Biodegradation of Azo Dyes by Using Soil Bacteria

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Abstract

Azo dyes are used in different industries such as food, textile, printing and pharmaceuticals. After using the dye, the remaining effluents get mixed with fresh water causing pollution. Biodegradation is a green friendly method used for the degradation of azo dyes. In this study, the degradation of azo dyes was carried out by using Bacillus species that were isolated from the soil obtained from the textile industry. Biochemical tests were carried out and identified as Bacillus species. Spectrophotometry analysis was carried out at 480 nm (methyl red) and 420 nm (orange dye) for analysis of dye degradation. The degradation percentage of methyl red dye (78.57%) and orange dye (47.59%) were compared. This study shows that the degradation percentage of methyl red was greater than orange dye by bacillus species screened from the textile area soil sample.

***Key words**: Biodegradation, bacillus species, spectrophotometer, azo dye*

1. Introduction

Azo dye is a type of synthetic organic dye that contains nitrogen as the azo group . The structures of azo dyes consists coupling of diazotized amine with either an amine or a phenol and also contain azo linkage(s) [2]. More than half the commercial dyes belong to this class. Azo dyes are used for colouring many different materials such as textile, leather, plastics, food, and pharmaceuticals and for manufacturing paints and for printing purposes [3]. Most of the azo dyes are toxic, carcinogenic, and mutagenic [1] and potentially toxic to aquatic life [8].

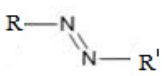

Figure 1: General structure of Azo dye

Removal of colour from dye bearing wastewater is a complex problem because of difficulty in treating such waste waters by conventional treatment methods [7]. Without adequate treatment these, dyes will remain in the environment for an extended period of time [10].

Microbialdegradation of pollutantshas intensified in recent years as humans strive to find sustainable ways to clean up contaminated environments [4]. Biodegradation is nature's way of recycling wastes, or breaking down organic matter into nutrients that can be used by other organisms. "Degradation" means decay, and the "bio-" prefix means that the decay is carried out by a huge assortment of bacteria, fungi, insects, worms, and other organisms that eat dead material and recycle it into new forms. The term is often used in relation to ecology, waste management, biomedicine, and the natural environment and is now commonly associated with environmentally friendly products that are capable of decomposing back into natural elements. The objective of the present work is to isolate and identify azo dye degrading mechanisms and to examine the percentage of degradation level of textile dye effluent by the microorganisms.

In most cases, bacteria disintegrate azo bonds of the dyes, which result in the formation of colourless amines and subsequently simpler compounds [11]. The elimination of a higher range of pollutants is an important need for the development of a better environment. Bacteria offers a cheaper and environment friendlier alternative for colour removal in textile effluents [10].

2. Materials and methods

2.1. Azo dyes

The Azo dyes used are methyl red dye and orange dye.

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2.2. Collection of soil sample

The industrial effluent soil samples were collected from an industry in Bangalore, Karnataka, India. The samples were collected and subjected to various microbiological studies.

2.3. Culture media

Minimal essential media was used for the isolation of dye degrading bacterial species. The media consists of 1g of Agar, 0.15g of potassium dihydrogen phosphate, 0.3g of Disodium hydrogen phosphate, 0.25g of sodium chloride, 0.1g of ammonium chloride.

2.4. Isolation of dye degrading bacteria

Pour plate technique was used for the isolation of dye decolourizing bacteria from dye effluent contaminated soil. Effluent samples were enriched by co-incubating in nutrient broth containing 0.1g of each dye at 37^{o}C for 24 hours. A minute volume of each enrichment culture was plated onto nutrient agar medium supplemented with 0.1g of respective dye. After incubation at 35^{o}C for 24 hours, the resulting bacterial colonies exhibiting clear zone around them were isolated on the basis of colony morphology and dimension of clear zone.

2.5. Physical and biochemical characterisation

The selected isolates were screened for their morphological properties and staining properties. The isolate was gram stained. For biochemical characterisation the isolate was subjected to Voges–proskauer, methyl red, indole, catalase and citrate utilization test.

2.6. Dye degradation assay

The dye degradation assay was initiated by incubating 5% (v/v) of the culture to the decolourization medium at 37^{o}C and 500 rpm. At regular intervals of 1st day, 2nd day, 3rd day, 4th day and 5th day, the culture was withdrawn, centrifuged at 5000rpm for 10 minutes. The supernatant was read by spectrophotometer at wavelength of 480 nm for Methyl red dye and 420 nm for Orange dye. Two control flasks (Dye + medium without inoculums and medium with inoculums without dye) were maintained. The percentage of dye degradation was calculated using the following equation,

$$\% \text{ of dye degradation} = \frac{\text{O.D (Initial)} - \text{O.D (Final)}}{\text{O.D (Initial)}} \times 100$$

3. Results and discussion

3.1. Isolation and identification of the bacteria

A number of azo dyes including reactive dyes are used in textile dying industry. This leads to effluent streams containing intense colour due to the presence of these dyes. Therefore, the removal of azo dyes from effluents is important. Both physicochemical and biological methods for removal of dyes have been studied. Keeping this in view, the textile dye effluents examined in this study appeared to harbour a diverse community of microorganisms.

Biodegradation of commercially available textile dyes namely methyl red dye and orange dye were studied against bacterial isolates which have been isolated from the dye effluent sample by pour plate method. This finding in this study is similar to some other previous studies [9].Of all the 5 isolates tested, 3 isolates were further screened for dye degradation and finally one bacterial isolate was selected on the basis of dye tolerance. The selected bacterial isolates were investigated for their morphological, physiological and biochemical features and the bacterial strains were identified as Bacillus species.

Table 1: Morphological, physiological and Bio chemical characteristics of the bacterial isolate

Sl.No	Characteristics	Result
1	Gram's staining	Rod shaped purple-blue colour
2	Indole test	cherry red colour ring formation
3	Methyl red test	bright red colour formation
4	Voges–proskauer test	formation of pink colour
5	Citrate utilization test	blue colour formation
6	Catalase test	Bubbles formation

3.2. Dyes degradation assay

Decolourization of azo dyes by bacteria has been reported by many earlier research workers [5][6]. The degradation study was carried out for 5 days and the percentage decolourization was monitored every alternate day. The maximum absorption wavelength for the selected dye was

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found to be Methyl red dye (480nm) and Orange dye (420nm). The above wavelength was taken for carrying out the photometric analysis of the culture samples. The blank value determined by using the minimal essential media with dye and without microorganism forMethyl red dye and Orange dye was 1.512 and 1.103 respectively. The absorbance values were determined for both the dyes up to 5 days and the values have been shown in Table 2. There was a gradual decrease in the absorbance values of both the Methyl red dye and Orange dye (Figure 2).

Table 2: O.D. value for degraded dyes

O.D. value		
Day	**Methyl red dye**	**Orange dye**
1st day	0.812	0.992
2nd day	0.681	0.876
3rd day	0.524	0.792
4th day	0.391	0.682
5th day	0.324	0.578

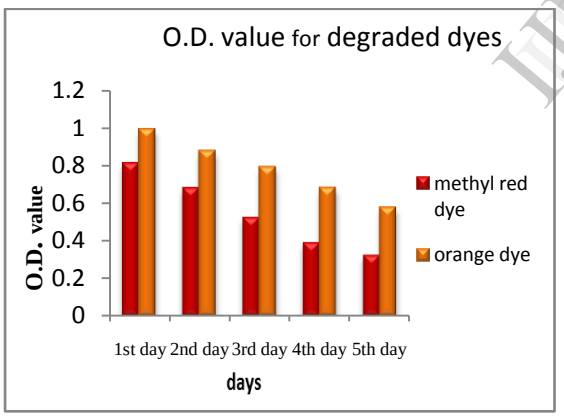

Figure 2: O.D value for degraded dyes

The percentage of dye degradation for Methyl red dye and Orange dye by the bacteria were calculated and the values were shown in Table 3. It was found that the isolated bacteria were efficient decolourizers of Methyl Red (78.57%) and Orange dye (47.59%). The comparison of dye degradation percentage of Bacillus sp. is shown in Figure 3.

Table 3: Percentage of azo dye degradation

Percentage of degradation		
Day	Methyl red dye	Orange dye
1st day	46.29	10.06
2nd day	54.96	20.58
3rd day	65.34	28.19
4th day	74.14	38.16
5th day	78.57	47.59

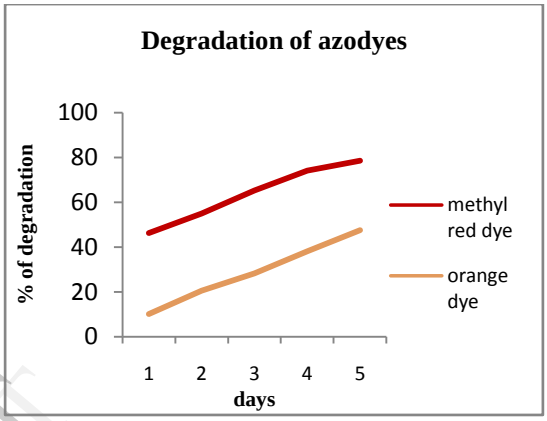

Figure 3: Degradation percentage of azo dyes

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4. Conclusion

Azo dyes are the largest and most widely used class of dyes, accounting for 50% of the dyes produced annually. Detoxification and disposal of sludge is a problem to textile dye units. Microbial treatment which involves enzymatic process was very promising for the degradation and detoxification of azo dyes. In the current study, the dyes which were used were Methyl red dye and Orange dye. Of these two dyes, the maximum degradation was observed in Methyl red dye. The percentage of dye degradation was about 78.57% for methyl red dye and 47.59% for Orange dye. Further, this study confirms that Methyl red dye was degraded more than the Orange dye by *Bacillus* species. It shows the ability of *Bacillus* species in degrading azo dyes present in the industrial effluents which are polluting aquatic life as well as equally harmful to human life and animals.

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