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Research Article

Evaluation Of *Spirulina*, *Nannochloropsis*, and *Chlorella* Microalgae Growth in Palm Oil Mill Effluent (POME) Medium with Variation of Medium Types and Time Adding Nutrient

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Abstract: POME is a kind of liquid waste produced by the crude palm oil industry. POME was not treated adequately, resulting in an issue for the environment owing to excessive levels of COD and BOD. Algae is a kind of bio-absorbent that may neutralize contaminants in liquid waste. Microalgae need carbon, nitrogen, and phosphorus-containing ingredients to flourish. These nutrients are necessary for photosynthetic carbon sources to be converted into biomass. POME includes a high concentration of C, N, and P; hence this research aims to investigate the potential of POME as a medium for the development of algae such as *Spirulina*, *Nannochloropsis*, and *Chlorella*. The experiment was carried out by varying the nutrients, water type, and time of nutrient feeding. Urea and sodium bicarbonate were the nutrition. Preparing the medium, culturing the microalgae, assessing biomass, counting the algae cells, and creating a calibration curve were the procedures in the experiment. The findings revealed that POME is the best medium for microalgae, that *Spirulina* grows better in POME than *Chlorella* and *Nannochloropsis*, and that providing nutrients every 2 days was better than introducing nutrients at the beginning and without adding nutrients.

Keywords: POME, microalgae, saline water

1. Introduction

Indonesia has been the world's largest producer of palm oil since 2006, when it dethroned Malaysia from the top spot (Isroi, 2010). This ever-increasing trend in the production of crude palm oil (CPO) has severe environmental repercussions. This is connected to water pollution produced by the improper disposal of palm oil mill effluent (POME) that pollutes the environment by not being handled effectively (Puteh, 2007).

Alternative that is effective and efficient is the growing of algae in trash. POME, which is rich in minerals such as N, P, K, and other minerals, is well suited for usage as a plant fertilizer or nutrient. In addition to optimizing waste treatment to become harmless waste, algal productivity may be boosted by treating POME as a fertilizer for algae. Therefore, more study must be conducted to identify the proliferation of algae in POME waste.

This project will evaluate the viability of POME trash as a microalgae growing medium. Three species of algae were employed, notably *Spirulina*, *Nannochloropsis*, and *Chlorella*. In this research, it will be determined the optimal conditions for microalgae growth in POME, the viability of microalgae in POME, and the concentration of N, P, and K elements in POME waste as a medium for the growth and development of microalgae.

2. Material and Method

2.1. Material

Microalgae *Spirulina*, *Nannochloropsis*, and *Chlorella* collected from the Jepara Brackish Water Cultivation Center (BPAP) and Palm Oil Mill Effluent (POME) obtained from PT. Perkebunan Nusantara VII, Lampung, Sumatra were

used as the raw materials. In addition to nutrition consisting of urea and sodium bicarbonate acquired at a Semarang chemical store.

2.2. Microalgae Cultivation

In a 1000 ml Erlenmeyer, 720 ml of distilled water and 180 ml of POME were mixed. Then, approximately 10 % of microalgae, or 100 ml, was introduced to an Erlenmeyer containing POME and other nutrients based on the variables. The microalgae culture was aerated, supplemented with appropriate light, and the surrounding temperature was set to between 23 and 27 °C. Every day for seven days, optical density was measured using a spectrophotometer at a wavelength of 680 nm.

2.3. Biomass and cell count

After weighing the blank filter paper (W1), the microalgae and the medium were filtered using a vacuum pump. The filter paper was dried in a 50 ° C oven. After drying, the filter paper (W2) was weighed, and the dry biomass (grams) was calculated by subtracting W2 from W1 (grams).

The microscope and hemocytometer were prepared for use and then cleaned with 70% alcohol. The sample was thereafter collected using a pipette and dripped onto the hemocytometer. Attached to the microscope, the hemocytometer counts the number of cells present.

2.4. Calibration Curve Creation

Create a calibration curve between optical density and biomass weight when the OD of each microalgal culture is 1. Then, the weight of the blank filter paper (W_1) is determined, and the microalgae biomass in POME is filtered using a vacuum pump. Following this, filter paper and biomass were dried in an oven. Weighing the filter paper and the dry biomass (W_2) yielded biomass (grams) = W_2 (grams) - W_1 (grams). OD values of 0.8; 0.6; 0.4; 0.2; 0 are likewise treated as described above.

When the OD of each microalgae culture is equal to 1, the number of cells for each species of microalgae in POME is determined. Then, repeat the preceding procedures for OD values of 0.8; 0.6; 0.4; 0.2; 0

3. Result and Discussion

3.1. Calibration curves of microalgae

A calibration curve is required to determine the relationship between the biomass (dry weight) generated and the number of cells in the solution for each optical density. Figures 1a-b show the experimentally determined calibration curves for the microalgae *Spirulina*, *Chlorella*, and *Nannochloropsis*.

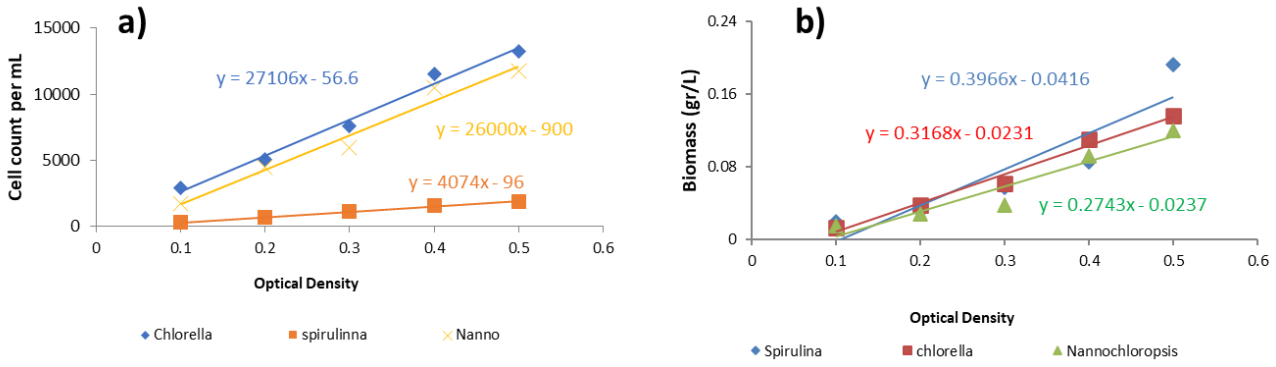

Figure 1. Calibration curves between OD and a) cell count per mL, as well as b) biomass

The Optical Density (OD) is directly related to the biomass and the number of cells, as seen in Figures 1 and 2. The empirical relationship for *Spirulina* is derived from the calibration curve shown above: Biomass (gr/L) = 0.396 x OD_{680} and Number of cells (per mL) = 4074 x OD_{680}. It can be deduced from these equations that the number of cells in *Spirulina* is directly related to the biomass produced:

$$\text{Biomass (g/L)} = (9.72 \times 10^{-5}) \times \text{(Number of cells/mL)}$$

The number of *Chlorella* microalgal cells was shown to be directly related to their biomass, as follows:

$$\text{Biomass (g/L)} = (1.16 \times 10^{-5}) \times (\text{Number of cells /mL}).$$

And based on the calibration curves in Figures 1a-b, the Biomass (gr/L), OD_{680}, and number of cells (mL) for the *Nannochloropsis* algae equals 274,000 x OD_{680}. The empirical connection between OD680 and the number of cells in *Nannochloropsis* is as follows:

$$\text{Biomass (g/L)} = (1.05 \times 10^{-5}) \times (\text{Number of cells/mL})$$

Figure 1 demonstrates that at the same optical density (OD), *Spirulina* produces the greatest amount of biomass, followed by *Chlorella*, and then *Nannochloropsis*. As seen in Figure 2, *Spirulina* contains the fewest cells per milliliter compared to *Chlorella* and *Nannochloropsis*. Due to the bigger size of *Spirulina* (200-300 microns in length and 5-70 microns in width) compared to *Chlorella* (1-2 microns in diameter) and *Nannochloropsis* (2-8 microns), the optical density or cell density of *Spirulina* is less than that of *Chlorella* and *Nannochloropsis*. According to these data, the biomass generated by *Spirulina* is more than that of *Chlorella* and *Nannochloropsis* at the same optical density. In contrast, *Chlorella* generates more biomass than *Nannochloropsis*; in addition to its bigger size/diameter, the number of *Chlorella* cells at the same optical density or density is more than that of *Nannochloropsis*.

3.2. Effect of Media on the Growth of Microalgae

In Figure 2, the impact of adding nutrients to the POME waste medium is compared to that of adding nutrients to fresh medium and saline water at the beginning of the experiment. In the experimental results graph, the growth of microalgae *Spirulina*, *Chlorella*, and *Nannochloropsis* in POME waste was greater than in fresh medium and salt water. This is because the nutritional needs of microalgae are relatively high, namely the ratio of mass C: N; P = 56: 9: 1 (Kim-Chong and Siew-Moi, 1988), whereas the POME waste utilized includes fairly high levels of nutrients (C, N, P) (Yusoff and Chan, 1997). In contrast, neither the fresh medium nor the salty water had the nutritional ingredients required for microalgae.

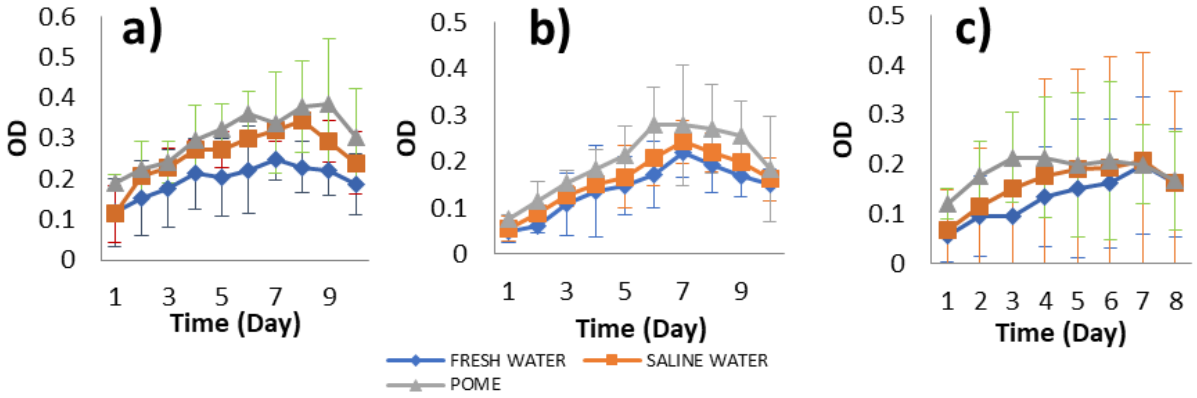

Figure 2. Media Effect on Microalgae Growth: (a) *Nannochloropsis*, (b) *Spirulina*, (c) *Chlorella*

The image also demonstrates that microalgae in salty water have a faster growth rate than those in fresh water. This is because these three species of microalgae thrive best in salt levels ranging from 5 to 15 ppm (Anon, 2009; Hirata, 1981). While the salty water used in the experiment had a salinity of 10 parts per million (ppm), the water was still able to support life. This situation increases the growth rate of microalgae that inhabit salty water.

Figure 3 demonstrates that the growth rate of microalgae residing in POME medium is greater than that of microalgae residing in saline water media. This is due to POME waste, which provides sufficient nutrients to satisfy the nutritional requirements of microalgae. Meanwhile, microalgae do not need the nutrients found in salt water. Thus, nutrients have a significant impact on the growth rate of microalgae.

3.3. Effect of Nutrient Addition Time on Microalgae Growth

Figure 3 shows that the timing of nutrient addition impacts the growth rate of microalgae. The growth rate was greater when nutrients were added every two days compared to when nutrients were added at the beginning or not at all. This is due to the fact that microalgal nutritional requirements are relatively high, namely the ratio of mass C: N; P

= 56: 9: 1 (Kim-Chong and Siew-Moi, 1988), however the nutrients in POME are insufficient for microalgal nutrient requirements, namely the ratio of mass C: N; P = 47: 7: 1 (Yusoff and Chan, 1997). With the addition of nutrients every two days, the microalgae's supply of extra nutrients is increased. The addition of nutrients every two days resulted in more algal growth than the addition of nutrients at the beginning or without nutrients.

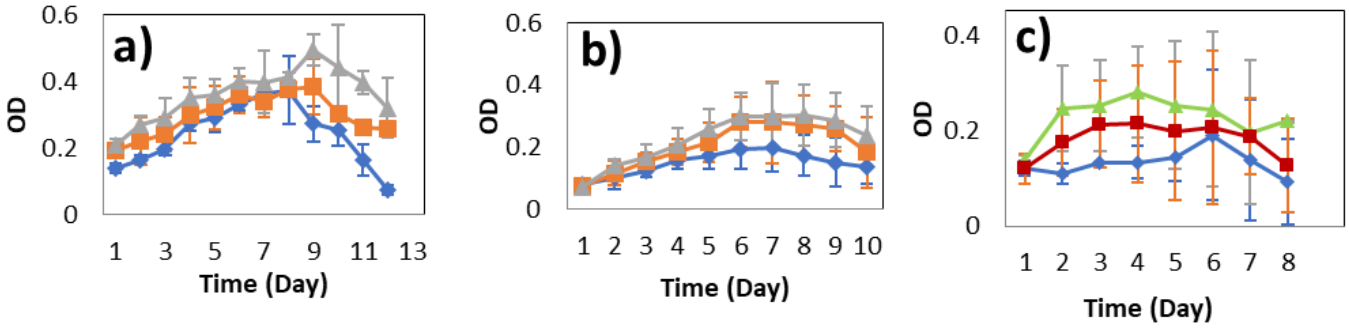

Figure 3. Effect of Nutrient Addition on Microalgae Growth: (a) *Nannochloropsis*, (b) *Spirulina*, (c) *Chlorella*

3.4. Effect of Nutrient Addition Time on Microalgae Growth

Microalgae need sunlight, carbon dioxide, and water to grow, much like other plants. Microalgae use sunlight to carry out the photosynthesis process. During photosynthesis, solar energy is used to transform inorganic chemicals into simple sugar molecules. The following equation describes the photosynthetic reaction of microalgae (Shelef, 1976; Edwards, 1980):

$$122\ CO_2 + 16\ NH_4 + PO_3^{3-} + 58\ H_2O \rightarrow C_{122}H_{179}O_{44}N_{16}P + 131\ O_2 + H^+$$

The maximum biomass production throughout the experiment was 0.2 grams. Assuming that the produced biomass is equivalent to the product $C_{122}H_{179}O_{44}N_{16}P$, the nutritional needs for carbon (in CO_2) and nitrogen (in NH4) are 0.102 and 0.016 grams, respectively.

Several treatments were applied in the experiment, including growing with POME medium, salt water, and fresh water. POME provides the essential microalgal nutrients C, N, and P. The employed POME medium has a C: N: P ratio of 47: 7: 1 (Yusoff and Chan, 1997), while the optimal development of microalgae on 20 % POME media requires a C: N: P ratio of 56: 9: 1. (1988, Kim-Chong and Siew-Moi). The ratio between the difference between the ratio of nutrient levels (C, N, P) and the ratio of the optimal nutrients multiplied by the needed nutritional needs must be added to determine the nutrient requirements.

In the POME medium, nutrients are received not only from the medium itself, but also from NaHCO3 and $CO(NH_2)$ added to the medium. In the first addition, 50 ppm $NaHCO_3$ and 25 ppm $CO(NH_2)_2$ were added such that the quantities of additional C and N could be determined by comparing the relative masses of each element, as shown in Table 1. During the addition of 30 ppm $NaHCO_3$ and 5 ppm CO(NH2)2 every two days, the levels of C and N may be estimated based on the ratio of the relative mass of each element. In the computation of nutrient additions every two days, the concentration is higher on the third addition (on the sixth or seventh day) because microalgal development is at its highest on that day.

Table 1. Quantity of Nutrients Present in Each Treatment

Medium	Nutrien ($NaHCO_3$ and $CO(NH_2)_2$)	Given weight (gram)	
		C	N
POME	Without addition	0.086	0.012
	Initial addition	0.098	0.024
	Added every 2 days	0.101	0.02
	Saline water	0.025	0.01
	Fresh water	0.025	0.01

In the medium of saline and fresh water, C, N, and P are absent, therefore pure microalgae must be supplemented with nutrients every two days to receive the nutrients they need. The addition of nutrients using 30 ppm $NaHCO_3$ and 5 ppm Urea $CO(NH_2)_2$ in order to quantify the additional C and N levels by comparing the relative mass of each element.

In the treatment without nutrients, it was seen that the nutrients were less able to fulfill the demands of microalgae, resulting in slower growth than in the treatment with an initial addition and additions every two days. In the first addition, nutrient C was undersupplied (0.100 grams < 0.102 grams), but vitamin N was oversupplied (0.024 grams > 0.016 grams). Due to the near-required amount of nutrient C, the growth of microalgae in the initial addition treatment is superior to that of microalgae in the medium without nutrients; nevertheless, the growth is inferior to that of microalgae in the nutrient addition every two days.

In line with calculations, the peak of microalgae growth occurs on the sixth/seventh day after the addition of every two days, since the nutrients required by microalgae are met under these circumstances (C needs of 0.102 grams and N needs of 0.016 grams). The next day, an overabundance of C and N nutrients inhibited growth and caused microalgae to enter the death phase. Thus, the growth of microalgae on the addition of nutrients every two days was greater than the growth of microalgae on the first addition of nutrients and in the absence of nutrients.

Medium saline and fresh water have the same quantity of additional nutrients, yet the growth of microalgae in saline water is superior to that in fresh water. This is because microalgae thrive in salty water, hence the growth of microalgae in saline medium is greater than in fresh water.

3.5. Microalgae Growth Rate

The growth rate of microalgae in POME medium with the addition of nutrients was greater than without the addition of nutrients, as shown in Table 2. Urea and sodium bicarbonate are given as nutrients to the medium. This nutrient is added to the microalgae cultivation medium since the nutrients in POME will diminish as the microalgae Growth.

Table 2. Growth rate of microalgae

Medium	Nutrient		μ (1/day)			OD max		
			N	S	C	N	S	C
POME	Initial addition	Urea	0.091	0.172	0.118	0.331	0.353	0.147
		NaHCO3	0.067	0.171	0.151	0.365	0.324	0.215
		Urea + NaHCO3	0.095	0.221	0.192	0.384	0.278	0.214
		Mean	0.084	0.188	0.154	0.36	0.318	0.192
	Added every days	Urea	0.059	0.201	0.169	0.333	0.318	0.268
		NaHCO3	0.056	0.199	0.133	0.422	0.29	0.251
		Urea + NaHCO3	0.108	0.229	0.201	0.496	0.302	0.28
		Mean	0.074	0.21	0.168	0.417	0.303	0.266
	Tanpa Nutrien		0.028	0.153	0.092	0.274	0.197	0.19
Fresh Water + Nutrien			0.085	0.159	0.118	0.249	0.22	0.197
Saline Water + Nutrien			0.087	0.165	0.128	0.278	0.242	0.206

Note : N: *Nannochloropsis*; S: *Spirulina*; C: *Chlorella*

Based on that table, *Spirulina* platensis microalgae exhibited the greatest growth, particularly in POME waste, as compared to *Nannochloropsis* and *Chlorella*. This is evident from the fact that the value of the greatest growth rate, OD, constantly grows with time.

This is because S. platensis is a microalgae capable of growing under a variety of environmental circumstances. Despite the fact that these cyanobacteria are photoautotrophic microorganisms, they are also capable of flourishing as mixotrophs and heterotrophs (Aiba & Ogawa, 1977; Marquez, 1993). In the latter two growth conditions, microalgae use sugar as a carbon source and protein hydrolyzate as a carbon and nitrogen source (Marquez., 1995). (Singh, 1995). POME waste contains a considerable amount of the nutrients required for microalgae. Carbon is the primary nutrient required for *Spirulina*, and POME waste contains a substantial amount of carbon (Vonshak, 1997).

Chlorella microalgae showed a greater growth rate than *Nannochloropsis* microalgae with the addition of the same quantity of $NaHCO_3$ feed. This is because *Chlorella* has a higher CO_2 demand tolerance than *Nannochloropsis* (Maeda, 1995). As with *Nannochloropsis*, excessive CO2 availability will impede its growth (Chiu, 2009).

3.6. Effect of POME Waste on Microalgae Nutrient Needs

According to the findings of the experiment, POME waste may minimize the nutritional needs of microalgae. POME waste supplies the nutritional components required for microalgae of sufficient size, namely C:N; P = 47: 7: 1. (Yusoff and Chan, 1997). Microalgae need huge quantities of nutrients such as carbon, nitrogen, and phosphorus, with a mass ratio of C:N; P = 56:9:1 (Kim-Chong and Siew-Moi, 1988). The calculation is as follows:

$$\text{Nutrient C} : \frac{47}{56}\ x\ 100\ \% = 83.9\ \%$$

$$\text{Nutrient N} : \frac{7}{9}\ x\ 100\ \% = 77.7\ \%$$

The calculation above demonstrates that the nutrient needs of microalgae that live in POME waste can be reduced by a high enough nutrient content in POME waste, specifically for nutrient C of 62.5 % and for nutrient N of 83.9 %, which helps supply the nutrient needs of microalgae so that POME can be used as a medium for microalgae growth more efficiently.

4. Conclusion

The microalgae calibration curve demonstrates that the OD is directly related to the cell number and biomass. The growth rate of microalgae in POME medium was more than that of microalgae in salty water, but it was greater than that of microalgae in fresh water. The growth rate of microalgae was greater when nutrients were added every two days than when nutrients were added at the beginning; however, the growth rate of microalgae was greater when nutrients were added at the beginning than when nutrients were not added. Compared to the microalgae *Nannochloropsis* and *Chlorella*, *Spirulina* microalgae saw the greatest growth. The presence of a high enough nutritional content in POME waste may minimize the nutrient requirements of microalgae, namely 83.9% for nutrient C and 77.7% for nutrient N. Further study is required to optimize the optimal circumstances for microalgae development in POME waste, specifically with a lower POME dilution and protein content tests, so that its commercial use may be realized.

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