Confirmatory Analysis of Biodegradation of Textile Azo Dye-Blue S Using Bacterial Isolates

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Abstract- *The textile dyes are wide in range with different colour shades which are very difficult to remove from the environment. The bacterial treatment is most successful in treating dye samples and effectively degrades them. Azo dye Blue S was selected for the biodegradation study using bacterial isolates such as Bacillus sp and E coli. Under optimized conditions, the dye was completely (100%) degraded by E coli and 96% degradation was found with Bacillus sp. The degradation was monitored and confirmed through various standard methods includes by UV-Vis, TLC, HPLC, zone of inhibition and phyto toxicity analysis. The bacterial treatment of textile azo dyes was found to be very effective in 24 hours and the process was found to be complete removal of colour and toxicity of the dye.*

Keywords- Biodegradation, HPLC, Phyto toxicity, TLC and toxicity.

I. INTRODUCTION

Wastewaters generated by textile industries are known to contain large amounts of toxic aromatic compounds, especially azo dyes [1]. It is well known that some azo dyes and their degradation products, such as aromatic amines, are highly carcinogenic [20]. Textile mill effluents are also characterized by high level of color caused by residual dyes [2].

The colored waste water released into the ecosystem is also a dramatic source of aesthetic pollution and perturbation in the aquatic life. The effluent must be treated before discharged into environment because of their recalcitrant nature and potential toxicity to animals and human. Dyes also obstruct light penetration and oxygen transfer that affects water bodies Biological treatment offers a cheaper and environment friendly alternative to dye decolorization and wastewater reutilization in industrial process [4, 9]

The general approach for bioremediation of textile effluent is to improve the natural degradation capacity of the indigenous microorganism that allows degradation and mineralization of dyes with a low environmental impact and without using potentially toxic chemical substances, under optimum conditions [6]. The present work deals with the bacterial degradation of azo dye at optimum conditions and various standard confirmatory analysis methods were performed to proof the complete and effective degradation by the bacterial isolates [8, 16, 17].

II. MATERIALS AND METHODS

Sample Preparation

The dye incorporated MSM broth was inoculated with the isolates and incubated under optimized conditions. After the incubation the broth was then centrifuged at 10,000 rpm for 20minutes [13]. Then the cells free supernatant was collected and used for following confirmatory analysis methods of dye degradation [21].

TLC

According to Krishnakumari and Thangavel (2017) and Barathi *et al*., (2015), TLC was performed. The supernatant was extracted with equal volume of methanol and concentrated with anhydrous Na_2SO_4 and spotted on silica gel coated TLC plate using with mobile phase solvent system of methanol, toluene, acetic acid and ethyl acetate (6:2:1:1) and results were observed under UV illuminator.

HPLC

HPLC analysis (Joshi *et al*., 2015; Roat *et al*., 2016) was carried out on C 18 reversed phase column (RPC – 18 phenomenex) equipped with dual wave length detector by the isocratic method. The mobile phase was methanol with a flow rate of 0.75 mL/min and having 10 min run time.

Zone of inhibition assay

Sterile MH agar plates were prepared and spreaded with soil bacteria such as *Bacillus sp, E coli, Pseudomonas sp and Staphylococcus sp* [14]. The plates were created with wells and filled with the bacterial treated dye sample and incubated at 37^0C for 24 hours and observed for zone of inhibition.

Phyto toxicity - Seed Germination Assay

The phytotoxicity assay was carried out [12] using two different seeds of *Eleusine coracana* (Finger millet) and *Cicer aritinum* (Chick pea). The test samples were sprayed on the seeds for 7 days in frequent intervals. The germination of the seeds was calculated by measuring the length of the root and the shoot formed. Germination of seeds was observed and the length of shoot and roots were measured after the incubation.

III. RESULTS

Thin Layer Chromatography (TLC)

The Rf value of untreated dye samples were found to be less than the treated samples. Maximum Rf value was found as 5.8. 5.0 and 3.5 cm with *E coli* treated dye sample when compared to *Bacillus sp* treated sample (2.4,2.1 and 1.8cm) and it confirmed that the biodegradation of dye and the bacterial isolates was found to be effective in degrading the azo dye.

HPLC

HPLC analysis of untreated dye shows 3 major peaks at RT 2.50, 4.415 and 6.020 seconds with some minor peaks and the treated dye chromatogram shows the disappearance of the major peaks at 4.415 and 6.020 and also certain new peaks with different RT as 2.677, 3.140 and 3.594, which confirmed that the biodegradation of azo dye by the both bacterial isolates were complete and effective.

Zone of inhibition assay

No zone of inhibition was found against all the test cultures indicates the complete detoxification of the dyes**.**

Phyto toxicity - Seed Germination Assay

The *Bacillus sp* and *E coli* respectively treated dye samples were supports the germination of seeds with shoot length 1.3cm and 1.6cm. The root length was found as 0.9cm and 0.8.

IV. DISCUSSION

The prepared TLC plates were spotted with bacterial treated dyes along with the untreated dyes [4, 13 and 14]. The plates were observed for the band formations [8, 11]. Additional bands were observed in treated samples when compared to the untreated dye sample which indicates the biodegradation of the dye samples by the bacterial isolates [6, 19]. The bands were found with different Rf values which indicates the ability of the isolates to the breaking down of the dye molecule into simpler compounds and strongly confirmed as the degradation of dye molecules [2, 3]. Similar to TLC method, HPLC technique was performed as confirmatory analysis of the dye degradation [5, 15]. The HPLC method was performed and the results were found as appearance of new peaks and disappearance of certain peaks indicates the biodegradation of the dye molecules by the isolates [1, 7]. The significant change in the position of peaks with varying RT found in treated samples when compared to the (control) untreated dye chromatogram, confirming the biodegradation of dyes has occurred [4, 10]. The efficiency of the biodegradation process of the dye and effluent samples were analyzed through the zone of inhibition assay to confirm the detoxification of dyes [13, 21]. The positive control (water) showed the better germination of the seeds with 0.8cm -3.1cm range, root and 2.2cm - 5.5cm range of shoot length [16, 17, 20]. The untreated dye and effluent samples completely inhibits the germination of the seeds thus indicates the presences of toxicity nature of the untreated samples [9, 12, 18].

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