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BACTERIAL DEGRADATION OF TEXTILE DYES BY *BACILLUS SPECIES*

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ABSTRACT

Rapid industrialization has necessitated the manufacture and use of different chemicals in day to day life. Water is life but now a day due to advantagement in industrialization, it is spoiling a lot. The dyes were collected from Tirupur textile industry, Tamil Nadu. Effect of physical and chemical parameters were analysed for effective degradation of the dye. *B.subtilis* was showed maximum activity at the pH (7), temperature (37^{o}C) and chemical source such as carbon source (sucrose) and nitrogen source (peptone) of dye degradation. The sample were collected from dye effluent contaminated soil of Tirupur textile industry, Tamil Nadu. The serial dilution method was followed for the isolation of bacteria. The isolated bacteria were identified as *Bacillus subtilis* and *Bacillus cereus* by cultural, Gram staining and biochemical characteristics. The identified bacteria were used for decolorization of dyes by broth method. *Bacillus subtilis* showed maximum decolorization on red color dye (65%) and blue colour dye (48%) compared with *Bacillus cereus*. *Bacillus cereus* showed decolorization on the red colour dye (51%) and blue color dye (38%). In this study, the bacterial isolates for the decolorization of dyes was performed by broth method. After incubation the degradation of samples were analysed by UV Spectrophotometer and High Performance Liquid Chromatography.

KEYWORDS: Textile Dyes, *Bacillus subtilis*, *Bacillus cereus*, Total solids, Total dissolved solids, Total suspended solids.

1. INTRODUCTION

Biodegradation is a chemical breakdown of different compounds into various byproducts with the help of the various enzyme's actions. Biodegradation fragments the synthetic dyes into simpler and smaller parts and also decolorizes. The breakdown of the chromophoric center of dyes results into the form of decolorization of the synthetic dyes (Kaushik *et al*., 2009) Bacteria, algae, fungi and yeasts are numerous types of microorganisms used for biodegradation and decolorization of synthetic dyes. These organisms have different capacity for different types of synthetic dyes. Among these organisms, some are preferred over others because of their great capacity for degradation. The effective degradation and decolorization depend on the activity of microorganisms selected for that purpose (Chen *et al.*, 2003).

Microbial degradation and decolorization is an environment friendly and cost-competitive alternative to chemical decomposition processes. Many microorganisms belonging to different taxonomic groups of bacteria, fungi, actinomycetes and algae have been reported for their ability to decolorize dyes (Saini *et a*l., 2005). Acid red is one of the dye which has large consumption rate in textile industry. Various parameters such as agitation, temperature, pH and different dye concentration required to achieve maximum dye decolorization were standardized.

A dye is a synthetic chemical used to impart color to materials of which it becomes an integral part. Dyes are carbon based organic compounds while pigments are normally inorganic compounds, often involving heavy toxic metals (Balakrishnan *et al*., 2008). Dyes are classified to their application and chemical structure. They are composed of a group of atoms responsible for the dye color, called chromophores, as well as an electron withdrawing or donating substituents that cause or intensify the color of the chromophores, called auxophores.

Various microorganisms (e.g. *Bacillus subtilis, Phanerochaete chrysosporium, Aeromonas hydrophila, Penicillium sp., Klebsiella pneumoniae*, *Proteus mirabilis* and *Pseudomonas cepacia)* have been isolated and have been shown to be very promising for degrading different dyes (Maulin *et al*., 2013; Blyskal, 2014). A number of microorganisms namely *Pseudomonas kurthia* (Zimmermann *et al*., 1982), *Aeromonas* (Chen *et al*., 2008), *Proteus mirabilis, Rhodococcus globerulus* (Joshi *et al.,* 1991), *Bacillus spp*., *Micrococcus cutes, Staphylococcus aureus* has already been reported of having the capability of decolorizing textile dye. This study highly intend to examine the physical and chemical characteristics of

the dyes effluent contaminated soil samples and to identify as many bacteria as present in the soil collected from major soil types of Tirupur, Tamil Nadu. To screen the dye degrading organisms by degradation assay, To optimize the dye degradation and the degradation of samples were analysed by UV Spectrophotometer and High Performance Liquid Chromatography.

2. MATERIALS AND METHODS

2.1 Collection of dyes

The textile dyes such as red and blue color were collected from Tirupur textile industry, Tamil Nadu, India. It was refrigerated at 4°C and used without any preliminary treatment.

2.2 Physico Chemical Properties Of The soil (Crini *et al*., 2006)

Physico chemical parameters such as Carbon, Nitrogen, Calcium, Potassium, Total solids, Total dissolved solids, Total suspended solids of the collected dyes effluent contaminated soil sample were analysed using the standard methods.

2.3 Screening of bacterial isolates for dye decolorization (Chen *et al*., 2003)

The decolorization of textile dyes by bacterial isolates was determined by plate assay technique. The plate assay was performed for the detection of decolorizaion activity of bacteria isolated and identified from the textile dyes contaminated site sample. The Nutrient agar and dyes (500 mg / l) was autoclaved at 121°C for 15 min. The bacterial cultures were inoculated on Nutrient agar plates containing dyes. The plates were wrapped with parafin and were incubated in 37°C for 4 days. The plates were observed for clearance of the dye surrounding the colonies.

2.4 Decolorization (degradation) assay (Waffa and Moawad, 2003)

The experiment were carried out in 250 ml conical flasks containing 200ml of nutrient broth to which 5 ml of dye from the stock solution was added. 1 ml of bacterial inoculum were also inoculated and incubated at 37°C. The dye medium was treated with two bacterial species individually and in different combination. 3 ml of supernatant from each bacterial culture were taken for 10 days and decolourization studies were performed.

The same amount of minimal medium containing dye was added to each culture, after each sampling to keep a constant volume in the culture flask. Six replicate flasks with the same dye concentration and inoculum size were used for the study and the results were reported as

an average of six samples. A control was also set up, 200 ml nutrient broth and 5 ml dye solution but without any bacterial inoculum. Care was taken to reduce variation induced by photo-degradation of due. The samples after 10 days of treatment were filtered through a 0.45m membrane filter prior to UV Spectrophotometer and HPLC analysis.

2.5 Optimization of dye degradation

A set of test tubes containing sterilized 10 ml Minimal salt medium with different concentration of dyes. In this experiment, different carbon, nitrogen, temperature and pH were used for optimization of dye decolorization.

2.6 Effect of pH on dye degradation (Kuo-chengchen *et al*., 2002)

A set of test tube containing sterilized 10 ml MSM medium and 0.1 mg dye prepared at different pH (5, 6, 7, 8, and 9) was inoculated with 18 hrs old culture and incubated at 37°C. The culture of test tube was harvested after 24 and 48 hours consecutively and content of the tubes were centrifuged at 7000 rpm for 15 min and supernatant was analysed spectrophotometrically for any residual dye at 254 nm.

2.7 Effect of temperature on dye degradation (Kuo-chengchen *et al*., 2002)

A set of test tube containing sterilized 10 ml MSM medium with 0.1 mg dye was inoculated with 18 hrs old culture and incubated at different temperature (25°C, 37°C, 40°C and 45°C.The culture of test tube was harvested after 24 and 48 hours consecutively and content of the tubes were centrifuged at 7000 rpm for 15 min and supernatant was analysed spectrophotometrically for any residual dye at 254 nm.

2.8 Effect of the dye concentration (Kuo- chengchen *et al*., 2012).

A set of the test tubes containing sterilized 10 ml MSM medium with different concentration of the dye (1 mg/ ml to 5 mg/ml) was inoculated with 18 hrs old culture and incubated at 37°C. A set of tubes were harvested after 24 and 48 hours consecutively and content of the tubes were centrifuged at 7000 rpm for 15 min and supernatant was analysed spectrophotoetrically for any residual dye at 254 nm

2.9 Effect of inoculum volume

At the optimal pH, temperature and dye concentration, the effect of inoculum volume towards dye decolorization was studied using the inoculum volume range from 0.25 ml to 1.25 ml.

2.10 Effect of culture condition

The decolorization studies were carried out both at static and agitator condition. The static condition was maintained by incubating the reaction mixture in a 30°C incubator whereas the agitator condition was maintained by placing the reaction mixture in the shaker at 100 rpm.

Optimization of carbon and nitrogen source in the medium

In this experiment, different carbon and nitrogen sources were used for optimizatiom but the dye concentration was same.

2.11 Effect of different carbon concentration for dye degradation (Chen *et al*., 2002)

A set of tubes containing different sugars (glucose, sucrose, mannitol, maltose, lactose and cellulose) were prepared. Control tubes were also prepared together with the experimental one. In control, inoculum was not added and dye concentration was same in all tubes. A set of tubes containing 10ml of medium and 0.1mg dye were sterilized at 121°C for 15 min. The tubes were inoculated with 18 hours old culture separately and incubated at 37°C. The tubes were centrifuged at 7000 rpm, 15 min and supernatant was analysed spectrophotometrically for residual dye at 254nm. The result for the decolorization of medium were observed for each tube containing different sugars.

Effect of organic and inorganic nitrogen concentration for dye degradation

A set of tubes containing different nitrogen sources (NH_4Cl, NH_4OH, $NaNO_3$, urea, peptone) were prepared. Control tubes were also prepared together with the experimental one. In control, inoculum was not added and dye concentration was same in all tubes. A set of tubes containing 10ml of medium and 0.1mg dye were sterilized at 121°C for 15 min. The tubes were inoculated with 18 hours old culture separately and incubated at 37°C. The tubes were centrifuged at 7000 rpm, 15 min and supernatant was analysed Spectrophotometrically for residual dye at 254nm.

2.12 UV–Vis Spectral Analysis (Chen *et al* ., 2013)

Using distilled water as a blank, Aliquots of sample 5-6ml volume of clear dye solution were prepared and absorbance was analyzed using UV- Visible Spectrophotometer. Decolorization can be determined with in absorbance of wavelength 200-800nm and by the reduction in area of peak for each dyes.

2.13 Statistical Analysis (Kannan 2013)

All analysis were performed in triplicate and results were presented here by the mean of triplicates ± standard deviation (SD).

2.14 High performance of liquid chromatography (HPLC) (Selva raj *et al., 2012)*

The separation and identification of compounds were made through High Performance of Liquid Chromatography. The extracts used for HPLC analysis were passed through a 0.45 µm filter (Millipore, MSI, Westboro, MA) before injection into a HPLC column of 150 mm length (Agilent technologies 1200 series). The mobile phase was acidified water containing 0.1% formic acid (A), and acidified acetonitrile containing 0.1% formic acid (B), eluted in gradient. The flow rate was 0.8 Ml/min and the wavelengths of detection were set at UV 300 nm, temperature at 30°C, injection volume = 20µl and analysis time was 60 min. Reference substances is a mixture of gallic acid, vanilic acid, ascorbic acid, quercetin, caffeic acid, catechin and coumaic acid (solutions in methanol, each of them 0.5 mg/ml).

HPLC ANALYSIS

Column Specification

Reverse phase HPLC (Cyberlab,USA) analysis was carried out in a C 18 column (250mm ×4.6mm) version (lake forest,CA USA)equipped with a c 18 curved column. The components were eluted with an isocractic elution of acetonitrile vs water at the flow rate of1 ml /min and absorption recorded at 680nm.

Sample preparation

One ml of the samples was centrifuged (at3000rpm for 15 minutes) and dissolved in specific solvent of HPLCgrade and filtered through 0.22 micro filter. The filtrate was collected and degassed using sonicator for 50 times at 4°c.

Solvent preparation

Solvent was prepared using aceto nitrile and water in the radio 65:35 and degassed using sonicator for 15 times at 4°c.

Column equilibration

Column equilibration was done using 65% aceto nitrile in water until zero base line.

Sample injection

Twenty micro litre of the sample was injected in to the injection head using injection needle. Required time and wavelength were set and the purification profiles were seen on the screen that shows the degraded components with its retention time.

3. RESULT AND DISCUSSION

3.1 Collection of Dyes

The textile dye such as red and blue color were collected from Tirupur textile industry, Tamil Nadu, India.

3.2 Physico-chemical parameters of the dye sample

Analysis of the physico -chemical parameters of the collected dye effluent samples using the standard methods. The dye pH (8.0), carbon (1.24%), nitrogen (68.4kg/ac), calcium (10.9kg/ac), and potassium (2.9kg/ac). In the present study, the dye is degradable under aerobic conditions with a concerted effort of bacteria. Nutrients (carbon and nitrogen source) and physico chemical parameters (pH, temperature) had significant effect on dye decolorization. Similarly Ponraj *et al*., (2011) the textile dyes was degraded under aerobic condition. Nutrients and physico -chemical parameters were significant on degradation.

3.3 Collection of soil sample

In the present study, bacterial species were isolated from the dye effluent contaminated soil samples collected from Textile industry, Tirupur, Tamil Nadu.

3.4 Identification of isolated bacteria

The selected two bacterial colonies were identified by cultural, morphological and biochemical characteristics and compared with standard mannuals. Based on the results, the isolated colonies were confirmed as *Bacillus subtilis* and *Bacillus cereus*.

Table 1: Biochemical characteristics of isolated microbes.

S.NO	TESTS	*Bacillus subtilis*	*Bacillus cereus*
isolated organism based on colony morphology			
1	Colony morphology	Dry, flat, irregular with lobatemargine.	Rough ad dry texture
2	Gram's staining	Gram positive	Gram positive
3	Shape	Rod	Rod
4	Motility	Motile	Motile
Biochemical characters			
5	Indole	-	-
6	Methyl red	-	-
7	Vogesprokauer	+	+
8	Catalase	+	+
9	Oxidase	-	+
10	Citrae utilization test	-	+
11	Urease	-	-

(+) – indicates positive

(-) – indicates negative

3.5 Screening for dye decolorization

The bacterial isolates were screened for the decolorization of red and blue color dyes by plate assay method. The identified bacterial isolates *Bacillus subtilis* and *Bacillus cereus* were used for plate decolorization assay. Maximum decolorization was recorded by *Bacillus subtilis* in the plate containing Red color (32mm) and Blue color (24mm), followed by *Bacillus cereus* in the plate containing Red color (28mm), and Blue color (22mm). The bacteria isolates were screened for the decolorization of red and blue color dyes by plate assay and results were presented. The identified bacterial isolates *Bacillus subtilis* and *Bacillus cereus* were used for plate decolorization assay. Maximum decolorization was recorded by *Bacillus subtilis* in the plate containing Red color (32mm) and Blue color (24mm) followed by *Bacillus cereus* in the plate containing Red color (28mm), Blue color (22mm). Similarly Saranraj and Sivasakthivela (2014) *Bacillus odyssey* in maximum decolorization for plate containing Reactive orange-16 when compared to other dyes.

3.6 Effect of pH on dye degradation

The maximum dye degradation was shown at pH 7. The minimum dye degradation was shown at pH 9(Table-2). In our study, the decolorization efficiency of *Bacillus subtilis* and *Bacillus cereus* was studied by measuring the optical density after 0, 4, 8, 12 and 16 days incubation and results were presented. It was noticed that there was a decrease in the optical density (OD) in all the two colors as the incubation period increased. *Bacillus subtilis* was

more effective followed by *Bacillus cereus*. The percentage of decolorization of colors by bacteria was also calculated (Uma maheswari and Sivagami, 2016). Our report correlated to the finding of Sriram *et al*., (2013), the efficiency of three species and five color were studied by measuring optical density *Pseudomonas fluorescence* was more effective followed by *Bacillus species* and *Echerichia coli.*

Table 2: Optimization of red color dye decolorization by *Bacillus species.*

Red color		OD of control	OD of sample	Percentage(%)
Carbon source	Glucose	1.440	0.250	84.5
	Sucrose	1.546	0.269	90.7
	Lactose	1.028	0.210	61.9
	Mannitol	1.251	0.200	72.5
Nitrogen source	Yeast extract	1.020	0.138	57.9
	Peptone	1.039	0.128	58.3
	Ammonium sulphate	1.056	0.10	57.8
	Ammonium chloride	1.068	0.069	56.8
Temperature (°C)	25	1.110	0.339	72.4
	37	1.130	0.506	81.8
	40	1.112	0.150	80.9
	45	1.200	0.065	63.2
Ph	5	1.150	0.240	69.5
	6	1.200	0.236	71.8
	7	1.318	0.315	81.6
	8	1.028	0.120	57.4
	9	1.020	0.125	57.2

Table 3: Optimization of blue color dye decolorization by *Bacillus species.*

Blue color		OD of control	OD of sample	Percentage (%)
Carbon source	Glucose	1.320	0.255	78.7
	Sucrose	1.269	0.215	74.2
	Lactose	1.040	0.320	68.o
	Mannitol	1.420	0.299	85.9
	Yeast extract	1.038	0.145	59
Nitrogen source	Peptone	1.045	0 .139	59.2
	Ammonium sulphate	1.058	0.125	59
	Ammonium chloride	1.098	0.188	64.3
Temperature (°C)	25	1.119	0.362	74
	37	1.145	0.505	82.5
	40	1.115	0.185	65
	45	1.212	0.068	64
pH	5	1.140	0.240	69
	6	1.320	0.352	83.6
	7	1.290	0.250	77
	8	1.089	0.128	60.8
	9	1.038	0.136	58

3.7 Effect of Temperature On Dye Degradation

The optimum temperature for the growth and dye degradation for *B.subtilis* was 37°C (Table-2).The minimum temperature for the growth and dye degradation for *B. subtilis* was 45°C.

3.8 Optimization Of Dye Concentration

Bacillus subtilis was showed maximum dye degradation at 1mg/ml concentration. The decrease in the decolorization efficiency was seen to be decreased with increase in the concentration of the dyes. Least dye degradation was observed in 5mg/ml concentration. Optimization of the dye degradation of the dye was carried out only for *B.subtilis* because maximum dye decolorization was (65%) noticed (Table -2).

3.9 Optimization Of Carbon And Nitrogen Source in the medium

Attempts were made to alter the composition of the medium by altering the carbon source, nitrogen source. The glucose and sucrose was found to be best suitable carbon source for the growth of *B.subtilis* (Table-2).

3.10 Effect Of Carbon And Nitrogen Source

Different carbon sources namely Sucrose, Glucose, Mannitol and Lactose were used for dye decolorization. In red dye color dye decolorization, Sucrose was showed maximum dye degradation activity (90.7%), followed by Glucose (84.5%), Mannitol (72.5%) and Lactose (61.9%). Similarly in nitrogen source, Peptone was showed maximum degradation activity (58.3%) followed by Yeast extract (57.9%), Ammonium sulphate (57.8%) and Ammonium chloride (56.8%) (Table -2).

In blue dye decolorization, Mannitol was showed maximum degradation activity (85.9%) followed by Glucose (78.7%), Sucrose (74.2%), Lactose (68%). Similarly in nitrogen source Ammonium chloride was showed maximum degradation activity (64.3%) followed by Peptone (59.2%), Ammonium sulphate (59.1%) and Yeast extract (50%) (Table-3).

3.11 Degradation Of Dyes by UV – Vis spectral analysis

Decolorization of red and blue color dye has been confirmed by UV –Vis spectral analysis. Spectrophotometrical analysis of the red and blue color dyes, showed maximum absorbance at 490 nm and the decrease in the absorbance of samples which was withdrawn after decolorization. This study was clearly highlight to *B.subtilis* and *B.cereus* was showed

maximum degradation ability on Red and Blue color. In over all aspect, Red and Blue color was effectively degraded by *B.subtilis* than *Bacillus cereus.*

3.12 Degradation of dyes HPLC analysis

The absorption spectra of the samples obtained at 300nm are presented in figures 7 and 8. The HPLC elution profile of the red color dye control showed 2 peaks with retention time (RT) of 3.557 and 5.613 for 20 minutes. The elution profile obtained for the bacteria treated samples significantly differed from the control in terms of number, height of peaks obtained and RT. The HPLC profile of red color dye treated with bacterial isolate *Bacillus subtilis* showed 2 peaks with RT 3.535 and 5.300. Blue color dye treated with bacterial isolate *Bacillus subtilis* showed 3 peaks with RT 5.550, 6.134 and 6.704.

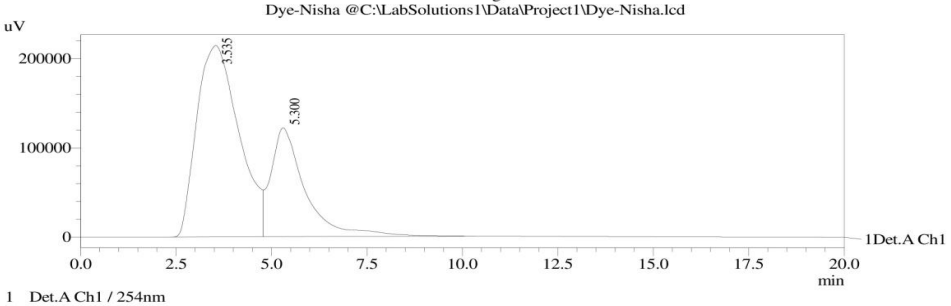
HPLC ANALYSIS REPORT

Sample Information

Acquired by	: Admin
Sample Name	: Dye-Nisha
Sample ID	:
Vail#	:
Injection Volume	: 20 uL
Data Filename	: Dye-Nisha.lcd
Method Filename	: SJC-Law-Method.lcm
Batch Filename	:
Report Filename	: Default.lcr
Date Acquired	: 30-03-2017 AM 11:58:12
Data Processed	: 30-03-2017 PM 12:18:19

Chromatogram

Dye-Nisha @C:\LabSolutions1\Data\Project1\Dye-Nisha.lcd



1 Det.A Ch1 / 254nm

PeakTable

Detector A Ch1 254nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	3.535	16464572	214703	68.308	63.755
2	5.300	7638894	122060	31.692	36.245
Total		24103466	336763	100.000	100.000

ANALYSED BY

APPROVED BY

HPLC ANALYSIS REPORT

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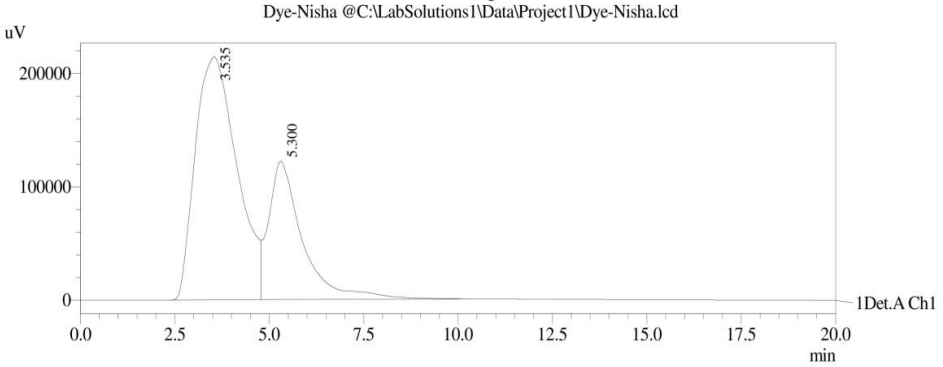
Chromatogram

Dye-Nisha @C:\LabSolutions1\Data\Project1\Dye-Nisha.lcd



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Total		24103466	336763	100.000	100.000

---------- ANALYSED BY

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4. CONCLUSION

The isolates used in the present study were *Bacillus subtilis* and *Bacillus cereus* were able to persist and flourish in the dye environment utilizing the dye as the sole energy source though other nutrient are limited. Though both the isolates were found to be excellent bio-agent for

the bioremediation of dye. *Bacillus subtilis* caused better decolorization than *Bacillus cereus* which indicates the possible use of these isolates in biodegradation of textile dyes and actual textile effluents. If the metabolites could be analyzed and the pathway of dye degradation explained the microbial degradation of textile effluent could be successfully performed on a commercial scale. Bacteria is a cheaper and better environment friendlier for color removal in textile dye effluents. Biological treatment has been effective in reducing dye house effluents and when used properly has a lower operating cost than other remediation decolorization process. Since they are cost effective and efficient it is highly recommended for the industries in making use of the consortium for the proper disposal of textile effluents.

5. ACKNOWLEDGEMENT

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