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Evaluating the Potential of *Pseudomonas Aeruginosa* in the Biodegradation of A Few Dyes

Jasmine R*[1], Arthy[1]

1. P.G.Dept of Biotechnology, Bishop Heber College, Trichy 620 017

ABSTRACT

Effluent discharge from textile and dyestuff industries to neighbouring water bodies and wastewater treatment systems is currently causing significant health concerns to environmental regulatory agencies. Color removal, in particular, has recently become of major scientific interest, as indicated by the multitude of related research reports. Microbial decolorization and degradation of dyes is seen as a cost-effective method for removing these pollutants from the environment. In this review we have examined biological decolorization of dyes used in textile industries. A simple spectrophotometric assay method was adapted to screen for the ability of *Pseudomonas aeruginosa* isolates from dye industry effluent to degrade the chosen dyes. They were checked for the extent of dye decolorization [Methyl Red, Methyl Blue & Malachite green] at three different concentrations as 40, 60 & 80ppm. Visual and spec decolonization indicated that decolorization was higher in 60ppm concentration of all three dyes, indicating that to be the optimum concentration for degradation by *Pseudomonas aeruginosa* isolates. We suggest that the addition of bacterium to the microbial mixture indicated that decolorization was higher in case of dyes in 60 ppm concentration. It can also reduce the bulking problems of the effluent by preventing the load of the organic matter from becoming too high.

Keywords: *Pseudomonas aeruginosa;* Methyl Red, Methyl Blue; Malachite green; effluent;

*Corresponding Author Email: jasminebhc@gmail.com

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INTRODUCTION

Over the last two decades considerable amount of research has been reported on the use of micro-organisms as bioremediation agents in the treatment of dye-containing waste water[1]. Although these dyes contribute a minor fraction to the usually high load of dissolved organic matter, they tend to be highly visible and must be removed before effluent that complies with government environmental legislation can be discharged into the ecosystem. The waste water characteristics from a dye house are highly variable from day to day, depending on the type of dye and concentration of chemicals used. Treatment of such waste waters is, therefore, essential but difficult. Physico-chemical treatment methods which include electrochemical oxidation and sorption are effective as tertiary treatments but are not economically viable as they result in the generation of secondary pollutants that require further treatment[2]. This has led to extensive research for development of cheaper alternatives such as biological treatment. Many of the world's textile manufacturers are equipped with their own waste water treatment plants which usually combine biological processes with physical – chemical process[3]. Studies have shown that several dyes, in particular azo dyes, are mutagenic as parent molecules or when they are metabolized[4]. As a result, the use of certain azo dyes in Germany in consumers goods was banned in 1996[5]. Independently on the microorganism used there are several parameters affecting the colour removal efficiency: medium composition, oxygen, temperature, pH, dye concentration, dye structure, electron donor, redox potential, redox mediators and the presence of inhibitory substances[6,7]. For instance oxygen, due to its high redox potential, can compete with the dye for the electrons during the reduction stage and, during cell growth, will affect the physiological characteristics of the cells[7,8]. Nevertheless there are reports of microorganisms capable of decolorizing dyes in aerobic conditions[9-12]. The conventional biological treatment systems like activated sludge are not capable of degrading the dyes, being mainly removed by physical adsorption[13], because under aerobic conditions azo dyes are not readily metabolised[14]. Also in these conventional systems, there are several substances like nitrate and nitrite, usually present in high levels in municipal wastewater that compete with azo dyes as electron acceptors[15]. The decolourisation of dyes by microorganisms is commonly done with bacteria or white-rot fungi. In bacteria the mechanisms of decolourisation can be: non-specific reduction by electron transporters from the cellular metabolic pathways (flavins, quinones), dye-specific reduction by azo reductase enzymes and chemical reduction by sulphide generated in sulphate reduction[16,17].

MATERIALS AND METHODS

Sterilization techniques

All glasswares were washed with detergent, rinsed thoroughly with distilled water and oven sterilized at 80^{o}C for 2 h. All polypropylene tubes and tips used as well as media and solutions prepared were sterilized by autoclaving at 121oC for 15 - 25 min. Inoculations were done with flame sterilized loops and all experiments were performed wearing sterile disposable hand gloves.

Dyes

Dyes as Methyl red, Methyl blue and Malachite green were used. The dyes were diluted and used in three concentrations as 40, 60 & 80ppm.

Isolation of pure culture

The bacteria isolated from the effluent sample contain a mixed population exhibiting diverse morphological and physiological characters. Single germinating spores were picked from the mixed culture containing several spores and subcultured. A pure culture was produced by repeated subculturing. The purified cultures were then transferred to nutrient medium and subcultured fortnightly which was then stored at 4^0 C until use.

Identification of bacteria

Bacteria were identified by several biochemical tests

Dye decolourization by the crude Bacterial culture

Procedure:

The three different dye solutions at different concentrations say 40ppm, 60ppm and 80ppm were prepared using the formula,

$$\textbf{Concentration of dye} = \frac{\textbf{Required ppm}}{\textbf{Stock}} \times \textbf{Required volume}$$

The three different dye solutions were subjected to various dilutions to a series of 1 ml, 2 ml upto 10 ml and made up with distilled water. 1 ml of bacterial culture was inoculated onto the tubes containing water samples respectively. The initial Optical density was assessed by measuring absorbance with the help of UV- Spectrophotometer at 560 nm. The samples were incubated for 24 hrs at room temperature. After 48 hrs incubation, final OD was measured at 560 nm, and was also repeated after 72 hrs incubation. Biodegradation of dye was calculated by using the formula:

$$\text{Percentage of decoolourization} = \frac{\text{Final OD- Initial OD}}{\text{Initial OD}} \times 100$$

Maximum Tolerable Concentration (MTC) of Dyes

To determine the maximum tolerable concentration of dyes, 1M stock solution of each of the dye was prepared and used to prepare appropriate dilutions (0.08, 0.1, 0.16, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75 and 2.0mM). Bacterial strains were streaked from log phase culture on Nutrient agar plates, the plates were then incubated at 37C and growth was observed after 48-72 hours. The isolate was considered sensitive if it could not grow on 0.08mM concentration of the dye.

RESULTS & DISCUSSION

Aerobic bacterial isolate (*Pseudomonas auruginosa*) from the dye-contaminated effluent was isolated and identified as in tables 1&2.

Table: 1 Identification by Morphology

Tests	Isolate 1 Morphology
Color	Dull white
Shape	Regular
Texture	Smooth
Elevation	Undulate
Magin	convex

Table: 2 identification by biochemical tests

S.no.	Biochemical tests	Isolate 1
1	Indole production	+
2	Methyl red	-
3	Voges proskaur	-
4	Citrate utilization	-
5	Catalase	+
6	Oxidase	+
7	Nitrate reduction	+
8	Urease	-
9	Glucose	+
10	Sucrose	+
11	Lactose	-
12	Mannitol	+
13	TSI	+

The identified isolate was *Pseudomonas aeruginosa*

It was found capable of growing in media containing dyes. They were checked for the extent of dye decolorization [Methyl Red, Methyl Blue & Malachite green at 40 ppm, 60ppm and 80ppm with 10 dilution of nutrient broth]. Visual and spec decolorization indicated that decolorization was higher in all the dyes at 60ppm. They were also checked for the extent of dye decolorization in concentrations of 40ppm and 80ppm, where in both the results was not significant as seen in Table 3.

Table 3A: Percentage Degradation Of Methyl Red By The Bacterial Strain- *Pseudomonas aeruginos*

S.No.	Time duration in hrs	Percentage of Decolourization		
		40ppm(%)	60ppm(%)	80ppm(%)
1	After 48hrs	84.85	47.36	32
2	After 72 hrs	85	57	36

Table: 3B Percentage Degradation Of Methyl Blue By The Bacterial Strain- *Pseudomonas aeruginosa*

S.No.	Time duration in hrs	Percentage of Decolourization		
		40ppm(%)	60ppm(%)	80ppm(%)
1	After 48hrs	81.3	52.8	41
2	After 72 hrs	85	52.8	40.5

Table:3C Percentage Degradation Of Malachite Green By The Bacterial Strain- *Pseudomonas aeruginosa*

S.No.	Time duration in hrs	Percentage of Decolourization		
		40`ppm(%)	60ppm(%)	80ppm(%)
1	After 48hrs	85.62	64	38
2	After 72 hrs	87.2	71	39.5

Since the isolate was able to degrade the above mentioned dyes, effort was taken to determine the maximum tolerance concentration of the isolate to the dyes chosen. It was observed that *Pseudomonas aeruginosa*. could efficiently tolerate 1.25mM concentration of methyl red and methyl blue, but a higher concentration of 1.75mM was tolerated for malachite green (Table 4).

Table: 4 Growth of Pseudomonas *aeruginosa* in various concentrations of dye(in mM)

Dyes	0.08	0.25	0.50	0.75	1.0	1.25	1.5	1.75	2.0
Methyl red	+	+	+	+	+	+	-	-	-
Methyl blue	+	+	+	+	+	+	-	-	-
Malachite green	+	+	+	+	+	+	+	+	-

+ Shows presence of growth, - Shows absence of growth

The results reveal that the isolated *Pseudomonas sp*. was not sensitive, but could efficiently grow in the presence of the dyes and could effectively degrade them, which could be employed to treat effluents loaded with such dyes.

Though these dyes are not generally toxic to the environment, they impart colour to the water bodies, which may hinder light penetration and in turn affects aquatic life. This limits utilization[18] Though *Pseudomonas auruginosa* isolates seem to have a high potential for dye degradation, yet the involvement of the extrachromosomal genes have not been yet studied.

CONCLUSION

It can be concluded that, the isolated bacteria *Pseudomonas auruginosa* is one of the most effective organisms to reduce the decolourization against the selected dyes at 40ppm, 60ppm and 80ppm with 10 dilution of nutrient broth.This has been a preliminary step in the trial of

P.aeruginosa strains to degrade a few dyes. Further work is in progress to determine the factors influencing the degradation and the exact mechanism involved.

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