JOURNAL OF APPLIED GEOSPATIAL INFORMATION

Vol 7 No 1 2023

http://jurnal.polibatam.ac.id/index.php/JAGI
ISSN Online: 2579-3608

Utilization of Palm Oil Mill Effluent (POME) Liquid Waste to Increase Density and Growth Rate of Microalgae *Chlorella Pyrenoidosa*

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Received: December 30, 2022
Accepted: February 02, 2023
Published: February 02, 2023

Abstract

Microalgae *Chlorella pyrenoidosa* is a microalgae that has the potential to have various roles, especially as a bioabsorbant for organic waste. The growth of the oil palm plantation industry is also directly proportional to the increase in waste generated from the CPO production process, namely POME Waste (Palm Oil Mill Effluent). POME waste has a high nutrient content so that it can be utilized by the microalgae *Chlorella pyrenoidosa*. This study aims to utilize POME waste to increase the growth of *Chlorella pyrenoidosa*. Microalgae cultivation had a significant effect on the 1:2 treatment and 1:3 treatment when compared to the control cultivation medium with the highest cell density values of 263(106cells/ml) and 279(106cells/ml) respectively. Injection of POME waste into microalgae rearing media will have an impact on increasing the growth of *Chlorella pyrenoidosa* microalgae.

Keywords: Microalgae, POME, *Chlorella pyrenoidosa*, Waste

1. Introduction

1.1 Sub Introduction

The development of research related to vegetable protein sources is quite intensively carried out in order to meet the needs of market demand in line with the rapid development of the industrial world. One of the potential and environmentally friendly raw materials is microalgae. Microalgae are aquatic plants and are at the base of the food chain structure because they are primary producers. The diversity of microalgae in the world is estimated to be in the range of millions of species, most of which are unknown and cannot be cultivated (self-propagated). It is estimated that 200,000-800,000 species live in nature, 35,000 species can be identified, and 15,000 chemical components that make up the biomass are known (Hadiyanto, et al., 2012).

Microalgae *Chlorella pyrenoidosa* is a microalgae that has the potential to have various roles that can be developed as a source of feed, food, and raw materials for the pharmaceutical, cosmetic, and biofuel industries (Hadiyanto and Azim M. 2012). The large potential of *Chlorella pyrenoidosa* so that this microalgae has high economic value. In addition, the microalgae *Chlorella pyrenoidosa* was chosen as a medium for absorption of POME waste because *Chlorella pyrenoidosa* can grow and multiply in dirty water. The ability of *Chlorella pyrenoidosa* to utilize nutrients is expected to reduce the content of toxic compounds such as nitrogen, phosphate and ammonia.

The growth of the oil palm plantation industry is also directly proportional to the increase in waste generated from the CPO production process, namely POME Waste (Palm Oil Mill Effluent). POME waste is palm oil liquid waste which is currently still a problem and has not been utilized optimally. POME waste is generally obtained from the rest of the Crude Palm Oil (CPO) manufacturing process where 1 ton of palm oil will produce 50-60% POME and 20% CPO.

In 2016, the share of world CPO production reached 40% of the world's total main vegetable oil, while soybean oil had a share of 33.18% (United States Department of Agriculture, 2016). It is estimated that this figure will increase to 43 million tons in 2019. The main problem with this liquid waste is the high concentration of COD, BOD and total solids, so that this waste cannot be disposed of directly into the environment. In addition to these

parameters, POME waste still contains a relatively high amount of total nitrogen, phosphorus and potassium, and can be used as nutrients in the process of photosynthesis for the growth of microalgae.

This study aims to determine the effect of POME waste on the density and growth rate of Microalgae *Chlorella pyrenoidosa* at various concentrations, 2. To determine the effect of POME waste on the growth phase of *Chlorella pyrenoidosa*, and 3. To determine the environmental factors that affect the density and growth rate of Microalgae *Chlorella pyrenoidosa*.

2. Methodology

The microalgae maintenance method in this study used a standard cultivation system with transparent jar maintenance containers placed in open spaces. As in Figure 1. Below

Fig 1. Microalgae *Chlorella pyrenoidosa* Cultivation System.

Cultivation of the microalgae *Chlorella pyrenoidosa* was carried out by mixing (injecting) POME waste into the cultivation medium. Injections were carried out with 4 treatments at each cultivation scale and 1 control, as shown below:

Table 1. Cultivation (injecting)

Treatment 1:1	1liter POME x 1liter microalgae
Treatment 1:2	1liter POME x 2liter microalgae
Treatment 1:3	1liter POME x 3liter microalgae
Treatment 1:4	1liter POME x 4liter microalgae

Control is a cultivation medium that is not given POME waste injection, so that it can be used as a comparison for the treatment to be applied. Treatment 1:1 is the amount of Pome Waste 1, and the number of seeds of the microalgae *Chlorella pyrenoidosa*.

Measurement of growth and water quality is carried out every day. The growth of the *Chlorella pyrenoidosa* microalgae is known from the density values measured using a haemacytometer with the equation (Kawaroe et al. 2010):

$$N=(n/4)\times 〖10〗^{6} \quad (1)$$

Information:
N = Density of microalgae (cells/ml)
n = number of microalgae observed

Furthermore, after the density data is obtained, the daily growth rate will be measured using the Wood et al. equation (2005):

$$K=(〖\ln〗^{Nt}- 〖\ln〗^{(N_0)})/(t- t_0) \quad (2)$$

Information:
K = Growth rate
N = Cell density at time – t
N0 = Initial cultivation cell density
T = Observation time at the end of cultivation
t0 = Initial cultivation time

Data on growth rate and POME effluent content were statistically analyzed using Analysis of Variance (ANOVA) with a 95% confidence interval (Steel & Torrie, 1989):

$$Yij = \mu + \tau i + \acute{\epsilon} ij \quad (3)$$

Where:
Yij = Type of organic matter treatment (i-th) on the j-th repetition
μ = Common mean
τi = Added value due to the type of organic matter treatment (i-th)
ɛ́ij = Experiment treatment error at the type of organic matter treatment (i-th) and j-th repetition.

Then Tukey's further test was carried out (Mattjik & Sumertajaya, 2002):

$$q_{\alpha\,;\,p\,;db_g} \qquad BNJ = q_{\alpha;p;dbg};S\gamma \quad (4)$$

Where:
BNJ = Honest Real Difference
P = Treatment
Dbg = Degrees free of error
= standard error of mean deviation
= Tukey table value at significant level α

3. Results and Discussion

Based on the graph of microalgae cell density as shown in Figure 2. where the growth of *Chlorella pyrenoidosa* microalgae in the cultivation media can be seen from the density measurements every day. The density of microalgae in the 1:1 treatment media experienced an increase in the number of cells from day 2 to peak growth on day 8 with a total density of 240(cells106cells/ml). Density decreased on day 9 with a density of 203(cells106cells/ml). Growth continued to decrease until the end of maintenance on day 11 of 154(cells106cells/ml).

Density growth in the 1:2 treatment cultivation medium experienced an increase in density starting on the 2nd day of the maintenance period of 40(106cells/ml). The increase in growth occurred until the 8th day of the maintenance period as the peak density in the 1:2 treatment was 263(106cells) /ml). on the 9th day it decreased in density with the number of cells 257 (cells106cells/ml). until the end of maintenance decreased by 203(cells106cells/ml).

In the 1:3 treatment, the cell density of microalgae *Chlorella pyrenoidosa* had the highest cell count of all POME waste injection treatments and controls. The highest number of cells was found on day 8, namely 279 (106 cells/ml). An increase in density also occurred on day 2 of maintenance by 39(cells106cells/ml). The density of microalgae cells in the 1:3 treatment cultivation medium decreased on day 9, namely 263(cells106cells/ml) to day 11 of 22 (cells106cells/ml).

The density in the 1:4 treatment also increased on the 2nd day of maintenance, namely 38(106cells/ml). The peak cell density in the 1:4 treatment occurred on day 8 with the number of cells 243(cells106cells/ml), then decreased on day 9 to day 11 of the maintenance period of 150(cells106cells/ml). The total cell density in the 1:4 treatment was similar to the cell density in the 1:1 treatment, where the peak density occurred on the same day, namely the 8th day, which was 240 (cells106cells/ml), and the number of cells at the end of the maintenance was not significantly different. between these two treatments, where the final density of the rearing period in the 1:1 treatment was 154(106cells/ml), in the 1:4 treatment it was 150(106cells/ml).

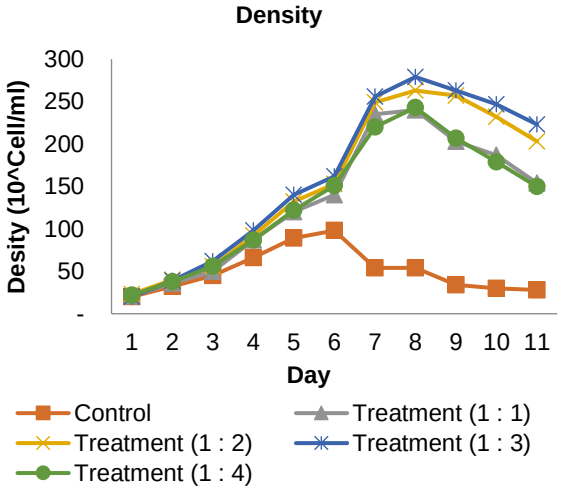

Fig 2. Graph of *Chlorella pyrenoidosa* Microalgae Cell Density

Growth density in the control cultivation medium experienced relatively lower density growth than all POME waste injection treatments. The increase in cell density of microalgae *Chlorella pyrenoidosa* experienced growth on day 2 of 32, this increase continued until day 6 of the maintenance period with the number of cells 98 as the peak density growth. then decreased from day 7 to 54, this decrease in density continued to occur consistently until the end of maintenance, namely on day 11 with a total of 28 cells. The total cell density in the Control cultivation medium was the lowest of all the cell numbers in the treatment media. Compared to the peak density of microalgae *Chlorella pyrenoidosa* in the control media, it was only 98, and was the lowest cell density of all the highest densities in each treatment. This is due to the nutrient content in POME waste which is injected into each treatment medium which has an impact on the growth of microalgae cells. POME waste is a source of additional nutrients in the rearing medium.

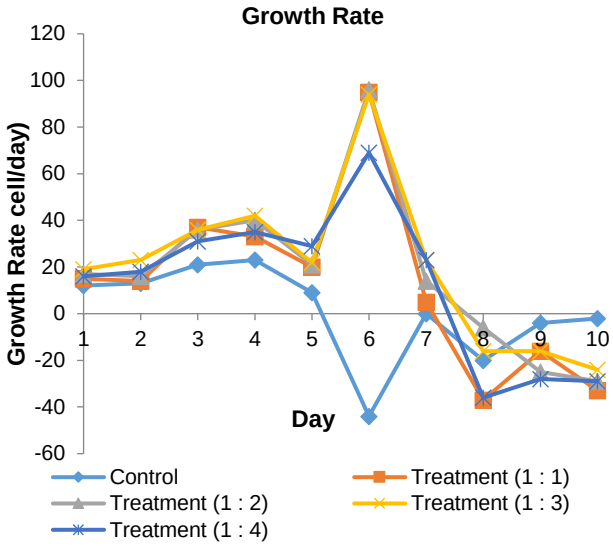

Fig. 3. Graph of Daily Growth Rate of Microalgae *Chlorella pyrenoidosa*

Based on the graph of the growth rate of microalgae in Figure 3, it shows that the growth pattern in all treatment media has the same pattern, namely experiencing a lag phase on the first day, and then experiencing a logarithmic phase on day 2 to day 8, then experiencing a phase of decreased growth. on the 9th day until the death phase on the 11th day. The growth phase was different in the control media where the lag phase occurred on the first day, then experienced a logarithmic phase on the 2nd to 6th day where the logarithmic phase in the control media occurred more short, namely for 5 days of rearing, that is, from day 2 to day 6. Meanwhile, in medium cultivation, the long treatment logarithmic phase was longer, namely 7 days starting from day 2 to day 8. Then the phase of decreased growth in the control media happened faster, starting on the 7th day, while in the treatment cultivation media the new growth decline phase occurred on the 9th day. he continued the death phase occurred on day 11. The death phase in all cultivation media occurred on day 11 indicated by the yellow color of the microalgae.

Microalgae are a type of aquatic plant that can grow in a relatively short time. During its life, microalgae go through 5 phases namely the lag phase, logarithmic phase, decreased growth phase, stationary phase and death phase. These five phases have their own characteristics as follows (Kawaroe et al., 2010):

- The lag phase or what is called the initial phase, the microalgae are still very small in number because they are still in the adjustment stage.
- Logarithmic phase, microalgae will experience a rapid increase in growth rates because they are able to adapt to the medium in which they live.
- Growth reduction phase, microalgae in this phase will experience a decrease in abundance until they can return to their initial growth rate due to a decrease in nutrient levels from the media.
- Stationary phase, microalgae experience a constant growth status and this phase is known to have a very short period of time.

- Death phase, microalgae in the growing media will change color from the original pigment color and usually will clump at the bottom of the cultivation medium.

Table 2. Annova Statistical Test Data

(i) Comparison		Sig.
Control	Treatment (1 : 1)	.100
	Treatment (1 : 2)	.022
	Treatment (1 : 3)	.011
	Treatment (1 : 4)	.099

Based on Table 1. it can be seen that Treatment 1:2 and Treatment 1:3 are cultivation media that have a significant effect when compared to Control media. This means that injection of POME waste in the 1:2 and 1:3 cultivation media had a significant effect on the growth of *Chlorella pyrenoidosa* microalgae cells, where the density of microalgae cells in these two rearing media experienced a significant increase compared to the density growth of microalgae cells in the control medium. . The composition of 1:2 and 1:3 between POME X waste Microalgae seeds are the best and have a significant impact on increasing density growth.

The nutrient content in the 1:2 and 1:3 treatment media can be utilized optimally by microalgae for their growth. This is different from the 1:1 treatment, because the content of POME waste is too high which increases turbidity which causes low sun intensity to enter the water column. While the 1:4 treatment did not experience significant growth because the number of microalgae seeds was too much compared to the volume and nutrient content of POME waste which was only injected at 1:4. In other words, that the rearing medium in Treatment 1:4 has little nutrient content when compared to the volume of microalgae seeds, this can be seen based on growth data, where the highest density is only 243(106cells/ml).

The effect of injection of POME waste on the rearing medium aims to provide additional nutrient content in the rearing medium. POME waste has a very high nutrient content, while microalgae need *Chlorella sp*. Will grow normally on media containing at least 19 types of nutrients which include macro nutrients (nutrients needed in large quantities) such as carbon, hydrogen, oxygen, nitrogen, phosphorus, sulfur, sodium, magnesium, calcium. Nitrogen, phosphorus, and potassium as well as micro-nutrients (nutrients needed in small amounts) such as carbon, hydrogen, oxygen, nitrogen, phosphorus, sulfur, sodium, magnesium, calcium. Nitrogen, phosphorus, and potassium as well as micro-nutrients (nutrients needed in small amounts) such as magnesium (Mg), iron (Fe), copper (Cu), manganese (Mn), zinc (Zn), silicon (Si), boron (B), molybdenum (Mo), vanadium (V) and cobalt (Co) (Garno, Y.S. 1992).

Table 2. Water Quality

Days-To	Treatment (1 : 1)			Treatment (1 : 2)		
	Temp	Do	pH	Temp	Do	pH
1	28	2.8	8.00	28	2	8
2	27	3.1	8	27	2.8	8.1
3	30	3.2	8.1	30	3	8
4	31	3.2	8	31	3	8
5	31	3	8.2	31	3	8
6	30	3	8.2	30	3.2	8.1
7	30	3.2	8.2	30	3.4	8.2
8	31	3.2	8	31	3.2	8.2
9	31	3.2	8.1	30	3	8
10	30	3.2	8	30	3	8.2
11	30	3	8.1	31	3.2	8.2

Days-To	Treatment (1 : 3)			Treatment (1 : 4)		
	Temp	Do	pH	Temp	Do	pH
1	28	2.8	9	28	8	8.8
2	27	3	8.2	27	2.8	8.1
3	29	3.4	8	29	3	8
4	28	3.2	8	28	3.4	8.2
5	29	3.4	8	29	3.2	8.2
6	30	3	8.1	30	3.4	8.2
7	30	3	8.1	30	3	8.1
8	31	3.2	8	31	3.4	8
9	29	3.4	8	28	3.2	8
10	28	3.3	8	27	3	8.2
11	29	3	8.1	30	3	8.1

Days-To	Control		
	Temp	Do	pH
1	27	2.8	8.2
2	28	3	8
3	27	3.1	8.2
4	29	3.1	8.2
5	28	3	8.1
6	29	3	8
7	30	3.2	8
8	30	3.2	8.2
9	31	3.1	8.2
10	29	3.1	8
11	30	3	8.2

Based on Table 2. that the water quality in all cultivation media is relatively stable and uniform, this is because the jars used as containers for cultivation are closed and placed in a transparent plastic covered shelf. This condition causes the

maintenance media water to not be disturbed by external environmental influences such as rain.

If seen from all the water quality parameters measured, namely temperature, Do, and Ph, they are still at the tolerance threshold for optimal microalgae growth.

The temperature in all cultivation media has the same value range, starting from 270C - 310C. This value range is still in the optimum microalgae growth tolerance. According to Reynolds (1990) the optimum temperature for microalgae growth is 25 - 400C.

Dissolved oxygen (Do) in all microalgae cultivation media is below the optimal value range for microalgae growth. The range of Do values in all cultivation media is between 2 - 3.4 ppm. according to Facta et al (2006) the optimal range of dissolved oxygen for microalgae is 5-7 ppm.

The pH of all microalgae cultivations ranges from 8 – 8.2, and this range is still within the optimal range for microalgae growth. The average pH for the culture of most microalgae species is between 7 - 9, with an average optimum pH ranging from 8.2 to 8.7 (Lavens & Sorgeloos, 1996).

4. Conclusion

Based on the results of the study, that injection of POME waste into microalgae cultivation media had a significant effect on the 1:2 treatment and 1:3 treatment when compared to the Control cultivation media with the highest cell density values of 263(106cells/ml) and 279(106cells/ml) respectively. ml). Injection of POME waste into microalgae rearing media will have an impact on increasing the growth of *Chlorella pyrenoidosa* microalgae.

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