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Research Article

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Decolorization of Acid Dyes by *B.cereus* and *P.aeruginosa* isolated from Effluent of Dyeing Industry

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ABSTRACT

The Bacillus subtilis and Pseudomonas aeruginosa were isolated from disposal site of tannery effluent and was tested for decolorization activity against commercially important dyes, acid red and acid blue in nutrient agar medium and minimal salt broth. In agar medium, decolorization began with the formation of zone of clearance around the colonies. Both bacteria are decolorized both the dyes to a great extent. In liquid medium, the bacterial inoculums were inoculated into flasks containing acid red and acid blue (100 mg/l) with trace amounts of yeast extract and glucose and then sterilized and incubated for 72 hrs. Both bacteria decolourized differently depending upon the strength of dyes present in the medium. Also, the different parameters such as various carbon source, nitrogen source, time and inoculum size were optimized for decolorization of both dyes by using bacterial isolates. Bacillus cereus and Pseudomonas aeruginosa showed maximum dye decolorization of 90% at the end of 72h under all optimum conditions. But the Pseudomonas aeruginosa was found to be more efficient in dye decolorization. High decolorization extent and facile conditions shows the potential for this bacterial strain to be used in the biological treatment of dyeing mill effluents.

Keywords: *Tannery effluent, Acid Dyes, Decolourisation, Optimisation.*

INTRODUCTION

Synthetic dyes are coloring agents mainly used in textile industries which generate a huge amount of wastewater in the process of dyeing. It is estimated that these industries discharge around 280,000 tonnes of dyes worldwide every year into the environment. A very small amount of dye in water (10-50 mg L-1) affects the aesthetic value, transparency of water and gas solubility of water bodies. The effluents from these industries are complex; contain a wide variety of dyes and other products such as dispersants, acids, bases, salts, detergents, humectants, oxidants, etc. The release of residual azo dye into industrial effluents deteriorates the water quality, and may cause a significant impact on human health due to mutagenic or carcinogenic effects of some azo dyes or their metabolites[1]. Therefore, the treatment of industrial effluents contaminated with dye becomes necessary prior to their final discharge to the environment [2.]

These dyes are poorly biodegradable because of their structures and treatment of wastewater containing dyes usually involves physical and / or chemical methods [3] such as adsorption, Coagulation flocculation, Oxidation, filtration and electrochemical methods[4]. Over the Past decades, Biological decolorization has been investigated as a method to transform, degrade or mineralize azo dyes[5]. Moreover, such decolorization and degradation is an environmentally friendly and cost competitive alternative to chemical decomposition possess[6]. Unfortunately, most azo dyes are recalcitrant to aerobic degradation by

bacterial cells[7]. However, there are few known microorganisms that have the ability to reductively cleave azo bonds under aerobic conditions[8,9,10,11]. Compared with chemical/Physical methods, biological processes have received more interest because of their cost effectiveness, lower sludge production and environmental friendliness. Various wood-rotting fungi were able to decolorize azo dyes using peroxides or lactases. But fungal treatment of effluents is usually time-consuming. Under static or anaerobic conditions, bacterial decolorization generally demonstrates good color removal effects [12].

This study aims to investigate the potential of bacterial cultures for decolorization of effluent dyes, Acid red and Acid blue and optimisation with respect to various nutritional sources (carbon and nitrogen), environmental parameters (time, inoculum size).

MATERIALS AND METHODS

Sample Collection and Reactive Acid Dyes Preparation

The reactive dyes Acid red and Acid blue were obtained from a dyeing industry in Chrompet, Chennai, Tamilnadu. A stock solution of the dye (1000mg L-1) was prepared in de-ionized water and used for all studies. Along with the dyes, the effluent sample was collected from the disposal sites and transported to laboratory for the isolation of dye decolorizing bacterial species. Samples were collected in sterilized glass bottles aseptically and transported to the laboratory in an ice bucket. Samples were analyzed within 6h of collection.

Screening of bacteria for dye decolourisation

Sample was serially diluted in sterile distilled water and plated onto Luria Bertani Agar (g L^{-1} Casein enzymic hydrolysate-10, yeast extract-5, NaCl-10, Agar-15) and then incubated for 48h at 30°C[13]. Discrete bacterial colonies that developed on agar plates were initially grouped on the basis of pigmentation, colony morphology followed by gram staining and motility. Selected bacterial isolates were further purified and sub-cultured. The pure cultures were identified based on their biochemical activity and by Bergey's Manual of determinative Bacteriology.

Dye decolorization on agar plate

The solid medium in Petri plates was prepared using Nutrient agar medium with an aliquot of an individual dye to a final concentration of 200µl. Along with control plates they were incubated at 37°C for 1-2 days. The extent of zone formation around the colonies was observed and recorded.

Dye decolorization in liquid media

The bacterial isolates were tested for de-colorization activity against commercially important dye of acid red and acid blue in broth cultures. The flasks containing Mineral salt medium and the dye (gL-1 K2HPO4: 1.73, KH2PO4: 0.68, MgSO4.7H2O: 0.1, NaCl: 0.1, FeSO4.7H2O: 0.03, NH4NO3: 1.0, Peptone: 1.0, CaCl2.2H2O: 0.02, Glucose: 5.0, and either acid red or acid blue: 0.1) was inoculated using loopful of isolated bacterial suspension. The culture flasks were incubated on orbital shaker with 120 rpm, at 30^{O}C. Uninoculated flasks served as controls to assess the abiotic de-colorization. OD values were measured spectrophotometrically at 540 nm to estimate the de-colorization process. The rate of de-colorization was calculated using the following formula as described[14].

$$\% \text{ Decolorization} = \frac{\text{Initial absorbance value - final absorbance value}}{\text{Initial absorbance value}} \times 100$$

Optimization for maximum decolorization of selected dyes under dfifferent culture conditions

Decolorization of both the reactive dyes by the two bacterial isolates was optimized with respect to the effect of carbon source (Dextrose, Sucrose and mannitol), nitrogen source (Beef extract, peptone, yeast extract), and inoculum volume (0.5%, 1% and 1.5%). Decolourization under different culture conditions was done by changing the factors one at a time. The basic conditions of culture being 30^{o}C, pH 7.0 under shaking conditions (120 RPM) and duration of the experiment was for three days. In all the experiments, medium without culture inoculum was served as control. At an interval of 24hrs, the samples were withdrawn and analyzed for percent decolorization of the dye.

Time course of decolorization

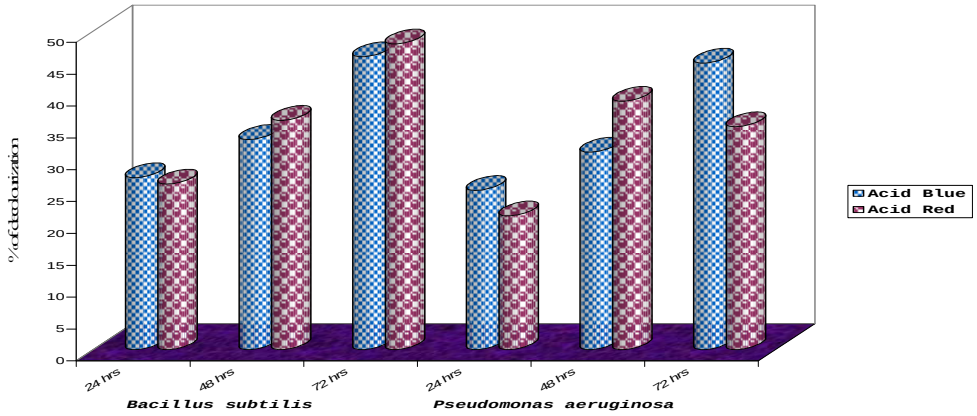
The time course of decolorization was carried out under optimum conditions obtained from above studies and the optimum conditions are: for *Bacillus subtilis* 1% sucrose, 0.25% peptone, pH 7, 37^{o}C and 0.5% inoculum for Acid red and 1% for Acid Blue), *Pseudomonas aeruginosa* (1% glucose, 0.25% yeast extract, pH 6, 37^{0}C, 0.5% inoculum for Acid red and 1% for Acid Blue). Flasks were incubated up to 72h at their respective temperature and samples were removed after every 24 h and analyzed for decolorization activity as described above.

RESULTS AND DISCUSSION

In this present study, two bacterial isolates that predominantly decolourises the reactive dyes at different concentration were isolated and utilized for the analysis. The pure colonies of the isolated strains were prepared and maintained in Nutrient Agar slants. The organisms were identified as *Bacillus subtilis* and *Pseudomonas aeruginosa* on the basis of various physiological and biochemical tests as described in Bergey's manual of determinative bacteriology (1974).

Dye decolorizing activity of *Pseudomonas aeruginosa*, and *Bacillus subtilis*, were tested against Acid blue and Acid red (100 mg/l) by the liquid culture method. The inhibitory effect was directly proportional to decreasing concentration of dyes. The decrease in dye absorbance was in the range of 26 to 48% (*B. subtilis*) and 31 to 45% (*P. aeruginosa*). Decolorization pattern of two reactive dyes are given (Fig.1).

Fig. 1. Dye Decolourisation in liquid media



The effect of different carbon sources on decolorization of acid red and acid blue by *B. subtilis* and *P. aeruginosa* is illustrated (Fig.2 and Fig.3). Sucrose has emerged as the ideal carbon source for both the strains of bacteria, recording highest rate of decolorization (90 and 94% respectively). Of the two bacterial strains tested, *Bacillus subtilis* exhibited highest activity recording 94 % reduction in color. Both the strains showed similar pattern with all the 'C' sources against Acid Blue decolourisation. *Pseudomonas* showed high rate of decolourisation of Acid Blue when compared to *Bacillus sp.* Similar trend has been reported by other authors[15]. In contrast, a decrease in decolorization of anthraquinone dye by *shawanella discolorations* S12 was reported when glucose, sucrose and dextrin were provided as additional carbon sources[16].

Fig. 2. Decolourisation of Acid red under different 'C' Sources.

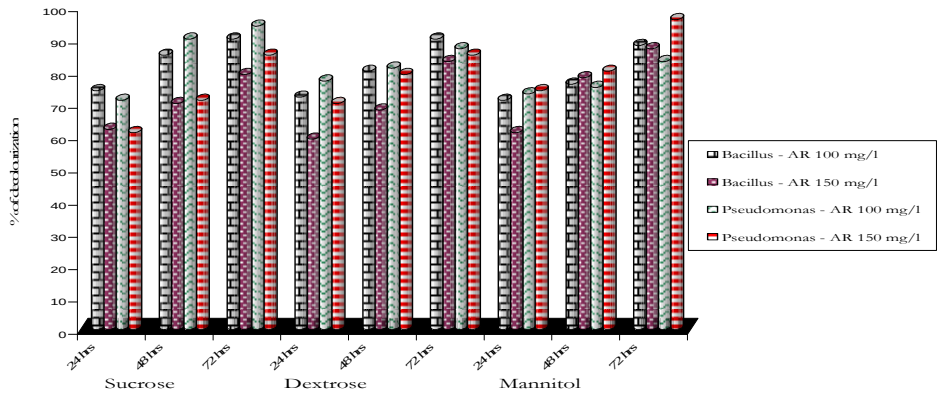
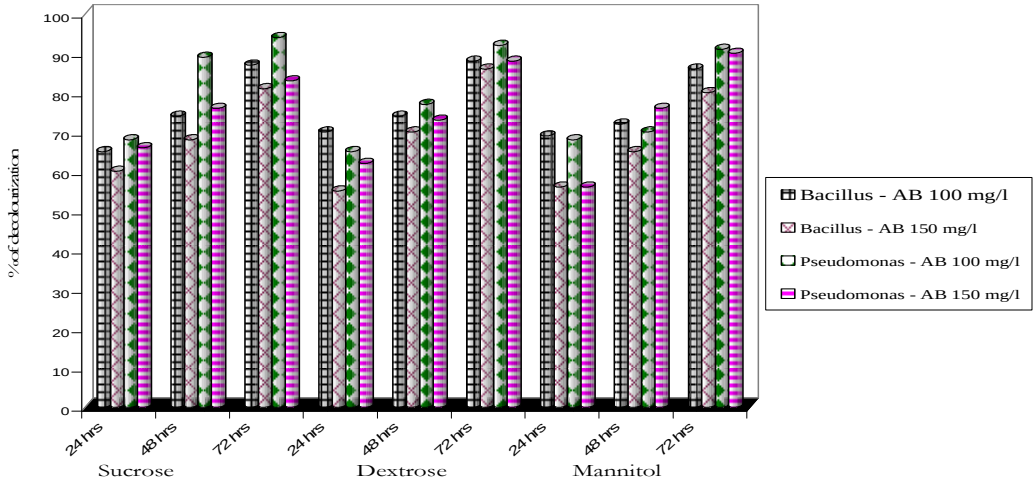


Fig. 3. Decolourisation of Acid Blue under different 'C' Sources



The effect of different nitrogen sources on decolorization of acid red and acid blue by *P. aeruginosa*, and *Bacillus subtilis* is represented (Fig.4 and Fig.5). Among the three nitrogen sources evaluated, peptone appeared to highest percentage of decolorization process by both the strains, recording 97% decolorization of acid red by *P. aeruginosa* and 90% decolorization of Acid blue dye by *Bacillus subtilis*. But both the bacterial strains exhibited moderate percentage of decolorization when beef extract and yeast extract were used as nitrogen source (below 90%). Among the different nitrogen sources used in this study, the maximum decolourization was found in peptone as a nitrogen source. Like that of our study, peptone gave the best color removal percentage for azo dye Red RBN by *Proteus mirabilis*[17].

Fig. 4. Decolourisation of Acid red under different 'N' Sources

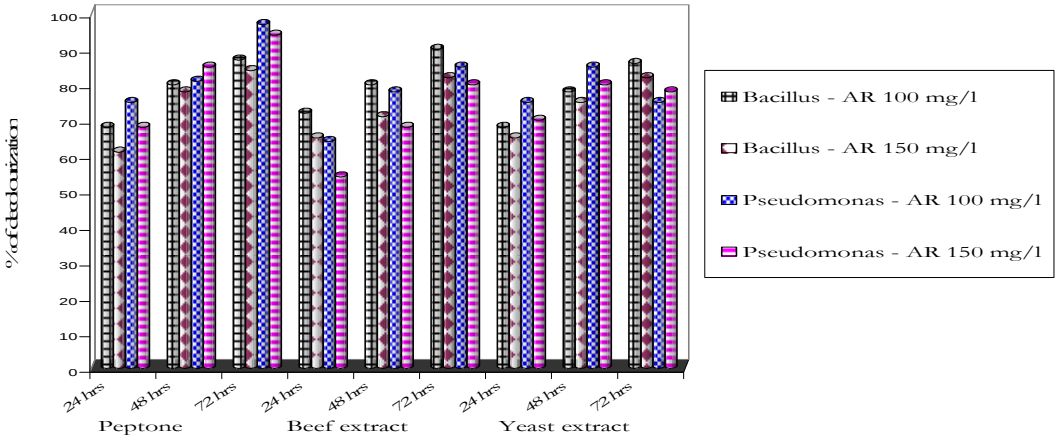


Fig. 5. Decolourisation of Acid Blue under different 'N' Sources.

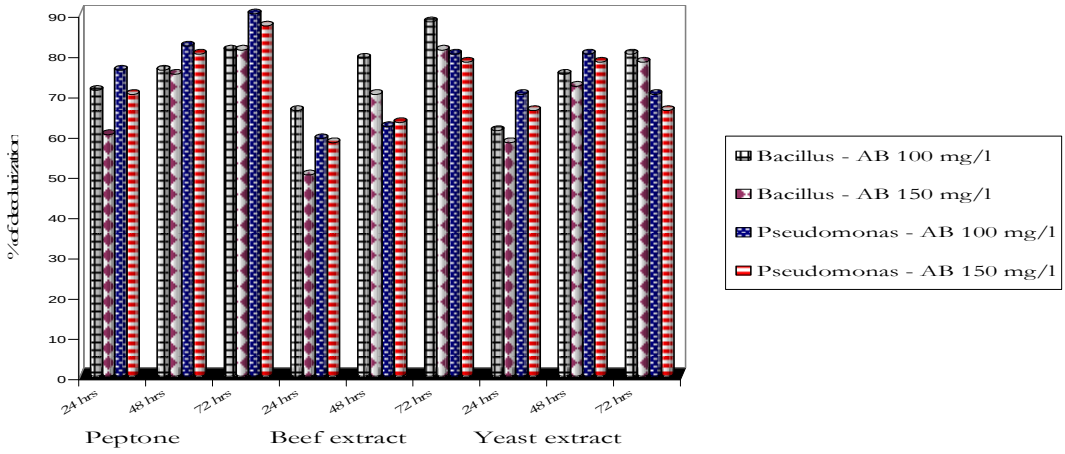


Fig. 6. Decolourisation of Acid red under different inoculum size

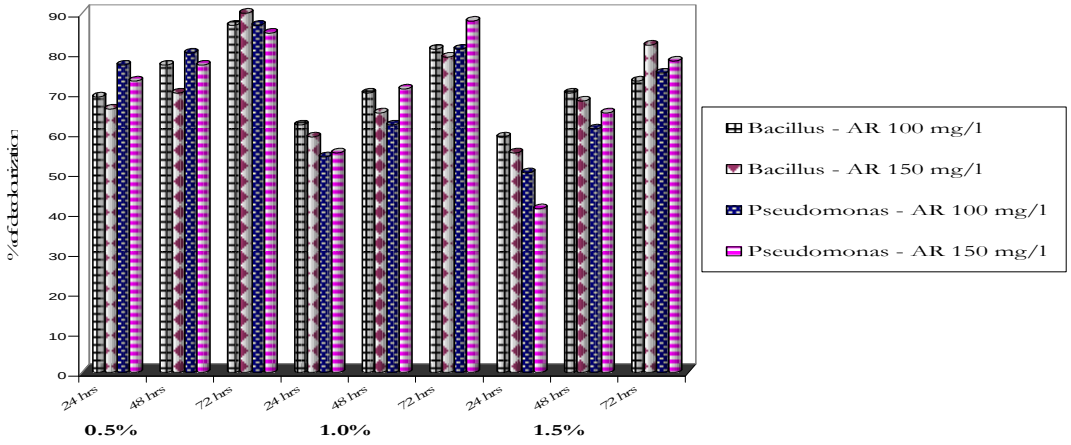
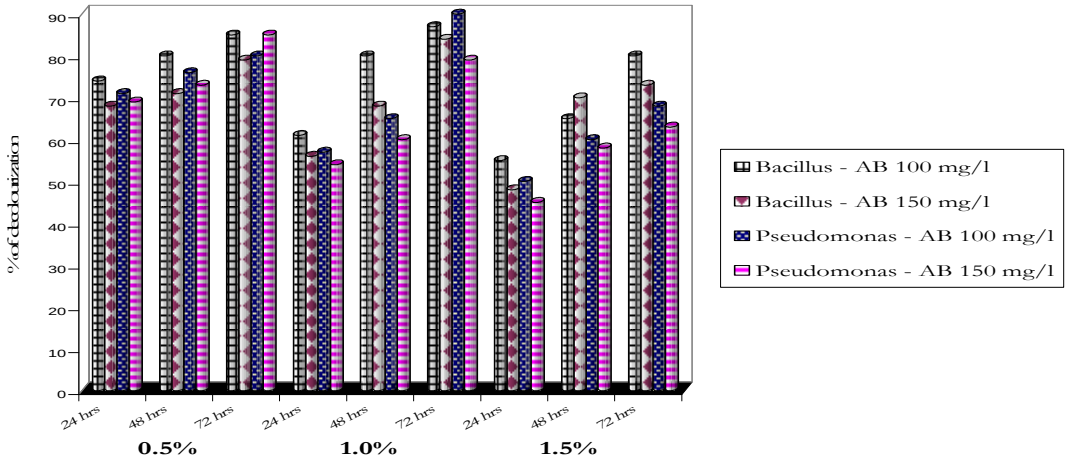


Fig. 7. Decolourisation of Acid Blue under different inoculum size



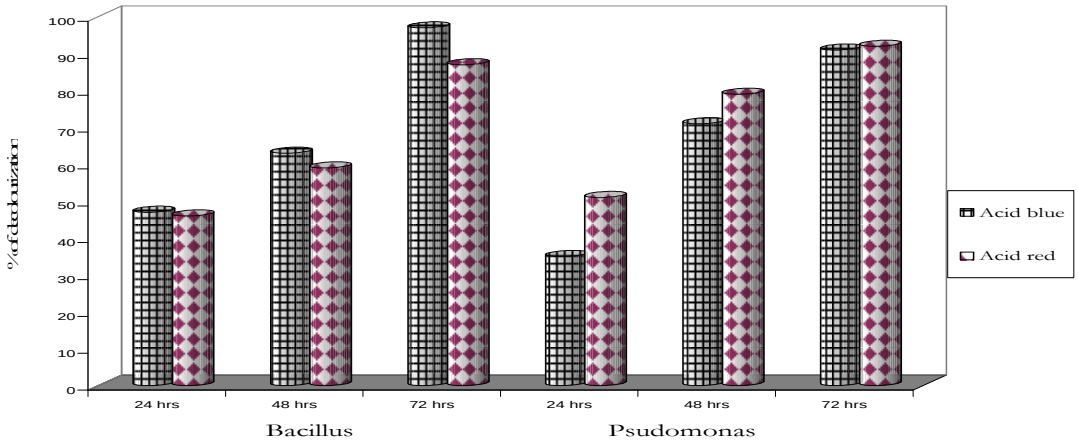
Evaluation of the effect of different inoculum size on dye decolorization with reference to acid red and acid blue by *P. aeruginosa*, and *Bacillus subtilis* is presented (Fig.6 and Fig.7). Among the three inoculum sizes evaluated, 0.5% showed high rate of decolourisation of Acid red with highest percentage of decolorization recording 90% of acid red by *B. subtilis* and 90% of acid blue by *P. aeruginosa* followed by 1.0% inoculum size. The increase in inoculum size shows decrease in rate of decolourisation. The maximum decolourization was found in the lower inoculum size of 0.5% than higher concentrations. Both Bacillus sp (87%) and Pseudomonas (90%) showed high rate of decolourisation of Acid Blue with 1% inoculum size. 0.5% inoculation shows neglible difference whereas 1.5% inoculum size showed a more decrease in rate of decolourisation of Acid Blue. Both the strains showed high rate of decolourisation of Acid red when compared to Acid Blue. In contrast to our result, it is reported that the Direct Red 81 decolorization rate was increased with increase in the inoculum size, reaching maximum (2.53 mg/l/h) at 20% (v/v) inoculum size[18].

Evaluation of the effect of different timing of incubation on dye decolorization with reference to, acid red and acid blue by *P. aeruginosa*, and *Bacillus subtilis* is presented (Fig. 8). In the present study, *P. aeruginosa* showed high rate of decolourisation of Acid red (92%) when compared to *Bacillus subtilis* (87%), while *Bacillus subtilis* shows high % of decolourisation (97%) of Acid Blue than *Pseudomonas* (91%) under optimized condition

Decolourization of synthetic dyes is the result of the cleavage of the chromophoric group which generates colourless metabolic intermediates. The intermediate metabolite of the dye substrate is aromatic amines. The cleavage of the chromophoric group of dyes is a reduction process which requires redox equivalents (electron donors) that transfer electrons to the chromophoric group (electron acceptors) of dye[19]. Bacterial

strains that can aerobically reduce azo dyes cannot use dye as the growth substrate and therefore require organic carbon sources. Aromatic amines resulting from the reductive cleavage of azo bond can be used as a carbon and energy source for bacterial growth. Like carbon source, a nitrogen source is also essential for decolorization process, with the exception of bacteria that can use azo dyes as a carbon source[20].

Fig. 8. Decolourisation of Acid Blue and Acid red under Optimized conditions



CONCLUSION

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 decolorization bioassay with least value of the final color. The continued development and application of biotechnologies for the biodegradation is limited primarily by physical factors such as pH, temperature and substrate concentration. Biotreatment offers a cheaper and environmentally friendlier alternative for colour removal in textile effluents. Thus the selection of microbial symbiont which was able to withstand high level of toxicity proves to be an essential tool for biodegradation of effluent dyes.

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