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Journal of Chemical and Pharmaceutical Research, 2013, 5(5):219-225

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Decolorization of synthetic textile dyes using *Pseudomonas putida*

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ABSTRACT

Some of the synthetic dyes are recalcitrant compounds that are difficult to degrade by most conventional methods. In this study, crystal violet, safranin and trypan blue were decolorized using Pseudomonas putida. The effect of pH, temperature, dye concentration, inoculum volume static/agitated conditions and different carbon and nitrogen sources was determined using One Factor At a Time (OFAT) approach to maximize the decolorization process. The optimum conditions were found to be pH 7, at 30°C, dye concentration of 200 mg/L, 0.5mL of inoculum for crystal violet and safranin whereas pH 7, at 40°C, dye concentration 100mg/L, inoculum volume 0.5 mL for trypan blue. The bacteria showed higher decolorization in static compared to agitated condition. The decolorization efficiency was higher when starch and ammonium sulphate were used supplements. On physiochemical parameter optimization, crystal violet, safranin and trypan blue were degraded by 50%, 30% and 80% respectively.

Keywords: recalcitrant, One Factor At a Time, crystal violet, safranin, trypan blue

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INTRODUCTION

Water pollution due to dye effluents is the major environmental threat of the present. Dyes are widely used as coloring material in textile, plastic, food, paper, printing, pharmaceutical and cosmetic industries [1, 2]. The unspent dyes are discharged into the aquatic environment and are the cause of environmental concern [3].

Dyes usually have a synthetic origin, complex aromatic molecular structures [2, 4] and contain substitution groups like azo, nitro and sulpho groups [5, 6] which make them more stable and more difficult to biodegrade [7]. They persist in water, inhibit sunlight from penetrating into the stream and reduce the photosynthetic reaction inhibit growth of aquatic biota and decrease recreation value of stream [8]. These colored compounds are not only aesthetically displeasing but some of them are also toxic and carcinogenic [9].

The colored effluents are usually treated by many physio chemical techniques like chemical oxidation, ozonation, ion exchange process, electrochemical process [10], electrolytic precipitation, coagulants [11], foam fractionation, membrane filtration, adsorption [12], photo catalytic degradation [2,13]. However, some of these processes are costly and cannot effectively be used to treat the wide range of dyes wastewater [14].

Biological methods are generally considered environmentally friendly as they can lead to complete mineralization of organic pollutants at low cost [15]. Bioremediation may be the most effective method of treating industrial dyes wastewater [16].

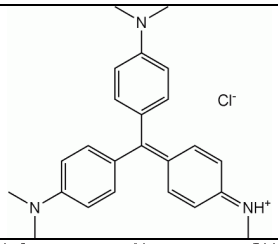
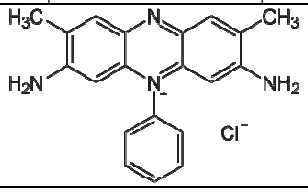
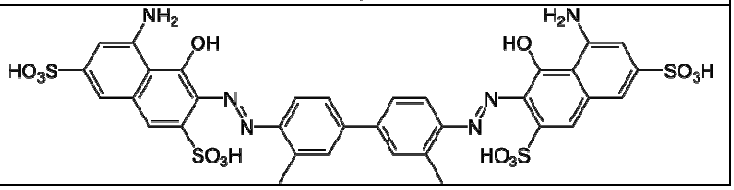
Crystal Violet
Crystal violet also known as methyl violet (N, N, N', N', N'', N''- hexamethylpararosalinine), is a triphenylmethane dye used extensively in veterinary and human medicine, as a biological stain and in various textile processes as a dye [17, 18]. Methyl violet is recalcitrant and hence long lived in the environment [19]. It is a potent clastogen responsible for causing tumor in some species of fishes [20].

Safranin
Safranin is a biological stain used in histology and cytology. Safranin is used as a counter stain in Gram staining protocols, coloring all cell nuclei red. It is also used for the detection of cartilage, mucin and mast cell granules. It is a dimethyl dye and is recalcitrant.

Trypan Blue
Trypan blue is an azo dye derived from toluidine. It is a vital stain used to selectively colour dead tissues or cells blue and hence is widely used in microscopy to check viability of cells. Azo dyes are considered to be recalcitrant, non biodegradable and persistent. They are sometimes fused with heavy metals on the structural interface and are considered to have relatively bad consequence on the surrounding environment due to their toxic and inhibitory nature.

In the present study the efficiency of *Pseudomonas putida* on the decolorization of synthetic dyes crystal violet, safranin and trypan blue was tested. Further the decolorization conditions were optimized for maximum efficiency using One Factor At a Time (OFAT) approach.

Table 1: Structure of the dyes used

Dye	Structure
Crystal violet	
Safranin	
Trypan blue	

EXPERIMENTAL SECTION

2.1 Microorganism and culture medium
Pseudomonas putida MTCC 102 was purchased from IMTECH, Chandigarh, India. The pure culture was maintained on nutrient agar and nutrient broth.

2.2 Dyes
Crystal violet 10B, safranin (dye content 80%) and trypan blue (dye content 40%) were obtained from Himedia, India. All the chemicals were of analytical grade.

2.3 Equipment
UV spectrophotometer of ELICO, India was used for measuring the absorbance in decolorization studies.

2.4 Decolorization studies
The effect of influencing variables such as pH, temperature, dye concentration, inoculum volume and static/agitated condition towards dye decolorization was investigated using One Factor at a Time (OFAT) approach. 100 mg/L of the dyes amended in nutrient broth were inoculated with 1 mL of *Pseudomonas putida* strain and incubated at 37^0C for 72 hours. After the incubation, the reaction mixture was centrifuged at 2000 rpm for 15 minutes and the supernatant was taken to measure the dye decolorization efficiency. Dye decolorization was measured by monitoring the decrease in absorbance of the dye in a double beam UV-Visible spectrophotometer [21]. The absorbance was read at 590nm, 520 nm and 610 nm for crystal violet, safranin and trypan blue respectively. Dye decolorization was expressed as percentage as follows: equation 1,

$$\text{Absorbance} = \frac{[A_0 - A_t]}{[A_0]} * 100 \quad \text{---- (1)}$$

Where
A_0 : initial absorbance
A_t : final absorbance

2.5. Effect of process variables
2.5.1. Effect of pH
Effect of pH on enzyme activity was studied in the pH range of 4-9 at constant 100mg/L dye concentration. The initial concentration of the reaction was kept at 100mg/L and the decrease in the absorbance after 72 hours was recorded.

2.5.2. Effect of temperature
The effect of temperature was studied in the range of 30°C - 70°C using the same above conditions, but at the optimal pH for 72 hours.

2.5.3.3. Effect of dye concentration
The effect of dye concentration was studied at optimal pH and temperature in concentration range of 100mg/L to 300mg/L was chosen using the same reaction conditions.

2.5.4. Effect of inoculum volume
At the optimal pH, temperature and dye concentration, the effect of inoculum volume towards dye decolorization was studied using the inoculum volume range from 0.25mL to 1.25mL.

2.5.5. Effect of culture condition
The decolorization studies were carried out both at static and agitated conditions. The static condition was maintained by incubating the reaction mixture in a 30°C incubator whereas the agitated condition was maintained by placing the reaction mixture in the shaker at 100 rpm.

2.5.6. Effect of carbon and nitrogen sources
1% of different carbon and nitrogen source was added as supplement in the nutrient broth and the decolorization efficiency was monitored. The different carbon sources used for the study were glucose, sucrose, maltose, starch and fructose. The different nitrogen sources used were ammonium sulphate, ammonium chloride, urea and tryptone.

RESULTS AND DISCUSSION

3.1. Effect of process variables
3.1.1. Effect of pH
pH has a major effect on the efficiency of dye decolorization and the optimal pH is usually in the range of 6-10 [22, 23]. The optimum pH for the decolorization of all the 3 dyes using *Pseudomonas putida* was found to be 7. The decolorization rate increased from pH 4 to 7 and on further increment in pH, the rate gradually decreased (Figure 1).

Majority of the azo dye reducing bacterial species reported [24, 25, 26] so far were able to reduce the dye at pH near 7.

3.1.2. Effect of temperature

The optimum temperature for decolorization was found to be 30°C for crystal violet and safranin whereas 40°C for trypan blue. The decolorization rate reduced significantly beyond 40°C (Figure 2). This might be due to loss of cell viability or deactivation of enzymes responsible for decolorization [27, 28].

3.1.3. Effect of dye concentration

The optimum dye concentration for decolorization was found to be 200 mg/L for crystal violet and safranin whereas 100 mg/L for trypan blue (Figure 3). The concentration of dye influences the decolorization through a number of factors including toxicity of the dye at higher concentrations and the ability of the enzyme to recognize the substrate efficiently at very low concentrations [29].

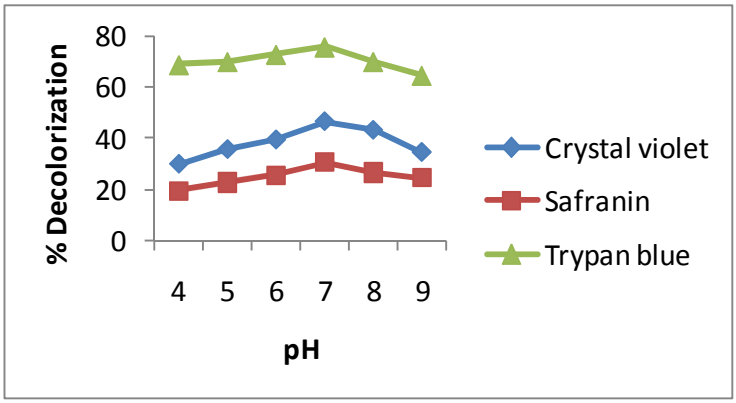


Figure 1: Effect of pH on decolorization

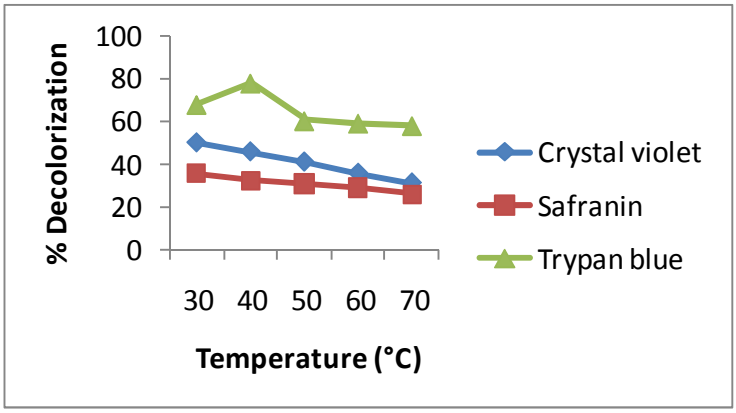


Figure 2: Effect of temperature on decolorization

3.1.4. Effect of inoculum volume

The decolorization rate increased as the inoculum volume increased from 0.25 to 1.25 mL. But beyond 0.5 mL there was just a mild increase (Figure 4). So the optimal inoculums volume was recorded as 0.5 mL. This can be explained on the basis that 200 mg/L of the dye acted as an inhibitor to bacterial growth at lower inoculum concentrations whereas beyond 0.5 mL inoculum could overcome the inhibiting action of dyes resulting in higher decolorization rates.

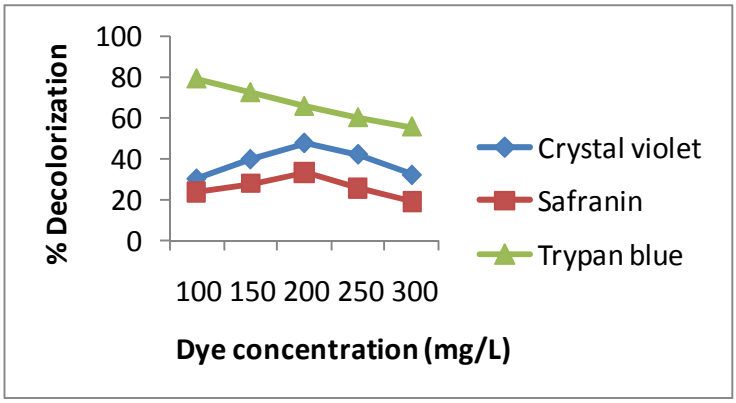


Figure 3: Effect of dye concentration on decolorization

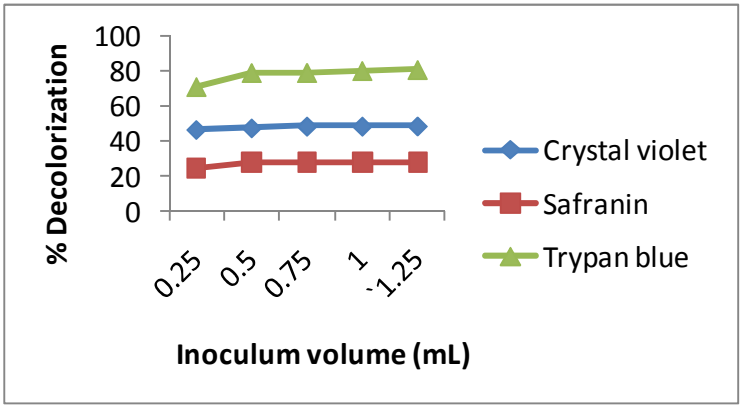


Figure 4: Effect of inoculum volume on decolorization

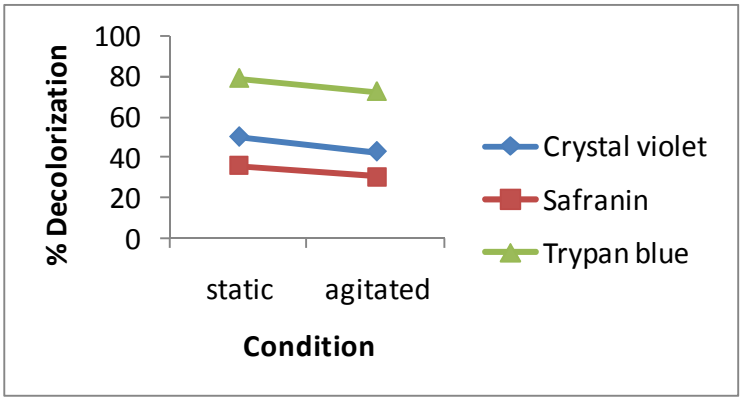


Figure 5: Effect of culture condition on decolorization

3.1.5. Effect of culture condition

Decolorization rates were found to be higher in static conditions than agitated conditions (Figure 5). It was found that under agitated conditions, presence of oxygen deprives the azoreductase from obtaining electrons needed for cleavage of azo dyes. Under static anoxic conditions, these electrons are available to azoreductase from NADH to decolorize azo dyes [30, 31, 32].

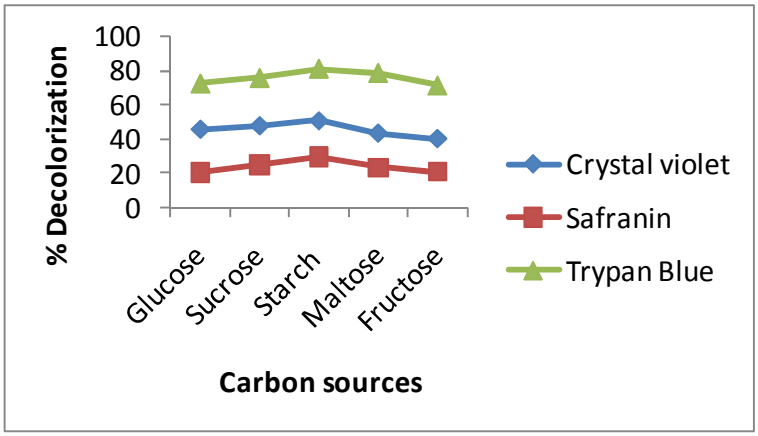


Figure 6: Effect of different carbon sources on decolorization

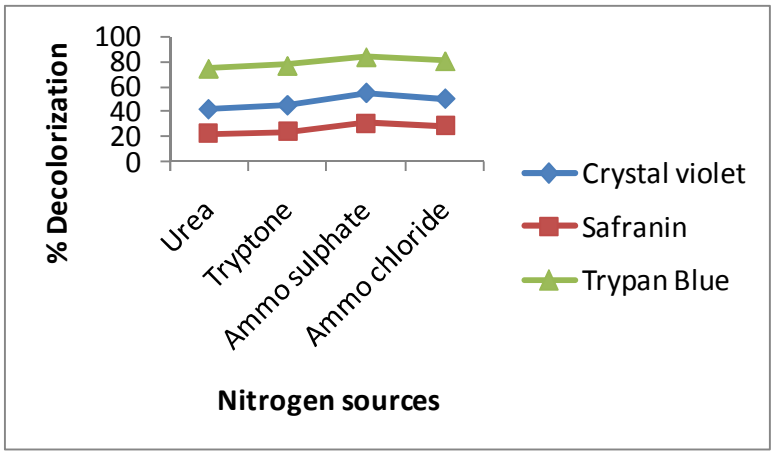


Figure 7: Effect of different nitrogen sources on decolorization

3.1.6. Effect of carbon and nitrogen sources

Maximum decolorization rate was observed when starch and ammonium sulphate were used as the carbon and nitrogen supplements respectively (Figure 6 and 7). *Bacillus subtilis* was also reported to exhibit higher decolorization rate when starch was used as a carbon supplement [33].

Acknowledgements

The authors are thankful to Dr. Jeppiaar, chairman, Jeppiaar Engineering College, Tamil nadu, India and Dr. Shaleesha A.Stanley, Head and Professor, Department of Biotechnology, Jeppiaar Engineering College for providing constant encouragement and support.

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