BIOPENDIX
JURNAL BIOLOGI PENDIDIKAN DAN TERAPAN

EFFECTS OF ADDITION BAGASSE DEGRADANT BY CELLULOLYTIC BACTERIA *Bacillus subtilis ON GROWTH MEDIA OF OYSTER MUSHROOM Pleurotus ostreatus*

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Abstract

Background: Sugarcane *(Saccharum officinarum* L.) serves as a valuable resource for the sugar and beverage industries, a significant quantity of its by-product, bagasse, remains underutilized. A promising strategy for increasing the value of bagasse involves improving its substrate quality through the application of cellulose-degrading bacteria, such as *Bacillus subtilis.*
Methods: The objective of this research is to investigate the influence of bagasse degradant supplementation on the growth of oyster mushrooms. A randomized experimental design was employed to evaluate growth media containing varying concentrations of bagasse degradant (500, 600, 700, and 800 gram) in comparison to a control group. The study assessed the impact of bagasse degradant on pin diameter, fresh weight, dry weight, and water content, using ANOVA and the Duncan's Multiple Range Test (DMRT) at a significance level of 5%.
Results: The study showed the addition of bagasse degradant affected the pin diameter, fresh weight, and dry weight of oyster mushroom. However, it is not effective towards the water content of oyster mushroom.
Conclusion: The addition of bagasse degradant affected the pin diameter, fresh weight, and dry weight, whereas the water composition did not affected the growth of *P. ostreatus*

Keywords: Bagasse, Cellulolytic Bacteria, Oyster Mushroom.

Abstract

Latar Belakang: Tebu (*Saccharum officinarum* L.) merupakan sumber utama bagi industri gula dan minuman, namun produk sampingannya yang berupa ampas tebu masih kurang dimanfaatkan. Strategi yang menjanjikan untuk meningkatkan nilai ampas tebu adalah dengan meningkatkan kualitas substratnya melalui penggunaan bakteri pengurai selulosa, seperti *Bacillus subtilis*.
Metode: Penelitian ini bertujuan untuk mengetahui pengaruh penambahan media hasil degradasi ampas tebu terhadap pertumbuhan jamur tiram putih. Pelaksanaan penelitian dilakukan menggunakan Rancangan Acak Lengkap digunakan untuk mengevaluasi media pertumbuhan yang mengandung berbagai konsentrasi pengurai ampas tebu (500, 600, 700, dan 800 gram) dibandingkan dengan kelompok kontrol. Penelitian ini menilai pengaruh degradasi ampas tebu terhadap diameter pin, berat segar, berat kering, dan kadar air, dengan menggunakan ANOVA dan Duncan's Multiple Range Test (DMRT) pada tingkat signifikansi 5%.
Hasil: Hasil penelitian menunjukkan bahwa penambahan media hasil degradasi ampas tebu mempengaruhi diameter tudung, berat basah, dan berat kering. Sedangkan kadar air tidak berpengaruh.
Kesimpulan: Penambahan media degradasi ampas tebu berpengaruh terhadap diameter tudung, berat basah, serta berat kering jamur tiram putih (*Pleurotus ostreatus*) pada komposisi 600 gram.

Keywords: Ampas Tebu, Bakteri Selulolitik, Jamur Tiram.

INTRODUCTION

Sugarcane (*Saccharum officinarum* L.) is a widely cultivated crop in Indonesia. It serves as a primary raw material for sugar production and various beverages. However, a significant portion of the sugarcane by-product, bagasse, still needs to be utilized. Bagasse contains a substantial amount of cellulose which potential resource for animal feed, biomass, and fungal cultivation. According to Christiyanto and Subrata (2005), bagasse contains 47% Carbon (C), 6.5% Hydrogen (H), 44% Oxygen (O_2), 2.5% ash, 2.5% crude protein, 43-52% crude fiber,33.2% hemicellulose, 40.3% cellulose, and 11.2% lignin. Based on its composition, bagasse has the potential to be used as a growth medium for fungi.

The quality of growth media for mushroom cultivation using bagasse can be enhanced by the application of cellulolytic bacteria, which can degrade cellulose and thus reduce the time required for the decomposition of organic materials. The addition of cellulolytic bacteria to bagasse-based media increases the cellulose content and provides additional nutrients for oyster mushroom growth. *Bacillus subtilis* is one example of a cellulolytic bacterium(Saskiawan, 2015).

This research aims to explore the potential bagasse by utilizing it as a sustainable growth medium for white oyster mushrooms (*Pleurotus ostreatus*). The study will investigate the impact of degraded bagasse on mushroom growth.

MATERIALS AND METHODS

Completely Randomized Design was used in this research. Treatments were adapted from Astuti and Kuswytasari (2013), comprising a control (sengon wood sawdust) and four levels of degraded bagasse media (500, 600, 700, and 800 g), with each treatment replicated five times. The study aimed to assess the influence of degraded bagasse as an alternative substrate for cultivating white oyster mushrooms. The composition of mushroom growth media in this study is as follows:

P0 = sawdust 800 g + barn 200 g + corn flour 50 g + $CaCO_3$ 10 g
P1 = bagasse 800 g + barn 200 g + corn flour 50 g + $CaCO_3$ 10 g
P2 = bagasse 700 g + barn 300 g + corn flour 50 g + $CaCO_3$ 10 g
P3 = bagasse 600 g + barn 400 g + corn flour 50 g + $CaCO_3$ 10 g
P4 = bagasse 500 g + barn 500 g + corn flour 50 g + $CaCO_3$ 10 g

The *B. subtilis* cultures were rejuvenated by streaking onto nutrient agar slants and incubating at 37°C for 32 hours, as per the protocol outlined by Korsten and Cook (1996). This method was employed to ensure the vitality and purity of the bacterial cultures used in the experiment.

Microbial suspensions made with a loopful of bacterial culture were aseptically transferred from a nutrient agar slant into 3 mL of sterile distilled water. The suspension was inoculated into nutrient broth supplemented with 12.5 g of bagasse (Saskiawan, 2015). The inoculated flasks were incubated on a rotary shaker at 100 rpm for 48 hours (Prasetya etal., 2012).

The cleaned bagasse was sun-dried to remove moisture. Subsequently, it was shredded using a grinder and adjusted to a humidity level of 60% by adding distilled water. A bacterial suspension of *B. subtilis* was prepared and aseptically inoculated into the bagasse media of 10 mL per 2 kg of bagasse. The inoculated mixture was composted for 6 days until a change in color and odor occurred (Saskiawan, 2015). The result of bagasse degradation product was subsequently mixed with supporting materials, including, bran and corn flour. The baglogs were sterilized in a sterilization bath at 122°C, 1 atm, for 8 hours.

The cooled baglogs were inoculated with white oyster mushroom spawn. The baglogs were incubated until the mycelium had completely covered the growth medium, the process taking 40-60 days. Baglog maintenance involved regular watering, three times per day. The baglogs were deemed ready for harvest when the oyster mushroom caps became visible.

The growth of *P. ostreatus* was assessed by measuring the cap diameter, fresh weight, dry weight, and moisture content of the cultivated mushrooms. Cap diameter was measured horizontally from the rightmost tip to the leftmost tip, passing through the center of the cap. Fresh weight was determined using analytical scales. Dry weight was obtained by

drying the mushrooms in an oven at 67°C for 24 hours and subsequently weighing them. Moisture content was calculated using the formula described below (Ulfa et al., 2014).

$$Water\ contain = \frac{Fresh\ weight - Dry\ weight}{Dry\ weight}\ x\ 100\%$$

RESULTS AND DISCUSSIONS

The data regarding the average cap diamter of P. ostreatus is shown in **Table 1**. Based on this research bagasse, rich in cellulose and lignin, provides an adequate substrate for white oyster mushroom growth. This finding aligns with previous research by Naem *et al.,* (2014) and Gocalves *et al.,* (2005), which the importance of cellulose and lignin for oyster mushroom cultivation. In comparison, sengon wood sawdust, while also containing cellulose and lignin, may not offer the same level of nutritional support.

Table 1. The average of Cap diameter of *P. ostreatus* in different baggase concentrations

Concentration of baggase degradant (g)	Code	Cap Diameter (cm)
500	P4	7.74[a]
600	P3	8.80[ab]
700	P2	10.14[b]
800	P1	9.76[b]
Control	C	9.88[b]

Note*:* The different letters in the same column are significantly different (p< 0.05) based on *Duncan's multiple range test (DNMRT)*

According to Diallo et al., (2017) *Bacillus subtilis* is a cellulolytic bacterium characterized by its high metabolic activity, enabling degrade cellulose efficiently. This degradation facilitates the absorption of cellulose by white oyster mushrooms. Further more, the inoculation of *B. subtilis* into a substrate has been shown to reduce the overall microbial population.

As reported by Brienzo et al., (2016), bagasse- contains approximately 42% cellulose. The other study state, sawdust used as the control, exhibits a cellulose content of 40% (Wahidah et al., 2015). Despite this slight difference in cellulose content, no significant variation was observed in the cap diameter of white oyster mushrooms cultivated on these media. This finding aligns with the research of Maulinda et al., (2015), which also indicated that the growth of white oyster mushrooms,pa rticularly cap diameter, was not significantly affected by variations in the growth medium.

The cellulose content of white oyster mushroom baglogs plays an important role in the formation of primordia, which subsequentl y develop into fruiting bodies. Cellulose in these baglogs is derived from sawdust and degraded bagasse. Diallo et al., (2017) reported that bagasse media inoculated with B. subtilis bacteria can significantly enhance the production of cellulose.

The primary components of the baglog media are sawdust and degraded bagasse, w hich serve as carbon sources. Additionally, br an plays a crucial role in supplementing nitrogen levels within the substrate. This aligns with the findings of Kalsum et al., (2011), who emphasized the significance of nitrogen content in supporting mycelial growth. While the nutritional composition of the baglog is a significant factor, media density also plays a role in influencing cultivation outcomes. Sawdust-based media generally exhibit a denser structure compared to bagasse, which can create a more optimal environment for mycelial growth.

The fresh weight is the weight of white oyster mushrooms at the time of harvest showed in Table 2. The fresh weight of white oyster mushrooms is determined by weighing the entire fruiting body of each mushroom in every experimental group. weight also serves as an indicator of the quality of white oyster mushrooms during the harvesting process (Matondang, 2018).

Table 2 The average of fresh weight of *P. ostreatus* in different baggase concentrations

Concentration of baggase degradant (g)	Code	Frash weight (g)
500	P4	55.15[a]
600	P3	67.44[ab]
700	P2	89.70[b]
800	P1	84.70[b]
Control	C	67.85[ab]

Note: The different letters in the same column are significantly different (p< 0.05) based on *Duncan's multiple range test (DNMRT)*

The fresh weight of white oyster mushrooms is closely correlated with the cap diameter and the number of fruiting bodies produced. The high cellulose content in the substrate can enhance nutrient availability, but an excess can hinder the mushroom's ability to optimally absorb all nutrients. High cellulose content is beneficial for fruiting body growth, leading to a greater quantity and potentially influencing the fresh weight of the mushrooms. According to Ikhsan and Ariani (2017), that excessive cellulose can impact the growth of oyster mushrooms by inhibiting the absorption of other nutrients in the substrate, thereby affecting cap diameter. Fur thermore, the number of fruiting bodies on a substrate can influence both cap diameter and fresh weight. A higher number of fruiting bodies may limit cap diameter growth but can increase the overall wet weight of the white oyster mushrooms.

Excessive bran supplementation in the substrate can adversely affect oyster mushroom growth. While bran increases the nitrogen (N) content of the substrate and can promote mycelial growth, an excessively high nitrogen level may lead to ammonia production, inhibiting fruiting body developme nt. This finding aligns with Estheria's (2008) assertion that elevated nitrogen levels can ge nerate mmonia, thereby hindering the growth of white oyster mushrooms.

Dry weight is a crucial parameter for assessing the moisture content of white oyster mushrooms. It represents the total dry mass of the mushroom, which remains after the removal of water. Muhandri (2017) explained that the dry weight reflects the accumulated biomass of the mushroom. The average dry weight values for *P. ostreatus* are detailed in Table 3.

Table 3 The average of dye weight of *P. ostreatus* in different baggase concentrations

Concentration of baggase degradant (g)	Code	Dry weight (cm)
500	P4	6.72[a]
600	P3	9.25[ab]
700	P2	18.60[c]
800	P1	16.27[c]
Control	C	12.95[bc]

Note: The different letters in the same column are significantly different (p< 0.05) based on *Duncan's multiple range test (DNMRT)*

Dry weight is a crucial parameter for assessing the nutritional quality and biomass of white oyster mushrooms. A higher dry weight indicates a greater accumulation of biomass, suggesting a nutrient richsubstrate. Conversely, a low dry weight is indicative of nutrient deficiencies. Ratri (2007) emphasized the direct relationship between nutrient availa bility and biomass accumulation in white oyster mushroom

There is a positive correlation between the wet and dry weights of white oyster mushrooms. A higher wet weight typically corresponds to a higher dry weight. Wahyuni (2018) explained that the water in the growth medium facilitates nutrient transport and contributes to biomass. A disproportionate relationship between wet and dry weights suggests inadequate nutrient uptake. The dry weight, representing the accumulated nutrient s in the mushroom, is a reliable indicator of its quality (Zuniar and Purnomo 2016).

The moisture content of white oyster mushrooms is calculated by comparing the fresh weight to the dry weight. It represents the proportion of water within the mushroom

and is a crucial factor influencing the nutritional quality. The average moisture content values for *P. ostreatus* are shown in Table 4.

Table 4 The average of water content of *P. ostreatus* in different baggase concentrations

Concentration of baggase degradant (g)	Code	Water content (%)
500	P4	87.08
600	P3	84.62
700	P2	79.94
800	P1	80.76
Control	C	81.3

Based on the ANOVA results, there was no significant difference ($p > 0.05$) in the moisture content of *P. ostreatus* due to the addition of sugarcane bagasse degradation media. Therefore, a post-hoc DMRT test was not performed. The results indicated that the moisture content of white oyster mushrooms was not significantly affected by the different treatments. This consistency can be attributed to the controlled environmental conditions in the cultivation room, including a relatively constant humidity level of around 70% (Trubus, 2014). The baglogs were subjected to the same environmental conditions and watering regime throughout the cultivation period. These findings align with the research of Tesfaw *et al.,* (2015), who highlighted the importance of humidity for optimal mushroom growth.

One factor that may influence the moisture content is a deficiency in the nutrient content of the substrate, leading to an accumulation of water within the fungal cells without contributing to biomass. This finding is in line with Wahyuni (2018), stated that the moisture content of oyster mushrooms significantly impacts their nutritional quality. A higher moisture content correlates with a lower nutritional value, while a lower moisture content indicates a higher quality mushroom.

CONCLUSION

The addition of sugarcane bagasse degradation products, processed by the cellulolytic bacterium *Bacillus subtilis*, as a growth media of *P. ostreatus* significantly affected the cap diameter, fresh weight, and dry weight of the mushrooms.

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