Application of Microalgae and Agrobost Symbiotic Mutualism Technology in Palm Oil Mill Effluent (POME) Processing

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Abstract: The purpose of this study was to get the selected treatment mutualism symbiosis between microalgae (Chlorella sp.) and agrobost, thus can decrease of pollution level of Palm Oil Mill Effluent (POME) according to standard quality. This research used a completely randomized design (CRD) with 5 treatments and 3 replications. The treatments in this addition was microalgae Chlorella sp. as much 800 ml/L (Abundance of cells 6.3 x 10^6 cell/ml) of POME with some variations concentrations of Agrobost (0%, 1%, 2%, 3%, and 4%). The data obtained were analyzed statistically using ANOVA and DNMRT at 5% level. The result showed that the addition of Agrobost had significant affect for COD, BOD, oil, pH and TSS. The treatment chosen from the result of this research was the P4 treatment showed the highest level of reduction which had COD 91.67%, BOD 95.93%, Oil 80.18%, TSS 71.69% and pH 12.08%.

Keywords: Palm oil mill effluent, microalgae Chlorella sp., Agrobost

1. Introduction

Crude palm oil (CPO) production in Riau Province is 8.06 million tons or around 25.94 percent of Indonesia's total production [1]. CPO production will produce palm oil liquid waste containing an average biochemical oxygen demand (BOD) ranging between 20,000-40,000 mg / L, chemical oxygen demand (COD) 25,000-50,000 mg / L, and total suspended solid (TSS) 2,000-5,000 mg / L [2]. The high content of BOD, COD, and TSS has above standard quality by PERMEN LH NO 5 year 2014.

One method of biological wastewater treatment that uses decomposes bacteria. Decomposing bacteria are biological agents that are effectively able to treat organic waste. However, bacteria have difficulty in degrading organic matter because the process is slow and requires a large amount of oxygen. Therefore, photosynthetic microorganisms such as microalgae are needed that can help supply oxygen to the decomposing bacteria.

Decomposing bacteria symbiotic mutualism with microalgae to regulate the balancing between dissolved oxygen and carbon dioxide in water. Based on research using 800 ml Chlorella pyrenoidosa microalgae in 1 liter palm oil liquid waste can reduce organic material in palm oil liquid waste within 9
days [3]. One effort that can be applied to speed up the decomposition process is the addition of decomposing bacteria.

Agrobost is a collection of decomposing bacteria that can be used to decompose liquid waste pollutants. Agrobost is an organic fertilizer containing *Azotobacter* sp., *Azospirillum* sp., and *Pseudomonas* sp. In addition, Agrobost also contains a mixture of liquid inoculants in the form of liquid, containing *Lactobacillus* sp., phosphate soluble microbes, cellulolytic microbes, and can produce indole acetic acid (IAA) growth hormone [4]. The resulting IAA growth hormone can be utilized by microalgae to speed up metabolism. IAA growth hormone applied to the algae *Chlorella* sp. with a concentration of 65 ppm can increase the growth rate with an average cell density of 2,100,000 cells / ml [5].

Several studies have used variations in the addition of decomposing bacteria in reducing levels of palm oil mill effluent pollution such as *Bacillus* sp [6] and EM-4 [7] with the addition of microalgae *Chlorella* sp. the same as 800 ml. Based on the description, conducted to research with title Application of Microalgae and Agrobost Symbiotic Mutualism Technology in Palm Oil Mill Effluent (POME) Processing.

2. Methodos

2.1 Materials and Tools

The material used is Palm Oil Mill Effluent (POME) obtained from PTPN V Sei Pagar, Agrobost obtained from PT SMS Indoputra, microalgae culture *Chlorella* sp. obtained from Prof. Dr. Ir. H. Tengku Dahril, M.Sc., as well as material for analysis. The tools used are jerry cans, aerators, hoses, stoves, aquariums, 2-liter plastic jars, microscopes, haemacytometers, hand counters, DO meters, Chemical Oxygen Demand (COD) tubes, burettes, magnetic stirrers, pH meters, desicators, incubators, 2 winkler bottles and reactor COD and others.

2.2 Research Methods

The method used in this study was an experimental method using Complete Randomized Design (CRD) consisting of five treatments and was repeated three times so that 15 experimental units were obtained. The treatment matrix in the experiment can be seen in Table 1.

| Material                          | Treatment |
|----------------------------------|-----------|
| Agrobost (ml)                    | P0  | 0  | P1  | 18 | P2  | 36 | P3  | 54 | P4  | 72 |
| Mikroalgae *Chlorella* sp. (ml)  |     | 800|     | 800|     | 800|     | 800|     | 800|
| Waste Liquid (ml)                |     | 1000|    | 982|    | 964|    | 946|    | 928|
| Total (ml)                       |     | 1800|    | 1800|   | 1800|   | 1800|   | 1800|

2.3. Research Procedures

2.3.1. Sterilization of tools

Sterilization is done to minimize the occurrence of bacterial contamination of the equipment used. Tool sterilization is done by means such as measuring cups, hoses, culture containers and others washed with soap and rinsed with water until clean, then sprayed with 96% alcohol and air dried [8].

2.3.2. Propagation of microalgae *Chlorella* sp.

Aquarium container with has capacity 20 liters sterilized. 10.500 ml of distilled water and 1.200 ml of nutrient solution were put into the aquarium, and then homogenized by stirring until they were clear or
evenly mixed. Microalgae *Chlorella* sp. as much as 300 ml was put into the aquarium and given aeration, then placed in a room exposed to indirect sunlight and incubated for 7 days until the liquid turns green with an abundance $10^6$ cells/ml. To get the concentration of *Chlorella* sp. accordingly, calculation of cell abundance is carried out every day, and then 12 liter stock culture is obtained [3].

### 2.3.3. Palm liquid waste sampling

Liquid waste sampling is done by random sample technique, which is taking at the same time and point; sampling is obtained from oil palm plantations in the third reservoir using jerry cans. The jerry cans are cleaned inside by rinsing with waste water to be taken. When the sample was taken at 10:00 WIB, this was done because the factory activity was already running.

### 2.3.4. Sterilization of palm oil waste

Sterilization of palm liquid waste refers heat used stove to a temperature of ± 100°C so that the waste is completely sterile. The process of sterilization of liquid waste aims to avoid contamination of waste with natural decomposing bacteria found in nature [8].

### 2.3.5. Measurement of the characteristics of palm oil waste

Palm liquid waste is analyzed by the content of Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD), Dissolved Oxygen (DO), oil, nitrate, phosphate, pH, and Total Suspended Solid (TSS) after the sterilization process. Characteristic measurements were carried out to determine the level of pollutant content in palm oil wastewater.

### 2.3.6. Palm oil liquid waste processing

Palm liquid waste is put into 15 containers of 2 liter transparent plastic jars with liquid waste capacity according to the treatment in each container, then agrobost inoculation is carried out on each sample according to treatment and stirred until homogeneous or the solution is mixed evenly. 800 ml microalgae were added to the sample and aerated using a hose connected to an aerator in each treatment container. Analysis of the abundance cells of *chlorella* sp., and pH was carried out on day 0 to day 7. Analysis of biochemical oxygen demand (BOD), chemical oxygen demand (COD), and total suspended solid (TSS) were carried out on day 0 and day 7.

### 3. Results and Discussion

#### 3.1. Abundance of *Chlorella* sp.

The results of variance showed that variations in the addition of Agrobost had a significant effect (P <0.05) on the abundance of *Chlorella* sp. The average value of the abundance of *chlorella* sp. from day 0 to day 7 can be seen in Table 2.

| Treatment | 0  | 1  | 2  | 3  | 4  | 5  | 6  | 7  |
|-----------|----|----|----|----|----|----|----|----|
| P0        | 6.25<sup>c</sup> | 11.1<sup>d</sup> | 11.4<sup>a</sup> | 33.6<sup>a</sup> | 43.2<sup>a</sup> | 55.5<sup>a</sup> | 62.4<sup>a</sup> | 65.6<sup>a</sup> |
| P1        | 6.23<sup>d</sup> | 9.9<sup>a</sup> | 11.6<sup>b</sup> | 34.1<sup>a</sup> | 45.9<sup>ab</sup> | 58.1<sup>ab</sup> | 65.1<sup>a</sup> | 67.2<sup>a</sup> |
| P2        | 6.11<sup>c</sup> | 10.6<sup>b</sup> | 12.5<sup>c</sup> | 35.2<sup>a</sup> | 47.5<sup>ab</sup> | 59.2<sup>ab</sup> | 67.2<sup>a</sup> | 68.8<sup>a</sup> |
| P3        | 6.08<sup>b</sup> | 11.4<sup>e</sup> | 17.3<sup>d</sup> | 36.8<sup>ab</sup> | 51.2<sup>bc</sup> | 60.8<sup>bc</sup> | 73.6<sup>k</sup> | 76.8<sup>b</sup> |
| P4        | 6.05<sup>a</sup> | 11.0<sup>c</sup> | 18.5<sup>e</sup> | 41.6<sup>b</sup> | 56.0<sup>c</sup> | 64.0<sup>c</sup> | 75.2<sup>b</sup> | 78.4<sup>b</sup> |
Note: numbers that are followed by small letters of the same unreal test according to different DNMRT at the 5% level.

Table 2 shows the change in the number of cell abundances from day 0 to day 7. *Chlorella* sp. the initial used was $6.3 \times 10^6$ cells / ml. The average value of cell abundance on day 0 after being treated ranged from $6.05 \times 10^6$ - $6.25 \times 10^6$ cells / ml, a decrease in the amount of cell abundance on day 0. This decrease is thought to occur due to changes in the concentration of *chlorella* sp. due to dilution by palm liquid waste. The abundance of cells on the 7th day increased with an average value ranging from $65.6 \times 10^6$ - $78.4 \times 10^6$ cells / ml. The more abundance of cells will be the higher the biomass of *chlorella* sp. resulting from. The increase in the amount of cell abundance is thought to occur because of the growth hormone Indole Acetic Acid (IAA) produced by bacteria contained in agrobost [9]. *Azospirillum* bacteria are bacteria that are able to produce growth hormones such as indole acetic acid.

### 3.2. Chemical oxygen demand (COD)

The average COD value of palm oil liquid waste after processing on the 0th day and after the 7th day can be seen in Table 3.

| Treatment | COD (mg/L) | Day-0 | Day-7 |
|-----------|------------|-------|-------|
| P0        | 2549.33    |       | 466.92 |
| P1        | 2443.67    |       | 390.96 |
| P2        | 2419.33    |       | 275.73 |
| P3        | 2393.33    |       | 236.96 |
| P4        | 2305.33    |       | 191.93 |

Table 3 shows that the concentration of *chlorella* sp. with the addition of agrobost variations can reduce the value of COD contained in palm oil liquid waste. The addition of agrobost causes the COD value decreases. This is due to the decomposition of organic matter by bacteria producing CO$_2$. Agrobost is a collection of aerobic bacteria that can decompose organic and inorganic materials contained in waste. One of the decomposing bacteria contained in agrobost is *lactobacillus* sp. which is able to decompose organic matter. [10] Bacteria *lactobacillus* sp. role to increase the acceleration of the overhaul of organic matter. These results are greater than the results of degradation using B-DECO3, EM-4, and *Bacillus* sp. in previous studies. Processing of palm liquid waste uses 20 ml B-DECO3 degrading agent and *Chlorella* sp. in treatment P4 was able to reduce the COD value by 82.78% [11]. Processing of palm liquid waste uses 20 ml EM-4 degradation agent and *chlorella* sp. the P4 treatment was able to reduce 74.55% COD [7]. Combination between *bacillus* sp and *chorella* sp was able to reduce 80.82% COD [6]. Based on this comparison, it can be concluded that the more addition of degradation material to palm oil liquid waste will cause the COD value to decrease.

### 3.3. Biological oxygen demand (BOD)

The variance results showed that the variation of agrobost addition significantly affected (P<0.05) the content of BOD value of palm oil waste. The average value of BOD content of palm oil waste in each treatment after processing on the 0th day and 7th day can be seen in Table 4.
Table 4. The average BOD value of palm oil liquid waste on day 0 and day 7

| Treatment | BOD (mg/L) | Day 0 | Day 7 |
|-----------|------------|-------|-------|
| P0        | 1095.43d   | 322.87e |
| P1        | 1060.40c   | 246.20d |
| P2        | 1025.10b   | 111.03c |
| P3        | 1018.07b   | 95.13b  |
| P4        | 1004.73a   | 40.90a  |

Note: numbers that are followed by small letters of the same unreal test according to different DNMRT at the 5% level.

Table 4 shows that the concentration of *chlorella* sp. with the addition of agrobost variations can reduce the BOD value contained in palm oil liquid waste. BOD value on the 7th day decreased, which ranged from 40.90 to 322.87 mg / L. The BOD value in the P0 treatment was significantly different from the P1, P2, P3, and P4 treatments. The highest BOD value on the 7th day was found in the treatment without the addition of agrobost in the amount of 322.87 mg/L, while the lowest in the treatment of agrobost concentration was 4% (v/v) in the amount of 40.90 mg / L. The decrease in BOD value is thought to have occurred due to the role of the *lactobacillus* sp. contained in agrobost. *Bacteria lactobacillus* sp. can ferment organic material contained in liquid waste into lactic acid compounds that function to accelerate the breakdown of organic matter [12].

These results are not much different compared to the results of degradation using B-DECO3, EM-4, and *bacillus* sp. in previous studies. Processing of palm liquid waste using 20 ml B-DECO3 degrading agent and *chlorella* sp. in treatment P4 was able to reduce 87.48% BOD [11]. Processing of palm liquid waste using 20 ml EM-4 degradation agent and *chlorella* sp. in treatment P4 was able to reduce 97.21% BOD [7]. Combination between *bacillus* sp. and *chlorella* sp was able to reduce 93.05% BOD [6].

3.4. Oil

The variance results showed that the variation of Agrobost addition significantly affected (P <0.05) the value of palm oil liquid waste. The average values of palm oil liquid waste oil on day 0 and day 7 are presented in Table 5.

Table 5. The average value of palm oil liquid waste oil on day 0 and day 7

| Treatment | Oil (mg/L) | Day-0 | Day-7 |
|-----------|------------|-------|-------|
| P0        | 12.43a     | 8.93e |
| P1        | 11.25a     | 8.10d |
| P2        | 10.95b     | 5.13c |
| P3        | 10.10b     | 2.87b |
| P4        | 9.75c      | 1.93a |

Note: numbers that are followed by small letters of the same unreal test according to different DNMRT at the 5% level.

Table 5 shows that the concentration of *chlorella* sp. with the addition of agrobost addition can reduce oil levels found in palm oil liquid waste. Oil levels on the 7th day showed significant results. The oil...
content in the P0 treatment was significantly different from the P1, P2, P3, and P4 treatments. The highest oil on the 7th day was in the treatment without the addition of agrobost in the amount of 8.93 mg/L, while the lowest in the treatment of agrobost concentration was 4% (v/v) in the amount of 1.93 mg/L. The results of this study have met the established quality standard that is 25 mg/L. The initial oil content of palm oil waste is 14.33 mg/L, also meets the quality standard. The low initial oil content is presumably due to the waste taken from the third pond which has experienced a process of decreasing the oil content in the previous ponds. Based on the results that have been obtained, oil levels have decreased from the initial oil content until the 7th day.

The higher the concentration of agrobost given to the waste can be seen a significant decrease in the value of oil. This decrease is thought to occur due to the role of the bacterium Pseudomonas sp. found on agrobost. Pseudomonas sp. able to utilize organic materials contained in waste by releasing enzymes to decompose organic compounds to produce byproducts such as carbon dioxide (CO₂), hydrogen (H₂) and water (H₂O), and energy as a support for metabolic activities.

Domestic waste water treatment using aeration and the addition of \textit{pseudomonas} sp. can reduce 93% oil [13]. Decrease in oil content occurs due to the presence of microbes that are able to produce lipase enzymes that play a role in reducing oil levels. Bacteria \textit{pseudomonas} sp. commonly found in palm oil liquid waste. Testing of the activity of extracellular lipase enzymes from the species is able to decompose triglycerides into free fatty acids [14].

Processing of palm liquid waste using 20 ml B-DECO3 and \textit{Chlorella} sp. was able to reduce 82.14% oil [11]. Processing of palm liquid with addition 20 ml EM-4 and \textit{chlorella} sp. can reduce 88.07% [7]. Combination between \textit{Bacillus} sp. and \textit{Chlorella} sp. in the processing of palm oil liquid waste can reduce 88.57% oil [6].

3.5. Degree of Acidity (pH)

Results of variance showed that variations in the addition of agrobost had a significant effect (P <0.05) on the pH of palm oil wastewater. The average pH of palm oil liquid waste after processing day 0 to day 7 is presented in Table 6.

| Treatment | Day       |       |       |       |       |       |       |
|-----------|-----------|-------|-------|-------|-------|-------|-------|
|           | 0         | 1     | 2     | 3     | 4     | 5     | 6     | 7     |
| P0        | 7.80*a    | 8.03*a| 8.40*a| 8.47*a| 8.57  | 8.63  | 8.70  | 8.77*a|
| P1        | 7.87*ab   | 8.20*b| 8.47*a| 8.53*ab| 8.63  | 8.67  | 8.80  | 8.83*a|
| P2        | 7.93*bc   | 8.27*b| 8.60*b| 8.67*ab| 8.67  | 8.70  | 8.77  | 8.87*ab|
| P3        | 8.00*cd   | 8.40*c| 8.63*b| 8.70*bc| 8.70  | 8.77  | 8.83  | 8.90*ab|
| P4        | 8.03*d    | 8.60*d| 8.67*b| 8.77*c | 8.77  | 8.83  | 8.87  | 9.00*bc|

Note: numbers that are followed by small letters of the same unreal test according to different DNMRT at the 5% level

Table 6 shows that pH tends to increase with increasing variation in addition of agrobost. The pH value on the 7th day when compared with the day the initial pH of the waste showed an increase, the initial pH of the waste was 8.6 increasing on the 7th day the pH rose to 8.77-9.00. The increase in pH is thought to be due to the activity of microorganisms that act as decomposers. Microorganism requires minimum and maximum pH for growth which is between 4 until 9 [15]. The optimum process of decomposition of
organic matter by microorganisms occurs between pH 6.5 to 7.5. Microbe cannot survive or die with low pH values. Aerobic bacteria survive at pH 6.5-8.5 while anaerobic bacteria survive at pH 6.6 - 7.6 [16].

Increasing pH value is a result of the activity of microalgae and bacteria oxidizing organic matter and cell components from insoluble complexes to simpler forms. Microalgae use carbon dioxide as a result of degradation of organic matter as the main carbon source for the synthesis of new cells and release oxygen through photosynthesis [17]. Chemical reactions in dissociated carbonate and bicarbonate ions support the continuous consumption of CO₂ by microalgae so that OH- accumulates and tends to increase acid level.

3.6. Total suspended solid (TSS)

The variance results showed that the variation of agrobost addition significantly affected (P <0.05) the TSS of palm oil liquid waste. The average TSS value of palm oil liquid waste after processing day 0 and day 7 is presented in Table 7.

| Treatment | TSS (mg/L) | Day-0 | Day-7 |
|-----------|------------|-------|-------|
| P0        | 854.67     |       | 467.67|
| P1        | 825.33     |       | 324.33|
| P2        | 785.00     |       | 264.33|
| P3        | 748.33     |       | 251.67|
| P4        | 730.00     |       | 206.67|

Note: numbers that are followed by small letters of the same unreal test according to different DNMRT at the 5% level

Table 7 shows that the TSS value tends to decrease with increasing variation in the addition of agrobost given. TSS value of the 0th day after the treatment was added ranged from 730.00 to 854.67 mg/L. On the 7th day there was a decrease in the value of TSS, which ranged from 206.67-467.67 mg/L. TSS values on day 0 showed significant results. The P0 treatment was significantly different from the P1, P2, P3, and P4 treatments. The decrease in TSS value on day 0 was occurred due to the dilution when adding chlorella sp. and decomposing bacteria. TSS value on the 7th day showed that the P0 treatment was significantly different from the P1, P2, P3, and P4 treatments, but the P2 treatment was not significantly different from the P3 treatment. P4 treatment with the addition of 4% (v/v) agrobost has the lowest TSS value of 206.67 mg/L. This value meets the established quality standard that is 250 mg/L.

The role of microalgae in reducing TSS is by utilizing mineral materials in palm liquid waste which is very needed and easily utilized by microalgae, so that the activity of microalgae in reducing TSS is higher. In addition, the decrease in TSS value can also be influenced by Lactobacillus sp. contained in agrobost. Principle of the formation of lactic acid by the fermentation process by Lactobacillus sp. is the breakdown of lactose into its monosaccharide form and from that monosaccharide with the help of enzymes produced by Lactobacillus sp. will be converted to lactic acid [18]. These bacteria function as hydrolytic bacteria (break down complex organic molecules).

3.7. Selected Agrobost Variation Treatments

Based on the characteristics that have been observed, it has been obtained results that meet the specified quality standards. Characteristics in the form of COD, BOD, DO, oil, pH and TSS that have met the standards by the Ministry of Environment No. 5 of 2014 can be seen in Table 8.
Table 8. Treatment of selected Agrobost variations

| Characteristics | Standard Quality | Treatments |
|-----------------|------------------|------------|
|                 |                  | P0         | P1         | P2         | P3         | P4         |
| COD (mg/L)      | Maks 350         | 466.92c    | 390.96d    | 275.73e    | 236.96b    | 191.93a    |
| BOD (mg/L)      | Maks 100         | 322.87e    | 246.20d    | 111.03c    | 95.13b     | 40.90a     |
| Oil (mg/L)      | Maks 25          | 8.93c      | 8.10d      | 5.13c      | 2.87b      | 1.93a      |
| pH              | 6-9              | 8.77a      | 8.83c      | 8.87ab     | 8.90ab     | 9.00b      |
| TSS (mg/L)      | Maks 250         | 467.67d    | 324.33c    | 264.33b    | 251.67b    | 206.67a    |

Note: Numbers in bold indicate the selected treatment.

Table 9 shows the selected treatment that has met the quality standard standards stipulated by the Minister of Environment Regulation No. 5 years 2014. The selected treatment was in the P4 treatment with the 4% (v/v) addition of agrobost, this is because the highest level of pollution reduction was found in the P4 treatment.

Table 9 shows treatments P2, P3 and P4 that have met the liquid waste quality standard for the established COD parameters. The lowest COD value was found in the P4 treatment with a value of 191.93 mg/L which was the chosen treatment. P3 and P4 treatments have met the liquid waste quality standard for predetermined BOD parameters. The lowest BOD value was found in the P4 treatment with a value of 40.90 mg/L which was the chosen treatment. The pH parameters of all treatments have met the waste quality standard. The pH value chosen in the P4 treatment is 9.00. P3 and P4 treatments have met the liquid waste quality standard for the specified TSS parameters. The lowest TSS value was found in the P4 treatment with a value of 206.67 mg/L which was the selected treatment.

4. Conclusions

Based on the results of the research that has been carried out, it can be concluded that the symbiosis of microalgae chlorella sp. with agrobost has a significant effect on the value of COD, BOD, oil, pH, and TSS in reducing levels of palm oil mill effluent pollution. The selected treatments in this study were P4 treatment with variations 4% (v/v) addition of agrobost, able to reduce COD pollutants by 91.67%, BOD by 95.93%, Oil by 80.18%, TSS by 71.69% and a pH value of 12.08%.

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