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Influence of some chemical parameters on decolorization of textile dyes by bacterial strains isolated from waste water treatment plant

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Bacterial species capable of decolorizing textile and laboratory dyes were isolated from textile effluent treatment plant. Two bacterial strains (one Gram negative and another Gram positive bacterial strain) were screened for their ability to decolorize Red H_5BL (reactive dye), Thymol blue (acid dye), Malachite green, Crystal violet (Triphenyl dye) and Congo red (azo dye). The highest decolorization was achieved for Red H_5BL (90%) and lowest for Thymol blue (26%) in 24 h incubation. The effect of different carbon sources and nitrogen sources were studied. The presence of dextrose in the culture medium suppresses the decolorization ability of Gram negative bacterial strain. In case of Gram positive strain, decolorization of Red H_5BL was achieved in the range of 75 to 86% for all the tested carbon sources. During the experiments of checking the effect of carbon sources it was found that shaking culture condition offers high biomass and less color removal of the dye. Decolorization of dyes was effective only under static culture condition. Inorganic or organic nitrogen source has no remarkable effect of decolorization process. Increased dye concentration and salt concentration has negative effect on the process of dye decolorization and biomass synthesis.

Key words: Biodegradation, decolorization, textile dyes, bioremediation.

INTRODUCTION

Textile dyes are of environmental interest because of their widespread use, their potential for formation of toxic aromatic amines and their low removal rate during aerobic wastewater treatment. It is estimated that over 10,000 different dyes and pigments are in common use, with 0.7 million tons of dyestuff being manufactured each year. Two percent of the dye is lost directly in effluent during its manufacturing process while around 10% dye is lost during coloration process (Pearce et al., 2003).

The ability of microorganisms to decolorize and meta-

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bolize dyes has long been known, and the use of bioremediation based technologies for treating textile wastewater has attracted interest (Kothari et al., 2005; Kunjadia et al., 2012). Several physicochemical techniques for textile effluent treatment including adsorption on materials, oxidation, precipitation, photodegradation and membrane filtration have been assessed, but these techniques have been less effective and economically restricted (Mutafov et al., 2007). Many bacterial strains with ability to degrade dyes under aerobic or anaerobic conditions have been isolated from wastewater treatment plant. This indicates that microorganisms may develop the ability of degrading azo components after an adaptation period.

Since microbial populations are responsible for terrestrial nutrient cycling transformations and contribute to the depuration of contaminated ecosystem. Response to stressful conditions may be reflected in change in the size and/or make up of the community, metabolic activity of the microbial biomass, in addition to changes in the taxonomic or functional make up of the community.

Efforts to isolate bacterial culture capable of degrading azo dyes started in 1970 with the reports of *Bacillus subtilis* (Horitsu et al., 1997). Azo dyes were long considered to be nearly non-biodegradable or untransformable by bacteria. Sequential anaerobic and aerobic condition for dye removal has been studied by many researchers. Switching between aerobic and anaerobic metabolic functions during anoxic operation facilitated the reduction of dye to its intermediate in anaerobic condition followed by their mineralization in aerobic condition (Venkat Mohan et al., 2013).

Treatment of textile wastewater is difficult mainly because of complex aeromatic structure and synthetic origin of dyes as well as presence of other organic and inorganic compounds required in dyeing process (Gul, 2013). Bioremediation has to signify the complete microbial breakdown or mineralization of complex material into inorganic constituents such as carbon dioxide, water, mineral components and cell biomass (Alexander and Lustigman, 1996).

In this study, we try to isolate microorganisms, especially bacterial species, capable of decolorization/degradation of various textile dyes present in waste water effluent generated by dyeing and printing industries of our study area. We investigated various levels of chemical and physical parameters that can influence the process of decolorization of selected dyes, in order to design optimum condition for decolorization process.

MATERIALS AND METHODS

Dyes and chemicals

Textile and laboratory dyes were procured from local manufacturer (GIDC, Ahmedabad, Gujarat, India). The dyes were Red H_5BL, Thymol Blue, Malachite Green, Crystal Violet, and Congo red. Red H_5BL dye was used as model for this study. Stock solution of each dye (10 g L^{-1}) were prepared in distilled water and sterilized by passing through 0.45 µm membrane filter. Yeast extract and peptone from Himedia while rest of the all other chemicals used in this study were from Merck.

Screening of organisms and culture condition

Samples collected from different stages of the Common Effluent Treatment Plant (CETP at Jetpur, Gujarat, India) were used for the isolation of bacterial species by enrichment culture technique. The Jetpur town has more than 100 dying and printing industries generating huge quantity of colored waste water. All the waste is carried through drainage line to CETP located 2 Km far from Jetpur. Samples were collected in sample collection bottle and maintained at 4°C during transportation to the laboratory and tested within 24 h. The effluent samples were inoculated in complete medium broth (CMB) containing 100 mg L^{-1} textile dye Red H_5BL. The CMB comprise of Glucose 0.2%; yeast extract 0.5%; Peptone 0.5%; KH_2PO_4 1.0%, $MgSO_4.7H_2O$ 0.02% in 100 ml of distilled water as described by Kothari et al. (2006). The pH of the medium was adjusted to 7. After incubation of 24 h at 30°C, 1 ml aliquot from flask containing effluent as inoculum, were serially diluted and then plated on complete medium agar plate in order to get well isolated colonies for screening of dye decolorizing bacteria (Joe et al., 2008). The pure culture stocks of these isolates were maintained on CM agar slant at 4 °C. CM agar slant comprise of Glucose 0.2%; yeast extract 0.5%; Peptone 0.5%; KH_2PO_4 1.0%, $MgSO_4$ $7H_2O$ 0.02%, and 2% agar agar in 100 ml of distilled water. Bacterial isolates were individually tested for their ability to decolorize dyes in liquid media containing 100 mg L^{-1} textile dye Red H_5BL.

Batch decolorization operation

A loopful of growth from CM agar slant was transferred in to the CMB and incubated at 30°C for 12 h. The cells were in middle of the exponential phase, and used as inoculum with 1 OD at 620 nm. Reaction mixture contained 100 ml CMB in 250 ml flask. 2 ml of the young culture was used as inoculum with 100 mg L^{-1} Red H_5BL dye concentration constant unless until required to change for all experiments. Flasks were incubated in incubator (Remi, RIS-24 BL) in static or shaking condition at 30°C. Periodically samples were withdrawn from reaction flask, centrifuged (Remi, RM-1214) for biomass separation, and supernatant was used to analyze decolorization of dyes using a UV visible spectrophotometer (Shimadzu UV-1800). The growth response of the organism was determined by resuspending the biomass in 2 ml sterile distilled water and OD was taken at 620 nm (Kothari et al., 2005). Decolorization was determined by measuring the absorbance of culture supernatants at the absorbance maxima of the respective dyes. The decolorization assay was calculated as Decolorization activity (%) = (A-B)/ A x 100; where A = initial absorbance and B = observed absorbance (Patil et al., 2008). Parameters of batch operations are summarized in Table 1.

Effect of C and N sources on dye decolorization

Glucose was omitted from the CMB medium and replaced by other carbon sources to be tested. Other carbon sources were Dextrose, Fructose, Galactose, Maltose, Sucrose, Glycerol, Fumeric acid, Starch, Citric acid, Malic acid, Carboxymethylcellulose, and Succinic acid. The combine effect of two carbon sources on dye decolorization was tested by offering combination like Succinic acid + Glycerol, and Succinic acid + Glucose on dye decolorization. These carbon sources were added in the reaction mixture with the 0.5 % final concentration, so in case of combination of carbon source each were added with 0.25 % final concentration. Organic and

Table 1. Summary of parameters of batch operations.

Factors investigated	Culture condition	C source	N source	Dye	Salt (g %)	Initial dye (mg L^{-1})
C source	Static and Shaking	14 different	Peptone	Red H_5BL	0.5	100
N source	Static	Glucose	8 different	Red H_5BL	0.5	100
Dye	Static	Glucose	Peptone	5 different	0.5	100
Salt	Static	Glucose	Peptone	Red H_5BL	0.2 - 3	100
Initial dye	Static	Glucose	Peptone	Red H_5BL	0.5	100 - 500

inorganic nitrogen sources like Peptone, Yeast extract, Ammonium nitrate, Ammonium sulphate, Ammonium tartrate, Ammonium chloride, Sodium nitrate, and Urea were tested for its effect on rate of decolorization of Red H_5BL under static condition. These nitrogen sources were added in reaction mixture with 0.5% final concentration.

Effect of Initial dye and salt concentration on dye decolorization

Various concentrations of Red H_5BL (100, 200, 300, 400, and 500 mg L^{-1}) were prepared in CMB medium and inoculated with stock culture. This experiment was performed in triplicate. The effect of different salt concentration on bacterial growth and its decolorization efficiency were tested by supplementing the reaction mixture with different salt concentrations in the range of 0.2, 0.5, 1, 2, and 3 g%.

Effect of Different Dyes on decolorization

Total of 5 dyes were studied for both the isolated species for their ability to decolorize it. All dyes were added with 100 mg L^{-1} final concentration in individual reaction mixture in three sets of experiment. Samples were collected from reaction mixture, centrifuged, and supernatant was used to analyze dye decolorization by UV-visible spectrophotometer at lambda max of respective dyes (Red H_5BL 512 nm, Thymol Blue 670 nm, Malachite green 622 nm, Crystal violet 588 nm, and Congo red - 505 nm).

RESULTS AND DISCUSSIONS

Screening of bacterial isolates

Experiments to isolate different bacterial species from CEPT effluent resulted in isolation of 33 bacterial species. These strains have been attempted to distinguish on the basis of some morphological, colony characteristics and Gram reactions. The strains have been screened for capability to decolorize dye Red H5BL under laboratory conditions. These strain showed different rates of reduction of color of the dye when grown under stationary batch culture. From these observations, we selected one Gram negative short rod shaped bacterial strain and another Gram positive regular rod shaped bacterial strain for further studies.

Effect of carbon and nitrogen sources on red H_5BL decolorization

In order to check the effect of various carbon sources on the process of dye decolorization by both the bacterial strain, as many as fourteen different carbon sources were tested. Each carbon sources were added at constant concentration in different reaction mixtures. The effect was observed under both the incubation condition – shaking and static condition. Figure 1 showed that there is no marked difference in decolorization pattern when Gram negative bacterial strain was subjected to different carbon sources. It seems that microaerophilic (static incubation) condition favors better decolorization. Isolated Gram positive strain when grown under stationary condition reduced the color of dye Red H_5BL almost completely (80 - 100 %) when grown with all carbon sources except dextrose. When the strain grew under shaking condition, the rate of decolorization was significantly less (4 - 45%). Sharp reduction in decolorization capacity was observed in case of dextrose as a carbon source. Higher concentrations of glucose are known to inhibit dye decolorization by microbial processes (Kumar et al., 2009).

Microbial reduction of dyes is an enzymatic reaction and linked to anaerobiosis because it is inhibited by oxygen. It is possible that non-specific enzymes may be exist in many anaerobic and obligate anaerobic strains to reduce dye under anaerobic condition (Hong and Gu, 2010). Therefore facultative or obligate anaerobes are necessary for azo dye reduction.

Figure 2 explained results of carbon sources effect on decolorization of Red H_5BL by Gram positive bacteria. There was no remarkable difference in decolorization pattern by Gram positive bacteria. This strain also prefer static condition for better decolorization of Red H_5BL, where as shaking condition favors bacterial growth by formation of heavy biomass but negligible dye decolorization was observed.

Organic and inorganic nitrogen sources have influence on the decolorization of various textile dyes under static conditions. Gram negative strain has less effect (57 to 77% decolorization) of nitrogen source whether it is organic or inorganic, where as Gram positive strain showed much influence of organic or inorganic nitrogen sources on decolorization process (43 to 84% decolorization). Results showed that yeast extract favors highest dye

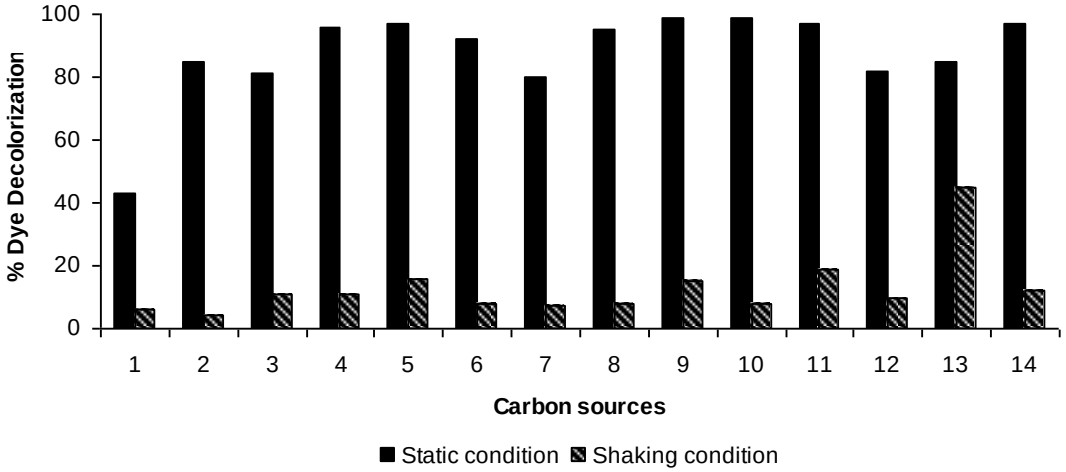

Figure 1. Influence of various carbon sources on decolorization of Red H_5BL by a Gram negative bacteria under static and shaking condition. Numbers on X - axis indicates different carbon sources as; 1. Dextrose, 2. Fructose, 3. Galactose, 4. Maltose, 5. Sucrose, 6. Glycerol, 7. Fumeric acid, 8. Starch, 9. Citric acid, 10. Malic acid, 11. Succinic acid + Glycerol, 12. Succinic acid + Glucose, 13. Carboxymethycellulose, 14. Succinic acid.

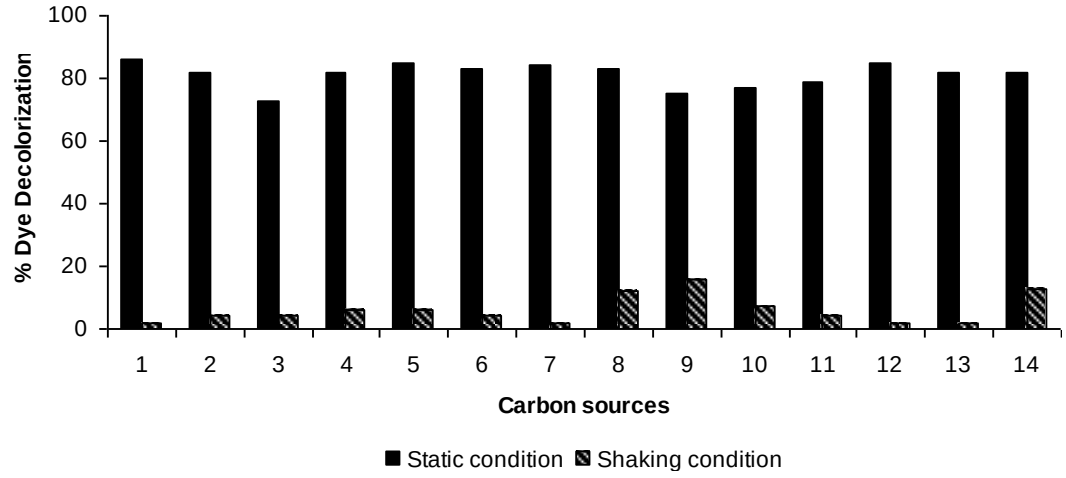

Figure 2. Influence of various carbon sources on decolorization of Red H_5BL by a Gram positive bacteria under static and shaking condition. Numbers on X - axis indicates different carbon sources as ;1. Dextrose, 2. Fructose, 3. Galactose, 4. Maltose, 5. Sucrose, 6. Glycerol, 7. Fumeric acid, 8. Starch, 9. Citric acid, 10. Malic acid, 11. Succinic acid + Glycerol, 12. Succinic acid + Glucose, 13. Carboxymethycellulose, 14. Succinic acid.

decolorization for both the strain; 77 and 84% for Gram negative strain and Gram positive strain respectively (Figure 3).

Effect of different dye on decolorization

Textile industry effluent contains wide variety of dye with different concentration in each batch. Various commercial and laboratory dyes were used to observe the rate of decolorization by both the strains. Both the strains showed highest decolorization of Red H_5BL, 90 and 88% by Gram negative bacteria and Gram positive bacteria respectively (Figure 4). All the three laboratory dyes malachite green, crystal violet, and congo red were decolorized more than 50% by both the strains. The lowest decolorization was of thymol blue (26%) by Gram positive bacterial strain among all tested dyes.

Effect of salt and dye concentration on dye decolorization

Effect of different salt concentration on the growth of the organisms and decolorization was studied by using different sodium chloride concentrations in CMB medium.

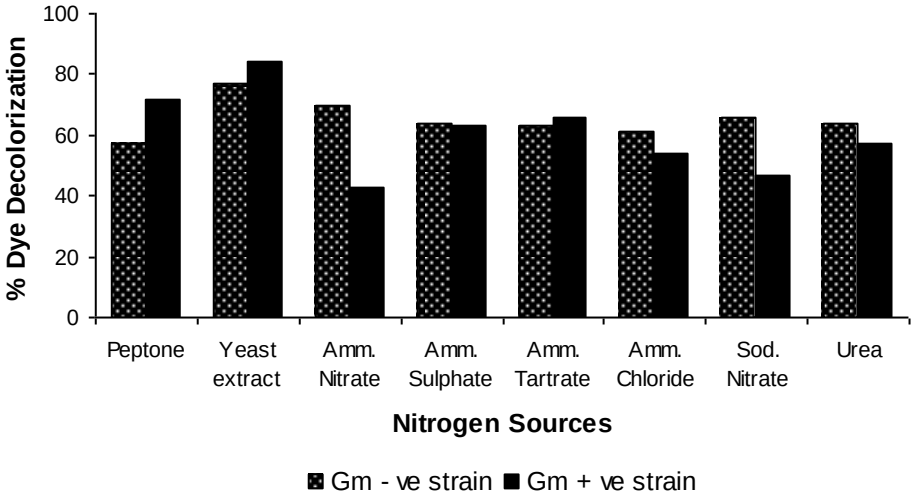

Figure 3. Effect of nitrogen sources on decolorization of Red H5BL by Gram negative strain and Gram positive strain under static condition.

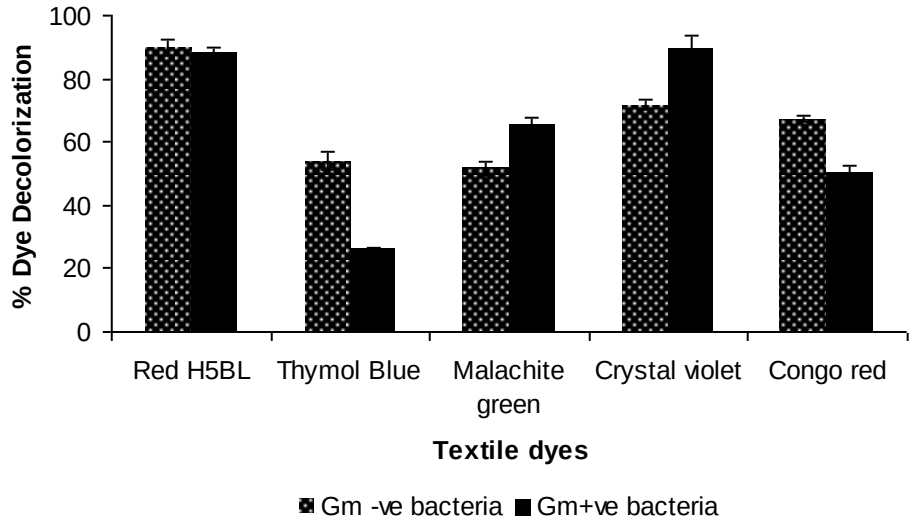

Figure 4. Various textile dye decolorization by both Gram negative strain and Gram positive strain.

Halophilic bacteria have also been reported to decolorize azo dye under high salt condition (Guo et al., 2007). For Gram negative bacterial strain, as the salt concentration was increased from 0.2 to 3 g%, the biomass was reduced by around 54%, while decolorization ability was drastically affected and reduced up to 72%. At high salt concentration, dye decolorization process was affected more than Gram negative bacterial growth (Figure 5). Salt concentration higher than 1 g% found inhibitory to the process. Sharp reduction in biomass and decolorization capacity was observed only after salt concentration higher than it. Almost same pattern of reduction in decolorization efficiency and biomass was observed in case of Gram positive bacteria. The results obtained are indicative of importance of plasma membrane in transport of molecules across the membrane, as at high salt concentration surrounding the cell, Na^+ / K^+ will affect the permeability of the membrane.

The decolorization activity for both the selected organisms was studied using Red H5BL at different initial concentration varying from 100 to 500 mg L^{-1}. Actual textile effluent contains around 200 mg L^{-1} dye concentration (Kumar et al., 2009). The dye concentration up to 200 mg L^{-1} does not affect the biomass synthesis but the 300 mg L^{-1} dye concentration onward, growth was decreased as the concentration of dye was increased in reaction mixture. A kind of regular pattern was observed in reduction of decolorization capacity as increase in initial concentration of dye in case of Gram negative organism (Figure 6). The result obtained from Gram positive

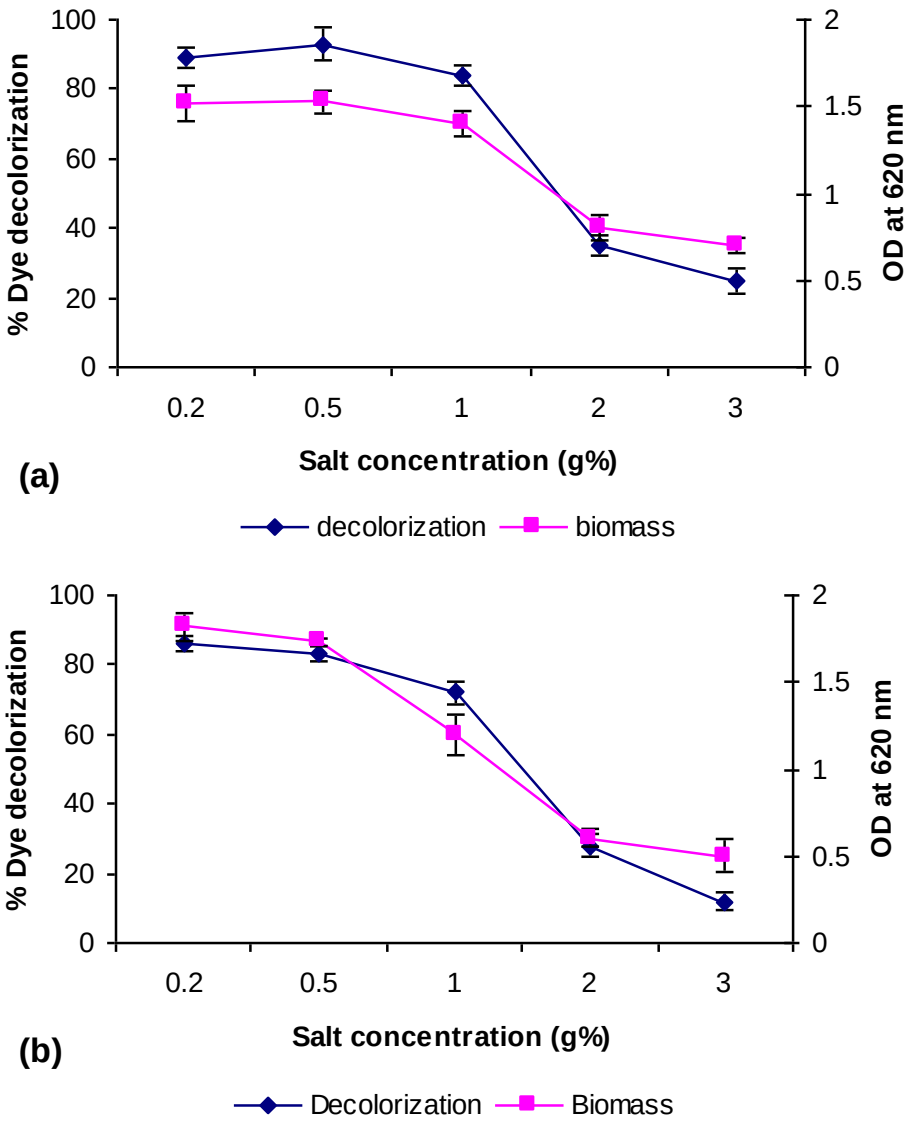

Figure 5. Effect of different salt concentration on bacterial biomass synthesis and rate of Red H_5BL decolorization: **a**. Gram negative bacterial strain, **b**. Gram positive bacterial strain.

organism was slight different from that of Gram negative as there was sharp decline in decolorization activity as well as biomass as the initial dye concentration was increased to 400 mg L^{-1}. As the initial dye concentration increased from 100 to 500 mg L^{-1}, the percentage of Red H5BL decolorization decreased from 90 to 35% for Gram negative bacterial strain and 86 to 12% for Gram positive bacterial strain. In both the cases the increased concentration of dye has inhibited the bacterial growth as evident from results. The decrease in decolorization efficiency might be due to the toxic effect of dyes (Ayed et al., 2009).

Dye containing wastewater treatment presents an arduous task. Wide ranges of pH, salt concentration and variety of chemical structures often add to the complications. Although decolorization is a challenging process to the textile industry, microbial decolorizing system show great potential for achieving total color removal with only hours of exposure.

Conclusion

The bacterial diversity isolated from wastewater effluent plant indicated presence of wide variety of organisms at the contaminated sites. All the isolated organisms showed different decolorization capacity. Both the strains under study showed much similarity in their pattern of Red H_5BL decolorization. No significant effect of carbon and nitrogen sources on dye decolorization process

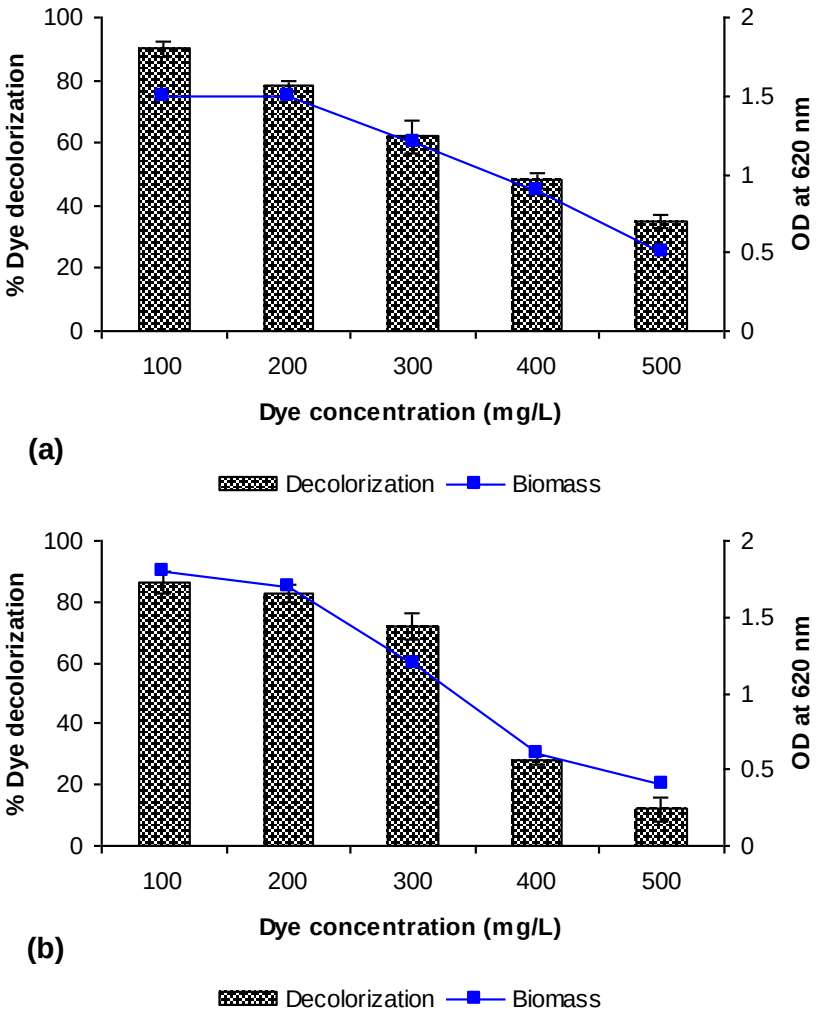

Figure 6. Effect of different initial dye concentration on bacterial biomass synthesis and rate of Red H_5BL decolorization: **a**. Gram negative bacterial strain, **b**. Gram positive bacterial strain.

indicated organism's adaptability to diversified environment. The difference in decolorization capacity of various dyes may be correlated to the difference in the dye structure and its complexity. Their ability to decolorize dye is due to their environment from where they have been isolated. This indicates that microorganisms may develop the ability of degrading azo components after an adaptation period. The Gram negative bacterial strain under study showed more tolerance to high concentration of initial dye concentration than Gram positive strain.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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REFERENCES

Alexander M, Lustigman BK (1996). Effect of chemical structures on microbial degradation of substituted benzenes. J. Agric. Food Chem. 14:410-413

Ayed L, Chaieg K, Cheref A, Bakhrouf A (2009). Biodegradation of triphenylmethane dye malachite green by *Sphingomonas paucimobilis*. World J. Microbiol. Biotechnol. 25:705-711

Gul U D (2013). Treatment of dyeing wastewater including reactive dyes (Reactive Red RB, Reactive Black B, Remazol Blue) and Methylene Blue by fungal biomass. Water SA 39:593-598.

Guo J, Zhou J, Wang D, Tian C, Wang P, Uddin M S (2007). A novel moderately halophilic bacterium for decolorizing azo dye under high salt conditions. Biodegradation 19:15-19

Hong YG, Gu J D (2010). Physiology and biochemistry of reduction of azo compounds by *Shewanella* strain relevant to electron transport chain. Appl. Microbiol. Biotechnol. 88 : 637-643 DOI 10.1007/s00253-010-2820-z

Horitsu H, Takada M, Idaka E, Tomoyeda M, Ogawa T (1997). Degradation of aminoazobenzenear by *Bacillus subtilis*. Eur. J. Appl. Microbiol. 4:217-224

Joe MH, Lim SY, Kim DH, Lee IS (2008). Decolorization of reactive dyes by *Clostridium bifermentans* SL186 isolated from contaminated soil. World J. Mcirobial Biotechnol. 24:2221-2226

Kothari RK, Kothari CR, Pathak SJ (2005). Microbial decolorization and degradation of textile dyes Golden HR and Magenta HB. Asian J. Microbiol. Biotehnol. Environ. Sci. 7(2):1-5.

Kumar K, Dastidar MG, Sreekrishnan TR (2009). Effect of process parameters on aerobic decolourization of reactive azo dye using mixed culture. World Acad. Sci. Eng. Technol. 58:962-965

Kunjadia PD , Patel FD , Nagee A, Mukhopadhyaya PN, Dave GS (2012). Crystal violet (triphenylmethan dye) decolorization potential of *Pleurotus ostreatus* MTCC 142. Bioresources 7(1):1189 - 1199.

Mutafov S, Avramova T, Stefanova L, Angelova B (2007). Decolorization of acid orange 7 by bacteria of different tinctorial type: a comparative study. World J. Microbiol. Biotechnol. 23:417-422

Patil PS, Shedbalkar UU, Kalyani DC, Jadhav JP (2008) Biodegradation of Reactive Blue 59 by isolated bacterial consortium PMB11. J. Ind. Microbiol. Biotechnol. 35:1181-1190

Pearce CI, Lloyd JR, Guthrie JT (2003). The removal of colour from textile wastewater using whole bacterial cell: a review. Dyes Pigm. 58:179-196

Venkat Mohan S, Suresh Babu P, Srikanth S (2013). Azo dye remediation in periodic discountinuous batch mode operation: Evaluation of metabolic shifts of the biocatalyst under aerobic, anaerobic and anoxic conditions. Sep. Purif. Technol. 118:196-208