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Optimization of dye degrading fungi in different environmental conditions

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ABSTRACT

Water-pollution is presently one of the major areas of scientific activity. Textile effluents are among the most difficult-to-treat waste waters, due to their considerable amount of recalcitrant and toxic substances. Microbial decolonization and degradation of dyes is seen as a cost-effective method for removing these pollutants from the environment. Fungal biosorption is viewed as a valuable additional treatment for degradation of industrial dyes. In this study the efficiency of dye degrading fungal biomass are treated against 7 different industrial dyes. The results showed that *Aspergillus spp* is a promising candidate for the degradating the different types of industrial dyes and its very competitive compared with conventional sorbents adopted in industrial process. Few microbes are resistant to the unfavorable conditions and degrade the strong industrial dyes.

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1. Introduction

The first human made synthetic dye, mauevin was discovered in 1856 that took over the natural dye quickly. Since then over 100000 dyes have been generated worldwide with an annual production of over 7×10^{5} metric tons. Synthetic dyes are widely used in textile, paper, Food, colour photography, paper printing, plastic, cosmetics, pharmaceutical, leather and toy industries (Zollinger, 1991).

The presence of dyes in surface and subsurface water is making them not only aesthetically objectionable but also causes many water borne diseases. Contamination to this aquatic system brings serious threat to the overall epidemic and socioeconomic pattern inside. Industrial effluents impart a minor fraction of chemical load to the environment; its integrity renders the environmental quality fairly Deplorable (Islam et al., 2011).

Dyes are released in to the environmental through industrial effluents from three major sources such as textiles, dyestuff manufacturing and paper industrial (Camarero et al., 2005). Green technologies to deal with this problem include adsorption of dyestuffs on bacterial and fungal biomass (Fu et al., 2002; Yang et al., 2009) or low-cost non-conventional adsorbents (Crini et al., 2006; Ferrero et al., 2007).

2. Materials and methods

2.1. Sample selection

Various industrial dye accumulated soil and water samples were collected for isolate a potential dye degrading microorganisms (fungi). Different types of industrial dyes are collected from the industry from 4 different places in Thirupur {Tamil nadu,India}. For the preparation of test solution 1 mg of each industrial dye powder is dissolved in 100 ml of suitable solvent which gives final concentration of 1% (1mg/ml).The desired dye concentration was prepared. Then 1ml of fungal Inoculum is added in each flask. Incubation is carried out in 30°C at 150rpm. Decolourization activity was determined by monitoring the decrease in absorbance on a spectrophotometer at 620nm.By measuring the initial and final absorbance, percent dye decolourization was calculated.

Dye reduction value analyzed and compared Based on the high or maximum percentage level of decolorization.

For calculating % decolourization of dye following formula was used % (Babita Rani et al., 2014).

Decolourization = (Initial absorbance – Final absorbance)/ (Initial absorbance) x 1000

Dye decolourization % = (Ao –At) (Ao) * 1000

2.2. Decolorizing activity in solid media using potent isolates

The industrial dye decolorization activity was easily detected in petri dishes with SD Agar plates. Each agar plated in 5 different concentrations of industrial dyes. The petri dish was inoculated with the potent isolate and it was incubated 7 days at room temperature. The fungal strains that exhibited decolorization activity experiment. The decolorizing trials used SD agar supplemented with each (7) dye. The fungi were cultivated during 7 days at room temperature. The decolorizing activity was described as positive when the solid media exhibited partial or total loss of color. The fungal strains with decolorizing activity were selected for the liquid media evaluation.

2.3. Optimization of parameter

2.3.1. Factors affecting the growth of Fungi

For the development of inoculam 100ml of SD Broth containing desire concentration of industrial dyes was inoculated with 1ml inoculums containing 7 days old culture of dye degrading fungi and incubated at 30°C at 150 rpm for growth and degrading desired concentration of industrial dyes. The selected process parameters were optimized *viz*. Inoculum level, Temperature, Agitation, Time of incubation and pH.

2.3.2. Effect of inoculum level on dye degradation

The effect of different concentration of seven days old inoculums level (0.2, 0.4, 0.6, 0.8, 1.0) of *Aspergillus spp* on degradation efficiency were determined by inoculating in the SD Broth medium containing desired concentration of industrial dye. After incubation for 7^{th} day of Sample were subjected to spectroscopic analysis.

2.3.3. Effect of agitation on dye degradation

The effect of agitation on dye degradation was determined optimal condition by incubating desired concentration of industrial dye on SD broth *Aspergillus spp* at various rpm (Revolution per minute) like 50,100,150 for 7^{th} day and degradation activity was studied by recording the initial and final absorbance of dye.

2.3.4. Effect of temperature of incubation on dye degradation

The SD Broth was used for determination of optimal temperature of 10,20,30,40 and 50 ºC – at desired concentration of industrial dye. The samples were withdrawn after 7^{th} day of incubation and analyzed by spectrophotometric analysis.

2.3.5. Effect of pH on dye degradation

The SD broth of different pH (2, 4, 6, 8 and 10) was used for determination of optimum pH. The influence of pH on dye degradation activity was studied by recording the initial and final absorbance of dye after incubated at 30ºC for 7^{th} day and absorbance were analyzed by spectrophotometric analysis.

2.4. Media composition

There is no doubt that media composition has an enormous effect on fungal growth and production of their decolourization systems. It must be noted that real industrial effluents vary with location and time, not to mention the often very complex composition with a lack of nutrients, compared to the usually well-defined media used in the research. Therefore, attention has to be focused on the supply of carbon and nitrogen sources together with mineral nutrients and other additives (Babita et al., 2014; Hao et al., 2000; Knapp et al., 2000; Singh et al., 2006).

2.4.1. Effect of carbon source of incubation on dye degradation

The SD Broth was used for determination different carbon sources of Fructose, Glucose and Sucrose 1mg/100ml was added on the each test sample and incubated at 30ºC for desired concentration of industrial dye. The samples were withdrawn after 7^{th} day and analyzed by spectrophotometric analysis.

2.4.2. Effect of Nitrogen source of incubation on dye degradation

The SD Broth was used for determination different nitrogen sources of yeast extract, peptone, urea and ammonium sulfate 1mg was added on the each test sample and incubated at 30ºC for desired concentration of industrial dye degradation. The samples were withdrawn after 7^{th} day and analyzed by spectrophotometric analysis.

3. RESULT AND DISCUSSION

Potential strain selection showed in the Table 1. Out of 51 fungal isolates, only one fungal strain (*Aspergilus spp)* was selected after comprehensive screening of the industrial dyes biodegradation for further studies. Based on the high or maximum percentage level of decolorization.

3.1. Optimization of parameter

3.1.2. Factors affecting the growth of fungi and dye degradation

In the present study, an attempt was made to optimize the degradation of industrial dyes by the potential A*spergillus* strain. Effect of various testing process like concentration of inoculums, agitation and aeriation, pH, temperature and media composition were studied. The efficiency of *Aspergillus spp* isolate was evaluated for the degradation of industrial dye. The effect of different parameters of factors affecting the growth of fungi and dye degradation was studied with an aim to determine the optimal conditions. *Aspergillus spp* dye degradation showed in Figure. 1.

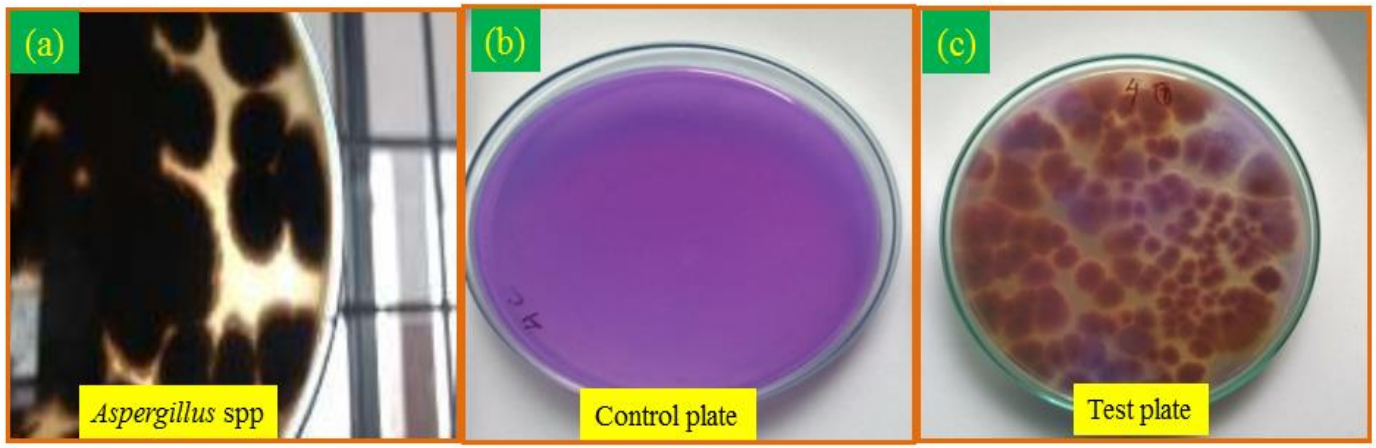

Fig 1 Decolorizing activity of *Aspergillus* spp in Sabouraud Dextrose Agar media

Table 1 Spectrometric analysis using potent dye degrading fungal strain (OD value)

Dye degrading fungi	Dye1	Dye2	Dye3	Dye4	Dye5	Dye6	Dye7
1. Aspergillus spp	969.37	880.10	693.12	247.88	182.16	111.78	81.729
2. Mucor sp	1883.1	1651.5	1339.7	1190.6	695.52	521.10	290.6
3. Curvularia sp	2120.0	2047.7	1938.3	1844.2	1374.9	1285.0	142.1
4. Alteraria spp	2120.0	1961.1	1939.3	1858.0	1507.5	1431.0	151.8
5. Fusarium spp	1142.2	1090.1	909.06	783.13	339.89	233.52	0.239
6. Candida spp	2070	1868.5	1116.2	510.44	491.79	211.05	176.70

7.Caldosporium spp	2120.0	2035.5	948.65	475.75	284.35	163.56	149.25
8. Penicillium spp	969.37	642.41	637.06	510.55	482.30	299.3	190.12
9. Rhizopus spp	2120.0	841.38	293.20	276.87	250.0	206.17	128.47
10.Aspergillus nigar	1651.5	534.19	438.36	338.78	131.97	666.7	890.36
11.Aspergilus terreus	98.15	105.29	153.86	83.39	92.83	100.85	74.39
12.Aspergillus flavus	112.0	107.85	253.59	132.76	296.35	166.51	142.16

3.1.3. Effect of inoculum level on dye degradation

Effect of inoculum level (0.2 – 1 ml) with days on degradation of industrial dye was represented in the Table 2. The result in table 2 depicts that, at every ml of inoculum, dye degradation increased with per day of incubation. The percent degradation of testing industrial dye was found to be 67% - 85%. When the inoculum level was increased up to 1 ml in 1000ml of test dye solution, the extent of maximum dye degradation increased to 85.99%. Therefore, 1 mL of *Aspergillus spp* inoculum was selected as optimum for the degradation of industrial dye.

3.1.4. Effect of agitation on dye degradation

Table 2 Inoculum concentration

Optimum concentration of inoculums of fungi						
S. No	**Concentration of inoculum**	**0.2**	**0.4**	**0.6**	**0.8**	**1ml**
1	control	0.495	0.495	0.495	0.495	0.495
2	Day 7	0.361	0.213	0.287	0.152	0.028

The percent degradation of industrial dyes was found to be 58% to 83% of considerable increase of the percent dye degradation was observed when the increased of agitation. The maximum dye degradation (83 %) was attained. Therefore, 150

rpm agitation of *Aspergillus spp* was selected as optimum for the degradation of industrial dye reduction showed in Figure 2.

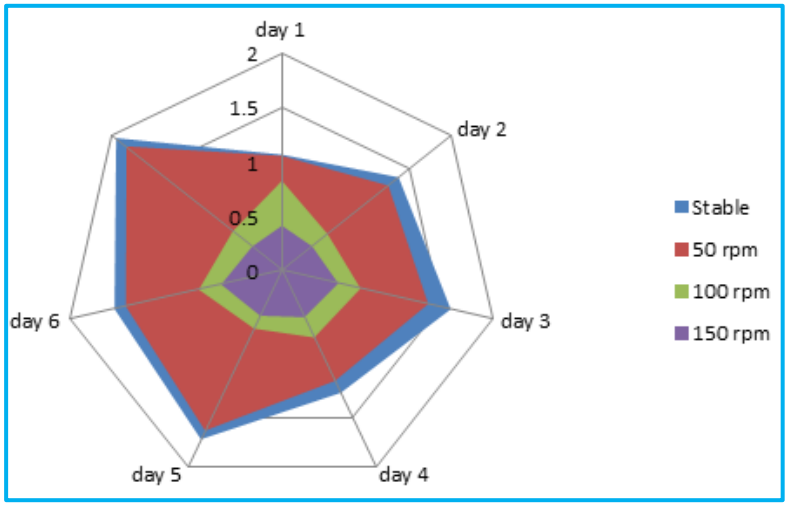

Fig 2 Effect of agitation for dye degradation

3.1.5. Effect of temperature and incubation on dye degradation

Figure 3 shows degradation of industrial dye by *Aspergillus spp* with time at different temperatures (10, 20, 30, 40 and 50°C). It was clear that, percent degradation of dye increased with an increase in temperature from 20 to 35 °C. The percentage removal of dye was decreased with further increase in temperature up to 50 °C. Degradation activity was significantly suppressed at 20 °C than other temperatures, which might be due to the loss of cell viability or deactivation of the enzymes responsible for degradation at 20 °C. Further, increase in the temperature resulted in the decrease in the percent degradation. This may be due to the higher temperatures, thermal deactivation to the enzyme responsible for degradation may occur. So the optimum temperature of the dye degradation was occurring in 30°C. This is shown in the figure 3.

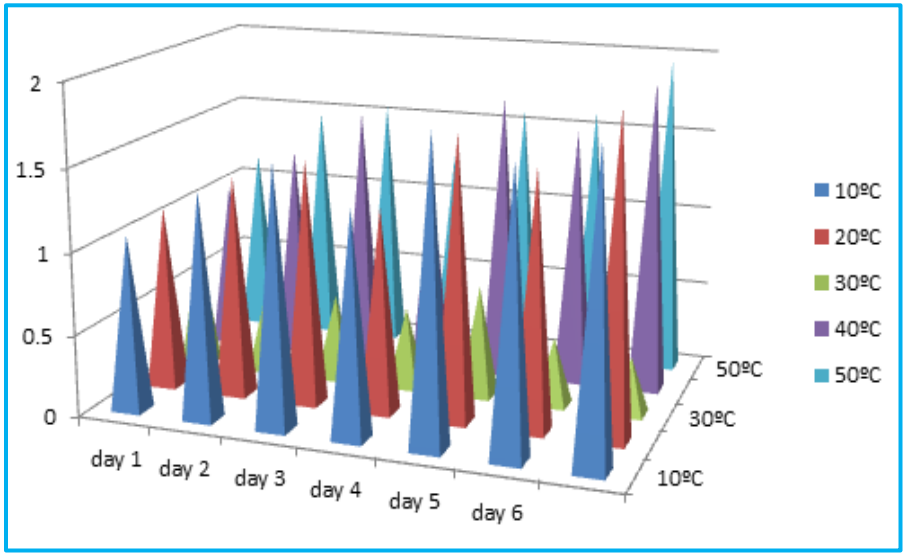

Fig 3 Effect of temperature and incubation for dye degradation

3.1.6. Effect of pH on dye degradation

Effect of pH (2.0 – 10.0) on the degradation of industrial dyes by *Aspergillus spp* was shown in Figure 4. In our study, it was noticed that, an increase in pH from 6 to 7 enhanced the rate of degradation significantly. However, degradation rate was the highest between 6 – 7 pH. Highest degree of degradation occurred at optimum pH 7.0. The results further revealed that, any deviation

in the pH from optimum, decreased the extent of dye degradation. From the Figure 4 it was clearly noted that, the percent degradation of industrial dye increased with increase in time irrespective of pH. The maximum percent degradation (62.90 %) of dye was found at pH 5 after incubation period. Good percent degradation (70.63 %) was observed at pH 6. Further, increase in pH from 7.5 to 8.0, decreased the percent degradation of industrial dye. It was clearly understood that, degradation was lower in acidic pH than alkaline pH.

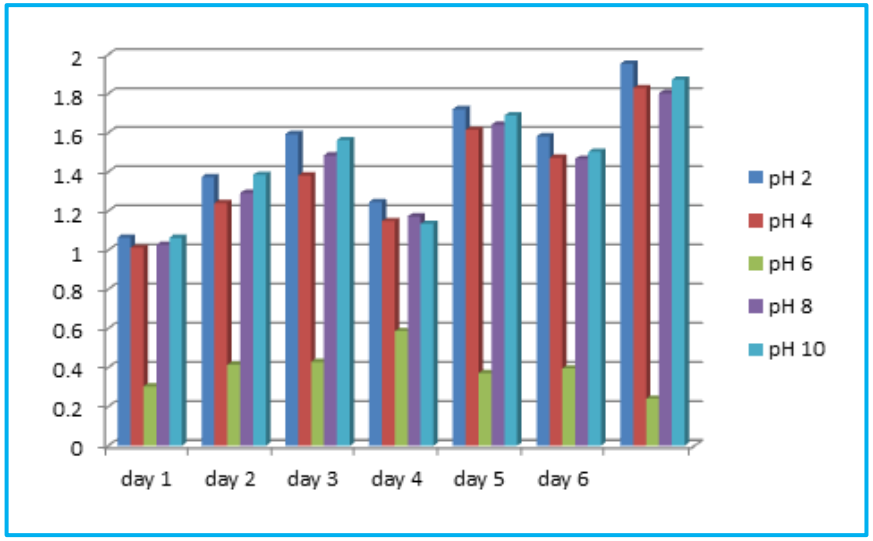

Fig 4 Effect of pH for dye degradation

3.2. Media composition

3.2.1. Effect of carbon source of incubation on dye degradation

To establish the effect of different carbon sources of Sucrose, Glucose, and fructose by adapting the experimental design of one factor at a time by keeping other factors constant was a valuable method used to investigate the role of single factor in an occurrence. Dye removal was more significant between growth of fungal isolates and selective carbon source. Results for effect of carbon source on removal of industrial dyes decolourization with fungi showed fructose (Figure 5) 82 % of dye reduction as the good nutrient source. In the contrast addition of carbon source, instead of increasing decolourization, reduce the rate possibly due to assimilation of added carbon source. So the carbon source was important for the metabolism of fungi.

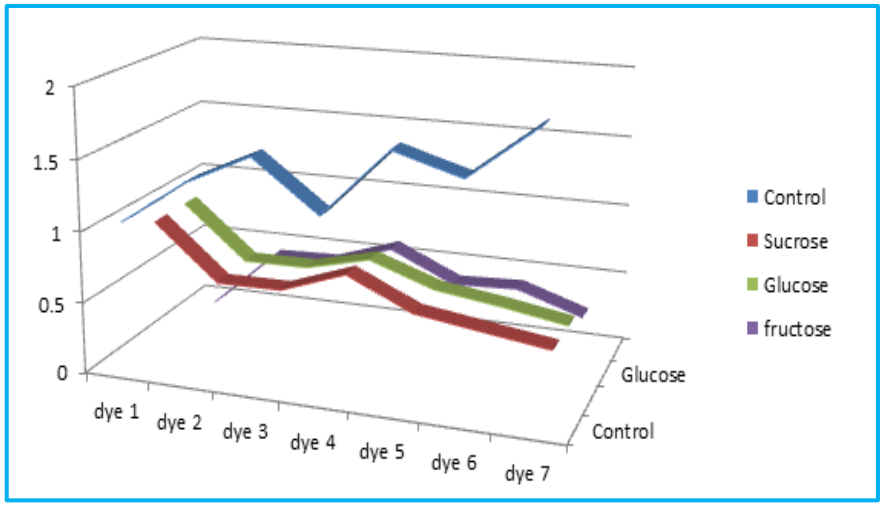

Fig 5 Effect of different carbon source for dye degradation

3.2.2. Effect of nitrogen source of incubation on dye degradation

All the industrial dyes showed evidence of maximum color removal on 7th day of incubation. The presence of yeast extract and urea increased the color removal. Maximum decolorization as observed (Figure 6) in 84 % urea as a important nitrogen source. Respectively in the medium incorporated with yeast extract. Effect of nitrogen source suggested that addition of organic nitrogen source yeast extract and urea favoured better decolorization. however, literature survey supports that urea and yeast extract as a best co-inducer.

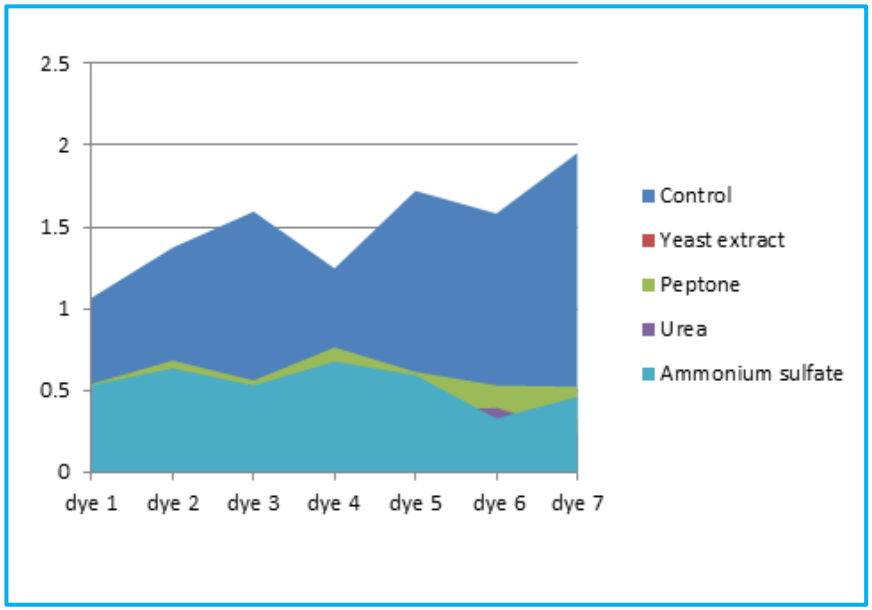

Fig 6 Effect of different carbon source for dye degradation

The use of these fungi, thus could offer a much cheaper and efficient alternative treatment of waters contaminated heavily with textile dyes (Abdel-Raheem and Shearer, 2002). Industrial waste water is complex and highly variable mixture of many polluting agents ranging from inorganic and low molecular weight organic compounds to polymers. In general dye decolourization progresses more slowly than radial growth and decolourization of some dyes did not occupy the entire diameter of the even when incubated more than 21 days.

The decolourization was faster in mixed culture of *Aspergillus terreus* compared to pure culture with approximately 90% colour reduction. The excellent performance of these fungi in the decolourization of dyes of chemical structures reinforces the potential of these fungi for environmental decontamination. *Aspergillus terreus*, a less common pathogen, present in worldwide, decomposting vegetation and dust. This is commonly used in industry to produce important organic acids, such as itaconic acid and cis-aconitic acid, as well as enzymes, lie xylanase. It was also the initial source for the drug mevinolin, a drug for lowering serum cholesterol. Soil fungi possess ligninolytic enzymes and play an important role in the degradation of lignocellulose in soil ecosystems (Okino et al., 2000; Wesenberg et al., 2003). These lignin-degrading enzymes are directly involved

not only in the degradation of lignin in their natural lignocellulosic substrates but also in the degradation of various xenobiotic compounds, including dyes. Moreover, ligninolytic enzymes have been reported to oxidize many recalcitrant substances such as chlorophenols, polycyclic aromatic hydrocarbons (PAHs), organophosphorus compounds, and phenols (Sudhspriyadharsini et al., 2008).

4. Conclusions

The decolourization of dyes was studied under stationary condition encouraging results were obtained after 3 days, but maximum decolourization of all the dyes were obtained after 9 days. In this study we have observed higher decolourization under the conditions of pH6, 30 as a temperature, fructose, urea, and 150 rpm speed will be maintained by *Aspergillus terreus.* which could be due to better oxygenation of the fungus and regular contact of secreted enzymes with dye molecules to decolorize it, moreover agitation also helps the fungus to grow better. Disappearance of dye colour may be due to biodegradation of chromophore in dye molecule because of extra celluar enzyme production by fungi along with absorption and adsorption. Due to the environmental friendly techniques it utilizes, bioremediation has been characterized as a soft technology. Its cost-effectiveness and the little disturbance in the environment render this technology a very attractive and alternative method of choice. The identification and research of new fungal strains with the aid of molecular techniques will further improve practical applications of fungi and it is anticipated that fungal remediation will be soon a reliable and competitive dye remediation technology. In future colour producing microbes and plants were used to control the pollution and save our nature for our future.

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Conflicts of Interest: None

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