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Biodecolourization of textile azo dye (methyl orange) by *Pseudomonas sp*. isolated from Aruppukkottai town

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Abstract

Rapid industrialization has necessitated the produce and use of different chemicals in day to day life. Synthetic dyes have been used progressively more in industries because of their simplicity and low cost. Methyl orange is a carcinogenic water soluble azo dye which is widely used in textile industries. Textile dye effluent sample was collected from dying unit, Aruppukkottai town, India. The soil samples were stored in a clean screw cap bottles. The aim of the present research is the isolation of bacterial species from naturally dyes contaminated soil samples which have the ability to decolorize azo dyes used in textile industry dying unit. *Pseudomonas sp*. were isolated and identified biochemically. Our result suggest a great potential for *Pseudomonas sp* used to be remove color from dye waste water.

Keywords: Decolourization, Azo dye, *Pseudomonas*

Introduction

Industrialization has necessitated the use of different chemicals in day to day life. The textile industry extensively uses synthetic chemicals as dyes. Wastewaters from textile industries pose a threat to the environment (Shah *et al*., 2013). Azo dyes (synthetic) have been used increasingly in industries because of their ease and cost-effectiveness in synthesis compared with natural dyes. However, most azo dyes are toxic, carcinogenic and mutagenic, reactive azo dyes the

largest chemical class of dyes with the greatest variety of colors, have been used extensively for textile dyeing and paper painting (Fang *et al*., 2004).

Textile industry is a major user of water, starting from washing raw wool or manmade fiber production up to garment manufacturing with diminishing water resources due to rapid population growth and industrial development. Reuse of industrial waste water and elimination of potential pollutant become more critical (Puvaneswari *et al*., 2006).

Methyl orange is a carcinogenic water soluble azo dye which is widely used in textile industries, in manufacturing printing paper, and in research laboratories. Methyl orange has a deep orange color and its presence in effluents possess an environmental threat (Ma *et al*., 2012). Methyl orange is stable, shows low biodegradability and is soluble in water hence it is difficult to remove from aqueous solutions by common water purification/treatment methods (Suciu *et al*., 2012). Perhaps the most predominant health problems related to dyeing and finishing processes arise from exposure to chemicals acting as irritants. These may cause skin irritation, itchy or blocked noses, sore eyes (Hassan and Nemr, 2017). The effluent even reduces the rate of seed germination and growth of crop plants (Nirmalarani and Janardhanan, 1988). Bioaccumulation of toxicants depends on the availability and persistence of the contaminants in water, food and physicochemical properties of the toxicants (Puvaneswari *et al*., 2006).

The effectiveness of microbial decolorization depends on the adaptability and the activity of selected microorganisms including bacteria, actionomycetes, fungi, yeasts and algae capable of degrading azo dyes (Daneshwar *et al*., 2007). Bacteria could produce azo reductase, showing maximum decolorization activity (Fu and Viraraghavan, 2001). Hence the present investigation "Biodecolourization of textile azo dye (methyl orange) by *Pseudomonas sp*. isolated from Aruppukkottai town effluent soil sample exhibited has been carried out.

Materials and Methods

Sample Collection

Textile dye effluent soil sample was collected from an industry in a dying unit nearby area of 118, main bajar, Vishalachi Street, Aruppukkottai town, Virudhunagar district, Tammilnadu, India.

Preparation of Soil Sample

Soil samples were air dried in shade by spreading on newspaper. They were cleaned by discarding plant residues, gravels, coarse materials, stones and other debris if present. The cleaned soil samples were stored in a clean screw cap bottles.

Isolation of dye decolourizing bacteria

1g of soil sample was inoculated in 100 ml sterile distilled water 250 ml Erleynmeyer Flask. Inoculated flask was incubated in shaker at room temperature for 24 hours. After the incubated solution was ten times diluted with the sterile saline solution and spread on nutrient agar plates, each plate containing dye and incubated at 35 ◦C for 24 hours and decolourization zone in each plate. The colony showing in each plate largest decolourization zone was picked up for further studies (Bayoumi *et al*., 2014).

Maintenance of bacterial isolates

The well growth colonies were picked and purified by streaking. The isolated strains were maintained on nutrient agar slants and stored at 4 ◦C.

Identification of bacteria

The isolated bacterial strains were identified on morphological, biochemical test using protocol given by bergeys manual of determinative bacteriology.

Conical flask assay

Conical flask assay was performed for the detection of decolorizing activity of bacteria. The nutrient broth containing methyl orange was autoclaved at 121 ◦C for 15 minutes. Inoculums of the selected culture showing maximum decolorizing activity was added to nutrient broth flasks containing methyl orange (250 mg/1). The flasks were covered with Aluminum foils and were incubated at 37 ◦C for 3 days. The flasks were observed for decolorization of the azo dye present in the medium (Tripathi and Srivastava, 2011).

Decolourization experiments

The bacterial cultures were transferred to fresh nutrient medium containing methyl orange (250 mg/l) and were incubated at 37 ◦C, under static condition for 5 days. After different time intervals aliquots (5ml) of the culture media were withdrawn, centrifuged at 10,000 g for 10 minutes in a centrifuge to separate the bacterial cell mass. The supernatant was used for analysis of decolorization. The supernatant was used for analysis decolourization. Then different time intervals were measured at the absorbance maximum wavelength for the dye methyl orange (λ max = 465 nm) in the visible region on a Shimadzu double beam spectrophotometer (UV 1601). The percentage of decolorization was calculated from the difference between initial and final values using the following formula:

$$\% \text{ decolourization} = \frac{\text{Initial absorbance} - \text{Final absorbance}}{\text{Initial absorbance}} \times 100$$

The bacterial strain giving maximum decolorization values was selected and used for further decolorization experiments (Tripathi and Srivastava, 2011).

Result and Discussion

Dyes, including many structurally different dyes, are extensively used in the textile industry because of their wide variety of color shades, high wet fastness profiles, ease of application, brilliant colors and minimal energy consumption (Shah *et al*., 2013). Azo dyes, which caused serious environmental pollution, especially in developing countries. Synthetic azo dyestuff is very difficult to remove and biodegrade due to its complex aromatic molecular structure, they are also highly colored, toxic and can heavily contaminate water source (Raffi and Hall, 1997).

The main aim of the present study is the isolation of bacterial species from naturally dyes contaminated soil samples which have the ability to decolorize azo dyes used in textile industry dying unit. Bacterial isolates that showing Gram negative and motility test on motile and methyl red, voges proskauer, and indole production test showing negative results and citrate utilization, oxidase and catalase test showing results on Table 1. Similar results was observed by Han (2012) and Jhon and Anyanwu, (2013).

Table 1: Biochemical characteristics isolated bacterial species

Biochemical test	Results
Methyl ret	Negative
Voges- Proskauer	Negative
Citrate utilization	Positive
Indole production	Negative
Oxidase	Positive
Catalase	Positive

The biodecolourization of Methyl orange was carried out by employing *Pseudomonas* sp. as the test organism. They were exposed to two different viz control experiments viz., control and experiment for 5 days. It is evident that *Pseudomonas* sp. that showing 92% of methyl orange after five days incubation (Table.2).

Table 2: Biodecolourization experiment of methyl orange

Dye	Incubation period (hours)	O.D value at 465 nm	Percentage of decolourization (%)
Methyl orange	Initial value	3.978	Nil
	24	3.311	21
	48	2.767	35
	72	1.535	76
	96	0.814	84
	120	0.512	92

Similar trends for the showed higher decolorization by methyl orange efficiently decolourized in static compared with shaken cultures. The bacterium remarkable colour removal capability over wide range of dye concentration 250 mg/1 and temperature 37 ◦C. the *Pseudomonas sp*. the decolourization rates of methyl orange 10-94% (Shah *et al*., 2013).

In the present study decolourization experiment supernatant was used for analysis decolourization. Then different time intervals were measured at the absorbance maximum wavelength for the dye methyl orange (λ max = 465 nm) in the visible region on a Shimadzu double beam spectrophotometer (UV 1601).

Similar trends decolourization quantitatively analyzed by measuring the absorbance of supernatant using a UV visible spectrophotometer at maximum wavelength λ max 465 nm for methyl orange, was calculated (Saratale *et al*., 2006).

Conclusion

Although decolorization is a challenging process in textile industry, the result of this findings suggest a great potential for *Pseudomonas sp* used to be remove color from dye waste water. The bacterial strain *Pseudomonas sp*. showed decolourizing activity through a degradation mechanism rather than adsorption. This observation has established that the *Pseudomonas sp*. is adaptive in nature and can degrade contaminants.

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