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BIODEGRADATION OF SYNTHETIC DYES BY *ASPERGILLUS TERREUS* INOCULATED ON SOLID MEDIA

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Abstract: Water pollution caused by industrial waste discharges has become an alarming trend worldwide, while dye industries are considered as the most polluting among all others. In recent years, bio-treatment took attraction in removing the unwanted colour and toxicity of dyes than other conventional treatment processes. The release of dyes in to environment is of great concerned due to color, toxicity, mutagenicity and carcinogenicity of the dye, considerable attention has been given in evaluating the capability of microorganism in decolourisation and degradation diazo dye. The present study fungi were inoculated on different solid media to attain biodegradability of a diazo dye used for the coloration of paper products. For the solid, to be employed as media, special characteristics are needed with regards to adsorption capacity for concentrating substrate within the cell environment and an adequate particle size and surface texture for assuring fungal colonization. These factors were in different pH, temperature, incubation period, inoculum sizes, carbon sources, nitrogen sources and different concentrations of yeast extract. In the present investigation the ability of heat killed fungus *Aspergillus terreus* to adsorb the dyes Methylene blue and Congo red was investigated. The removal of the dyes Congo red and Methylene Blue from aqueous solutions on the fungal *A. terreus* was demonstrated by a series of batch experiments.

Keywords: Decolorization, Biodegradation, Congo red, Methylene Blue and *Aspergillus terreus*.

I. INTRODUCTION

Environmental pollution has been recognized as one of the major problems of the modern world. The increasing demand for water and dwindling supply has made the treatment and reuse of industrial effluents an attractive option. One of the most important environmental pollution problems is the color in water courses, although some of this color is normally present and of "natural" origins (e.g. the color originates from the activity of some microorganisms in ponds), a considerable proportion, especially in the lower reaches of rivers draining large industrial conurbations, originates from industrial effluents. Some colored effluents are associated with the production and use of dyestuff.

Dye wastewaters are highly visible even at very low concentrations of dyes (less than 1.0 mg/l for some dyes). When they are discharged into receiving water bodies, they make the water aesthetically unpleasing, affect water transparency and gas solubility in water bodies which cause adverse impacts on aquatic life and may be toxic to aquatic life[1]. Wastewaters have been known to be detrimental to the microorganisms involved in biological wastewater treatment; thus dye wastewaters cause low removal efficiency or failure of the treatment plants[2]. To comply with environmental legislation restricting the discharge of wastewater, the textile industry is attempting to develop technologies for wastewater remediation. Dye removal is of particular concern because it is largely unaffected by conventional treatment systems[3].

Synthetic dyes are extensively used in the textile industry. Due to inefficiencies of the industrial dyeing process, 10–15% of the dyes are lost in the effluents of textile units, rendering them highly colored[4-5]. It is estimated that 280,000 tons of textile dyes are discharged in such industrial effluents every year worldwide[6]. Direct discharge of these effluents causes formation of toxic aromatic amines under anaerobic conditions in receiving media. In addition to their visual effect and their adverse impact in terms of chemical oxygen demand, many synthetic dyes are toxic,

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mutagenic and carcinogenic[7]. The efficient removal of dyes from textile industry effluents is still a major environmental challenge[8]. The frequently high volumetric rate of industrial effluent discharge in combination with increasingly stringent legislation, make the search for appropriate treatment technologies.

Degradation of dyes, especially azo dyes, which comprise about 70% of all dyes used, is difficult due to their complex structure and synthetic nature[6,9]. Currently, various chemical, physical and biological treatment methods are used to remove colour[10-11]. Because of the high cost and disposal problems, most of the chemical and physical methods for treating dye wastewater were not widely applied in the textile industries[12-13]. Because synthetic dyestuffs are resistant to biological degradation, color removal by bioprocesses is also difficult[14-15]. Decolorization generally occurs by the adsorption of dyestuffs on bacteria, rather than oxidation in aerobic systems. Some bacteria can biodegrade dyestuffs by azoreductase activity. However, the effluent at the end of biotransformation of dyestuffs could be toxic[7]. These problems limit large-scale application of bacterial decolorization.

There are a number of methods to treat dye waste waters. These can be classified into two general categories: biological treatment and physical/chemical treatment. Since dyes are designed to be bio-resistant, conventional aerobic biological processes generally are not efficient to biodegrade dyes. These processes remove dyes primarily through adsorption to the biomass[16]. An anaerobic biological pretreatment followed by an aerobic treatment may represent a significant advancement in biological treatment for dye wastewaters[17]. Loyd[18] studied anaerobic /aerobic processes for a dye wastewater treatment and produced much better color reduction than aerobic treatment alone. However, this innovative process is in a developmental stage.

Due to the low removal efficiency of biological treatment process, dye wastewaters are usually treated by physical/ chemical processes. There are six distinct groups: coagulation, adsorption, membrane techniques, electrochemical technology, reduction and oxidation[17,19].

Biosorption has been studied since 1980s in removing heavy metals and dyes as well as other organic pollutants from wastewaters. It is a promising alternative to replace or supplement present treatment processes. Biosorption may be defined as the removal of metal and metalloid species, compounds and particulates from solution by biological materials, called biosorbents[20]. Various mechanisms of biosorption range from physico-chemical interactions, such as adsorption, deposition, ion-exchange, to processes dependant on cell metabolism. Living and dead cells as well as derived products, such as cell wall constituents, are able to function as biosorbents[20]. Compared with the living cells, dead cells posses various advantages. They may be stored or used for extended periods. Their biosorptive capacities may be greater, equal, or less than those of living cells. Their operation is easier and they can be regenerated by certain methods[21]. Therefore, dead cells are preferred than living cells as biosorbents.

Fungus *Aspergillus terreus* is used in industrial processes to produce citric acid, kojic acid, cellulases, lipases and glucanases[22]. These industries can serve as an economical and constant source of fungal biomass, which means that *A. terreus* could be used in practice to remove color from dye wastewater in the future. At the same time, the amount of available information on a systematic evaluation of the potential of *A. terreus* to remove dyes from dye wastewaters and the explanations for the mechanisms are limited. By keeping all the above facts in mind, the present investigation was carried out to know the biosorption efficiency of *Aspergillus terreus* on dyes. In this paper, we studied the location of the dye degrading enzyme, decolorization of other direct and basic dyes, and the ability of the isolates to decolorize Azo dye in microcosm.

II. MATERIALS AND METHODS

Sources of Study Materials

Aspergillus terreus received from National Center for Industrial Microorganisms (NCIM), Pune, India. The strain was maintained on PDA (potato, dextrose and agar) medium slant. The slant was inoculated and incubated at 30°C for 7–8 days and then stored at 4°C and periodically sub-cultured.

Dyes Used For Experiments

Congo red (Merck) and Methylene blue (Sigma) were used.

Solid Cultures

Solid media are used for the growth of fungi and for storage of cultures *Aspergillus terreus* was grown in potato dextrose agar (PDA) petridishes and was incubated for 7 to 10 days at room temperature (28° C). The strain was

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routinely transferred every 7 to 10 days to fresh PDA petridishes by streaking. *A.terreus* was always stored in sealed plastic bags to prevent the loss of moisture from agar.

Preparation of Dye Solutions

The two dyes used in this study were Basic blue or methylene blue ($C_{16}H_{18}ClN_3S.3H_2O$) is commonly available as a chloride salt and soluble in water. It is widely used in textile industry. It is often used as a biological strain and as antidote for cyanide poisoning in humans and animals. Congo red ($C_{32}H_{22}N_6Na_2O_6S_2$) is soluble in water. It is a pH indicator which is blue and red at pH 3.0 and 5.2 respectively. Congo red has an affinity for both proteinaceous and cellulosic substrates. It can dye cotton directly and wool out of a neutral bath.

Name	Classification	Color index number	Molecular Weight	Solubility in water (mg/L)
Congo Red	Anionic direct diazo	22120	696.7	40
Methylene blue	Cationic thiazine	52015	373.9	50

In this study the initial concentration of each dye solution was fixed at 50.0 mg/l. This concentration is in the range of concentrations usually observed in actual dye wastewaters. Dye solution (50 mg/l) was prepared by dissolving accurately 50.0 mg dye in 1 L distilled water.

Calibration of Dye Solution

The dye solutions were initially calibrated for concentration in terms of absorbance units. Each of the standard dye solution (50.0 mg) was diluted with distilled water to concentrations of 0, 0.5, 1.0, 5.0, 10.0, 15.0, 20.0, 30.0, 40.0, 50.0 mg/l, respectively. The pH of the dye solutions with different concentrations was adjusted to 7.6 by using dilute HCl or NaOH solution. Each concentration of solutions was measured for its absorbance value at its corresponding λ_{max}. The absorbance values versus concentrations were then plotted. All calibration plots were straight lines passing through the origin (0, 0).

Concentration Calculation

In order to compare removal of dye on the same basis, the pH of all samples was adjusted to 7.6 before measurement. The pH value of 7.6 was selected based on the Standard Methods for examination of water and wastewater[23]. Dilute HCl or NaOH was used for pH adjustment. The absorbance values of the samples were determined by a spectrophotometer operating at the corresponding maximum absorbance wavelength (λ_{max}) on absorbance mode. The dye concentration was obtained by interpolating the measured absorbance values on the linear portion of its calibration plot. If the absorbance values of the samples were outside of the linear range of the calibration plot, the samples were diluted before absorbance measurements.

III. RESULT AND DISCUSSION

Growth on Solid Medium

The growth of the fungus *Aspergillus terreus* on petri dishes was observed after four days. In six days, the surface of PDA solid medium in the petri dishes was covered by brown colored spores. In eight to ten days, a brown colored mat covered the whole surface of PDA solid medium in petri dishes. Figure 1 shows a picture of a PDA petridish covered by *A. terreus*. Previous reports on azo dye degradation by bacteria investigated a certain enzyme which is the azo reductase as responsible for the reduction of azo dye. According to Russ et al. [(24)], enzymatic reduction can occur both intracellularly and extracellularly. In a study of the anaerobic reduction by whole cells, cell extracts and cell membranes of *Sphingomona*s sp. strain BN6, enzymatic azo dye reduction activity was found to be located in the cytoplasm as well as in the membrane fraction but it was suggested that azo dye reduction by whole cells is mainly related to the membrane fraction[25].

Dyes Maximum Absorbance Wavelengths (λ_{max})

Figure 2 shows the absorbance of Methylene Blue solution versus the wavelength in the visible range (400 – 700 nm) at a pH of 7.6 for different concentrations. It was observed that the values of λ_{max} varied slightly with concentrations. The solution with a high concentration had a slightly higher value of λ_{max}. The differences were in a range of 10- 20 nm when the dye concentrations were changed from 10 mg/L to 50 mg/L. In this study, λ_{max} of Methylene Blue solution with a concentration of 10 mg/l was used. So the value of λ_{max} for Methylene Blue was 660 nm.

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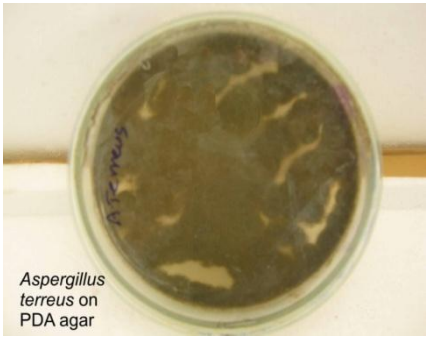

Fig. 1. The growth of the fungus *Aspergillus terreus* on PDA Agar

Figure 3 shows the absorbance of Congo red solution versus the wavelength in the visible range (400 – 700 nm) at a pH of 7.6 for different concentrations. It was observed that the values of λ_{max} were same at different concentrations. The value of λ_{max} for Congo Red was 500 nm. Culture medium and culture conditions employed in Congo red decolorization studies were utilized. Aerobic and anaerobic conditions were provided. Absorbance of Methylene Blue solution versus the wavelength in the visible range (400 – 700 nm) at a pH of 7.6 for different concentrations. Absorbance of Congo red solution versus the wavelength in the visible range (400 – 700 nm) at a pH of 7.6 for different concentrations. No decolorization was observed in the aerobic set-up with six months of incubation. All the isolates and consortia showed positive growth in all the dyes as evidenced by the presence of gas in the Durham tubes except for methyl violet in which no gas was produced.

Figures 4 and 5 show the calibration plots for the dyes Methylene Blue and Congo Red. The calibration equations based on linear regression. Only direct dyes were decolorized by the isolates and consortia. The isolates and consortia were enriched in medium with Congo red which is a direct dye. This could explain why the isolates were only able to decolorize the direct dyes. *Aspergillus* strain was able to decolorize a wide range of direct azo dyes at different rates but was not able to decolorize basic dyes and acid dyes[26]. Direct dyes have different components than basic and acid dyes. The ability of microorganisms to decolorize different dyes varies depending on the structure and complexity of the dye[27]. In the study of Paszczynski et al. [(28)] on the mechanism of azo dye oxidation by lignin peroxidase produced by the white rot fungi *P. chrysosporium*, it was concluded that the susceptibility of a particular compound to degradation depends on both structure and culture conditions. Under different culture conditions, the fungus may secrete different isozymes of its peroxidases.

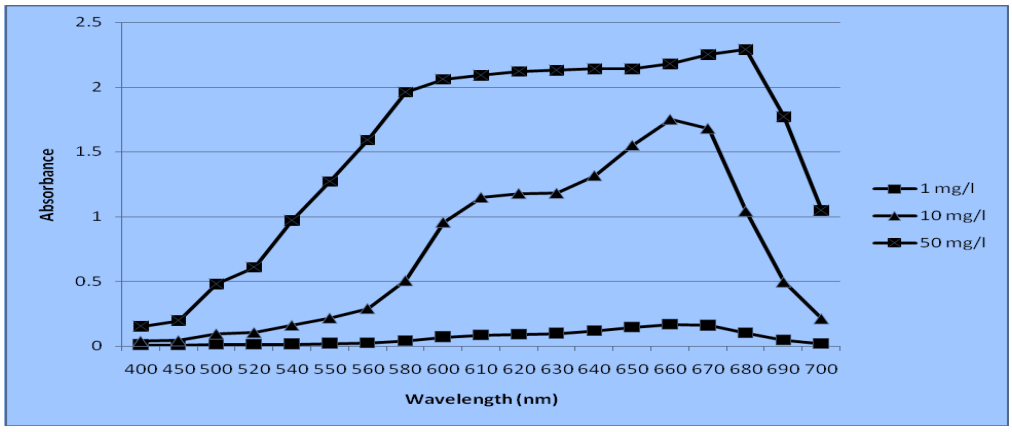

Fig. 2. Absorbance of Methylene Blue solution versus the wavelength in the visible range (400 – 700 nm) at a pH of 7.6 for different concentrations

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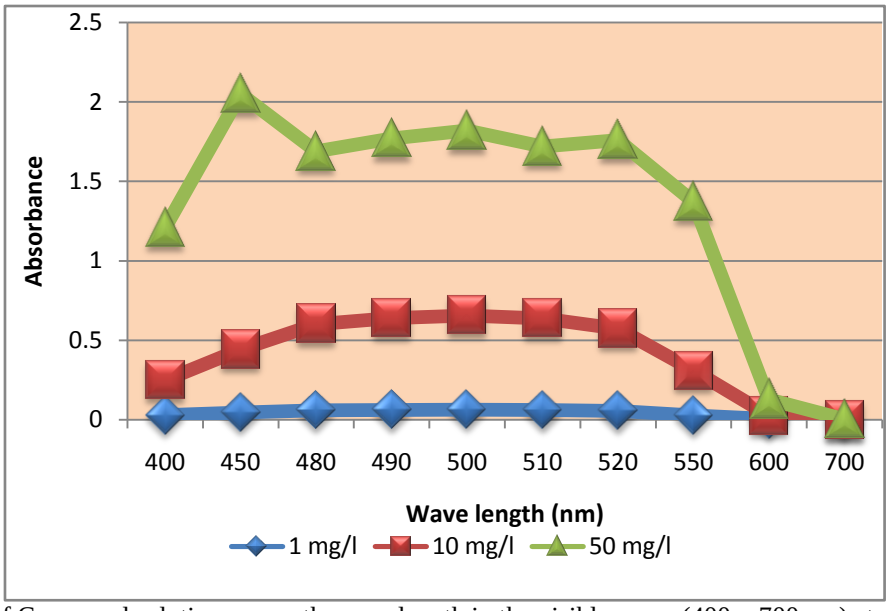

Fig. 3. Absorbance of Congo red solution versus the wavelength in the visible range (400 – 700 nm) at a pH of 7.6 for different concentrations

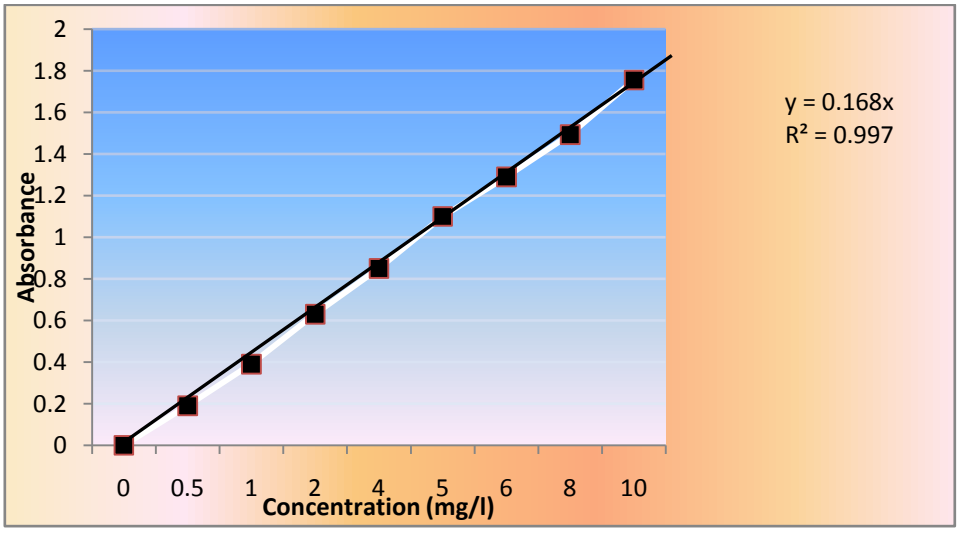

Fig. 4. Calibration plot for Methylene Blue at 660 nm

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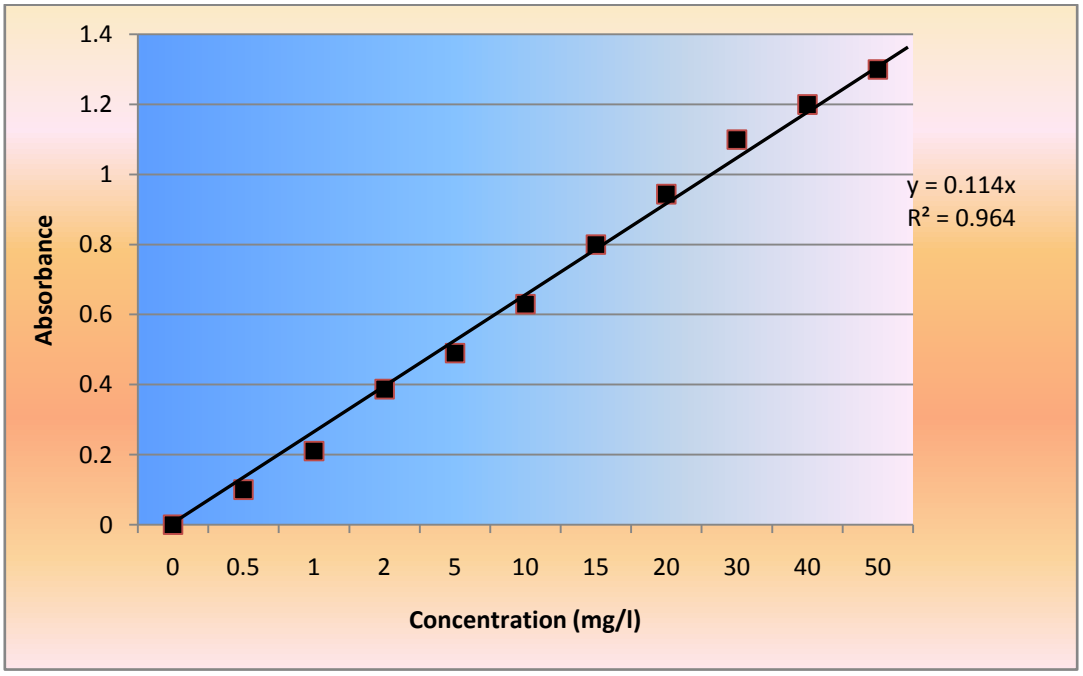

Fig. 5. Calibration plot for Congo Red at 500 nm

For the removal of dyes from wastewaters, a combination of the anaerobic cleavage with an aerobic zone to degrade the amines formed was generally accepted. Two strategies have been developed: sequential and simultaneous processes. Sequential processes combine the anaerobic and the aerobic steps either in the same reaction vessel or alternatively in a continuous system in separate vessels. The simultaneous processes utilize anaerobic zones within basically aerobic bulk phases, such as observed in biofilms[29], granular sludge or biomass immobilized in other matrices[30-31]. In the sequential and simultaneous processes, auxiliary substrates are required, for supplying bacteria with a source of reduction equivalents for the cleavage of the azo bonds.

In this research we demonstrate that the addition of a fungal culture on the surface of solid media produces dye decolorization. Particle size and surface texture have played an important role on dye biodegradation because substrate has to be concentrated in the proximity of the cells (adsorption capacity of the solid) and an irregular solid surface and a proper particle size are required. In conclusion, biodegradation of recalcitrant compounds as azo dyes can be facilitated by the presence of surfaces that have convenient pores to promote formation of microaerophilic niches and permit microbial growth. Furthermore, they should have adsorptive capacity of the pollutant and nutrients to assure fungal feeding. In this sense, some agroindustrial residues could be used as convenient supports. Fungal decolorization was found to be promising alternative to replace or supplement present treatment process. However, using fungal biomass to remove color in a dye wastewater is still in an early stage. More studies are needed to develop a practical application.

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