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Decolorization of acridine red dye by the fungi *Aspergillus* species

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Abstract

Acridine red dye was selected for decolorization studies by the microorganism isolated from textile dye contaminated soil samples. Enrichment and isolation was carried out in simulated medium using Bushnell-Haas broth embedded with 100 ppm of filter sterilized dye, pH 7.0±2. Six bacterial and one fungal colony was isolates on same agar media after one week incubation at room temperature. Screening test was carried out on the basis of zone of solubilization and qualitative decolorization of dye in liquid broth which indicates that fungi was most efficient than the bacterial colonies. A decolorization study with this fungal strain was performed by measuring percentage of decolorization, pH and biomass change in the simulated media. Optimization of various parameters like pH in the range of 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, addition of 1.0% of co-substrates like glucose, sucrose as carbon source, effect of dye concentration in the range of 100, 120, 130, 140, 150 and 200 ppm were carried out in shaking conditions. Identification of fungus was carried out by routine microbiological lactophenol blue method by which it was tentatively identified as *Aspergillus* species. In our study it was found that, our isolate was able to decolorize 95 to 98% dye in Bushnell Haas medium with 1% glucose, sucrose, pH 7.0, 8.0 at room temperature with 100 to 120 ppm concentration of dye within 48 to 72 hrs on shaking conditions.

Keywords: Decolorization, Acridine red dye, Screening, Bushnell-Haas medium, *Aspergillus* species.

Introduction

Gujarat State is famous for its textile business both in terms of production of gray fabrics and processing the same. Due to this many textile dying units present in different cities like Surat, Vapi etc. The textile effluents, usually highly colored, when discharged in open waters present an obvious aesthetic problem. Moreover, the dyes without an appropriate treatment can persist in the environment for extensive periods of time and are deleterious not only for the photosynthetic processes of the aquatic plants but also for all the living organisms since the degradation of these can lead to carcinogenic substances.[1, 2] Dye is a chemical compound having auxochrome and chromophore group. Dyes are substrate capable of coloring fabrics to such an extent that the color cannot be removed by rubbing or washing. Due to complex chemical structure of synthetic organic pigments in dyes, they are resistant to breakdown.[3] Numerous physio-chemical methods used for wastewater decolorization have shortcoming due to high costs and operational problems with less efficiency. Nowadays, effective biological processes would be of great value due to their inexpensive, eco-friendly nature and lesser sludge producing properties.[4] Various

microbes present in the environment having indigenous capacity to degrade complex compounds in nature like aerobic and anaerobic bacteria for example Pseudomonas pseudomodle, Bacillus subtilis, Pseudomonas cepacia and fungus like *Aspergillus* species, Hirschioporus larincinus, Inonotus hispidus, Penicillium species, Cynthus bulleri which can utilize dye as sole carbon source have been isolated from textile and dye effluent sample.[5-7] Related to fungus it can capable to decolorize or degrade or absorb dye from the liquid media. With respect to this laboratory work was done for isolation of microbes which remove color from waste water stuff.

Materials and Methods

Chemicals

Acridine orange dye was purchased from Sudarshan dying industry, Surat, Gujarat. Bushnell & Haas Medium was purchased from Himedia Company India. Other chemicals from S.D. fine chemicals, India.

Collection of Samples, Enrichment and Isolation

Soil samples contaminated with textile dyes was collected from Sudarshan dying Industry, Surat. Homogenization of soil sample was carried out by inoculation of 1.0 gm of soil sample into 100 ml of sterile distilled water. It was kept on shaker for 02 hours at 120 rpm. After homogenization of samples 5.0ml of supernatant was inoculated in the laboratory level simulated media. Simulated media was made up of Bushnell Haas broth which contains mainly salts (grams/litre); Magnesium sulphate (0.2); Calcium chloride (0.02); Monopotassium phosphate (1.0); Dipotassium phosphate (1.0); Ammonium nitrate (1.0); Ferric chloride (0.05) and 100 ppm filter sterilized acridine red dye, pH 7.0±2. It was kept at room temperature for seven days on shaker at 120 rpm for enrichment process. For isolation, this enriched sample was serially diluted in sterile distilled water and each diluent was spread on same agar media. It was also kept at room temperature for seven days. Six bacterial and one fungal colony was observed and coded as bacterial strains B1, B2, B3, B4, B5, B6, for fungal strain as F1. Each of these colonies was purified by repeatedly streaking on same agar media by routine four flame method of microbiology (Patel, 2008). Each pure colony of bacteria was stored on nutrient agar slants while fungus was stored on potato dextrose agar slants in refrigerator.

Screening of potential microbes

To screen the potential microbes which remove acridine red dye from the medium rapidly, zone of solubilization on agar media and qualitative decolorization study in liquid broth was performed according to Mane *et al*. and Syed *et al*. respectively.[8, 9] To study zone of solubilization, a loopful of each bacterial and fungal pure culture was placed on the center of the same agar plates within the area of 0.5 mm and incubated for 27±2ºC. Zone of solubilization was observed for 24, 48, 72 and 96 hrs (Table 1). The solubilization zone was determined by subtracting the diameter of colony from the diameter of total zone. Qualitative test was carried out in liquid assay, in which, one ml of 24 hrs old each bacterial and spore suspension of fungus was inoculated in 5.0 ml of Bushnell-Haas medium containing different concentration of dyes which was in the range of 20, 40, 60, 80 and 100 ppm in the 10.0 ml capacity screw cap tubes. All tubes along with its control were kept at for five days at room temperature. Spore suspension was prepared by flooding the fungal colony with sterile distilled water containing tween 80 on Potato dextorse agar media. Microbe with rapid removal of dye within short period of time was selected for further studies.

Decolorization study

In our study, fungal strain F1 was found to be more effective than the bacterial strains during screening technique. Thus, decolorization study of acridine red dye in the simulated medium was carried with F1 strain only. For that, 5.0ml of spore suspension of isolated fungus was inoculated in 100ml of simulated broth containing 100 ppm filter sterilized acridine dye, pH 7.0±2. At specific time interval of 24 hours, 10.0 ml of the samples was withdrawn and centrifuged at 10,000 rpm for 15 minutes. Supernatant was collected and use to observe maximum absorbance of dye and pH. Cell pellet was used to determine the biomass content. These parameters were studied continuously for five days by keeping the flasks at room temperature on shaker at 120 rpm. Control was also kept by providing same conditions except it was not inoculated with fungus. The percentage of decolorization was carried out according to following formula.

$$\% \text{ Decolorization} = \frac{\text{Initial absorbance-Final absorbance}}{\text{Initial absorbance}} \times 100$$

Effect of different Physiochemical factors

Studies with different parameters like pH 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, co-substrates like 1%glucose, sucrose as carbon

source and dye concentration in range of 50, 100, 120, 130, 140, 150 and 200 ppm were carried out to know the effect of various physiochemical conditions on the decolorization rate of dye.[10]

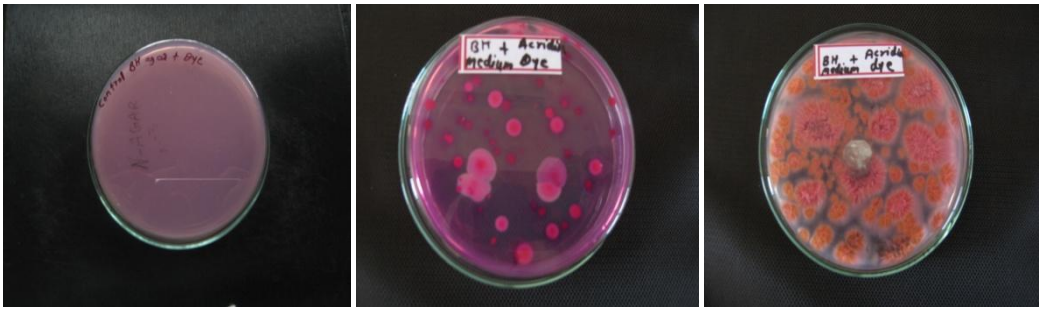
Analytical Methods and Identification of the fungus

Decolorization study was monitored using U.V. spectrophotometer (Shimatzu, UV 1800) by measuring absorbance maxima at 540 nm. Dry weight method and pH electrode was used to measure the cell biomass and pH of the medium respectively.[11] Each of above experiments was performed in triplicate with uninoculated control. Identification of the fungus was done by microscopic examination using Lactophenol blue method.[12]

Results and Discussion

Enrichment and Isolation of microbes

Soil sample collected from the Sudarshan dying industries had shown the presence of growth of microbes in the simulated media containing acridine red dye during enrichment technique. Total seven isolates was purified which was able to grow in the presence of dye in Bushnell Haas agar embedded with 100 ppm dye. It was observed that among seven isolates, six were bacterial and one was fungal colony (Figure no.1). As a simulated medium, Bushnell Haas medium was selected because it contains only minerals and lack in carbon and nitrogen sources. In nature, continuously exposure of complex compounds makes indigenous microbes to adapt themselves to survive in such adverse physiochemical conditions. Thus, various microbes have ability to break down complex compounds including dye containing compounds.[13] In our study, the presence of growth in the medium indicates that these microbes might be use dye as a substrate for its growth. Finally, these seven isolates were further used to screen the most potential microbe which can remove the dye of the color within short period of time.



(i) Control **(ii) Bacterial colonies** **(iii) Fungi**

Figure 1: Different isolates growing on Bushnell Haas agar medium with 100 ppm acridine red dye, in which (i) is a control plate, while (ii) and (iii) are showing colonies of bacteria and fungal respectively.

Screening of potential microbes

According to screening methods mentioned above, potential microbe was isolated on the basis of zone of clearance on Bushnell Haas agar plate with 100 ppm dye concentration as well as qualitatively removal of color of dye in liquid media with the different concentrations. Results shown in the table no. 1, indicates that fungal colony F1 found to be more effective than the bacterial colonies.

Qualitative test was performed to know the efficiency of each isolates to decolorize the dye with different concentrations. It was found that fungas (F1) was efficiently remove color upto 100 ppm concentration of dye within 48 to 72 hours Bacterial isolates B3 and B4 were able to remove the color but it was less significant compare to fungal isolate. Other isolates were not given satisfactory results to remove the dye color from the medium. Table no. 2 indicate the rate of removal of dye by fungas(F1) with the different concentration of dye and incubation time at room temperature. Data for bacterial isolates was not shown in this paper.

According to the results of table no.1 and 2, it was clearly indicated that fungal isolate was found to be more potential than the bacterial isolates. As fungus having more biomass or effective enzymes, it can more able to absorb or decolorize or degrade the dye more efficiently than the bacteria.[14]

Table 1: Data represents the diameter of zone of solubilization (in millimeter) by various isolates on media containing dye

Sr. No	Isolates	Zone of clearance (mm) with different incubation time			
		24 hours	48 hours	72 hours	96 hours
1.	B1	-	-	0.1	0.1
2.	B2	-	-	-	-
3.	B3	0.1	0.16	0.16	0.17
4.	B4	-	-	0.20	0.24
5.	B5	-	-	-	0.13
6.	F1	-	-	**4.2**	**5.6**

Table 2: Data representing the qualitative removal of dye color in the medium by Fungus (F1)

Sr. No	Concentration of dye (ppm)	Rate of decolorization of dye by Fungus (F1)			
		24 hours	48 hours	72 hours	96 hours
1.	20	-	+	+++	+++
2.	40	-	+	+++	+++
3.	60	-	+	++	+++
4.	80	-	-	++	+++
5.	100	-	-	++	+++’

Study of dye decolorization

Decolorization study was performed only with fungal strain with 100 ppm concentration of dye in Bushnell Haas media, pH 7.0+2 in shaking condition. The rate of removal of dye from the medium which was predicted by calculating percentage of dye decolorizing, pH change and increased biomass at specific interval of time of 24 hours. According to figure 2(i), decolorization of fungi (F1) was found to be 41.66%, 58.33% and 98.33% with the duration of time 24, 48 and 72 hours, respectively. Within the same duration, continuous increased biomass with rapid acidic pH was observed. Extreme low pH of the medium was found to be inhibitory for the growth of fungus, but up to that almost whole color of the medium was removed by fungal biomass. These parameters indicate that fungus (F1) was successfully able to remove the color of the dye from the medium. Removal of the color might be due to absorption or degradation of the dye. Prediction of absorption is due to observation of red colored biomass of fungi which was clearly appeared in figure no.1. While increase biomass and acidic pH indicates degradation because it might due to breakdown of the dye which leads to produce acidic end products but to confirm it further studies are require.

Effect of Physiochemical parameters on decolorization rate

In nature, various physicochemical parameters like temperature, pH, presence of other substrates etc. can affect on pattern of growth as well as its ability to breakdown any substance. Thus, with respect to that effect of different parameters were carried out which results were shown in figure 2(ii), 2(iii) and 2(iv). Figure 2(ii) indicates the effect of pH on decolorization rate. With the pH 7.0 and 8.0, the decolorization rate was found to be 95% to 98% within 72 hours while pH 6.0 found to be less significant because within the duration period rapid acidic pH leads to inhibition of growth. Growth was not observed within the pH of too acidic (4.0 and 5.0) and alkaline (9.0).

Maximum decolorization was observed within 48 hours, when addition of 1.0% glucose or sucrose was added in the medium as carbon sources (Figure no. 2(iii)). It is due to rapid growth of organisms due to utilization of simple sugars before act of complex structure like dye.[10] Studies with different concentration of dye showed in the figure no. 2(iv) indicating decrease decolorization rate with increase in the dye concentration by the microbe. An isolate can able to remove 80 to 98% of dye color with 100 to 140ppm concentration within 72 hours, which followed

by about 50-60% decolorization with 150 ppm. Reproducible results were not found with higher concentration of dye. Thus, addition of 1.0% glucose, sucrose, pH 7.0 and 8.0, 100 to 120 ppm dye concentration found to be optimized condition to remove maximum color from the medium.

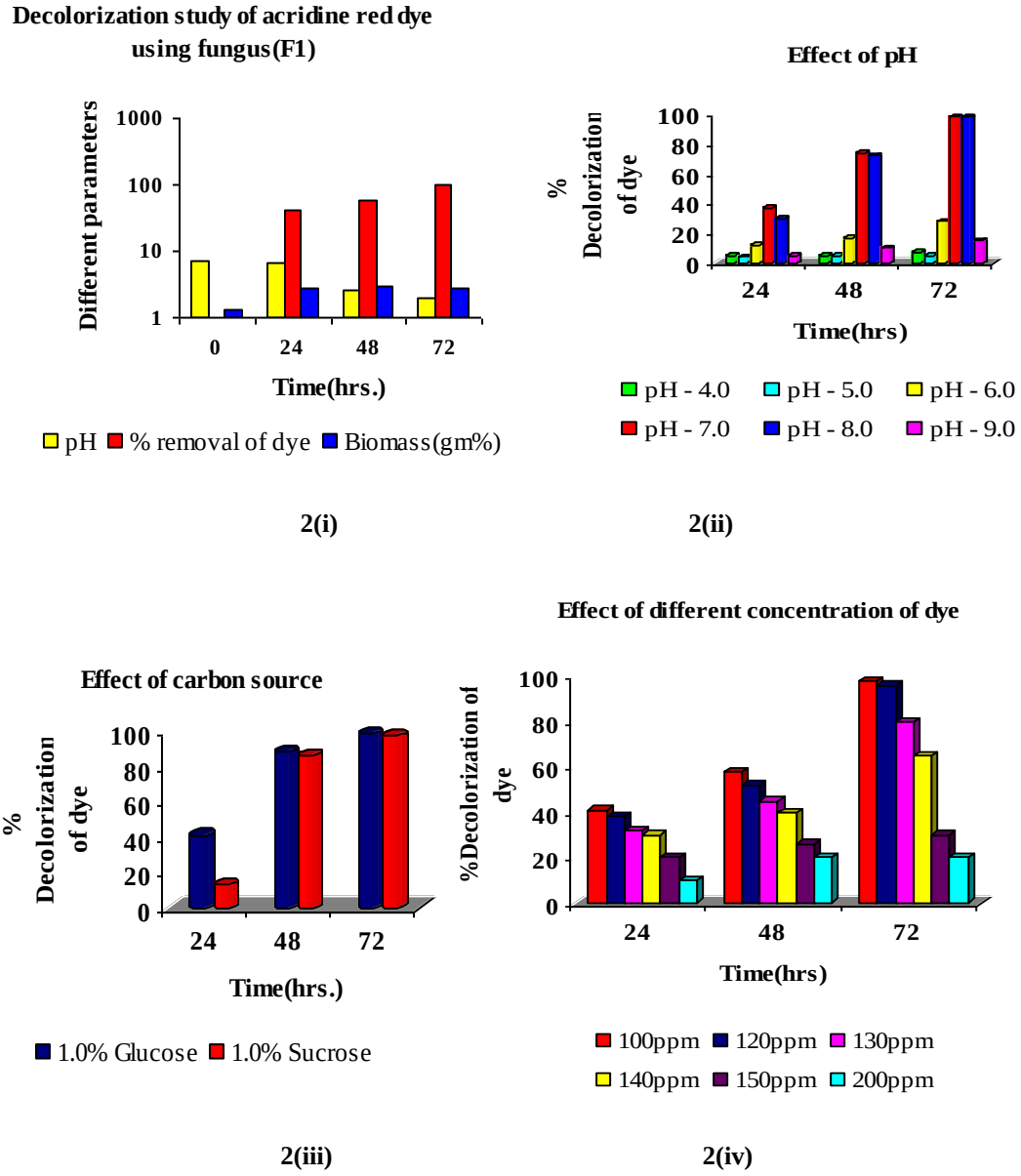


Figure 2: Graphical representation of (i) Decolorization studies (ii) Effect of different pH (iii) Effect of 1.0% Glucose and Sucrose as carbon source and (iv) Effect of different concentration of acridine red dye

Identification of the fungus

Slide culture technique and careful morphological study under microscope using lactophenol blue indicate the presence of conidiophore arising from the foot cell, basipetal conidia on phialides (1 to 2 series) on vesicle. Growth of colonies was found very rapid on potato dextrose agar within 4 days with black spores. These characters gives tentatively idea that fungi belongs to *Aspergillus* species but for further identification require.[15]

Conclusion

Biodecolorization studies were carried out with the dye acridine red dye in the Bushnell-Haas media using indigenous fungus isolated from the dye contaminated soil sample. In our study it was found that, our isolate was able to decolorize 95 to 98% dye in Bushnell Haas medium with 1% glucose, sucrose, pH 7.0, 8.0 at room temperature with 100 to 120 ppm concentration of dye within 48 to 72 hrs on shaking conditions. Identification of the fungus with lactophenol blue gives tentatively idea that the fungus

belongs to *Aspergillus* species. Further studies should be require to use this indigenous fungal for treatment of textile unit effluents, where acridine red dye are used.

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