



Microbial Decolorization of Leather Dye Effluent

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ABSTRACT

As we know 71% of Earth's Surface is covered with water. Water is one of the most essential element for the persistence of life on this planet. Pure and contamination free water is the dire necessity of every living being present on Earth but today water pollution has posed great threat to the existence of life. The discharge of effluents from various industries into the water bodies are mainly responsible for water pollution specially effluents from leather industry are the most contaminating as along with the harmful chemicals they contain organic matter as well. The dyes disposed by the leather industry are the most harmful for the environment. The conventional physicochemical methods used for the treatment of leather dyes in the contaminated water i.e. coagulation, precipitation, oxidation etc have disadvantages and limitations. This study presents microbial decolorization of leather dye effluents from *Shigella* sp. isolated from effluent samples collected from leather industry. Different parameters such as temperature and pH were optimized for decolorization of Methylene Blue, Crystal Violet, Eosin Yellow, Safranin dyes by using bacterial isolates. Optimum temperature for decolorization was observed to be 30°C, the optimum pH range for decolorization was found to be from pH6-pH8. All the samples were incubated at 30°C 150 rpm. The decolorization was measured as decrease in absorbance maxima at 663 nm, 590 nm, 518nm, 530 nm for methylene blue, crystal violet, eosin yellow, safranin respectively.

Keywords: *shigella* sp. Crystal Violet, Methylene Blue, Eosin Yellow, Safranin

INTRODUCTION

Potable water is one of the primary necessities for the existence of life on this earth. Water is essential for daily human activities may it be for domestic purposes, industrial or agricultural purposes. With the increase in urbanization and industrialization the environment has been remarkably affected by man. With the increase in population we have witnessed a rapid increase in pollution mainly water pollution. The discharge of effluents from industries into the water bodies has emerged out to be the main culprit of water pollution. Indian tannery industry is one of the leading tannery industries in the world but with that it also one of highly polluting industry. Tannery effluents lead to both water as well as air pollution. On the basis of volume of tannery effluents and its composition it has been regarded as most polluting among all [2], [4], [13], [15]. The studies have reported that waste water discharged from tannery industry have greater values of BOD, COD, color and pH [14], [17].

The colored components of tannery effluent along with organic load causes ecological imbalance in the receiving water body [9]. Tannery waste water discharges large amount of dye during washing operations [8]. These dyes are mainly xenobiotic and also carcinogenic in some cases [3]. Studies have also reported allergic effect of these dyes [11], [12]. If not

treated properly these dyes persist in the environment Use of synthetic dye is more prevalent than natural dyes as they are cost effective their production is easy, large variety of shades can be produced moreover due to comparative fastness of colour than natural dyes. More than 100,000 dyes are commercially available and about one million ton of these dyes are produced annually worldwide [1]. During industrial process, approximately 10-15% of these dyes are released into the environment [6] due to their chemical nature these dyes pose great threat to environment.

Addition of colour to water is the main indication of the water pollution. Highly coloured synthetic dyes block the entry of sunlight in the receiving water body, hence are fatal for flora and fauna living in it. Moreover dyes and their breakdown product are cytotoxic and carcinogenic [7]. A number of physiochemical methods such as coagulation, filtration, precipitation, oxidation etc. have been used to treat effluent containing synthetic dyes, but these methods have many disadvantages and limitations. It is therefore important to develop efficient and cost effective methods for decolorization and degradation of dyes in tannery effluents.

In situ degradation of dyes by microorganisms is a novel method for treatment of effluent. In this method, the microorganisms isolated from the site of pollution can be used for the treatment of effluent [9]. The present study aims to isolate dye decolorizing microorganisms from the tannery effluents. As the microbial isolates will be isolated from the tannery wastewater, they can easily adopt the prevailing environment. Such microorganisms can be used to develop effective biological treatment system for waste water contaminated with leather dyes. Further present research aims to study the decolorization capacity of such microorganisms by screening them from effluent, isolation and optimization of decolorization conditions (temperature, pH etc.), hence to produce a system to treat leather dyes in an ecofriendly manner with the help of microbes.

MATERIALS AND METHODS:

➤ Chemicals and Media

Dye effluents were collected from a Leather Complex Jalandhar Punjab. All microbiological media and medium ingredients were purchased from HiMedia Laboratories (Mumbai, MH, India).

for a long duration of time.

➤ Isolation dye degrading Bacteria

The Leather Dye containing effluent sample was collected from a Leather industry located at Jalandhar, Punjab. One ml of effluent was transferred into 9 ml of distilled water in sterile test tubes. Serial dilution was done up to 10 by thorough mixing. 0.1 ml of sample from each dilution was spread on Nutrient Agar plates containing with the help of sterile L-rod. The petridishes were incubated at 30⁰ C for 24 hours from these bacterial colonies were isolated by Streak Plate Method.

➤ Preservation and maintenance

Pure bacterial isolates were obtained on the Nutrient agar plates were stored in refrigerator and served as stock cultures. Subcultures were routinely made every 7 to 10 days.

➤ Screening of decolorizing Bacteria

All the isolates were selected for screening of decolorizing activity of dye. Inoculums each isolate were added to 100 ml of Nutrient Broth and were incubated at 30⁰ C for 48 hours. 1% of dye solution was serially diluted from 10⁻¹ to 10⁻⁵. These solutions were inoculated with 1% of nutrient broth and incubated at 30⁰ C for 6 days. After 6 days, effective decolorization was seen visually. Those isolates showing decolorization of textile dye effluent were selected for further studies with decolorization of synthetic dyes methylene blue, crystal violet, eosin yellow, safranin. The bacterial strain found to be potential in dye decolorization was selected and best decolorization results were observed in 10⁻⁴ dilution.

➤ Biochemical characterization

Furthermore the microbial isolate selected on the basis of optimum decolourization activity was characterized biochemically as well as physiologically up to species level.

➤ Decolorization assay

Decolorization activity in terms of percent decolorization was determined by following method described by (Ponraj et.al). 10 ml of sample was centrifuged at 1000 rpm for 10 minutes. Spectrophotometer was used for absorbance measurement. The decrease in absorbance was monitored at 663 nm, 590 nm, 518nm, 530 nm for Methylene Blue, Crystal Violet, Eosin Yellow and Safranin respectively. Decolorization activity was

calculated according to the following formula: $D = \frac{A - F}{A} \times 100$

Where: D, decolorization; A initial absorbance; F final absorbance (ponraj et .al)[10].

➤ Dye Decolorization Optimization

Decolorization of methylene blue, crystal violet, eosin yellow, safranin by selected bacterial isolate was optimized with respect to the effect temperature (4⁰C, 27⁰C, 37⁰C, 60⁰C) and pH(2,4,6,8,10).Dye solution without culture served as control. All the test tubes were incubated at 30 ⁰C under shaking conditions 100 rpm for 6 days.

RESULTS AND DISCUSSION

The complication faced during treatment of water pollution is the degradation of synthetic dyes disposed in the water bodies through industrial effluents. Tannery effluents being most polluting need to be paid most attention. Indian tanning industry is one of the leading tanning industries in the world. The tanning of leather involves use of many toxic chemicals to process and dye the leather. These chemicals cause life threatening problems like cancer, damage to lungs and kidneys etc. to the living being coming in contact with them. So there is an urgent requirement for the treatment of the water polluted by the effluents from various industries specially the dye containing effluent from the leather industry. The method devised has to be eco-friendly and cost effective so as to make it viable on large scale. In the present study microbial decolorization of leather dye effluent was carried out using the bacterial isolate obtained from the leather dye effluent. Leather dye effluent samples were collected from the disposal site of effluent for screening efficient microorganisms, (bacteria).

➤ Screening of dye decolorization Bacteria

The obtained bacterial isolates showed decolorization of dyes after 6 days of incubation at 30⁰C under shaking (100 rpm). In the present investigation the rate of color removed increased with incubation periods.



➤ Bacterial isolated by streak plate method



Decolorization of Crystal Violet dye after 6 days incubation

Biochemical Characterization

Biochemical characterization of the bacterial isolate revealed the following results:-

TEST	RESULT
H2S (TSI)	-
Urease	-
Indole	Variable
Methyl red (37 ⁰ C)	+
Voges–Proskauer(37 ⁰ C)	-
Simmons’ citrate	-
Christensen’s citrate	-
Sodium acetate	-
Mucate	-
KCN	-
Motility	-
Sodium malonate	-
Lysine decarboxylase	-
Arginine dihydrolase	-
Ornithine decarboxylase	-
Phenylalanine deaminase	-
Galactosidase (ONPG)	-
Catalase	+
Oxidase	-
Glucose (acid)	+
Glucose (gas)	-
Lactose	-
Sucrose	-
Mannitol	+
Salicin	-
Adonitol	-
Dulcitol	-
Inositol	-
Arabinose	+
Sorbitol	+
Raffinose	-
Rhamnose	-
Maltose	-
Xylose	+
Trehalose	+

Cellobiose	-
Mannose	+
Glycerol	+
Esculin	-

The potential strain was identified as *Shigella* sp.

➤ **Optimization of dye Decolorization**

For the maximization of decolourization of the dyes Methylene Blue, Crystal Violet, Eosin Yellow, Safranin by the selected bacterial isolates, experiments were conducted for optimization of temperature and Ph.

➤ **Effect of Temperatures**

Different temperatures used were as refrigerator temperature (4°C), room temperature (27°C), incubator temperature (37°C) and extreme temperatures (60 °C). The maximum decolorization was observed 27 C followed by 37 C and 4 C, least decolorization was observed at 60°C as cells does not remain viable at high temperatures(Figure- 1). Similar results were obtained by Ponraj et.al [10] who used by different bacterial strains for studying decolorization.

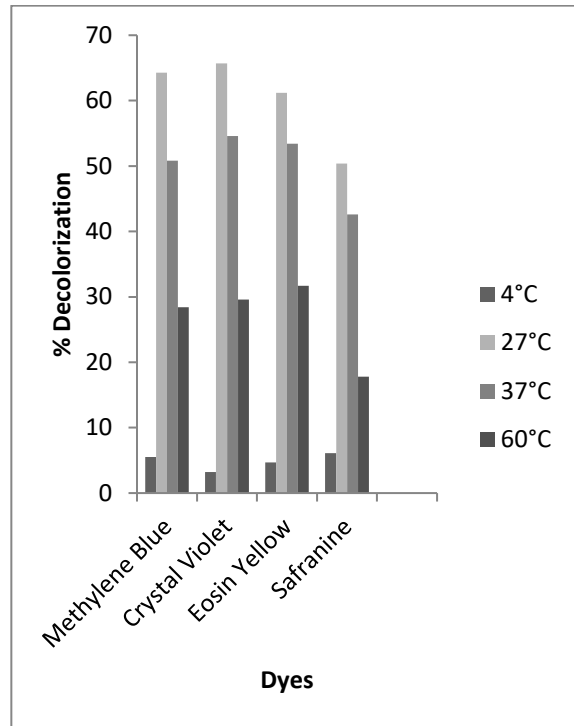


Figure -1 Effect of temperature on decolorization of dyes by *Shigella* Sp.

➤ **Effect of pH**

The five bacterial isolates were grown in medium with different pH (2,4,6,8,10). The pH of the medium was adjusted with HCl and NaOH. The maximum percentage decolorization was observed between pH 6 to 8. (Figure-2) Similar results were seen in studies carried by Wong et.al and Hu et.al [16], [5].

Temperature	% Decolorization			
	Methylene Blue	Crystal Violet	Eosin yellow	Safranin
4°C	5.5	3.2	4.7	6.1
27°C	64.3	65.7	61.2	50.4
37°C	50.8	54.6	53.4	42.6
60°C	28.4	29.6	31.7	17.8

pH	% Decolorization			
	Methylene Blue	Crystal Violet	Eosin yellow	Safranin
2	2.6	3.5	4.9	6.9
4	24.8	25.5	21.6	23.6
6	50.8	54.6	53.4	42.5
8	58.4	59.4	55.7	58.7
10	16.7	17.6	18.7	19

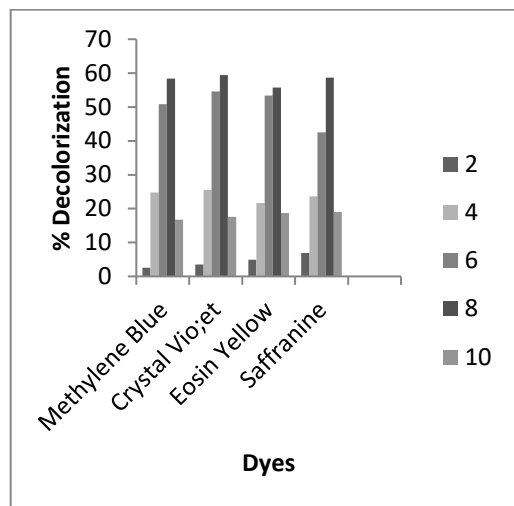


Figure -2 Effect of pH on decolorization dyes by *Shigella Sp.*

CONCLUSION

The present study reveals that all the four dyes methylene blue, crystal violet, eosin yellow, safranin are degradable under aerobic conditions with *Shigella* isolated from textile dye effluent. The bacteria exhibited maximum decolorization ability between pH6-pH8. Whereas temperature did not show considerable effect on decolorization. 27°C-37°C showed best decolorization results based on these results we can carry out further studies and devise an eco friendly cost effective and efficient system for the degradation of leather dyes in the industrial effluents.

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