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RESEARCH ARTICLE

ISOLATION AND OPTIMIZATION OF SOIL BORNE FUNGI TO DECOLORIZE BROMOTHYMOL BLUE AND METHYLENE BLUE [JENIFFER SYLVIA D]

JENIFFER SYLVIA D, KAVITHA J

Dept. of Biotechnology, Anna University Regional Centre, Coimbatore

Abstract

The waste generated during the process and operation of the dyes, contains inorganic and organic contaminant leading to the hazard to ecosystem and biodiversity causing impact on the environment. The physico-chemical treatment does not remove the color and dye compound concentration. The decolorization of the dye takes place either by adsorption on the microbial biomass or and enzymatic degradation. As soil with leaf litter are rich in lignin degrading organisms 4 different soil samples containing leaf litter were analyzed for potential microbes for the decolourization of Dyes. The decolorization of dyes was studied in Potato Dextrose Agar (PDA) medium, while a fixed amount of dye solution (0.5%) in each case was used in culture medium. The decolorization of dyes was observed by the change in original colour and visual disappearance of colour from the treated Petri plates.Physico-chemicalparameters like carbon source, temperature, and pH are optimized for the decolorizationprocess by changing one parameter at a time. Four isolates showed dye decolorization and one isolate was found to be laccase producing organism. The study has confirmed the potential of the above isolates in the decolorization of dyes such as Methylene Blue and Bromothymol Blue and opened scope for future analysis of their performance in the treatment of textile effluent.

Keywords: Decolorization, Enzymatic degradation, Effluent

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**Corresponding Author*

JENIFFER SYLVIA D
KAVITHA J

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INTRODUCTION

In recent years, Environmental pollution has been one of the major problems of the modern world. Various chemical substances discharged from the industries become an environmental contaminant. Due to rapid

industrialization and urbanization, a lot of chemicals including dyes, pigments, and aromatic molecular structural compounds were widely used in several industrialapplications such as textiles, printing, pharmaceuticals, food, toys, paper, plastic and cosmetics [Shymala *et al*., 2014].

Textile processing industries were found in most of the countries and their numbers have been increased. Textile dyes constitute a major source of pollution. Textile industries consume a major share of dyes in India [Kumar *et al*., 2012].The dyes includes such as acidic, reactive, basic, disperse, azo, diazo, anthraquinonedyes which causes a considerable environmental pollution problems. The effluent from the dyeing processes contributes to the high colorant content and chemical oxygen demand of the total drainage. Effluent that release from the production process of textiles is not properly disposed, can cause grave environmental pollution, sometimes to levels that can threaten human health, livestock, wildlife, aquatic lives and collapse the entire ecosystem. Presence of dyes in the effluent causes an unpleasant appearance by imparting the color and its breakdown products are toxic, carcinogenic and mutagenic [Chacko *et al*., 2011].

MICROBIAL DEGRADATION OF DYES

FUNGAL BIODEGRADATION

A group of fungal organisms have an ability to decolorize wide range of dyes. Fungus is capable of degrading dioxins, polychlorinated, biphenyls (PCBS) and chloro- organics. White- rot fungi is capable of decolorizing dyes e.g., *Coriolus versicolor*, *Tramates versicolor*, *Funaliatrogii*, *Umbelopsisisabellina* and *Penicillium geastrivous*, *Aspergillus foetidus,Rhizopus oryzae* can decolorize or biosorb various dyes.

Major group of fungus produce lignin modifying enzymes like laccase, manganese peroxidase (MnP) and lignin peroxidase (Lip) to mineralize/ degrade synthetic lignin or dyes. The decolorization was a secondary metabolic activity linked to the fungus Ligninolytic degradation activity. The degradation of some xenobiotic by other white- rot fungi is known to occur under non- ligninolytic conditions and would mainly be through the laccase enzyme activity. Basidiomycetes fungi not only decolorize but also degrade and mineralize dye structure and it also degrade numerous toxic organic and recalcitrant compounds. The enzyme system of basidiomycetes fungi is nonspecific in the degradation of pollutants and even acts on mixtures of pollutants.

BACTERIAL DEGRADATION

Many organisms are reported to decolorize various triphenlymethane and azo dyes. There are a few reports on the biodegradation of theses dyes by bacteria. Biodegradation of synthetic dyes by *Pseudomonas pseudomallei* is reported. In general the decolorization of the dyes is not related to their molecular weights and the octanol- water coefficients of the dyes [Ruchi., 2012]. *Bacillus subtili*s, *Aeromonas hydrophilia*, *Bacillus cereus*, *Pseudomonas sp*, *E. Coli* degrades dyes efficiently. Under aerobic conditions azo dyes are not readily metabolized, intermediates formed during degradation resulted in disruption of metabolic pathways and the dyes were not mineralized.

ALGAL BIODEGRADATION

Algal culture also has an ability to degrade azo dyes through azoreductase. *Chlorella* and *Oscillatoria* were capable of degrading azo dyes to aromatic amines and further to simple organic compounds. *Synechocystis* sp and *Phormidium* sp have a capacity to remove reactive dyes such as Reactive Red, Remazol Blue, and Reactive Black B [Shymala *et al*., 2014].

The present study is to isolate dye decolorizing microorganisms from the litter samples as they are rich in lignolytic enzymes and to monitor the effect of dye decolorization at different parameters and to analyze the presence of enzymes.

2. METHODOLOGY

COLLECTION AND CULTIVATION OF MICROBIAL STRAINS

4 Soil sample of decomposed leaf litter were collected. 1g of soil sample were mixed with 10mL of distilled water in test tubes. The stock solution is serially diluted to get the concentration up to 10^{-7}. 0.1mL of sample

from each dilution was spread on Potato Dextrose Agar plates with the help of L rod. Pure fungal isolates were obtained on the PDA plates by sub culturing. The isolates were further sub-cultured on PDA plates and incubated at room temperature. After sufficient growth of the colonies, the plates were stored in refrigerator and served as stock cultures. Subcultures were routinely made every 15 days.

PREPARATION OF DYE SOLUTIONS

The two dyes used in this study were Bromothymol Blue ($C_{27}H_{28}Br_2O_5S$) and 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. It can dye cotton directly and wool out o f a neutral bath.

Table 1: Dyes Used

Name	pH	Molecular weight [g/mol]	Absorption peak
Methylene Blue	6	319.85	600nm
Bromothymol Blue	6	624.40	440nm

Dye solution (0.5%) was prepared by dissolving accurately 0.5 g dye in 100mL distilled water. This procedure was adopted as per [Ramamurthy *et al*., 2013] for preparation of dye solution.

SCREENING SAMPLES FOR DYE DEGRADATION

Dye degradation was examined through primary screening by plating method and secondary screening by submerged method. The dye decolorization assay was done by absorbance method. The screening was done to measure its ability to degrade dyes accordingly.

PRIMARY SCREENING BY PLATING METHOD

The PDA medium was prepared and sterilized. After autoclaving, the medium is cooled. 0.05% of Methylene Blue was added to the medium and poured in respective plates. The plates are incubated at 30 °C for 1-3 days. The plates which shows the zone of clearance of dyes was selected for secondary screening. This was done according to the procedure followed by [Gopinath *et al*., 2013].

SECONDARY SCREENING BY SUBMERGED METHOD

The secondary screening was carried out in Potato Dextrose Broth (PDB) in an Erlenmeyer flask. The Strain which showed positive results i.e, zone of clearance at primary screening undergo secondary screening. The Mycelial disc of size approximately 5 mm diameter was cut from the selected petriplates of a week old culture, and placed in flask containing 0.05% of Bromothymol Blue and Methylene Blue along with Potato Dextrose Broth. They are incubated at 30 °C in rotary shaker at 100 rpm. After 7 days of incubation, effective decolorization was visualized. Screening results in isolation of fungi capable of degrading Dyes. Those isolates showing decolorization of dyes were selected for further studies on optimization of physio-chemical parameters.

DECOLORIZATION ASSAY

Decolorization activity in terms of percent of decolorization was determined by measuring the absorbance of the initial and residual medium. A 2.0mL aliquot of the decolorized culture broth was placed in Eppendorf tubes and centrifuged at 5000 rpm for 15 minutes. The supernatant was recovered and analyzed spectrophotometrically at a wavelength corresponding to the maximum absorbance of both the dyes which is 600 nm for Methylene Blue and 440nm for Bromothymol Blue. The un-inoculated medium which contains dye was used as blank. Decolorization activity was calculated according to the following formula.

$$\text{Percent Decolorization} = \frac{[\text{OD initial - OD final}]}{\quad} * 100 \qquad [2.1]$$

OD initial

The experimental procedure adopted was as per [Gopinath *et al*., 2013] for the decolourization of malachite green.

EFFECT OF EXTERNAL PARAMETERS ON DYE DECOLORIZATION

These optimization process was carried out with reference to Kumar *et al*., 2012.

EFFECT OF CARBON SOURCE

Decolorization of Methylene Blue and Bromothymol Blue dye (0.05%) in broth by selected fungal isolates were optimized with respect to the effect of 2% of different Carbon sources such as Glucose, Dextrose and Sucrose, keeping the other parameters constant such as pH 7, temp 35°C in rotary shaker [100 rpm]. The samples were centrifuged at 5000 rpm for 5 minutes and the supernatant was used to measure the absorbance. The effect of carbon source on the dye decolorization was monitored.

EFFECT OF TEMPERATURE

The PDB medium was prepared and incubated at different temperature such as 28°C, 35°C and 42°C on a rotary shaker [100 rpm]. The effect of temperature on dye degradation was monitored. The samples were centrifuged at 5000 rpm for 5 minutes and the supernatant was used to measure the absorbance. The effect of temperature on dye decolorization was monitored.

EFFECT OF pH

The PDB medium was prepared at different pH such as 6, 7, and 8 using appropriate acid or base. They are sterilized and the fungal strains are inoculated. The medium was incubated at 35 °C on a rotary shaker [100rpm]. The samples were centrifuged at 5000 rpm for 5 minutes and the supernatant was used to measure the absorbance. The effect of the pH on dye decolorization was monitored.

3. RESULTS AND DISCUSSION

ISOLATION AND SCREENING OF SAMPLES FOR DYE DECOLORIZATION

Various single colonies were observed in spread plate method. Those were picked to analyze the dye decolorizing capacity by primary plating assay. Among them 4 strains showed zone of clearance of Methylene blue on primary screening by plating method were represented in Fig 1.

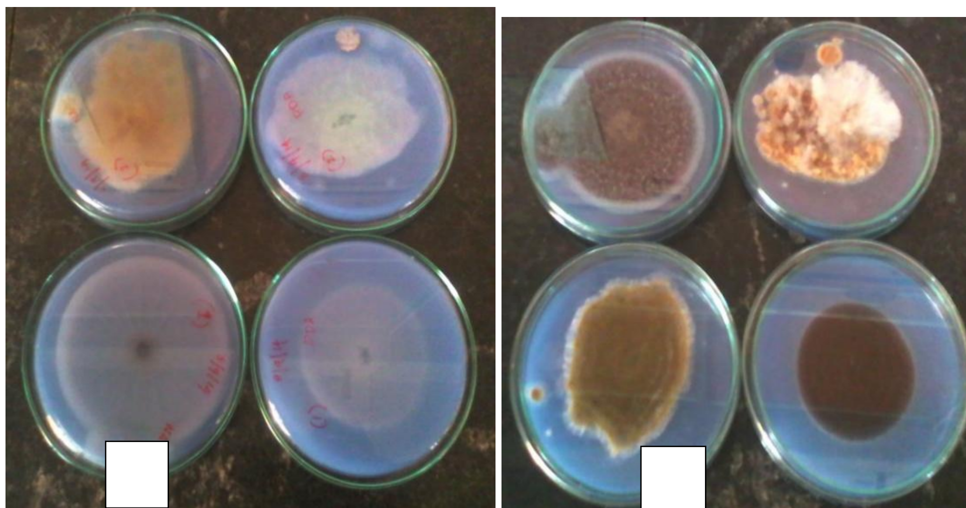

Figure 1: Primary Plating Assay [A], [B] Showing Four Selected Isolates

Those isolates were selected to further monitor the capability of decolorizing dye through submerged method. Absorbance was measured for all the four samples on their 4th and 8th day of incubation. Decolorized medium of both dyes were represented in Fig 3.2 & 3.3. When compared to the results obtained in the work of [Gopinath et al., 2013], the organisms showed almost same decolorization percentage in decolorization assay.

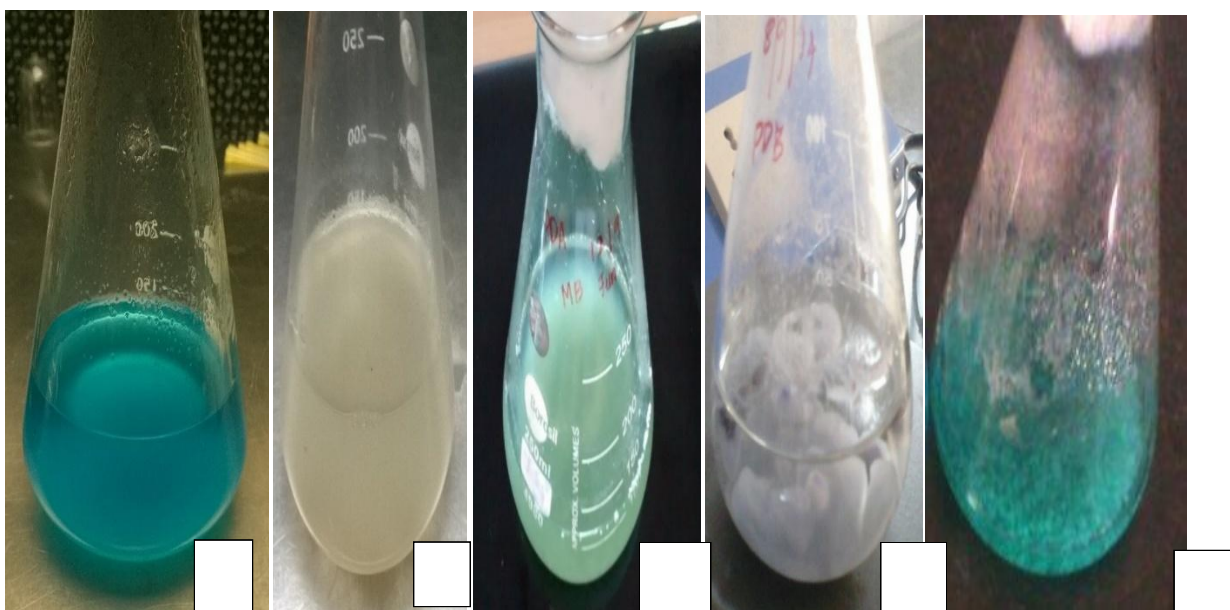

Figure 2: Screening by Submerged Method [A]. [1] Control, [2], [3], [4], [5] Shows Decolorization of Methylene Blue after 8 Days Incubation by Isolates A, B, C, &D Respectively

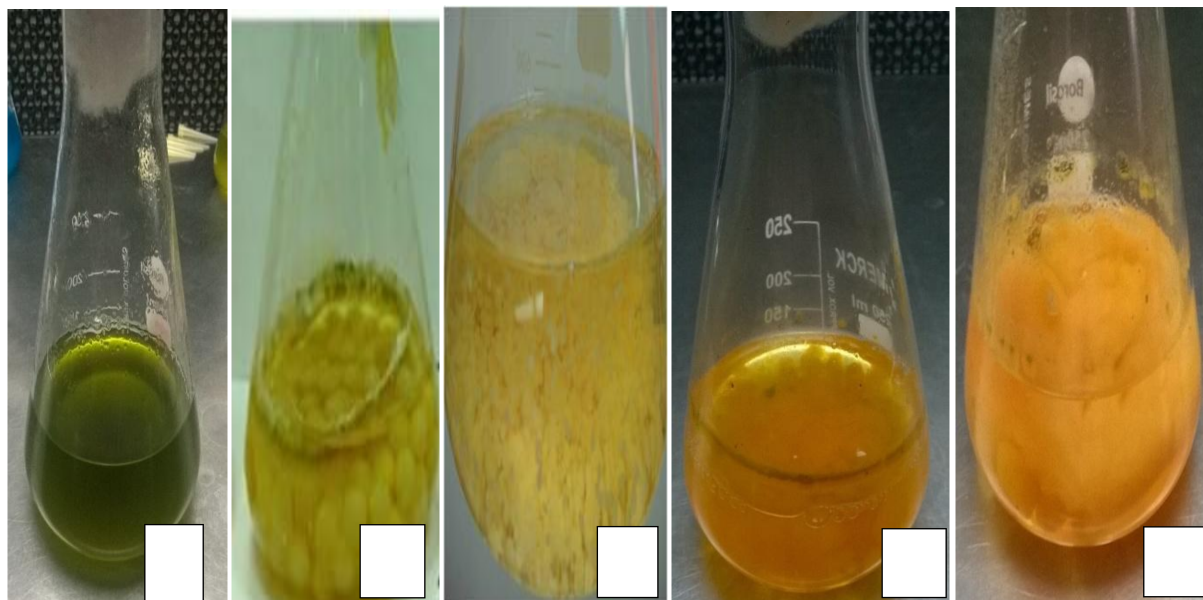

Figure 3: Screening by Submerged Method [B] [1] Control, [2], [3], [4], [5] Shows Decolorization of Bromothymol Blue after 8 Days Incubation by Isolates A, B, C, &D Respectively

DECOLORIZATION ASSAY

This assay was done by measuring the absorbance of the medium at initial and residual stage. The percent decolorization was calculated according to the formula stated. On comparing the four isolates, isolate A and D showed maximum decolorization of methylene blue on 8th day of incubation. The isolates A and D showed white and pale blue color formation.

Table 2: % Decolorization for Methylene Blue

S.No	Isolates	% Decolorization	
		4th Day	8th Day
1	A	36.5	82.6
2	B	57.6	67.3
3	C	46.1	61.5
4	D	65.3	76.9

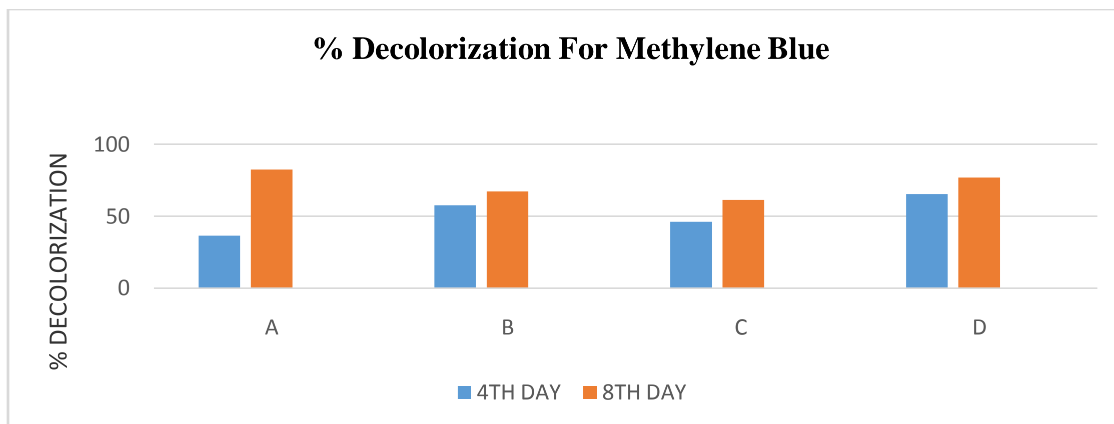

Figure 4: % Decolorization for Methylene Blue

Isolates A and B showed maximum decolorization after incubation of eight days for Bromothymol Blue. The percent decolorization was pictorially represented in Fig 4 & 5. The green color of dye transferred into pale yellow.

Table 3: % Decolorizaton for Bromothymol Blue

S.No.	Isolates	% Decolorization	
		4th Day	8th Day
1	A	51.1	81.3
2	B	48.8	76.7
3	C	34.8	53.4
4	D	55.8	67.4

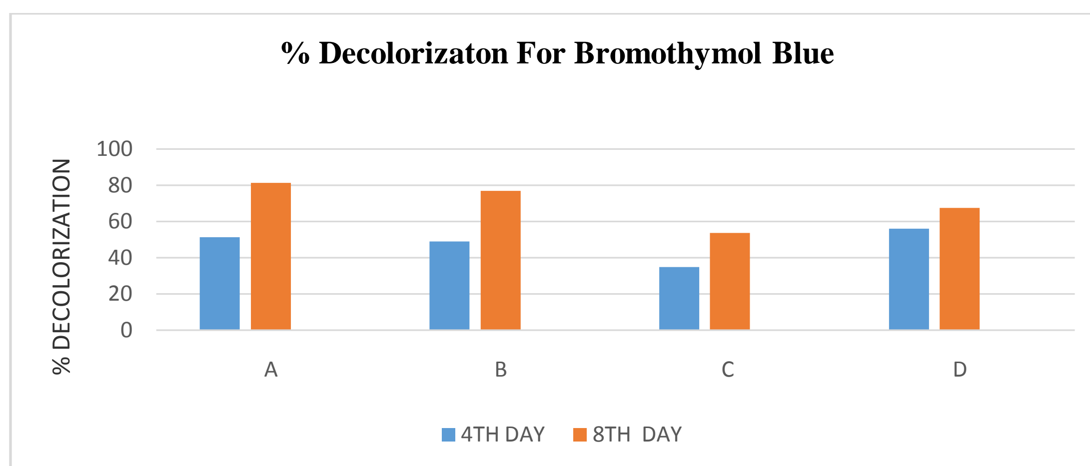

Figure 5: % Decolorizaton for Bromothymol Blue

EFFECT OF EXTERNAL PARAMETERS

EFFECT OF CARBON SOURCE

To explore the effect of the carbon source, experiments were carried out with different carbon source such as Glucose, Sucrose, Dextrose, keeping the other conditions constant [pH 7, dye conc. 0.05%, Temp 35°C]. When compared to the other two, 2% glucose showed maximum decolorization at 8th day of incubation. The average decolorization percentage of both dyes was represented in Fig 6. When the results were compared to the work of [Kumar et al., 2012] % decolorization were more for glucose supplemented medium as per the results cited.

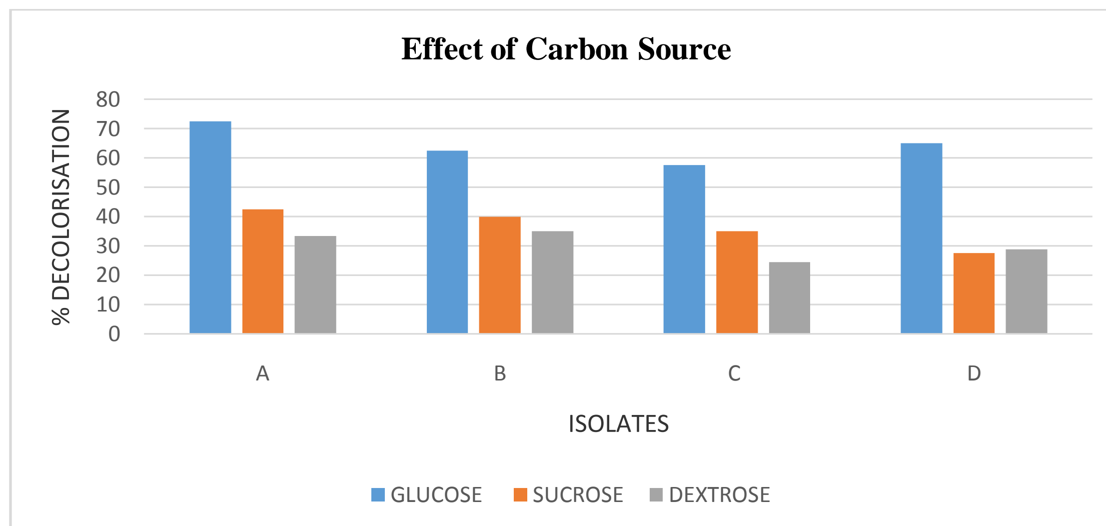

Figure 6: Effect of Various Carbon Source in Decolorization

EFFECT OF TEMPERATURE

The experiment was performed with different temperatures [28°C, 35°C, 42°C] keeping the other conditions constant [pH 7, dye conc. 0.05%], Maximum decolorization was found at 35°C at 8th day of incubation for isolates A, B, & C, whereas for isolate D showed maximum decolorization at 28 °C. The average decolorization percentage was represented in Fig 7.

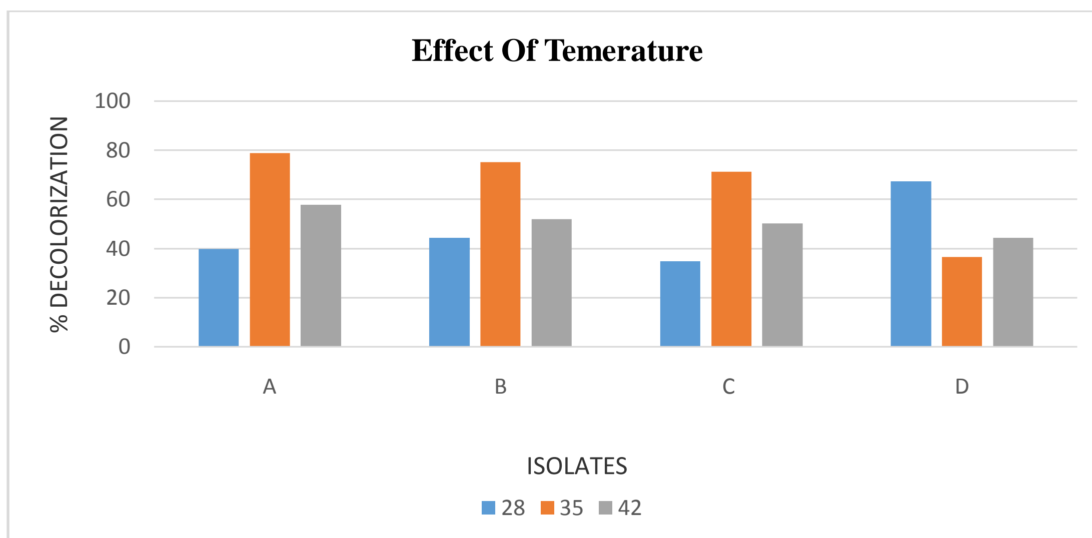

Figure 7: Effect of Different Temperature in Decolorization

EFFECT OF pH

The effect of pH was investigated on various pH 5,7, 9 respectively and the other parameters were kept constant [dye conc. 0.05%, temp 35°C] on the 8th day of incubation there was color decolorization in almost all the pH but pH 9 showed higher percentage of decolorization. The average decolorization percentage was represented in Fig 3.8.

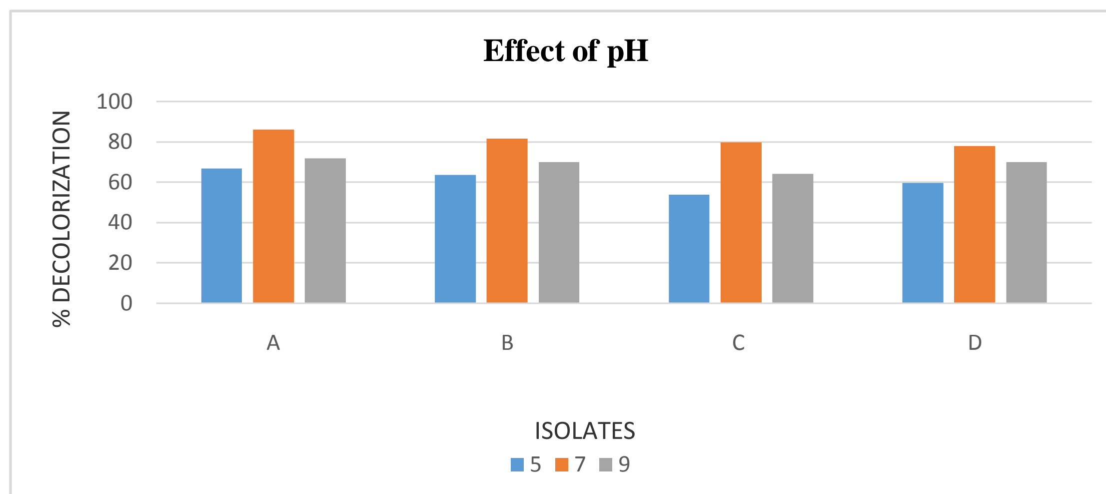

Figure 8: Effect of Different pH in Decolorization

The % decolorization were more for isolate A in glucose supplemented medium and the optimization temperature and pH were found to be 35 °C and pH 7 as per the results cited in the work of Kumar et al., 2012. In case of isolate D decolorization percent were high at 28°C.

FUNGAL IDENTIFICATION

This procedure was adopted according to the method followed by [Gopinath et al., 2013].

Table 4: Fungal Identification

ISOLATES	CULTURAL CHARCTERISTCS	ORGANISM
A	Cotton like growth with green color covered with black spores.	*Aspergillus niger*
B	Initially white and fluffy, later produced pigmented spores turn into bluish green.	*Penicillium chrysogenum.*
C	Rapid growth of colonies was observed by the Velvety in texture, yellowish green in color.	*Aspergillus flavus*
D	White center green yellow periphery	*Aspergillus oryzae*

The isolate A *Aspergillus niger* was found to have more dye decolorizing capability and can also produce laccase enzyme. This was in accordance to the work done by Kiiskinen et al., 2004. Thus it can be concluded that isolate A can be further used to treat effluent by immobilization technique.

CONCLUSION

In this study, addition of a fungal culture on PDA plates produces dye decolorization was demonstrated. From the present study isolated microbes are capable of degrading the dyes such as Methylene Blue and Bromothymol Blue was analyzed. Gas chromatography- mass spectroscopy analysis can be carried out for confirmation of degradation [Thorat et al., 2010]. The present study has an impact of soil borne fungi to be used in textile dye wastewater decolorizing process even in large scale, it is a boon for the water recycling and water purification processes. Even the enzymes isolated and purified from the waste water can be used further in effluent treatment [Mutambanengwe et al., 2008]. Immobilization of the enzymes or the whole organisms can be done for the effective way of decolorizing or degrading the textile wastewater treatment [Aishwarya et al., 2012]. Thus any bioprocesses based dye removal system using such type of fungus should be designed on the basis of these parameters for successful operation.

REFERENCES

1. Aishwarya, Abhijit Limaye, Amit Vernekar, Vandana Jain And Prof. Zabin K. Bagewadi (FS) (2012),"Design For Effective Degradation Of Industrially Important Textile Dyes By Microorganisms Isolated From Activated Sludge", Motorola Scholar Programme.

2. Mutambanengwe C, Oyekola O, Togo And Whiteley (2008), "Production Of Enzymes For Industrial Wastewater Treatment: Proof Of Concept And Application To The Textile Dye Industry", Report To The Water Research Commission On The Project "The Production Of A Cocktail Of Hydrolytic Enzymes From An Anaerobic Sulphidogenic Bioreactor Fed With Sulphate Reducing Bacteria And Municipal Sludge For The Treatment Of Biological And Industrial Wastewater: Proof Of Concept.".

3. Joshni Chacko. T., Kalidass Subramaniam, (2011),"Enzymatic Degradation of Azo Dyes – A Review", International Journal of Environmental Sciences Volume 1, No 6.

4. Gopinath S M, Kalleshappa. T. K. Narasimhamurthy. T. P, Ashwinipatil. G. M (2013)" Isolation And Screening Of Fungi For Decolourization Of Azo Dye", International Journal Of Latest Research In Science And Technology, Volume 2, Issue 2.

5. Praveen Kumar G.N. And Sumangala K. Bhat (2012), "Fungal Degradation of Azo Dye- Red 3BN and Optimization ofPhysico-Chemical Parameters", ISCA J. Biological Sci., Vol. 1(2), 17-24.

6. Ramamurthy V, Umamaheswari G (2013), "Biodegradation of Synthetic Dyes by *Aspergillus Terreus* Inoculated On Solid Media", International Journal of Innovative Research in Science, Engineering and Technology, Vol. 2, Issue 12.

7. Ruchi (2012), "Decolorization Of True Blue And Congo Red Using Two Soil Borne Non-Ligninolytic Fungal Species Viz. *Aspergillus Niger* and *Rhizopus Nigricans*", CIBTech Journal Of Microbiology, Vol. 1 Pp.42-47.

8. Gowri Shyamala R, Vijayaraghavan R and Meenambigai P (2014)," Microbial Degradation of Reactive Dyes- A Review", Int. J. Curr. Microbiol. App. Sci, 3(3): 421-436.

9. Thorat P.R & Sayyad M (2010), "Microbial Decolourization and Degradation of Crystal Violet by Aerobic Bacteria", Save Nature to Survive, 5(4) 591-594.