COMPARATIVE SCREENING OF DYE DEGRADATION USING BACTERIA AND FUNGI

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ABSTRACT

India's dye industry produces every type of dyes and pigments.Production of dye stuff and pigments in India is close to 80,000 tons.India is the second largest exporter of dye stuffs and intermediates after china .the textile industry account for the largest consumption of dyestuffs.During drying process 5-10% of dyes are released in textile wastewater streams which is ultimately, reach to the receiving natural wastebodies . Textile industries are reported to use the largest amount of dyes (60-70%) compared to other industries. In this present study the isolates obtained from waste water collected from textile industry was characterized morphologically and biochemically. Based on the comparison of biochemical characteristics with standard description the bacterial isolates was identified as *Bacillus* species and fungal isolates was identified as *Aspergillus.* The isolate such as bacillus and Aspergillus species exhibit different pH, temperature and inoculums response to the decolorization activity.In pH high level of decolourization was observed in pH6 and pH7. In temperature high amount of decolourization is seen best at 25ºC. In inoculums decolourization high dye decolorization was observed in 500µl. In our present study shown better effect in inoculums, temperature, pH and showed 80% of decolorization in both bacteria and fungi.

Keywords: Dye decolorization, pH, temperature, inoculums.

INTRODUCTION

For centuries ,mankind has been using dyes and by Neanderthal man the very first proof of dye use came about 18,000 years back.Azodyes are the most commonly used dye in the textile ,food, leather, cosmetic industries.Industrialization plays a vital role in nation's economy and it also serves as a vehicle for development and growth of the nation. However there are associated problems resulting from the introduction of industrial wastes into the environment. There are a number of industries in India and most of the available industries (textile, paper, leather, food, cosmetics) directly release their untreated or partially treated wastewater into nearby rivers, drains, ponds, lagoons or lakes . Among the various industries, textile industries discharge large volume of wastewater after dyeing process. It is considered to be one of the largest generators of toxic chemical wastes in India .

In the present study was focused on decolourization of dye using bacteria and fungi isolated from textile dye effluent and determine the parameters like effect of different inoculums, effect of different pH, effect of different temperature.

METHODOLOGY

Sample collection

The untreated effluent was collected . The sample were collected in sterile plastic bottles, transported to the laboratory within 1hour and stored at 4ºc.

Enrichment of samples

The collected samples were enriched by inoculating 5 ml of sample into 100ml of nutrient broth with 0.002 gm/l methylene blue and phenol red.The flasks were incubated for 48h under shaking condition**.**

Isolation of dye decolourizing bacteria

After 48h of incubation, 1 ml of culture broth was diluted and plated on agar plates. Plates were incubated at 37ºc for 24h. The morphologically distinct bacterial isolates showing clear zones around their colonies due to decolourization of dye were selected for further studies. The pure cultures were stored below 10ºC on nutrient agar slopes.

Morphological characterization

Gram staining

- A smear of bacterial culuture was prepared, air dried and heat fixed.
- The smear was flooded with crystal violet solution for 30 seconds and rinsed in tap water for 2 seconds.
- The smear was then flooded with iodine solution for 30 seconds and rinsed with tap water.
- The smear was washed with 95% ethanol or acetone for 10-30 seconds and rinsed in tap water for 2 seconds.
- The smear flooded with saffranin solution for 30 seconds and rinsed in tap water for 2 seconds.
- It was air dried and observed under the microscope**.**

Biochemical characterization

Indole production test

- Tryptophan water was prepared and sterilized and incubate the tubes at 37ºc for 48 hours.
- After incubation, kovac's reagent was added and result were noted.

Methyl Red Test

- To detect the acid production by the test organism.
- The isolate was inoculated into sterilized MR-VP broth and incubated the tubes at 37ºc for 48 hours.
- After the incubation period 2-3 drops of methyl red reagent was added.
- The results were noted.

Voges- Proskauer test

- To detect the production of non-acidic end product by the test organism.
- The isolates were inoculated into MR-VP broth and incubated the tubes at 37ºc for 48 hours.
- After the incubation period add 2-3 drops of barrits reagent was added.
- The result were observed.

Citrate Utilization Test

- To detect the ability of citrate utilization by the test organism.
- Simmon citrate agar medium was prepared and sterilized.
- The slants was prepared and inoculated with bacterial culture and incubated for 24 hours.
- The results were noted.

Fungal isolation and identification

Isolation of dye decolourizing fungi

Textile wastewater was collected in a sterile container. Decolourization activity of fungi was detected by plate using Potato Dextrose Agar (PDA). The Potato Dextrose Agar medium were prepared and sample were inoculated. The plates were incubated at room temperature for 4-6 days at 25-30ºC.

Identification of fungi

- A clean glass slide was taken.
- A drop of lactophenol cotton blue stain was placed on the glass slide.
- By using a sterile needle a fungal mycelial mat was placed on the glass slide.
- A coverslip was placed over the slide.
- Observed it under low and high power objective microscope.

Decolourizationof dyes by individual strains under static and static incubation

Nutrient broth medium was prepared and 100ml of the medium was dispensed in 250ml of Erlenmeyer flasks. The medium was sterilized at 1 atmospheric pressure for 15 minutes. All the dye methylene blue and phenol red were filtered and sterilized and added to the nutrient broth medium individually at the concentration level of 0.5g/L in aseptic manner. To this, 1.0ml of the bacterial and fungal culture were inoculated and in 37ºc for 24 hours and 30ºC for 3 days. Control flask containing without inoculums were also maintained. The samples were then analyzed for percent decolourization after incubation.

Measurement of percentage decolourization

After incubation samples were filtered and centrifuged at 3500 rpm for 10 minutes and the suspended biomass was separated. The absorption spectra was measured at the lambda max of the dyes for the clear supernatant using a spectrophotometer. Medium containing dyes without the inoculum was maintained as control. Percent decolourization was calculated with the following formula taking into consideration in the initial and the final absorbance value of the dye.

Percentage decolourization =

Initial absorbance – final absorbance

$$\frac{\text{Initial absorbance value}}{\text{Initial absorbance value}} \times 100$$

Effect of different inoculum on growth and decolorization

25 ml of the decolourizing media was prepared and adjusted for pH 7 and sterilized at 1 atmospheric pressure for 15 minutes. The flasks were inoculated with different inoculums 125µl, 250µl, 375µ, 500µl of bacterial cultures were incubated at 37ºc under static condition for 24 hours. Appropriate control flask without inoculum was maintained. After incubation percentage of decolorization and growth were estimated.

Effect of pH on growth and decolourization

25 ml of the decolourizing medium was dispensed in 100ml of flask and pH was adjusted to 5, 6, 7, and 8 adding 0.1N HCL or 0.1N NAOH, autoclaved at 1 atmospheric pressure for 15 minutes. Methylene blue and phenol red was added in separate flask and inoculated with 0.25ml of inoculums. Control flasks containing dyes without inoculums were maintained. The flask was incubated in static condition at 27ºc for 24 hours. After incubation period percent decolonization and growth were calculated. The optimum PHfor growth in terms of optical density and decolorization of the dyes were observed

Effect of different temperature for dye decolorization

25 ml of the decolorizing medium was dispensed in 100ml of flask and different temperature like 25ºC, 30ºC, 40ºC. Inoculation of culture dye concentration was concentrate used. Methylene blue and phenol red was added in separate flask and inoculated with 0.25ml of inoculums. All the flask were incubate at temperature like 25ºC, 30ºC, 40ºC for 24 hours. After incubation decolourization and growth was calculated.

RESULT

Table : 1 Dye decolourization and growth in different inoculums – Bacteria

Dye concentration	% of inoculums concentration	Methylene blue			Phenol red		
		OD value			OD value		
		Initial value	Final value	% decolorization	Initial value	Final value	% decolorization
0.01	1.0	1.45	0.147	89.97			
0.01	1.5	1.96	0.434	77.81			
0.01	2.0				1.568	0.458	88.37
0.01	2.5				1.256	0.347	72.37

Table: 2 Dye decolourization and growth in different pH – Bacteria

pH levels	% of inoculums conc.	Methylene blue			Phenol red		
		OD value					
		Initial	final	% of decolorization	Initial	Final	% of decolorization
5	0.5g	1.460	0.88	39.72			
6	0.5g	1.460	0.190	86.98			
7	0.5g				0.566	0.176	68.90
8	0.5g				0.566	0.194	65.72

Table :3Dye decolourization and growth in different temperature – Bacteria

Temperature	% of inoculums conc.	Methylene blue			Phenol red		
		OD value			OD value		
		Initial conc.	Final conc.	% of decolorization	Initial conc.	Final conc.	% of decolorization
25ºC	0.5g	1.468	1.460	0.544	0.567	0.07	87.47
35ºC	0.5g	1.468	0.348	76.29	0.567	0.07	86.4
45ºC	0.5g	1.468	0.451	69.27	0.567	0.08	85.53

Table :3Dye decolorization and growth in different inoculums – fungi

Dye conc.	% of decolorization	Methylene blue			Phenol red		
		OD value			OD value		
		Initial	final	% of decolorization	Initial	final	% of decolorization
0.001g	1.0	1.507	0.55	64.96			

0.001g	1.5	1.110	0.45	40.540			
0.001g	2.0				1.622	0.582	64.11
0.001g	2.5				1.300	0.468	64.38

Table :4Dye decolorization and growth of different pH – Fungi

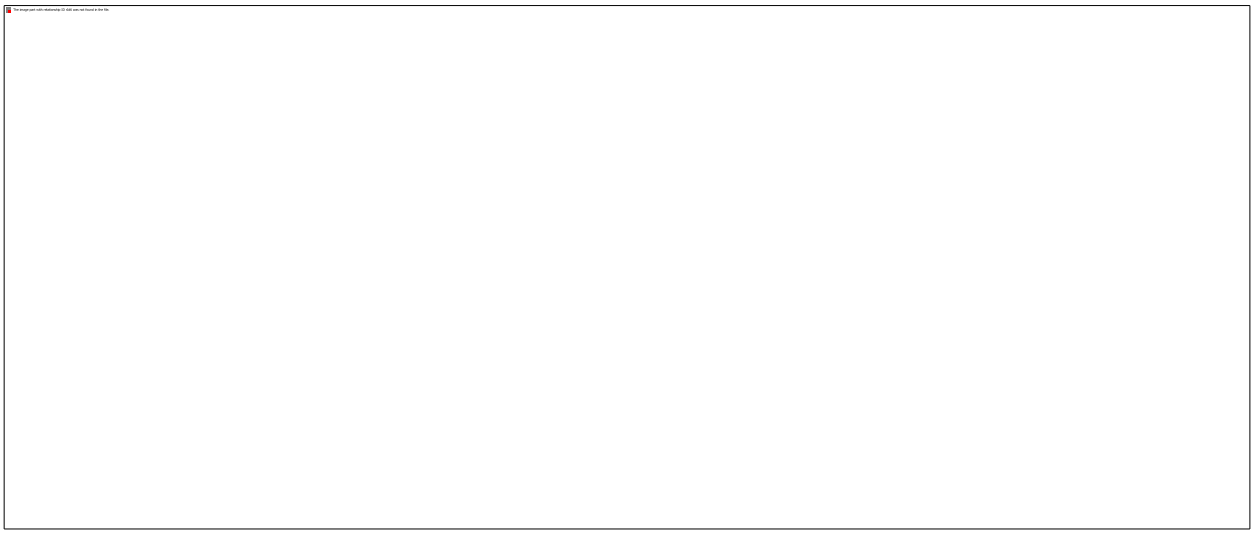
pH levels	% of inoculums conc.	Dye conc.	Methylene blue			Phenol red		
			OD value			OD value		
			Initial	Final	% of dye decolorization	Initial	Final	% of dye decolorization
5	0.5%	0.01g	1.501	0.92	38.7			
6	0.5%	0.01g	1.501	0.80	46.2			
7						0.572	0.187	67.30
8						0.572	0.197	65.55

Table : 5 Dye decolorization and growth in different temperature – Fungi

Temperature	% of inoculums in conc.	Dye Conc.	Methylene blue			Phenol red		
			OD value			OD value		
			Initial	final	% of decolorization	initial	final	% of decolorization
25ºC	0.5	0.01g	1.470	0.40	72.78	0.570	0.572	0.34

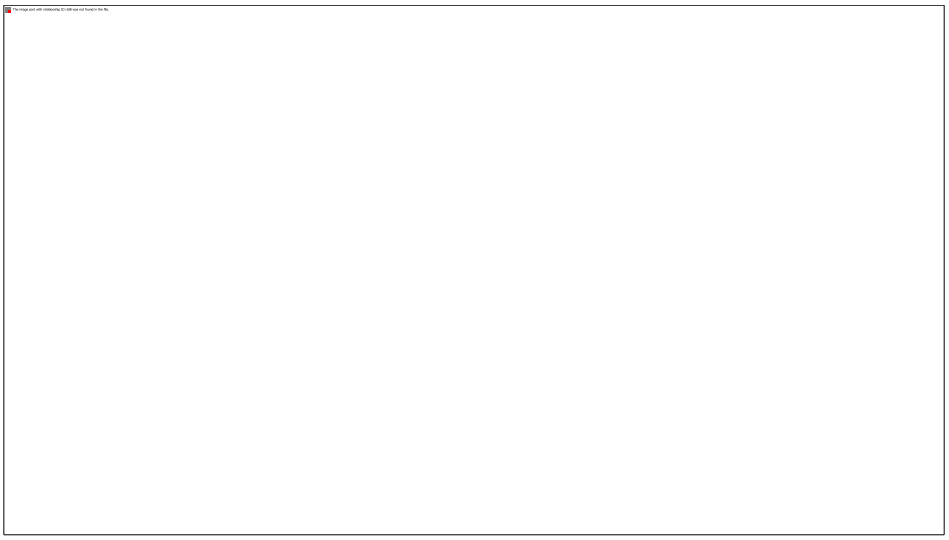
35ºC	0.5	0.01g	1.470	0.40	74.14	0.570	0.562	1.40
45ºC	0.5	0.01g	1.470	0.40	71.42	0.570	0.462	18.42

Enrichment of sample

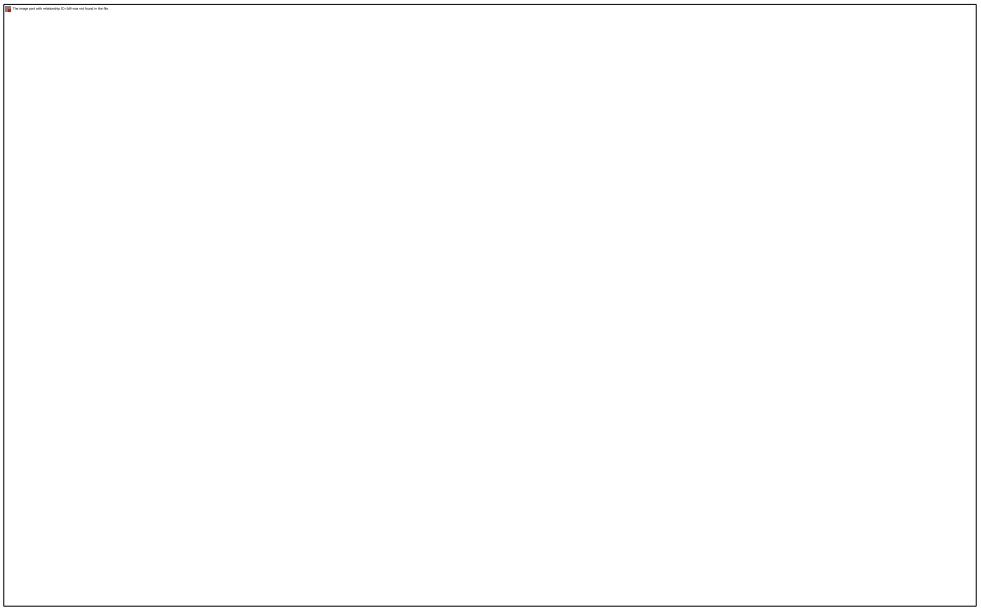


Effect of different inoculums on growth and decolourization–Bacteria

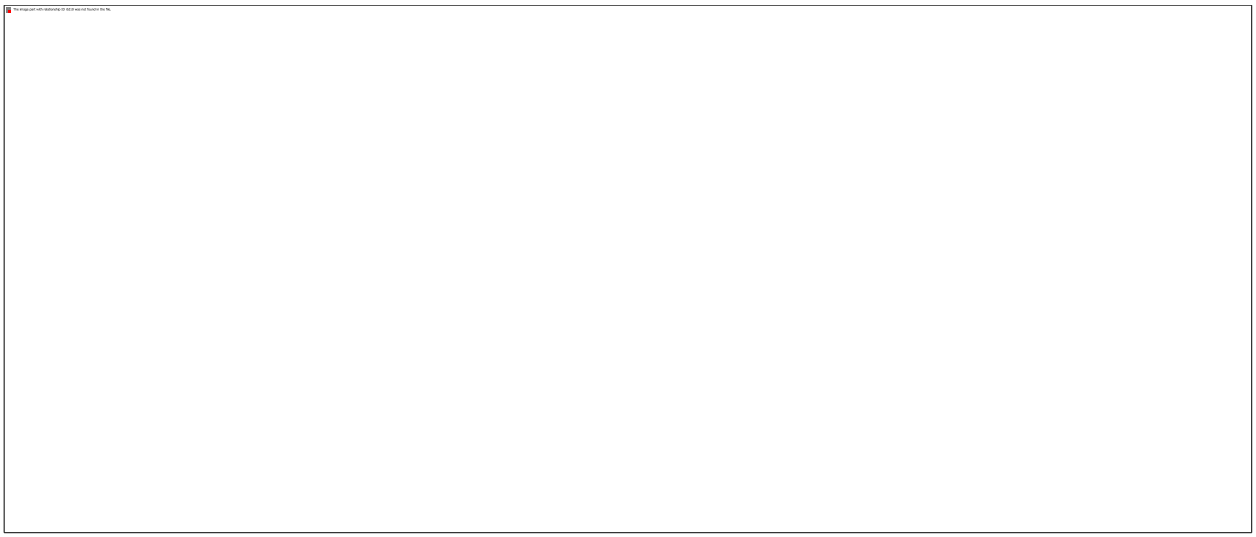
Effect of different temperature on growth – Bacteria



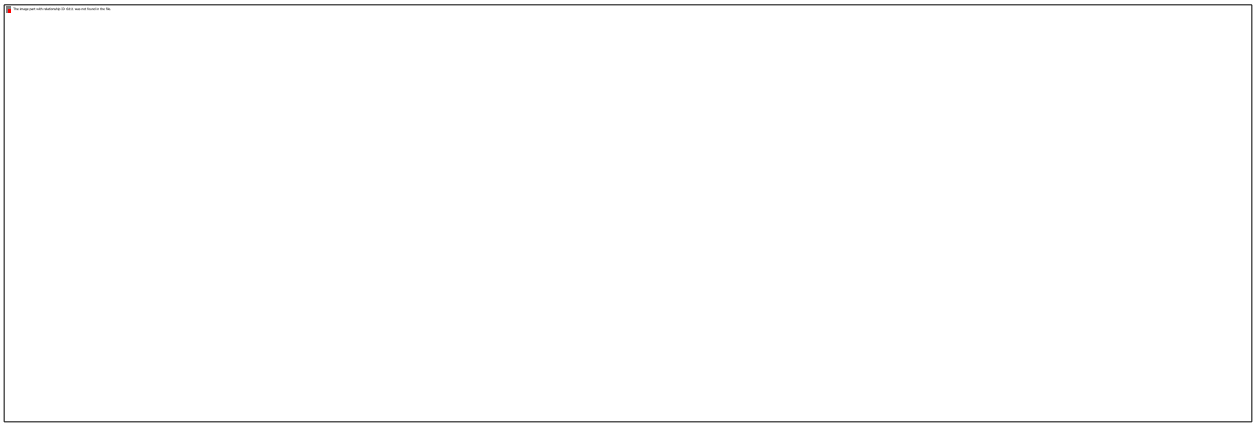
Effect of different inoculums on growth and decolourization –Bacteria



Effect of different inoculums on growth and decolorization – Fungi



Effect of different temperature on growth and decolorization fungi



DISCUSSION

In this present study the isolates obtained from waste water was characterized morphologically and biochemically. Based on the comparison of biochemical characteristics with standard description the bacterial isolates was identified as *Bacillus* species and fungal isolates was identified as *Aspergillus.* The isolate such as bacillus and Aspergillus species exhibited different pH, temperature and inoculums response to the decolorization activity.

The decolorization of methyl red and navy blue by using different inoculums the maximum decolorization shown in both methyl red and navy blue maximum of 90.2% and 80.1%.the effect of decolorization depends upon the inoculums concentration because of inoculums level of decreased the decolorization level also decreased. studied the effect of pH dye decolorization.

The pH tolerance of bacteria is quite important because of azo dye bind to cotton fibres by addition and at high temperature.the result was compared with azo dye reducing species like *bacillus, citrobacter, pseudomonas.*

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