

## **OPTIMIZATION AND DECOLORIZATION OF TEXTILE DYE WITH BACTERIAL STRAINS**

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**Abstract:** Textile dyeing and printing industries play a vital role in nation's economy and environmental sector. The textile dyeing industries has been condemned as being one of the world's worst offenders in terms of pollution. They have a huge volume of bilge, show high impact on environment, mainly on water ecosystem. This study is aimed to isolate the bacteria from dye contaminated water bodies in and around Tirupur District, Tamilnadu. Bacterial strains are isolated based on their morphological characteristics and are subjected to screening for their ability to decolorize the textile dyes. Screening results in five isolates were found to posses decolorization against three different commonly used dyes (red F3, Blue FB & black B). All parameters studied in this paper were found to be effective for all isolates. The results reported here warrant further investigation to establish the usefulness of these isolates for bioremediation and biodegradation application such as waste water treatment. The results indicated that *Pseudomonas* sp. and *Bacillus* sp. Showed maximum dye decolorization capability within 48hrs at an incubation at minimum temperature of 30°C in the medium with high carbon source. But the *Bacillus* sp. was found to be more efficient in dye decolorization.  
**Keywords:** Textile dye, Decolorization, Textile effluent, Bacterial isolates.

### **Introduction**

Due to rapid industrialization, a lot of chemicals including dyes are manufactured and used in day to day life. The textile dyeing industry consumes large quantities of water and produces large volumes of wastewater from different steps in the dyeing and finishing processes. Dyes are commonly used in food, cosmetic and textile industry. Wastewater from printing and dyeing units is often rich in color, containing residues of reactive dyes and chemicals, such as complex components, many aerosols, high chroma, high COD and BOD concentration as well as much more hard-degradation materials. The textile industry is one of the largest water users and polluters that threaten the environment with its high coloured discharge in to the surface and ground water source. According to recent statistics, China's annual sewage has already reached 390 million tons, including 51% of industrial sewage, and it has been increasing with the rate of 1% every year. Each year about 70 billion tons of waste water

from textile and dyeing industry are produced and requires proper treatment before being released into the environment (State Environmental Protection Administration, 1994).

Dyes are classified into acidic, basic, disperse, azo, diazo, anthraquinone and metal complex based on their structure. On the basis of the dyeing process, textile dyes are classified as reactive, direct, disperse, acid, basic and vat dyes (Campos et al. 2001). Textile industries utilize large amounts of water during processing and also generate substantial amounts of wastewater (Hutton 1972). About 10–15 % of the dyes are lost in the wastewater during the dyeing process (Zollinger 1987). Coloured wastewater from the textile industries is one of the most obvious indicators of water pollution. Coloured dye wastewater causes severe effects on aquatic environment even in small amounts. Apart from the colour, the dischargeable dye wastewater also contains other pollutants like degradable organics, nutrients, pH altering agent, salts, sulphur, toxicants and refractory organics (Somasiri et al. 2008; Haroun and Idris 2009). As a result, it is an urgent task to reduce the pollutants in the receiving water. In general, physical, chemical and biological methods are used to treat the textile industry wastewater. Physical and chemical methods include adsorption, chemical precipitation, flocculation, photolysis, chemical oxidation and reduction, electro-chemical treatment and ion-pair extraction (Azmi et al. 1998; Moreria et al. 2000; Rajeshkannan et al. 2010, 2011). These methods are mostly ineffective, expensive, produce side reactions, high sludge and by-products, not suited to degrade all dyes, etc. (Krull et al. 1998; Verma and Madamwar 2003). Hence, researchers have focused on biological treatment as the best alternative. The colour removal is due to the chemical reductive cleavage of azo-bonds within the dye molecules under anaerobic conditions.

A review of the different treatment technologies and techniques and their efficiency towards degradation of xenobiotics has been given by Mastumoto et al., (1994) and Banat et al., (1996) and concluded that not one specific treatment process seems to be handle decolorization of textile waste waters. Generally, a customized process, which probably involves the combination of different methods, will be more applicable. Anaerobically degraded products of azo-dyes subsequently be degraded under aerobic or anaerobic conditions by mixed bacterial community (Seshadri et al., 1994 and Flores et al., 1997). Anaerobic decolorization of azo-dyes by bacteria, the reduction equivalents generated by the oxidation of auxiliary substrates, i.e organic carbon complexes, as electron donor via NAD(P) reduce the azo bond through chiefly assuming electron carriers (coenzymes) flavin nucleotides (FMN, FAD) or riboflavin as cofactor (Rafii et al., 1990, Chung and Stevens 1993, Field et al., 1995 and

Georgiou et al., 2003). Present work has been carried out to isolate the most potent dye decolorizing microbes from dye contaminated water bodies and to optimize the nutrient requirements for decolorization in culture conditions using specific isolated pure strains of bacteria.

## **Materials and Methods**

### **Sample collection:**

Samples for isolation of bacteria was collected from the sludge of textile dye contaminated water resource in and around Tirupur, Tamilnadu, India.

### **Selection of Textile Dye:**

Commercially available textile dyes such as blue FB, red FB and black B collected from textile industry in Tirupur district were used for this study.

### **Media Preparation:**

Medium composition

The first media is prepared by the combination of  $\text{NaH}_2\text{PO}_4$  - 4gm,  $\text{KH}_2\text{PO}_4$ -1gm,  $\text{KH}_2\text{PO}_4$ -1gm,  $(\text{NH}_4)_2\text{SO}_4$ -2gm,  $\text{KCl}$ -2gm,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ -2gm. The second medium contains  $\text{Na}_2\text{HPO}_4$ -2gm,  $\text{KH}_2\text{PO}_4$ -1gm,  $(\text{NH}_4)_2\text{HPO}_4$ -4gm,  $\text{NaCl}$ -2gm,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ -2gm. The third media is the combination of first medium plus yeast extract (1gm)+Glucose(2gm). The fourth media is prepared by adding yeast extract (1gm)+Sucrose(2gm) with the second medium. All these media were autoclaved at 15lbs for 30minutes.

### **Isolation of dye decolorizing bacteria:**

Isolation of bacterial population was carried out with enriched process and isolation technique was carried out from the collected water sample in third medium. 100ml of the solution is autoclaved at  $120^\circ$  for 30 minutes in which glucose and dye were added from the stock of each dye. After incubation it was streaked and incubated. Six different types of bacterial colonies were formed and are differentiated according to their morphology.

### **Optimization of culture media:**

The medium composition was set in such a way the ingredient added to satisfy all the minerals, carbon, nitrogen, phosphorous and sulphur would act as buffers. The screened isolates were taken for standardization for the optimum concentration of nutrient in medium for the highest decolorization efficiency of each strain.

### **Results and Discussion:**

The present study was attempted to investigate bacterial strains present in textile dye contaminated water bodies, and to isolate the efficient bacterial strains present in the

contaminant habitat, many microbial species must be present and which has competent to degrade a wide variety of xenobiotics. The ultimate aim is to characterize under laboratory conditions with respect to eco-physiological requirements.

#### **Isolation of dye decolorising bacteria:**

Here six bacterial species were isolated from the sludge of textile dye contaminated habitat using enriched media with dyes. The bacterial isolates were differentiated by morphological characteristics and designated as SOL-1,2,3 respectively. As indicated by Lorimer et.al., (2001), it is carried out to know whether the isolates are having potency of degree of decolorization, if screened for. The enrichment technique involved in incubating a source of microorganisms is sludge by adding carbon as energy source as well as nitrogen, phosphorous and sulphur sources in the presence of dye in the medium (Banet et al., 1996).

#### **Screening:**

The result obtained in this work were recorded in terms of overall degree of decolorization as good (++++), moderate (+++), low (++) , very low(+). Three isolates showed high degree of decolorization due to the combinations of the three medium. The isolates 1&6 showed complete degradation, 3&4 is of moderate respectively of the dyes used.

The results of this experiment in culture conditions confirmed that static batch culture system is highly favourable for decolorization. From the results it was understood that combination of the media showed the presence of glucose, which supported the growth as well as the creation of fermentative condition in the broth. Glucose and yeast extract as essential to supply growth factors that enhanced the process (Nigam Et al., 2009).

#### **Optimization of medium condition:**

Decolorization process had been considered as a special case where in a order of decolorization to occur, the isolates required essential nutrients growth factor, which must be added in the basal medium. Several nutrient ingredients were tested in medium 2&3, in which carbon source was either in presence of glucose or sucrose respectively and represented in table 3. Each ingredient tested in the media showed clear relationship with the ingredients added such as yeast, multivitamin solution, beef extract and the decolorization activity reduction of the dye colour ranged between 16% and 90%. However among all the presence of yeast and beef extract were resulted in the highest activity of decolorization. It can be stated that these nutrient ingredients are classified as growth factors, essential for decolorization. Among the various mediums medium 3&4 more favouring in terms of decolorization. To testify the effect of varying concentrations of sugars on decolorization, the

third medium and fourth medium with varying concentrations of sugar from 0.1% to 0.2% were taken in the experiments for each six isolates separately and percentage of decolorization after incubation of 48hrs. It was believed that as concentration of sugar increased, percentage of decolorization activity also increased at about 0.5% except the isolates 2,4&5 which required 1.0% for maximum activity, where the increased concentration of the sugar increased the decolorization activity except fourth isolate, where the increases concentration of sugars educed the decolorization activity, thus suggesting that isolates behave differently for the energy metabolism and decolorization.

In the present study, successful isolation of desired organisms was made, that showed effective decolorization of three different types of dyes. Thus, there was strong influence of growth promoting medium ingredients. From the results obtained of the nutrient combination in the broth showed that the isolates when subjected to grow in the presence of both yeast extract and glucose showed adequate growth, generation of fermentative condition and high percentage of dye decolorization. This clearly shows that dye decolorization process can only be initiated, provided the inoculated organisms are given enough carbon energy, nitrogen and other growth factors that enhance the decolorization activity.

**Table: 1** Over all degree of decolorization of selected dyes by bacterial isolates under various nutrient conditions (48hrs)

Dye used	Degree of decolorization					
	I	II	III	IV	V	VI
Red	++++	++++	+++	+++	++++	++++
Blue	++++	++++	+++	++++	++++	++++
Black	++++	+++	++++	++++	+++	++++

[++++=good; +++ = moderate].

**Table 2:** Secondary selection of bacterial isolates based on their degree of decolorization in II medium (48hrs)

Isolate	Degree of decolorization		
	Med1+Glucose (0.5%)	Med1+Yeast	Med1+ Glucose+Yeast
1	++++	+++	++++
2	++++	++++	+++
3	++++	++++	+++
4	+++	++++	++++
5	+++	++++	++++
6	++++	++++	+++

[++++=good; +++= moderate].

**Table 3:** Percentage of decolorization of dye by isolate bacterial strains in the presence of various nutrient ingredients in media

Nutrient ingredients	Isolate Con.	% of decolorization					
		I	II	III	IV	V	VI
Yeast extract	1g/L	70	96	97	95	98	92
Trace element solution	10ml/L	18	19	22	25	16	18
Tryptose	2g/L	40	76	21	48	55	56
Peptone	2g/L	45	42	44	76	54	45
Beef extract	1g/L	95	90	92	98	85	49
Riboflavin	1mg/L	40	60	71	90	75	40

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