Palm Oil Milling Effluent (POME) Waste Processing by Using Microalgae *Chlamydomonas* sp.

Riky Yonasa, Uray Irzandia, Hantoro Satriadia, W. Widayata, H. Hadiyantoa,b,*, Marcelinus Christwardanac,d

^a Department of Chemical Engineering, Faculty of Engineering, Diponegoro University, Jl. Prof. Sudarto SH, Tembalang, Semarang-50275, Indonesia ^b School of Postgraduate Studies, Diponegoro University, Jl. Imam Bardjo SH, Pleburan, Semarang-50241, Indonesia

^c Department of Chemistry, Faculty of Science and Mathematics, Diponegoro University, Jl. Prof. Soedarto SH, Semarang- 50275, Indonesia

^d School of Postgraduate Studies, Master Program of Energy, Diponegoro University, Jl. Imam Bardjo SH, Pleburan, Semarang-50241, Indonesia

* Correspondence: hadiyanto@live.undip.ac.id;

Received: 4th June 2022 Accepted: 13th July 2022 Published: 28th July 2022



Copyright: © 2022 by the authors. Submitted for possible open access publication under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/license s/by/4.0/). Abstract: Along with the growth in oil palm output, the amount of trash produced will also increase. Every palm oil mill is responsible for the disposal of liquid waste known as palm oil mill effluent (POME). POME includes very high levels of BOD and COD, which may hinder the development of microalgae. Before POME may be utilized as a medium for the growth and development of microalgae, a detailed investigation is required to establish the pretreatment measures necessary to reduce the BOD and COD levels. The purpose of this investigation of POME waste as a substrate for the growth and development of microalgae is to examine the POME processing procedure utilizing wild microalgae. The experimental technique consisted of adding POME and microalgae to the Erlemeyer in accordance with the required proportion. Research demonstrates that POME pond IV waste may be utilized as a substrate for the development of wild microalgae to lower POME waste BOD and COD levels. The variables used were the ratio of POME to microalgae volume and the quantity of nutrients supplied. Microalgae growth at a ratio of 1:4 produced the greatest decreases in BOD and COD, namely 61.66 ppm and 173.33 ppm from 110.6 ppm and 496.67 ppm, respectively. The impact of adding nutrient C at a concentration of 120 ppm led to the greatest decrease of BOD and COD, namely 65.33 ppm and 186.67 ppm, whereas adding nutrient N at a concentration of 40 ppm led to the greatest reduction of BOD and COD, namely 55.41 ppm and 158.33 ppm.

Keywords: POME, microalgae, COD, BOD

1. Introduction

Palm oil has played a significant part in the Indonesian economy throughout the years and is one of the most important export commodities. Production of palm oil tends to rise annually. Along with the rise in oil palm output, the amount of trash produced also rises (Chen et al., 1996). Each palm oil mill emits liquid waste known as palm oil mill effluent (POME), gas emissions from boilers and incinerators, solid waste materials such as empty fruit bunches, fiber and shells, and products such as potassium carbonate and palm kernel. This creates a serious environmental hazard if it is not disposed of properly (Sulaiman and Chea, 2004).

POME is a colloidal suspension made up of 95 to 96 % water, 0.6 to 0.7 % oil, and 4-5 % fat and total solids. POME is extracted from the industry as a brown liquid with a discharge temperature between 80 °C and 90 °C and a pH value between 4.0 and 5.0. Typical POME includes 6,000 mg/l of oils and fats on average (Low et al., 2021). The typical POME comprises BOD (Biological Oxygen Demand) in the range of 8,200-35,000 mg/Land COD (Chemical Oxygen Demand) in the range of 15,103-65,100 mg/L, which, if dumped directly into open waterways, would constitute pollution (Lee et al., 2019)

Poh and Chong (2009) have characterized POME processing, namely anaerobically, aerobically has the benefit of low energy usage (no aeration), and the creation of a great deal of methane in the product, while anaerobic processing has the drawback of a lengthy start-up period. Aerobic treatment has the benefit of being reasonably quick and efficient for dealing with hazardous waste, but it has the disadvantage of requiring a great quantity of energy for aeration. Treatment with membranes provides the advantages of steady production and high-quality generated water, while the

disadvantage is a limited membrane lifespan. Evaporative treatment has the benefit of being able to treat effluents with high solids concentrations, but the disadvantage of requiring a great deal of energy. These techniques are extensively employed in the sector of CPO processing. The problem of these approaches is that they only lower the concentrations of BOD and COD, although the concentrations of other components such as N, P, K, and several other minerals remain high enough for further processing.

In this research, the optimal pretreatment procedures for lowering BOD and COD levels while preserving POME's mineral content (N, P, K) will be determined. Comparing the amount of POME to that of wild algae such as Chlamydomonas was performed as part of the pretreatment. In this research, various things will be determined, including the optimal ratio and quantity of nutrients to lower the concentrations of BOD and COD while maintaining a high enough mineral content (N, P, K) to use POME as a medium for the growth and development of microalgae.

2. Material and Method

2.1. Material

POME, NaHCO₃, urea, and wild algae are some of the materials used. The POME was acquired from the PT. Nusantara VII Plantation in Lampung, Sumatra. The Waste Treatment Laboratory, Department of Chemical Engineering, UNDIP, provided the wild algae in the meantime. Chlamydomonas microalgae have been identified based on visual observations.

2.2. Microalgae Cultivation and Analysis

This study was done by acclimating POME and microalgae using urea and NaHCO₃ as nutrients. This procedure is performed using a stirrer at scale 4 speed. Optical Density and microalgae count using hemacytometer (Figure 1) was used to conduct a microalgae concentration study. The procedure is repeated until the OD value stays constant (14 days). After fourteen days, do a biomass analysis. Then, separate the microalgae in the mixture with 30 ppm alum and adjust the pH to 11 using 2M NaOH. The mixture was allowed to precipitate for a day. The COD and BOD content of the filtrate was tested, whereas the oil content of the precipitate was assessed.



Figure 1. Microalgae cell count

3. Result and Discussion

3.1. Calibration curve correlating the quantity of biomass cells to the optical density (OD) value

To determine the number of cells in the utilized microalgae, a relationship curve is constructed between the number of cells and the OD value. Using a counting chamber counting method and spectrophotometry, the OD value is determined by calculating the number of cells. The microalgae sample was not diluted prior to measuring the number of cells using the counting chamber since the number of cells was not excessive and could be measured without dilution. Because it was apparent which towns were simple to count, only particular boxes were picked, and computations were performed twice to ensure accuracy. After obtaining the number of cells and the OD value, the two values are related through a graph such that an equation may be derived to determine the number of cells from the OD value. The gathered data on the number of cells and the OD value are shown below, along with the equation.



Figure 2. Graph of relationship between cell number and OD value

The graph that follows depicts the link between the number of cells and the OD value. Using the technique of least squares, the following equation is obtained: y = 50.79 + 560.13x with $R^2 = 0.974$. Using the above equation, the number of cells in a sample may be calculated using the OD value. So, it is known that POME has microalgal growth. R^2 values close to 1 imply that the percentage of variation in the value of the y variable (number of cells) can be explained by a linear connection with the value of the x variable (OD value). The remaining variance (0.026) may be accounted for by other variables, such as observational errors.

3.2. Cell Growth in Relationship to Algae Volume Ratio to POME Variation

Using the change of the microalgae-to-POME volume ratio, the data displayed in Figure 3 is generated. Based on Figure 3, the growth of microalgae tends to rise when the ratio of algal volume to POME increases. This is because there are more cells as time progresses. The adaptation period of microalgae to grow in POME growth medium lasts from the first to the sixth day, during which the growth of microalgae is negligible. From the sixth through the fourteenth day, microalgae develop rapidly during the lag phase.



Figure 3. The effect of variations in the volume ratio of microalgae on POME

The composition of the ratio of microalgae to POME has an influence on the growth of microalgae, as shown by the findings of the previous experiment. From Figure 3, it is evident that the oscillations in the development of microalgae cells are highly favorable, namely at ratios of 1:1, 1:2, and 1:3. The variations in microalgae growth were comparable in all three studies. In the meanwhile, the development of microalgae seems gradual and not excessive for ratios of 1:4 and 1:5. The concentration comparison between microalgae and POME has a significant impact on the growth of microalgae, as shown by the data shown above. This impact is caused by the initial amount of cells present in the mixture. The more starting cells microalgae have, the greater their potential to divide. This is because the rise in the number of cells is not accompanied by an increase in nutrients for these cells at a 1:1 ratio. In order for the microalgae cells to perish from lack of nutrition. After determining that the 1:3 ratio yields the most increase, it is then used to the subsequent variant.

3.3. Cell Growth with Variations in Urea as Nutrients

Figure 4 depicts the fluctuation of Urea feeding on the development of microalgae cells as observed in studies (as shown). 12 % of the protoplasm of microalgae and 5 to 6 % of the protoplasm of molds or microbes are composed of nitrogen (Fogg & Thake, 1987). Nitrogen will be present as ammonia nitrogen in wastewater, with the amount depending on the rate of breakdown of organic matter. According to this hypothesis, the addition of nitrogen has a significant effect on the development of microalgae.

Figure 4 illustrates that the slower the cell development, the greater the amount of urea injected. This is due to the fact that if the ratio of carbon to nitrogen is too low (the quantity of nitrogen is too high), an excess of NH₃ will be produced, which might ultimately lead to the acidification process (Lee et al., 2019). This acidification process will inhibit the development of microalgae because it affects the stability of the optimal pH, as shown by the presence of urea at concentrations of 40, 45, and 50 parts per million. The stagnant period of their development curves tends to shrink in these variants (Chisti, 2007). Due to the high acidity of nitrogen molecules, more microalgae perish than proliferate. In optimal nutrient ratios of 20 and 30, the development of microalgae was neither inhibited by excessive nitrogen acidification or reduced cell formation caused by an excessive N ratio. With proper microalgae growth, the process of pollutant degradation is able to proceed efficiently (Sing et al., 2008).

Nitrogen is a crucial component of amino acids and nucleic acids, making it vital for all forms of life. As a result, nitrogen is crucial for the metabolism of microbes. Amino acids make up proteins, while nucleic acids are one of the components of DNA and RNA. In addition, the carbon-to-nitrogen ratio is dependent on the pollutants to be degraded, microalgae, and the kind of nitrogen employed. According to Shewfelt et al. (2005), if ammonium-nitrogen is employed, the rate of hydrocarbon breakdown will accelerate.



Figure 4. The effect of variations in the addition of nutrient urea on microalgae growth

3.4. Variations in Cell Growth Due to the Addition of the Nutrient NaHCO3

Figure 5 depicts the results of an experiment on the effect of NaHCO3 feeding on the proliferation of microalgae cells. The addition of NaHCO3 as a supplement increases the C concentration in POME medium. Carbon influences the photosynthetic process of microalgae in accordance with the following chemical equation:

 $CO_2 + H_2O + [Light] Energy \longrightarrow (CH_2O)N + O_2$

According to the graph, the impact of differences in NaHCO₃ addition tends to rise. This is because the passage of time increases the number of cells. Figure 5 depicts the optimal nutrient concentration at 100 ppm. This is due to the fact that the addition of 100 ppm NaHCO₃ produced an excellent comparison of the nutritional content of C, N, and P in POME medium for the microalgal photosynthesis process.



Figure 5. The effect of variations in the addition of NaHCO3 on microalgae growth

3.5. COD and BOD Measurements

From the experimental findings of POME waste treatment, the BOD and COD values obtained from the beginning and final samples are provided in Tables 1, 2, and 3, respectively. In the study of POME waste, modifications in the volume ratio of POME and microalgae do not appreciably alter the COD and BOD results. At a volume ratio of 1:4, the COD and BOD readings were reduced the most. The counting chamber is utilized as the basis for calculating the number of cells in the 1:4 volume ratio variation compared to variations of 1:1, 1:2, and 1:3. This is because the counting chamber is the foundation for calculating the number of cells. Where in this counting method, both live and nonliving cells are tallied (Lokman et al., 2021). Therefore, a greater number of microalgae are still active in this 1:4 volume ratio variation than in the others. Because, once again, the activity of live microalgae influences the lowering BOD and COD levels.

days		
POME : microalgae Ratio	BOD (mg/l)	COD (mg/l)
1:0	110.6	496.67
1:1	79	330
1:2	89.53	368.33
1:3	84.27	388.33
1:4	61.66	173.33
1:5	65.58	181.67
Waste discharge parameters	75	150

Table 1. BOD and COD values with variations in comparison of POME and microalgae volumes after aclimited for 14

Table 2. BOD and COD values with variations in the addition of urea nutrients after aclimited for 14 days in a 1:3

 POME and microalgae volume comparison

Urea (ppm)	BOD (mg/l)	COD (mg/l)	
0	110.6	496.67	
20	66.58	181.67	
30	84.27	388.33	
40	55.41	158.33	
45	67.08	191.67	
50	67.50	190.00	
Waste discharge parameters	75	150	

As demonstrated in Table 2, changes in the addition of the nutrient UREA decreased the values of COD and BOD in the analysis of POME waste. In the variant where UREA was added at a concentration of 40 ppm, COD and BOD values decreased the most. This is because microalgae are effective in degrading the sample's chemicals. According to Table 3, modifications in the addition of the nutrient NaHCO3 may reduce COD and BOD levels. The addition of 120ppm of the nutrient NaHCO3 produced the greatest decrease in COD and BOD levels. This is because microalgae are effective in degrading the sample's chemicals. Based on all BOD and COD analyses, the final COD value is insufficient to be released into the environment. According to Regional Regulation No. 10 of 2004 for the Province of Central Java, the acceptable COD disposal level is 150 mg/l. The threshold for BOD values that may be released into the environment is 75 mg/l, hence the BOD values obtained by the majority of samples are acceptable to the environment.

Concentration of NaHCO ₃	BOD (mg/l)	COD (mg/l)	
0	110.6	496.67	
50	68.08	191.67	
80	68.25	195.00	
100	84.27	388.33	
120	65.33	186.67	
150	68.83	196.67	
Waste discharge parameters	75	150	

Table 3. BOD and COD values with variations in the addition of NaHCO3 nutrients after aclimited for 14 days in	ı a 1:3
POME and microalgae volume comparison	

3.6. Microalgal Oil Content Analysis

The value of the oil analysis of the dry microalgae cultivated was determined by experimentation. Table 4 presents the value of the oil as shown below. According to the statistics shown above, the ratio of pome volume to microalgae volume of 1:3 has the maximum oil concentration. This is due to the fact that in this setting, photosynthesis of microalgae is more efficient, allowing a greater quantity of microalgae to generate more oil (Mohammadi et al., 2015).

No.	POME: microalgae Ratio	Oil content (%)
1.	1:1	1.46
2.	1:2	11.17
3.	1:3	18.18
4.	1:4	11.78
5.	1:5	7.56

Table 4. Oil content analysis on variations in comparison of POME volume with microalgae volume

4. Conclusion

The following are the conclusions that can be drawn from this study's findings. POME pond IV waste may be utilized as a medium for the development of wild microalgae to lower POME waste BOD and COD levels. The optimal development of microalgae was achieved by adjusting the volume ratio of POME and microalgae to 1:3 and adding 100ppmNaHCO₃ nutrient and 30ppm urea nutrient. The most effective reduction of BOD and COD occurred when the volume ratio of POME and microalgae was 1:4. The obtained BOD and COD values were 61.66 ppm and 173.33 ppm, respectively. Providing vitamin C at a concentration of 120 ppm led to the greatest reductions in BOD and COD, namely 65.33 ppm and 186.67 ppm, while providing nutrient N at a concentration of 40 ppm led to the greatest reductions in BOD and COD, reaching 55.41 ppm and 158.33 ppm.

References

- Chen, F. (1996). High cell density culture of microalgae in heterotrophic growth. Trends in biotechnology, 14(11), 421-426.
- Chisti, Y. (2007). Biodiesel from microalgae. Biotechnology advances, 25(3), 294-306.
- Fogg, G. E., & Thake, B. (1987). Algal cultures and phytoplankton ecology. Univ of Wisconsin Press.
- Haryanti, A., Norsamsi, N., Sholiha, P. S. F., & Putri, N. P. (2014). Studi pemanfaatan limbah padat kelapa sawit. Konversi, 3(2), 57-66.
- Lee, Z. S., Chin, S. Y., Lim, J. W., Witoon, T., & Cheng, C. K. (2019). Treatment technologies of palm oil mill effluent (POME) and olive mill wastewater (OMW): A brief review. Environmental technology & innovation, 15, 100377.
- Lokman, N. A., Ithnin, A. M., Yahya, W. J., & Yuzir, M. A. (2021). A brief review on biochemical oxygen demand (BOD) treatment methods for palm oil mill effluents (POME). Environmental Technology & Innovation, 21, 101258.
- Low, S. S., Bong, K. X., Mubashir, M., Cheng, C. K., Lam, M. K., Lim, J. W., ... & Show, P. L. (2021). Microalgae cultivation in palm oil mill effluent (POME) treatment and biofuel production. Sustainability, 13(6), 3247.
- Mohammadi, M., Kazeroni, N., & Baboli, M. J. (2015). Fatty acid composition of the marine micro alga Tetraselmis chuii Butcher in response to culture conditions. J. Algal. Biomass Utln, 6, 49-55.
- Shewfelt, K., Lee, H., & Zytner, R. G. (2005). Optimization of nitrogen for bioventing of gasoline contaminated soil. Journal of Environmental Engineering and Science, 4(1), 29-42.
- Song, D., Fu, J., & Shi, D. (2008). Exploitation of oil-bearing microalgae for biodiesel. Chinese Journal of Biotechnology, 24(3), 341-348.
- Sulaiman, N. M. N., & Chea, K. L. (2004). Membrane ultrafiltration of treated palm oil mill effluent (POME). Jurnal Teknologi, 113â-120.