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Isolation and screening of dye decolorizing bacteria from industrial effluent

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ABSTRACT

Acidic dye is a dye which is salt of a sulphuric, carboxylic or phenolic organic acid .It is used on wool, other animal fibers and some manufactured fibers. These dyes when disposed into the environment causes pollution and serious irreversible damage to the ecosystem. In the present study various sample is collected from effluent of industry in the Vapi region. Four isolates SP1, SP2, SP3 and SP4 found to be effective decolorizer of Acid Maroon V dye. Among these isolate, SP4 shows maximum decolorization.

1. INTRODUCTION

A dye or dyestuff is usually a coloured organic compound or mixture that may be used for imparting the colour to a substrate such as cloth, paper, plastic, or leather in a reasonable permanent manner."A dye may be defined as an organic compound containing chromophore and auxochrome group linked to the benzene ring." A chromophore group imparts property of colour. Compounds of benzene containing chromophore radicals are called chromogensss. Such a compound, even though coloured, is not a dye, because it possesses nor affinity for, nor the ability to unite with the fibers and tissues [1].

Dyes are an important class of synthetic organic compounds, widely used in textile, leather, plastic, cosmetic and food industries and are therefore common industrial pollutants. Dyes may also significantly affect photo synthetic activity in aquatic life by reducing light penetration intensity and may also toxic to some aquatic flora and fauna which is a salt of a sulphuric, carboxylic or phenolic organic acid [5]. The salts are often sodium or ammonium salts. Acid dyes are typically soluble in water. Some acid dyes are used as food colorants [6]. Biodegradation processes are environmentally, friendly and cost effective and are also alternative to chemical decomposition process. Microorganism plays a very important role in the biodegradation and mineralization of these dyes which is of great significance [3, 4]. In present study screening for dye decolorization bacteria was performed using various dye contaminated sample.

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2. MATERIALS AND METHODS

2.1 Dye and chemicals

Dye such as Acid Maroon V, Colorent Red BS, Carzol Brilliant Blue RN, Red FRR, T Blue M5G, and Acid Red-2 were collected from Narayan Processor Pandesara, Faze limited chemical industries Surat. Glucose, Ammonium Sulphate, NaCl, K_2HPO_4, KH_2PO_4, and $MgSO_4$ All media components and chemicals used in the study were of high purity and analytical grade.

2.2 Isolation and Screening of Dye Decolorizing Microorganisms

Isolation of dye decolorization was carried out by inoculating 1g of soil sample in 100 ml of MSM medium (Mineral Salt Medium containing (g/l) glucose5.0; ammonium sulphate 1.0; K_2HPO_4 6.0; KH_2PO_4 1.0; $MgSO_4$ 0.1; NaCl 5.0;) containing 100 ppm of dye into250 ml of Erlenmeyer flask.

The inoculated medium was incubated at 30°C under static condition and observed for the dye decolorization. After decolorization, the enriched media is serially diluted and spreaded on dye containing plate. The isolates obtained were purified by subculturing on nutrient agar plates. All the isolates were studied for decolorization on dye containing plate as well was in liquid medium (MSM). The isolate giving better decolorization was selected for further study. The pure form of isolated bacteria was streaked on nutrient agar slants and incubated at 30°C for 48 hours. The pure culture is then stored in refrigerator at 4°C and subcultured periodically.

2.3 Decolorization Experiment

2.3.1 Inoculum Preparation

The preserved culture was transferred in 100 ml Erlenmeyer flask containing 50 ml nutrient broth. The flasks were incubated at 30°C for24 hours. The freshly grown 24 hours old culture with 1.0 O.D. at 600nm is used as inoculum for decolorization study.

2.4 Dye Decolorization Study

The sterilized medium was inoculated with 100 ppm dye and 1% (v/v) of 24 hours old culture. The inoculated flask was allowed to incubate at 30°C for 72 hours under static condition. The sample was withdrawn at regular time interval and supernatant was subjected to centrifuge at 5,000 rpm for 20 minutes and decolorization was determined.

2.5 Analytical method for dye decolorization study

Decolorization was quantitatively analyzed by measuring the absorbance of the supernatant at maximum absorption wavelength, λmax of respective dyes. Decolorization was calculated by using the equation:

$$\% \text{ Decolorization} = (A-B)/A \times 100$$

Where, A is initial absorbance of control dye (initial absorbance) and B is observed absorbance of degraded dye (final absorbance).

2.6 Time course study

The time course was done by inoculating the MSM medium with 100 ppm dye, 1 % inoculum and incubated at 30° C for 72 hours under static condition. The sample were withdrawn from both medium flasks at interval of 12 hours and centrifuged at 5,000 rpm for 20 minutes and supernatant was used to determining decolorization.

2.7 Effect of medium composition on dye decolorization process

Different medium were used for evaluation of decolorization and each medium (100 ml) were inoculated with 100 ppm dye, 1% inoculums and incubated at 30°C for 48 hours under static condition. After 48 hours of incubation, 5 ml of sample were withdrawn from each flask and centrifuged at 5,000 rpm for 20 minutes. The supernatant was used for determining decolorization. The medium used in present study are as follows:

Medium 1: MSM (Mineral Salt Medium) containing (g/L) glucose 5.0; ammonium sulphate 1.0; K_2HPO_4 1.0; KH_2PO_4 1.0; $MgSO_4$ 0.1; NaCl 5.0

Medium 2: NDM (Normal Decolorization Medium) containing (g/L) glucose 2.0; ammonium sulphate 2.5; yeast extract 2.5; KH_2PO_4 5.0; $MgSO_4$ 0.5; $CaCl_2$ 0.13

Medium 3: BSM (Basal Salt Medium) containing (g/L) NH_4Cl 0.5; K_2HPO_4 1.2; KH_2PO_4 0.4; glucose 0.2; peptone 0.2

Medium 4: BHM (Basal and Haas Medium) containing (g/L) $MgSO_4$ 0.2; $CaCl_2$ 0.02; K_2HPO_4 1.0; KH_2PO_4 1.0; $NaNO_3$ 1.0; $FeCl_3$ 0.05

Medium 5: BMM (Basal Mineral Medium) containing (g/L) NaCl 7.0; $CaCl_2$ 4.0; $MgSO_4$ 2.0; glucose 10.0; yeast extract 1.0

3. RESULTS AND DISCUSSION

3.1 Isolation and screening of dye decolorizing bacterial isolates

Various sample from Faze chemical effluent, Dye contaminated soil, and Sludge sample was used to isolate dye decolorizing microorganisms. A total of 15 bacterial strains were isolated and purified by subculturing on nutrient agar plates. The morphological and cultural characteristics of the bacterial isolate are shown in Table 1. All the isolated cultures were further screened for dye decolorization in liquid medium. The screening of all the isolate for dye decolorization is shown in Table 2. The result obtained shows that maximum decolorization (76.07%) of Acid Maroon V dye by bacterial isolate SP4 was obtained after 48 hours of decolorization process. Patel at al., (2016) reported maximum decolorization (87.33%) of Red H8B dye by isolate R5 within 72 hours of incubation.

Fig. 1: Decolorization of Acid Maroon V dye by isolate SP4.

Table 1: Morphological and cultural characteristics of isolates.

Sr. No	Sample	Isolate	Colony characteristic	Gram staining	motility	Capsule staining
1	Faze chemical industry soil	SP1	Medium, circular, entire, smooth, moist, opaque	Gram positive cocci	Non-motile	Positive
2	Faze chemical industry sludge	SP2	Large, round, entire, smooth, mucoid, opaque	Gram negative rods	Non- motile	Negative
3	Faze chemical industry soil	SP3	Medium, round, entire, smooth, moist, opaque	Gram positive cocci	Non-motile	Negative
4	Faze chemical industry sludge	SP4	Small, round, smooth, irregular, opaque	Gram positive rods	Motile	Negative
5	Polyster chemical industry soil	SP5	Large, round, irregular, moist, opaque	Gram positive cocci	Non- motile	Negative
6	Polyster chemical industry soil	SP6	Medium, irregular, smooth, butyrous, opaque	Gram positive thin rods	Non-motile	Positive
7	Polyster chemical industry sludge	SP7	Small, round, entire, smooth, translucent	Gram positive cocci	Motile	Positive
8	Faze chemical industry soil	SP8	Large, round, entire, smooth, opaque	Gram negative short rods	Non-motile	Positive
9	Polyster chemical industry soil	SP9	Small, round, circular, mucoid, opaque	Gram negative short rods	Non-motile	Negative
10	Faze chemical industry soil	SP10	Medium, irregular, smooth, butyrous, opaque	Gram positive cocci	Non-motile	Positive
11	Dye chemical industry soil	SP11	Medium, round, entire, smooth, translucent	Gram positive rods	Motile	Negative
12	Dye chemical industry sludge	SP12	Large, irregular, smooth, butyrous, opaque	Gram negative rods	Non-motile	Negative
13	Polyster chemical industry soil	SP13	Small, circular, entire, smooth, opaque	Gram negative short rods	Non-motile	Positive
14	Faze chemical industry soil	SP14	Medium, entire, moist, translucent,	Gram positive cocci	Non-motile	Positive
15	Dye chemical industry sludge	SP15	Large, round, irregular, mucoid, opaque	Gram negative rods	Non-motile	Negative

Table 2: Screening of bacterial isolates for dye decolorization (%).

Sr. No	Isolates	Decolorization (%)					
		Acid maroon v	Colorent Red Bs	Carzol Brilliant Blue RN	Red FRR	T Blue M5G	Acid Red-2
1	SP1	27.09	11.23	12.67	24.76	54.89	19.89
2	SP2	19.45	34.15	40.34	30.66	66.65	58.90
3	SP3	54.12	49.19	45.45	37.87	59.87	24.36
4	SP4	76.07	48.47	68.76	70.37	62.26	68.76
5	SP5	68.90	55.32	63.36	59.00	70.00	70.54
6	SP6	23.32	67.34	58.56	63.86	64.28	57.65
7	SP7	65.87	56.23	60.00	62.98	69.43	61.32
8	SP8	67.32	30.54	63.43	63.87	31.67	39.98
9	SP9	9.25	21.11	30.45	42.76	54.23	26.43
10	SP10	54.43	58.76	69.45	63.23	58.71	61.66
11	SP11	59.87	55.43	58.54	65.76	51.43	59.32
12	SP12	8.32	6.1	13.12	32.11	41.98	21.54
13	SP13	66.52	64.69	61.62	56.43	62.55	59.25
14	SP14	34.24	39.54	49.34	38.34	37.25	40.65
15	SP15	59.35	53.54	55.95	41.12	50.43	58.65

3.2 Optimization of cultural condition for dye decolorization study

In present study a bacterial isolate 15 selected as reference culture and Acid Maroon V dye as reference dye for the dye decolorization study.

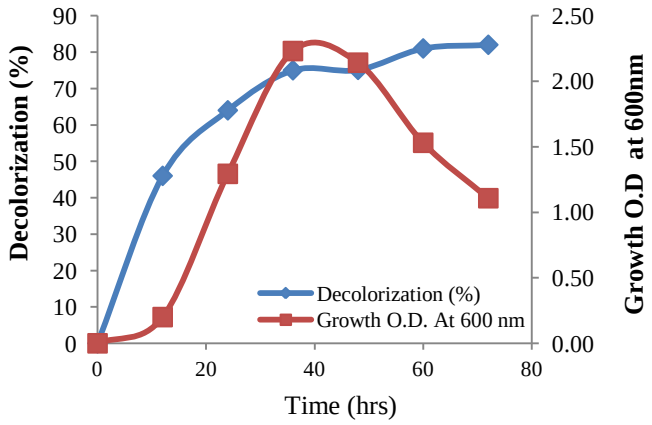

Fig. 2: Time course study.

3.3 Effect of incubation period on dye decolorization

The decolorization of Acid Maroon V dye was determined at various incubation time (i.e. 0, 72 hours). The result obtained shows that decolorization was increased as incubation time increased and maximum decolorization was obtain at 48 hours of incubation (75.11%), however, further incubation of decolorization flask does not enhance the decolorization process.

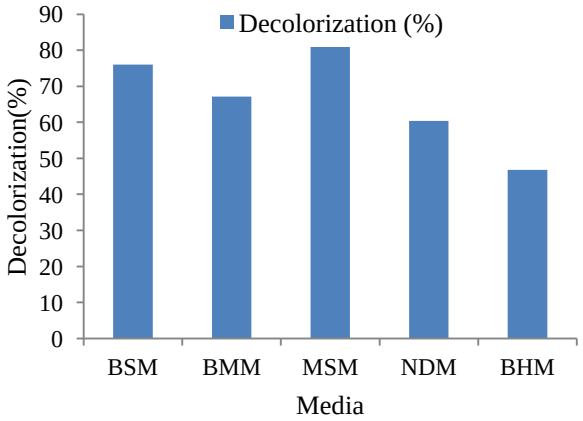

Fig. 3: Effect of different medium composition on dye decolorization.

3.4 Effect of medium composition on dye decolorization

In present study decolorization of dye was studied using various media composition such as NDM, BMM, BHM, MSM, and BSM. The result obtained shows that after 48 hours of

incubation at 30°C under static condition, the percent decolorization obtained was 60.04%, 67.09%, 48.08%, 91.00% and 76.00% of NDM, BMM, BHM, MSM, and BSM respectively. The MSM medium gives maximum decolorization, thus MSM medium was selected as reference medium for further decolorization study. Patel et al reported MSM medium for 88.60% decolorization of Red H8B dye by isolate R5.

4. SUMMARY AND CONCLUSIONS

The present study reveals that the isolate SP4 showed consistent decolorization and degradation of textile dye (Acid Maroon V) throughout the study and could be effectively utilized for the treatment of textile effluent containing high concentration of dyes before discharge into the environment.

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