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Efficiency of *Paecilomyces variotii* in Bioremoval of Reactive Black Dye from Tannery Effluent

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ABSTRACT

The present work investigates the efficiency of *Paecilomyces variotii* upon degrading Reactive Black dye which has been termed a recalcitrant variety of synthetic dye. In this research, initially a predominant fungal species, *Paecilomyces variotii* was isolated from the tannery effluent sample. The study was carried out by assessing the ability of fungi to decolorize the dye under various parameters like pH (5,7 and 9), Temperature (7°C, 30°C, and 45°C), Dye concentration (200, 300, and 400 mg/L) for different incubation or exposure time interval (3, 5 and 7 days). From the experimental study, it was found that *Paecilomyces variotii* showed a maximum percentage of dye decolorization at 7°C at pH 9 with 75%, at 30°C at pH 7 with 85%, at 45°C at pH 5 with 82% and a maximum period of incubation with 7 days in 200 $mg.L^{-1}$ concentration. This result conveys that the strength of *Paecilomyces variotii* in decolorizing the synthetic dye is effective at a moderate temperature with neutral pH for maximum exposure time. So *Paecilomyces variotii* could be a good candidate of choice for the biodegradation of various synthetic dyes when manipulated wisely. Also, the result sparks a positive attribute toward decreasing industrial wastewater pollution.

INTRODUCTION

These days, water has become an essential resource for humans and other species in the world for existence and survival (Wang et al. 2020). Wastewater from various factories and industries poses a great threat to water quality. Especially, tannery and textile effluent release a wide range of hazardous compounds containing heavy metals and synthetic dyes into nearby water systems which impart deleterious effects on surrounding flora and fauna. Every year, about 7×10^7 tons of synthetic dyes are produced worldwide, which have been used by various industries like textile, tannery, food, cosmetics, etc. (Chandanshive et at. 2020). These dyes are highly reactive, chemically stable, and resistant to the degradation process (Martínez-Huitle & Brillas 2015). Among the dye used in tannery and textile industries, Reactive Black 5 (RB5) accounts for 50% of the azo and becoming a recalcitrant to natural aquifers (Nabil et al. 2014). Leather industries make use of these azo dyes in large proportion because of their strong binding ability towards the collagen in respective pH ranges. But the fate of unbounded dyes present in untreated effluents greatly spoils nearby water streams (Rocha et al.2017). Normally, industrial effluents correspond to high levels of BOD, COD, pH, and color which is lethal to all forms of life associated with water. Among other pollutants, color is one of the vivid signs of pollution caused by various synthetic dyes that a water body can receive (Nigam et al. 1996). The carcinogenic effect of these dyes poses a big threat to living organisms also the intensity of different dyes interferes with the photosynthetic activity of water flora that indirectly affects the food chain (Weisburger 2002). Everything considered, the deleterious sequel of dye on the ecosystem, discarding and subsequent decolorizing of the dyes should be the prime concern (John et al. 2020).

Several physico-chemical methods such as electrolysis, adsorption, ion exchange, ultrafiltration chemical oxidation, ozonation, electrochemical degradation, etc., have been employed for dye decolorization as well as the degradation process (Jagadeesan et al. 2013). Anyway, all these procedures retain a few intrinsic factors such as expensiveness, formation of harmful by-products, and exhaustive energy requirements (Aravindhan et al. 2007). The biological way of removing the dyes from wastewater through bioaccumulation and biodegradation has been proven to be more effective against the above conventional procedures since it is economical and environmentally friendly also the treatment process

can be carried out on site itself with very less or no by-products (Jagadeesan et al. 2013). So, recently scientists are significantly addressing the issue of effluent treatment through biological methods (Vijayaraghavan et al. 2008). Among the biological methods, microbial degradation of dyes is proven to be one of the prime methods, since most of the bacterial, fungal, and algae species present in the contaminated area were recorded as effective in dye decolorization and degradation. It was observed that during the decolorization process by fungi and algae, dye adsorption plays a major part (Slama et al. 2021). Scientific studies have stated that microbes possessing genes that code for various enzymes like laccase, peroxidase, and azoreductase cleave the aromatic ring present in dyes for its effective degradation (Chen et al. 2003, Babu et al. 2015). Fungi species such as *Cladosporium, Chaetomium globosum, Fusarium solani, Alternaria,* and *Aspergillus niger* secrete manganese peroxidase, lignin peroxidase, and laccase enzymes extracellularly having the ability to degrade various complex synthetic dyes (El-Gendi et al. 2021). In this study, predominant fungi present in the tannery effluent were isolated and their efficiency in decolorizing the reactive black B dye was analyzed under various parameters like different pH, Temperature, time, and concentration of dye.

MATERIALS AND METHODS

Media and Chemicals

Fungal growth media, namely potato dextrose agar (PDA), potato dextrose broth (PDB), and Czepak's dox broth were purchased from HiMedia. Reactive Black 5 (RB5) (Fig. 1) dye was procured from Sigma Aldrich (Bangalore).

Collection of Tannery Effluent Sample and Its

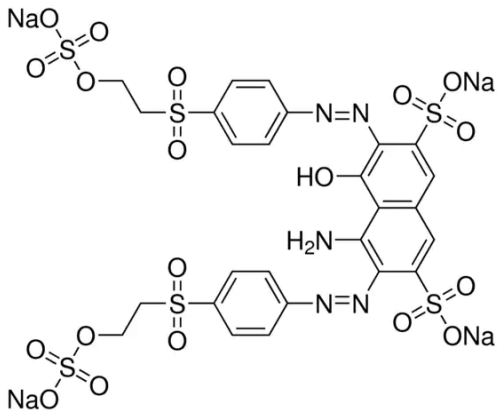

Fig.1: Chemical structure of Reactive Black 5 azo Dye.

Physico-Chemical Property Analysis

The tannery effluent was collected in a sterile sample container tube from the leather industry located in Nagalkeni, Chennai (Lat Long (12.961140, 80.135430) GPS Coordinates (12°57□40.104□-N80°8□7.548□ E) and taken to the laboratory for further analysis. The physicochemical parameters of the effluent such as color, odor, pH, Electrical conductivity (EC), Total dissolved solids (TDS), Total suspended solids (TSS), BOD, COD, Copper, Chromium, Chloride, and sodium were analyzed following the standard approved by CPCB (1995) (Table 1).

Fungal Isolation from Tannery Effluent Sample and Culture Conditions

From the tannery effluent sample, the predominant fungal species were identified by serial dilution technique followed by inoculation in potato dextrose agar (PDA) by pour plate technique subsequently incubation was done at 37°C for 3-4 days. After the incubation period, the colonies were identified based on morphological, microscopic observations, and cultural characteristics and identified up to the species level (Gilmann 1971, Subramanian 1971, Ellis 1971, Udaya Prakash 2004). A fungal species was selected based on its prevalence and dominance and used to evaluate its efficiency in the bioremoval of reactive black dye. Initially, the efficiency of dye decolorizing property of predominant fungal species was determined by cultivating in Czepak's dox broth containing Reactive Black dye (100 $mg.L^{-1}$). After 3 days of incubation, the culture sample was read using UV-spectrophotometer at 470 nm (Khatid et al. 2008). The fungal species which showed maximum efficiency was selected for further studies. To improve its dye decolorizing efficacy, the process was studied under various parameters

Table 1. Analysis of physicochemical parameters of untreated tannery effluent.

S. No.	Parameters	Observations	Reference value (CPCB-195)
1	Color	Blackish	Colorless
2	Odor	Disagreeable odor	Odorless
3	pH	8.5	5.5-9.0
4	EC	12013 $\mu s.cm^{-1}$	400 $\mu s.cm^{-1}$
5	TDS	3021 $mg.L^{-1}$	2100 $mg.L^{-1}$
6	TSS	151 $mg.L^{-1}$	100 $mg.L^{-1}$
7	BOD	829 $mg.L^{-1}$	30 $mg.L^{-1}$
8	COD	1131 $mg.L^{-1}$	250 $mg.L^{-1}$
9	Chromium	4.6 $mg.L^{-1}$	2 $mg.L^{-1}$
10	Copper	5 $mg.L^{-1}$	3 $mg.L^{-1}$

like pH, temperature, time, and concentration of dye sample.

Optimization of Conditions

Effect of Dye Concentration

The commercially available dye was taken in three different concentrations such as 200 $mg.L^{-1}$, 300 $mg.L^{-1}$, and 400 $mg.L^{-1}$in separate flasks containing czepak's dox broth. One milliliter of the fungal inoculum was added to each bottle aseptically and incubated for 3 days at 37°C. The absorbance was measured and calculated periodically to evaluate the effect of concentration and percentage of decolorization.

Effect of pH

The pH of the medium with different dye concentrations (200 $mg.L^{-1}$, 300 $mg.L^{-1}$, and 400 $mg.L^{-1}$) was maintained at 5, 7, and 9 individually. The pH was adjusted using 0.1 M hydrogen chloride (HCL) and 0.1 N Sodium hydroxide (NaOH) before inoculation of fungi. It was followed by incubation for 3-4 days at 37°C. The absorbance was measured and calculated periodically to evaluate the effect of concentration and percentage of decolorization. Based on the absorbance, the percentage of dye decolorization at various pH was calculated.

Effect of Temperature

The temperature was maintained at 7°C, 30°C, and 45°C for different dye concentrations i.e., 200 $mg.L^{-1}$, 300 $mg.L^{-1}$, and 400 $mg.L^{-1}$, and various pH levels (5, 7, and 9). The temperature is maintained by keeping flasks in the refrigerator setting the temperature at 7°C, room temperature at 30°C, and moderately high temperature at 45°C by incubating them at respective temperatures in incubators. After the incubation period, the absorbance was recorded and calculated.

Effect of Time

The Czepak's dox broth amended with different concentrations of the dye at different pH was studied for the removal of dye when inoculated with fungal isolate. The absorbance of the solutions was measured at different periods, viz., 72, 120, and 168 h. All the above experiments were performed in triplicates to maintain the reliability of the procedures.

Percentage of Decolorization

For analyzing the decolorizing efficacy of isolated fungal species, after incubation of respective parameters, the sample was centrifuged at 3,000 rpm for 30 min. Using a supernatant solution absorbance was measured through a UV spectrophotometer. A sample with the same experimental condition but without fungi was taken as a control.

The percentage of decolorization was calculated using the formula:

$$\% \text{ removal} = \frac{(\text{Control absorbance} - \text{Test absorbance})}{\text{Control absorbance}} \times 100$$

Where, control is the absorbance of the initial dye solution and it is constant which is equal to 0% decolorization. Test absorbance is studied after the experimental parameter. Decolorization percentage refers to the percentage mean of decolorization percentage of three replicas.

Statistical Analysis

All the data from dye decolorization assays were tested for statistical significance by comparing the mean of different test conditions using One-way ANOVA with the Dunnett Multiple Comparisons Test. The data were considered significant if $p < 0.05$, and highly significant if $p < 0.001$.

RESULTS AND DISCUSSION

Diversity of Fungi from Leather Effluent

On serial dilution of the contaminated tannery effluent followed by the pour plate method, after incubation different colonies of fungi were observed. The predominant colonies were subcultured in PDA and identified. A total of 23 different colonies were recorded among which *Paecilomyces variotii* alone dominated with more than 50% of fungal species. This

Fig. 2: Organisms isolated from tannery effluents.

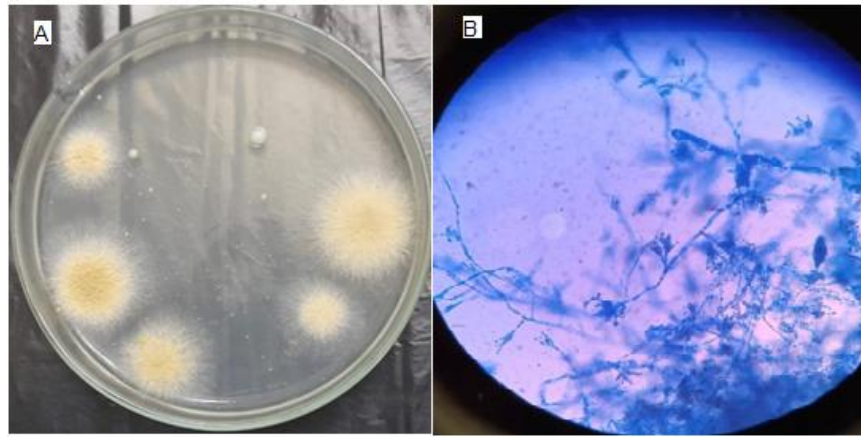

Fig. 3: A-Plate containing a pure culture of *Paecilomyces variotii* and B-Microscopic observation of *Paecilomyces variotii.*

was followed by *Aspergillus flavus, Penicillium* sp., and a non-sporulating colony. Fig. 2 shows the fungal colonies with varied morphologies. Fig. 3 reveals the *Paecilomyces variotii* culture growth in SDA and its microscopic structure when viewed at 40X magnification through the microscope.

Removal of Reactive Dye Black at the Temperature of 7°C

For culture conditions at 7°C in 200 mg.L^{-1} concentration in pH 5, there is a 35%, 42%, and 50% dye decolorization rate that happened in 200 mg.L^{-1} concentration for 3rd, 5^{th,} and 7th day of incubation consequently. For conditions at pH 7, there are is33%, 46%, and 55% decolorization rates that happened on the 3rd, 5^{th,} and 7th day of incubation respectively. While maintained at pH 9, there is 55%, 68%, and 75% decolorization rates occurred on the 3rd, 5^{th,} and 7th day of incubation respectively. The efficiency of *Paecilomyces variotii* dye degradation was compared between three different concentrations and at three different pH conditions for different time intervals. Among these conditions, *Paecilomyces variotii* maintained at 7°C, exhibits its maximum efficiency in alkaline pH of 9 exposed for 7 days in a dye concentration of 200 mg.L^{-1} with 75% dye decolorization rate (Fig. 4). This proves that at low

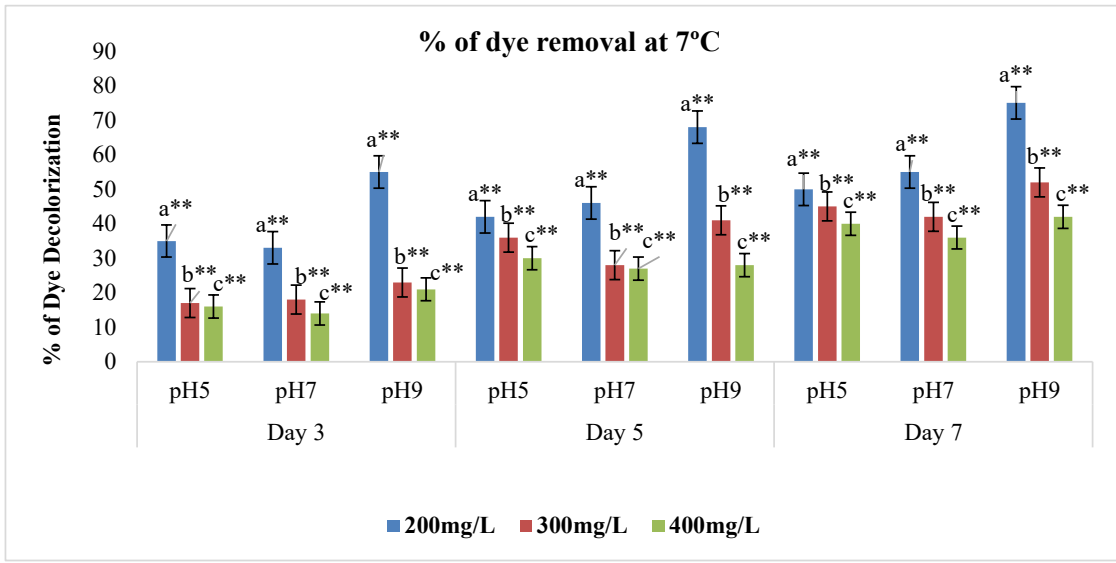

Fig. 4: Values are expressed in mean ± SD (n = 3), statistically significant test for comparison was done by ANOVA followed by Dunnet's 't-test. Comparison of dye decolorization % between a – Control vs dye concentration at 200 mg.L^{-1} in different pH ranges and time intervals, b – Control vs dye concentration at 300 mg.L^{-1} in different pH ranges and time intervals, c - Control vs dye concentration at 400 mg.L^{-1} in different pH range and time intervals, *p < 0.05, **p < 0.01 and ns – Non-Significant.

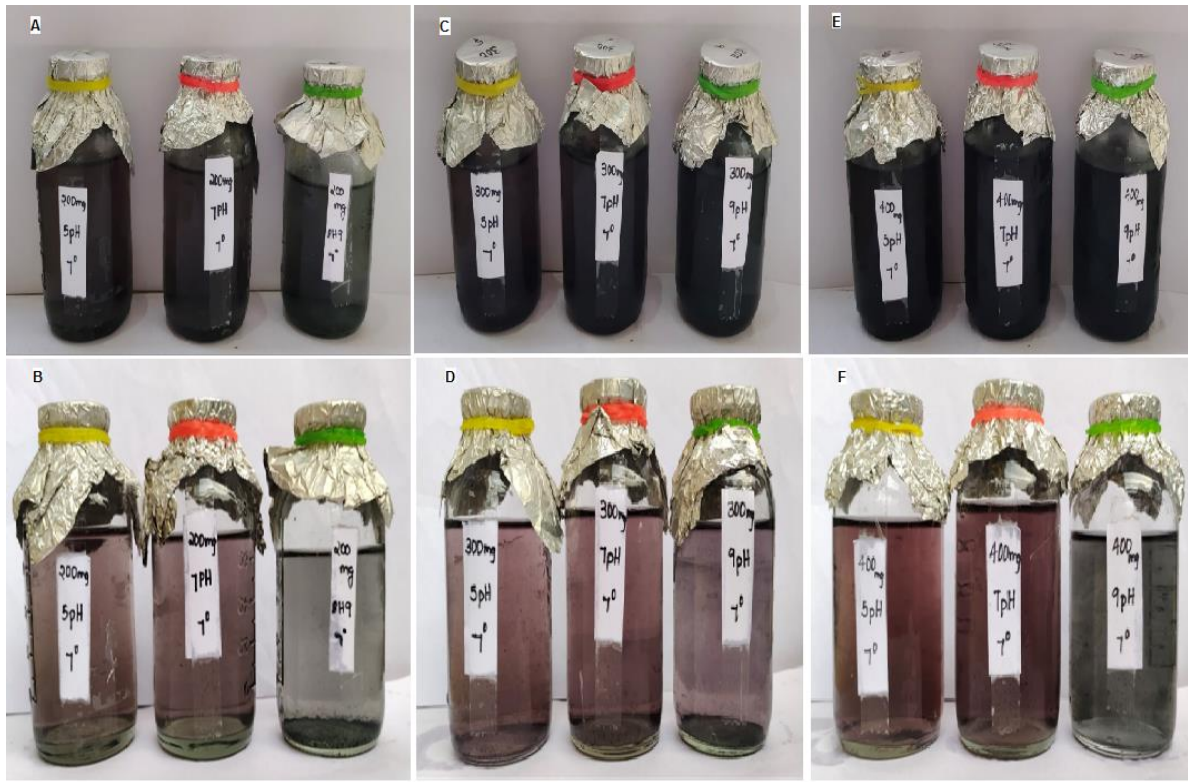

Fig. 5: photograph of dye with concentration 200 mg.L^{-1} at 7^{o}C before (A) and after processing at pH 5, 7, and 9 (B). Photograph of dye with concentration 300 mg/L at 7^{o}C before (C) and after processing at pH 5, 7, and 9 (D). Photograph of dye with concentration 400 mg.L^{-1} at 7^{o}C before (E) and after processing at pH 5, 7 and 9 (F).

temperatures the enzymes needed for dye degradation works at alkaline pH. The demand for fungi demand in the dye-degrading process can be achieved greater by accelerating their metabolism through various factors. Fungi usually secrets intracellular and extracellular enzymes which enhance their metabolism in treating dye effluents (Rania et al. 2022). Fig. 5 shows the vivid difference in color change by the action of *P. variotii* grown at different concentrations, times, and pH by keeping the temperature constant at 7^{o}C.

Removal of Reactive Dye Black at the Temperature of 30°C

Keeping the temperature of 30^{o}C at constant, the dye bio-removal efficacy of *Paecilomyces variotii* was studied at various pH ranges (5, 7 and 9). Also, it was done at different day intervals of the 3rd, 5^{th,} and 7th day with increasing concentrations of 200, 300, and 400 mg.L^{-1}. During 30^{o}C, the function of *Paecilomyces variotii* becomes less effective at pH5 with 20%, 38%, and 55% of dye color reduction with 200mg/L concentration on the 3rd, 5^{th,} and 7th day of incubation respectively. But in pH7, it shows maximum efficiency with 50%, 62%, and 85% on the 3rd, 5^{th,} and 7th day of incubation respectively. Also at pH 9 for the same culture conditions, it gives tough with 48%, 60%, and 80 % of dye decolorization from the above results (Fig. 6). It is clear that in moderate temperature the enzymes become active at neutral pH of 7 exhibiting its maximum efficiency in dye degradation with increased period of incubation (Radha et al. 2005). It is found that at low concentrations of dye, the degradation process is faster which corresponds to other studies also where 80% decolorization occurred with 75 mg.L^{-1} concentration (Puentes-Cárdenas et al. 2012). Also in another study decolorization was recorded at 97% with an RB5 concentration of 1 mg L-1 after 150 min of treatment (Chong et al. 2014). Fig. 7 shows the vivid difference of color change by the action of *P. variotii* grown at different concentrations, times, and pH by keeping the temperature constant at 30^{o}C. From the above experimental condition, it is stated that *P. variotii* showed a maximum of 85% dye decolorization effect when compared with other prevailed conditions.

Removal of Reactive Dye Black at the Temperature of 45°C

The bio-removal efficacy of *Paecilomyces variotii* was

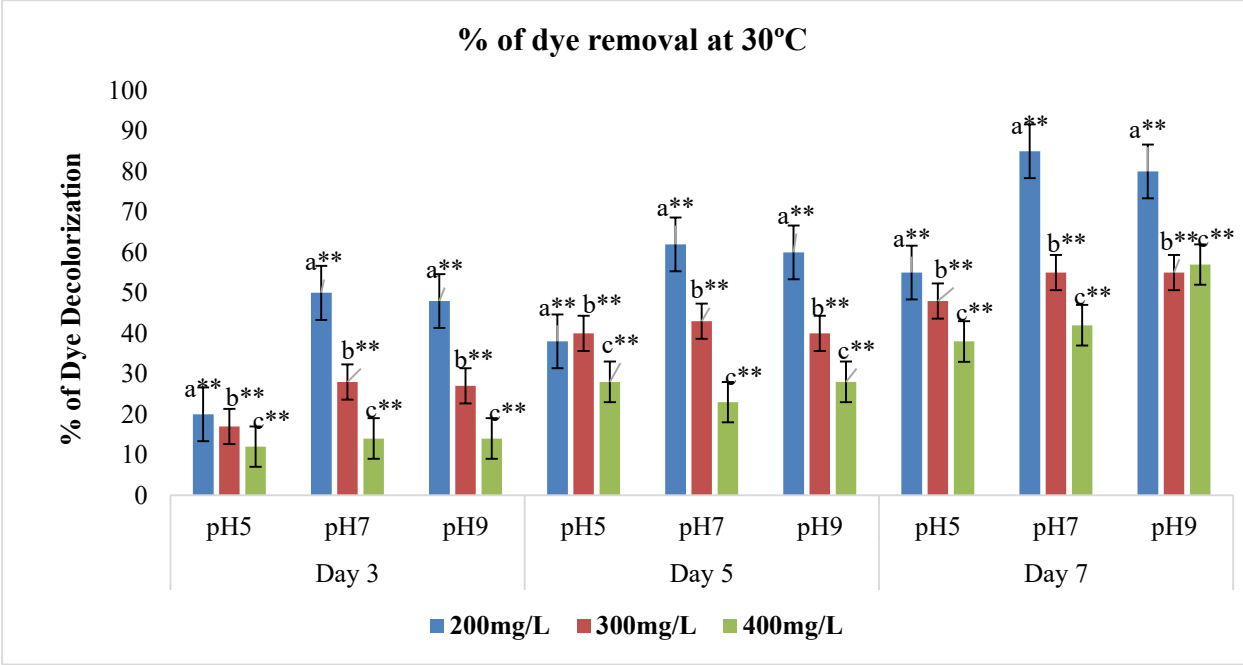

Fig. 6: Values are expressed in mean±SD (n=3), statistically significant test for comparison was done by ANOVA followed by Dunnet's 't-test. Comparison of dye decolorization % between a – Control vs dye concentration at 200 mg.L^{-1} in different pH ranges and time intervals, b – Control vs dye concentration at 300mg.L^{-1} in different pH range and time intervals, c - Control vs dye concentration at 400 mg.L^{-1} in different pH range and time intervals, *p < 0.05, **p < 0.01 and ns – Non-Significant.

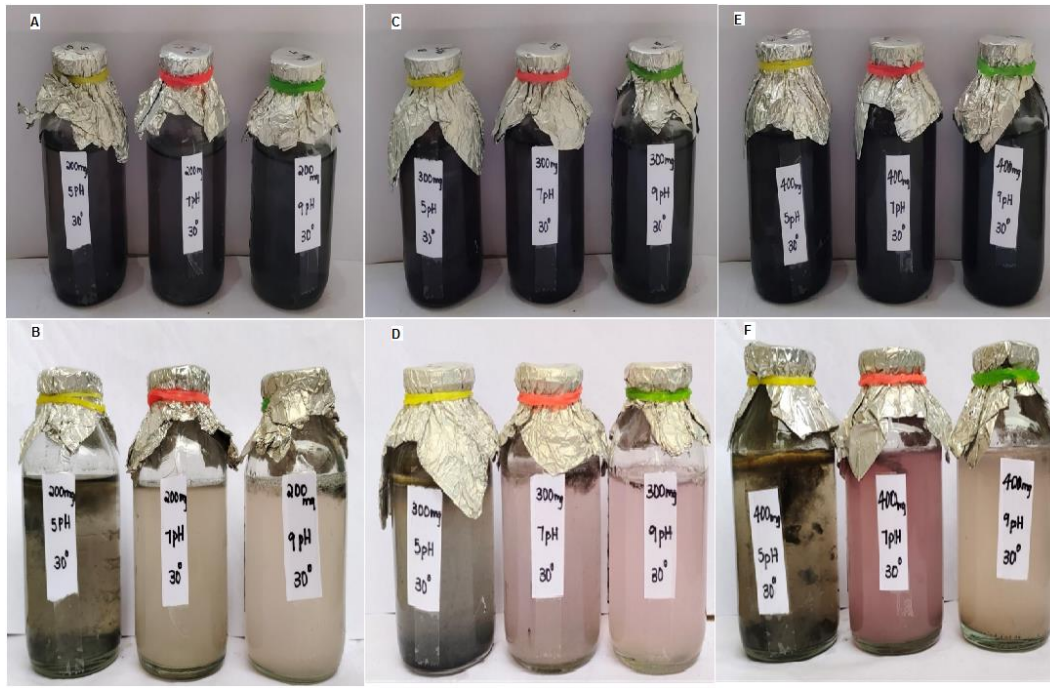

Fig. 7: Photograph of dye with concentration 200 mg.L^{-1} at 30°C before (A) and after processing at pH 5, 7, and 9 (B). Photograph of dye with concentration 300 mg.L^{-1} at 30°C before (C) and after processing at pH 5, 7, and 9 (D). Photograph of dye with concentration 400 mg.L^{-1} at 30°C before (E) and after processing at pH 5, 7 and 9 (F).

studied at various pH ranges (5, 7, and 9). Also, it was done at different day intervals of the 3rd, 5th, and 7th day with increasing concentrations of 200, 300, and 400 mg.L^{-1} by maintaining the temperature at 45°C. In experimental conditions at 45°C, pH 5 for 200 mg.L^{-1} concentration, the dye degradation efficiency of *Paecilomyces variotii* was recorded at 55%, 60%, and 82% during the 3rd, 5th, and 7th days of incubation respectively. For the condition availed at 45°C and pH 7 for 200 mg.L^{-1} concentration the efficiency of *Paecilomyces variotii* in dye degradation was found to be 38%, 52% and 71% during the 3rd, 5th and 7th days of incubation respectively. With the conditions at 45°C and pH 9 for 200 mg.L^{-1} concentration, the dye decolorization effect was found to be 20%, 38%, and 55% during the 3rd, 5th and 7th days of incubation respectively (Fig. 8). The results from above experimental conditions revealed that when the fungi allowed to grow in increased temperature, the enzymes become active at acidic pH (9). Here at pH 5, the *Paecilomyces variotii* showed maximum efficiency with 82% dye decolorization property. Fig. 9 shows the vivid difference of color change by the action of *P. variotii* grown at different concentrations, time, and pH by keeping the temperature constant at 45°C. There is a significant decline rate in decolorization efficiency at 7°C. This finding correlates with the other research studies where it is found that low temperatures could significantly inhibit dye decolorization. Comparatively, in this study, *P. variotii* still showed a potential decolorizing capability at higher temperatures like 30°C and 45°C, suggesting its valuable potential in the bioremediation of azo dyes.

Presently scientists have turned towards bioremediation using fungal communities due to their applicability of high biomass rather than employing bacterial species (Rani et al. 2014). Researchers have proposed that the filamentous nature of fungi aids in greater uptake and conversion of pollutants to other metabolites with lesser or no environmental impact. Recent research studies already proved the effectiveness of various fungal species in the biodegradation of volatile organic compounds. Also when compared to bacterial species, fungi can be handled effectively in extreme conditions due to their resistive nature (Kennes & Veiga 2001, 2004). The fungi, *P. variotii has* already been studied for their competence in the biodegradation of hydrocarbons in soils polluted with petroleum products (Nrior & Jirigwa 2017). The result from the above study also conveys that *P. variotii* will be a good candidate for bio removal of reactive dyes in a moderate temperature range (30-35°C) and at moderate pH of 7. So the bio removal of dye could be made possible

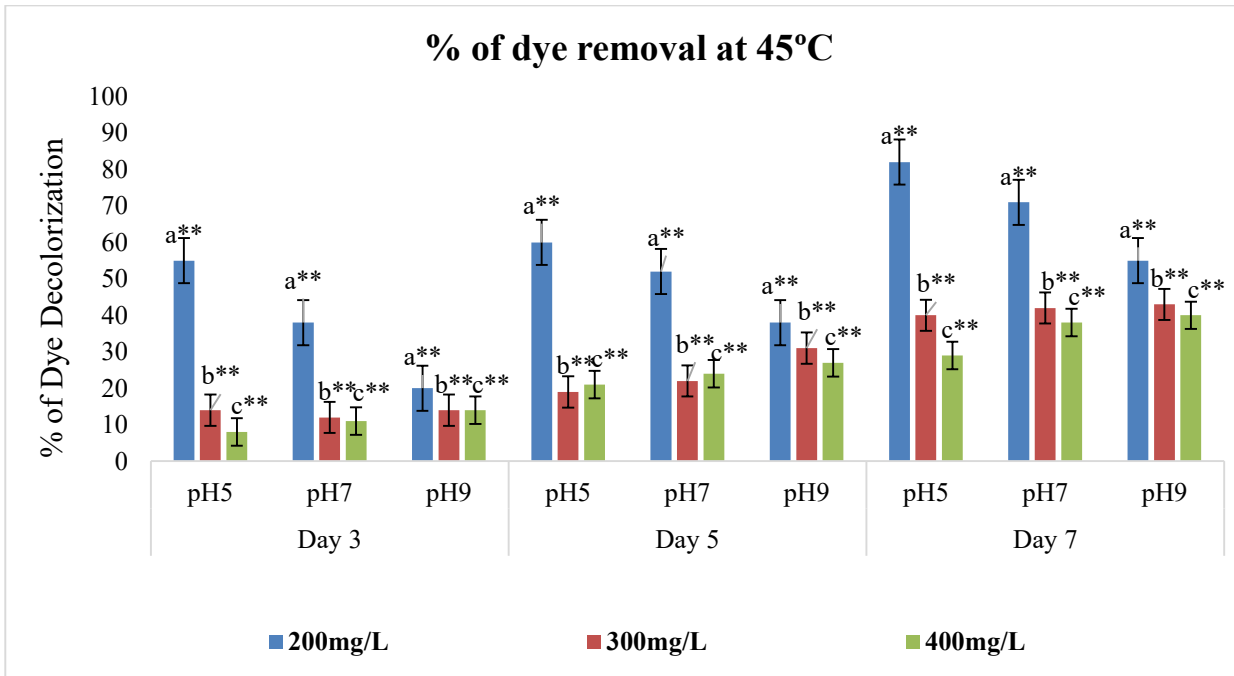

Fig. 8: Values are expressed in mean±SD (n=3), statistically significant test for comparison was done by ANOVA followed by Dunnet's 't-test. Comparison of dye decolorization % between a - Control vs dye concentration at 200 mg.L^{-1} in different pH ranges and time intervals, b - Control vs dye concentration at 300 mg.L^{-1} in different pH ranges and time intervals, c - Control vs dye concentration at 400 mg.L^{-1} in different pH range and time intervals, *p < 0.05, **p < 0.01 and ns – Non-Significant.

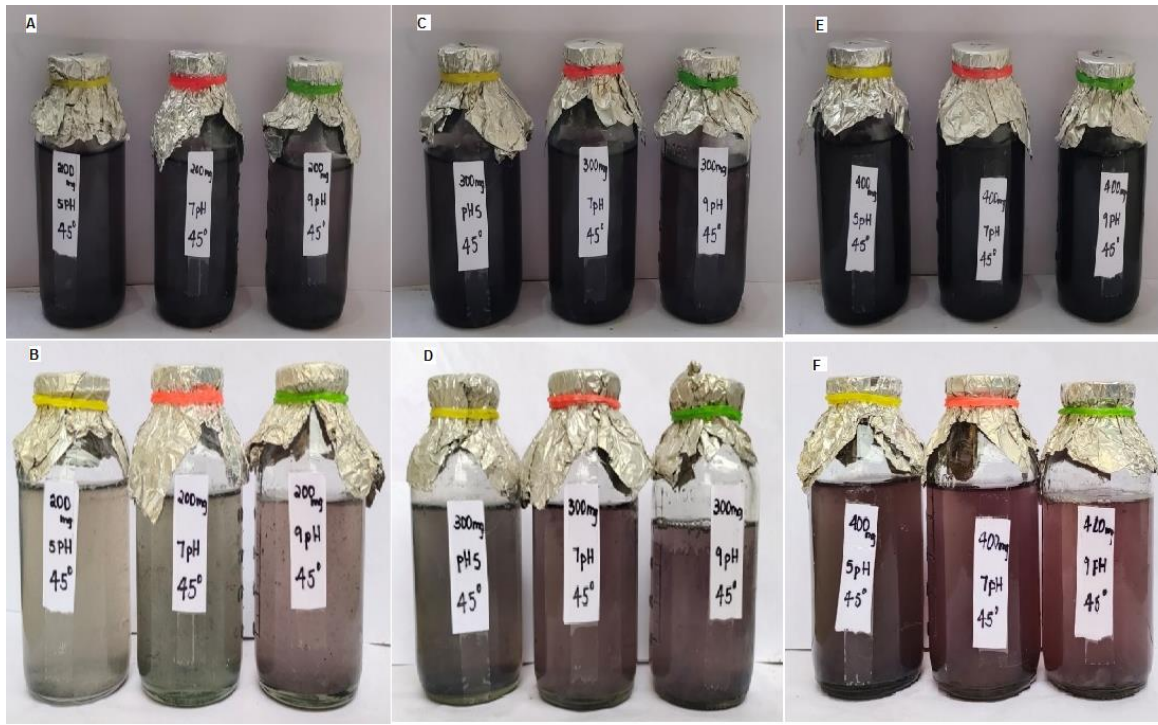

Fig. 9: Photograph of dye with concentration 200 mg.L^{-1} at 45°C before (A) and after processing at pH 5, 7 and 9 (B). Photograph of dye with concentration 300 mg.L^{-1} at 45°C before (C) and after processing at pH 5, 7 and 9 (D). Photograph of dye with concentration 400 mg.L^{-1} at 45°C before (E) and after processing at pH 5, 7 and 9 (F).

even at normal temperatures for the effect result. Even when it is grown at a slightly high temperature of 45°C, the fungi works well at acidic pH of 5 with an 82 % decolorization effect. When it is studied at a very low temperature of 7°C, it is showing 75% effectiveness in reducing the dye color to an alkaline pH of 9. It was studied that decolorization and biosorption of dyes take place by forming complexes, making precipitation, and finally reserving them in the interior walls of mycelia. The higher dye absorption capability of fungi relates to their augmented cell-to-surface ratio while in contact with pollutants in the environment (Yeddou-Mezenner 2010, Fu & Viraraghavan 2002). When planned accordingly *P. variotii* not only severs to reduce the color even it will also act as a good choice for bioaugmentation of dye by removing the contaminant from the polluted site.

CONCLUSION

In this research work, the efficacy of *P. variotii* in dye decolorization was studied at various parameters like pH (5,7 and 9), Temperature (7°C, 30°C, and 45°C), Dye concentration (200, 300, and 400 mg.L^{-1}) and Time (3, 5 and 7 days). Fruitful results were attained with a maximum percentage of dye decolorization at 30°C, pH 7, and when the fungi is grown for 7 days. This productive nature of fungi in the case of bioremediation of dyes could efficiently be increased by altering their metabolism when grown in different carbon sources. Since Extracellular Fungal enzymes play a critical role in dye decolorization, the enzyme secretion could be highly influenced by growing them in different carbon sources to achieve, a higher percentage of dye decolorization. The usage of living sources or biological products creates very less impact on the environment in the case of bioremediation processes. The cost-effective and environmentally friendly attributes of the above kind create a best-preferred approach for bioremediation. Furtherly, the competent fungal strains can be genetically improved and more than one fungal strain can be co-cultured for aiming effective results in dye biodegradation in adverse environmental conditions.

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