White Rot Fungi Consortium Treatment Enhanced Recalcitrant Organic Pollutant Removal in River Water

Devi Kusuma Wardani
Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Indonesia

Idris, Idris
Research Center for Applied Microbiology, National Research and Innovation Agency, Indonesia

Purnaningsih, Ismu
Directorate of Culture Collection, National Research and Innovation Agency, Indonesia

Toga Pangihotan Napitupulu
Research Center for Applied Microbiology, National Research and Innovation Agency, Indonesia

他

https://doi.org/10.5109/7160870

出版情報：Evergreen. 10 (4), pp.2092-2104, 2023-12. Transdisciplinary Research and Education Center for Green Technologies, Kyushu University
バージョン：

White Rot Fungi Consortium Treatment Enhanced Recalcitrant Organic Pollutant Removal in River Water

Devi Kusuma Wardani[1], Idris Idris[2*], Ismu Purnaningsih[3], Toga Pangihotan Napitupulu[2], Indriati Ramadhani[4], Masrukhin[4], Yustian Rovi Alfiansah[2], Atit Kanti[4], Wibowo Mangunwardoyo[1], I Made Sudiana[2]

[1]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Indonesia, Indonesia

[2]Research Center for Applied Microbiology, National Research and Innovation Agency, Indonesia

[3]Directorate of Culture Collection, National Research and Innovation Agency, Indonesia

[4]Research Center for Biosystematics and Evolution, National Research and Innovation Agency, Indonesia

*Author to whom correspondence should be addressed:
E-mail: idri002@brin.go.id

(Received July 27, 2023; Revised December 5, 2023; accepted December 12, 2023).

Abstract: The Sentiong River, located in Indonesia, has been polluted by various organic compounds originating from household and small-scale industries. Sentiong River water has a pungent odor and blackish color. The study aimed to determine the feasibility of five white-rot fungi species to degrade the organic pollutant compounds in the Sentiong River water hence avoiding the production of a pungent odor and blackish sediment. White rot fungi are well known for their ability to degrade or mineralize a wide range of organic pollutants. The study was started by a screening of potential fungi isolates to obtain the best ligninolytic fungi. A compatibility test was performed to verify the interspecies interaction between the isolates as a consortium. The biodegradation of organic compounds in river water was performed in two scenarios, biodegradation by a single culture using the best ligninolytic fungi isolate and by a consortium of five fungal isolates. The parameters observed include total carbon, ligninolytic enzymes activity (lignin peroxidase, mangan peroxidase, and laccase), and profiling the reduction of organic compounds analyzed by gas chromatography-mass spectrometry (GC-MS). The screening results showed that all five fungal isolates showed ligninolytic activity and *Pycnoporus sanguineus* IN004 has the best activity compared to the others whose averaged ligninase activity of 55.86 U/mL. Our study revealed that the fungal consortium degraded recalcitrant organic compounds more rapidly than a single fungal isolate The fungal consortium is able to remove about 63.46% of total dissolved carbon in 14 days of incubation, while the single isolate *Pycnoporus sanguineus* IN004 only has 42.33% of total dissolved carbon. However, at the final incubation period (21 days), both treatments yielded the quite similar results. Fungal ligninolytic enzymes (lignin peroxidase, mangan peroxidase, and laccase) play a role in the recalcitrant organic pollutant degradation process that was proved by the increase of ligninolytic enzymes activity along the treatments. This study revealed that this fungal consortium was able to act in synergism toward organic pollutant biodegradation in river water and was preferable to reduce recalcitrant substances in polluted river water.

Keywords: fungal consortium, ligninolytic; polluted river; mycoremediation; recalcitrant organic pollutant; white rote fungi

1. Introduction

Anthropogenic activities and an increase in the global population are causing an increase in water quality problems throughout the world[1)]. Some of organic pollutants are recalcitrant and belong to the main groups of environmental contaminants, which cause significant effects on human health and aquatic ecosystems[2)]. Pollutant compounds are generally resistant to degrade by microbial activities[3)].

Sentiong River is one of the rivers in Indonesia that has been polluted by various organic pollutant compounds from household and small-scale industrial wastes. Sentiong River water has a pungent odor and blackish color. The pungent odor in the river may be caused by the presence of hydrogen sulfide (H_2S)

compounds, while the blackish color is caused by the presence of metal sulfide particles that are formed when H_2S reacts with metal ions in the water. This condition is caused by the metabolic process of organic carbon compounds by sulfate-reducing bacteria[4]. Ko et al. (2009) mentioned that lignin and other recalcitrant organic compounds (ROC) cannot be degraded well by sulfate-reducing bacteria under anaerobic conditions[5]. Various efforts have been made by the government and community groups, including the installation of plasma nanobubble generator systems, blowers, aerators, bioaugmentation with lactic acid bacterial inoculums (*Lactobacillus* spp.), and odor removal powder (containing fungal inoculum *Omphalina* sp.). However, various bioremediation techniques have not been optimal in eliminating the pungent odor and pollutant compounds found in the Sentiong River. Therefore, study to find the best method to solve this problem are still needed.

Various methods have been developed to deal with recalcitrant pollutant, including physical, chemical, and biological method[6,7]. Biological approaches or bioremediation are favored due to their low cost and environmental friendliness[8]. Bioremediation uses microorganisms to remove pollutant compound in environment[6].

This study tried to examine the feasibility of white rot fungi to remove organic pollutant in polluted water river. White rot fungi (WRF) are well known for their ability to degrade or mineralize a wide range of organic pollutant, particularly persistent organic pollutant that has a similar structure to lignin molecules, such as polyaromatic hydrocarbon, polychlorinated biphenyl, synthetic polymers, and synthetic dyes by involving the activity of ligninolytic enzymes[9]. Indonesian Culture Collection (InaCC), National Research and Innovation Agency, West Java has several collections of WRF cultures, but its ability to degrade pollutant compounds has not been evaluated yet. This study aimed to obtain the best ligninolytic white-rot fungus isolates and evaluate the ability of the best ligninolytic isolate, and a consortium of five isolates on mycoremediation of polluted river water. The sample collection for this study took place at Sentiong River, Jakarta, Indonesia.

2. Materials and methods

2.1 Source of microorganism

Microorganisms used in this study were *Pleurotus ostreatus* InaCC F109, *Agaricus campestris* InaCC F126, *Trametes versicolor* InaCC F200, *Amauroderma rugosum* IN002, and *Pycnoporus sanguineus* IN004. The fungal cultures were obtained from the Indonesian Culture Collection (InaCC), National Research and Innovation Agency. All of fungi cultures were maintained in potatoes dextrose agar (PDA) prior use of experimental works[10].

2.2 Sampling location

Four river water samples from Sentiong River, Jakarta, were collected at 09.00-11.00 AM on sunny weather with a temperature range of 29-31°C. Each water sample was collected from different locations, Location 1 (-6°09’09.35” S, 106°51’39.82”E), Location 2 (-6°08’38.59”S, 106°51’26.74”E), Location 3 (-6°08’05.22”S, 106°51’18.43”E), and Location 4 (-6°08’56.57”S, 106°51’30.77”E). A total of 1 L of water samples from each location point was collected into a sterile glass bottle using a stainless-steel bucket. Water samples were mixed and homogenized.

2.3. Screening for ligninolytic activity of white rot fungi isolates

2.3.1 Growth ability

Growth ability was conducted in minimal media lignin (MM-L). Solid MM-L was made with alkali lignin as a carbon source. Alkali lignin powder obtained from oil palm empty fruit bunch powder (OPEFB) was prepared according to Barapatre et al.[11]. The composition of solid MM-L/1000 mL was 100 mL of Stock I, 1 mL of Stock II, 10 g of lignin powder, 20 g of agar, and 900 mL of distilled water. Stock solution I (macronutrients) was made of 45.5 g/L K_2HPO_4, 5.3 g/L KH_2PO_4, 5.0 g/L, $CaCl_2.2H_2O$, 5.0 g/L $MgSO_4.7H_2O$ and 5.0 g/L NH_4NO_3. While Stock Solution II (micronutrients) consisted of 1 g/L $CuSO_4.5H_2O$, 1 g/L $FeSO_4.7H_2O$, 1 g/L $MnSO_4.H_2O$, and 1 g/L $ZnSO_4.7H_2O$. The mixture was then autoclaved at 121°C for 15 minutes[12].

One disc (5 mm diameter) fungal inoculum obtained from the edge of the 7 days old colony was placed on the centre of solid media. Fungi isolates were grown on the PDA medium as a comparison. Petri dishes were incubated at 30°C and observed on the 7th day. The isolate growth on solid MM-L was determined by radial growth and fungal biomass and assessment of lignin degradation zone scores. Radial growth measurements and fungal biomass were carried out according to Ajdari et al.[13].

2.3.2 Guaiacol oxidation test

Qualitative detection of ligninolytic activity was conducted by inoculating fungi on the PDA supplemented with 100 mM of guaiacol. One disc (5 mm diameter) of inoculum was taken from the edge of the colony. The isolate was incubated at 30°C and observed on the 7th day of incubation. Positive results showed by the reddish-brown color appearance surrounding the fungal colony[14].

2.3.3 Ligninolytic enzyme activity assay

Ten fungal mycelia discs were inoculated in liquid MM-L, then incubated at 30°C in static conditions. Data collections on ligninolytic enzyme activity were carried out on days 3, 5, and 7. Crude enzyme (CE) extraction was carried out by taking 1.5 mL of liquid MM-L, then

centrifuged at 10000 rpm for 15 minutes. Supernatants were used to measure ligninolytic enzyme activity[15]. Lignin peroxidase (LiP) activity measurement was carried out by mixing 0.1 mL of 8 mM veratryl alcohol; 0.2 mL acetate buffer pH 3 50 mM; 0.45 mL of distilled water; 0.05 mL of 5 mM H_2O_2 and 0.2 mL of CE filtrate; the mixture was put into the cuvette. The manganese peroxidase (MnP) activity was tested by mixing 0.1 mL of lactate buffer pH 4.5 50 mM; 0.1 mL guaiacol 4 mM; 0.3 mL of distilled water; 0.1 mL H_2O_2 1 mM; 0.2 mL of 1 mM $MnSO_4$ and 0.2 mL of CE filtrate; the mixture was added to the cuvette. While laccase activity measurement was carried out by mixing 0.5 mL acetate buffer pH 5 0.5 M; 0.1 mL of 1 mM guaiacol and 0.4 mL of the CE filtrate; the mixture was added to the cuvette. The solution was shaken slowly. Absorbance was measured at 0 and 5 minutes, at a wavelength (λ) 310 nm for LiP activity and 465 nm for MnP and laccase.

The formula for calculating enzyme activity (U/L) is shown in Eq. 1, according to Baltierra-Trejo et al.[16].

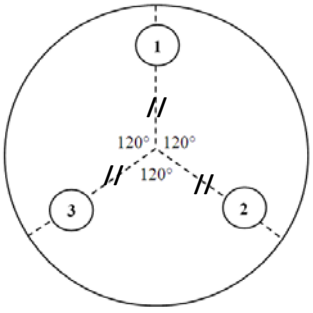
$$\text{Activity (U/L)} = \frac{(\text{At} - \text{Ao})\lambda \text{ x } V_{\text{reaction}} \text{ x } 10^6}{\varepsilon_{\text{max}} \text{ x d x } V_{\text{enzyme}} \text{ x t}}$$

Where Ao is the value of initial absorbance and At is the absorbance at t; λ is the wavelength (310 nm for LiP; 465 nm for MnP and laccase); εmax is the molar absorptivity (for guaiacol is 12100/M cm; veratryl alcohol is 9300/M cm); d is the cuvette thickness (cm); Venzyme is the volume of crude enzyme (mL); Vreaction is the total volume of reaction (mL); t is the reaction time (min).

For LiP activity, one unit (U) is defined as the amount of enzyme required to oxidize 1 μmole veratryl alcohol per minute. For MnP activity, one U is defined as the amount of enzyme required to oxidize 1 μmole guaiacol per minute involved H_2O_2. For laccase activity, one U is defined as the amount of enzyme required to oxidize 1 μmole guaiacol per minute.

2.4 Compatibility test of white rot fungi isolates

The compatibility test was carried out according to Mohammad et al.[17]. The interaction was tested in combination with every 3 isolates into 4 different PDA Petri dishes (Figure 1). Fungi isolates were divided into two groups; slow-growing fungi and fast-growing fungi. The fast-growing fungi group is WRF isolates that are able to form hypha colonies to meet Petri dishes 9 cm in diameter on the seventh day after inoculation, while the slow-growing fungi group consists of isolates that need more than 7 days incubation period. Based on this statement, isolates included in the slow-growing fungi group were InaCC F109, InaCC 126, and IN002; while InaCC F200 and IN004 isolates were included in the fast-growing fungi group. Fast-growing fungal inoculums were inoculated 3 days after hyphal growth in slow-growing fungi has been observed. Petri dishes were incubated at 30°C and the observation was done until the interactions between isolates were observed.



length // = 2 cm

Fig. 1: Fungal isolates compatibility test on PDA petri dish

2.5 River water mycoremediation test

Preparation of river water samples was carried out by biostimulation; the addition of 1% glucose, 4 mL stock I, and 0.04 mL stock II into each 330 mL glass bottle containing 40 mL of river water sample. The mixture was autoclaved at 121°C for 15 minutes. Each system (batch) was used for one-time data retrieval. Inoculum preparation was done by growing isolates on solid MM-L. The isolate was incubated at 30°C for 7 days. A total of 10 discs of fungal inoculum (5 mm diameter) were inoculated into each glass bottle containing a sample of river water prepared. There are three treatment groups, Control (Not inoculated by WRF); Treatment I (Inoculated by a single WRF isolate with the best ligninolytic ability); Treatment II (Inoculated by WRF consortium (InaCC F109, InaCC F126, InaCC F200, IN002, and IN004).

The three treatment groups were incubated for 21 days under static conditions at 30°C. The parameters were measured on days 3, 5, 7, 14, and 21; pH, total dissolved carbon (TDC), ligninolytic enzyme activity (LiP, MnP and laccase), and fungal dry biomass. Types and abundance of organic compounds were known based on GC-MS analysis on the 14th and 21st days of incubation. Measurement of TDC levels in mycoremediation samples was carried out using Shimadzu TOC VCSH and was filtered with 0.22 μm before analysis. pH measurement was carried out using a pH meter. The enzyme extraction and the measurement of ligninolytic enzyme activity were carried out according to the method listed on the screening of fungi isolates on liquid MM-L. Organic compounds analysis was carried out using the Shimadzu GC MS-QP 2010 Ultra instrument. All peaks were identified from their mass spectra by comparison with spectral NIST 02 MS libraries.

2.6 Data collection and analysis

Experiments were set up in two replications for the abundance of organic compounds (GC-MS) data, or three replications for data on radial growth and fungi dry

biomass, ligninolytic enzyme activity, TDC, and pH. Data presented are the mean value ± standard error (SE). Data analysis and conclusion making were carried out descriptively.

3. Results

3.1 Ligninolytic Activity Assay of White Rot Fungi Isolates

All fungi were able to grow on solid MM-L with different colony diameters and biomass (Figure 2). Mycelia was thin with low density and had a white transparent color. The largest colony diameter isolate on solid MM-L was IN004 (9 cm); followed by F200 (7.9 cm); F126 (6.3 cm); IN002 (6.2 cm) and F109 (5,3 cm). The radial growth of mycelium of F109, F126, and F200 was more optimal on PDA than MM-L. IN004 has the same colony diameter when grown on solid MM-L and PDA. While the IN002 colony diameter on MM-L was larger when compared to PDA. However, based on morphological observations, IN002 had the lowest mycelia density compared to the other four isolates.

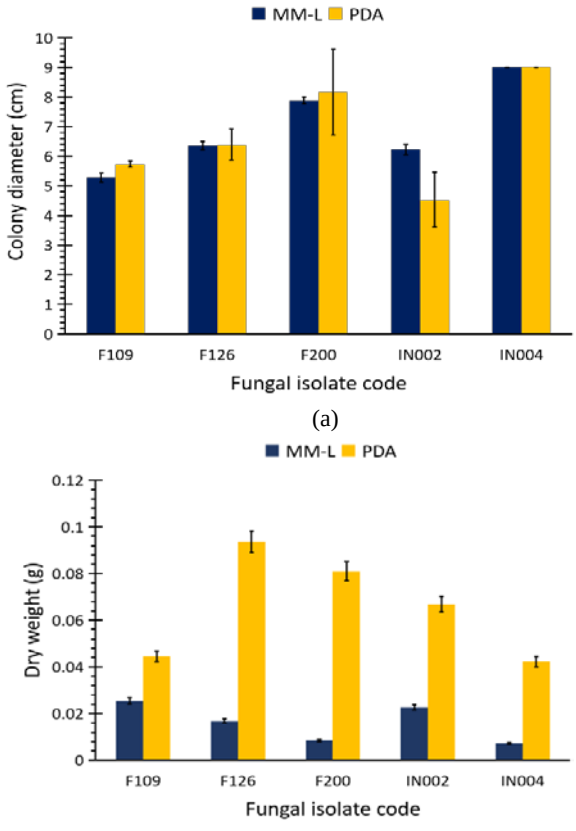


Fig. 2: The growth of white rot fungi isolates (InaCC F109, InaCC F126, InaCC F200, IN002 and IN004) on solid MM-L and PDA medium after 7 days incubation period: (a) Radial growth, (b) Dry biomass.

All of the fungal isolates showed the decrease of growth in MM-L medium implied by the low dry weight of fungal mycelia compare to PDA medium (Figure 2b). F126 (0.0937 g) has the biggest mycelium dry weight isolate on PDA; followed by F200 (0.0809 g); IN002 (0.0668 g); F109 (0.0445 g); and IN004 (0.0422 g). While the biggest dry weight isolate on solid MM-L was F109 (0.0254 g); followed by IN002 (0.0227 g); F126 (0.0168 g); F200 (0.0085 g) and IN004 (0.0072 g). All fungi isolates had greater dry weight when grown on PDA medium compared to MM-L.

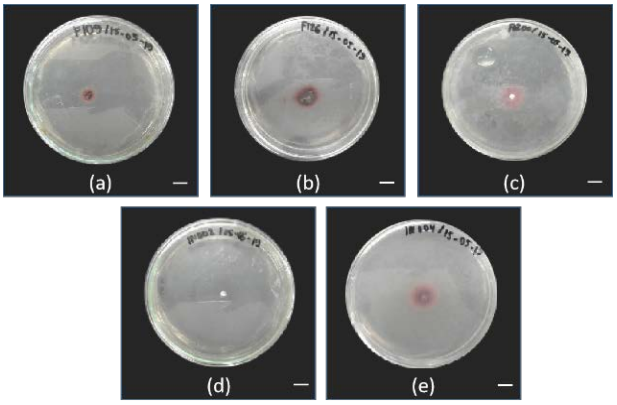


Fig. 3: Fungal isolates on PDA-Guaiacol medium after 7th days incubation. (a) InaCC F109, (b) InaCC F126, (c) InaCC F200, (d) InaCC IN002, (e) IN004 isolate (surface colony), (f) IN004 isolate (reverse colony). Bar = 1 cm.

All fungal isolates except IN002 had the reddish-brown zone around the fungal mycelia on medium supplemented with guaiacol (Figure 3). These results implied that all fungi isolates except IN002 have ligninolytic activity, particularly laccase enzyme. The highest value of LiP activity on days 3, 5, and 7, respectively, was observed in IN004, IN002, and IN004 (Figure 4a). While the highest MnP activity on liquid MM-L on days 3, 5, and 7 respectively were F109, IN004, and F200 (Figure 4b). Laccase activity of all isolates on liquid MM-L on the 3rd day of incubation was high with a range of 29-31 U/L and then decreased on the 5th day in the range of 0-2.8 U/L (Figure 5c). The results of measurements of LiP, MnP, and Laccase activity were averaged as the activity of ligninolytic enzymes (Figure 4d). The highest ligninolytic enzyme activity (LEA) values on days 3 and 5 were IN004 and IN002, respectively. On the last day of the incubation period (day 7), IN004 had the highest value with LEA of 55.86 U/L; followed by F126 (52.73 U/L); IN002 (43.45 U/L); F109 (24.92 U/L); and F200 (23.15 U/L).

3.2 Compatibility test of white rot fungi isolates

Inhibition interactions occurred between InaCC F109 and InaCC F200; InaCC F109 and IN004; and InaCC F200 and IN004. While the inhibition with distance interaction occurred between InaCC F109 and InaCC F126, also InaCC F126 and InaCC F200. The initial invasion occurred between IN002 and IN004, InaCC F126 and IN002, also InaCC F126 and IN004. However, InaCC F200 and IN002 and InaCC F109 and IN002 were

thought to interact partially compatible or invasion at the initial stage. Some isolates undergo mycelium pigmentation in the interspecies interaction zone (Figure 5). The mycelia of InaCC F109 becomes thicker and was surrounded by InaCC F126 and InaCC F200, resulting in a deadlock region.

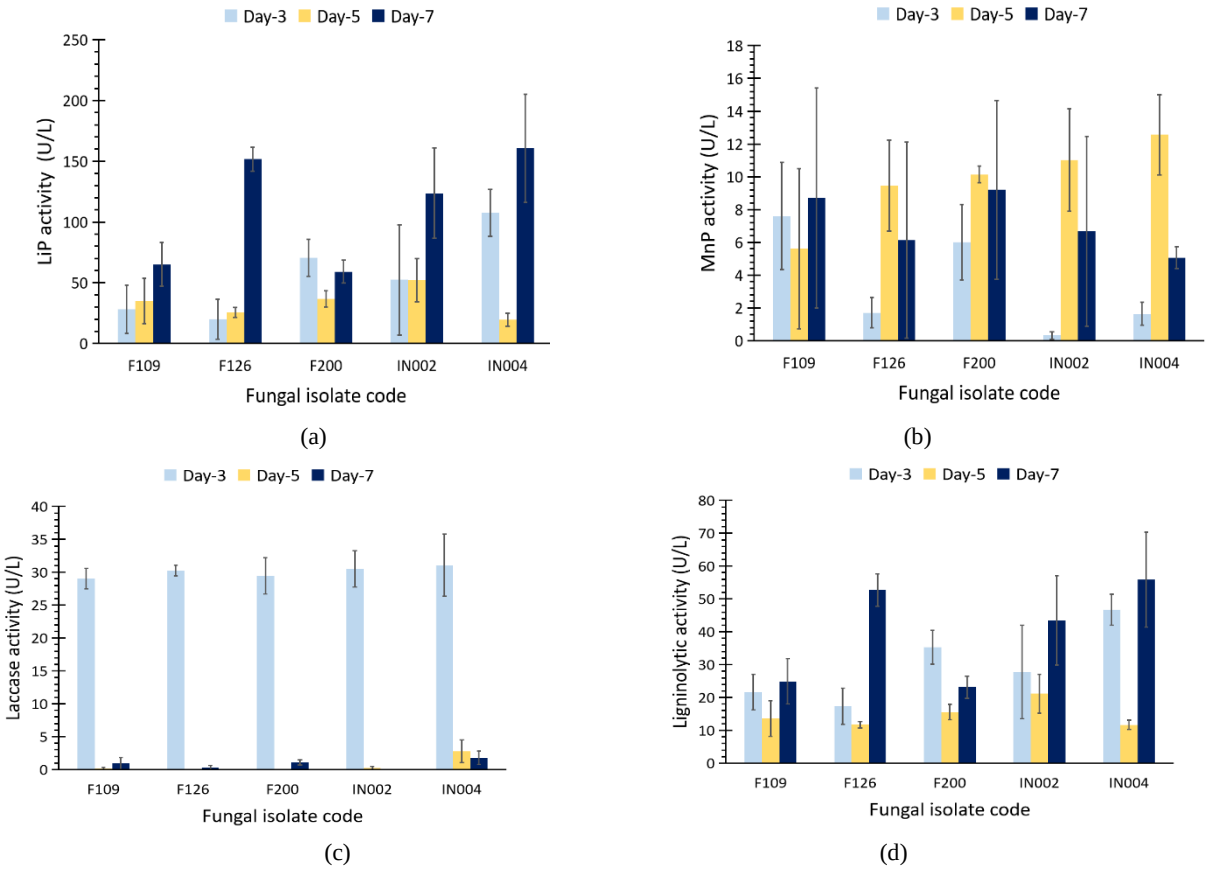


Fig. 4: Ligninolytic enzymes activity (LiP, MnP and Laccase) of five white rot fungi isolates (InaCC F109, InaCC F126, InaCC F200, IN002 and IN004) on liquid MM-L. (a) LiP, (b) MnP, (c) Laccase, (d) Ligninolytic Enzyme Activity.

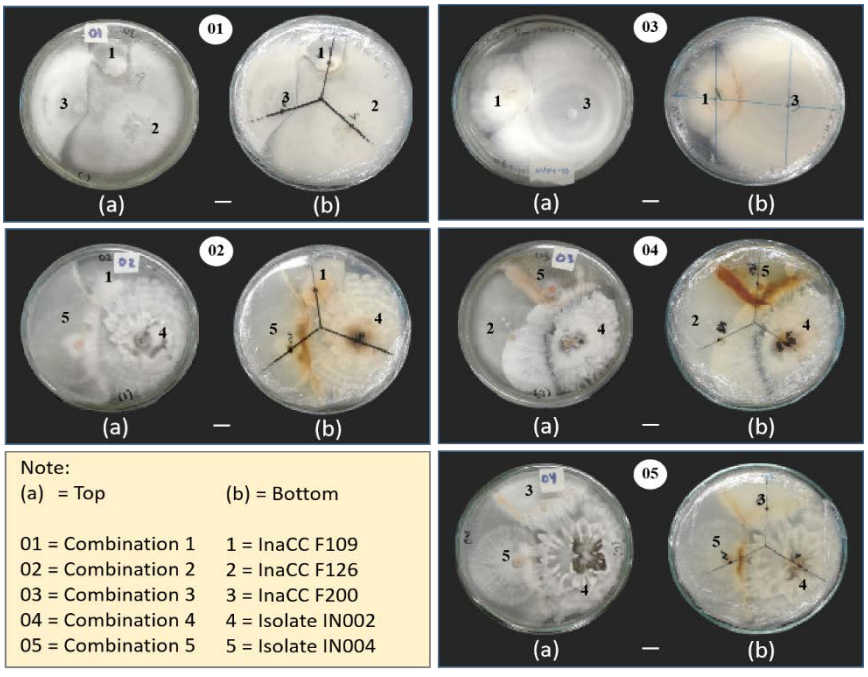
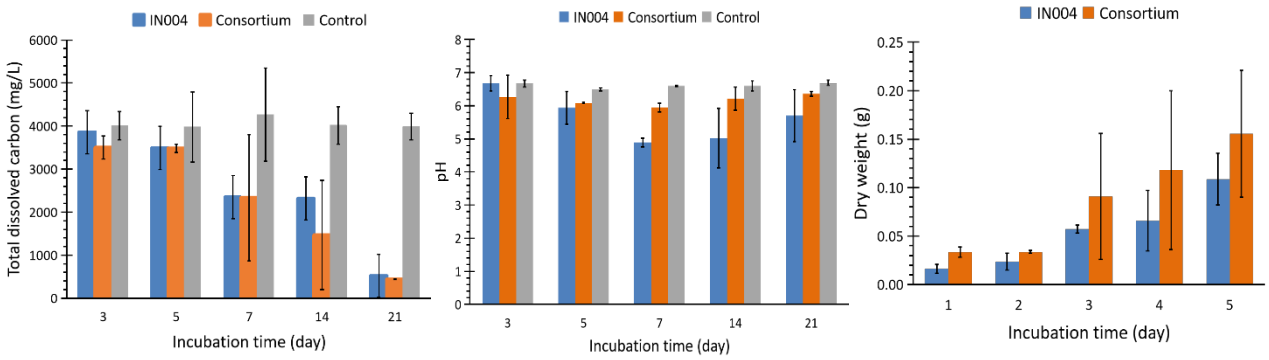


Fig. 5: Compatibility test of white rot fungi isolates on PDA petri dishes. Bar = 1 cm

3.3 River water mycoremediation

Mycoremediation of river water was carried out in two treatments, namely single isolate (*Pycnoporus sanguineus* IN004 isolate only) and consortium (consortia of all fungal isolates in this study). Mycoremediation treatment of river water was evaluated by measuring the total carbon value, pH, biomass, and ligninolytic enzymes activity as well as untargeted GC-MS analysis to profiling the compounds in the sample river water. The mean value of total dissolved carbon (TDC) in both mycoremediation treatments was decreased during the incubation period. The TDC value on the 14th and 21st day of the consortium treatment was lower than the treatment of a single isolate (Figure 6a). Up to 63,46% of TDC have been removed by fungal consortium in 14 days incubation and prolong incubation until 21 days increase the TDC removing up to 88,96% relative to control (un-treatment). On other hand, single isolate able to remove up to 42,33% and 86.94% of TDC in 14 and 21 days of incubation, respectively. The pH values of the two treatments were decreased from 6.68 to 4.89 on days 5 and 7, then increased up to 6.21 and 6.36 on days 14 and 21, respectively (Figure 6b). The fungi in both treatments were still experiencing a growth phase as shown by increasing the fungal dry biomass of both treatments during the incubation period (Figure 6c). The highest biomass was given by fungal consortium approximately 0.16 g of dry weight.



(a) (b) (c)

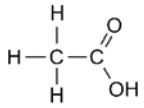
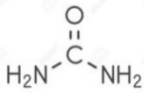
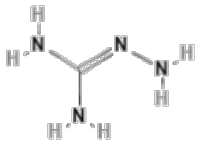
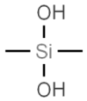
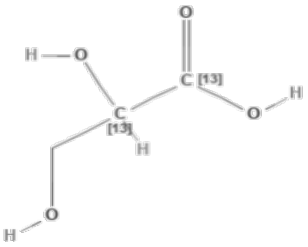
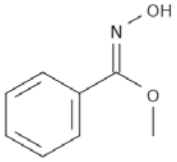
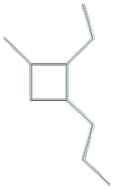
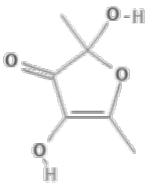
Fig. 6: Mycoremediation of Sentiong River Water with Treatment I (Pycnoporus sanguineus IN004) and Treatment II (fungal consortium). (a) Total Dissolved Carbon (TDC), (b) pH, (c) Fungal Dry Biomass.

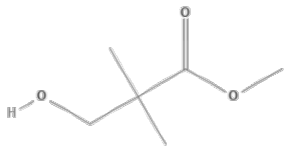
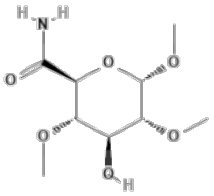
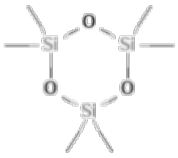
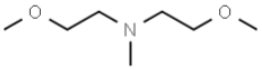
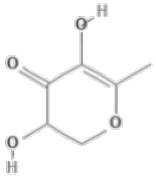
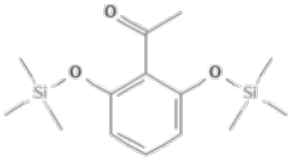
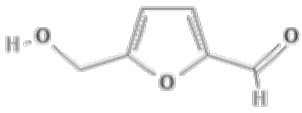
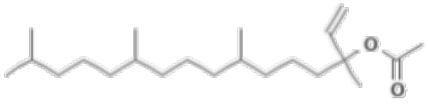
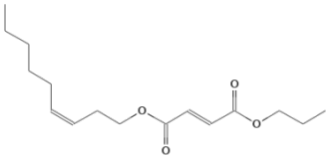
Based on GC-MS analysis of all treatment on the 14- and 21-days incubation, there were 12 types of organic compounds detected in the control river water sample and there were 7 types of organic compounds that was produced along treatment (Table 1). Comparing the composition of organic compounds detected by GC-MS analysis, fungal consortium (Treatment II) showed a better ability in removing or reducing organic compounds than the single isolate IN004 (Treatment I). Fungal consortium able to completely remove acetic acid, urea, aminoguanidine, 2,4-dihydroxy-2,5-dimethyl-3(2H)-furan-3-one, ethanamine, 2-methoxy-N-(2-methoxyethyl)-N-methyl-, and methyl 2,2-dimethyl-3-hydroxypropionate only in 14 days incubation, whiles single isolate needs 21 days incubation to remove it completely. Moreover, fungal consortium able to completely remove cyclotrisiloxane, hexamethyl- and 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl that single isolate unable to remove both of it. There were two compounds that were still exist in the water sample after treatment, silanediol, dimethyl and oxime-, methoxy-phenyl-. Nevertheless, their yields are reduced comparing to control and fungal consortium treatment showed the highest removing activity than single isolate treatment.

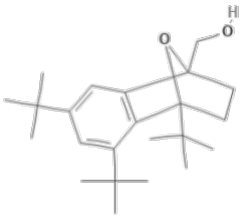
Several organic compounds were detected in Treatment I on day 21 that were not found in the control; propanoic acid, 2,3-dihydroxy; anhydro 5-mercapto-3-methyl-1,2,3,4-oxatriazolium hydroxide; and 2',6'-dihydroxyacetophenone, bis(trimethylsilyl) ether. Fumaric acid, cis-non-3-enyl propyl ester was found on the 14th day and 21st day by Treatment II. While 1,4-epoxynaphthalene-1(2H)-methanol, 4,5,7-tris(1,1-dimethylethyl)-3,4-dihydro- was found in Treatment II on the 21st day.

Ligninolytic enzyme activity was also observed during the mycoremediation treatment. LiP activity in treatments tended to increase (Figure 7b), corresponding to a decrease in TDC (Figure 6a) and an increase in fungal biomass (Figure 6c). The increase in LiP activity was in line with the increase in biomass of white-rot fungi isolates. MnP activity of treatment II was higher than the treatment I. The value of MnP activity of treatment II increased from day 3 to day 7 and there was a decrease in activity on days 14 and 21 (Figure 7b). Based on the LEA (the average activity of LiP, MnP, and Laccase), fungal consortium treatment was higher than single isolate treatment (Figure 7d).

Table 1 Detected organic compounds in the river water by GC-MS analysis along mycoremediation process.

No.	Organic compounds (Molecular formula)	Retention times	Molecular structure	C	Day-14		Day-21	
					T I	T II	T I	T II
1	Acetic acid (CH_3COOH)	2.087-2.649		+	+	-	-	-
2	Urea (CH_4N_2O)	2.150-2.512		+	+	-	+	-
3	Aminoguanidine (CH_6N_4)	2.341-2.535		+	+	-	-	-
4	Silanediol, dimethyl ($C_2H_8O_2Si$)	2.438-2.867		+	+	+	+	+
5	Propanoic acid, 2,3-dihydroxy ($C_3H_6O_4$)	3.392		-	-	-	+	-
6	Anhydro 5-mercapto-3-methyl-1,2,3,4-oxatriazolium hydroxide	3.737		-	-	-	+	-
7	Oxime-, methoxy-phenyl- ($C_8H_9NO_2$)	4.828--5.007		+	+	+	+	+
8	Cyclobutane, 2-ethyl-1-methyl-3-propyl- ($C_{10}H_{20}$)	5.190		-	+	-	-	-
9	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one ($C_6H_8O_4$)	6.211-6.345		+	+	-	-	-

No.	Organic compounds (Molecular formula)	Retention times	Molecular structure	C	Day-14		Day-21	
					T I	T II	T I	T II
10	Methyl 2,2-dimethyl-3-hydroxypropionate ($C_6H_{12}O_3$)	7.609-7.641		+	+	-	-	-
11	.alpha.-D-Glucopyranosiduronamide, methyl 2,4-di-O-methyl- ($C_9H_{17}NO_6$)	7.677		+	-	-	-	-
12	Cyclotrisiloxane, hexamethyl- ($C_6H_{18}O_3Si_3$)	8.171-8.190		+	+	-	+	-
13	Ethanamine, 2-methoxy-N-(2-methoxyethyl)-N-methyl- ($C_7H_{17}NO_2$)	9.489-9.490		+	+	-	-	-
14	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl - ($C_6H_8O_4$)	9.645-9.667		+	+	-	+	-
15	2',6'-Dihydroxyacetophenone, bis(trimethylsilyl) ether ($C_{14}H_{24}O_3Si_2$)	11.250		-	-	-	+	-
16	5-Hydroxymethylfurfural ($C_6H_6O_3$)	11.498		+	-	-	-	-
17	Isophytol, acetate ($C_{22}H_{42}O_2$)	29.233		-	+	-	-	-
18	Fumaric acid, cis-non-3-enyl propyl ester ($C_{16}H_{26}O_4$)	29.233-29.238		-	-	+	-	+

No.	Organic compounds (Molecular formula)	Retention times	Molecular structure	C	Day-14		Day-21	
					T I	T II	T I	T II
19	1,4-Epoxynaphthalene-1(2H)-methanol, 4,5,7-tris(1,1-dimethylethyl)-3,4-dihydro- ($C_{23}H_{36}O_2$)	30.745		-	-	-	+	-

Note:
C = Control, T I = Single isolate treatment (*Pycnoporus sanguineus* IN004), T II = Fungal consortium treatment (*Pleurotus ostreatus* InaCC F109, *Agaricus campestris* InaCC F126, *Trametes versicolor* InaCC F200, *Amauroderma rugosum* IN002, and *Pycnoporus sanguineus* IN004), + = compounds detected on GCMS chromatogram, - = compounds that are not detected on GCMS chromatogram.

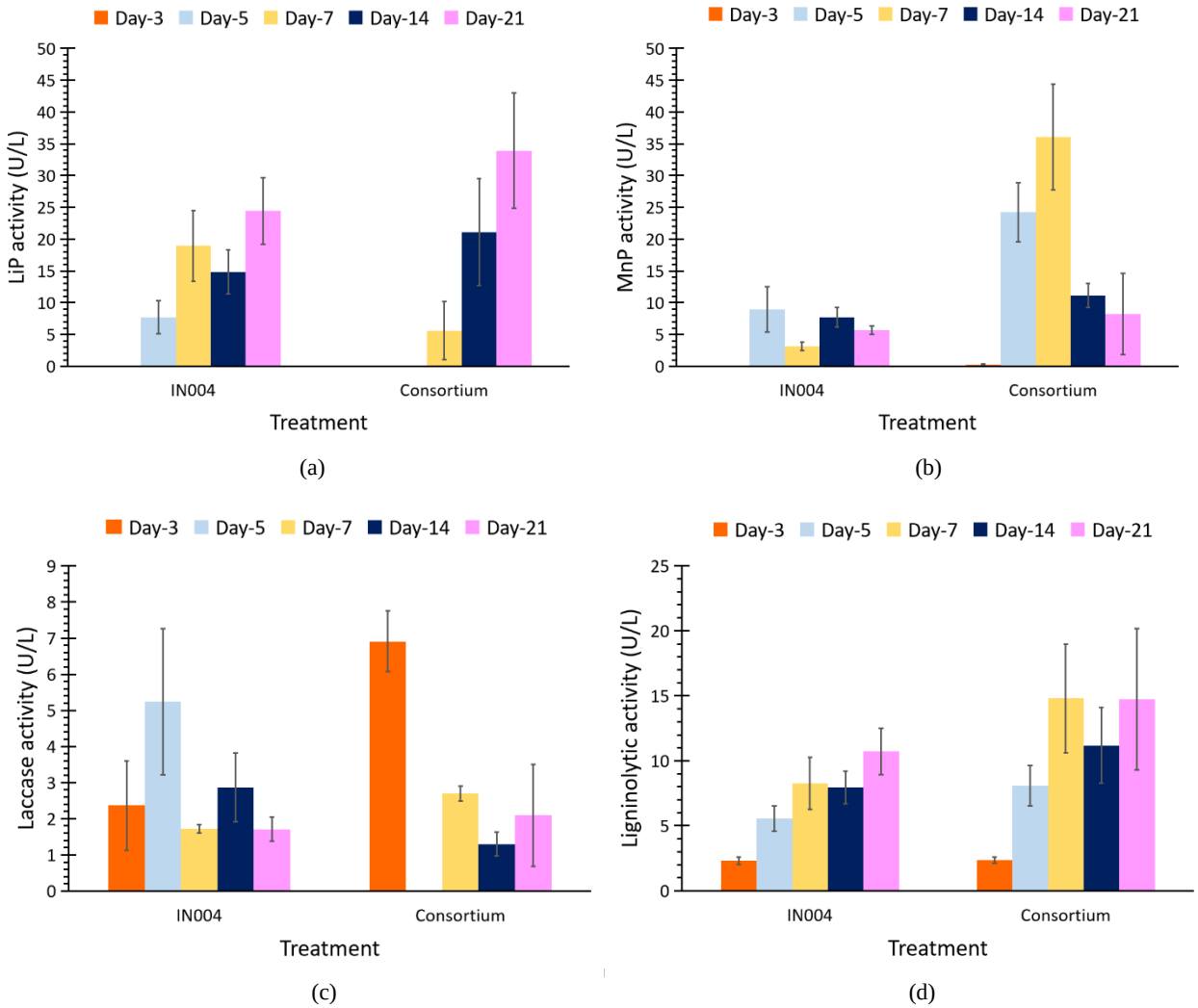


Fig. 7: Ligninolytic enzymes activities of Treatment I (*Pycnoporus sanguineus* IN004) and Treatment II (fungal consortium) on mycoremediation test. (a) LiP, (b) MnP, (c) Laccase, (d) Avarage ligninolytic activity.

4. Discussion

White rot fungi (WRF) are well known for their ability to degrade or mineralize a wide range organic pollutant, including complex compounds. These fungi produced several types enzymes that play key role on catalyzing the degradation reaction, such as lignin peroxidase (LiP), mangan peroxidase (MnP) and laccase. Therefore, it was necessary to confirm the ligninolytic activity of each fungal isolate prior to be be applied on bioremediation. Our results showed that all of fungal isolates showed ligninolytic activity. All of fungal isolates able to grow in MML implied that these fungi could use lignin as a sole carbon source to support biomass growth, in which the metabolic process of recalcitrant compounds is generally known as a co-metabolism process[18]. In guaiacol oxidation test, all fungal isolates except IN002 had the reddish-brown zone around the fungal mycelia on medium supplemented with guaiacol. The reddish-brown zone was due to the presence of biphenoquinone produced during the degradation of the guaiacol substrate by the laccase enzyme[19]. These results implied that all fungal isolates except IN002 have ligninolytic activity, particularly laccase enzyme.

The presence of LiP and MnP activity by the five isolates in liquid MM-L without H2O2 biostimulation showed that all white-rot fungal isolates in this study were able to produce monoculture extracellular enzymes producing H_2O_2[20]. Based on the screening results, *Pycnoporus sanguineus* IN004 was determined as the white rot fungi isolate with the best ligninolytic ability compared to the other isolates (*Pleurotus ostreatus* InaCC F109, *Agaricus campestris* InaCC F126, *Trametes versicolor* InaCC F200, and *Amauroderma rugosum* IN002. Therefore, the IN004 isolate was chosen as an inoculum for Treatment I (single isolate) in the mycoremediation test of the Sentiong River water, Jakarta. Gutiérrez-Soto et al.[21] stated that *Pycnoporus sanguineus* had the highest ligninolytic enzyme activity compared to *Phanerochaete chrysosporium*, *T. hirsuta*, and *T. versicolor*, indicated by a greater degradation zone on Poly R-478 and syringaldazine medium. *Pycnoporus sanguineus* species was also capable of degrading polycyclic aromatic hydrocarbon compounds such as phenanthrene and benz[a]anthracene, producing metabolites in the form of 2-methylphenol; 2,3-dihydroxy-3-phenylpropanoid acid, and citramalic acid. The species also had the highest laccase activity compared to *Coriolus versicolor*, *Pleurotus ostreatus*, and *T. versicolor* [22].

In order to get the best consortia, compatibility test should be performed before construct the fungi consortium. Interactions that may occur between fungi are compatible (mutual intermingling), partial compatible (partial mutual intermingling), invasion (replacement) at the initial stage, invasion (replacement) at the final stage, inhibition (deadlock) with and without distance[18]. Based on our observation results, it was concluded that the interaction among five fungal isolates is partial compatible. Partial compatible interaction is characterized by the growth of an isolate that is inhibited by the growth of another isolate. It might be possible to combine all of fungal isolate into one consortium. The effectiveness of this fungal consortium was evaluated further in this study.

Our finding showed that mycoremdiation using fungal consortium treatment were given the best result than single isolate treatment. The TDC value on the 14th day of the consortium treatment was lower than the treatment of a single isolate (Figure 6a). This indicates that the fungal consortium was able to remove organic compounds and/or recalcitrant compounds fatser than a single isolate treatment. However, the TDC value obtained in this study does not represent persistent organic pollutants only but also includes other carbon compounds such as glucose, as glucose was added in this study. Therefore, further GC-MS analysis was conducted to ensure the biodegradation of persistent organic pollutants has occurred.

The pH of river water also was change along incubation period. At the first stage of incubation the pH was decreased, while at the end of incubation time the pH was increased. A decrease in pH values indicates the production of organic acid metabolites. However, the increase in pH can be caused by alkaline metabolites, the result of mineralization of proteins or other organic compounds[23]. The acetic acid compound was detected in a river water sample based on GC-MS analysis with a very high abundance. This compound is known as one of the odors in the wastewater[4]. Fortunately, both of treatments able to remove it completely, which fungal consortium treatment removed this pollutant faster than single isolate treatment. Acetic acid is a natural degradation and deterioration product of wood so that all fungal isolates in both treatments are able to use acetic acid as a carbon source[25]. Some fungal species are able to use urea as a source of nitrogen to support their growth. Metabolism of urea will produce a product in the form of ammonia that is alkaline. This was indicated by an increase in pH by the two mycoremediation treatments that take place during the incubation period on days 14 and 21 (Figure 6b). Based on GC-MS analysis, urea compounds were still detected in single isolate treatment on the 14 and 21 days of incubation. While fungal consortium treatment showed the best performance that able to use 100% urea in the water sample starting from the 14 days of incubation.

The growth of fungal was indicated by the increase of its biomass. Increased fungal biomass showed the rate of utilization of organic carbon compounds. Co-metabolism of organic pollutant compounds in river water with glucose as the primary carbon source was indicated by an increase in fungal biomass and a decrease in TDC value. This is similar to Sari et al.[26], who found that Trametes versicolor was able to co-metabolize DDT as indicated

by an increase in mycelium biomass and glucose consumption during the incubation period. Bankole et al.[27] also observed the increase in biomass in the monoculture and co-culture of *Daldinia concentrica* and *Xylaria polymorpha* were correspond to an increase in the percentage of decolorization of cibacron brilliant Red 3B-A dyes. Increased biomass concentrations can also increase or decrease the production of ligninolytic enzymes, depending on the fungi strain used[28]. Based on the LEA (the average activity of LiP, MnP, and Laccase), treatment II was higher than treatment I. Increased production of ligninolytic enzymes by both treatments can be caused by the addition of glucose as a primary carbon source[29]. Fungal consortium causes oxidative metabolic stress due to the increasingly limited availability of nutrients. This causes other fungi isolates to switch using secondary metabolism, thereby increasing the production of certain enzymes[30].

The degradation process of pollutant compounds can take place through a mechanism of transformation or mineralization. The transformation process occurred by produced several metabolite compounds that has a different molecular structure from the parent structure[30]. Metabolite compounds such as cyclobutane, 2-ethyl-1-methyl-3-propyl-; isophytol, acetate; and fumaric acid, cis-non-3-enyl propyl ester may be an aliphatic compound, which is derived from the breakdown of aromatic groups[27]. While the methyl 2,2-dimethyl-3-hydroxypropionate compound might be oxidized to a propanoic acid compound, 2,3-dihydroxy, which was detected on the chromatogram of treatment I on the 21st day.

The decrease in the abundance of organic compounds corresponds to the decrease in TDC and an increase in ligninolytic enzymes activity, proved that fungal ligninolytic enzymes in this study play a role in the organic pollutant degradation process in Sentiong River sample, Jakarta. Ligninolytic enzyme activity that was observed during the mycoremediation test implied that these fungi involved ligninolytic enzyme to grow. Moreover, the mycoremediation by fungal consortium gave the best result, as revealed by the higher decrease of TDC that was in line with its higher value of ligninolytic enzyme activity. These results revealed that this fungal consortium in this study having the capacity to act in synergism toward organic pollutant biodegradation in river water. Fungal consortium proved to be more effective in degrading pollutant compounds compare to single isolate. The results of the study correspond to Bankole et al.[27] which stated that the use of fungal consortium was more effective in biodegradation of recalcitrant organic compounds compared to a single isolate. The co-culture of *Daldinia concentrica* and *Xylaria polymorpha* had a higher decolorization percentage of cibacron brilliant Red 3B-A dye compared to its monocultures.

5. Conclusion

All of five fungal isolates in this study showed the ligninolytic activity and *Pycnoporus sanguineus* IN004 has the best ligninolytic ability compared to the other four isolates. The fungal consortium has a better performance organic compounds removal than a single isolate revealed by a higher and faster reduction of total carbon. The fungal consortium able to remove about 63.46% of total dissolved carbon in 14 days of incubation, while the single isolate only 42.33% of total dissolved carbon. The untargeted GC-MS anyalisis ensure this finding showed that several organic compounds have been removed completely by fungal consortium but these compounds were still found in single isolate treatment or control. In this study, fungal ligninolytic enzymes (lignin peroxidase, mangan peroxidase, and laccase) play a role in the organic pollutant degradation process that was proved by the increase of ligninolytic enzymes activity along treatment. In final incubation period (21 days), the mycoremediation of polluted river water by fungal consortium able to remove up to 3587.8 mg/L of total dissolved carbon or equal to 88,96% relative to control (un-treatment). This finding proved that fungal consortium was more preferable to reduce recalcitrant substances in polluted river water. However, a compatibility test should be performed beforehand to make sure the synergism among fungal species. This study still involved glucose as a co-substrate to promote fungal growth in the early stages of treatment. Further research is needed to minimize the addition of glucose as it becomes an issue in real-world applications when used in high doses. Finding a low-cost carbon source as an alternative to replace glucose is necessary to make this technology more feasible in practical applications.

Acknowledgements

The authors acknowledge the facilities, scientific and technical support form National Research and Innovation Agency through E-Layanan Sains. Greatly acknowledge Mr. Ilyas and Indonesian Culture Collection (InaCC), National Research and Innovation Agency, for providing the fungal isolates used for this study.

References

1) A.R. Bizhanova, A.S. Koshkinbayeva, G.A. Zhunisova, G.Z. Osmanova, D. Belkhozhayeva, D.S. Baisymakova, “Regulatory issues of depollution in Kazakhstan,” *Evergreen*, **9**(4) 903–908(2022). doi:10.5109/6622876.
2) R.P. Schwarzenbach, T. Egli,T.B. Hofstetter, U. von Gunten, and B.Wehrli, “Global water pollution and human health,” *Annual Review of Environment and Resource*, **35** 109–36 (2010). doi:10.1146/annurev-environ-100809-125342.
3) R.K. Rothmel, A.M. Chakrabarty, “Microbial

degradation of synthetic recalcitrant compounds," *Pure Applied Chemistry* **62**(4) 769–79 (1990). doi:/10.1515/iupac.62.0011.

4) Z. Liang, M. Siegert, W. Fang, Y. Sun, F. Jiang, H. Lu, G.-H. Chen, S. Wang, "Blackening and odorization of urban rivers: A bio-geochemical process," *FEMS Microbiology Ecology,* **94**(3) 1–12 (2018). doi:10.1093/femsec/fix180
5) J.-J. Ko, Y. Shimizu, K. Ikeda, S.-K. Kim, C.-H. Park, S. Matsui, "Biodegradation of high molecular weight lignin under sulfate reducing conditions: Lignin degradability and degradation by-products," *Bioresource Technology,* **100**(4) 1622–7 (2009).doi:10.1016/j.biortech.2008.09.029.
6) R. Narmanova, A. Tapalova, R. Zhapparbergenov, N. Appazov, "Biological products for soil and water purification from oil and petroleum products," *Evergreen*, **10**(2) 688–695 (2023). doi: 10.5109/6792815
7) R.S. Sinsinwar, M. Verma, "Analysis of pH value of water for treatment plant of Kekri and Surajpura (Rajasthan) India," *Evergreen*, **10**(1) 324–328 (2023). doi:10.5109/6781087.
8) R. Muhammad, "Biosorption of brilliant green dye from synthetic wastewater by modified wild algae biomass," *Evergreen*, **9**(1) 133–140 (2022). doi:10.5109/4774228.
9) M. Ohkuma, Y. Maeda, T. Johjima, T. Kudo, "Lignin degradation and roles of white rot fungi : Study on an efficient symbiotic system in fungus-growing termites and its application to bioremediation", *Microbiology* **42**(December) 39–42 (2001).
10) Hauser JT. 2006. *Techniques for Studying Bacteria and Fungi*. Carolina Biological Supply Company.
11) A. Barapatre, K.R. Aadil, B.P. Tiwary, H. Jha, "*In vitro* antioxidant and antidiabetic activities of biomodified lignin from *Acacia nilotica* wood. *International Journal of Biological Macromolecul*, **75**(April) 81–9 (2015). doi:10.1016/j.ijbiomac.2015.01.012.
12) C. Casciello, F. Tonin, F. Berini, E. Fasoli, F. Marinelli, L. Pollegioni, E. Rosini, "A valuable peroxidase activity from the novel species *Nonomuraea gerenzanensis* growing on alkali lignin," *Biotechnology Reports,* **13**(March) 49–57 (2017). doi:10.1016/j.btre.2016.12.005.
13) Z. Ajdari, A. Ebrahimpour, M. A. Manan, M. Hamid, R. Mohamad, A.B. Ariff, "Nutritional requirements for the improvement of growth and sporulation of several strains of *Monascus purpureus* on solid state cultivation," *Journal of Biomedicine and Biotechnology* 2011. doi:10.1155/2011/487329.
14) K. Fu, S. Fu, H. Zhan, P. Zhou, M. Liu, H. Liu, "A Newly Isolated Wood-rot Fungus for Laccase Production in Submerged Cultures," *BioResources*, **8**(1) 1385–97 (2013).
15) E. Erden, M.C. Ucar, T. Gezer, N.K. Pazarlioglu, "Screening for ligninolytic enzymes from autochthonous fungi and applications for decolorization of remazole marine blue," *Brazilian Journal of Microbiology,* **40**(2) 346–53 (2009). doi:10.1590/S1517-83822009000200026.
16) E. Baltierra-Trejo, L. Márquez-Benavides, J.M. Sánchez-Yáñez, "Inconsistencies and ambiguities in calculating enzyme activity: The case of laccase," *Journal Microbiological Methods*, **119**(December) 126–31 (2015). doi:10.1016/j.mimet.2015.10.007.
17) N. Mohammad, Z Alam, N.A. Kabashi, O.S. Adebayo, "Development of compatible fungal mixed culture for composting process of oil palm industrial waste," *African Journal of Biotechnology,* **10**(81) 18657–65 (2011). doi:10.5897/AJB11.2735.
18) H. Yemendzhiev, M. Gerginova, A. Krastanov, I. Stoilova, Z. Alexieva, "Growth of *Trametes versicolor* on phenol," *Journal of Industrial Microbiology and Biotechnology*, **35**(11) 1309–12 (2008). doi:10.1007/s10295-008-0412-z.
19) S. Devasia, A.J. Nair, "Screening of Potent Laccase Producing Organisms Based on the Oxidation Pattern of Different Phenolic Substrates," *International Journal of Current Microbiology and Applied Science*, **5**(5) 127–37 (2016). doi: 10.20546/ijcmas.2016.505.014.
20) S.K. Shin, Y.J. Ko, J.E. Hyeon, S.O. Han, "Studies of advanced lignin valorization based on various types of lignolytic enzymes and microbes," *Bioresource Technology,* **289**(October) 121728 (2019). doi:10.1016/j.biortech.2019.121728.
21) G. Gutiérrez-Soto,G.E. Medina-González, E.A. García-Zambrano, J.E. Treviño-Ramírez, C.E. Hernández-Luna, "Selection and characterization of a native *Pycnoporus sanguineus* strain as a lignocellulolytic extract producer from submerged cultures of various agroindustrial wastes," *BioResources* **10**(2) 3564–76 (2015). doi:10.15376/biores.10.2.3564-3576.
22) X. Li, Y. Pan, S. Hu, Y. Cheng, Y. Wang, K Wu, S. Zhang, S. Yang, "Diversity of phenanthrene and benz[a]anthracene metabolic pathways in white rot fungus *Pycnoporus sanguineus* 14," *International Biodeterioration and Biodegradation,* **134**(October) 25–30 (2018).doi:10.1016/j.ibiod.2018.07.012.
23) A. Ohtaki, N. Sato, K. Nakasaki, "Biodegradation of poly-ε-caprolactone under controlled composting conditions," *Polyme Degradation and Stability,* **61**(3) 499–505 (1998). doi:10.1016/S0141-3910(97)00238-3.
24) M. Toledo, R. Muñoz, "Odour prevention strategies in wastewater treatment plants: A pilot scale study of activated sludge recycling and oxidized nitrogen recycling," *Journal of Environmental Chemical Engineering*, **11**(5),110366 (2023). doi.org/10.1016/j.jece.2023.110366.
25) P. Barbara, M. Humar, A. Ulčnik, D. Jurc, F.

Pohleven, “Fungal wood decay processes as a basis for bioremediation. In: Goltapeh EM, Danesh YR, Varma A (eds) *Fungi as Bioremediators*. Berlin, Heidelberg, Springer Berlin Heidelberg, pp 51–74 (2013). doi: 10.1007/978-3-642-33811-3_3.

26) A.A. Sari, S. Tachibana, K. Itoh, “Determination of co-metabolism for 1,1,1-trichloro-2,2-bis(4-chlorophenyl) ethane (DDT) degradation with enzymes from Trametes versicolor U97,” *Journal of Bioscience and Bioengineering,* **114**(2) 176–81 (2012). doi:10.1016/j.jbiosc.2012.03.006.
27) P.O. Bankole, A.A. Adekunle, S.P. Govindwar, “Biodegradation of a monochlorotriazine dye, cibacron brilliant red 3B-A in solid state fermentation by wood-rot fungal consortium, *Daldinia concentrica* and *Xylaria polymorpha*: Co-biomass decolorization of cibacron brilliant red 3B-A dye,” *International Journal of Biological Macromolecul,* **120**(Part A) 19–27 (2018). doi:10.1016/j.ijbiomac.2018.08.068.
28) W.C. Cupul, G.H. Abarca, D.M Carrera, R.R. Vázquez, “Enhancement of ligninolytic enzyme activities in a Trametes maxima-Paecilomyces carneus co-culture: Key factors revealed after screening using a Plackett-Burman experimental design,” *Electronic Journal of Biotechnology,* **17**(3) 114–21 (2014). doi:10.1016/j.ejbt.2014.04.007.
29) R.C. Bonugli-Santos, L.R. Durrant, M. da Silva, L.D. Sette, “Production of laccase, manganese peroxidase and lignin peroxidase by Brazilian marine-derived fungi,” *Enzyme and Microbial Technology,* **46**(1) 32–7 (2010). doi:10.1016/j.enzmictec.2009.07.014.
30) Y. Chi, A. Hatakka, P. Maijala, “Can co-culturing of two white-rot fungi increase lignin degradation and the production of lignin-degrading enzymes?,” *International Biodeterior.ation and Biodegradation,* **59** (1) 32–9 (2007). doi:10.1016/j.ibiod.2006.06.025.
31) A.H. Neilson, A.-S. Allard, “Degradation and Transformation of Organic Fluorine Compounds,” In: Neilson AH (ed) Organofluorines. Berlin/Heidelberg, Springer-Verlag, pp 137–202 (2002).