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DECREASING LEVELS OF BOD, COD AND OIL IN PALM OIL MILL EFFLUENT (POME) USING *SCALE UP EXPERIMENT* OF SYMBIOSIS MUTUALISM TECHNOLOGY BETWEEN MICROALGAE *CHORELLA* SP AND AGROBOST

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Abstract. *Biological processing liquid sago waste is carried out by utilizing microlagae which are symbiotic with decomposer bacteria. The aim of this research was to get chosen treatment of microalgae Chlorella sp. as a reducing BOD, COD and Oil content in Palm Oil Mill Effluent with symbiotic mutualism between microalgae Chorella sp and Agrobost. This research used a Complete Randomized Design (CRD) with treatment 5 times and replication 3 times. This research was microalgae 800 ml/L (with abundance 6.883.000 cell/ml) in 6000 ml of total treatment solution with 5 treatments of Agrobost (0% v/v, 4% v/v, 8% v/v, 12% v/v, and 16% v/v). Data processing using ANOVA and DNRT 5%, the results showed that the concentration of Agrobost had significant affects for BOD, COD, oil and pH. The chosen treatment from the result of this research was 16% v/v which had the value BOD 89.10 mg/L, COD 297.67 mg/L, oil 2.85 mg/L, and pH 9.05*
Keywords: *palm oil mill effluent; agrobost; microalgae chlorella sp.*

1. Introduction

The largest oil palm plantation in Indonesia is located in Riau Province. Statistical data on Indonesian plantations shows that in 2017 the oil palm plantations area in Riau province had reached 2.5 million hectares or 20.32% of the oil palm plantations total area in Indonesia. The growth rate of oil palm plantations in Riau Province is marked by an increase in crude palm oil (CPO) production from 2015 to 2017, amounting to 6.6 million tons (Statistics Indonesia, 2017).

Oil palm plantations in Riau Province play a major role in expanding employment, increasing people's income, increasing the country's foreign exchange, meeting consumption needs and domestic raw material industry. However, in the process of processing it produces a liquid residue that has the potential to become an environmental pollution material. Palm Oil Mill Effluent is generally produced from a sterilization process of 15-20%, clarification and centrifugation of 40-50%. Every one ton of palm oil will produce 2.5 tons of Palm Oil Mill Effluent, equivalent to 60% of the processing capacity of the plant, in the form of organic waste originating from hydro cyclone water, boiled condensate water and clarified drab water (Ahuat, 2005). Therefore, if the production of palm oil in Riau in 2017 is 8.72 million tons, it is estimated that it will produce 21.8 million tons Palm Oil Mill Effluent.

Palm Oil Mill Effluent has an average biological oxygen demand ranging from 1004.73-1095.43 mg/L, chemical oxygen demand 2305.33-2549.33 mg/L and total suspended 730.00-

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854.67 mg/L (Restuhadi dan Zalfiatri, 2020). Through the Ministry of Environment, the Government has issued regulation No. 5 year 2014 concerning wastewater industry for quality standards. The maximum allowable BOD, COD, and TSS content are 100 mg /L, 350 mg /L, and 250 mg /L. The palm oil processing industry is currently processing palm oil wastewater in a number of ways such as using membranes, evaporation, aerobes and anaerobes. This method will only reduce BOD and COD content but cannot utilize other components such as N, P, K, and various other minerals whose levels are still high.

Wastewater treatment technology in general can be done physically, chemically and biologically. The simplest treatment and does not require large costs is biological treatment. Biological treatment is carried out by utilizing decomposing microorganisms such as bacteria, mold and protozoa as agents for the degradation of liquid waste. However, decomposing microorganisms require high amounts of oxygen to accelerate the process of degradation of liquid waste pollutants. One effort that can be done to supply oxygen to decomposing microorganisms is to use photosynthetic microorganisms, microalgae.

Microalgae are a photosynthetic microorganism that is currently widely used in wastewater treatment. Microalgae will provide oxygen (O_2) to decompose microorganisms by photosynthesis and form biomass with the help of sunlight, carbon dioxide (CO_2), nitrogen and phosphorus. While decomposing microorganisms in their activities to break down organic matter will produce carbon dioxide (CO_2) so that symbiosis occurs between the decomposing bacteria and microalgae.

In order to accelerate the process of degradation of palm oil waste, a symbiosis was made between microalgae Chlorella sp. and decomposer bacteria. Previous research has used many microalgae mutualism symbiosis with decomposer bacteria, such as *Bacillus* sp (Prasetyowati, 2017), EM4 (Restuhadi *et al.*, 2017a), and Starback (Restuhadi *et al.*, 2017b), B-DECO3 (Zalfiatri *et al*., 2017) and Agrobost (Restuhadi & Zalfiatri, 2020)

Research on a laboratory scale was conducted by Restuhadi and Zalfiatri (2020) by utilizing the symbiosis of *Chlorella* sp. and Agrobost to reduce pollutant level from palm oil mill effluent. The Agrobost selection is based because it contains *Azospirilium* sp. which producing indole acetic acid (IAA) as growth hormones. Growth hormone produced can accelerate the growth of microalgae *Chlorella* sp. in accordance with the results of Retnowati *et al*., (2015), 65 ppm growth hormone had applied to *Chlorella* sp. increasing growth rates with 2,100,000 cells/ml average cell density . The rate of the process of decomposition of waste requires the right concentration of Agrobost addition to symbiosis with microalgae *Chlorella* sp. so as to reduce liquid waste pollution in accordance with the quality standard PERMEN LH No.5.

Scale-up is a study that processes and transfers laboratory-scale research data to a larger scale regarding the design of the operation process or and the design of equipment buildings

(Stoica *et al*., 2015). Increasing the scale is important because if it is only done on a laboratory scale, the microalgae activity is still the same. Upscaling includes upgrading a new, larger system, as well as designing and composing a larger system based on experimental results using a smaller model (Oncel & Sabankay, 2012). Scale up experiment will connect between the laboratory scale and the industrial scale. According to Ismiyati (2013), increasing the pilot scale can develop a laboratory scale and provide information that can be used for decision making in designing industrial scale. The results of research conducted by Restuhadi and Zalfiatri (2020) on a laboratory scale showed significantly different results from each variation of agrobost addition. The chosen treatment is P4 with a variation of agrobost addition of 4% (v / v) and the volume of palm liquid waste as much as 1000 ml can reduce COD, BOD, oil, nitrate, phosphate and TSS sequentially namely 91.67%, 95.93% , 80.18%, 77.63%, 61.06%, 71.69% and were able to increase DO by 566.56% and pH by 9. Graph of percentage reduction in the value of contamination shows that these results can still decrease, so it is necessary an experiment scale was increased by increasing the agrobost concentration to 4%, 8%, 12% and 16% in 6000 ml of palm oil waste to obtain the best results before reaching the industry scale. In addition, scale up experiment can also increase biomass production, so that all products produced can be utilized. Based on this description, a study will be conducted scale up experiment palm oil liquid waste treatment using symbiosis mutualism technology between *chlorella* sp. and agrobost.

2. Methods

2.1. Materials and Tools

The materials needed was microalgae *Chlorella* sp. originating from collection culture Prof. Dr. Ir. H. Tengku Dahril, M.Sc, Palm Oil Mill Effluent from PTPN V Sei Galuh, Agrobost was from PT. SMS Indoputra. The tools used are liquid waste jerry cans, aerators, gallons, stoves, aquariums, 15 gallons of 6 liter size, microscopes, hymacytometers, hand counters, DO meters, pH meters, vacuum pumps, spectrophotometers, drop pipettes, desiccators, analytical scales, petri dishes, spatulas, aluminum scales, erlenmeyers, beaker glass, COD reactors and other supporting tools

2.2. Research Methods

This study used the Non-factorial Complete Randomized Design (CRD) method which consisted of 5 treatments and 3 replications in order to obtain 15 experimental units. The general model of the experimental design is as follows (1):

$$Yij = \mu + \tau i + \sum ij \qquad (1)$$

Information :
Yij: Results of treatment observations in an experimental unit
μ: Common mean

τi: Effect of treatment at level-i

∑ij: Experimental error on treatment i and jth repetition

This treatment refers to Restuhadi and Zalfiatri (2020), which is only the addition of Agrobost which changes according to the treatment, while the microalgae Chlorella sp. with an abundance of ≥2,100,000 cfu.ml-1 and palm oil waste is made permanent. The treatments in the experiment can be seen as follows:

P0 = Without adding Agrobost

P_1 = Agrobost 4% v / v (240 ml) in 6000 ml of total treatment solution

P2 = Agrobost 8% v / v (480 ml) in 6000 ml of total treatment solution

P3 = Agrobost 12% v / v (720 ml) in 6000 ml of total treatment solution

P4 = Agrobost 16% v / v (960 ml) in 6000 ml of total treatment solution

2.3. Research Procedure

2.3.1. Liquid waste sampling

POME collection refers to Restuhadi and Zalfiatri (2020). The sampling technique uses the grap method, which is taking at the same time but at different points. Sampling was carried out in the third pond using jerry cans that had been cleaned and greased with POME. The time of collection is around 10.00 WIB, when factory activity is running.

2.3.2. Tools and liquid waste sterilization

Sterilization of tools and liquid waste refers to Yolanda (2016). Microalgae culture requires conditions that must be sterile both space, equipment and the entire set of work so as not to be contaminated with harmful bacteria that can inhibit or interfere with the growth of *Chlorella* sp. Tools that are sterilized are measuring cups, hoses, culture containers and others. The tool is washed with soap and rinsed with flow water until clean. After that, it is sprayed with 96% alcohol. As for the sterilization of palm oil waste, heating is done using stove.

2.3.3. Preparation of microalgae isolates *Chlorella* sp

Propagation of microalgae Chlorella sp. referring to Habibah (2011). Prepare 1 container for the aquarium that has been sterilized. 14000 ml of distilled water and 1600 ml of nutrient solution is put into the aquarium, after which it is stirred until it is homogeneous. Then add 400 ml of chorella sp microalgae and given oxygen using an aerator. The aquarium was placed in a room exposed to sunlight and incubated for up to 7 days. The color of the liquid will turn solid green, the abundance of microalgae cells is measured every day.

2.3.4. Palm oil waste treatment process

On the first day, palm oil waste was taken, after the sterilization process, an analysis of COD, BOD, oil and pH was carried out. Furthermore, the sample of palm oil liquid waste was put into a bucket of 78 L then *Chlorella* sp. as much as 12 L. After homogeneous wastewater and microalgae, then put into a translucent gallon container according to treatment, Then add Agrobost according to the treatment, then homogenize it by stirring and then given oxygen using an aerator. Analysis of BOD, COD, Oil and pH on days 1, 3, 5 and 7.

3. Results and Discussion

3.1. Biological Oxygen Demand (BOD)

BOD analysis is performed to determine the amount of O_2 needed by a number of bacteria to decompose the substances that are in water. Oxygen used to break down organic substances makes other biota that needs O_2 is deficient. As a result, biota that requires O2 cannot live. According Ginting (2007), the higher the BOD more difficult the aquatic biota to live. The results of variance indicate that the Agrobost addition variation has a significant effect (P <0.5) on the BOD value of palm oil mill effluent. The average BOD value of palm oil mill effluent in each treatment after processing on the 0, 1, 3, 5, and 7 days can be seen in Table 1.

Table 1. Decreasing BOD value on 0,1,3,5,and 7 days

Treatment	Day				
	0	1	3	5	7
P0	1359.53^{d}	1342.93^{d}	1105.47^{e}	576.53^{e}	430.67^{e}
P1	1240.90^{c}	1217.00^{c}	884.40^{d}	445.10^{d}	316.23^{d}
P2	1175.33^{b}	1133.90^{b}	781.90^{c}	291.20^{c}	219.10^{c}
P3	979.67^{a}	935.70^{a}	566.70^{b}	182.40^{b}	108.40^{b}
P4	969.40^{a}	923.87^{a}	483.20^{a}	122.30^{a}	89.10^{a}

Note: Numbers followed by different letters in the same column show significantly different (P <0.5).

Table 1 show that the Agrobost addition in concentration of *Chlorella* sp. can reduce the value of BOD in palm oil mill effluent. BOD analysis on days 0, 1, 3, 5, and 7 showed significant results. The highest BOD was on the 7th day without the addition of Agrobost in the amount of 1359 mg /L, while the lowest BOD was 89.10 mg / L on the 7th day with the 16% (v/v) Agrobost addition. Based on these data, can be seen that the decrease in BOD value is in line with the increase in the concentration of Agrobost added and the length of time of wastewater treatment.

The analysis of the characteristics of raw materials in laboratory scale research obtained an initial BOD of 1043 mg/L then after processing it decreased to 40.90 mg/L (Restuhadi and Zalfiatri, 2020). While in a scale up study the BOD value of raw materials was higher, namely 1370 mg/L, then after processing it decreased to 316.23 mg/L. Based on these data, it is known that the processing of palm oil wastewater on a laboratory scale and scale up with the same concentration of addition 4% (v/v) Agrobost results in a different decrease in BOD. This is due to differences in the source of raw materials, where on a laboratory scale the BOD of raw materials is lower than scale up.

Microalgae can work together with decomposing bacteria in the treatment of organic waste. According to Hadyanto *et al.*, (2012), microalgae will utilize the content of organic compounds and nutrients that are still remaining in the waste then produce oxygen which can reduce levels of

COD and BOD in the waste through the help of bacteria that decompose organic substances. Technically, microalgae photosynthesis will produce O_2 which has a role for respiration in the growth of waste oxidizing bacteria, on the contrary the oxidation reaction or decomposition of waste carried out by bacteria produces CO_2 which can support the growth of microalgae so that there is a symbiosis of mutualism between microalgae with decomposing bacteria. Increased microalgae cell abundance also indicates that the biomass produced is increasing. While the decrease in BOD will occurs because of an increase in the amount of dissolved oxygen in the waste.

Oxygen will be used by decomposing bacteria to remodel organic compounds into simpler compounds so as to reduce the BOD value. Hadiyanto and Azim (2012) stated that microalgae are able to utilize carbon dioxide as the main carbon source for the synthesis of new cells and release oxygen through the mechanism of photosynthesis. The carbon dioxide obtained is the result of a breakdown of decomposing bacteria and dissolved oxygen produced by microalgae used by aerobic bacteria for the decomposition process.

3.2. Chemical Oxygen Demand (COD)

COD analysis is performed to determine the value of water contamination by organic substances which can naturally be oxidized with $K_2Cr_2O_7$ or $KMnO_4$ through microbiological processes and result in reduced dissolved oxygen in water (Muhajir, 2013). The results of variance showed that Agrobost addition had given significantly affected ($P < 0.05$) the COD value. The average value of COD content of palm oil mill effluent after processing on days 0, 1, 3, 5, and 7 showed in Table 2.

Table 2. Decreasing COD value on 0,1,3,5,and 7 days

Treatment	Day				
	0	1	3	5	7
P0	2568.00^{d}	2537.00^{e}	1229.67^{b}	941.00^{d}	779.67^{d}
P1	2445.67^{dc}	2279.67^{d}	1461.67^{b}	960.67^{d}	709.00^{c}
P2	2377.00^{cb}	2167.33^{c}	1498.67^{ba}	795.33^{c}	593.00^{b}
P3	2298.00^{b}	2056.00^{b}	997.67^{a}	571.33^{b}	360.67^{a}
P4	2144.33^{a}	1912.67^{a}	844.67^{a}	435.67^{a}	297.67^{a}

Note: Numbers followed by different letters in the same column show significantly different ($P < 0.5$).

Table 2 shows that the concentration of microalgae and addition of Agrobost can reduce the COD contained in palm oil mill effluent. COD values from day 0 to day 7 showed significant results. Increasing the concentration of Agrobost which can increase the COD value decreases. This happens because of the CO_2^- producing bacteria that make the process of decomposing organic matter. Agrobost is aerobic bacteria that are able to break down both organic and inorganic compound contained in palm oil mill effluent. The COD decrease line with Restuhadi and Zalfiatri (2020) laboratory scale research where the higher Agrobost concentration added and the longer processing time will be the greater COD decreasing.

The results of Restuhadi and Zalfiatri (2020), the characteristics of raw materials in laboratory scale studies obtained an initial COD value of 2587 mg/L then after processing it dropped to 191.93 mg/L, whereas in the research scale up the COD value of the raw material was higher at 2690 mg/L, then after do the processing down to 709.00 mg /L. Based on these data it is known that the processing of palm oil mill effluent at a laboratory scale and scale up with the same Agrobost additive concentration of 4% (v/v) results in a different COD decrease. This is due to differences in the characteristics of raw materials, which in the research scale up the COD value of raw materials is higher than the laboratory scale.

3.3. Oil

Oil is one of the contaminants which is included in the class of solids that float on the surface of the water. A layer of oil on the surface of the water can block the entry of sunlight into the water so that it can interfere with the process of photosynthesis. The layer will also prevent the diffusion of oxygen from the air into the water, so that dissolved oxygen will be reduced (Widyaningsih, 2011). The results showed the Agrobost addition variation had significantly ($P < 0.05$) on oil value. The average value of palm oil waste oil after being processed on the 0th, 0, 1, 3, 5, and 7 days can be seen in Table 3.

Table 2. Decreasing COD value on 0,1,3,5,and 7 days

Treatment	Day				
	0	1	3	5	7
P0	14.28^{e}	13.70^{e}	12.74^{e}	11.79^{d}	10.58^{e}
P1	12.76^{d}	12.14^{d}	10.52^{d}	9.21^{c}	8.55^{d}
P2	12.30^{c}	11.48^{c}	9.43^{c}	7.53^{b}	6.49^{c}
P3	11.20^{b}	10.27^{b}	7.30^{b}	5.47^{a}	3.84^{b}
P4	10.75^{a}	9.86^{a}	6.89^{a}	5.23^{a}	2.85^{a}

Note: Numbers followed by different letters in the same column show significantly different ($P < 0.5$).

Table 3 shows that the increasing concentration Agrobost can reduce the oil content in liquid waste. The results of this study have met the quality standards for palm oil industry, which is 25 mg /L. The small of oil content in palm oil liquid waste is suspected because waste liquid sample was obtained from the third pond. This is thought to occur because of the bacteria *Pseudomonas* sp. which utilizing organic matter in waste as supporting metabolic activities. According to Rahardja *et al*., (2010), the bacterium *Pseudomonas* sp. able to produce lipase enzymes. Lipase enzyme will break down triglycerides into free fatty acids.

Comparison of the results of the reduction in palm oil mill effluent oil content between laboratory scale and scale up at the same Agrobost concentration of 4% (v/v) obtained different results. Base on Restuhadi and Zalfiatri (2020), the oil content at the laboratory scale is 1.93 mg/L, while the scale up is greater at 8.55 mg/L. This is thought to occur because the measurement results of raw material characteristics at scale up are higher at 15.4 mg /L compared to the laboratory scale of 14.3 mg /L. The volume of liquid waste used in the research scales up more so that the oil content is also more, thus causing the dissolved oxygen content to be low and can inhibit sunlight which causes disruption of the process of photosynthesis of microalgae.

3.4. Degree of Acidity (pH)

The degree of acidity or potential of hydrogen (pH) is a value indicating the activity of hydrogen ions in water. The pH value of a waters characterizes the balance between acids and bases in water and is a measurement of the concentration of hydrogen ions in water (Alashty et al., 2011). The variance results showed that the Agrobost addition variation had significantly affected (P <0.05) the pH value. The decreasing pH of liquid waste from intial to 7th day can be seen in table 4.

Table 2. Decreasing COD value on 0,1,3,5,and 7 days

Perlakuan	Day				
	0	1	3	5	7
P0	7.45^{a}	7.50^{a}	7.68^{a}	7.83^{a}	8.08^{a}
P1	7.66^{b}	7.76^{b}	8.13^{b}	8.35^{b}	8.54^{b}
P2	7.98^{c}	8.09^{c}	8.68^{c}	8.85^{c}	8.91^{c}
P3	8.14^{cd}	8.64^{d}	8.81^{d}	8.92^{cd}	8.98^{cd}
P4	8.22^{d}	8.84^{e}	8.85^{d}	8.89^{d}	9.05^{d}

Note: Numbers followed by different letters in the same column show significantly different (P <0.5).

Table 4 shows that the pH of palm oil mill effluent tends to increase with increasing Agrobost given. The pH value from day 0 to day 7 showed significant results. Based on the results obtained, it is known that palm oil mill effluent with the addition Agrobost 12% (v/v) does not provide a significant difference to the pH produced in palm oil mill effluent with the addition Agrobost 16% (v/v) in 6000 ml palm oil mill effluent and microalgae *Chlorella* sp. This is thought to occur because the CO_2 contained in the liquid waste in the treatment is not much different. In line with Arifin's research (2012), that an increase in the pH value from acid to neutral and alkaline can be associated with a decrease in CO_2 levels in a waters. This is thought to be due to the microalgae *Chlorella* sp. utilizing CO_2 for support the photosynthesis process, so there is no formation of carbonic acid which reducing pH value of palm oil mill eflluent.

Increasing of pH value is thought to be due to the activity of decomposer bacteria symbiotic with microalgae *Chlorella* sp. According to Suriani *et al*. (2013), effect of pH on bacterial growth is related to enzyme activity. Enzymes are needed by some bacteria to catalyze reactions related to bacterial growth. Effendi (2003) states that the presence of free mineral acids and carbonic acid can be increase acidity. Bacteria will grow well at neutral and alkaline pH, while fungi prefer low pH.

In line with Restuhadi and Zalfiatri (2020), an increase in pH value indicates the decomposition of organic materials by microalgae *Chlorella* sp. symbiotic with decomposed bacteria. Comparison of the results of laboratory scale pH measurement of palm oil liquid waste with scale up at the same Agrobost concentration of 4% (v / v) obtained different results. After processing until the 7th day, the pH value on the laboratory scale was higher, namely 9.00, while in the scale up study it was only 8.54. This is thought to have occurred due to differences in the characteristics of the raw materials used where the initial pH value on the laboratory scale was higher, namely 8.6 while in the scale-up study it was only 7.58.

3.5. Selected Agrobost Additions

All treatments for the addition of Agrobost have met quality standards. The observed parameters of BOD, COD, oil, and pH that have met the standards by the Minister of Environment Regulation No.5 of 2014 can be seen in Table 5.

Table 5. Selected Agrobost Additions

Parameters	Quality Standar	Treatment				
		P0	P1	P2	P3	P4
BOD (mg/L)	Max 100	430.67[e]	316.23[d]	219.10[c]	108.40[b]	**89.10[a]**
COD (mg/L)	Max 350	779.67[d]	709.00[c]	593.00[b]	360.67[a]	**297.67[a]**
Oil (mg/L)	Max 25	10.58[e]	8.55[d]	6.49[c]	3.84[b]	**2.85[a]**
pH	6-9	8.08[a]	8.54[b]	8.91[c]	8.98[cd]	**9.05[d]**

Note: The numbers followed by different letters in the same column, show significantly different (P <0.05)

Based on Table 5, P4 treatment is the selected treatment with the addition of Agrobost concentration of 16% (v/v), this is because the highest decrease in levels of pollutants from palm oil liquid waste. Table 5 shows that the P4 treatment has a BOD value of 89.10 mg / L, which has met the quality standard. The lowest COD pamater in treatment P4 with a value of 297.67 mg / L, the selected treatment was selected. The lowest oil pamater was in P4 treatment with a value of 2.85 mg / L, which was the chosen treatment. Finally, the highest pH parameter was in P4 treatment with 9.05 value.

4. Conclusions

The conclusion that can be drawn from this research is P4 with the addition 16% (v/v) Agrobost became selected treatment for reduce pollutant in Palm Oil Mill Effluent. All of parameters this study have met the quality standards for liquid waste for industrial. There is still a tendency to decrease pollutant parameters, so further study is needed for optimal treatment so that it can be applied on an industrial scale.

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