and reactive blue 222 by selected bacterial isolates. It is evident (Fig. 4) that when the temperature was raised from 25 to 35°C there was same trend in decolorization by different isolates. *Pseudomonas sp.* (D4), *Salmonella sp.* (D7), *Aeromonas sp.* (D11), *Klebsiella sp.* (D22) and *Bacillus sp.* (D31) isolates showed gradual increase in decolorization from 25 to 35°C with maximum decolorization at 35°C. As the temperature increased further from 35°C to 45°C, there was sharp decline in

decolorization capacity in all the isolates. Maximum decolorization for reactive black 5 and reactive blue 222 was observed with *Pseudomonas* sp. (D4) with 83% and 70% respectively at 35°C and it is followed by *Aeromonas* sp. (D11) (79% and 63%) > *Bacillus* sp. (D31) (66 and 61%) > *Salmonella* sp. (D7) (64 and 60%) > *Klebsiella* sp. (D22) (61 and 60%) at the same temperature. The decreasing in decolorization activity with increasing the temperature from 35°C to 45°C for the selected bacterial isolates might be due to the loss of cell viability or deactivation of the enzymes responsible for decolorization at higher temperature [34]. Previous study, Bayoumi *et al.* [1] reported that the optimal incubation temperature for decolorization process was 35°C. Decolorization percentage was decreased as temperature was decreased lower than 35°C or increased over this particular value. This finding was consistent with previous studies reported by Kannan *et al.* [32], where the best decolorization was achieved at temperature 35°C and 40°C with 94.25% and 83.65% decolorization respectively in 48 h. This could be owing to a greater production of enzymes and maximal growth conditions of the bacterial culture for its dye decolonization ability.

CONCLUSION

In conformity with similar literatures, decolorizing bacterial isolates was found to successful in the decolorization and mineralization of dyes from textile effluents. The biological treatment of textile effluent not only decolorized the effluent but also reduced the cost of effluent treatment process. In this study bacterial isolates *Pseudomonas* sp. (D4), *Salmonella* sp. (D7), *Aeromonas* sp. (D11), *Klebsiella* sp. (D22) and *Bacillus* sp. (D31) were selected for the decolorization studies based on their higher potentials to decolorize reactive black 5 and reactive blue 222. Maximum decolorization was shown by *Pseudomonas* sp. (D4) for both dyes (83% and 70%) within 24h., followed by *Aeromonas* sp. (D11) (79% and 63%), *Bacillus* sp. (D31) (66 and 61%), *Salmonella* sp. (D7) (64 and 60%) and *Klebsiella* sp. (D22) (61 and 60%), respectively during screening. Further research on these strains could explore new tools and techniques to evolve viable and eco-friendly microbial solutions for treatment of dyeing industrial effluent. It also is becoming easier to determine the detailed mechanisms of biodegradation and their enzymatic property using the tools of biochemistry and molecular biology. This information could be useful to produce an important textile dye degrader.

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REFERENCES

1. Bayoumi, R.A., Sh.M. Husseiny, A.S. Bahobil, S.S. Louboudy and T.A. El-Sakawey, 2010. Biodecolorization and Biodegradation of Azo Dyes by Some Bacterial Isolates. *Journal of Applied Environmental and Biological Sciences*, 1(1): 1-25.
2. Ponraj, M., K. Gokila and V. Zambare, 2011. Bacterial Decolorization of Textile Dye Orange 3R. *International Journal of Advanced Biotechnological Research*, 2: 168-177.
3. Asad, S., M.A. Amoozegar, A.A. Pourbabae, M.N. Sarbolouki and S.M.M. Dastgheib, 2007. Decolorization of Textile Azo Dyes by Newly Isolated Halophilic and Halotolerant Bacteria. *Bioresource Technology*, 98: 2082-2088.
4. Aksu, Z. and S. Tezer, 2005. Biosorption of Reactive Dyes on The Green Alga *Chlorella vulgaris*. *Process Biochemistry*, 40: 1347-1361.
5. Daneshvar, N., M. Ayazloo, A.R. Khataee and M. Pourhassan, 2007. Biological Decolorization of Dye Solution Containing Malachite Green by Microalgae *Cosmarium* sp. *Bioresource Technology*, 98: 1176-1182.
6. Bayoumi, M.N., R.S. Al-Wasify and S.R. Hamed, 2014. Bioremediation of Textile Wastewater Dyes Using Local Bacterial Isolates. *International Journal of Current Microbiology and Applied Sciences*, 3(12): 962-970.
7. Santos, A.B, 2005. Reductive Decolorization of Dyes by Thermophylic Anaerobic Granular Sludge. Doctoral Thesis, Wageningen University, Wageningen, the Netherlands.
8. Hao, O., H. Kim and P. Chiang, 2000. Decolorization of wastewater: Critical Reviews. *Environmental Science Technology*, 30: 449-505.
9. Shah, M.P. and K.A. Patel, 2014. Microbial Degradation of Reactive Red 195 by Three Bacterial Isolates in Anaerobic-Aerobic Bioprocess. *International Journal of Environmental Bioremediation and Biodegradation*, 2(1): 5-11.

10. Maier, J., A. Kandelbauer, A. Erlacher, A. Cavaco-Paulo and M.G. Gubitza, 2004. A New Alkali-thermostable Azoreductase from *Bacillus* sp. Strain SF. Applied Environmental Microbiology, 70: 837-844.
11. Kurniawan, T.A., G.Y.S. Chan, W.H. Lo and S. Babel, 2006. Physico-chemical Treatment Techniques for Waste Water Laden with Heavy Metals. Chemical Engineering Journal, 118: 83-98.
12. Mondal, P.K. and R. Ahmad, 2009. Aerobic Biodegradation and Adsorption of Industrial Sludge Containing Malachite Green by Sequential Batch Reactor. Proceedings in International Conference on Energy and Environment, March 19-21.
13. Chen, K.C., J.Y. Wu, D.J. Liou and S.C.J. Hwang, 2003. Decolorization of the Textile Azo Dyes by Newly Isolated Bacterial Strains. Journal of Biotechnology, 101: 57-68.
14. Kapdan, I.K. and B. Erten, 2007. Anaerobic Treatment of Saline Wastewater by *Halanaerobium lacusrosei*. Process Biochemistry, 42: 449-453.
15. Dhanve, R.S., U. Shedbalkar and J.P. Jadhav, 2008. Biodegradation of Diazo Reactive Dye Navy Blue HE2R (Reactive blue 172) by an Isolated *Exiguobacterium* sp. RD3. Biotechnology and Bioprocess Engineering, 13: 53-60.
16. Pokharia, A. and S.S. Ahluwalia, 2013. Isolation and Screening of Dye Decolorizing Bacterial Isolates from Contaminated Sites. Journal of Textiles and Light Industrial Science and Technology, 2(2): 54-61.
17. Mabrouk, M.E.M. and H.H. Yusef, 2008. Decolorization of Fast Red by *Bacillus subtilis* HM. Journal of Applied Sciences and Res., 4: 262-269.
18. Clesceri, L.S., A.E. Greenberg and R.R. Trussel, 2005. Standard Method for the Analysis of Water and Wastewater. American Public Health Association.
19. Cappuccino, J. and N. Sherman, 1996. Microbiology: A Laboratory Manual. The Benjamin Cummings Publishing Company Inc., California.
20. Ali, N., A. Hameed and S. Ahmed, 2009. Physicochemical Characterization and Bioremediation Perspective of Textile Effluent, Dyes and Metals by Indigenous Bacteria. Journal of Hazardous Materials, 164: 322-328.
21. Sneath, P.H.A., N.S. Mair, M.E. Sharpe and J.G. Holf, 1984. Bergey's Manual of Systematic Bacteriology. Vol. 1, Willams and Wilkins, Baltimore, U.S.A.
22. Mahmood, S., M. Arshad, A. Khalid, Z.H. Nazli and T. Mahmood, 2011. Isolation and screening of azo dye decolorizing bacterial isolates from dye-contaminated textile wastewater. Soil Environment, 30(1): 7-12.
23. Kaushik, P. and A. Malik, 2009. Microbial Decolorization of Textile Dyes through Isolates Obtained from Contaminated Sites. Journal of Scientific and Industrial Research, 68: 325-331.
24. Khadijah, O., K.K. Lee, F.M. Faiz and H. Abdulla, 2009. Isolation, Screening and Development of Local Bacterial Consortia with Azo Dyes Decolorizing Capability. Malaysian Journal of Microbiology, 5: 25-32.
25. Prasad, A.S.A. and K.V. Bhaskara Rao, 2010. Physico-chemical Characterization of Textile Effluent and Screening for Dye Decolorizing Bacteria. Global Journal of Biotechnology and Biochemistry, 5: 80-86.
26. Wang, H., J.Q. Su, W.X. Zheng, Y. Tian, X.J. Xiong and T.L. Xheng, 2009. Bacterial Decolorization and Degradation of the Reactive Dye Reactive Red 180 by *Citrobacter* sp. CK3. International Biodeterioration and Biodegradation, 63: 395-399.
27. Leena, R. and D.S. Raj, 2008. Bio-decolorization of Textile Effluent Containing Reactive Black-B by Effluent-adapted and Non-adapted bacteria. African Journal of Biotechnology, 7: 3309-3313.
28. Telke, A., D. Kalyani, J. Jadhav and S. Govindwar, 2008. Kinetics and Mechanism of Reactive Red 141 Degradation by a Bacterial isolate *Rhizobium radiobacter* MTCC 8161. Acta Chim. Slov., 55: 320-329.
29. Khehra, M.S., H.S. Saini, D.K. Sharma, B.S. Chadha and S.S. Chimni, 2005. Decolorization of Various Azo Dyes by Bacterial Consortium. Dyes and Pigments, 67: 5-61.
30. Abd El-Rahim, W.M., H. Moawad and M. Khalafallah, 2003. Microflora Involved in Textile Dye Waste Removal. Basic Microbiology, pp: 167-174.
31. Sponza, D.T. and M. Isik, 2005. Reactor Performances and Fate of Aromatic Amines Through Decolorization of Direct Black 38 Dye under Anaerobic/ Aerobic Sequential. Process Biochemistry, 40: 35-44.
32. Kannan, S., K. Dhandayuthapani and M. Sultana, 2013. Decolorization and Degradation of Azo dye - Remazol Black B by Newly Isolated *Pseudomonas putida*. International Journal of Current Microbiology and Applied Sciences, 2(4): 108-116.
33. Smali, P.L., M.M. Mahajan, D.P. Patil and M.V. Kulkarni, 1999. Biodecolorisation of Members of Triphenylmethane and Azo Groups of Dyes. Journal of Scientific and Industrial Res., 59: 221-224.
34. Cetin, D. and G. Donmez, 2006. Decolorization of Reactive Dyes by Mixed Cultures Isolated from Textile Effluent under Anaerobic Conditions. Enzyme and Microbial Technology, 38: 926-930.