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The Application of *Rigidoporus* sp J12 and *Stenotrophomonas maltophilia* BM in the Degradation of Batik Waste

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Abstract. The batik industry in Indonesia produces batik waste which pollutes the environment. This waste can be degraded using laccase-producing microorganisms. The microorganisms used in the research were the fungus *Rigidoporus* sp J12 and the bacteria *Stenotrophomonas maltophilia* BM. This research aims to determine the ability of *Rigidoporus* sp J12 and *Stenotrophomonas maltophilia* BM and their consortium in producing laccase, observing their ability to degrade Poly R-478 which is an indicator of phenoloxidase activity and batik waste. Microorganisms are grown in growth media and then placed in media containing Poly R-478 or batik waste. Inducers are added to increase laccase activity. The inducers used were 15 g/L sucrose, 200 µM $CuSO_4$ and 40 mM veratryl alcohol. The results showed that *Rigidoporus* sp J12 and *Stenotrophomonas maltophilia* BM produced laccase in PDB and NA media. The highest laccase activity was found in the enzyme produced by *Rigidoporus* sp J12 in PDB media at a temperature of 40°C, media pH 6.0 and the addition of sucrose. *Rigidoporus* sp J12 degraded batik waste by 39.38% and increased by 2.12 times after adding sucrose and incubation for 15 days. These bacteria and fungi can be used to degrade batik waste in order to prevent environmental pollution. Using the fungus *Rigidoporus* sp J12 purely is more profitable than using it with *S. maltophilia* BM bacteria.

Keywords: *batik waste; degradation; laccase; Rigidoporus; Stenotrophomonas maltophilia*

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INTRODUCTION

The batik industry has recently grown rapidly in Indonesia. Almost every region has a batik center and its own batik icon. Several well-known batik centers are: Sentra Batik Tulis Giriloyo, Yogyakarta; Kampung Batik Laweyan Solo; Kampung Batik Kauman Pekalongan; Kampung Batik Trusmi Cirebon, and Kampung Batik Lasem Rembang East Java. Batik comes from the Javanese language, namely "amba" meaning to write and "nitik" meaning point. It's about writing with wax (Nuriyanto, 2022). Batik is a fabric specially made by writing or applying wax, then processing it in a certain way that has its own unique characteristics. Many people develop and promote Batik due to its high economic and cultural values. The process of making hand drawn batik includes: preparation of raw materials (mori fabric), removal of starch from mori, draw directly on clean mori fabric in various pattern, covering the part of the fabric that is not layered with wax (night); dyeing waxed fabrics by dipping in fabric dye, removing wax and washing.

Liquid wastes are produced during the process of batik making. According to Apriyani (2018), liquid batik waste is in the form of dyes produced from residual dyes, washing and rinsing processes. Commonly used dyes include: naphtol, indigosol, rapid and indanthrene. Based on data from the Badan Lingkungan Hidup (BLH) of Pekalongan City in 2014, as much as 73,878 m^3 of waste was discharged monthly into rivers in Pekalongan City. Batik waste contains COD: 4951.75 mg/L, TSS: 448 mg/L, Cr: 7.0 mg/L, pH: 6.9. This waste is toxic. Cr ions contained in batik waste are also harmful to humans. Lung exposure to Cr ions causes DNA damage, tissue irritation, inflammation, cytotoxicity, and, ultimately, lung cancer (Shin et al., 2023).

Some microorganisms are able to produce laccase, *Marasmius* sp., *Trametes hirsuta*, *Trametes versicolor* and *Phanerochaete crysosporium* (Risdianto et al., 2012), *Bacillus*

subtilis MTCC 1039 (Mishra and Srivastava, 2016). This enzyme decomposes dyes. Kanagaraj et al (2015) reported that laccase degrades azo dyes rapidly at optimum growth media pH of 7.0, at 37°C temperature and 72 hours incubation. The percentage of degradation for CI Acid black 210 is 96.4% and for CI Acid black 234 is 92.2%. Naphthol used in the batik dyeing process was also degraded by microbes. Esmaeili and Fazeli (2012) reported that *Aspergillus niger* degraded 1-naphtol by 75% within 5 days at a concentration of 75 mg/L and the addition of Tween 80 can increase the degradation process by 5%. *Trametes versicolor* F200 and *Aspergillus* sp NS were also able to degrade Indigosol Blue dye. *Trametes versicolor* F200 has a higher ability than *Aspergillus* sp NS and degraded as much as 97.21% of the dye (Pertiwi et al, 2020). White-rot fungus *Rigidoporus* sp FMD21 is a fungus that produces lignin-degrading enzymes that degrade dioxins. It generates extracellular enzymes including laccase and Manganese Peroxidase that can be used for myco-remediation (Dao et al, 2021). Galai et al (2012) reported that *Stenotrophomonas maltophilia* AAP56 is a laccase-producing bacterium, its laccase activity is influenced by several environmental factors. The use of the fungus *Rigidoporus* sp J12 and the bacterium *Stenotrophomonas maltophilia* BM either individually or in a consortium for batik waste degradation has never been done. The fungus *Rigidoporus* sp J12 and the bacteria *Stenotrophomonas maltophilia* BM have the ability to produce laccase enzymes so they can be used to degrade dyes in batik waste. Therefore this research was carried out to obtain data on the ability of Rigidoporus sp. J12 and S. malthophilia BM to degrade batik waste. Batik waste, which is usually thrown into rivers and causes river water pollution, can be processed first using these microorganisms so that environmental pollution can be avoided.

METHODS

Microorganism. The microorganisms used consisted of the fungus *Rigidoporus* sp J12 and the bacteria *Stenotrophomonas maltophilia* BM. These microorganisms were obtained from the Microbiology Section of the Research Center of Biology (LIPI) which were stored in PDA and NA media at -20°C. Furthermore, the inducers used included: sucrose 15 g/L, $CuSO_4$ 200 µM, and veratril alcohol 40 mM, while the batik waste used was taken from Batik Village in Pekalongan, Central Java.

Media. NB consists of: 1.0 g Beef Extract; 5.0 g Bacto Peptone; 2.0 g Yeast Extract; 5.0 g Sodium Chloride, while the PDB medium consists of 4.0 g Potato starch; 20.0 g Dextrose. The composition of Poly R-478 is 0.60 g KH_2PO_4; 0.50 g $MgSO_4.7H_2O$; 0.40 g K_2HPO_4; 0.22 g (NH4)2 tartrate; 40.0 g sorbose; 0.20 g Poly R-478 (Sigma); then added 10.0 mL of stock mineral solution and added distilled water to 1 L. In addition, the composition of the stock mineral solution consisted of: 7.4 g $CaCl_2$. 2H2O; 1.2 g Ferric citrate; 0.7 g ZnSO4.7H2O; 0.5 g MnSO4.4H2O; 0.1 g CoCl2.6H2O; 10.0 mg Thiamin HCl, then add aqudest to 1 L (Glenn and Gold, 1983).

Laccase activity in *Rigidoporus* sp J12 and *S. maltophilia* BM

Laccase activity from *Rigidoporus* sp J12 and *Stenotrophomonas maltophilia* BM was calculated using the method of Papinutti et al. (2003). The reaction is based on the oxidation of ABTS by the enzyme laccase. The reaction mixture consisted of 0.5 mL citrate buffer at pH 6.0, 0.1 mL 1 mM ABTS and 0.4 mL enzyme supernatant. The tube was then shaken gently to mix all the ingredients and left to stand for 15 minutes at room temperature. Absorbance was measured at a wavelength of 420 nm

The effect of temperature on laccase activity of *Rigidoporus* sp J12 and *S. maltophilia* BM

The reaction mixture consisted of 0.5 mL citrate buffer at pH 6.0, 0.1 mL 1 mM ABTS and 0.4 mL enzyme supernatant from *Rigidoporus* sp J12 or *S. maltophilia* BM. The tube was then shaken gently so that all the ingredients were mixed and left for 15 minutes at a temperature of 27ºC, 30ºC, 40ºC, 50ºC, 60ºC, 70ºC, 80ºC. Absorbance was measured using a spectrophotometer at a wavelength of 420 nm

The effect of media pH on laccase activity in Rigidoporus sp J12 and S. maltophilia BM

The ingredient mixtured of 0.5 mL citrate buffer at pH 6.0, 0.1 mL 1 mM ABTS and 0.4 mL enzyme supernatant from *Rigidoporus* sp J12 or *S. maltophilia* BM. The pH of the culture is adjusted to 3, 4, 5, 6, 7, 8, 9 by adding HCL or NaOH. The tube was then shaken gently to mix all the ingredients and left to stand for 15 minutes at room temperature. Absorbance was measured using a spectrophotometer at a wavelength of 420 nm

The degradation of Poly R-478 by *Rigidoporus* sp J12 and *S. maltophilia* BM in the presence of inducer

A total of 5 ml of *Rigidoporus* sp J12 or *S. maltophilia* BM suspension or a mixture of both were added to 45 ml of Poly R-478 media as a control (1). Then 5 mL of *Rigidoporus* sp. J12 or *S. maltophilia* BM suspension or a mixture of both were added to 45 mL of Poly R-478 + 15g/L sucrose (2). The third treatment was a suspension of mycelium or bacterial cells or a mixture of 5mL + 45 mL Poly R-478 + 200 µM $CuSO_4$ (3). The last treatment was 5 mL suspension of mycelium or bacterial cells or mixture + 45 mL Poly R-478 + 40 mM veratryl alcohol (4). The culture was incubated on a shaker at 112 rpm at room temperature for 10 days. The absorbance was read at 520 nm wavelength.

The degradation of batik waste by *Rigidoporus* sp J12 and *S. maltophilia* BM in the presence of inducer

Isolate of *Rigidoporus* sp J12, *S. maltophilia* BM or a mixture of both with the addition of $CuSO_4$ or sucrose, and the optimum pH was used to degrade batik waste. The treatments tested were 1) 45 mL of batik waste 2). 45 mL of batik waste + 200 µM $CuSO_4$. 3) 45 mL of batik waste + 15g/L sucrose. The experiment was carried out three times. All media were sterilized and chilled. A total of 5 ml mycelium suspension, bacterial cells or a mixture of both were inoculated into 1-3 treatment media. The culture was incubated on a shaker at 112 rpm in a room temperature for 10 days. Samples were then taken and centrifuged to measure the degree of degradation. The absorbance of supernatant was read at a wavelength of 600 nm using a spectrophotometer.

Data analysis

In this study, each treatment used 3 replications. The data was then analyzed by comparing the mean and standard deviation between treatments.

RESULTS AND DISCUSSION

In this study, *Rigidoporus* sp J12 and *Stenptrophomonas maltophilia* BM were used either individually or in combination to degrade batik waste.

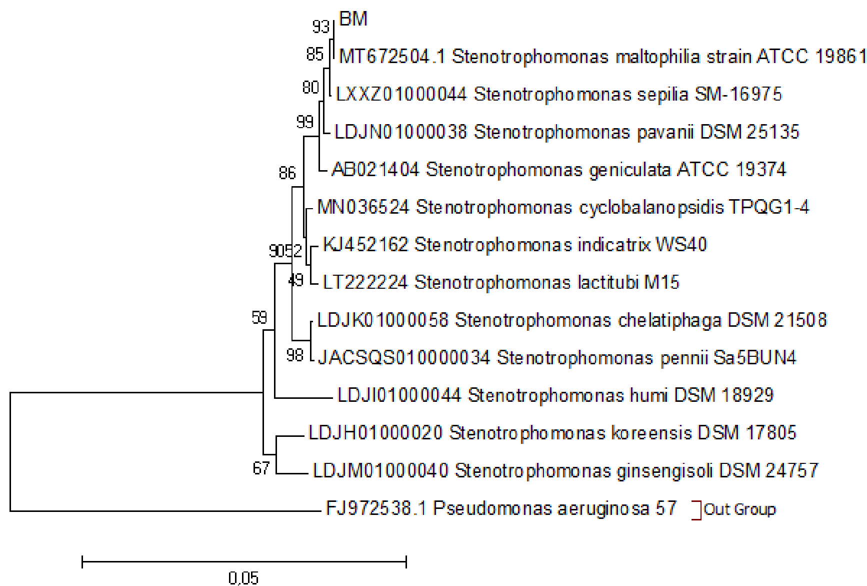

Figure 1. Phylogeny of *Stenotrophomonas maltophilia* BM.
Neighbor-Joining (NJ) Tree Based on 16S sequences of the stenotrophomonas bacteria genera. Bootsrap values above 50% are recorded at the nodes with 1000 replication and pseudomonas was used as the outgroup. The neighbor joining showed that the sequence of isolates code BM which was isolated from the petiole of Mitragyna speciosa is closely related to Stenotrophomonas maltophilia strain ATCC 19861, with bootstrap value 93%.

These two microbes were able to produce laccase. *Rigidoporus* sp J12 grown on PDB media produce laccase enzyme with the highest activity of 1721.98 U/mL, occurred on the 4th day of incubation period while the smallest activity, 94.90 U/mL, occurred on the 1st day of incubation period (Figure 2A). *Stenotrophomonas maltophilia* BM bacteria which derived from

Kratom plant roots (*Mitragyna speciosa*) produced 256.47 U/mL laccase enzymes and the highest enzyme activity was achieved within 28 hours of incubation while the lowest activity, 134.02 U/mL, was achieved within 26 hours of incubation period (Figure 2B).

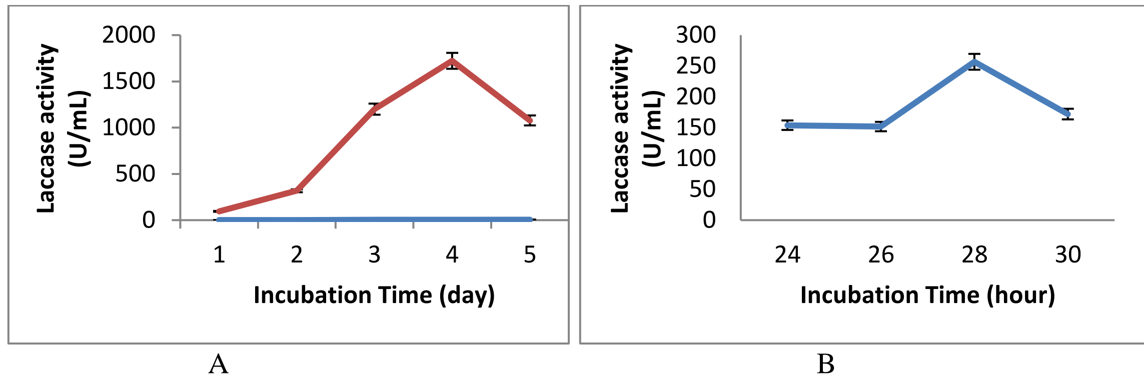

Figure 2. A. Laccase activity of *Rigidoporus* sp J12 on media PDB.
B. Laccase activity of *S. maltophilia* BM on media Nutrien Agar

The highest activity of the laccase enzyme in *Rigidoporus* sp J12 occurred on the 4th day of incubation period. This result is consistent with Dao et al (2021) research which screened 70 isolates of white rot fungus from Vietnam forests to determine the activity of lignin-decomposing enzymes. Fungus that produces high laccase activity, *Rigidoporus* sp. from southern Vietnam forests, also produces Manganese peroxidase. In PDSRb media, *Rigidoporus* sp laccase activity was 238800 U/L after 10 days of incubation while the highest MnP activity, which was around 40 U/L, occurred on the 4th day of incubation. *Stenoprophomonas maltophila* BM was able to produce laccase enzymes with the highest activity obtained at the 28th hour incubation with the amount of 256.47 U/mL.

Room temperature affects the activity of enzymes including laccase enzymes produced by *Rigidoporus* sp J12. On PDB media, this fungus produces 182.28 U/mL laccase with the highest activity at 40°C and the lowest at 80°C with the amount of 85.64 U/mL laccase (Figure 3A). Similarly, *Stenotrophomonas maltophilia* BM is affected by room temperature when secreting laccase. During incubation on NA media, these bacteria produced 222.33 U/mL laccase enzymes with the highest activity at 30°C and the lowest at 80°C with the amount of 32.63 U/mL (Figure 3B).

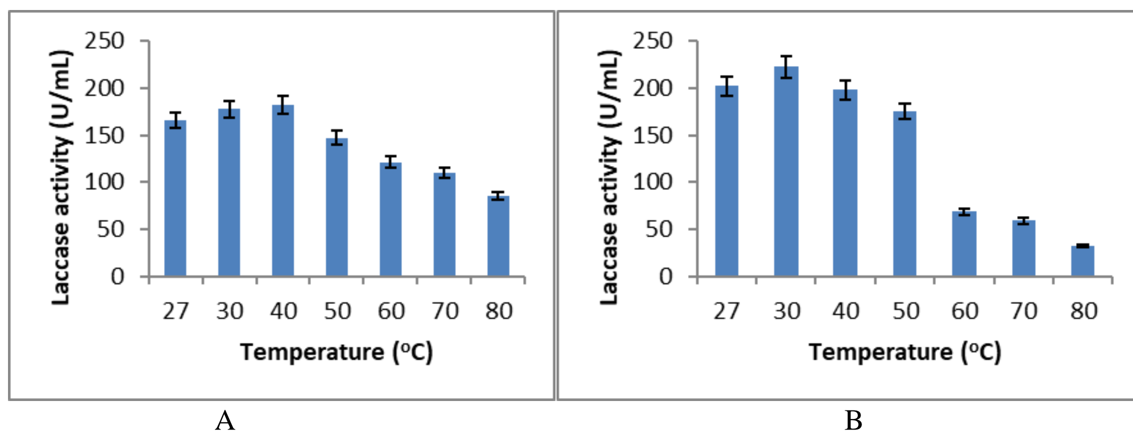

Figure 3. A. The effect of temperature on laccase activity of *Rigidoporus* sp J12
B. The effect of temperature on laccase activity of *Stenotrophomonas maltophilia* BM

Temperature affects the activity of laccase enzymes in *Rigidoporus* sp J12. According to Mazlan and Hanifah (2017) the activity of the immobilized laccase enzyme is influenced by higher pH and temperature than the free enzyme. In this study, the optimum temperature for

Rigidoporus sp is 40ºC. On the otherhand the optimum temperature for *S. malthophilia* BM is 30ºC which is lower than the results of Galai et al (2014) study which reported that the optimal temperature for producing laccase in *Stenotrophomonas maltophilia* AAP56 with ABTS substrate was 40ºC.

Media pH also affects the production of laccase enzymes in microbes. The highest enzyme activity in *Rigidoporus* sp J12 grown on PDB media was 200.80 U/mL at pH 6 and the lowest was 39.46 U/mL at pH 3 (Figure 4A). The same thing goes for *S. maltophilia* BM grown on NA media. The highest laccase activity was 200.92 U/mL which obtained at pH 7 and the lowest was 30.09 U/mL at pH 3 (Figure 4B).

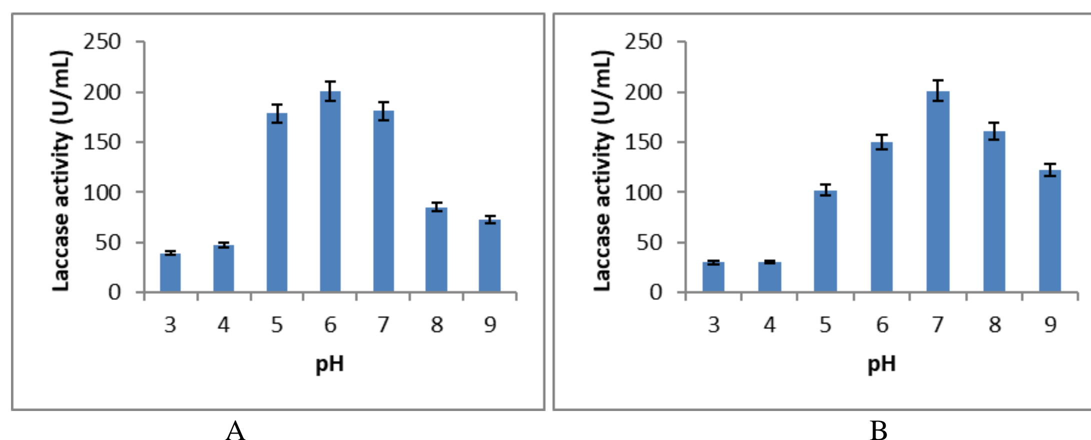

Figure 4. A. The effect of media pH on laccase activity of *Rigidoporus* sp J12.
B. The effect of media pH on laccase activity of *Stenotrophomonas maltophilia* BM.

Media pH affected the production of laccase in the *Rigidoporus* sp J12. The optimal pH for laccase production in some fungi is ranging between 5.0 to 6.0. Strong (2011) reported that the highest laccase activity in *Fomes sclerodermeus* occurred in the media with a pH of 6.0. *S maltophilia* BM produce laccase enzymes maximally at pH 7. This result is in accordance with the research conducted by Unuofin (2020) which reported that the optimum pH of *Stenotrophomonas maltophilia* BIJ16 to produce laccase enzymes was 7.0.

A number of materials also take effect on laccase enzymes production in microbes. In the experiment of *Rigidoporus* sp J12, the addition of three kinds of inducers can increase laccase activity. The highest laccase activity (183.79 U/mL) was obtained with the addition of 15 g/L sucrose while the lowest one (112.84 U/mL) was obtained with the addition of 200 µM $CuSO_4$ (Table 1).

Table 1. The effect of inducer on laccase activity of *Rigidoporus* sp J12

No	Inducer	Laccase activity (U/mL)	Increase of laccase activity after addition of inducers (fold)
1	Control	172.68 ± 8.72	-
2	Sucrose	183.79 ± 4.36	1.06
3	$CuSO_4$	112.84 ± 4.26	0.65
4	Veratryl alcohol	147.21 ± 3.02	0.85

The addition of sucrose to *Rigidoporus* sp J12 can increase laccase activity 1.06 times compared to the control. These results correspond with Umar and Ahmed (2022) research, they reported that glucose and sucrose were good carbon source to enhance the laccase synthesis. The addition of 15 g/L sucrose to the media could also increase *Coltricia cinnamomea* laccase activity by 40.80%, which was grown on PDB media (Subowo and Sugiharto, 2021).

Inducer experiment to increase laccase activity were carried out on *S. maltophilia* BM cultures at NA media as well. After incubated, the highest laccase enzyme activity was obtained with the addition of $CuSO_4$ 200 µM at 197.68 U/mL and the lowest was in the control (143.16 U/mL) (Table 2).

Table 2. The effect of inducer on laccase acivity of *Stenotrophomonas maltophilia* BM

No	Inducer	Laccase activity (U/mL)	Increase of laccase activity after addition of inducers (fold)
1	Control	143.16 ± 6.98	-
2	Sucrose	148.83 ± 16.05	1.03
3	$CuSO_4$	197.68 ± 5.73	1.38
4	Veratryl alcohol	183.79 ± 14.93	1.28

The addition of $CuSO_4$ inducer to the culture of *S. maltophilia* BM also able to increase laccase enzyme activity by 1.38 times compared to the control. These results are consistent with Galai et al. (2012) study that the laccase activity produced by *S.maltophilia* AAP56 is a $CuSO_4$ induced activity. The laccase activity of *S. maltophilia* greatly decreased with decreasing $CuSO_4$ concentration in the medium.

Poly R-478 is a model compound used to observe the ligninolytic activity generated by microbes. The laccase enzyme produced by microbes can decompose Poly R-478, which characterized by the decreasing color density of the compound. *Rigidoporus* sp J12 and *S. maltophilia* BM were able to degrade Poly R-478 either as a single isolate or a mixture. The addition of an inducer also able to increase the degradation process of Poly R. The greatest color degradation of Poly R-478 with the amount of 66.45% occurred at the mix treatment of *Rigidoporus* sp. J12 and *S. maltophilia* BM with the addition of the inducer $CuSO_4$ at media pH 6 in 10 incubation days. The smallest color degradation of Poly R-478, by 17.16%, occurred in the treatment of *S. maltophilia* with the addition of a sucrose inducer at media pH 7. The addition of $CuSO_4$ inducer to a mixture of fungi and bacteria can increase the degradation process by 1.62 times (Table 3).

Table 3. Degradation of Poly R-478 by *Rigidoporus* sp J12 and *S.maltophilia* BM

No	Microorganism	Inducer	pH media	The decrease of Poly R-478 color (%)	Inducer increase by fold
1	*Rigidoporus* sp J12	Control		15.21±2.49	
		Sucrose	6.0	49.10±2.83	3.22
			7.0	40.00±4.22	2.62
		$CuSO_4$	6.0	59.08±2.00	3.88
			7.0	47.16±1.69	3.10
2	*S. maltophilia* BM	Control		13.29±1.51	
		Sucrose	6.0	28.20±9.03	2.12
			7.0	17.16±2.15	1.29
		$CuSO_4$	6.0	17.93±4.26	1.34
			7.0	20.63±5.23	1.55
3	*Rigidoporus* sp J12+ *S.maltophilia* BM	Control		40.99±1.04	
		Sucrose	6.0	50.87±4.65	1.24
			7.0	53.68±7.68	1.30
		$CuSO_4$	6.0	66.45±4.91	1.62
			7.0	56.82±3.98	1.38

The mix treatment of *Rigidoporus* sp J12 and *S. maltophilia* BM with the addition of $CuSO_4$ was able to degrade Poly R-478 with the highest percentage of color loss compared to the single isolate treatment. This shows that both microbes able to coexist, work together, and not inhibit each other in the degradation of Poly R. Laccase and other enzymes produced by the two microbes degrade Poly R together. This dynamic was also reported by Melloul (2016) who used *Stenotrophomonas maltophilia* and *Aspergillus fumigatus* together to make biofilms. Thus, these two microbes sinergism. Mawad et al (2020) used *Pseudomonas aeruginosa* and *Aspergillus flavus* separately and consortia for the degradation of Disperse Blue 64 (DB 64) and Acid Yellow 17 (AY 17). The results showed that the fungal and bacterial consortium produce a higher percentage of dye degradation than individual strains, even at a high concentration of 300 mg/L.

Stenotrophomonas maltophilia BM can degrade Poly R when used individually. This

bacterium can reduce the Poly R by 28.20%. The addition of sucrose could increase degradation process by 2.12 times compared to control. This result is consistent with Rajeswari et al (2013) research which reported that the bacterium *Stenotrophomonas maltophilia* RSV-2 can degrade mixed dye substance up to 2100 ppm as much as 58% within 67 hours. *Rigidoporus* sp J12 can degrade Poly R as well when used independently, the addition of $CuSO_4$ increased degradation process by 3.88 times.

Batik waste generated by the batik industry is the residual liquid from dyeing batik cloth which contains synthetic textile dyes. In addition, synthetic textile dyes consist of napthol, indigosol and rapid. According to Apriyani (2018), coloring is a very important process in the batik industry which cannot be left out. This process uses textile dyes which produce waste. In this experiment, batik waste was degraded using fungi, bacteria and a mixture of both. The greatest color reduction occurred in *Rigidoporus* sp J12 treatment with the amount of 39.38% sucrose inducer after being incubated for 15 days. The addition of 15 g/L sucrose inducer as carbon source can increase the degradation process by 2.12 times. The smallest color reduction occurred in *S. maltophilia* BM treatment with the addition of $CuSO_4$ inducer by 16.78% in media pH 6.0 (Table 4).

Table 4. *Rigidoporus* sp J12 and *S. maltophilia* BM ability to degrade batik waste

No	Microorganism	Inducer	Batik waste decolorization (%)	Inducer increase by fold
1	*Rigidoporus* sp J12	Control	18.55±1.39	-
		Sucrose	39.38±0.57	2.12
		$CuSO_4$	34.36±0.52	1.85
2	*S. maltophilia* BM	Control	10.96±0.12	-
		Sucrose	26.90±3.47	2.45
		$CuSO_4$	16.78±2.59	1.53
3	*Rigidoporus* sp J12+	Control	28.75±2.86	-
	S.maltophilia BM	Sucrose	33.19±1.58	1.15
		$CuSO_4$	28.67±0.13	0.99

Rigidoporus sp J12 was able to degrade dyes in batik waste that result in the highest color loss. These results correspond with Sridhar et al (2013) study which utilised laccase enzyme produced by *Rigidoporus lignosus* for the decolorization of nine textile and non-textile dyes. As a result, laccase enzyme can degrade an amount of 69.8% Acid Blue 113; 45.07% Reactive Blue 19; 36.61% Reactive Orange 122; 30.55% Acid Red 88; 24.59% Direct Blue 14; 18.48% Reactive Black B; 16.49% Reactive Black Blue RGB and 11.66% Acid Blue 9. Similarly, *Rigidoporus ulmarius* was able to reduce color by 83.39% (Poornima et al., 2014). Eichlerová and Baldrian (2020) reported that the most efficient Basidiomycetes member for decolorization of Orange G and Remazol Brilliant Blue R (RBBR) and the highest laccase producer were found in Polyporales and Agaricales Orders. Strains with high MnP activity were found mainly in Polyporales, Agaricales, Hymenochaetales, and Russulales Orders.

Furthermore, *S. maltophilia* BM were able to degrade dyes in batik waste with the addition of 15 g/L sucrose that the color was reduced by 26.90%. The addition of a carbon source evidently could increase the activity of laccase so that the color loss increases 2.45 times compared to the control. Alaya et al (2021) reported that the *Stenotrophomonas maltophilia* isolated from neem oil seed compost was able to degrade Malachite green. Another bacteria which also degrade dye substance, *Bacillus thuringiensis*, able to degrade 3 textile dyes namely Reactive blue 13, Reactive red 58, Reactive yellow 42 and dye waste up to 80-95% within 6 hours (Olukanni et al, 2013).

Rigidoporus sp J12 and *S. maptophilia* utilized in a consortium can also degrade batik waste. This microbial mixture was able to reduce the color of batik waste by 33.19% with the addition of sucrose. Lade et al (2016) used the *Providencia rettgeri* strain HSL1 bacteria and the *Aspergillus ochraceus* NCIM 1146 fungus both separately and consortium for the decolorization of textile waste. *Aspergillus ochraceus* NCIM 1146 and *Providencia rettgeri* HSL1 separately were able to reduce the color of the American Dye Manufacturing Institute textile mill effluent by 6% and 32% in 30 hours at 30±0.2ºC while the consortium could reduce 92%.

Fungus Rigidoporus sp has never been used to degrade batik waste. Considering its laccase producing ability, research on its effectivity on batik waste degradation needed to be done. Hence, this research aimed to provide the data on it. The combination of fungus Rigidoporus sp J12 and the bacteria Stenotrophomonas maltophilia BM for batik waste degradation was invaluable groundwork for future research as well. The data obtained on the ability of the fungus Rigidoporus sp J12 and the bacteria S. maltophilia to degrade batik waste allowed the use of these microorganisms to overcome environmental pollution caused by batik waste.

CONCLUSION

Rigidoporus sp J12 and the *Stenotrophomonas maltophilia* BM were able to produce laccase enzymes in PDB and NA media respectively. *Rigidoporus* sp J12 was able to degrade batik waste by 39.38% with the addition of sucrose. The consortium of microorganisms was able to degrade batik waste by 33.19% with the addition of sucrose.

The batik waste used in this research is a mixture of several dyes used in the batik making process so the data obtained is not specific. Further research can be carried out on each dye so that data on the ability of microbes to degrade dyes is more specific.

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