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# CHARACTERIZATION AND BIODECOLORIZATION OF AZO DYE USING *BACILLUS KOCHII* MH152512 FROM TEXTILE DYE EFFLUENT FROM TAMIL NADU

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**ABSTRACT**

**The present investigation was focused to decolorize the dye effluent by bacteria. The isolated bacterium was identified as *Bacillus kochii* MH152512 on the basis of 16S rDNA analysis. Several steps were initiated in treating the dye effluents and the degradation activity of these effluents by *B.kochii* was determined by the physical and chemical property of the effluent. This strain was identified to show maximum decolorization as compared to other bacterial strains isolated from the effluent. The *B. kochii* strain was investigated for various parameters such as effect of pH, temperature along with carbon and nitrogen sources as supplement nutrients. The concentration of the dye degraded activity of the effluent was confirmed using UV- spectrophotometer, which is recorded continuously for 3 days. Complete decolorization was observed at pH 6 and at temperature 37°C. Phytotoxicity studies revealed that there is a reduction in toxicity of the decolorized dye by bacteria. Also cleavage of bond after decolorization was confirmed using FT-IR. Then the medium optimization was done through Response Surface Methodology, in which optimum pH was obtained at 6 and temperature at 37°C and then the model was predicted through Box-Behnken method.**

**KEY WORDS :** *Bacillus kochii,* UV-Spectrophotometer, FT-IR, Response Surface Methodology and dye degradation.

## INTRODUCTION

Textile industries are blooming almost everywhere in the world. The reason behind is, in 1856 the first synthetic dye was discovered by mauevin and because of which more than 10,000 dyes were generated now (Asad *et al.,* 2006). On the upcoming years it has been noted that there is an increased industrialization such as textile, paper, plastic, pharmaceutical and leather industries which utilizes huge amount of dyestuffs. The growth and development of those industries had uplifted the overall growth of Country's Economy. In India one fourth of the textile industries cover the western parts of the Tamil Nadu. The major drawback of these industries is the discharge of synthetic dyes to the environment. Even though after the proper treatment of dye effluent, some of the dyes namely azo dyes get mixed with the water. The major cause is that during the process in dying some dyes tend not bind to fibers and releasedinto the water (Zouringer, 1991; Abdulla *et al.,* 2000). A certain kind of industrial activities affects the aquatic lives. The fact is that nearly annual consumption of this industrial waste accounts for about 0.7 million tons were reported. Even though textile industries follow the standard effluent treatment procedures, some of the textile dyes are discharged to the ecosystem. The reason is that 90% of textile azo dyes are released during the process as the dye can't able to bind with

the fiber and enters into the waste water. The recent survey says that nearly millions of tons of colored dyes are produced in textile industry as well as dying industries. The reason behind this is untreated dye contains several aromatic compounds known as mutagens. These are carcinogenic to the human being. Hence the reason for the complexity of dye degradation is due to the strong nitro amine triple bond. One of the major reasons behind the depletion of aquatic form is due to the accumulation of large amount of these coloring dyes which in turn decreases the photosynthetic activity (Cralieu *et al.,* 1995). Hence several steps has been developed to trounce such problems in a economic way in which biological method is applicable in many cases as compared to the chemical way of treatment (Chen *et al.,* 2014). Dyes are considered to be most stable to light, heat and some of the chemical oxidant (Zumriye *et al.,* 2005). Noyyal is an Indian river in the western Tamil Nadu in Tiruppur. This river is the major source for the agriculture activities. But at present scenario the condition of this river is fully polluted as it is surrounded by chemical and textile industries (Tezer *et al*., 2005). The population density has been mainly affected by the polluted dye effluent (Shashi *et al.,* 2017).

Several textile industries in India as well as all over the world utilize azodyes as their ease since it is cost effective during its synthesizing process. The major challenges are to degrade the textile dyes using bacteria. It has come to known that microorganisms such as actinomycetes, fungi and bacteria plays a major role in decolorizing these dyes (Saratale *et al.,* 2010). Economically certain steps can be taken to degrade these dyes by chemical or physical-chemical treatment methods. Experimentally, this issue can be solved by some of the physical-chemical analysis such as Chemical oxygen demand, biochemical oxygen demand, total dissolved solids and by total suspended solids (Sathian *et al.,* 2013, Ansari *et al.,* 2006; Rajeshkannan *et al.,* 2015). Biological method of bacterial species has the capability of decolorizing the metal dyes and Nitro aromatic dyes. The present study focuses on isolating the new bacterial species and to decolorize those dyes with the isolated bacteria. A fact that bacterial species are capable of degrading the dairy effluent,promised that bacterial species contributes in removing dye. In this paper various biological and physical methods of degrading dye are discussed. It has been noticed most of these dyes contain aromatic groups therefore some of the bacterial species has the tendency to break such groups present in dye effluent (Vishal *et al.,* 2009).

## MATERIALS AND METHODS

### Sample collection

Effluents samples were collected from the river of Noyyal, where it is surrounded by large and small scale industries such as leather, paper and textile industries. It is brought to notice that the collected effluents have to be studied in a deep manner as this effluent may contain different species of microorganisms and other toxic compounds. Sterile glass bottles were taken in which the dye effluent from the slurry was transferred to the bottle and kept in a thermocol box containing ice cubes for the period of three days at 4 °C. The river water was filled with colored effluents and this colored dye effluent is used as the parent source for the present study. To analyze the physical and chemical properties of this effluent, physico-chemical tests were undergone by using standard protocols.

### Analyzing the Physico-chemical properties

The collected dye effluent samples were analyzed to check the physico-chemical parameters. It is necessary to check the quantitative and qualitative assays of the collected dye effluent and to notify whether the constituents present in the effluent are not toxic to the flora and fauna in the environment. This can be determined via parameters such as Temperature, Total dissolved solids (TDS), Total suspended solids (TSS), Chemical oxygen demand (cod), Biochemical oxygen demand (BOD), sulphate and chloride concentration were performed in laboratory scale using the standard protocols.

### Isolation and screening of dye degrading bacterial isolate

In order to quantify the different microbial culture in dye effluent and its activity in the decolorizing as well as in degradation process, the concentration of the dye effluent was reduced. The process is followed by pour plate technique in which thousands of microbes were present. In that case one among them is bacteria *B. kochii*. This is proved by carrying out staining and the biochemical test for the bacterial confirmation. Before the qualitative test serial dilution was performed to the concentration of $10^{-6}$ folds. At this concentration rate it has been noted that there is a formation of some bacterial colonies formed during the period of 24 hours and at

incubation rate of 37 °C. Pure cultures were isolated by streaking the cells repeatedly on the agar plate. The grown bacterial cultures are used for further screening and stored at 4 °C.

**16S rRNA Sequence and analysis**

The newly isolated bacterial species were screened for the 16S rRNA sequencing. Physiological and morphological characterization was performed. Different bacterial strains were isolated in which one of the best isolate was identified using 16S rRNA sequencing and the isolated strain was given to gene sequence in Rajiv Gandhi centre for biotechnology. The partial gene sequence was done with locus MH152512 1152 base pair. The obtained gene sequence compared with the BLAST and noted as *B.kochii* strain PS17 16S ribosomal RNA gene.

**Decolorization of azo dyes by dye removal assays**

Potentially Isolated bacterial species were selected and has been analyzed for the dye absorption property and by characterizing their degrading activity by the specific bacterial strains through dye effluents. To analyze the bacterial role in degradation role, 250 ml Erlenmeyer flask was taken and the five different textile dyes have been collected and subjected to their dye degrading activity of bacteria. The initial pH was observed and the molasses medium was supplied with approximately 15 ml/l of dye by varying pH at 6, 7 and 8. The Erlenmeyer flask contains 100 ml of the nutrient broth and 5 ml of the particular dye was added to the flask. Then it was autoclaved at 121 °C for 15 minutes. Then the inoculation was done from the obtained culture of thedye effluent.

Then at the final stage the inoculated medium was kept in an orbital shaker where the agitation rate was 100 rpm. The incubation temperature was maintained at 30° ± 1C. During the soaking period, the degradation activity of bacteria was confirmed by the percentage of degradation through absorbance value. Therefore, the bacterial biomass were taken in a falcon tube and centrifuged at 5000 RPM for 5 min at 4 °C (Saratale, *et al*., 2015). By centrifuging the cells were separated and take the supernatant then discard the pellet. In order to determine the effect of percentage of decolorization of the dye, the absorbance values of Remazol and cure black textile dyes were noted by a UV - spectrophotometer at a range of 580 and 600 NM.

The percentage decolorization was calculated by the equation:

$$\% \text{ Decolorization} = \frac{\text{Initial OD} - \text{Final OD}}{\text{Initial OD}} \times 100$$

The effective culture medium was grown in a sterilized medium. Parameters such as pH and different carbon sources were taken into consideration so as to determine the initial level, pH on the growth and dye degrading capacity of the isolated bacterial species from the dye effluent. For the microbial action to take place energy Dextrose, Glucose and starch were added to the medium containing bacterial biomass and along with the dye effluent. This activity focused to identify the optimized condition in which the bacterial inoculum growth rate will be maximized and some biochemical changes take place at different growth condition such as intake of carbon source and nitrogen source at different pH levels.

**Effect of pH on decolorization activity**

The dye removal activity of the bacteria is mainly affected by the pH values.Analyzing the acidic and basic nature of the bacteria in degradation of the dye effluent is taken into consideration for the optimization. In this study the pH was adjusted to 6, 7 and 8 were inoculated with sterilized 50 ml nutrient broth and 7 ml of the dye effluent. Then it was incubated at 37 °C at orbital shaker of 200 rpm. Decolorization assay was measured as quoted earlier.

**Effect of carbon and nitrogen source on dye decolorization**

During the dye degradation activity of bacteria, it has been observed that up to the certain period of time all the nutrient constituents get depleted. Hence, the dye degrading activity comes to the ground state and no degradation of dye occurs. In order to control this activity, 1% of carbon and nitrogen sources are supplemented to the medium constitutes of bacterial inoculums containing dye effluent. Carbon source such as lactose, glucose, starch and sucrose, yeast extract, peptone and urea respectively. 7ml of dye was added and then sterilized (Sathian *et al*., 2013).

**Phytotoxicity studies**

Phytotoxicity studies were undergone to assess the toxic activity of the dye effluent. The phytotoxicity experiment was carried out in which the seed was taken and sterilized. The seed was grown in a MS medium along with the some of the nutrient

supplements. The seeds were inoculated in a petri plate containing the MS medium. Then 2ml of decolorized dye effluent were spread and kept under the light for 2 days (Shashi Prabha, 2017).

**Statistical analysis though Response Surface Methodology: Box-Behnken design**

In this study parameters such as temperature,pH and agitation speed were taken for optimization studies. RSM is the standard method in which experimental data given and assumed before the experiment carried out. In order to run the experiment following such as temperature, pH and agitation rate Box-benhken method was applied for the further studies to optimize % decolorization. RSM method was applied by using Design expert software followed by a range of tests F-Test lack of fit and other tests were performed. Then the best model has been selected (Thoker Farook Ahmed, *et al*., 2012).

## RESULTS AND DISCUSSION

**Physico-chemical analysis of dye effluent**

From Table 1 it is clear that that the collected dye effluent performs its maximum chemical noxious activity greater than the standard laboratory Indian standard limits tested in the seed Enviro lab. As these dye effluents contain various types of dyes and varying dye concentration. This is owing to the discharge of aqueous chemical waste released from industries such as paper, leather, textile and pharmaceuticals without the proper treatment. In this study the following results demonstrate that this collected dye effluent is toxic to the environmental flora and fauna (Vishal *et al.,* 2009). The pH was recorded at 27 °C and noted that it is alkaline in nature is: pH-10.06 (limit= 5.50-9.0) and pH in thislimit reported (Kalyani *et al.,* 2008).

**Biochemical characteristics of bacteria**

By Figure 1 it has come to discern that the decolorizing activity of the bacterial culture shows the positive results towards the dwindling level of decolourization. It is clear that the capability of decolourization had raised from 10-100 mg/l. From the graph it has been concluded that at 90 mg/l the decolourizarion is inactive in the state, excluding at 10 and 20mg/l, the decolourization activity is enormous. All this action is conceded out by the isolated bacteria followed by microbial culture conditions of various parameters. Hence this type of

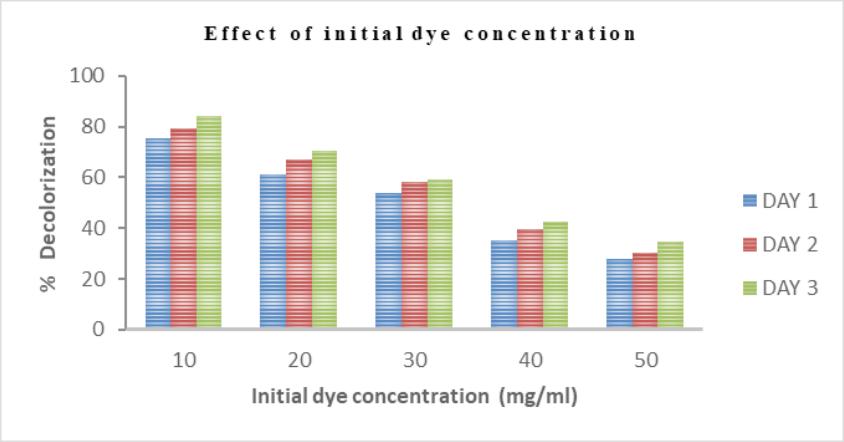


**Fig. 1.** Effect of initial dye decolorization

**Table 1.** Physico-Chemical property of dye effluent

| S.No | Parameter | Results | Limits | Test Method |
|---|---|---|---|---|
| 1. | pH @27°C | 10.06 | 5.50-9.0 | IS:3025(Part 11):1984 |
| 2. | Total dissolved solids | 32,250.0 mg/l | 210.0 mg/l | IS:3025(Part 16):1984 |
| 3. | Total suspended solids | 590.0 mg/l | 100.0 mg/l | IS:3025(Part 17):2002 |
| 4. | Chromium | 1.50 mg/l | 2.0mg/l | 38 of IS:3025:1964 |
| 5. | Chloride | 15314.40 mg/l | 1000.0 mg/l | IS:3025(Part 32):1988 |
| 6. | Biochemical oxygen demand | 1280.0 mg/ml | 30.0 mg/l | IS:3025(Part 44): 1993 |
| 7. | Chemical oxygen demand | 6000.0 mg/l | 250.0 mg/l | IS:3025 (PART 58):2006 |

microorganisms shows enormous activity and can be designed to engage in small and large levelling dying industries, so as to safeguard the aquatic organisms from this venomous nature of these dyes (Saratale *et al*., 2009).

**Table 2.** Biochemical, morphological and physiological characteristics of *B.kochii* sp.

| Type of Test | Result |
|---|---|
| **Morphology** | |
| Bacterial Cell Shape | Rod |
| Colony Color | White |
| Colony Formed | Irregular |
| Gram Staining | + |
| Endospore Staining | + |
| **Biochemical Test** | |
| Catalase test | + |
| Oxidase test | + |
| **Physiology** | |
| Temperature range (°C) | 25-37 |
| Optimum temperature (°C) | 31 |
| pH range | 6-8 |
| Optimum pH | 6 |

**Effect of pH on decolorization activity**

Graphical depiction of the following graph illustrates that *Bacillus kochii* showed their maximum decolourization activity at pH 6-7. The variable comparison was performed to spot the optimum growth conditions in which isolated bacterial strain will grow at different levels of pH. Hence the pH has been opted for 6, 7 and 8 in which the optimum results had been obtained at a pH- 6 when compared to (pH 7 and 8). The decolorizing action of the dye was determined via the absorbance at the wavelength of 580nm via UV-spectrophotometer. It had come to the conclusion that the isolated bacterial strains hows its decolourization activity at alkaline in nature (Madhuri *et al.*, 2014).

**Effect of carbon and nitrogen source on decolorization**

To enhance the percentage decolorization of dye effluent, nutrient supplements were given in form of carbon and nitrogen source. From the Figure it came to be known that maximum decolorization experiential in yeast extract (72%) whereas less in

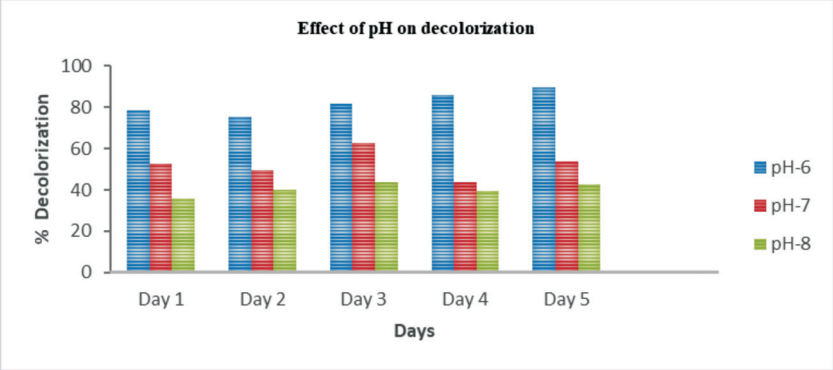


**Fig. 2.** Effect of pH on dye decolorization

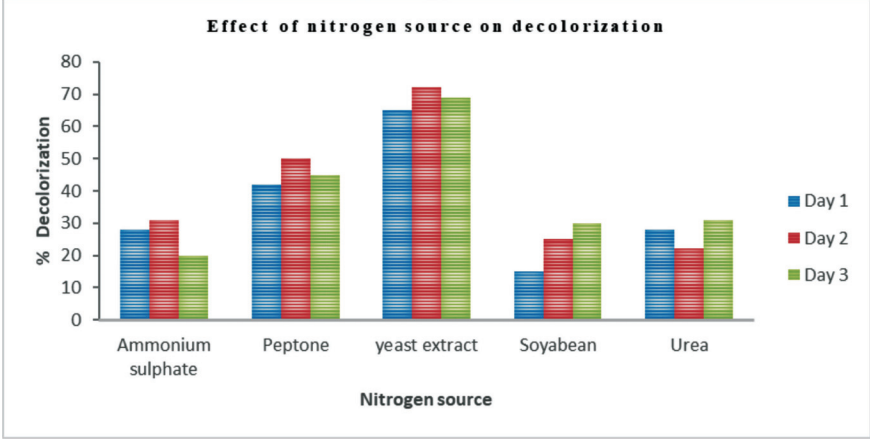


**Fig. 3.** Effect of nitrogen source on decolorization

soyabean (30%). These activities were seen at second day of decolorization and peptone (50%) showed the negligible decolorization next to yeast extract (Abdulla *et al.*, 2000).

**Analysis of degraded product by Liquid FTIR**

From the FTIR results of control and sample after decolorization showed some of the peaks. A peak at 3339.19cm$^{-1}$, 3678cm$^{-1}$ and 1634.12cm$^{-1}$ indicates –NH stretching vibration for asymmetric stretching and –OH stretching vibration.

**Response surface methodology**

In order to predict the optimized value of dye decolorization 17 runs were experimented to get the exact model by varying with factors such as Temperature, pH with respect to the actual factor is taken as agitation speed (Zollinger, 1991; Zumriye Aksu, 2005).

The results were analyzed by using variance (ANOVA) and were given in the Table 4.

The anova is the quadratic model. The Model F-value of 305.65 implies the model is significant. P-values less than 0.0500 indicate model terms are significant. In this case B, C, AB, $B^2$, $C^2$ are significant model terms. Values greater than 0.1000 indicate the model terms are not significant (Thoker *et al.*, 2012). The Lack of Fit F-value of 1.20 implies the Lack of Fit is not significant relative to the pure error. There is a 41.69% chance that a "Lack of Fit F-value" this large could occur due to noise. The 3D surface model for optimum decolorization is given in Figure 7. Hence

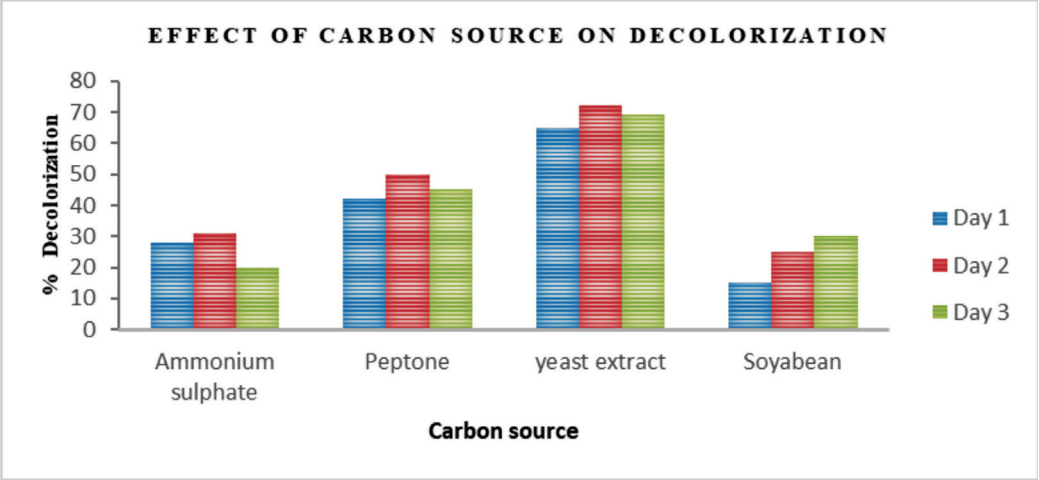


**Fig. 4.** Effect of carbon source on decolorization

**Table 3.** RSM for percentage decolorization of dye effluent of various parameters

| | Run | Temperature | pH | Agitation speed | %Decolorization |
|---|---|---|---|---|---|
| 16 | 1 | 31 | 6 | 125 | 78.9 |
| 6 | 2 | 37 | 6 | 100 | 76.5 |
| 10 | 3 | 31 | 8 | 100 | 55.4 |
| 5 | 4 | 25 | 6 | 100 | 72.7 |
| 11 | 5 | 31 | 4 | 150 | 36.5 |
| 12 | 6 | 31 | 8 | 150 | 57.89 |
| 3 | 7 | 25 | 8 | 125 | 56.84 |
| 13 | 8 | 31 | 6 | 125 | 77.35 |
| 1 | 9 | 25 | 4 | 125 | 39.57 |
| 9 | 10 | 31 | 4 | 100 | 35.89 |
| 4 | 11 | 37 | 8 | 125 | 63.56 |
| 15 | 12 | 31 | 6 | 125 | 78.54 |
| 14 | 13 | 31 | 6 | 125 | 76.24 |
| 7 | 14 | 25 | 6 | 150 | 76.5 |
| 8 | 15 | 37 | 6 | 150 | 78.34 |
| 17 | 16 | 31 | 6 | 125 | 76.25 |
| 2 | 17 | 37 | 4 | 125 | 34.9 |

the optimum decolorization obtained via RSM was 73.4% and pH-6, and the optimum temperature noted as 31 °C.

Apparently from the isolation of different strains were done in which the *B.kochii* showed the maximum bio degradation of dye effluent as compared to other strains. Physico-chemical parameters were determined followed by pH activity and temperature factors were considered. Hence the optimization studies were carried out for the approximate temperature and pH. Based on initial dye concentration the decolorization activity

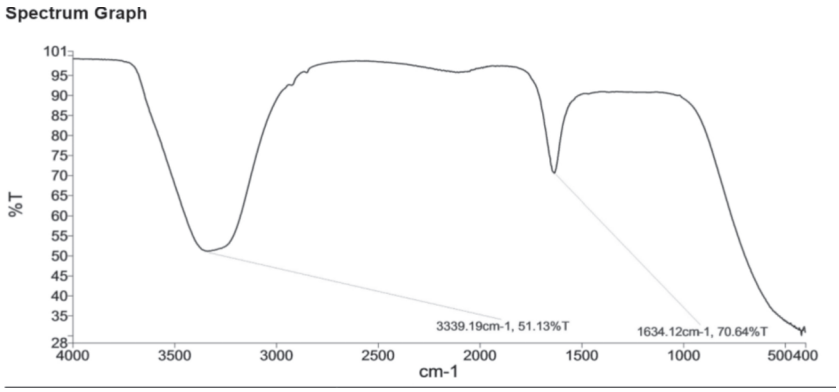


**Fig. 6.** FTIR spectral analysis of treated Dye effluent

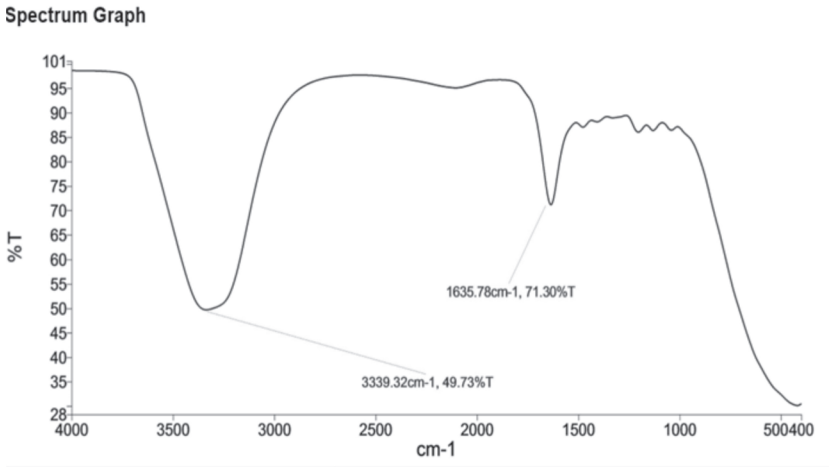


**Fig. 5.** FTIR spectral analysis of control Dye effluent

**Table 4.** ANOVA for Quadratic model

| Source | Sum of Squares | df | Mean Square | F-value | p-value | |
|---|---|---|---|---|---|---|
| **Model** | 4631.07 | 9 | 514.56 | 305.65 | < 0.0001 | Significant |
| A-Temperature | 7.39 | 1 | 7.39 | 4.39 | 0.0744 | |
| B-pH | 942.43 | 1 | 942.43 | 559.80 | < 0.0001 | |
| C-Agitation | 9.55 | 1 | 9.55 | 5.67 | 0.0488 | |
| AB | 32.43 | 1 | 32.43 | 19.27 | 0.0032 | |
| AC | 0.9604 | 1 | 0.9604 | 0.5705 | 0.4747 | |
| BC | 0.8836 | 1 | 0.8836 | 0.5249 | 0.4923 | |
| $A^2$ | 0.7632 | 1 | 0.7632 | 0.4533 | 0.5224 | |
| $B^2$ | 3581.28 | 1 | 3581.28 | 2127.26 | < 0.0001 | |
| $C^2$ | 14.75 | 1 | 14.75 | 8.76 | 0.0211 | |
| **Residual** | 11.78 | 7 | 1.68 | | | |
| Lack of Fit | 5.58 | 3 | 1.86 | 1.20 | 0.4169 | Not Significant |
| Pure Error | 6.20 | 4 | 1.55 | | | |
| **Cor Total** | 4642.85 | 16 | | | | |

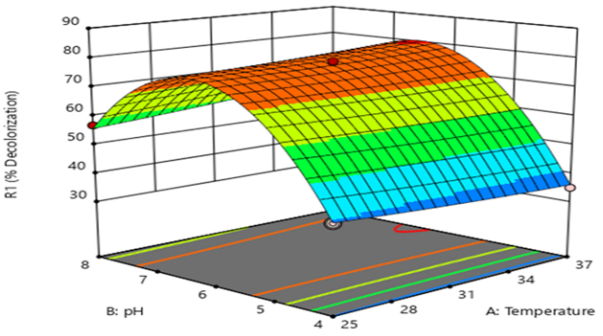


**Fig. 7.** 3D plot showing the interactive effect of pH and temperature with respect to Decolorization of textile dye effluent

was noted. *B.kochii* is environmental friendly as it showed positive results towards the phytotoxicity studies (Manikandan *et al.,* 2012). The various functional groups were analyzed by FTIR analysis to check the vibration between the azo groups. A chemical method of treatment may also cause side effects to the environment. On the other side the microbial survival rate will be higher in river water containing this polluted dye effluent (Zhanmei *et al.,* 2009; Draper *et al.,* 1988). Hence it had come to the notice that some of the specific micro-organism shows their tendency to degrade these azo groups and other chemical compounds in the dye effluent (Ahmadi *et al.,* 2005). Fungus and bacteria were reported to have the inclination to decolorizing the dye in which bacteria showed the maximum potential in decolorizing various other textile dyes.

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