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Comparison of the Rate and Level of Biodegradation of Leaf Waste by Lignocellulolytic Molds from Universitas Negeri Semarang Campus

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Abstract

Biodegradation is a physical decomposition of the substrate caused by the activity of microorganisms by producing products that have benefits for humans. Lignocellulose degrading fungi are microorganisms that play a role in the process of decomposing organic waste. The biodegradation rate of each type of lignocellulose degrading mold is different. The purpose of this research is to comprehensively analyze and compare the rate and level of biodegradation of leaf waste in lignocellulolytic molds isolated from the campus environment of Universitas Negeri Semarang, as well as to identify and evaluate the type of lignocellulolytic mold that shows the highest efficiency in the rate and level of biodegradation of leaf waste. The method used in this research is quantitative method, by measuring the mass ratio and pH of leaf waste media before inoculation and after inoculation. The results of this study are the rate of leaf waste biodegradation based on the ratio of leaf waste mass by *Trichoderma koningiopsis* mold isolate of 0.015 grams/day, while by *Trichoderma erinaceum* mold isolate of 0.002 grams/day. Then the rate of leaf waste biodegradation based on the pH change value of leaf waste by *Trichoderma koningiopsis* mold isolate is higher with a pH change value of 0.3 when compared to the *Trichoderma erinaceum* mold isolate with a pH change value of 0.1. The superior performance of *Trichoderma koningiopsis* highlights its potential application in optimizing organic waste decomposition systems and provides a scientific basis for selecting lignocellulolytic fungi in sustainable waste management programs.

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INTRODUCTION

Environmental pollution, which frequently occurs in various regions, is partly caused by waste. Under current conditions, waste is no longer merely a regional issue but has become a national and even global concern. The increasing volume of waste can be attributed to several factors, one of which is the inefficiency of existing waste management systems. The Universitas Negeri Semarang campus occupies a vast area with abundant vegetation throughout its grounds. This vegetation produces a daily accumulation of fallen leaves, which can pollute the environment if not properly managed. These leaves are regularly cleaned by maintenance staff, but some remain on the ground and decompose naturally. This decomposition is partly facilitated by the activity of lignocellulolytic fungi.

Waste is the solid residue of daily human activities and/or natural processes. Waste requires special management due to its nature, concentration, and/or volume. (PP No. 27 of 2020). These residues must be properly managed to avoid adverse impacts on the surrounding environment.

Nearly all activities and locations inevitably generate waste, some of which can be classified as organic waste. Organic waste is derived from biological materials that are biodegradable by microorganisms. It includes commercial waste, garden waste, household waste (Chattopadhyay & Singha, 2022).

Collected organic waste can be repurposed as animal feed or processed into compost fertilizer. Organic waste such as food scraps can often be directly fed to livestock like cattle. Meanwhile, organic waste that is processed into agricultural fertilizer requires microbial assistance for composting. Microorganisms play a crucial role in the composting process, which thrives under warm and moist conditions that accelerate the breakdown of organic matter (Rahim & Selintung, 2014).

In the Universitas Negeri Semarang environment, a significant portion of the leaf waste originates from mahogany trees. Mahogany (*Swietenia mahogany*) is a tropical tree species in the Meliaceae family, native to the West Indies, commonly found growing wild in teak forests, coastal areas, or planted along roadsides as shade trees (Qodri et al., 2014). Mahogany is one of the tree species that produces a substantial amount of leaf litter. According to research by Aryani et al., (2019) mahogany leaf litter contains 9.90 ± 0.65% ash, 24.83 ± 0.79% crude protein, 11.37 ± 1.05% crude fat, 65.14 ± 4.77% volatile matter, 7.66 ± 0.71% charcoal, and 6.61 ± 0.69% moisture.

The lignocellulose biodegradation process is influenced by several factors, including fungal strains, environmental conditions, and nutrient availability. Fungi are multicellular microorganisms commonly found in diverse environmental conditions. According to Purwadaria et al., (2003) fungi exhibit more effective cellulose and hemicellulose degradation capabilities compared to bacteria. Environmental factors such as temperature and pH also significantly affect lignocellulolytic fungal activity. These fungi have an optimal temperature range for efficient functioning. At low temperatures, enzyme activity decreases and microbial growth slows, while at high temperatures, enzymes may denature and microorganisms may perish. Generally, the ideal temperature for fungal growth ranges between 25–30°C (Pujiati & Widiyanto, 2017).

Lignocellulose-degrading fungi found in the Universitas Negeri Semarang campus environment exhibit varying levels of biodegradation activity. Therefore, analyzing and comparing the rate and extent of leaf litter biodegradation using lignocellulolytic fungal isolates from the campus serves as an effort to preserve the environment while also contributing to the effective management of leaf waste.

METHODS

This research was conducted at the Microbiology Laboratory of the Faculty of Mathematics and Natural Sciences, Universitas Negeri Semarang. The fungal samples used in this study were lignocellulolytic fungi isolated from the campus environment of Universitas Negeri Semarang,

located in Gunungpati District, Semarang City, Central Java Province. The fungal species used in this study were *Trichoderma erinaceum* and *Trichoderma koningiopsis* (Sulaiha et al., 2022)

Inoculum Preparation

The inoculum was prepared using a mixture consisting of 80% rice flour, 5% soybean flour, and 5% corn flour. These ingredients were homogenized with the aid of water and steamed for 1 hour to sterilize the components. Once cooled, the fungal cultures—previously suspended in NaCl to facilitate even mixing—were inoculated into the substrate (Safitri et al., 2011).

Inoculation of Lignocellulolytic Fungi on Leaf Waste

Prior to inoculation, the leaf waste substrate was shredded. A total of 100 grams was weighed and placed into Erlenmeyer flasks, which were then covered with heat-resistant plastic. The media were sterilized by autoclaving for 15 minutes. After cooling, pure fungal colonies were inoculated into the substrate and incubated at room temperature for 7, 14, 21, and 28 days (Rahim et al., 2015).

Measurement

After 28 days of incubation, the mass of the leaf waste was re-measured using an analytical balance to determine the biodegradation rate, which was calculated using the following formula:

$$V = \left[\frac{W1-Wn}{\Delta t}\right]$$

Notes:
V = Biodegradation rate (mg/day)
W1 = Initial weight of the leaf waste
Wn = Final weight of the leaf waste after day *n*
Δt = Time elapsed for biodegradation (Sari et al., 2019)

Data Analysis

The data were analyzed using the t-test method. Several assumptions must be fulfilled for the data to be considered suitable for t-test analysis, including a normal distribution. Normality was tested using the Shapiro-Wilk test, which is appropriate for small sample sizes ($n \leq 50$). In the Shapiro-Wilk test, data are considered normally distributed if the significance value (p) exceeds 0.05 ($p > 0.05$).

If the data do not meet the assumption of normality, non-parametric testing using the Mann-Whitney U test is conducted instead. A significance value (2-tailed) below 0.05 indicates a statistically significant difference between the tested groups, while a value above 0.05 suggests no significant difference.

RESULTS AND DISCUSSION

Biodegradation Rate Analysis

Descriptive analysis of the biodegradation rate is presented in Table 1, which shows the weight of the degraded leaves over time.

Table 1. Degraded leaf weight (g)

Fungal Species	Replicate	Leaf Weight			
		Day 1	Day 7	Day 14	Day 28
TE	1	115,29	115,25	115,22	115,21
	2	117,53	117,50	117,50	117,45
TK	1	120,04	120,03	120,01	119,94
	2	123,73	123,05	123,02	122,97

Notes:
TE : *Trichoderma erinaceum*
TK : *Trichoderma koningiopsis*

Following data collection, a normality test was conducted using the Shapiro-Wilk test (Table 2)

Table 2. Shapiro-wilk normality test results

		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Kapang	Statistic	df	Sig.	Statistic	df	Sig.
Biodegradatio	TE	.315	8	.019	.687	8	.002
n	TK	.318	8	.017	.751	8	.008

a. Lilliefors Significance Correction

The significance (p) values for both *T. erinaceum* and *T. koningiopsis* were less than 0.05, indicating that the data were not normally distributed. Therefore, the non-parametric Mann-Whitney U test was applied.

Table 3. Mann-whitney test results

Test Statistics[a]	
	Biodegradation
Mann-Whitney	0,000
Wilcoxon W	36,000
Z	-3,363
Asymp. Sig. (2-tailed)	**0,001**
Exact Sig. [2*(1-tailed Sig.)]	0,000[b]

The Mann-Whitney U test showed a significance value below 0.05, indicating a statistically significant difference in the biodegradation rate of leaf waste between the two fungal species isolated from the Universitas Negeri Semarang campus environment.

The observation period in this study was only conducted for 28 days. A 28-day observation period provides only a limited view of lignocellulose biodegradation. Based on the observations, the biodegradation process requires a longer duration to be observed optimally. Therefore, the results of this study should be interpreted with the note that a longer observation period is necessary to obtain a better

understanding of the lignocellulose biodegradation capability of the two observed lignocellulolytic fungal isolates. Extending the observation period may yield more accurate data regarding the changes that occur. According to the literature review, cellulose is one of the most abundant biopolymers in nature, found along with lignin and hemicellulose in plant-derived substances. Cellulose present in plant waste is difficult to degrade naturally and requires 4–5 months (Pötzinger et al., 2017). The fungal species with the highest biodegradation rate is shown in Table 4. *T. koningiopsis* demonstrated a higher lignocellulose biodegradation rate of 0.015 g/day compared to *T. erinaceum*, which showed a rate of 0.003 g/day.

Table 4. Lignocellulose biodegradation rate calculation

Fungal Species	Replicate	Leaf Waste Weight Loss (g)	Avg. Weight Loss	Biodegradation Rate (g/day)
TE	1	0,08	0,08	0,003
	2	0,08		
TK	1	0,10	0,43	0,015
	2	0,76		

Biodegradation Level Analysis

The degree of lignocellulose biodegradation was assessed by measuring the pH change of the media, as shown in Table 5. *T. koningiopsis* showed a greater pH reduction (0.3) than *T. erinaceum* (0.1), indicating a higher biodegradation level.

Table 5. Media pH changes

Fungal Species	Replicate	Initial pH	Final pH	pH Change	Avg. pH Change
TE	1	6,7	6,6	0,1	0,1
	2	6,7	6,6	0,1	
TK	1	6,7	6,4	0,3	0,3
	2	6,8	6,5	0,3	

According to de Assis et al., (2024), *T. erinaceum* menghasilkan produces 16 types of Plant Cell Wall-Degrading Enzymes (PCWDEs) to degrade cellulose. Cellobiohydrolases I and II exhibit the highest secretion levels on sugarcane straw (SCS) and energy cane bagasse (ECB). The enzyme secretion profile varies depending on time and substrate. Cellobiohydrolase II is secreted more abundantly at 96 hours, while Cellobiohydrolase I peaks at 120 hours. Endoglucanases are detected at low concentrations. One β-glucosidase was identified in both SCS and ECB, with the highest concentration at 120 hours. One lytic polysaccharide monooxygenase is also produced, showing a different secretion pattern between ECB and SCS. However, there is no scientific evidence yet indicating that *T. erinaceum* is capable of producing lignin-degrading enzymes.

The species *T. koningiopsis* produces various enzymes such as endoglucanase, cellobiohydrolase, and β-glucosidase, which function in the process of cellulolysis. These enzymes work synergistically to break down cellulose, with each enzyme having a specific role in the process. Endoglucanase acts on cellulose, while cellobiohydrolase and β-glucosidase convert cellulose into glucose. These enzymes can

function independently or in combination, exhibiting a synergistic effect in the presence of the other enzymes (Tripathi et al., 2013).

T. koningiopsis also produces the enzyme lignin peroxidase (LiP), which enhances its overall effectiveness. LiP is capable of breaking down lignin found in leaf litter (Gendokesumo et al., 2022). LiP is an extracellular peroxidase enzyme whose activity depends on H_2O_2. It oxidizes a variety of substrates, including phenolic and non-phenolic compounds, through a single-electron transfer process. This results in the formation of phenoxy radicals and cation radicals. Subsequently, the enzyme undergoes spontaneous reactions with water and oxygen molecules, leading to a phenomenon known as enzymatic combustion. This process involves cleavage of C–C and C–O bonds, depolymerization of polymer compounds, and opening of aromatic rings. Therefore, the fungus *T. koningiopsis* is more effective in degrading lignocellulose, as it demonstrates a higher rate and extent of lignocellulose biodegradation compared to *T. erinaceum* over time in decomposing leaf litter.

CONCLUSION

T. koningiopsis has a higher lignocellulose biodegradation rate, with a value of 0.015 grams/day, whereas *T. erinaceum* has a lignocellulose biodegradation rate of 0.002 grams/day. In addition, *T. koningiopsis* shows a higher level of lignocellulose biodegradation, indicated by a pH change of 0.3, compared to *T. erinaceum*, which shows a pH change of 0.1. These findings indicate that *T. koningiopsis* has strong potential to be utilized as an effective biodegradation agent for managing leaf waste on campus scales, thereby supporting environmentally friendly waste reduction strategies

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