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Biodegradation of Textile Azo Dyes using Fungi

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Abstract

Dyes are widely used in the Textile, rubber product, paper, printing, color photography, Pharmaceuticals, Cosmetics and many other industries. Amongst these, azo dyes represent the largest and most versatile class of synthetic dyes. Textile dyes enhances the quality of human lifestyle on an extent. Nowadays, there are more than 100,000 commercially available dyes with over 7.105 tons of dyestuff produced annually. Textile industries are found in most countries and their number had been increased. A large number of dyes are azo compounds (-N-N-), which are linked by an azo bridge. These dyes are poorly bio-degradable because of their structures and treatment of wastewater containing dyes usually involves physical and / or chemical methods such as adsorption, Coagulation, flocculation, Oxidation, filtration and electrochemical methods. The present study was undertaken to isolate and characterize fungi from soil sample. The synthetic dyes used in textile industry causes hazardous effect in the environment. By using certain fungi, eco - friendly degradation of azo dyes can be carried out to remove toxicity and thus can help in keeping the environment free from pollution.
Keywords: Ethanol, *Camellia sinensis* extract (CSE), RBCs, Hb%, PCV, TLC

Contents

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1. Introduction

The first human-made (synthetic) organic dye, mauveine, was discovered serendipitously by William Henry Perkin in 1856. Many thousands of synthetic dyes have since been prepared **(N. Sri Kumaran *et al*., 2011).** Synthetic dyes quickly replaced the traditional natural dyes. They cost less, they offered a vast range of new colors, and they imparted better properties to the dyed materials. Dyes are now classified according to how they are used in the dyeing process **(Tripathi A. and Srivastava S.K., 2011)**. Almost all the colors that you see today are Synthetic dyes. Synthetic dyes are used everywhere in everything from clothes to paper, from food to wood. Synthetic dyes today have evolved into a multibillion dollar industry. They are widely used for dyeing and printing in a broad range of industries **(Sidra Ilyas, Skinder Sultan and Abdul Rehman 2012)**. There are over 10,000 dyes, and the annual

production globally, exceeds over 7 × 105 metric tones. Cheaper to produce, brighter, more color-fast, and easy to apply to fabric, these synthetic dyes changed the playing field. Scientists raced to formulate gorgeous new colors and before long, dyed fabric was available to all, and natural dyes had become obsolete for most applications **(S. Senthil Kumar *et al*., 2013).**

The synthetic dyes can be named according to the chemical structure of their particular chromophoric group. For example, diphenylmethane derivatives, triphenylmethane compounds oxazine compounds, xanthene compounds, Azo dyes are one of the most popular varieties of synthetic dyes. Today it is being used upto 90% in the dyeing units, as they are versatile and simple to synthesize. Most of the synthetic dyes with a few exception are aromatic organic compounds which can be divided into groups like non-ionic (oil soluble), cationic, and anionic. A typical example of Cationic dye is Methyl violet while Azo dyes are anionic dyes (**Wong, Y., Yu, J., 1999)**.

Azo Compound

Azo compounds are compounds bearing the functional group R-N=N-R', in which R and R' can be either aryl or alkyl. IUPAC defines azo compounds as "Derivatives of diazene (diamide), HN=NH, wherein both hydrogens are substituted by hydrocarbyl groups, e.g. PhN=NPh azobenzene or diphenyldiazene." The more stable derivatives contain two aryl groups. The N=N group is called an *azo group*. The name azo comes from *azote*, the French name for nitrogen that is derived from the Greek *a* (not) + *zoe* (to live) **(Mustafa Isik *et al.*, 2003).**

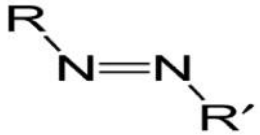

Figure 1. Chemical formula of azo dyes

Characterization of Azo Dyes

Azo colorings are the most versatile class of dyes. Their structure has been intensely studied and many spectral data analyses have already been reported (**Table 1**). The dyes have been most widely used in fields such as dying textile fibers, biomedical studies, advanced applications in organic synthesis and high technology areas like lasers, liquid crystalline displays, electro-optical devices and ink-jet printer (**Syed, *et.al.*, 2009)**.

Types of Synthetic Dyes

- Azoic (or Naphthol) Dyes
- Acid Dyes
- Basic Dyes
- Chrome (or Mordant) Dyes
- Developed (or Diazo) Dyes
- Direct Dyes
- Disperse (or Acetate) Dyes
- Reactive (or Fiber-reactive) Dyes
- Sulphur Dyes
- Vat Dyes

Properties of Azo Dyes

The properties of Azo dyes are resistance to heat, resistance to weather condition, resistance to ultraviolet light (UV), they are water soluble, conducts electricity, contain reinforcing fibers and free from heavy metals **(Anjali, *et.al.*, 2007).**

Organisms used in Dye Degradation

Many microorganisms belonging to different taxonomic groups of bacteria, fungi, actinomycetes and algae have been reported for the ability to decolorize the azo dyes. Fungus used in degradation are *Aspergillus niger, aspergillus flavus, mucormucedo, Cladosporium sp, Aspergillus fumigatus, Fusarium oxysporu ,Tricoderma viride, Pleurotus ostreatus, Pleurotus sajor-caju, Daedalea flavida* and white rot fungus *Phanerocheate chrysosporium* (Swamy *et al*., 1999; Robinson *et al*., 2001). The fungus *Trichoderma harzianum* has also been reported earlier for the degradation of textile dyes (Singh and Singh, 2010).

Problems of Textile Industry

Although cotton textile is one of the most important industries of India, it suffers from many problems. Some of the burning problems are briefly described as under:

Scarcity of Raw Cotton- Indian cotton textile industry suffered a lot as a result of partition because most of the long staple cotton growing areas went to Pakistan. Although much headway has been made to improve the production of raw cotton, its supply has always fallen short of the demand **(Gaanappriya Mohan *et al*.,2012).** Consequently, much of the long staple cotton requirements are met by resorting to imports.

Obsolete Machinery - Most of the textile mills are old with obsolete machinery. This results in low productivity and inferior quality. In the developed countries, the textile machinery installed even 10-15 years ago has become outdated and obsolete, whereas in India about 60-75 per cent machinery is 25-30 years old. Only 18-20 per cent of the looms in India are automatic whereas percentage of such looms ranges from 100% in Hong Kong, 99% in USA, 92% in Canada, 83% in Sweden, 76% in Norway, 70% in Denmark, 60% in Australia, 60% in Pakistan and 45% in China.

Erratic Power Supply - Power supply to most cotton textile mills is erratic and inadequate which adversely affects the production.

Low Productivity of Labour - Labour productivity in India is extremely low as compared to some of the advanced countries. On an average a worker in India handles about 2 looms as compared to 30 looms in Japan and 60 looms in the USA. If the productivity of an American worker is taken as 100, the corresponding figure is 51 for U.K. 33 for Japan and only 13 for India **(N. Sri Kumaran *et al*., 2011**. **Strikes -** Labour strikes are common in the industrial sector but cotton textile industry suffers a lot due to frequent strikes by a labour force. The long drawn strike in 1980 dealt a severe blow to the organised sector. It took almost 23 years for the Government to realise this and introduce legislation for encouraging the organised sector.

Stiff Competition - Indian cotton mill industry has to face stiff competition from power loom and handloom sector, synthetic fibres and from products of other countries.

Sick Mills - The above factors acting singly or in association with one another have resulted in many sick mills. As many as 177 mills have been declared as sick mills. The National Textile Corporation set up in 1975 has been striving to avoid sick mills and has taken over the administration of 125 sick mills. What is alarming is 483 mills have already been closed. Thus the growth of dye sector in the future continues to depend on the performance of end user industries like paints, textiles, printing inks, paper, plastics and foodstuffs **(K. Perumal *et al*., 2012).** The changing customer preferences, boom and expansion of infrastructure in certain parts of the world creates new market opportunities for the dye industry. To achieve global standards the industry needs to put efforts in critical areas so as to adopt aggressive growth and focus on exports, R&D, co-marketing alliances, up-gradation of manufacturing facility, contract manufacturing with companies having established markets, identification of areas of core competence, consolidation, collaboration by cluster development, outsourcing, environmental consciousness and cost reduction (**Lokendra Singh and Ved Pal Singh 2010)**.

2. Materials and Methodology

Microorganisms Used

Aspergillus niger, Aspergillus flavus

Isolation of Fungi

PDA Composition

- Water – 1000 mL
- Potatoes – 200g (boiled in water and made into an infusion)
- Dextrose – 20g
- Agar – 20g

Soil samples were collected randomly from college premises and 1g of the soil was dissolved in 10ml of distilled water in a test tube. Three plates of PDA were prepared for two organisms each and one was used as a control. 1.17g of PDA was weighed and added to 30ml of distilled water, autoclaved at 121°C for 15minutes and poured onto the petriplates and allowed to solidify before inoculating inside the UV chamber. Then the soil solution (0.1 ml) was spread onto the Potato Dextrose Agar (PDA) plates with the help of a sterile spreader. The contaminated almond and stored rices are used for isolating *Aspergillus flavus*. The spoiled almond or stored rice are crushed by using mortar and pestle and are added to Potato Dextrose Agar. All the PDA plates were then kept in an incubator at 28°C for 4-5 days and observed for fungal colonies.

Characterization for Fungi

After colony identification, to study the microscopic features of the fungi Lacto Phenol Cotton Blue (LPCB) staining technique was performed

LPCB Composition(45mL)

- Phenol (pre weigh) – 10g
- Methyl blue – 0.04g
- Lactic acid – 10mL
- Glycerol – 20mL
- Distilled water – 10mL

The stain was prepared by dissolving the chemicals with gentle heating for complete dissolution. The stain was added to phenol and transferred to brown bottle. A clean glass slide was taken and a drop of 70% alcohol (ethanol)

was placed on it. The specimen of fungi was placed onto it using a sterile inoculation loop. The specimen was gently teased for even distribution with the help of a sterile teaser. 1 or 2 drops of LPCB was added to stain before the alcohol dries. A coverslip was placed on top of the slide and observed under 40X and 100X oil immersion. The fungus *Aspergillus flavus* and *Aspergillus niger* was used for the degradation of congo red and bromophenol blue which is extensively used in textile industry.

Preparation of PDA Containing Congo Red and Bromophenol Blue

The congo red and bromophenol blue was selected for decolorization *Aspergillus flavus* and *Aspergillus niger*. PDA media was prepared. 0.26 gm of dye powder or 1.0% dye solution was added in water and after it gets solubilized was followed by the addition of PDA powder and autoclaved at 121ºC for 15 minutes.

Pouring the Modified PDA Plate

The media of about 20ml was poured into previously sterilised petriplates, which were allowed to cool inside the UV chamber. Solidification of media was attained after 30 miutes. The petriplates of modified PDA + congo red and PDA + Bromophenol blue were inoculated with the help of inoculation loop and 5 days old mycelium was used as inoculant. All inoculated plates were incubated at 28ºC.

Preparation of Controls

For the present study two types of controls were used. The first without dyes and second with dyes were used. The first control was used to compare the fungal growth in the medium with and without dyes. The second control was used to compare the visual disappearance of colour from the inoculated petriplates.

Optimization of Modified PDA

Optimization was carried out by adding 2 gm each of sucrose and peptone powder along with 0.26 gm of dye powder or 1.0% of dye solution in sterilized distilled water and solubilized. It was followed by addition of PDA powder and autoclaving. A plate of optimised PDA+dye+sucrose+peptone was prepared for inoculation along with the control.

Inoculation of Fungus

Inoculation of stored *Aspergillus flavus* and *Aspergillus niger* was carried out in the modified (PDA+dye) petriplate and optimized (PDA+dye+sucrose+peptone) plate. The plates was streaked using sterilised techniques. The two control modified PDA plate and optimized (PDA+dye+sucrose+peptone) were un-inoculated by fungus.

Monitoring for Decolorization

Decolorization of dyes from the *A.flavus* and *A.niger* treated petriplates were assessed by the change in original color (as compared to control) and by the visual disappearance of colour from the petriplates. The radial growth of the fungal mycelium and change in colour was measured after a fixed interval (72 hours).The culture plates containing dyes were examined for the visual disappearance of colour from the media of the petriplates when compared to their respective controls. The decolorization was observed earlier (3 days) in case of optimised PDA containing dye, sucrose and peptone, as compared to that in modified PDA containing only dye (8 days).

Percentage of Inhibition

Percentage of inhibition of fungal growth during degradation/decolorization was measured and calculated by using the following formula:

$$I=C-T/C \times 100$$

Where, I = Percentage of inhibition in fungal growth, C= growth in terms of colony diameter in control and T=Growth in terms of colony diameter in the sample.

Thin Layer Chromotography

Conformation of dye degradation /decolorization of dyes was done as follows: Different solvent system were used for TLC where the following ratios were followed. Water : ethanol : methanol (4:3:3) and ethanol : methanol (5:5) and water : methanol (4:3). Proper separation was obtained in ethanol : methanol (5:5) solvent system for detection of degradation of the dyes. The confirmation of dye degradation was observed by the Retention factor R_f which was calculated using the formula

$$R_f = \frac{\text{Distance travelled by the compound}}{\text{Distance travelled by solvent front}}$$

Application In The Degradation of Azo Dye In Fabric

Different types of clothes (cotton, nylon and silk cotton) were used in the degradation. The piece of clothes was soaked in the dye and was boiled for 5 minutes and the clothes were dried for 30 minutes. The nutrient broth was prepared and the organism such as *Bacillus subtilis* and *Pseudomonas spp.* were inoculated in the nutrient broth and incubated for 24 hrs. The mordant fabrics with the dye were inoculated in the broth and incubated at 32ºC for 24 hours.

Table 1. Application Categories of Dyes

Type of dye	Characteristics	Substrates
Acid	When in solution are negatively charged; bind to the cationic NH_3^+-groups present in fibres	Nylon, wool, polyamide, silk, modified acryl, paper, inks and leather
Reactive	Form covalent bonds with OH-, NH- or SH- groups	Cotton, wool, silk and nylon
Metal complex	Strong complexes of one metal ion (usually chromium, copper, cobalt or nickel) and one or two dye molecules (acid or reactive)	Silk, wool and polyamide
Direct	Large molecules bound by Van der Waals forces to the fibre	Cellulose fibres, cotton, viscose, paper, leather and nylon
Basic	Cationic compounds that bind to the acid groups of the fibre	Synthetic fibres, paper and inks
Mordant	Require the addition of a chemical that combines with the dye and the fibre, like tannic acid, alum, chrome alum, and other salts of aluminium, chromium, copper, iron, potassium, and tin	Wool, leather, silk, paper, modified cellulose fibres and anodised aluminium
Disperse	Scarcely soluble dyes that penetrate the fibre through fibres swelling	Polyester, polyamide, acetate, acrylic and plastics
Pigment	Insoluble, non-ionic compounds or insoluble salts that retain their crystalline or particulate structure throughout their application	Paints, inks, plastics and textiles
Vat	Insoluble coloured dyes which on reduction give soluble colourless forms (leuco form) with affinity for the fibre; on exposure to air are reoxidised	Cellulose fibres, cotton, viscose and wool
Azoic and Ingrain	Insoluble products of a reaction between a coupling component and a diazotised aromatic amine that occurs in the fibre	Cotton, viscose, cellulose acetate and polyester
Sulphur	Complex polymeric aromatics with heterocyclic S-containing rings	Cellulose fibres, cotton and viscose
Solvent	Non ionic dyes that dissolve the substrate to which they bind	Plastics, gasoline, varnish, lacquer, stains, inks, oils, waxes and fats
Fluorescence brightners	Mask the yellowish tint of natural fibres	Soaps and detergents, all fibres, oils, paints and plastics
Food	Non-toxic and not used as textile dyes	Food
Natural	Obtained mainly from plants	Food, cotton, wool, silk, polyester, polyamide and polyacrylonitrile

3. Results and Discussion

Isolation of fungi and Bacteria

The pure culture of *Aspergillus niger* was isolated from soil sample and *Aspergillus flavus* was isolated from Almond or stored rice using PDA and NUTRIENT AGAR (**Figures 1 - 2**).

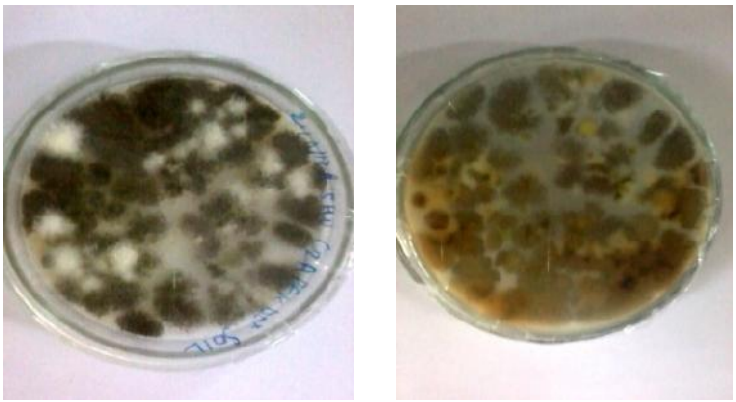

Figure 1. *Aspergillus Niger*

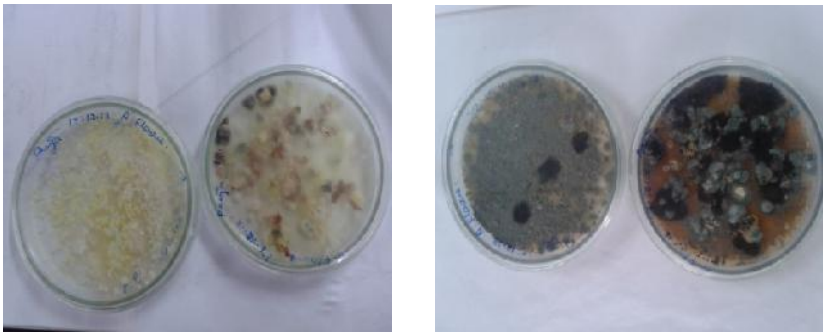

Figure 2. *Aspergillus Flavus*

Microsopic Characterization of Fungi

For *Aspergillus niger* colonies on potato dextrose agar at 25°C were initially white which turned to black colonies with conidial production. The hyphae are septate and hyaline. Conidial heads are radiate initially, splitting into columns at maturity. The species is biseriate (vesicles produces sterile cells known as metulae that support the conidiogenous phialides). Conidiophores are long, smooth, and hyaline, becoming darker at the apex and terminating in a globose vesicle. Metulae and phialides cover the entire vesicle. Conidia are brown to black, very rough and globose (**Figure 3 & 4**).

Aspergillus niger *Aspergillus flavus*

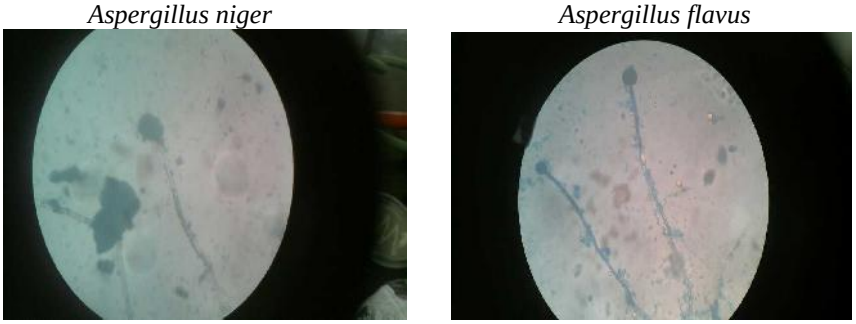

Figure 2. Microscopic View of Fungus

The *Aspergillus flavus* colonies on potato dextrose agar at 25ºC were light sparase grey green to pale blue green or parrot green, mycelium fluffy creamy white to dull white color and exudates were present on surface, reverse uncolored to yellowish or orange and wrinkled mycelial growth, soluble pigments were absent, very few sclerotia were present in wheat brown color (**Figure 4**). In the present study, results for dyes degradation/decolourization by *A. Flavus* and *A.niger* were positive, and the accumulation of dyes by the fungus also took place. The disappearance of colour and change in original colour in the fungus-treated medium were slightly observed. The evaluation of degradation/decolourization was assessed as the disappearance of colour from the Petri plate, during the growth of the fungal mycelium. For the dye Bromophenol blue, the applied fungus has shown positive result for biodegradation and the blue colour of this dye was turned into yellow and finally, a zone of yellow colour was present around the mycelium. A small fraction of dye was also accumulated by the applied fungus, and its mycelium turned into blue colour. In case of fungal degradation of Congo red also, the results were positive, but it was degraded more efficiently than Bromophenol blue and the disappearance of colour from the Petri plate was slightly observed in the fungus-treated dye. The inhibition of fungal growth in dye-containing medium was also observed during dye degradation. Biodegradation of textile dyes, Bromophenol blue and Congo red by *A. flavus* and *A. niger* percentage inhibition of growth of this fungus is in response to these dyes in Potato Dextrose Agar (PDA) medium (**Tables 2 - 3 & Figures 5 - 9**).

Table 2. Percentage Inhibition for *Apsergillus Flavus*

Dyes tested	***Aspergillus flavus***			**Inhibition (in%)**	
	Control (in cm) C	PDA + Culture (in cm) T	PDA +Culture+Sucrose+Peptone (in cm) T	PDA + Culture (in cm) T	PDA +Culture+Sucrose+Peptone (in cm) T
Bromo phenol blue	4.4	3.2	3.1	27.27	29.54
Congo Red	4.4	4.4	3.5	0	20.45

Table 3. Percentage Inhibition for *Apsergillus Niger*

Dyes tested	***Aspergillus niger***			**Inhibition (in%)**	
	Control (in cm) C	PDA + Culture (in cm) T	PDA +Culture+Sucrose+ Peptone (in cm) T	PDA + Culture (in cm) T	PDA +Culture+Sucrose+P eptone (in cm) T
Bromo phenol blue	4.4	4.2	4.3	4.54	2.27
Congo Red	4.4	4.1	4.0	6.81	9.09

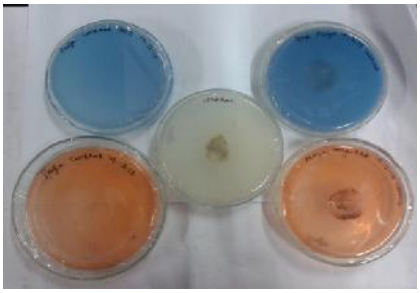

Figure 5. Control and Inoculated Plates

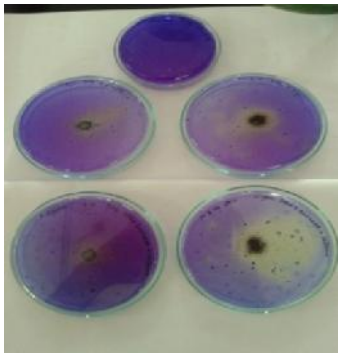

Figure 6. Visual Decolourizarion of Bromophenol Blue

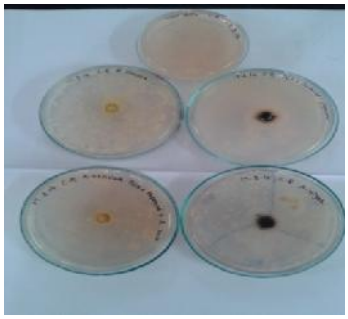

Figure 7. Visual Decolourizarion of Congo Red

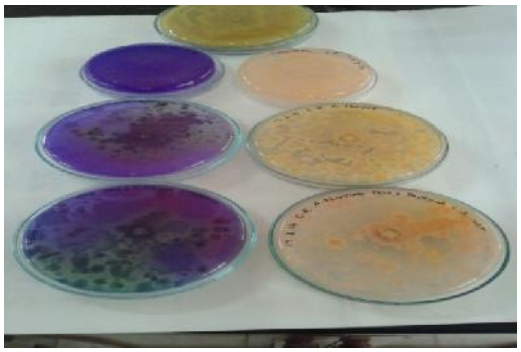

Figure 8. Visual Decolourizarion of Congo Red by *Aspergillus Flavus* in Modified PDA And Optimised PDA Containing Sucrose And Peptone

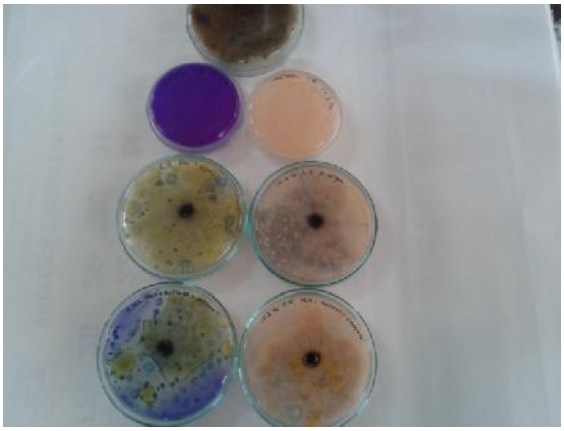

Figure 9. Visual Decolourizarion of Congo Red By *Aspergillus Niger* in Modified PDA and Optimised PDA Containing Sucrose And Peptone

The time taken for decolorization in modified PDA (containing dye) is 8 days (192 hrs) and the time taken for decolorization in optimised PDA(containing dye+sucrose+peptone is 3 days (72hrs) A zone of different colour (yellow to pale) around the fungal colony was also observed which might be due to the production of extracellular enzymes by the applied fungus, during the biodegradation of tested dyes.

Effect of Different Dye Concentrations on Decolourization

The decolourization performance of Congo red and Bromophenol blue by *A. niger* and *A. flavus* was studied at various increasing dye concentration (50, 100, 250mg/L). It was observed that the rate of decolourization varies at different increasing concentration. At 200 mg/L of congo red dye concentration, showed 74% and 80% decolourization with *Aspergillus niger* at static and shaking condition was observed. In case of *A. flavus* percentage of decolourization was observed only 77% and 84% in static and shaking condition respectively. The time required for decolourization varied from 1-5 days. Thus it is revealed that *Aspergillus flavus* showed an increased percentage of decolourization of about 84% in shaking condition on congo red dye and it is also proved that *Aspergillus* species are most suitable for the decolourization of harmful synthetic dyes.

Table 4 & 5 comprises of the OD values of Congo red dye decolourisation under static and shaking condition of *Aspergillus flavus* at 530nm and *Aspergillus niger* at 520 -540nm. **Table 6 & 7** comprises of the OD values of Bromophenol blue dye decolourisation under static and shaking condition of *Aspergillus flavus* at 530nm and *Aspergillus niger* at 520 -540nm.

Table 4. OD Values of Congo Red Dye Decolourisation Under Static And Shaking Condition of *Aspergillus Flavus (530 Nm)*

Days	50 mg/l		100 mg/l		200 mg/l	
	Static 1	Shaking 1	Static 2	Shaking 2	Static 3	Shaking 3
1	0.06	0.05	0.07	0.08	0.08	0.07
2	0.02	0.01	0.02	0.03	0.02	0.03
3	0.06	0.03	0.04	0.03	0.05	0.04
4	0.22	0.08	0.01	0.06	0.12	0.01
5	0.06	0.12	0.07	0.12	0.03	0.14

Table 5. OD Values of Congo Red Dye Decolourisation Under Static And Shaking Condition of *Aspergillus Niger (520 -540nm)*

DAYS	50 mg/l		100 mg/l		200 mg/l	
	Static 1	Shaking 1	Static 2	Shaking 2	Static 3	Shaking 3
1	1.06	0.02	0.06	0.10	1.04	0.05
2	0.09	0.06	0.09	0.07	0.09	0.10
3	0.11	0.07	0.09	0.06	0.08	0.09
4	0.09	0.01	0.09	0.08	0.09	0.06
5	0.01	0.08	0.07	0.01	0.03	0.07

Table 6. OD Values of Bromophenol Blue Dye Decolourisation Under Static and Shaking Condition of *Aspergillus Flavus (530 Nm)*

DAYS	50 mg/l		100 mg/l		200 mg/l	
	Static 1	Shaking 1	Static 2	Shaking 2	Static 3	Shaking 3
1	1.17	0.03	1.20	0.16	1.42	1.27
2	0.48	0.51	0.11	0.46	0.26	0.48
3	0.12	0.11	0.12	0.12	0.06	0.09
4	0.18	0.03	0.01	0.03	0.09	0.01
5	0.13	0.07	0.17	0.08	0.23	0.27

Table 7. OD Values of Bromophenolblue Dye Decolourisation Under Static And Shaking Condition of *Aspergillus Niger (520-540nm)*

DAYS	50 mg/l		100 mg/l		200 mg/l	
	Static 1	Shaking 1	Static 2	Shaking 2	Static 3	Shaking 3
1	1.09	0.03	0.62	0.16	0.50	1.67
2	1.09	0.04	0.10	0.46	0.04	0.48
3	1.05	0.11	0.03	0.12	0.07	0.28
4	0.03	0.05	0.01	0.02	0.09	0.22
5	0.04	0.02	0.13	0.11	0.05	0.16

Thin Layer Chromotography

The result of biodegradation of Acid Orange 10, Methyl red, Congo red, Bromophenol blue and Carbol Fuchsin was confirmed by Thin Layer Chromatography (TLC) as spots with different RF values which were obtained and were compared to spots of standard dye sample i.e. Control (**Figures 10 & 11 and Table 8**). The azo dyes have immense application as an eco – friendly degradent of fabrics (**Figures 12 - 15**).

The Retention factor (R_f) was then calculated using the formula:

$$R_f = \frac{\text{Distance travelled by the compound}}{\text{Distance travelled by solvent front}}$$

Figure 10. TLC Setup on Different Solvent Systems

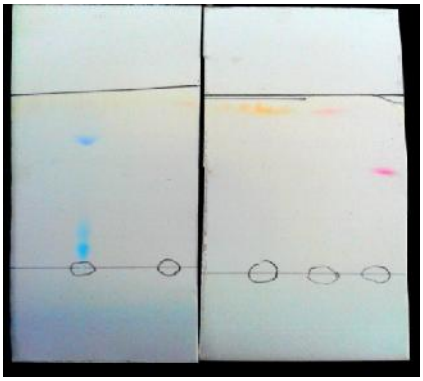

Figure 11. Presence of Dye Concentration is Identified as Spots on TLC Plate

Table 8. Thin Layer Chromotography

Azo Dye	Solvent Used	Retention Factor (R_f)
ACID ORANGE 10	Ethanol:Methanol (5:5)	0.94
METHYL RED	Ethanol:Methanol (5:5)	0.9
CARBOL FUCHSIN	Ethanol:Methanol (5:5)	0.58
BROMOPHENOLBLUE	Ethanol:Methanol (5:5)	0.71
CONGO RED	Ethanol:Methanol (5:5)	0.9

Figure 12. Dyeing of Fabric in Acid Orange 10

Figure 13. Decolourization of Dyed Fabric

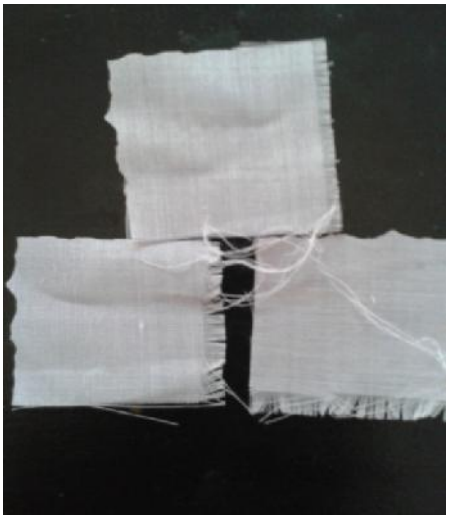

Figure 14. Control

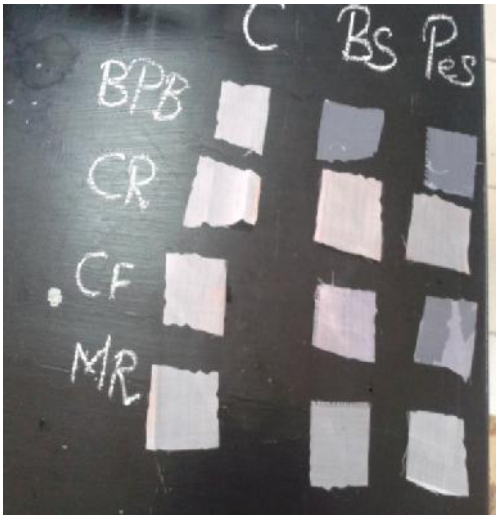

Figure 15. Degradation of dye was observed in dyed fabrics

4. Conclusion

Fungal organism *Aspergillus nige*r were isolated from soil sample whereas *Aspergillus flavus* were isolated from contaminated Almond or stored rice. The fungal mycelium was also tested for percentage inhibition in the dye inoculated plates. Sugar concentration analysis was done for *Aspergillus flavus* and *Aspergillus nige*r using peptone and sucrose. Concentration degradation of dye was analysed in static and shaking condition for fungus. Bioautography of the dye was done by TLC with ethanol and methanol as solvent system. The time required for decolourization varied from 1-5 days. *Aspergillus flavus* showed an increased percentage of decolourization of about 84% in shaking condition on congo red dye and was proved that *Aspergillus* species are most suitable for the decolourization of harmful synthetic dyes.

5. References

1. Anjali P, Poonam S, Leela I. Bacterial decolorization and degradation of azo dyes. *Int Biodet Biodegr.*, **2007**, 59:73–84.
2. Gaanappriya Mohan, Logambal. K. and Ravikumar. R. Investigation on the removal of direct red dye using *aspergillus niger* and *aspergillus flavus* under static and shaking conditions with modeling., **2012**, 1(3): 144 - 153

3. Perumal K., Baby Malleswari R., A. Catherin A., and T.A. Sambanda Moorthy T.A. Decolourization of Congo Red dye by bacterial consortium isolated from dye contaminated soil, Paramakudi, Tamil Nadu,*J. Microbiol. Biotech. Res.*, **2012**, 2(3):475-480
4. Lokendra Singh and Ved Pal Singh. Biodegradation of Textile Dyes, Bromophenol Blue and Congored by Fungus *Aspergillus Flavus*,Environ. We *Int. J. Sci. Tech.*, **2010**, 5: 235-242
5. Mustafa Isik, Delia Teresa Sponza. Effect of oxygen on decolorization of azo dyes by *Escherichia coli* and *Pseudomonas sp.* and fate of aromatic amines, **2003**, pp: 1183-1192.
6. Sri Kumaran1 N., and Dharani G. Decolorization of textile dyes by white rot fungi *phanerocheate chrysosporium* and *pleurotus sajor-caju, International peer-reviewed journa.l*, **2011**, 1(4): 361-370.
7. Robinson T, McMullan G, Marchant R, Nigam P. Remediation of dyes in textile effluent: a critical review on current treatment technologies with a proposed alternative. *Bioresour. Technol.*, **2001**, 77: 247–255.
8. Senthil Kumar S., Balasubramanian P., Swaminathan G., Degradation Potential Of Free And Immobilized Cells Of White Rot Fungus Phanerochaete chrysosporium on Synthetic Dyes, CODEN(USA): IJCRGG, 2013, 5(2), pp. 565-571.
9. Sidra Ilyas, Skinder Sultan and Abdul Rehman, Decolourization and degradation of azo Dye, Synozol Red HF6BN, by *Pleurotus ostreatus*, *African Journal of Biotechnology*., **2012**, 11(88), pp.15422-15429
10. Singh, L., Singh, V, P. Microbial degradation and decolourization of dyes in semisolid medium by the fungus–*Trichoderma harzianum*. *Environment & We: International Journal of Science & Technology*., **2010**, 5(3):147-153.
11. Swamy, J., Ramsay, J.A., The evaluation of white-rot fungi in the decolourization of textile dyes. *Enzyme and Microbial Technology*, **1999**, 24, 130-137.
12. Syed M.A. , Sim H.K., Khalid A.,and Shukor M.Y. A simple method to screen for azo-dye-degrading bacteria, *J. Environ. Biol*. **2009**, 30(1): 89-92
13. Tripathi A. and Srivastava S.K.. Ecofriendly Treatment of Azo Dyes: Biodecolorization using Bacterial Strains, *International Journal of Bioscience, Biochemistry and Bioinformatics*, **2011**, 1(1).
14. Wong, Y., Yu, J., Laccase-catalyzed decolorization of synthetic dyes. *Water Research.*, **1999**, 3, 3512-3520.