Decolourization of Congo Red dye by bacterial consortium isolated from dye contaminated soil, Paramakudi, Tamil Nadu

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ABSTRACT

Congo Red (CR) is a carcinogenic azo dye utilized in paper and textile industries. Five bacterial strains EDS-S1, EDS-S2, EDS-S3, ETS-SB1, ETS-SB2 and consortium isolated from dye contaminated soil of Emaneswaram textile dyeing unit, Paramakudi, Tamil Nadu, decolourized CR and identified as Proteus sp, Klebsiella sp, Salmonella sp and Pseudomonas sp by biochemical tests. Among various concentrations (50 to 250ppm) of CR, 200ppm was decolourized by five bacterial strains individually and also as consortium and the decolourization rate was recorded as EDS-S1, 67%; EDS-S2, 81%; EDS-S3, 90%; ETS-SB1, 77%; ETS-SB2, 65% and consortium 98%, by static conditions within 48 hours under pH 7 at 37°C. The decolourization abilities of bacteria were due to the secretion of azoreductase, which cleaves the azo bond of CR dye. This study confirms the ability of consortium as effective tool to develop a commercial scale biodecolourization plant to treat CR polluted effluent water and also future scope for producing commercial azoreductase enzyme by utilizing modern biotechnological methods.

Key words: Biodecolourization, Congo Red, Proteus sp, Klebsiella sp, Salmonella sp, Pseudomonas sp.

INTRODUCTION

Azo dyes are the largest class of synthetic dyes due to the ease and cost effectiveness of their synthesis and the highest range of colors [1]. Congo Red is an azo dye with a structure 3, 3′-((biphenyl)-4,4′-diylbis(azo))-bis(4-amino-1-naphthalenesulphoniacid) disodium salt. It is intended primarily for the coloration of paper products, used in medicine (as a biological stain) and as an indicator since it turns from red-brown in basic medium to blue in acidic, used to color textile and wood pulp. It is a recalcitrant and act as potent carcinogen and mutagenic because of the presence of aromatic amine group [2]. Minute amounts of reactive group of azo dyes shows their visual effect with adverse impact in terms of total organic carbon (TOC) and chemical oxygen demand (COD) and thus causing severe environmental problems worldwide [3]. Azo dyes have long been recognized as a human urinary bladder carcinogen and Tumorigenic in animals [4] cyanogenic in fishes, reduction in seed germination and induce dwarfism in plants [5].

Due to more excessive use of CR in various industries posses a high threat to environment. Varieties of microorganisms including bacteria, fungi, yeasts, actinomycetes and algae are capable of degrading azo dyes, among which bacterial cells represent an inexpensive and promising tool for the removal of various azo dyes from textile dye effluents [6]. In the current scenario, microbial or enzymatic treatment offers an indispensable, ecofriendly and cost-effective solution towards restoring azo dye polluted ecosystems [1]. Bacteria capable of dye decolorization, either in pure cultures or in consortia, have been reported [8].

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The mode of degradation of the azo dyes is by production of azoreductase enzyme which cleaves azo bond and converts azo compounds to colourless amines [7] and functional groups.

The present study deals with the isolation of bacterial strains from dye contaminated soil of textile dye unit, to evaluate their ability to decolourize CR at different concentrations and to standardize the optimum conditions.

MATERIALS AND METHODS

Congo Red
An analytical grade CR obtained from S.D. Fine chemicals Ltd was utilized in this study. A known concentration of CR dye (50 to 250 ppm) added to nutrient broth, sterilized and decolourization study was carried out.

Collection of dye contaminated soil and sludge
A dye contaminated soil and sludge sample were collected from Emaneswaram textile dyeing unit, Paramakudi, Tamil Nadu. The soil and sludge were labeled as EDS (Emaneswaram Dye Soil) and ETS (Emaneswaram Textile Sludge), brought to the laboratory and stored at room temperature.

Isolation and Identification of bacterial strains
Isolation and identification of bacteria were carried out by plate counting technique. One gram of soil and sludge were weighed individually and suspended in 99ml of sterile distilled water. One ml was pipetted and serially diluted with 10 ml distilled water up to 10^{-6} dilutions. From this 0.1ml of was taken and spread onto nutrient agar medium containing the following chemicals: Beef extract 3gmL^{-1}, Nacl 5gmL^{-1}, Peptone 5gmL^{-1}, Agar 20gmL^{-1} and incubated at 37^0C for 24hrs. Five different bacterial colonies were observed, further purified by streaking method and were identified by biochemical tests.

Determination of bacterial growth
The maximum growth rate of five bacterial isolates and consortium were measured by spectro photometrically. Five loops of 48 hrs old culture was inoculated in to conical flask containing 100 ml sterile nutrient broth and incubated in an incubator at 37^0C. The bacterial growth curve was measured by turbidometric method, reading at 660 nm in regular time intervals.

Screening of bacteria for azo dye decolourization
Inoculum size:
Five loops of 18 hrs old bacterial pure isolates namely two Proteus sp, Klebsiella sp, Salmonella sp and Pseudomonas sp were used as inoculants to degrade the CR dye (50 to 250ppm).

Effect of Static and shaking conditions for dye decolourization
In an attempt to study the effect of static and shaking (120 rpm) conditions on CR decolourization, five bacterial monocultures cultures and consortium were inoculated in nutrient broth, amended with 50 ppm of CR dye. Maximum decolourization was observed in static condition.

Effect of pH on dye decolourization
Eighteen hrs old culture was inoculated in conical flasks containing 100 ml nutrient broth maintained at pH (3-13) and each flasks were amended with 50 ppm of Congo Red dye and incubated at 37 ^0C. At different time intervals aliquot (5ml) of the culture medium was withdrawn and supernatant obtained after centrifugation was used for analysis of % decolourization by measuring Optical Density at 497nm.

Effect of Bacterial monocultures and consortium on dye decolourization
Five pure cultures two Proteus sp., Klebsiella sp., Salmonella sp., and Pseudomonas sp individually and as a consortium were tested for their ability to decolourize CR by spectrophotometric analysis [9]. A known concentration of CR (50 to 250 ppm) dye was added to the nutrient broth in 250 ml conical flask, sterilized, inoculated with 5 loops of individual bacterial culture and decolourization study was carried out. At every 12hrs interval 5 ml aliquot of the decolorized culture broth was collected and centrifuged at 10,000 rpm for 5 minutes. The supernatant was recovered and analyzed spectrophotometrically at 497 nm. The uninoculated medium with CR dye was served as blank. A standard curve of known concentrations (5 to 20 ppm) of pure CR was prepared and
Congo Red dye decolourization % = \[
\frac{\text{Initial conc. of dye} - \text{residual conc. of dye}}{\text{Initial conc. of dye}} \times 100
\]

RESULTS AND DISCUSSION

Isolation and identification of bacterial strains

Five bacterial strains were isolated from two soil samples collected from Emaneswarem textile dyeing unit of Tamil Nadu. The pure bacterial isolates EDS-S1, EDS-S2, EDS-S3, ETS-SB1 and ETS-SB2 are identified as *Proteus* sp., *Klebsiella* sp., *Salmonella* sp., and *Pseudomonas* sp., by respectively biochemical characteristics (Table 1). The bacterial strains were cultured individually and as a consortium in 250 ml conical flask with 100 ml of nutrient broth. Bacterial strains showed maximum growth at 18 hrs.

Five monocultures and consortium were inoculated in a nutrient broth with pH range from 3-13 at 50 ppm CR. At pH 7, 98% CR decolourization was observed in consortium followed by 89% and 70% in EDS-S2 and ETS-SB2 respectively. At pH 5, consortium showed 70%, EDS-S2 gave 67% decolourization, and maximum decolourization was observed in neutral pH (Table 2). Similar observations were reported that the *Bacillus subtilis* showed maximum CR decolourization at pH 8 [10]. 94 % of decolourization of reactive violet 5, was observed at pH 7 [11]. *Pseudomonas putida* was the best decolourizer of Acid Orange 10 with a percentage decolourization value of 90%, *Bacillus cereus* and *Pseudomonas fluorescens* with an efficiency of 65%, 54% and 39% respectively was reported [12].

Under static conditions % decolourization of CR was maximum (97%) by consortium [8]. Decolourization of Acid Orange 10 was 90 % at static conditions within 24 hrs as compared to 32 % under agitation [12]. In shaking conditions the oxygen and the reducing equivalents enters to oxidative phosphorylation. Azoreductases catalyze the cleavage of azo bond in presence of reducing equivalents like FADH and NADH. Azo bonds get reduced by accepting electrons form NADH, which leads to bacterial decolourization of azo dyes, the utilization of NADH by oxidative phosphorylation would result in a negative effect on the azo reductase driven decolourization [13]. Oxygen inhibition is more likely to be a metabolism dependent event [14].

<table>
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<th>S.No</th>
<th>Monocultures</th>
<th>Indole</th>
<th>Methyl red</th>
<th>Vogues-proskauer</th>
<th>Citrate</th>
<th>Urease</th>
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<td>ETS - SB1</td>
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Fig.1 Growth curve of Monocultures and consortium

Fig 2: Decolourization of Congo Red (50 ppm) by monocultures and consortium

Fig 3: Decolourization of Congo Red (100 ppm) by monocultures and consortium

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Fig 4: Decolourization of Congo Red (150 ppm) by monocultures and consortium

![Graph showing decolourization of Congo Red (150 ppm) by monocultures and consortium.]

Fig 5: Decolourization of Congo Red (200 ppm) by monocultures and consortium

![Graph showing decolourization of Congo Red (200 ppm) by monocultures and consortium.]

Fig 6: Decolourization of Congo Red (250 ppm) by monocultures and consortium

![Graph showing decolourization of Congo Red (250 ppm) by monocultures and consortium.]

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Effect of dye concentration on decolourization

The decolourizing activity of the bacterial consortium was studied using CR at different initial concentrations varying from 50 to 250 ppm. The maximum decolourization was observed up to 200 ppm. Bacterial consortium showed 98% decolourization at 200 ppm concentration of Congo Red dye at pH 7 and temperature 37°C. EDS-S1 monoculture showed 90% decolourization at 200 ppm. At 250 ppm % dye decolourization was dropped to 20%.

CONCLUSION

In this present study, *Proteus* sp (EDS-S1, ETS-SB2), *Klebsiella* sp (EDS-S2), *Salmonella* sp (EDS-S3) and *Pseudomonas* sp (ETS-SB1) were decolorized CR dye and consortium showed maximum decolorization. The azoreductase which catalyzes the degradation of dye can be purified, commercially produced and added into the effluent to degrade azo dye. The commercial biotechnological production of azoreductase enzyme may be the promising field in decolourization of azo dye. Moreover, further research on these strains could explore new tools and techniques to evolve viable and eco-friendly microbial solutions for treatment of dyeing industrial effluent.

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REFERENCES