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Isolation and Screening of Azo Dye Decoloizing Bacterial Isolates from Shrimp Shell Contaminated Soil in Thoothukudi Coast

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Abstract: ***Two bacterial isolates were obtained from shrimp shell contaminated soil in Thoothukudi coast and they were identified by biochemical tests as Acinetobacter sp. and Bacillus sp. These isolates were checked for their dye decolourization potential by growing them in Mueller Hinton broth containing various concentrations (100ppm to 1000ppm) of the textile dyes (Congo red, Acid Orange 5 and Black 7984). The absorbance was found out for the three dyes at the appropriate wavelength i.e. congo red dye at 523.20 nm, for acid orange 5 and black 7984 dyes at 450 nm using UV-Visible spectrophotometer. The percentage of decolourization was found out using the formula; Percentage decolourization = (Initial O.D. - Final O.D) / Initial OD X 100. Acinetobacter sp showed a maximum percentage decolourization of 93.98% when grown in broth containing 100 ppm solutions of the acid orange dye 5 and Bacillus sp. showed the highest percentage decolourization (92.4%) for acid orange 5 at 300 ppm concentration respectively in just 48 hours of static incubation. FTIR spectral comparison between dyes and its products formed after decolourization by bacterial strains, confirmed biodegradation of the dye into different metabolites.***
Key words: Azo dye, Acinetobacter sp, Bacillus sp, decolourization, congo red, acid orange 5, black 7984.

I. INTRODUCTION

The dyes are natural and synthetic compounds that make the world more beautiful through colored products. The textile dyes represent a category of organic compounds, generally considered as pollutants, presented into wastewaters resulting mainly from processes of chemical textile finishing [1, 2]. Because of their synthetic nature and structure mainly aromatic, the most of dyes are non-biodegradable, having carcinogenic action or causing allergies, dermatitis, skin irritation or different tissular changes [3]. One of the main sources with severe pollution problems worldwide is the textile industry and its dye-containing wastewaters [4, 5 and 6]. The textile organic dyes must be separated and eliminated from industrial wastewaters by effective and viable treatment includes different separation processes (sedimentation, filtration, membrane separation), and some physico-chemical treatment (i.e. adsorption; coagulation-flocculation with inorganic coagulants and organic polymers; chemical oxidation; ozonation; electrochemical process, etc.). The partial and complete mineralization or decomposition of pollutants can be achieved by biological and chemical processes (biological processes in connection with the activated sludge processes and membrane bioreactors, advanced oxidation with ozone, H_2O_2, UV) [7, 8, 9 and 2]. Methods like chemical processes have considerable disadvantages such as complex structural set-up, huge chemical and power consumption and formation of a large volume of sludge. The physico-chemical sludge is highly toxic and troublesome to safe disposal. In contrast, remediation of dyeing industry effluent by using microorganisms has proved to be the best solution since numerous bacterial species including *Bacillus, Pseudomonas, Enterobacter, Halobacter,* and *Aeromonas* have been reported to exhibit tremendous capability to decolourize and detoxify a wide range of azo dyes composed of phenylamine, benzenediazonium chloride or phenol. The present work is focused with the isolation, identification and screening of bacterial species capable to decolorize variety of dyes as an alternative for degradation for waste water treatment.

II. MATERIALS AND METHODS

A. Collection of Sample

Soil sample was collected aseptically from shrimp shell waste contaminated area and preserved in refrigerator at 4°C and they were tested within 24 hours of collection.

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B. Dyes

Three types of dye including Congo Red, Acid Orange and Black 7984 were used for all these dye decolourization assay dyes were bought from the small textile industry in Erode. The main cause for choosing these three dyes, as because, they are widely used in the different textile industry.

C. Isolation, Identification and Maintenance of Dye Decolorizing Bacteria

Total bacterial count of the sample was determined by serial dilution followed by pour plate method using Mueller Hinton agar as medium. The isolated strains were maintained on Mueller Hinton agar slants & stored at 4˚C further it was utilized for dye decolourization process.

D. Screening of Bacterial Isolates for Textile Dye Degradation Inoculum Preparation

Isolates were individually tested for their growth and decolourization ability on Muller Hinton broth. Broth was supplemented with increasing concentrations of the dye (100ppm, 200ppm, 300ppm, 400ppm, 500ppm, 600ppm, 700ppm, 800ppm, 900ppm, 1000ppm). All the dyes were prepared separately and each of the cultures was tested against a single dye. The broths were incubated at 37± 2 ˚C.

E. Dye Decolourization Experiments

Dye decolourization experiments were carried out in 10ml test tubes containing 0.6 ml of Congo Red, Acid Orange 5, Black 7984 (500 mg/l) with 10ml of Mueller Hinton broth. The pH was adjusted to 7 ± 0.2 using sodium hydroxide and hydrochloric acid solution. Then, the flasks were autoclaved at 121°C for 15 minutes. The autoclaved flasks were inoculated with 0.1ml of bacterial inoculums of each isolates. The flasks were kept in shaker and incubated at 37°C ± 2 for 2 days. Samples were drawn after 48 hours for observation. Then the broth was filtered and centrifuged at 8000 rpm for 20 minutes. Decolourization was assessed by measuring absorbance of the supernatant with the help of spectrophotometer at wavelength maxima (λm) of respective dye.

F. Decolourization Assay

Decolourization assay was measured in the terms of percentage decolorization using UV- Spectrophotometer, The percentage decolourization was calculated from the following equation,

% Decolourization= (Initial OD-Final OD* 100) / Initial OD.

III. RESULTS

A. Identification of Dye Degrading Bacteria

Two isolates were isolated from soil sample based on morphological and biochemical characters and they were identified as Acinetobacter sp. and Bacillus sp. (Plate 1).

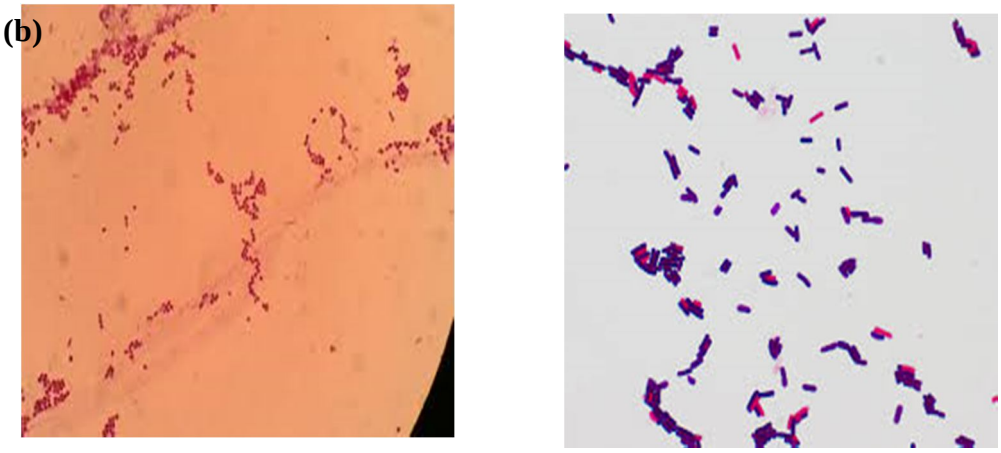

Plate 1: Bacterial isolates from shrimp shell waste contaminated soil.
(a) *Acinetobacter sp* (b) *Bacillus sp*

B. Decolorization of Azo Dye

The two isolated bacterial strains showed their potentiality to degrade all the three dyes used during present investigation.

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Acinetobacter sp showed a percentage decolourization of 93.98% and 84.61% respectively when grown in broth containing 100 ppm solutions of the acid orange dye and 400 ppm solutions of Congo red dye presented in Fig. 1.
After incubation of Bacillus sp with the individual azo dyes solutions, the percentage decolourization for Congo red, acid orange 5 and black 7984 was shown in Fig. 2. The results were found to be 85 % (300ppm), 92.4% (300ppm) and 81.39% (300ppm) respectively in just 48 hours of static incubation.

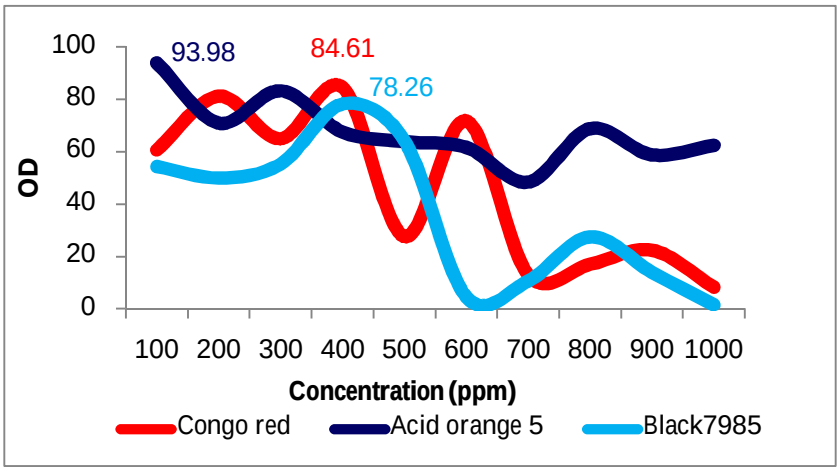

Fig. 1: Decolourization of dyes by Acinetobacter sp in different concentration after 48 hrs.

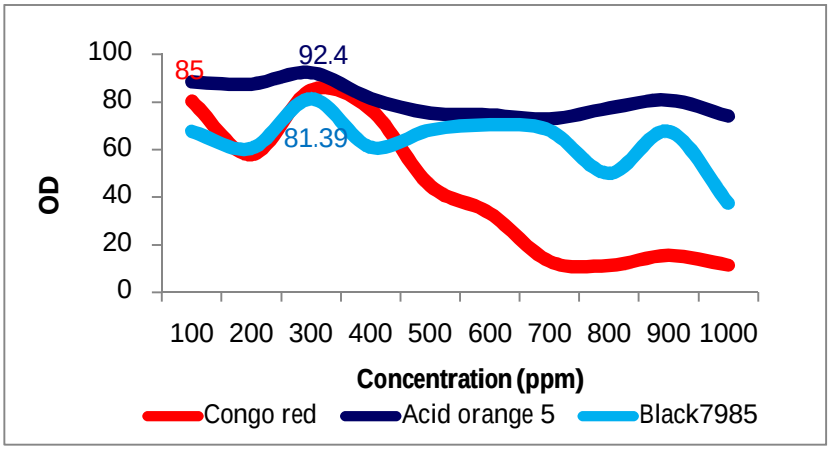

Fig. 2: Decolourization of dyes by Bacillus sp in different concentration after 48 hrs.

C. Discussion

Based on preliminary tests, plating a selective media and Biochemical tests, they are identified as Acinetobacter sp. and Bacillus sp. Shrimp shell waste adapted bacterial isolates belonging to the genera Acinetobacter and Bacillus [10].
The dye decolourization was studied using spectroscopic analysis. The decolourization was expressed in terms of percentage decolourization. Acinetobacter sp (93.98%) was the best decolourization of Acid Orange 5 at 100ppm concentration and Bacillus sp showed a highest percentage decolourization of 92.4% at 300ppm concentration of Acid orange 5.
The bacterial isolates like Acinetobacter sp., Bacillus sp, and Legionella sp. had potential for colour removal [11]. Aerobic reduction has also been reported to occur in several bacteria such as Pseudomonas sp., Bacillus sp. and Klebsiella pneumonia [12].
Findings suggest that, aerobic conditions are required for the complete mineralization of the reactive dye molecule. Microorganisms capable of using the dye molecules as a sole source of carbon, nitrogen, and energy are of special interest and significance because they consume the dye for their growth and activities [13]. Bacteria usually degrade azo dyes under anaerobic conditions to colorless

toxic aromatic amines, of which some are readily metabolized under aerobic conditions [14]. Except for a few, the aromatic amines formed from decolorization of azo dyes are recalcitrant to biodegradation under anaerobic conditions [15].

IV. CONCLUSION

Although bioremediation/degradation is a challenging process to textile industry, the result of the present study suggests a great potential for bacteria to be used to remove pollutants from textile effluents. Interestingly, the evidence for bacterial bioremediation of effluent from textile wastewaters was established. These findings established that the bacteria were adaptive in nature and can degrade contaminants. The ability of the bacteria to adapt and degrade effluents from textile at high concentration gives it an advantage for treatment of effluents from textile industry. Bioremediation of textile effluents represent a promising tool for application in biodegradation of textile industries effluents at large scale.

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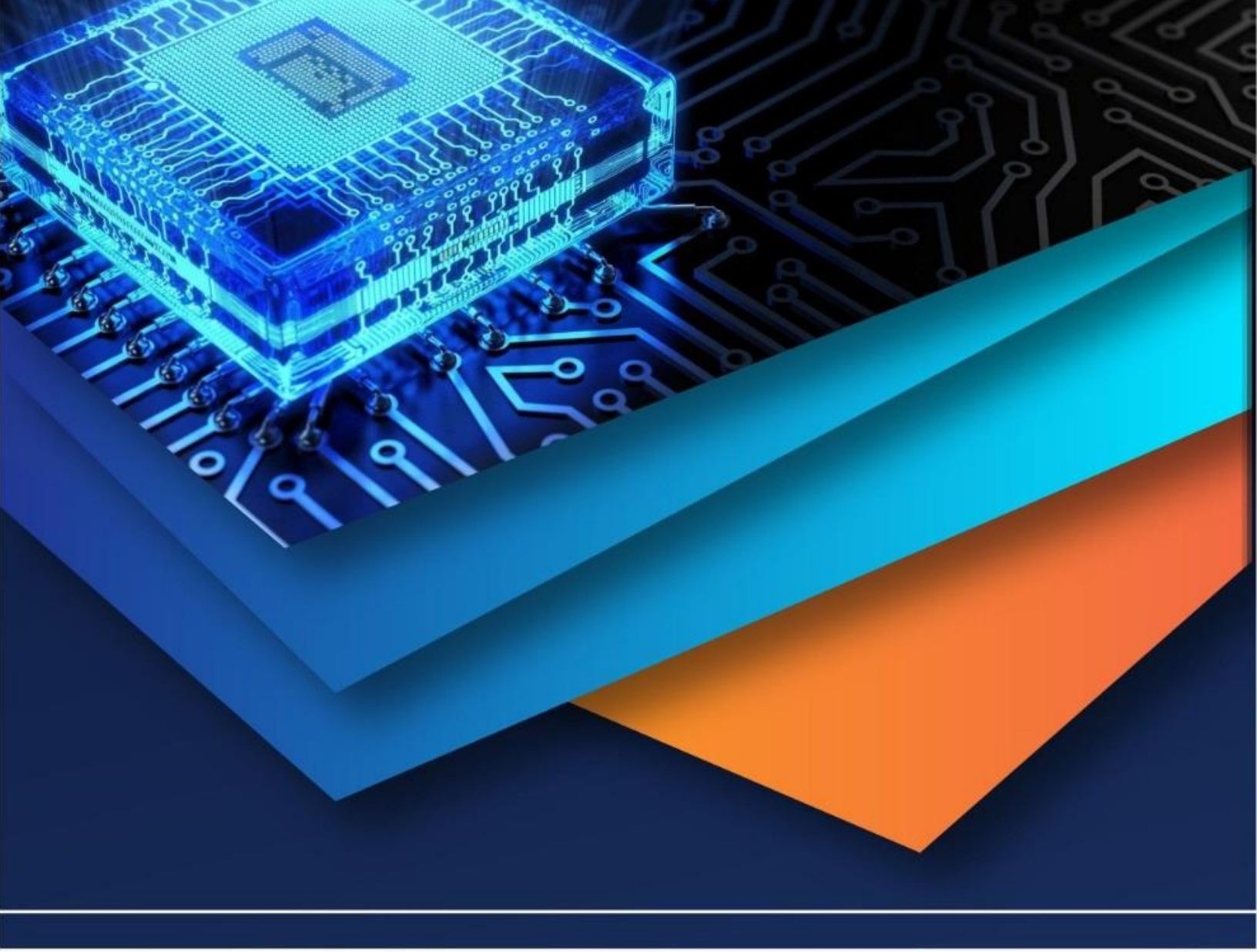

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