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# Capacity of a newly isolated fungus *Pleurotus eryngii* from Tunceli, Ovacik for chemical oxygen demand reduction and biodecolorization of Azo-Dye Congo Red

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**Abstract**

Biodecolorization of Congo red dye in both agar-plate and agitated liquid culture mediums by newly isolated white rot fungus *Pleurotus eryngii* has been studied. This fungus isolated from Tunceli-Ovacik province of Turkey. We have also examined the chemical oxygen demand reduction after decolorization under agitated liquid culture medium. For agar plate screening the decolorization capacity of *P. eryngii*, growth and decolorization halos were determined on saboroud dextrose agar (SDA) plates containing 0.05, 0.1, 0.5, 1 and 2 g/l of Congo red. *P. eryngii* showed certain decolorization capacities and was able to decolorize all studied concentrations of Congo red, but not to the same extent. Our results indicated that the new isolate *P. eryngii* had maximum decolorization (87% at 100 mg/l initial dye concentration) and chemical oxygen demand reduction (82% at 25 mg/l initial dye concentration) activities after 7 days under agitated submerged culture conditions. This new isolate could be an effective bioremediation tool for treatment of Congo red containing textile wastewater.

***Key words:*** Biodecolorization, chemical oxygen demand reduction, *P. eryngii*, Congo red.

## Introduction

Synthetic organic dyes are extensively used in various industries, e.g., in area of the textile industry (1), of the leather and paper production industry (2,3,4), in food production technologies (5). Wastewater in textile industry with high amount of chemical oxygen demand (COD) was also reported to be toxic to living organisms in aquatic environments. The intensive color causes some ecological problems to the aquatic life (6).

Congo red (sodium salt of benzidinediazobis1 naphtylamine4 sulfonic acid) has been reported to be a carcinogenic direct diazo dye used for colouration of paper products (7,8). The dye contaminated soils are harmful to the growth of plants also. Extensive study has been carried out on the pollution problems associated with the discharge of dye effluent from industries to various environments. It has been reported that the safe method for azo dye biodegradation is combined aerobic treatment (9,10). Many organisms such as some bacteria and a group of fungi, yeast have been studied for their decolorization of Congo red dye (11,8).

Physical and chemical technologies for treatment of textile wastewaters are not cost effective and commercially unattractive (12,13). Biological degradation is an alternative to these technologies which is environmentally friendly, more economical way of treatment and do not produce large quantity of sludge or second pollutant (14,15).

White rot fungi are able to degrade a wide variety of recalcitrant organic pollutants in aerobic conditions, including various types of synthetic dyes (16). Because of the low specificity of ligninolytic enzymes, individual azo, triphenylmethane, anthraquinone, phthalocyanine and heterocyclic dyes (17,18), as well as complex industrial effluents (19,20) are efficiently decolorized. The *Pleurotus eryngii* belongs to the family of oyster mushrooms (*Pleurotaceae*). The wild species of *Pleurotus eryngii* can be found in large areas of Europe. Its natural habitat is on the dead root of the weed *Eryngium campestre* (21).

In the present work, we have investigated fungal decolorization and COD reduction of azo dye Congo red, using an indigenous fungus, *Pleurotus eryngii* isolated from Tunceli province of Turkey.

## Materials and methods

### *Chemicals*

All chemicals used were of analytical grade and purchased from Sigma and Merck Company and Congo red (Figure 1) were obtained from Sigma-Aldrich Company. Preparation of dye solution is carried out by mixing azo dye namely “Congo red” powder with distilled water.

### *Organism*

White rot fungus *P. eryngii* used in this study. Fungus was collected from Ovacik-Tunceli province of Turkey at May 2011. This fungus described by Prof. Dr. Abdunnasir Yildiz from Dicle University from Turkey. The strains were maintained on SDA slants at 4°C in refrigerator.

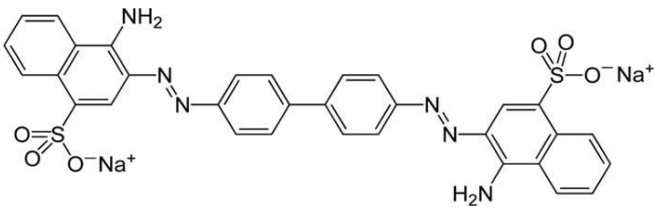


**Figure 1.** Chemical structure of azo-dye Congo red.

***Agar-Plate Screening for Congo red Decolorization***

Decolorization efficiency of fungus was tested on solid media. For this aim, mycelial plugs (5 mm diameter) were inoculated into the center of Petri dishes (90 mm diameter) containing 0.05, 0.1, 0.5, 1 and 2 g/l of Congo red, in triplicate. The plates were incubated at 25 ºC in the dark until they were completely colonized with the fungus or for a maximum period of 20 days. The diameters (cm) of the decolorization and growth halos were determined in two perpendicular directions of the plate. Plates containing the dye but not inoculated served as control.

***Preparation of inoculum and submerged medium***

*P. eryngii* were cultured at 25˚C on SDA slants in glass tube. After 1 week of incubation, conidial suspensions were prepared and used for the preparation of inoculum. 10 ml of the suspension was transferred into a 250 ml flask containing Sabouraud dextrose Broth (SDB) and agitated on a rotary shaker at 140 rpm for 10 days at 28˚C. After incubation, flasks homogenized and then these homogenized mycelium cultures were used as inoculum for submerged decolorization studies. 10 ml homogenized mycelium culture was transferred into 250 ml flasks containing SDB and 5, 25, 50 and 100 ppm Congo red on a rotary shaker incubator at 140 rpm for 7 days at 28 ˚C in triplicate. After incubation, all flasks filtered and then were used in decolorization and COD reduction assays.

***Decolorization and COD reduction assays***

Decolorization of Congo red in submerged liquid medium was measured in culture filtrates (tree replicate flasks) after removing the mycelia by filtration through filter paper, and monitored spectrophotometrically at the maximum wavelength of absorbance (490 nm). The systems without the fungi served as abiotic controls.

$$\text{Decolorization (\%)} = \frac{A0 - At}{A0} \times 100$$

Where,
A0 = Absorbance of the blank (dye solution)
At = Absorbance of the treated dyes solution after incubation.

The concentration of chemical oxygen demand (COD) was analyzed by a DR/890 portable colorimeter (HACH Co., Ltd., USA).

$$\text{COD reduction (\%)} = \frac{Ax - Ay}{Ax} \times 100$$

Where,
Ax = COD concentration of the blank (dye solution)
Ay = COD concentration of the treated dyes solution after incubation.

***Statistically analaysis***

All statistical analyses were performed with SPSS (SPSS Inc., Chicago, IL, USA). The data presented are the averages of the results of three replicates with a standard error. To compare the decolourization ability of fungus, the data were analyzed by analysis of variance (ANOVA).

**Results and Discussion**

The ability of white rot fungi to decolorize various synthetic textile dyes has been extensively studied (22,23,24,25).

*P. eryngii* was tested for decolorization and radial growth rate on saboroud dextrose agar plates containing 0.05, 0.1, 0.5, 1 and 2 g/l of Congo red. *P. eryngii* was able to grow on solid media in the presence of the Congo red dye. It is found that the highest decolorization zone as 8.5 cm at solid media containing 0.05, 0.1, 0.5, g/l dye within 20 days (Table 1)

Biodegradation of Congo red by *Gliocladium virens* (26), various hazardous dyes likes, Congo red, Acid red, Basic blue and Bromophenol blue, Direct green by the fungus *Trichoderma harzianum* (27) and biodegradation of plant wastes materials by using different fungal strains has been studied earlier (28).

The decolorization studies were carried out in 250 ml flasks containing SDB and 5, 25, 50 and 100 ppm Congo red on a rotary shaker incubator at 140 rpm for 7 days at 27 ˚C in triplicate under agitated condition. The decolorization rates of *P. eryngii* for Congo red after 3 and 7 days of cultivation are summarized in Figure 2. Maximum decolorization was found to be 87.32% at 100 ppm initial dye concentration on 7th days of incubation.

Knapp et al. (29) was reported that adsorption of dyes to the microbial cell surface is the primary mecha-

**Table 1.** Decolorization of Congo red on agar plate.

| Concentration ($gl^{-1}$) | Day | Radial Growth (cm) | Decolorization (cm) | Mycelium density |
|---|---|---|---|---|
| **0.05** | **8** | 6.30±0.05 | 3.10±0.01 | +++ |
| | **20** | 8.50±0.00 | 8.50±0.00 | +++ |
| | | | | +++ |
| **0.1** | **8** | 6.00±0.10 | 4.50±0.03 | +++ |
| | **20** | 8.50±0.00 | 8,50±0.00 | +++ |
| | | | | +++ |
| **0.5** | **8** | 6.3±0.11 | 4.5±0.15 | +++ |
| | **20** | 8.5±0.00 | 8.5±0.00 | +++ |
| | | | | +++ |
| **1** | **8** | 6.5±0.12 | 4.8±0.15 | +++ |
| | **20** | 8.5±0.00 | 8.0±0.22 | +++ |
| | | | | +++ |
| **2** | **8** | 7.5±0.05 | 4.5±0.05 | +++ |
| | **20** | 8.5±0.00 | 8.0±0.20 | +++ |

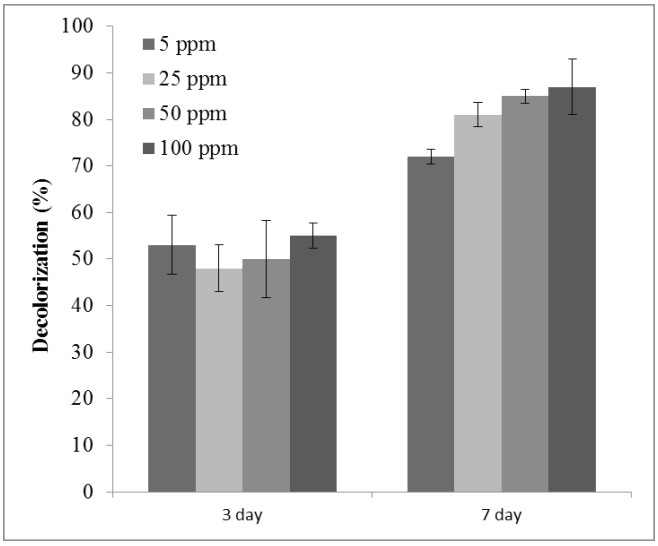


**Figure 2.** Decolorization of Congo red by *P. eryngii* under agiated conditions at 140 rpm.

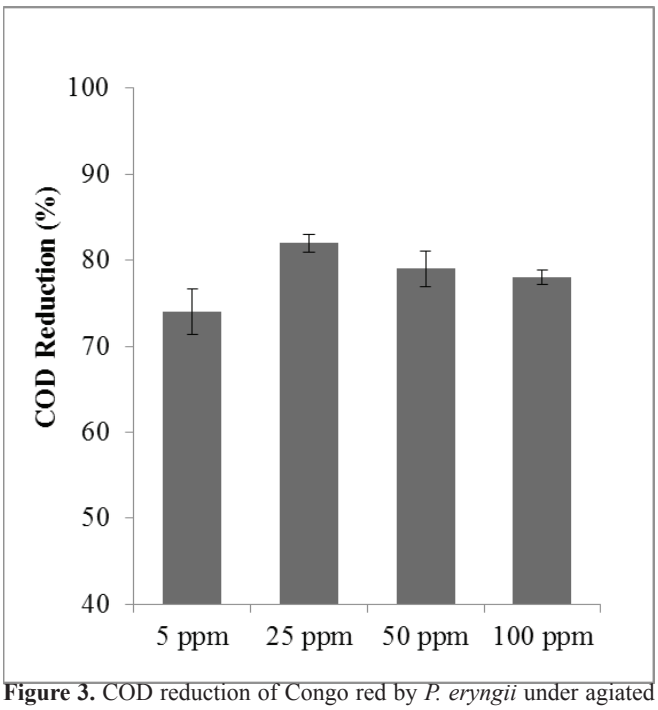


**Figure 3.** COD reduction of Congo red by *P. eryngii* under agiated conditions at 140 rpm.

nism of decolorization. In our study, the adsorption of Congo red by the fungal mycelium was observed, as it was confirmed by the change in the color of fungal mycelium in tested dye.

The a newly isolated *P. eryngii* from Tunceli-Ovacik has a great potential for decolorizing and COD reducing activity of Congo red dye. The COD reduction rates of *P. eryngii* for Congo red after 7 days of cultivation are illustrated in Figure 3. Maximum COD reduction rate was found to be 82% at 25 ppm initial dye concentration on 7th days of incubation. Cao (30) reported that COD removal is carried out by the white rot fungus rapidly. Similarly a fungal isolated from textile industry *Aspergillus terreus* SA3 was efficiently used for the removal of dye such as Sulfur black from textile effluent in the Stirred tank reactor system (STR) were removed by 75.24% (31). Pourbabaee et al. (32) observed similar reductions in COD during the study of aerobic decolorization of Terasil Black in textile effluent by a newly isolated *Bacillus* sp. Reductions in COD level indicates the formation of new metabolites during the process of decolorization.

Biodecolorization of Congo red by fungi has been investigated by a few authors. Shin Shin et al., (33) reported 77% decolorization of Congo red by fungal strain *Pleurotus ostreatus*. Novotny et al. (34) investigated biodegradative ability of 103 strains of wood rot fungi. It is worth mentioning, however, that the diazo dye, Congo red appeared to be one of the dyes that were comparatively more resistant to degradation. *Irapex lactus* caused only 58% decolourisation of Congo red during 14 days of investigation.

In this research, based upon these findings, it can be predicted that this new isolate fungus could be an effective bioremediation tool for treatment of Congo red containing textile wastewater. The identification of new fungal strains will further improve application of fungi in textile wastewater remediation technology.

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