



## Textile industry Dye degrading by bacterial strain *Bacillus* sp.

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### Abstract

The dye effluents collected from the dyeing industry at ERODE. The Physico chemical characters of the sample was analysed on pH, Temperature, Color and Odour viz., 8-8.5, 30°C, Blue and Foul. Dye degrading bacterial strains were isolated and identified by using traditional microbiological techniques. The isolate were identified based on the generic level as *Bacillus* sp. The effective bacterial strains was optimized with Various inoculums concentration, Different dye concentration, Temperature, pH, Carbon and Nitrogen sources.

**Keywords:** dye effluents, Dye degrading, Physico chemical characters, *Bacillus* sp.

### Introduction

Environmental pollution has been recognized as one of the major hazard of the morden world. Due to the rapid industrialization, lot of chemicals including dyes manufactured and used in day to day life (Moorthi *et al.*, 2007). The presence of very small amount of dye in water (<1 ppm) is highly visible, affecting the aesthetic merit, water transparency and gas solubility in lakes, rivers and other water bodies (Couto *et al.*, 2009). The effluents from these industries are complex, containing a wide variety of dye products such as dispersant, acids, bases, salts, detergents and oxidants. Discharge of these colored effluents into the rivers and lakes reduce dissolved oxygen concentration, thus creating anoxic conditions that are lethal to resident organisms (Wang *et al.*, 1991).

A considerable amount of waste water is generated having strong color, a large amount of suspended solids, a highly fluctuating pH, salts, heavy metals, sulphides, chlorine, temperature and COD concentration (Gurnham (1965). The disposal of untreated textile wastewater is a serious threat to the

environment. It accounts for 15-20% of total wastewater in the country (Gopal B (1994). The strong color of discharged dyes even at very small concentrations has a huge impact on the aquatic environment caused by its turbidity and high pollution strength. Additional toxic degradation products can be formed (Pourbabaee *et al.*, 2006).

Dyes usually have a synthetic origin and complex aromatic molecular structures which make them more stable and more difficult to biodegraded (Aksu, 2005). First synthetic dye was reported in 1856. There are more than 40,000 dyes and pigment with some 7000 different chemicals structure, out of which more than 3500 dyes practical use. Consumer of the dyes includes textile, tannery, paper, pulp, paint, electroplating and leather processing industries. Based on the chemical structure of the chromophoric group, the synthetic dyes are classified as azo dyes, nitroso dyes, triphenylmethane dyes, xanthane dyes and anthraquinone dyes (Shenai, 1994). Most synthetic dyes are highly resistant to degradation due to their

complex chemical structures(Lin *et al.*, 2010). Besides dyes, the wastewater contains acids/alkalis, common salt (NaCl), heavy metals, sulphides, chlorine and mineral oils. As a result, the dye wastewaters are extremely toxic to both aquatic fauna and flora, crop plants, including human beings. These dyes are regarded as relatively persistent pollutants because they are extremely stable when exposed to light and aerobic condition(Anlinker, 1997).

Some of these methods are effective but are quite expensive because they generate significant amounts of chemicals sludge waste whose disposal in a secure landfill increases process cost. Also, there is disposal problem of such waste materials to a proper place that also limit the use of these methods(Aguedach *et al.*, 2005; Sanghi *et al.*, 2007; Hernandez *et al.*, 2008).

Microbial degradation and decolourization of dyes is seen as a cost-effective method for removing these pollution from the environments(Banat *et al.*, 1996; Pearce *et al.*, 2003). Many bacterial, fungal and algal species have the ability to adsorb and/or degrade azo dyes(Stolz, 2001; Don Santoz, 2007). Moreover, bacterial decolourization is normally faster compared to fungal system with regard to the decolourization and mineralization of azo dyes(Banat *et al.*, 1996). Since numerous bacterial species including *Bacillus*, *Pseudomonas*, *Enterobacter*, *Halobacter*, and *Aeromonas* have been to decolorized and detoxify a wide range of azo dyes compared of phenylamine, benzenediazonium chloride or phenol(Telke *et al.*, 2008; Mendes *et al.*, 2011; Feng *et al.*, 2012).

The present study focused on isolation and optimization of the bacteria from the effluent to processing the strong decolorization capacity of the *Bacillus* sp.

### **Aim and Objective**

To collect the effluent sample from the dye industry

To study the physicochemical parameter of the effluent sample

Isolate and identification bacteria from the effluents.

To screen the potential dye decolorizing bacteria.

To optimize the potential strains with the influence of various inoculum concentration, Different dye concentration, Different temperature, Various pH, Different carbon and Nitrogen sources at various concentration.

## **Materials and Methods**

### **Collection of effluent sample:**

The effluent sample was collected in a clean collection bottles from the ARUNA TEXTILE industry in Erode district of TamilNadu. The sample were transferred immediately to the laboratory for further analysis. This effluent sample was used for the parental sources .

### **Selection of dyes:**

Methylred and Navy blue are the dyes used in this study. The stock solution of the dyes 1ppm was prepared by dissolving 0.5g/L of distilled water and this stock solution was preserved and used for the further study.

### **Physico-chemical properties of effluents**

To analyse the physicochemical properties of effluent viz., pH, Temperature, Color and Odour.

### **Isolation of dye degrading bacteria from effluent:**

Bacterial strains were isolated from the effluent samples. The Nutrient broth were prepared 25ml and add 1ml effluent sample. The flask were incubated at 27c for 24 hrs. After incubation growth was occur. These culture were streaked on steril nutrient agar plate. The plate were incubated at 27c for 24 hrs. After incubation the colony was growth. The single colony was transfer to the dye solution contain nutrient broth media and without culture inoculated flask serve as a control. The flask were incubated at 27c for 24hrs. after incubation the dye solution were decolorized. The bacterial culture was used for the further study and maintained for the nutrient agar slant.

### **Morphological characterization:**

Colony characterization Gram staining, fermentation of sugars were performed for identification of potential isolates of bacteria. Cultural characteristics of the colony such as margin, size, shape, type of colony, nature of colony (mucous, rough, smooth, transparent etc).

**Biochemical characterization: (Bargey's manual)**

**Gram staining:**

**Procedure:**

Air dry or heats fix the bacteria smear. Flood the slide with Crystal violet staining reagent for 1 minute.

Wash the smear in a gentle and direct stream of tap water for 2 seconds.

Flood the slide with iodine mordant for 1 minute.

Wash the slide in a gentle and indirect stream of tap water for 2 seconds.

Blot the smear dry with absorbent paper.

Immerse the smear in 95% ethanol for 30 seconds with gentle agitation.

Blot the smear dry with absorbent paper.

Immerse the smear for 2 minutes with counter stain.

Wash the smear in a gentle and indirect stream of water until no color appears in the wash water.

Blot the smear dry with the absorbent paper.

Examine under the microscope.

**Motility test (hanging drop method)**

Bacterial isolates were inoculated into the peptone water and incubated at 37°C for 24 hrs. Hanging drop preparation is useful for microscopic examination of living microorganisms, especially bacteria without staining them to see their motility due to flagella.

**Procedure:**

1. Clean and flame a hanging drops slide and place it on the table with the depression uppermost.
2. Spread a little Vaseline or petroleum jelly around the cavity of the slide.
3. Clean a cover slip and apply petroleum jelly on each of the four corners of the cover slips, using a match stick.
4. Place the cover slip on a clean paper with the petroleum jelly side up.
5. Transfer one loopful of culture in the center of the cover slip.
6. Place the depression slide on to the cover slip, with the cavity facing down so that the depression covers the suspension.
7. Press the slide gently to form a seal between the cover slip and the slide.
8. Lift the preparation and quickly turn the hanging drop preparation cover slip up so that the culture drop is suspended.
9. Examine the preparation under low - power objective with reduced light.

10. Switch to the high - power objective and examine the preparation again.

11. Place a drop of oil on the cover slip and examine the preparation under oil immersion objective.

**IMVIC Test**

**In dole Test**

To detect the tryptophase enzyme production by the test organisms.

The isolates were inoculated into the peptone water tubes and the tube were kept for incubation at 37°C for 24 hrs after the incubation period 2-3 drops of KOVAC'S reagent containing P-dimethyl amino benzaldehyde-5g, Amyl alcohol-75, Hcl-25ml was added and the results were observed.

**Methyl Red (MR) Test**

To detect the acid production by the test organisms.

The isolates were inoculated into sterilized MR-VP broth and incubated the tubes at 37° C for 48 hrs. After the incubation period 2-3 drops of methyl red reagent containing Methyl red-0.04g, Ethyl alcohol-40.0ml and Distilled water-60 ml was added and the results were observed.

**Voges-Proskauer (VP) Test**

To detect the production of non-acidic end product by the test organisms

The isolates were inoculated into MR-VP broth containing (g/L) of Peptone-7.0, Potassium phosphate-5.0, Dextrose-5.0 and incubated at 37° C for 48 hrs. After the incubation period 2-3 drops of Barrits reagent a containing Alpha-naphthol-5.0ml, Ethanol-95.0ml and B containing NaoH-40.0, creatine-0.3, Distilled water-100.0ml were added into the culture tubes and the results were observed.

**Citrate Utilization Test**

To detect the ability of citrate utilization by the test organisms.

In Simmon Citrate Agar (SCA) medium containing (g/L) of Ammonium dehydrogenate phosphate-1.0g, Dipotassium hydrogen phosphate-1.0, Sodium chloride-5.0, Magnesium sulphate-0.2, Bromothymol blue-0.08 and Agar-12.0. Then the isolates were

streaked on the agar slants and incubated for 18 -24 hrs at 37° C. After the incubation period the result were observed.

### Urea's Test

In the Christenson urea medium containing (g/L) of Peptone-0.1, NaoH-5.0, Potassium Monohydrate Phosphate-2.0, Glucose-1.0, Phenol red (0.02% solution) - 6.0ml and Urea (20% aqueous solution)-100.0ml. Then the isolates were streaked on the agar slants and incubated for 18-24 hrs at 37°C.

After the incubation period the result were observed.

### Triple Sugar Iron (TSI) Test

To detect the ability of the test organisms to ferment Glucose, Lactose, Sucrose and H<sub>2</sub>S production.

The isolates were stabbed on the TSI medium. The inoculated tubes were incubated at 37°C for 24 hrs. After the incubation period the results were observed.

### Screening :

1 gram of dye was dissolved in sterile distilled water and made up to 1000 mg/L. from the stock solution, required concentration of dye was prepared and filter sterilized using 0.5 µ membrane filter and used for further study. The selected strains must be screened to know the properly of degrading the dye, since they adapt to grow in the industrial effluent. The percentage of degradation observed by using UV-spectrophotometry.

### Decolourization of dyes by individual strains under static and static incubation:

Nutrient broth medium was prepared and 100 ml of the medium was dispensed in 250 ml of Erlenmeyer flasks. The medium was sterilized at 1 atmospheric pressure for 15 minutes. All the dyes (navy blue and Methyl red) were filtered and sterilized and added to the nutrient broth medium individually at a concentration level of 0.5g/L in an aseptic manner. To this, 1.0 ml of bacterial culture was inoculated and in a 37°C for 24 hours. Control flasks containing without inoculum were also maintained. The samples were then analyzed for percent decolourization after incubation. The bacterial strains that exhibited maximum percent decolourization were selected for further studies.

### Measurement of percent decolourization:

After incubation samples were filtered and centrifuged at 3500 rpm for 10 minutes and the suspended biomass was separated. The absorption spectra was measured at the lambda max of the dyes for the clear supernatant using a spectrophotometer. Medium containing dyes without the inoculum was taken as control. Percent decolourization was calculated with the following formula taking into consideration the initial and the final absorbance value of the dye.

Percent decolourization =

$$\frac{\text{Initial absorbance} - \text{Final absorbance value} \times 100}{\text{Initial absorbance values}}$$

After overnight growth, 0.25ml of cultures were taken in separate flask and 25ml of the azo dye (Navy Blue, Methylene blue, Blue, Methyl red, Yellow, Brilliant green) was added to each flask. Similarly the procedure was followed for the other culture. The flask was kept in the shaker at 37°C. The nutrient broth containing the dye of same concentration was taken as the control.

### Effect of different inoculum on growth and decolourization:

25 ml of the decolorizing media was prepared and adjusted to pH 7 and sterilized at 1 atmospheric pressure for 15 minutes. The flasks were inoculated with different inoculums (125µl, 250µl, 375µl, 500µl) of bacterial cultures and incubated at 37°C under static condition for 24hrs. Appropriate control flask without inoculums was maintained. After incubation percentage of decolonization and growth were estimated.

### Effect of dye concentration on growth of decolourization:

100ml of decolorizing media was prepared by using different dye concentration such as 0.025g/500ml, 0.05g/500ml, 0.1g/500ml, at a concentration level. The pH was adjusted to 7.0 and sterilized at 1 atmospheric pressure for 15 minutes. The flasks were inoculated with 0.25ml of inoculums at 27 for 48 hrs. Medium without any salts was maintained as control. The samples were drawn after the incubation time and were monitored for growth and percent decolonization.

**Effect of pH growth and decolourization:**

25ml of the decolorizing medium was dispensed in 100ml of flask and pH was adjusted to 5, 6, 7, and 8 adding 0.1N HCl or 0.1 N NaOH, autoclaved at 1 atmospheric pressure for 15 minutes. Methyl red and Navy blue was added in separate flask and inoculated with 0.25ml of inoculums. Control flasks containing dyes without inoculums were maintained. The flask was incubated under static condition at 27°C for 24hrs. After the incubation period the percent decolonization and growth were calculated. The optimum pH for growth in terms of optical density and decolonization of the dyes were observed.

**Effect of temperature on growth and decolourization:**

25 ml of the decolorizing media was prepared and adjusted to pH 7 and sterilized at 1 atmospheric pressure for 15 minutes. The flask was inoculated with 0.25 ml of bacterial cultures and incubated at 10°C, 27°C, 37°C under static condition for 24hrs. Appropriate control flask without inoculums was maintained. After incubation percent decolonization and growth were estimated.

**Effect of various carbon source on growth and declorization :**

25ml of decolorizing medium without glucose was prepared and adjusted to pH 7.0 and sterilized at 1

atmospheric pressure for 15 minutes. Different carbon sources such as sucrose, mannitol, and fructose were filter sterilized and added to the decolorizing media 0.5, and 1 %. To this, filter sterilized dyes methyl red and nevy blue at the concentration of 50mg/l was added separately, and incubated at 27°C under static condition for 24 hours. The sterilized decolorizing medium without any carbon source was maintained as control. After the incubation period, percent of decolonization was observed.

**Effect of various nitrogen source on growth and decolourization:**

Nitrogen sources such as potassium nitrate, casein and ammonium sulphate were used in place of peptone in decolorizing media. 25ml of decolorizing media containing the corresponding nitrogen sources at 0.5 and 1 % level were prepared and pH was adjusted to 7.0 and sterilized at 1 atmospheric pressure for 15 minutes . Methyl red and nevy blue were added to sterilized media, inoculated with 0.25ml of the inoculums and incubated at 27°C under static condition. The sterilized synthetic medium without any nitrogen source was maintained as control.

**Results and Discussion**

**Physicochemical parameter of the effluent sample**

The data on physico chemical analysis of effluent samples were presented in **Table-1**

PARAMETER	VALUE
Ph	8-8.5
Temperature	30°C
Color	Blue
Odour	Foul

**Determination of odour**

The sample of effluent was filled into the wide-mouth container closed with glass stopper and vigorous shaking for 2 to 3 seconds. The stopper was removed and odour was quickly observed.

**Isolation and identification dye decolorizing bacteria from effluents**

The effluent sample were collected from the aruna textile industry from erode district. Since the effluent

having microbes ability to decolorize the dyes effluent is very high. The textile and dyeing industry are one of the industry, which contribute to the soil and water pollution. There are about 700 dyeing and bleaching units located in and around Tiruppur and Coimbatore in TamilNadu state.(Jothimani *et al.*,2003). The species were identified tentatively up to genera level based on morphological and biochemical characters were identified observation as shown in Table and identified as *Bacillus* sp. Similar kind of observation by (Ponraj *et al.*, 20 )

Table-2

Test	Result
Gram's Staining	+
Shape	Rod
Motility	Motile
Indole Production	-
Methylred	-
Voges-Proskauer	-
Citrate utilization	+
Catalase	+
Oxidase	-

### Screening for potential dye degrading bacteria

The isolated bacterial strain was inoculated into the dye containing NB medium. It was incubated at 30°C for 24hrs (Narsinge and Hamde, 203). After incubation the the colour changes was obtained and the OD value(Bacterial growth) was measured by UV-Spectrophotometer. The table 2 shows the screening of selected bacterial decolorization.

### Effect of different inoculum for dye decolorization

Culture inoculum was various for decolorization study like 0.5% , 1%, 1.5% and 2% against methylred and navyblue dyes. Initially optical density value of methylred and navyblue dye was recorded 1.470 and 0.567 respectively. Percentage of decolorization was gradually increased when various bacterial concentration, maximum 90.2% and 80.1% reduction was recorded in methylred and navyblue dye respectively. 0.5% bacterial inoculum was finally taken for further work. The table 1 shows the decolorization percentage of methylred and navyblue at different inoculums concentration. The effect of decolorization depend upon the the inoculum concentration because of inoculum level of decreased the decolorization level also decreased. This study is corroborated with the finding of Kumar *et al.*,(2009).

### Effect of different dye concentration for dye decolorization

Three different concentration of dyes were used for decolorization estimation like 0.025g, 0.05g and 0.1g. In methylred maximum 89.9% was reduced and 77.7% decolorization in 0.025g. In methylred was decolorized at above 70% at all the concentration level, and also navy decolorization was above 60% at all the concentration. The table 2 shows the decolorization percentage of methylred and navyblue. These result indicate the toxicity of the Methyl red and Navy blue. The similar case was seen with (Karunya *et al.*, 2013).

have reported that after 48h, the dye was completely decolorized up to 200mg/l, by *Pseudomonas aeruginosa* but above this concentration there was much change in the decolorization level.

### Effect of different pH for dye decolorization

Percentage of decolorization was absorbed at different pH concentration like pH 5, 6, 7, and 8. In this study inoculum concentration and dye concentration was constantly used 0.5% and 0.025 g respectively. Above 86% and 68.3% decolorization was recorded in pH 7 at methylred and navyblue, it was reduces at pH 8. The table 3 shows the decolorization percentage of methylred and navyblue at various pH level. The pH tolerance of decolorizing bacteria is quite important because reactive azo dyes bind to cotton fibers by addition or substitution mechanisms under alkaline condition and at high temperature(Asku, 2003). The result were comparable with other azo dye reducing species like Bacilus, citrobacter and Pseudomonas report so far (Kalm *et al.*, 2007; Chang *et al.*, 2001; Suzuki *et al.*, 2001; Wang *et a.*,l 2009)which gave the similar result. Also, report the same result of (Karunya *et al.*, 2013).

pH is among the other most important factors for any microbial activity . Each microorganism possesses a pH range for its growth and activity of metabolite production with a optimal value in between the range. The pH of culture medium plays a critical role for the optimal physiological performance of microbial cells and the transport of various nutrient components across the cell membrane. Bayoumi *et al.*, (2010) concluded the decolorization at pH 8 was suitable for decolorization of textile wastewater. It should be mentioned that most of collected textile wastewater samples used in this study were slightly alkaline so in economic view decolorization at pH 8 is an advantages.

Decolorization of azo dyes in alkaline medium was recorded by many studies, Asad *et al.*, (2007) found that decolorization rate of remazol black B increased as pH increased. Also Dafale, *et al.*, (2008) found that, the specific decolorization rate increased with increasing pH from 5 to 7, which remained approximately the same for pH 7–8. This seems to indicate that neutral and slightly basic pH values would be more favorable for decolorization process of remazole black B by a bacterial consortium containing *Pseudomonas aeruginosa*. In contrast to the present results pH 7 was the optimum pH for the decolorization of reactive red 195 by *Enterobacter* sp and the decolorization percentage decreased as pH increased (Kalyanee *et al.*, 2008).

### Effect of different temperature for dye decolorization

Percentage of decolorization was studied at different temperature like 10 °C , 30° C and 40° C. Inoculation of culture as dye concentration was concentrate used. Above 76% and 86% reduction was recorded in methylred and navyblue dyes at 30°C, in 40°C maximum 69.3% and 86% was recorded. No color reduction was recorded in 10°C in both dyes. The table 4 shows the decolorization percentage of methylred and navyblue at different temperature. Similar result were reported on *Bacillus subtilis* (RA 29) showed ((95.67%) congo red decolorization at 37°C (Kumar and Sawhney , 2011).The decresed in decolorization at higher temperature may be due to the loss of cell viability or deactivation of enzyme resonsible for decolorization at 40 C (Cetin and Donmenz 2006; Panswad and Luangdilok, 2000).

Temperature control is very important for any bacterial process, since growth and production of enzymes are usually sensitive to high temperature (Sani *et al.*, 1992; Babu and Satyanarayana 1995). It must be noted that, the optimum temperature for production of an enzyme (in this case azo reductase enzyme) does not always coincide with that for growth (Sodhi *et al.*, 2005).

Bayoumi *et al.*, (2010) reported the optimum incubation temperature for maximum color removal percentage for the two azo dyes Acid orange 7 and Direct blue 75 was 35 °C when decolorized by any of the two strains *Com. acidovorans*-TM1 or *Bur. cepacia*TM5. Results recorded indicated that this temperature was also the optimum for growth. This result is in complete accordance with the study by (Asad *et al.*, 2007) where the optimum temperature for

decolorization of remazol black B by *Halomonas aquamarina* was also 35 °C. The decolorization percentage and growth represented by protein content increase with temperature increase until 35 °C, further increase in temperature resulted in marginal reduction in decolorization activity of bacterial strains because the reduction in cell growth and the enzyme azo reductase may be deactivated.

Mezohegyi *et al.*, (2007) investigated the anaerobic reduction of azo dye Acid Orange 7 (AO7) in a continuous up flow packed bed reactor (UPBR) containing biological activated carbon (BAC) and the temperature was adjusted at 35°C. This result also is more related to many results those reported by many authors, where Dafale *et al.*, (2008) found that, 37°C was the optimal temperature for decolorization of remazol black-B (RB-B) by a bacterial consortium containing *Pseudomonas aeruginosa*. In contrast to the present results, HU *et al.*, (1994) incubated *Pseudomonasluteola* at 28°C to obtain maximum decolorization power of textile wastewater.

### Effect of carbon and nitrogen source for dye decolorization

Bacteria needs nitrogen source to degradation the dye stuffs, in this study we used three different nitrogen source like potassium nitrate, ammonium sulphate and casein . In this experiment use concently used percentage of bacterial inoculam (0.5%), percentage of dye concentration (0.025g), Ph 7 and 30°C temperature. Maximum decolorization was 89.9% and 83.3% observed in methylred and navyblue colour reactively using ammonium sulphate nitrogen source. Only 60% was observed in casein substrate. Narsinge *et al.*, 2013 reported the similar findings of ammonium salts supporting decolorization of reactive golden yellow 84 by *Rhizobium sp.* F5. The increased in decolorization percentage after addition of carbon and nitrogen sources aer attributed to the fact that the dyes are deficient in carbin and nitrogen content and biodegradation without any carbon and nitrogen sources are difficult (Padmavathi *et al.*, 2003 ) The table 5 shows the decolorization percentage of methylred and navyblue at different concentration nitrogen sources.

Bayoumi *et al.*, (2010) Concerned the effect of the addition of different nitrogen sources for the purpose of decolorization of the two azo dyes acid orange 75 and direct blue 75 by the two strains *Com. acidovorans*-TM1 and *Bur. cepacia*-TM5, it was found that organic nitrogen source peptone was the

best inducer for the decolorization of the two dyes by the two strains. In contrast to the case of carbon source, peptone was the best nitrogen source for growth in addition to decolorization. Presence of peptone as the best nitrogen source was proved by many azo dyes bioremediation studies.

Chen *et al.*, (1999) found that peptone gave the best color removal percentage for azo dye Red RBN by *Proteus mirabilis* and the substitution of inorganic nitrogen (NH<sub>4</sub>Cl) for peptone gave poor cell growth and low color removal. Also HU, (1994) used medium containing peptone as a nitrogen source in decolorization of reactive azo dyes by *Pseudomonas luteola*. In contrast to this results inorganic nitrogen sources (NH<sub>4</sub>Cl) was used in anaerobic treatment of azo dye Acid Orange 7 under fed-batch and continuous conditions Mendez-Paz *et al.*, (2005).

In carbon source fructose, sucrose and mannitol have used a substrate both dyes. The highest decolorization was recorded in sucrose at both dyes 78.7 and 68.3. the concentration was increased the decolorization was reduced. Same observation done by the Narsinge *et al.*, 2013 as reported sucrose as found the maximum degradation at 1% level and also he reported the best degradation of using with fructose (70.5%). Similar to this work, Wang *et al.*, 2009 reported that a *Citrobacter* sp. Decolorized 96.2% of reactive red 180 dye with 4g/l of glucose as a carbon source. The table 6 shows the decolorization percentage of methylred and navyblue at different concentration carbon sources.

Bayoumi *et al.*, (2010) studied the effect of introducing some carbon sources on the decolorization process of acid orange 7 and direct blue 75 by the selected most potent two bacterial strains *Com. acidovorans*-TM1 and *Bur. cepacia*-TM5, it was found that decolorization percentage increased after addition of most carbon sources but decreased after addition of other sources of carbon when compared with the control. But the most promising results for decolorization were obtained when starch was used as a carbon source although starch was not the optimum co-metabolite for growth.

The increase in decolorization percentage after addition of carbon sources is attributed to the fact that the dyes are deficient in carbon content and biodegradation without any extra carbon sources is difficult (Padmavathy *et al.*, 2003). The decrease in decolorization percent after addition of some carbon sources and the ability of some carbon sources to

induce growth without increase in decolorization may attributed to that, the sugars may inhibit the decolorization of azo dyes because its effect as catabolite repression (Chang *et al.*, 2001).

Presence of starch as the best co-metabolite in decolorization of azo dyes was supported by many studies Padmavathy *et al.*, (2003) found that starch was the best carbon source in azo dye biodegradation from synthetic waste water under aerobic co-metabolite conditions also Georgiou *et al.*, (2005) suggested the use of potato-starch industrial wastes to increase the decolorization of textile waste water in large scale. Also starch was added by Olukanni *et al.*, (2006) in studying the textile effluent biodegradation potentialities of textile effluent-adapted and non-adapted bacteria. In contrast to the present study, glucose was used as a carbon source in decolorization of reactive azo dyes by *Pseudomonas luteola* (Hu, 1994). Also Asad *et al.*, (2007) used glucose in decolorization of remazol black B by halotolerant and halophilic isolates.

Wang *et al.*, (2009) studied the addition of glucose enhanced the decolorization of Reactive Red 180 by *Citrobacter* sp. CK3 (Fig. 3a). However, lack of glucose inhibited the decolorizing activity of *Citrobacter* sp. CK3 since only 26.72% color removal was observed after 120 h incubation. In experiments with glucose supplementation, *Citrobacter* sp. CK3 exhibited strong decolorizing activity with about 90% decolorization extent in 48 h, except that when the glucose concentration was 0.5 g l<sup>-1</sup> or 12 g l<sup>-1</sup>, the decolorization efficiencies (64.19% and 67.23% in 120 h, respectively) were much lower. The reason why low decolorization extent appeared when the glucose concentration was 0.5 g l<sup>-1</sup> may be that low glucose concentration could not meet the growth requirements of the bacteria. When the glucose concentration was much higher, such as 12 g l<sup>-1</sup>, the bacteria could utilize glucose preferentially, thus resulting in lower decolorization extent.

**TABLE-3**  
**EFFECT OF DIFFERENT INOCULUM CONCENTRATION**

Dye concentration (g)	% of inoculums concentration	METHYL RED				NAVY BLUE			
		OD Value				OD Value			
		Initial Value	Final Value	% of Transmission	% of Decolorization	Initial value	Final Value	% of Transmission	% of Decolorization
0.025g	0.5%	1.470	0.149	70.9	89.9	0.567	0.126	74.6	77.7
0.05g		1.960	0.438	54.8	77.6	1.256	0.348	62.3	72.3
0.1g		2.453	0.692	44.4	71.8	1.961	0.689	36.8	64.9

**TABLE-4**  
**EFFECT OF DIFFERENT CONCENTRATION OF DYES**

pH Levels	inoculum Conc.	Dye Conc (g)	METHYL RED				NAVY BLUE			
			OD Value				OD Value			
			Initial Value	Final Value	% of Trnsmission	% of Decolorization	Initial Value	Final Value	% of Transmission	% of Decolorization
5	0.5%	0.025g	1.470	0.88	81.5	40.1	0.567	0.236	57.9	58.4
6				0.192	64.2	86.9		0.232	58.5	59.1
7				0.194	63.9	86.8		0.180	66	68.3
8				0.780	60.5	47		0.196	63.5	65.4

**TABLE-5**  
**EFFECT OF DIFFERENT pH**

Temperature at (°C)	% of Inoculum Conc.	Dye Conc. (g)	METHYL RED				NAVY BLUE			
			OD Value				OD Value			
			Initial Value	Final Value	% of Transmission	% of Decolorization	Initial Value	Final Value	% of Transmissio	% of Decolorization
10°C	0.5%	0.025g	1.470	1.470	0.00	0.00	0.567	0.567	0.00	0.00
30°C				0.350	44.6	76.3		0.071	84.2	86.9
40°C				0.452	35.2	69.3		0.077	83.7	86.4

**TABLE-6**  
**EFFECT OF TEMPERATURE**

Nitrogen Sources	Mol.Wt (g)	% of Inoculum Concentration	% of Dye Concentration (g)	pH	Temperature (°C)	METHYL RED						
						OD Values						
						Initial Value	Final Value		% of Transmission		% of Decolorization	
							0.5%	1%	0.5%	1%	0.5%	1%
Casein	---	0.5%	0.025g	7	30°C	1.470	0.573	0.818	26.6	15.1	61	44.4
KNO <sub>3</sub>	132.13						0.181	0.370	65.8	42.6	87.7	74.9
Ammonium Sulphate	101.10						0.149	0.289	70.9	50.9	89.9	80

**TABLE-7**

**EFFECT NITROGEN SOURCES**

Nitrogen Sources	Mol.Wt (g)	% of Inoculum Concentration	Dye Concentration (g)	pH	Temperature (°C)	NAVY BLUE						
						OD Values						
						Initial Value	Final Value		% of Transmission		% of Decolorization	
							0.5%	1%	0.5%	1%	0.5%	1%
Casein	---	0.5%	0.025g	7	30°C	0.567	0.223	0.293	59.8	50.8	60.6	48.3
KNO <sub>3</sub>	132.13						0.126	0.133	74.6	73.6	77.6	76.4
Ammonium Sulphate	101.10						0.95	0.131	80.3	73.8	83.2	77

**TABLE-9**

**EFFECT OF CARBON SOURCES**

Carbon Sources	Mol.Wt (g)	% of Inoculum Concentration	Dye Concentration (g)	pH	Temperature (°C)	METHYL RED						
						OD Values						
						Initial Value	Final Value		% of Transmission		% Of Decolorization	
							0.5%	1%	0.5%	1%	0.5%	1%
Fructose	180.16	0.5%	0.025g	7	30°C	1.470	0.348	0.272	44.7	53.4	76.3	81.5
Sucrose	342.30						0.313	0.551	48.6	28.8	78.7	62.5
Maltose	360.32						0.551	0.350	28.8	44.6	62.5	76.2

## Conclusion

Now a days Bioremediation is promising technology to remediate the environmental contaminants which is an eco-friendly, cost effective, easily approach technology. The isolated and identified bacterial strains were found to be more effective and having enormous potential of textile degradation under versatile environmental condition. They can be used to excellent bioremediation for the bioremediation of the textile dyes. The bacterial strain were showed effective decolorization at optimized condition. The observation showed that the bacteria are adaptive in nature and decolorize contaminants. The ability of the strain to tolerate, decolorize textile dyes at high concentration gives it an advantage for treatment of textile industry waste water.

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