Application of myco-light expanded clay aggregate for real textile wastewater treatment in rotating drum biological contactor

Fenny Clara Ardiati1*, Dede Heri Yuli Yanto1**, Sita Heris Anita1, Kharisma Panji Ramadhan1, Raden Permana Budi Laksana1, Susila Tri Harsono2, Yandes Panelin2 and Widiyatno2

1 Research Center for Biomaterials, National Research and Innovation Agency (BRIN) Jl. Raya Bogor Km. 46, Cibinong Science Center, Cibinong, Bogor 16911, Indonesia
2 PT. Jababeka Infrastruktur, Jl. Jababeka IV Blok B No. 12, Jababeka Industrial Estate Cikarang Bekasi 17530, Indonesia

E-mail: *fenny.clara.ardiati@brin.go.id; **dede.heri.yuli.yanto@brin.go.id

Abstract. Textile effluent could endanger human health and the water environment, but it is very challenging to be treated due to its complex composition. Biological methods for textile wastewater treatment by using fungi has been extensively studied in a lab-scale yet the investigation on a larger scale is still limited. In this study, a preliminary investigation of immobilized Trametes hirsuta D7 in light expanded clay aggregate (myco-LECA) application was conducted to treat the real textile wastewater in a rotating drum biological contactor. The undiluted wastewater without any addition of carbon and nutrients was used in the experiment and treated for 72 hours in the batch-mode bioreactor. The results revealed the maximum decolorization of 74.62% along with the highest laccase activity observed at 107 UL-1. Moreover, the pH was successfully reduced from 12.94 to 8.57 while the chemical oxygen demand still fluctuated. In terms of nutrients, 57.4% of phosphorus (PO4-P) removal was achieved but no observation of the nitrification process. Based on the toxicity assay using Artemia salina larvae, the treatment could reduce the toxicity level and performed a total chromium removal up to 36.5%. These findings showed the promising abilities of myco-LECA for textile wastewater treatment prior to the full-scale application.

Keywords: Bioreactor; light expanded clay aggregate; mycoremediation; rotating drum biological contactor; textile wastewater; Trametes hirsuta

1. Introduction
The textile industry has been one of the essential supports for the basic needs of society. However, it was reported as the most polluting business among all industrial sectors due to the enormous wastewater generation and complex composition [1]. Textile wastewater not only consists of a 2–60% residual mixture of dyes but is also characterized by a high and fluctuating chemical oxygen demand (COD), pH, heavy metals, toxic and resistant organic and inorganic chemicals [1,2]. Therefore, discharging the improperly treated textile wastewater can result in aesthetic nuisance and harm the aquatic ecosystem by inhibiting the photosynthetic activities and decreasing the available amount of dissolved oxygen in...
the water [3]. In addition, various diseases in humans such as cancer, dermatitis, and even an attack on the nervous system can be caused by the textile dyes through biomagnification effects [4].

Compared to the physical and chemical methods, biological treatment is viewed as a sustainable alternative for wastewater treatment by the absence of chemicals and sludge, lower cost of energy, and more environmentally friendly [4,5]. Among biological agents, white-rot fungi (WRF) have gained much attention with their ability to produce extracellular lignin-modifying enzymes (LMEs) which are directly involved in biodegradation from lignin to xenobiotic compounds, including dyes [6]. Many research reported the ability of different WRF strains to decolorize synthetic dyes such as *Trametes hirsute* [7,8], *Trametes polyzona* [9], and *Aspergillus niger* [10]. Moreover, the usage of immobilized culture has been explored since it enhances biosorption capacity and offers reusability, easier separation of solids and liquids, resilience to pH changing and toxic compounds, as well as the protection for fungal cells [5,11]. Despite the high decolorization of synthetic dyes by WRF in the flask experiment, its application for the real textile wastewater in the reactor is another challenge yet the study is still limited [1,6]. It requires the optimized operational conditions of the reactor, appropriate fungal strains and depends on the complex constitution of textile effluents [6].

Previous studies of immobilized *T. hirsuta* D7 in light expanded clay aggregate, so-called by myco-LECA, to decolorize synthetic dyes by using the fungal cells were performed [12,13]. The results revealed the enhanced decolorization by the fungi together with the LECA as the carrier, up to 96% of anthraquinone dyes removal for 24 hours. Furthermore, the detoxification of the dyes was reported as the result of the fungal treatment [13]. Thus, these promising outcomes were further investigated by using the real textile effluent within a bioreactor. In this study, a preliminary study of myco-LECA applied in a bench-scale rotating drum biological contactor (RDBC) was done. Besides the colour and enzyme activities, monitoring of pH, COD, ammonia (NH$_3$-N), phosphate (PO$_4$-P), total chromium, and toxicity assays were performed. The aim was to evaluate the ability of immobilized *T. hirsuta* D7 to treat textile wastewater with more comprehensive parameters on a larger scale. The results are expected to be beneficial for the better understanding of textile wastewater treatment by myco-LECA prior to the full-scale application.

2. Materials and Methods

2.1. Organism, raw material, and chemicals

*Trametes hirsuta* D7 (NCBI GenBank, accession No. KX444204) was isolated from a peat swamp forest in Bengkalis, Riau, Indonesia [14]. LECAs as the carriers for fungal immobilization were purchased from a local company in Ciamis, West Java, Indonesia. Malt extract agar, malt extract, glucose, peptone, tartaric acid, and other chemicals for culturing process were purchased from Wako Pure Chemical Industries, Ltd (Japan). For enzyme assay, the 2,2’-azino-di-[3-ethyl-benzothiazoline-(6)-sulfonic acid] (ABTS) 98% was purchased from Sigma-Aldrich (USA) while chemicals for wastewater monitoring were purchased from Merck, Germany. *Artemia salina* which was used for the toxicity test was purchased from the local company in Indonesia.

2.2. Wastewater characteristics

The wastewater sample was obtained from one of the textile industries within the industrial areas, located in Bekasi, West Java, Indonesia. Interest characteristics of the textile wastewater were tested (Table 1). Freshly taken textile wastewater without any dilution was used directly for the experiments.

2.3. Fungal culture preparation

*T. hirsuta* D7 was cultured on malt extract agar (MEA) and then incubated at room temperature (25−30 °C) for 7 days. Every four agar plugs (Ø 5 mm) of actively growing fungus on MEA were inoculated into 20 mL of medium malt extract-glucose-peptone (MGP) broth in the Erlenmeyer flask. The MGP medium consists of malt extract (20 g/L); glucose (20 g/L); and peptone (1g/L) which was adjusted to pH 4.5 by using tartaric acid and sterilized in an autoclave for 15 min at 121 °C. The
Erlenmeyer flask was capped with cotton to allow passive aeration and static incubation was done at room temperature for seven days. Afterward, the fungal culture was homogenized, called homogenate, using a Waring blender (Waring Commercial) and ready to use for immobilization purposes.

Table 1. Composition of textile wastewater used in the study.

| Parameter                          | Value      |
|------------------------------------|------------|
| Chemical oxygen demand (COD)       | 2320 mg/L  |
| pH                                 | 12.94      |
| Ammonia (NH₃-N)                    | 149.59 mg/L|
| Phosphate (PO₄-P)                  | 8.71 mg/L  |
| Total chromium (total Cr)          | 0.167 ppm  |

2.4. Preparation of LECA
Prior the study, granular LECAs were screened for the sizing around 10–16 mm for further pre-treated by using steam activation at 800–900 °C for 1 hour and washed with distilled water to remove the dust [12,15]. Then, the LECAs were ready to use after the drying process in the oven at 60 °C for 24 hours to remove extra water and moisture.

2.5. Experimental set-up of RDBC and fungi immobilization
Previous designs of RDBC have been applied [16–18] although it was still rarely reported for wastewater treatment. A bench-scale RDBC adapted from the previous study [18] was used and illustrated in Figure 1. The RDBC tank was equipped with a 6 L single drum made from stainless steel mesh (mesh size = 3 mm). The drum was supported by a shaft which was connected to a motor, rotating the drum with an adjustable speed between 1 to 10 rotations per minute (rpm). Table 2 shows the detailed design and operational conditions of RDBC.

For fungi immobilization, the LECAs were prepared within a 5 L Erlenmeyer flask and submerged by the MGP medium. These LECAs were sterilized in an autoclave for 15 min at 121 °C. Afterward, the LECAs were filled into the drum within the RDBC tank along with the residual MGP medium, and the homogenate of fungal culture poured into the RDBC tank around 20% (v/v) of the medium. The drum was rotated at 4 rpm for 7 days to let the fungi grow and penetrate the LECAs. Next, the myco-LECA was ready for the subsequent experiments by changing the spent medium into textile wastewater without any dilution.

Table 2. Design and operational conditions of RDBC.

| Parameter             | Value                                      |
|-----------------------|--------------------------------------------|
| Drum dimensions       | diameter = 15 cm; length = 32 cm           |
| Tank dimensions       | length = 40 cm; width = 22 cm; height = 20 cm |
| Submergence of wastewater | 40%                                 |
| Rotational speed      | 4 rpm                                      |
2.6. Analytical assays
The filtered samples were used for determining the color by measuring the absorbance scanned at 515.5 nm wavelength using a UV-Visible spectrophotometer (Hitachi U-5100, Japan). Laccase activity was observed by using a spectrophotometer to monitor the oxidation of 1 mM 2,2-azino-bis-[3-ethyl benzothiazoline-6-sulphonic acid] (ABTS) in 0.05 M acetate buffers pH 4.5 at 420 nm for 1 minute at room temperature. The mixture consisted of 100μL sample, 400μL acetate buffers 0.1 M, and 500μL ABTS 2 mM. The enzyme activity (U/L) was calculated by using equation (1) [19]. The toxicity of wastewater before and after treatment was compared and determined according to the brine shrimp lethality test (BSLT) in terms of the mortality rate [20].

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\text{Enzyme activity (U/L)} = \frac{(\Delta \text{Abs}) \times V_{\text{mixture}} (L)}{t \times \varepsilon \times d \times V_{\text{enzyme}} (L)}
\]  

where: \(\Delta \text{A}\) = change in absorbance; \(t\) = 1 minute; \(\varepsilon\) = molar extinction coefficient for laccase = 36,000 M\(^{-1}\) cm\(^{-1}\); \(d\) = optical trajectory = 1 cm.

The pH of wastewater was measured by a pH meter (Hach Sension-3). The COD of the samples was determined based on the standard methods [21]. For nutrients analysis, ammonia (NH\(_3\)-N) concentration was tested by using colorimetric-phenate methods based on national standards [22] while phosphate (PO\(_4\)-P) concentration was measured by using the colorimetric-stannous chloride method [21]. An atomic absorption spectrophotometer (AA-7000, Shimadzu, Japan) was used to determine total chromium (Cr) concentration [21]. The removal percentage of parameters was calculated by using equation (2). The presented values of measurements were counted as the average of duplicates.

\[
\text{Removal percentage (\%) = } \frac{\text{initial concentration} - \text{final concentration}}{\text{initial concentration}} \times 100\%
\]

3. Results and Discussions
3.1. Wastewater decolonization and laccase activity
In this study, myco-LECA was used to treat real textile wastewater by using RDBC operated in a batch mode for three days. The results showed that the decolorization reached 74.62 ± 0.004% after 72 hours of treatment. A previous study by Pakshirajan & Kheria (2012) conducted similar experiments by using immobilized \textit{P. chrysosporium} in the RBC [23]. However, it reported a treatment failure of undiluted colored industry wastewater due to the high COD and dyes concentrations. Instead, the optimum treatment was achieved by using diluted and pH adjusted wastewater with minimum additional glucose of 5 g/L. Under this condition, around 83% decolorization was obtained within 2 days of operation. Comparing to this, a promising performance of myco-LECA in this study was shown since the textile wastewater was used without any dilution, no additional carbon source, and no pH adjustment.
Dyes decolorization by myco-remediation has been reported to occur through three principal mechanisms: biosorption, bioaccumulation, and biodegradation [24]. Through biosorption, the dyes molecules are rapidly adsorbed onto the surface of fungal cells while bioaccumulation happens by the absorption of dyes within the actively growing fungal cells where the dyes will be accumulated inside the cytoplasm [25]. In this study, myco-LECA was formulated by the application of the immobilization technique of T. hirsuta D7 through the entrapment of fungal biomass within the granulated LECA (36.4 mg biomass dry weight in 25 g LECA [13]). Thus, it could be assumed that the immobilized fungal cells contributed to the dye’s removal. In addition, the activated LECA as the carrier of immobilized fungi might also have a role in enhancing the sorption of the dyes. LECA is made from clay heated in high temperature (1100 °C−1300 °C) and it was porous, inert, and resilient to toxic conditions which were then found to be a potential adsorbent for dyes [26,27]. This is supported by another study of granular activated LECA with larger pores which could decolorize 35% of Remazol Brilliant Blue R (RBBR) dyes (100 ppm) within 24 hours [12].

For biodegradation, the dyes molecules are broken down and degraded mediated by enzymes secreted by the fungi with concentrations that may be higher than the tolerance limit of biosorption by the living fungal cells [25]. T. hirsuta D7 is classified as white-rot fungi (WRF) which has been intensively studied for the dyes decolorization due to its production of extracellular lignin-modifying-enzymes (LMEs) [28]. Among the LMEs, laccase was reported as the foremost utilized ligninolytic enzymes and commonly highlighted for only need the oxygen as the oxidizing agent and less sensitivity to the substrates [29]. Therefore, laccase activity measured in the wastewater was monitored along the operation period, reaching the highest at 107 U/L (Figure 2). An increasing trend of enzyme activity was observed although it was slightly declined at certain points. Reflecting on the increased decolorization, the enzymatic degradation could be assumed to involve within the dyes removal process which was similarly demonstrated by previous studies [9].

![Laccase activity of T. hirsuta D7 immobilized within myco-LECA during experiment (the laccase activity was taken from the treated wastewater).](image)

**Figure 2.** Laccase activity of T. hirsuta D7 immobilized within myco-LECA during experiment (the laccase activity was taken from the treated wastewater).

### 3.2. Monitoring of pH and COD removal

After 72 hours of treatment, the pH progressively decreased from 12.94 to 8.57 (Figure 3). This value was able to comply with the local regulation based on the regulation of the minister of environment and forestry of the Republic of Indonesia No. 5/2014 with a pH range of 6−9. Indeed, the growth of WRF
was reported to decrease the pH of the environment through its metabolic regulation [30]. Srikanlayanukul et al. (2016) demonstrated similar findings of pH reduction from 8.22 to 3.91 within 48 h of real textile wastewater by using immobilized *C. versicolor* RC3 in an air-bubble bioreactor [31]. However, many studies found an optimum pH for decolorization by WRF in a much lower pH of 4–6 [32] with the maximum laccase activity observed [33]. Therefore, pH adjustment is suggested to reach maximum decolorization and COD removals for textile wastewater treatment [1].

A high COD concentration after 72 hours of treatment is depicted in Figure 3, around 3040 mg COD/L. It increased from the initial concentration of 2320 mg COD/L although some removals were observed at the end of day-1 and day-3. The results were pertinent to the findings by Anastasi et al. (2012) which showed an increase of COD in a range of 742 to 1504 mg/L after 48 hours of textile wastewater treatment by three best fungal strains [34]. This was assumed due to the addition of immobilized fungal biomass to the bioreactor after comparing the results with the abiotic controls. Thus, the COD analysis was started after the biomass was included. In this case, our results could also reveal a slightly COD reduction (9.5%) within 3 days. Another possibility might be due to the higher contribution of inorganic chemicals such as ammonia that was also observed in this study (Figure 4). This was by the fact that COD measures the oxygen concentration used for the decomposition of both organic matter and oxidation of inorganic chemicals from wastewater [35]. Similarly, Faraco et al. (2009) investigated a colored wastewater treatment by *P. chrysosporium* and *P. ostreatus* and there was no COD reduction observed due to higher nutrients concentration [36].

![Figure 3. COD removal and pH changes from textile wastewater by myco-LECA in RDBC.](image)

In contrast, many studies reported higher decolorization along with COD removals by using myco-remediation. Pakshirajan & Kheria (2012) observed an agreement of 64% decolorization efficiency with 73% COD reduction of diluted textile wastewater by immobilized *P. chrysosporium* within 2 days [23]. Moreover, other research showed similar relation with longer treatment days between 12–70 days in the optimum treatment conditions and/or by using synthetic dyes [37,38]. A recent study by Dalecka et al. (2020) also claimed that the pH value adjustment to 5.5 revealed stable organic removals while the unsteady reduction of total organic carbon concentration was observed within 72 hours without the pH adjustment [39]. Therefore, the optimum environments of pH, dilution of wastewater, and additional media might support the enhancement performance of myco-LECA for further investigation.
3.3. Removal of phosphorus and ammonia-nitrogen

In terms of nutrients, the phosphate (PO$_4$-P) concentration decreased by 57.4% after 72 hours of treatment while an increase of ammonium was observed around 61.4% (Figure 4). According to Indonesian regulation (Decree of P.16/Menhk/Setjen/Kum.1/4/2019) for textile wastewater effluents standard, phosphate is not regulated while ammonia (NH$_3$-N) is limited by 8 mg/L. Based on the results, ammonia concentration was over the limit whereas the phosphate concentration was relatively lower than the effluent standard mentioned by global regulation [40] at 10 mg/L. High ammonia nitrogen in textile wastewater was reported due to nitrogen-based substances which commonly utilized. This should be a further concern since the excessive of these nutrients promote eutrophication and water quality problems [41].

The removal of nutrients by white-rot fungi from textile wastewater in a bioreactor has been less investigated. Recently, similar findings were reported by Dalecka et al. which used two fungal species for phosphorus and ammonia nitrogen removal from non-sterile municipal wastewater [39]. After 8 days operation, PO$_4$-P concentration was able to reduce with more than 80% removal within 22 days by using a fluidized bed bioreactor. On the contrary, NH$_3$-N concentration increased within 72 h in the batch experiment and within 19 days in the bioreactor with a flow of 0.11 L/min. In this case, the ammonium increment was hypothesized due to the lower pH caused by the fungi ability which was similarly observed in this study. Moreover, it was believed that the fungi were not capable to remove nitrogen in wastewater through their metabolic pathway which was similarly observed in this study. However, fungal nitrogen treatment has been reported as a promising technique compared to a bacteria-based system.

![Figure 4. phosphorus and ammonia nitrogen removal by myco-LECA in RDBC.](image)

Various fungi can perform nitrogen removal through nitrification, denitrification, or both processes [42]. These fungi were able to oxidize ammonia for their energy and nutrient source, but it strongly depended on the fungal strains [43]. For instance, *Phanerochaete chrysosporium*, *Aspergillus niger*, and *Penicillium chrysogenum* could carry out the nitrification process [44]. Moreover, some fungi were also found to significantly reduce phosphate concentration such as *Geotrichum* sp. and *Mucor* sp., although it might not along with higher ammonium reduction, meaning that a combination of various strains might be needed [45]. To the extent of our knowledge, this might be the first study to report the ability of *Trametes hirsuta* for nutrients removal from wastewater. Among *Trametes* sp., *T. versicolor* has been demonstrated to remove phosphorus around 28-80% [39,46] and total nitrogen around 43% but the ammonium (NH$_3$-N) concentration remained unaffected [46] or increased [39]. Another study reported the immobilized *T. menziesii* that could perform BOD and COD removal but without ammonia removal.
observed [47]. Despite the absence of nitrification process during this study, nitrogen removal by *T. hirsuta* is worth being further evaluated by various environmental and wastewater conditions.

### 3.4. Toxicity assays and total Cr removal

Synthetic dyes in textile wastewater are often characterized as toxic, mutagenic, and carcinogenic substances [1,10]. In some cases, the toxicity of textile wastewater is persistent even after the treatment. For instance, Almeida and Corso (2014) reported biodegradation of azo dye by *A. terreus* which produced toxic metabolites, resulting in 100% mortality of *A. salina* larvae for the treated effluent despite the 98% decolorization [10]. Therefore, the toxicity assay was conducted to investigate the harmful effects of textile effluent before and after the fungal treatment.

The results revealed that the raw textile wastewater had a 60 ± 10% *A. salina* larvae mortality rate. However, the toxicity was successfully reduced to 10 ± 10% after 72 hours of treatment. This finding showed a promising result of decolorization as well as detoxification of textile effluent by myco-LECA in the bioreactor. Previous studies of immobilized *T. hirsuta* D7 in LECA have also shown a similar observation of toxicity reduction [13]. Alam *et al.* (2021) did the cytotoxicity assay on human dermal fibroblast (HDF) for three anthraquinone dyes (100 mg/L) before and after the fungal treatment. The results demonstrated a reduced toxicity level by increasing the cell viability up to 94% for the cytotoxicity assessment.

Another threat of textile wastewater is the heavy metals composed in the synthetic dyes [1]. Among the heavy metals, total chromium (total Cr) has been a concern as the carcinogen substance and for its impact on skin irritation [48]. In this study, the total chromium concentration of raw textile wastewater was already below the limit value of 1 ppm based on local regulation (Decree of P.16/Menhk/Setjen/Kum.1/4/2019). However, the total Cr of wastewater was slightly decreased from 0.167 ppm to 0.106 ppm (36.5%) after 72 hours of treatment. The ability of *T. hirsuta* to perform a simultaneous removal of dyes and chromium was recently reported by Liu *et al.* (2020) through the transformation mechanisms [8]. It was found that the toxic Cr (IV) was removed up to 96% while promoting the decolorization percentage of RB5 dye from 57.15% to 83.65%.

### 4. Conclusion

The study demonstrated the promising results of real textile wastewater treatment by immobilized *T. hirsuta* D7 in LECA (myco-LECA) within the RDBC. This application was able to decolorize the textile effluents without any dilution, no additional carbon source, and no pH adjustment. Furthermore, it decreased the pH, phosphate, and total chromium concentration although the organic removal still fluctuated, and no nitrification process was observed. The treatment was also reduced the toxicity level and thus, a further investigation is recommended to optimize the environmental, wastewater, and operational conditions of the bioreactor to reach optimum performance.

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### Declarations

**Author contributions**

F C Ardiati and D H Y Yanto contributed equally as the main contributor of this paper. All authors read and approved the final paper.

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