

## Optimization of Parameters for Decolorization of Reactive Dyes using Bacterial Isolates

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The present study was carried out to degrade the textile Reactive azo dyes by using bacteria isolated from dye contaminated soil. The effect of pH, temperature, carbon, nitrogen sources, inoculum size and incubation time was studied with an aim to determine the optimal conditions required for maximum decolorization. The bacterial strains used in the study were *Bacillus licheniformis* and *Pseudomonas putida*. The optimum pH for decolorization of RR 195 by *P. putida* was 7.0. It shows good decolorization efficiency even in alkaline region. The optimum temperature was 37°C. Glucose and Yeast extract has emerged as the ideal carbon and nitrogen sources for both bacteria. In different inoculum size and incubation time evaluated, 3.0% v/v and 120 hrs appeared to highest percentage of decolorization process by both bacterial strains.

**Key words:** Decolourization, Reactive Azo Dyes, Optimization, *Bacillus licheniformis* and *Pseudomonas putida*.

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Color in the effluent is one of the most obvious indicators of water pollution and the discharge of highly colored synthetic dye effluents is aesthetically displeasing and can damage the receiving water body by impeding penetration of light. During dyeing processes, up to 50% of the dyestuff does not bind to the fibers and is therefore released into the environment (O'Neill *et al.*, 1999 and Tan *et al.*, 2000). Azo dyes constitute the largest and versatile class of synthetic dyes used in the textile, industries and represent major components in wastewater. Azo dyes and their metabolites are carcinogenic and mutagenic as well (Myslak and Bolt, 1998). Germination rates and biomass production of several crop species is

seriously affected with application of this dye contaminated wastewater (Pourbabaee *et al.*, 2006).. Thus, treatment of this dye contaminated wastewater is necessary to make it safe for disposal to aqueous system or to reuse it. Various physiochemical and microbial approaches are in use to treat the textile effluent.

Microbial degradation and decolorization is an environment friendly and cost-competitive alternative to chemical decomposition processes. Some studies have documented the ability of certain strains of bacteria, fungi and yeast to degrade azo dyes (Stolz, 2001). However, only a limited number of microbes can directly utilize azo dyes as source of carbon and nitrogen and quite often, it is a co-metabolic process requiring a co-substrate (source of carbon, nitrogen & reducing equivalents). The rate of degradation increases in the presence of co-substrates for those microbes utilizing azo dyes as source of C and N (Massovi

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*et al.*, 2005; Saraswathi and Balakumar, 2009). Some other factors such as pH and temperature also affect rate of biodegradation of azo dyes (Jadhav *et al.*, 2007; Khan *et al.*, 2009; Prasad *et al.*, 2011). Also microbes are very sensitive to temperature and pH, some strains have the potential to perform their activities over wider range. Therefore, there is need to determine optimum pH and temperature conditions for maximum effectiveness of microbes for removing dye from wastewater. Hence the present investigation has been selected to study optimization of four reactive dyes decolorization.

## MATERIALS AND METHODS

The *Bacillus licheniformis* and *Pseudomonas putida* bacterial strains were isolated from industrial effluent water collected from Erode effluent discharge points located in Kauvery River at March 2012. Isolated bacterial strains were tested for their highest decolorization activity against commercially important dyes such Reactive red 195, Reactive Yellow 17, Reactive Orange 72 and Reactive Blue 36 in MSM broth.

### Optimization of environmental factors

Various factors were optimized to achieve the highest decolorization rate of four reactive dyes by the selected isolates of bacteria. All the experiments were conducted in triplicate.

### pH

Effect of different pH ranging from 5 to 11 was examined on the decolorization efficiency of the selected two bacterial isolates. MSM that was enriched with RR 195, RY 17, RO 72 and RB 36 at the concentration of 50 mg L<sup>-1</sup> was used. Incubation temperature was maintained at 37°C. The pH was adjusted by using 0.01 M HCl or 0.01 M NaOH

### Temperature

Decolorization of RR 195, RY 17, RO 72 and RB 36 by the selected bacterial isolates was studied at different temperatures such as 37, 40 and 45°C. Medium used for this purpose was mineral salt media (MSM), which was enriched with abovementioned dyes at the concentration of 50 ppm and pH of the MSM was maintained at 7.

### Carbon source

In order to assess the effects of different carbon sources on bacterial decolorization, four carbon sources, namely Xylose, glucose, Sucrose,

maltose and starch were used at the rate of 4 g L<sup>-1</sup>. The MSM spiked with RR 195, RY 17, RO 72 and RB 36 at the concentration of 50 ppm was used. Incubation temperature was 37°C.

### Nitrogen source

The effect of various organic nitrogen sources on the growth of *Bacillus licheniformis* and *Pseudomonas putida* and decolorization was studied by using different nitrogen sources; Yeast extract, Beef extract, Peptone, potassium nitrate, urea and Soybean powder. 100ml of MS media with 0.1% of respective organic nitrogen source was added with 50 ppm of RR 195, RY 17, RO 72 and RB 36.

### Inoculum size

Effect of different inoculum size ranging from 1.0 to 3.0 v/v were examined on the decolorization efficiency of the selected two bacterial isolates. MSM that was enriched with RR 195, RY 17, RO 72 and RB 36 at the concentration of 50 ppm were used. Incubation temperature was maintained at 37°C.

### Incubation time

The incubation time varying from 24 to 120 were examined for the detection of optimum incubation time required for the degradation of abovementioned dyes. MSM that was enriched with RR 195, RY 17, RO 72 and RB 36 at the concentration of 50 ppm were used. Incubation temperature was maintained at 37°C for 24 hrs to 120 hrs.

## RESULTS AND DISCUSSION

### Effect of pH and temperature on percentage of Bacterial degradation of different dyes

The decolorization efficiencies of *P. putida* and *B. licheniformis* were compared across a range of pH values. After 24h of incubation, the maximum decolorization was achieved at pH 7 (87.49±0.81%) in *P. putida* tested against RR 195 and the minimum decolorizing activity was recorded at pH 5 (33.93±1.30%) in *P. putida* tested against RO 72 (Fig 1). For different temperature tested, *P. putida* showed maximum decolorization at 37°C (77.87±0.36), while the minimum level of decolorization was achieved in 45°C (64.69±1.35%) at *P. putida* tested against RR 195 (Fig 2). Temperature and pH are very important for any bacterial process, since growth and production of

enzymes are usually sensitive to high temperature. The pH of culture medium plays a critical role for the optimal physiological performance of microbial cells and the transport of various nutrient

components across the cell membrane. Thus, the pH of the decolorization medium has a marked effect on the cell growth and enzyme production (Bibi *et al.*, 2012).

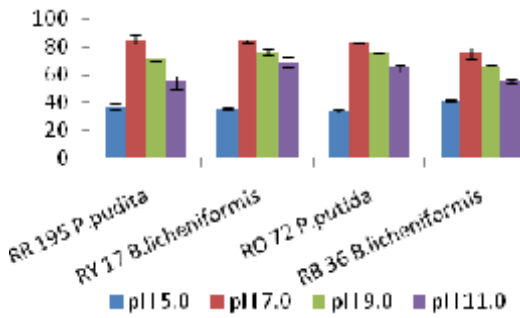


Fig.1 (a)

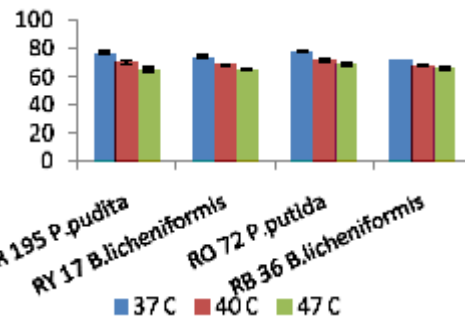


Fig.1 (b)

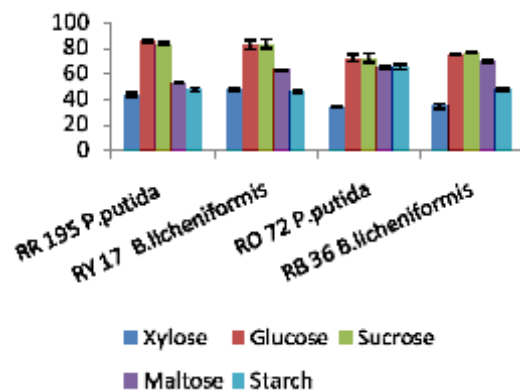


Fig. 2(a)

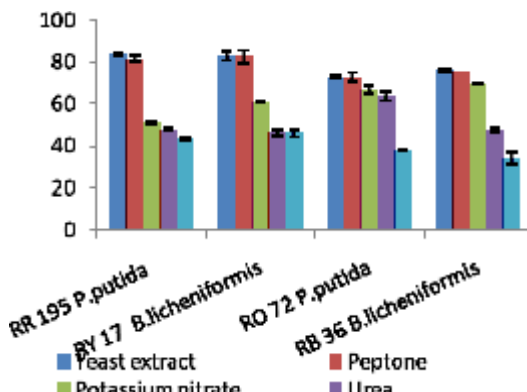


Fig. 2(b)

Fig. 1-2. Effect of pH and temperature on percentage of bacterial degradation

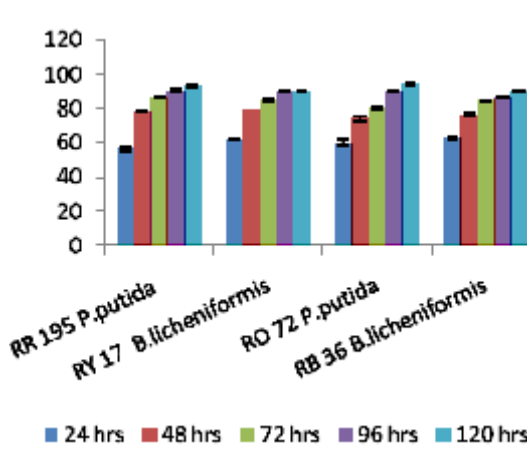
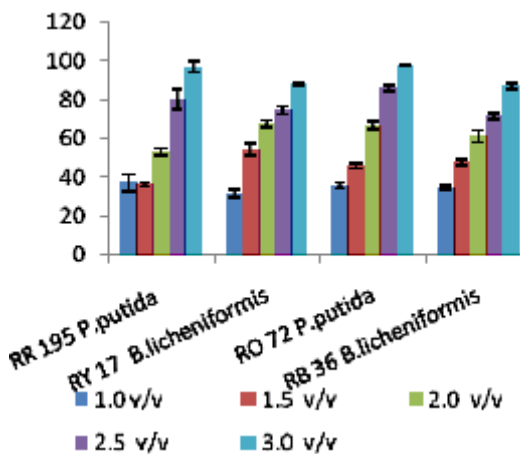


Fig. 3

### Effect of Carbon and nitrogen sources on percentage of bacterial degradation

Fig 3 and 4 illustrates the effect of different carbon and nitrogen sources on decolorization of RR 195 and RO 72 by *P. putida* and RY 17 and RB 36 by *B. licheniformis*. Glucose has emerged as the ideal carbon source for both bacteria, all recording highest rate of decolorization (85.07 and 71.90, 82.21 and 74.82% respectively), but xylose recorded least percentage decolorization by both bacteria (below 48%).

Among the five nitrogen sources evaluated, yeast extract appeared to highest percentage of decolorization process by both bacterial strains (72 – 84%). But both the bacterial strain exhibited lowest percentage decolorization when soyabean powder was used as nitrogen source (below 46%). The increase in decolorization percentage after addition of carbon and nitrogen sources are attributed to the fact that the dyes are deficient in carbon and nitrogen content and biodegradation without any extra carbon and nitrogen sources are difficult (Padmavathy *et al.*, 2003).

### Effect of different inoculum size in dye degradation

Among the five inoculum concentrations evaluated, 3.0% v/v appeared to highest percentage of decolorization process by both bacterial strains (72 to 97%). But both the bacterial strain exhibited lowest percentage decolorization when 1.0% v/v was used as a inoculum size (Fig 5). The incubation time varying from 24 to 120 were examined for the detection of optimum incubation time required for the degradation of dyes, and the results showed optimum to be 120 hrs (above 90%) and minimum was observed at 24 (below 63%) (Fig 6). Similar results were also found in Bayoumi *et al.*, 2010. This study recommended the application of the two most potent bacterial strains in the decolorization of the reactive azo dyes generally and RR 195, RY 17, RO 72 and RB 36 specifically in the industrial effluents under all nutritional and environmental conditions.

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