

Physicochemical Analysis of Textile Dye Effluent and Screening the Textile Dye Degrading Microbial Species

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Abstract: The dye degradation and decolorization processes, which include many physical and chemical methods having inherent drawbacks, like costing, economical unfeasible (require more energy and chemicals), unable to remove some of the recalcitrant dyes and production of large amount of sludge which if not properly treated, in turn can cause secondary pollution. So, biological degradation, being eco-friendly and inexpensive method, is considered as an effective method for the removal of toxic azo dyes. Our present study is therefore aimed to isolate azo dye decolorizing bacteria from dyeing industry effluent and to assay their dye decolorization capability in order to use them as an efficient bio agent for decolorizing and mineralizing toxic azo dyes. In this research we also tried to determine the physicochemical analysis of textile dye effluent. We tried to determine the BOD and COD of the collected effluent samples, which were found to range from 50 to 100 ppm and 200 to 400 ppm. Temperature of the three collected samples was found to vary from 26°C to 36°C and pH was in the range of 8.6 to 9.7. Total bacterial count was found within the range of 1.3×10^5 (CFU/ml) to 9.2×10^5 (CFU/ml). For the purpose of studying dye decolorization assay, twelve bacterial colonies were isolated on the basis of their unique colony features from the collected effluent samples and from them seven bacterial isolates were selected by further screening method and observed their degradation on Novacorn Orange C₃R dye.

Keywords: BOD, COD, Microbial Species, Novacorn Orange, Textile Dye.

I. Introduction

The utilization of manufactured chemical dyes in different industrial methods, including paper and mash producing, plastics, coloring of material, printing, leather treatment etc. has expanded significantly throughout the last few years, bringing about the arrival of dye containing mechanical effluents into the soil and sea-going environments. Since a large portion of these dyes are dangerous in nature, their vicinity in modern effluents is of major natural concern in light of the fact that they are typically extremely unmanageable to microbial degradation [1]. At times, the dye solution can likewise experience anaerobic debasement to structure possibly cancer-causing intensifies that can wind up in the food chain [2]. Besides, very colored waste waters can obstruct the entrance of daylight and oxygen, key for the survival of different sea-going structures [3]. Material commercial enterprises e.g. textile industries use significant volumes of water and chemicals for wet-handling of textiles. These chemicals, varying from inorganic components to polymers and organic products are utilized for scouring, fading, coloring, printing, and finishing [4].

Chemical pollutants present in textile wastewater are dyes containing various chemical substances including carcinogenic amines, toxic heavy metals, pentachlorophenol, chlorine-bleaching, halogen carriers, free formaldehyde, biocides, fire retardants and softeners [5]. One of the major classes of dyes used in textile industry are azo dyes and the use of azo dyes have been growing tremendously as of their ease and cost effectiveness in synthesis than natural dyes [6-7]. Around the world, more or less 10,000 separate dyes and pigments are utilized industrially and in excess of 0.7 million tons of synthetic dyes are produced every year. China, Korea and Taiwan are solid players in scatter dyes while India leads underway of reactive dyes because of availability of intermediates like vinyl sulphone in the nation (Bhimani, 2005). Azo (monoazo, diazo, triazo, polyazo), anthraquinone, phthalocyanine, and triarylmethane dyes are quantitatively the most important groups. The greater parts of the industrially accessible azo dyes are actually formulations of various components with a specific end goal to enhance the technical properties of the dyeing procedure (Palacios, 2009).

Use of synthetic dyes has an adverse effect on all forms of life. Presence of sulphur or vat dyes makes the textile effluent highly toxic. Other harmful chemicals present in the water may be formaldehyde based dye fixing agents, chlorinated stain removers, hydrocarbon based softeners, non-biodegradable dyeing chemicals. These organic materials react with many disinfectants especially chlorine and form by products (DBP's) that are

often carcinogenic and therefore undesirable [8]. Many of these show allergic reactions. The colloidal matter present along with colors and oily scum increases the turbidity, gives the water a bad appearance and foul smell and prevents the penetration of sunlight necessary for the process of photosynthesis. This in turn interferes with the oxygen transfer mechanism at air-water interface, which in turn interferes with marine life and self-purification process of water. This effluent if allowed to flow in the fields' clogs the pores of the soil resulting in loss of soil productivity. It allowed flowing in drains and rivers, it affects the quality of drinking water in hand pumps making it unfit for human consumption [9].

Textile dye effluents are complex, containing a wide variety of dyes, natural impurities extracted from the fibers and other products such as dispersants, leveling agents, acids, alkalis, salts and sometimes heavy metals. Dye removal strategies consist mostly of a combination of different techniques [9]. The majority of physical, chemical and biological color removal techniques work either by concentrating the color into sludge, solid supports, or by the complete destruction of the dye molecule. However, the goal of this study was to investigate the textile dye effluent and screening the textile dye degrading microbes.

II. Method And Materials

2.1. Sampling site

For the present study textile effluent samples were collected from 3 different sites of KDS Textiles Ltd. Unit- Fabrics and Dye, Oxygen, Chittagong.

2.2 Collection of samples

Effluent samples from three different selected sites of the textile mill were collected. During sample collection, sterile glass bottle, marker pen, field note book, polythene bags, thermometer, pH meter etc. were taken to the sampling sites for the purpose of sampling. Samples were collected from the discharge point from the textile. The time, date and location of sampling, pH, color of the samples were recorded carefully at the time of sample collection. The samples were taken in sterile glass bottles and were taken to the lab in a cooler box for proper preservation. Temperature of the samples collected from the selected industry was determined in the laboratory with a mercury thermometer immediately after collection of the samples. pH of the samples collected from the selected industries were determined in the laboratory with an electric pH meter (pH Hanna Instrument Ltd. & 3310, pH meter Jenway, UK) immediately after collection of the samples.

2.3.Determination of Biological Oxygen Demand (BOD) of the sample

For the determination of the BOD of collected samples, two bottles were filled with the sample. At first, the dissolved oxygen (D. O.) for one BOD bottle was calculated and the rest was remained in the dark condition for five days. After five days, the D. O. for the dark bottles was calculated. Then the BOD level of the sample was determined from the difference between the initial and final D.O.

Calculation:

BOD was calculated using the following formula:

$$\text{DO (mg/L)} = \frac{8 \times C \times V_B - V_A}{V_S} \times 1000$$

Where,

C= Normality of Sodium thiosulfate

V_A= Volume of initial titration amount of Sodium thiosulfate

V_B= Volume of last titration amount of Sodium thiosulfate

V_S= Volume of Sample

The final amount BOD (mg/l) = Initial D. O. – Final D. O.

2.4. Determination of Chemical Oxygen Demand (COD) of the sample

COD refers to the oxygen consumed by the oxidisable organic substances. The chemical oxidant such as K₂Cr₂O₇ or KMnO₄ is used to measure the oxidisability of the organic matter of water where the oxidants oxidize constituents. Then KI is added. The excess amount of oxygen reacts with KI & liberates iodine. The excess amount of oxygen is equal to the amounts of oxygen.

To determine COD of the effluent sample, 50ml of water was poured in a 100ml conical flask. Similarly 50ml distilled water was taken in a flask as control. 5ml K₂Cr₂O₇ solution was poured separately in both the flask. The flasks were incubated at 100⁰C for 1hr keeping in a water bath. There after the flasks were removed to cool for 10 minutes. 5ml KI solution & 10ml of H₂SO₄ solution was mixed in each flask. 0.1M Sodium thiosulphate solution was transferred in burette fitted in titration assembly & titrated with both the

sample in flasks till pale yellow color was disappeared. In each case the amount of sodium thiosulphate was noted. 1ml of starch solution was added to both flasks. The colour was turned blue. Again these were titrated with sodium thiosulphate till complete disappearance of blue color. The volume of sodium thiosulphate used was noted for the both samples.

Calculation

COD was calculated using the following formula:

$$\text{COD (mg/L)} = \frac{8 \times C \times V_B - V_A}{V_S} \times 100$$

Where, C= Concentration of titrant.

VB= Volume of titrant (ml) used for water samples

VA= Volume of titrant (ml) used for control

VS= Volume of water sample taken.

2.5. Determination of TS, TDS and TSS

250 ml effluent sample filtered through whatmannno. 44 filter paper. Then the filtrate was dried in a beaker at 105-120°C. After cooling, the residue was weighted and TDS in ppm was calculated.

For TSS, 250 ml effluent sample was taken in a beaker and evaporated to dryness on a hot plate. After cooling, the residue was weighted and TS in ppm was calculated.

Calculation

$$\text{TS (in ppm)} = \frac{\text{wt.of residue} \times 1000000}{\text{Amount of sample (ml)}}$$

$$\text{TDS (in ppm)} = \frac{\text{wt.of residue} \times 1000000}{\text{Amount of sample (ml)}}$$

$$\text{TSS (in ppm)} = \text{TS} - \text{TDS}$$

2.6. Media and techniques for enumeration and isolation of bacteria

Media used

Nutrient agar media were used for the enumeration and isolation of bacteria from the collected industrial effluents. pH of the media was adjusted to the pH of the collected samples.

Techniques employed

Three different techniques were applied for the enumeration and isolation of bacteria: dilution plate, pour plate and streak plate methods. For the enumeration and isolation, serial dilution was carried out. 1 ml of sample was taken into a conical flask containing 99 ml sterile distilled water and mixed well. The mixture was then used to prepare serial dilution. 1 ml of this suspension was transferred to 9 ml of sterile distilled water for tenfold (1: 9) dilution and further diluted up to 10⁶ dilutions. The inoculated media were incubated at 37°C for 24 to 48 hours.

2.6.1. Enumeration of bacteria

After incubation, the plates having well-spaced colonies were selected for counting. The selected plates were placed on a colony counter (Stuart Scientific U. K.) and the colonies were counted. The colonies or viable bacterial count per ml were calculated by multiplying the average number of colonies per plate by reciprocal of the dilution. The calculated results would be as colony forming units (CFU) per ml of sample.

2.6.2. Isolation of discrete colonies

Isolation of well discrete bacterial colonies was done immediately after counting. On the basis of colony morphology, different unique colonies were selected for isolation. Characters of the colonies were recorded as color, form, elevation, margin, surface etc. Then the marked observed colonies were transferred to nutrient agar slant for screening.

2.6.3. Screening and selection for dye decolorizers

Bacterial isolates from textile effluent were inoculated via stab inoculation into loosely-capped microbiological test tubes containing the screening medium, semi-solidified with 5.0 g l⁻¹ of agar. The medium used was a modified version of Hayaseet al., 2000 [10] and contained 2.34 g K₂HPO₄, 1.33g KH₂PO₄, 0.20 g of MgSO₄.7H₂O, 1.00 g of (NH₄)₂SO₄, 0.50 g of NaCl, 0.10 g of yeast extract, 1.00 g of glucose and 1.0 ml of trace element solution per liter, adjusted to the final pH of 7.0 with 3MNaOH and HCl. The trace element solutions contained 11.90 mg l⁻¹ of CoCl₂.6H₂O, 11.80 mg l⁻¹ of NiCl₂, 6.30 mg l⁻¹ of CrCl₂, 15.70 mg l⁻¹ of CuSO₄.5H₂O, 0.97 g l⁻¹ of FeCl₃, 0.78 g l⁻¹ of CaCl₂.2H₂O and 10.00mg l⁻¹ of MnCl₂.4H₂O. Bacteria from effluent samples were tested for their abilities to decolourise Novacron Orange C₃R.

III. Result And Discussion

Initially collected sample were analyzed for various parameters, those are Color, Temperature, pH, BOD, COD, TSS, TDS, TS, TVC (Total viable count).

Screening Results:

A total of 12 bacterial colonies were isolated from the collected effluent samples on basis of their colony morphology like size, shape, elevation, margin and color of the samples. Only one isolates were observed to significantly decolorize the dye novacorn orange C₃R in the screening test. The selected isolates were coded as I-2.

Morphological characters:

Various morphological properties i.e., size, shape and arrangement of the vegetative cells, as well as staining properties, i.e., Gram and Endosporing staining of the selected isolates were summarized microscopically in Table 04.

IV. Discussion

For this research work, the samples were collected from three different discharge locations of textile industry. The name temperature, pH and color of collected effluent samples were recorded and listed in **Table 01**. While BOD, COD, TDS, TSS and total bacterial count of the collected effluent samples are represented in **Table 02**.

The total bacterial count of the sample from first equalization tank was found to be 2.1×10⁵ (cfu/ml) after washing 2.5×10⁵ (cfu/ml), (cfu/ml) and after complete treatment 2.9×10⁵.

From the collected sample twelve bacterial colonies were isolated from three different effluent samples on the basis of their unique morphological and cultural characteristics. Out of these, one isolate was selected for further decolorization study on the basis of their dye decolorizing ability on screening media containing Novacron Orange C₃R by screening method, which is shown in **Fig.01** and the isolates were coded I-2. The selected isolate was observed microscopically. Size, shape and arrangement of the vegetative cells, Gram reaction, spore staining were observed under microscope after proper staining of the isolated bacteria. The isolates were Gram positive and spore former. Vegetative cells were coccus and short rod. Isolate I-2 was found to be short rod the rests were cocci. The aim of identification is to place the microorganisms into a specific class or group, so that characteristics of these unknown organisms can be compared with others. The morphological characters include size and shape, arrangement of the cells, presence or absence of spores, irregular forms, acid fast reaction, gram reaction etc. which in details are shown in **Table 04**

V. Figures And Table

Table 01: Color, temperature and pH of textile dye effluent

| | Color | Temperature | pH |
|----------------------------|--------------------------|-------------------|-----|
| Initial effluent pipe line | Blakish, Grey | 29 ⁰ C | 8.3 |
| Equalization tank | Grey | 28 ⁰ C | 8.4 |
| Treatment effluent | Cloudy, Colorless, Clear | 30 ⁰ C | 8.1 |

Table 02: Physicochemical and microbiological parameters of effluent of samples

| Parameter | Unit | Specification | Standard specification [DoE-GoB] | |
|-----------|------|---------------|----------------------------------|----------------|
| | | | In-land surface water | Irrigated land |
| BOD | mg/l | 411 | 50 | 100 |
| COD | mg/l | 1309 | 200 | 400 |
| TSS | mg/l | 95.59 | 50 | 200 |
| TDS | mg/l | 463.35 | 2100 | 2100 |

Table 03: Microbiological Parameters

| | | | |
|--------------------|------------|-------------------|-------------|
| Total Coliforms | CFU/100 ml | 2.1×10^5 | Not defined |
| Total viable count | CFU/100 ml | 3.9×10^3 | |

Table 04: Microbiological enumeration

| Isolate | Vegetative Cell | | Staining Properties | |
|---------|-----------------|---------------------|---------------------|--------------|
| | From | Arrangement | Gram | Spore |
| I-2 | Short rod | Single, pair, chain | Positive | Spore former |



Fig. 01: I-2 is the selected strain which is able to degraded the Novacorn Orange C₃R.

VI. Conclusion

In this Investigation, one isolate has been selected after screening for decolorization of textile azo dyes. As this is an issue of environmental pollution, the isolates can play an important role in the prevention of pollution. It can be concluded that the dye decolorizers can be a solution for the treatment of waste water and textile effluents which are alarming for the environment. At future studies, the characterization, optimization and molecular investigation of the azo dye degradation by the isolate can also give vital information in this field to solve the arising problem. We can also develop molecular biology technique and commercially can produce such enzyme to protect environment from the pollution.

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