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Research Article

Cultivation of Microalgae *Spirulina platensis* in Palm Oil Mill Effluent (POME) Media with Variations of POME Concentration and Nutrient Composition

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Abstract: Indonesia and Malaysia are recognized as the world's leading producers of palm oil. Along with the growth of the palm oil industry in Indonesia, the amount of Palm Oil Mill Effluent has increased. Palm Oil Mill Effluent (POME) is a liquid byproduct of the palm oil production process. POME has been treated using aerobic and anaerobic ponds to lower Chemical Oxygen Demand (COD) and Biochemical Oxygen Demand (BOD) levels, but it still includes nutrients like as C,N,P that are beneficial to the development of microalgae. On this study, *Spirulina platensis* was grown in POME medium with 20%, 40%, and 60% V. concentrations. Every two days, the nutrients Urea, $NaHCO_3$, and TSP were administered. Seven days of aeration and 24-hour lights are used throughout the cultivation phase. The results indicate that POME with a 20 % concentration is the optimal medium for plant growth. Add 25 ppm Urea, 50 ppm TSP, and 200 ppm $NaHCO_3$ for the optimal nutritional composition. At the same treatment on various medium, the highest growth rate of *Spirulina platensis* is determined to be $\mu = 0.128\%$ per day, with an optical density of 0.648. Carbon reductions range from 83.03 % to 84.10 %, while Nitrogen savings range from 78 % to 79.55 % when POME is used as a growing medium. This study also shown that the C, N, and P concentrations of POME fall by 93 to 98 %, 99 to 99.5 %, and 92 to 97 %, respectively.

Keywords: POME, microalgae, nutrient, Spirulina

1. Introduction

Crude Palm Oil, or what is often referred to as palm oil, is one of Indonesia's primary agricultural exports that generates foreign currency. Indonesia and Malaysia are well-known as major producers of palm oil (CPO). Indonesia and Malaysia generate over 80% of the palm oil circulating on the global market (Isroi, 2010). Additionally, the rise of the palm oil sector has detrimental environmental consequences (Puteh, 2007). Palm Oil Mill Effluent (POME) is a liquid byproduct of palm oil production. POME is often exclusively treated using aerobic and anaerobic pond techniques to minimize COD and BOD levels, despite the fact that POME still includes nutrients such as nitrogen, phosphorus, and potassium that are essential for microalgae development.

This research will evaluate the viability of POME as a microalgae culture medium. The microalgae used in this study is *Spirulina platensis*, which will be grown at different concentrations on POME medium. This research will determine the optimal concentration of POME for microalgal development, the maximum growth rate, the percentage of nutrients that can be conserved by utilizing POME as a culture medium, and the decreasing levels of carbon, nitrogen, and phosphorus in POME media after microalgal *Spirulina platensis* cultivation.

2. Material and Method

2.1. Material

POME acquired from PTPN VII Lampung, microalgae *Spirulina platensis* obtained from the Center for the Development of Brackish Water Cultivation (BBPBAP) Jepara, urea, $NaHCO_3$, and TSP were utilized in this study. The research was conducted in the Bioprocess Laboratory of the Department of Chemical Engineering at Diponegoro University, while the analysis of the reduction in levels of Carbon, Nitrogen, and Phosphorus was conducted in the Environmental Engineering Laboratory and the Analytical Chemistry Laboratory.

2.2. Microalgae Cultivation and Analysis

Spirulina platensis was cultivated in batches utilizing a 1000 ml Erlenmeyer as a reactor, aeration and illumination for 24 hours using an aerator and a TL lamp as a light source, and the addition of Urea, TSP, and $NaHCO_3$ every two days during the culture period. POME was employed with concentrations of 20%, 40%, and 60% as growing medium, and the remainder was *S. platensis* on a 1000 ml basis with varying nutrient additions: Urea 25 or 50 ppm, TSP 25 or 50 ppm, and $NaHCO_3$ 200 or 400 ppm. Experimental set-up can be shown in Figure 1.

The optimal growth rate for *S. platensis* was established using a spectrophotometer with a wavelength of 680 nm to measure optical density. The density of *S. platensis* (cells/ml) was estimated using a hemocytometer at each specified optical density. After seven days of culture, *S. platensis* was harvested using filter paper and a vacuum pump to get its dry weight. Therefore, a calibration curve between optical density and biomass weight might be generated.

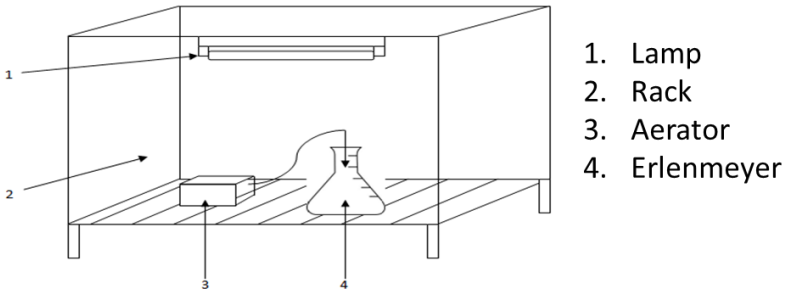

Figure 1. Experimental set-up of microalgae cultivation

3. Result and Discussion

3.1. Spirulina platensis microalgae calibration curve

To calculate the number of cells and weight of the biomass based on the observed Optical Density, a calibration curve demonstrating the relationship between Optical Density and the number of cells and weight of the biomass of *Spirulina platensis* is required. The equation y = 202277.29 x - 84.84 is derived from Figure 2a so that the number of cells in the daily Optical Density measurement may be determined. In a previous study, it was reported that the number of *Spirulina platensis* cells cultivated in Schlosser synthetic media reached 44.73 95 x 103 cells/ml on the 14th day of cultivation (Suantika & Hendrawandi, 2009); however, in this study, the number of *Spirulina platensis* cells was 13 x 104 cells/ml on the 7th day of cultivation; thus, it can be In Figure 2b, the equation y = 0.95 x - 0.03 is derived such that the daily biomass weight on Optical Density may be determined. The mass of the biomass is directly related to the number of *Spirulina platensis* cells, therefore the greater the number of cells generated, the greater the mass of the biomass produced (Hadiyanto et al., 2010).

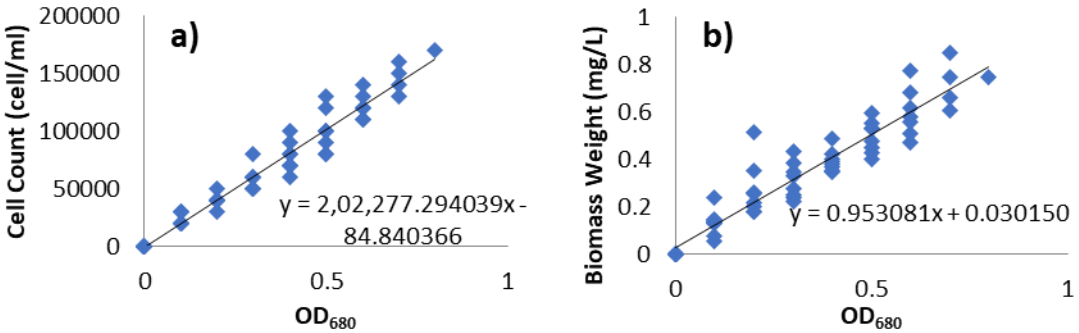

Figure 2. Correlation between optical density versus a) cell count and b) biomass weight

Figures 3a and 3b demonstrate that in the same treatment, *Spirulina platensis* grown on 20% POME media had the maximum growth in terms of cell number and biomass. This was because the nutritional content of this medium was optimal for algal development (Ahsan et al. al.,2008). On POME 40% and POME 60% medium, the proliferation of *S. platensis* cells was somewhat decreased. This is owing to the fact that the nutritional content of the medium exceeds the nutrient needs. In earlier research, it was reported that microalgal development was restricted in medium with too much nutrients or minerals because the microalgae need more time to adjust (Suminto & Hirayama, 1996).

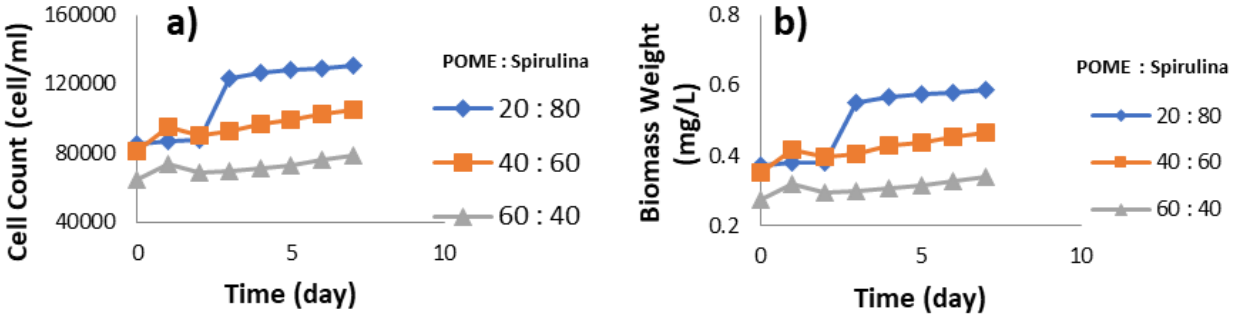

Figure 3. Microalgae growth during 7 days of culture based on a) cell count and b) biomass weight

3.2. The Effect of Nutrient Addition on Spirulina platensis Growth

Spirulina platensis needs the nutrients C, H, O, N, P, and K for photosynthesis. In stoichiometry, the following equation (1) represents the nutritional needs for photosynthesis (Moi et al.,1988):

$$122\ CO_2 + 16\ NH_4 + PO_3^{3-} + 58\ H_2O \rightarrow C_{122}H_{179}O_{44}N_{16}P + 131\ O_2 + H^+ \quad (1)$$

While nutrients are added every two days, this is because the nutritional needs of microalgae are relatively high: 56.3 % carbon, 8.6 % nitrogen, and 1.2 % phosphorus on a weight basis. Ahsan et al. (2008) noted that POME has a CNP ratio (weight basis) of 99.12:4.7:1.0; if the POME 20% has a CNP ratio of 46.5:7.16:1, then extra nutrients are required to obtain the desired CNP. The ratio comparison is derived from the requirements for carbon, nitrogen, and phosphorus, thus to determine how many nutrients have been met by POME, Table 1 presents a comparison of POME concentrations.

Table 1. Nutrient Content in POME

Parameter (mg/l)	20%	40%	60%
C	1592.06	3214.91	4819.51
N	245.1	481.56	717.76
P	34.2	68.4	102.2
CNP ratio	46.5:7.16:1	47.0:7.04:1	47.1:7.02:1

From the comparison of C:N:P on POME and the stoichiometry of nutrient requirements in microalgae, calculations were performed so that the results of adding nutrients to supply the deficiency of nutrients required by microalgae in POME media were Urea (25 or 50 ppm), TSP (25 or 50 ppm), and $NaHCO_3$ (25 or 50 ppm) (200 or 400 ppm). Figure 4 presents the 7-day growth rate for microalgal growth in POME medium, which may be used to determine the optimal nutrient ratio for microalgal development.

Figure 4 shows that the ratio of POME: *Spirulina platensis* = 20:80 has the greatest average growth rate, particularly in the fifth experimental sample, which reached = 0.128/day with 25 ppm Urea, 50 ppm TSP, and 200 ppm $NaHCO_3$. This is in conformity with the nutritional needs of microalgae, which consist of a ratio of C:N:P = 56:9:1, with carbon being the most critical nutrient for microalgal development (Costa et al., 2002). While the ratio of POME:*Spirulina platensis* = 40:60 has comparable growth to the ratio of POME:*Spirulina platensis* = 60:40, the ratio of POME:*Spirulina platensis* = 60:40 has the lowest growth rate value, especially in the sixth experimental sample, which only reached = 0.013 with 25 ppm urea, 50 ppm TSP, and 400 ppm $NaHCO_3$. *Spirulina platensis* requires nutrients for photosynthesis; if the demands are satisfied, the growth will be maximized; however, if the supply of nutrients is supplied in excess, it will precipitate into poison because it is not absorbed adequately, resulting in a reduction in growth (Mun et al., 1989).

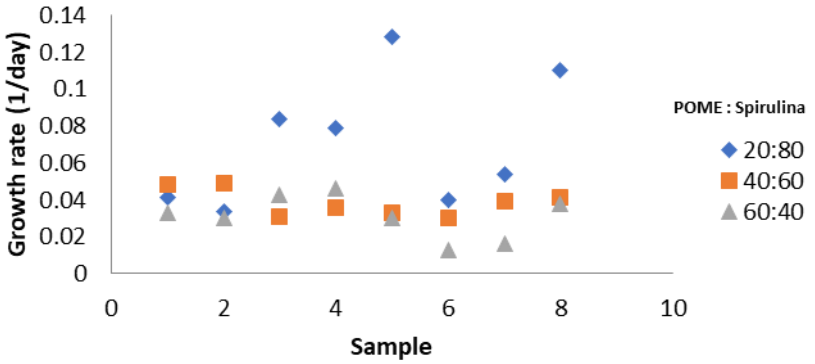

Figure 4. Growth rate on the growth of *S. platensis* in POME media

3.3. Growth Rate (μ) of Spirulina platensis

In the discussion on the growth rate (μ), we compared 6 experimental points: 3 with the greatest Optical Density findings on each culture medium and 3 with the identical treatment on each culture medium. Table 2 reveals that the growth rate of microalgae *Spirulina platensis* on a culture medium containing 20 % POME is the greatest compared to 40 % POME and 60 % POME cultivation media. These phenomena may occur if the medium utilized by *Spirulina platensis* for photosynthesis, consisting of 40 % POME and 60 % POME of light, is inhibited by media that is too dark. The absence of incoming light intensity hindered the development of *Spirulina platensis* cells on culture medium containing 40% and 60% POME (Phang & Kim-Ong 1988). This lack of light intensity also causes *Spirulina platensis* to take longer to reach its stationary phase, therefore the growth rate of *Spirulina platensis* is greater in 20 % culture medium that is more transparent (Anton et al., 1988). As a point of comparison, we compiled the growth rate statistics provided in the Table 2 below from past research.

Table 2. Growth rate of *Spirulina platensis* on each cultivation medium

Equal Treatment on Every Media						Highest OD on Any Media				
Media	**Nutrien (ppm)**			**μ**	**OD**	**Nutrien (ppm)**			**μ**	**OD**
	Urea	**TSP**	**$NaHCO_3$**			**Urea**	**TSP**	**$NaHCO_3$**		
20 % POME	25	50	200	0.128	0.648	50	50	400	0.110	0.810
40% POME	25	50	200	0.033	0.521	50	25	400	0.050	0.550
60% POME	25	50	200	0.030	0.389	50	25	400	0.046	0.421

Table 3. Comparison of Growth Rate (μ) values of *Spirulina platensis* cultivated in different media

Source	Media	Reactor	Cultivation Time (day)	μ
Costa et al, 2002	Fresh water	Open Raceway Pond	15	0.157
Dianursanti et al, 2007	Cowny medium	Photobioreactor	5	0.139
Goksan et al, 2006	Zarrouk medium	Open Raceway Pond	10	0.200
This research	POME	Erlenmeyer	7	0.128

In this investigation, the greatest growth rate observed in a sample of 5 POME culture medium with a 20 % concentration was 0.128% per day. Compared to the findings reported in Table 3, the growth rate of *Spirulina platensis* grown on POME medium was much lower. This occurs because the medium, POME, is composed of trash that still contains toxins, therefore microalgae need more time to adapt. In addition, the cultivation time in this study was limited

to 7 days, whereas the obtained results (appendix 1) indicated that *Spirulina platensis* was still in the exponential growth phase; therefore, if the cultivation time was extended, *Spirulina platensis* was still capable of reaching the stationary growth phase.

3.4. Potential of POME waste as Microalgae Nutrient Source

Based on the findings of the experiments, it is known that POME waste includes C,N,P, which may be utilized as a substrate for microalgae development while lowering the demand for externally provided nutrients for photosynthesis. POME at a certain concentration has a CNP ratio (Ahsan et al., 2008), while microalgae need huge quantities of nutrients, with a CNP ratio of 56:9:1 (Phang & Kim-Ong, 1988). So that the following outcomes may be achieved:

Table 4. Percentage of available nutrients that may be reduced

	POME 20%	**POME 40%**	**POME 60%**
CNP ratio	46.5 : 7.16 : 1	47.0:7.04:1	47.1:7.02:1
Nutrien C	83.03 %	83.92 %	84.10 %
Nutrien N	79.55 %	78.22 %	78 %

POME may be utilized as a growth medium for microalgae, particularly *Spirulina platensis*, since, in addition to decreasing direct waste disposal, it can save nutrient supply for microalgal demands (Table 4).

3.5. Evaluation of Final CNP Levels on POME Media

After 7 days of usage in the culture of *Spirulina platensis*, the final CNP analysis was conducted to assess the residual Carbon, Nitrogen, and Phosphorus content in POME medium. The percentage decrease in CNP levels may be calculated using the initial CNP levels in POME medium and the findings of the end CNP levels analysis:

Table 5. The percentage reduction in CNP levels after cultivation

Ratio	**Sample Name**	**C (%)**	**N (%)**	**P (%)**
POME 20%	Sample 5	93.94	99.28	93.45
	Sample 8	93.94	99.43	92.57
POME 40%	Sample 5	96.12	99.07	96.44
	Sample 4	97.43	99.26	97.54
POME 60%	Sample 5	98.79	99.35	93.13
	Sample 4	98.50	99.51	97.15

According to Table 5, the 60 % POME ratio resulted in the greatest carbon reduction, while the 20 % POME ratio resulted in the least carbon reduction. The reduction in carbon content reflects the utilization of carbon for photosynthesis, as a source of nutrient for *Spirulina platensis* throughout the culture period in POME medium, which influences its development. In this case, the 20 % POME ratio decreases by 91.66 % when compared to sample 5 and the initial carbon concentration of 1592.06 mg/L, based on the Growth rate at 20 % POME ratio demonstrating the greatest growth, as *Spirulina platensis* will experience good growth if its nutritional needs are met and in accordance with its needs.

At a POME ratio of 60 %, the presence of excessive carbon consumption by *Spirulina platensis* is indicated, which may lead to the formation of a toxin that inhibits the development of *Spirulina platensis*. Although carbon is one of the most important components that impacts the development and composition of microalgae biomass, if it is utilized in excess, it will hinder the growth of microalgae (Costa et al., 2002). Table 5 further demonstrates that the 60 % POME ratio results in the greatest decrease of nitrogen from the starting concentration of 481.56 mg/L in sample 5 to 99.35 %. This suggests that in addition to carbon, *Spirulina platensis* needs nitrogen for growth, with a 20 % POME ratio and a 40 % POME ratio requiring the most nitrogen during culture on POME medium. It was stated that any addition of urea below a concentration of 1.5 g/L was the best source as an additional supply of nutrients for microalgal needs (Richmond, 1990). The decrease in Phosphorus in the three POME ratios based on the sample with the highest Optical density in each variable indicates that sample 4 with a 40% POME ratio requires more phosphorus to achieve the highest Optical density of 0.55 than the sample with a 60% POME ratio at an Opt. While achieving the greatest optical density of 0.81

at a 20 % POME ratio has the least loss in phosphorus compared to 40 % POME and 60 % POME ratios, this has no effect on the development of *Spirulina platensis* since a 20 % POME ratio has the highest growth rate. 40 % or 60% POME ratio.

4. Conclusion

Based on the research conducted, it was determined that a 20 % concentration of POME was the optimal culture medium for *Spirulina platensis*. Furthermore, nutrients were added as a supply of microalgae needs for the growth of *Spirulina platensis* during the cultivation period, so as to obtain the optimal composition of nutrient additions for this study. Urea at a concentration of 25 ppm, TSP at a concentration of 50 ppm, and $NaHCO_3$ at a concentration of 200 ppm. During the culture period, an analysis was conducted to determine the maximum growth rate that *Spirulina platensis* could attain in this research, which was = 0.128/day with an optical density of 0.648 in the same treatment for every growing medium. The use of POME as a culture medium can reduce microalgae nutrient requirements by 83.05 % C and 79.55 % N at a concentration of 20 % POME, 83.92 % C and 78.22 % N at a concentration of 40 % POME, and up to 84.10 % C and 78 % N at a concentration of 60 % POME. The drop in CNP levels in POME medium after culture is achieved by using a POME ratio of 20-60 % with a Carbon reduction of 93% -98.3%, Nitrogen reduction of 99.9% -99.9%, and Phosphorus reduction of 92.7% -97.7%.

In microalgae production, aeration management is required to prevent an excess of oxygen from entering the growth media. Prior to being utilized as culture medium, POME waste must be screened so that the particulates included in POME do not interfere with the spectrophotometry analysis process. Careful drying of *Spirulina platensis* biomass is also required for more accurate findings, and more research is required for the continuing commercial cultivation of Spirulina platensis.

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