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Identification of Bacteria and Molds In The Process of Bioethanol Production Using A Mixture of Sheep Manure and Empty Palm Oil Bunches.

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Abstract

Empty palm oil bunches have the potential to be converted into second-generation bioethanol which does not compete with human food needs. EFB as a substrate is mixed with sheep feces which contains bacteria and cellulolytic mold to help degrade lignocellulose so that glucose can be produced. This research aims to determine the number, and characteristics of bacteria and mold in the process of making bioethanol from a mixture of sheep feces and EFB. The research used an exploratory method with 3 treatments (P1= 60% sheep feces: 40% TKKS, P2= 70% sheep feces: 30% TKKS, and P3= 80% sheep feces: 20% TKKS) with 3 repetitions. Data were analyzed using descriptive methods. The best proportion that produces the best bacteria and mold is P1 with an average number of bacteria of 84-167 x10^{8}FU/g. The research results showed that the bacteria found in the initial decomposition phase were mesophilic bacteria. The pH that occurs during decomposition tends to be high between pH 8-9. Rod-shaped bacteria (bacilli) and Gram-positive bacteria dominated in all treatments and replications. The cellulolytic bacteria that grow include those from the genus Bacillus, Streptomyces, and Paenibacillus. White mold and septate hyphae were present in almost all treatments and replications. Cellulolytic molds that grow include those from the genera Aspergillus andTrichoderma.

Keywords: *Sheep Feces, Empty Palm Oil Bunches, Bioethanol, Bacteria, Molds*

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I. Introduction

Indonesia is an agricultural country with a vast and diverse livestock and agriculture sector. Waste from the livestock sector can cause problems in the environment if not handled properly, one of which is livestock waste from sheep farming. Livestock waste on a large scale can cause water and soil pollution, as well as cause health problems and greenhouse gases.

When producing bioethanol from lignocellulosic feedstock, lignin content is a major obstacle in bioethanol conversion. Cellulose is coated by lignin, making it difficult to hydrolyze into glucose. Therefore, it is necessary to pre-treat the lignocellulosic feedstock. For this reason, to reduce the lignin contained, pretreatment stages are carried out by means of physical, chemical, and biological treatments. Pretreatment can accelerate fiber separation, improve biomass conversion, and hydrolyze polysaccharides into monosaccharides. Pretreatment also reduces lignin content, cellulose crystallinity, and energy consumption (Darojati *et al*., 2022).

The use of NaOH was chosen in the chemical pretreatment stage because this compound is more effective in dissolving lignin compared to using acids, quite effective in increasing the hydrolysis yield and relatively cheap compared to other reagents. One of the effects of NaOH is to dissolve the lignin content in the material, making it easier to separate lignin and fiber. (Permatasari *et al*., 2014).

In the hydrolysis stage, initial decomposition is carried out. Initial decomposition is carried out by mixing TKKS and sheep feces. This is done because sheep feces contain lignolytic bacteria that are useful for damaging lignin. TKKS contains cellulolytic molds and sheep manure is a substrate containing cellulolytic bacteria that can be used as hydrolysis material. Cellulolytic bacteria are able to hydrolyze cellulose into oligosaccharides and eventually into glucose(Nurrochman, 2015). Cellulolytic bacteria produce cellulase and hemicellulose enzymes that are responsible for breaking down the chemical bonds in cellulose and hemicellulose structures.

II. Methodology

The research was conducted at the Laboratory of Microbiology and Animal Waste Management, Faculty of Animal Husbandry, Padjadjaran University. The biological pretreatment process was carried out by cutting and chopping the TKKS into 20-30 mesh size. After that, chemical pretreatment was carried out using NaOH and biological pretreatment and hydrolysis stages with initial decomposition using a mixture of sheep feces. The tools used are digital scales, bucket tubs, ovens, plastic polybags, thermometers, pH meters, colony counters, microscopes, and stationery, while the materials used are sheep feces, TKKS, NaOH, and straw.

This research was conducted in the following stages: a) Soaking the TKKS that has been sized 20-30 mesh with 10% NaOH solution. b) Putting it in the oven by setting the temperature at 90^{o}C for 2 hours. Then wash the TKKS with clean water until the pH is neutral, then put it back into the oven for 24 hours at 60^{o}C. c) Weighing TKKS and sheep feces according to the predetermined scales. Then mix the two materials with the previously calculated scales until they are evenly mixed into a polybag that has been given straw on the base and lid. After mixing and solidifying, store in a closed place. The initial decomposition process lasted for 14 days. d) Calculation of parameters including the number of bacterial and mold colonies, macroscopic characteristics of bacteria and molds, microscopic characteristics of bacteria and molds was carried out at the end of the study, while temperature and pH observations were made on days 1, 7, and 14.

This study aims to determine the number, characteristics of bacteria and molds in the process of making bioethanol mixed with sheep feces and TKKS. This research used explorative method with 3 treatments (P1 = 60% sheep feces: 40% TKKS, P2 = 70% sheep feces: 30% TKKS, and P3 = 80% sheep feces: 20% TKKS) with 3 repetitions. Data were analyzed using descriptive method.

III. Results and Discussion

Temperature Dynamics in the Bioethanol Manufacturing Process

One of the factors that affect bacterial growth is temperature. Bacteria can be classified based on the optimal temperature range for their growth. Psychrophile bacteria grow optimally at cold temperatures (0-20°C), while psychotropic bacteria can grow at a wider temperature range (0-35°C). Mesophile bacteria grow optimally at moderate temperatures (20-45°C). Thermophilic bacteria grow optimally at high temperatures (45-65°C), and hyperthermophilic bacteria grow optimally at very high temperatures (above 90°C), and some can even survive up to 113°C. (Sabzevar, 2023).

The treatment of various decomposition balances on temperature changes that occurred was observed for 3 days in each replicate, namely on days 1, 7, and 14. Temperature changes that occur are factors that affect the growth of bacteria contained in the decompost. The temperature dynamics in the early stages of decomposition of the mixture of sheep feces and oil palm empty fruit bunches can be seen in Figure 1.

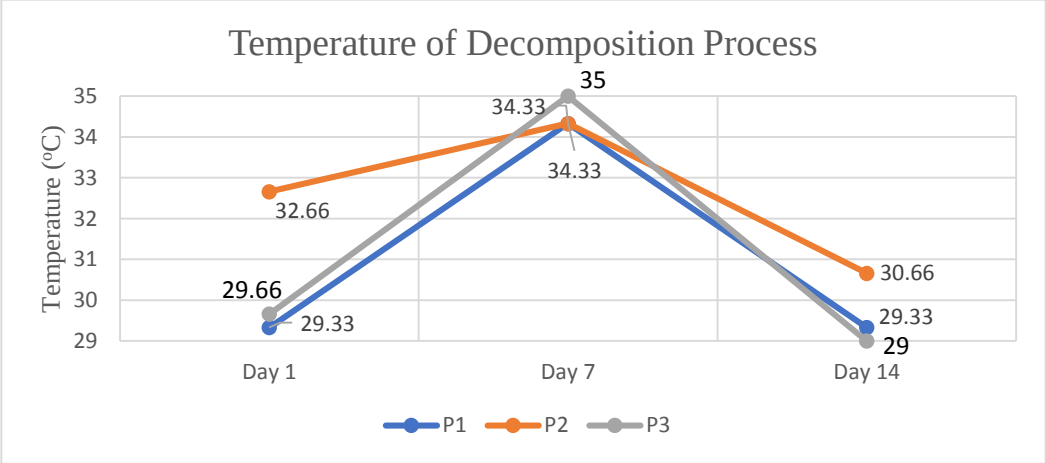


Figure 1. Chart of Temperature Change

The results of this study indicate that mesophilic bacteria are the dominant bacterial group that plays a role in the decomposition process in all treatments (P1, P2, and P3). These mesophilic bacteria can grow optimally at a temperature of 20-45°C, in accordance with the temperature range observed during the study (29-35°C). At the beginning of the study (day 1), the relatively low temperature (29-32°C) allowed the growth of various types of mesophilic bacteria, including Bacillus, Streptomyces, Cellulomonas, and Paenibacillus. These bacteria are known to degrade complex organic components found in oil palm empty fruit bunches and sheep manure, such as cellulose and hemicellulose.

As time goes by (day 7), the temperature tends to increase slightly (34-35°C). This increase in temperature did not significantly affect the dominant bacterial species, but Bacillus and Paenibacillus bacteria were more prominent than Streptomyces and Cellulomonas. This is due to the better adaptability of Bacillus and Paenibacillus at slightly higher temperatures. At the end of the study (day 14), the temperature again dropped to the initial range (29-30°C). This decrease in temperature did not cause significant changes in the dominant type of bacteria. Bacillus, Streptomyces, Cellulomonas, and Paenibacillus remain the main groups of bacteria that play a role in decomposition.

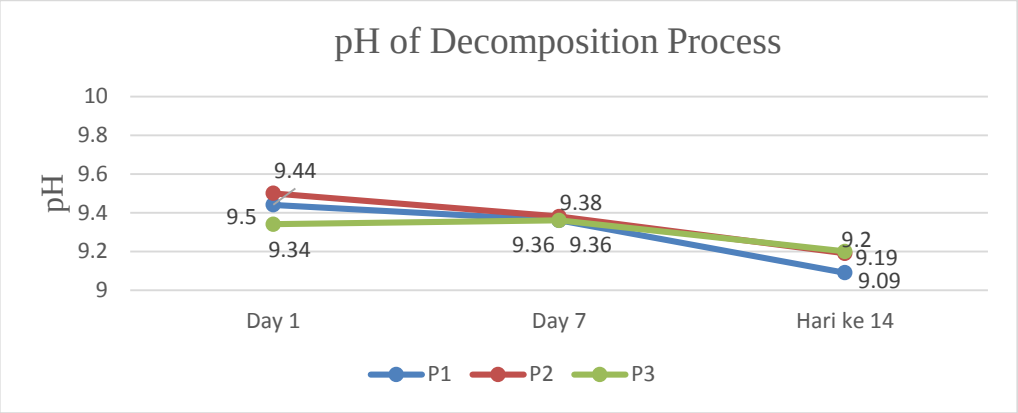
Overall, this study provides an overview of the population dynamics of mesophilic bacteria during the decomposition process of the mixture of empty bunches of oil palm and sheep feces. Bacillus and Paenibacillus bacteria appear to be dominant in this process, especially at slightly higher temperatures. However, the contribution of Streptomyces and Cellulomonas is also not negligible, especially at lower temperatures.

pH Dynamics in Bioethanol Manufacturing Process

The pH value is also one of the factors in the growth of bacteria. Bacteria need an optimal pH (6.5 - 7.5) for ideal growth. Generally, the minimum and maximum pH ranges that most bacterial species can tolerate are 4 and 9. The effect of pH on bacterial growth is related to the activity of enzymes that play an important role in catalyzing bacterial growth reactions. Suboptimal pH conditions can interfere with the work of these enzymes, thereby inhibiting the growth of bacteria(Suriani et al., 2013). The optimum pH of cellulolytic bacteria ranges from 5- 7(Fauziah & Ibrahim, 2020).

The treatment of various decomposition balances against the pH changes that occurred was observed for 3 days on each replicate, namely on days 1, 7, and 14. The pH changes that occur are factors that affect the growth of bacteria found in the decompost. The pH dynamics of the initial decomposition stage of the mixture of sheep feces and empty oil palm bunches can be seen in Figure 2.

Figure 2.Chart of pH Change



The decomposition of the mixture of empty bunches of oil palm and sheep feces showed significant changes in pH during the 14 days of observation. At the beginning of decomposition (day 1), the pH was recorded to be very alkaline, ranging from 9.34 to 9.5. This alkaline condition favors the growth of Bacillus bacteria that are known to be tolerant of high pH. As time went on (day 7), the pH decreased slightly to around 9.36 to 9.38. This decrease in pH indicates bacterial activity and acid production. Although Bacillus still dominates, the population of Paenibacillus and Streptomyces bacteria begins to increase due to slightly more neutral pH conditions.

At the end of the observation (day 14), the pH decreased further, ranging from 9.09 to 9.2. This condition indicates increased decomposition activity and higher acid production. At this stage, the Bacillus population begins to be replaced by Paenibacillus and Streptomyces which prefer a neutral to slightly alkaline pH. Overall, this study showed that there was a shift in the dominant cellulolytic bacteria type during the decomposition process of the mixture of empty oil palm bunches and sheep feces. At the beginning of the decomposition, the high pH-tolerant Bacillus bacteria dominate. However, as the pH decreases to neutral to slightly alkaline, populations of Paenibacillus and Streptomyces bacteria that prefer these conditions become more dominant.

Total Bacterial Colonies in the Bioethanol Manufacturing Process

The initial decomposition process between the mixture of sheep feces and TKKS is accompanied by the growth of bacteria in the decompost. Changes in the number of bacteria are presented in table 1 and figure 3.

Table 1. Total Bacterial Colonies of Sheep Feces Decomposition and TKKS

Repetition	Day 1		
	P1	P2	P3
	……..…….....10^8CFU/g……		
1	24,50	45,00	85,00
2	150,50	79,00	76,00
3	78,00	33,00	92,50
Average	84,33	52,33	84,50
SD	63,23	23,86	8,26
CV	74,98%	45,59%	9,77%
	Day 7		
	P1	P2	P3
	……..…….....10^8CFU/g……		
1	67,00	78,00	72,00
2	73,00	83,00	141,50
3	86,00	42,50	41,50
Average	75,33	67,83	85,00
SD	9,71	22,08	51,25
CV	12,89%	32,55%	60,29%
	Day 14		
	P1	P2	P3
	……..…….....10^8CFU/g……		
1	324,00	195,00	104,50
2	108,50	61,00	107,00
3	70,00	231,50	43,00
Average	167,50	162,50	84,83
SD	136,89	89,78	36,25
CV	81,72%	55,25%	42,73%

Note :P1 = Sheep Feces (60%) and TKKS (40%);
P2 = Sheep Feces (70%) andTKKS (30%);
P3 = Sheep Feces (80%) and TKKS (20%).

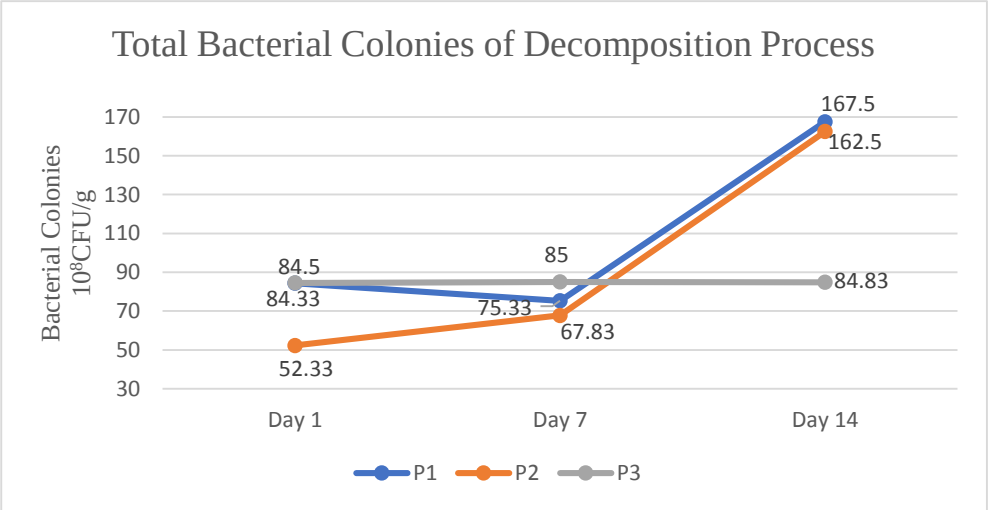


Figure 3: Chart of Bacterial Colony Growth

On day 1 P1 and P3 showed a relatively similar average number of bacteria and higher than P2. This indicates that the proportion of sheep feces of 60% and 80% may be more supportive of bacterial growth in the early stages of decomposition compared to 70%. P1 had the highest SD, indicating that the number of bacteria

on the P1 replica varied widely. In contrast, P3 had the lowest SD, showing more consistent results between tests. This could mean that P3 creates more stable decomposition conditions in the early stages. P1 has the highest CV, indicating that the variation in the number of bacteria is very large compared to the average. P3 has the lowest CV, showing small variation and more uniform results.

On day 7, P3 showed the highest average number of bacteria, followed by P1 and P2. This suggests that the proportion of sheep feces of 80% may be most supportive of bacterial growth on day 7 of decomposition. On the 7th day the number of P1 bacteria decreased slightly, this was due to the increase in temperature to 34ºC with a pH of 9.36. In P2, with the temperature rising to 34ºC and pH at 9.38, the number of bacteria also increased. Meanwhile, in P3, even though the temperature has increased, the number of bacteria has not changed.P3 had the highest SD, indicating that the number of bacteria on the P3 repeat varied greatly on day 7. In contrast, P1 had the lowest SD, showing more consistent results between reps. This could mean that P1 creates more stable decomposition conditions on day 7. P3 had the highest CV, indicating that the variation in the number of bacteria was very large compared to the average on day 7. P1 has the lowest CV, showing small variation and more uniform results. This strengthens the suspicion that P1 had the most stable decomposition condition on day 7.

On day 14, P1 and P2 showed a relatively similar average number of bacteria and were higher than P3. On the 14th day, the number of P1 bacteria increased, along with a decrease in temperature to 29ºC and pH 9.09. Similar to P1, P2 also experienced an increase in the number of bacteria with a decrease in temperature to 30ºC and a pH of 9.19. Meanwhile, in P3, even though the temperature has decreased, the number of bacteria has not changed. This suggests that sheep feces proportions of 60% and 70% may be more supportive of bacterial growth on day 14 decomposition compared to 80% proportions. The bacteria that grow are mesophilic bacteria whose possible optimal temperature for growing is 29 – 30ºC. P1 had the highest SD, indicating that the number of bacteria on the P1 replica varied greatly at day 14. In contrast, P3 had the lowest SD, showing more consistent results between tests. P1 had the highest KV, indicating that the variation in the number of bacteria was very large compared to the average on day 14. P3 has the lowest KV, showing small variation and more uniform results.

Total Mold Colonies in the Bioethanol Manufacturing Process

The initial decomposition process between the sheep feces mixture and TKKS is not only accompanied by bacterial growth, but also mold growth in the decompost. Changes in the number of molds are presented in the following table 2 and figure 4.

Table 2. Total Mold Colonies Decomposition of Sheep Feces and TKKS

Repetition	Day 1		
	P1	P2	P3
	…….…….....10^4CFU/g……		
1	22,00	21,00	14,00
2	108,50	48,00	98,50
3	99,50	48,00	93,50
Average	76,66	39,00	68,66
SD	47,55	15,58	47,40
CV	62,02%	39,97%	69,04%
	Day 7		
	P1	P2	P3
	…….…….....10^4CFU/g……		
1	58,00	41,00	44,50
2	34,00	27,50	24,50
3	39,00	61,00	102,00
Average	43,66	43,16	57,00
SD	12,66	16,85	40,23
CV	28,99%	39,04%	70,58%
	Day 14		
	P1	P2	P3
	…….…….....10^4CFU/g……		
1	61,50	53,00	109,00
2	59,50	65,50	48,50
3	25,50	23,50	40,00
Average	48,83	47,33	65,83

SD	20,23	21,56	37,62
CV	41,43%	45,56%	57,15%

Note : P1 = Sheep Feces (60%) and TKKS (40%);
P2 = Sheep Feces (70%) and TKKS (30%);
P3 = Sheep Feces (80%) and TKKS (20%).

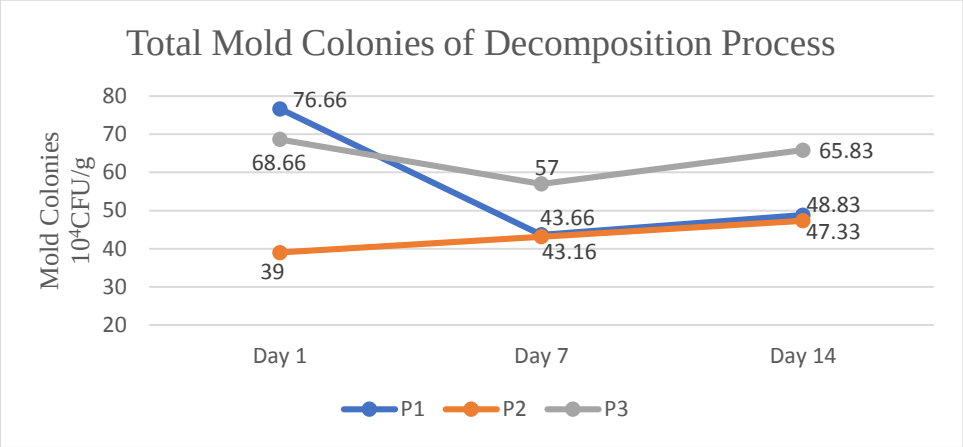


Figure 4: Chart of Mold Colony Growth

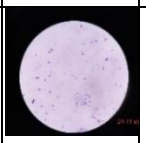
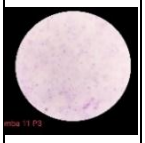
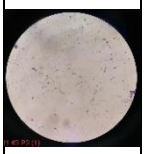
On day 1, P1 had the highest average number of molds, followed by P3 and then P2. This indicates that the lower proportion of sheep feces in the mixture provides more optimal conditions for mold growth in the early stages of decomposition. P1 and P3 showed high standard deviations, indicating a large variation in the number of molds between replicates in the treatment. In contrast, P2 has a lower standard deviation, indicating more uniform data. P3 has the highest coefficient of variation, indicating that the data on the number of molds is the most diverse compared to the average. P2 has the lowest KV, showing the most uniform data. According to Sudjana (1996) in Novianti, et al. (2021) stated that if the coefficient of variation < 20%, the data is considered uniform and if the coefficient of variation > 20%, the data is considered non-uniform.

On day 7, P1 and P2 had a very similar average number of molds, while P3 had the highest average. This is in contrast to the first day where P1 had the highest average. On the 7th day, the number of P1 molds decreased, this was due to the increase in temperature to 34ºC with a pH of 9.36. In P2, with the temperature increase to 34ºC and pH at 9.38, the number of molds also increased slightly. Meanwhile, in P3 there was a decrease, this was due to the increase in temperature to 35ºC with a pH of 9.36. P3 has the highest standard deviation, indicating large variation in data between replicates. P1 has the lowest standard deviation, indicating more uniform data. P3 has the highest coefficient of variation, indicating that the data on the number of molds is the most diverse compared to the average. P1 has the lowest KV, showing the most uniform data.

On the 14th day, P3 had the highest average number of molds, followed by P1 and then P2. The number of molds in P1 increased again, along with the decrease in temperature to 29ºC and pH 9.09. Similar to P1, P2 also experienced an increase in the number of molds with a decrease in temperature to 30ºC and a pH of 9.19. In P3, it also increased again, this was due to a decrease in temperature to 29ºC with a pH of 9.2. P3 has the highest standard deviation, indicating a large variation in the number of molds between replicates. P3 has the highest coefficient of variation, indicating that the data on the number of molds is the most diverse compared to the average. P1 has the lowest coefficient of variation, this indicates that mold in P1 is the most uniform.

Macroscopic and Microscopic Characteristics of Bacteria Found in the Bioethanol Manufacturing Process

Table 3. Results of Observation of Macroscopic and Microscopic Characteristics of Bacteria

Isolation Code	Sightings	Macroscopic Characteristics					Macroscopic Characteristics		
		Shape	Surface	Edge	Color	Size	Sightings	Cell Shape	Grams
P2H1 U1A		Irregular	Flat	Undulate	White	Moserat		Basil : Bacilli	+
P3H1 U1A		Irregular	Flat	Lobate	White	Moserat		Basil : Bacilli	+
P3H7 U3A		Circular	Convex	Entire	Cream	Moserat		Basil : Bacilli	+

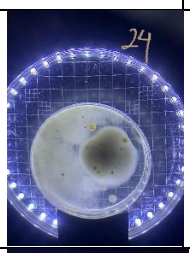
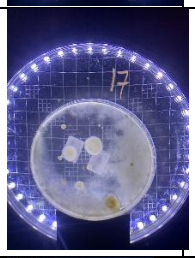
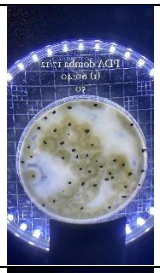
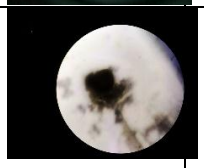
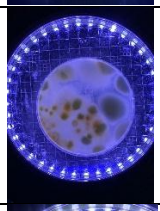
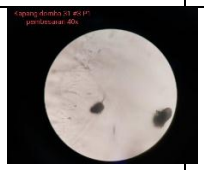
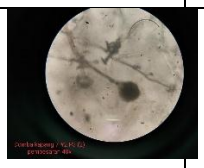
Note : P1 (Treatment1), P2 (Treatment 2), P3 (Treatment 3); H1-H7 (Day); A, B, C (IsolationCode)

Table 3. showing macroscopic and microscopic characteristics of bacteria with the dominance of bacteria of the genus Bacillus. The results of observation of bacterial colonies showed that P2H1 and P3H1 had an irregular colony shape with a flat surface, white, rod-shaped bacteria (bacilli) and Gram-positive. In P2H1 it has wavy colony edges, while in P3H1 isolate it has notched colony edges. This characteristic corresponds to the genus Bacillus or Paenibacillus. Bacillus has a milky-white or beige colony color, while for microscopic characters, it is Gram-positive (Royanti et al., 2023).According to Corbin (2004), Bacillus colonies have the general characteristics of having a whitish-beige color as well as a round, irregular colony shape. According to Hatmanti (2000), Bacillus bacteria have a wide variety of flat and uneven colony edges, the surface is rough and not slimy, there is even a tendency to dry and powdery, large and non-glossy colonies. The convex and flat surface of the colony is one of the characteristics of Bacillus bacteria. The optimum temperature required for bacteria to grow properly is at a temperature of 30-37°C with a pH of 2-8. Examples of common species in this genus include Bacillus subtilis, Bacillus licheniformis, and Bacillus amyloliquefaciens. These three species are known for their ability to produce cellulase enzymes to break down cellulose, even Bacillus amyloliquefaciens also produces amylase enzymes to break down starch (Mukminin, 2014).

Meanwhile, in P3H7 it has a round colony shape with a convex surface, beige in color, rod-shaped bacteria (bacilli) and Gram-positive. The edges of the P3H7 isolate colonies appear flat. These morphological characteristics correspond to the genus Bacillus, Streptomyces, or Paenibacillus. Streptomyces have white or gray colonies and have flat to wavy edges. Members of the genus Streptomyces are characterized by a branched stem shape, purple color and Gram positive (Ambarwati *et al.*, 2019).Streptomyces grows optimally at a temperature of 25°-35°C with a pH of 6.5-8.0 (Retnowati, 2007).Paenibacillus has a stem shape and is Gram-positive with white or light brown colonies that mostly show optimum growth at a neutral pH in the temperature range of 28–40°C (Patowary & Deka, 2020).

Macroscopic and Microscopic Characteristics of Mold Found in the Bioethanol Manufacturing Process

Table 4. Results of Observation of Macroscopic and Microscopic Characteristics of Mold

Isolation Code	Sightings	Macroscopic Characteristics		Microscopic Characteristics			
		Shape	Color	Sightings	Types of Hyphae	Spore Head Shape	Asexual Spores
P1H1U1		Round like Cotton or Wool	Grayish-white		Septat	Round	Conidiospores
P2H1U1		Velvety round	Brownish yellow		Septat	Round	Conidiospores
P1H7U1		Velvety round	Brownish white		Septat	Round	Conidiospores
P1H7U2		Round with irregular edges	Greenish-gray, yellow, and brownish-yellow		Septat	Round	Conidiospores
P3H14U2		Round with irregular edges	White, yellow, and greenish-gray		Septat	Round	Conidiospores

Note : P1 (Treatment 1), P2 (Treatment 2), P3 (Treatment 3); H1-H7 (Day); A, B, C (IsolationCode)

Table 4. show the macroscopic and microscopic characteristics of molds with the dominance of molds of the genus Aspergillus. In P1H1U1, the colonies are rounded in shape resembling cotton or wool with a grayish-white color. Hyphae are clearly visible with septats. The spore head is spherical and produces asexual spores in the form of conidiospores. This characteristic leads to the possibility of the genus Aspergillus. In P2H1U1, the colony is round in shape with a velvety texture and is brownish-yellow in color. Hyphae are pinnate and the spore head is spherical and produces conidiospores. These characteristics indicate a possible genus Trichoderma. Trichoderma has the morphological characteristics of yellowish-white and brown, hyphae colonies. Conidium is round, slightly rounded to oval (Rina et al., 2019).

In P1H7U1, the colonies are round in shape with a velvety texture and brownish-white in color. The hyphae are clearly visible. The spore head is spherical in shape and produces conidiospores. This characteristic leads to the possibility of the genus Aspergillus. In P1H7U2, the colonies are rounded with irregular edges,

displaying greenish-gray, yellow, and brownish-yellow colors. The hyphae are clearly visible. The spore head is spherical in shape and produces conidiospores. These characteristics indicate a possible genus Aspergillus. In P3H14U2, the colonies are rounded with irregular edges, white, yellow, and greenish-gray in color. The hyphae are clearly visible.The spore head is spherical in shape and produces conidiospores. This characteristic leads to the possibility of the genus Aspergillus. Aspergillus mold predominates as a cellulolytic mold with characteristic white, white, yellow, yellowish-brown, brown or black, and green colonies. Conidia are round and dark brown in color, have erect, long, unbranched conidiospores. Aspergillus has a large and dense conidia carrier head, rounded and black in color (Rina *et al.,* 2019).

IV. Conclusion

Based on the results of the research and discussion, it can be concluded as follows:

1. The number of bacteria in the bioethanol manufacturing process at the decomposition stage is:

Day 1 : (P1) 84,33 x 108CFU/g, (P2) 52,33 x 108CFU/g, (P3) 84,50 x 108CFU/g.
Day 7 : (P1) 75,33 x 108CFU/g, (P2) 67,83 x 108CFU/g, (P3) 85,00 x 108CFU/g.
Day 14 : (P1) 167,50 x 108CFU/g, (P2) 162,50 x 108CFU/g, (P3) 84,83 x 108CFU/g.

While the number of molds in the bioethanol manufacturing process at the decomposition stage is:

Day 1 : (P1) 76,66 x 104CFU/g, (P2) 39,00 x 104CFU/g, (P3) 68,66 x 104CFU/g.
Day 7 : (P1) 43,66 x 104CFU/g, (P2) 43,16 x 104CFU/g, (P3) 57,00 x 104CFU/g.
Day 14 : (P1) 48,83 x 104CFU/g, (P2) 47,33 x 104CFU/g, (P3) 65,83 x 104CFU/g.

2. Overall, the macroscopic characteristics of the bacteria show variations in the shape, surface, and edges of the colony, but have a color that is limited to white and beige. While in molds, the majority of colonies are rounded, but there are some that have irregular edges. The texture of the colony varies, ranging from resembling cotton or wool to velvety. The colors of the colonies are very diverse, including white, gray, yellow, brown, and green. Some colonies even show different color combinations.

3. Rod-shaped bacteria (bacilli) and Gram-positive dominate in all treatments and replicates. Cellulolytic bacteria that grow include those of the genus Bacillus, Streptomyces, and Paenibacillus. Meanwhile, molds, molds with hyphae with septic (septic), spherical spore head shape, and conidiospore asexual spores are present in almost all treatments and replicates. Cellulolytic molds that grow are from the genus Aspergillus and Trichoderma, among others.

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