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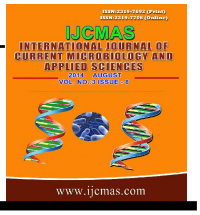


Original Research Article

Microbial Decolourization of Dye Reactive Blue 19 by Bacteria Isolated from Dye Effluent Contaminated Soil

Deepti Gulati* and Isha Jha

Department of Biotechnology, Dolphin (PG) Institute of Biomedical & Natural Sciences, Dehra Dun-248007, India
**Corresponding author*

Keywords

Decolourizing efficiency, *Enterococcus sp*., *Klebsiella sp.*, Optimization, Reactive Blue 19 dye

A B S T R A C T

The present study was carried out to decolourize the textile Reactive Blue 19 dye, by bacteria isolated from dye contaminated soil obtained from Paltan Bazar, Dehra Dun. Sixteen bacterial strains were isolated by serial dilution, spread plate techniques and streaking; out of which fifteen were capable to decolourize Reactive Blue 19, tested with decolourization efficiency varying in the range of 55 to 95%. Two isolates were selected on the basis of decolourizing efficiency and were characterized further by staining and different biochemical tests. The strains were identified to be *Enterococcus sp. and Klebsiella sp.* For maximum decolourization, the cultures were optimized on different parameters such as incubation time, dye concentration, temperature and pH. It was investigated that the decolourization activity was shown during 24 to 96 hours of incubation with optimum decolourization at 72 hours for both the isolates. The decolorizing activity was seen with increasing dye concentration from 20 to 900mgl^{-1} for *Enterococcus sp.* and 20 to 600mgl^{-1} for *Klebsiella sp*. in Reactive Blue 19 dye, with optimum activity at 100mgl^{-1} for both the isolates. The isolates were able to decolourize the dye at varying pH, ranging from 5-11 with optimum pH 7 for both isolates. Similarly the decolourizing activity was observed at various temperatures ranging from 30^{O}C to 70 ^{O}C with optimum temperature 37^{O}C for both isolates.

Introduction

Environmental pollution has been recognized as one of the major hazard of the modern world. Due to rapid industrialization, lots of chemicals including dyes are manufactured and used in day to day life (Moosvi, Haresh and Madamwar, 2005). Dyes usually have a synthetic origin and complex aromatic molecular structures which make them more stable and more difficult to biodegrade (Aksu and Tezer, 2005). The three most common groups of dyes are azo, anthraquinone and phthalocyanine (Alexander and Lustigman, 1996), most of which are toxic and carcinogenic. In present days, reactive dyes are the most widely used dyes because of

their broad variety of colour shades, brilliant colour, ease of application, minimal energy consumption etc. Within the manufacturing of various synthetic dyes many potentially hazardous organic compounds have been introduced into various components of the environment. This process is going on till date with exponential increase. Several chemical and physical decolourization methods such as adsorption, precipitation, coagulation/flocculation, oxidation, electrolysis and membrane extraction are available. These conventional methods have their own disadvantages at large scale (Keharia and Madamwar, 2003). These techniques are effective for colour removal but are energy intensive and introduce chemicals which are not wanted in the first place. They also concentrate the pollutants into solid or liquid side streams which require additional treatment or disposal thus escalating cost of effluent treatment (Sieminatycki et al., 2000). In the past few decades, there is an immense effort to develop a cost effective and eco-friendly alternative to conventional waste treatment methods. Therefore, bioremediation has emerged as the most desirable approach to clean up the environment and to restore its original status. Bioremediation is the use of biological systems (mainly microorganisms and plants) for the treatment of polluted air, aquatic or terrestrial component of environment. Microorganisms can breakdown most compounds for their growth and/or energy needs. In some cases, metabolic pathways, which organisms follow for their own normal growth and development, may also be used to break down pollutant molecules. Therefore, the overall aim of this research was to employ microorganisms to degrade dyes released from the textile processes, in a cost efficient and environmentally safe manner. The main components of the study were isolation, screening and selection of bacterial isolates capable of decolourizing reactive blue 19 dye, characterization of the isolates and optimization of process parameters for maximum decolourization.

Materials and Methods

Selection of dye

Reactive Blue 19 dye was used for the decolourization studies.

Sampling

All lab ware and sampling apparatus were pre-soaked in distilled water for a day prior to experiment. Four soil samples were collected from dye contaminated area from four different dye shops at Paltan Bazar, Dehradun, in sterile plastic bags and named as S1, S2, S3 and S4. All the soil samples were from the surface (0-15 cm depth). The samples were transferred immediately to the laboratory for further analysis. These plastic bags were maintained at $4^{o}C$ to ensure minimal biological activity. To provide homogenized soil samples the soil was thoroughly mixed.

Isolation of bacteria

Soil suspension was prepared by mixing 5gm of soil sample in 45ml distilled water. It was then stirred and allowed to settle. This allowed the microorganisms to come in water phase. The suspension was then serially diluted from 10^{-1} to 10^{-5}. Undiluted suspension and dilutions were used for isolation of microorganisms. Undiluted suspension and dilutions, each 100µl were spread on the surface of nutrient agar plates and incubated for 24hrs at $37^{\circ}C \pm 1°C$. The colonies so obtained on the plates were marked and numbered. They were then streaked on their respective medium from which they were isolated. Bacterial pure

cultures were maintained on NAM slants and stored at 4˚C and sub cultured every month.

Screening for dye decolourization ability

Liquid culture assay

For liquid culture dye decolourizing experiment, sterile Nutrient Broth was supplemented with 100mg/l dye. 250 ml conical flask containing 100ml media was inoculated with the bacterial cultures and incubated for 72hrs at 37^{o}C.

Spectrophotometric estimation of dye decolourization

Extent of dye decolourization was assessed by spectrophotometer at the absorbance maxima of 590nm at time interval of 24 hrs for 3 days. The rate of decolourization was calculated using the following formula as described by Sani and Banarjee, 1999:

$$\%Decolorization = \frac{Initial O.D. \;\; Final O.D. \;\; 100}{Initial O.D.}$$

The bacterial isolates showing maximum decolourization were selected and characterized.

Characterization of bacterial isolates

Isolated pure strains were identified on the basis of morphological and physiological characteristics in nutrient agar plates, slants and broth and by biochemical tests. Colony size, Margins, Forms, Texture, Elevation and Colour was studied. Simple and Gram staining was carried out. Catalase test, citrate utilization test, indole production test, methyl red-Voges Proskeur (MR-VP) test, Nitrate Reduction test, H_2S production test, oxidase test and urease tests were carried out for the identification.

Optimization of culture conditions

The reactive blue 19 dye decolourization using the isolated test organisms was optimized under different conditions and parameters. Media was prepared with different concentrations of dye at varying pH and temperature and extent of decolourization was assessed spectrophotometrically for the best dye degraders.

Effect of incubation period on dye decolourization

Nutrient media with dye concentration 100mg/l was taken in conical flasks and inoculated with the test organisms. The cultures was incubated for 96 hours at 37^{o}C. The decolourization was measured spectrophotometrically at varying time intervals.

Effect of dye concentration on decolourization

The decolourization was studied at different concentrations of the dye (20mg/l to 1500mg/l). Culture media was withdrawn at different time intervals. Aliquot was centrifuged at 10000rpm for 5 minutes to separate the bacterial cell mass and clear supernatant was used to measure the decolourization at the absorbance maxima of 590nm. Controls were carried out in the same conditions but without inoculum.

Effect of pH on dye decolourization

Nutrient media prepared at different pH (5, 6, 7, 8, 9, 10 and 11) was taken in conical flask with each dye concentration 100mg/l and inoculated with seed culture. The result of decolourization was observed spectrophotometrically after 72 hours.

Effect of temperature on dye decolourization

Nutrient media was prepared at optimum pH, supplemented with dye (100mg/l), and inoculated. The cultures at optimum pH were incubated for 72 hours at different temperatures ranging from 30^{o}C to 100^{o}C. The result of decolourization was observed spectrophotometrically.

Results and Discussion

Isolation of bacterial strains

A total of 16 bacterial strains were isolated from four soil samples obtained from different dye shops at Dehra Dun. The samples were serially diluted and spread on Nutrient Agar plates. Isolates
which showed different morphology on plates were purified and preserved as shown in figure 1.

Screening

Liquid culture assay technique was used to screen for the dye degrading bacteria. OD at 590nm was measured at 24 hrs interval and percentage of decolourization was calculated. 15 isolates were capable of decolourizing the dye, but isolates BI-10 and BI-11 showed best results and therefore were selected for further experiments. Result obtained are presented in table 1 and figure 2 (a & b).

Identification of the bacterial isolates

Isolated pure strains were identified by Bergey's Manual of Determinative Bacteriology, on the basis of morphological and physiological characteristics. The results obtained are shown in table 2.

Optimization of culture conditions

Reactive Blue 19 dye degradation using the isolated test organisms was optimized under different conditions and parameters as given below.

Effect of incubation period on dye decolourization

The decolourization efficiency of BI-10 and BI-11 was studied by measuring the optical density at 590nm after 4, 24, 32, 40, 48, 56, 64, 72, 80, 88 and 96 hours of incubation and the results were as shown in table 3 and figure 3. It was noticed that there was a decrease in the optical density in two species as the incubation period increased. BI-10 gave maximum decolourization of 93% and BI-11 gave maximum decolourization of 95% at 72 hours of incubation.

Effect of dye concentration on decolourization

The decolourization efficiency of BI-10 and BI-11 was studied by measuring the optical density at 590nm at different concentrations of Reactive Blue 19 dye (20mg/l to 1500 mg/l). The result obtained are depicted in table 4 and figure 4.

The decolorizing activity was seen with increasing dye concentration from 20 to 900 mgl^{-1} for *Enterococcus sp.* and 20 to 600 mgl^{-1} for *Klebsiella sp*. in Reactive Blue 19 dye, with optimum activity at 100 mgl^{-1} for both the isolates.

Effect of pH on dye decolourization

The decolourization efficiency of BI-10 and BI-11was studied by measuring the optical density at 590nm at different pH (5, 6, 7, 8, 9, 10, 11) and the results were as shown in table 5 and figure 5. BI-10 and BI-11 showed maximum decolourization at pH 7 after 72 hours of incubation.

Effect of temperature on dye decolourization

The decolourization efficiency of BI-10 and BI-11was studied by measuring the optical density at 590nm at different at temperatures ranging from 30 to 100^{O}C. The result obtained is shown in table 6 and figure 6. BI-10 and BI-11 gave maximum decolourization at 37^{o}C after 72 hours of incubation.

Khadijah, Lee and Abdullah, 2008 studied nine out of 1540 isolates and found the consortia to be able to degrade 70-100% colour within 72 hours compared to 60-97% colour removed by individual isolates. A microbial consortium labelled C15 showed good growth in agitation culture but the colour removal was best in static culture with 80-100% colour removed in less than 72 hours. Pourbabaee, Malekzadeh, Sarbolouki and Najafi (2005) reported maximum of 90% decolourization by a *Bacillus sps*. Majority of the azo dye reducing species of *Bacillus* and *Pseudomonas* reported (Kalme, Parshetti, Jadhav and Govindwar, 2007; Chang, Chou, Lin, Ho and Hu, 2001; Suzuki, Yoda, Ruhul and Suguira, 2001) so far are able to reduce the dye at pH near neutrality. It has been reported by Yu, Wang and Yue, 2001 that in microbial physiology, temperature changes lead to a sudden alteration of the activation energy. The effects of temperature on the growth rate, biomass yield and reaction mechanism have also been reported Blaga, Tatyana, Lilyana and Sava, 2008.

Table.1 % Decolourization of Reactive Blue 19 Dye by Bacterial Isolates at 24 hr. Interval.

Acc. No.	Reactive Blue 19 Dye (% decolourization)		
	24	**48**	**72**
BI-01	36	57	81
BI-02	27	42	80
BI-03	32	56	66
BI-04	29	50	70
BI-05	27	46	65
BI-06	25	59	86
BI-07	21	52	57
BI-08	26	76	80
BI-09	26	52	55
BI-10	40	69	93
BI-11	22	54	95
BI-12	07	09	09
BI-13	38	69	75
BI-14	17	38	59
BI-15	28	49	64
BI-16	17	29	69

Table.2 Morphological and Physiological Characteristics of the Bacterial Isolates.

Characteristics	***Enterocoocus sp. (BI-10)***	***Klebsiella sp. (BI-11)***
Gram Reaction	+	-
Morphology	Coccus	Rod
Arrangement	Pairs	Single
Motility	-	-
Oxidase	-	-
Indole	-	-
MR	-	[-]
VP	+	-
Citrate	[-]	+
Urease	-	+
H_2S	-	-
Nitrate Reduction	-	+
Catalase	+	+
Starch	+	+
Glucose	+	+
Lactose	+	+
Sucrose	+	+

Table.3 Effect of Incubation Period on % Decolourization of Reactive Blue 19 Dye.

Hours	**% Decolourization of Reactive Blue 19 Dye**	
	BI-10 (*Enterocoocus sp.*)	**BI-11 (*Klebsiella sp.)***
4	1	1.5
24	40	22
32	49	23
40	62	38
48	69	54
56	78	59
64	82	70
72	93	95
80	92	95
88	92	94
96	92	94

Table.4 Effect of Dye Concentration on % Decolourization of Reactive Blue 19 Dye after 72 hrs.

Dye conc in mg/l	**% Decolourization of Reactive Blue 19 dye**	
	BI-10 (*Enterocoocus sp.*)	**BI-11 (*Klebsiella sp.)***
20	92	94
50	93	94
100	93	95
200	84	57
400	83	49
600	72	21
800	68	9
900	20	6
1000	8	2
1500	7	2

Table.5 Effect of pH on % Decolourization of Reactive Blue 19 Dye after 72 hrs.

pH Values	**% Decolourization of Reactive Blue 19 Dye**	
	BI-10 (*Enterocoocus sp.*)	**BI-11 (*Klebsiella sp.)***
5	20	23
6	40	49
7	90	93
8	73	37
9	71	22
10	65	22
11	50	25

Table.6 Effect of Temperature on % Decolourization of Reactive Blue 19 Dye after 72 hrs.

Temperature (^{o}C)	% Decolourization of Reactive Blue 19 Dye	
	BI-10 (*Enterocoocus sp.*)	**BI-11 (*Klebsiella sp.)***
30	80	73
40	88	86
50	37	56
60	16	12
70	12	4
80	6	4
90	3	3
100	2	3

Fig.1 Effect of Incubation Period on % Decolourization of Reactive Blue 19 Dye.

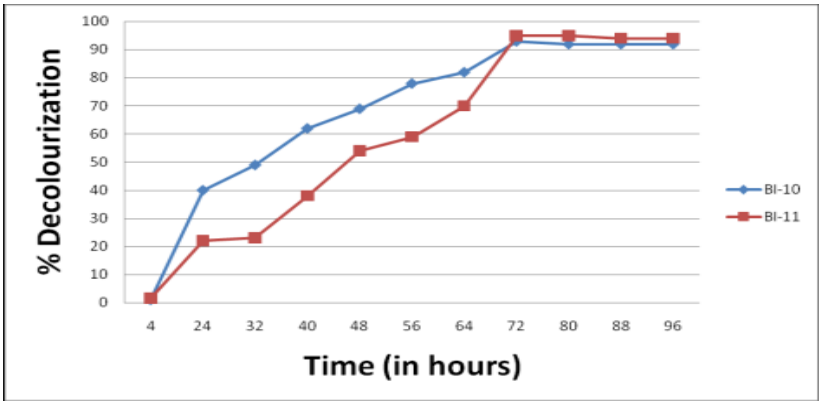


Fig.2 Effect of Dye Concentration on % Decolourization of Reactive Blue 19 Dye after 72 hrs

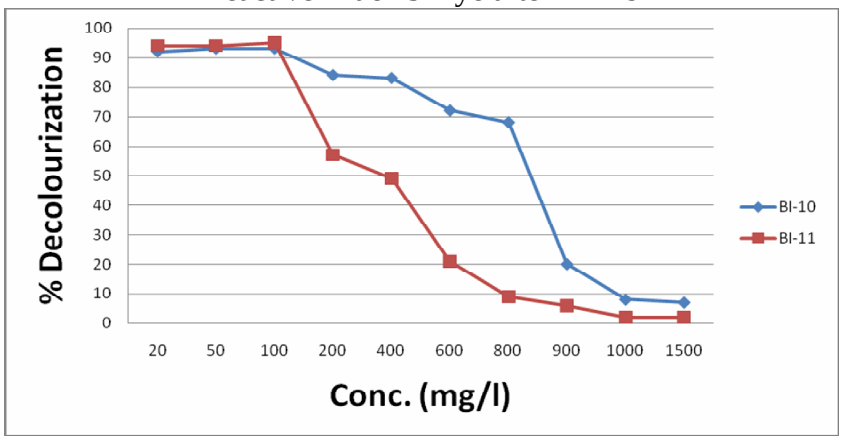


Fig.3 Effect of pH on % Decolourization of Reactive Blue 19 Dye after 72 hrs

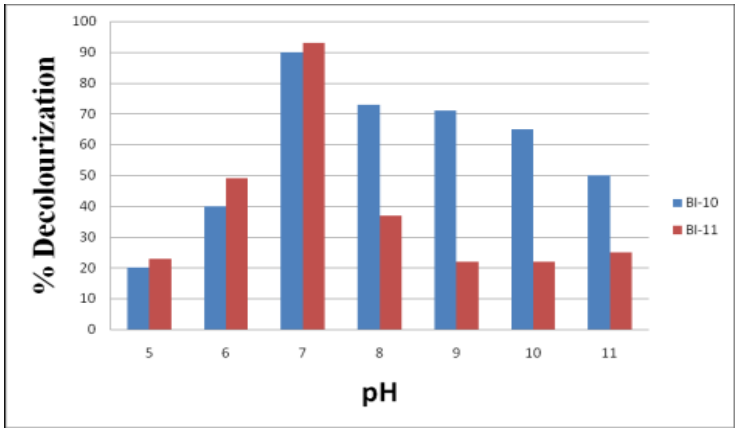
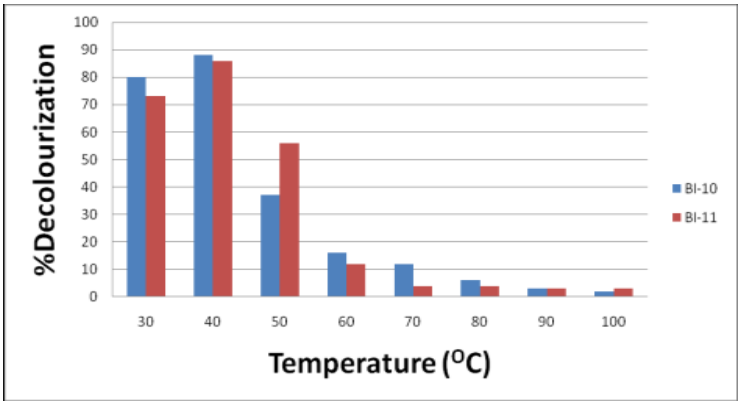


Fig.4 Effect of Temperature on % Decolourization of Reactive Blue 19 Dye after 72 hrs.



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