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Cultivation of *Chlorella sp.* as Biofuel Sources in Palm Oil Mill Effluent (POME)

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ABSTRACT: Renewable energy is essential and vital aspect for development in Indonesia especially less oil reserve for coming 15 years. Biodiesel has received much attention as renewable energy in recent years. One of potential biodiesel is produced from microalgae. Due to high content of nutrients in Palm Oil Mill Effluent (POME), this waste is a potential for nutrient growth for microalgae. *Chlorella* is one of high potential for biodiesel since it has high lipid content (20-30%). The objective of the research is to determine growth rate and biomass productivity in *Chlorella* Sp cultured in POME. Chlorella Sp was cultured in 20%, 50%, 70% POME using urea concentration 0.1gr/L (low nitrogen source) and 1gr/l (high nitrogen source) at flask disk, pH 6.8-7.2; aerated using aquarium pump and fluorescence lamp 3000-6000 lux as light. Medium was measured using spectrophotometer Optima Sp-300 OD at 680 wave length in 15 days to calculate specific growth rate. At end of cultivation, *Chlorella* sp was filtered and measured as dry weight. Result indicated that *Chlorella* sp at 50% POME 1gr/L urea showed higher specific growth rate (0.066/day). Factor affecting growth rate of microalgae is CNP ratio, POME concentration, and urea concentration.

Keywords: biofuel, *chlorella sp*, growth rate, microalgae, POME

1. Introduction

Indonesia is a largest producer of coconut palm in the world. In 2008, Indonesia produced 44% coconut palm of shared demand from around the world **[1]** and from 2005 to 2008 the production rose up to 8.88%. Moreover, the production is still increasing as predicted from 2010 to 2014; it will grow in about 5.22% per annum (Table 1).

Table 1
Commodities of Indonesia agriculture 2010-2014 **[2]**

Commodities	Year			Growth /annum
	2011	2012	2013	
Coconut Palm	24.429	25.046	27.046	5.22%
Rubber	2.711	2.741	2.771	1.10%
Coconut	3.290	3.317	3.348	0.86%

About 1 ton fresh fruit bunch (FFB) can be converted to 0.2 ton CPO, while 0.66 ton will be released as palm oil mill effluent (POME). This enormous amount of POME produced due to high amount of water used. Almost of POME in Indonesia is treated by using open anaerobic pond to reduce COD and BOD content. The raw POME from palm mill has high COD 50000 mg/L, BOD 25000 mg/L, while the pond is only able to reduce up to 1400 mg/L and 700 mg/L for COD and BOD, respectively.

The characteristic of POME before and after treated using anaerobic pond is listed in Table 2. From the composition, it is noted that POME still high content of nutrient such as Nitrogen and Phosphor. This nutrient is highly potential for nutrient of microalgae growth especially to support the photosynthetic reaction to produce biomass. The biomass which depends on composition can be converted to other valuable product such as lipid for biofuel, protein for feed supplement or carbohydrate for bioethanol.

The need of renewable energy in Indonesia cannot be avoided since within 15 years, the oil reserve will be diminished. Therefore, renewable energy is really essential and vital aspect for Indonesia. One of the

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potential renewable energy resources is microalgae. The productivity of microalgae as compared to terrestrial plant is higher **[3]** while the lipid concentration is varied between 30-60%. Therefore, the objective of this paper is to utilize POME as growth medium of microalgae especially *Chlorella sp*.

Table 2
POME before and after digestion

Parameter*	POME	POME digested
pH	3.91-4.9	4-6
COD	83356	1400
TSS	49233.57	700
Total N	1494.66	456
NH_3-N	50.42	34.2
PO_4-P	315.36	68.4

*all in ppm except pH **[4][5]**

2. Experimental Method

2.1. Cultivation Medium

Medium for cultivation is POME collected from PTPN VII Lampung. To be used as medium, POME was pretreated to reduce its BOD contents. The additional nutrient was added such as urea, TSP and NPK to enhance the requirement of N,P and K for photosynthetic.

2.2. Culture Chlorella sp

Chlorella sp was collected from BBPAP Jepara and cultivated in modified medium 40ppm urea, 30ppm TSP, 10ppm ZA, 1ppm $FeCl_3$ and 25 µg/l of vitamin B12. The medium was cultivated for control variable. Glucose (200ppm) are also added as carbon source as comparison variable.

2.3. Cultivation condition

Chlorella was cultivated in different POME concentration (20%, 50%, and 70%) and different low-high urea concentration (0.1gr/l, and 1gr/l). Light intensity was maintained in 3000 lux, pH 6.8-7.2, 28^{0}C temperature, and aerated using aquarium air pump to mix the medium in 1L glass flask disk.

2.4. Measurement

The concentration of biomass was measured using spectrophotometer Optima sp-300, 680nm wavelength for 15 days in every day. The optical density was plotted in biomass to make regression between optical density and biomass. Specific growth rate was calculated using equation from exponential growth (Eq 1).

$$\mu = \frac{\ln(x_t) - \ln(x_0)}{t - t_0} \quad (1)$$

Biomass was harvested in the end of cultivation using control pH 11 **[4]**. Biomass was dried at 55^{0}C tray dryer for 2 hours and the productivity (X) (mg/l/d) was calculated using Biomass produced divided by cultivation time.

3. Results and Discussion

3.1. Biomass vs Optical density

Relationship between DW (dry weight) and OD (optical density) in this research was described in Fig. 1.

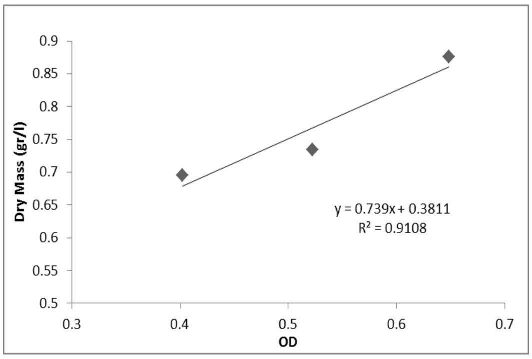


Fig. 1 Correlation between OD and Dry Mass *Chlorella* sp cultivated in POME at 680nm

The result was described in linear equation y=0.739x + 0.3811 (R^2=0.910). Another correlation between OD and DW also described from literature **[5]** y = 1.5343x, (R^2= 0.977) 680nm wavelength.

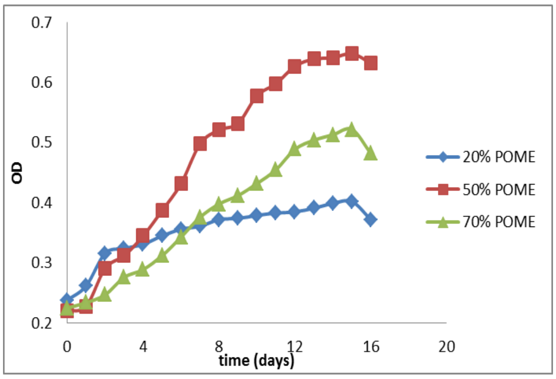
Table 3
Specific growth rate (day $^{-1}$) *Chlorella* sp in different POME and urea concentration

Urea	POME concentration		
	20%	50%	70%
0.1gr/l	0.036	0.057	0.058
1gr/l	0.020	0.066	0.059

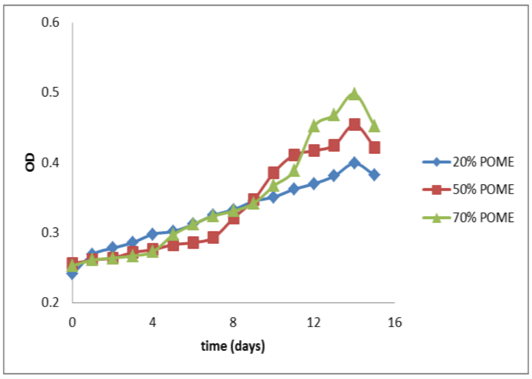
Table 4
Biomass productivity (mg/l/d) in different concentration of POME and urea

Urea	POME concentration		
	20%	50%	70%
0.1gr/l	37.8	38.2	42
1gr/l	46.3	58.4	48.93

Specific growth rate was determined from calculation in exponential growth rate. The result indicates that 50% POME and 1gr/l urea has highest specific growth rate (µ) than other variables. Biomass productivity (mg/l/d) was calculated and the result is tabulated in Table 4.



(a)



(b)

Fig. 2 Growth phase *Chlorella* sp at 1gr/l (a) and 0.1 gr/L (b) urea in different POME concentration

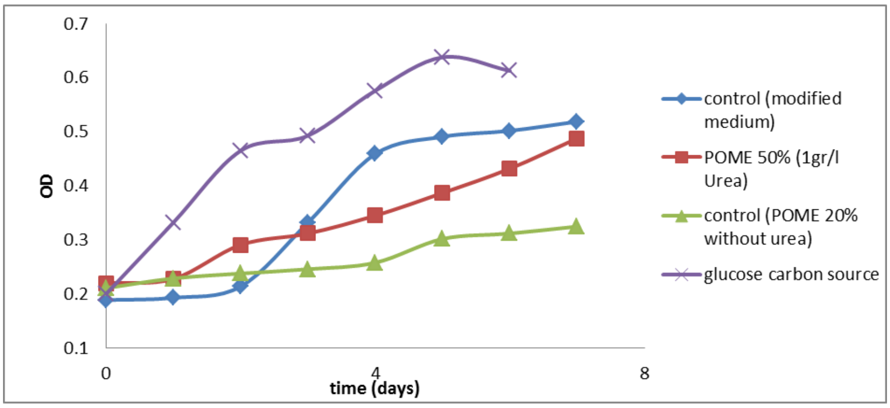


Fig. 3 Comparison growth phase Chlorella sp in different medium

The result shows that *Chlorella* sp in this experiment has lower specific growth rate but has higher biomass productivity as compared to literature **[6]** (0.084/day & 5.9mg/l/day), respectively. A theoretical of carbon, nitrogen and phosphor in POME was calculated from Habib, et al **[7, 8]** for 20%, 50%, and 70% concentration.

Table 5
Concentration carbon, nitrogen, and phosphate in POME in different concentration

	POME		
	20%	**50%**	**70%**
Carbon(ppm)	1591	4017	5622.4
Nitrogen (ppm)	245	600	837
Phosphor(ppm)	34.2	85.3	119.1

High specific growth rate was found in 50% POME 1gr/l urea, followed by 70% POME 1gr/l urea are shown in Table 3. The CNP ratio in medium limits microalgae growth rate **[9]** with recommended ratio of 56:9:1. According to equation of POME after adding urea, CNP ratio has changed (Table 6).

For 70% POME and 1gr/l additional urea showed that it is closest to recommended CNP ratio according to Edwards et al. **[9]** i.e 56:9:1 of weight ratio. However, high specific growth rate was found in 50% POME 1gr/l.

Table 6
CNP ratio in POME after additional urea

Urea	**CNP ratio POME**		
	20%	**50%**	**70%**
0.1gr/l	46.5:8:8.5:1	47.09:7.5:1	47.2:7.4:1
1gr/l	46.5:20.6:1	47.09:12 :1	47.2:10:1

Habib et al **[8]** informed that higher POME concentration influences dark brown color in medium which may from tannic acid **[10]**. Tannic acid can inhibit shading in light intensity and photosynthetic reaction. As compared to 50% POME (1gr/l urea), it has higher nitrogen ratio, but Chlorella still grow in higher specific growth rate. Lower tannic acid in 50% POME influence growth of microalgae, although nitrogen source is high but Chlorella still can tolerance to it. However *Chlorella* sp has lower specific growth rate in POME 20% although the medium has lower tannic acid and has more light intensity. This lower growth rate is caused by high nitrogen content. The nitrogen can be a toxic if it cannot be utilized to form biomass.

Putri, et al. **[6]** investigated several microalgae growth in diluted POME (250mg/l COD) and found that *Chlorella sorokiniana* has higher specific growth rate According to this research, higher urea concentration also influences in specific growth rate of *Chlorella* sp. Urea influences in Chlorella `s growth **[11]** and the concentration also influences in biomass production **[12]**,**[13]**. Wijanarko **[14]**, in his research also studied influences urea as nitrogen source and conclude that urea has potential nutrient for Chlorella than nitrate. It also can increase growth rate and biomass productivity. The biomass forming *Chlorella vulgaris* based the research is:

$$CH_{3.3}N_{0.203}O_{0.322}P_{0.041} + 1.11\ H_2O + HCO^{3-} + 0.041\ H2PO^{4-} + 0.203\ NO^{3-} \rightarrow 2CH_{3.3}N_{0.203}O_{0.322}P_{0.041} + 2.03\ O2$$

Wijanarko **[14]** also reported that high urea concentration that exceed in medium could inhibit growth rate caused by toxicity.

According to several researches, *Chlorella* sp cultivated in wastewater has different lipid content. Agwa et al., **[15]** informed that the lipid content obtained at the end of an artificial illumination with aeration recorded the highest lipid content (11.19%) from poultry waste; the others were as follows: grass cutter waste 9.71%; cow dung waste 6.60%; pig waste 3.38% and goat waste 2.60%, respectively. Meanwhile in Putri et al, **[6]**, lipid content was highest obtained from *Botryococcus studeticus* 30.83%. Several factor limits lipid forming in biomass. Cheirsilp & Torpee **[16]** investigated the enhancement of growth and lipid productivity of several microalgae in different condition, autotrophic, mixotrophic, and heterotrophic.

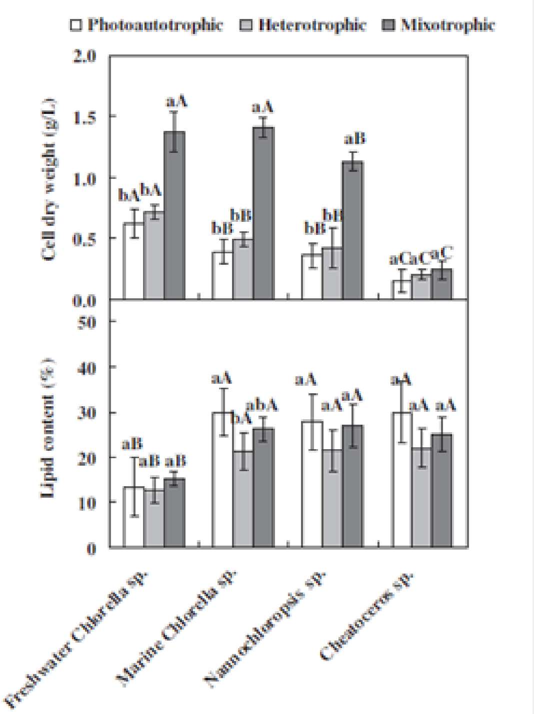


Fig. 4 Lipid content and dry weight in different cultivation condition microalgae **[16]**

The result indicates that most of microalgae have high lipid productivity in mixotrophic condition. The results are also shown in Fig. 3 which carbon source from glucose give high growth rate. Meanwhile Widjaja et al. **[17]** reported that nitrogen starvation can increasing lipid content in *Chlorella vulgaris* but it also may lower biomass productivity. Another factor such drying temperature, lipid extraction, and CO_2 supply in medium also affect lipid productivity. Wijanarko **[14]** also reported that different nitrogen source may affect in lipid productivity. Nitrate source is good for lipid content in *Chlorella vulgaris*, but compared to urea, although it can lower lipid content, urea has influence biomass productivity than nitrate.

4. Conclusion

Cultivation of *Chlorella sp* was done in different POME concentration and different additional urea concentration. Highest specific growth rate and biomass productivity was recorded at 50% POME and 1gr/l urea. Factor affecting growth rate of microalgae is CNP ratio, POME concentration, and urea concentration.

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