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RESEARCH ARTICLE

Isolation, Characterization and Optimization of Azo Dye Degrading Bacteria and Its Application In Textile Industry.

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Abstract

Soil enriched with dye from Textile industry (Baddi) Dupatta house, Nalagarh, Dupatta staining house, Baddi Textile industry, kharuni, Nalagarh was taken. Isolation of bacteria from different soil samples was done. Morphological Characterization of isolates was done with the help of Gram Staining. Biochemical characterization of isolates was reported with the help of Indole, Methyl red, Catalase, Nitrogen reductase & Urease, Citrate Hydrolysis, Hydrogen sulfide test. γ λ_{max} of methyl orange dye was determined. Optimization of the decolonization condition was done by optimizing its pH & temperature as well as rate of Decolourization of methyl orange dye was determined. Screening of the most efficient bacterial strain for dye decolourizaion was done with the help of Nitrate reduction test This test is based on the detection of nitrite and its ability to form red color compound when it reacts with sulfanilic acid (Reagent A) to form a complex (Nitrite-sulfanilic acid) which then reacts with α-Naphthylamine (Reagent B) to five red precipitates (prontosil).

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Introduction:-

Dyes are colored & aromatic compounds which show an affinity towards the substrate to which it is being applied. Most of the dyes are persistent environmental pollutants that are not removed from industrial effluents by conventional treatments (1).Tough controls are being applied to remove dyes from industrial effluents(2).there are many different types of dyes, but all of these,the azo aromatic ones are the most widespread dyes in the industry(3).Among azo dyes methyl orange is an important dye. It is prepared by coupling diazotized sulphanilic acid with dimethylaniline.It releases toxic & carcinogenic products in the environment (4).The bacterial degradation is much faster than fungal degradation (5).Many bacteria,actinomycetes,yeast & fungi are able to decolorize dyes (6).*Lactobacillus casei* TISTR 1500 from soli is selected as the most active azo dye degrader (7).Methyl orange was used to understand the mechanism of biodegradation by *Pseudomonas sp.*SUK1(8).Titanium dioxide showed higher activity for degrading the methyl orange(9) The azo dyes and their degradation intermediates such as aromatic amines are mutagenic and carcinogenic (10).

Therefore considerable attention has been given to evaluating the fate of azo dyes during waste water treatment and in the natural environment (11).Their widespread use ,coupled with the fact that azo dyes are not readily degraded in most biological treatment systems makes this class of chemicals a significant environmental problem(12). Removal of dyes from these wastewaters has been reviewed with respect to biological decolourization as well as complete biodegradation of the dyes molecules(13).

Material and Methods:-

Collection of soil sample:-

To isolate methyl orange dye degrading bacteria the soil samples were collected from different locations preferably nearby textile industries located in and around Baddi, Distt. Solan (H.P). 5 g of soil was collected from each location.

S.No.	Description	Location
1	Soil enriched with dye	Textile industry(Baddi)
2		Dupatta House(Nalagarh)
3		Dupatta staining house(Baddi)
4		Textile industry (Nalagarh)

Isolation of bacteria from different soil samples:-
It was carried out by serial dilution agar-plate method. In this method a known amount (1g) of soil sample was suspended in a known volume (10 ml) of sterile or saline water to make microbial suspension. Serial dilutions 10^2,10^3, …….. $10^{-7}$was made by pipetting measured volumes (usually 1 ml) into additional dilution blanks (having 9 ml sterile/saline water). The contents of tubes were mixed by rolling the tube back and forth between hands for uniform distribution of organisms. From the first dilution, 1 ml of suspension was transferred to the dilution blank 10^{-2} with a sterile 1ml pipette. The procedure was repeated until the original sample has been diluted 10^{-7} times. Poured autoclaved agar medium (supplemented with dye) to 7 Petri dishes and allowed the plates to solidify. 0.1 ml of suspension was transferred to Petri dishes from each dilution and spread with the spreader. These plates were incubated in an inverted position for 24-48 hours at 37°C. Streaking (quadrant) was done on the nutrient agar plates for isolation of pure microbial strains.

Morphological Characterization of isolates:-
After growth the bacterial colonies which appeared on the medium were examined for morphological characterization. Gram staining was performed on each isolate to determine the shape of cells and to know the type of isolate (14)

Biochemical characterization of isolates:- Bacterial isolates were biochemically characterized by Indole, Methyl red,Voges ,Catalase,& Hydrogen sulfide test (Berges Manual ,2010)
$\gamma/_{max}$ of methyl orange dye: Maximum wavelength (γ_{max}) of methyl orange dye was obtained by observing 1 ppm dye in UV-VIS spectrophotometer

Screening of bacterial strains for their Decolourization ability against Methyl orange:-
50 ppm of Methyl orange dye was added to nutrient broth .The above solution was inoculated with different bacterial strains with help of sterilized loop and incubated at 37°C for 24 hours. Observed absorbance (OD) of Methyl orange dye at its γ_{max}.

$$\% \text{ decolorization} = C-T/C*100$$

Where
C-Absorbance of control
T-Absorbance of test

Optimization of the Decolourization condition:-It includes pH optimization & Temperature optimization.

pH optimization:-
Nutrient broth was prepared and 100 ml was added to each beaker. The pH of the broth was set at in the range of 1.0 -11.0 and labeled the flask according to the pH. Transferred the broth from the beakers to the test and control flask in equal amount, add 0.005g methyl orange azo dye in each flask and autoclaved. After cooling the flasks they were inoculated with most efficient bacterial strains and incubated at 37^0 C for 48 hrs. The dye decolourizaion was observed for bacterial strain at the lamda max of dye. Final pH was also recorded.

Temperature optimization:-

Nutrient broth was prepared (pH 6.0).The flasks were labeled with temperature as 25^0 C, 30^0 C,37^0 C,40^0 C and 45^0 C for both test and control flasks. Transferred 100 ml of broth into each flask, Added 0.005g Methyl Orange azo dye into each flask and autoclaved these. Inoculated the test flasks with bacterial strain and then incubated at respective temperatures for 48 hrs. After that, dye decolourizaion was observed at the lamda max of dye.

Rate of Decolourization of methyl orange dye:- Rate of Decolourization was calculated using the formula:

Decolourization rate=C% Decolourization/100t (15).
Where C=Concentration of dye used (mg/l)
T=Time (h)

Results and Discussion:-

We isolated eight different strains & named them as DPI,DPII-----DPVIII.
455.6nm lambda max was observed on UV-Visible spectrophotometer when 1ppm methyl orange dye was scan from 400 nm to 900nm.

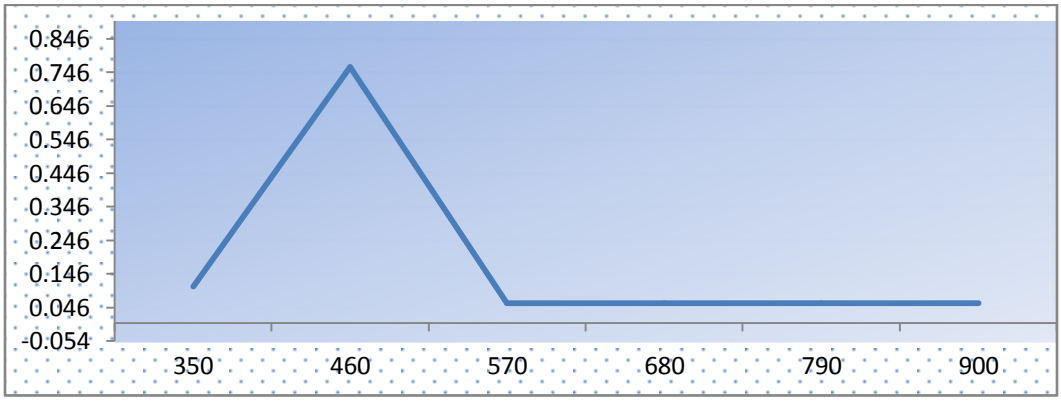

Figure 1:- 455.6nm lambda max was observed on UV-Visible spectrophotometer screening of the most efficient bacterial strain for dye Decolourization.

8 different isolates were then screened to get the best strain to show maximum decolourizaion of methyl orange. The percentage decolourizaion of bacterial strains was calculated after an incubation of 48 hours. It was observed that the strain designated as DP IV showed maximum decolourizaion of methyl orange within 48 hours i.e. 82.15% and the strain designated as DP VI showed minimum decolourizaion (9%).In previous researches many azo dyes have been reported to be degraded by *Bacillus species*, *Bacillus subtilis*showed 80 % decolourization of disperse yellow 211 (16) However 100% decolourizaion of golden yellow dye by *Bacillus species*. 98% decolourizaion of an azo dye, remazol black B was shown by *Paenibacillus species*(17).

Table 1:- Showing percentage decolourization of Strains.

S.No.	Strain code	Media +dye	Incubation	Absorbance after 48 hours		% decolourization
				Control	Test	
1	DP I		At 37°C for 48 hours	1.597	0.536	66.40%
2	DP II			1.597	0.748	53%
3	DP III	50ml		1.597	0.633	60%
4	DP IV			1.597	0.285	82%
5	DP V			1.597	0.965	39%
6	DP VI			1.597	1.451	09%
7	DP VII			1.597	1.405	12%
8	DP VIII			1.597	1.278	19%

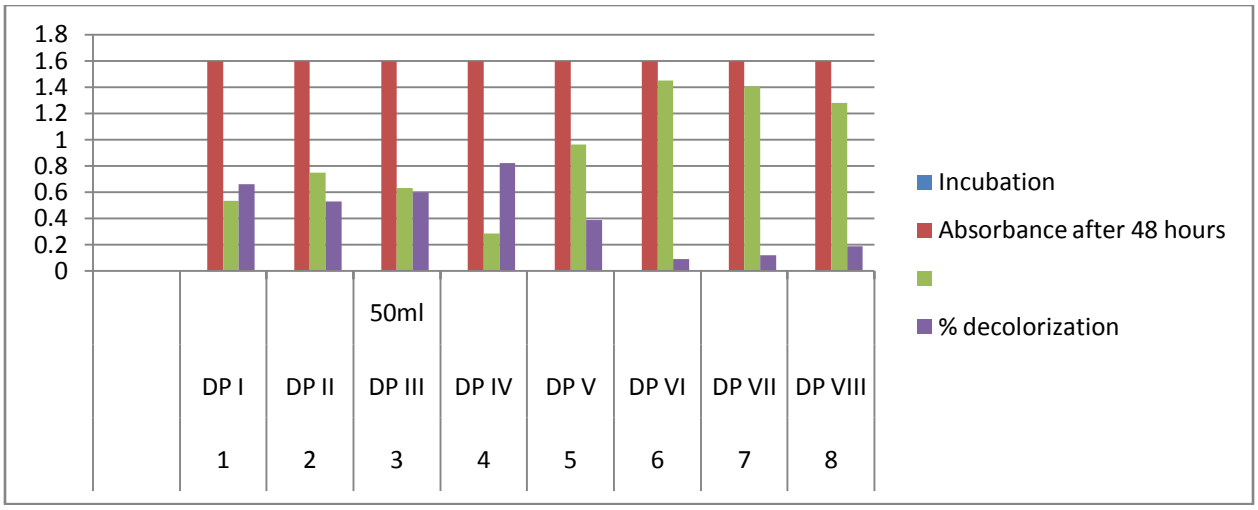

Figure 2:- Graphical presentation of percentage Decolourization of different strains Morphological Characterization of Bacterial Isolates:

TheGram staining method is a useful method for identifying & classifying bacteria into two major groups. The bacterium which retains dark blue color is called Gram positive & those that appear pink with counter stain safranin are referred as Gram Negative. The results of morphological characterization are as below in table.

Table 2:-Morphological Characterization of Bacterial Isolates.

Isolates	Shape	Gram's Reaction
DP I	Rod	-ve
DP II	Coccus	+ve
DP III	Rod	+ve
DP IV	Rod	-ve
DP V	Coccus	+ve
DP VI	spiral	+ve
DP VII	Rod	-ve
DP VIII	spiral	+ve

Biochemical Characterization of Bacterial Isolates: for further identification of bacterial isolates various biochemical tests were performed. Results are given below

Table 3:- Biochemical Characterization of Bacterial Isolates.

Isolates	Indole test	Mr/vp test	Catalase test	Urease test	H2s test	Nitrate test	Citrate test
DP I	-ve	+ve/-ve	+ve	-ve	+ve	+ve	-ve
DP II	-ve	-ve/+ve	-ve	+ve	+ve	+ve	-ve
DP III	+ve	-ve/+ve	+ve	-ve	+ve	+ve	+ve
DP IV	+ve	+ve/-ve	+ve	+ve	+ve	+ve	-ve
DP V	-ve	-ve/+ve	-ve	+ve+ve	-ve	+ve	+ve
DP VI	+ve	+ve/-ve	+ve	+ve	-ve	-ve	+ve
DP VII	-ve	+ve/-ve	+ve	-ve	-ve	+ve	-ve
DP VIII	+ve	-ve/+ve	+ve	-ve	+ve	+ve	-ve

Optimization of Decolourization conditions:-

DP IV the best degrader of Methyl Orange was subjected to optimization studies.The decolourization of Methyl Orange was found to be 87.13% at pH 6.0.100% decolourization of methyl orange was observed at pH optimized at 6.8.(18).However 92% decolourization was observed by *Bacillus cereus*at pH 7.0 was observed (19)

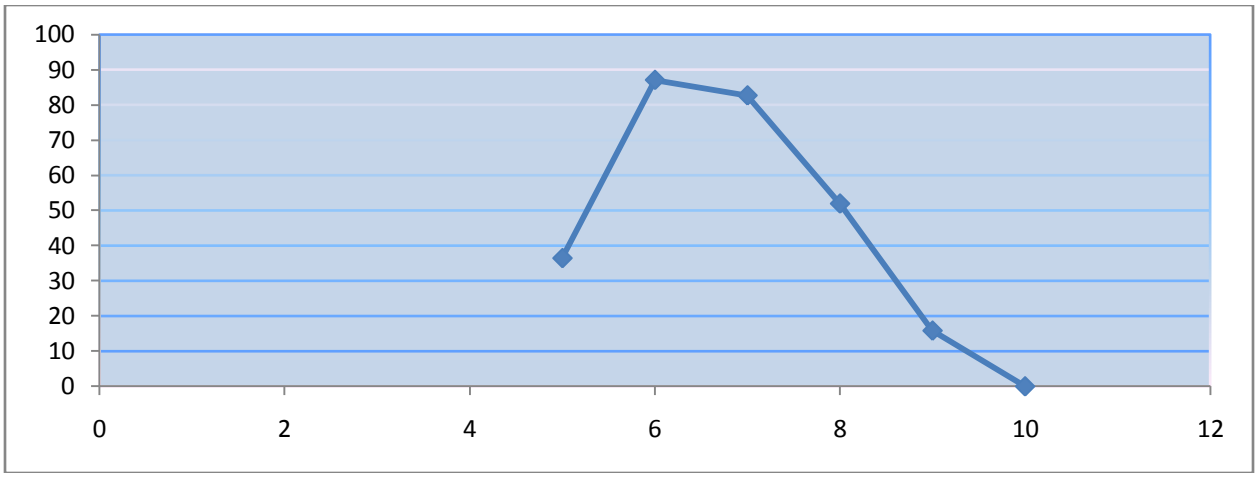

Figure 3:- pH Optimization.

Temperature Optimization: The maximum decolourization of methyl orange reached 94.23% at 37°C with pH 6.0.Another research showed decolourization of methyl orange by *Bacillus sp*.at30°C(20). However maximum decolourization of methyl orange by *Lactobacillus casei*at temperature optimized at 35 °C

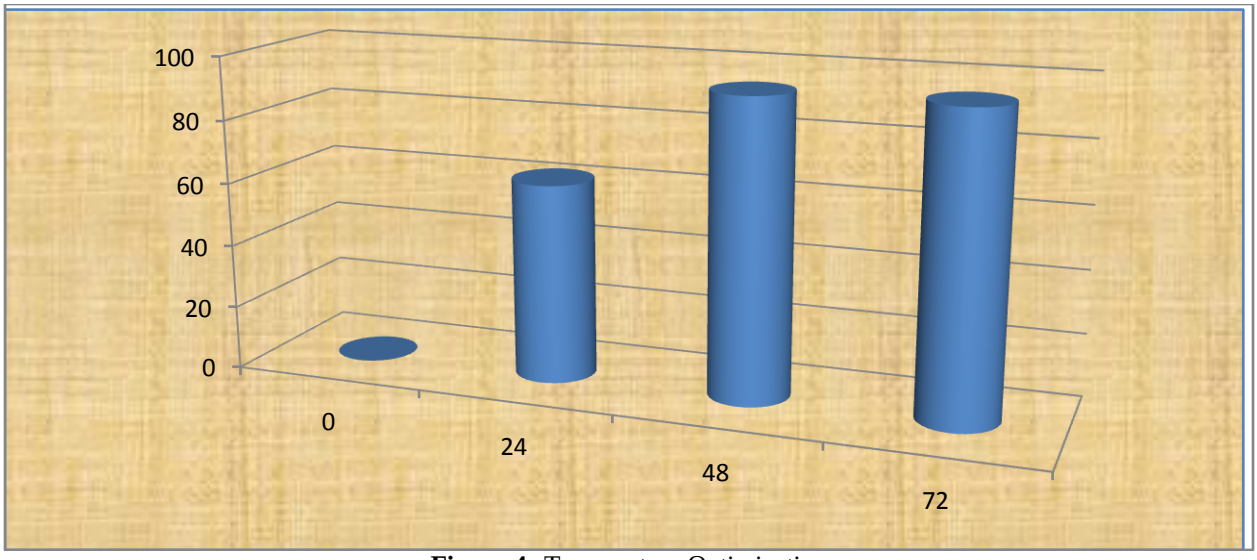

Figure 4:-Temperature Optimization

Rate of Decolourization of Methyl Orange Dye: As shown in table no.4

Table 4:-%age Decolourization at different time intervals: as shown in table below

S.No.	Incubation	Absorbance at 455.6nm		% Decolourization
		Control	Test	
1	0	1.579	1.579	0
2	24	1.589	0.589	62.40
3	48	1.597	0.092	94.23
4	72	1.623	0.082	94.94

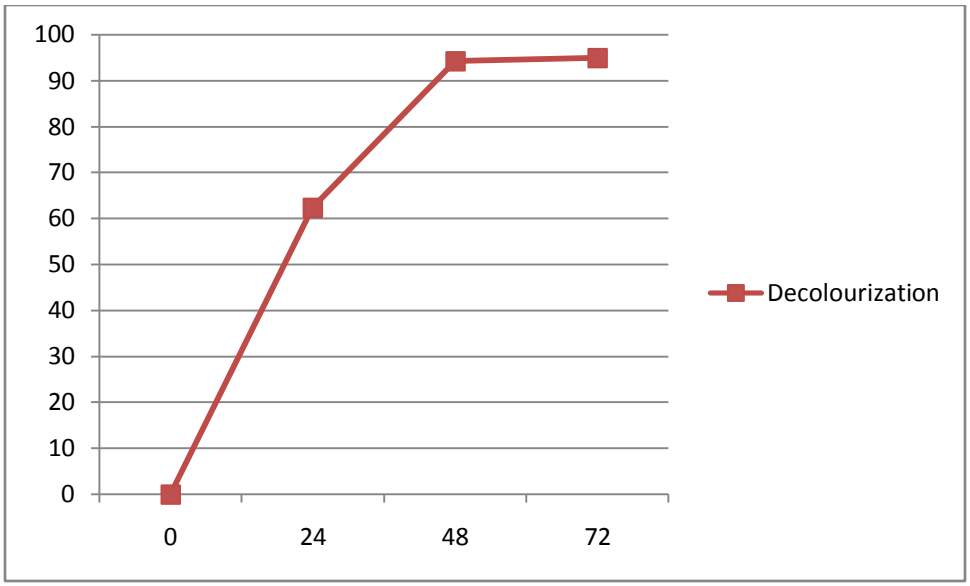

Figure 5:-Percentage Decolourization

The rate of Decolourization of methyl orange was calculated as 0.66mg/lt/hr.Thus it is evident from cited results that Bacillus sp. could decolorize methyl orange approximately 94.94%& temperature 37°C with decolorizing rate of 0.66mg/lt/hr. thus can be used as a tool for decolourizaion & further research with respect to its commercial application

Conclusion:-

Dyes are widely used in textile, rubberproduct; paper cosmetics& many other industries,Methyl orange represent the largest &most versatile class of synthetic dyes.Some of the dyes are harmful, dye containing wastes important environmental problem.These dyes are poorly biodegradable because of their structures& treatment of wastewater contain dyes usually involves physical & chemical methods such as adsorption, oxidation& electrochemical methods. Pseudomonas sp.shows higher activity on dyes selected (21), this was the first time in which growth on textile dyes of these microorganisms was reported. *Staphylococcus areta*could only decolorize the dyes effectively when the medium was supplemented with yeast extract (22)

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