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MICROBIAL DECOLORIZATION OF TEXTILE DYE EFFLUENT

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ABSTRACT

Synthetic dyes are used in several industries including textile dyeing printing and cosmetic industries. Due to thier complex structure they are resistant to be decomposed by conventional treatment technologies. Hence microbial decolorization of dyes could be a viable option as low cost ecofriendly waste water treatment system from these industries. This study presents microbial decolorization of textile dye effluents from five microbial isolates isolated from five different effluent samples collected from fabric industry. Different parameters such as carbon source, temperature, pH and inoculum size were optimized for decolorization of Methylene Blue, Crystal Violet, EosinYellow, Safranine dyes by using bacterial isolates. Among carbon sources glucose was found to be the best decolorizer, 27°C-30°C of temperature was observed to be optimum for decolorization, the optimum pH range for decolorization was found to be from pH6-pH8 whereas 6% (v/v) of inoculum showed best decolorization results. All the samples were incubated at 30°C 100 rpm. The decolorization was measured as decrease in absorbance maxima at 663 nm,590 nm,518nm, 530 nm for mehtylene blue, crystal violet, eosin yellow, safranine respectively.

INTRODUCTION

Water is life but water pollution now a days is posing a great threat to human survival. Textile and dye stuff industries have the major contribution to this. The effluents from these industries are complex; contain a wide variety of dyes and other products such as dispersants, acids, bases, salts, detergents, humectants, oxidants, etc. Discharge of these colored effluents into rivers and lakes results into reduced dissolved oxygen concentration, thus creating anoxic conditions that are lethal to resident organisms.Many reports indicate that textile dyes and effluents have toxic effect on the germination rates and biomass concentration of several plant species which play many important ecological functions such as providing the habitat for wildlife; protecting soil from erosion and providing bulk of organic matter that is significant to soil fertility. The toxicity of effluent is because of the presence of dye or its degraded products which are mutagenic or carcinogenic(Conneely *et al* 1999;Xu *et a*l.,2005). Therefore, the treatment of industrial effluents contaminated with dye becomes necessary prior to their final discharge to the environment. Various kinds of physico-chemical methods(Anastasios et al 2005;Theodra et al.,2006) are in use for the treatment of wastewater contaminated with dye such as ozonization, photooxidation, electrocoagulation , froth floatation etc but these physiochemical methods are less efficient, costly, of limited applicability and produce waste which are difficult to dispose off(Pak & Chang 1999;Kabita *et al* 2001). Biological methods using various microbes could be a viable option as a low cost and ecofriendly waste water treatment system. This study presents microbial decolorzation of textile dye through bacterial isolates isolated from the effluent samples collected from fabric industry. Different parameters such as carbon source, temperature ,pH and inoculum size were optimized for decolorization of four dyes methylene blue,crystal violet,eosin yellow,safranine

MATERIALS AND METHODS

Chemicals and media

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

Isolation dye degrading Bacteria

The dyeing industry effluent sample was collected from a fabric 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 L-rod. The petridishes were incubated at room temperature for 24 hours.from these bscterial 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 30 to 60 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 30 C for 48 Hours.1% of dye solution was serially diluted from 10^{-1} to 10^{-5}. These solutions were inoculated with 1% of nutient broth and incubated at 30 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, safranine. Five Bacterial strains were found to be potential in dye decolorization and best decolorization results were observed in 10^{-4} dilution.

Decolorization assay

Decolorization activity in terms of percent decolorization was determined by following method described by.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 for methylene blue, 590 nm for Crytal Violet, 518nm for eosin yellow, 530 nm for safranine. Decolorization activity was calculated according to the following formula .

$$D= [A -A)/A] x 100$$

Where, D, decolourization; A , initial absorbance; A , final absorbance

Dye decolorization optimization

Decolorization of methylene blue,crystal violet, eosin yellow,safranine by all five isolates was optimized with respect to the effect of 1%, carbon sources (glucose, lactose, sucrose), Inoculum size(2%,4%,6%,8%,10%) and temperature (4, 27, 37,60 C).Dye solution without culture served as control. All the test tubes were incubated at 30 C under shaking conditions 100 rpm for 6 days.

RESULTS AND DISCUSSION

Decolorization of textile dye effluent is serious environmental problem, which is evident from the magnitude of research done in this field in the last decade. Treatment of textile dye effluent by physical and chemical methods have a high cost potential and a high sludge problem, whereas biological process convert organic compounds completely into water and carbon dioxide, have low cost and are easy to use . In the present study microbial decolourization of textile dye effluent was carried out using the bacterial isolates obtained from the textile dye effluent.Textile dye effluent

samples were collected from the disposal site of effluent for screening efficient microorganisms, (bacteria).

Isolation and Decolorizing Bacteria

Different types of Bacterial isolates were obtained from the textile dye effluent sample.Among them five bacterial isolates were found to have high Decolorization potential and were selected for further studies

Screening of dye decolorization

The obtained bacterial isolates showed decolorization of dyes 6 days of incubation at 30°C under shaking(100 rpm). Only the rate of decolorization of dye and final percent color removal varied for each isolates. In the present investigation the rate of color removed increased with incubation periods.

Optimization of dye decolorization

For the maximization of decolourization of the dyes methylene blue,crystal violet,eosin yellow,safranine by the selected bacterial isolates, experiments were conducted for optimization of carbon source, inoculum size and temperature.

Effect of carbon sources

All the bacterial isolates showed higher percent decolorization than control showing that all the three sugars could be utilized effectively as carbon source by these isolates. The highest decolorization was observed in glucose among the carbon sources in all the three dyes as it is simplest sugar can be easily assimilated by bacteria .Nosheen *et al* used glucose and starch as carbon sources for optimizing the maximum decolorization of azo dyes Reactive Black B and Reactive Orange 16. Also Parshetti *et a*l used molasses and sucrose as carbon sources for decolorization of Malachite Green (91%) using MTCC 1532.

Methylene Blue

Carbon source	% Decolorization				
	S1	**S2**	**S3**	**S4**	**S5**
Glucose	78	44	86	67	46
Lactose	20.6	45	37.4	60.6	56
Sucrose	68	37	46.4	61.7	41.2

Effect of carbon source on decolorzation of Methylene Blue dye by bacterial isolates

Crystal Violet					
Carbon source	**% Decolorization**				
	S1	**S2**	**S3**	**S4**	**S5**
Glucose	65.9	42.8	51.3	61.7	47.3
Lactose	34.2	37.7	47.2	24.2	21.6
Sucrose	42.5	30	24.8	55.2	33.3

Effect of carbon source on decolorzation of Crystal Violet dye by bacterial isolates

Eosin Yellow					
Carbon source	**% Decolorization**				
	S1	**S2**	**S3**	**S4**	**S5**
Glucose	56.5	26.7	48.8	36.6	35.2
Lactose	47.6	23.9	43.6	31.7	29.1
Sucrose	41.3	25.6	40.7	24.8	31.3

Effect of carbon source on decolorzation of Eosin Yellow dye by bacterial isolates

Safranine					
Carbon source	**% Decolorization**				
	S1	**S2**	**S3**	**S4**	**S5**
Glucose	51.9	32	24.3	18.6	15.2
Lactose	42.3	24	18.6	15.1	12.6
Sucrose	39.8	21.3	15.2	12.6	11.2

Effect of carbon source on decolorzation of Safranine dye by bacterial isolate

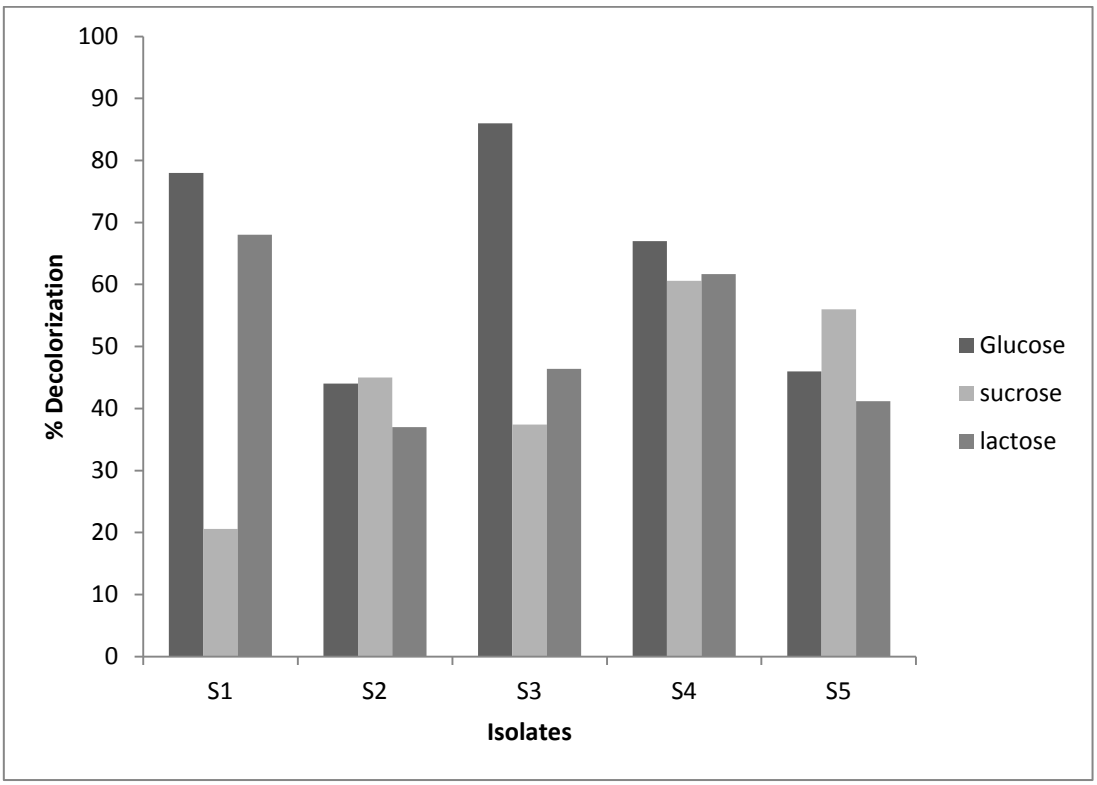

Figure -1 Effect of carbon source on decolorzation of Methylene Blue dye by bacterial isolates

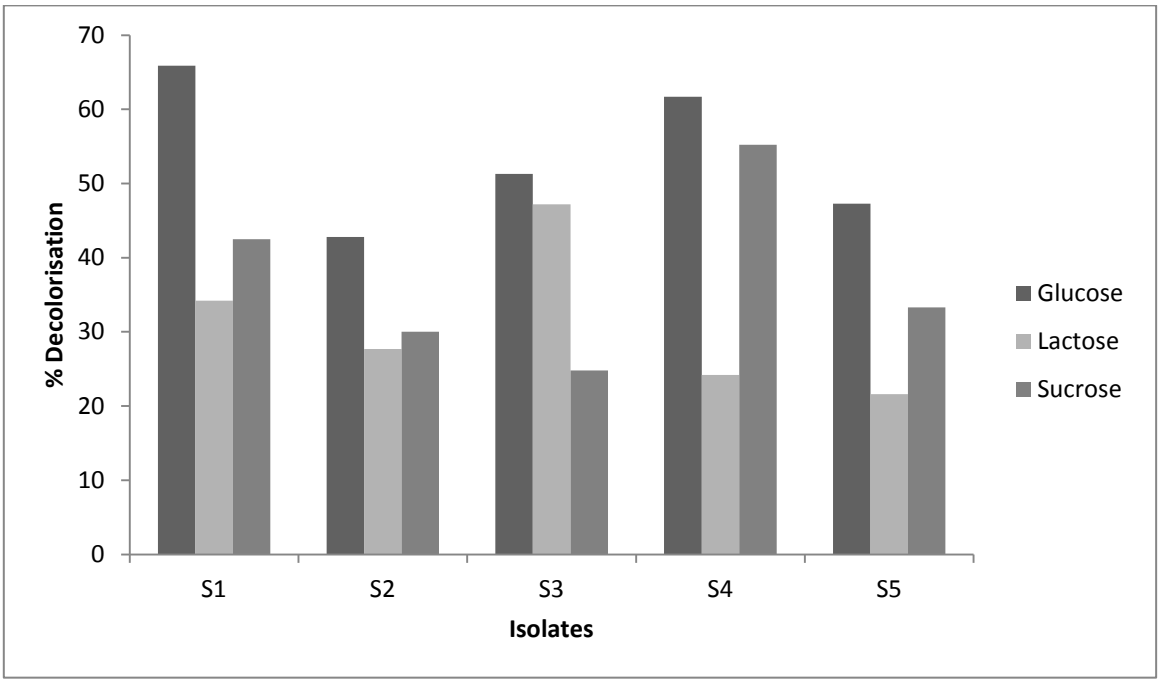

Figure -2 Effect of Carbon Source on Decolorization of Crystal Violet Dye by bacterial Isolates

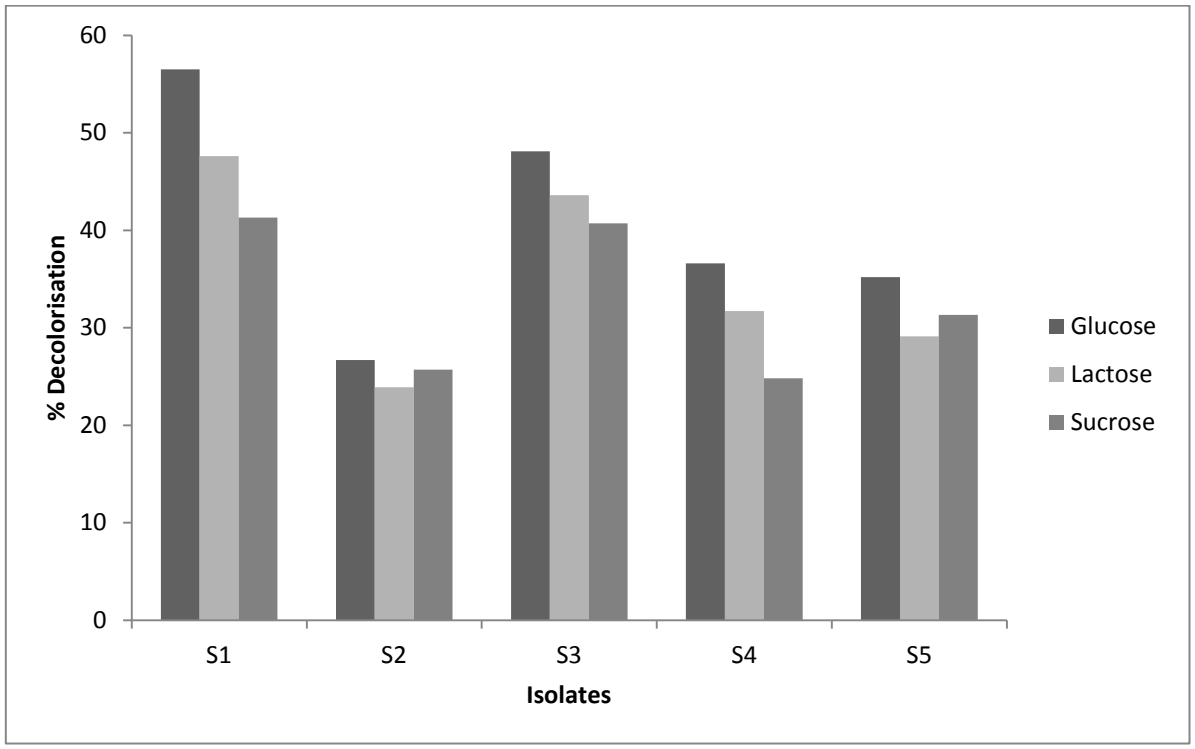

Figure -3 Effect of Carbon Source on Decolorization of Eosin Yellow Dye by bacterial Isolates

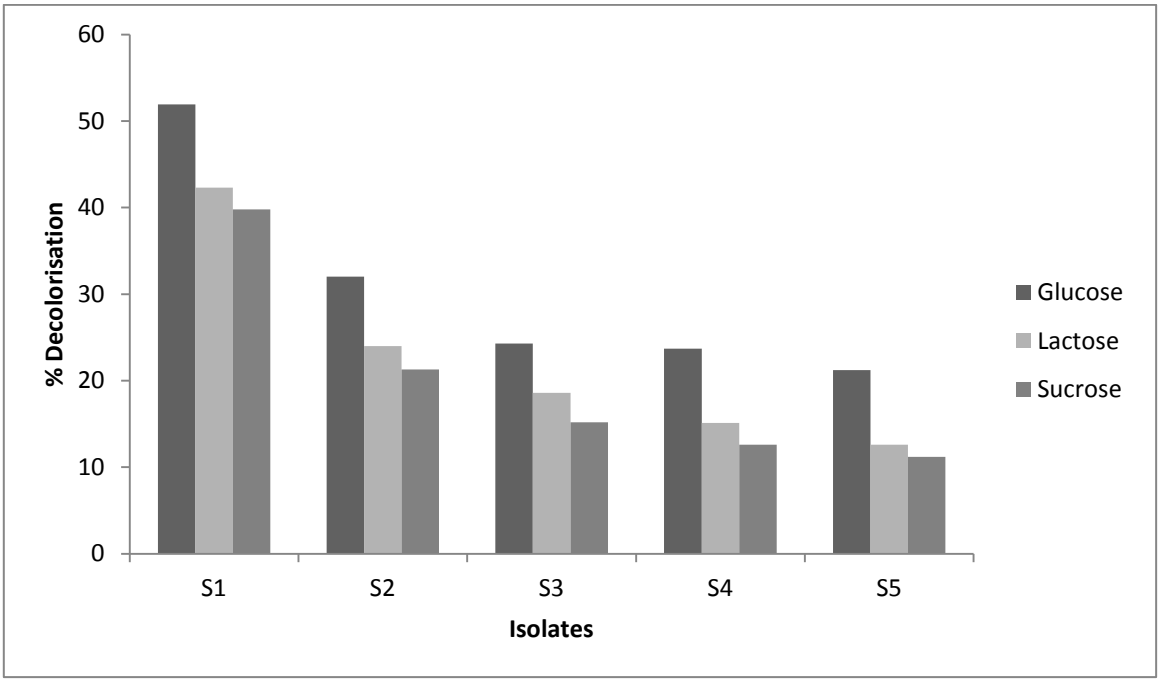

Figure -4 Effect of Carbon Source on Decolorization of Safranine Dye by bacterial Isolates

Effect of carbon source(Glucose, Lactose,Sucrose respectively) on decolorization of Methylene Blue dye

Effect of carbon source(Glucose, Lactose,Sucrose respectively) on decolorization of Crystal Violet dye

Effect of carbon source(Glucose, Lactose,Sucrose respectively) on decolorization of Eosin Yellow dye

Effect of carbon source(Glucose, Lactose,Sucrose respectively) on decolorization of Safranine dye

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 decolorizaton was observed at 60°C as cells does not remain viable at high temperatures(Fig. 4). Similar results were obtained by M.Ponraj et al who used by different bacterial strains for studying decolorization.

Methylene Blue

Temperature	% Decolorization				
	S1	**S2**	**S3**	**S4**	**S5**
4°C	58.5	61.2	53.6	46	37
27°C	59.3	64.7	59	49	40.2
37°C	56.2	59.6	52	44.2	36.4
60°C	29.3	32.6	35	26	17

Effect of temperature on decolorization of Methylene Blue dye by bacterial isolates

Crystal Violet

Temperature	% Decolorization				
	S1	**S2**	**S3**	**S4**	**S5**
4°C	44.6	21.9	37.6	28.4	39.3
27°C	49.6	27.4	41.2	33.6	47.7
37°C	47.4	25.3	43.6	26.6	42.1
60°C	29.6	18.1	25.1	22.6	28.3

Effect of temperature on decolorization of Crystal Violet dye by bacterial isolates

Eosin Yellow					
Temperature	**% Decolorization**				
	S1	**S2**	**S3**	**S4**	**S5**
4°C	42.6	26.6	41.2	33.9	31
27°C	49.6	30.5	45.2	37.6	29.2
37°C	44.1	29.3	39.6	35.1	27
60°C	24.6	19.1	27.2	22.7	18.1

Effect of temperature on decolorization of Eosin Yellow dye by bacterial isolates

Safranine					
Temperature	**% Decolorization**				
	S1	**S2**	**S3**	**S4**	**S5**
4°C	41.2	20.6	35.2	29.5	34.1
27°C	42.3	26.5	39.6	31.8	41.8
37°C	39.8	21.2	37.8	32.5	21.2
60°C	24.6	19.8	24.3	21.2	25.3

Effect of temperature on decolorization of Safranine dye by bacterial isolates

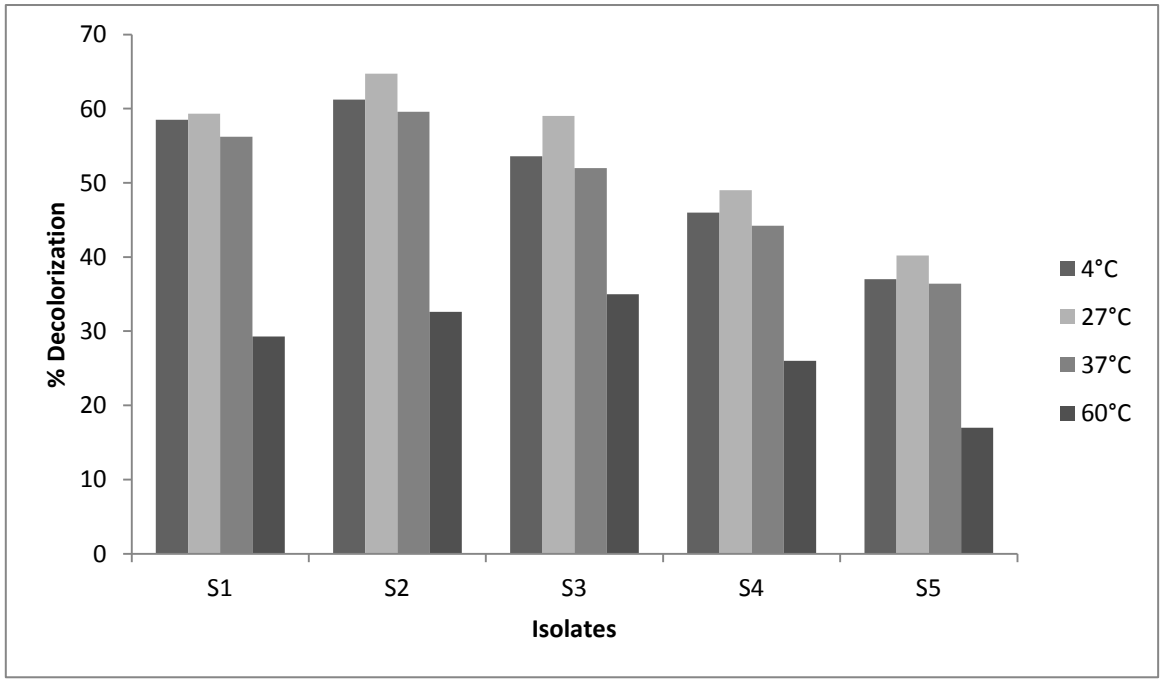

Figure -5 Effect of temperature on decolorization of Methylene Blue dye by bacterial isolates

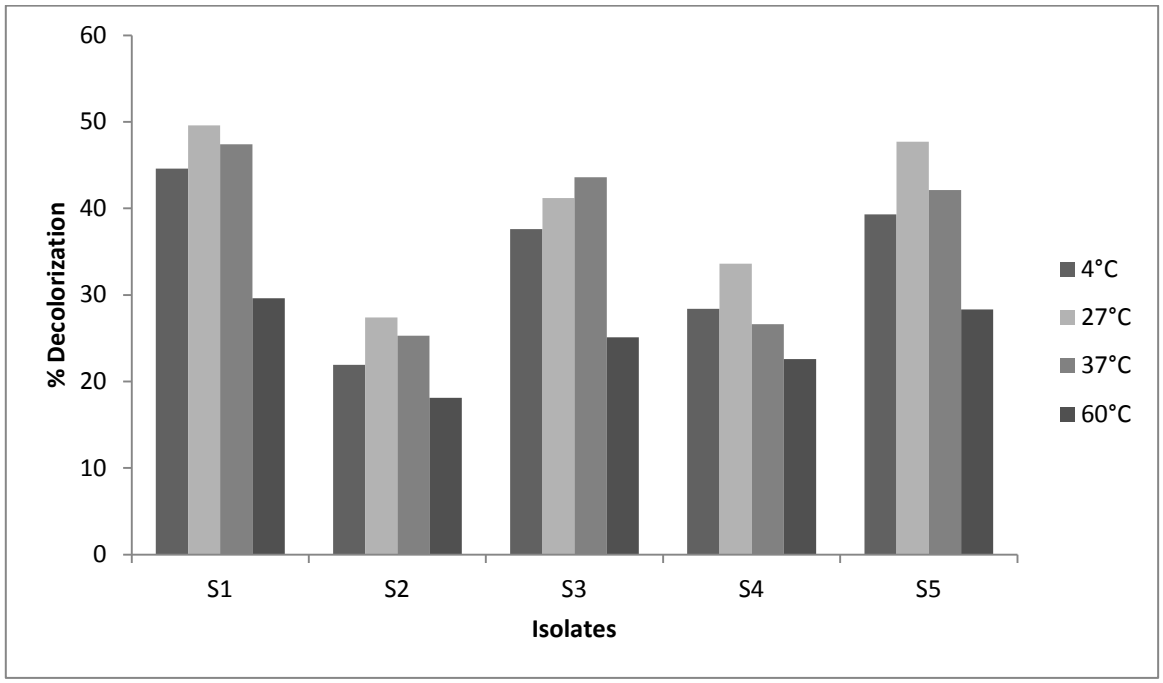

Figure -6 Effect of temperature on decolorization of Crystal Violet dye by bacterial isolates

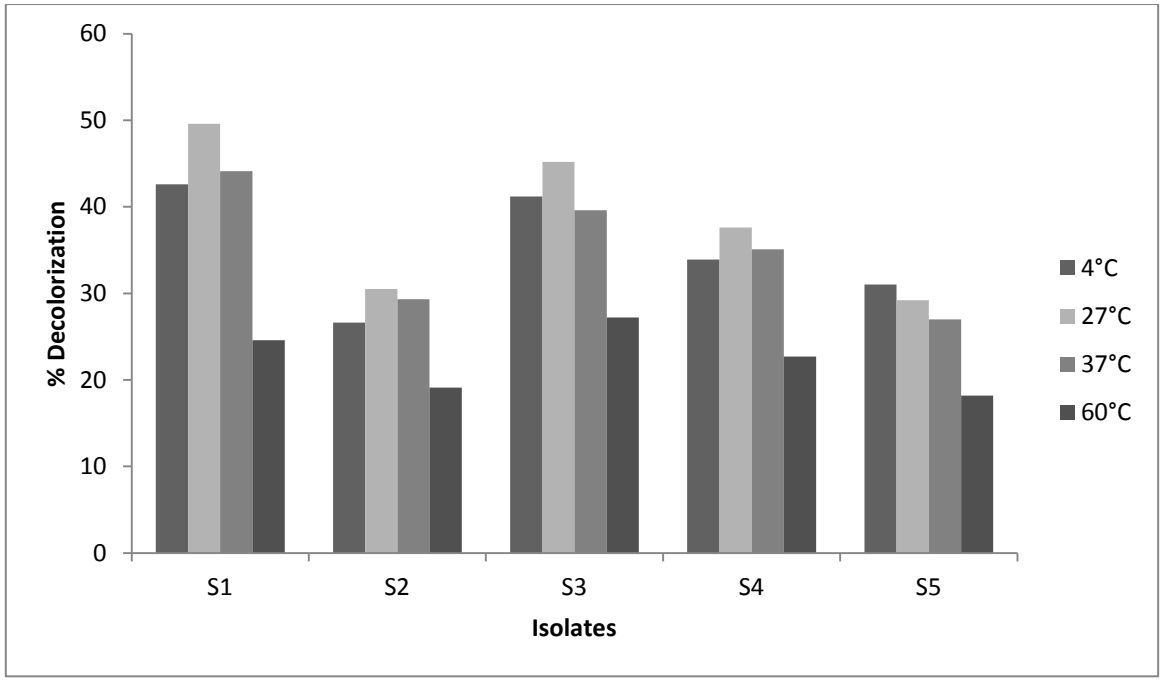

Figure -7 Effect of temperature on decolorization of Eosin yellow dye by bacterial isolates

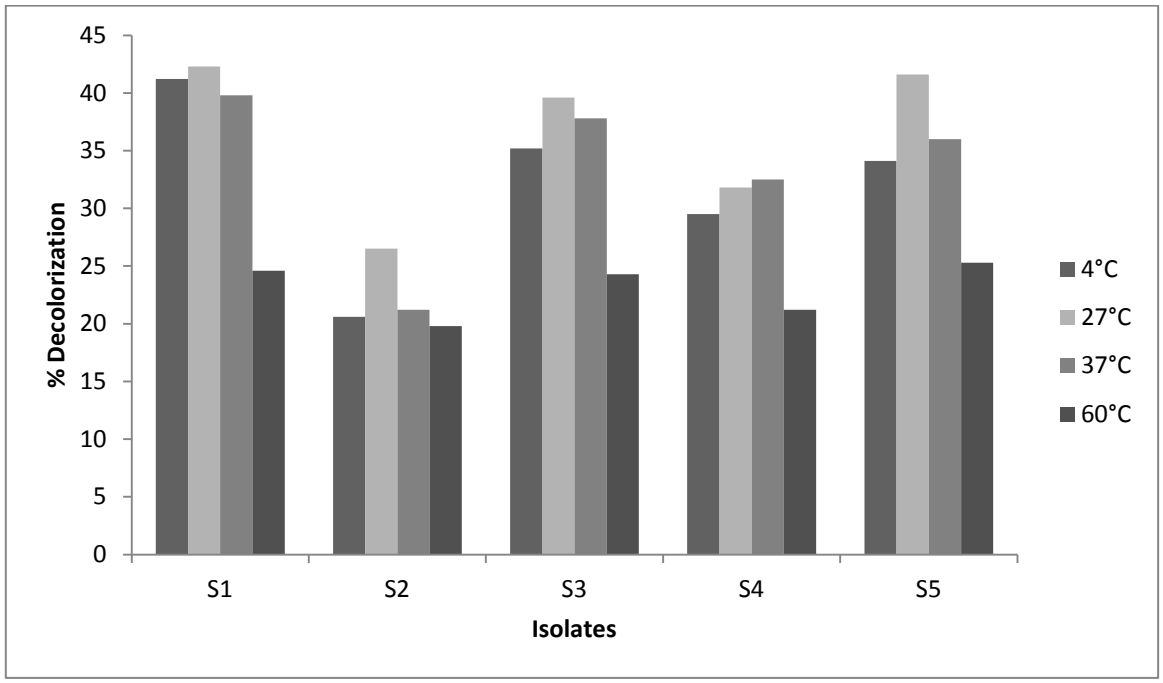

Figure -8 Effect of temperature on decolorization of safranine dye by bacterial isolates

Effect of Inoculum Size

The 2%, 4%, 6%, 8% and 10% of inoculums were used for the five bacterial isolates to degrade the dyes solution (Fig. 9,10,11,12). Decolorization activity of bacterial isolates was found to be best in 6% of inoculums. M.Ponraj. *et al* used four different bacterial isolates for studying the effect of Inoculum size on decolorization reported high decolorization activity of *Klebsiella* sp. (67.19%) and *Salmonella* sp (53.91%) in 6% of inoculums.

Methylene Blue					
Inoculum Size	**% Decolorization**				
	S1	**S2**	**S3**	**S4**	**S5**
2%	24	46.7	17	18.6	21
4%	20.3	5	12	13.1	23
6%	32	9	21	26.6	38.6
8%	22.3	14	19.1	8.5	23.2
10%	15	34	17.4	23.4	16

Effect of inoculum size on decolorzation of Methylene Blue dye by bacterial isolates

Crystal Violet					
Inoculum Size	**% Decolorization**				
	S1	**S2**	**S3**	**S4**	**S5**
2%	48.7	46.6	35.2	31.4	43.2
4%	51.2	41.1	46.1	18.2	21
6%	57.4	51.3	48.3	3737.2	44.2
8%	32.4	26.7	31.2	31.6	28.4
10%	19.5	18.6	29	28.8	23.6

Effect of inoculum size on decolorzation of Crystal Violet dye by bacterial isolates

Eosin Yellow					
Inoculum Size	**% Decolorization**				
	S1	**S2**	**S3**	**S4**	**S5**
2%	45.7	47	43.2	35.4	41.3
4%	49.8	45.1	27	66.5	33.1
6%	52.4	49.3	48	39.2	43.2
8%	34.4	27.6	23.7	21.7	38.7
10%	19.5	18.6	29	28.8	23.6

Effect of inoculum size on decolorzation of Eosin Yellow dye by bacterial isolates

Safranine					
Inoculum Size	**% Decolorization**				
	S1	**S2**	**S3**	**S4**	**S5**
2%	40.7	45	31.4	20.6	18.3
4%	30.3	26.6	23.03	14	16.7
6%	43.5	36.7	44.8	37.2	27.6
8%	27.6	17.2	19.1	30	20.6
10%	22.4	20.6	19.8	15.2	17.1

Effect of inoculum size on decolorzation of Safranine dye by bacterial isolates

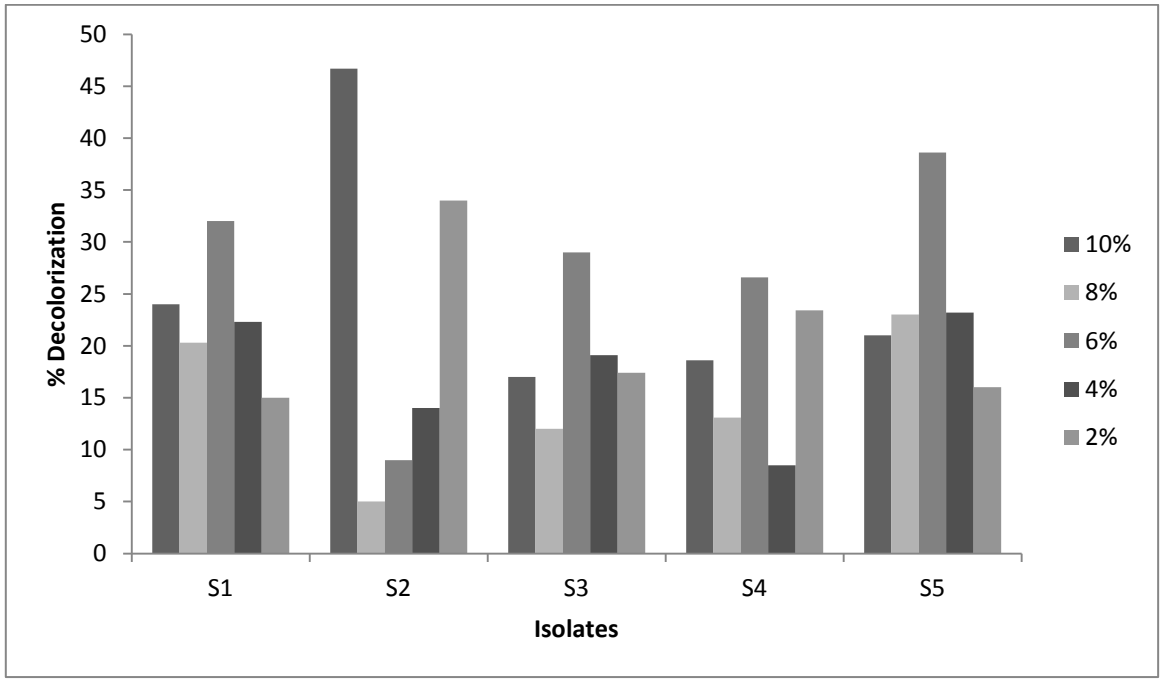

Figure -9 Effect of inoculum size on decolorzation of Methylene Blue dye by bacterial isolates

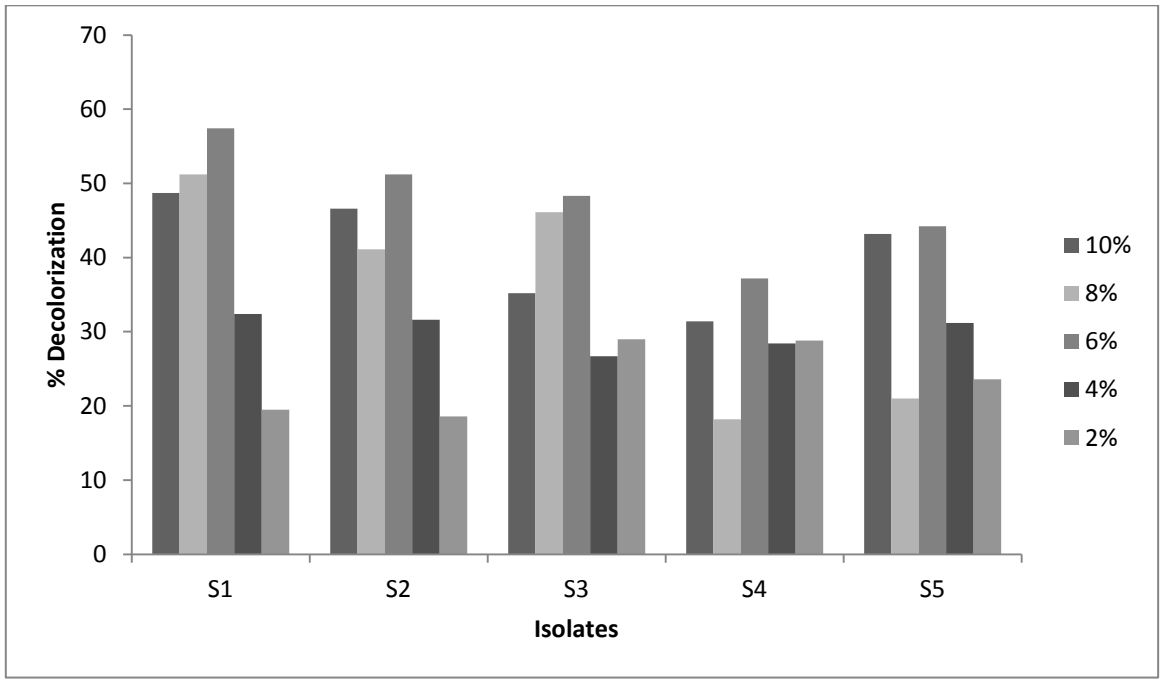

Figure- 10 Effect of inoculum size on decolorzation of Crystal Violet dye by bacterial isolates

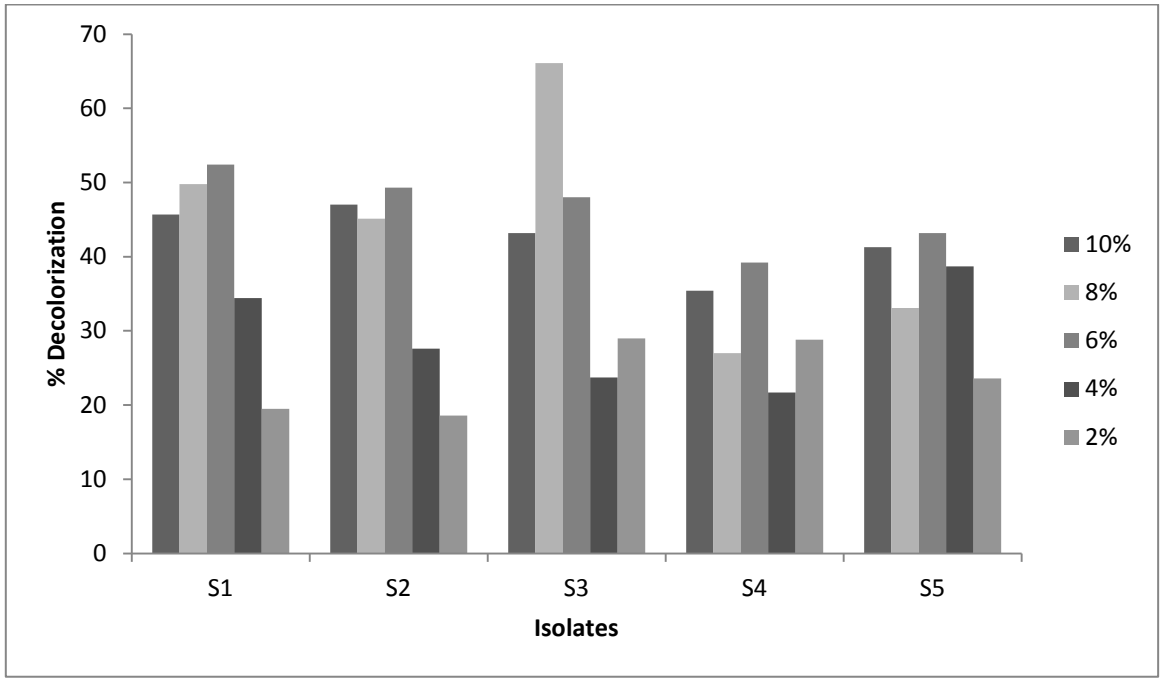

Figure -11 Effect of inoculum size on decolorzation of Eosin Yellow dye by bacterial isolates

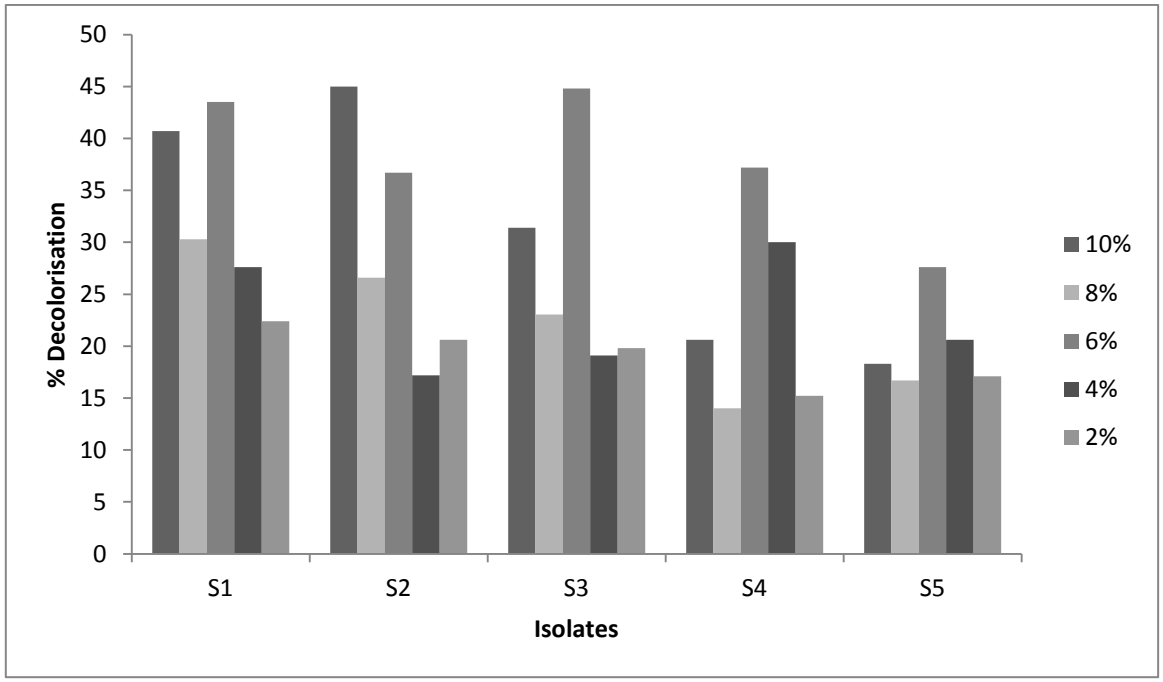

Figure- 12 Effect of Inoculum size on Decolorization of Safranine dye by Bacterial Isolates

CONCLUSION

The present study reveals that all the four dyes methylene blue, crystal violet, eosin yellow, safranine are degradable under aerobic conditions with concerted effort of bacteria isolated from textile dye effluent. The bacterial isolates exhibhited maximum decolorization ability between pH6-pH8. Among the carbon sources isolated bacteria inoculted in 1% glucose exhibited better decolorization. 6% (v/v) inoculum showed better decolorization ability. Whereas temperature did not showed considerable effect on on decolorization 27°C-37°C showed best decolorization results. On the basis of the results of present study suitable strategy can be developed for the treatment of waste water contaminated with dye.

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