

Bioremediation of Textile Effluent Direct Orange-102 by Means of *Pseudomonas fluorescens* Strains

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Abstract:- Large quantity of water and chemicals are used in textile industries for processing, even the traces of dye present in the effluent is not permissible. Many dyes are difficult to decolorize due to their chemical structure and synthetic origin. In recent years there has been an alternative research on microbial degradation of dye present in wastewater. Biodegradation method has been proved to be very effective in treatment of this pollution in an environmentally friendly and cost effective way. The extent of decolourization of direct orange dye by microbial degradation by employing *Pseudomonas fluorescens* need to be investigated in detail and an attempt was made to develop a cost effective method for dye decolourisation using bacteria *pseudomonas fluorescens* with respect to various nutritional sources (carbon and nitrogen) experimental parameters (temperature, PH, inoculum size) has been experimented in the present study.

Keywords: Bioremediation, Decolorization, Biodegradation, Orange Dye, Textile Wastewater, Spectrophotometer, Effluent, *Pseudomonas fluorescens*.

1. INTRODUCTION

Textile industry was the oldest industry and it was dated back many centuries. The development of textile industry is directly linked to the economic part of our country. These textile industries releases large amount of colored effluent in to the environment without any proper treatment which causes many environmental problems.

The degradation of synthetic azo dye can be done in different possible methods namely Physico-Chemical process and Biological Degradation. The methods involved in Physico-chemical processes are Coagulation, Precipitation, Flocculation, Adsorption and Electro Chemical Destruction, etc. The biological degradation is classified as 1. Fungal Degradation, 2. Bacterial Degradation and 3. Bacteria-Fungus Degradation.

In this paper, bacterial degradation is mainly focused and the possible methods involved are Bacterial Enzyme, Whole Bacterial Cell, Bacterial Consortium and Nanoparticle-enzyme conjugate.

Textile waste water includes a large variety of dyes and chemicals that make the environmental challenge for textile industry. The characteristics of textile wastewater are illustrated in Table 1:

Table 1: Characteristics of Textile Wastewater

Parameters	Value
pH	7.0– 9.0
Biochemical Oxygen Demand (mg/L)	80 – 6,000
Chemical Oxygen Demand (mg/L)	150 – 12,000
Total Suspended Solids (mg/L)	15 – 8,000
Total Dissolved Solids (mg/L)	2,900 -3,100
Chloride (mg/L)	1000 – 1600
Nitrogen (mg/L)	70 – 80
Colour	50-2500

As textile plants are in remote areas and the treatment methods are expensive scientists are forced to look for cost effective & eco-friendly methods to decolorize dyes in effluent. The microbial organism has the ability to decolorize and *Pseudomonas* *Flourescens* strains are used in this study as a cost effective way.

2. MATERIALS AND METHODS

2.1 Sample Collection

Dye effluent was collected from a dyeing unit in Erode, Tamilnadu, India. The sample was collected in a brown bottle. Prior to the collection the sample bottle was rinsed thoroughly with the sample effluent. Then the sample was stored in refrigerator at 4°C and used without any pretreatment.

2.2 Materials and Methods Employed

2.2.1 Microorganism and Culture Medium

Pseudomonas fluorescens were obtained from the culture collection centre of Camrit Biosolutions at Kochi, Kerala, India. Pure cultures were maintained on nutrient agar slants. Composition for nutrient medium (g l⁻¹) used for decolorization studies is provided in the table.

Table 2: Composition of Nutrient Medium

NaCl	5g
Peptone	10g
Beef extract	10g
Distilled Water	1 L

2.2.2 Dyes and Chemicals

The textile dye Direct Orange 102(direct dye orange (C.I. 25430) used throughout the study were obtained from Sigma Aldrich, India. All the chemicals were of highest purity available and were of analytical grade.

2.3 Analysis of Physicochemical Parameters of Textile Effluent

The physicochemical parameters of the collected textile dye were determined using standard methods in the laboratory.

2.3.1 Decolourisation Experiments

Decolourisation of direct orange dye by *Pseudomonas fluorescens* in the culture supernatants was determined using UV Vis spectrophotometer. The effect of static and shaking condition of the bacterial culture on Direct Orange 102 were studied. 1ml of bacterial cultures were transferred into separate 100 ml conical flask containing fresh nutrient medium containing Direct Orange 102 (250 mg/l) and were incubated at 30°C, under static condition. One set of flask was incubated under agitation at 180 rpm and temperature of 30°C while the second set was incubated under stationary condition at 30°C for a period of 48 hours. The uninoculated dye Medium supplemented with respective dye served as the experimental. The control consisted flask without any microorganisms. All experiments were done in triplicates.

2.3.2 Analytical Methods for Dye Decolourisation Studies

After 3 day aliquots (5ml) of the culture media were withdrawn, centrifuged at 10,000 g for 10 minutes in a refrigerated centrifuge (Ependorf centrifuge) at 4°C. Decolourisation was quantitatively analyzed by measuring the absorbance of the supernatant using a UV-visible spectrophotometer. Maximum wavelength for the Direct Orange 102 ($\lambda_{\text{max}}=480$ nm) in the visible region on a UV-Vis double beam spectrophotometer (systronics P 2202). The percentage of decolorization was calculated from the difference between initial and final values.

Change in Chemical Oxygen Demand (COD) and Biological Oxygen Demand (BOD) was also studied using Standard Methods for Examination of Water and Wastewater APHA, 1995.V2-253.

2.4 Plate Assay

Plate assays were performed to observe Decolourisation of Direct orange dye. Fresh nutrient agar plates incorporated with direct orange dye inoculated with *Pseudomonas fluorescens* were incubated at 30°C for 48 hrs Fresh nutrient agar plates incorporated with direct orange dye (uninoculated) were used as control plate. The plates were observed after 48 hours.

2.5 Dye Decolorization Optimization

To determine the effect of pH on decolorization, the full grown culture was inoculated in conical flasks containing 100 ml nutrient broth of varying pH (4-10) and was amended with 250 mg/l of Direct Orange 102. pH was adjusted using either HCl (0.1M) or Na₂CO₃ (0.1M). In the similar fashion, the optimum temperature of dye decolorization by selected bacterium was determined by evaluating the dye decolorization at 20, 30, 37, 40, 50 °C.

After different time intervals aliquot (5ml) of the culture media was withdrawn and supernatants obtained after centrifugation was used for analysis of decolorization by UV Vis double beam spectrophotometer (Systronics P 2202).

2.6 Concentration of Dye

Various concentrations of dye (50, 100, 200, 300, 400 and 500mg/l), and inoculum sizes of 10% (1 ml), 20% (2 ml), 30% (3 ml) and 40% (4 ml) were used to examine the effect of initial dye concentration and inoculum size on the decolourisation rate. *Pseudomonas fluorescens* was cultivated for 48 h in conical flask containing 100 ml nutrient broth. Incubation was done at 30°C.

2.7 Decolourisation Assays

The UV and visible spectra of the samples were measured in ethanol with a UV-Vis double beam Spectrophotometer. Quartz cells (1 cm square) having 1.0 cm path length were used for the determination.

Hydrogen discharge tungsten filament lamp was used as a source of light and maximum absorbance was recorded.

The optical densities (OD) measured were then converted to the dye concentrations using the respective standard curves. The efficiency of colour removal was expressed as the percentage ratio of the decolourised dye concentration to that of initial one based on the following equation ,

$$\text{Colour removal (\%)} = \frac{\text{Dye (i)} - \text{Dye (r)}}{\text{Dye (i)}} \times 100\%$$

Where, Dye (i) = initial dye concentration (mg/L), Dye (r) = residual dye concentration (mg/L).

3. RESULTS AND DISCUSSION

3.1 Textile Wastewater Characteristics

Textile wastewater used for the study is characterized by measurements of biochemical oxygen demand (BOD), chemical oxygen demand (COD), suspended solids (SS) and dissolved solids (DS). The COD values of composite wastewater are extremely high compare to other parameters. In most cases BOD/COD ratio of the composite textile wastewater is around 0.25 that implies that the wastewater contains large amount of non-biodegradable organic matter.

Table 3: Comparison of the physicochemical properties of the textile effluent treated with *Pseudomonas fluorescens*

Physicochemical property	Untreated effluent	Effluent treated with <i>Pseudomonas fluorescens</i>
pH	8.8	7.2
Decolourisation rate	0% (Dark orange)	80%
COD (mg/L)	750	108
Total suspended solids(mg/L)	386	91
Total Dissolved Solids (mg/L)	15124	2000
Biochemical Oxygen Demand (mg/L)	480	44
Chloride (mg/L)	6900	892

3.2 Decolourisation Rate

The concentration of dye is proportional to the efficiency of dye removal, by the way of a combination of factors such as the toxicity of the dye. Incubation of the dye direct Orange 102 with *Pseudomonas fluorescens* resulted in 60 % removal of colour during the first day itself. The decolourisation rate of dye by *Pseudomonas fluorescens* at different dye concentration under static and agitated conditions shows that using 1 mg dye concentration, the % decolourisation rates are 75 and 89% respectively for agitated and static conditions. Similarly using dye concentration at the levels of 4 and 5 mg, the percentage of dye decolourisation rates are 71, 74, 88 and 91 % respectively for the agitated and static conditions. It is found that static conditions are more efficient than the agitated conditions for the dye decolourisation.

The dye samples that show clear solution were from initial concentrations of 1 and 2 mgms. The results shows that highest rate of agitation tends to decrease the bacterial growth and activities of some biologic substrates such as enzymes which play an important role in decolourising the dye.

Table 4: Decolourisation of orange dye by *Pseudomonas fluorescens* at different dye concentrations

Dye concentration mg/100ml	% of dye degradation	Agitation (%)	Static(%)
1	2	75	89
2	2	78	90
3	3	78	92
4	4	74	91
5	5	71	88

3.3 Optimization of Factors affecting Dye Decolorization

a) Effect of pH on decolorization: 100 ml of nutrient broth inoculated with *pseudomonas fluorescens* was amended with 250 mg/l of Direct Orange 102. Varying pH from 5 -9 were experimented. Results clearly indicates that the optimum pH for colour removal is at slightly alkaline pH value of 8 and the rate of colour removal tends to decrease rapidly at strongly acid or strongly alkaline pH values.

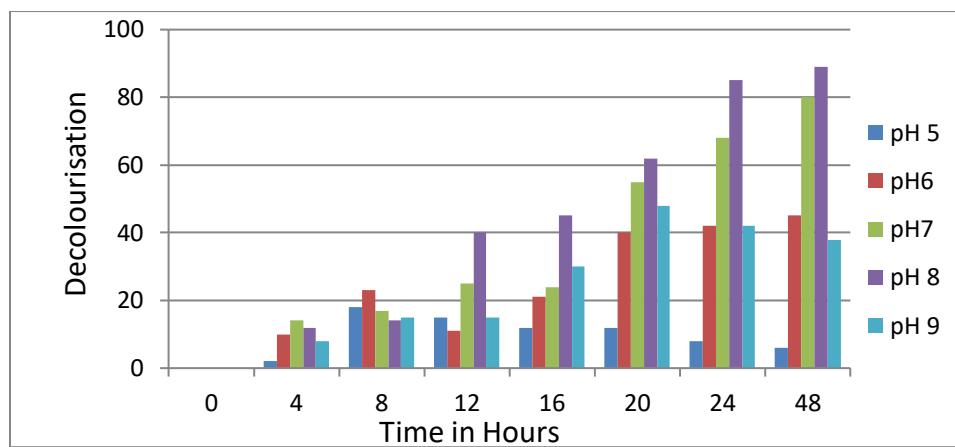


Fig.1 Direct Orange 102 decolorization at different pH

b) Effect of temperature on dye decolorization: 100 ml of bacterial culture amended with 250 mg/l of Direct Orange 102 under different temperature (20, 30, 37, 44, 50°C) were determined. The rate of colour removal increases with increasing temperature, within a defined range of 35-37°C. The temperature required to produce the maximum rate of colour removal tends to correspond with the optimum cell culture growth temperature of 30-37°C.

4. SUMMARY AND CONCLUSION

The biodecolorization process was studied and presents a feasible and economical method of treating coloured effluents. Results show that the textile wastewater having diverse characteristics could be decolorized effectively using *Pseudomonas fluorescens*. The textile dye (Direct Orange) is degradable under aerobic conditions with a concerted effort of bacteria isolated from textile dye effluent. Nutrients (carbon and nitrogen sources) and physical parameters (pH, temperature and inoculum size) had significant effect on dye decolorization. *Pseudomonas fluorescens* showed highest decolorization of Orange dye effectively during optimization and *Pseudomonas fluorescens* showed consistent decolorization of textile dye (Direct Orange) throughout the study.

The bacterial species *Pseudomonas fluorescens* which is used in decolorization of Direct Orange dye in this study gives remarkable results. As the strain is capable to decolorize dyes at high concentration, it is highly recommended in textile industries. However, potential of the strain needs to identify, and more experiments are recommended. Thus further research can enhance understanding, control and application of this process.

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