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Comparative Indigenous Study of Azo Dye (Blue, Black, Red, Yellow) Using Pseudomonas and Bacillus Species

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Abstract:
Bioremediation is the microbial clean up approach , microbes can acclimatize themselves to toxic waste and new resistant strain develop naturally , which can transform various toxic chemical to less harmful forms . Bacterial degradation of azo dyes is often initiated by cleavage of azo bonds by azo reductase which are followed by the aerobic degradation .

Key Words : Toxic , Bacteria , Degradation, Azo , Dye , Bioremediation , Reductase .

1. INTRODUCTION:

Azoreductases are the enzymes which catalyses the reductive cleavage of azo bonds to produce aromatic amine products . Azoreductases are observed in many organism , including the rat liver enzyme , rabbit liver . Azoreductase is the key enzyme responsible for the reductive azo dye degradation in bacterial species .
Synthetic dyes are widely used in textile , paper making , colour printing , food leather and cosmetic industries . The three most common group of dyes are azo , anthraquinone and phthalocyanine which are considered toxic to human beings and environment as well .
Thousands of azo dyes are available on the market, and more than 500 of them contain possibly carcinogenic aromatic amines in their chemical makeup. When these chemicals reach the human body through eating, skin contact, or injection, azoreductases in the gastrointestinal system and mammalian liver convert them to aromatic amine. (2) A survey indicated that 90% of the 4,000 dyes studied had L.D 50 values more than 2000 mg/kg, with basic and diazo dyes having the highest toxicitie

2. Materials and Method

Isolation of bacteria from soil:
The isolation of the bacteria was done by collecting the soil sample .The soil sample collected was gone under serial dilution .In serial dilution 1gm of soil is measured and 7 test tubes are taken .In the first tube the soil sample is mixed with the water and mixed well after mixing 1ml is poured in to the 2nd test tube the same procedure is followed till the last test tube and from the last test tube the water is thrown out .From the last 4 test tube 1 ml is mixed in the petri plate which contains nutrient medium .From the 4 petri plates the 5th plate is considered as control which only contains nutrient medium .
After the plates are ready they are sealed tightly and kept in incubator for 24 hrs .After 24 hrs the incubation the plate are removed and are seen whether the bacteria growth happened or not .

Identification of soil flora by phenotypic method :
The isolated soil bacteria are identified and Colonies of the bacteria on the nutrient plate were identified to the genus level using cultural morphology (surface appearance , size, shape, pigmentation , gram staining reaction, a biochemical test scheme for gram positive(catalase, coagulase, starch hydrolysis) test are done .

To identify gram negative bacteria the following test are as follows (urease, methyl red, vogues Proskauer and sugar fermentation). test are done.

The various test to identify gram negativeare as follows:

S.NO	TEST	RESULT
1	Gram -stain	-
2	Oxidase test	+
3	Catalase test	+
4	Pigments production	+
5	Haemolysis	+
6	Indole test	-
7	Methyl -red	-
8	Voges- Proskauer	-
9	Simmon's citrate	+
10	Urease production	-
11	H_2S production	-

The test to identify gram positiveare as follows:

S.NO	CHARACTERISTICS	RESULT
1	Gram-positive	+
2	Catalases	+
3	Reduction of nitrate	+
5	Acid from: Sucrose	+
6	Fructose	+
7	Glucose	+
8	Lactose	+
9	Indole production	-
10	Motility	+
11	Citrate utilization	-
12	H_2S production	-

Gram negative bacteria can cause many serious infections such as Pneumonia,Peritonitis (inflammation of the membrane that lines the abdominal cavity), Urinary tract infection ,bloodstream infections ,wound or surgical site infection ,meningitis.

Gram negative bacteria are becoming resistantly to antibiotics. Bacteria may be resistant because of any of the following :

- They are naturally resistant to certain antibiotics .
- They acquire genes from bacteria that have become resistant .
- Their genes mutate

Gram – negative bacterial infections include the following :

- Brucellosis
- Campylobacter infections
- Cat-scratch diseases
- Cholera
- Escherichia coli. Infections
- Plaque
- Pseudomonas infection
- Salmonella
- Typhoid fever

Gram positive bacteria are increasingly becoming resistant to antibiotics .For example , methicillin-resistant staphylococcus aureus .Bacteria are resistant to most antibiotics that are needed to penicillin .Methicillin is a type of penicillin .MRSA strains are commonly involved in infections acquired in health care facilities and cause infections acquired outside health care facilities .

Gram -positive bacterial infection include the following :

- Anthrax
- Diphtheria
- Enterococcal infections
- Erysipelothricosis
- Listeriosis
- Pneumococcal infections
- Staphylococcal aureus infection
- **Application of azo – reductase producing bacteria for the removal of azo dye (blue, black ,red and yellow).**

Pseudomonas aeruginosa

After identification of the bacteria in a conical flask all the four types of the dye that is azo (red , blue , black and yellow)are mixed with 100ml of nutrient broth which contains *Pseudomonas* species and all these mixture are kept in the shaking incubator for 12 hrs .

After incubation of 12hrs the colour change is observed and a O.D value is taken in the spectrophotometer and the lambda max is identified .The same procedure is followed for another species that is *Bacillus.*

Bacillus subtilis

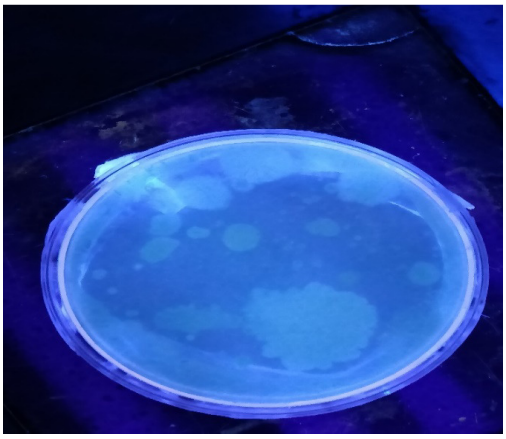
The same procedure is followed for the *Bacillus* species .The *Bacillus* species is mixed with the 4 types of different dye in the conical flask and it is kept in the shaking incubator for 12 hrs .

After 12 hrs the conical is observed for the colour change and the colour change is observed in blue and yellow colour .The yellow colour is changed in to pale yellow and in the blue colour it is changed in to the brown colour and the following colour change is observed under spectrophotometer for O.D value .

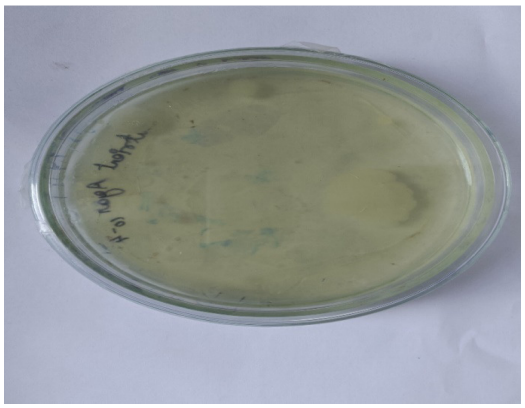
- **Result and Discussion:**

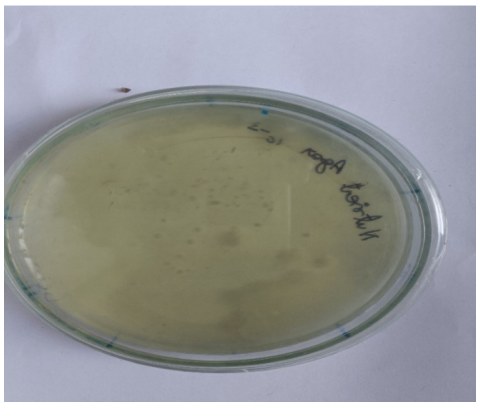
The following result for this study are as follows :

Isolation of bacteria

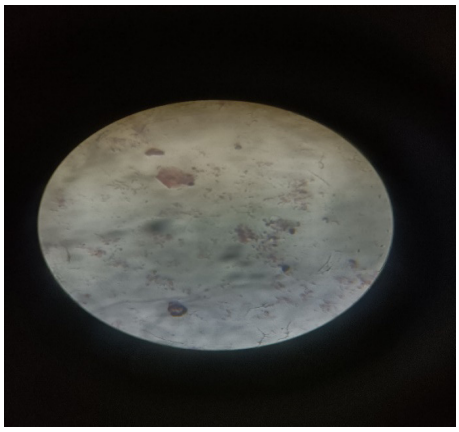


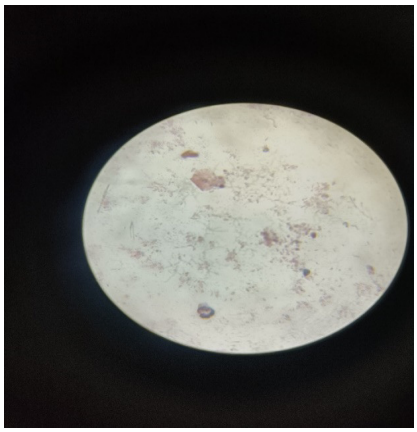
Growth of bacteria





Gram Staining of bacteria – Negative stain





Mixture Of Dye with Bacteria(*Pseudomonas)*

Mixture Of Dye With Bacteria *(Bacillus)*

Change of colour from blue to brown.

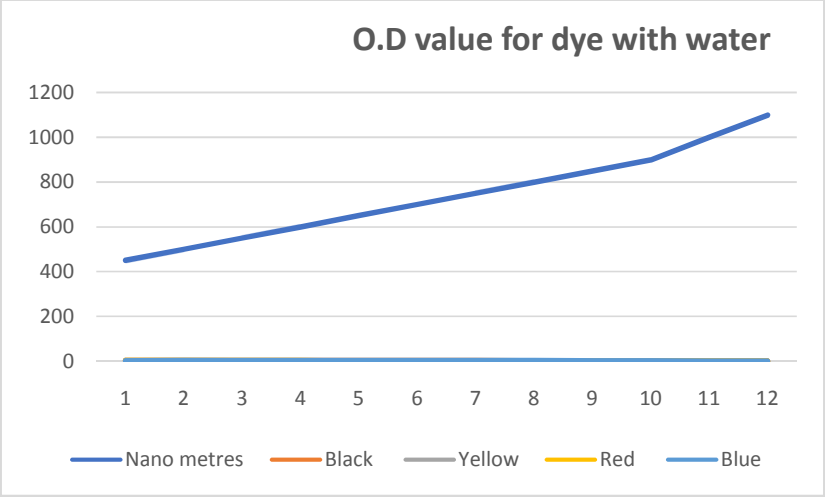
Change of colour from yellow to pale yellow

- **Assay of Azoreductase activity:**

Assays were carried out in cuvettes with the total volume of 1ml using Ultrospec 2100 UV-VIS Spectrophotometer .The reaction mixture consist of 400 ml of potassium buffer with 200 ml of sample, and 200ml of reactive dyes(500mg/L). The reaction was started by addition of 200ml of NADH (7mg/L) and was monitored photometrically at 502 nm. The linear decrease of absorption was used to calculate the azoreductase activity. One unit of azoreductase can be defined as the amount of enzyme require to decolorize 1mol of acid red per minute.

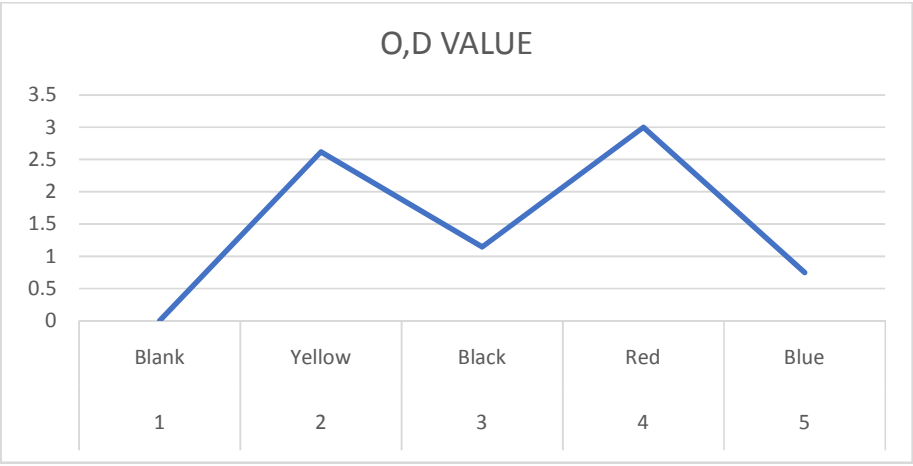
O.D values of the dye with water before treatment

S.NO	Nano metres	Black	Yellow	Red	Blue
1	450	2.352	2.536	2.550	2.512
2	500	2.704	2.709	2.750	2.698
3	550	2.726	0.829	2.567	2.753
4	600	2.804	0.489	2.524	2.863
5	650	2.941	0.387	0.438	2.919
6	700	3.000	0.321	0.216	3.000
7	750	3.00	0.283	0.143	3.000
9	800	1.317	0.261	0.108	3.000
10	850	0.731	0.245	0.091	2.197
11	900	0.566	0.231	0.073	1.855
12	1000	0.409	0.201	0.54	1.374
13	1100	0.348	0.189	0.048	1.095



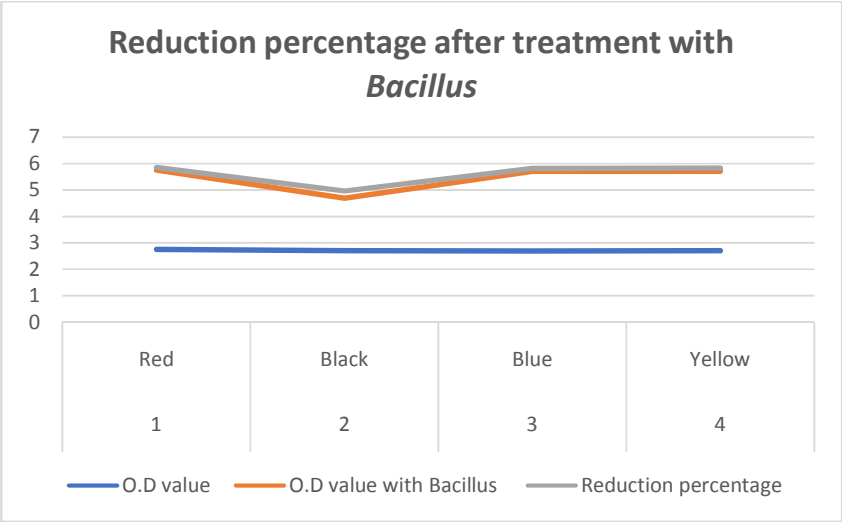
O.D value for NADPH and potassium phosphate buffer

SNO	COLOUR	O,D VALUE
1	Blank	0
2	Yellow	2.618
3	Black	1.141
4	Red	3
5	Blue	0.746



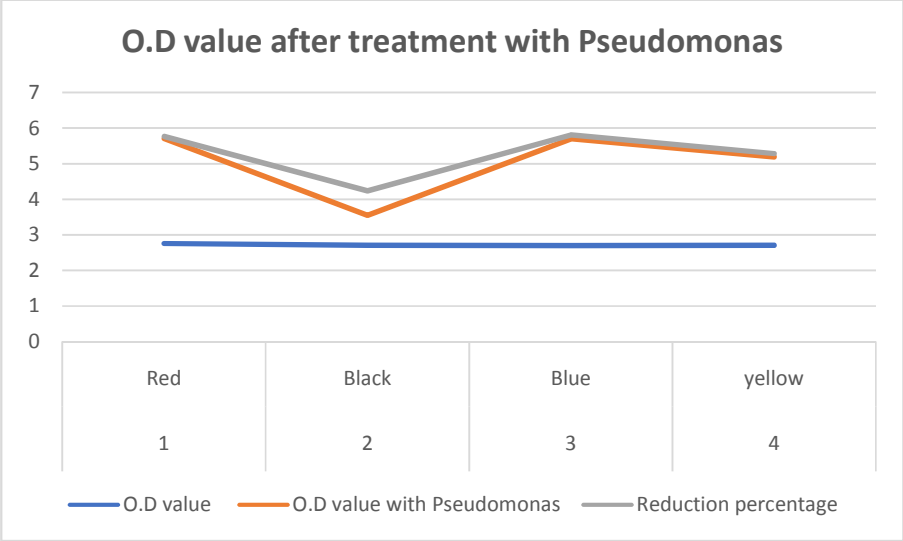
Reduction percentage after treatment

S.NO	Positive control	O.D value	O.D value with Bacillus	Reduction percentage
1	Red	2.753	3	8.97%
2	Black	2.704	1.984	26.6%
3	Blue	2.698	3	11.4%
4	Yellow	2.709	3	10.7%



Reduction percentage after treatment with *Pseudomonas aeruginosa*

S,NO	Positive control	O.D value	O.D value with Pseudomonas	Reduction percentage
1	Red	2.753	2.951	6.70%
2	Black	2.704	0.85	68.5%
3	Blue	2.698	3	11.19%
4	yellow	2.709	2.484	9.05%



- **Conclusion:**

The present study concluded that collected effluent were good sources of dye degrading bacteria. Out of four dye sample*Bacillus*was good for the treatment of black dye . Whereas the *Pseudomonas* was suitable for yellow azo dye.

- **Acknowledgement :**

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