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Original Research Paper

Bacterial Decolourisation of Diazo Monochlorotriazine Fibre Reactive Dyes Under Optimized Physico-Chemical Conditions

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ABSTRACT

Large volumes of effluent are generated at different stages of textile manufacturing as a result of the use of copious amounts of dyes. Several tons of textiles, required to meet up with societal demands, are produced daily in this industry. Azo monochlorotriazine (MCT) fibre reactive dyes, an important class of dyes, are commonly used to dye cellulosic fibres and appear in wastewater due to their lower fixation on fibre. Coloured textile water requires efficient treatment before its discharge. An efficient dye decolourising Gram negative bacterial strain of *Enterobacter gergoviae* was isolated from coloured textile effluent after enrichment of sample. Biodecolourisation study of reactive red 141 and reactive red 120 was carried out in Bushnell Hass medium where dyes were used as sole source of carbon and nitrogen. Various parameters like addition of carbon source, nitrogen source, pH and temperature, were optimized for maximum decolorization of dyes. Up to 95% of dye decolorization was observed when dye was used as source of carbon and nitrogen. Addition of glucose and urea enhanced the rate of decolorization at pH 7 and 31°C. These findings show that isolated *Enterobacter gergoviae* could be effective for the treatment of dye-containing industrial effluents.

INTRODUCTION

Synthetic dyes are widely used in textile, rubber products, enamel, plastic, cosmetic and many other industries (Guang et al. 2005, Jo & Chia 2001). Textile processing industries is a widespread sector in developing countries. Among various processes in the textile industry, dyeing process uses large volume of water for dyeing, fixing and washing process (Husseiny 2008). Almost 10^6 tons of dyes are produced annually around the world, of which azo dyes, characterized by one or more azo groups (R1–N = N–R2) linking substituted aromatic structures, represent about 70% by weight (Guang et al. 2005). Azo monochlorotriazine (MCT) fibre-reactive dyes are commonly used for batch dyeing of cellulosic fibres, but typically exhibit lower exhaustion and fixation levels than other classes (Brent et al. 2006). Excess dyestuff in process water is highly undesirable because of environmental concerns (Andreas et al. 2004, Khalid et al. 2008), health hazards and aesthetical aspects. Colour is the first contaminant in the wastewater, which should be recognized and has to be removed before it is discharged in the environment (Handaayani et al. 2007). Removal of colour from dye containing wastewater is current issue of discussion and regulation in many countries because of awareness that water is valuable asset which should be protected (Casieri et al. 2008). Nowadays physico-chemical methods used for colour removal of the effluents are effective, but they show disadvantages in terms of operational problems, high cost, and sludge production (Kapdan et al. 2000, Lopez et al. 2004, Kodam et al. 2005). Moreover, those methods use more energy and chemicals than biological processes. Because of these disadvantages, in recent years a number of studies have focused on microbial decolorization and degradation of azo dyes (Handaayani et al. 2007). In the present study two diazo monochlorotriazine dyes, namely reactive red 141 and reactive red 120, were used. The objectives of the present study were to isolate efficient dye decolourising bacteria and optimizing various parameters for dye decolorization.

MATERIALS AND METHODS

Dyes and chemicals: Reactive red 141 and reactive red 120 were procured from National Chemicals, Surat, Gujarat. The various chemicals used in this study were of analytical grade and procured from Hi-Media Pvt. Ltd., Mumbai.

Sample collection: Highly coloured effluent was collected from a dyeing unit in the GIDC, Pandesara, Surat. The pH of the effluent was 7.5. The effluent was collected in airtight sterile plastic container and filtered through ordinary filter paper to remove large suspended particles.

Enrichment of sample: The effluent sample was inoculated with 50 mg/L of reactive red 141 and reactive red 120 and

incubated on rotary shaker (100 rpm) at 30°C. After 24 h 5% of inoculum was transferred to fresh effluent along with reactive red 141 and reactive red 120. Three such transfers were made.

Screening of dye decolourising microorganisms: After third transfer, cell suspension from last enriched flask was plated on the Bushnell Hass agar medium for screening of dye decolourising microorganisms. Composition of BH agar medium was (g/L), MgSO4 0.2, CaCl2 0.02, KH_2PO_4 1.0, (NH_4) NO3 1.0, $FeCl_3$ 0.05 supplemented with reactive red 141 (200 mg/L), pH 7.4. From that five bacterial colonies were selected on the basis of formation of decolorization zone surrounding the colonies.

Isolation and characterization of decolourisers: Out of selected five decolourising colonies most promising bacterial colony was selected on its capacity to produce largest decolorization zone on BH agar plate containing dye. The isolated bacteria were characterized by various morphological and biochemical tests according to Bergey's Manual of Systematic Bacteriology.

Dye decolorization experiments: Dye decolorization by isolated bacteria was tested in 250 mL Erlenmeyer flasks with 100 mL BH medium containing 200 mg/L of each dye. The sterilized medium was inoculated with the isolated bacterial culture of uniform cell density (optical density 1.0 at 550 nm). The medium-to-inoculum ratio (v/v) was 50:1. Inoculated medium was incubated at 30°C on rotary shaker at 100 rpm.

Spectrophotometric assessment of microbial dye decolorization: After 24 h of incubation, 3 mL of medium was withdrawn from each flask. Aliquots were centrifuged at 10,000 rpm in a centrifuge for 15 min to separate cell mass, clear supernatant was used to measure the decolorization at absorbance maxima of dye (544 nm). Uninoculated mediums were incubated as a control to check abiotic decolorization. Experiment was performed in triplicate. Decolorization efficiency was expressed as percentage of decolorization and was calculated using equation,

$$\text{Decolorization (\%)} = A_C - A_T / A_C \times 100$$

Where A_C is the absorbance of the control and A_T is average absorbance of the test samples.

To ensure that the change in pH of the dye solution had no effect on the decolorization, the visible spectrum was recorded between pH 5.0 to 11.0, in which the pH did not show any effect in spectrum.

Optimization of physico-chemical conditions for maximum decolorization of dyes

Effect of different carbon sources on decolorization: Three different carbon sources, i.e. glucose, lactose and sucrose were tested for decolorization at various concentrations, i.e. 0.2%, 0.5%, 1.0% (w/v). 2 mL of inoculum was inoculated in 100 mL BH medium along with respective dyes and different concentrations of carbon source. All flasks were incubated at 30°C on rotary shaker. Aliquot was removed from each flask for the determination of decolourising activity at different time intervals.

Effect of nitrogen sources on decolorization: Two nitrogen sources were tested for decolorization of dyes. The concentration of organic nitrogen (urea) and inorganic nitrogen source (ammonium chloride) were 0.2%, 0.5%, 1.0% (w/v). 2 mL of inoculum was added to 100 mL of BH medium along with respective dyes, 0.5% glucose and different concentrations of nitrogen source. All flasks were incubated at 30°C on rotary shaker. Aliquot was removed from each flask for the determination of decolourising activity at different time intervals.

Effect of pH and temperature on decolorization: Effect of pH and temperature on decolorization was observed by growing the isolate in the BH medium containing respective dyes having pH range from 5.0 to 11.0. In the same way the effect of temperature was examined by growing cultures at 25°C, 27°C, 29°C, 31°C, 33°C, 35°C, 37°C, 39°C, 41°C by keeping the pH of the medium 7.4 for 7 days. Samples were withdrawn at different time intervals and decolourising activity was determined as described earlier.

RESULTS AND DISCUSSION

From the effluent sample collected from a dyeing unit, a promising decolourising bacterial strain was isolated. This strain formed a distinct clear zone on BH agar plate containing dye. To identify this bacterium, we investigated its morphological and physiological properties using various biochemical media. On the basis of results the isolate was identified as *Enterobacter gergoviae* (Table 1).

Microbial dye decolorization: The isolated strain was tested for its capacity to remove the two dyes. Dyes were added to BH medium at concentration of 200 mg/L as sole source of carbon and nitrogen. The results indicate that the strain is capable of decolourising the dyes up to 95% in 7 days. Rate of decolorization is depicted in Fig. 1. The results show that the isolated strain is effective in decolorization.

Optimization of culture condition: For the maximization of decolorization of the dyes by the isolated strain, experiments were conducted for the optimization of carbon source, nitrogen source, pH and temperature.

Effect of different carbon sources: Three different carbon sources, glucose, lactose and sucrose were tested for maximum decolorization by the isolated strain. Each carbon source

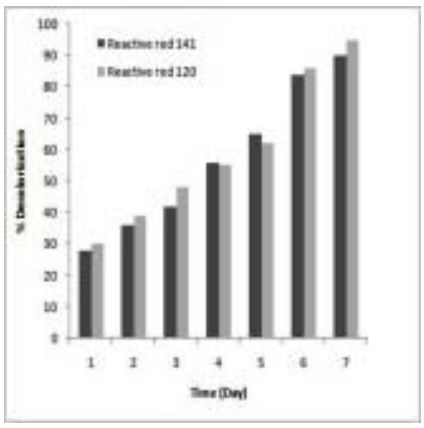

Fig.1: Microbial decolorization.

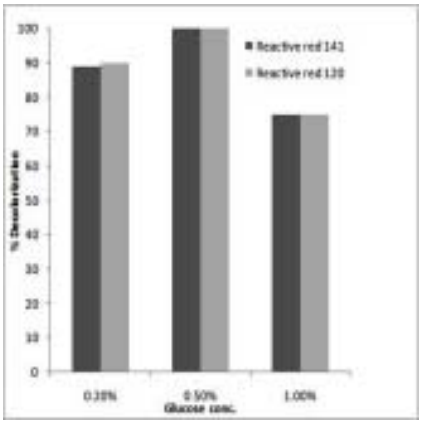

Fig. 2(a): Effect of glucose.

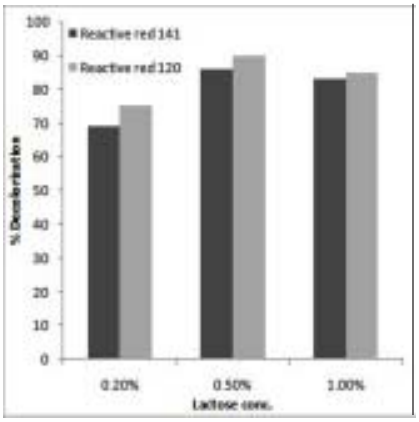

Fig. 2(b): Effect of lactose.

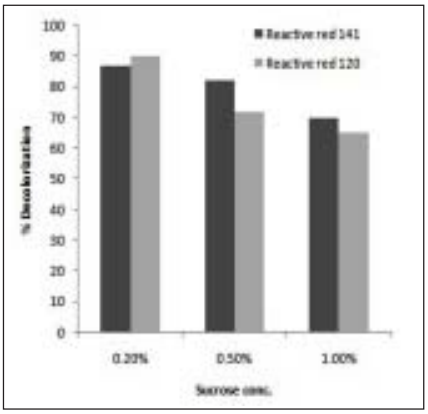

Fig. 2(c): Effect of sucrose.

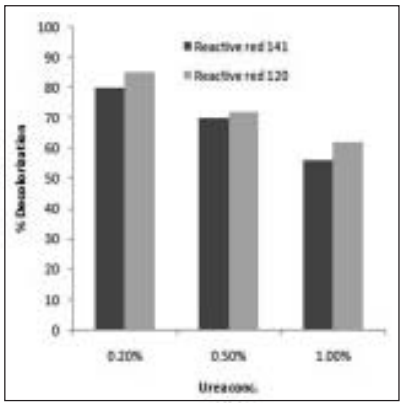

Fig. 2(d): Effect of urea.

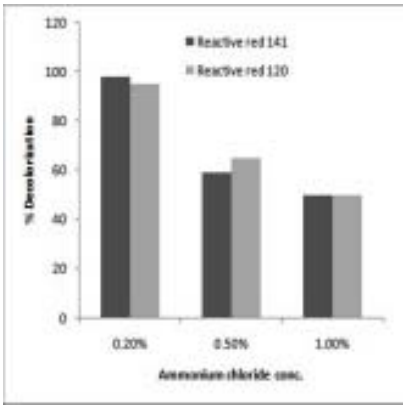

Fig. 2(e): Effect of ammonium chloride.

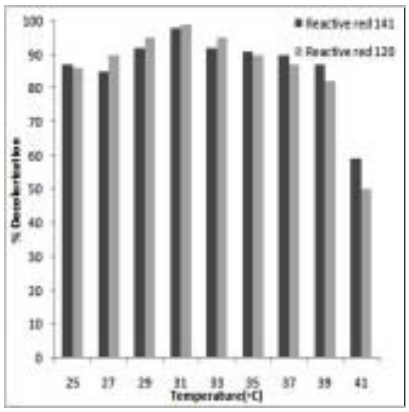

Fig. 2(f): Effect of temperature.

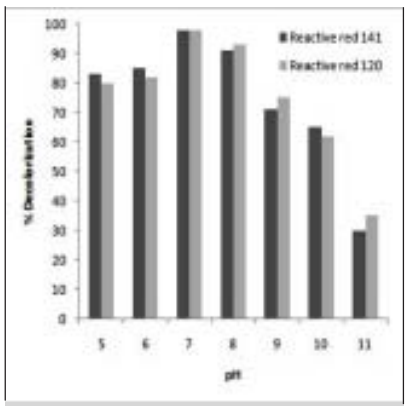

Fig. 2(g): Effect of pH.

was added at 0.2%, 0.5% and 1.0% in BH medium containing respective dye at 200 mg/L.

The strain is capable of decolourising the dyes in the presence of glucose at various concentrations. Complete decolorization was observed when there was addition of 0.5% of glucose (Fig. 2a).

There was no increase in the rate of decolorization when lactose and sucrose were added (Figs. 2b & 2c). Maximum percentage decolorization was observed when glucose was used as carbon source at 0.5%.

Table 1: Physiological and biochemical characterization of the isolated bacteria.

Sr. No.	Characteristics	Result	Sr. No.	Utilization of	Result
1	Gram Reaction	Negative	13	L-Arabinose	Positive
2	Cell Morphology	Short Rods	14	Cellobiose	Positive
3	Motility	Positive	15	Dulcitol	Negative
4	Pigmentation	Negative	16	Glycerol	Positive
5	Spore Formation	Negative	17	Lactose	Positive
6	Urea hydrolysis Test	Positive	18	Maltose	Positive
7	Indole Production Test	Negative	19	Mannitol	Positive
8	Methyl Red Test	Negative	20	Raffinose	Positive
9	Voges Proskauer Test	Positive	21	Sucrose	Positive
10	Gelatin Hydrolysis Test	Negative	22	Trehalose	Positive
11	Phenyl Alanine Deaminase Test	Negative	23	Xylose	Positive
12	Glucose Dehydrogenase Test	Positive	24	D- Sorbitol	Negative

Effect of different nitrogen sources on decolorization: Two nitrogen sources, urea and ammonium chloride, were tested for decolorization of dyes by the isolated strain, the results of which are depicted in Figs. 2(d) & 2(e). BH medium containing respective dyes was supplemented with 0.5%, and 0.2%, 0.5% and 1.0% of urea and ammonium chloride respectively. Results suggest that the strain showed maximum decolorization at concentration 0.2% of urea and 0.2% of ammonium chloride. The best decolorization was observed at 0.2% of ammonium chloride.

Effect of temperature and pH on dye decolorization: The effect of temperature and pH on the dye decolorization was tested. It was found that a temperature of 31°C was optimum for maximum decolorization (Fig. 2f). Decline in decolorization activity at higher temperature more than 39°C can be attributed to the loss of cell viability. Optimum pH for maximum dye decolorization was 7.0 (Fig. 2g).

The present study has resulted in the isolation of a bacterial strain which has capacity of decolourising diazo mono- cholorotriazine dyes, reactive red 141 and reactive red 120, thus showing the potential to be exploited as possible candidate for bioremediation. Decolorization activity can be enhanced by addition of glucose. The isolated strain can decolourise dyes under wide range of pH and temperature, which is the nature of effluent from dyeing industries.

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