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Decolorization of Azo Dye by *Bacillus mesophilus*

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Abstract:

Azo dyes are more harmful and it's against biodegradation, causing environmental problems. Based on the suitable environmental conditions, microorganisms will convert dyes to non-coloured products or mineralize the solution. In this current study the azo dye reactive red (RED ECO) was selected for decolorization by *Bacillus mesophilus*. The parameters optimization of dye decolorization was studied under shaking condition. Decolorization rate was monitored by spectrophotometry under different conditions. TheReactive red decolorization by *Bacillus* was found to be 93% at 250mg/L within 48 hours in shaking condition. The optimum temperature and pH for decolorization was30-35^{o}C and 9.0 respectively. The results suggest that the isolated organism *Bacillus mesophilus* has the potential activity to degrade the effluents which contains the reactive dyes.

Keywords — **Reactive red, Bacillus, Azo dye, shaking condition.**

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I. INTRODUCTION

The textile industry is the onewhich extensively use synthetic chemicals as dyes. The Wastewaters discharged from textile industries gives more threat to the environment, as large amount ofchemical dyes is used. A significant proportion of these dyes enter the environment via wastewater [7]. The textile finishing generates a large amount of waste water containing dyes and represents one of the largest causes of water pollution as 10- 15% of dyes are lost in the effluent during the dyeing process [8].

The textileindustry consumes about 100 litres of water to process about 1 Kg of textile material. Treatment of dye waste water involves chemical/physical methods, among which are coagulation, precipitation, and adsorption by activated carbon, oxidation by ozone, ionizing radiation and ultrafiltration. Chemical or physical-chemical methods are very costly, produce wastes which are difficult to dispose of, are less efficient and of limited applicability. As a viable alternative, biological processes have received increasing

interest owing to their cost effectiveness, ability to produce less sludge, and eco-friendly [4]. Azo dyes consist of a diazotized amine coupled to an amine or a phenol, and contain one or more azo linkages. Azo dyes are the largest class of dyes with the greatest variety of colours. Azo dyes have been used increasingly in industries because of their ease and cost effectiveness in synthesis compared to natural dyes.

The new closed-loop technologies such as the reuse of microbial or enzymatic treatment of dyeing effluents could help reducing this enormous water pollution [9].However, most azo dyes are toxic, carcinogenic and mutagenic [10]. Therefore, economic and safe removal of the polluting dyes is still an important issue. The Bioremediation through microorganisms has been identified as a cost effective and environment. In recent years number of studies has focussed on several microorganisms capable of degrading and absorbing dyes from waste water. This study deals with the isolation of textile dyes degrading bacterium from contaminated soil and its ability to degrade the reactive dyes.

II. MATERIALS AND METHODS

Isolation of Microorganism

Bacillus was isolated from the polluted soil at Erode. To obtain pure colonies the soil sample was serially diluted and plated on nutrient agar plates. Pure colonies were isolated and stored on the nutrient agar plates for further analysis. The composition of nutrient agar and broth used for this decolorization analysis are given below.

Medium Composition

Peptone – 5g/L

NaCl – 5g/L

Beef extract 1.5g/L

Yeast extract 1.5g/L

Agar – 15g/L and pH 7.

Identification of the culture

The selected isolate was subjected to biochemical tests and gene sequencing followed by BLAST analysis for species confirmation. Biochemical characterization was done based on *Bergey's manual of determinative bacteriology.* The DNAextraction was done using High Pure PCR template kit (Roche). DNA was amplified by using PCR primer set, 27F-5'AGAGTTTGATCMTGGCTCAG3'and 1492R-5'TACGGYTACCTTGTTACGACTT3'. This amplified product was sequenced using automated sequencer with the primer set 518F-5'CCAGCAGCCGCGGTAATACG3' and 800R-5'TACCAGGGTATCTAATCC3'. The sequence obtained is aligned and ran through BLAST to identify the organism, only sequence obtained with 1200 base pairs or above is considered to give assured result in BLAST.

Decolorization by various dye concentrations

The initial dye concentration was measured under shaking conditions.In nutrient broth medium 50- 250 mg/L of reactive red was added and inoculated with 10% of *Bacillus mesophilus*. The flasks were incubated at 37°C under shaking conditions. After 24hours the decolorization percentage was measured. Non inoculated flasks (controls) were always included. The decolorization percentage was calculated [6].

Influence of pH in Dye Decolorization

Nutrient broth withvarious pH [4-9] was inoculated with 10% of *Bacillus mesophilus*and incubated under shaking conditionat 37°C.50mg/L of dye was the initial concentration. Non inoculated flasks (controls) were always included. The decolorization percentage was measured and pH was adjusted using1N HCl & 1N NaOH.

Dye Decolorization on different temperatures

Nutrient broth was inoculated with 10% of *Bacillus mesophilus* and dye 50 mg/L concentration was added. The nutrient broth was incubated at 25, 30, 35, 40, 45 and 50°C. Non inoculated flasks (controls) were always included. The decolorization percentage was measured.

Dye decolorization with different Carbon Sources

The decolorization of reactive red was done with different carbon sources in nutrient broth medium. 1% of glucose, sucrose, lactose and maltose, was respectively added to the medium.50mg/L of dye was the initial concentration. Non inoculated flasks (controls) were always included. For every analysis dye should be added before the sterilization process.

III. RESULTS AND DISCUSSION

Identification of organism

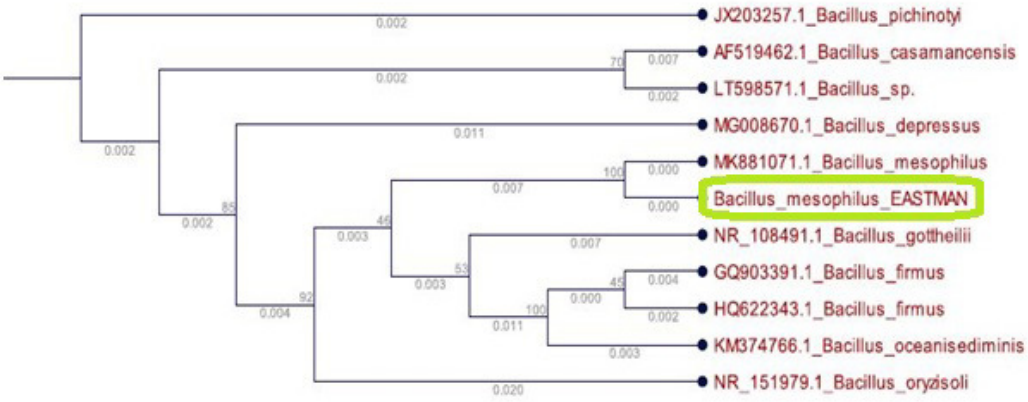
Thebacterial isolation was carried out by using nutrient broth medium. The carbon and nitrogen are the main source for the growth of bacteria and also for the decolorization process. The isolate was found to be *Bacillus mesophilus* by Biochemical results (Table-1) and conformed by gene sequencing.

Table 1. Biochemical test results of *Bacillus mesophilus*

S.No	Biochemical tests	Results
1	Gram Staining	Positive
2	Shape	Rod
3	Capsule staining	Negative
4	Spore	Negative
5	Oxidase test	Positive
6	Catalase	Negative

7	Pigment	Positive(orange)
8	Citrate utilization	Negative
9	TSI test	Negative
10	Litmus milk test	Positive
11	MR	Negative
12	VP	Negative
13	Indole	Negative
14	Starch hydrolysis	Negative
15	Gelatin hydrolysis	Negative
16	Casein hydrolysis	Negative
17	Salt tolerance	Positive
18	Motility	Positive
19	Lipase	Positive
20	Urease	Positive
21	Kings media	Negative
22	Cetrimide agar	Negative

Figure 1. *Bacillus mesophilus* conformation by gene sequencing



In gene sequencing, a sequence of 1862 base pairs were obtained. And on running through BLAST, 100% identity was observed for *Bacillus mesophilus* (Figure-1).

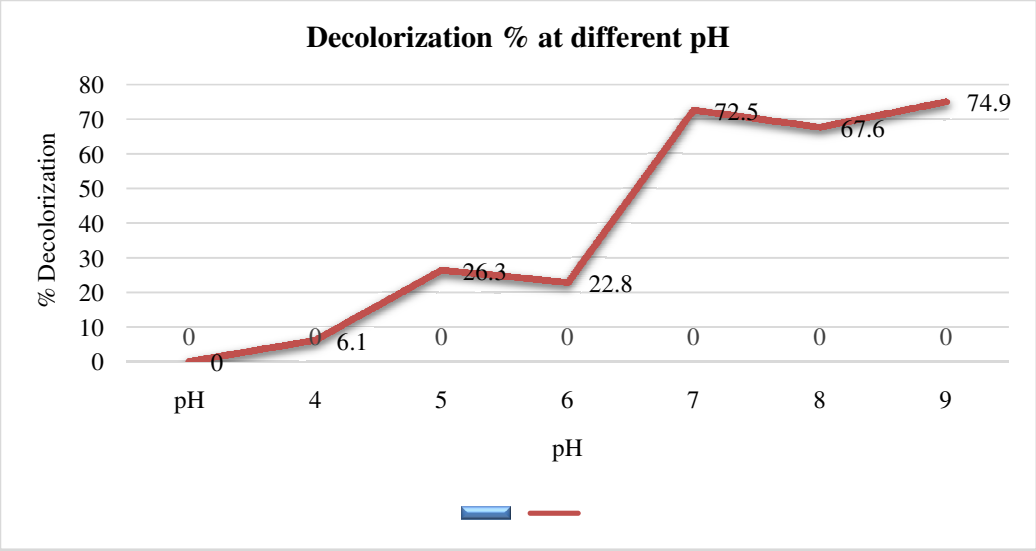
Physiochemical conditions for decolorization

The various physiochemical conditions such as temperature, pH, dye concentration and different carbon sources on decolorization of reactive red by this isolate was studied. All analysis was studied under shaking conditions at 37°C. 10% of inoculum with O.D.530 nm was used at 50mg/L dye concentration.

Effect of pH

Themaximum decolorization of microorganisms will occur at neutral pH only. In this present study this culture shows maximum decolorization performance in the range of pH 9. At pH 4 and 5 decolorization was observed less than 30% respectively. The isolate showed maximum decolorization (75%) at pH 9 (Figure-2). This isolate shows decolorization ability from the range of pH 7-9. Most of the microorganisms will not degrade in alkaline conditions but this isolate works in alkaline nature

Figure 2.Decolorization effect at different pH



Effect of Temperature

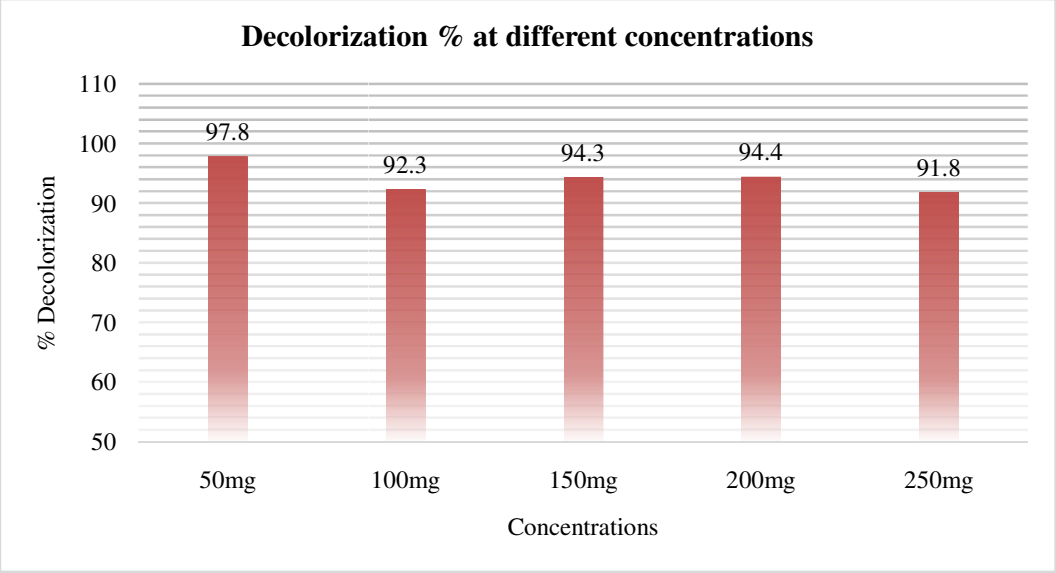
There are several important parameters plays a major role in degradation of reactive dyes. The most important parameter for decolorization is temperature. The decolorization of dye was measured with six different incubation temperatures (25, 30, 35, 40, 45 and 50°C).Decolorization performance was low at 25°C, the decolorization level was increased to a certain level from 30°C to 35°C. Decolorizing process was suppressed after 45°C, which might be due to the loss of cell viability or enzyme deactivation which is

responsible for decolorization at 45°C [12]. This isolate exhibited maximum decolorization at 30-35°C. No decolorization was observed at 50°C. Based on substrate metabolism the optimum temperature will be varied for decolorization.

Effect of Dye Concentration

Decolorizing performance of the culture was studied with different dye concentration from 50 – 250 mg/L (Figure-3). The isolatedecolorizes 98% of 50 mg/L of the dye in less than 24 hours where as it took 48 hours to decolorize 93% of 250 mg/L. The rate of decolorization has been reduced after 200 mg/L concentration. The efficiency of microbial decolorization through a combination of factors including the toxicity imposed by dye at higher concentration [11]. The isolate has the capacity to decolorize dye up to the reported concentrations and it can be implemented for waste water treatment.

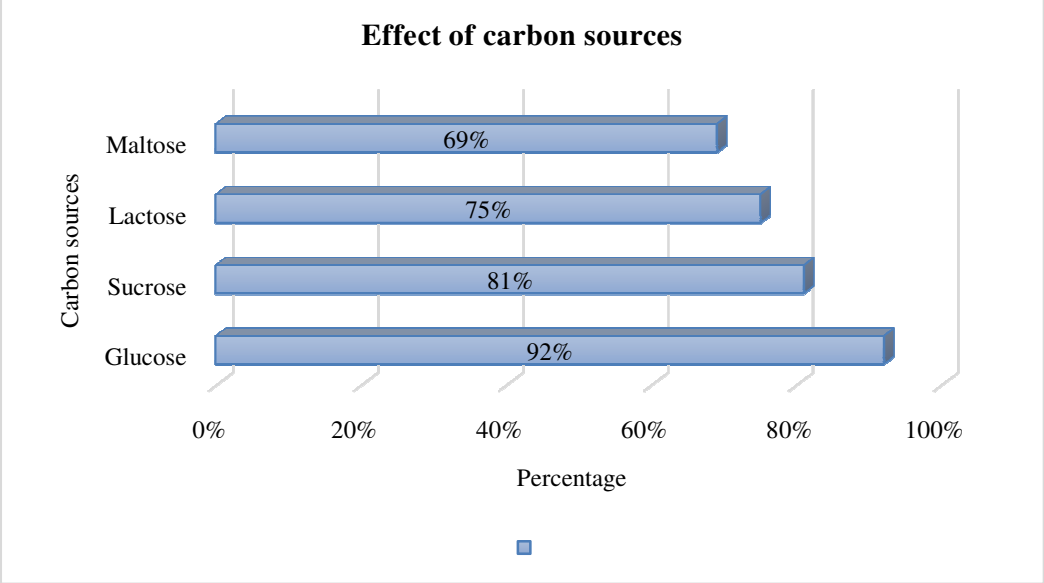
Figure 3. Effect of dye concentrations



Effect of various carbon sources

The effect of various carbon sources glucose, sucrose, lactose and maltose (1%) on decolorization was performed at 50 mg/L concentration of dyes with 10% of inoculum concentration under shaking condition. Figure – 4 represents the maximum removal of dye 92% by using the glucose and the co-substrate sucrose shows 81% of dye decolorization and other carbon sources are relatively low when compared with glucose. This shows that the carbon source glucose will meet up all growth requirements of this bacterium.

Figure 4. Effect of carbon sources



IV. CONCLUSION

This current study concludes that dye degrading microorganism *Bacillus mesophilus* from a polluted soil have the potential capacity for decolorization. The organism has the ability to decolorize and degrade reactive azo dye at high concentration and it gives more benefits for textile industry waste water. The bacteria showed the highest decolorization rate 98% of 50 mg/L and 93% of 250 mg/L under shaking conditions. High decolorization was observed in alkaline pH, moderate temperature (35^{o}C) and glucose as a carbon source. This biological method can be promoted to degrade the variety of reactive dyes from the textile industries [13]. This potential organism *Bacillus mesophilus* may be used for the industrial effluent's treatment and also for all other polluted water.

REFERENCES

[1] Bio decolourisation of textile dyes by local microbial consortia isolated from dye polluted soils in ngaoundere (Cameroon) Ngwasiri pride Ndasi, Mbawala Augustin, Tchatchueng Jean Bosco, 2011

[2] Biological methods for textile dye removal from wastewater: A review Deepika Bhatiaa, Neeta Raj Sharmaa, Joginder Singh and Rameshwar S. Kanwara

[3] Decolorization of Wastewater Oliver J. Hao a,Hyunook Kim a & Pen-Chi Chiang a Department of Civil and Environmental Engineering, University of Maryland, College Park,MD 20742, U.S.A.

[4] Microbial decolorization of azo dyes by Proteus mirabilis K-C Chen, W-T Huang, J-Y Wu Department of Chemical Engineering, National

Tsing Hua University, Hsinchu, Taiwan

[5] Microbial Degradation and Decolorization of Reactive Orange Dye by Strain of Pseudomonas SppMaulin P Shah*, Kavita A Patel, Sunu S Nair, A M Darji Applied & Environmental Microbiology Lab, Enviro Technology Limited (CETP), Gujarat, India Received August 23, 2013; Revised August 31, 2013; Accepted September 02, 2013

[6] Pant D, Adholeya A. (2009). Nitrogen removal from biomethanated spentwash using hydroponics treatment followed by fungal decolorization. Environ. Eng. Sci, 26(3), 559.

[7] Moorthi P.S, Selvam S.P, Sasikalaveni A, Murugesan K, and Kalaichelvan P.T (2007) Decolorization of textile dyes and their effluents using white rot fungi, African J Biotech, 6(4), 424-429.

[8] Zollinger H (1991) Colour Chemistry Synthesis Properties and Application of Organic Dyes and Pigments, VCH New York, 92- 102.

[9] Abadulla E, Tzanov T, Costa S, Robra K, Cavaco A. and Gubitz G (2000) Decolorization and detoxification of textiles dyes with Laccase from fromtrameteshirsuta, Appl Environ Microbial, 66(80), 3357-62

[10] Pinherio H.M, Touraud E, and Tomas O (2004) Aromatic amines from azo dye reduction: status review with emphasis on direct UV spectrophotometric detection in textile industry wastewater, Dyes and Pigments, 61(2), 121-139

[11] Pearce, C.I., Lloyd, J.R., Guthrie, J.T. (2003). The removal of colour from textile wastewater using whole bacterial cells: a review. Dyes and Pigments, 58, 179-196

[12] Cetin, D., Donmez, G. (2006). Decolorization of reactive dyes by mixed cultures isolated from textile effluent under anaerobic condition. Enzyme and Microbial technology. 38: 926-930.

[13] Microbial decolorization of Reactive azo dyes by Bacillus spp. ETL-1949 under anaerobic condition. International Journal of Environmental Bioremediation & Biodegradation, 2014,30-36.

