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Research Article

# DECOLOURIZATION OF AZO DYE BY NATIVE MICROBIAL CONSORTIUM

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## ABSTRACT

The present study was focused on decolourization of textile azo dyes by using native bacterial consortium isolated from textile dye effluent. The bacterial isolates were screened for dye decolourising capability in Bushnell Hass medium. Potent five isolates (KTE7, KTE28, KTE48, KTE64 and KTE83) out of 95 total isolates were selected for efficient dye decolouration using spot assay method. Best dye decolouration was noted with KTE7 and KTE 28. These five isolates were subjected for microbial characterization, which reveals the presence of *Bacillus cereus* (KTE7), *Pseudomonas fluorescence* (KTE28), *Staphylococcus aureus* (KTE48), *Escherichia coli* (KTE64) and *Lactobacillus* sp., (KTE83). The decolorization was optimized under different parameters like growth temperature, incubation time and pH. The nutritional supplementation was carried out using various carbon and nitrogen sources to enrich the production medium to enhance microbial decolouration. Mixed cultures in 10 combinations and consortium of all five strains also used for assessing dye decolourizing efficiency. Among the mixed culture combination Bacillus + Pseudomonas combination produced 97.84% dye decolouration within 8 days of incubation. Similarly corsortium of five microbial agents efficiently decolourized azo dye up to 83.26 within four days of incubation, which is evidenced in FTIR report.

**Keywords:** Microbial Consortium, Azo Dye, Dy decolorization, Remzol dye

## 1. INTRODUCTION

Textiles industry is one of the largest and oldest industries present globally. The synthetic fibers used in textile industry are coloured by making use of acid based azo dyes. Textiles industries produce large amounts of liquid wastes with Azo dyes [1]. This would cause lots of ecological and health related issues to the peoples of that surroundings. It is estimated that these industries discharge around 280,000 tonnes of dyes worldwide every year in to the environment. A very small amount of dye in water [10-50mg liter] affects the aesthetic value, transparency of water and gas solubility of water bodies. Azo compounds constitute the largest and the most diverse group of synthetic dyes and are widely used in a number of industries such a textile, food, cosmetics and paper printing [2]. Several factors determine the technical and economic feasibility of each single dye removal technique. These include dye type and its concentration, waste water composition, operation costs [energy and material], environmental fate and handling costs of generated waste products. However, a great deal of expense is a prerequisite in screening, selection and application of right organisms against specific dyes under different physic-chemical condition to achieve to achieve optimum results [3].

Many microorganisms belonging to different taxonomic group of bacteria, fungi Actinomycetes and algae have been reported for their ability to decolourize azo dyes. Bacterial degradation of these dyes was carried out by their intracellular uptake [4]. A verity of microorganisms used including bacteria such as *Escherichia coli, Bacillus cereus, B. coagulans, B. subtilis and Pseudomonas pseudomalli* are capable in decolourizing a wide range of dyes through aerobic, anaerobic and sequential anaerobic-aerobic treatment process [5]. Biological dye removal technique are either based on partial or complete biodegradation of dyes by pure and mixed cultures of bacteria, fungi and algae [3]. A textile industry uses different types of Azo dyes, which brings serious threat to human society. Considering the impact of dyes on environment and significant crops, and the ability of microorganisms to metabolizing azo dyes, in the present study Remazol Yellow was subjected to bacterial attack at optimized conditions.

## 2. MATERIALS AND METHODS

### 2.1. Sample Collection

The Effluent samples were collected from textile dye effluent run off site of textile unit located in Kovilpatti, Thoothukudi district, Tamil Nadu, India using sterile containers. All the samples were transported to the laboratory for the isolation of efficient azo dye degraders.

### 2.2. Isolation of dye degrading bacteria [6]

The dye decolourizing bacteria was isolated from the effluent sample of textile dye effluent run-off site by serial dilution and plating appropriate dilutions on modified Bushnell Hass agar medium with azo dye. All the inoculated plates were incubated at 37°C for 24 h. Following incubation, clearance of dye colour around the colonies were selected and enumerated. These selected colonies were considered as predominant dye degraders.

### 2.3. Secondary Screening of dye decolourizers [7]

A total of 95 strains were selected in the primary screening. Spot inoculation method was performed to isolate effective dye degraders. Dye added Bushnell hass medium was inoculated with test organisms as spot, incubated at 37°C for 72 hrs. Following incubation, clearance of dye colour around the colonies were selected. One the basis of higher zone of clearance a total of five test organisms were selected and named as KTE7, KTE28, KTE48, KTE64 and KTE83.

### 2.4. Dye decolourization evaluation [6]

Decolourization activity was performed in 90 mL of Bushnell Hass medium containing 0.02g of Remazol Yellow dyes inoculated with 10% (v/v) inoculum of each isolate. Uninoculated medium with dyes at similar concentration (0.02g) served as separate controls. Inoculated medium and control was incubated at 37°C for 3 to 12 days under shake culture condition. About 10 mL samples were withdrawn aseptically and centrifuged at 8,000 rpm for 15 min. The clear supernatant was used for measuring absorption at 590 nm for Remazol Yellow dye using UV-VIS spectrophotometer. The percentage decolourization of dyes was determined by using the formula:

$$D = [(A_0-A_1)/A_0] \times 100$$

Where, D, decolourization in %; $A_0$, initial absorbance; $A_1$, final absorbance

### 2.5. Identification of Dye Degrading Bacteria [8]

KTE7, KTE28, KTE48, KTE64 and KTE83 strains were identified by making use of selective cum differential agar, microscopy and Biochemical tests with reference to Bergeys manual of Determinative Bacteriology.

### 2.6. Optimization of dye decolourization using different parameters [2]

The parameters considered for optimization is supplementation of the growth medium with carbon source and nitrogen source. Apart from this inoculam size, time, pH and temperature are other factors that influence the decolourisation and therefore these conditions were also optimized. All the optimization analyses were conducted in triplicates and the results were presented as the mean of triplicate ± Standard deviations (SD).

### 2.7. Dye decolouration using Mixed culture and Microbial consortium [9].

**Mixed culture** was prepared in ten combinations as following:

*Lactobacillus and Escherichia coli*
*Staphylococcus aureus and E. coli*
*Staphylococcus aureus and Lactobacillus*
*Bacillus and E. coli*
*Bacillus and Lactobacillus*
*Bacillus and Staphylococcus aureus*
*Pseudomonas and E. coli*
*Pseudomonas and Lactobacillus*
*Pseudomonas and Staphylococcus*
*Pseudomonas and Bacillus*

**Consortium** was prepared with the mixture of all the five isolates (KTE7, KTE28, KTE48, KTE64 and KTE83).

### 2.8. FTIR analysis

FTIR analysis of *Pseudomonas* inoculated filtrate and *Bacillus* inoculated filtrate were done at National College, Tiruchirappalli. Samples were mixed with 200mg of spectroscopic grade KBr. FTIR Spectra were recorded using a Nicolet 520P Spectrometer with detector at 4cm-1 resolution and 20 scans per sample.

## 3. RESULTS

Azo dye decolourizing bacteria were isolated from effluent samples. A total of 95 dye decolourizers were assessed in secondary screening which showed five effective azo dye decolourizers and are denoted as KTE7, KTE28, KTE48, KTE64 and KTE8. These strains were characterized as *Bacillus cereus* (KTE7), *Pseudomonas fluorescence* (KTE28), *Staphylococcus aureus* (KTE48),

*Escherichia coli* (KTE64) and *Lactobacillus* (KTE83). These strains were subjected for optimization study. Optimization study revealed that variable nutrients were needed for better decolourization efficiency. Among the strains KTE7 and KTE28 decolourized azo dye within shorter duration when compared to other strains tested. Overall *Pseudomonas aeruginosa* produced 95.4% azo dye degradation in the presence of lactose, peptone with 10% inoculum, pH 7, incubation temperature 37°C on 10th day of incubation. *Bacillus* cereus also produced 90.8% dye discolouration with the similar condition used for *Pseudomonas aeruginosa* with sucrose as a carbon source (Table 1). *E. coli* produced least dye decolouration ability with 52.8% ability. Nature of microorganisms influence on bioremediation process in an ecosystem.

**Table 1: Optimized conditions of wild strains on azodye degradation**

| Category | KTE7 | KTE28 | KTE48 | KTE64 | KTE83 |
|---|---|---|---|---|---|
| **Basal Medium** | Bushnell Hass Medium | | | | |
| **pH** | 7 | 7 | 7 | 7 | 6 |
| **Temperature** | 37°C | 37°C | 37°C | 37°C | 37°C |
| **Carbon** | Sucrose | Lactose | Mannitol | Glucose | Glucose |
| **Nitrogen** | Peptone | Peptone | Peptone | Peptone | Peptone |
| **Inoculum Size** | 10% | 10% | 10% | 10% | 10% |
| **Days Of incubation** | 10 | 10 | 12 | 12 | 12 |
| **Percentage of Degradation** | 90.8 | 95.4 | 60.9 | 52.8 | 64.2 |

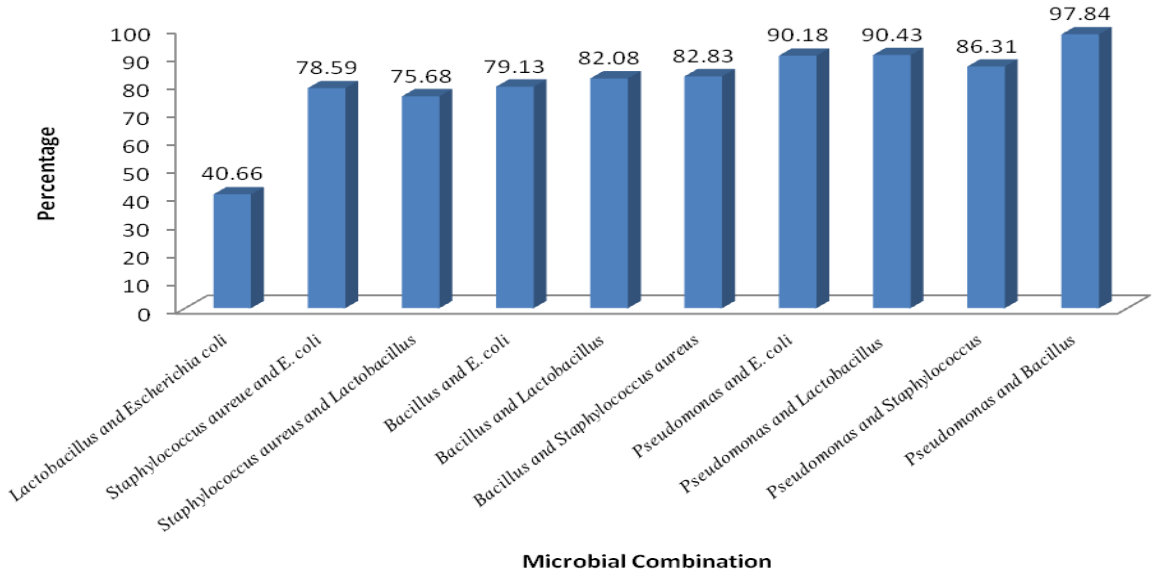


**Fig. 1: Azodegrandation by Combined wild strains of Bacteria under optimized Condition - Days of Incubation 8**

Combination of microbial species also used to study decolouration ability of the azo dye. Ten different combinations were done to assess dye decolouration. Among combination, *Pseudomonas* + *Bacillus* produced 97.84% of dye discolouration within six days of inoculation. Similarly morethan 90% dye decolouration was done by *Pseudomonas*+ *Lactobacillus and Pseudomonas*+*Escherichia coli I within six days. Lactobacillus* + *Escherichia coli* combination produced only 40.66% dye discolouration effect (Fig. 1) on 6th day of incubation. Hence incubation stopped on 6th day for all test organisms and assessed dye degradation activity.

Consortium of gram positive as well as gram negative microorganisms also prepared using *Bacillus* + *Staphylococcus*+ *Lactobacillus* and *Pseudomonas* + *E. coli*. Results revealed that gram positive group produced 93.97% dye decolouration (fig. 2) when compared to gram negative group (90.18%). Here incubation was done upto 6 days.

Consortium of five different microbial species isolated in this study also assessed for its azo dye degradation effect. Here all the five strains were mixed as consortium under optimized medium and ecological conditions. Microbial consortium produced upto 83.26% azo dye discolouration within 4 days of incubation. This result indicated the efficiency of microbial consortium. Organisms used in this study (*Pseudomonas*+*Bacillus*, *Pseudomonas*+ *E. coli* and *Pseudomonas*+ *Lactobacilli*) may used in bioremediation process (Fig. 3).

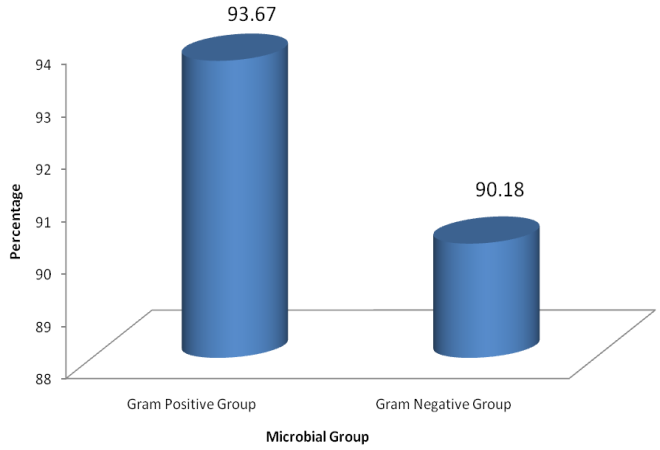


**Fig. 2: Effect of Azodegradation by Consortium of microorganisms with reference to Grams nature - Incubation 6 days**

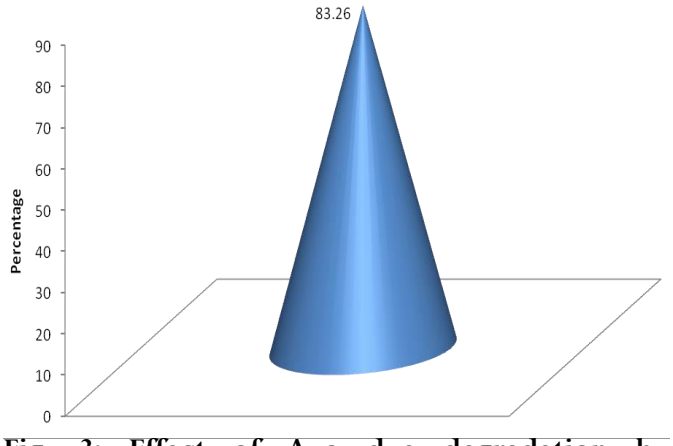


**Fig. 3: Effect of Azo dye degradation by Consortium of All Microbial Strains - days of Incubation 4**

From the FTIR analysis it was concluded that the decolourization of azo dye. It was by the means of degradation which causes changes in the molecular orientation of the pure dye molecule, it results in the formation of the different fragments indicated by the formation of new peaks in FTIR spectra. It was concluded that the action of the selected *Pseudomonas* species and *Bacillus* on dye molecules resulted in to the formation of dye products, which may be non toxic to the ecosystem.

## 4. DISCUSSIONS

Microbial bioremediation is one among the best practices to overcome the problems of chemical and other treatments of waste. Scientists throughout the world work on the prevention of pollution by biological means. This study also add few insights to the researchers those who are using native microbial species isolated from dye mixed effluent. Efficient dye decolourizes were identified as *Bacillus cereus* and *Pseudomonas fluorescence*. NurHazirah *et al.,* [5] also identified decolourization ability of *Pseudomonas* sp., *Bacillus* sp, *Lactobacillus* sp, and *E. coli* . Nachiyar *et al.,* [10] also studied four strains of *Bacillus* sp, *Pseudomonas* sp, *Lactobacillus* sp and *E.coli* as dye decolourizer, these stains utilize the dye as a sole source. Wastewater treatment using biological approach is one of the technologies apply in textile wastewater, through physical and chemical approach [5, 11]. The ubiquitous nature of bacteria makes them invaluable tools in effluent biotreatment. The genus *Bacillus* and *Pseudomonas*, which were beneficial for the degradation of toxic constituents present in the effluents, was confirmed by the decolourization bioassay with least value of the final colour. The continued development and application of biotechnologies for the biodegradation is limited primarily by physical factors such as pH, temperature and substrate concentration [12, 13]. Palanivelan *et al.,* [9] pointed out that the most of the strains shows clearing zone is formed surrounding the bacterial culture which grown on LB agar plates for dye concentration of 100 mg/l. According to them *Pseudomonas* sp showed high level degradation in 200µl textile dye, *Bacillus* sp and *Lactobacillus* sp showed moderate level degradation and lastly *E.coli* has low level degradation at normal temperature and pH 7 [14]. Our study also showed bacillus and Pseudmonas produced effective dye decolouration. Bacterial degradation of these dyes was carried out by their intracellular uptake [4]. Maulin *et al.,*[15] indicated the importance of sucrose, glucose and Lactose carbon souce in dye discolouration. These carbon increases dye decolorization. A microbial consortium consisting of three bacterial *Pseudomonas* sp. originally obtained from dye contaminated sites was capable of decolourizing textile effluent and dye faster than the individual bacteria under static conditions [16]. The similar thing also indicated in this study. Stephy *et al.,*[12] also supported the work related to dye degradation.

Tandon *et al.,* [17] reported that an aerobic bacterial consortium consisting of two isolated strains and a strain of *Pseudomonas putida* was developed for the aerobic degradation of a mixture of textile azo dyes and individual azo dyes at alkaline pH (9-10) and salinity (0.9-3.8 g/l) at ambient temperature (28±2°C). The degradation efficiency of the strains in different media and at different dye concentrations was studied. The enzyme present in the crude supernatant was found to be reusable for the dye degradation. Shah *et al.,* [14] worked on biodegradation of an azo dye AR-97 using anoxic aerobic sequential bioreactor. A consortium of four bacterial strains belonging to *Pseudomonas* sp., and *Bacillus* sp., isolated from waste disposal sites of Heleco "2005", textile processing industries was used as inoculum for developing the sequential bioreactors. The anoxic conditions in the column provided suitable environment for decolourization of dye, as the chromophore of azo dyes is susceptible to reduction under anaerobic conditions [18, 19].

## 5. CONCLUSION

It is concluded that *Pseudomoans fluorescence* and *Bacillus cereus* were able to decolourize Remazol Yellow dye upto 97%. Bioremediated dye containing effluent doesnot prevent germination of seed and growth of the plant. Our results suggested that these organisms could be considered as a good dye degrading strains, suitably used for pollution control. They are also considered as a ecofriently strains as these strains are considered to be a plant growth promoting rhizobacteria. Good combination Microbial consortium may be developed with these strains and utilized properly for bioremediation process.

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