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Research Article

Decolorization Study of Remazol Black B Textile Dye Using Local Fungi of *Ganoderma* sp. and Their Ligninolytic Enzymes

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Abstract

Background and Objective: Azo dyes were most commonly used for dyeing in the textile industry. Wastewater with high azo dyes concentration could potentially risk to environment because it was difficult to decompose naturally in the environment. Decolorization of remazol black B was investigated using wood decaying fungus strain of *Ganoderma* sp. and their ligninolytic enzymes. **Materials and Methods:** The fungus was collected from a coffee plantation in Bali, Indonesia. In this research, operating parameters affecting the color removal efficiency examined include pH, dye concentration and incubation time. **Results:** The results showed that the color removal of remazol black B is more effective using ligninolytic enzymes from *Ganoderma* sp. than directly use fungus strain of *Ganoderma* sp. The optimum pH condition required *Ganoderma* sp. to decolorize remazol black B was 6, whereas crude ligninolytic enzymes at pH 4 with dye concentration of 70 mg L⁻¹ within 7 days incubation time. Color removal efficiency of remazol black B using the *Ganoderma* sp. and their crude ligninolytic enzymes was 89.23 and 90.82%, respectively. **Conclusion:** Ligninolytic enzyme is playing important role in degradation of dye, in which the color removal efficiency is dependent on pH, dye concentration and incubation time. The fungus strain *Ganoderma* and their ligninolytic enzymes have a great potential to develop as alternative technology for textile wastewater treatment.

Key words: Remazol black B textile dye, ligninolytic enzyme, *Ganoderma* sp., color removal efficiency

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

INTRODUCTION

Textile wastewater is commonly characterized as high color intensity water which contain a large amount of organic and inorganic pollutants which are harmful to the environment. The total dye consumption for world textile industry is more than 10^4 t/year¹ and estimated about 10-50% of dye used in dyeing process will be loose to the environment². Discharge of this effluent directly into the water bodies without treatment cause potential serious environmental problems. The presence of dyes in the water raises aesthetic problems as well as inhibits the penetration of sunlight into the water, thus, disrupting the photosynthetic activities of water organisms. The further impact is reduced availability of oxygen in water that triggers the anaerobic activities producing unpleasant odors compounds. Approximately 60-70% of synthetic dyes produced in the world are of azo groups³. Azo dyes are more widely used in dyeing because they are easy to obtain, varied in color and cheaper than natural dyes. However, some azo dyes are mutagenic and carcinogenic and also recalcitrant by chemical or photolytic treatments. For this reason, textile wastewater must be treated before disposed into the environment⁴.

Various methods have been developed in order to serve the effective technology for the removal of textile dyes from wastewater which include chemical methods such as electrochemical oxidation, ozonation and advanced oxidation process⁵⁻⁷, physical methods, e.g. photo oxidation and activated carbon⁸⁻⁹ and biological methods use bacteria, algae, ligninolytic enzyme, fungi and bacteria¹⁰⁻¹⁴. The most chemical and physical methods which are used for textile wastewater treatments have limitations such as high operating cost and produce a large amount of sludge during these processes.

Biological method has been shown to be one of the most promising and widely used for the removal of textile wastewater. Microorganisms can use the organic and inorganic matters from wastewater as one of nutrition for their growth. Fungi especially wood decaying fungi have been reported as potential bioremoval agents for textile wastewater treatment. The ability of fungi to decolorize various kinds of dyes through their extracellular ligninolytic enzymes comprised lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase. White rot fungi such as *Pleurotus ostreatus*, *Coriolus versicolor*, *Lentinula edodes*, *Agrocybe aegerita*, *Agrocybe* sp. *Coprinus comatus*, *Gloeophyllum trabeum*, *Meripilus giganteus*, *Rigidoporus ulmarius* and *Tricholoma caligatum* have great ability to produce ligninolytic enzyme and prospective developed to reduce environmental pollution¹⁵⁻¹⁷.

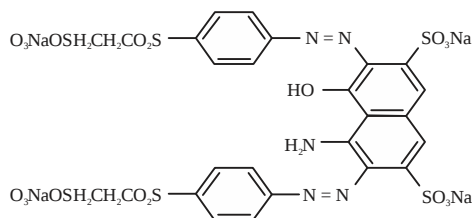


Fig. 1: Chemical structure of remazol black B

Two fermentation methods widely employed for the various enzymes production from fungi are submerged fermentations (SmF) and solid-state fermentations (SSF). In SmF technique, the fungi were cultivated in liquid nutrient broth, whereas in the SSF method, the fungi grow on a solid material in a very limited amount of free water. Solid materials which used in SSF can be divided into two groups: Inert materials are the materials only as attachment place for the microbial whereas, non-inert material as inducer to enhance ligninolytic enzymes production. Agricultural waste such as corn, stover, corn cobs, sugarcane bagasse, wheat straw, rice straw, sawdust and banana skin can be used as a solid support for the production of ligninolytic enzymes¹⁸⁻¹⁹.

The present study focused on the investigation of *Ganoderma* sp. fungus and their ligninolytic enzymes for color removal of the synthetic azo dyes, remazol black B (Fig. 1). The ligninolytic enzyme production from *Ganoderma* sp. used SSF method with sawdust as a solid support.

MATERIALS AND METHODS

All the experiments were carried out at Laboratory of Biochemistry, Chemistry Department, Universitas Pendidikan Ganesha, Singaraja, Bali, Indonesia in 2016.

Equipments and chemicals: The main equipment used in this research was UV-Vis Shimadzu type of 1700, 3H16R1 centrifuge, a Hanna Instrument pH meter was used to measure pH, remazol black B (used for degradation study) was purchased from commercial market of Bali. Potato dextrose agar (PDA) media for culturing fungi in a 1000 mL beaker consist of 20 g sucrose, 200 g potatoes and 20 g agar and chloramphenicol tablet whereas media for ligninolytic enzyme production was Czapek-Dox liquid medium with the following composition: 15 g L⁻¹ sucrose, 3.0 g L⁻¹ sodium nitrate, 0.5 g L⁻¹ potassium chloride, 0.5 g L⁻¹ magnesium sulfate, 0.01 g L⁻¹ iron (II) sulfate and 1.0 g L⁻¹ potassium dihydrogen phosphates. The fungus used for production of



Fig. 2: Macroscopic performance of *Ganoderma* sp.

ligninolytic enzyme was a *Ganoderma* sp. collected from decaying wood around of Bali. The macroscopic performance of *Ganoderma* sp. fungi is shown in Fig. 2.

Production of crude ligninolytic enzyme by *Ganoderma* sp.:

Ligninolytic enzyme from *Ganoderma* sp. fungi was produced by solid state fermentation method with sawdust as solid support. A 25.0 mL of spore suspension was inoculated into 250 mL autoclaved Erlenmeyer flask containing 200 g of sawdust. The mixture was added with Czapek-Dox medium to achieve moisture level of 80% w/v and kept at pH 5. The inoculated flask was then incubated in automatic shaking incubator at room temperature ($28 \pm 2^\circ\text{C}$), at 100 rpm for 12 days to achieve full colonization.

Qualitative analysis of crude ligninolytic enzymes: After fermentation, the crude enzyme product was taken from the substrate by shaking it in automatic shaking incubator at 250 rpm for 15 min. The extract was filtered through Whatman No.1 filter paper and culture filtrate was then centrifuged at 6000 rpm for 15 min. The supernatant was again filtered through Whatman No.1 filter paper. The crude ligninolytic enzymes were tested for lignin peroxidase and manganese peroxidase. Lignin peroxidase (LiP) enzyme was qualitatively tested by adding 0.6 mL of supernatant with 0.3 mL of 100 mM n-propanol, 0.3 mL of 250 mM tartaric acid and 0.3 mL of 10 mM H_2O_2 . The positive test for LiP enzyme was showed by formation of transparent yellowish color. Manganese peroxidase (MnP) enzyme was tested by adding 0.6 mL of supernatant with 0.3 mL of 0.1 mM MnSO_4 , 0.3 mL of 0.1 mM phenol red, 0.3 mL of phosphate buffer (pH = 5) and 0.3 mL of 50 mM H_2O_2 . Formation of brownish brown color indicates positive test for MnP enzyme.

Degradation study of remazol black B with *Ganoderma* sp. and their ligninolytic enzymes:

A volume of 46 mL of liquid Czapek-Dox medium was put into Erlenmeyer 200 mL flask and then filled with 3 mL suspense of *Ganoderma* sp. The Erlenmeyer flask was incubated for 3 days while shaken with an automatic shaker at 200 rpm. After 3 days of incubation, the Erlenmeyer flask was added with 1 mL of 2500 mg L^{-1} remazol black B. Amount of 46 mL of liquid Czapek-Dox medium, 3 mL of ligninolytic enzymes from *Ganoderma* sp. were put into other Erlenmeyer flask, then filled with 1 mL of 2500 mg L^{-1} remazol black B (total concentration of remazol black B on treatment was 50 mg L^{-1}). Both Erlenmeyer flasks incubated for 7 days while being shaken using an automatic shaker at 200 rpm. At the end of treatment, the mixture was filtered with Whatman filter paper, then its filtrate was centrifuged at 4000 rpm for 30 min using a 3H16R1 centrifuge. The absorbance of each supernatant was measured with a double beam Shimadzu-1800 UV-Visible spectrophotometer at 600 nm. The color removal efficiency was calculated with the following formula:

$$\text{Color removal (\%)} = \frac{A_0 - A_t}{A_0} \times 100$$

Where, A_0 and A_t are the values of absorbance before and after treatment, respectively. The influence of parameters on color removal of dye to be studied was dye concentration, pH and incubation time.

RESULTS AND DISCUSSION

Production of ligninolytic enzymes: Production of ligninolytic enzyme from *Ganoderma* sp. was carried out by solid state fermentation (SSF) method using Czapek-Dox medium containing 200 g sawdust. After 12 days of incubation, the fungus has produced ligninolytic enzyme indicated by the appearance of yellowish color in both media (Fig. 3).

The color of ligninolytic crude enzymes which was produced by SSF method using sawdust as a solid material appears brownish due to the effect of the solid material as shown in Fig. 3. The use of agricultural waste as solid materials in the production of ligninolytic enzymes has advantages over inert materials, since sawdust beside as a place of fungal growth also serves as an inducer to increase enzyme activity. The role of agricultural waste in SSF method was as a solid support and inducer for stimulated and accelerated the ligninolytic enzyme production. Various types of agricultural waste have been reported as a solid

substrate for ligninolytic enzymes production in SSF method, e.g. wheat straw, corn cobs, coconut husk, wheat bran and rice bran²⁰. Sukarta and Sastrawidana¹⁸ also reported that the enzyme activity of laccase, MnP and LiP produced without the addition of solid material in succession is 20.5, 25.7 and 75.4 $\mu\text{mol mL}^{-1} \text{min}^{-1}$, whereas by adding banana skins, the resulting enzyme activity increased to 139, 116 and 654 $\mu\text{mol mL}^{-1} \text{min}^{-1}$, respectively.

Qualitative analysis of crude ligninolytic enzyme:

Qualitative test was conducted for MnP using phenol

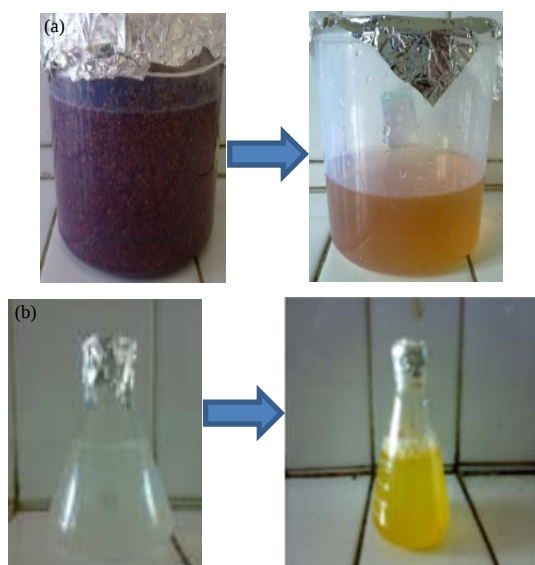


Fig. 3(a-b): Crude ligninolytic enzymes for *Ganoderma* sp. after fermented 12 days incubation on Czapek-Dox medium (a) With sawdust as solid support and (b) Without solid support

red as a substrate while, for LiP, with n-propanol as the organic substrate.

The positive test for MnP was indicated by the formation of brownish color after addition of phenol red as shown in Fig. 4. This color provides absorption at a maximum wavelength of 610 nm. Test for LiP enzyme was done by using n-propanol as the organic substrate to produce colorless compound of propionaldehyde.

Decolorization of remazol black B

Effect of pH medium: The effect of pH on decolorization of remazol black B using wood decaying fungi *Ganoderma* sp. and their ligninolytic enzymes was examined at range from 3-8 with a dye concentration of 50 mg L⁻¹ and an incubation time of 7 days. The color removal efficiencies are illustrated in Fig. 5.

The color removal capability of *Ganoderma* sp. and their ligninolytic enzymes was pH dependent as shown in Fig. 5. The color removal increased with increase in pH medium up to pH 6 for *Ganoderma* sp. whereas up to pH 4 for their ligninolytic enzymes. This result is consistent with Jayasinghe *et al.*²¹ and Singh and Srivastava²², who reported that the pH optimum for *Ganoderma* sp. growth was at

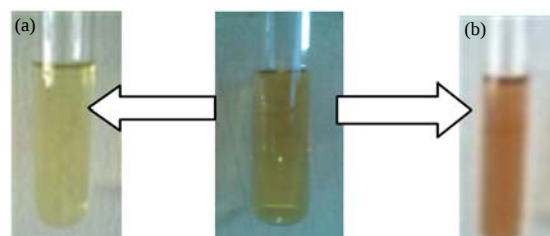


Fig. 4(a-b): Qualitative analysis of LiP and MnP enzymes, (a) Test for LiP and (b) Test for MnP

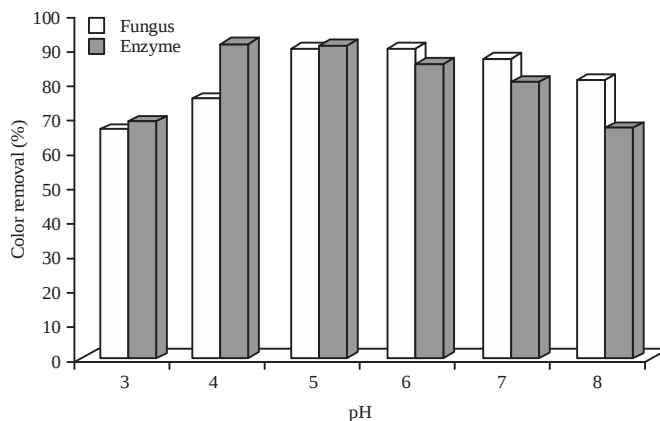


Fig. 5: Effect of pH on color removal of remazol black B

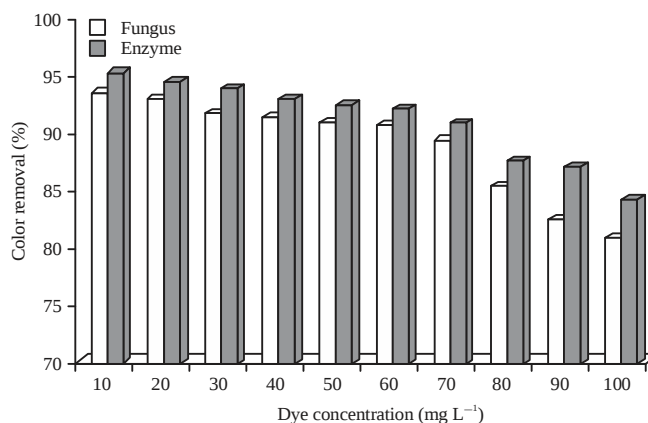


Fig. 6: Effect of dye concentration on color removal of remazol black B

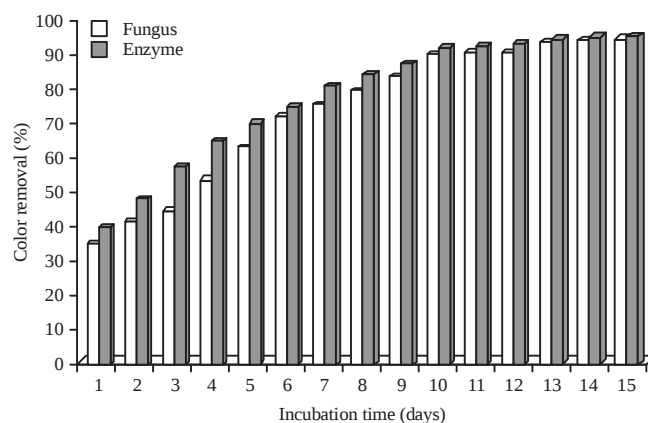


Fig. 7: Effect of incubation time on color removal of remazol black B

range 5-6. However, the optimum pH for ligninolytic enzymes activity such as manganese peroxidase (MnP) and lignin peroxidase (LiP) range obtained was around 4.2-4.5²³. Cheng *et al.*²⁴ reported that the azo-based dye was more effectively degraded by *Corioloopsis* sp. under an acidic condition. These results agreed with Hefnawy *et al.*²⁵, who reported that the optimum pH was needed by *Aspergillus flavus* and *Penicillium canescens* to decolorize direct blue dye obtained at pH 4 and 5, respectively within 7 days.

Effect of initial remazol black B concentration: The effect of remazol black B concentration on color removal was investigated by increasing the dye concentration from 10-100 mg L⁻¹. Decolorization of dye using fungus was conditioned at pH 6 whereas with crude ligninolytic enzymes was adjusted at pH 4. The profile of color removal efficiencies is presented in Fig. 6. The capability of *Ganoderma* sp. and their enzymes to decolorize remazol black B decreases gradually with an increase in dye concentration was shown in Fig. 6.

Color removal efficiency of remazol black B by the ligninolytic enzymes was obtained up to 90.82% whereas through fungus up to 89.23% with a dye concentration of 70 mg L⁻¹ within 7 days incubation time. Decolorization of dye by fungus takes place through two mechanisms: Adsorption by fungal biomass and enzymatic degradation process. The fungi growth was decreased with increase the dye concentration due to the toxic effect of the dye to the fungus. On the other hand, the active side of the enzyme becomes saturated so its activity decreased with increase the substrate concentration. This finding was in line with the results of the research conducted by Permpornsakul *et al.*²⁶ that the white rot fungus *Phanerochaete sordida* PBU 0057 completely decolorized reactive black 5 at 100 ppm within 72 h and it was decreased at the higher concentration.

Effect incubation time: Different incubation time used was range between 1-15 days. The result presented in Fig. 7

indicated that optimum color removal efficiency in 10 days incubation time was observed of 89.91 and 91.84% for *Ganoderma* sp. and their crude ligninolytic enzymes, respectively.

The optimum time required to decolorize dye significantly depend on the type of dye and fungus used. This was demonstrated by Bergsten-Torralba, *et al.*,²⁷ that incubation time required by the *Penicillium simplicissimum* INCQS 40211 to decolorize completely reactive red 198 (monoazo) and reactive blue 214 (diazo) was 7 days whereas reactive blue (phtalocyanine) within 2 days incubation time. Differences in the decolorization ability of three kinds of dyes may be depending on dye structure.

CONCLUSION

Color removal efficiency of diazo dye, remazol black B using *Ganoderma* sp. and their crude ligninolytic enzymes was 89.23 and 90.82%, respectively in liquid medium with dye concentration of 70 mg L⁻¹ within 7 days of incubation time at pH 6 for *Ganoderma* sp. whereas at pH 4 for crude ligninolytic enzymes. The fungus of *Ganoderma* sp. or their enzyme is potentially used for textile wastewater treatment.

SIGNIFICANCE STATEMENTS

This study discovers the ability of *Ganoderma* sp. and their ligninolytic enzymes to degrade remazol black B textile dyes at variation environmental conditions (pH, incubation time and dyes concentration). The study will help the researchers to uncover the critical areas of biotechnology development for textile wastewater treatment using the wood decaying fungi.

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