

Isolation and Identification of Magenta Dye Decolorizing Bacterium *Citrobacter* sp

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ABSTRACT: We have isolated 20 bacterial strains as a dye decolorizing micro-organisms. Out of 20 we have selected one bacterial strain for further study. The selected bacterial strain was identified as *Citrobacter* sp. on the basis of morphological and biochemical tests. The UV-Vis analyses and colorless bacterial cells suggested that *Citrobacter* sp. exhibited decolorizing activity through biodegradation, rather than inactive surface adsorption in liquid medium. The *Citrobacter* sp. showed high decolorization activity in static condition as compared to shaking condition and the effect of inoculum size on the decolorization of Magenta (100 ppm) by *Citrobacter* sp. indicated that increase the inoculum size from 1-10 mL progressively decreases the time requirement for complete dye decolorization. These results suggest that the isolated organism *Citrobacter* sp. as a useful tool to treat waste water containing reactive dyes.

KEYWORDS: *Citrobacter* sp., Decolorization, Magenta.

I. INTRODUCTION

More than 100,000 commercially available dyes are known and close to one million tons of these dyes are produced annually worldwide [1]. When compared to natural dyes, reactive dyes are extensively used in the textile industry due to their easy use, cost effectiveness in synthesis, stability, variety of colors and better dyeing processing conditions [2, 3]. Disposal of these dyes into the environment causes serious damage, since they may significantly affect the photosynthetic activity of hydrophytes by reducing light penetration [4] and also they may be toxic to some aquatic organisms due to their breakdown products [5,6].

Dyes can be removed from waste water by chemical and physical methods including adsorption, coagulation-flocculation, oxidation and electrochemical methods [7]. However, both the physical and chemical methods have many disadvantages in application, such as high-energy costs, high-sludge production, formation of by-products [8]. Conversely, bioprocessing can overcome these defects because it is cost saving and environmentally benign. Adsorption rather than degradation plays a major role during the decolorization process by fungi and algae, as a result, the dyes remain in the environment. It is well known that bacteria can degrade and even completely mineralize many reactive dyes under certain conditions [9, 10]. Biological methods are specific, less energy intensive, effective, environmentally safe and they result in complete bioconversion of organic pollutants to stable and nontoxic end products. Hence the present investigation was undertaken isolation and identification of dye decolorizing microorganism.

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II. MATERIALS AND METHODS

Dyes

The azo dye Magenta (Basic Red-9) was obtained from Shailaja textile Industry Sholapur, Maharashtra, India.

Isolation of dye decolorizing microorganisms

Dye decolorizing bacteria were isolated from different samples such as dye amended soil, textile mill effluents and other soil samples from MSK Mill, Gulbarga, Karnataka. The 1g of soil dissolved in physiological saline and inoculated into Bushnell-Hass (BH) containing 0.1 g of dye Magenta ($MgSO_4 \cdot 7H_2O$ 0.2 g, $CaCl_2$ 0.02 g, KH_2PO_4 1 g, K_2HPO_4 1 g, $FeCl_3 \cdot 3H_2O$ 0.05 g, peptone 1.5 g, pH 7.0 – 7.4) and the flasks were incubated at 37 °C under static condition for 48 h. The decolorized flask culture was transferred to fresh BH medium to screen the strain having color removing ability and the screening process was repeated in 5 times. The isolated bacterial strains were tentatively characterized by cultural, morphological and biochemical tests and maintained on nutrient agar at 4 °C.

Dye decolorization studies of Bacterium

Decolorization of the dye was monitored spectrophotometrically by reading the decrease in absorbance of the culture supernatant containing dye at its respective absorbance maxima. Uninoculated control was used to compare abiotic color loss during the experiment. Decolorizing activity is expressed in terms of percentage of decolorization of the dye [11].

$$D = [(A_0 - A_1) / A_0] \times 100$$

Where, D, % of decolorization; A_0 , initial absorbance; A_1 , final absorbance

Spectrophotometric analysis

Aliquots of cell-free decolorized supernatant collected at regular intervals of time were used for Spectrophotometric analysis (ANTHELE-SECOMAM France) at wavelength of 300- 700 nm. The dye Magenta absorbance maximum was at 557 nm and the efficiency of decolorization was expressed as the percentage ratio of the decolorized dye concentration to that of the initial one.

Effect of shaking, static and inoculum size on dye decolorization

The 250 mL Erlenmeyer flasks having 100 mL of MM medium containing 100 ppm of dye were inoculated with selected bacterium. The effect of shaking, static and various sizes (1-10 ml) of inoculum on dye decolorization were studied and uninoculated controls were prepared in parallel in all experiments.

III. RESULTS AND DISCUSSION

Isolation and identification

We have isolated 20 bacterial strains as a dye decolorizing micro-organisms (Table 1). Out of 20, strain BRM-10 was found to be most efficient in that it decolorized 100 ppm of Magenta within 18 h of incubation. This isolated bacterium was showed morphology such as round, white, small, creamy growth on nutrient agar, gram negative, motile and positive biochemical tests such as catalase, H_2S , Urease, Methyl red, Citrate tests (Table 2). Based upon the growth characteristics, staining reactions and biochemical tests, the isolated bacterium was identified as *Citrobacter* sp. according to Bergey's Manual of Determinative bacteriology [12].

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Table 1: Bacterial isolates, their sources and time for complete decolourization of Magenta.

| Isolate | Source | Time for complete Decolourization |
|---------|------------------------|-----------------------------------|
| BRM-1 | Sewage | 48 |
| BRM-2 | Garden soil | 44 |
| BRM-3 | Red soil | 72 |
| BRM-4 | Black soil | 68 |
| BRM-5 | Black soil | 36 |
| BRM-6 | Compost | 32 |
| BRM-7 | Sand | 56 |
| BRM-8 | Dye amended soil | 28* |
| BRM-9 | Textile mill effluents | 32 |
| BRM-10 | Textile mill effluents | 18*** |
| BRM-11 | Textile mill effluents | 60 |
| BRM-12 | Dye amended soil | 72 |
| BRM-13 | Dye amended soil | 62 |
| BRM-14 | Textile mill effluents | 32 |
| BRM-15 | Textile mill effluents | 24** |
| BRM-16 | Pond water | 64 |
| BRM-17 | Jagath tank | 32 |
| BRM-18 | Jagath tank | 72 |
| BRM-19 | Sewage | 60 |
| BRM-20 | Sewage | 56 |

** - Efficient in decolorization, *** - Most efficient in decolorization

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Table 2: Morphological and biochemical characters of BRM-10 strain used for decolonization.

| Tests | BRM-10 isolate |
|----------------------------------------------------------------------------------------|--------------------------------------|
| A. Colony character Size Shape Colour | Small Round White |
| B. Morphological Character Grams staining Motility Cell shape and arrangement | Negative Motile Bacilli |
| C. Sugar fermentation Lactose Maltose Glucose Sucrose | + A/-G + A/-G + A/+G + A/-G |
| D. IMViC Indole Methyl Red VogesProskaur Citrate | - ve + ve - ve + ve |
| E. Urease production | + ve |
| F. H ₂ S production | + ve |
| G. Catalase activity | + ve |
| H. Growth on McConkey | Creamy |
| I. Growth on EMB agar | Creamy |
| Tentatively Identified as | <i>Citrobacter</i> sp. |

Decolorization of Magenta dye by *Citrobacter* sp.

The complete Magenta dye decolorized medium was centrifuged, to separate the spent medium and bacterial cell biomass. The spent medium and bacterial cell biomass were independently inoculated into the fresh medium containing dye Magenta and incubated at 37 °C under static condition. The decolorization of Magenta was observed in inoculated bacterial cell biomass not in inoculated spent medium indicates that decolorization takes place by bacterium *Citrobacter* sp. (Data not shown).

Decolorization assays

A spectrophotometric scanning (300–700 nm) of the Magenta dye showed a single peak at 557 nm. The dye decolorization was represented by a decrease in absorbance and the absorbance spectral pattern of each recovered dye solution was not similar to that of the initial one. Furthermore, after decolorization, at 16 h the bacterial cell mass was slightly colored and the same bacterial cell mass was clear at 18 h (Fig. 1). It clearly indicated that the decolorization was initiated by adsorption then degradation takes place and this indicates that the observed dye [13, 14].

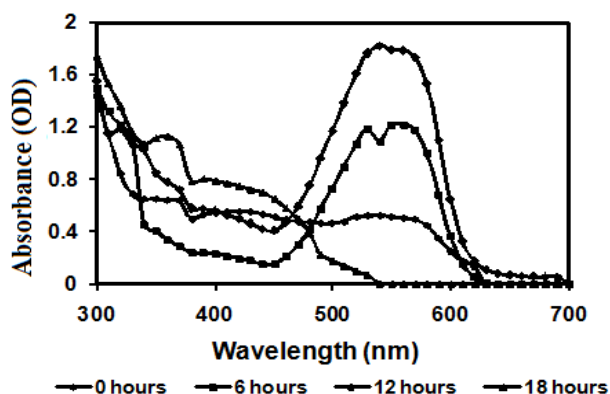


Fig. 1. UV-Visible scanning of (a) Magenta and (b) decolourisation of Magenta by *Citrobacter* sp. at different intervals of time.

Effect of shaking, static and inoculum size on dye decolorization

The effect of shaking on decolourization of Magenta (100 ppm) by *Citrobacter* sp. was investigated and the results are shown in Fig. 2. There was no advantage of shaking or aeration during the decolourization of Magenta by *Citrobacter* sp. Continuous shaking increased the time required for complete decolourization. At 50 rpm it required 32 h for complete dye decolourization and at 100 rpm required 48 h of incubation. This clearly indicated that shaking is a disadvantage for decolourization of Magenta compared to the static conditions. The aerobic decolorization of Magenta (100 ppm) by *Citrobacter* sp. was much faster at static conditions compared to shaking conditions. The several studies reported at static conditions for decolorization by *Proteus mirabilis* and *Enterbacter agglomerans* [15, 16] and Nermeen, et al., [17] reported decolorization of Red dye by *Streptomyces globosus* under static and shaking condition.

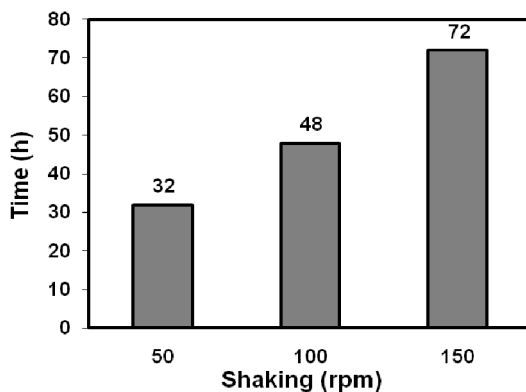


Fig. 2. Effect of shaking on decolourisation of Magenta by *Citrobacter* sp.

The observations on the effect of inoculum size on the decolourization of Magenta (100 ppm) by *Citrobacter* sp. indicated that increase the inoculum size from 1-10 mL progressively decreases the time requirement for complete dye decolourization (Fig. 3). The 1 mL inoculum size required 24 h to complete decolourization of Magenta and as the

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inoculum size increased time taken for complete decolourization of the dye decreases from 24 h at 1 mL to below 12 h at 8 mL inoculum. The time taken for complete decolourization of dye above 8 mL inoculum size was constant indicating that increase in inoculum size will reduce incubation only up to 8 mL.

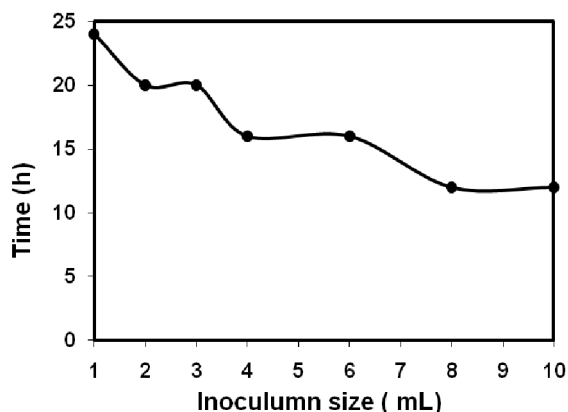


Fig. 3. Effect of Inoculum size on decolourisation of Magenta by *Citrobacter* sp.

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