International Journal of Innovative Technology and Interdisciplinary Sciences
www.IJITIS.org
ISSN: 2613-7305
Volume 4, Issue 3, pp. 716-732, 2021
DOI: https://doi.org/10.15157/IJITIS.2021.4.3.716-732
Received April 24, 2021; Accepted June 30, 2021

Valorization of Waste Textile Dyeing by Immobilized Fungus Daedalopsis Eff. Confragosa in Sawdust

Ngurah Mahendra Dinatha*[a], James Sibarani[b] , I Gede Mahardika[c]
[a]Department of Natural Science Education, STKIP Citra Bakti, Jl. Trans Flores, Malanuza, Kec. Golewa, Ngada Flores NTT 86461, Indonesia.
[b]Faculty of Math and Science, Udayana University, Jl. Raya Kampus Unud No.9, Jimbaran, South Kuta District, Badung Regency, Bali 80361, Indonesia.
[c]Faculty of Animal Husbandry, Udayana University, Bukit Jimbaran Campus, Jimbaran, South Kuta District, Badung Regency, Bali 80361, Indonesia
*[a]ngurahm87@gmail.com; [b]james_sibarani@unud.ac.id; [c]mahardikagede@unud.ac.id

ABSTRACT

This study aims to determine optimal valorisation of textile dyeing by immobilized fungus Daedalopsis Eff. Confragosa in sawdust as well as the quality of the valorisation, which covers Chemical Oxygen Demand (COD), Biological Oxygen Demand (BOD), Total Suspended Solids (TSS), pH, colour and level of toxicity. This fungus was obtained from plantation areas in Negara, Bali. Textile dyeing wastewater was taken from the Mama & Leon textile dyeing industry, Tabanan Bali. The optimum condition needed to process the dyeing waste was at pH 4 with eight days of incubation. Field-scale textile waste treatment in a reactor containing the immobilized fungus Daedaleopsis eff. confragosa in sawdust for eight days can reduce colour, TSS, COD, and BOD by 79.78%, 779.11%, 88.307%, and 82.932%. Furthermore, textile waste processing results in a lower level of toxicity compared to the one before processing. The level of toxicity of wastewater before it was treated was mildly toxic. Meanwhile, the level of toxicity after treatment was non-toxic. In this paper we have tested the results on a large scale to ensure that this method was applicable for a practical purpose.

Keywords: Immobilized fungus, textile waste, wastewater toxicity, total suspended solids.

1. INTRODUCTION

The textile waste produced by dyeing industries has the potential to pollute the environment. This was because the textile wastewater contains very complex pollutants and has high colour intensity [1]. The value of biological oxygen demand (BOD) and chemical oxygen demand (COD) for textile waste ranges from 80-6,000 mg/L and 150-12,000mg/L [2]. This value exceeds the quality standard threshold of textile industry wastewater when viewed from the Minister of Environment Decree No. 51/MENLH/10/1995. The existence of textile waste in waters can interfere with the penetration of sunlight. This leads to the life of organisms in the waters will be disturbed and, at the same time, threatening the sustainability of the aquatic ecosystem. Thus, textile waste disposal to the environment must be preceded by certain processing actions [3].

Several methods have been developed to reduce textile waste pollution. Those methods include chemical methods such as electrochemical oxidation, ozonisation, and the oxidation process [4]–[6], physical methods, namely photo-oxidation and activated carbon [7], [8], and biological methods by using algae, ligninolytic enzymes, fungi and bacteria [9]–[16]. Chemical and physical textile waste processing was considerably effective in removing color, but they were not efficient in cost. Moreover, the use of chemicals causes a lot of sludge. Accordingly, utilizing microorganisms was a rapidly growing method to solve textile waste processing problems. Besides, the advantages of using microorganisms were low cost, environmentally friendly, and can be used repeatedly if it was utilizing immobilized microbes [17].

Wood-degrading fungi can degrade wood components, particularly lignin and cellulose. The group of wood-degrading fungi that were reported to be able to degrade lignin was white-rot fungi. Apart from being useful for degrading lignin compounds, white-rot fungi were also useful for degrading textile dyes [18]. 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 were* reported to have great ability break down textile dyes [19]–[21]. Besides, *Daedaleopsis eff. confragosa* was a type of white-rot fungus that was reported to effectively degrade textile waste [22].

The ability of wood-degrading fungi to remodel textile waste was closely related to the extracellular lignolytic enzymes produced by these fungi, namely lignin peroxidase (LiP), manganese peroxidase (MnP), and lacase [18]. Lignolytic enzymes can tear down aromatic compounds, synthetic polymers, and dyes through redox reactions, where the enzyme will completely oxidize carbon compounds into CO_2 and H_2O [13].

The valorisation of textile waste using wood degrading fungi was influenced by environmental factors such as pH, concentration, and unsuitable waste disposal period. The biological process of tearing down azo dyes was strongly influenced by pH and incubation period [23], [24]. The valorisation of the Reactive Blue dye with a 100 mg / L concentration by the fungus *Polyporus rubidus* causes a decrease in colour by 37%, 54%, and 61% respectively during 2, 3, and 5 days of incubation period [24]. Accordingly, it was necessary to determine the valorisation's optimum condition to obtain an efficient textile waste degradation by wood degrading fungi.

The valorisation of textile waste by wood degrading fungi can be done in two ways, either using free (suspended) or attached (immobilized) fungi. It shows that using microorganisms with an embedded growth process provides higher efficiency of transformation compared to using microorganisms with a suspended growth process [23].

Agricultural wastes such as corn, stover, corn cobs, bagasse, wheat straw, rice straw, sawdust, and banana peels can be used as an attached growth medium for white-rot fungus to produce lignolytic enzymes [25]. Sawdust was a potential material used as a supporting solid for immobilizing fungi. This was feasible since sawdust was easy to find and cheap. Another advantage of using wood sawdust as a supporting solid was the content of lignin, cellulose, and hemicellulose in sawdust which function as an inducer in degrading dyes by lignolytic enzymes.

In the context of controlling environmental pollution by industrial waste, the Republic of Indonesia's Government has issued the Minister of Environment Decree No. 51/MENLH/10/1995 regarding quality standards for industrial waste. This law requires every business or activity to carry out waste treatment until it meets the standard requirements for the quality of wastewater before being discharged into the environment. The quality standard parameters of wastewater for the textile industry include BOD5, COD, TSS, total phenol, total chromium (Cr), oils and fats, and pH. To determine whether the results of textile waste valorization by the immobilized fungus *Daedaleopsis eff. confragosa* in sawdust has met the quality standard requirements, tests were carried out which included BOD_5, COD, TSS, pH, and color tests.

The toxicity of the dye according to EU criteria for hazardous substances was classified as low. Van der Zee (2002) reported that only 2% of the 300 dyes tested had Lethal Concentration (LC_{50}) values for fish lower than 1.0 mg/L. Although the dye's toxicity level was low, there was a possibility that the valorization process was likely to produce a more toxic product than before. Therefore, toxicity testing was important to see how big the waste's toxic effect was on the affected organism.

Based on the problems mentioned above, this research will study the optimum conditions for textile waste valorisation by the immobilized fungus *Daedaleopsis eff. confragosa* in sawdust and the quality of its valorization results, including COD, BOD, TSS, pH, color, and level of toxicity.

2. METHOD AND MATERIALS

This research was an experimental study that aims to determine the optimum conditions and quality of textile waste after valorization using immobilized *Daedaleopsis eff. Confragosa* in sawdust.

2.1 Fungus *Daedaleopsis eff. confragosa*

The fungi used in this study were wood degrading fungi taken from plantation areas in Negara, Jembrana District, Jembrana Regency, where this fungus grows on dead tree trunks. Based on the identification results at the Plant Taxonomy Laboratory, Department of Biology, Faculty of Mathematics and Science, Udayana University, the fungus used was *Daedaleopsis eff. confragosa*. This mushroom has the characteristics of a fruiting body in the form of a fan and was rather hard, as shown in Figure 1.

The fungi were then reproduced on Potato Dextrose Agar (PDA) media by following the method used by Ali and Muhammad [26]. *Daedaleopsis eff. confragosa* was crushed and put into a test tube filled with sterile water while shaking it. Next, 1 mL of liquid containing spores was put into a petri dish containing PDA media and incubated for seven days until white threads grow on the surface of the PDA. 1 liter of PDA media consists of 200 grams of potatoes, 20 grams of dextrose, and 20 grams of agar and 1 tablet of chloramphenicol to prevent bacterial growth. The mushroom mycelium was then transferred to modified Czapek liquid media. The mycelium of fungus *Daedaleopsis eff. confragosa* was put into a 500 mL Erlenmeyer flask containing 250 mL of liquid of Czaspek media. The mixture was incubated for seven days while being shaken using a shaker. Further, 1 liter of liquid Czaspek medium contains 15 g of sucrose, 3 g $NaNO_3$, 0,5 g KCl, 0,5 g $MgSO_4$ $7H_2O$, 0,01 g $FeSO_4$ 7 H2O dan 1 g KH_2PO_4.

Scientific Classification
Divisio: Basidiumycota
Class: Basidiomycetes
Order: Polyparales
Family: Polyporaceae
Genus: *Daedaleopsis*
Species: *Daedaleopsis eff. confragosa*

Figure 1. *Daedaleopsis eff. Confragosa*

2.2 Determining the Optimum Growth Period for *Daedaleopsis eff. Confragosa*

A total of 10 mL of liquid media was inserted into the test tube, and 1 mL of the mushroom suspension was added to the media. After that, the test tube was closed and incubated for

1 day while shaking it using a shaker. The absorbance of the mixture was measured using a 20^+ spectronics at a wavelength of 360 nm. Similarly, the fungus *Daedaleopsis eff. Confragosa* was grown at 2, 3, 4 to 10 days of incubation. The growth curve was made by plotting the absorbance data against the growth time.

2.3 The Immobilization of Fungus *Daedaleopsis eff. confragosa* on Wood Sawdust

The method used to immobilize the fungus *Daedaleopsis eff. confragosa* on wood sawdust follows the method used by Srikanlayanukul, Khanongnuch, & Lumyong (2006). For a 100 mL Erlenmeyer flask, the researcher adds 3 grams of sterile sawdust, 5 mL of mushroom suspension, and 5 mL of Czapek liquid media. The mixture sits for four days while shaking it using a shaker. After four days, the liquid in the Erlenmeyer flask was poured, and water was added again to remove the sawdust's mobilized fungus.

2.4 Determining the Optimal Conditions for the Valorization of Synthetic Textile Waste

The textile waste samples used were made by mixing Remazol Red, Remazol Blue, Remazol Golden Yellow, and Remazol Black B dyes with the same mass ratio to obtain a total concentration of 5000 mg / L. Synthetic waste was prepared by considering the concentrations at 25, 50, 75 and 100 mg / L.

2.5 The Efficiency of Synthetic Waste Reshuffle at Variations in pH

A total of 50 mL of synthetic waste with a 50 mg / L concentration was put into a 100 mL Erlenmeyer containing the immobilized fungus Daedaleopsis eff. confragosa. The synthetic waste was conditioned at pH 3 by adding a solution of HCl. The Erlenmeyer flask was closed and incubated for one week while shaking it using a shaker. After one week, the liquid was centrifuged at 4000 rpm for 30 minutes and filtered using filter paper. The absorbance of the filtrate was measured using spectronics 20^+ at a wavelength of 595 nm. Similarly, the treatment of pH 4, 5, 6, 7, and 8 was carried out. Each treatment was repeated three times. A negative control was made in the same way using 3 grams of sawdust without any mold.

2.6 Valorisation Efficiency at Variety of Dyestuff Concentrations

A total of 50 mL of synthetic waste with a 25 mg / L concentration was put into a 100 mL Erlenmeyer containing the immobilized fungus *Daedaleopsis eff. confragosa*. Synthetic waste was conditioned at an optimum pH obtained from pH treatment. Erlenmeyer flask was tightly closed and incubated for one week while shaking it using a shaker. After one week, the liquid was centrifuged at 4000 rpm for 30 minutes and filtered, and then the absorbance was measured using a 20+ spectronics at a wavelength of 595 nm.

Similarly, synthetic waste valorisation was carried out with concentrations of 50, 75, and 100mg/L. Each treatment was repeated three times. A negative control was made in the same way by using 3 grams of sawdust without mold.

2.7 Valorization Efficiency at Variation in Length of Contact

A total of 50 mL of synthetic waste with the optimum concentration was obtained from treating the concentration into 100 mL Erlenmeyer containing the immobilized fungus *Daedaleopsis eff. confragosa*. The synthetic waste was conditioned to an optimum pH obtained from pH treatment and then closed and incubated for one day while shaking it using a shaker. After one day, the liquid was centrifuged at 4000 rpm for 30 minutes and filtered, and then the absorbance was measured using 20+ spectronics at a wavelength of 595 nm. Similarly, synthetic waste's valorisation was carried out during some period starting from 2, 3, 4, 5, 6, 7, 8, 9, and 10 days. Each treatment was repeated three times.

A negative control was made in the same way but using 3 grams of sawdust without the fungus.

2.8 Immobilization of the fungus Daedaleopsis eff. confragosa. on wood sawdust

The reactor was made of glass, with dimensions of length, width, height of 10 cm, 10 cm, and 30 cm. After adding 200 grams of sawdust, the effective volume of the reactor for waste was 2500 mL. The reactor design was presented in Figure 2.

A total of 200 grams of sawdust was added to the reactor, then it was added more with 250 mL of fungus *Daedaleopsis eff. confragosa* as well as 250 mL of growth medium. The mixture was allowed to set for four days while being aerated. After four days, the liquid in the reactor was flowed through the tap and was added with more water to remove the fungus that was not attached to the sawdust. The immobilized fungus was then used to valorise textile waste.

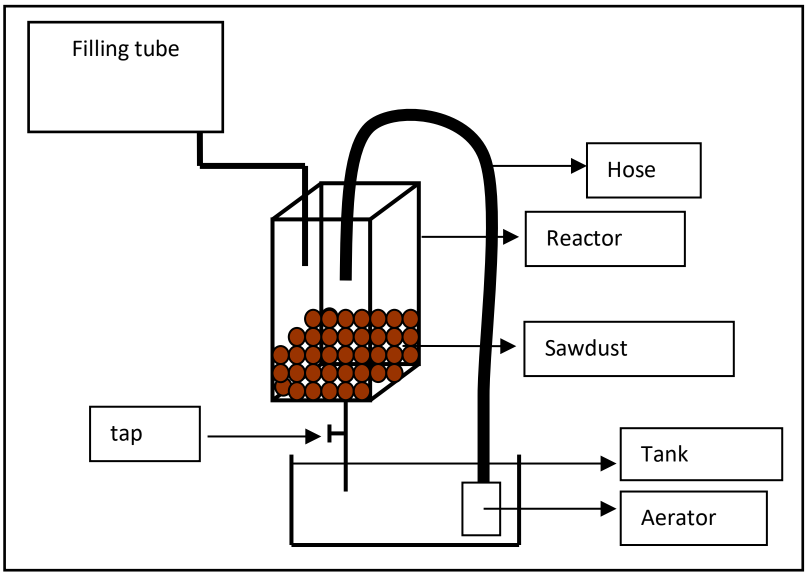

Figure 2. Reactor Design for Textile Industrial Wastewater Processing Using Immobilized Fungus *Daedaleopsis eff. Confragosa* on Sawdust

2.9 Waste Treatment Process in Reactors

Textile dyeing wastewater was taken from the Mama & Leon textile dyeing industry, Tabanan Regency, Bali. The waste must not be disposed of in the processing tank to be used in this study. A total of 2500 mL of wastewater was put into a filling tub, and 7.5 grams of sucrose was added, and the pH conditions were adjusted to the optimum pH. Further, the equalized wastewater was flowed into the reactor basin. Wastewater was treated in the reactor during the optimum contact time obtained from synthetic textile waste treatment. During its process, wastewater was recirculated repeatedly using an aerator to let the valorisation process takes place aerobically. After this process, the liquid was centrifuged at 4000 rpm for 30 minutes. Its quality was tested by measuring several parameters of waste quality, including BOD, COD, TSS, pH, and colour, as well as the level of toxicity. The wastewater treatment was repeated three times. A negative control was carried out by the same procedure but using sawdust without adding the fungus.

2.10 Waste Quality Test Before and After Valorisation

The waste quality test before and after valorisation aims to assess the efficiency of textile dyeing waste treatment using immobilized *Daedaleopsis eff. confragosa* and the feasibility of treated wastewater to be disposed into the environment. The waste quality parameters tested and their measuring methods were presented in Table 1.

Table 1. Wastewater Quality Parameters and Measurement Methods

No	Parameters	Unit	Measurement Methods
1	pH	-	Electrochemistry
2	Color	CU	Spectrophotometry
3	TSS	mg/L	Gravimetry
4	BOD	mg/L	Titration
5	COD	mg/L	Titration

2.11 Wastewater Acute Toxicity Test Before and After Valorisation

The acute toxicity test for wastewater before and after valorisation was carried out using Tilapia[28]. The tilapia fish were previously acclimatized for seven days and fed once a day. The toxicity test was carried out by setting the concentration of liquid waste to 100%, 50%; 25%; 12.5% , and 6.25% as much as 2000 mL. The pH of the water was set to pH 7 and aerated for 30 minutes. A total of 10 Tilapia fish with a length of ± 2.00 cm were added to each concentration. The observations of dead tilapia were conducted for the next 96 hours of exposure. The immobilization of Tilapia (%) was compared to the immobility of controls with the Abbot formula:

$$P = \frac{P_0 - P_c}{1 - P_c} \quad (1)$$

P was the percentage of immobilized Tilapia Nilotica after correction, P_0 was the percentage of immobilized Tilapia nilotica because of treatment, and Pc was the percentage of immobilized Tilapia nilotica in control. On the other hand, the Pc value cannot be more than 10%. The calculation of the value of LC_{50} at 96-hour observation for the waste samples before and after treatment was determined by the linear regression method.

2.12 Data Analysis

The data obtained in this study were quantitative in the form of the efficiency of synthetic waste valorisation in various environmental conditions and several parameters of the quality of wastewater before and after processing. A formula determines the efficiency of valorisation of the synthetic waste in each process:

$$efficiency = \frac{(A - B)}{A} \times 100\% \quad (2)$$

where A was the value before processing while B was the value after processing.

Data on the efficiency of the valorisation of synthetic waste in various environmental conditions (pH, dye concentration, and incubation time) were presented in a curve form which was then analysed descriptively. An analysis of the quality of processing results was carried out by comparing all parameters of the quality of the waste as measured by the quality standards of textile industrial wastewater from the Minister of Environment Decree No. 51 / MENLH / 10/1995. If the waste quality parameters were below the quality standard, this means valorizing the textile waste using immobilized *Daedaleopsis eff. confragosa* was effective and efficient.

The acute toxicity measurement for waste was based on the classification of LC_{50} value for textile waste according to Coleman and Qureshi (1985). The value of LC_{50} with a scale of LC_{50}> 100% = non-toxic, LC_{50}> 75% -100% = slightly toxic, LC_{50}> 50% -75% = toxic and LC_{50}> 25% = very toxic.

.

3. FINDING AND DISCUSSIONS

The fungus *Daedaleopsis eff. confragosa* was grown in Potato Dextrose Agar (PDA) media using a petri dish and being incubated for seven days. Its mycelium that grew on PDA media was transferred aseptically to liquid Czaspek medium. Fungus *Daedaleopsis eff. confragosa* on PDA and Liquid Czaspek was presented in Figure 3.

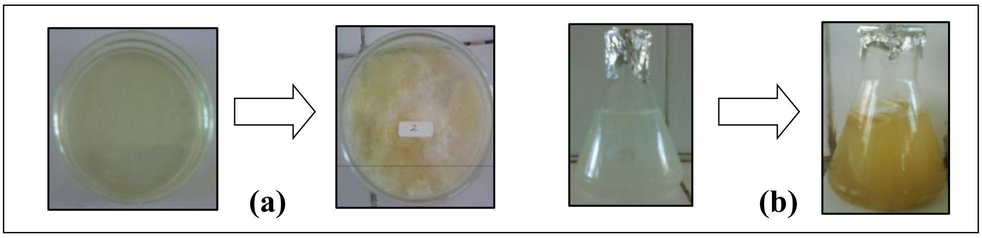

Figure 3. Cultivation of Daedaleopsis eff. confragosa, (a) on PDA Media, (b) on Czaspek Liquid Medium

Figure 3 shows the cultivation of the fungus Daedaleopsis eff. confragosa on PDA and Czapek liquid medium. Mycelium was formed in the form of white threads on PDA and Czapek liquid medium. The colour of PDA and Czapek liquid also turned into a brownish yellow color. The brownish-yellow color in the growth of wood-degrading fungi in PDA and Czapek liquid medium was due to the excretion of extracellular lignolytic enzymes by these fungi.

Determining the growth of the fungus *Daedaleopsis eff. confragosa* was intended to know the time required for the fungus to grow optimally. The growth curve was created by plotting the incubation time (days) with the absorbance. The growth curve of fungus *Daedaleopsis eff. confragosa was* presented in Figure 4.

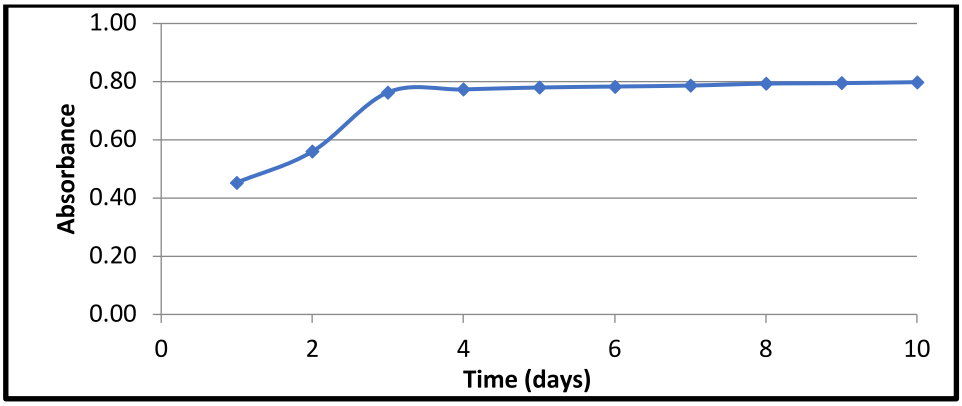

Figure 4. The Curve of Fungus *Daedaleopsis eff. Confragosa* Growth

Figure 4 shows that the absorbance of the fungal suspension increased from day 1 to day 3, and it tends to be stable in terms of the growth from 4 to 10 days. The optimum growth time for the fungus *Daedaleopsis eff. Confragosa* was on the 3rd day with an absorbance of 0.76 at a wavelength of 360 nm.

Measuring the growth time of the fungus *Daedaleopsis eff. confragosa* was used to determine the time required for the fungus to reach its optimum growth. Fungal growth was directly proportional to the fungal suspension's absorbance—the greater the absorbance of the fungal suspension, the greater the fungal growth. In the early stages, the fungus undergoes an adaptation phase, where the fungus adapts to its environment and makes its growth was relatively small. This can be seen from the low absorbance value on day 1, which was 0.45. After adapting to the environment, the fungus begins to grow until it reaches its optimum growth[29]. The optimum growth of fungi occurs on the 3rd day with an absorbance of 0.76. During days 4 to 10, fungal growth was relatively constant in which the number of dead and living fungi was equal. This was due to reduced nutrients and the build-up of metabolic waste. This phase was called the stationary phase, with a relatively constant absorbance value ranging from 0.77 to 0.80.

3.1 Determining the Optimal Conditions for Valorization of Synthetic Waste by Immobilized Fungus *Daedaleopsis eff. Confragosa* in Wood Sawdust

3.1.1 Immobilization of Daedaleopsis eff. Confragosa on Sawdust

The immobilized fungus *Daedaleopsis eff. confragosa* on sawdust after four days of incubation was presented in Figure 5. The mycelium of the fungus *Daedaleopsis eff. confragosa* appears to cover the surface of wood sawdust. This indicates that a fungal biofilm has formed on it. The immobilized fungus was then used to valorise synthetic waste in various environmental conditions.

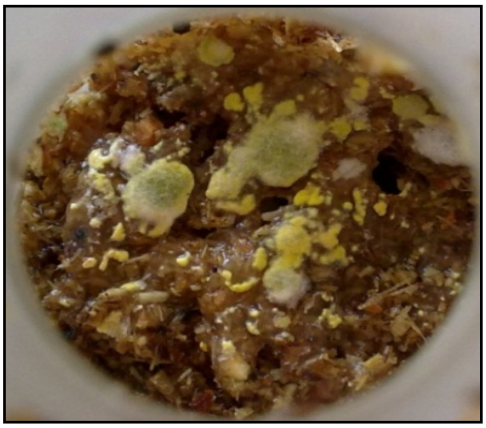

Figure 5. Immobilized Fungus *Daedaleopsis eff. Confragosa* on Wood Sawdust

The process of forming a fungal biofilm on the sawdust surface was through adsorption. The fungus initially approaches the surface of the sawdust, and then the cell adsorption process occurs. Fungi can excrete the Extracellular Polymer Substance (EPS). The presence of EPS strengthens the attachment of the fungus to the sawdust as well as maintaining the stability of the fungus population. The use of sawdust as solid support has several advantages, namely more easily to find and cheap. For those reasons, some studies have been conducted to design a low-cost wastewater treatment using sawdust [27]–[32]. Another advantage was lignin, cellulose, and hemicellulose content in sawdust function as an inducer in valorising the dyes by lignolytic enzymes.

3.1.2 Valorization Efficiency at Variations of pH

The valorization of synthetic waste at various pH conditions aims to determine the optimum pH of synthetic waste valorization using immobilized *Daedaleopsis eff. confragosa*. The data of 50 mg / L of synthetic waste for seven days of incubation at various pH conditions by the immobilized fungus *Daedaleopsis eff. confragosa* was presented in Figure 6. The optimum valorization occurs at pH 4 with an efficiency of 71.574%.

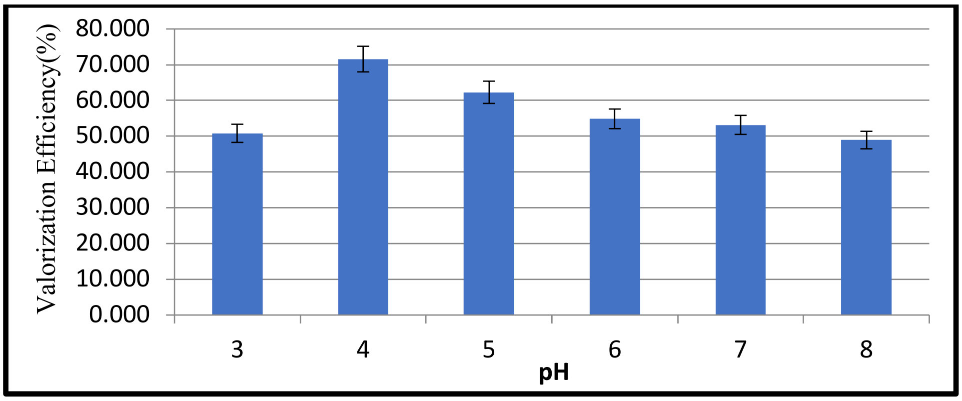

Figure 6. Valorization Efficiency of 50mg/L of Synthetic Waste during 7 Days of Incubation in Various pH Conditions

Biological changes in dye were strongly influenced by the pH of the environment [23]. The conversion efficiency of 50 mg / L of synthetic waste by the immobilized fungus *Daedaleopsis eff. confragosa* in sawdust for seven days of incubation increased when the pH conditions vary from pH 3 to 4, then decreased at pH 5 to 8. The valorization of 50 mg / L synthetic waste occurs optimally at pH 4 with an efficiency of 71.574%. The results of this study support Darayam and Dasgupta conclusions about textile waste remodeling using the fungus Polyporus rubidus [24]. It shows that the optimum pH for the valorisation was between pH 4-7.

The difference in dyeing's valorisation efficiency over a variety of pH was caused by differences in fungal growth and enzyme activity. Generally, molds thrive at an acidic pH. The range of pH for moulds to grow was between pH 1-9, and the optimum pH was between 4-6 [33]. In unfavourable pH conditions, the growth of the fungus was disturbed. The disruption of fungal growth causes the produced enzyme to be less optimal and low efficiency of valorisation. Apart from fungal growth, environmental pH conditions also affect the effectiveness of the enzymes involved in the degradation. Enzymes were proteins that catalyse reactions in biological systems. The enzyme effectiveness will decrease if the pH condition was inappropriate. This was due to a change in ionization of the groups on the active side. At the optimum pH, the enzyme activeness will be maximized to provide a large valorisation efficiency. This study finds out that the optimum change occurs at pH 4. This indicates that the lignolytic enzymes produced by the fungus *Daedaleopsis eff. confragosa* works optimally at pH 4.

3.1.3 Efficiency of the Valorization in Various Synthetic Waste Concentrations

The data on synthetic waste valorization efficiency at various concentrations (25-100mg/L) was presented in Figure 7. It shows that the efficiency of synthetic waste valorization increases with the rising concentration of synthetic waste from 25mg/L to 75mg/L, then decreases at a concentration of 100mg/L. The optimum concentration obtained was 75mg/L with an efficiency of 84.29% at pH 4 for seven days of incubation.

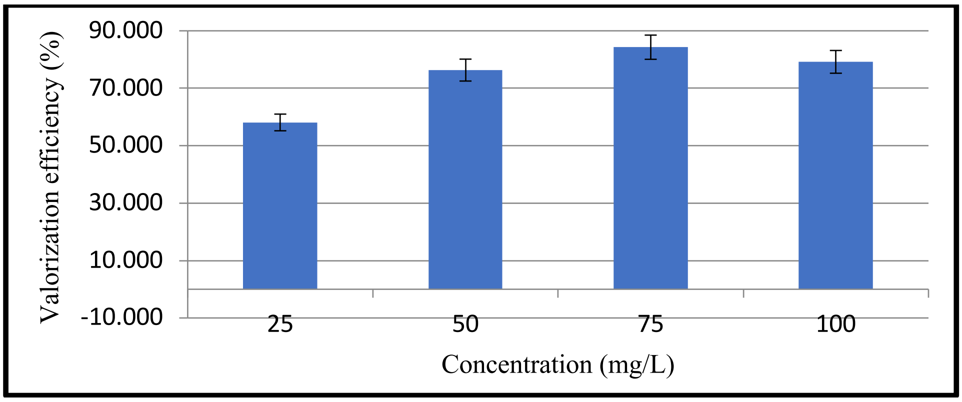

Figure 7. The Efficiency of Dyestuff Change at pH 4 for seven days of Incubation at Various Concentrations of Synthetic Waste

The change of dyes by fungi was influenced by the large amount of dye concentration valorised [34]. The valorisation efficiency increases with the rising concentration of synthetic waste from 25mg/L to 75mg/L, then decreasing at a concentration of 100mg/L. The optimum concentration for the valorisation of synthetic waste at pH 4 for seven days of incubation was 75mg/L with an efficiency of 84.29%. The difference in the efficiency of changeover at various concentrations was related to the kinetic reactions of azo dyes and the toxicity[2].

The valorisation of synthetic waste by the immobilized fungus *Daedaleopsis eff. confragosa* was an enzymatic reaction. Essentially, enzymatic reactions for substrates in a low concentration range will lead to rapid degradation along with the increasing substrate concentration. The efficiency of the dye's degradation tends to increase to a certain concentration level and then decrease along with the saturation of the active groups of the enzyme. This finding was in line with the study by Demir et al., who reported that Remazol Yellow RR Gran dye's degradation efficiency by Phanerochaete chrysosporium fungi at concentrations of 10mg/L and 25mg/L was 64% and 74%, respectively. In contrast, at concentrations 50 mg/L, the efficiency drops to 67% [35].

3.1.4 Efficiency of Valorisation at Various Incubation Period

Valorisation at different incubation periods was conducted to determine the optimal amount of time needed by immobilized fungus *Daedaleopsis eff. confragosa* in degrading synthetic waste. It was found out that the optimum period for degrading synthetic waste was eight days, with efficiency at 87.033%. The data of the valorisation was presented in Figure 8.

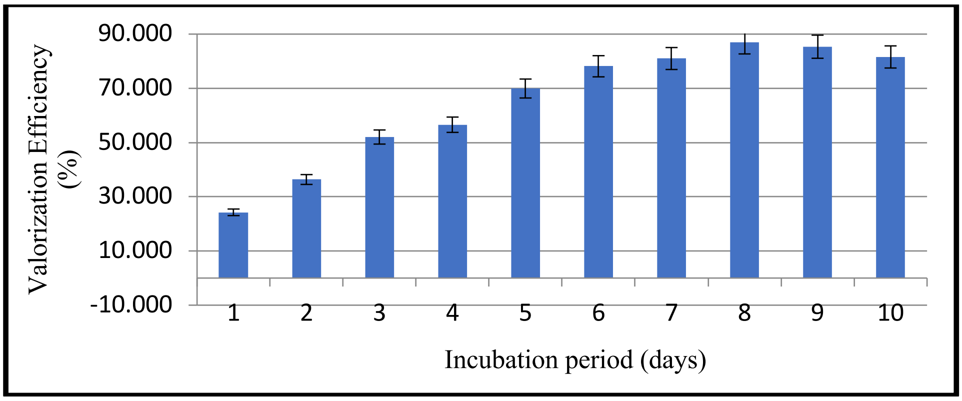

Figure 8. Valorisation Efficiency of 75mg/L Dyes with 1-10 Days of Incubation Period

The fungus's activeness in degrading dyes was also influenced by the contact duration between the fungus and dyes [36]. According to Wiloso [37], in his study that focuses on degrading reactive red 3 dyes by utilizing fungus *Penicillium* sp shows that contact duration between the fungus dyes has a significant effect on enhancing the efficiency of the valorisation. The efficiency of waste degradation tends to improve during the 1-8 days of the incubation period. The efficiency on day 1 of incubation was at 24.25%, day 2 at 36.35%, and after eight days of incubation, it improves to 87.03%. On the other hand, during the 9-10 days of incubation, the efficiency tends to lower 85.35% and 81.57%, respectively. The fungal growth causes the difference in valorisation efficiency with different incubation periods. In the early stages, the fungus was in the adaptation phase. This phase enables the fungi to adapt to the environmental conditions and causes non-optimal fungal growth.

This was indicated by the low efficiency of degradation on day 1, which was at 25,309%. Furthermore, the fungus undergoes an exponential growth phase, which causes it to grow more rapidly until it reaches its optimum growth. The optimum exponential growth occurs on day 8, then on day 10, the fungus undergoes a death phase. Apart from the death phase, the decrease in the valorisation efficiency in various incubation periods can also be caused by the fact that the fungal mycelium was already in a saturated state for a long contact time. This state causes fungal activeness to weaken because toxins have begun to form.

3.2 Textile Wastewater Treatment Using Immobilized Daedaleopsis Mushroom eff. Confragosa in Sawdust

Textile dyeing wastewater was drawn from the textile industry named CV. Mama & Leon in Tabanan, Bali. The characteristics of the textile waste before and after treatment were presented in Table 2.

Table 2. Textile Wastewater Characteristics before and after Treated by Using Immobilized *Daedaleopsis eff. Confragosa* in **Sawdust**

Parameter	Units	Initial Waste Characteristics	Negative control	Waste Characteristics after Treatment			
				Cycle			Average
				1	2	3	
Color	TCU	1140	1050	206	213	218	212,33±6,03
pH	-	10,60	4,5	6,70	6,89	6,50	6,68±0,19
COD	mg/L	1840	1430	167,6	165	169	167,20±2,03
BOD	mg/L	461	415	67,50	70	75	70,83±3,81
TSS	mg/L	445	300	63,00	61	64	62,67±1,53

Table 2 shows that the initial conditions of textile dyeing waste have the characteristics of color, pH, COD, BOD, and TSS as much as 1140 CU, 10.6, 1840 mg / L, 461 mg / L, and 445 mg / L, respectively, while after eight days of processing it decreased to 212.33 CU, 6.68, 167.2 mg / L, 70.83mg / L, and 62.67 mg / L. After comparing the waste quality parameters to the Ministry of Environment and Forestry Decree No. 51 / MENLH / 10/1995, the pH, COD, and BOD values are below the quality standard, while the TSS value was still above the quality standard. Data on the spectrum analysis of textile dyeing waste before and after treatment using UV-Vis were presented in Figure 9.

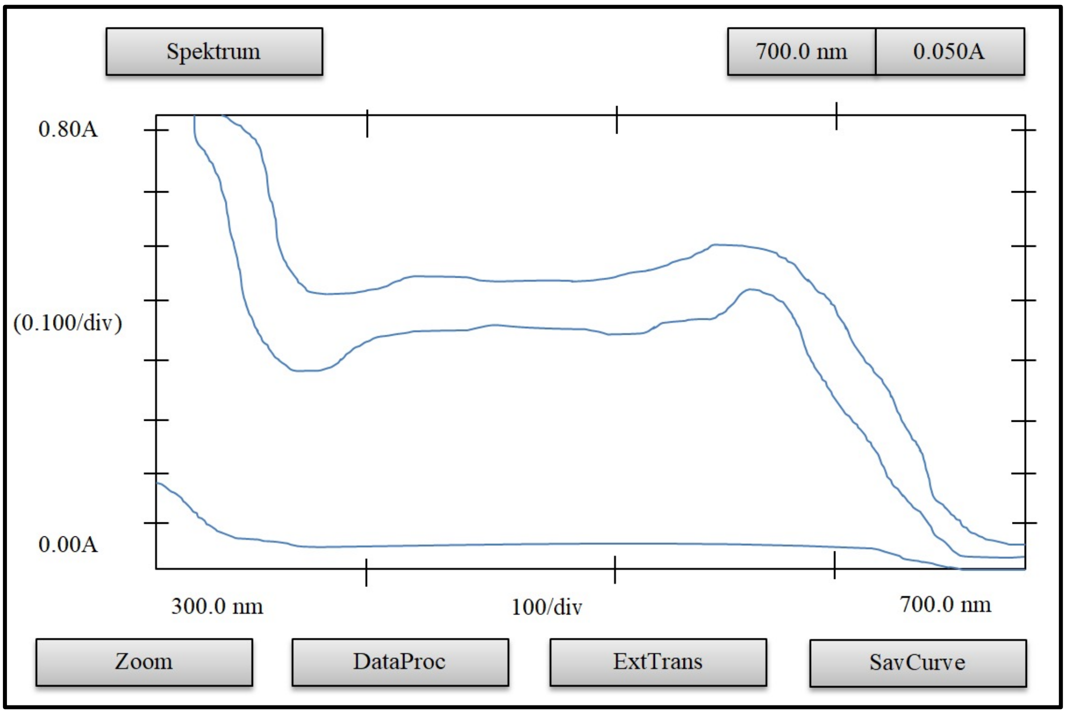

Figure 9. Textile Dyeing Waste Spectrum, (a) Initial Waste, (b) Negative Control, (c) Valorization Results

The textile dyeing wastewater used has a colour concentration of 1140 CU. After being processed using the immobilized fungus *Daedaleopsis eff. confragosa* for eight days, the colour concentration decreases to 212.33 CU. In other words, there was a valorisation efficiency of 79.778%. Color was not listed as one of the parameters of quality standard requirements in the Minister of Environment Decree No.51 / MENLH / 10/1995. Colour was not harmful to health directly, yet it harms aquatic ecosystems.

Coloured water inhibits the penetration of sunlight into the water so that it can interfere the photosynthetic activity. Lack of oxygen in the water can also trigger anoxic-anaerobic microorganisms to be active and produce odour's.

Textile wastewater was generated from the textile dyeing process at CV. Mama & Leon Tabanan Bali has a high acidity (pH) of 10.60. NaOH, Na2CO3, or detergent cause the high pH of the waste in the textile dyeing process. Before being treated, the wastewater was conditioned at pH 4 to optimize fungi's activeness in valorizing the waste. After the process of using the immobilized fungus *Daedaleopsis eff. confragosa* for eight days of incubation, the pH of the wastewater was 6.69. This pH condition, if reviewed based on the Minister of Environment Decree No. 51/MENLH/10/1995 has met the quality standard requirements for textile industry waste to be disposed into the environment.

The Total Suspended Solid (TSS) from textile dyeing wastewater was 445mg/L. High TSS was bad for the environment because it can block sunlight from penetrating water. High water turbidity can also interfere with the growth of aquatic organisms. After eight days of processing, the TSS value fell to 62.67 mg/L or an efficiency of 79.11%. When viewed from the Minister of Environment Decree no.51/MENLH/10/1995, TSS value does not meet the requirements because the required TSS threshold was 60 mg/L.

The textile dyeing wastewater used has BOD_5 and COD values of 461 mg/L and 1840 mg/L, respectively. The values of BOD_5 and COD indicate the organic matter content in the wastewater. The main constituents of organic matter were usually polysaccharides (carbohydrates), polypeptides, and fats. The high level of organic matter in textile wastewater was caused by detergents and synthetic dyes in the textile production process. After being processed with the immobilized fungus *Daedaleopsis eff. confragosa* for eight days, the BOD_5 value decreases to 70.833mg/L or 82.932% of efficiency. The COD value decreased to 167.203 mg/L or 88.307% of efficiency. This shows that the decomposition of organic materials was efficient. The BOD_5 and COD values resulting from this process were already below the textile wastewater quality standards required by the Minister of Environment Decree No. 51/MENLH/10/1995. Thereunto, it was safe to dispose of into the environment.

Changes in the absorbance spectrum of textile dyeing waste before and after treatment were observed using UV-Vis Zhimadsu 1700. Textile dyeing waste before processing shows the maximum absorption was at 572.5 nm and 412 nm. The negative control has relatively unchanged peaks of absorption; they were 572 nm and 410 nm. However, the absorbance was lower than that of the waste before treatment. The decrease in absorbance in negative control was caused by the fact that sawdust can adsorb dyestuff. The results of sewage processing for eight days show maximum absorption at 558 nm. The change of maximum absorption before and after treatment indicates that the waste has been decomposed by the immobilized fungus *Daedaleopsis eff. confragosa*.

3.3 Acute Toxicity Test

Evaluation of textile waste's toxic effects before and after processing was carried out using Tilapia as a testing animal. The curves of the correlation between waste concentration and mortality rate of tilapia during 96 hours of exposure before and after treatment were presented in Figures 10 and 11.

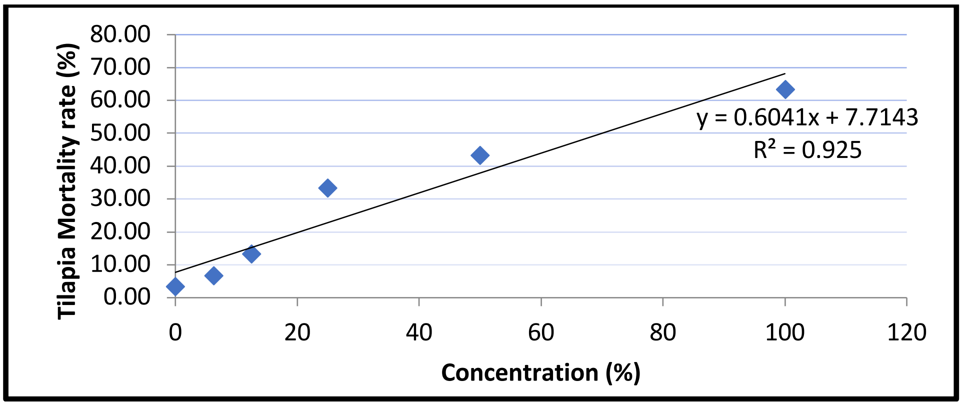

Figure 10. The curve of the Relationship of Concentration of Waste Before Treatment on Tilapia Mortality Rate Waste during 96 hours of exposure

Based on the curve above, the equation for the line y = 0.604x + 7,714 was obtained. It was known that y = 50 since the value of LC^{50} was being looked for. By entering the y value in the equation, it was obtained that the LC^{50} value = 70.01%.

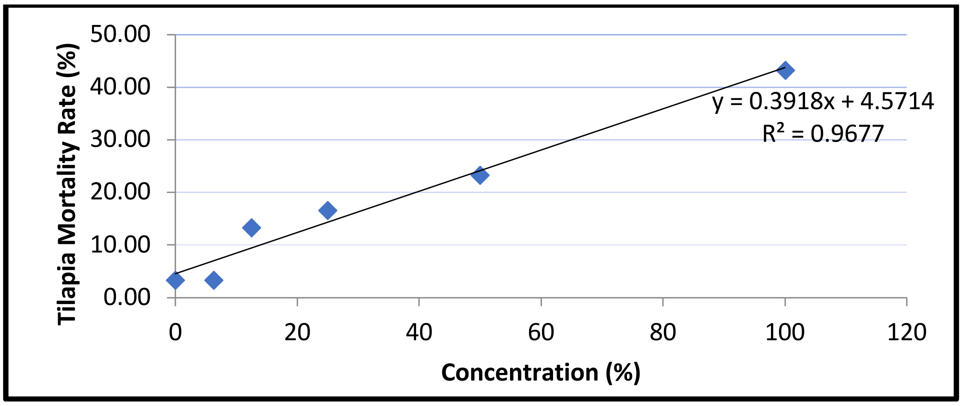

Figure 11. The Curve of the Relationship of Waste Concentration after Treatment on Tilapia Mortality Rate during 96 Hours of Exposure

Based on the curve above, the equation for the line y = 0.391x + 4.571 with R^2 = 0.967. Since we were looking for the value for LC_{50}, then y = 50. However, y = 50 was excluded from the equation above. Thus, the LC_{50} value is> 100% effluent concentration. The measurement of waste toxicity using LC_{50} focuses on the effect of the concentration that causes the test animal's death at 96 hours of exposure. The relationship curve above also reflects the relation of waste dilution rate to the mortality rate of Tilapia (Tilapia nilotica) before and after processing.

In the Figure 10 was obtained the linear regression of the curve that has a coefficient of determination R2 of 0.925. This means that pollutants cause 92.5% of tilapia deaths in the waste. This shows a strong linear relationship between the increase in the concentration of pollutants (textile dyeing waste) and the number of dead Tilapia within 96 hours of exposure. The LC_{50} value of textile dyeing waste before processing was 70.01%. Waste water will be categorized as toxic if the LC50 > 50-75% [38]. Thus, it means the textile dyeing waste of CV. Mama & Leon, Bali fall into the toxic category.

The result of textile dyeing waste treatment after being processed using the immobilized fungus *Daedaleopsis eff. confragosa* for eight days of incubation, causing tilapia mortality to decrease to less than 50% during the 95 hours of exposure at 100% concentration. This indicates that the LC_{50} value is> 100%. These findings indicate that the LC_{50} value increases and. According to Coleman & Qureshi [38], if the LC50 > 100%, it means that the result of textile dyeing waste treatment uses the immobilized fungus *Daedaleopsis eff. confragosa* was categorized as non-toxic.

This study's results were in line with previous studies on the toxicity of azo by previous researchers. Ramsay and Nguyen [39] conducted a toxicity study of the remazol Briliant Blue and Reactive Blue dyes. The toxicity of Remazol Briliant Blue before treatment was categorized into the toxic category (75%> LC20> 50%), while Reactive Blue was in the very toxic category (50%> LC20> 25%). After being treated by Trametes versicolor fungus, the level of toxicity decreases to become non-toxic

4. CONCLUSION

This study concluded that the optimum condition needed to process the dyeing waste was at pH 4 with eight days of incubation. Field-scale textile waste treatment in a reactor containing the immobilized fungus Daedaleopsis eff. confragosa in sawdust for eight days was able to reduce colour, TSS, COD, and BOD by 79.78%, 779.11%, 88.307%, and 82.932%. Besides, textile waste processing results show a lower level of toxicity than textile waste before treatment. The level of toxicity of wastewater before it was treated was mildly toxic, and it changes to a non-toxic level after treatment. This study needs further study on a larger scale to ensure that this method was ready to be implemented in a real situation in the field.

ACKNOWLEDGMENT

The researcher would like to express the highest gratitude to the advisors, James Sibarani, Ph.D. and Prof. Dr. Ir. I Gede Mahardika, M.S who have contributed to this study by giving suggestions, guidance, and supervision so that this study can be completed. The researcher also would like to thank Prof. Dr. I Made Dira Swantara who has provided the researchers with equipment and tools in the laboratory to support this study.

CONFLICT OF INTEREST

The authors confirm that there is no conflict of interests associated with this publication and there is no financial fund for this work that can affect the research outcomes.

REFERENCES

[1] Sudiana I.K., Sastrawidana I.D.K, Sukarta I.N. Decolorization study of remazol black B textile dye using local fungi of Ganoderma sp. and their ligninolytic enzymes. *J Environ Sci Technol.,* 2018; 11(1); 16-22.

[2] Azbar N., Yonar T., Kestioglu K. Comparison of various advanced oxidation processes and chemical treatment methods for COD and color removal from a polyester and acetate fiber dyeing effluent. *Chemosphere*. 2004; 55(1); 35-43.

[3] Pandey A., Singh P., Iyengar L. Bacterial decolorization and degradation of azo dyes. *Biodegradation.,* 2007; 59(2); 73-84.

[4] Nordin N., Amir S.F., Riyanto., Othman M.R. Textile industries wastewater treatment by electrochemical. *Int J Electrochem Sci.,* 2013; 8; 11403-11415.

[5] Mustapha A. Colour removal technology using ozone in textile industrial wastewater effluent: an overview. *Int J Inno Scientific & Eng Tech Res.,* 2015; 3(2); 45-51.

[6] Hassaan MA, Nemr AE. Advanced oxidation processes for textile wastewater. Int J Photochem Photobiol. 2017;2(3):85–93.

[7] Deshannavar U., Murgod A., Golangade M., Koli P., Samyak B., Naik N. Photo-oxidation process – application for removal of color from. *Res J Chem Sci.,* 2012; 2(10); 75-79.

[8] Igwegbe C.A., Onyechi P.C., Onukwuli O.D., Nwokedi I.C. Adsorptive treatment of textile wastewater using activated carbon produced from mucuna pruriens seed shells. *World J Eng Technol.*, 2016; 4(1); 21-37.

[9] Sastrawidana I.D., Lay B.W., Fauzi A.M., Santosa D.A. The utilizing of local bacteria consortia for bioremediation of textile wastewater under combined anaerobic-aerobic system. *Ber Biol.*, 2008; 9(2); 123–132.

[10] Bayoumi M.N., Al-Wasify R.S., Hamed S.R. Bioremediation of textile wastewater dyes using local bacterial isolates. *Int J Curr Microbiol Appl Sci.*, 2014; 3(12); 962-970.

[11] Shasha D., Mupa M., Muzarabani N., Gwatidzo L. Removal of congo red from

aqueous synthetic solutions using silica gel immobilized chlorophyta hydrodictyon africanum. *J Environ Sci Technol.*, 2015; 8(2); 83-90.

[12] Santos Y.V., Freire D.A., Pinheiro S., Fontão L., Souza J.V., Cavallazzi J.R. Production of laccase from a white rot fungi isolated. *Sci Res essays.*, 2015; 10(4); 132–136.

[13] Hartikainen E.S., Miettinen O., Hatakka A., Kähkönen M.A. Decolorization of six synthetic dyes by fungi. *Am J Environ Sci.,* 2016; 12(2); 77–85.

[14] Ali S.S., Al-Tohamy R., Koutra E., El-Naggar A.H., Kornaros M., Sun J. Valorizing lignin-like dyes and textile dyeing wastewater by a newly constructed lipid-producing and lignin modifying oleaginous yeast consortium valued for biodiesel and bioremediation. *J Hazard Mater.,* 2021; 403; 123575.

[15] Oliveira S.F, da Luz J.M.R., Kasuya M.C.M., Ladeira L.O, Correa Junior A. Enzymatic extract containing lignin peroxidase immobilized on carbon nanotubes: Potential biocatalyst in dye decolourization. *Saudi J Biol Sci.*, 2018; 25(4); 651-659.

[16] Sastrawidana D.K., Rachmawati D.O., Sudiana K. Color removal of textile wastewater using indirect electrochemical oxidation with multi carbon electrodes. *Environment Asia.* 2018;11(3); 170–81.

[17] Banat I.M., Nigam P., Singh D., Marchant R. Microbial decolorization of textile-dyecontaining effluents: A review. *Bioresour Technol.,* 1996; 58(3): 217–227.

[18] Zhao X. Analysis of fungal degradation products of azo dyes. [Georgia]: The University of Georgia; 2004.

[19] Montoya S., Sánchez Ó.J., Levin L. Production of lignocellulolytic enzymes from three white-rot fungi by solid-state fermentation and mathematical modeling. *African Journal of Biotechnology.* 2015; 14(15): 1304–1317.

[20] Dhliwayo-Chiunzi P.D., Rwafa R., Mugayi L., Siwanja F., Ngurube T., Hudson G. Screening and evaluationof ligninolytic dye decolourisation capacity of Pleurotus ostreatus. *J Biodivers Environ Sci.* 2015; 6(3); 165–173.

[21] Swamy H.S., Ramalingappa. Lignolytic enzymes production from selected mushrooms. *Int J Appl Sci Biotechnol.* 2015; 3(2); 308–313.

[22] Dinatha N.M, Sibarani J., Mahardika I.G. Degradation of textile waste using the white weathered fungus Daedaleopsis eff. confragosa. *Bumi Lestari.* 2013; 13(2); 288–296.

[23] Fang H, Wenrong H, Yuezhong L. Biodegradation mechanisms and kinetics of azo dye 4BS by a microbial consortium. *Chemosphere.,* 2004; 57(4); 293–301.

[24] Dayaram P., Dasgupta D. Decolorisation of synthetic dyes and textile wastewater using Polyporus rubidus. *J Environ Biol.* 2008; 29(6); 831–836.

[25] Munir N., Asghar M., Tahir I.M., Riaz M., Bilal M., Shah S.M. Utilization of agro-wastes for production of ligninolytic enzymes in liquid state fermentation by Phanerochaete Chrysosporium. *Int J Chem Biochem Sci.,* 2015; 7; 9–14.

[26] Ali H., Muhammad S.K. Biodecolorization of acid violet 19 by Alternaria solani. *African J Biotechnol.,* 2008; 7(6); 831–833.

[27] Khdair A., Abu-Rumman G., Sustainable Environmental Management and Valorization Options for Olive Mill Byproducts in the Middle East and North Africa (MENA) Region. *Processes,* 2020; 8; 1-22.

[28] Dutournié P., Jeguirim M., Khiari B., Goddard M.L., Jellali S. Olive Mill Wastewater: From a Pollutant to Green Fuels, Agricultural Water Source, and Bio-Fertilizer. Part 2: Water Recovery. *Water,* 2019; 11(4); 1-13.

[29] Moustakas K., Loizidou M. Waste and biomass management and valorization. *Environ Sci Pollut Res Int.,* 2021; 28(19); 24224-24229.

[30] Daâssi D., Zouari-Mechichi H., Frikha F., Rodríguez-Couto S., Nasri M., Mechichi T. Sawdust waste as a low-cost support-substrate for laccases production and adsorbent for azo dyes decolorization. *J Environ Heal Sci Eng*., 2016; 14; 1-12.

[31] Dkhissi O., Chatoui M., El Hakmaoui A., Abouri M., Kadmi Y., Akssira M. Valorization of Opuntia ficus-Indica Pads and Steel Industry FeCl(3)-Rich Rejection for Removing Surfactant and Phenol from Oil Refinery Wastewater Through Coagulation-Flocculation. *J Heal Pollut.* 2020; 10(28); 201204.

[32] Alidadi H., Dolatabadi M., Davoudi M., Barjasteh-Askari F., Jamali-Behnam F., Hosseinzadeh A. Enhanced removal of tetracycline using modified sawdust: Optimization, isotherm, kinetics, and regeneration studies. *Process Saf Environ Prot.,* 2018; 117; 51–60.

[33] Kusnadi. Microbiology. Bandung: Biology Education of Indonesian University of Education; 2003.

[34] Ramya M., Anusha B., Kalavathy S., Devilaksmi S. Biodecolorization and biodegradation of reactive blue by Aspergillus sp. *African J Biotechnol.,* 2007; 6(12); 1441–1445.

[35] Demir G., Ozcan H., Tufekci N., Borat M. Decolorization of remazol yellow RR gran by white rot fungus. *J Environ Biol.,* 2007; 28(4); 813–817.

[36] Dewi R.S, Mumpuni A, Tsabitah NI. Batik Dye Decolorization by Immobilized Biomass of Aspergillus sp. *IOP Conf Ser Earth Environ Sci.,* 2020; 550(1); 012020.

[37] Wiloso E.I. Handling of colored liquid waste containing compounds Azo in Biology, especially by the fungus Penicillium sp. L2. Jakarta; 2002.

[38] Coleman R.N, Qureshi AA. Microtox and spirillum volutans tests for assessing toxicity of environmental samples. *Bull Env Contam Toxicol.,* 1985; 35(4); 443–451.

[39] Ramsay J.A, Nguyen T. Decoloration of textile dyes by trametes versicolor and its effect on dye toxicity. *Biotechnol Lett.,* 2002; 24; 1757–1761.