RESEARCH PAPER | BIOTECHNOLOGY | Volume : 6 | Issue : 3 | March 2016 | ISSN - 2249-555X | IF : 3.919 | IC Value : 74.50

Biodegradation of Congo Red, a synthetic dye by bacterial species and its bioremediation potential using seed germination efficiency.

KEYWORDS: Phytotoxicity, Congo Red, Vigna radiata, Cicer Arietinum

Shweta Nakul
Department of Biotechnology, Govt. Holkar Science College, Indore.

Gunjan Singh
Department of Biotechnology, Govt. Holkar Science College, Indore

ABSTRACT *To decolourize congo red dye by soil bacterial isolates, Ecoli and streptococcus species. Soil sample collected from dyeing unit, Collectorate, Indore District, Madhya Pradesh for screening Congo red dye degrading bacterial isolates. Two bacterial isolates IS-1 and IS- 2 screened based on their ability to decolorize the Congo red , 1S-1 decolorized up to 94% followed by IS-2 with 91%. Both isolates were identified as IS-1 is E.coli species and IS-2 is streptococci species after microscopic and biochemical characteristics. Phytotoxicity test performed on two crop plants Vigna radiata and Cicer Arietinum for 10days. With phytotoxicity test, found that dye is toxic to the growth of agricultural crops, can treat polluted dye soil through micro-organisms, identified as a cost effective and environment friendly.*

Introduction:
Ever since the beginning of mankind, people have been using colorants for painting and dyeing their surroundings, their skins and their clothes. Now there are more than 1, 00,000 commercially available dyes whilst over 7 x 105 metric tons of dyestuffs are produced annually (Wong and Yu, 1999). Dyes are used in textile industry, leather tanning industry, paper production, food technology, agricultural research, light-harvesting arrays, photo electrochemical cells, hair coloring and cosmetics Color present in the dye effluent gives a straight forward indication of water being polluted (Nigam et al., 1996). Waste water from textile industry is a complex mixture of many polluting substances ranging from organochloride based waste pesticides to heavy metals associated with dyes and dyeing process (Correia *et al.*, 1994). Toxic compounds from dye effluent get into aquatic organisms, pass through the food chain and ultimately reach humans, leading to various physiological disorders like hypertension, sporadic fever, renal damage, cramps, etc., Plant growth parameters namely germination percentage, seedling survival and seedling height have been taken as criteria to assess plant response to specific pollutants. Dyes used are considered as carcinogenic and mutagenic and the effluents reduce the rate of 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 physico-chemical properties of the toxicants (Puvaneswari et al., 2006). Bioremediation of textile effluents has been of considerable significance since it is inexpensive, eco-friendly. Considering the advantages and potential applications of bioremediation processes, the present investigation targets on the bioremediation of Congo red, a synthetic azo dye by a Bacterial species isolated from dyeing unit soil.

Materials and Methods:
Soil sample was collected from dye unit of Collect rate, Indore District, Madhya Pradesh for screening dye degrading bacterial isolates.A common dye used in textile industries; Congo red was selected based on its usage in clothes.

Methodology
Screening of Dye-Decolourizing Bacteria:
Dyeing unit soil sample are used to isolate bacteria having potential to degrade congo red dye. Nutrient broth having 0.01% Congo red dye prepared and add serial dilutions of soil sample, then tubes are incubated at 37°C for 24-96 hours. . Following incubation, the resulting tubes exhibiting decolourizaion .The tube showing more decolourization is choosen for isolating bacteria and was plated on to nutrient agar medium supplemented with 0.01% congo red dye.

Identification of selected isolates
The selected isolates were examined for their morphological properties, such as size, Shape, cell arrangement and gram staining . Cultural properties including form, color, elevation, margin, surface of colonies on nutrient agar plate and slant were also recorded. Physiological and biochemical characteristics of the isolates were evaluated by, methyl red test, indole test, catalase test. The following selective media MacConkey agar, EMB agar and blood agar medium are used to grow and identify bacterial isolates. The isolates were identified up to species level based on comparative analysis of the observed characteristics with the standard description of bacterial strains in Bergey's Manual of Determinative Bacteriology.

Dye Decolourization Assay:
Bacterial Inoculums was prepared by incubating loopful bacterial suspension in nutrient broth containing 0.01%congo red dye for 24- 72 hours at 37°C. At defined intervals of 2nd, 3rd, 4th and 5th day, the culture was withdrawn, centrifuged at 8000g at 10°C. for 15 min, and the supernatant was examined for absorbance at 540 nm under visible light in a\spectrophotometer (UV-VIS spectrophotometer). The extent of decolourization was expressed as percent (%) decolourization and estimated as

Decolourization (%) D = [A0-A1)/A0] x 100
Where, D= decolourization in %, A0= initial absorbance, A1= final absorbance

Volume : 6 | Issue : 3 | March 2016 | ISSN - 2249-555X | IF : 3.919 | IC Value : 74.50

Bioassay for dye toxicity/ phytotoxicity: Seed germination test (Durve *et al.*, 2012)

In this experiment, the effect of congo red dye at the concentration of 0.01 gm was evaluated on germination of seeds of two crops, chana (Cicer Arietinum) and Moong (*Vigna radiata*). The seeds were germinated in disposable flasks containing 10 g of field soil .Different sets of 10 seeds of both vigna radiata and cicer arietinum were treated every 24 hours with 10 ml of dye solution and degraded product of Congo red dye by bacterial isolates and tap water (Control) separately. All pots were kept under shade near sunlight for the period of 10days. Germination of seeds treated with dye and degraded dye solutions was calculated after comparing with control. At the end of the germination experiment, the shoot length and root length of seedlings was measured separately for dye, degraded dye product and control.

Result and Discussion

Isolation, screening and identification of dye degrading bacteria

Two isolates were obtained after screening dye degrading organisms from soil. All the isolates

were evaluated for their dye decolorizing ability. The two isolates, IS-1and IS-2showed decolourization percentage of 94% and 91% at 72 hrs respectively. Based on microscopic, morphological and biochemical parameters, the two dye decolorizing isolates (IS-1and IS-2) were identified as *Escherichia coli* sp and *streptococci* sp.

Time course of dye decolourization

Under this optimized conditions the time course of dye decolourization experiment was carried out using the two isolates. We examine the dye decolourization after every 12hrs and found that at 72 hours incubation, we get maximum decolourization percentage for both the bacterial isolates. . In this study of two Bacterial isolates ,Ecoli Species shows more decolourization percentage(94% at 72 hrs) than Streptococci species(91% at 72 hrs) and having more potential for congo red dye degradation .After 96 hours both the bacterial isolates showed no further decolourization and it becomes stable .(Figure-1).

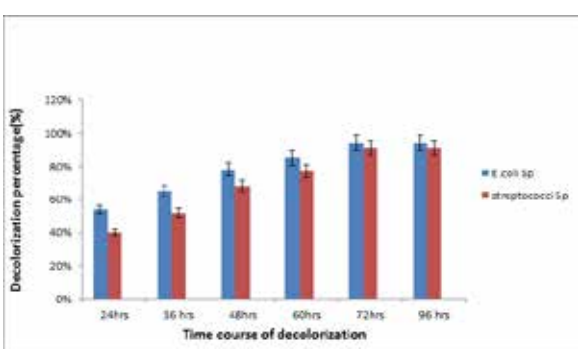


Figure 1: Time course of dye decolourization

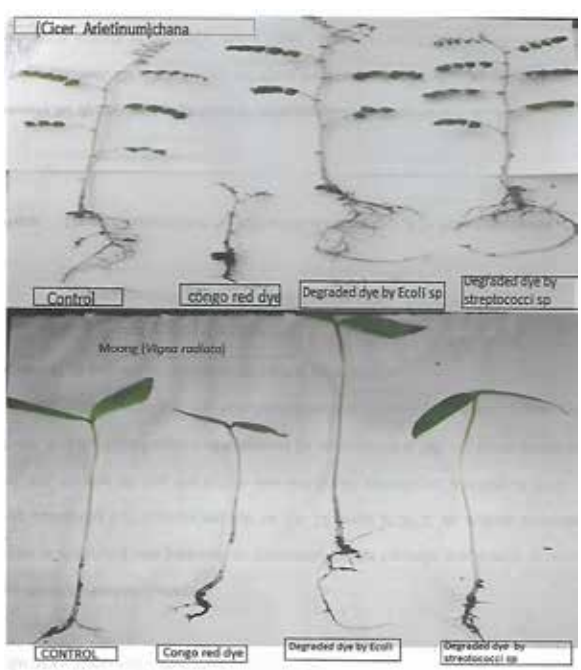
Bioassay for dye toxicity Seed germination test

The bioassay for dye toxicity or phytotoxicity was based on measuring the effect of congo red dye on seed germination, plant shooting and root elongation. The phytotoxicity of the dye was estimated by measuring the ability of dye and degraded dye to germinate the m oong (Vigna radiate) and chana (Cicer Arietinum) seeds used as test plants (Table1). Phytotoxicity of dye and degraded dye was evaluated after 10 days of study. Good germination, shoot length ,and root length of the plants were observed for both degraded dye product of bacterial isolates after comparing with the dye and control (figure2). The isolates from the soil dyeing unit sites showed a potential of degrading dyes at faster rate with application good seed germinating efficiency. These properties thus found useful for the bioremediation of various textile industrial effluents and soil contaminated with dye , saving the ecosystem from harmful effects of various dyes.

Table.1: Bioassay of dye and degraded dye toxicity

Seed Type	Samples	Shoot length (cm)	Root Length **(cm)**
chana (Cicer Arietinum)	Control (water)	18	9.8
	Dye(Congo Red	6	3.7
	Degraded dye product by IS-1	19	15
	Degraded dye product by IS-2	17	14.3
Moong (*Vigna radiata*)	Control (water)	14.6	3.2
	Dye(Congo Red)	13.2	2.5
	Degraded dye product by IS-1	18	9.5
	Degraded dye product by IS-2	16.8	4.5

Figure2: Bioassay for dye toxicity Seed germination test for Cicer arietinum (chana) and Vigna radiate (moong)



Acknowledgement: We are thankful to Dr. kiran billore for providing research facilities in the Department of Biotechnology, Govt. Holkar Science College, Indore(m.p).

References

1. Correia, V.M., T. Stephenson and Judd, S.J.1994. Characterization of textile wastewaters A review. Environ. Technol. 15:917.
2. Durve, A.A., R. Arvind and Sayali R.Naphade. 2012. Decolourization of Textile Dyes and Biological stains by Bacterial strains isolated from Industrial effluents .Adv. Appl. Sci. Res. 3(5): 2660-2671.
3. Nigam. P.,I.M.Banat, D.Singh and R.Marchant. (1996).Microbial processfor

Volume : 6 | Issue : 3 | March 2016 | ISSN - 2249-555X | IF : 3.919 | IC Value : 74.50

the decolorization of textile effluent containing azo, diazo and reactive dyes. Proc. Biochem., 31(5):435-442.

4. Nirmalarani, J., and Janardhanan, K. 1988.Effect of South India Viscose factory effluent on seed germination seedling growth and chloroplast pigments content in five varieties of Maize (Zea mays l)Madras. Agric. J. 75: 41.
5. Puvaneswari, N., J.Muthukrishnan and P.Gunasekaran. (2006). Toxicity assessment and microbial degradation ofazo dyes. Ind. J. Exp. Biol., 44:618-626.
6. Wong, Y. and J.Yu. (1999). Laccase-catalysed decolorization of synthetic dyes. Water Res., 33:3512-3520.