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Research Article
Azo Congo Red Dye Decolorization by Oyster Mushroom (*Pleurotus ostreatus*) F209

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Abstract

Background and Objective: *Pleurotus ostreatus* is known for its high potential as a decolorization and degradation agent for colored organic liquid waste. This study aimed to determine the degradation ability of various types of azo textile dyes by *Pleurotus ostreatus* strain F209 on azo Congo red dye. **Materials and Methods:** This research was conducted using an exploratory method. Observations were made on the ability of *Pleurotus ostreatus* strain F209 to degrade azo Congo red dye with a concentration of 50 ppm dissolved in mineral salts medium (MSM) which was incubated for 15 days. **Results:** The Congo red decolorization was qualitative, measured through the clear zone formed and had a diameter of 6.78 cm and a colony growth diameter of 6.32 cm. Meanwhile, quantitatively, *Pleurotus ostreatus* F209 was able to degrade 56% of 50 ppm Congo red. **Conclusion:** *Pleurotus ostreatus* F209 was successful in increasing the decolorization percentage of the Congo red dye after 15 days of incubation.

Key words: Azo Congo red dye, decolorization, *Pleurotus ostreatus*, wastewater treatment, degradation

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The textile industry is currently experiencing a significant increase due to the rapid development in the fashion industry. Thus, spurring the production of more various dyes to meet the requirements for coloring various fabrics. However, the textile industry caused environmental pollution which is liquid waste originating from the dyeing process, as well as procedures related to the textile industry and leads to environmental issues along with other industries[1,2].

Now-a-days, the textile industry generally uses synthetic due to their stability. Even the production of dyes for dyeing and printing in the textile industry is more than one million tons annually and produces 93% of liquid waste, which contains colors agent, organic compounds and heavy metals and will cause adverse effects if not managed properly. One of the problems is colored organic waste, which is difficult to decompose and causes the low-quality of wastewater as well as enhances the potential of toxicity and turbidity[3].

Conventional dye-based waste treatment can produce complex aromatic structures and cause toxicity to microorganisms around the industrial environment. Conventional biological waste treatment converts waste into dyes containing nitrogen into aromatic amines, which are more dangerous than the main waste[4]. Processing of color waste by bio-decolorization can minimize waste problems. Waste treatment by bio-decolorization is the utilization of the ability of bacteria, fungi, yeast and algae to degrade color in waste both in aerobic and anaerobic conditions[5]. The bio-decolorization method of treating waste is safe for the environment, cost-effective and less sludge produced. A rotting fungus that can be developed in the decolorization of waste in the textile industry is the white oyster mushroom (*Pleurotus ostreatus*).

Oyster mushrooms are a group of white rot fungi that generate ligninolytic and extracellular enzymes such as lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase[6]. White rot fungi can degrade complex organic compounds through catalysis. Mushrooms also have a high and efficient ability to adapt and remove aromatic compounds and can degrade various organic and inorganic contaminants[7]. Ligninolytic enzymes are known to have an essential role in degrading lignin on lignocellulosic substrates, which also can degrade different recalcitrant compounds and complex pollutants such as dyes[8,9]. Oyster mushroom has the potential as a bio-decolorizing agent because it has a large cell surface that is efficient as a biosorbent. The mushroom cell wall is composed of polysaccharide polymers so that the fungus can absorb contaminants in the environment, such as heavy metals and synthetic dyes.

Optimization of the decolorization ability of oyster mushrooms (*Pleurotus ostreatus*) can be supported by data on the optimal ability of oyster mushrooms to degrade waste from several types of azo dyes and can be used an alternative as an effective method to decolorize certain dyes. This study aimed to determine the degradation ability of *Pleurotus ostreatus* strain F209 in azo textile dyes.

MATERIALS AND METHODS

This research was conducted in the Laboratory of Biotechnology and Microbiology of Agricultural Products and Central Instrumentation Laboratory, Faculty of Agricultural Technology, Andalas University from March to September, 2022.

The materials used for this study were mineral salts medium (MSM) media, azo Congo red dye and *Pleurotus ostreatus* mushroom isolates obtained from the Indonesian Institute of Sciences (LIPI) Culture Collection (InaCC) and potato dextrose agar media (PDA). This research was conducted using an exploratory method. Observations were made to determine the optimal ability of color degradation by oyster mushrooms against several azo colors. After obtaining the optimum color that can be degraded by oyster mushrooms, a color degradation growth curve will be made against the incubation time of oyster mushrooms.

Decolorization of several types of azo colors by *Pleurotus ostreatus* on solid media: Solid MSM media containing 100 mg L^{-1} of various types of azo dyes according to the treatment. A pure culture of *Pleurotus ostreatus* in PDA media was taken with a size of 1×1 cm and inoculated into solid MSM media containing azo dye according to the treatment. Incubated for 12 days at 37°C with an interval of 3 days sampling was carried out to monitor the condition of color degradation by *Pleurotus ostreatus*. Color degradation by *Pleurotus ostreatus* during incubation was observed for the percentage of decolorization quantitatively and qualitatively. Quantitatively what will be observed is the decolorization index by measuring the clear zone on the media. Qualitative measurement by measuring the concentration of Congo red using a spectrophotometer (Shimadzu UV-1800, Japan). Different dyes and dye wastewaters were tested for decolorization which was read spectrophotometrically at 540 nm[1,10,11]. The formula used was:

$$\text{Decolorization (\%)} = \frac{\text{Initial concentration (ppm)}}{\text{Final concentracion (ppm)}} \times 100$$

Determination of index decolorization: During the incubation period, all cultures were counted for the diameter of the mycelium produced and their decolorization using a ruler every day. Mycelium measurements were carried out on the surface of the agar plate while decolorization measurements were carried out on the bottom of the agar plate. As a positive control, fungal culture was grown on an agar plate without dye, while for the negative control, it was an agar plate with MO without fungus[12]. The diameter of the clear zone and the diameter of the colony were observed quantitatively to obtain the index decolorization. To calculate the decolorization index according to the equation:

$$\text{Index decolorization} = \frac{\text{Diameter of clear zone (cm)}}{\text{Diameter of colony (cm)}}$$

The decolorization index was determined by the development of clear zones on agar medium during a certain time incubation treatment.

RESULTS AND DISCUSSION

Qualitative decolorization of azo dyes: Research on the biodegradation of Congo red by *Pleurotus ostreatus* began with an agar plate containing MSM and Congo red at a concentration of 50 ppm as a growth medium. The aim was to qualitatively prove the ability of *Pleurotus ostreatus* to degrade Congo red and to determine the incubation time of the degradation process by *Pleurotus ostreatus*.

Qualitative decolorization of azo dyes can be carried out by measuring the clear zone of MSM which contains Congo red dye. The decolorization method using *Pleurotus ostreatus* F209 was able to reduce the concentration of azo Congo red dye. A decrease in color concentration during the 15 days incubation period on MSM media was shown in Fig. 1. *Pleurotus ostreatus* could decolorize 96% of dye from the medium after 24 days of incubation[13]. The decrease in Congo red color can be seen from the greater the growth of the *Pleurotus ostreatus*, the greater the formation of clear zones as a result of color fading. The discoloration is an indication that *Pleurotus ostreatus* has the ability to degrade azo dyes. This result shows promising bio-decolorization of dyeing wastewaters by a microbial enzyme from *Pleurotus ostreatus*. Another study also reported that *Pleurotus ostreatus* cultivation was successfully discolored black chromo-reactive mix (BRM) and the discoloration increased from 20-80% in 2-110 hrs[14]. On the other hand, it was found that the rate of dye decolorization increased (96%) after 24 days of incubation[15]. This suggests that *Pleurotus ostreatus* is showing a promising result as a decolorization agent.

A positive correlation between the growth of *Pleurotus ostreatus* and the decolorization of Congo red where with an increase in colony growth there is an increase in decolorization which can be seen from the increase in the clear zone formed on MSM containing Congo red as shown in Table 1. To determine the ability of *Pleurotus ostreatus*, to degrade Congo red, index decolorization (ID) was calculated by comparing the diameter of the clear zone with colony diameter the during the incubation period.

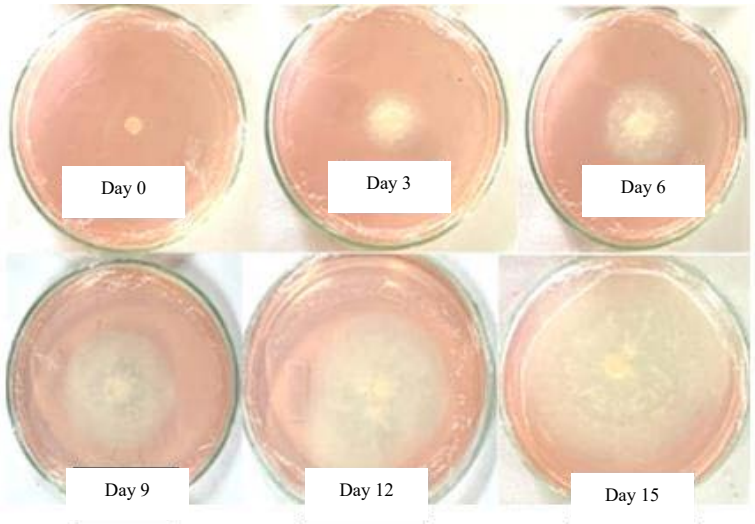

Fig. 1: Decolorization of Congo red dye by *Pleurotus ostreatus* with an incubation time of 15 days

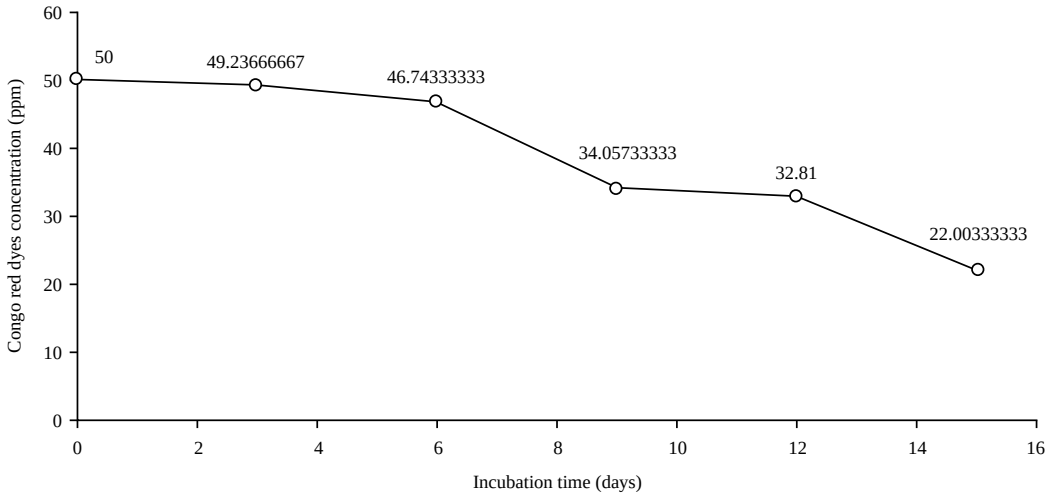

Fig. 2: Degradation of Congo red dye with variation of incubation time

Table 1: Qualitative decolorization Congo red dye by *Pleurotus ostreatus*

Incubation time (hrs)	Colony diameter (cm)	Clear zone diameter (cm)	Index of decolorization
3	1.82	1.94	1.06
6	2.22	2.69	1.20
9	4.48	5.89	1.24
12	5.45	6.96	1.26
15	5.56	7.23	1.30

The initial stage of Congo red decolorization occurs, namely the *Pleurotus ostreatus* mycelium will grow on the surface of the MSM media but has not decolorized, as the mycelium grows, the Congo red decolorization will gradually spread to the bottom of the media. The mechanism of decolorization of Congo red by *Pleurotus ostreatus* can occur enzymatically and non-enzymatically[16]. The decolorization mechanism is enzymatic, which means *Pleurotus ostreatus* produces the laccase to degrade the substrate. The laccase enzyme modifies the structure of the azo dye by removing its chromatographic structure[17]. The ability of the laccase enzyme can be observed qualitatively on the surface of solid media containing Congo red dye where the color of the media will fade as the *Pleurotus ostreatus* mycelium grows.

Non-enzymatic decolorization occurs because the cell wall of *Pleurotus ostreatus* contains a matrix consisting of organic compounds such as enzymes, proteins and polysaccharides. The cell wall will produce an adhesive that can absorb the color of the media. The *Pleurotus ostreatus* mycelium is hydrophobic and the Congo red dye is hydrophilic, so it will form hydrophilic and hydrophobic interactions that cause color absorption by the *Pleurotus ostreatus* mycelium.

Quantitative dyes decolorization: Decolorization of Congo red by *Pleurotus ostreatus* shows that increasing incubation time causes an increase in the percentage of dye degradation, which means the longer the incubation time, the less Congo red dyes concentration contained in the liquid waste as shown in Fig. 2.

Incubation time is one of the factors that can affect the process of ligninolytic enzymes produced by isolates to decolorize Congo red dyes. The length of incubation times will increase the decolorization percentage. A study that was conducted by Hashmi showed that *P. ostreatus* was able the ability to decolorize 32, 48 and 94% of the dye Synazol red from dye-contaminated industrial effluents after 10, 20 and 30 days[15]. Another finding observed the decolorization of Congo red using various fungal isolates including *Pleurotus ostreatus*. It was shown that *P. ostreatus* increased the percentage of decolorization of Congo red from around 10-20% after 1-4 incubation[18]. Recently, Jamil *et al.*[19] also studied the biodegradation of synthetic textile dyes by chitosan beads cross-linked laccase from *Pleurotus ostreatus* IBL-02 and found that chitosan cross-linked laccase from *Pleurotus ostreatus* decolorized five different synthetic textile dyes efficiently.

Pleurotus ostreatus mycelium can grow because it utilizes the nutrients and lignin contained in the medium. The optimal incubation time was obtained in 15 days, this is because, on the 15th day, *Pleurotus ostreatus* experienced a peak of nutrient absorption. The mechanism of waste

Table 2: Percentage of decolorization of Congo red by *Pleurotus ostreatus*

Incubation time (days)	Initial concentration (ppm)	Final concentration (ppm)	Decolorization (%)
0	50	50.00	0.00
3	50	49.24	1.52
6	50	46.74	6.52
9	50	34.06	31.88
12	50	32.81	34.38
15	50	22.00	56.00

treatment by fungi is carried out through a process of degradation and adsorption. The degradation process is the process of decomposing complex compounds into simple compounds by organisms, while the adsorption process is the absorption of waste by the gel in the mycelium. White rot fungi can use the dye as a carbon source, causing the concentration of the dye to decrease or run out. The mechanism of dye degradation by fungi might be enzymatically by the enzymes lignin peroxidase (Lip), manganese peroxidase (MnP) and laccase. Oyster mushrooms belong to a group of white rot fungi that produce ligninolytic and extracellular enzymes such as lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase[6].

Bio decolorization of azo dyes based on enzymes synthesized by microorganisms. Enzymes synthesized by microorganisms to reduce azo dyes can only synthesize certain dyes[5]. The effectiveness of *Pleurotus ostreatus* in degrading dyes can be determined from the calculation of the percent decolorization. In this study, it was seen that *Pleurotus ostreatus* was able to synthesize Congo red dye which was seen during an incubation period of 15 days able to reduce Congo red dye by 56%. Table 2 concluded the longer the incubation time, the more reduced the Congo red dye by *Pleurotus ostreatus*. This result was also supported by Ilyas *et al.*[13], who reported that *P. ostreatus* was able to decolorize azo dye, synazol red HF6BN by 40, 66, 82 and 96% from the medium 6, 12, 18 and 24 days, respectively. Another investigation showed that enzymatic decolorization percentages from *Pleurotus ostreatus* were up to 100 for azure B (heterocyclic dye) and indigo carmine (indigoid dye), 74.5 for malachite green (MG) (triphenylmethane dye)[20]. Meanwhile, Dewi *et al.*[21] also reported that ligninolytic immobilized from *Pleurotus ostreatus* has decolorized about 94.867% of batik wastewater within 24 hrs under a static condition.

Based on the research results, it was found that there was an effect of incubation time on the bio decolorization of azo Congo red dye by *Pleurotus ostreatus*. The decolorization time of color waste by *Pleurotus ostreatus* is an important thing that must be studied because each rot fungus has a different optimal time. The optimal time is different because the growth phase of each microorganism is different. Incubation time determines the ability of *Pleurotus ostreatus* to reduce the concentration of azo compounds contained in textile industry wastewater. The longer the incubation time, the higher the ability of *Pleurotus ostreatus* to reduce Congo red compounds.

This research was conducted through scientific research procedures, but this research has the following limitations such as (1) This research identified the ability of *Pleurotus ostreatus* F209 only on one type of azo, namely Congo red, while there are many other types of azo dyes in textile waste which can be reduced by *Pleurotus ostreatus* F209 and (2) In this study just discussed the optimal incubation of the *Pleurotus ostreatus* F209 in the bio decolorization of azo dyes (Congo red). The ability bio decolorization azo dyes by *Pleurotus ostreatus* is influenced by several factors including the pH of the media, incubation temperature and the concentration of azo dyes contained in textile wastewater. Wastewater treatment including for textiles is a significant concern nowadays because of the high level of pollution that occurs as a result of industrial developments[22]. The finding of this study will guide the practitioner in wastewater treatment to utilize another source material that is natural for reducing azo contaminant in water. Further research on real application needs to be considered in the near future.

CONCLUSION

Oyster mushrooms belong to a group of white rot fungi that produce ligninolytic and extracellular enzymes such as lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase. Qualitative testing showed that *Pleurotus ostreatus* F209 was able to reduce the concentration of azo Congo red dye during the 15 days incubation period on MSM media. The effectiveness of *Pleurotus ostreatus*, in degrading dyes can be determined from the calculation of the percent decolorization. In this study, the ability of *Pleurotus ostreatus* to synthesize Congo red dye was seen during the 15 days incubation period to reduce Congo red dye by 56%. It can be concluded that the longer the incubation time, the more reduced the Congo red dye by *Pleurotus ostreatus*.

SIGNIFICANCE STATEMENT

This research has resulted in the potential ability of oyster mushrooms (*Pleurotus ostreatus*) as a bio-decolorizing agent. The ability of oyster mushrooms to bio-decolorize is measured by the ability of oyster mushrooms to degrade colors from textile industry waste. This research will examine the optimum time for oyster mushrooms to degrade the azo Congo red color compound. The incubation time of 14 days was able to reduce the Congo red concentration by 56%.

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