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AZO Dye Degrading Bacteria Isolated From Cow Dung in Trichy Districrt, Tamilnadu, South India

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Abstract: *Dye degrading efficiency of bacteria isolated from Cow dung in Trichy District. Four different bacterial strains Enterobacter sp., Klebsiella sp., Serratia sp., and Bacillus sp. were isolated. The dye degrading efficiency was ranging between 24.03% to 61.52%. As the days of incubation were increasing from 1 to 7 days the degradation efficiency was also increasing to a greater extent. When compared to all the bacterial isolates Bacillus sp. is a highly promising agent, which could act as an efficient dye degrader. Because, from the first day onwards, the degradation was high (34.10%), and after that, at every day incubation the degradation was increasing profoundly. 46.31%, 54.26% and to a maximum of 98.44% at the end of 7 days. Almost hundred % of degradation was effected by this isolate. The entire cell Supernatants also showed equal dye degrading ability. FT –IR, HPLC results confirmed that, the complex, toxic azo dyes are degraded in to simple, non toxic compounds. Due to the removal or the complete degradation the environment is protected from Azo dyes.*

Keywords: *Bacteria, Azo dye, Degradation, FT –IR and HPLC*

INTRODUCTION

Synthetic dyes are colouring agents mainly used in textile industries which generate a huge amount of wastewater in the process of dyeing. Different dyes used in textile industry usually have a synthetic origin and complex aromatic molecular structures which make them more stable and more difficult to be biodegraded. Due to their ease of manufacturing methodology, azo dye statement for almost 82% of annual production of commercial dyes all over the world. Dyes are coloring pigment that imparts color to the substrate when they are in solution form. Dyes are derived synthetically from raw materials like hydrocarbons, benzene, toluene, naphthalene and anthracene using coal tar obtained from distillation of coal. Both organic and inorganic materials are needed to make dyes and intermediates (Patovirta *et al.,* 2003). The raw material sequence for making dye is petroleum to hydrocarbons to intermediates to dyes. Textile dyes effluents have toxic effect on the germination rates and biomass concentration of several plant species which play many important ecological functions such as providing the habitat for wildlife: protecting soil from erosion and providing bulk of organic matter that is significant to soil fertility. The toxicity of effluent is because of the presence of dye or its degraded products which are mutagenic or carcinogenic. Therefore, the treatment of industrial effluents contaminated with dye becomes necessary prior to their final discharged to the environment (Elisangela *et al.,* 2009). Biological processes provide an alternative to existing technologies because they are more cost-effective, environmentally friendly, and do not produce large quantities of sludge. biodegradation by microorganisms is a promising approach for treating dyes contained in wastes. The effectiveness of decolorization depends on the adaptability and the activity of selected microbes. In the present study was to investigate degradation of Azo dye by bacteria isolated from cow dung.

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MATERIALS AND METHODS

Sample Collection

Fresh Cow dung was collected from Kuzhumani, Trichy district, Tamilnadu, South India. The sample was collected with sterilized spatula and kept in sterile polyethylene bags. The samples were transported to the laboratory in an icebox and processed within 6hrs of collection.

Isolation of Bacteria

Nutrient agar medium was prepared and autoclaved. Then the medium was poured into sterile petri plates. The collected cow dung sample was diluted up to 10^{-6} and 0.1 ml of the diluted samples was spread over the agar plates. The plate was incubated at 37°C for 24 Hrs. After incubation the colonies was purified by using streak plate method then the bacterial cultures were stored at 4°C for further investigation (Teo *et al*., 2011).

Characterization of Bacteria

Selected individual colonies from nutrient agar were subjected to microscopic observations of gram staining, motility test and biochemical tests (indole, MR-VP, Citrate utilization, Urea production, Triple sugar iron and oxidase test) for further identification (Shrivastava and Mishra, 2011).

Collection of Dye

The dye sample was graded and supplied by the dealers of global dyes, Tiruppur, Tamilnadu, and South India. Azo dye used in this study is reactive 2B (λm= 5m).

Bacterial in Coulum Preparation

All the isolates cultures were inoculated in Nutrient broth and incubated for 24 hrs. 18 – 24 hrs fresh cultures were used. Fresh cultures were prepared for every time (Chang and. Lin, 2001).

Dye Decolorization (Waghmode *et al.,* 2011a)

Dye decolorization were carried out in 500 ml flask containing 250ppm of azo dyes in 250 ml of C-limited Czapek-Dox broth. The pH was adjusted to 7.3± 0.2 using sodium hydroxide and hydrochloric acid solution. Then, the flasks were autoclaved at 121^{O}C for 15minutes. The autoclaved flasks were inoculated with 1ml of all the bacterial inoculums separately. The flasks were kept in a mechanical shaker at room temperature for 7-15 days. Samples were drawn at 24 hrs intervals for observation. 10ml of the dyes solution was filtered and centrifuged at 5000rpm for 20minutes. Decolorization was assessed by measuring absorbance of the supernatant with the help of UV spectrophotometer at wavelength maxima (□m□) of respective dye (580nm).

Assay on the dye degrading Efficiency of supernatants

From 24 Hrs broth cultures, the supernatant was collected by the following procedure. 10ml of the culture was centrifuged at 10000 rpm for 30 minutes. The supernatant was collected separately, pellet was discarded. The supernatants of all the cultures were utilized separately for the dye degradation analysis (Waghmode *et al.,* 2011a).

FT-IR spectral analysis

The samples were dried and grinded with KBr pellets and analyzed on a Shimadzu FT-IR Affinity model in the diffuse reflectance mode operating at a resolution of 4 cm−1 (Vaijayanthi *et al.,* 2012).

HPLC ANALYSIS

Reverse phase HPLC (Cyberlab, USA) analysis was carried out in a C 18 Column (250 mm X 4.6 mm) version (Lake Forest, CA, USA) equipped with a C18 guard column. The compounds were eluted with an isocratic elution of Acetonitrile vs water at the flow rate of 1 mL/ min & absorbance recorded at 680 nm (Baiocchi *et al*., 2002).

RESULT AND DISCUSSION

In the present study cow dung sample was collected from Trichy district, Tamilnadu, South India A total of 4 different bacterial isolates were isolated from the cow dung sample of trichy district, and they were characterized based on the morphological, biochemical and cultural properties. Morphologically, all the isolates were rod shaped and 3 is motile and one is non motile bacteria. Among the 4 isolates 3 isolates were identified as Gram negative and one identified as Gram positive bacteria. Biochemically, indole was positive by 3 isolates and 1 isolate is negative followed by citrate utilization. Whereas, the

results of other biochemical tests were varied between the isolates. Among 4 bacteria, each one isolate was belonged to *Enterobacter* sp., *Klebsiella* sp., *Serratia* sp. and *Bacillus* sp. (Table-1). Earlier studies, Shunyao *et al*., 2017 was identified E2-degradating bacterium E2S was isolated from the activated sludge of a STP in Nanjing, China. E2S is rod shaped with a size of 0.5 μm × 1.8 μm and lacks a flagellum. The analysis of the physiological and biochemical characteristics of E2S show that it is gram negative; it lacks the ability to produce indol or H2S, hydrolyze starch, use sodium citrate, decompose glucose to produce pyruvate, or hydrolyze gelatin. By contrast, the glucose fermentation, nitrate reductase and methyl red test were positive. Similarly, Sharma *et al.,*(2009)., Waghmode *et al*.,2012 stated that the decolorization of textile dye waste water is a major aspect of research , to solve the problem of environmental pollution.

Dye degrading efficiency of bacteria was assayed against dye. At every 24 Hrs intervals sample was derived and analyzed spectrophotometrically. The dye degrading efficiency was ranging between 24.03% to 61.52%. As the days of incubation were increasing from 1 to 7 days the degradation efficiency was also increasing to a greater extent. The bacterial isolates *Bacillus* sp., 78.44% (Table-2) followed by *Serratia* sp., 65.11%, (Table-3) *Klebsiella* sp., 62.9% (Table-4) and *Enterobacter* (61.52%). When compared to all the bacterial isolates *Bacillus sp* is a highly promising agent, which could act as an efficient dye degrader. Because, from the first day onwards, the degradation was high (34.10%), and after that, at every day incubation the degradation was increasing profoundly.*i.e* 46.31%, 54.26%. To a maximum of 78.44% at the end of 7 days (Table-5). Previously, Aldoury *et al.,* (2014) reported that when *plicatilis* was cultured in malt extract medium containing 50mg/L RB 19, the percentage decolorization increased over time, reaching a peak of 99% decolorization after 15 days of incubation. similarly, Elisangela *et al.,* 2009 studied that *pleurotus* sp. it is capable of efficient decolorization of a wide range of chemically different dyes. In this study the dye degradation which could be available in the supernatant was also subjected for the degradation of dye. All the supernatants showed good results similar to that of whole bacterial cells the result was given in fig-1. *Bacillus* sp., is a dominant isolates of dye degradation when compared to other isolates so, it was selected for FT-IR and HPLC analysis. Many authours reported the similar result such as Chen *et al*., 2005; Modi *et al.,* 2010; Phugare *et al.,* 2011 and Lade *et al.,* 2015).

Table 1: Characterizations of cow dung bacteria

Name of the test	***Enterobacter***	***Klebsiella***	***Serratia***	***Bacillus sp***
Gram staining	-	-	-	+
Shape	Rods	Rods	Rods	Rods
Motility	+	-	+	+
Indole	+	+	-	+
Methyl red	+	-	+	-
Voges proskaur	-	+	+	+
Citrate utilization	-	+	+	-
TSI (H_2S)	acidic	-	-	acidic
Oxidase	-	+	-	+
Urease	+	-	-	+

Table 2: Dye Degrading Efficiency of *Enterobacter* sp.

Days of Incubation	**Control(OD)**	**OD (590 nm)**	**Degradation Efficiency (%)**
1	1.29	0.98	24.03
2	1.29	0..93	30.83
3	1.29	0.88	33.09
4	1.29	0.83	40.63
5	1.29	0.76	42.10
6	1.29	0.66	48.83
7	1.29	0.59	61.52

Table 3: Dye Degrading Efficiency of *Klebsiella pneumoniae*

Days of Incubation	Control(OD)	OD (590 nm)	Degradation Efficiency (%)
1	1.29	0.93	33.09
2	1.29	0.86	33.33
3	1.29	0..82	36.43
4	1.29	0.79	43.42
5	1.29	0.72	44.18
6	1.29	0.68	47.28
7	1.29	0.56	62.90

Table 4: Dye Degrading Efficiency of *Serratia* sp.,

Days of Incubation	Control(OD)	OD (590 nm)	Degradation Efficiency (%)
1	1.29	0.88	33.09
2	1.29	0.83	35.56
3	1.29	0..79	38.75
4	1.29	0.76	43.42
5	1.29	0.71	46.31
6	1.29	0.60	53.48
7	1.29	0.45	65.11

Table 5: Dye Degrading Efficiency of *Bacillus sp*

Days of Incubation	Control	OD (590nm)	Degradation Efficiency (%)
1	1.29	0.99	23.25
2	1.29	0.93	33.09
3	1.29	0..82	36.43
4	1.29	0.74	65.11
5	1.29	0.68	47.28
6	1.29	0.61	52.71
7	1.29	0.53	**58.9**

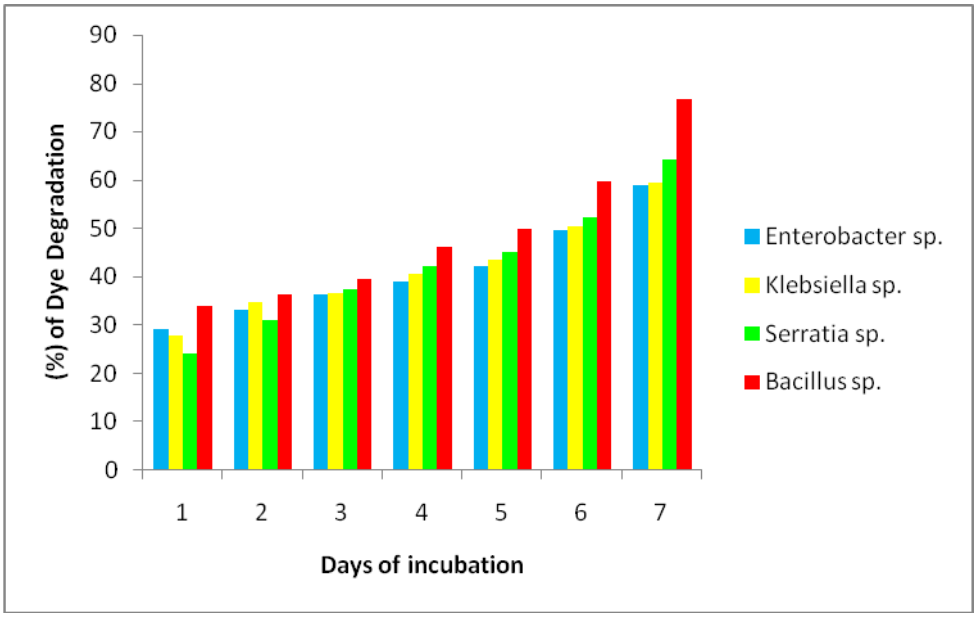

Figure 1: Dye degradation of bacterial supernatant

FTIR spectrum of control and samples obtained after decolourization of both dyes showed various peaks. A new peak at 1384 cm-1 represented –N=N- stretching vibration. The C- H deformation showed at 1112 cm-1. The peak at 1384 cm-1 showed N-H stretching vibration. . The significant change in the FTIR spectrum of metabolites compared to control spectrum suggest the biotransformation of complex dyes present in the mixture into simple form. The FTIR spectrum of control Dye 6 displays peak at 3449 for intramolecular hydrogen bonding and O-H stretching. Peaks in the control dye spectrum represented symmetric stretching at 1384 cm-1and asymmetric stretching at 1114 cm-1for C–N. C–N

stretching at 1637 cm- 1represented nature of aromatic amine group present in parent dye; 3449 cm-1and 2075 cm- 1represented the presence of free NH group of parent dye. Whereas peak at 1637 cm-1represented –N=N– stretching of azo group. In degraded extracted metabolites, a new peak at 435 cm-1represented C–H deformation of alicyclic CH2 whereas a peak at 685 cm-1was observed for substituted anilines fig 2. Zhou (2002), reported that, the sample treated with both *E. cloacae* and *H. alvei* obtained a new peak with 2.61 RT along with 2 other peaks with similar RT as those observed in case of two previously mentioned treatments. Comparison between the polarities of the samples showed that the control and *H. alvei* treated samples have the peak with shortest RT 1.98. From the retention times, it can be concluded that the control has the highest polarity. Polarity refers to the number of functional groups in the dye Zhou H, (2002). Highest retention time of 2.6 and thus the lowest polarity was obtained by the sample treated with the two bacterial isolates. All the treated samples showed peaks almost similar to that of control. But the peak with RT 2.94 did not appear in any of the treated samples.

In this present study HPLC analysis of control azo dye and *Bacillus* sp degraded sample of dye were depicted in fig 5. The absorption spectra of the samples obtained at 680 nm are presented in. The HPLC elution profile of the Azo dye (Control) showed 5 peaks with retention time (RT) of 1.98, 2.18, 2.37, 2.59 and 2.94 minutes. The elution profile obtained for the bacteria treated samples significantly differed from the control in terms of number, height of peaks obtained and RT. The HPLC profile of azo dye treated with bacterial isolate *Bacillu Sp* showed 3 peaks with RT 1.98, 2.18 & 2.39. Kalyani *et al*., 2008 reported the HPLC analysis of control dye showed the presence of one major peak at retention time of 2.702 min and three minor peaks at retention times of 2.125, 2.801, and 3.394 min fig 4. After the dye decolorization process, the disappearance of peaks as seen in case of the control and the formation of completely different three major peaks at retention times of 2.521, 3.241, and 3.564 min and two minor peaks at retention time of 3.123 and 3.910 min were observed.

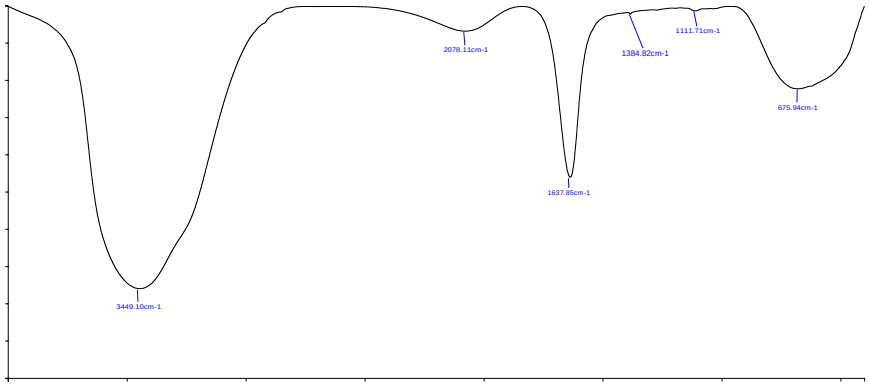

Figure 2: FTIR analysis of dye (control)

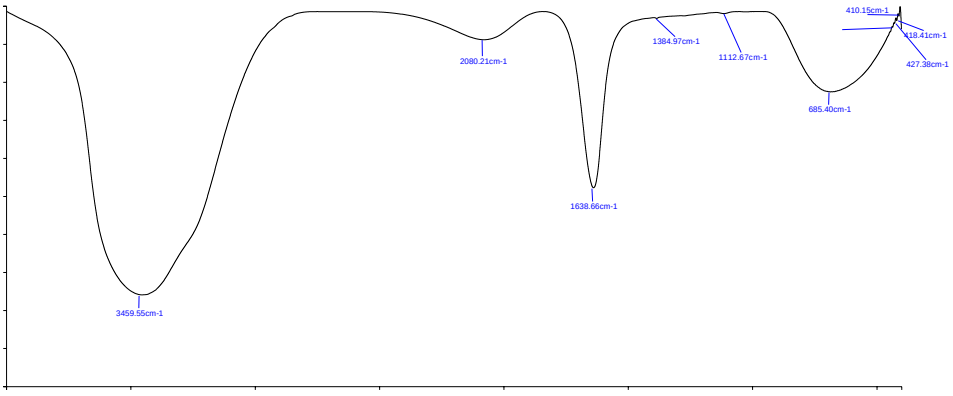

Figure 3: FTIR Analysis of Azo dye degradation by *Bacillus* sp.

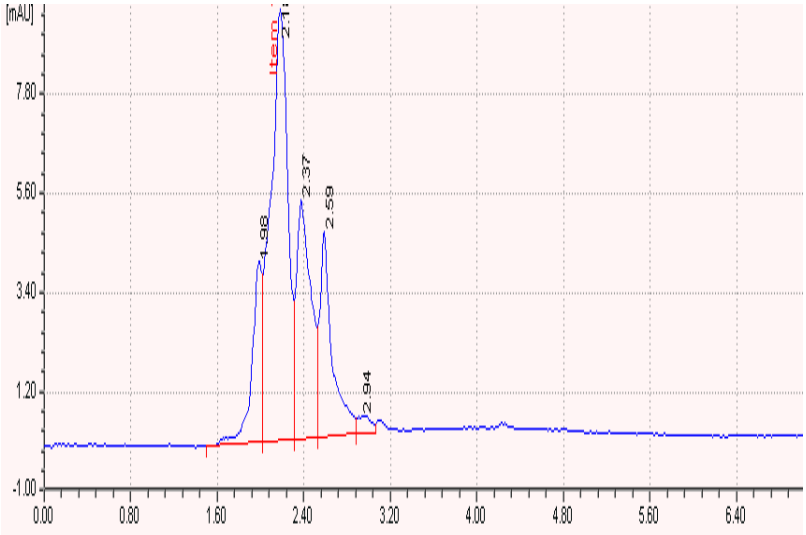

Figure 4: HPLC chromatogram of Azo dye (Control)

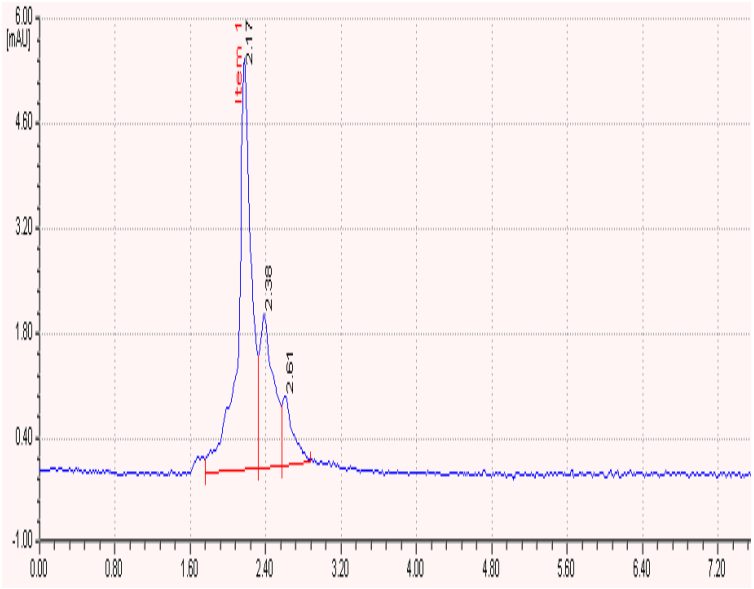

Figure 5: HPLC chromatogram of Azo dye degraded by the *Bacillus* Sp.

CONCLUSION

To conclude, the complex chemical azo dyes could be degraded by the whole cell, and supernatant of *Bacillus sp* isolated from Cow dung. Similarly, instead of using hazardous, time consuming and costly chemicals, we could protect our environment from dyes by using these types of natural bacterial isolates and their supernatant.

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