DECOLORIZATION AND DETOXIFICATION OF REACTIVE RED 152 DYE BY PSEUDOMONAS SP., ISOLATED FROM TEXTILE EFFLUENT

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ABSTRACT

Reactive dyes are one of the most used dyes in textile industries for dyeing of cellulosic fiber. As per current trends in fashion, cotton fibers are widely utilized. Due to its chemical nature, about 40- 50% of the dyes are remaining unfixed and finally appear in the effluent. For the treatment of this effluent various chemical and physical methods are available but due its limiting factors they are not widely used. Bioremediation using bacteria is nowadays becoming an attractive alternative for the treatment of textile effluents containing dyes. In the current research work, decolorization of Reactive Red 152, a very important synthetic dye was investigated. A potential dye degrading organism, Pseudomonas sp. was isolated from textile effluent. Optimization of various physicochemical parameters like pH, temperature, various carbon source and nitrogen source were carried out for maximum dye decolorization. Under optimized condition 98% decolorization of dye is observed. The dye can be used as sole source carbon and energy source for cell growth. Phytotoxicity on wheat was tested and treated samples were found to be non-toxic. These results suggest that isolated organism Pseudomonas sp. is suitable bacterium for the bioremediation of textile waste water.

Key words: Bioremediation, Decolorization, Reactive Red 152, Reactive dye, Phytotoxicity

I. INTRODUCTION

Dyes are the coloring chemical compound having wide application in various industries like food, plastic, rubber, pharmaceutical, textile, enamel, leather, cosmetics and paper Industries[1,2]. Synthetic dyes are classified in to several group viz., direct dye, Reactive dye, disperse dye, vat dye, sulphur dyes, acid dye, basic dye etc.. Due to new fashion trends, cotton is gaining importance in the fashion industries. Reactive dyes belong to the most important group of synthetic colorants and are used extensively in the textile industries. They are used predominantly on cellulose, cellulose acetate, and acrylic fibres. Due to its low fixation on the fiber, generally 40-50% of the dyes appear in the effluent. They are generally considered as the xenobiotic compounds, which are very recalcitrant to biodegradation[3]. Unfortunately, effluent treatment facilities are not enough capable to remove the dye from the dye effluents and thus contributing toxic effects to various habitats[4]. Various alternative treatments aimed at removing Reactive dye from wastewater have been investigated, like chemical processes (Fenton oxidation and reduction)[5,6], physical precipitation and flocculation, photolysis, adsorption, electrocoagulation[7], advanced oxidation, reverse osmosis and biodegradation[8]. It is known that conventional aerobic wastewater treatment processes, such as activated sludge, cannot efficiently remove the azo dyes. Thus, there is still a need to develop novel and effective

biological decolorization processes for the cleanup of azo dyes[9]. Biological processes have gained a great attention because, due to its more cost-effectiveness and environment friendly nature than physical and chemical treatment methods, and they produce less sludge [10,11,12]. Thus, biodecolorization study of Reactive Red 152, a widely used dye is carried out. Phytotoxicity of treated and untreated samples had been studied.

II. MATERIALS AND METHODS

2.1 Chemical

Reactive Red 152 was purchased from local market of Surat textile market. Other chemicals, used in this study were of analytical grade and obtained from Hi-media, India.

2.2 Sample Collection

For sample collection various site near Surat were visited and samples were collected in sterile plastic container from Sachin GIDC, Gujarat, India. Effluent and soil samples were immediately transferred to our laboratory and stored at 4°C in refrigerator until use.

2.3 Isolation, Screening and Partial Identification of Dye Decolorizing Bacteria

10 ml of sample was mixed with 100 ml normal saline and was kept rotary shaker (100 rpm) for 1 h. After 1 h supernatant was spread on nutrient agar plate (composition : 0.5% peptone; 0.3% beef extract; 1.5 % agar; 0.5% NaCl; pH was adjusted to 7.2) and incubated at 30°C for 24 h for isolation of microorganism. Isolated organism were streaked on the BH Agar Media (Composition: TABLE 1) containing 100 ppm dye. After incubation of 72 h, potent dye decolorizing organism was selected on the basis of dye decolorization zone surrounding the colony. Isolated bacteria were maintained on nutrient agar plate. The organism was partially identified on the basis of its metabolic and morphological characteristics by BD Phoenix™.

Table: 1 Composition of Bushnell Hass (BH) Media

Component	Concentration (grams per liter)
$MgSO_4$	0.2
$CaCl_2$	0.02
KH_2PO_4	1
$(NH_4)NO_3$	1
$FeCl_3$	0.05
pH	7.4

2.4 Dye Decolorization Studies and Optimization of Physicochemical Condition

Dye decolorization experiments were carried out in 250 ml Erlenmeyer flask containing 100 ml sterile BH media containing 100 ppm dye. 5% of inoculums was transferred in the flask and kept on rotary shaker (100 rpm) at 30°C. Next day, 5 ml of sample was removed and centrifuged at 10,000 rpm for 15 min to separate biomass and supernatant. Absorbance of supernatant at λmax was recorded. Dye decolorization was measured in percent(%) dye decolorization according to following formula. An un-inoculated flask was kept as control to check the abiotic decolorization.

Decolorization (%) = $A_C - A_T / A_C \times 100$

Where, A_C is the absorbance of the control and A_T is average absorbance of the test samples.

To ensure that the change in pH of the dye solution had no effect on the decolorization, the visible spectrum was recorded between pH 5.0 to 11.0, in which the pH did not show any effect in spectrum.

Three different carbon sources, i.e. glucose, lactose and sucrose, were tested for decolorization at various concentrations i.e. 0.2%, 0.5%, 1.0% (w/v). 2 ml of inoculum was inoculated in 100 ml BH medium along with dye and different concentration of carbon source. All flasks were incubated at 30°C on rotary shaker. Aliquot was removed for the determination of decolorizing activity at different time intervals. In the same way, two nitrogen sources were tested for decolorization of dye. The concentration of organic nitrogen (urea) and inorganic nitrogen source (ammonium chloride) were 0.2%, 0.5%, 1.0% (w/v). Effect of pH (5 to 11) and temperature (25-41°C), on dye decolorization, was also studied in the BH media containing 0.5% glucose, 0.5% ammonium chloride and dye.

2.5 Phytotoxicity Study By Seed Germination Study

To study seed germination experiment, 10 uniform sized seeds of *Phaseolus mungo* were placed in sterilized glass petridishes lined with two filter paper discs. Such three petridishes (A, B, C) were prepared. These filter discs were then moistened with 10 ml of water for control (C) and with the same volume of untreated (A) and treated (B) dye samples followed by incubation at 28°C in a BOD incubator for a period of six consecutive days to record the percent germination. The experiment was performed in triplicate[13].

III. RESULT AND DISCUSSION

3.1 Isolation, Screening and Identification of The Dye Decolorizing Bacteria

A promising dye decolorizing bacterial strain was isolated from the textile effluent sample. This strain formed a distinct clear zone on BH agar plate containing dye. To identify this bacterium, we investigated its morphological and physiological properties using various biochemical media. On the basis of results the isolate was identified as *Pseudomonas sp*. BD Phoenix is one of the reliable microbial identification system [14].

3.2 Dye Decolorization and Optimization of Physicochemical Condition

The isolated strain was tested for its capacity to remove Reactive Red 152 dye. BH media containing 100 ppm dye (as a sole source of carbon and nitrogen) was inoculated by bacterial culture. The results indicate that the strain is capable of decolorizing the dye up to 80% in 7 days. Rate of dye decolorization is presented in Fig.1. The results show that the isolated strain is effective in decolorization. Many investigators reported that reactive dyes can be used as a carbon source [15,16] and rate of the decolorization of Reactive Red 152 can be increased under optimized condition [17]. But on the other hand, some reports suggest that Reactive Red 2 could not be utilized as sole source of carbon [18]. Our results showed that Reactive Red 152 can be used as sole source of Carbon.

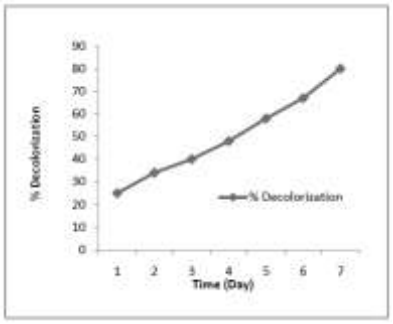

Figure 1 Decolorization of Dye

To optimize various physicochemical conditions i.e., optimization of carbon source, nitrogen source, pH and temperature, for maximum dye decolorization by isolated strains, various experiments were carried out in triplicate.. For maximum decolorization of Reactive Red 152 by the isolated strain, three different carbon sources viz., glucose, lactose and sucrose were tested. Each carbon sources were added at 0.2%, 0.5%, and 1.0% in BH medium containing 100 ppm Reactive Red 152 dye. There was increase in decolorization of Reactive Red 152 in the presence of glucose at various concentrations. Additional lactose and sucrose were not showed any significant effect on decolorization of dye.. Maximum percentage decolorization was observed when glucose was used as carbon source at 0.5% as shown in fig. 2. Two nitrogen sources urea and ammonium chloride were tested for decolorization of dyes by the isolated strain. BH medium containing Reactive Red 152 dye was supplemented with 0.2%., 0.5%, and 1.0% of urea. In the same way, experiments were carried out at 0.2%., 0.5%, and 1.0% of ammonium chloride. The best decolorization was observed when BH media was supplemented with 0.5% of ammonium chloride and 100 ppm Dye. Results are depicted in fig. 3. Effect of additional carbon and nitrogen sources were studied by many authors[19]. Our results were also supported by some author [20,21]. Our results were supported by some researcher that degradation of Reactive Red BS is increased in the presence of the glucose, peptone and yeast extract [22]. Additional nitrogen source yeast extract also showed increase in decolorization by halophilic *Pseudomonas spp*. RA20 [23].

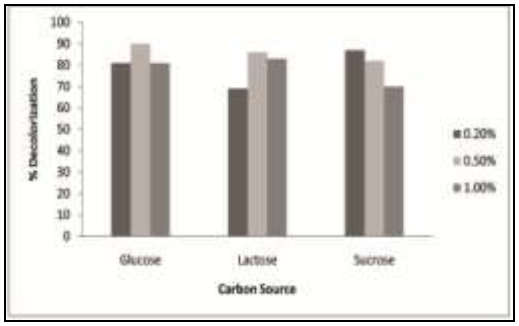

Figure 2 Effect of Carbon source on on decolorization of Dye

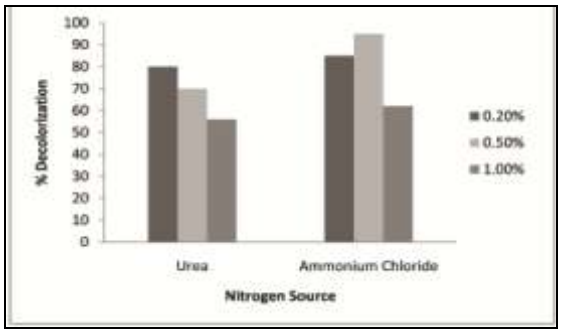

Figure 3 Effect of Nitrogen source decolorization of Dye

The effect of temperature and pH on the dye decolorization was tested. It was found that a temperature of 31°C was optimum for maximum decolorization (Fig. 4). Decline in decolorization activity at higher temperature more than 39°C can be attributed to the loss of cell viability. Optimum pH for maximum dye decolorization was 7.0 (Fig. 5). Results of some researcher support our results that Reactive Navy Blue HE2R degraded best at temp 30°C and pH 7[24].

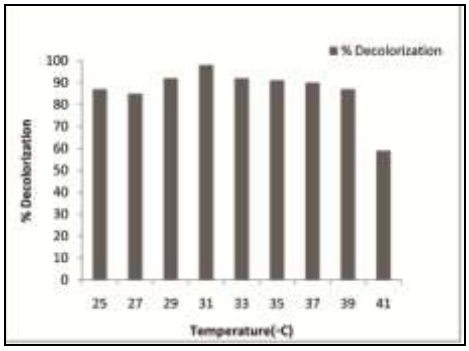

Figure 4 Effect o Temperature On Decolorization of Dye

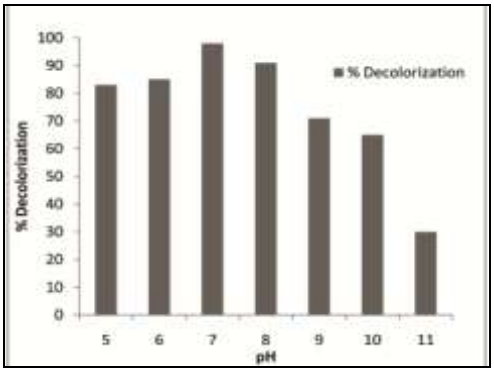

Figure 5 Effect of Ph On Decolorization of Dye

3.3 Phytotoxicity Study

Study of phytotoxicity of treated sample provides the information about the toxic nature of metabolites [25].Untreated samples exhibited 30% germination at a same time control and treated samples 100% germination.

IV. CONCLUSION

The present study has resulted in the isolation of a bacterial strain that has capacity of decolorizing Reactive Red 152, thus show the potential to be exploited as possible candidate for bioremediation. Decolorization activity can be enhanced by addition of glucose. The isolated strain can decolorize dyes under wide range of pH and temperature, which is the nature of effluent from dyeing industries.

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