Improving white oyster mushroom productivity by biogas sludge and its potential as functional foods

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Abstract. White oyster mushroom (Pleurotus florida) is widely consumed by Indonesian people in daily life. It contains high nutrients, especially its protein content. Protein can be generated from meat but it is expensive and gives an economic burden to some people, particularly in this pandemic condition. The aim of this research was to improve the productivity of white oyster mushrooms using sludge biogas as biofertilizer and to provide alternative protein sources. The mushroom medium used in this research consisted of M1, M2, and M3, and each medium was made in three replications. Parameters observed were harvesting age, fresh weight, number of caps, stalk length, and diameter of caps. The data were analyzed using one-way ANOVA and the difference between means was determined by DMRT. All media with the sludge addition improved the growth and production of the mushroom. M2 medium demonstrated the best improvement in productivity. The increasing productivity in the M2 medium included fresh weight (118%), diameter of cap (22%), number of caps per cluster (225%), and stalk length (50%). In conclusion, the sludge biogas could be able to enhance the productivity of white oyster mushroom. The increasing production of this mushroom will affect the availability of alternative protein sources and support food safety in this pandemic situation.

1. Introduction

White oyster mushrooms are edible mushrooms that many farmers would like to cultivate them due to their benefits compared to other mushrooms. White oyster mushrooms as require a short growth time in the cultivation. Their fruiting body is resist to diseases and pests. They also can be grown in a simple and cheap way with high yield, wider substrate utilization, sporelessness, wide temperature, chemical tolerance and environmental bioremediation. In addition, these mushrooms are good nutrient sources with beneficial effects for the human body [1,2]. The white oyster mushrooms degrade cellulose, hemicellulose, and lignin of wood whereas brown rot fungi only degrade cellulose and hemicellulose [2,3]. According to Velioglu and Urek [4], in basidiomycete fungi, extracellular laccases are constitutively produced in small amounts and the lignocellulolytic enzymes are affected by medium composition, pH, and temperature as well as aeration rate.

Medium composition is one of the most important factors in the growth of oyster mushrooms. Medium with a complete and rich nutrition for the growth will produce high productivities of the mushrooms.
In contrast, low nutrition media will decrease the production of mushrooms. In commercial media, the growth of the mushroom is still not maximal and needs an improvisation in media composition such as a biofertilizer that is friendly environment. Rice bran can be added into media and can enhance the fresh weight of oyster mushrooms [5]. However, this material can increase the production cost of the mushrooms. Therefore, it needs a biofertilizer that can foster the production of mushrooms with low production cost.

Biogas sludge has a potential to be used as a mixture of rice bran in mushroom media. Generally, the mushroom media uses rice bran as a source of nutrients. Hidayat et al. [6] stated that rice bran contains around 30.39% carbohydrates. Biogas sludge contains 34-35% of the crude fiber as source of carbohydrates. It is known that sludge has the crude fiber content comparable to the rice bran. Thus, a mixture of biogas sludge and rice brain as a substitution can be used as mushroom media. Suparti and Lismiyati [7] stated that the protein content of white oyster mushrooms can be influenced by the composition of their growing media such as cellulose, hemicellulose, lignin, and additional nutrients. In addition, biogas sludge is generated from an anaerobic fermentation of agricultural waste. Therefore, sludge biogas has a potential as biofertilizer with the value added and supports the environmentally friendly agriculture. Pratama et al. [27] demonstrated that agricultural wastes could be processed with using appropriate technologies to increase the value added in agricultural wastes and enhance the economic value [28].

Another factor that plays an important role in the mushroom growth is the seed of the mushrooms. Hamdiyati [8] stated that the superior seed will produce the high quality fruit body of mushrooms and be able to adapt with the environment. Therefore, to get a better mushroom production, the selection of the superior mushroom seed is necessary so that it will produce a better quality of mushrooms and provide alternative nutrients, particularly the protein intake for the human body. Moreover, this protein source has a potential in the substitution of meat protein, due to the expensive meat protein.

It has been reported that the use of rice brain as a mixture of mushroom media is still not effective yet. It needs an appropriate biofertilizer to increase the production of oyster mushrooms such as the biogas sludge. Biogas sludge is supposed to foster the mushroom growth with a low production cost. Therefore, the objective of this research was to improve the productivity of white oyster mushrooms using biogas sludge as bio-fertilizer and to provide alternative nutrient sources, especially protein to support food safety during the pandemic situation.

2. Materials and Methods

2.1 Preparation of biogas sludge
Biogas sludge was dried under the sun for 3 days until its shape resembled soil. It was then divided into 3 parts, each was 40 g and put into a bucket. Then, the biogas sludge obtained, each was 5% of the total weight of the mushroom medium. M1 was 40 g biogas sludge. M2 was a mixture of 34 g sludge plus 6 g of manure meal. M3 was a mixture of 22 g of sludge plus 18 g of manure meal. M1, M2, and M3 were mixed into mushroom media with 3 replications.

2.2 Preparation of mushroom media (baglog)
Sawdust 664 g (83%) in each grow bag was soaked with 60% clean water in a container for one night and it was then drained. M1 medium was a mixture of sawdust 664 g (83%), rice bran 80 g (10%), sludge 40 g (5%), CaCO3 8 g (1%) of, and 8 g (1%) of gyps and they were then mixed and stirred until becoming a completed mixture. While M2 medium used was 34 g of sludge plus 6 g of manure meal and M3 medium used was 22 g of sludge plus 18 g of manure meal. For other materials from the
M2 and M3 medium was same with M1 medium. Each treatment was repeated three times. The next process was followed by grow bag preparation and sterilization, seed inoculation, incubation, growing and maintenance and mushroom harvesting.

2.3 Sterilization of baglog
The raw materials of the mushroom media were put into a polypropylene plastic bag. The plastic bag was filled with more or less ¾ parts, then the ¼ parts were bent inside and given a pralon ring and then covered with cotton. Baglog was inserted into a drum with a flame to be sterilized. The sterilization process was carried out for 5 hours at a temperature of 95°C. After the baglog sterilized, it was cooled down for 24 hours in the sterilization room.

2.4 Inoculation and incubation
Seeds of oyster mushrooms were first destroyed by using a long spatula that had been sprayed with alcohol and burned on the Bunsen fire. About 15 g of seeds were then put on the planting medium by opening the pralon ring, cotton and plastic cover first. After the seeds were inserted, the pralon ring was closed again using cotton. Baglog was then incubated at 25-30°C with a humidity of 65-70%. Baglog was left for 40 days until the harvesting time. The mycelium growth could be known about 1 week after the inoculation. After the mycelium was full, the baglog was ready to be moved to the growth room (kumbung).

2.5 Growing, maintenance and harvesting
The growth room (kumbung) was cleaned first and sprayed with 70% alcohol. Baglog was arranged horizontally on a rack that had been cleaned and the baglog covering cotton was opened slowly. Maintenance was done by watering the floor of the kumbung and spraying water with a sprayer to maintain temperature and humidity. Harvesting was done by pulling all parts of the mushrooms until the root. Harvesting was done in the morning or afternoon to maintain the freshness.
2.6 Biological parameters of oyster mushrooms

The biological parameters of mushroom productivities were included harvesting age, fresh weight, number of caps, stalk length, and diameter of caps. The harvesting age was recorded from the seed inoculation until the first mushroom harvesting. Fresh weight was measured by an analytical scale after the harvesting. The number of caps on the mushrooms was calculated manually in each fruiting body after harvesting. The stalk length was done by measuring the three highest stalks of mushrooms and then they were averaged. The diameter of caps was measured in three widest mushroom caps and then averaged. The research had been conducted at the Agrotechnology Innovation center (PIAT) Universitas Gadjah Mada Yogyakarta, Indonesia. Environmental conditions of the mushroom production were made at the average temperature of 28.98°C and the average humidity of 55.94%.

2.7 Statistical analysis

The data obtained were processed with the variance analysis (one-way ANOVA) and the difference between the means was analysed with Duncan's Multiple Range Test. The data were presented as means ± standard deviation (SD) and all data were in triplicate experiment. The statistical analysis was performed using SPSS Statistics 21 (IBM, Chicago, USA).

3. Result and Discussion

3.1 Biological parameters

The productivity of white oyster mushrooms using biogas sludge could be seen in the table 1. The biological parameters such as age harvesting, fresh weight, number of caps, stalk length and diameter of caps were evaluated after harvesting of the mushrooms. Generally, the productivity of the mushrooms increased with a shorter harvesting time.

Table 1. Productivity of white oyster mushrooms

| Parameters                  | MC*  | M1        | M2          | M3          |
|-----------------------------|------|-----------|-------------|-------------|
| Harvesting age (days)       | 58.33±1.15 | 43.67±0.58  | 42.67±1.15  | 44.33±0.58  |
| Fresh weight (g)            | 55.33±5.80 | 76.30±9.37  | 111.95±18.15| 119.07±12.11b|
| Number of caps              | 9.00±2.00 | 11.67±5.13  | 20.67±8.33  | 32.00±6.25b |
| Stalk length (cm)           | 4.07±0.48 | 5.15±0.33  | 5.32±0.64   | 6.79±2.07   |
| Diameter of cap (cm)        | 9.26±1.08 | 9.37±1.15a  | 9.54±1.27b  | 11.08±1.01b |

Notes:
(a, b, c): Different superscripts on the same row indicate significant differences in terms of medium
(*) : Pertiwiningrum et al. (2018)
MC: Commercial media (without sludge addition)
M1: Mushroom medium with sludge addition
M2: Mushroom medium with the addition of sludge and manure
M3: Mushroom medium with the addition of sludge and manure

3.2 Harvesting age

The harvesting age tended to be shorter with the addition of biogas sludge as biofertilizer. M1 medium had the average harvesting age of 43 days, while M2 and M3 medium had respectively the average harvesting age of 42 days and 44 days (Table 1). Compared to CM medium (58 days), sludge biogas was able to make a shorter harvesting age of the mushrooms in the medium of M1, M2 and M3. Parlindungan [9] stated that the entire surface of the grow bag will be full of white mycelium within
40-60 days. In this research the grow bag had been filled with mycelium in less than 40 days (data not shown) then the cover of the grow bag was opened. Based on the results, the time required for the mycelium to grow was faster than the time mentioned in the literature. It was due to the addition of biogas sludge in the media that can increase the nutrient content of media.

Parlindungan [9] stated that in a period of 1-2 weeks after the grow bag was opened, shoots would grow within 2-3 days then they would form the fruit body. In this study, the shoots began to appear 1 week after the cover of the grow bag was opened. They then became the fruit body and 1 week later, they were ready to be harvested. The time required for the shoots to grow was slightly faster than the time mentioned in the literature. The sludge biogas may also affect the shoot growth during the cultivation due to its nutritional supports. Nurilla [10] stated that the contamination and the presence of pests in media are one of factors affecting the harvesting age. Ediningtias and Utami [11] also reported that the quality of mushroom seeds will decrease when they are more than 4 weeks old after the inoculation process (planting). Therefore, it is necessary to know the date to start seeding.

3.3 Fresh weight
The Fresh weight of white oyster mushrooms significantly increased after the addition of biogas sludge in the mushroom media. Table 1 showed that the highest fresh weight was in the M3 medium (119.07 g) and the lowest was in the MC medium (76.30 g). The Fresh weight in M3 medium was 118% higher than the MC medium. According to Djarijah [12], the weight of oyster mushrooms during the first harvesting is ranged between 50-75 g. The fresh weight of the mushroom is an indicator of the increasing mushroom productivity. The fresh weight of the mushrooms in this study was higher than the weight mentioned in the literature. The fresh weight of the mushrooms obtained in media with sludge addition was higher than the fresh weight of white oyster mushroom obtained from Pertiwiningrum et al. [1] with its highest fresh weight value (59.66 g).

According to Yanuati [13] stated, the media in which the mycelium has covered the growing media faster will supply the nutrients earlier than the growing media that is not full of mycelium. The growing media with full mycelium will collect energy for the formation of the fruit body. The formation of mycelium is the early phase in the development of mushroom before the formation of the pinhead (primordia) or the mushroom fruit. Onyango et al. [14] stated that white oyster mushrooms could be grown in a variety of agricultural waste. Therefore, the biogas sludge as the agricultural waste could be applied for the mushroom media.

3.4 Number of caps
The addition of the biogas sludge to mushroom media could affect the number of oyster mushroom caps. Based on the table 1, the highest average number of caps was in M3 medium (32 caps) and the lowest was in MC medium (9 caps). The number of caps in M3 medium was 225% higher than in MC medium. It showed that, compared to the MC medium, the M3 medium was optimal for the productivity of the mushroom related to the number of caps. According to Soenanto [15], the number of caps is an indicator of the increasing mushroom productivity. The number of caps of white oyster mushroom obtained in media with sludge addition was higher than the number of caps of white oyster mushroom obtained from Pertiwiningrum et al. [1] with the highest number (11 caps).

3.5 Stalk length
The addition of biogas sludge to the mushroom media increased the length of oyster mushroom stalks. The highest average stalk length was in M3 medium (6.79 cm) and the lowest was in MC medium (4.07 cm). The length of the mushroom stalk in M3 medium was 50% higher than in the MC medium. According to Gunawan [16], the length of white oyster mushroom stalk is about 0.5-4.0 cm. Based on the results of the research, the stalk length in the media with the addition of biogas sludge was longer
than the stalk length mentioned in the literature. The stalk length of white oyster mushrooms obtained in the media with the addition of biogas sludge was higher than the stalk length of white oyster mushrooms obtained from Pertiwiningrum et al. [1] where the highest stalk length value was 4.68 cm.

3.6 Diameter of caps
The addition the biogas sludge could affect the diameter of oyster mushroom caps. Table 1 showed that the widest diameter of cap was found in M3 medium (11.08 cm) and the lowest was in the MC medium (9.66 cm). The diameter of mushroom cap in M3 medium was 22% higher than in the MC medium. The diameter of the mushroom cap was influenced by the moisture level. This is in accordance with the statement of Seswati [17] which reported that the optimum water content on oyster mushroom media is about 60%. White oyster mushroom has the cap diameter about 10-13 cm [1,18]. The diameter of mushroom caps in M3 media was in accordance with the literature. Essential nutrients, especially cellulose from media substrate, will be absorbed by the spores to grow into mycelium and to develop into adult mushrooms [1,15].

3.7 Potential of white oyster mushroom as functional foods
White oyster mushrooms have high nutrient contents such as protein with beneficial effects in body and health-promotion actions. The oyster mushrooms contain around 19/100 g to 39/100 g in the dry weight basis with [19,20] with low calorie values and rich in vitamins, essential minerals, and trace elements [21]. The high protein and low fat content make the mushrooms as sources of good dietary foods. Recent researches have demonstrated the mushrooms with the active components against some diseases such as hypertension, diabetes, and cancer [21]. They also have been used as antibacterial, anti-inflammation and antioxidant [22].

The components that are responsible for the health-supporting actions would be like the bioactive compounds such as bioactive peptides derivative from the mushroom protein. During digestion, the mushroom protein would be degraded by protease enzymes and release short peptides that would be absorbed into the blood system. Sato [23] reported that pyroglutamyl peptides were short peptides derivative from food protein directly absorbed into the blood and have shown biological functions as
in vitro and in vivo studies. Bioactive peptides in body generated during digestion and absorption will be transferred by the blood system to the tissue [24]. In addition, hydrophobic amino acid contents may affect the health-supporting actions [25]. It also reported that short sequence peptides had the ACE inhibitory activity [26]. For this reason, bioactive components in the oyster mushrooms need to be explored further.

4. Conclusion

It can be concluded that the addition of biogas sludge into the media could generally enhance the productivity of white oyster mushrooms. The fresh weight, diameter of caps, total number of caps, and stalk length increased after the addition of the biogas sludge. Also, the harvesting age was shorter compared to the commercial medium (MC). The biogas sludge may contribute to providing nutrients for the mushrooms and support for the mushroom growth. In addition, the use of biogas sludge as biofertilizer as also decreased the agricultural waste with the value added. It can be the alternative way to cultivate white oyster mushroom and increase the income of farmers with the low production cost. Moreover, with the increasing of the mushroom productivity, it will provide more the alternative nutrient sources with beneficial effects in body and support the food safety during this pandemic condition. Therefore, the next research will be the bioactive peptides in the white oyster mushrooms.

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