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FUNGAL BIODEGRADATION: A STUDY ON PROCESS OPTIMIZATION FOR DECOLORIZATION OF INDUSTRIAL DYES

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ABSTRACT: Textile Dyes are difficult to remove from industrial effluents by conventional Wastewater treatments since they are designed to resist physico-chemical and biological fading. Environmental pollution caused by such textile effluents results in adverse effects on flora, fauna, and cause severe contamination of surface and underground water. Soil Fungi have been reported to remove dyes and dye colors from effluents, leading to a decrease in their toxicity and resulting in a safer discharge. The decolorization and degradation rate seems to be dependent on variable culture conditions. The removal of textile and industrial dyes using fungi isolated from soil has been an important area of bioremediation research and the present work was carried out to study dye removal by fungal isolates of industrial area soil. The effect of independent variables such as time, temperature, pH, agitation on decolorization efficiency was studied using textile dyes in a shake flask experimental setup. Dye removal/Biodegradation of textile dyes was indicated by decolorization of culture medium, the extent of which was determined by monitoring the decrease in absorbance at λ max of the dye.

KEYWORDS: Bioremediation, Dye decolorization, Fungi, Industrial effluent.

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1.INTRODUCTION

Removal of dyes and color residues from the textile wastewater prior to environmental discharge poses a significant environmental challenge. Textile dyes are heterogeneous group of chemically synthesized organic compounds, which are meant to be stable structures. Environmental legislations are being imposed to control the release of dye stained effluents owing to their recalcitrance potential toxicity and probable carcinogenicity. Small amount of dye in water (10-50mg mg/l) affects light

transparency and gas solubility of water. Industrial dyes include a wide spectrum of chemical structures, primarily based on substituted aromatic and heterocyclic groups and azo compounds predominate [1]. Bioremediation and environmental cleanup involves degradation or removal of organic pollutants to concentrations that are either undetectable or below thresholds considered safe by regulatory agencies [2]. Over the past decade, studies have reported that filamentous fungi could be used in clean-up of complex hydrocarbon contamination. Therefore, fungi have been investigated for their potential to decolorize effluents and removal of toxic dyes from wastewaters, in diverse lab-scale setups [3,4]. The present study was conducted with an aim to investigate textile dye decolorization consequent to the growth of fungi under varying conditions in Shake flask culture setup. Principal objectives of present study were to study absorbance characteristics of Textile dye samples, to make a broad assessment of Dye removal capability of the isolated fungal strain using textile dyes and to study various factors like time, pH and agitation that play important role in dye degradation.

2. MATERIALS AND METHODS

a) Potato Dextrose Agar (PDA) medium

PDA medium was amended with 60µg/ml Ampicillin was used for spread plate method and Nutrient Broth for Shake flask experiment. Textile dyes used were Navy Blue FB, Yellow Dye, Red dye and Black Dye.

b) Lactophenol cotton blue stain was used for the morphological identification of isolated fungal strains from soil [1].

c) Isolation of fungus from soil:

Soil samples were collected from Textile industry vicinity establishments receiving treated industrial effluent. 0.1g soil sample weighed and sterile blanks were prepared containing 9ml distilled water and serially diluted up to 10^{-6} using sterile blanks [1]. 1ml of aliquot from 10^{-4} dilution was spread plated on PDA plates and incubated at 37°C in inverted position for 5 days. The isolated fungi were subcultured and maintained as slant cultures at 4°C. Morphological identification of isolated fungal strains was done using Lactophenol cotton blue stain. A portion of mycelial mat from the colony was transferred into a drop of stain on the slide with the help of flamed needle and observed microscopically [5].

d) Standardization of Absorbance characteristics of Textile dyes:

Stock solutions of dyes were prepared (100µg/ml). 10mg was weighed and dissolved in 10ml distilled water. Dyes were scanned in UV- Visible Spectrophotometer to ascertain the maximum wavelength and maximum absorbance. The working concentration range was determined by scanning different concentrations (0.1-1µg/ml) of dyes in spectrophotometer and absorbance recorded. A plot of absorbance v/s concentration was plotted for each dye [6].

e) Assessment of Dye Removal based on Decolorization (Spread Plate Method)

PDA plates containing 1µg/ml and 0.1µg/ml concentrations of selected Textile dyes were prepared and streaked with isolated fungal strain. Petriplates were incubated at 27°C for 4-5 days, to observe decolorization [7,8].

i. **Shake Flask Experiment:** Four discs from the periphery of fungal mat were inoculated in each culture flask containing nutrient broth (pH-5.5) with or without textile dye (4µg/ml) (Test and Controls) . All flasks were incubated at 37°C. Readings were taken for 6-7 days at λmax of dye ([1,9,10].

ii. **Effect of Agitation:** Flasks were kept static or on 120 rpm, to observe agitation effect for 6 days and absorbance of the centrifuged aliquot was recorded [11].

iii. **Effect of Time:** Test and Control Flasks were put up and Absorbance of aliquot supernatant was recorded from day 1 to day 7, at 505 nm [13].

iv. **Effect of Temperature:** Each flask was incubated at 4^0 C, 27^0C, 37°C and 50^0C along with control for 5days and absorbance was recorded on each day.

v. **Effect of pH:** Nutrient broths of varying pH, at defined temperature and time, were used to carry out the experiment.

3. RESULTS AND DISCUSSION

a. Isolation of fungal strain from the soil:

Fungal strains were isolated from Industrial area soils by employing serial dilution technique and examined microscopically followed by staining. A randomly selected strain was used for the study. The fungus was observed to be rapidly growing with moist colonies becoming cottony with aerial hyphae (Figure 1). Elliptical spores (conidia) were observed held together in clusters at the tips of conidiophores (Figure 2).

Figure 1: Petriplate showing fungal growth

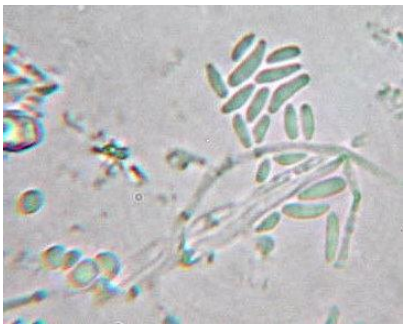

Figure 2: Morphological features of Soil Fungal Isolate

b. Absorbance characteristics of textile dyes:

Dilutions were prepared and absorbance was measured at respective λmax for different concentrations of each dye. Standard absorbance curves were plotted .Absorbance maxima i.e. 505 nm for Navy Blue FB (Figure 3), 515 nm for Red F3B (Figure 4), 565 nm for Black B (Figure 5), 400 nm for Yellow F4G (Figure 6) were determined by scanning over a wide range of wavelengths, using UV-Visible spectrophotometer.

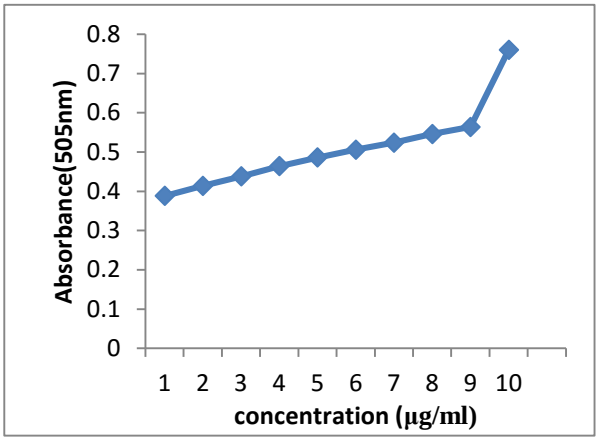

Figure 3: Standard absorbance curve for Navy Blue FB

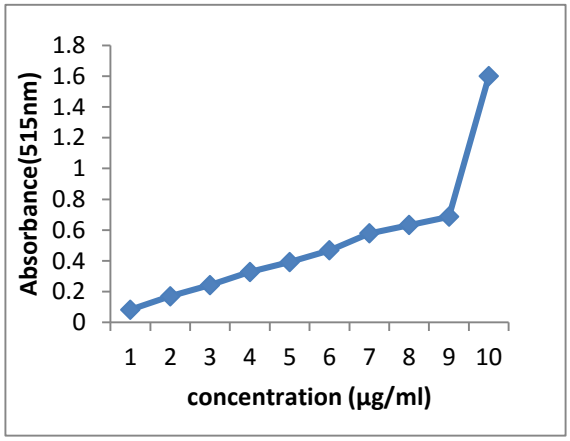

Figure 4: Standard absorbance curve for RedF3B

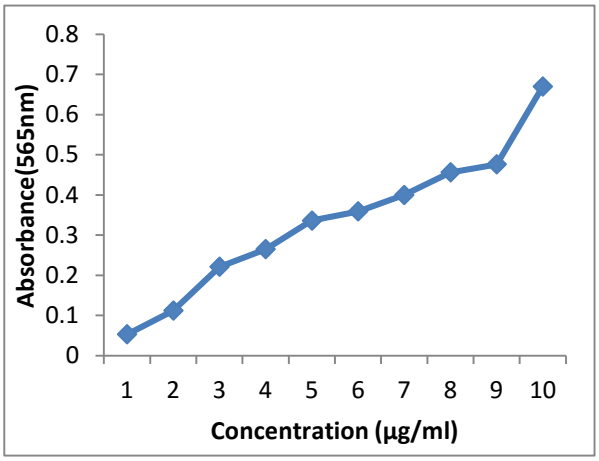

Figure 5: Standard absorbance curve for Black B.

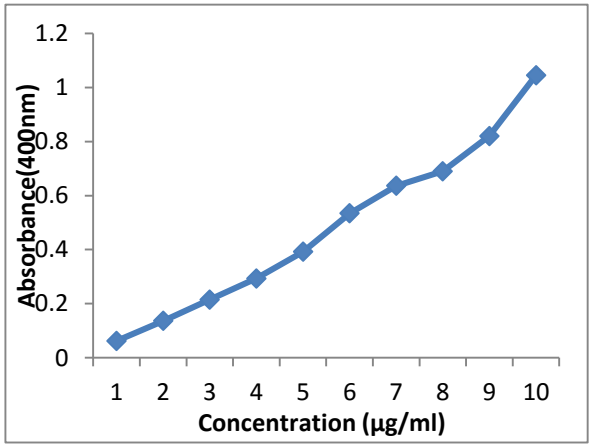

Figure 6: Standard absorbance curve for Yellow F4G.

c. Dye degradation ability of the isolated fungal strain

Different concentrations of Navy Blue FB-Textile dye were used (Figure 7). A visible removal of Dye as indicated by decolorization was observed at all the concentrations but was most clearly visible at concentration 1µg/ml and 4µg/ml.4 µg/ml of Navy Blue FB was used for Flask experiments and observations made (Figure 8). A visible removal of Dye as indicated by decolorization was observed (Figure 9). Thus, dye degradation ability of the isolated fungal strain was confirmed.

(a.) (b.)

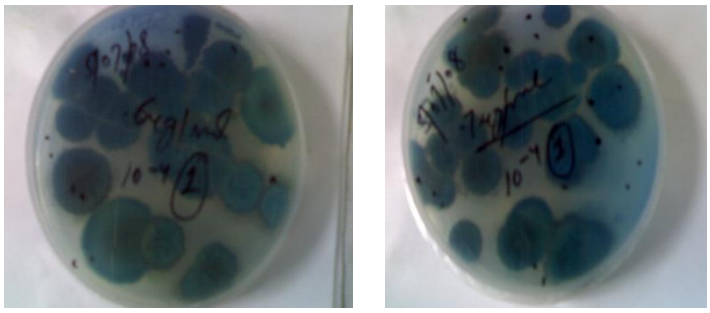

Figure 7: Representative pictures of Petriplates showing Fungal growth on PDA with different concentration of Navy blue FB. (a.) 6µg/ml ; (b.) 7 µg/ml

(a) **(b)**

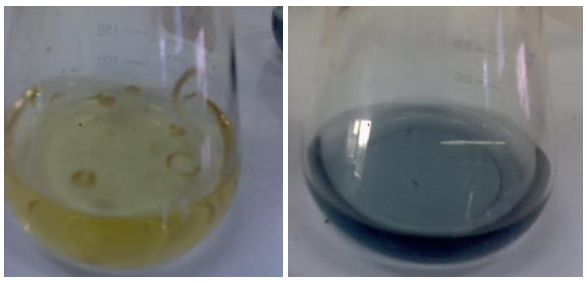

Figure 8: Flask containing Nutrient Broth (a) with isolated fungal strain (Control); (b) with Navy Blue FB 4µg/ml (Control 2)

(a) **(b)**

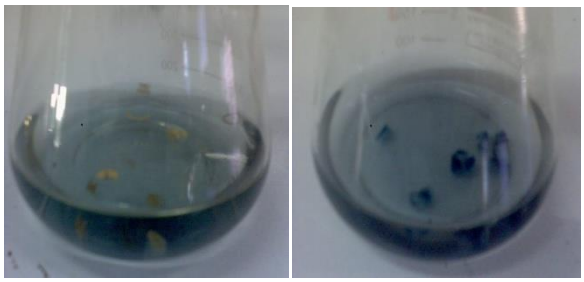

Figure 9: (a) Flask showing decolorisation of Navy Blue FB; (b) Flask showing fungal strain absorb color.

d. Effect of time on the degradation of the navy blue dye in nutrient broth by fungus

Up to day 3-4, absorbance decreased due to ability of isolated fungal strain to absorb and assimilate dye from the medium (Figure 10, Table 1). It was indicated by the decrease in dye color. Absorbance increased after day 5, which might be due to media depletion and excretion of metabolites by fungus into the medium causing turbidity, leading to an increase in absorbance. Differences in the capacity of dye decolorization between fungi have been related to inter- and intraspecific variations, the molecular complexity of the dye and culture conditions [13,14]. Initial concentration of dye also has an effect on the rate of degradation of dye by fungi. Rate of dye degradation was more at 4µg/ml as compared to 2µg/ml indicating that higher dye concentration showed higher rate of removal. Dye degradation at higher concentration tends to be more toxic as reported by others. Initial concentration of the dye was shown to influence the decolorization capacity of *T. villosa* where decolorization rates of 34 and 55% were observed at dye concentrations of 2 and 4 mg /L, respectively. About 70% decolorization was obtained at concentrations higher than 6 mg /L [15].

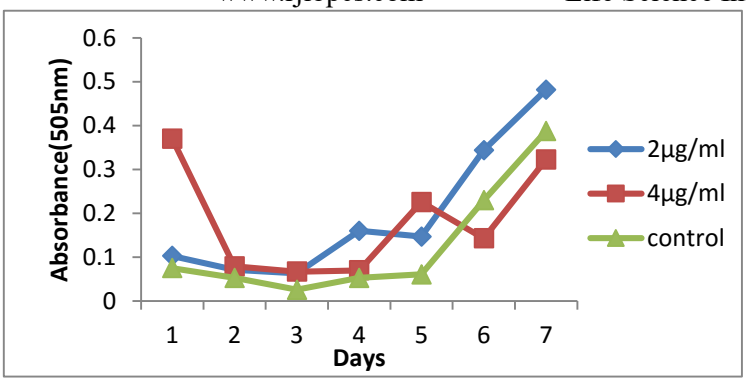

Figure 10: Effect of Time on the degradation of Navy Blue

e. Effect of pH on the degradation of the navy blue dye in nutrient broth by isolated fungal strain(strain 1)

Data represented graphically in Figure 11 and 12 showed effect of pH on the efficiency of fungi to reduce color intensity of dye solution (Navy Blue) within the pH ranged between 5 to 9.It was clear that, the maximum degradation was at pH 6. The minimum degradation of dye was observed at pH 9 (Table 2 and 3) Supporting evidence comes from studies showing that microbial ability to decolorize dyes in terms of percentage reduction of color was observed effectively in the pH range 4-7. Maximum dye decolorization was reported to be 93.73% and 78.4%, at pH 4.5 with *Aspergillus niger* and *Penicillium* spp respectively. These results suggested that better fungal growth usually occurs at low pH values [16]. Sukumar *et al* (2007) reported that higher color reduction of Bismarck Brown by *Phanerochaete sp*. was obtained at pH 6 and recorded the lowest value at pH 9 [1]. Efficiency of biodegradation of xenobiotic compounds by the white-rot fungus *Trametes trogii* have been reported to be more at low pH where both growth and ligninolytic activity of fungus were higher [17].

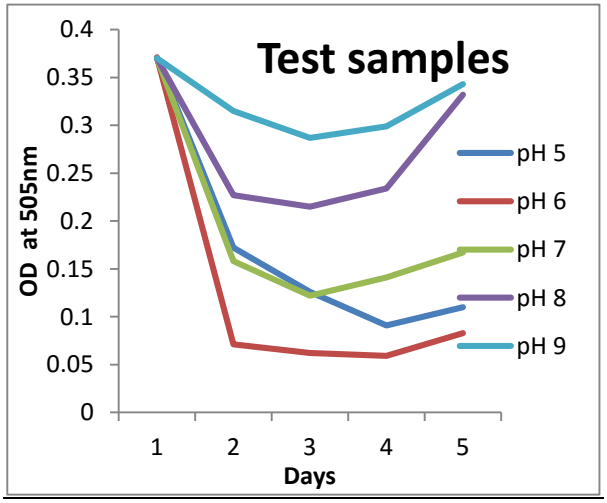

Figure 11: Effect of pH on the degradation of the navy blue dye (Test sample)

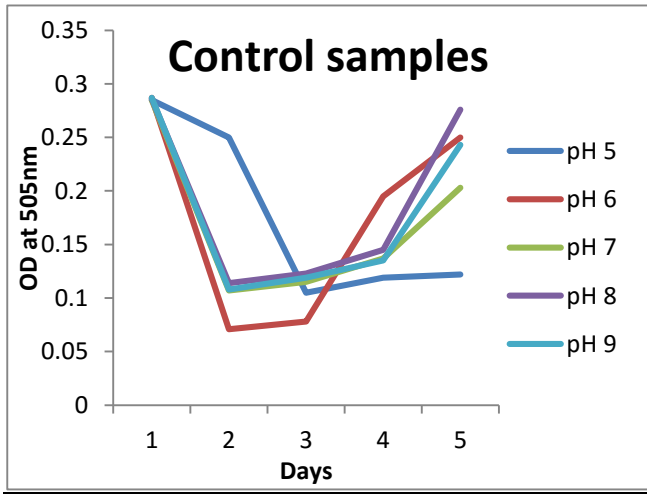

Figure 12: Effect of pH on the degradation of the navy blue dye (control sample)

f. Effect of agitation on the degradation of the navy blue dye in nutrient broth by isolated fungal strain

It was found that static conditions were more efficient in dye decolorization than the shaking conditions. The high rate of the agitation might decrease the fungal growth and the activities of some biological substances such as enzymes, which play an important role in Dye removal (Figure 13 and 14) (Table 4 and 5). Similar results were obtained in earlier studies where Kirby *et al* (2000) observed better dye decolorization by *Phlebia tremellosa* under static conditions [18]. *Phanerochaete sp*. was observed to give highest color reduction under static condition for Bismarck Brown whereas agitation tended to decrease the color reduction [1]. These results are similar to those obtained by Daneshvar *et al* (2007), using another type of microorganism and can be discussed in terms of the high rate of the agitation decreases the fungal growth and the activities of some biological substances such as enzymes which play an important role in the decolorization of the dye [19].

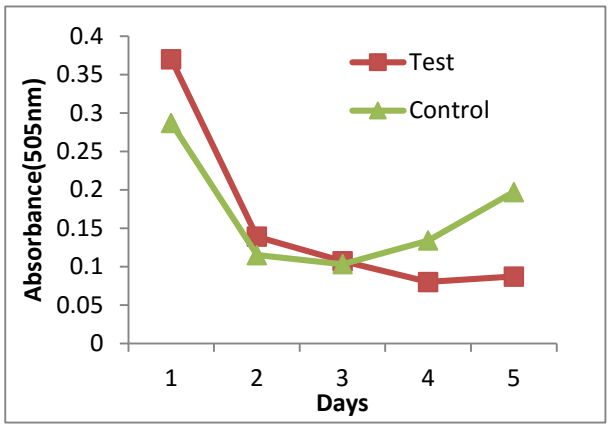

Figure 13: Effect of static conditions on degradation of Navy Blue

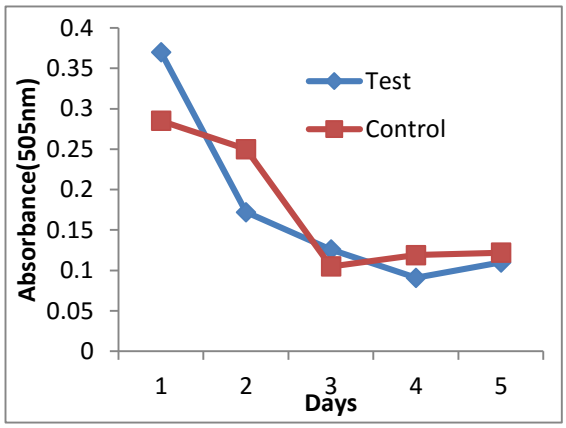

Figure 14: Effect of agitation on degradation of Navy Blue

g. Effect of temperature on the degradation of navy blue dye:

There was no visible fungal growth at 4° C and 50 °C and no major decrease in absorbance (Figure 15 and 18) (Table 6). At 27° C and 37 °C, visible growth and dye decolorization had taken place since there was a gradual decrease in absorbance with time. (Figure 16 and 17) (Table 6). Temperature dependent dye degradation studies by other researchers had shown similar results. Husseiny (2008) had reported maximum color reduction with *Aspergillus niger* after 4 days of incubation period at temperatures 28 ºC and 35 ºC [12]. These results are similar to those obtained by Assadi*et* al (2001) and Mcmullan*et al* (2001) with *Penicillium sp* [16,20]. In another study, at temperature of 30°C was found favorable for good growth and enzyme activity of *Coriolus versicolor* [1]. Differences in the capacity of dye decolorization between fungi have been related to

inter- and intraspecific variations, the molecular complexity of the dye and culture conditions [9,13,14].

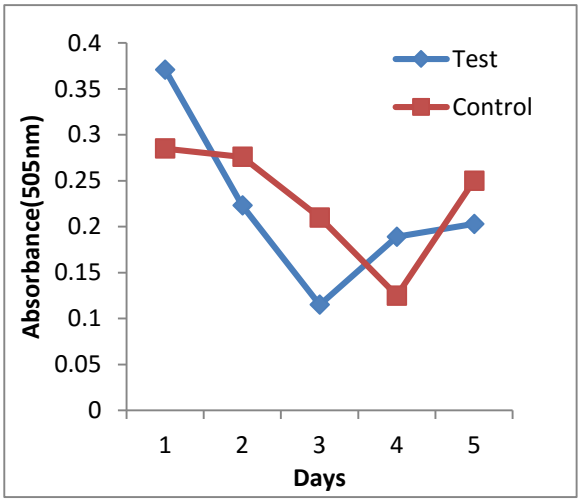

Figure 15: Effect of temperature(4°C) on degradation of Navy Blue

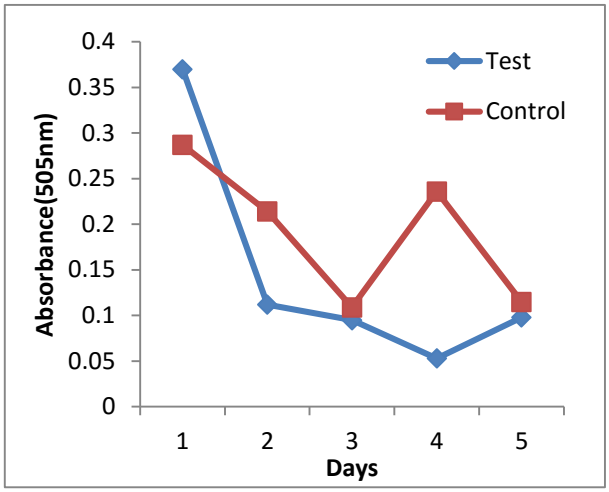

Figure 16: Effect of temperature(27°C) on degradation of Navy Blue

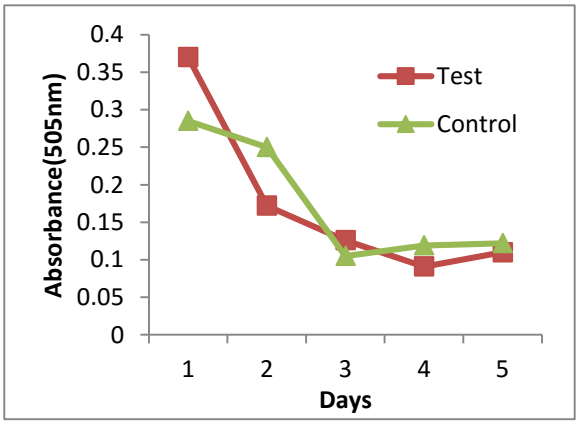

Figure 17: Effect of temperature (37°C) on degradation of Navy Blue

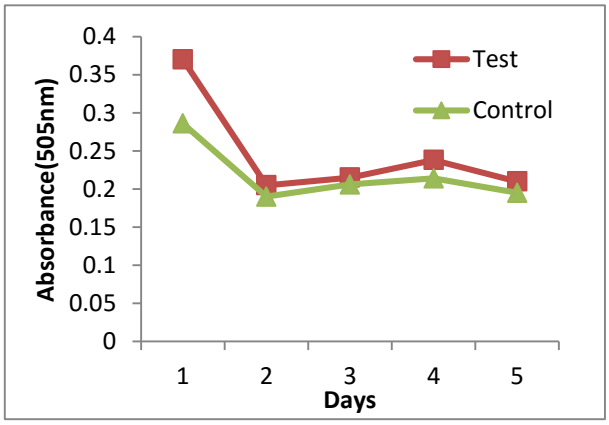

Figure 18: Effect of temperature (50°C) on degradation of Navy Blue

4. CONCLUSION

Decolorization of Industrial dye, Navy Blue by isolated fungal strain was studied and effect of independent variables such as pH, temperature, time, agitation on the decolorization efficiency was observed. Decrease in absorbance was taken as an indicator of dye removal and decolorizationIt can be concluded that the isolated fungal strain has the potential to decolorize industrial dyes like Navy Blue, over the experimental period. The color reduction increased linearly with increase in incubation time. Maximum decolorization activity of the isolated strain for the Navy Blue FB was under static conditions at pH 6, 27°C and within the incubation time of 3-4 days.

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CONFLICT OF INTEREST

No conflict of interest exists.

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