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**Original Research Paper**

### **ENHANCED DECOLORIZATION AND DEGRADATION STUDY OF RED H8B DYE BY BACTERIAL ISOLATE R5**

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#### **ABSTRACT**

Azo dyes are largest class of synthetic dye, widely used in textile industries which are the major source of pollution. Azo dyes are toxic, carcinogenic and mutagenic. Therefore, azo dye decolorization has been of great research area nowadays. Several microorganisms have the ability to transform dyes to colorless products and can mineralize them. In present study, various environmental samples were used for the screening of dye decolorizing bacteria. Isolate shows highest decolorization was further studied to optimize various process parameters and nutrients components. The bacterial isolate showed maximum decolorization of azo dye Red H8B (95.83%) under static condition within 72 hrs. The optimum temperature and pH for the decolorization was found to be 30°C and pH-7 respectively. The TLC and FTIR analysis confirms the degradation of dye.

**Keywords:** Azo dyes, Biodegradation, Decolorization, Microorganisms, Red H8B DYE, Bacterial isolate R5.

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#### **INTRODUCTION**

Dyes are chromogenic substance that imparts color to the substrate or material. Dyes are classified as natural and synthetic. Natural dyes are obtained from natural sources such as animals, plants and minerals. Whereas, synthetic dyes are man – made. Dye consists of two groups Chromophore and Auxochrome. A chromophore group imparts color to the dye and chromogen without auxochrome cannot act as a dye. Dye are widely used to color the substrate like textile fiber, paper, leather, hair, fur, plastic material, wax, a cosmetic base and food stuff (Hanan, 2008; Zollinger, 1987). The first synthetic dye, Mauveine, was synthesized by William Henry Perkin in 1856. In 1868, Alizarin was the first natural dye to be produced synthetically and in 1880 indigo was synthesized (Zollinger, 1987). Most of the dyes used in textile industries are synthetic dyes and of which 60-70% constitute azo dyes. Azo dyes consists of aromatic ring linked by azo (–N=N–) bond and sulphonic

electron withdrawing groups making the compound difficult to be degraded by bacteria aerobically. Therefore, azo dyes are usually degraded under anaerobic conditions. The presence of dyes in receiving water bodies can be toxic to aquatic life. It reduces light penetration and thus affects the photosynthetic aquatic life because dye absorbs most of the light that the organism needs to survive (Stolz, 2001). Physicochemical methods can be used to treat dye containing effluents but these methods are costly, produces large amount of sludge and results in byproduct that can be hazardous. Therefore, Biological method that is quite effective and eco-friendly can be used as an alternative option (Shukla and Patel, 2012). In present study, various soil samples from contaminated industrial sites were utilized for the screening of dye decolorizing bacteria. The isolated bacteria were further utilized for the dye decolorization study.

The degraded product of the dye was further analyzed by TLC and FTIR analysis.

## **MATERIALS AND METHODS**

### **Dye and Media**

Dye such as Orange HR, Yellow H4G, Red Brown H4R, Golden yellow HR and Red H8B were collected from Narayan Processor Pandesara, Surat. All media components and chemicals used in the study were of high purity and analytical grade.

### **Sample Collection**

Soil sample was collected from contaminated site of Aarti Chemical waste effluent, Garage soil, Garbage soil and Petroleum soil, Vapi, Gujarat (India).

### **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) glucose 5.0, 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 into 250 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 as 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 hrs. The pure culture is then stored in refrigerator at 4°C and subcultured periodically.

### **Decolorization Experiment**

#### *Inoculum Preparation*

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

### *Dye Decolorization Study*

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

#### *Analytical Method for Dye Decolorization Study*

Decolorization was quantitatively analyzed by measuring the absorbance of the supernatant at maximum absorption wavelength,  $\lambda_{max}$  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).

### **Optimization of Process Parameters for Decolorization Study**

For decolorization study, Reactive Red H8B dye was selected as reference dye. Decolorization of dye was done using bacterial isolate R5 as reference culture. Various process parameters such as carbon source, nitrogen source, pH, temperature, medium and incubation period were optimized. Decolorization study was done at 520nm.

#### *Effect of Incubation Period on Dye Decolorization Process*

In present study the effect of incubation period was determined, In a 250 ml Erlenmeyer flask with 100 ml of MSM medium and 100 ppm dye. The flasks were inoculated with 1% inoculum and incubated at 30°C under static condition. A sample of 5ml was withdrawn from the inoculated medium at different incubation periods (0 to 96 hrs) and subjected to centrifugation at 5,000 rpm for 20 min and supernatant was used for the determination of decolorization.

#### *Effect of pH on Dye Decolorization Process*

The effect of pH on dye decolorization was studied by adjusting medium pH in the range of 4 to 9. The respective flasks were inoculated with 1% inoculum, 100 ppm dye and incubated for 72

hrs under static condition at 30°C. The samples were withdrawn after 72 hrs of incubation, centrifuged at 5,000 rpm for 20 min and percent decolorization was determined.

#### *Effect of Temperature on Dye Decolorization Process*

The effect of temperature was studied by incubating inoculated medium at a temperature range of 15°C to 50°C. The samples were withdrawn after 72 hrs of incubation, centrifuged at 5,000 rpm for 20 min and percent decolorization was determined.

#### *Effect of Carbon Source on Dye Decolorization Process*

The decolorization was done by taking different carbon sources such as sucrose, glucose, fructose, maltose, lactose, and starch in the medium. The respective flask were then inoculated with 1% inoculum, 100 ppm dye and incubated for 72 hrs under static condition at 30°C. The samples were withdrawn after 72 hrs of incubation, centrifuged at 5,000 rpm for 20 min and percent decolorization was determined.

#### *Effect of Nitrogen Source on Dye Decolorization Process*

Nitrogen such as peptone, ammonium sulphate, urea, ammonium chloride, yeast extract and beef extract were used to determine the effect of various nitrogen sources on dye decolorization process. The decolorization flask with respective nitrogen source in MSM medium was inoculated with 100 ppm dye and 1% inoculum and incubated for 72 hrs under static condition at 30°C. The samples were withdrawn after 72 hrs of incubation, centrifuged at 5,000 rpm for 20 min and percent decolorization was determined.

#### *Effect of Different Concentrations of Dye on Decolorization Process*

To determine the efficiency of dye concentrations on decolorization by the bacterial isolate. The decolorization experiment was carried out by inoculating the medium with different concentration of azo dye Red H8B (100 ppm, 200 ppm, 300 ppm, 400 ppm, 500 ppm, and 600 ppm) with 1% inoculum which were then incubated for 72 hrs at 30°C under static condition. The

samples were withdrawn after 72 hrs of incubation, centrifuged at 5,000 rpm for 20 min and percent decolorization was determined.

### **Degradation Analysis**

#### *Sample Preparation*

The decolorized broth obtained by decolorization process was centrifuged at 5,000 rpm for 20 min and the supernatant obtained was dried in oven at 60°C. The dried powder of dye degraded product was used for further degradation analysis.

#### *Thin Layer Chromatography (TLC)*

The control dye (Original dye) and degraded product was dissolved in distilled water to obtain 100 ppm concentration. Silica gel 60 F<sub>254</sub> was used in thin layer chromatography to confirm biodegradation of the Reactive Red H8B dye from the collected supernatant. Propanol: ethyl acetate: distilled water in 6: 1: 3 proportions were used as a solvent system for the TLC.

#### *Fourier Transform Infrared Spectroscopy (FTIR)*

The biodegradation of Red H8B dye was further characterized by FTIR spectroscopy. The FTIR analysis was carried out in the mid IR region of 400-4000 cm<sup>-1</sup>. The control and degraded dye samples were mixed with spectroscopically pure KBr in the ratio of 5:95 to form a uniform pellets, which was then fixed in sample holder of FTIR spectrometer, and the analysis was carried out.

## **RESULTS AND DISCUSSION**

### **Isolation and Screening of Dye Decolorizing Bacterial Isolates**

Various samples from Aarti Chemical effluent, Garage soil, Garbage soil and Petroleum soil was used to isolate dye decolorizing microorganisms. A total of 14 bacterial strains were isolated and purified by subculturing on nutrient agar plates. All the isolated cultures were studied for dye decolorization on plate assay and liquid medium. The bacterial isolate R5 shows maximum decolorization (75.69 %) in liquid medium at 30°C after 72 hrs of incubation under static condition. The isolate R5 was found to be gram negative and motile giving greenish pigmentation on nutrient agar plate and shows slimy growth.

## Optimization of Cultural Condition for Dye Decolorization Study

### *Effect of Incubation Period on Dye Decolorization*

The decolorization of Red H8B was determined at various incubation time (i.e.0, 24, 48, 72 and 96 hrs). The result obtained shows that decolorization was increased as incubation time increased and maximum decolorization was obtained at 72 hrs of incubation (87.33%), however, further incubation of decolorization flask does not enhances the decolorization process. Neelam and Rao, (2013) reported maximum decolorization (92.63%) of Remazol Red dye by isolate IB3 within 72 hrs of incubation.

### *Effect of pH on Dye Decolorization*

The decolorization study of dye was determined at a pH range of 4-9. As the medium pH increases from pH 4 to 7.0, the extent of decolorization was increased and maximum decolorization obtained at neutral pH-7.0 (88.76%). Above pH-7 decolorization decreased gradually. Prasad and Rao, (2014) reported maximum decolorization of azo dye Acid Black-24 was about 90% at pH-9 by *Bacillus halodurans*. Pandey and Dubey., (2012) reported that the optimum pH for decolorization of Reactive Red BL by *Alcaligenes* sp. AA09 was pH-7.0.

### *Effect of Temperature on Dye Decolorization*

The dye decolorization experiment was performed in a temperature range of 15°C to 50°C. The result obtained shows that as the incubation temperature increases from 15°C to 30°C, the % decolorization was increased and maximum decolorization was obtained at 30°C of incubation temperature(89%). However, incubation of decolorization flask above 30°C results in decreased decolorization process. Birmole *et al.*, (2014) reported maximum decolorization of 78% of Reactive Blue-172 by *Shewanella haliotis* DW01 at 35°C in 12 hrs. Prasad and Rao, (2014) reported optimal temperature for decolorization of azo dye Acid Black-24 by *Bacillus halodurans* was 37°C.

### *Effect of Carbon Source on Dye Decolorization*

The decolorization study involved the use of different carbon sources such as sucrose, glucose, fructose, maltose, lactose and starch on decolorization process. Maximum decolorization obtained when glucose (89.67%) was used as sole source of carbon in MSM medium. Decolorization flask supplemented with fructose (50.92%) and sucrose (70.48%) also shows comparable results. Whereas, starch showed least decolorization (17.56%) when used as sole source of carbon in MSM medium. Sahasrabudhe and Pathade., (2011) showed minimum decolorization in the presence of glucose (42.58%) and maximum decolorization in presence of starch (94.98%). Pavani, (2014) reported sucrose as best carbon source exhibiting maximum decolorization of 85%.

### *Effect of Nitrogen Source on Dye Decolorization*

The decolorization process was also studied using different nitrogen source on decolorization medium. Maximum decolorization (93.73%) was obtained when urea was used as nitrogen source in decolorization medium. The other nitrogen sources such as Ammonium chloride, Ammonium sulphate, Yeast extract, Beef extract and Peptone showed 87.02%, 92%, 40.88%, 55.08% and 30.45% of decolorization, respectively. Sahasrabudha and Pathade., (2011) reported urea exhibiting less decolorizing ability. Pavani, (2014) reported peptone as best nitrogen source for decolorization process.

### *Effect of Dye Concentrations on Dye Decolorization*

The decolorization was studied at different dye concentration. The result obtained shows that as the dye concentration increased, the decolorization process was decreased gradually. Maximum decolorization (95.83%) was obtained at 100 ppm dye concentration within 72 hrs of incubation. However, the decolorization was above 85% in all the dye concentration tested (100 ppm to 600 ppm). Birmole *et al.*, (2014) showed that maximum percent decolorization (88%) was achieved at 100ppm of Reactive Blue-172 by *Shewanella haliotis* DW01. Pandey and Dubey, (2012) showed maximum decolorization

of azo dye Reactive Red BL at 50 ppm in 48 hrs by *Alcaligenes* sp. AA09.

## Degradation Analysis

### Thin Layer Chromatography (TLC) Analysis

The degradation of azo dye study was further characterized by TLC analysis using Propanol: ethyl acetate: distilled water in 6: 1: 3 proportions as a solvent system. The control (Red H8B) dye sample shows single band on TLC plate with Rf value of 0.89. The decolorized sample shows 3 bands with Rf value of 0.85, 0.89 and 0.96 respectively which may support the degradation pattern of Red H8b dye when exposed to R5 bacterial isolate. Gahlout *et al.*, (2013) reported four different spots with Rf values of 0.90, 0.85, 0.73 and 0.27 of degraded sample as compared with Rf value (0.93) of original dye (Reactive violet 1) when exposed to *G. cupreum* AG-1.

### Fourier Transform Infrared Spectroscopy (FTIR) Analysis

The FTIR spectrum of control dye compared with degraded product is shown in figure 9a and 9b. In case of control dye, peak around  $1469.88\text{ cm}^{-1}$  and  $1411.44\text{ cm}^{-1}$  assigned to (N=N) azo bond vibration and azo linkage on aromatic ring. The peaks at the region of  $1207.29\text{ cm}^{-1}$  and  $1047.54\text{ cm}^{-1}$  assigned to CO, CN or phenolic C-O

vibration. The FTIR spectrum of degraded structure product displays a peak at  $1639.64\text{ cm}^{-1}$ , which indicates the formation of C=O group. The peak generated at  $1138.17\text{ cm}^{-1}$  represents C-O-C stretching. The FTIR of degraded product does not have any peak in the region of  $1500\text{ cm}^{-1}$  and  $1400\text{ cm}^{-1}$ , which indicates loss or degradation of azo bond by bacterial isolate. Similar results have been observed other researchers (Jadhav *et al.*, 2007; Kumar *et al.*, 2010; Gahlout *et al.*, 2013).

## CONCLUSIONS

The present study reveals that the isolate R5 showed consistent decolorization and degradation of textile dye (Red H8B) 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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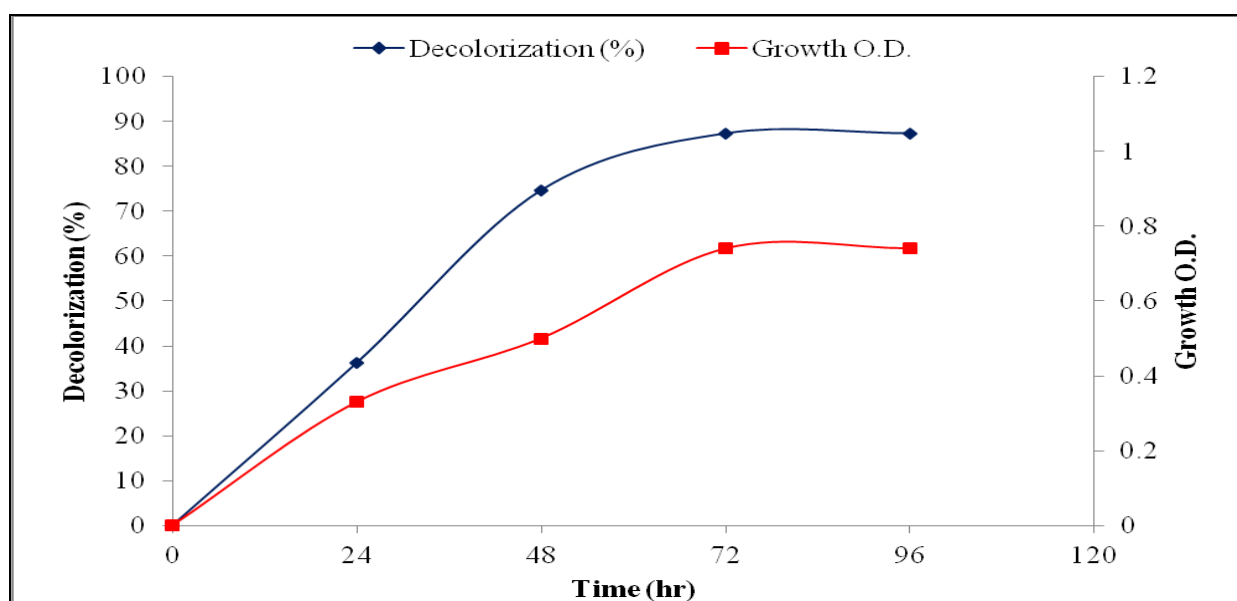


Figure 1: Time course study

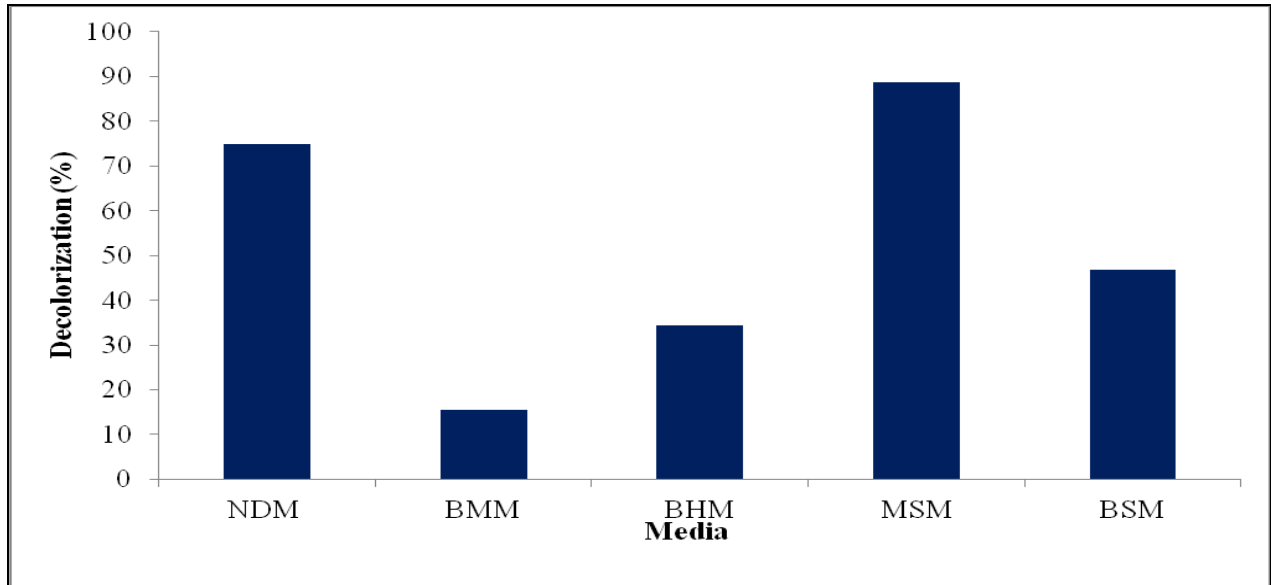


Figure 2: Effect of medium composition on dye decolorization

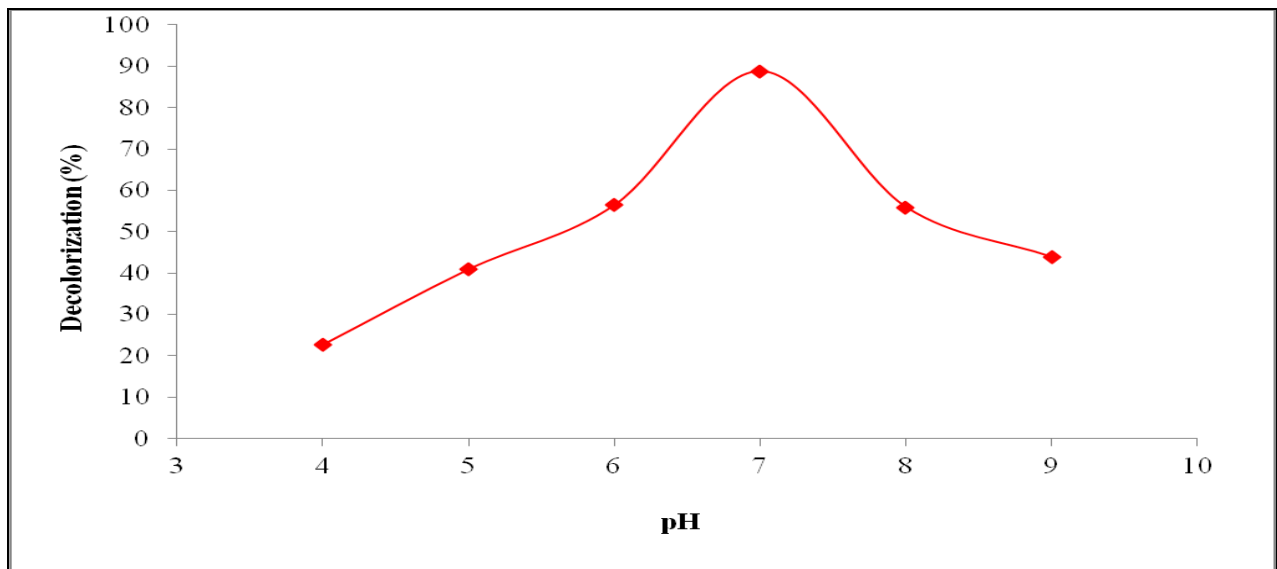


Figure 3: Effect of pH on dye decolorization

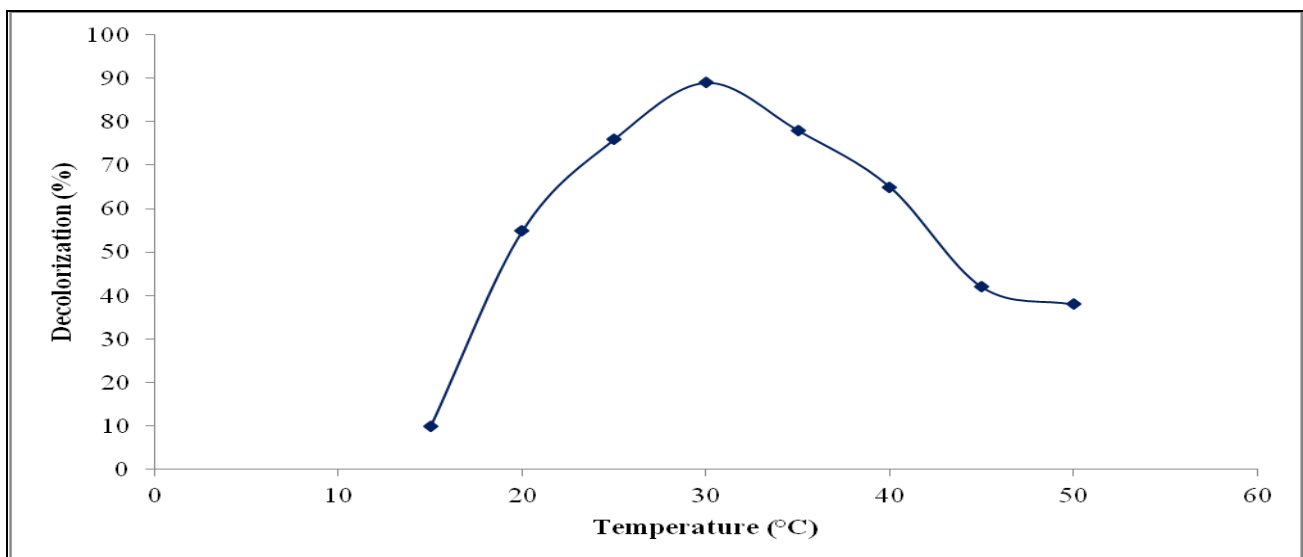


Figure 4: Effect of temperature on dye decolorization

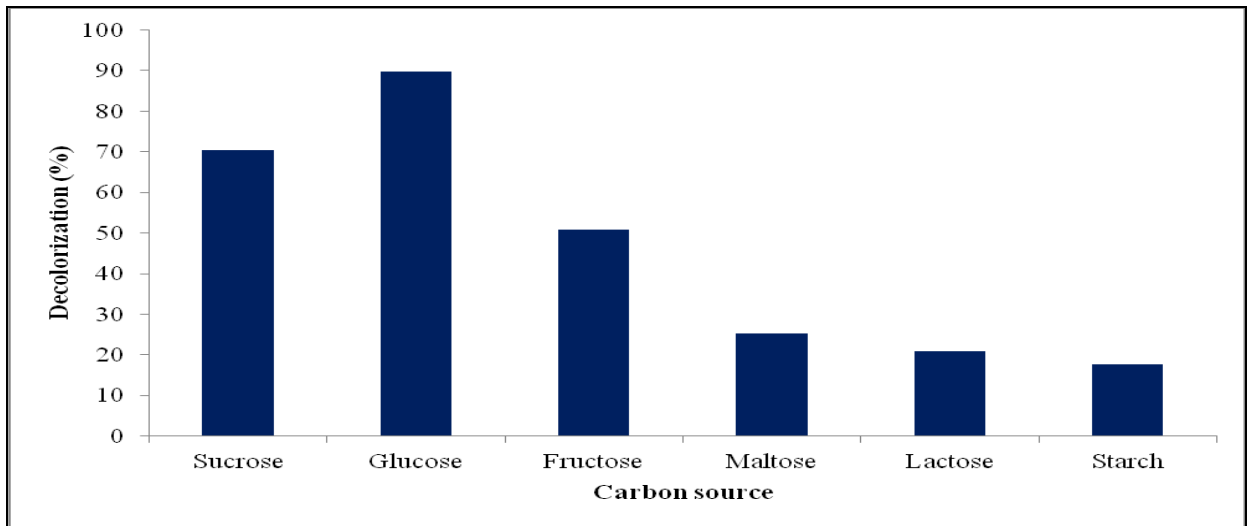


Figure 5: Effect of different carbon source on dye decolorization

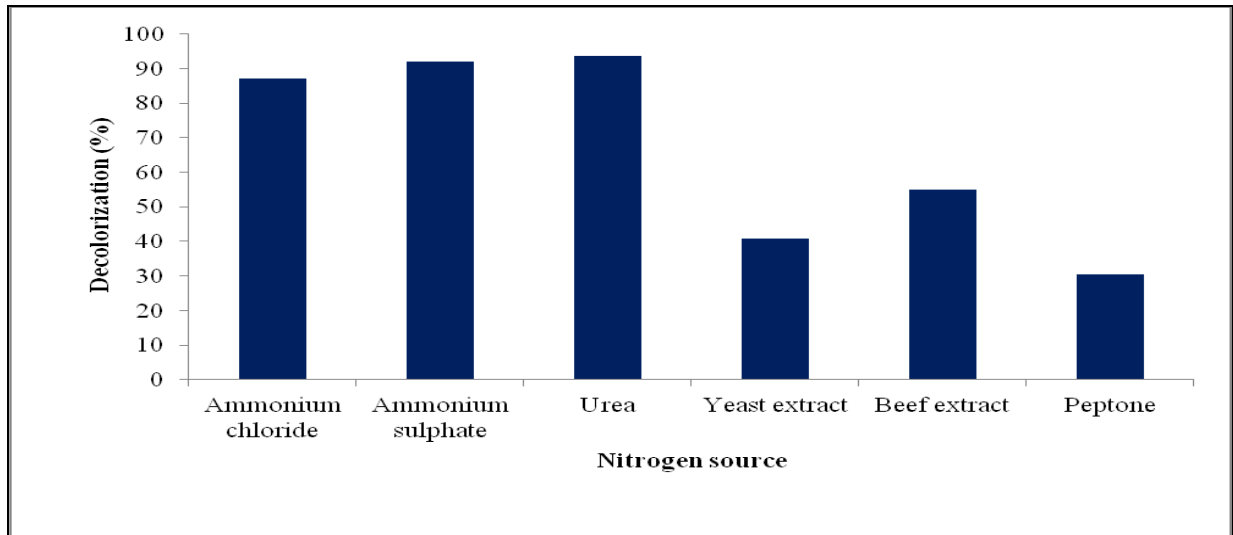


Figure 6: Effect of different nitrogen source on dye decolorization

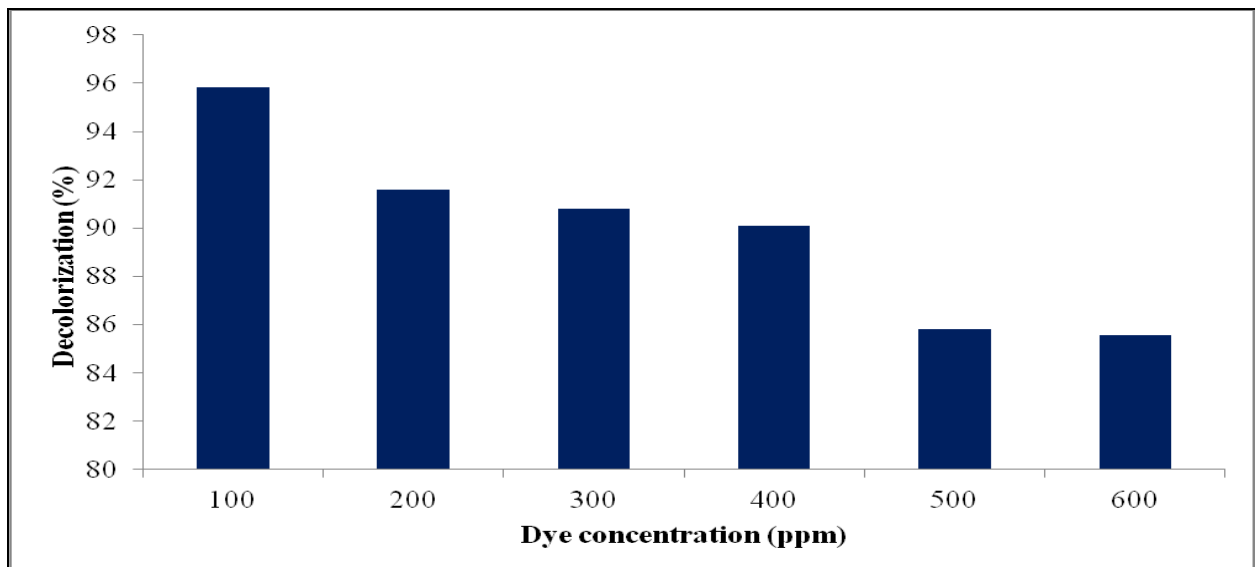


Figure 7: Effect of dye concentrations

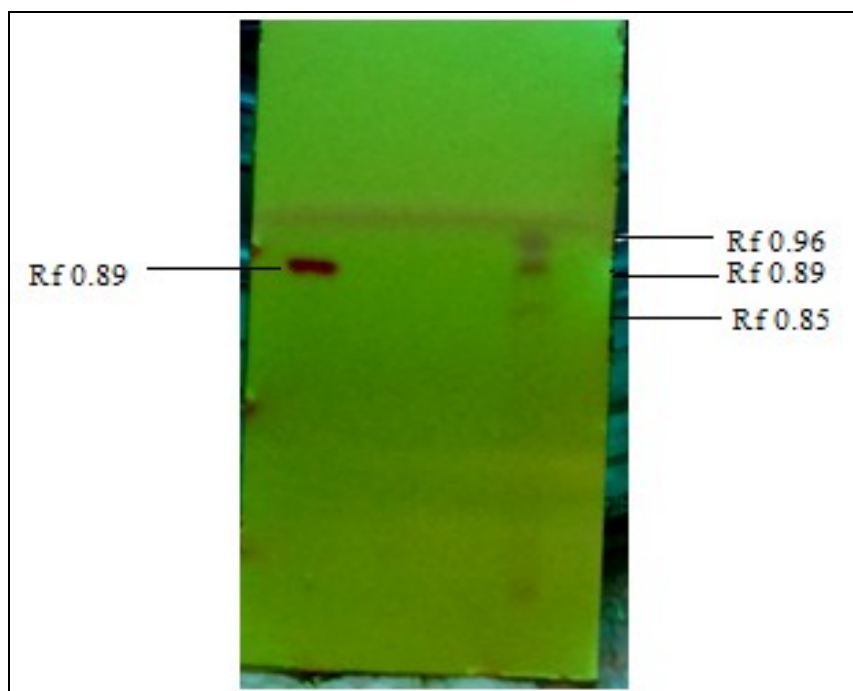


Figure 8: Degradation of Red H8B dye by TLC analysis

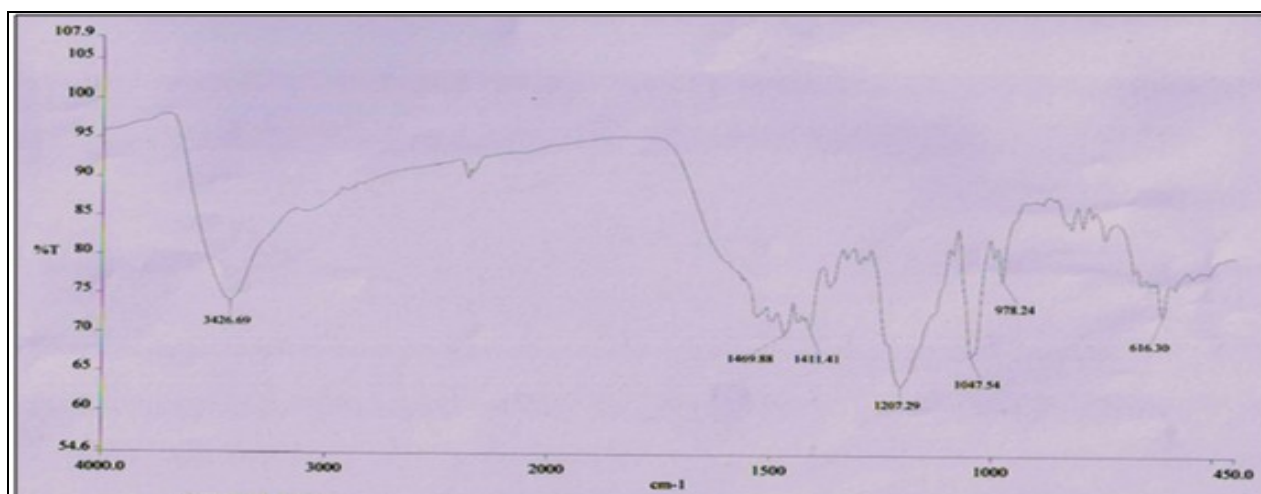


Figure 9a: FTIR analysis of Red H8B dye Control

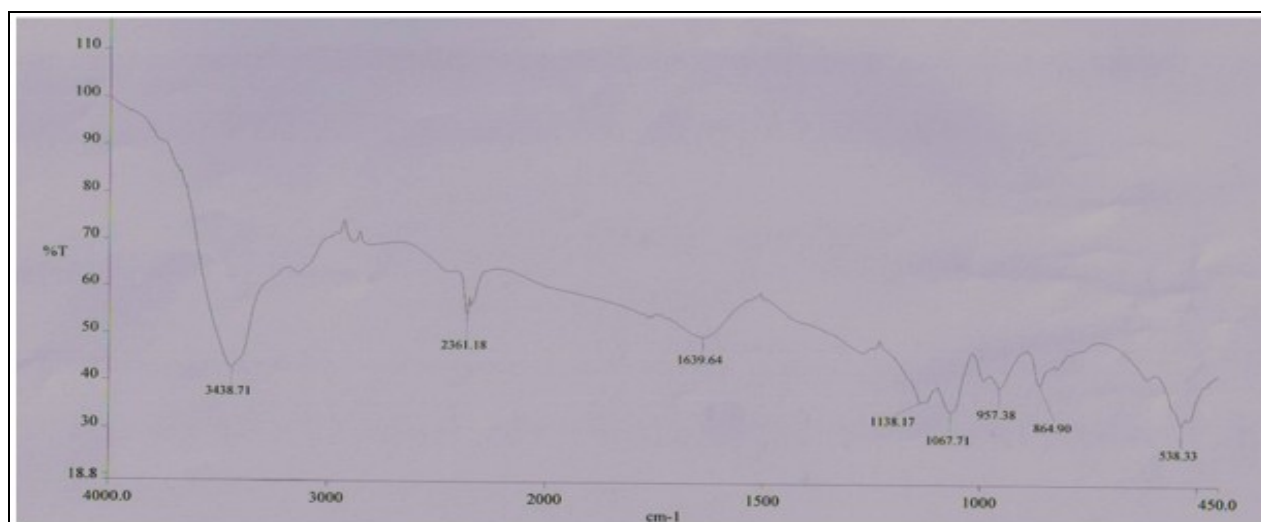


Figure 9b: FTIR analysis of degraded product by isolate R5



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