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Decolorization and Degradation Study of Azodye 151 with Stress Tolerant Bacteria

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***Abstract*:- The development of reliable process with effective bacterial strains for the decolorization of azo dye Acid Red151 is an important aspect of bioremediation. The present study aims to characterize the biological potential of bacterial isolates isolated from cowdung. The isolates were tested for salt tolerance, pH tolerance and temperature tolerance along with the Azo dye degradation and decolorization. Five potential stress tolerant bacterial isolates were screened and shown excellent results with azo dye decolorization at the concentration of 10µg/ml and 20µg/ml both.**

Keywords: Azodye, cowdung, salt tolerant, pH tolerant, screening; decolorization

INTRODUCTION

Azo dyes are one of the most widely used synthetic colorants, constitute the largest class of synthetic dyes used in commercial applications (Zollinger, 1991). Due to rapid industrialization and urbanization, a lot of chemicals including dyes, pigments, and aromatic molecular structural compounds were widely used in several industrial applications such as textiles, printing, pharmaceuticals, food, toys, paper, plastic and cosmetics. They are still widely used in textile dying, paper printing, cosmetics and food industries (Singh et al., 2012). Textile industries consume a major share of dyes in India. Further, the textile industry of India also contributes nearly 14% of the total industrial production of the country because the dyes used in the textile manufacturing does not adhere to the fibres during dyeing process and results in the production of large amounts of waste water exhibiting intense coloration. Therefore, when the wastewater from textile is directly released in the surface water without any treatment, causes a rapid depletion of dissolved oxygen which can leads to a great environmental damage (Lalnunhlimi & Krishnaswamy, 2016). When dyes are mixed in the water system, the penetration of sunlight greatly reduced into deeper layers, which effects photosynthetic activity. That ultimately results in water quality deterioration, dropping the gas solubility, and also causes severe toxic effects on aquatic flora and fauna. Most of the dyes which are released from wastewater are toxic, carcinogenic, or mutagenic to humans and other life forms. This should be removed before release into natural water streams.

During conventional aerobic wastewater treatment, Azo dyes are not degraded. However, Azo linkages are easily reduced under anaerobic conditions, with digester sludge, anaerobic granular sludge or sediments. There are physical and chemical methods for removal of colourization from the water. Physical methods based on coagulation–flocculation of dyes are effective for the removal of mainly sulphur and disperse dyes, but show very low coagulation–flocculation capacity for acid, direct, reactive and vat dyes. Chemical oxidation methods facilitate the decomposition of dye molecules. These approaches use various oxidizing agents, viz. ozone, hydrogen peroxide and permanganate. Modification in the chemical composition of a compound or a group of compounds takes place in the presence of these oxidizing agents, and thus the dye molecules become susceptible to degradation. But due to some or other drawback of both the physical and chemical methods biological method has thought to be a good alternative.

Use of microbial or enzymatic treatment method for the complete decolorization and degradation of azo dyes from textile effluent have many advantages: viz. environmentally friendly, cost effective, yielding end products that are non-toxic, produces less sludge, and requiring less water consumption compared to physicochemical methods. The effectiveness of microbial decolorization depends on the adaptability and the activity of the selected microorganisms. Therefore, a large number of species has been tested for the decolorization and mineralization of various dyes in recent years. The isolation of potent species and thereby their degradation is one of the interesting biological aspects of effluent treatment. A wide variety of microorganisms are capable of decolorizing of a wide range of dyes including; bacteria, fungi and algae.

Decolorization of synthetic dyes is due to the cleavage of the chromophoric group which generates colorless metabolic intermediates (Agarwal & Singh, 2012). These intermediate metabolites of the dye substrates are aromatic amines (Ganesh R, 1992; Brown & Stephen, 1993). The cleavage of the chromophoric group of dyes is a reduction process which requires redox equivalents (electron donors) that transfer electrons to the chrophoric group of dyes (Russ et al., 2000).

The process of bacterial decolorization of azo dye is affected by various physicochemical operational parameters, such as temperature, pH, dye concentration, supplementation of different carbon and nitrogen sources etc. These factors directly influence the bacterial decolorization performance of azo dyes. Thus, to make the process more efficient, faster and practically applicable, prior determination of the effect of each factor on the bacterial decolorization of azo dyes is essential.

In the present study potent bacterial isolates were screened and characterized, for temperature resistance and

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sensitivity, salt tolerance and tolerance of pH. Further the isolates were tested for decolorization of azo dye Acid Red 131 at two different concentrations.

MATERIAL AND METHODS

Sample collection and preservation:
Two different samples of cowdung was used for the isolation of bacteria. Bacteria were isolated from cowdung samples by serial dilution technique (Dubey and Maheshwari, 2011). Bacterial colony appearing on nutrient agar (NA) were screened, identified by Gram's staining, colony morphology, and biochemical analysis (Holt et al., 1994).

Dye stock preparation:

Composition:

- Nutrient Broth Media
- Beef extract
- Peptone
- Nacl
- Dye stock: 1mg/ml

The media was autoclaved separately. After cooling 1 ml of dye stock was added aseptically to 300 ml NB media supplemented with 10µg/ml or 20µg/ml dye according to the requirements.

Analysis of bacterial stress tolerance:
The fifteen isolated bacterial isolates were checked for stress tolerance at different pH (from pH 6 to 13), salt concentrations and temperatures.

Isolation and Characterization of pH tolerant bacteria
In order to screen pH tolerant bacterial strains, pH range of media has been varied from pH 6 to pH 13. All the bacterial isolates were tested on all the pH range and the plates were observed upto 72 hrs for the growth.

Isolation of Temperature tolerant bacteria:
All the isolated bacterial isolates were tested at different temperatures which were 4˚ C, 25˚ C, 37˚ C, 45 ˚C and 55 ˚C and observed for 72hrs for the growth.

Screening for Salt Tolerant bacteria:
Stress tolerance was checked at different salt concentration i.e 2M, 3M and 5M. The salt tolerance test was done to check the ability of the bacteria to grow in the presence of variable amount of Sodium Chloride (NaCl) has been used to characterize several bacteria.
The NA media was supplemented with the selected salt concentrations. The bacterial cultures were incubated at 37ºC for 48 hrs. to observe the growth.

Screening of bacterial strains for dye degradation
Primary Screening of dye degradative effect:
Five bacterial isolates were screened from cowdung samples and selected on the basis of salt tolerance, temperature tolerance and pH tolerance analysis. These cultures were used for decolorization and degradation study of Acid Red 151 dye.

Two concentrations of Acid Red 151 i.e. 10 µg/ml and 20 µg/ml were tested for decolorization and degradation analysis. The experiment was set up with 25ml of NB media and10µg/ml and 20µg/ml concentration of azo dye Acid Red 151 and inoculated with each bacterial strain in flask. The culture flasks were maintained at 37ºC for 7 days and optical density was observed and calculated for each day. Optical density is measured through spectrophotometer at a wavelength of 485nm. Thereafter the percentage of dye decolorization was calculated by the formula (Oranusi and Mbah, 2005).

*% Decolorization per day = $(OD_{control} - OD_{test})/OD_{control}$ * 100*

Standard Graph:
In order to analyze the actual concentration of the dye present in the media, the standard graph was plotted for Acid Red 151. For this NB media supplemented with known concentration of dye was made and finally the OD of each sample was analysed at 485nm. The best possible standard graph was plotted depending on the readings obtained.

RESULT :

Total fifteen different bacterial isolates were screened and characterized on the basis of biochemical reaction. Five bacterial strains i.e. CD1, CD4, CD5, CD8 and CD12 were isolated and glycerol culture was preserved at -80 degree Celsius.

PH TOLERANCE:

pH tolerance by all the isolates was monitored up to 72 hrs in nutrient agar medium at 37˚C. Five bacterial strains, CD1, CD4, CD5, CD8 and CD12 were found to tolerate pH 13.

SALT TOLERANCE:

The halophiles are extremophiles that can tolerate heavy salt concentrations. Those which can tolerate salt concentration from 0.5 M to 2.5 M are comes under moderate halophilic category. The bacterial isolates tested and again five CD1, CD4, CD5, CD8 and CD12 were shown tolerance up to 3.0 M salt concentration. Though CD1 has shown mild halophilic response.

TEMPERATURE TOLERANCE:

The bacterial strains tested for temperature tolerance, three bacterial isolates CD1, CD4 and CD8 were found to grow up to 50˚C in 48hrs.

Decolorization Study of Acid Red 151
The decolorization of the nutrient media with dye concentration of 10µg/ml and 20 µg/ml (Fig. 1) indicate the five stress tolerant bacterial strains CD1, CD4, CD5, CD8 and CD12 were able to utilize the azo dye Acid Red 151as a source of carbon and nitrogen. Thus they were able to decolorize and degrade the dye used in study (Fig.2).

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Fig 1: CONTROL Day 1- Flask after inoculation of bacterial culture in NB media

Fig. 2: Decolorization of Acid Red 151 after 7 days of inoculation in all the five bacterial cultures.

STANDARD GRAPH:

The standard graph was plotted for all five bacterial strains for azo dye Acid Red 151 at concentration of 10µg/ml (Fig. 3) 20µg/ml (Fig. 4) at 485mn to observe the degradation efficiency and decolorization of dye.

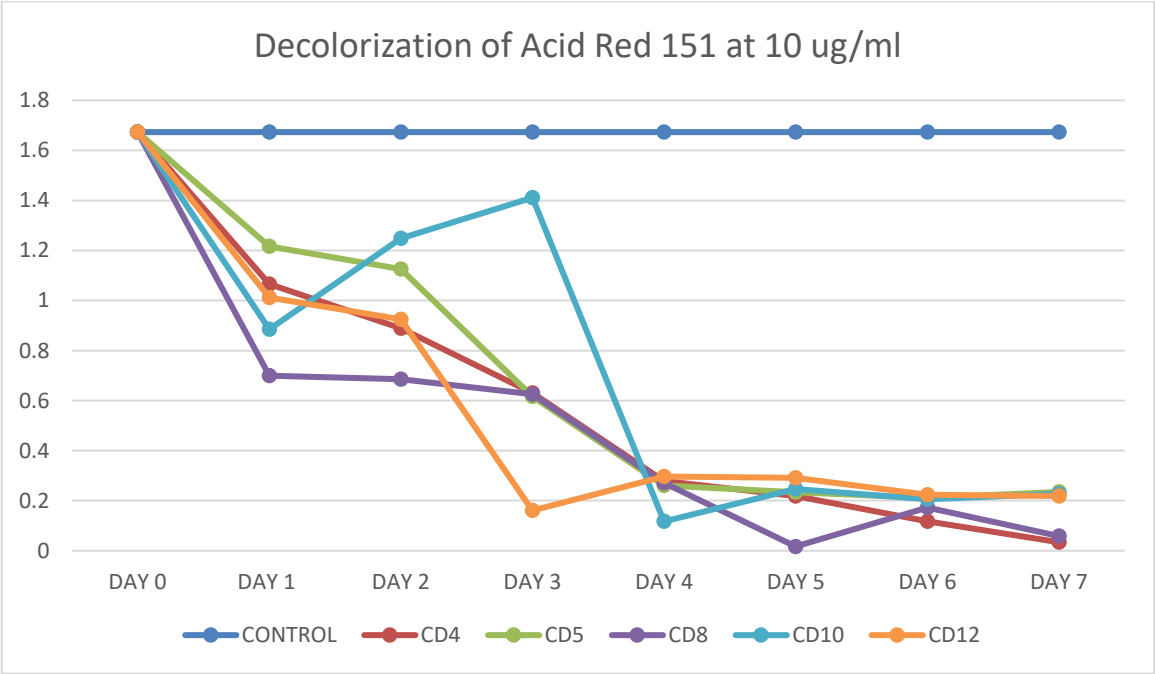

Fig.3: Decolourization of Acid Red 151 (10ug/ml) with all the five bacterial strains. Optical density at485 nm.

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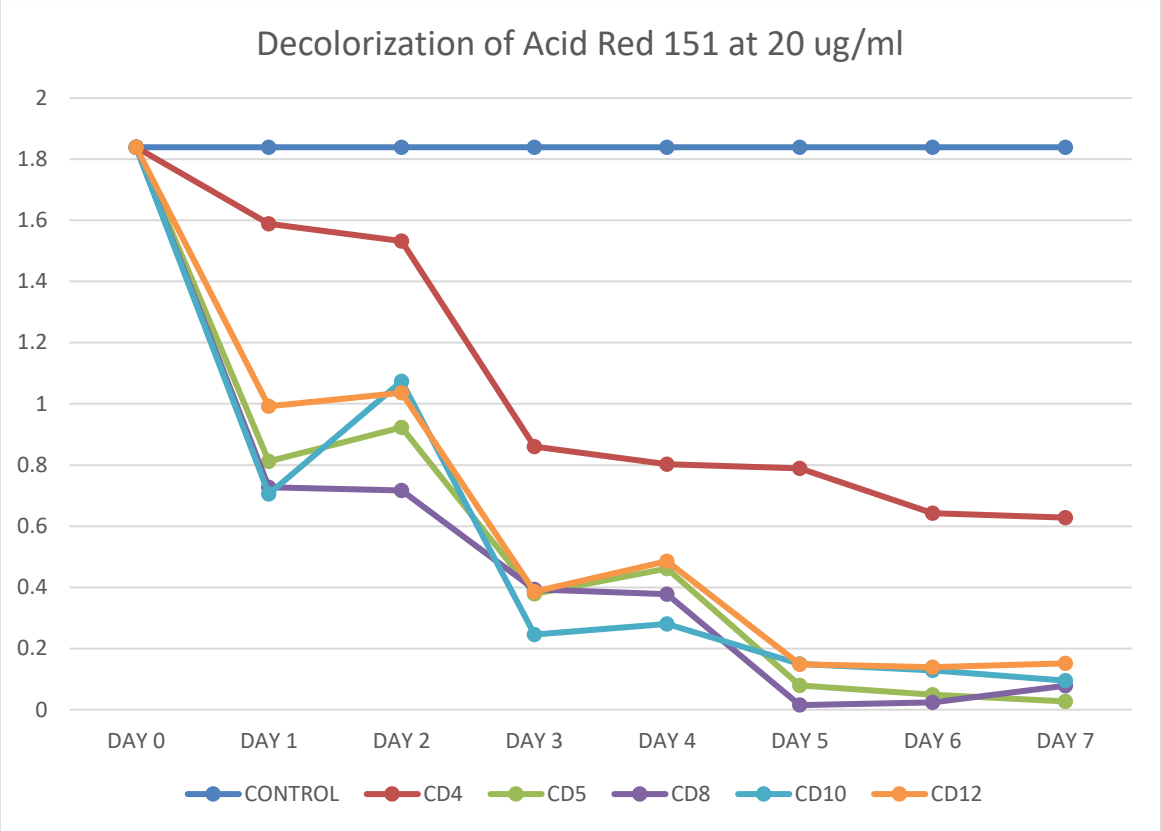

Fig.4: Decolourization of Acid Red 151 (20ug/ml) with all the five bacterial strains. Optical density at485 nm.

DISCUSSION

Acid Red 151 is an azo dye due to the presence of a unique azo group. It is used in the textile industry to impart colour to various items like wool, fabric, paper, etc. Thus, it becomes an important part of the industry. Dye after use is usually flushed out in the environment through water bodies. Water is also an essential component of the industry which is present in both the ingredients and the by products. Dye causes both soil and water pollution. It can also enter into the food chain and cause toxicity and cancers.

In the current study the objective was to identify the bacteria wich could resist at extreme conditions of salt, pH and temperature and at the same time should be able to decolorize anth azo dyes at different concentrations. The study has given very promising results with respect to isolation of stress tolerant bacteria and dye decolorization. All the five stress tolerant isolates have shown good decolorization at both the concentrations of dye within 48hrs. As all the five isolates are non pathogenic and showing good results they can be further used in other bioremediation process. This preliminary study has given a breakthrough in biodegradation of Acid Red 151, this can be extended by scaling up the process for large scale production of bacterial cultures and discover new dimensions of these isolates.

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