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Research Article

MICROBIOLOGY

Biodegradation of textile dyes waste water by *Pleurotus eryngii*

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KEY WORDS

Pleurotus eryngii, dyes, decolorization, immobilization

ABSTRACT

The potential of *Pleurotus eryngii* was studied for decolorization ability of different types of textile dyes which were screened on liquid medium. During decolorization study on seven textile dyes, it was found that *P. eryngii* had the most efficient decolorization on reactive Blue 21 with decolorization percentage (98.4%). On the other hand, the immobilized *P. eryngii* on wheat straw, rice straw, sugar cane waste and wheat bran had biodegradation ability of Blue 21 dye. The immobilization of fungus on sugar cane waste gave more efficient decolorization of Blue 21 with decolorization percentage (83.5%). Also it was found that the decolorization percentage in case of free *P. eryngii* was (50.8%). The fungus has the ability to produce lignin-degrading enzymes as laccase, Mn peroxidase, peroxidase and catechol oxidase enzymes. Immobilized *P. eryngii* on sugar cane waste exhibited the ability to treat industrial textile effluent, 85 to 90% reductions of various nutrients including biological oxygen demand (BOD) and chemical oxygen demand COD were achieved and color removal reached to 96%. So, it was suggested that the immobilized *P. eryngii* might be applicable to textile effluent system. It was found that the reuse of the treated textile effluent with immobilized *P. eryngii* on sugar cane waste for irrigation of Basil plant and good result similar to that obtained by tap water.

1. Introduction

Major classes of synthetic dyes, including azo, anthraquinone and triarylmethane dyes, are toxic or even carcinogenic compounds with long turn over times **(Hartman *et al.* 1978).** Azo dyes are the most important group of synthetic colorants. Synthetic azo dyes are the largest class of dyes, and represent more than half of the dyes as 1 million tons **(Stolz 2001).** Dye-containing effluents represent enormos problem because of their high chemical oxygen demand (COD) and biological oxygen demand (BOD), suspended solids and the content of toxic compounds which consider agreat problems in ecosystem with releasing in the environment, they often exhibit toxic effects on different organisms. Threating ecosystems by reducing sunlight penetration, this reduces photosynthetic activity and dissolved oxygen concentration **(Banat *et al*., 1996)**. Textile wastewaters are rated as the most pollutant among all industrial sectors. Important pollutants in textile effluents are mainly recalcitrant organics, colors, toxicants and inhibitory compounds, surfactants, chlorinated compounds and salts (**Sen and Demirer, 2003). Sudarjanto *et al*., (2006)** stated that some of the dyes and/or products are carcinogenic and mutagenic. Therefore, textile wastewater containing dyes must be treated before discharging into the environment **(Tantak and Chaudhari, 2006).**

There are many methods for the treatment of dye-containing effluents like physical and chemical methods, including adsorption, coagulation, precipitation, filtration and oxidation. Adsorption is the method most widely used at present due to its convenience and efficiency **(Nigam *et al.* 1996).**

Some microorganisms, including bacteria, fungi and algae can degrade or absorb a wide range of dyes **(Robinson *et al*. 2001).** Biological approach using fungi to treat dye-containing effluents has received much attention recently. The white rot fungi have proven its capability to degrade synthetic chemicals, such as azo dyes from textile industry **(Zhao and Hardin, 2007**). Most of the previous studies reported focused on the lignin-degrading enzymes of *P. chrysosporum* and *Tramotus versicolor*. Lately, there has been a growing interest in studying the lignin-modifying enzymes of a wide array of white rot fungi searching for better lignin-degrading systems **(Revankar and Lele 2007).** Many studies have demonstrated that white rot fungi can degrade a wide variety of structurally diverse dyes such as azo, anthraquinone, heterocyclic, triphenylmethane and polymeric dyes due to the lignin degrading system enzymes: lignin-peroxidase (LiP), manganese-peroxidase (MnP) and laccase (**Cripps *et al.* 1990; Vicuna *et al*. 1993; Heinfling *et al*. 1998**) The ligninolytic system of *Pleurotus* sp. has been extensively studied, and it appears to be an effective alternative for the bioremediation of resistant pollutants (**Cohen *et al*. 2002).** Also immobilization of ligninolytic fungi on solid supports was used for cultivation and enzyme production (**Kasinath *et al*., 2003, Saparrat and Guillén, 2005 and Šnajdr and Baldrian, 2007**), dye decolorization, and pollutant degradation (**Shin *et al*., 2002, Novotný *et al*., 2004, Šašek *et al*.,**

2006, Svobodová, *et al*., 2006 and Przysta *et al,* 2018).

The aim of this work is testing the decolorization by biodegradation process of seven azo dyes and the textile dyeing wastewater by immobilized *Pleurotus eryngii* on sugar cane waste. Additional phytotoxicity assays were investigated by irrigation of basil plant with treated waste water to know its safety in irrigation of the plant.

Materials and methods

Dyes and chemicals

Seven dyes were used in this study which were reactive blue 21, reactive yellow 160, orange 122, direct congo red, direct yellow 5g, acid methyl orange and Acid Eriochrome black T. These dyes were purchased as a powder from Egypt Company for the textile industry in El-Mahalla El-Kubra, Gharabia, Governomate, Egypt.

Organism

Pleurotus eryngii somycel France was obtained from mushroom laboratory culture collection (MLCC), El-Giza, Egypt. It was maintained in Petri dishes containing Potato Dextrose Agar (PDA) medium at 30°C.

Decolorization of dyes

Two fungal discs of 0.5 cm diameter on solid medium were removed with a sterile cork borer and inoculated in 250 ml conical flasks containing 100 ml sterilized PDA liquid medium. The inoculated media containing 50 mg/l for each tested dye were incubated at 30°C and 150 rpm, un-inoculated sterile growth medium served as control. The difference in color intensity of inoculated flasks was observed until 10 days for decolorization of dye **(Yang *et al*., 2009).**

Decolorization assays

Decolorization of dyes was detected using the culture supernatant after centrifugation at 3,000 rpm for 30 min. Control was performed using the same medium but without the tested fungus. Assays were performed by using visible recording spectrophotometer (labomed, Inc) at as pecific λ length for each dye.

Color removal was reported as:

Decolorization (%) = (A_o _ A_t)/A_o x 100

Where A_o is the absorbance of initial dye solution, which is constant for each dye and equals 0% decolorization, and A_t is the absorbance at cultivation time, t. Decolorization percentage reading refers to the percentage mean of decolorization percentage of three replicas.

Efficiency of decolorization of reactive Blue 21 dye using free and immobilized fungal biomass

Biomass immobilization:

Pleurotus eryngii was grown in glass bottles on different substrates like rice straw, wheat straw, sugar cane waste and wheat bran. The moisture content was adjusted to 75%. This substrate was steam sterilized at 121ºC for 15 minutes and inoculated with *P. eryngii*. The cultures were incubated at 28ºC until the mycelium covered the substrates.

Decolorization of reactive Blue 21 dye

The substrates covered with fungal mat were evenly homogenized under sterile conditions and equal amounts (5g of immobilized substrate) were used to inoculate 100 ml of sterilized tap water

containing 50 mg/l of reactive Blue 21 dye which was selected as the most decolorized dye more than other dye. Control with substrates without fungal mat. Incubation was carried at different periods (4, 6, 8, 24, 48, 72 and 96 hours), agitation rate and temperature were 150 rpm and 30ºC, respectively **(Yang *et al.*, 2003).**

Screening different enzymes activity of *Pleurotus eryngii* for decolorization of reactive Blue 21 dye

Extraction of Extracellular enzymes

Samples of sugar cane straw covered with fungal mat were extracted in phosphate buffer (pH 6.0) for ligninolytic enzymes. Filtrated extract was used for enzyme assay according to **Wood and Goodenough (1977)**.

Enzyme assay

Laccase

Guaiacol has been reported as efficient substrate for laccase assay, the intense brown color development due to oxidation of guaiacol by laccase can be correlated to its activity. The reaction mixture was prepared in test tubes each containing a volume of 3 ml acetate buffer (10 mM pH 5.0), 1ml guaiacol (2 mM) and 1 ml enzyme extract and enzyme blank contained 1ml of distilled water instead of enzyme source. The total volume of the reaction mixture was 5ml, the mixture was incubated at 30ºC for 1 hours and absorbance was read at 450 nm using UV spectrophotometer, enzyme activity was expressed in units/ml/min (**Sandhu and Arors, 1985).**

Manganese dependent peroxidase

Manganese peroxidase (MnP) activity was determined according to **Bonnen *et al.* (1994).** The assay solution contained 1 mM guaiacol, and 1 mM $MnSO_4$ in 10 mM citrate phosphate buffer (pH 5.5). The reaction was initiated by the addition of 0.5 ml of H_2O_2 (1 mM). After incubation at room temperature for 10 minutes, the change in absorbance due to the oxidation of guaiacol was monitored at 465 nm.

Peroxidase

The activity of peroxidase was assayed according to **Kato and Shimizu (1987)**. A sample of 3 ml of the reaction mixture containing 1.5 ml of 0.1 M sodium phosphate buffer (pH 5.8), 0.7 ml of 7.2 mM guaiacol, 0.7 ml of 11.8 mM H_2O_2 and 0.1 ml enzyme extract was used for the assay. By the addition of H_2O_2, the reaction was initiated and the change in the absorbance was measured at 470 nm. Enzyme activity was expressed in units of mM/g /min.

Catechol oxidase

Three ml buffered catechol solution was added to the clean cuvette of spectrophotometer (0.01 M catechol freshly prepared in 0.1 M phosphate buffer pH 6). The spectrophotometer was adjusted to read absorbance at 495 nm. The cuvette chamber of spectrophotometer and absorbance was adjusted to zero. 1ml of the enzyme extract was added to the cuvette, the changes in absorbance were recorded for every 5 min up to 30 min. The increasing absorbance values were plotted from the linear phase of the curve, the increase was determined in optical density per/minute.

FTIR analysis

FTIR spectra were obtained in transmission mode using Gasco model 4100 LE device, in the mid infra-red region of 400–4,000 cm^{-1} at the Micro-analytical unite, Faculty of Science, Tanta University, Tanta, Egypt. The samples

before and after treatment with the tested fungi were ground with KBr and pressed into transparent disk and analyzed.

Application of immobilized *Pleurotus eryngii* on industrial textile effluents:

The ability of *Pleurotus eryngii* mycelia immobilized on sugar cane waste to remove color of single dye efficiently does not indicate the suitability of this organism in treatment of colored textile effluents. In order to detect the efficiency of *P. eryngii* to decolorize textile effluents, the textile effluents were collected in sterile containers from a company for textile dyeing and printing located in El-Mahalla El-Kubra, Gharabia Governomate, Egypt. Samples were obtained at the end of a dying process of cotton, pH 10 and were stored at 4°C for use within 24 h. The absorbance maximum was detected using a spectrophotometer T80. Parameters as pH, total dissolved solid (TDS), total suspended solid (TSS), electric conductivity (EC), chemical oxygen demand (COD) and biological oxygen demand (BOD) were measured in textile effluent before and after treatment with immobilized *P. eryngii* mycelium on sugar cane waste.

Efficiency of using treated textile effluent on basil plant germination:

After germination and reach to yield stage, whole basil plant was washed thoroughly to get rid of any soil particles. The morphometric parameters as root and shoot length were measured, root and shoot were separated and weighed immediately (fresh weight) then dried in oven at 40°C for five days to determine dry weight, also inflorescence length and inflorescence fresh weight were measured. Biochemical parameters as total carbohydrates, photosynthetic pigment and total protein were estimated in plant.

2.11. Statistical analysis

The obtained results were statistically analyzed by one way and Duncan analysis of variance (ANOVA) to determine the degree of significance between the treatments using SPSS 18 software. Significance was set at $P < 0.05$ and all results were triplicated (**Pipkin, 1984).**

Results and discussion

Dye decolorization activity of *Pleurotus eryngii* was screened for seven dyes with the final dye concentration of 50 mg l^{-1}. **Fig (1)** show the efficiency of *P. eryngii* to decolorize different kinds of dyes: reactive Blue 21, reactive Yellow 160, reactive Orange 122, direct Congo red, direct Yellow 5G, acid Methyl orange and acid Eriochrome black T and the ability to decolorize textile dyes were screened on liquid medium. The dye Blue 21 was decolorized more than the other six dyes where the decolorization percentage of Blue 21, Yellow 160, Orange 122, Congo red, Yellow 5G, Methyl orange and Black T were 98.4%, 96.8%, 97.1%, 97.8%, 76%, 61.1% and 96.9% respectively. These results were correlated with that of **Omar (2016)** who reported that *Aspergillus niger* isolated from the soil samples around the textile distillery industries was evaluated for its efficiency in decolorization of different textile dyes; reactive red 198, reactive orange122, reactive yellow 160, reactive blue 21 and reactive blue 19**.** Fungal biomass walls were composed of macromolecules which contain carboxyl amino, sulphates, and hydroxyl groups which act as metal sorption sites (**Coulibaly, *et al*., 2003**), and they are

able to produce an array of enzymes to enable them to grow under a variety of conditions. The decolorization efficiency of fungi can be due the presence of chitin with hydroxyl and amino groups in their cell wall, which make them an efficient adsorbent of dye effluent (**Manikandan *et al*., 2012).** Difference in the capacity of dye decolorization between fungi had been related to inter and intraspecific variations, the molecular complexity of the dye and culture conditions (**Ramya *et al*., 2007**).

Decolorization efficiency of reactive Blue 21 dye using free and immobilized fungal biomass

The decolorization of Blue 21 dye by free and immobilized *Pleurotus eryngii* on different substrates were increased with increasing incubation periods up to 84 h and the maximum decolorization was reached after 72 h. **Fig (2)** shows the decolorization percentage in case of free *P. eryngii* was 50.8%, but decolorization percentage increased using immobilized mycelia of *P. eryngii.* The decolorization percentage of *P. eryngii* immobilized on wheat straw, rice straw, sugar cane waste and wheat bran were 57.5, 32.5, 83.5 and 62.2 % respectively. **Przysta *et al.* (2018)** were used different natural and synthetic solid supports for immobilization of fungal biomass. Among the different tested supports the best growth of *P. ostreatus* was observed on the sawdust, brush and polypropylene washer because the supports may take part in the decolorization process and the level of adsorption on them was estimated for tested dyes. Fungal immobilization represents several applicative advantages such as treatment of large volumes of waste water and allowing the persistent in competition with faster growing species (**Tavcar *et al.,* 2006)**.

Screening different enzymes activity of *Pleurotus eryngii* for decolorization of reactive Blue 21 dye

Use of *Pleurotus eryngii* as dye biodegrader or decolorizer has been studied in this report and the efficient decolorization may be attributed to either through the action of extracellular enzymes such as laccase, Mn peroxidase, peroxidase and catechol oxidase and biosorption by the fungal biomass. **Fig (3)** showed that fungus *P. eryngii* produced laccase, Mn peroxidase, peroxidase and catechol oxidase enzymes. The activity increased with increasing incubation periods, a maximum activity of enzymes observed after 12 days of incubation and their activity were as follow 4.55, 1.37, 0.023 and 0.035 U/min/ml respectively.

Many studies have demonstrated that white rot fungi can degrade a wide variety of structurally diverse dyes such as azo, anthraquinone, heterocyclic, triphenylmethane and polymeric dyes due to the lignin degrading system enzymes: lignin-peroxidase (LiP), manganese-peroxidase (MnP) and laccase (**Cripps *et al*. 1990; Vicuna *et al*. 1993; Heinfling *et al*. 1998**).

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), organo-phosphorus compounds, and phenols (**Wesenberg *et al*., 2003**).

FTIR analysis

Fourier transform infrared spectroscopy (FTIR) analysis of decolorization products showed that there were significant differences between the spectra of pure blue 21 dyes and those after exposed to fungal treatment (**Fig. 4**).

FTIR analysis of the pure dye Blue 21 showed peak at υ3455.81 cm^{-1} for the –OH stretching indicating presence of alcohol group and peaks at 3190 and 1467.56 cm^{-1} indicating presence of aromatic rings. Peak at υ 2924.52 cm^{-1} indicates the presence of - $-CH_2$ aliphatic gp presence in original dye. Also peak at υ 1635.34 cm^{-1} indicate stretching for C=N attached to aromatic ring (pyrol ring) but peak at υ 1500.35 indicate presence of C=N not attached to aromatic ring (azo-methine gp). The peak at υ1191.79 cm^{-1} was for $–SO_3$ stretching. FTIR analysis indicates that the pure dye molecule was complex molecule with presence of OH, SO3, -C=C, -C=N, groups (**Fig. 4 a**).

after treatment with *Pleurotus eryngii* FTIR analysis of the product gave a band at υ 3,365 cm^{-1} that corresponds to the NH group that is not present in the spectrum of and the band at υ 1500.35 of C=N azo-methine gp was disappeared this confirm that the C=N was broken and NH was formed. Also analysis showed that there was disappearance of the peaks at υ 2924.52, υ 1191.79 and υ 1467.56 cm^{-1} of $-CH_2$, $–SO_3$ and C=C of aromatic ring respectively (**Fig. 4 b**).

From the FTIR analysis it was concluded that the decolorization of reactive Blue 21 dye was by means of degradation which causes changes in the molecular orientation of the pure dye molecule, it results in the formation of the different fragments indicated by the formation of new peaks and disappeared of some peaks in FTIR spectra

It was concluded that all changes in υ of the original dye of reactive Blue 21 treated with *P. eryngii* indicated the ability of fungi not only to decolorizes the azo dye but also degrade dyes.

Jadhav *et al.* (2007) concluded from FTIR analysis that *S. cerevisiae* MTCC 463 effectively decolourizes methyl red in to the fragments by biodegradation

Application of immobilized *Pleurotus eryngii* on industrial textile effluent:

Among the various industrial effluents, the textile and the dye industry waste play a significant role in water pollution. Discharge of these effluents into aquatic systems not only causes formation of toxic aromatic amines under anaerobic conditions but also have an adverse effect in terms of chemical oxygen demand in the receiving media. Further, many of these dyes are toxic, mutagenic and carcinogenic besides interfering with the transmission of sunlight and reducing photosynthetic activity (**Chung and Stevens, 1993 and Campos *et al*., 2001)**

In the present investigation, *Pleurotus eryngii* significantly reduced the color from the dye effluent at immobilized conditions; about 96% removal of color was recorded. Also nearly 85 to 90% reductions of various nutrients including BOD and COD were achieved and the removal efficiency of TSS, TDS, and EC were 79, 37 and 38% respectively. These results indicate that all the parameters recorded a decrease in their levels compared to the control. This shows that immobilized *P. eryngii* on sugar cane waste have the ability to treat textile waste water **fig (5).** In parallel to our results **Arumugam and Sivakami**

(2016) studied the treatment of dye industry effluent using immobilized bacteria and fungi and the study indicated promising results for removal for many of the parameters estimated.

Efficiency of using treated textile effluent on basil plant germination:

This experiment aimed to investigate the ability of reusing treated textile effluents in irrigation of plants The treated effluent by *Pleurotus eryngii* was used for irrigation of basil plant and controls represented irrigation by untreated effluent (-ve) and with tap water (+ve). **Fig (6) and (7)** revealed that the effect of treated effluent on plants compared to controls. It was clearly noticed that all treated effluent had pronounced effect on all measured physical parameters comparing to untreated wastewater. Irrigation with treated effluent by *P. eryngii* reflect a significant increase in shoot and root lengths, on other hand shoot and root fresh and dry weights, inflorescence length and inflorescence fresh weight increased comparing to irrigation with untreated effluent. Also **Fig (8)** revealed the effect of treated textile effluent on pigment content of basil plant comparing with controls. The reuse of treated textile effluent by *P. eryngii* revealed a significant increase in chlorophyll (a), chlorophyll (b), carotenoid and total pigments comparing to untreated textile effluent. Effect of irrigation water types on protein and carbohydrate content of shoot and root of basil plant were studied. **Fig (9)** revealed that the plants were affected by treated oil wastewater compared to controls. It was obvious that all investigated treated oil waste water significantly increase root and shoot protein comparing to untreated oil waste water.

The decreasing in the growth parameters, in some cases, of the basil plant under the effect of irrigation by textile effluent may attribute to the presence of dyes in wastewater which inhibition in growth and germination may have been due to the presence of heavy metals that cause toxicity at cellular as well as at the whole plant level **(Kadar and Kastori, 2003)**. Furthermore, presence of heavy metals in the growth medium also causes reduction in uptake of other essential nutrients thereby resulting in reduced growth. Also it could be observed that the irrigation by treated textile effluent enhanced the growth and productivity of basil plant compared to the untreated one. This may be due to the presence of nutrients in treated wastewater which may be considered as fertilizers capable of improving the crop yield but the use of untreated oil wastewater may present serious toxicity and sanitary problems **(Griffin., 1981).**

Conclusion

The current study indicates that the *Pleurotus eryngii* has a great potential to decolorize and degrade dyes in the free and immobilized cases, also the fungus has significant effect in treatment industrial textile effluents in immobilized case. The treated textile effluent was reused in irrigation of basil plant and it gave a good result which was nearly similar comparing to tap water.

Figures

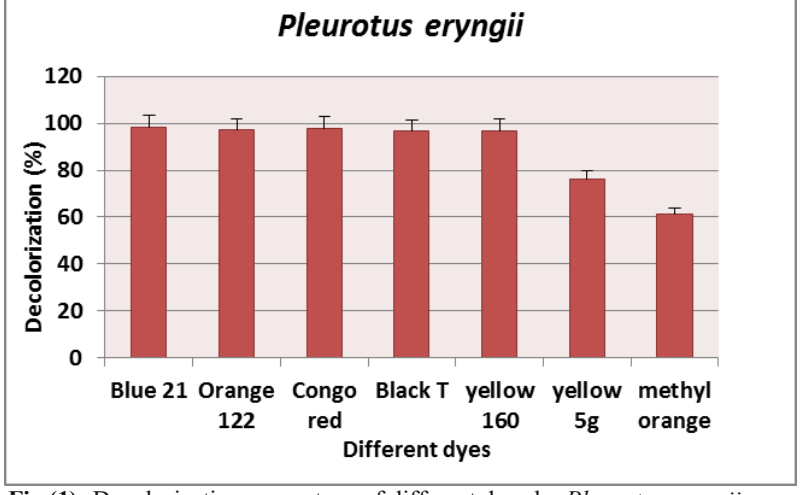

Fig (1): Decolorization percentage of different dyes by *Pleurotus eryngii*

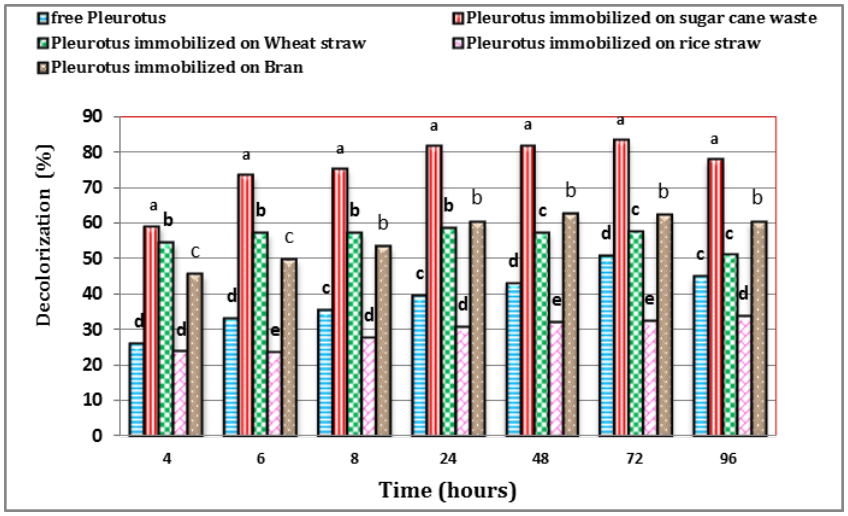

Fig 2: Decolorization efficiency of Blue 21 dye using free and immobilized *Pleurotus eryngii* on different substrate at different incubation periods**.**

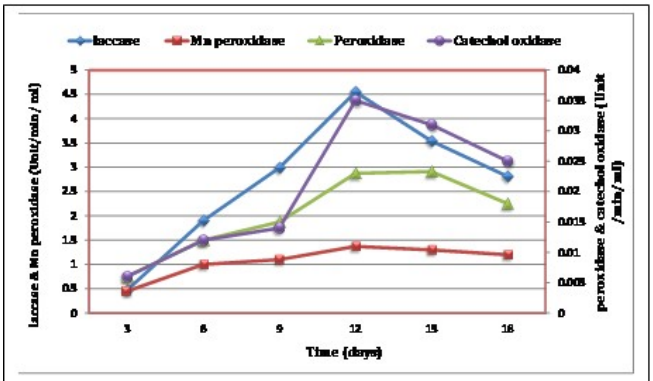

Fig 3: Enzymes activity of *Pleurotus eryngii* on different periods of incubation

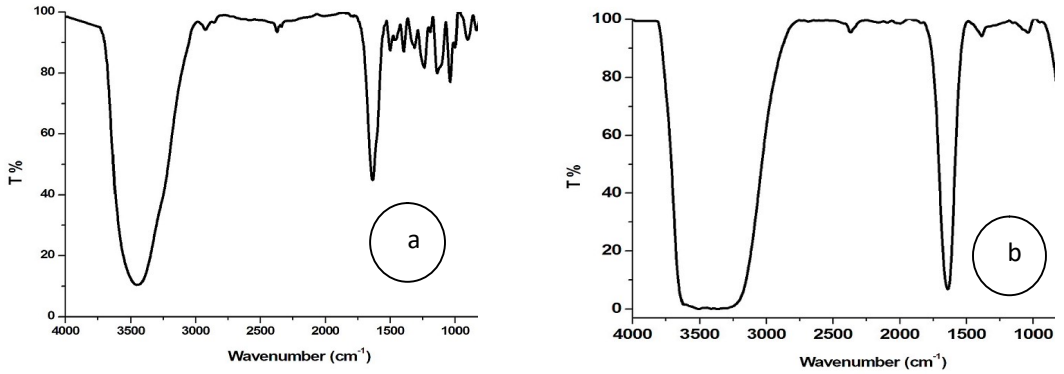

Fig 4: Infrared (IR) spectra of Reactive Blue 21. a Control, b Reactive Blue 21 treated with *Pleurotus eryngii*.

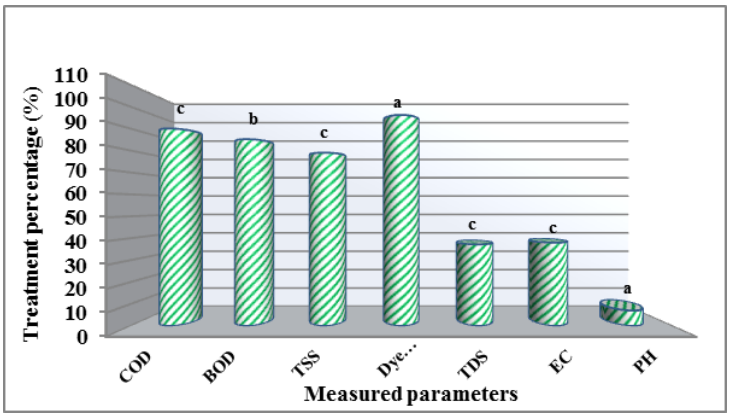

Fig 5: Treatment of textile effluent by immobilized mycelia of *P. eryngii* on sugar cane waste

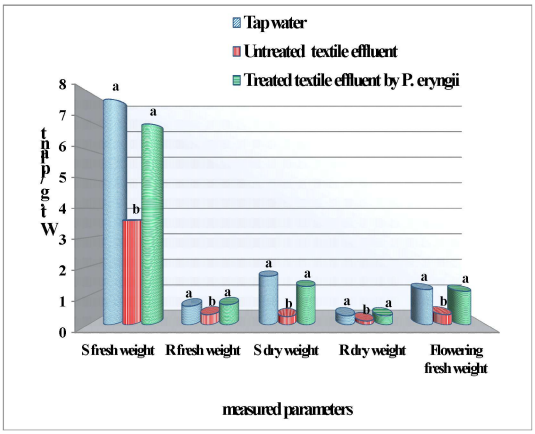

Fig 6: Effect of treated textile effluent by *Pleurotus eryngii* on physical parameter of growth of basil plant.

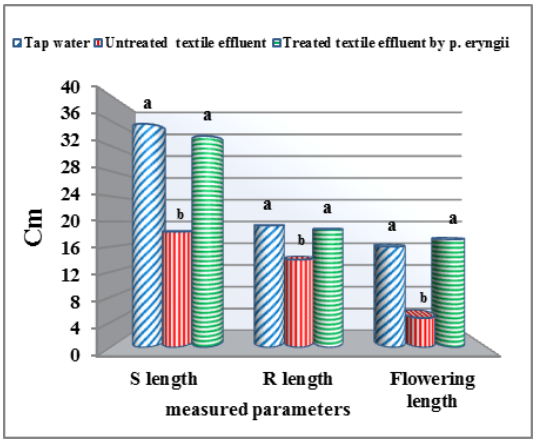

Fig 7: Effect of treated textile effluent by *Pleurotus eryngii* on physical parameter of growth of basil plant.

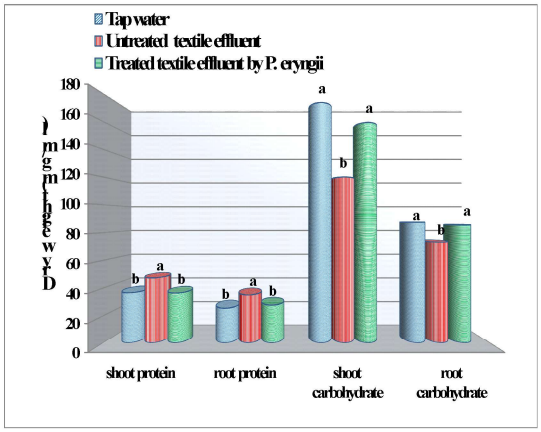

Fig 9: Effect of treated textile effluent by *Pleurotus eryngii* on shoot and root protein and carbohydrate of basil plant

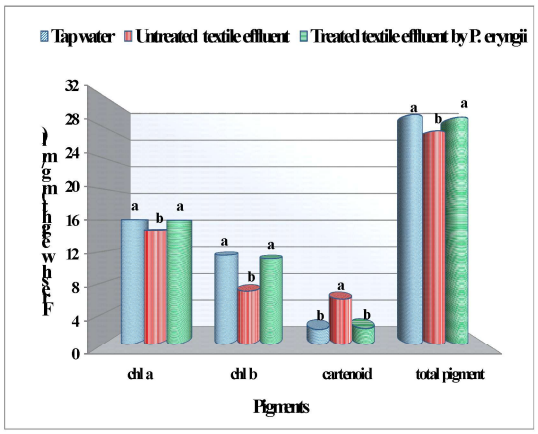

Fig 8: Effect of treated textile effluent by *Pleurotus eryngii* on pigments of basil plant.

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