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Biodegradation of Azo Dye (Congo Red) by Bacterial Isolate from the Textile Effluent Contaminated Soil

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ABSTRACT
In current situation, azo colors have grown largely popular than natural dye as a synthetic dye for colourants. The need for dye colorants in industry is growing, posing major health and environmental risks. Congo Red (CR) is an azo dye that has carcinogenic qualities and proposed into large variety in printing areas. This printing equipment remove large quantity of sewage water containing these colours, posing an environmental threat, environmental contamination. The dye degradation and decolorization processes, which employ a variety of physical and chemical strategies. The biological degradation is regarded a viable technique for eliminating nephrotoxic radical dyes. Bacillus subtilis, Aeromonas hydrophilia, and Bacillus cereus have been reported to have color removing activities. In the present research, at varied aggregations of CR dye (25 to 200 mg/L), red colour breakdown from the isolated bacteriological strain was observed. Soil sample collected from textile industry of surat. Soil sample having types of bacteria, soil sample inoculated in nutrient medium supplemented with the Congo red dye and then incubated. The dye degrading bacteria were isolated and the optimization was done for the growth of bacteria for degradation of dye. Optimization was done for different temperature, different pH and different dye concentration. According to the findings of this investigation, the bacteria Bacillus sp. might propose as an excellent bacterial weapon included in sewage management, particularly from the natural humiliation of printing color waste under oxidative circumstances and also use as commercially for removal of dye.
Keywords: *- Dye Degradation, Bacillus sp., Environmental pollution, Congo Red Dye*

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INTRODUCTION

Printing, colour, textiles, rubber products, paper, and pharmaceuticals are just a few of the industries that use dyes. Because of their complex chemical composition, artificial colors have more effective to decolorization. Around 280,000 types for printing colors have emitted in textile corporate waste per annual all over the universe [1]. Azo colors were identified from the appearance of azo bonds (-N=N-), and due to their simplicity of use and low cost, the description through equal to 70% of coloring matters used for printing processer. In their synthesis, they seek stability and cost-effectiveness. A greater range of colours is available than with organic colors. As a minimum 10% to 15% of which colour proposed in the process has released within the environment. Available areas as a source of wastewater providing a risk to the environment [2]. In 1856, English chemist William Henry Perkin created a black precipitate from aniline and found out reddish colour, whoever stained velvet quickly and has far further strong in visible wavelength rathe then any else (organic) reddish color at the time [3].

Even a little amount of dye hues, such as 10-50 mgL-1, can pollute the environment. Because azo dyes are made up of polyaromatic molecules, they are reactive dyes that are very water soluble, this is the case. Additionally, because the color in the wastewater is highly noticeable, it may pose a threat to photosynthetic aquatic plants by decreasing light penetration in water bodies [4]. A substantial amount of waste water is produced, with a bright colour, a high concentration of suspended particulates. Dyes are often synthetic and feature complex aromatic chemical structures, making them more lasting and

biodegradable. Pigments are coloured organic compounds that have the ability to transfer their colour to other substances, such as textile fibers. They can be natural or synthetic. When compared to natural dyes, artificial colors were widely proposed in printing fabric, printing of the paper, coloring of leather, color photographs, also in petroleum manufacturing due to that kind of manufacture, very resistance to sunlight, heating, laundry, and natural organisms, and colour types.

The treatment of dye wastewater often involves comprised and substantive processes like filtration of water, chromatography by using ions, absorbance, congelation, sedimentation, depositing and Fenton's oxidization. Decolorization and degradation by biological methods appear to have a number of advantages over chemical and physical procedures, including being ecologically benign, cost-competitive, producing less sludge, and being easier and more appealing to degrade all colours [5].

Bioremediation, which has evolved into a vital microbiological tool for dealing with a variety of contaminants, is a major research topic in environmentalism. A variety of bacteria, fungi, yeasts, algae, and actinomycetes have demonstrated the capability to decolorize azo dyes. Bacteria had importantly commonly used as biological agent for azo dye removal because they can reproduce quickly in aerobic, anaerobic, facultative, and harsh environmental conditions such as high salinity and extensive pH and temperature fluctuations. Heavy metals can be concentrated, accumulated, and absorbed by microbes within cells or cell walls [6].

The goal of this investigation was to see if various bacterial strains specified through textile effluent, soil, and water poisoning with petroleum oil may decolorize a Congo Red azo dye solution. In addition, the effects of several decolorization-related parameters were investigated.

MATERIAL AND METHODS

Collection of samples

Collection of soil sample was done from the Textile Industry, Surat, Gujarat, 394221. Dye degrading bacteria highly present in the textile waste material contaminant soil, therefore soil sample collection was done from this site.

Dye

Congo red is a diazo dye, or primary dye, which contains 2 azo groups. It's prepared by mixing two napthionic acid molecules with tetrazotised benzidine (4-aminonapthalene-1-sulphonic acid).

Enrichment and Isolation of Dye Degrading Bacteria

Enrichment

The soil sample collected from the fabric discharge polluted site was placed in a flask containing 50 ml nutritional media with 30 mg/L Congo red dye to increase the microbes present in the soil. The flask media was rotated at 180 revolution per minute after 3-4 days under 37 °C.

Isolation and Characterization of Dye Degrading Bacteria

The enrichment flask showed some signs of dye removal of dye. This culture was streaked on nutritional medium that included 20gl $^{-1}$ agar for solidification and 30 mg/L dye. The plates were incubated for 24 hrs. The basic morphological features of dye-degrading bacteria were identified and described.

Determination of Degradation by the Optical Density (OD)

To separate the bacterial cell mass, the culture was centrifuged for 5 min at 10,000 rpm at room temperature. The top layer was removed and separated, which was used for color removal and other investigations. The maximum removal process was identified at 600 nm and the absorbance of the resulting was measured using different wavelengths with in Visual Spectrophotometer.

The calculation of degradation number calculated by the formula given below

$$\% \text{ decolorization} = \frac{\text{primary absorbance rate - terminal absorbance rate}}{\text{Primary absorbance rate}} \times 100$$

[5].

Morphological and Biochemical Characterization

Morphological characterization

Gram's staining technique was used to characterize the morphology of the bacterial isolate from the 24-hour old bacterial culture.

Biochemical characterization

The plate containing the isolated bacteria from the textile waste was chosen and subjected to a series of biochemical tests, including the Indole test, Catalase test, Oxidase test, Methyl Red test, Voges-Proskauer test, Citrate Utilization test, Carbohydrate Fermentation for Sugars, Lactose, and Mannitol sugar, and the Triple Sugar Iron (TSI) test, to investigate the characteristic features of the isolate.

Optimization of Dye Decolorization

Effect of different Congo Red Dye Concentration on Decolorization

It was carried out to observe the effects of optimum dye concentrations degraded by bacterial isolate. The flasks with various dye concentrations of 50, 100, 150, and 200 mg/L and nutrient broth medium were inoculated by isolated bacterial culture. Further, they were incubated at 37°C for 24 to 48 hours at 180 revolutions per minute. The findings had been seen after the incubation period.

Effect of different pH on the decolorization

For the purpose of examining the effects of various pH levels on degradation, several pH levels were used (5, 6, 7, 8, 9, 10, 11, 12). Acidic and Basic solutions use for pH calibration. The isolated bacterial culture was inoculated and incubated for 24–48 hours at 37°C at 180 rpm/min in a medium containing nutrient medium added with the stain and controlled also with varied pH. The results had been seen after the incubation period.

Effect of the different Temperature

Temperatures of 25°C, 37°C, and 55°C were used to evaluate the effects of varying temperatures on deterioration. The flask containing nutrient media was dyed and inoculated with the isolated bacterial culture, which was then incubated at various temperatures for 24–48 hours at 180 rpm/min. The results have been seen after the incubation.

RESULTS AND DISCUSSION

Isolation of Congo Red Dye Degrading Bacteria

Bioremediation research has advanced after the discovery of a bacterial population and diversity in textile wastewater [8]. Bacteria and fungus, such as *Actinomycetes*, may break down azo colors [9]. The efficiency of degradation reduced when color increases concentrations due to the dyes poisonous for biomass either/rather because greater color efficiency requires larger biological intensity [10]. According to a previous study, when the dye concentration was raised, the efficacy of decolorization decreased and the time required for ultimate decolorization [11]. Textile dye manufacturing effluents are one of the most environmentally dangerous pollutants. In addition to impacting water quality, it has a deleterious influence on the microbiota and aquatic habitats. The dyeing industry has been identified as one that contributes to pollution.

Textile Industry, Surat was the dye wastewater polluted land. Colored effluent from the textile industry is discharged into the environment without being treated. For the enrichment of the soil organism first soil sample collected from the textile effluent added into the nutrient broth containing Congo red dye and incubated at 37 ˚C for 3-4 day at 180 rpm/min shaking condition. After incubation period bacterial growth was observed and it was found that bacterial was successfully able to degrade the dye of the medium (Figure 2).

The enriched bacterial culture was streaked on the nutrient plate supplemented with the agar and Congo red dye showed the decolorization on the plate (Figure 3). From the enriched soil medium bacteria which were able to degrade the dye isolated and used for the further analysis.

To test the rate of deterioration, the medium was centrifuged and then supernatant was utilised. The isolated bacteria from textile effluent polluted soil decolorized the Congo Red dye. The degradation rate of the dye increased day by day and after 4 days of incubation. However, the degradation was observed on the 1st, 2nd and 3rd day of inoculation with lower percent rate but the maximum dye decolorization was observed on 4th day with 73%. (Figure 4).

Morphological Characterization

By the use of isolated bacterium gram staining was done to identify the morphological characters. As shown if Figure 5, the isolated bacteria from the textile effluent soil is Gram positive, small in size, rod shape and single in arrangement.

Biochemical Characterization

The biochemical tests of bacterial isolate reveals that the bacterium is Indole, Methyl Red and Voges-Proskauer negative. However, catalase, citrate and oxidase positive. The fermentation tests reveals that bacteria is able to ferment sucrose and mannitol but unable to ferment lactose. The motility test showed that bacterial isolate is motile (Table 1).

Optimization of Dye Decolorization

To increase the dye degradation process by the organism, the temperature, pH, dye concentration, was optimized.

Effect of Temperature on Dye Degradation

To check the effect of temperature on the dye degradation activity by isolate, the isolate was inoculated with the congo red dye and incubated at various temperatures such as 25°C, 37°C and 55°C. The maximum decolorization was observed at 37˚C among all the temperatures which reveals that it is the optimum temperature for the growth of bacteria and degradation activity. Also, the bacterial growth and

degradation was observed at 25°C and 55°C but it was in very less amount may be due the metabolic inactivity of bacteria at these temperatures (Figure 6).

Effect of pH on Dye Degradation

To observe the effect of pH on bacterial growth and dye degradation, the bacterial isolate was inoculated into the various nutrient medium containing congo red dye and pH levels such as 5, 6, 7, 8, 9, 10, 11, and 12 respectively. The decolorization was noticed after the incubation period. As shown in Figure 7, It was observed that the isolate has a low degradation rate at low pH and a low very high pH value. At pH 6, the most decolorization was achieved. As a result, it may deduce that the bacterium isolated from textile waste is slightly acidophilic.

Effect of Dye Concentration on Dye Degradation

To identify the optimum concentration of dye degraded by the isolate, the dye-degrading bacteria from the soil sample was inoculated in the nutrient medium supplemented with varied Congo Red dye concentration (25, 50, 100, 150, and 200 mg/L). The medium was incubated for the specific period. After incubation period decolorization observed. At the low concentration of dye the decolorization was less but as the concentration was increased the isolate was able to degrade the higher concentration of dye. As shown in Figure 8, the maximum dye degraded by the isolate was 150 mg/L. The bacterium was able to decolorize the congo red dye upto 200mg/L. Therefore, we can conclude that the isolate is efficiently able to degrade the textile dye.

The bacterial isolate of waste water had shown to have the prospective to remove Congo Red color to the greatest extent possible, indicating that there are effective methods for treating textile effluent that reduce pollution in the environment.

Figure 1. The Sample Collection Site

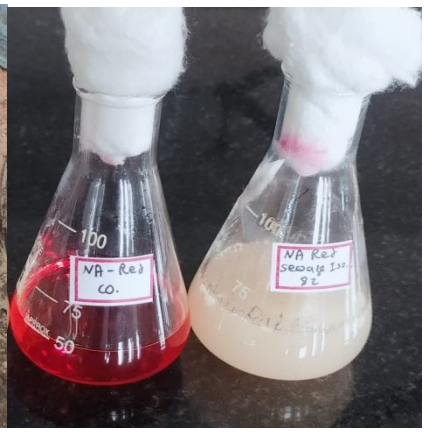

Figure 2. Dye Degradation in the medium

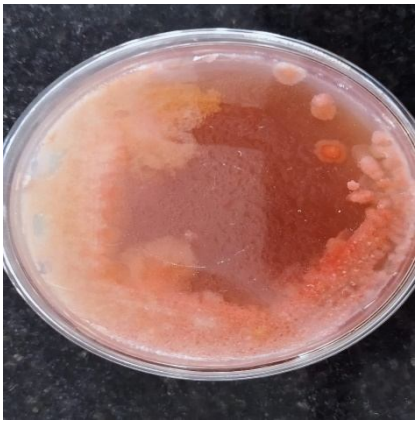

Figure 3. Bacteria isolated from waste water sample

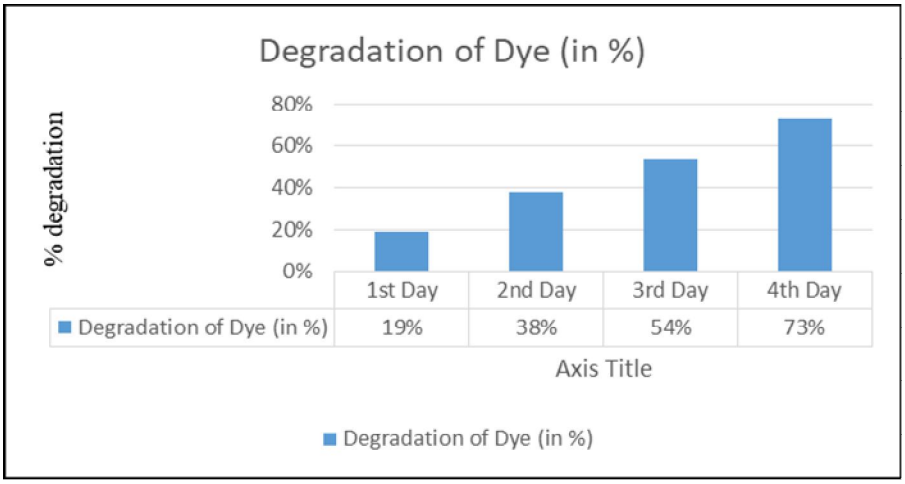

Figure 4. Percentage Decolorization observation

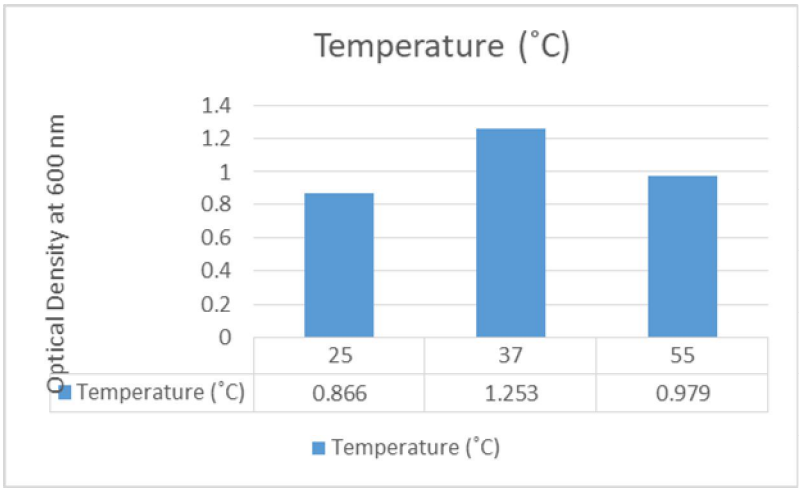

Figure *5*. Optimization of Temperature

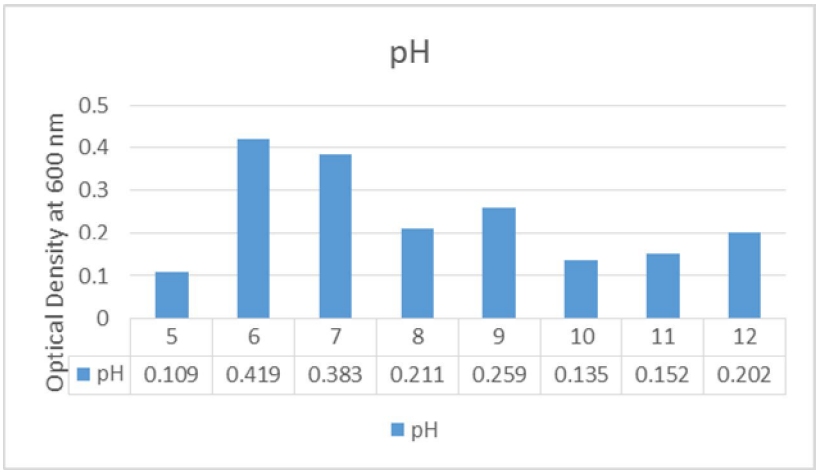

Figure 6. Optimization of pH

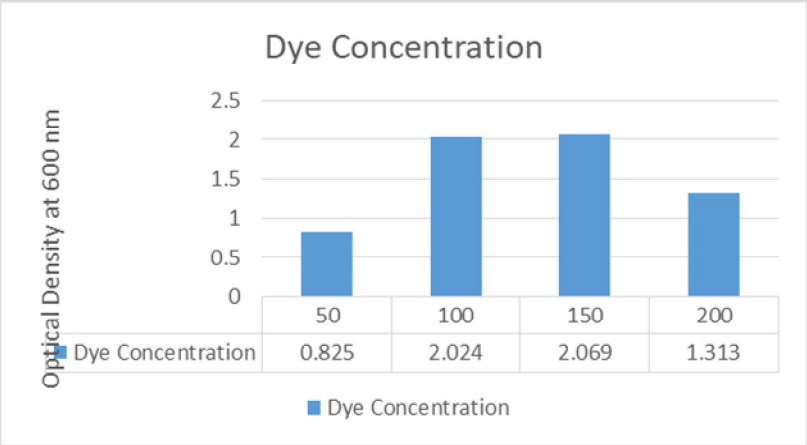

Figure 72. Optimization of Various Congo Red Dye Concentration

Table 1 : Showing the Biochemical test Results of isolated Bacillus sp.

Biochemical characterization	Results
Gram's staining	+
Size	Small
Shape	Rod
Indole Production	-
Methyl Red Test	-
Voges-Proskauer Test	-
Citrate Utilization Test	+
Triple Sugar Iron Test	+
Carbohydrate Fermentation (Lactose)	-
Carbohydrate Fermentation (Mannitol)	+
Carbohydrate fermentation (Sucrose)	+
Motility	Motile
Catalase Test	+
Oxidase Test	+

CONCLUSION

Textile effluent discharged into the natural environment without treatment causes concerns for the ecosystem, human, and other populations. As a result, we must treat the effluent before to disposal. Physical and chemical treatments are used for this, which are costly, time-consuming, and create a considerable volume of sludge. As a result, we must proceed with the biological therapy. In this work, we have isolated bacteria that can breakdown Congo Red dye and contribute to environmental cleanup. The species of bacteria, the content of the medium, and the physical conditions all influence the rate of degradation. It was deduced from the optimization results that the isolated bacterium showed the maximum rate of decolorization when incubated for 4 days at 37°C, pH -6. This research work give the contribution in clean the environment from the pollutants of the industry and other sources also.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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