

# Selection of White-Rot Fungi for Decolorization of Palm Oil Mill Effluent and Evaluation of Biodegradation and Biosorption Processes

Sanhathai Riddibud\*, Nuttika Suwannasai\*\*, Apichaya Sawasdee\*\*\*†, Verawat Champreda\*\*\*\*(\*\*\*\*\*), Cherdchai Phosri\*\*\*\*\*, Sarper Sarp\*\*\*\*\*, Nipon Pisutpaisal\*(\*\*\*\*\*) and Siriorn Boonyawanich\*(\*\*\*\*\*)

\*Department of Agro-Industrial, Food and Environment Technology, Faculty of Applied Science, King Mongkut's University of Technology North Bangkok, Bangkok, 10800, Thailand

\*\*Department of Microbiology, Faculty of Science, Srinakharinwirot University, 114 Sukhumvit 23, Watthana, Bangkok, 10110, Thailand

\*\*\*Program in Innovation of Environmental Management, College of Innovative Management, Valaya Alongkorn Rajabhat University Under the Royal Patronage, Pathumthani Province, 13180, Thailand

\*\*\*\*BIOTEC-JGSEE Integrative Biorefinery Laboratory, Innovation Cluster 2 Building, Thailand Science Park, Phaholyothin Road, Khlong Luang, Pathumthani 12120, Thailand

\*\*\*\*\*Biorefinery Technology and Bioproducts Research Group, National Center for Genetic Engineering and Biotechnology, 113 Thailand Science Park, Phaholyothin Road, Khlong Luang, Pathumthani 12120, Thailand

\*\*\*\*\*Department of Biology, Faculty of Science, Nakhon Phanom University, 124 Moo 12, Ard-Samart Subdistrict, Muang District, Nakhon Phanom, 48000, Thailand

\*\*\*\*\*Centre for Water Advanced Technologies and Environmental Research (CWATER), College of Engineering, Swansea University, Swansea SA1 8EN, UK

\*\*\*\*\*The Biosensor and Bioelectronics Technology Centre, King Mongkut's University of Technology, North Bangkok, Bangkok, 10800, Thailand

†Corresponding author: Apichaya Sawasdee; apichaya.s@vru.ac.th

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## ABSTRACT

Ten species of white-rot fungi were evaluated for their ability to decolorization of palm oil mill effluent. The highest decolorization efficiency was found with *Trametes elegans* (PP17-06), followed by *Ganoderma* sp.2 (PW17-06) and *Ganoderma* sp.2 (PW17-177), respectively. *T. elegans* was further evaluated for the long-term performance of decolorization for 24 d. The optimal retention time for the decolorization was 8 d, with a color removal efficiency of 47.7%. Beyond 18 d of incubation, decolorization efficiency was reduced due to the autolysis of enzymes. During the biodegradation process, manganese peroxidase enzyme activities reached a maximum of 36.03 U.L<sup>-1</sup>. However, no significant laccase and lignin peroxidase activities were observed. *T. elegans* was also assessed for decolorization performance through biosorption on mycelial biomass. The synthesis of the enzyme was prevented by exposing the mycelium to HgCl<sub>2</sub>. Within an optimal contact time of 2 d, decolorization efficiency reached 12.5% with ADML reduction from 4259.0 (±20.1) ADML to 3727 (±104.04) ADML. Results indicate that the adsorption capacity was reached at this time, and no significant color removal can be achieved by biomass. Results obtained in this study showed the potential of *T. elegans* in decolorizing palm oil mill effluent.

## INTRODUCTION

Palm oil is gaining high demand in developing countries as a high-quality oil source for cooking. Due to the increasing demand, the palm oil industry is expanding across countries in Asia, Africa, and Latin America (Mohammad et al. 2021). Palm oil mill effluent (POME) is a major environmental concern due to the high amount of organic compounds

resulting in high concentrations of biological oxygen demand (BOD) and chemical oxygen demand (COD) (Saad 2020). Treatment of POME is necessary before discharge into the natural water bodies. Currently, various chemical, physical, and biological methods, such as coagulation, adsorption, anaerobic biodegradation, aerobic biodegradation, and advanced oxidation processes, are used in treating wastewater from palm oil mills (Sani et al. 2020).

Biological treatment methods are widely applied to remove organic carbon concentrations. However, despite bacteria's ability to easily remove most biodegradable compounds, benzene, and aromatic structures are difficult to degrade using bacteria. POME usually has a dark brownish color due to the presence of various color compounds such as phenolic, carotene, lignin, and pectin (Abdulsalam et al. 2018, 2020, Saad 2020). The COD of 1586 mg.L<sup>-1</sup>, total solid of 3840 mg.L<sup>-1</sup>, suspended solid of 2170 mg.L<sup>-1</sup>, total phenol of 43 mg.L<sup>-1</sup>, and color value of 2417 PtCo were reported in the effluent of the final anaerobic pond (Rakamthong & Prasertsan 2011). Achieving satisfactory color removal using aerobic or anaerobic bacteria is challenging (Rakamthong & Prasertsan 2011). On the other hand, chemical and physical treatment methods are expensive compared to biological systems (Kutty et al. 2019).

White-rot fungi provide a promising solution in decolorizing various types of industrial wastewater due to their ability to degrade lignocellulose compounds by producing ligninolytic enzymes such as laccase, manganese peroxidase (MnP), and lignin peroxidase (LiP) (Fang et al. 2020, Grelska & Noszczyńska 2020, Zhang et al. 2017). The white-rot fungus of *Trametes hirsuta* AK04 was reported removal efficiencies between 78 and 98% for ten phenolic compounds (Phenol, gallic acid, protocatechuic acid, 4-hydroxybenzoic acid, 4-hydroxyphenylacetic acid, caffeic acid, ferulic acid, syringic acid, p-coumaric acid, and vanillic acid) that can be found in POME. It was also reported that the presence of glucose and yeast extract in the fungi growth medium could enhance the degradation rates of phenolics (Kietkwanboot et al. 2020). *Phanerochaete chrysosporium* ATCC 24725 mycelium achieved a decolorization efficiency of 83.4% in 3 d and a phenol removal efficiency of 61.22% in 6 d using the decanter effluent as the supplement nutrient source for the final effluent of a palm oil mill (Rakamthong & Prasertsan 2011).

In addition to the color removal by biodegradation of compounds through enzyme activities, fungi can remove color by biosorption. White-rot fungus, *Haematonectria haematococca* BwIII43, showed a sorption capacity of 247.47 and 161.00 mg.g<sup>-1</sup> for Alizarin Blue Black B and alkali lignin, respectively (Rybczyńska-Tkaczyk & Korniłowicz-Kowalska 2016). The biosorption capacities were correlated with the molecular structure of dye compounds, and the ionic radius of dyes was found to be influenced by the biosorption capabilities (Maurya et al. 2006).

The application of white-rot fungi in the decolorization of POME has not been widely investigated in previous studies. There is a lack of studies to characterize the mechanisms involved in the decolorization of POME. Therefore, this

study investigated the biodegradation and biosorption processes involved in the decolorization of POME. To characterize the biodegradation, enzyme activities of isolated fungi were monitored. To determine the biosorption capacities, mycelial growth was prevented by exposing it to the HgCl<sub>2</sub> solution. Thus, the enzyme biodegradation process ceased, and color removal mainly occurred through the biosorption of biomass. American Dye Manufacturing Index (ADMI) weighted ordinate method was used to measure the color of wastewater.

## MATERIALS AND METHODS

### Palm Oil Mill Effluent

POME was collected from the effluent of a palm oil mill after the treatment in the final aeration pond at Suksomboon Group, Chonburi, Thailand. The wastewater was kept at a temperature of 4°C and sterilized by autoclaving at 121°C and 15 psi for 15 min before use. The composition of glucose yeast extract (GYE) medium was supplemented in POME with concentrations (g.L<sup>-1</sup>) of 10.0 glucose, 1.0 K<sub>2</sub>HPO<sub>4</sub>, 0.5 MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.5 KCL, 0.01 FeSO<sub>4</sub>·7H<sub>2</sub>O, and 1.75 NH<sub>4</sub>NO<sub>3</sub> (Vaithanomsat et al. 2010). However, the original color of POME was not changed when GYE medium was added.

### Fungal Culture and Inoculum Preparation

The seven species, including ten strains of white-rot fungi as listed in Table 1, were obtained from the Department of Microbiology, Faculty of Science, Srinakharinwirot University, Thailand. These fungi species were originally collected from mixed deciduous and deciduous dipterocarp forests in the northeastern provinces of Nakhon Phanom, Sakon Nakhon, Udon Thani, and Chaiyaphum in Thailand.

Table 1: The seven species (ten strains) for the decolorization of palm oil mill effluent.

Species	Strains	GenBank Accession No.*
<i>Corioliopsis aspera</i>	NP17-02	MK589289
<i>Ganoderma lingzhi</i>	PW17-43	MK589272
<i>Ganoderma sp.2</i>	PW17-06, PW17-154, and PW17-177	MK589273, MK589275, and MK589276
<i>Microporus sp.1</i>	PP17-04	MK589279
<i>Microporus sp.2</i>	PP17-17 and PP17-20	MK589281 and MK589282
<i>Trametes elegans</i>	PP17-06	MK589285
<i>Pseudolagarobasidium sp.</i>	PP17-33	MK589289

\*The accession numbers are unique identifiers assigned to a record in GenBank (NCBI Nucleotide database).

Fungi were cultured in potato dextrose agar (PDA). The PDA contained ( $L^{-1}$  of distilled water) potatoes 200 g, glucose 20 g, and agar 15 g. Cultures were preserved on the PDA medium at 4°C. Preserved cultures were incubated in a fresh PDA medium for 7 d at room temperature to prepare inoculums for experiments.

### Evaluation of Decolorization Efficiencies of Isolated Fungi

From 7-day-old cultures, five active fungal mycelial plugs (5 mm in diameter) from each species were extracted and inoculated into Erlenmeyer flasks containing 50 mL of POME. An abiotic control experiment was also conducted without adding any fungal inoculum. The flasks were placed on an orbital shaker and incubated at 150 rpm and room temperature for 5 d. To measure the ADMI, samples were filtered through the Whatman filter paper (grade 1).

Based on the decolorization results obtained in the preliminary investigation, the fungal isolate that showed the highest decolorization efficiencies for POME was selected for further experiments. The incubation was conducted for 24 d to assess the decolorization efficiencies and enzyme activities.

### Evaluation of Biosorption Capabilities of Fungi Biomass

The fungal biomass was immersed in 0.7% (w/v)  $HgCl_2$  solution for 30 min during shaking. After that, the fungal biomass was filtered and rinsed with sterile distilled water. A piece of mycelium that was exposed to  $HgCl_2$  was placed on the PDA medium to ensure no synthesis of fungal enzymes (Argumedo-Delira et al. 2021). Then, the fungal biomass was inoculated to 50 ml POME and shaken for 24 d at room temperature. Samples from POME were taken every two days to measure ADMI. The color removal efficiencies by biosorption were calculated. The experiment was conducted in triplicates.

### Analytical Methods

**Color:** The color was determined in the ADMI unit using a spectrophotometer (Hach DR3900). A wavelength range of 320–1100 nm was applied, and the manufacturer's instructions were followed for the 2120 F. ADMI Weighted-Ordinate Spectrophotometric Method (APHA 23<sup>rd</sup>, 2017).

**Laccase, manganese peroxidase, and lignin peroxidase assays:** Samples of enzymes obtained from the POME were analyzed for the laccase, MnP, and LiP activities. Laccase enzyme activity was assessed by monitoring the oxidation of 2,2'-Azino-bis-3-ethylbenzthiazoline-6-sulphonic (ABTS) acid at a wavelength of 420 nm (Extinction coefficient;  $\epsilon = 36,000 M^{-1}.cm^{-1}$ ) (Machado & Matheus 2006). MnP

enzyme activity was determined by monitoring the oxidation of 2,6-dimethoxyphenol (DMP) at a wavelength of 469 nm ( $\epsilon = 10,000 M^{-1}.cm^{-1}$ ) (Silva et al. 2014). The oxidation of veratryl alcohol (3,4-dimethoxybenzyl alcohol) to veratraldehyde in the presence of  $H_2O_2$  at a wavelength of 310 nm ( $\epsilon = 9300 M^{-1}.cm^{-1}$ ) was monitored to determine the LiP enzyme activity (Tien & Kirk 1988). One unit (U) of the activity of ligninolytic enzymes is defined as the amount of enzyme that transforms 1  $\mu$ mol of substrate per minute (Thamvithayakorn et al. 2019).

**Other parameters measured:** Gravimetric analysis was used to determine biomass generation. The weight of the dry biomass was measured after drying the samples at 50 °C for 24 h in a temperature controller. A pH meter (PH100 EXTECH ExStik) was used to monitor the changes in pH during the treatment period.

### Calculations

**Color removal efficiencies and rate:** The percentage of color removal (CR%) was calculated using the color value measured from the control at the beginning of the experiment ( $ADMI_0$ ) and the color value measured from the fungi-inoculated samples at the end of the retention time of T ( $ADMI_T$ ). The color removal efficiencies were calculated according to equation (1).

$$\text{Color removal efficiency (\%)} = \frac{ADMI(T_0) - ADMI(T_5)}{ADMI(T_0)} \times 100 \quad \dots(1)$$

Where  $ADMI(T_0)$  is the Color from control at  $T_0$  and  $ADMI(T_5)$  is = Color from treatment at  $T_5$ .

**Enzyme activity:** Enzyme activity ( $U L^{-1}$ ) was calculated using followed the equation (2)

$$\text{Enzyme activity} = \frac{\Delta Abs \times V \times D \times 10^3}{\epsilon \times V \times t} \quad \dots(2)$$

Where  $\Delta Abs$  is the difference between the absorbance value before and after the reaction, V is the total amount of substance in the reaction (ml), D is the dilution level,  $\epsilon$  is the absorbance constant ( $M^{-1} cm^{-1}$ ), v is the volume of the crude extracted enzyme (ml), and t is curing time (min).

**Biomass growth rate:** Biomass growth rate ( $BM_r$ ) was calculated according to the equation (3)

$$\text{Biomass growth rate } (BM_r) = \frac{BM_{C2} - BM_{C1}}{T_2 - T_1} \quad \dots(3)$$

Where  $BM_{C1}$  and  $BM_{C2}$  are biomass concentrations measured at time  $T_1$  and  $T_2$ , respectively.

**Statistical analysis:** The mean value ( $\pm$  standard deviation) of three independent replicates was calculated for all parameters measured during the experiments. One-way

analysis of variance (One-way ANOVA) method was used for the statistical analysis at 0.05 probability level.

## RESULTS

### Preliminary Selection of Fungal Strains for Decolorization of Palm Oil Mill Effluent

The decolorization efficiencies obtained with isolated fungi are illustrated in Fig. 1. No color removal was found with three fungal species, *Ganoderma lingzhi* (PW17-43), *Coriopsis aspera* (NP17-02), and *Microporus* sp.2 (PP17-20). The highest decolorization efficiencies of 58.0 and 49.1% were detected with *T. elegans* and *Ganoderma* sp.2 (PW17-06), respectively. Other species showed decolorization efficiencies between 12.11-23.78% during the retention time of 5 d.

### Long-term Performance Evaluation of *T. Elegans* for Decolorization, Enzyme Production, Biomass Generation, and pH Changes

The variations of ADMI and pH are shown in Fig. 2a. Color removal efficiencies and enzyme activities with respect to time are plotted in Fig. 2b. The initial color (at 0 d) of POME was 4506 ( $\pm 143.54$ ) ADMI. No significant changes in the ADMI were observed until 6 d. Hence, no decolorization efficiencies were obtained. However, the color rapidly decreased to 2356 ( $\pm 361.01$ ) ADMI at 8 d. During this period (between 6 to 8 d), the color removal rate was 900 ADMI  $d^{-1}$ . As a result, a decolorization efficiency of 47.7( $\pm 6.94$ ) % was reached at 8 d in POME. The MnP enzyme activities

were gradually increased during the initial period and reached 34.63 ( $\pm 6.07$ ) U.L $^{-1}$  at 6 d. The rapid decolorization after 6 d could be attributed to enzyme production. The initial pH of 7.18 ( $\pm 0.02$ ) was decreased to 3.24 ( $\pm 0.19$ ) at 8 d with the decolorization of POME.

However, no significant changes in decolorization efficiencies were observed within the duration of 8 to 18 d. Similarly, pH in the POME was not significantly varied during this period. The MnP enzyme activities were also decreased, suggesting that the color removal was attributed to the MnP enzymatic biodegradation. The highest MnP production was 36.03 U.L $^{-1}$  in 10 d. MnP was not found on day 18, and color values tended to decrease. The color observed in POME at 14 d is shown in Fig. 3. It was within the period that fungi achieved the highest decolorization efficiencies. The dark brownish color found in the original POME was observed in the abiotic control. The biotic experiments showed a comparatively light brownish color. The original color of the fungal biomass observed at 0 d was changed to a dark brownish color when inoculated to the POME, as shown in Fig. 4. Thus, these observations indicate that fungi were in charge of the biosorption of color compounds.

The variation of fungal biomass concentration with respect to time is presented in Fig. 4. The initial biomass concentration was 0.55 ( $\pm 0.07$ ) g.L $^{-1}$ . No biomass growth was observed until 6 d. At 8 d, a biomass concentration of 2.29 ( $\pm 0.49$ ) g L $^{-1}$  was detected. During the period between 6 to 8 d, the biomass growth rate was 0.57 g.L $^{-1}$ .d $^{-1}$ . Similarly, higher decolorization rates were observed during this period, as

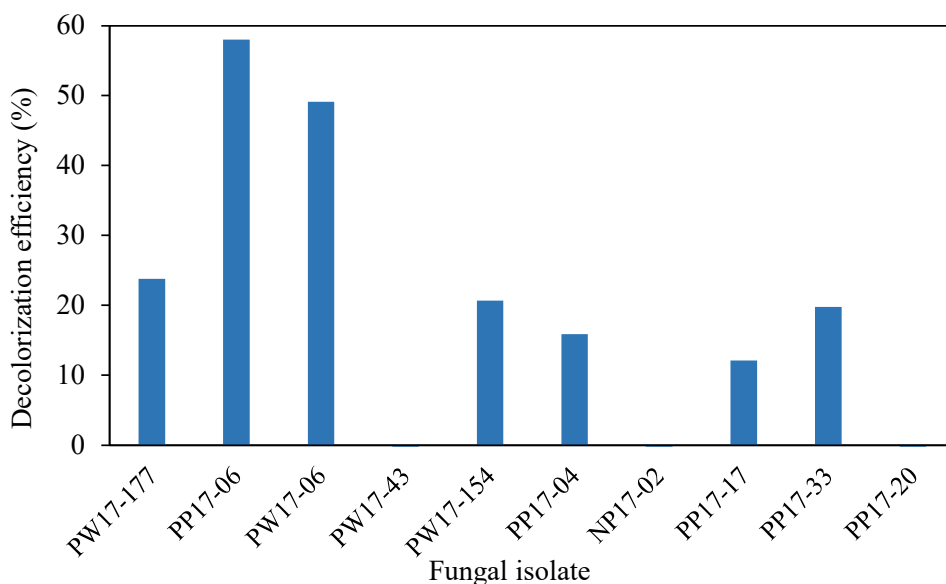


Fig. 1: The decolorization efficiencies of 10 isolated fungi after 5 d of incubation in palm oil mill effluent (POME).

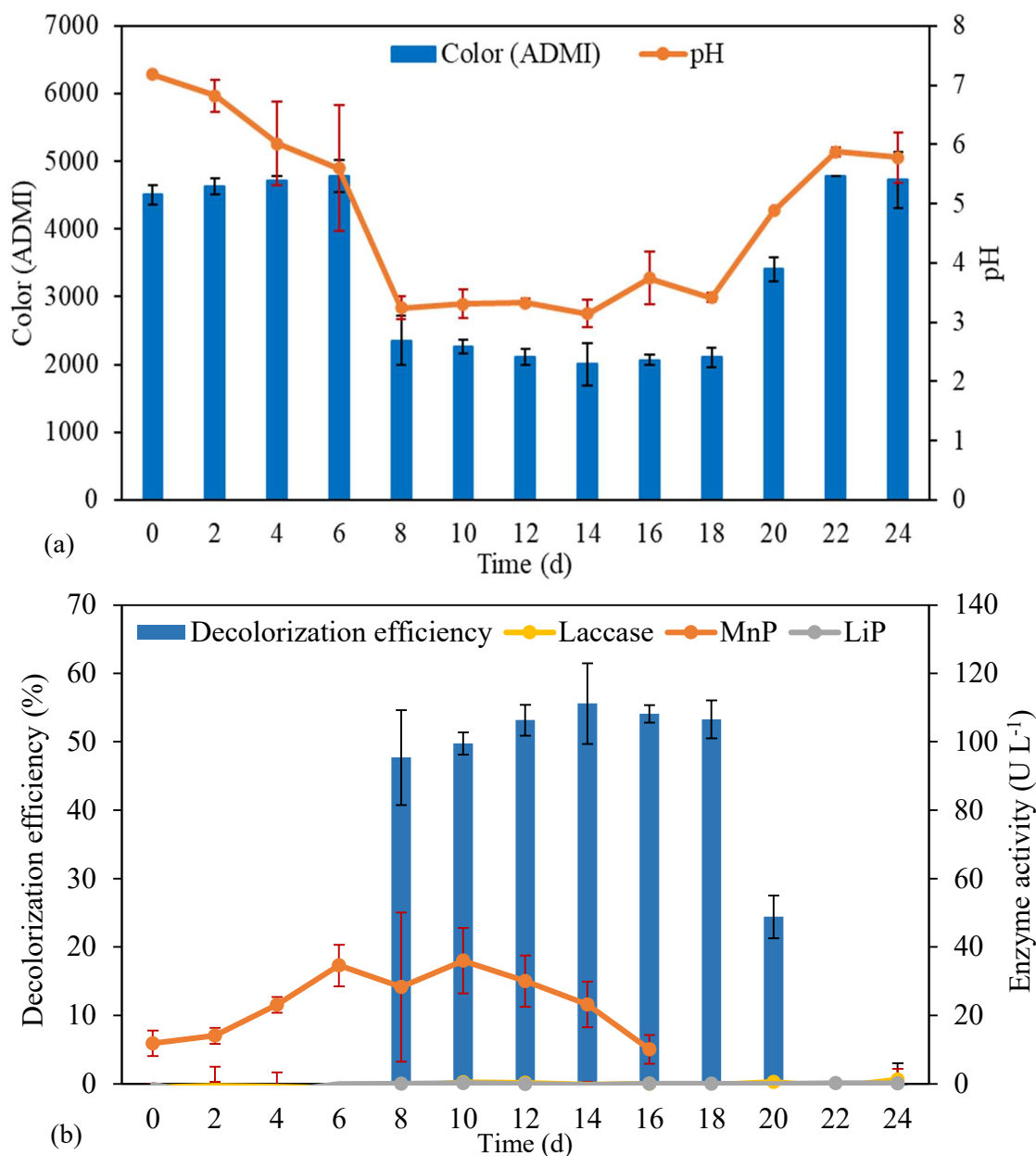


Fig. 2: Variations of (a) color and pH and (b) decolorization efficiency and enzyme activities with respect to time using *T. elegans*. The respective color of the column or line chart is used in the letters. A comparison of means of enzyme activities is provided only for MnP. Bars indicate the standard deviation of the mean ( $\pm$ SD).

discussed above. Thus, the decrease in color could be attributed to the consumption of color compounds in the POME during fungal biomass growth. However, despite no significant changes in color after 8 d, the biomass concentration gradually increased and reached a peak of  $7.24 (\pm 0.79) \text{ g.L}^{-1}$  at 18 d.

#### Evaluation of Biosorption Capabilities of *T. elegans* Biomass

The variations of color and decolorization efficiency with

respect to time are illustrated in Fig. 5. The initial color measured in the POME was  $4259.0 (\pm 20.1)$  ADMI. It was reduced to  $3727 (\pm 104.04)$  ADMI at the optimal time of 2 d, reaching a corresponding decolorization efficiency of  $12.50 (\pm 2.44) \%$ . After 2 d, no significant increase in the decolorization efficiency was detected.

#### DISCUSSION

Various color removal efficiencies were obtained with



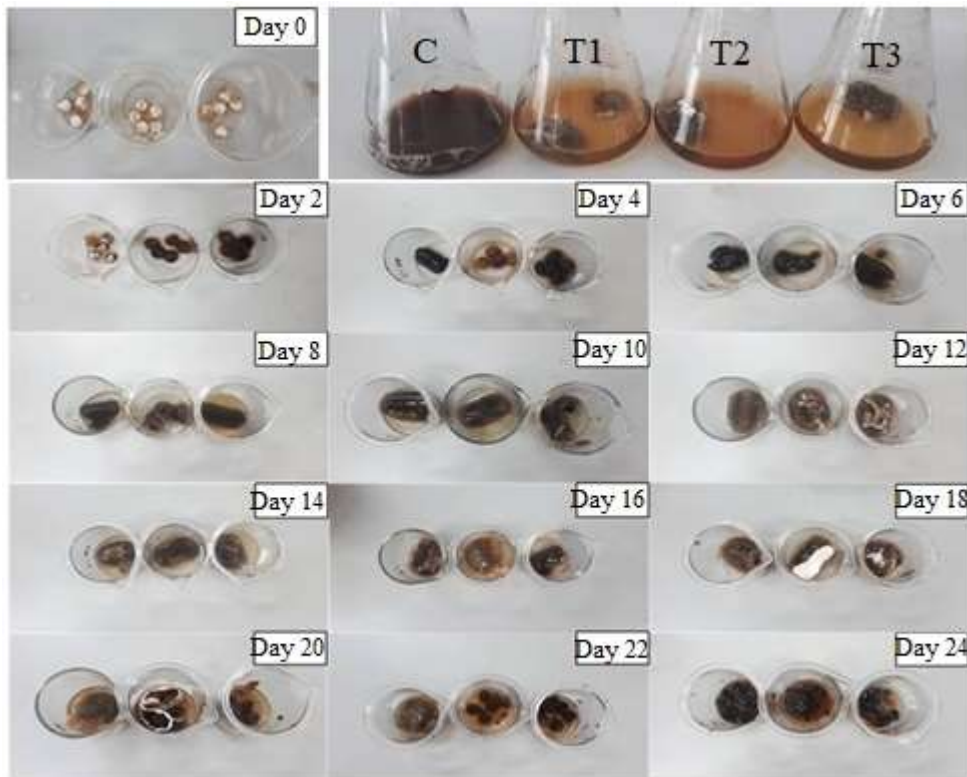


Fig. 3: Visual appearance observed in *Telegans* biomass during the palm oil mill effluent (POME) treatment period of 24 d. A comparison of color observed in POME at 14 d is shown in the upper right corner. C is the abiotic control. T1, T2, and T3 are biotic triplicates inoculated with *Telegans*.

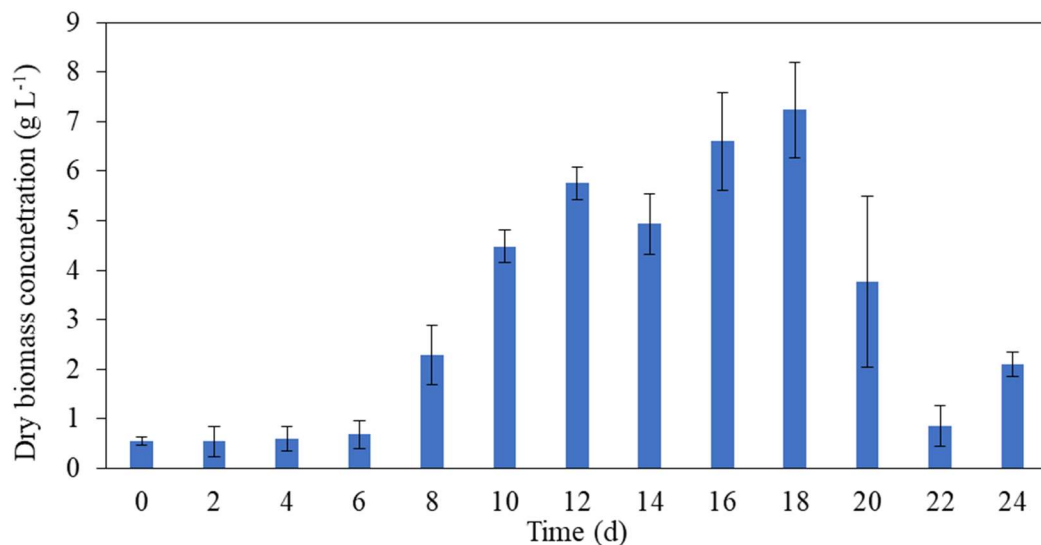


Fig. 4: Variations of dry *Telegans* biomass concentration with respect to time. Bars indicate the standard deviation of the mean ( $\pm$ SD).

different strains of fungi. In degrading a certain compound, the biological activities of different species can be varied (Sagar et al. 2020). Thus, the compounds available in POME could be easily degraded by some fungi species, while the

degradation process could be hindered in other species. Among the ten isolated fungi, seven isolates of white-rot fungi were able to decolorize POME. Results indicate that the ligninolytic enzymes, including laccase and manganese

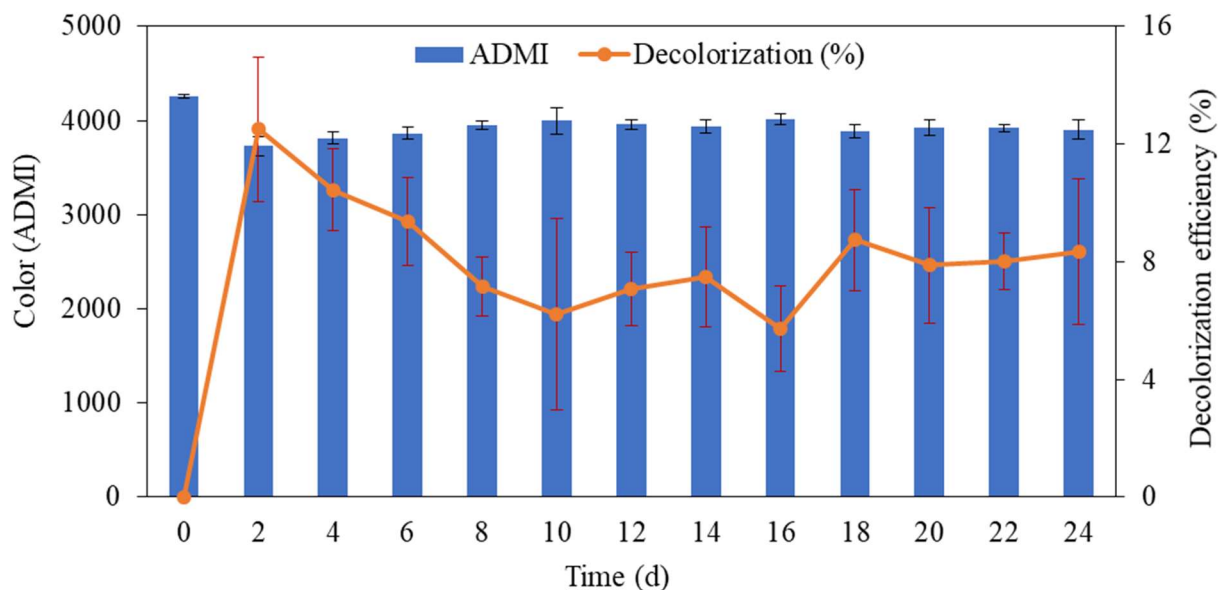


Fig. 5: Variations of color and decolorization efficiency with respect to time in treating palm oil mill effluent through biosorption on *T.elegans* biomass. Bars indicate the standard deviation of the mean ( $\pm$ SD).

peroxidase (MnP), can degrade color compounds that are unable to reduce during aerobic or anaerobic treatment (Raghukumar et al. 1996, Srinivasan & Viraraghavan 2010). Thus, white-rot fungi provide a promising solution for the further treatment of POME. However, the removal efficiencies were not satisfactory during 5 d of treatment time. To evaluate the decolorization performance in long-term applications, *T.elegans*, which showed the highest decolorization efficiency, was further tested for an extended period of 24 days.

However, extending the retention time did not elevate the decolorization efficiencies. The optimum retention time for the color removal was 8 d. After 18 d, decolorization efficiency was decreased. A similar phenomenon was reported with *Aspergillus flavus* and *Penicillium canescens* reaching maximum decolorization efficiencies of 97 and 92% at 7 d. No elevation in decolorization efficiency was reported, further extending the retention period to 14 d (Hefnawy et al. 2017). *Aspergillus* 2BNL1 and *Aspergillus* 1AAL1 reported maximum decolorization efficiencies between 81–95% at an optimal retention time of 1 d, while *Lentinus edodes* UEC2019 reached similar levels at 3 d (Souza et al. 2005). When the retention time increases, available nutrients in the growth medium are insufficient for the growth of fungi. As a result, the enzyme digests its enzymes, which is referred to as autolysis, releasing intracellular chromophore compounds. These compounds can further increase the color of the growth medium (Souza et al. 2014, 2005).

In this study, the highest decolorization efficiency reached by *T.elegans* was 55.61 ( $\pm$ 5.89) %. Decolorization efficiencies reported with different types of wastewater are summarized in Table 2. In decolorizing POME, *Trametes hirsuta* AK 04, which was immobilized onto oil palm fibers, reached decolorization efficiencies up to 87.1% after 8 d of incubation. It was reported immobilized fungi are more resistant to inhibitory compounds in POME than in the mobilized bacteria (Kietkwanboot et al. 2015).

Despite no significant changes monitored in color between the period of 8–18 d, the fungal biomass concentration gradually increased and reached the maximum of 7.24 ( $\pm$ 0.96) g.L<sup>-1</sup> at 18 d. POME contains various compounds that are added through the processes of palm oil production. Easily biodegradable compounds were utilized by fungi during the initial period, contributing to the color removal. However, some compounds in POME were reluctant to fungal biodegradation. Thus, these compounds still existed in the POME, contributing to the color in the later period. However, despite no degradation of color compounds occurring, the fungal biomass was gradually increased due to the consumption of other compounds. Generally, the fungal biodegradation process occurs through primary and secondary metabolisms. Fungi utilized a co-substrate such as glucose in the primary metabolisms. In the secondary metabolisms, laccase, MnP, and LiP enzymes carry out the color degradation (Pazarlıoğlu et al. 2005, Pedroza et al. 2007, Ramsay et al. 2005). In this study, POME was enriched by 10 g.L<sup>-1</sup> of glucose to enhance the decolorization process.

Table 2: Decolorization efficiencies reported with different types of wastewater using fungi.

Fungal species	Wastewater	Treatment system	T <sup>a</sup> [d]	DE <sup>b</sup> [%]	Reference
<i>Trametes Hirsuta</i> AK 04	Palm oil mill effluent	Two-stage biological process	8	87.1	Kietkwanboot et al. (2015)
<i>Phanerochaete flavido-alba</i>	Olive oil mill wastewater	Laboratory-scale bioreactor	18.75	70	Blázquez et al. (2002)
<i>Pleurotus ostreatus</i>	Apple processing residues	Packed-bed reactor	1	50	Iandolo et al. (2011)
<i>Aspergillus flavus</i> F10	Pulp mill effluent	Biological treatment	10	66.32	Barapatre and Jha (2016)
<i>Fibrodontia</i> sp. RCK783S	Pulp mill effluent	NR*	5	52.2-54.8	Kreetachat et al. (2016)
<i>Penicillium glabrum</i> Pg1	Textile wastewater	Packed bed bioreactor	3	98.2	Arikan et al. (2019)
<i>Phanerochaete velutina</i>	Textile wastewater	NR*	14	55	Zafiu et al. (2021)
<i>Saccharomyces cerevisiae</i>	Synthetic melanoidin wastewater	Packed bed bioreactor	0.25	70	Tsiakiri et al. (2020)
<i>T. elegans</i>	Palm oil mill effluent	Laboratory-scale reactor	8	47.72	This study

a: Treatment time, b: Decolorization efficiency, NR\*: Not reported

In this study, the isolated fungi showed significantly high MnP production compared to laccase. These findings indicate that the laccase and LiP enzymes were not active during the biodegradation of color compounds. The redox potential of laccase is lower compared to MnP. Thus, laccase degrades compounds with low redox potential. MnP enzyme can oxidize more recalcitrant aromatic compounds due to the higher redox potential (Fang et al. 2018, 2020). The higher MnP production could be attributed to the presence of recalcitrant aromatic compounds in the POME.

In addition to the enzymatic biodegradation of color compounds, part of the color removal could be attributed to the biosorption of fungal biomass. In the next experiment, fungal biomass was exposed to HgCl<sub>2</sub> to kill the mycelium and avoid the further synthesis of fungal enzymes. Thus, biosorption can act as a mechanism in the decolorizing of POME. In this study, the optimum decolorization through biosorption occurred at 2 d. Further increase in the contact time did not enhance the color removal. Similar behavior was observed (Kabbout & Taha 2014) with *Aspergillus fumigatus*. Biosorption of methylene blue reached a maximum of 71% at 210 min. After that, no significant increase in biosorption occurred.

## CONCLUSIONS

*T. elegans* showed decolorization of palm oil mill effluent utilizing both biodegradation and biosorption mechanisms. The optimal retention period for the color removal by biodegradation was 8 d, reaching a decolorization efficiency of 47.4%. MnP enzymes were useful in degrading complex color compounds. A decolorization efficiency of 12.5% was achieved during the optimal contact time of 2 d during biosorption on fungal biomass. Despite fungi showing the potential for decolorization of palm oil mill effluent, the

decolorization efficiencies were not satisfactory. The treated palm oil mill effluent was not sufficient to the purified levels that can be discharged into natural water bodies. Fungi-based decolorization can be coupled with other advanced treatment technologies to develop more efficient, sustainable, and economical methods.

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## ORCID DETAILS OF THE AUTHORS

Apichaya Sawasdee: <https://orcid.org/0000-0001-5947-5797>