The potential of Biogas Sludge Dairy Cattle with Fortification of Expired Milk Powder Waste and Eggshell as a Planting Media of White Oyster Mushroom (Pleurotus florida) on Mycelium Growth

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Abstract. This study aimed to determine the nutrient content of the growing media of sludge biogas waste from dairy cattle and expired milk waste and the growth of white oyster mushroom mycelium (Pleurotus florida). This study uses mixed media from dairy cattle biogas sludge waste and expired milk powder with different concentrations divided into 4 media treatments, 4 treatments then performed 3 repetitions to obtain 12 experimental units, the treatment is given code P0, P1, P2, P3 which has a media composition in the form of sawdust, biogas sludge, expired milk powder waste and eggshell, each treatment is distinguished in different concentrations of sludge and expired milk powder with different proportions namely P0 (0%:0%), P1 (50%:50%), P2 (25%:75%), P3 (75%:25%), P0 is used as a control because it does not use the two combination ingredients but uses bran as a source of nutrients and is a proportion commonly used by oyster mushroom farmers. Each media was then tested for its chemical content including water content, organic matter, crude fiber, C-organic, nitrogen, P-Total, and potassium. Then for the growing mycelium variable observed were the mycelium length, the percentage of growth of oyster mushroom mycelium, the first time to grow, and the time to fulfill mycelium. The data was processed using analysis of Variance-Completely Randomized Design Unidirectional and the average difference was tested using Duncan's Multiple Range Test (DMRT). Mushroom media nutrient content test results showed that the best results were in the planting media of sludge biogas waste from dairy cattle and expired milk waste in P3 treatment (75% dairy biogas sludge:25% of expired milk waste) was able to increase nutrient in a c-organic variable, crude fiber, C / N ratio, and potassium are 19.61%, 57.43%, 3.179%, and 0.887%. The best results of mycelium growth in this study were the planting media of dairy cattle biogas sludge waste and expired milk waste in P2 treatment (25% of dairy cattle biogas sludge:75% of expired milk waste) which was able to produce the best growth of the average variables of the daily mycelium length, percentage of growth mycelium, first time to grow and time to fulfill mycelium is 3.73 cm, 27.42%, 4.33 days and 26.33 days. In this study, it can be concluded that the planting media from dairy cattle biogas sludge waste with the fortification of expired milk powder waste can be potentially used as an alternative media other than bran at the same time can increase the nutrient content in the mushroom media and stimulate the growth of mycelium better.

Keywords: White Oyster Mushroom, Biogas Sludge, Expired Milk Powder, Quality of Media, Mycelium Growth
1. Introduction
The increasing number of human populations in the world also increases food needs, especially the need for animal protein that must be fulfilled. Animal protein comes from livestock, as a biological effort of livestock each produces waste in the form of feces and urine, and if it is not processed it will, of course, cause waste which is, of course, harmful to the environment or human health itself, the university as one axis of knowledge must be able to solve problems this with research innovations with the theme of handling waste so that it is not only safe for the environment but also useful to provide economic added value and also benefit the community. So far, livestock waste such as feces is usually processed into various products such as compost or biogas. Biogas installation certainly can utilize and add value-added from livestock waste, but by processing feces into biogas does not mean solving a problem, biogas still has waste that is sludge which if it’s not resolved it will create new problems in the environment. Sludge itself still contains many nutrients can be a source of carbon for microbes, and is very well used as a nutrient carrier because it contains various elements needed for microbial growth such as P, Mg, Ca, K, Cu, and Zn [1]. In addition, sludge has a potential to be used as mushroom media (2). However, previous studies using dairy cattle sludge as an oyster mushroom medium have not yielded significant results on the productivity of oyster mushrooms [1]. The appropriate processing of agriculture waste can enhance the value added (3) and economic value (4). It is expected that the addition of expired milk powder and eggshell as a fortification agent and as added nutrition can give better results so that it can be basic research for the future.

Mushroom growing media usually use bran as a source of added nutrition in commercial media currently used by mushroom farmers, bran itself is rich in nitrogen, carbon, and carbohydrates, usually used as a source of additional nutrients for the growing media of oyster mushrooms, but the material is also an ingredient animal feed so if viewed from an economic standpoint it will even add to production costs, expired milk one of waste still has a rich nutrient content as well as bran, expired milk powder also is a source of carbohydrates and protein, which is an essential nutrient needed by oyster mushrooms, it is carbohydrate component provides nutrition to mycelium, hyphae threads break down carbohydrate material into simple compounds such as sugar that can be used as energy to be metabolized so that mycelium can quickly grow and appear on log [5]. Waste of expired milk is a consequence of time-limited production codes, besides that, it is also high in product returns (return product), and is usually only handled by burning, even though the expired milk waste still has a high nutrient content, this material is potentially used as an ingredient compost and decomposer enrichment, expired milk is used as a compost enrichment material can provide better quality compost in the total C-Organic content, N-total, P$_2$O$_5$, and when the compost applied to Pakchoi vegetables produced a higher crop weight height, increase soil fertility and topsoil content in the soil [6].

One of the other ingredients needed by oyster mushrooms is calcium, usually in the cultivation of white oyster mushrooms, lime is used as a calcium source, and usually, mushroom farmers have to buy it in agricultural shops, and of course, this adds to the production costs even though there are alternative media that can be utilized and abundant availability. It is an underutilized household waste, which is an eggshell, the eggshell can be used as a substitute for lime in making mushroom growing media because it contains calcium, calcium is an important nutrient needed for oyster mushrooms to grow [7]. Oyster white mushrooms have heterotrophic properties that cannot synthesize their food, oyster mushrooms take food substances such as cellulose, glucose, lignin, proteins, and starch compounds from other organisms [8]. In this research, the use of sludge and expired milk waste is expected to be an alternative nutrient supplementary media for white oyster mushroom, to be one of the solutions for processing biogas sludge from dairy cattle and expired milk powder that is environmentally friendly, increasing the productivity of white oyster mushrooms, useful to support the concept of the system integrated agriculture, as well as an alternative media for growing oyster mushroom that is easily available and abundant in the environment.
2. Material and methods

This research was conducted at the leather science and technology, byproducts and livestock waste laboratory, Faculty of Animal Science, Universitas Gadjah Mada and House of organic mushroom unit Faculty of Animal Science, Universitas Gadjah Mada.

2.1. Materials

**Tool.** The tools used in this study are buckets, sterilization drums, shovels, plastics, polypropylene plastics, analytical scales, drums, stoves, filters, bottles, log rings, cotton, rubber, sacks, spatulas, Thermo hygrometers, gloves and masks, Erlenmeyer tubes, measuring cup, test tube, stirrer, porcelain cup, laminar airflow, petri dish, pH meter, gooch crucible, filter paper, cotton plack, dropper pipette, micropipette, spatula, Bunsen lamp, sprayer, set of destructors, vortex mixer, oven, furnace, spectrophotometer.

**Materials.** The materials used for this research were fresh dairy cattle sludge, sengon sawdust, expired milk powder, oyster mushroom F3 seeds, water, eggshell, methylated spirits, aqua dest, H$_2$SO$_4$, NaOH, selenium mixture, tartrate buffer, Na phenate, NaOCl 5%, HNO$_3$, HClO$_4$, ascorbic acid, and concentrated reagents.

2.2. Methods

Biogas sludge of dairy cattle was dried, then it was crushed using grinding machine, and filtered to obtain small particles, then other ingredients are prepared such as dried eggshells, expired milk powder, sawdust, bran, then the ingredients are formulated into four treatments and carried out three times and then repeated, each consisting of different proportions presented in Table 1 below.

| Materials for the media | $P_0$ (g) | $P_1$ (g) | $P_2$ (g) | $P_3$ (g) |
|-------------------------|-----------|-----------|-----------|-----------|
| Sawdust                 | 750       | 750       | 750       | 750       |
| Bran                    | 200       |           |           |           |
| Biogas Sludge           |           | 100       | 50        | 150       |
| Expired milk powder     |           | 100       | 150       | 50        |
| Eggshell                | 50        | 50        | 50        | 50        |
| Total (%)               | 1000      | 1000      | 1000      | 1000      |

The ingredients are then mixed and allowed to stand for a night, then just put into the plastic baglog with a thickness of 0.5 mm, the plastic is compacted and given a log ring and then sterilized in a sterilization drum for 8 hours with a temperature of about 95 °C. Then the baglogs were silenced for one night then after a cold the next day they were ready to be inoculated F3 white oyster mushroom seeds, inoculation was carried out aseptically so that there were no contaminants and fast in a sterile inoculation room, the seeds in the bottle were crushed and by using a sterile spatula with pouring it on the ring that has been opened cotton, the spatula is first burned using a methylated lamp, and the media is also sprayed briefly with methylated, as soon as possible cover using cotton, then incubated in a special room with a temperature of 22–28 °C, for two until four weeks until the mycelium is evenly white covering the entire surface of the to the bottom of the baglog.

2.2.1. Analysis of nutrients values. After making the media then testing the quality content of materials and planting media used. Testing the nutrient content of mushroom media and the main ingredient of this research is the sludge carried out is the measurement of water content, organic matter content, crude fiber, nitrogen content, C-organic content, nitrogen content, C/N ratio, phosphorus and potassium levels. Tests of water content and organic matter, and crude fiber using proximate analysis methods, nitrogen content testing using spectrophotometry, and phosphorus testing carried out using the UV Visual Spectrophotometer, while the potassium test was tested using the Atomic Absorption Spectrophotometry (AAS) method.
2.2.2. **Measurement of mycelium growth.** Measurements of mycelium growth include the length of mycelium propagation, the percentage of the baglog which is covered by mycelium. When it first grew and the time of mycelium fulfillment.

2.2.3. **Data Analysis.** all data included analysis of a nutrients values dan measurement of mycelium growth in this study were analyzed using a Completely Randomized Pattern Complete Analysis (CRD) analysis. Then proceed with Duncan's Multiple Range Test (DMRT) tests for significantly different data.

3. **Result and Discussion.**

3.1 **Nutrient Level of Biogas Sludge and Expired milk powder**

The main ingredients in this research are biogas dairy cattle sludge waste and expired milk waste tested for nutrient content which includes water content, organic matter, crude fiber, c-organic, nitrogen, P$_2$O$_5$, potassium. The result obtained from the test is shown in Table 2.

| Variables       | Biogas Sludge (%) | Expired milk powder (%) |
|-----------------|-------------------|-------------------------|
| Water Content   | 40.62%            | 2.93%                   |
| Organic Matter  | 48.80%            | 93.79%                  |
| Crude fiber     | 24.49%            | 2.64%                   |
| C-Organic content| 13.67%            | 14.301%                 |
| Nitrogen (N)    | 7.119%            | 7.429%                  |
| C/N Ratio (C/N) | 1.921%            | 1.926%                  |
| P$_2$O$_5$ (P)  | 1.367%            | 0.378%                  |
| Potassium       | 0.4509%           | 0.0623%                 |
| Carbohydrate    | -                 | 75.43%                  |
| Lipid           | -                 | 2.64%                   |
| Protein         | -                 | 15.54%                  |

Source: Analysis Result.

The biogas sludge content in this study contained many nutrients in the form of 40.62% water content, 48.80% organic matter, crude fiber 24.49%, C-organic 13.67%, Nitrogen 7.119%, C/N ratio 1.921%, phosphorus 1.367%, and potassium 0.4509%. When viewed from the biological content, even though it has become sludge waste it still has a high nutrient content, namely organic matter, crude fiber, carbon, nitrogen, phosphorus and potassium, the biogas fermentation process produces sludge (sludge) which can be used as organic fertilizer [9]. Sludge can be used as a source of carbon for microbes, it is also very good as a nutrient carrier because it contains various elements needed by microbial growth such as P, Mg, Ca, K, Cu, and Zn. The content can be used as a mushroom media [1].

While the expired milk used in the form of milk waste originating from the Milk Processing Industry Waste and applied to the oyster mushroom growing media, the results of the analysis of the nutrient content of the expired milk used in this study were in the form of the water content of 2.93%, organic matter 93.79%, fat 2.64%, Protein 15.54%, Carbohydrate 75.43%, crude fiber 2.64%, C-organic 14.301%, Nitrogen 7.429%, C/N ratio 1.9626%, phosphorus 0.378%, potassium 0.0623%. Milk waste contains high protein, carbohydrate and phosphate, and potassium as well as other elements that can be complementary [6]. Milk powder waste can be used as a decomposer and can also be used for compost enrichment, if it is used for compost it will produce better nutrient content, such as total C-Organic, N-total, P$_2$O$_5$, and higher K$_2$O, both rejects and sludge biogas have high nutrient content which is a substance needed for oyster mushrooms in its growth [6].
3.2. Nutrient level of white oyster mushroom media

Analysis of nutrient content in mushroom media made includes water content, organic matter, Nitrogen, Organic-C, C/N ratio, Potassium, Phosphorus. The nutrient content of oyster mushroom growing media can be seen in Table 3.

| Variables                  | P0          | P1          | P2          | P3          |
|----------------------------|-------------|-------------|-------------|-------------|
| Water Contents             | 72.31±0.85a | 74.07±1.39b | 76.42±1.04a | 76.14±0.75a |
| Organic Material           | 23.14±0.59a | 23.10±1.67a | 21.62±0.42a | 21.49±0.79a |
| Crude Fiber                | 55.27±1.24a | 56.69±1.76ab| 54.67±0.86ab| 57.43±0.70b |
| Organic-C                  | 16.68±0.59b | 16.89±0.13b | 19.11±0.27c | 19.61±0.14a |
| Nitrogen                   | 6.00±1.06a  | 6.34±0.38a  | 6.44±0.23a  | 6.17±0.22a  |
| C/N Ratio                  | 2.84±0.53a  | 2.66±0.13a  | 2.96±0.10a  | 3.17±0.10a  |
| P2O5                       | 0.44±0.26b  | 1.17±0.26a  | 0.98±0.06c  | 1.15±0.05b  |
| Potassium                  | 1.19±0.15a  | 0.59±0.04b  | 0.50±0.05b  | 0.88±0.05c  |

Source: Analysis Result.

3.2.1. Water content. The results of the analysis showed that the water content of the oyster mushroom media in P2 and P3 treatments with results of 76.42 and 76.14 gave a significant effect (P <0.05) showing a real difference to the control given while P1 and P0 with successive results 72.31% and 74.07%. The lowest water content was in the P0 (control) treatment that was 72.31% and the highest water content was 76.42% in the P2 treatment. The water content contained in the mushroom media is around 60–65%, this is conditioned so that the mycelium can grow well and can absorb media nutrients properly [10]. In a previous study, the water content of oyster mushroom growing media with a mixture of 20% of dairy cattle sludge produced a water content of 51.93% [1]. The water content of the treatment results showed the water content of the mushroom media was very high because the sludge in this study already contained as much as 52.07% water it was suspected the sludge material made increase the water content.

3.2.2. Organic material. The results of statistical analysis showed that all treatments P1, P2, P3 are 23.10, 21.62, and 21.49% did not show any significant difference (P>0.05) when compared with the P0 control i.e., 23.14, the most organic matter low was found in treatment P1 which was 23.10% while the highest levels of organic matter were shown in treatment P2 which was 21.62%. The result was thought to be influenced by the addition of milk whose milk itself contained very high organic matter, amounting to 96.3%. As for the lowest organic matter, it is thought to be due to the high-water content factor in P3, 76.14%. Organic matter has important properties if it is added to the growing media, among others, it can be used as a material to improve soil physical properties and ensure the growth of plant roots through good drainage and aeration [11].

3.2.3. Crude fiber. The results of the statistical analysis showed that the crude fiber content of oyster mushroom growing media treatment of P1, P2, P3 obtained results of 56.69, 54.67, and 57.43% which showed a significant difference (P<0.05) when compared with PO (control) is 55.27%. The highest level of crude fiber was found in P3 at 57.43%, while the lowest treatment was in P2 is 54.67%. The high crude fiber content in P3 is thought to be due to the addition of sludge which has a crude fiber content of 24.49% while the lowest content in P2 is also thought to be due to the most ingredient given is expired milk which has a very low crude fiber content of only 2.67%. The biogas sludge added is a source of crude fiber. The content of crude fiber in an ingredient affects crude fiber in a medium [10]. Another literature which is a source of crude fiber in this study is Sengon sawdust, sawdust has a crude fiber content of 53.33%) [12].
3.2.4. C-organic. The results of the statistical analysis showed that the C-organic content of the mushroom planting media, P2 and P3 treatments namely 19.11% and 19.61% showed significant differences (P <0.05) when compared with P0 (control) 16.68% that is, whereas for P1 is 16.89% had no significant effect compared to existing controls. The lowest C-organic content was found in the P0 (Control) treatment that was 16.68% while the highest C-organic content was in the P3 treatment which was 19.6%. It was assumed that the content was due to the addition of sludge which had a C-organic level of 13.67%. C-organic is an important parameter and is an indicator of soil quality or planting media because it affects the physical and chemical quality parameters [13]

3.2.5. Nitrogen. The results of the analysis show that the total nitrogen content of all treatments P1, P2, P3 respectively 6.34, 6.44, and 6.17% did not show any significant difference (P >0.05) compared to P0 (Control) 6.00%. The lowest nitrogen level is P0 (control) because the control is a commercial baglog not given any treatment while the highest nitrogen content is in P2 treatment which is 6.44% because the treatment is due to the influence of giving expired milk which has a nitrogen level of 7.429%, is higher than the nitrogen sludge level of 7.119%, then followed by P1 with 6.24% and P3 which is 6.17%, this is thought to be due to the reduction in the level of expired milk so that it affects the nitrogen content of the media. the same range due to the nitrogen content of the sludge material and expired milk does not differ much that is in the same range of 7%. The results of nitrogen levels in this study were still higher than the previous study which stated that the nitrogen content of biogas sludge growing media 20% was 0.93% [1]. Nitrogen itself is needed on the mycelium media used by microorganisms for protein formation

3.2.6. C/N ratio. The analysis showed that the C/N ratio or ratio of carbon and nitrogen levels in all treatments P1, P2, P3 in a row that is 2.66, 2.96, and 3.17% did not show any significant difference (P>0.05) compared to P0 (Control) which is 2.84%. The lowest C/N levels were found in P1 treatment is about 2.66%, while the highest treatment was in P3 is 3.17%. The C/N ratio content in the research is low because the nitrogen content is quite high and the carbon content is low so it produces a low ratio too, a material containing a high C element then C/N the ratio is high if the N content is high, the C/N value will be low, the C/N balance affects the decomposer process, carbon is needed for microorganisms for energy while nitrogen is needed for protein formation [14].

3.2.7. P2O5. The results of the analysis showed that the levels of phosphorus treatment P1, P2, and P3 namely 1.17, 0.98 and 1.15% showed significant differences (P<0.05) when compared with P0 (control) is 0.44%. phosphorus content the highest is the P1 treatment that is equal to 1.17%, while the lowest treatment is the treatment P0 (control) that is 0.44%. The phosphorus level in P1 is thought to be influenced by the administration of sludge which has a high enough phosphorus level, which is 1.367%. The results of this study are still higher than the results of the previous study namely the addition of 20% of dairy cattle biogas sludge and resulting in a phosphorus level of 1.10%. [1]. The addition of phosphorus fertilizer during composting is very important as a nutrient for mushroom growth, accelerates the growth of oyster mushroom mycelium, accelerates the emergence of a pinhead, if phosphorus deficiency can cause slow growth, stunted and root development is inhibited [15].

3.2.8. Potassium. The analysis showed that the potassium levels of treatments P1, P2 and P3 namely 1.19%, 0.59%, and 0.88% showed significant differences in all treatments showed significant differences (P<0.05) compared to P0 (control), all treatments were below the treatment range P0 (control), the highest potassium content was produced by P3 planting media with 75%:25% sludge biogas treatment and milk treatment that is 0.88% and the lowest is 0.50% in the ratio of sludge and Expired milk treatment 25%:75% namely P2 planting media. The high potassium content of P3 media is because P3 media is the media with the highest addition of sludge, while the sludge itself has a potassium content of 0.4509%, then for media with the lowest potassium content because the media has the largest proportion of expired milk while the expired milk in this study only contains potassium levels
of 0.0623. According to the previous study, the oyster mushroom growing media containing 20% sludge of dairy cattle can produce potassium as much as 0.22% [1]. Potassium has many essential functions, namely in protein synthesis, carbohydrate breakdown, the process of providing energy to plants, addressing disease disorders, fruit formation, increasing resistance to unfavorable climate [16]

3.3 Mycelium growth of white oyster mushroom media.  
3.3.1. Mycelium length daily after inoculation. Biological parameters observed were mycelium growth variables which included the length of daily mycelium, the percentage of daily mycelium achievement, the first time to grow, and the time of mycelium fulfillment. Following the average length of mycelium can be seen in Table 4.

| Treatment  | Average Length According to Age (cm) | Daily average |
|------------|--------------------------------------|---------------|
|            | 7 Days  | 14 Daysa | 21 Daysa | 28 Daysa | Daily average  |
| P0 (0%:0%) | 2.50±0.36b | 3.40±0.52a | 3.50±0.69a | 3.80±0.45a | 2.78±0.29b |
| P1(50%:50%)| 2.83±0.28ba | 3.93±0.49a | 3.43±0.64a | 3.60±0.17a | 3.55±0.50a |
| P2(25%:75%)| 3.03±0.20ba | 3.63±0.63a | 4.33±0.41a | 3.30±0.91a | 3.73±0.57a |
| P3(75%:25%)| 2.76±0.05a | 3.26±0.30a | 3.66±0.11a | 3.30±0.79a | 3.50±0.60a |

Source: Analysis Result.

The results of the analysis of variance showed that the planting media had a significant effect (P<0.05) on the control (P0), only on the seventh day, namely the treatment of planting media with a ratio of biogas sludge and expired milk with a ratio of 25%:75% (P2) which is 3.03 cm, while on the following day, the treatment did not show any significant difference (P> 0.05) to the control (P0), from observations made of the composition of the material given the material affected the length of growth of oyster mushroom mycelium white (Pleurotus ostreatus) per unit time, mycelium itself is the initial stage of growth of white oyster mushrooms. Based on observations of mycelium growth velocity showed different responses to the composition of the media, it was allegedly due to differences in the absorption of mycelium against the media substrate so that the growth of mycelium can grow faster.

Observation of the seventh day showed the fastest and longest growth in the growing media with a ratio of biogas sludge and expired milk with a ratio of 25%:75% (P2) is 3.03 cm compared to other media, while on the fourteenth day the longest growth was achieved by planting media biogas sludge and expired milk with a ratio of 50%:50% (P1) which is 3.93 cm, then observation on the twenty-first day, the fastest growth with mycelium length 4.33 cm on media with a ratio of biogas and milk sludge reject rate 25%:75% (P2), and at the last observation that is at the observation of the twenty-eighth day the fastest growth in the growing medium with a ratio of biogas sludge and milk with a ratio of 25%:75% (P2) with mycelium length is 3.30 cm.

Mushroom growth on daily observations in this study the fastest and the longest growth rate in the comparison of 25% sludge biogas treatment and 75% expired milk ie plant media (P2) was 3.73 cm. Previous research conducted who examined the effect of composting and different levels of rice bran administration on oyster mushroom media stated that rice bran is a source of carbohydrates, carbohydrate components provide nutrition to fungi, hyphae threads break down carbohydrate ingredients into simple compounds such as sugar that can be used as energy that is metabolized so that mycelium can quickly grow and appear on baglog [5]. In this study, it can also be concluded that the media given the highest addition of rejects milk produces the fastest and longest growth, namely the planting media with a ratio of sludge and expired milk 25%:75%, namely the planting media P2, as is known in this study milk is a source of carbohydrate that is equal to 75.43%.

3.3.2. The average percentage of growth achievement of mycelium after inoculation
The next biological parameter is the percentage of mycelium growth, this variable will be known to what extent the ability of mycelium can fill baglog per day, the percentage of mycelium growth can be seen in Table 5.

**Table 5.** Average percentage growth achievement of mycelium after inoculation

| Treatment       | 7 Days | 14 Days<sup>bc</sup> | 21 Days<sup>bc</sup> | 28 Days<sup>bc</sup> | Daily average |
|-----------------|--------|-----------------------|-----------------------|-----------------------|--------------|
| P0 (0%:0%)      | 18.88±1.02 | 25.74±3.11         | 26.35±2.51         | 29.01±5.00         | 20.48±1.55<sup>b</sup> |
| P1(50%:50%)     | 20.50±1.60 | 28.57±4.35         | 24.81±4.10         | 26.10±1.68         | 26.22±3.50<sup>a</sup> |
| P2(25%:75%)     | 21.21±1.42 | 25.42±4.58         | 30.31±3.08         | 23.04±6.26         | 27.42±3.30<sup>a</sup> |
| P3(75%:25%)     | 21.32±1.38 | 25.17±2.62         | 28.24±1.38         | 25.26±5.07         | 25.85±4.67<sup>a</sup> |

<sup>a</sup>Source: Analysis Result.

Results of analysis of variance showed daily growth observations showed no significant effect (P>0.05) on controls (P0). Observation of percentage on the seventh day showed that treatment with P3 code showed the highest percentage of mycelium growth achievement was 21.32%, on the fourteenth day treatment with P1 code recorded the highest time with 28.57%, then on the twenty-first day of treatment P2 produced the highest percentage of mycelium growth with a rate of 30.31%, and at the last observation on the twenty-eighth day mycelium growth percentage was treated with P2 code with 27.42%. The percentage of mycelium achievement is strongly influenced by the growth of mycelium, in particular, that is the speed and length of mycelium compared to and the length of the baglog media. At the percentage of mycelium achievement, the average growth rate with the best daily percentage is obtained, with a ratio of 25%:75% of biogas sludge and expired milk (P2) to the average data obtained in daily observations with a percentage of 27.42%. An important factor influencing the percentage of mycelium achievement is that the nutrient factor also affects one of the carbohydrates. Carbohydrate or sugar content is important in mushroom growth, as the addition of sugar (sucrose) which serves as an energy source for the growth and development of white oyster mushrooms, added that high levels of lignin in the media will inhibit the growth and formation of oyster mushrooms, this is because lignin is difficult to degrade both chemically and enzymatically [17].

**3.3.3. First time to grow and time to fulfill mycelium after inoculation**

The next variable is the first time to grow and the time of mycelium fulfillment after inoculation, in these variable observations are made by noting the time when the mycelium first appeared and when the mycelium can fill all parts of the baglog, the results of the first growing time and the time of mycelium fulfillment can be seen in Table 6.

**Table 6.** Average of the first time to grow and time to fulfill mycelium after inoculation (days)

| Treatment       | First time to grow (days) | Time to fulfill(days)<sup>c</sup> |
|-----------------|---------------------------|-----------------|
| P0 (0%:0%)      | 6.33±0.57<sup>a</sup>     | 26.66±1.15     |
| P1(50%:50%)     | 4.66±0.57<sup>bc</sup>    | 26.33±0.57     |
| P2(25%:75%)     | 4.33±0.57<sup>b</sup>     | 26.33±0.57     |
| P3(75%:25%)     | 5.66±0.57<sup>c</sup>     | 26.66±0.57     |

Source: Analysis Result.

The results of the analysis of the variance of the mean of the first time growing showed that all treatments namely P1, P2, and P3 obtained results of 4.66, 4.33, and 5.66 days showed significant differences (P<0.05) against P0 (control) namely is 6.33 days. The fastest-growing average of the first time was found in the ratio of biogas sludge and 25%:75% of expired milk that is 4.33 days in P2 planting media, while the longest time was produced by the control treatment (P0) which was 6.33 days. Whereas for the results of the average variance of mycelium fulfillment time, the results of all
treatments, namely P1, P2, P3, obtained results 26.33, 26.33, and 26.66 days, these results did not show any real difference. The results obtained by the fastest growing media in fulfilling mycelium are P2 and P1 planting media which have the same mean of 26.33 days. The first time to grow and the time of mycelium fulfillment is certainly influenced by nutrient factors due to the nature of the saprophytic fungus and its growth depends on the planting media, planting media with P2 media treatment get the fastest results on the first time variable growing and the time of mycelium fulfillment, this is presumably because P2 media is the media with the highest nitrogen content. Nutrients have been contained in the growing media but still lacking the amount needed for growth, the planting media must contain essential nutrients so that the growth of oyster mushrooms can be optimal where these nutrients play a role triggering the formation of the fruit body, and fruit body development requires nitrogen supplied from protein degradation [18]

4. Conclusion

The best quality of white oyster mushroom growing media in this study is the P3 media which uses a composition of 75% g of sludge biogas waste and 25% of expired milk waste because it can increase nutrients in the parameters of c-organic, crude fiber, C/N ratio, and potassium. The best quality for the best growth of mycelium in this study is in the treatment using P2 media using the composition of 25% of sludge biogas waste and 75% of expired milk waste shows the best results in mycelium growth which includes the length of daily mycelium, the percentage of achievement of mycelium growth, first time to grow and time to fulfill mycelium. Biogas sludge waste from dairy cattle and expired milk waste can potentially be used as an alternative media other than bran while increasing the nutrient content in mushroom media and triggering better growth of mycelium

References
[1] Umardi, Hamad 2017 Addition of Slurry or Sludge Composting Results in Media to Oyster Mushroom Productivity (Pleurotus Ostreatus). Thesis. Animal Husbandry Study Program. Faculty of Animal Husbandry Brawijaya University Malang.
[2] Pertiwiningrum, A., Dianita Juli Hapsari, Palupi Ratnaningrum, Asih Kurniawati, Rochijan and Ramdhani D. N. 2018. Effects of adding chicken blood meal and fishmeal to sludge biogas as white oyster mushroom media. Pak. J. Biol. Sci., Vol. 21 (1) 29-37.
[3] M Pratama et al. 2018. The effect of mimosa and syntan mixture on the quality of tanned red snapper leather. IOP Conf. Ser. Earth Environ. Sci. 139 012048
[4] L Sahubawa, A Pertiwiningrum and Y Rahmadian. 2018. Enhancing the economic value and consumer preferences of commercial mondol stingray (Himantura gerardi) leather creative products. IOP Conf. Ser. Earth Environ. Sci. 139 012049.
[5] Rochman Nur, M Agus, Malik I, Abu Salim 2017. Efforts to Increase Growth of White Oyster Mushrooms (Pleurotus Ostreatus) by Treatment in the Composting and Addition of Rice (Growth Efforts of White Oyster Mushroom (Pleurotus Ostreatus) with Treatment in Composing and Addition of Bran. ITEKIMA Journal. Vol 2 (1) pp 36-51
[6] Harjo S, Akhmad A. A, Syaiful A 2014 The Potential and Utilization of Milk Powder Waste for Compost Photrification in Organic Vegetable Agriculture. Journal of Natural Resource Management and Environment. Vol 4 (2) pp 103-110
[7] Zenika, Naufal. A. D. 2018. Effect of Cretaceous and Egg Shell Substitution on Beef Cattle Biogas Mud as Mushroom Media on Productivity of White Oyster Mushroom (Pleurotus florida). Thesis. Faculty of Animal Husbandry. Gadjah Mada University. p.2
[8] Amalia L, R Budiasih, A Samsul 2018 Effect of baglog plastic opening position and concentration of phosphorus fertilizer on growth and yield of white oyster mushroom (Pleurotus ostreatus) Cultivation Journal Vol 17 (1) pp 582 - 586.
[9] Hidayati, Yuli H, Ellin Harlia, Eulis Tanti Marlina 2010 Detection of the Total Number of Bacteria and Coliform in Sludge Resulting from Gasbio Formation from Dairy Cow Feces. Journal of Animal Sciences Vol. 10 (2) pp 17-20
[10] Hanifah E 2014 *Growth and Yields of White Oyster Mushrooms (Pleurotus ostreatus) in Different Composition of Sawdust, Sugar Cane, and Banana Heart Media Composition*. Publication manuscript Faculty of Teacher Training and Education, University of Muhammadiyah Surakarta. Surakarta

[11] Bellapama A, Kus H R A, Diana W 2018 *Effects of Organic Fertilization on Baglog Fungus Waste and NPK Fertilizing Fertilization on Growth and Production of Packcoy (Brassica Chinensis L)* Journal of Tropical Agriculture Vol 3 (3): pp 327-331

[12] Ibrahim, Y El-Ladan, E A Olofin 2013 *Proximate and Mineral Analyses of Variously Treated Sawdust as a Potential Livestock Feed*. International Journal of Pure and Applied Sciences and Technology. Vol 19 (1) pp 44 – 48

[13] Nardi S, F Morari, A Berti, M Tosoni, L Giardini 2004 *Soil organic matter properties after 40 years of different use of organic and mineral fertilizers* European Jurnal of Agronomy. Vol 21 pp 357 - 367

[14] Purnawanto, Agus M, Bambang, Nugroho 2015 *The Effectiveness of Waste Oyster Mushroom Growing Media Compost as Organic Fertilizer in Shallot Cultivation in Ultisol Land* Agritech Vol (17) pp 97-105.

[15] Draski Hardyan, Ernita 2013 *Effect of a media type and phosphorus dose on the growth of oyster mushrooms (Pleurotus ostreatus)* Journal of Agricultural Dynamics Vol 28 (3) pp 203-210.

[16] Kusuma Warta 2014 *Nitrogen (N), Phosphorus (P) and Potassium (K) content of Baglog Oyster Mushroom (Pleurotus Ostreatus), and Ear Fungus (Auricularia Auricula) for Utilization as a fertilizer* Thesis Faculty of Animal Husbandry. Hasanudin University. Makasar

[17] Sitompul Fritz T, Elza Zuhry, Armaini 2017 *Effect of Various Growing Media and Addition of Sugar (Sucrose) to the Growth of White Oyster Mushroom (Pleurotus Ostreatus)* Jom Faperta. Vol 4 (2) pp 1-15.

[18] Puspitasari, Eka F 2015 *The Effect of Coconut Fiber as Oyster Mushroom (Pleurotus ostreatus) Growth Media on mineral and vitamin B content* Thesis Department of Chemistry Faculty of Math and Science November 10 Institute of Technology. Surabaya.