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Research Article

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Biological Degradation of Textile Dyes using Marine *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-days due to advancement in industrialization, it a spoiling a lot. In the present study, an attempt was made to examine the isolation and identification of Bacillus species from marine soil, Nagapattinum seashore, Tamil Nadu, India. Dyes are collected from in around Tirupur dyeing unit, Tamil Nadu, India namely Reactive purple, Malachite green, Reactive red M5B, Reactive pink MB. Out of 5 Bacillus isolated, 2 of them are selected for our study based on the decolourization screening test. Percentage of dye degradation and physical and chemical parameters were optimized. In dye samples TS,TDS,TSS, calcium, phosphorus were estimated. It was therefore calculated that Bacillus subtilis is an remedial strain for the dye effluent treatment in Reactive pink dye degradation. Compared to Bacillus cereus the Bacillus subtilis was showed maximum dye degradation 70.8% at P^H 7, Temperature 37°C and carbon (glucose) nitrogen(yeast extract) for Reactive pink MB. Dye decolourization can use measured in terms of optical density.

Key words: *Marine soil, Dye, B.subtilis, B.cereus, Total solids, Total dissolved solids, Total suspended solids.*

INTRODUCTION

Textile industries are the major source of dye effluent. More than 10,000 different textile dyes are commercially available worldwide with an estimated production of 7×10^5 metric tons. Huge amount of dye and water are used in textile industry for dyeing. The textile industry annually discharges 30,000 to 150,000 tons of dyes in water bodies causing severe pollution. The toxicity of dye containing waste water various with the type of dye used in the textile industry[1]. Dye imparts color to water and is thus visually identifiable in water. Color causes hindrance in light penetration, which subsequently inhibits the process of photosynthesis. This may cause depletion of dissolved oxygen (DO) and deterioration of water quality, and causes severe toxic effects on aquatic life[2].

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In addition, dyes also cause adverse impact on total organic carbon (TOC), biological oxygen demand (BOD) and chemical oxygen demand (COD) of water bodies[3]. Eventually there is increase in the organic load, which leads to eutrophic condition in water bodies. It badly affects the biotic growth and solubilizes metal ions leading to the toxicity in plants, animals and other microorganism. Dye contains mutagenic and carcinogenic compounds, which enter the water bodies continuously and affect the aquatic and human life[4-5]. Degradation of dye is not easy due to its complicated aromatic structure and stability to sunlight, oxidizing agents and microorganism[6]. The present study was focused on prevalence of marine bacterial isolates in textile dye effluent and its dye degrading efficiency.

MATERIALS AND METHODS

Sample collection

The soil samples were collected marine environment from Nagapattinam seashore area (Latitude 11º 29´ N; Longitude 79º 46´ E) in sterile containers. Then the sample was brought to the laboratory as early as possible and was subjected for various microbiological studies.

The textile dyes such as Reactive purple, Malachite green, Reactive red M5B, and Reactive pink MB were collected from Tirupur, Tamil Nadu, India. It was refrigerated at 4ºC and used without any preliminary treatment.

Physico-chemical parameters of the dye sample

physico-chemical parameters were analysis such as (Carbon, Nitrogen, Calcium, Potassium, TS, TDS, TSS)of the collected dye sample using the standard methods[7-9].

Isolation and identification of bacteria

Isolation of microorganism from serial dilution technique. The isolated strains were identified on the basis of their morphological and biochemical characteristics according to Bergey's manual of determinative Bacteriology[10].

Reactive dyes employed

100ml of dye solutions of different concentrations were prepared to nutrient media components were added. The flask were sterilized and inoculated. Samples were centrifuged at 6000rpm for 10min to separate the cells. Supernatant was analyzed for reactive dyes using UV-vis spectrophotometer at λ_{max} (540nm) [11]. Decolourization studies and percent decolourization was found using the formula

$$\% \text{ Dye decolorization} = \frac{(\text{initial OD} - \text{final OD})}{(\text{Initial OD})} \times 100$$

Dye decolourization

50ml of Nutrient agar sterile medium was separately the dyes (200 mg/l) and inoculated with 2% bacterial suspension. The suspension contained 2.5×10^6 cfu/ml spores. The flasks were incubated at 30±1ºC for 8 days. Samples were kept for 2 days for observation. The Samples were centrifuged at 10000 rpm for 10 minutes and decolourization was assessed by spectrophotometer. Two control flasks (Dye + medium) without inoculums and medium with inoculums without dye were maintained[12].

Screening for decolourization of reactive dyes by plate assay

The decolourization of textile dyes by bacterial isolates was determined by plate assay technique. This technique was performed for detection of decolourization activity of bacteria isolated and identified bacteria. The Nutrient agar and Reactive dyes (500 mg/l) was autoclaved at 121ºC for 15 min. The bacterial cultures were platted on Nutrient agar plates. The plates were wrapped with parafilm and were incubated at 37ºC for 4 days. The plates were observed for clearance of the dye surrounding the colonie[13].

Peroxidase method

1ml of crude enzyme is extracted from the sample and centrifuged at 6000 rpm for 10 min followed by addition of 1 ml of methylene blue dye solution of 20 ppm and incubated for 10 min at 30ºC. The change in colour to green

shows the presence of peroxidase enzyme and it is quantitatively measured at 662 nm using UV-Vis spectrophotometer[14]. The amount of enzyme units was calculated using the formula

$$\text{Enzyme units} = \frac{(OD_{control} - OD_{sample})}{(\text{reaction time})} \times 100$$

Optimization of dye degradation

A set of test tubes containing sterilized 10ml MSM medium with different concentration of dyes. In this experiment different carbon, nitrogen, temperature and p^H were used for optimization of the dye concentration[15].

Statistical analysis

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

RESULT AND DISCUSSION

In our study the the decolurization of four different reactive dyes Reactive purple, Malachite green, Reactive red M5B, Reactive pink MB sample were collected from Tirupur, Tamil Nadu. The physico-chemical properties were analyzed. Screening for decolourization of reactive dyes by plate assay technique. The clearance of dye degradation was noticed in (Table-3)

Isolation and Identification of microorganisms

Nutrient agar media with minimal salt were used for the isolation of bacteria .They are two types of the bacteria were isolate from marine soil. The selected two bacterial colonies were identified by cultural, morphological and biochemical characteristics and compared with standard manuals. Based on the results the isolated colonies were confirmed as *Bacillus subtilis* and *Bacillus cereus* respectively (Table-1).

In this study the bacterial isolates were screened for the decolorization of reactive dyes by plate assay. The Reactive pink MB, the bacterial isolates showed maximum zone of inhibition in the plate containing Reactive purple followed by Malachite green and Reactive red M5B. Similarly *Bacillus odyssey* in maximum declourization for plate containing Reactive orange-16 when compared to other dyes.

Screening for decolourization of reactive dyes by plate assay

The bacterial isolates were screened for the decolourization of reactive dyes by plate assay and the results were tabulated in (Table-3). The identified bacterial isolates *Bacillus subtilis* and *Bacillus cereus* were used for plate decolourization assay. Maximum decolorization was recorded by *Bacillus subtilis* in the plate containing Reactive pink MB (36mm) followed by *Bacillus cereus* (28mm). Next to Reactive purple (32mm), Reactive red M5B (26mm), and Malachite green(34mm), (Table-3)

In P^H were noticed termed of 8.19, 7.91, 7.41, 7.12 for Reactive purple, Malachite green, Reactive red M5B, Reactive pink MB. Total solids, Total dissolved solids, Total suspended solids were noticed (Table-2)

In Reactive pink MB was effectively degraded at P^H 7, temperature 37°C, glucose as carbon source, yeast extract as nitrogen source using *B.subtilis* as a treatment organism. (Table-4).

Our study report revealed that biological degradation of reactive dyes by bacteria isolated from dye effluent contaminated soil. The different bacterial isolates such as *Pseudomonas, Bacillus subtilis, E.coli* . from that *Pseudomonas* was more effective followed by *Bacillus subtilis, E.coli*[16].

Table- 1 Biochemical Characteristics of Isolated Microbes

Tests / characters	***Bacillus subtilis***	***Bacillus cereus***
Colony morphology	Dry, flat, irregular with lobate margins.	Rough and dry texture
Gram's staining	Gram positive	Gram positive
Shape	Rod	Rod
Motility	Motile	Motile
Indole	–	–
Methyl red	–	–
Voges proskauer	+	+
Catalase	+	+
Oxidase	–	+
Citrate	–	+
Urease	–	–

(+) – indicate positive
(-) – indicate negative

Table- 2 Physico-chemical Parameters of Dye Sample

Name of the parameters	**Reactive purple**	**Malachite green**	**Reactive red M5B**	**Reactive pink MB**
P^H	8.19	7.91	7.41	7.12
Carbon (%)	1.34	0.53	0.68	1.45
Nitrogen (Kg/ac)	68.8	75.8	56.9	80.43
Calcium (Kg/ac)	11.9	18.7	9.1	19.2
Potassium(Kg/ac)	3.6	4.42	6.32	7.76
TS	1.29	3.01	3.31	5.7
TDS	4.87	2.43	5.66	7.49
TSS	7.69	3.91	5.01	8.56

Table-3 Screening of Bacterial Isolates for Dye Degradation by Plate Assay

Zone of inhibition (mm)				
Bacterial isolates	**Reactive purple**	**Malachite green**	**Reactive red M5B**	**Reactive pink MB**
Bacillus subtilis	34	34	26	36
Bacillus cereus	25	21	18	28

Table -4 Effect of Carbon and Nitrogen Source of Reactive pink MB

Reactive pink MB		OD of control	OD of sample	Percentage
Carbon source	Glucose	1.550	0.267	90.8%
	Sucrose	1.546	0.268	90.7%
	Lactose	1.038	0.310	67.4%
	Mannitol	1.261	0.212	73.6%
Nitrogen source	Yeast extract	1.032	0.137	58.4%
	Peptone	1.039	0.128	58.3%
	Ammonium sulphate	1.046	0.10	57.3%
	Ammonium chloride	1.068	0.068	56.8%
Temperature	25ºC	1.102	0.336	71.9%
	37ºC	1.127	0.502	81.4%
	40ºC	1.112	0.148	63%
	45ºC	1.211	0.069	64%
pH	5	1.148	0.245	72.2%
	6	1.219	0.237	72.8%
	7	1.318	0.316	81.7%
	8	1.074	0.128	60.1%
	9	1.023	0.126	57.5%

Table-5 Dye Decolouration of *Bacillus subtilis* in MSM Medium

DYE	Intial OD	Final OD	Percentage of degradation
Reactive purple	0.87	0.56	58.5%
Malachite green	0.58	0.40	49%
Reactive red M5B	0.74	0.43	71.5%
Reactive pink MB	0.68	0.89	78.5%

Table-6 Dye Decolouration of *Bacillus cereus* in MSM Medium

DYE	Intial OD	Final OD	Percentage of degradation
Reactive purple	0.65	0.34	49.4 %
Malachite green	0.48	0.28	38.1%
Reactive red M5B	0.35	0.69	72.5%
Reactive pink MB	0.67	0.78	52.%

CONCLUSION

From our present study concluded that the isolated *Bacillus subtilis* had high decolorization efficiency (78.5%) when compared *Bacillus cereus*. This strains also recommended for decolourized in textile dye. Among the four dyes tested, the dye Reactive pink MB showed maximum degradation which compared to other Reactive dyes. Although, several bacteria are capable of degradation, very few strains can withstand the condition of dyeing effluents in terms of the above mentioned extremes of parameters. The study contributes of the efforts of being out the

phenomena of bacterial remediation of reactive dyes nearly from laboratory condition to commercially applied in field conditions. Hence, economical, eco-friendly techniques using bacteria offer cheaper, easy and effective alternative for color removed of textile dyes.

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