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Microbial Decolourization of Dyes by Indigenous Bacteria Isolated from Industrial Waste

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Abstract

Azo dyes are the largest class of dyes commercially used in the textile industries. The presence of dye in water even at very low concentration is highly visible and affects the quality, water transparency and gas solubility of water bodies and also contribute to soil pollution in a major way. The present study deals with the isolation and screening of bacterial species capable of decolourize variety of dyes. Seven different dyes were used out of which five dyes were decolourized namely Acid Black 210, Acid Red 18, Acid Orange 10, Malachite Green, Reactive Black 5. Out of seventeen decolourizing bacterial isolates, nine morphologically distinct isolates were selected. These nine bacterial strains namely A1, A2, B1, D1, F1, F2, F3, G1 and G2 were isolated through enrichment technique by using different dyes having concentration of 100ppm in Nutrient Broth. Among these dyes, Malachite green and Acid Red 18 dyes were decolourized at highest percentage. Different parameters were used for optimization such as different carbon (Glucose, Fructose, Lactose), nitrogen source (Peptone, Yeast extract, Beef extract), inoculum size (1%, 3%, 5%), temperature(10°C, 32°C, 50°C), pH (4, 7, 10) and dye concentration (200ppm, 400ppm, 600ppm). The results obtained with spectrophotometric data prove the efficiency of bacteria suggesting its possible use in treating dye containing effluents.

Inroduction Dye, substance used to impart colour to textiles, paper, leather, and other material such that the colouring is not readily altered by washing, heat, light, or other factors to which the material is likely to be exposed. Dye molecules are comprised of two key components: the chromophores, responsible for producing the colour, and the auxochromes, which can not only supplement the chromophore but also

render the molecule soluble in water and add an enhanced affinity toward the fiber. Azo dyes comprises a diverse group of synthetic chemicals that are widely used in textile , paper, food,

cosmetics and pharmaceutical industries(Zollinger,1987;Careliell et al.,1995).The inefficiency in dyeing processes has resulted in 10-15% of unused dyestuff entering the wastewater directly(Zollinger, 1987; Spadarry et al.,1994).Their discharge into surface water also leads to aesthetic problems, obstructing light penetration and oxygen transfer into water bodies (Slokar and le Marechal 1998; Bae and Freeman 2007). Without adequate treatment, these dyes are stable and can remain in the environment for an extended period of time. Therefore, this effluent must be treated before discharge into natural water streams. The general structural characteristics of these compounds feature substituted aromatic rings that are joined by one or more azo groups (-N=N-).They are generally recalcitrant to biodegradation due to their complex structures and xenobiotic nature. Many azo dyes and their degradation intermediates are mutagenic and carcinogenic (Chung and Cerniglia 1992; Ozturk and Abdullah 2006).As a viable alternative, biological processes have received increasing interest owing to their cost effectiveness, ability to produce less sludge and environmental benignity (Banat et al., 1986).The first stage involves cleavage of azo bond under microaerophilic (static) or anaerobic conditions, leading to formation of aromatic amines. This step leads to the decolorization of azo dyes. Further degradation of aromatic amines generally requires aerobic condition (Singh et al.,2007, Van der Zee and Villaverde, 2005).

Materials and Method

Chemicals: Total 7 dyes and their symbols given: Acid Black 210 – A**,** Acid Red 18 – B,Acid Red 52 (Rhodamine) – C, Acid Orange 10 – D, Green B (Acid green 16) – E, Malachite Green – F, Reactive Black 5 – G were collected from "Adorn Specialist, Naroda, GIDC, Ahmedabad. All other chemicals used were of analytical/reagent grade.

Cultural medium: Nutrient Broth, Potato Dextrose Broth (PDB) for fungi, Glucose Yeast Extract (GYE) Broth for Yeast.

Collection of Samples: Effluent samples were obtained from various sources such as :

1)EF1 = 100MLD CETP Narol Project, L & T construction, Gyaspur village.

2)EF2 =Adorn Speciality, NarodaGIDC ,Naroda.

3)EF3 =S 6, Om arcade, Nr. Vastral cross road, S P ring road, Vastral.

All the samples were collected in sterile glass-screw cap tubes and preserved at 4°C in refrigerator.

Enrichment and isolation of microbial dye decolourizers:

All effluent samples were used for isolation of dye decolourizing microbial cultures by enrichment techniques using Nutrient Broth, GYE Broth and PDB containing respective 7 dyes with the final dye

concentration of 100 ppm. The enrichment was carried out in 100ml of above mentioned broths in 250 ml Erlenmeyer flask [98 ml sterilemedia (Nutrient broth, GYE and PDB)+1 ml respective dyes +1 ml effluent(EF1,EF2 and EF3)]. The culture flasks were incubated at 32±2°C for 48 hours under static condition. Bacteria, fungi and yeasts were not showing results

in EF2 and EF3 containing flasks. Fungi and yeasts were also not showing result in EF1 flasks.

Primary Screening:

For primary screening, different dilutions(10^{-2} to 10^{-5}) of supernatant from the flasks showing decolourization of an added dye were spreadon nutrient agar plates containing 100 ppm of respective dyes and incubated at 32 ± 2°C for 48 hours.The morphologically distinct bacterial isolates showing a clear zone of the decolourization in respective dye were selected.

Secondary Screening:

Secondary screening studies were conducted with selected distinct isolates by inoculating 10 ml of dye containing broth medium in test tubes with one loopful of activated culture.The Nutrient broth having respective dyes which gives decolourization during conformation test was used further. The nutrient agar plates having dyes were streaked by direct streaking method from the broth giving decolourization and incubated at 32±2°C for 24 hours. Next day observe the plates.

The colonies having different morphological characters and showing zone of decolourization were selected.

Growth and Colony Charactristics:

To determine the colony characteristics of the isolates obtained from the decolourized tubes were streaked by four flame method on Nutrient agar plates and incubated at 32±2°C for 24 hours.

Gram Reaction:

Prepare suspension of the colony from the Nutrient agar plate and perform gram staining using Gram staining kit.

Assay of Decolourization:

Decolourization activity was expressed in terms of percentage decolourization and was determined by monitoring the decrease in absorbance at absorption maxima (λ_{max}) of respective dyes. The uninoculatedN.Broth supplemented with respective dye was used as control. The culture suspension was centrifuged at 10,000 rpm for 10 minutes for removal of the biomass. The degree of decolourization of the tested dye was measured at its respective maximum absorbance wavelength using supernatant by UV-visible spectrophotometer. The decolourization assay was calculated according to the following formula.

Decolourizatiion activity (%) = (A-B)/A x 100, where A= initial absorbance,B= observed absorbance

Parameter for Optimization:

Ideal condition for growth of bacteria: Carbon source= Glucose (5g/l),

Nitrogen source= Peptone (5g/l),

pH= 7±0.2,

Temperature= 32±2°C at static condition,

Dye concentration= 100 ppm,

Inoculum size= 1%.

Effect of Carbon sources:

Each of the carbon source such asGlucose, fructose, lactose (0.4g%) were added in 100ml N.broth having respective dyes and other parameters remain constant according to ideal conditions.

Effect of Nitrogen sources:

Each of the nitrogen source such as Peptone, yeast extract, beef extract (0.4g%) were added in 100ml N.broth having respective dyeand other parameters remain constant according to ideal conditions.

Effect of Temperature:

100ml N.broth having respective dyes were kept at different temperatures such as 4°C ,32°C and 50°C and other parameters remain constant according to ideal conditions.

Effect of pH:

100ml N.broth having respective dyes were kept at different pH such as 4, 7 and 10and other parameters remain constant according to ideal conditions.

Effect of Inoculum size:

100ml N.broth having respective dyes were added with differentinoculum size such as 1%, 3% and 5% and other parameters remain constant according to ideal conditions.

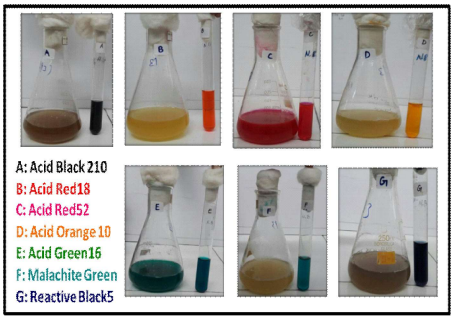
Effect of Dye concentration:

100ml N.broth having respective dyes were kept at different dye concentration such as 200 ppm, 400 ppm and 600 ppmand other parameters remain constant according to ideal conditions.

Result and Discussion

Enrichment and isolation of microbial dye decolourizers:

Only bacterial growth has been obtained in EF1 effluent sample.No bacterial, fungal and yeast growth have been obtained in EF2 and EF3 effluent samples.



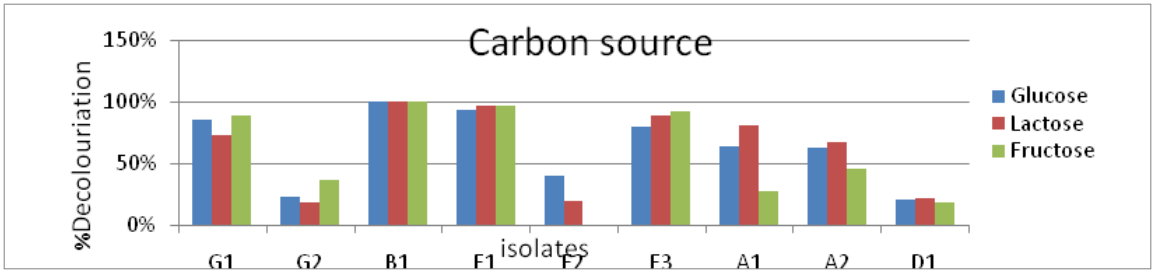
Primary and Secondary Screening:

Seventeen decolorizing bacterial isolates were obtained. When screening was carried out, nine morphologically distinct isolates namely A1, A2, B1, D1, F1, F2, F3, G1 and G2 were obtained.

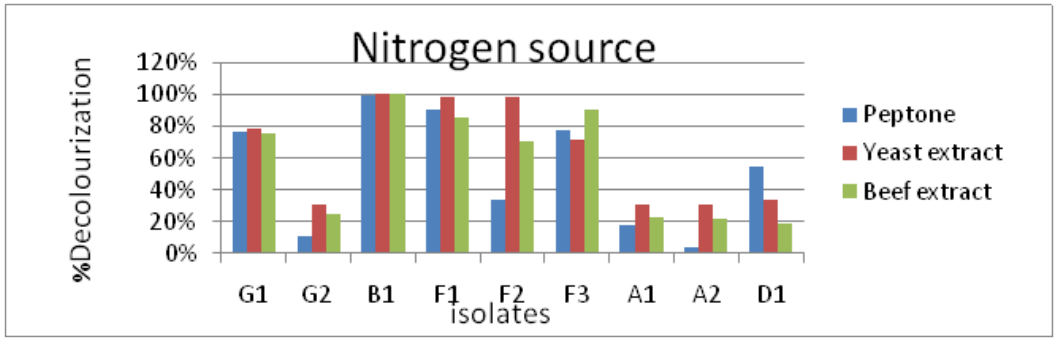
Gram Reaction:

Based on Gram staining results, out of 9 isolates, 8 were Gram negative and 1 was Gram positive.

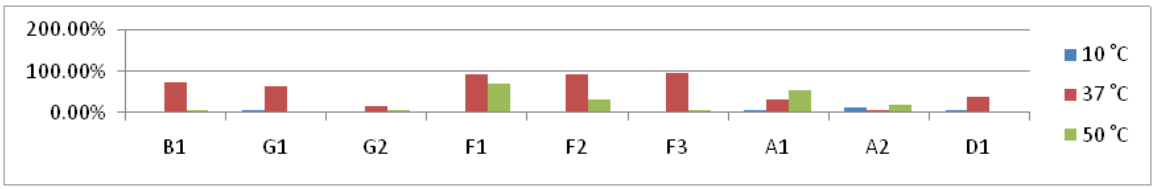
Effect of Carbon sources:



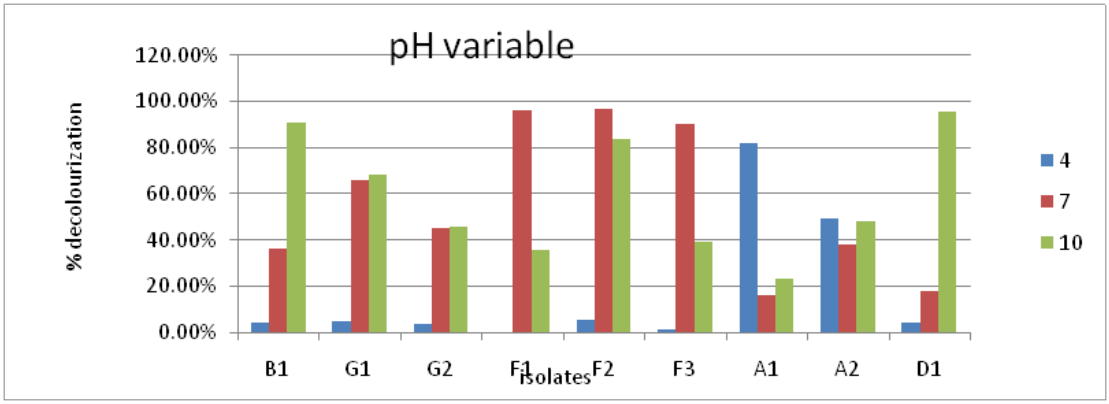
Effect of Nitrogen sources:



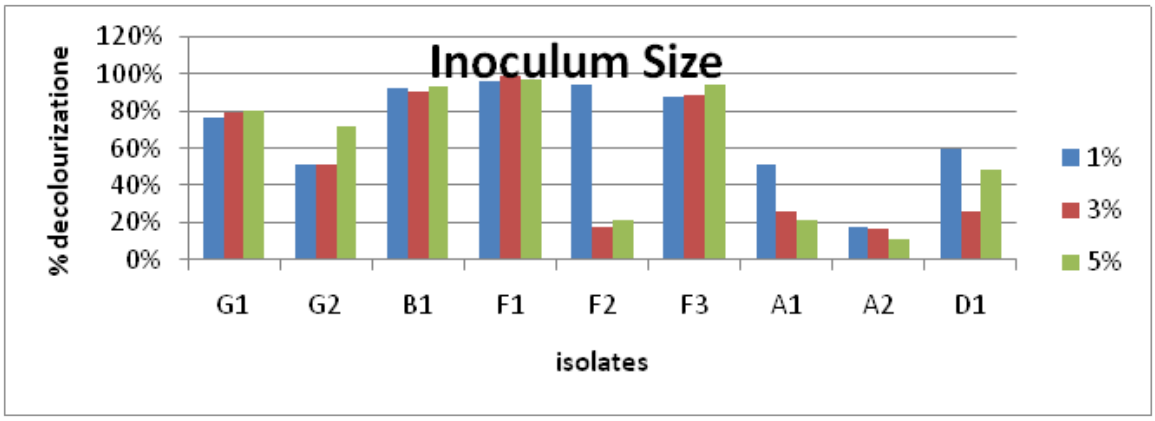
Effect of Temperature:



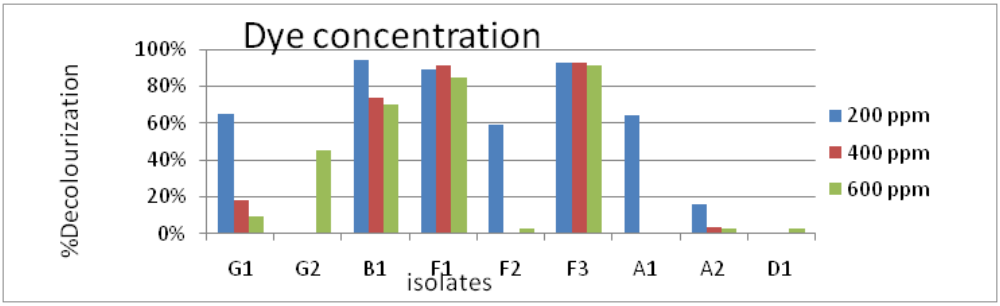
Effect of pH:



Effect of Inoculum size:



Effect of Dye concentration:



Conclusions

From the above results, it is concluded that best results are observed at these following conditions :

G1: 1% inoculum size, fructose*, yeast extract*, 200ppm, 37°C, pH 10.

G2: 5% inoculum size, fructose*, yeast extract*, 600 ppm, 37°C, pH 10.

B1: 5% inoculum size, fructose*, beef extract*, 200 ppm, 37°C, pH 10.

F1: 3% inoculum size, fructose*, yeast extract*,400 ppm, 37°C, pH7

F2: 1% inoculum size, glucose*, yeast extract*,200 ppm, 37°C, pH 7 (*)= 4 g/l

F3: 5% inoculum size, fructose*, beef extract*, 400 ppm, 370°C, pH 7

A1:1% inoculum size, lactose*, yeastextract*, 200 ppm, 50°C, pH 4

A2:1% inoculum size, lactose *, yeast extract*, 200 ppm,°C, pH 4

D1:1% inoculum size, lactose *, peptone*, 600 ppm, 37°C, pH 10

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