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Evaluation of Growth and Biomass Productivity of Marine Microalga *Nannochloropsis* sp. Cultured in Palm Oil Mill Effluent (POME)

Hadiyanto Hadiyanto*, **Danny Soetrinanto, Silviana Silviana**, **Muhamad Zaini Mahdi**, and **Yasinta Nikita Titisari**

Department of Chemical Engineering, Diponegoro University,
Jl. Prof. Soedarto, SH-Tembalang, Semarang 50275 Indonesia

The objective of this study was to evaluate the growth and productivity of marine algae *Nannochloropsis* sp. cultured in palm oil mill effluent (POME) medium. The POME was varied in concentration of 10%, 30%, and 50% (vol POME/vol water) while the comparison with fresh and saline medium was also investigated. The relative performance of the different concentrations of fresh POME were investigated with respect to their productivity, specific growth rate and biomass production. *Nannochloropsis sp.* cultured in 30% (v/v) fresh POME had significantly (p<0.05) higher growth rate (0.31 ± 0.06) d^{-1} and productivity (0.034 ± 0.01) g · L^{-1} d^{-1}) as compared to fresh medium and other treatments (10% and 50% v/v). These results indicated the potential of microalga *Nannochloropsis* sp. for biomass production and POME nutrients removals. Further research on optimizing biomass productivity and nutrients removal in POME medium should be done prior to its scale up for industrial application.

Key words: biomass, growth rate, marine algae, *Nannochloropsis* sp., POME

INTRODUCTION

In the last decade, Indonesia has produced and exported about 45% world's palm oil. However, this large production has eventually caused detrimental effects on the environmental due to palm oil mill effluent (POME) produced during processing and untreated discharge into water bodies. It was estimated that 5 tonnes of water was required to process the palm fruit bunches (PFB) in order to yield 1 tonnes of crude palm oil (Ahmad et al. 2003). Besides containing high chemical oxygen demand (COD), and biological oxygen demands (BOD), fresh POME also contains organic compounds such as carbohydrate, protein, ammonium (as N sources), and phosphate (Singh et al. 2010, Kumar et al. 2011). These organic matters can be potentially utilized as nutrient sources for photosynthetic plants, especially for microalgae (Markou et al 2011, Hadiyanto et al. 2013).

Microalgae are photosynthetic microorganisms that can be readily cultivated on a large scale for the benefits of humans (e.g., anti-cancer, antioxidant, food supplements). Some important products have been extracted from algae biomass (e.g., lipids, proteins, vitamins, carotenoids, antioxidants, fatty acids). Microalgae are generally grown in high salinity system in which access to light, water, carbon dioxide and inorganic salts are sufficient (Ahmad et al 2003, Danesi et al 2004). The major components of media used for cultivation of microalgae include inorganic nitrogen sources and both macro and micro elements.

The previous studies showed that POME contains a range of important nutrients and has been utilized for the cultivation

*Corresponding author: hadiyanto@live.undip.ac.id

of microalgae *Chlorella* sp. (Hadiyanto & Nur 2014). The application was considered an important way of pre-treating POME before its discharging into the environment. The important finding of this technology was that algae biomass has negative correlation with organic contents in POME. This may be due to nutrients uptake by algae during photosynthetic and metabolism reactions.

Among the marine microalgae, *Nannochloropsis* sp., has been widely used as the most promising sources to replace the eicosapentaenoic acid [EPA, 20:5(n-3)] content in fish oil. The main advantage of *Nannochloropsis* over other unicellular algae is its high content in EPA (Tocher 2010). Due to its advantages, it is worth to compensate the economic value of *Nannochloropsis* sp. cultivation by utilizing alternate sources of nutrients.

Several studies have been undertaken to enhance the biomass and growth rate of microalgae such as optimizing the medium composition, culture conditions, and supplementing organic and inorganic nutrients. Therefore, this study will evaluate the growth and biomass productivity of alga *Nannochloropsis* sp. in POME medium.

MATERIALS AND METHODS

Palm oil mill effluent

Palm oil mill effluent (POME) was collected from National Plantation Company VII Lampung, Indonesia. The POME was pretreated according to the method of Ding et al. (2016) to remove its impurities prior to its use as algae medium. The POME characteristic was depicted in Table 1.

Table 1. Characteristic of POME.

Parameter	Value
pH	8.29
COD (mg · L^{-1})	1 620
Turbidity (NTU)	375
BOD (mg · L^{-1})	809
Total N (mg · L^{-1})	284
NH_4^+-N (mg · L^{-1})	197
PO_4^{2-} (mg · L^{-1})	80

Chemical Oxygen Demand (COD) analysis

The COD was analyzed by using Hach DR 2400 spectrophotometer (Hach Co, Loveland, CO, US). Since the COD is very high (>50 000 mg · L^{-1}), then the sample needs to be diluted to approximately 2,000 mg. L^{-1} before they can be analyzed.

Nitrogen and phosphorous analysis

The analysis of total nitrogen in POME was carried out by the Kjeldahl method as described by Bremner and Mulvaney (1982), while total phosphorus in POME was determined by the perchloric acid digestion method described by Olsen and Sommers (1982).

Nannochloropsis sp. culture

Pure culture of marine algae *Nannochloropsis* sp. was provided by Center of Brackish Water Cultivation Development Jepara and pre-cultivated in a 10 L tanks containing 5 L of following modified medium: fresh water, saline water and P10 (10% v/v POME), P30 (30% v/v POME), P50 (50% v/v) POME. These POME concentrations were prepared by diluting POME in freshwater. Sodium bicarbonate (100 mg · L^{-1}) was added for each medium as carbon source of algae cultivation. The culture of *Nannochloropsis sp.* with 10% volume and OD = 1 was initially inoculated into each respective treatment. The *Nannochloropsis* sp. cultures were maintained for 10 days and agitated at 150 rpm using aquarium aeration pump. The culture density was measured colorimetrically by using spectrophotometer Optima 300 at 625 nm.

Growth measurements

The growth of algae was investigated by measuring its absorbance at 625 nm. About 1 mg · L^{-1} of $FeCl_3$ and 25 µg · L^{-1} of vitamin B12 were added for every trial to enhance micronutrient demand. Light intensity was maintained at 75-100 µmol · m^2 · s^{-1} using fluorescent lamp as light source, pH was adjusted by using NaOH and HCl in the pH range of 6.8-7.2, temperature was maintained at 30° C. Medium was aerated using air pump to mix the medium.

The specific growth rate (µ) was calculated by the following formula (Markou et al. 2012):

$$\mu = \frac{(\ln(\mu_m) - \ln(X_i)}{t} \qquad (1)$$

where: *Xi* = initial biomass concentration (g · L^{-1}), $\underline{X_m}$ = maximum biomass concentration (g · L^{-1}), t = cultivation time between X_i and X_m (d)

Productivity

The productivity of microalgae was predicted using the following equation (Danesi et al. 2011):

$$P = \frac{(X_{\max} - \ln(X_i)}{t} \qquad (2)$$

Where P = productivity (g · L^{-1} d^{-1}), X_i = initial biomass concentration (g · L^{-1}), X_{max} = maximum biomass concentration (g · L^{-1}), t = cultivation time related to the maximum biomass concentration (d)

Statistical analysis

All statistical analysis was done by using Statistica 6.0 software package. The analysis from experimental data and significance differences in the means were calculated by paired t-test with p-value < 0.05 considered as significant.

RESULTS AND DISCUSSION

Growth of *Nannochloropsis* sp.

The culture of *Nannocholoropsis* sp was evaluated for its optical density, number of cell and biomass concentration. A linear correlation has been found between optical density and biomass ($R^2 = 0.979$) as well as number of cell ($R^2 = 0.9292$), respectively. From this correlation, the direct equation between biomass and number of cell can be obtained as follows:

$$\text{Biomass } (g \cdot L^{-1}) = 1.014 \times 10^{-5} \text{ Number of cell } (\text{cell} \cdot mL^{-1}) \quad (3)$$

While the biomass concentration was in the order of 0.28-0.3 $g \cdot L^{-1}$ at $OD_{625} = 1$:

$$\text{Biomass } (g \cdot L^{-1}) = 0.282\ OD_{625} \quad (4)$$

Where OD_{625} is optical density at 625 nm.

These correlations are used for prediction of amount of biomass by measuring optical density in the later discussion.

Evaluation of *Nannochloropsis* sp. growth in various POME concentration

Figure 1 shows the growth profile of *Nannochloropsis* sp. in three culture mediums with different dilutions of POME (10%, 30%, 50% v/v). High turbidity (>375 NTU) in the POME content led to poor light penetration into the culture. Moreover, there was also tannin acid that gave the POME dark colour and inhibited the light penetration.

Nannochloropsis sp. cultivated in 10% and 30% POME could survive with highest optical density of 0.23 and 0.42, respectively. This value is equivalent to the biomass concentration of 0.065 $g \cdot L^{-1}$ and 0.091 $g \cdot L^{-1}$ for 10% and 30% POME, respectively. In contrast, at 50% POME, the growth of algae showed slight increase of up to OD = 0.15 (equivalent to 0.04 $g \cdot L^{-1}$) after 4 days of cultivation, and decrease afterwards. This was probably due to high turbidity (350-911 NTU for POME concentration of 10-50%), which may have reduced the light intensity, caused by high content of tannin in the culture. In this case, *Nannochloropsis* sp. might use heterotrophic regime for its photosynthetic metabolism, where light is determinant factor in the algae growth.

As shown in Table 2, *Nannochlropsis* sp. cultivated in the media with POME concentration of 10%, 30%, and 50% (v/v) showed different growth rate constant and productivity. Moreover, the lag time of growth curve was also varied that might be due to the nutrient content in each medium (Figure 1). At lowest concentration (10% v/v), the amount of required nutrients for photosynthetic reaction (N and P) was low, therefore the algae need longer lag time before they reach exponential stage. On the other hand, at higher concentration, the required amount of nutrients for photosynthetis was sufficient thus resulting to shorter lag time. The higher POME concentration led to darker color of the POME solution, which reduced the light intensity penetrating the medium. Ding et al. (2016) suggested that lower POME concentration will have sufficient nutrient for algae to grow and enhance the penetration of the light into medium. These factors will simultaneously enhance the growth until the nutrients in POME were fully utilized and the light penetration decreased due to the density of the biomass produced. *Nannochloropsis* sp. grown in the 50% (v/v) showed a lower growth rate constant as compared to 30% POME concentration. This was due to high turbidity content in the medium that reduced the penetration of the light and therefore inhibited biomass production.

Table 2. Growth rate and productivity of *Nannochloropsis* sp in different medium.

Medium	μ ($1 \cdot d^{-1}$)*)	OD max	Productivity*) ($g \cdot L^{-1} \cdot d^{-1}$)
Fresh Water	0.17±0.08	0.22	0.018±0.0025
POME (10 % v / v)	0.16±0.05	0.23	0.026±0.0036
POME (30 % v / v)	0.31±0.07	0.33	0.034±0.0075
POME (50 % v / v)	0.11±0.08	0.14	0.014±0.0083
Saline Water	0.38±0.04	0.42	0.048±0.0035

Note: means ± SD

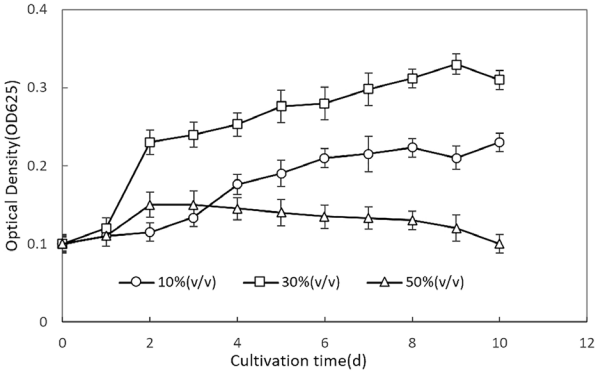

Figure 1. The growth of microalgae *Nannochloropsis* in three different POME concentration.

Evaluation of *Nannochloropsis* sp. growth in different media

The growth of *Nannochloropsis* sp. has been evaluated in three different media: saline water (salinity: 10-12 mg · L^{-1}), fresh water, and POME (Fig. 2). The growth of *Nannochloropsis* sp. in saline medium was the highest compared to POME and fresh water (Table 2). The origin of this species was from saline medium, and therefore both productivity and growth rate constant were the highest.

Nannochloropsis sp. cultured in 30% POME medium showed a better growth rate than in fresh water medium. In this case, the growth of algae was promoted by the availability of nutrient in the POME. In order to complete the photosynthetic reaction, the algae require mass ratio of C:N:P = 56:9:1 (Kim-Chong & Siew-Moi 1988), whereas the POME has already C:N:P ratio of 47:7:1 (Yusoff & Chan 1997). The nutrient was limiting factor for this cultivation, while the light penetration was kept constant for three media.

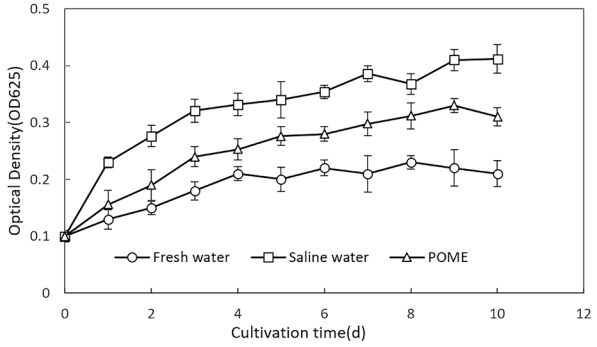

Figure 2. The growth of microalgae *Nannochloropsis* sp. in three different medium: fresh water, saline water and 30% (v/v) POME.

Nutrient removal

Table 3 shows that COD removal efficiency ranged from 27.2% to 34.7% among the different POME dilutions (10-50% v/v). This indicated that *Nannochloropsis* sp. has consumed COD in POME as organic carbon sources in POME. Ding et al. (2016) suggested that acetate is the main carbon sources in POME due to biological reaction during methanogenesis reaction. It means that *Nannochloropsis* sp. was grown in mixotrophic regime. Furthermore, Ding et al. (2016) also suggested that acetate was formed from an incomplete reaction of methanogenesis during anaerobic digestion.

Table 3 also shows that the COD removal efficiencies in 50% (v/v) POME media were higher than in the 10% and 30% (v/v) POME media, which indicates that *Nannochlorpsis* sp. adopted a mixotrophic strategy that requires less light intensity. The growth rate of microalgae in the higher POME concentration was almost constant, which also indicates that the reduction of COD in the POME was due to organic compound released through CO_2 bubbles to the surface (Ji et al. 2015; Abreu et al. 2012).

The most essential nutrient for microalgae photosynthetis is nitrogen. The nitrogen sources consist of NH_3^+-N and this compound is the most preferable for microalgae growth (Ding et al. 2016). Furthermore, Table 3 depicts that at lower POME concentration, the efficiency of total nitrogen was higher than they were in higher POME concentration. This indicates that microalgae growth requires less energy and high light intensity, thus consuming nitrogen sources rapidly (Cai et al. 2013). At higher POME concentration, microalgae was in compensatory growth as it was inhibited due to slow photosynthetic reaction and probably inhibited by toxicity of ammonium (>40 ppm) (Yuan et al. 2011). Another crucial nutrient for microalgae photosynthetic is phosphorous. In this experiment, the removal efficiencies of phosphorous ranged from 29.3% to 85.9% in the

Table 3. Nutrients removal in different dilutions of POME by *Nannochloropsis* sp.

Parameter		10 % (v/v) *)	30 % (v/v) *)	50 % (v/v) *)
COD	Initial (mg · L^{-1})	165.5 ± 12.1	478.6 ± 23.4	810.8 ± 42.2
	Final (mg · L^{-1})	120.5 ± 22.6	324.5 ± 32.6	529.1 ± 18.3
	% Removal	27.2	32.2	34.7
Total N	Initial (mg · L^{-1})	26.5 ± 2.4	86.2 ± 14.6	151.7 ± 21.7
	Final (mg · L^{-1})	2.1 ± 0.2	34.2 ± 1.5	100.5 ± 10.9
	% Removal	92.1	60.3	33.7
Total P	Initial (mg · L^{-1})	7.8 ± 1.6	18.3 ± 2.8	34.5 ± 4.2
	Final (mg · L^{-1})	1.1 ± 0.7	7.6 ± 1.3	18.1 ± 2.3
	% Removal	85.9	58.5	29.3

Note: means ± SD

POME media with concentration of 10-50% (v/v). The efficiency of removal of this compound in all media was lower compared with nitrogen removals. This implies that nitrogen was not the limiting nutrient for microalgae metabolism and photosynthetic and therefore phosphorus is to be the limiting nutrient for the algae growth in POME medium (Garcia et al 2005).

CONCLUSIONS

This study has been carried out to evaluate the feasibility of POME as medium of microalgae growth. The result revealed that POME has potential for use as medium for microalgae *Nannochloropsis* sp. with significant saving in treatment costs. The growth rate of microalgae *Nannochloropsis* sp. was significantly higher in 30% (v/v) POME although was still lower than the growth in the saline environment. The increase of POME concentration in medium lead to lower growth rate due to nutrient inhibitory effects and less light intensities.

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