Bacterial Decolourization of Textile Azo Dye Direct Red-28 under Aerobic Condition

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Bacterial strains (DRS-1, DRS-2,DRS-3,DRS-4) isolated from dye contaminated sludge,decolorized an azo dye namely Direct Red-28.Of these,DRS-1 & DRS-3 were selected for further studies because of their high efficiency to decolorize the dye. Effect of Physio-chemical parameters, such as aeration/agitation, concentration of glucose, peptone and $P^{\rm H}$ of the culture medium on dye decolourization by isolates DRS-1 & DRS-3 were analyzed.DRS-1 had higher dye decolorizing ability than DRS-3.Under optimal conditions, DRS-1 decolorized Direct Red-28 (100ppm) upto 94.80%.The highest decolourization ability of isolate DRS-1 can thus be exploited in the treatment of industrial effluent containing azo dyes.

Key words: Azo Dye, Congo Red, Decolourization, Direct Red-28, Industrial Effluent.

Waste water from textile industries creates a great pollution problem due to the dye content, There are more than 10⁵ kinds of commercially available dyes with over 7x10⁵ metric tones of dyestuff produced annually¹. 2% of these dyes are directly discharged as aqueous effluents and 10% are subsequently lost during textile colouration process² Colour is one of the most obvious indicators of water pollution, and discharge of highly coloured synthetic dye effluents can be damaging to the receiving water bodies.³ Among these, azo dyes are prominent

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class of colourants used in tattooing, cosmetics, printing, consumer's products as well as in textile dyeing because of their chemical stability and versatility.4 Azo dyes constitute a major class of environmental pollutants. These compounds are characterized by aromatic rings linked by an azo group, N=N.5 The azo linkage of azo dyes may undergo metabolic cleavage resulting in free aromatic amines which are recognized as possible human carcinogens6. Some of the azo dyes or their break down products also have a strong toxic and mutagenic influence on the living organisms.7 The discharge of highly coloured dye effluents can result in serious environmental damages. Thus, Colour elimination in wastewater is today the principal problem concerning the textile industries, since it is the first contaminant recognized in textile waste water and has to be removed before discharging into receiving water bodies.

In recent years, a number of studies have been focused on microorganisms which are able to decolorize and degrade dyes in waste water.⁸A variety of microorganisms capable of decolorizing a wide range of dyes include some bacteria, fungi and algae⁹. The use of microorganisms for the removal of synthetic dyes from industrial effluents offers considerable advantages. The process is simple and relatively inexpensive, the end products of complete mineralization are nontoxic¹⁰. Many studies on the decolorizing capability of microorganisms especially fungi and bacteria have been reported and reviewed. In this study, bacterial strains capable of decolorizing Direct Red-28 were isolated. Bacterial strain DRS-1 decolorized the dye Direct Red – 28, efficiently than other isolates. The effect of various physio-Chemical factors were determined and compared.

MATERIAL AND METHODS

Microorganisms used

Soil samples were collected from dye polluted areas and different sources like textile mill effluents.^{11,12} Approximately 1gm of sludge/ soil was suspended in 10ml saline and mixed thoroughly and left for 20 min to settle down. The supernatant was serially diluted with saline. Aliquots of 0.1ml of 10⁻¹, 10⁻², 10⁻³ dilutions were spread onto nutrient agar with 50 ppm of Direct red-28. All plates were incubated at 37°C for 3-4 days. Colonies surrounded by halo (decolorized) zones were picked and inoculated into 100 ml of nutrient broth containing 50ppm of Direct Red-28 to confirm their abilities to decolorize the dye.13 Four isolates were selected based on their efficiency of decolourization and they were named as DRS-1, DRS-2, DRS-3, DRS-4.

Dye

The azo dye Direct Red - 28 was procured from a textile company. The common name, class, maximum absorbance is given in Table 1 and its chemical structure⁹ is shown in (Fig. 1).

Inoculum Preparation

Pure stock cultures of these four isolates were prepared by growing a single colony in 250ml conical flasks containing 100ml of nutrient broth and 50ppm of Direct Red -28 under shaking (150 rpm) at 37 °C for 24 hrs.

Comparing dye removing efficiency of Microbial Cultures

Experiments with bacterial isolates DRS-1, DRS-2, DRS-3, DRS-4 were performed on flasks containing 100 ml of nutrient broth and each were autoclaved for 20 min at 121°C and 15 1bs. 1ml of 24 hrs cultures $(3.6 \times 10^9 \text{cfu ml}^{-1})$ of these isolates were inoculated aseptically to experimental flasks with the dye Direct Red- 28 (100ppm) respectively. Suitable control without any inoculum was also run along with experimental flasks. Samples were withdrawn at every 12 hrs for 3 days, centrifuged at 10,000 rpm for 15 min and absorbances of the supernatant were measured spectrophotometrically at 500nm(the absorption maxima of Direct Red-28). All experiments were done in triplicates. The decolourization percentage was calculated ¹⁴as follows:

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

Where,(i) is the initial dye concentration and Dye (r) is the residual dye concentration. **Decolourization in different Culture Conditions**

Decolourization in uniferent Culture Conditions

The best performing isolate was selected for further studies. The effect of various Physiochemical factors such as P^{H} , agitation/aeration, peptone, glucose concentration on decolourization of Direct Red-28 were examined by inoculating 1ml of 24 hrs cultures in 100ml of nutrient broth with 100 ppm of the dye.

RESULTS AND DISCUSSION

The four bacterial isolates DRS-1, DRS-2, DRS-3, & DRS-4 showed decolourization ability, by forming halo zones around the colonies on nutrient agar containing 50ppm of the dye.On inoculation into 100 ml of nutrient broth containing 50 ppm dye, their efficiencies were

Table 1. Stucture and class of Direct Red-28

Name	Common name of the dye	λ Max (nm)	Class
Direct Red 28	Congo Red	500nm	Azo dye

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determined quantitatively. After 72 hrs of incubation DRS-1,DRS-2,DRS-3&DRS-4 showed decolourization of about 85.50%, 51.85%, 77.88% and 49.98% respectively (Fig.2). DRS-1 showed 85.48% of decolourization after 36 hrs while DRS-3 showed 77.87% of decolourization after 48 hrs,

appreciable decolourization was not found after further incubation Since these two isolates showed high decolourization efficiency in short duration, they were selected for further experiments. The effect of various culture conditions on dye decolourization of these isolates were analysed and



Fig. 1. Chemical Stucture of Direct Red -28



Fig. 2. Screening of 4 Bacterial isolates for their ability to degrade Direct Red-28



Fig. 3. Effect of P^H on decolourization of Direct Red-28

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Fig. 4. Effect of Shaking/aeration on decolourization of Direct Red-28



Fig. 5. Effect of various concentrations of Glucose on decolourization of Direct Red-28



Fig. 6. Effect of various concentrations of Peptone on decolourization of Direct Red-28.

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the results discussed below were taken at 36 hrs of incubation.

Effect of P^H

An optimal P^H for decolourization by DRS-1 & DRS-3 were selected from the range of (6-10). Although the isolates showed excellent decolourization efficiency over a wide P^H range, the most effective P^H for DRS-1 & DRS-3 were found to be 7 &7.5 respectively (Fig.3).This P^H range is suitable for practical utility in a waste water treatment system. At P^H10; a slight and significant increase in decolourization was seen than at P^H 8 and 9 respectively. This supports the concept that P^H 10 was detrimental to the bacteria and caused release of enzymes to cause dye reduction or the dye might have been reduced by alkaline hydrolysis.¹⁵

Effect of agitation

The comparison study between the efficiency of static and shaking conditions (150rpm) for decolourization of Direct Red- 28 by DRS-1 & DRS-3 were shown in fig.4. It was found that shaking condition is more efficient than stationary for both the isolates; suggesting that dye decolourization by DRS-1 & DRS-3 were aerobic process. This is in contrast to results given by chen et al¹⁶ who reported that, although Aeromonas hydrophilia displayed good growth in aerobic or agitated cultures, colour removal was best in anoxic condition than agitated/aerobic cultures. The recent reports on aerobic degradation of azo dyes indicates that microbes posses more than one mechanism of dye degradation.17

Effect of glucose

It was observed that increase in glucose concentration increased the rate of decolourization (fig.5) i.e. 3gms of glucose decolorized the dye upto 94% and 89.80 % by the isolates DRS-1 & DRS-3 respectively. This may be because the isolates could not be able to utilize the dye as a sole carbon & energy sources.¹⁵ Further increase in the concentration of glucose did not appreciably alter the decolourisation process. This may be because when the concentration of glucose was higher, the bacteria could utilize glucose preferentially and resulting in lower decolourisation¹⁸.

Effect of peptone

The effect of different peptone

6.

concentration showed significant results on colour removal of dye effluent. The fig. 6 shows that increase in the concentration of peptone accelerates the dye decolourization. This may be because organic nitrogen sources are considered to be essential medium supplements for the regeneration of NADH, which acts as the electron donor for the reduction of azo bonds¹⁹.

CONCLUSIONS

DRS-1 was considered to be the efficient isolate when compared to DRS-3, with the ability to decolourize Direct-Red-28 upto 95% within 36 hrs under optimal cultural conditions. Physiochemical parameters like,aeration,P^H, Conc of glucose and peptone had a significant effect on the decolourization activity of Direct Red-28. The present study indicates that the Bacterial strain DRS-1 can decolourize the dye aerobically which is in contrast to other reports.²⁰ Further, it is suggested that apart from solving the problem of maintaining anaerobic condition for decolourization, this bacterial isolate could remediate the textile effluent efficiently and costeffectively.

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