

Analysis of Degrading Potentital of Pseudomonas Fluorescens Isolated From Textile Effluent Contaminated Soil

G.Dhanalakshmi , A.Reniprabha , S.Muralidharan, S.M.Thoulath Basha

PG and Research, Department of Zoology, Chikkaiah Naciker College, Erode, Tamil Nadu. India.

ABSTRACT: When the effluent was treated with *Pseudomonas* there was a significant decrease in all physico-chemical parameters including the dye .The percentage of reduction was proportional to the incubation period .The dye degradation efficiency was also found to be maximum at 72 hrs of treatment .Therefore , *Psuedomonas fluorescencs* from effluent contaminated soil can be used for biodegrading of textile effluent .

KEYWORDS : Degrading potential , Pseudomonas fluorescens , Textile Dyeing effluent .

I.INTRODUCTION

The textile industry is a key factor in India from the perspective of generating employment and earning foreign exchange .However this industry poses a serious environmental problem by discharging the untreated effluent to the environment . This textile effluent is characterized by high chemical oxygen demand , strong colour , high salinity , large amount of dissolved solids and suspended solids.[1] .

During textile processing, inefficiencies in dyeing results in large amount of dye being lost to the waste water which ultimately finds its way into the environment. The amount of dye lost is dependent upon the type of dye application varying from 2 % loss when using basic dyes to a 50 % loss when certain reactive dyes are used [2].So the dyes discharged from textile dyeing industry are a priority pollutant for the regulation authorities and water utilities because of their visibility at low concentrations. These dyes and their by products have a strong toxic and mutagenic influence on the living organisms [3].Hence textile waste water is rated as one of the most polluting among the industrial sectors considering both volume and composition of the effluent . [4] .If the textile effluent is not properly disposed , can cause serious environmental pollution that can threaten human health , livestock , wildlife , aquatic lives and the entire ecosystem .[5].

In view of these adverse effects, the textile industry effluent has to be treated and discharged according to the standard prescribed under Central Water (prevention and control of pollution) Act, 1974. To ensure the safety of effluents, proper technologies need to be used by treatment facilities .Previous research reports focused on various biological , chemical and physical techniques for treating dye wastes . However chemical treatment is often cost and application limited , while physical removal cam lead to extra solid wastes and increased overload . Biological treatment has been effective in reducing the pollution of dye wastes and when used properly has a lower operating cost than other remediation process. The bio remediation process is reported to be the possible solution due to its low-cost, environmental friendly and publicly accepted technology.

To date, although research on bio degradation and bio remediation by microbial consortia have been established internationally, limited studies on capability of locally isolated bacterial strains in reducing pollutional load in the textile effluent have been reported and their potential in treatment of effluent should therefore be investigated . Hence an attempt has been made to analyse the degrading potential of locally available *Pseudomonas* species.

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II. MATERIAL AND METHODS

Effluent Source: Highly colored textile effluent was collected from the out let of a textile dyeing unit located at Manickampalayam , Erode of Tamil Nadu .The effluent from the textile unit was collected in a 10 lit airtight plastic can. The effluent was filtered through ordinary filter paper to remove large suspended particles .

Sterilization technique: All glassware's were initially washed with detergent and rinsed thoroughly with distilled water followed by sterilization in hot air oven at 120⁰C for 1.5 hours. Inoculations were performed with flame sterilized loops and the entire experiment was carried out under strict sterile conditions.

Source and isolation of Microorganisms :

Isolation of bacterial species was carried out from soil samples taken from a textile unit area located at Manickampalayam , Erode .Soil samples were subjected to serial dilution and inoculated into sterile nutrient agar plates with the nutrient medium composed of beef extract (3 g/L),Peptone (5 g/L) , Sodium chloride (5 g/L) and agar (15 g/L) at a natural pH and 30 ± 1⁰C for 3 days. Bacterial colonies were identified and characterized based on Bergey's Manual of Determinative Bacteriology .[5].Counting and identification procedures were carried out under a stereo binocular microscope .Stock cultures of the isolated bacterial species were preserved on agar slants at 4⁰C.

Preparation of bacterial inoculum:

Standard inoculum was prepared by sub culturing a single colony of the *P.fluorescens* from the stock culture in 125 ml conical flasks containing 10 ml modified Minimal Medium (MM) (Sodium Chloride 4.0 , Magnesium sulphate ,0.42 , Potassium chloride 0.29 ,D – glucose 0.1 ,Ammonium sulphate , 0.1 Potassium phosphate dibasic 1.27 , Calcium chloride 0.02 , Ammonium nitrate 1.0 , Sodium carbonate 00.1 , Yeast extract 0.6 , EDTA 0.5 g/L) [6].at 30⁰ C in a rotary incubation shaker (200 rev /min) for 3 days .

Inoculation of bacteria into dye medium:

One ml aliquot of isolated bacterial species was inoculated into 500 ml conical flask containing 100 ml MM with 100 ml /L of the dye (Direct Orange – 102) and the pH was adjusted to 7 to 8.5 and incubated at 30⁰C for 10 days.Control flasks contained only dye medium and nutrients but received no bacterial inoculums.

Introduction of bacteria into effluent medium:

100 ml of the filtered effluent was taken in 500 ml conical flasks. 1ml of aliquot of the bacterial species was inoculated into the effluent medium. Raw effluent without bacterial inoculums was treated as control .Incubation at 30 ± 2⁰ C for 10 days in static condition .One hour shaking was given at 2 days interval on a mechanical shaker .In both the sets of experiments , incubations were performed in triplicate and results were expressed as mean ± SD.

Decolourisation assays :

Decolourisation of the individual dye was determined at their respective maximum absorption wave length in the culture supernatants using a spectrophotometer (Thermo spectronic spectrophotometer , Genesys 20). A sample of culture broth was withdrawn daily and about 1 ml was centrifuged at 10000 rpm for 15 minutes (Eppendorf centrifuge 5415) , before its optical density was measured .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 the of initial once based on the following equation . [7].

$$\text{Dye(i) - Dye(r)}$$

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

$$\text{Dye (i)}$$

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

III. RESULTS AND DISCUSSION

The soil samples collected from the local textile dyeing unit were screened for bacteria and the species *Pseudomonas fluorescence* was identified by microscopic, biochemical characters based on standard manual .

A perusal of literature indicated that *Pseudomonas* species was well studied and documented for their decolorisation and degradation potentials[8] and this is one of the dominant species isolated from the effluent contaminated soil.[9]

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All physico-chemical characteristics of textile dyeing effluent were determined by following standard methods and all were found to exceed the permissible limits. It was found that there was a significant decrease in all physico-chemical parameters including the dye in the raw textile effluent treated with *Pseudomonas fluorescens*. The percentage of reduction was found to be proportional to the incubation period as shown in Table 1 and also it is significant.

The present results coincided with the findings of several previous works reported by [10, 11, and 12] who found *Pseudomonas* as an organism with dye decolorisation potential. Though the bacterial inoculate was capable of decolorising the dye to some extent, the degrading potential for other pollutants was not much explored in locally isolated bacterial strains in textile effluent contaminated soil. The efficiency of degradation of other pollutants such as BOD, COD, TSS, TDS, Chlorides, Alkalinity, pH and Organic matter by *Pseudomonas fluorescens* was well evident from the present investigation.

The results pertaining to various physico-chemical characteristics of textile effluents, the pH of effluent samples ranged between 7.0-7.2. These results are in accordance with the results of [15] who reported that textile wastes are alkaline and pH of effluent affects physico-chemical properties of water which in turn adversely affects aquatic life, plants and human. Total dissolved solids (TDS) values fall within the range 1800-3100 mg/L. TDS values of samples are much higher than the permissible limits which predicts the presence of excess of materials and dissolved matter in textile effluents. High TDS is one of the major sources of sediments which reduce the light penetration into water and ultimately decrease the photosynthesis. *Pseudomonas fluorescens* treatment considerably reduced the TDS by 58%.

The chloride value was 36.4 mg/L in the textile effluent which was alarmingly high. High chloride contents are harmful for metallic pipes as well as for irrigation purposes. The chlorides also reduced in the treated samples (26.4 mg/L). COD value of textile was found to be in the range of 532 mg/L. These results are in agreement with the (16) and (17) who also reported a high COD which produce unaesthetic color and endanger water systems. [18].

The percent reduction of all physico-chemical parameters was gradually increased from the 3rd day of incubation period to 6th day incubation period as represented in Fig. 1. The impact of reduction in the values of pollutants from the effluent depends on the survival, adaptability and activity of selected organisms. [19]. The colour removal may be due to hydrogenase activity that utilize NADPH and NADH as cofactors.

The dye degradation efficiency was studied at different time intervals such as 24, 48, 72 and 96 hrs. It was found that the degradation efficiency was maximum at 72 hrs of treatment as seen in Fig. 2. These breakdown products are found to be non-toxic as evaluated [20].

Most studies on the metabolism of organic contaminants have been performed with bacteria especially in the context of bio remediation [21]. Bacteria generally are easier to culture and they grow quickly. They are amenable to molecular genetic manipulations. They are able to metabolize chlorinated and other organic contaminants.

Based on this, in the present study *Pseudomonas* species is selected for the bioremediation process to treat the textile dyeing effluent. It was reported that mutant forms of *P.aeruginosa* giving better results than the normal types. The mutational treatment resulted in increase percentage of survival due to the presence of nucleotide excision repair and base pair mechanism [22]. This mutational modifications in augmenting the degrading potential of *P.fluorescens* can be further explored. The genetic engineering studies on *P.fluorescens* may improve the treatment process if the amplification studies are carried out.

IV. CONCLUSION

Thus we can conclude that *Pseudomonas fluorescens* was a potential organism and would serve as a potential biological agent for the treatment of textile dyeing effluent. So as a future approach towards the problem, we have planned for isolation, amplification and enhancement of gene and its impact on treatment. This may give a solution to the present day problem in the dyeing industry sector.

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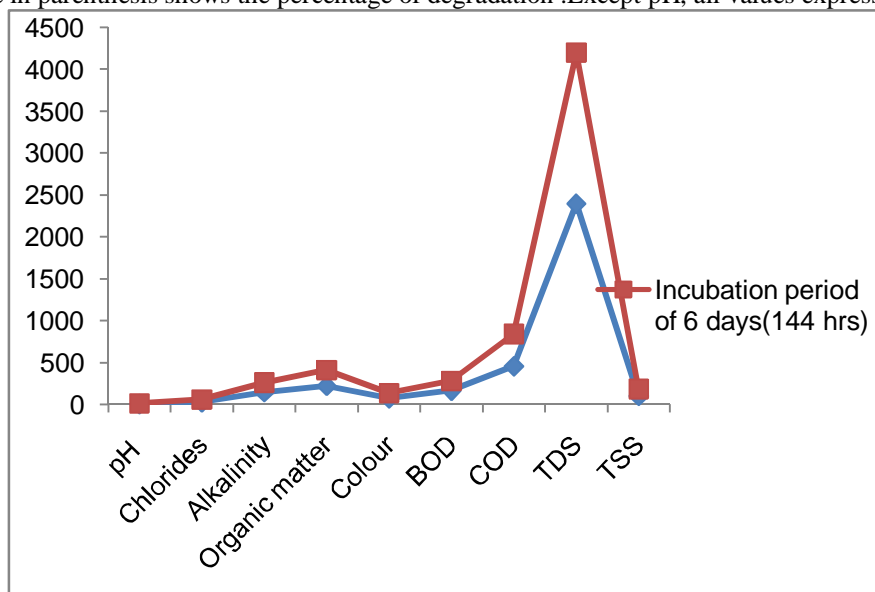
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Table .1 Properties of textile dye effluent before and after treatment with *Pseudomonas fluorescens*

S.NO	Parameters (mg /l)	Control (Raw effluent)	Treated with <i>Pseudomonas fluorescens</i>	
			Incubation period of 3 days (72 hrs)	Incubation period of 6 days(144 hrs)
1	pH	7.2	7.1 (98%)	7.0 (97 %)
2	Chlorides	36.4	30.4 (83 %)	26.4 (72 %)
3	Alkalinity	163	148.3(90 %)	113.4(69 %)
4	Organic matter	264	224 (84 %)	186.5 (70 %)
5	Colour	98.4	74.3 (75 %)	60.2 (61%)
6	BOD	227	164(72 %)	115 (50 %)
7	COD	532	458 (86 %)	385 (72 %)
8	TDS	3100	2400 (77 %)	1800 (58 %)
9	TSS	114	102 (89 %)	84 (73 %)

The value in parenthesis shows the percentage of degradation .Except pH, all values expressed in mg/l.



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