



BIOREMEDIATION OF TEXTILE AZO DYE ORANGE F2R BY BACTERIAL ISOLATES

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ABSTRACT

Microorganisms have been exploited for the treatment of waste water and bioremediation of environmental pollutants. The present research work deals with the decolorization and degradation of harmful textile dye Orange F2R by using bacteria isolated from acclimatized samples. All the promising isolates were studied for decolorization of dye by optimizing various cultural conditions. It was observed that the organisms were able to completely decolorize the dye (98.99%) at 30°C to 37°C temperature and pH 7.0. The effect of Inoculum size and dye concentration was also studied. Effect of various Carbon and Nitrogen studies revealed that these promising isolates were able to decolorize highest amount of dye in presence of Glucose and Yeast Extract.

KEY WORDS

Orange F2R, Bioremediation, Promising isolates, Decolorization.

1. INTRODUCTION:

Near about 10000 different types of dyes are used in textile industries and near about 280000 tons of dyes are discharged without any treatment into the environment and water resources due to inefficient processing (Hsueh *et al.*, 2005). These dyes are recalcitrant in nature hence persist in environment for longer period of time. Most of them are Azo dyes. Azo dyes contain N=N in their structure. Due to this bond Azo dyes are not easily biodegradable (Minussiet *et al.*, 2001; Gharbaniet *et al.*, 2008). These dyes form major constituents in all dyes of about 60%-70%. Out of which 2-50% of dyes get loose due to inefficient process and disposed along with waste water (Olukanniet *et al.*, 2009). These dyes affect badly on photosynthetic efficiency of aquatic plants by decreasing penetration of sunlight and produces deep coloration of water (Mesteret *et al.*, 2000; Duran *et al.*, 2000; Wu *et al.*, 2011). If these dyes are treated properly they remain in environment for longer period of time (Olukanniet *et al.*, 2006). These dyes can be treated by physical or chemical methods but due to

some limitations with these methods they cannot be applied for treatment of textile waste water (Chen *et al.*, 2003). Now a day's biological methods by using microorganisms are in great demand as these methods are cost effective, ecofriendly, easy and requires less experimental setup (Mossaviet *et al.*, 2005). Varieties of microbes are having ability to decolorize textile dyes viz. Actinomycetes, Fungi, Bacteria and Yeasts (Olukanniet *et al.*, 2006). These microorganisms decolorize and degrade the dyes enzymatically (Pandeyet *et al.*, 2007). Various factors and cultural conditions are responsible for dye decolorization activity. Recently the dye decolorization by using various potential microorganisms has been increased great attention. Various species of bacteria are exploited either in single species or as consortia for bioremediation of textile dyes (Vermaet *et al.*, 2003; Mossviet *et al.*, 2007).

The present research has been undertaken for bioremediation of harmful textile azo dye Orange F2R by using promising bacterial isolates. The optimization

of various cultural conditions was carried out for maximum decolorization of dye.

2. MATERIALS AND METHODS:

2.1. Dye: Azo dye Orange F2R (λ max- 494 nm).

2.2. Collection and Acclimatization of samples:

The soil and water samples were collected from waste disposal site around textile industries, ETP, compost and manure. These samples were collected in sterile polythene bag and bottle and brought to laboratory.

The samples were further mixed and homogenized properly and acclimatized by increasing concentration of dye Orange F2R upto one month. These acclimatized samples were then used for isolation of bacteria.

2.3. Isolation and screening of dye decolorizing bacteria:

By using serial dilution of acclimatized samples, the isolation of bacterial species was carried out on nutrient agar. The isolated and well grown bacterial colonies were used for further study.

The screening of dye decolorizing bacteria was carried by using nutrient agar having 100 ppm concentration of dye. Each of bacterial isolate was tested against Orange F2R. After incubation period plates were observed for zone of decolorization. Those bacterial isolates showing maximum zone of decolorization were selected for further studies.

2.4. Optimization of various cultural conditions:

2.4.1. Effect of Temperature, pH, Inoculum size, Dye concentration:

To achieve maximum dye decolorization the optimization of various cultural conditions was carried out. To study the effect of Temperature, the tubes containing 30 ml nutrient broth and 100 ppm dye concentration were inoculated with promising isolates. The tubes were kept for incubation at various temperatures viz. Room temperature, 37°C, 45°C, 55°C and 65°C. The effect of pH was studied by using 30 ml nutrient broth with 100 ppm dye concentration. The pH of medium was adjusted at the range 6.0-8.0. Then the tubes were inoculated with promising isolates and kept for incubation. Similarly, the effect of inoculum size was studied by inoculating promising isolates in various volumes viz. 1%, 2%, 3% and 4% in 30 ml nutrient broth having 100 ppm dye concentration. In order to check the efficiency of isolates for dye decolorization the tubes with 30 ml nutrient broth were added with various concentration of Orange F2R in the range 100

ppm to 1000 ppm. Further the tubes were inoculated with promising isolates and kept for incubation. After incubation period the tubes were observed for dye decolorization spectrophotometrically.

2.5. Effect of Carbon and Nitrogen sources:

The isolates were examined for dye decolorization ability in presence of various carbon and nitrogen source. 30 ml Minimal medium containing 100 ppm dye concentration was added with different Carbon sources (Glucose, Sucrose and Starch) and Nitrogen source (Peptone, Yeast extract and Meat extract). Tubes were inoculated with promising isolates and kept for incubation for 24 hours. After incubation tubes were observed for decolorization. Decolorization was monitored by spectrophotometer.

2.6. Percent decolorization study:

The determination of percent dye decolorization was carried out at λ max by spectrophotometer (Systronics-106 model) and calculated by using following formula.

$$\% \text{ Decolorization} = [(A_0 - A_1) / A_0] \times 100$$

Where, A_0 = Initial absorbance; A_1 = Final absorbance

3. RESULTS:

3.1. Isolation and screening of dye decolorizing bacteria:

Total 12 isolates were isolated. These isolates were further screened for dye decolorization ability. Among 12 isolates total 5 isolates were having highest efficiency for decolorization of Orange F2R within 24 hours. Hence these 5 promising isolates were used for further used study.

3.2. Optimization of various cultural conditions:

3.2.1. Effect of Temperature, pH, Inoculum size, Dye concentration:

The promising isolates exhibited complete decolorization of Orange F2R at temperature 37°C. These isolates showed higher decolorization of dye at pH range 6.0-8.0. Specifically, it was found that the pH 7.0 was optimum pH. The isolates exhibited maximum decolorization when inoculated with 1% inoculums size. Further it was observed that these isolates were able to decolorize dye Orange F2R up to 1000ppm concentration. However, the maximum activity of isolates was observed at 800ppm of dye concentration. Above 800ppm concentration the activity was slightly decreased, which was calculated by spectrophotometer at λ max 494 nm. The results are shown in Fig. 1

3.3. Effect of Carbon and Nitrogen sources:

It was observed that all the promising isolates were showing highest decolorization of Orange F2R in

presence of 1% Glucose as carbon source 1% Yeast extract as Nitrogen source. The results are shown in Fig. 1.

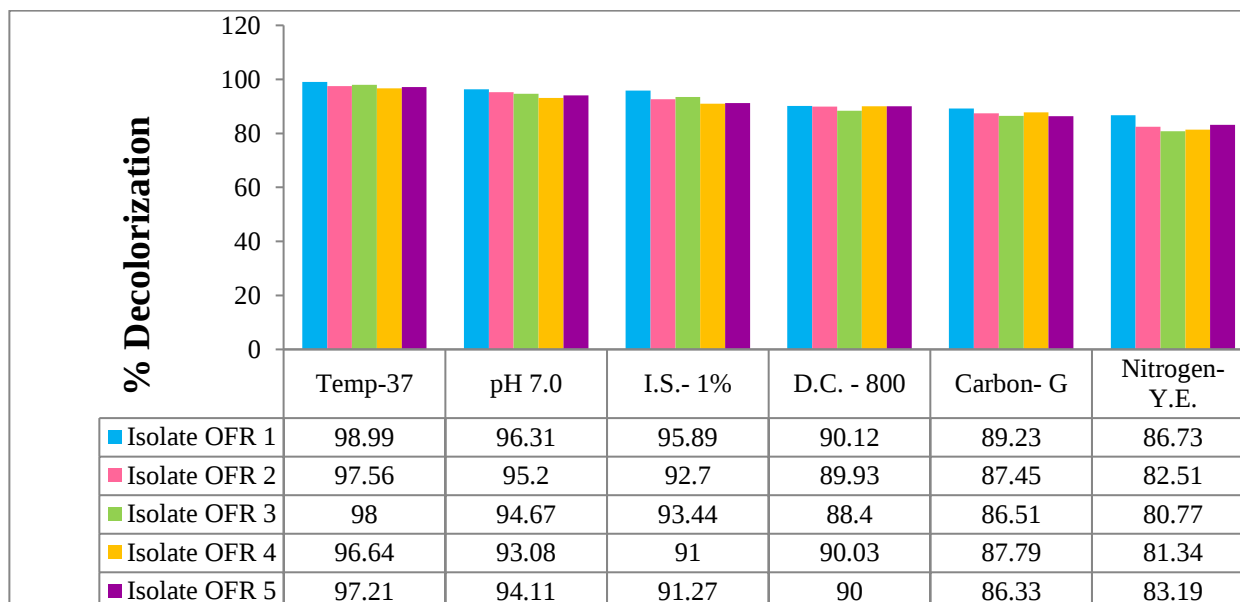


Figure 1: Effect of various Cultural conditions on decolorization of Orange F2R by 5 promising isolates in 24 hours. Where: Temp- Temperature; I.S- Inoculum size; D.C.- Dye concentration; G- Glucose; Y.E.- Yeast Extract.

4. DISCUSSION:

The present research work reveals the efficiency of 5 promising isolates which were isolated from acclimatized samples. These 5 isolates were designated as OFR-1, OFR-2, OFR-3, OFR-4 and OFR-5. The optimum temperature was 37°C which showed near about complete decolorization of dye (98.99%) at 24 hours. This could be because optimum cultural conditions and maximum enzyme production by the bacteria. These results were exactly similar with Bhatt et al. (2012) and Saratale et al. (2010). They revealed that 37°C is optimum temperature for dye decolorization by bacterial consortium. The efficiency of isolates for dye decolorization was gradually decreased above 45°C and this could be due to decrease in cell growth and enzyme activity (Cetin et al., 2006; Panswadet al., 2000). The pH is also important factor for activity of dye decolorization of bacteria. Generally, the pH in the range 6.0 to 10.0 is optimum for maximum decolorization of textile dyes (Chen et al., 2003; Guo et al., 2007; Kilic et al., 2007). In this research work the pH 7.0 was optimum pH for highest decolorization (96.31%) of dye. These results exactly in accordance with Kannan et al. (2013) who suggested that *Pseudomonas putida* was able decolorize dye Ramazol Black B at 7.0 pH upto 93.23% in 48 hours.

The organisms utilize textile dyes as their Carbon source but upto certain limit. Above that limit the increasing concentration of dye reduce the ability of organisms due to toxic nature of dye. In present research work the organisms could decolorize Orange F2R upto 800 ppm concentration above that the decolorization efficiency of isolates reduced. This was earlier proved by Gopinath et al. (2009) that *Bacillus sp.* isolated from tannery industrial effluent was able to decolorize Congo red dye at initial concentrations but the higher concentrations of dye inhibits the bacteria. The present research work exhibited that Glucose and Yeast extract were best carbon and nitrogen sources for maximum decolorization of dye. These results were strongly in accordance with Jagwaniet al., (2003) who suggested that decolorization of ROM2R (100 ppm) was maximum in presence of 1% Glucose and Yeast extract by six bacterial isolates namely VCS, NAS, SAS, VSS, VWS and SWS.

5. CONCLUSION:

From the present study it can be concluded that 5 promising bacterial isolates could be exploited for bioremediation of textile azo dye Orange F2R which is a

cost effective and ecofriendly approach and will lead to a green technology.

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