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ISOLATION AND CHARACTERIZATION OF REACTIVE BLACK DYE DECOLORIZING BACTERIA FROM TEXTILE INDUSTRY

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Abstract: Textile dye industry waste is one of the most serious problems in the environment. The dye house waste water is severely deleterious to surface water bodies. The present stud was an attempt for the assessment of different physico-chemical parameters such as pH, temperature, Total Solids (TS), Total Suspended solids (TSS), Dissolve Oxygen (DO), Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Total alkalinity, Total hardness and Chloride content of this effluent. Degradation of textile dye is a challenging task for microorganisms as the effluents contain complete chemical, highly toxic compounds and heavy metal. In the present research dye decolorizing bacterial species have been isolated on solid and liquid media. The optimum growth and decolorizing parameters, biochemical characterization and degradation are studies. Identification of the isolates organism based on biochemical test VITEK 2 system, identify mostly Enterobacter species.

Key words: Textile dye, Effluent, Decolourization, Degradation, surface water, heavy metal, BOD, COD, Chloride content

1. Introduction:

In modern life, rapid industrialization and urbanization resulted in the discharge of large amount of waste in to the environment, which in turn creates pollution. Water is essential for survival and existence of life on planet earth. The waste water and sewage are released from the industries, that wastes are entering into the water bodies, it is one of major source of environment toxicity [Arminder Kaur,2010], it also affect the soil micro flora and aquatic ecosystem [Thoker Farook Ahmed,2012]. The most environmental problem faced due to the textile dyeing industry is that the industry produces large volumes of high strength of aqueous waste effluents.

India's dye industry produces every type of dyes and pigments. Production of dye stuff and pigments in India is close to 80,000 tones. India is the second largest exporter of dyestuffs and intermediates (developing countries) after China. The textile industry accounts for the largest consumption of dyestuffs, at nearly 80% [Lorimer 2001]. Industrialization is vital to a nation's economy because it serves as a vehicle for development. However, there are associated problems resulting from the introduction of industrial waste products into the environment. Many of these products are problematic because of persistence (low biodegradability) and toxicity.
Amongst these, azo dyes represent the largest and most versatile class of synthetic dyes [Raffi F., Hall J.D.& Cernigila C.E. 1997],. Approximately 10 - 15% of the dyes are release into the environment during manufacturing and usage [Keharia H., Patel H. & Madamwar D. 2004]. Since some of the dyes are harmful, dye-containing wastes pore an important environmental problema [Spadarry J. T., Isabelle L. and Renganathan V. 1994]. These dyes are poorly biodegrabale because of their structures and treatment of wastewater containg dyes usually involves physica and / or chemical methods [Kim S.J. & Shoda M. 1999] such as adsorption, coagulation, flocculation, oxidation, filtration and electrochemical methods [Calabro V., Drioli E. & Matera F. 1991].

2. Materials and methods:

- **Sample collection:**

Soil Samples were collected from in and around Jetpur Common Effluent Treatment Plant. Samples were collected from different places, such as drainage canal that carry textile effluent to CETP located about

2 km far from dying industries, various stages of CETP. Samples were in the form of liquid untreated effluent, treated effluent. All the samples were collected in sterile glass-screw cap tubes and preserved at $4^{o}C$ in refrigerator.

- **Dyes:**

Azo dye Remazol black B were procured from local dye manufacturing unit GIDC, Ahmedabad. The main dye in this study Reactive Black 5 also known as Remazol Black B has H-acid core molecule with two vinylsulphone groups in its structure. All other chemical for preparing various media were purchased from Hi-media Pvt. Ltd. Mumbai, SRL India.

- **Chemicals:**

The Mineral Salt Medium (MSM) consisted of (g l^{-1}) $Na_2HPO_4 . 7H_2O$ 3.6; $(NH_4)_2SO_4$ 1.0; KH_2PO_4 1.0; $MgSO_4$ 1.0; $Fe(NH)_4$ citrate 0.01; $CaCl_2 . 2H_2O$ 0.1; and 10 ml of trace element solution per liter. The trace element solution contained (mg l^{-1}): $ZnSO_4 . 7H_2O$ 10; $MnCl_2 . 4H_2O$ 3; $CoCl_2 . 6H_2O$ 1; $NiCl_2 . 6H_2O$ 2; $Na_2MoO_4 . 2H_2O$ 3; H_3BO_3 30; $CuCl_2 . 2H_2O$ 1. The pH of the medium was adjusted to 7.

- **Physico-chemical property analysis:**

The collected effluent samples have been analyzed to determine its physico-chemical parameters. The various parameters viz., Temperature, pH, Colour, Odour, Total dissolved solid (TDS), Total suspended solids (TSS), Chemical oxygen demand (COD), Biological oxygen demand (BOD), Dissolved Oxygen (DO), Total Hardness, Chloride, Ca Hardness and Mg Hardness were analyzed in the laboratory by the standard protocol.

- **Isolation of dye decolorize bacteria:**

The bacterial isolates present in the effluent were isolated by serial dilution (Pour plate) technique. In this method, 1 ml of sample was thoroughly mixed with 9 ml of sterile N-saline and then it was serially diluted by standard procedure up to concentration of 10^{-6}. Then, 0.1 ml of serially diluted samples from each concentration of samples were transferred to MSM supplemented with glucose (0.5%) and yeast extract (0.5%) containing 50 ppm Reactive Black 5 dye and evenly distributed throughout the plates. The plates were incubated at 37°C for 24 hours. One uninoculated plate was kept as control. After incubation, the bacterial colonies were isolated and purified from the plates. The well grown bacterial cultures used for further screening technique and stored at 4°C.

- **Decolorization and growth measurement:**

At 0 hour and later on at the interval of the days, 3 ml sample had been harvested from flask containing MSM supplemented with glucose, yeast extract and dye. These samples were collected in centrifuge tube and centrifuge at 6000 rpm for 10 minutes and cell free supernatants were collected and analyzed for residual dye by determining absorbance at 557 nm of dye using UV-visible spectrophotometer. The pellets of bacterial cells were resuspended in 3 ml sterile distilled water and their absorbance was measured.

- **Assay of decolorization:**

Decolorization activity was stated in terms of % decolorization and was determined by observing the decrease in absorbance at absorption maxima (λmax) of respective dyes (i.e. 557 for Reactive Black 5). The culture suspension was centrifuged at 6,000 rpm for 15 min for removal of the biomass. The degree of decolorization of the tested dye was measured at its respective maximum absorbance wavelength using supernatant by UV-visible spectrophotometer. The decolorization assay was calculated according to the following formula.

Decolorization activity (%) = (A-B)/A x 100

Where A = initial absorbance

B = Observed absorbance

- **Spectral analysis:**

Spectral analysis of the dyes before and after decolorization was performed using UV-Vis spectrophotometer (Shimadzu UV-Vis 1601, Japan). The control and experimental (partially and completely decolorized) samples were scanned in the 300-800 nm range. The spectra of decolorized dyes were monitored for 0, 24, 48, 72 and 96 h for textile dyes and for laboratory dyes till 72 h against control spectra consisting mixture of dye and medium but without inoculation with seed culture.

3. Results and discussion:

- **Physico-chemical characterization of collected samples:**

Table -1: Physico - chemical characterization of textile dye effluent samples

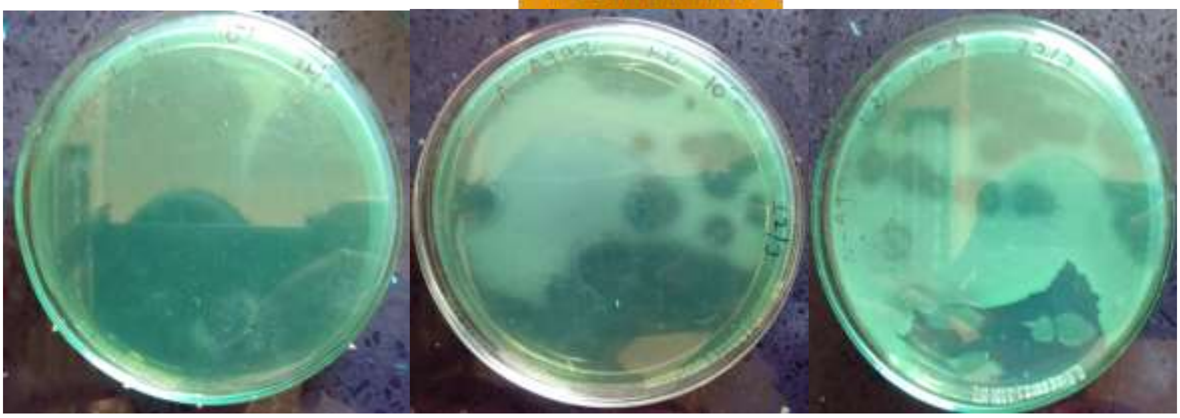
Sr. No	Name of the Parameters	Result
1	Temperature(^{0}C)	30-35^{o}C
2	pH	9.3
3	Color	Bluish black
4	Odor	Unpleasant
5	Total dissolved solid (mg/l)	2208
6	Total suspended solids (mg/l)	147
7	Chemical oxygen demand (mg/l)	813
8	Biological Oxygen Demand (mg/l)	202
9	Dissolved oxygen (mg/l)	10
10	Total Hardness (mg/l)	305
11	Chloride (mg/l)	1155
12	Ca Hardness (mg/l)	187
13	Mg Hardness (mg/l)	61.3

The samples were collected in sterilized container from sites. The color, temperature and pH of the sample were recorded on the site and samples were transported to the laboratory by storage at 4^oC. Other physico-chemical characteristics like BOD, COD, TSS, TDS etc. were measured on the same day of collection of sample as per table 1. The raw sewage was dark green in color because of the types of dyes generally used.

As the stages of treatment progressed, the color of effluents changed from greenish blue- blue- sky blue-brown. The green and bluish color of the incoming effluent is due to wide use of green and blue color dye in dyeing and printing industries, thus, it contributes more to the effluent’s color compared to other dyes. The light brown color of the finally released effluent after treatment may be due to the dirty water condition. The pH of the untreated effluent was 9.3, which reduced during treatment to near neutral 7.7.

- **Isolation of dye decolorizing bacteria:**

Sample was used for isolation of dye decolorizing bacterial cultures by enrichment culture techniques using MSM medium. After inoculation of serially diluted effluent sample on N-agar plates by spread plate method, 2 isolates has been observed which are potent dye decolorize.



Control **Black-1** **Black-2**

Figure 1: Dye decolorize bacterial isolates from textile effluent

- **Morphological characterization:**

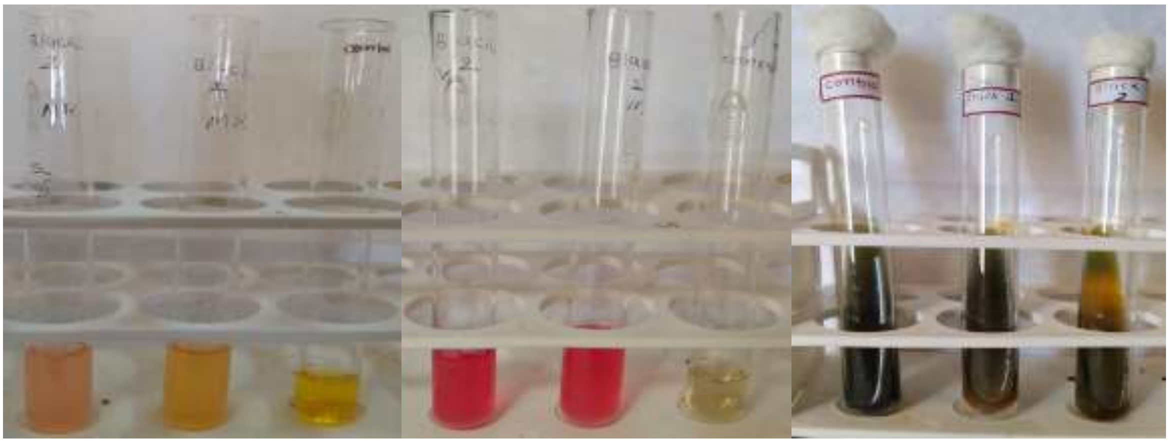
Table 2: Morphological characteristics of isolates were determined on N-agar plates and is shown.

Sr. No.	Shape	Size	Margin	Texture	Elevation	Opacity	Pigmentation
Black 1	Round	Small	Entire	Smooth	Raised	Opaque	Dirty White
Black 2	Round	Medium	Entire	Rough	Raised	Opaque	White

- **Biochemical tests:**

Table 3: Results of biochemical tests of the bacterial isolates

Sr. No.	Methyl red test	Vogues-Proskauer's test	Citrate utilization test	Oxidase test	Catalase test	Gelatin hydrolysis test
Black 1	Negative	Positive	Positive	Positive	Positive	Positive
Black 2	Positive	Positive	Positive	Positive	Positive	Positive



MR test **VP test** **Citrate utilization test**

Oxidase test **Catalase test** **Gelatin hydrolysis test**

Figure 2: Results of biochemical tests

- **Spectral analysis:**

Table 4: O.D. value for degraded dyes

Day	O.D. at 557 nm
1	0.256
4	0.199
6	0.171
10	0.167
13	0.137
17	0.103

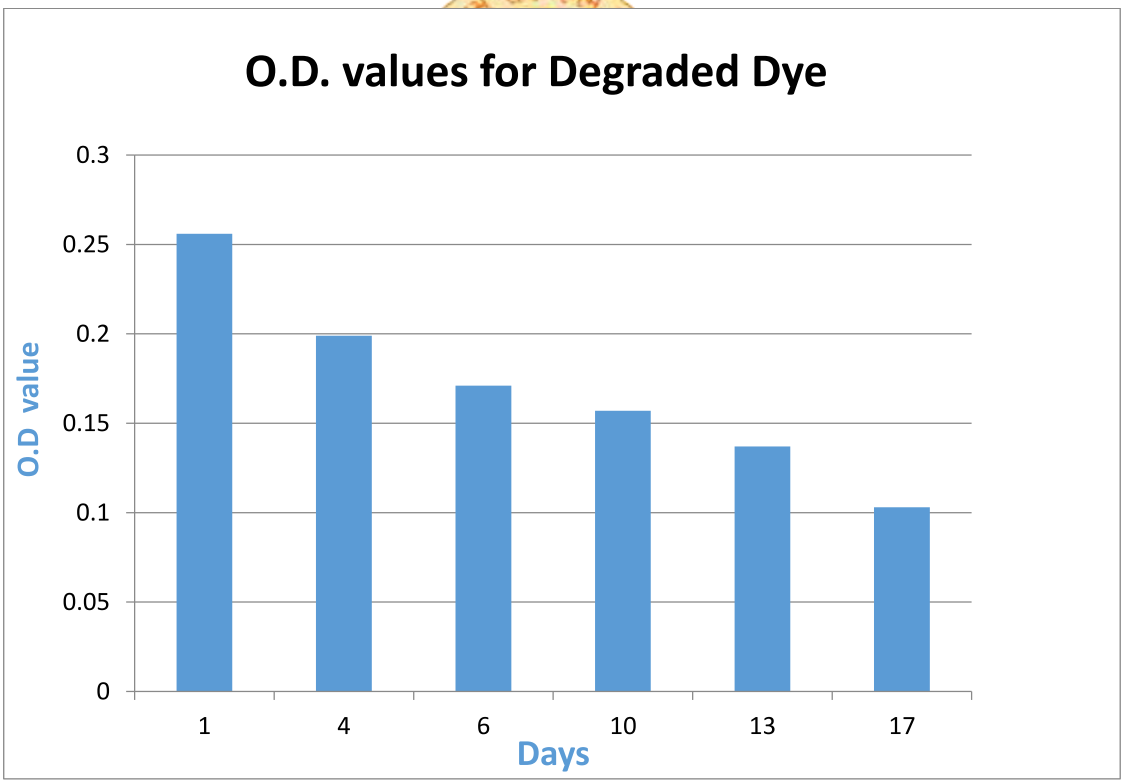


Figure 3: O.D value for degraded dyes

The percentage of degradation of Black dye by bacteria, were calculated and the values are shown in Table 3. It was found that the isolated bacteria were efficient decolorizers of Black dye (59.76%). The dye degradation percentage of *Enterobacter cloacae* complex sp. is shown in figure 3.

Table 7: Percentage of azo dye degradation

Day	% degradation of Black Dye
1	00.00
4	22.26
6	33.20
10	34.76
13	46.48
17	59.76

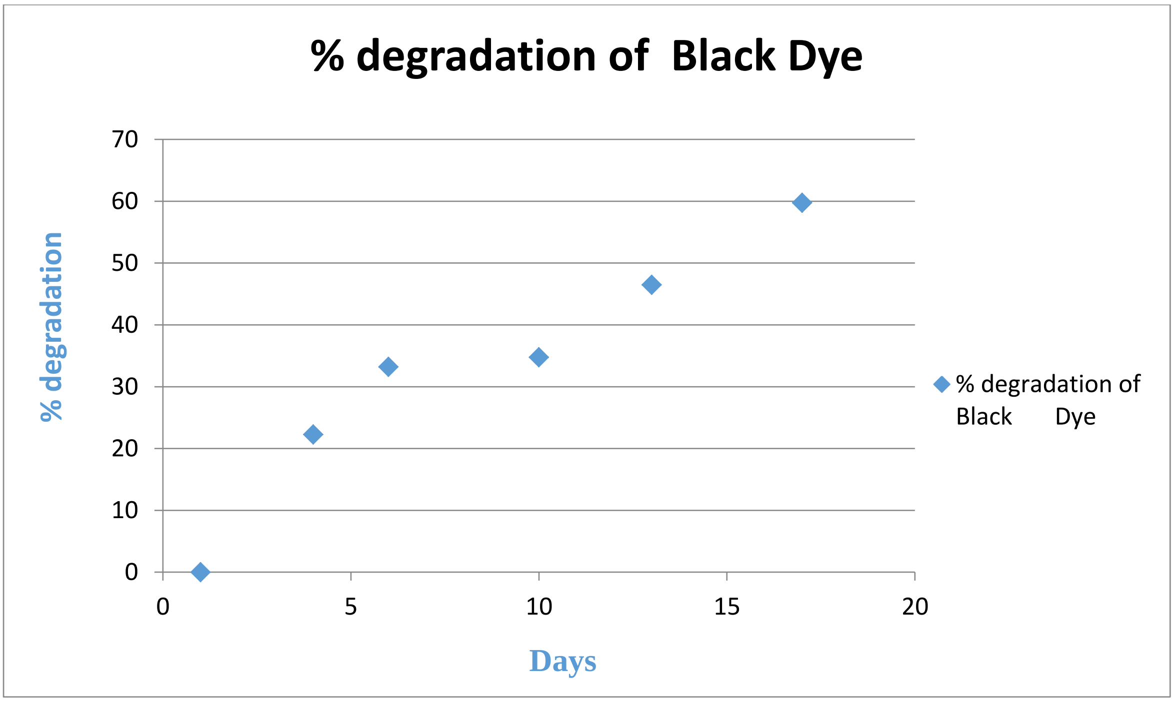


Figure 4: % Degradation of Black dyes

4. Conclusion:

Azo dyes are the largest and most widely used class of dyes, accounting for 50% of the dyes produced annually. Detoxification and disposal of sludge is a problem to textile dye units. Microbial treatment which involves enzymatic process was very promising for the degradation and detoxification of azo dyes. In the current study, the dyes which were used were Reactive Black 5. The maximum degradation was observed in Reactive Black dye. The percentage of dye degradation was about 59.76%. Further, this study confirms that Black dye was degraded by Enterobacter species. It shows the ability of Enterobacter species in degrading azo dyes present in the industrial effluents which are polluting aquatic life as well as equally harmful to human life and animals.

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