PROCEEDING

BOOK I

bio energy chemicals materials

BioEnChe 2013

International Seminar on Chemical Engineering
In conjunction with
Seminar Teknik Kimia Soehadi Reksowardojo (STKSR)2013

"Biorenewable Resources Utilization for Energy, Chemicals, and Materials"

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PREFACE

This BioEnChe-STKSR2013 proceeding contains of the collections of research works presented in International Conference of Bio Energy Chemicals and Materials 2013 (BioEnChe 2013) that was held in Institut Teknologi Bandung. The conference is in conjunction with annual Chemical Engineering Seminar of Soehadi Reksowardjojo (Seminar Teknik Kimia Soehadi Reksowardojo, STKSR 2013).

The international conference provides an opportunity to publicize research works which done or in ongoing ones in many research institution. As the use of fossil base energy and other derivate become harmful for human life, the uses of renewable resource become more interest in our daily life. Therefore, the science and technology for utilization of those resources become enhanced to get more effective and efficient process to produce their products.

We have expectation in this occasion is not only a good place to exchange and discuss the progress of their research in bioenergy, biochemical and biomaterials, but also a venue to collect and to disseminate the most updated technologies and the researches of regional issue and public interest in order to contribute to the community and to draw support from the industrial and the governmental sectors. As this conference has main theme of biorenewable resources utilization for bioenergy, biochemical and material, hopefully this conference will contribute to enhance the utilization of renewable resources for many uses.

We would like to grateful to all participants and sponsors who has contributed to the conference, to the organizing committee for their commitment in their busy days so that the conference is possible to be held and conducted successfully.

Thank you,

Dr. Tirto Prakoso, MEng.
Conference Chairman

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PROGRAM

No.	Time	Program						
Thursday, 10 October 2013								
1	07.30-08.30	Registration						
2	08.30-09.00	Opening						
3	09.00-09.45	Keynote Speech: Prof. Dr. Gunther Kolb - *Institut fur Mikrotechnik Mainz, Germany*						
4	09.45-10.00	Coffee Break						
5	10.00-10.45	Keynote Speech: Dr. Hyung Keun Song - *Korea Institute of Science and Technology (KIST), Republic of Korea*						
6	10.45-11.30	Keynote Speech: Prof. Dr. Tatang Hernas - *Chemical Engineering, Institut Teknologi Bandung (ITB), Indonesia*						
	11.30-11.40	Sponsor Company Profile Presentation: PT. Pupuk Indonesia						
7	11.40-13.00	Lunch Break (ISHOMA) + Poster Flash Presentation (10)						
8	13.00-14.30	Parallel Session I						
	22 paper	No	Room I	Room II	Room III	Room IV	Room V	
	3 invited	1	**Invited Speaker: Dr. Angelo Moreno**	**Invited Speaker: Prof. Dr. Shigeyuki Uemiya**	**Invited Speaker: Prof. Norikazu Nishiyama**	**BE.01**	**BD.01**	
		2				**BE.02**	**BD.02**	
		3	**FC.01**	**BH.01**	**OT.07**	**BE.03**	**BD.03**	
		4	**FC.02**	**BH.02**	**OT.16**	**BE.04**	**BD.04**	
		5	**FC.03**	**BH.03**	**OT.18**	**BE.05**	**BD.05**	
		6	**FC.04**		**OT.21**		**BD.06**	
9	14.30-14.45	Coffee Break						
10	14.45-15.45	Parallel Session II						
	20 paper	No	Room I	Room II	Room III	Room IV	Room V	
		1	**FC.05**	**BL.01**	**BM.01**	**BR.01**	**BD.07**	
		2	**FC.06**	**BL.02**	**BM.02**	**BR.02**	**BD.08**	
		3	**OT.14**	**BL.03**	**BM.03**	**BR.03**	**BD.09**	
		4	**OT.15**	**BL.04**	**BM.04**	**BR.04**	**BD.10**	
11	15.45-16.15	Break						
12	16.15-17.15	Parallel Session III						
	19 paper	No	Room I	Room II	Room III	Room IV	Room V	
		1	**BG.01**	**BHC.01**	**BM.05**	**BR.05**	**BD.11**	
		2	**BG.02**	**BHC.02**	**BM.06**	**BR.06**	**BD.12**	
		3	**BG.03**	**BHC.03**	**BM.07**	**BR.07**	**BD.13**	
		4	**BMG.01**		**BM.08**	**BR.08**	**BD.14**	
13	17.15-19.00	Break						
14	19.00-21.00	Gala Dinner						

No.	Time	Program					
Friday, 11 October 2013							
1	07.30-08.30	Registration					
2	08.30-09.15	Keynote Speech: Prof. Dr. Kunio Yoshikawa - *Tokyo Institute of Technology (TIT), Japan*					
3	09.15-10.00	Keynote Speech: Prof. Dr. Mohammad Nazri bin Mohd Ja'afar - *Faculty of Mechanical Engineering, Universiti Teknologi Malaysia (UTM), Malaysia*					
4	10.00-10.15	Coffee Break					
5	10.15-11.15 20 paper	Parallel Session IV					
		No	Room I	Room II	Room III	Room IV	Room V
		1	**BEL.01**	**BPS.01**	**BM.09**	**BD.23**	**BD.15**
		2	**BMG.02**	**BPS.02**	**BM.10**	**BL.05**	**BD.16**
		3	**BMG.03**	**BPS.03**	**BM.11**	**BPS.05**	**BD.17**
		4	**BMG.04**	**BPS.04**	**BM.12**	**BMG.05**	**BD.18**
6	11.15-13.00	Lunch Break (ISHOMA) + Poster Flash Presentation					
7	13.00-14.00 20 paper	Parallel Session V					
		No	Room I	Room II	Room III	Room IV	Room V
		1	**OT.01**	**OT.05**	**OT.10**	**OT.17**	**BD.19**
		2	**OT.02**	**OT.06**	**OT.11**	**OT.19**	**BD.20**
		3	**OT.03**	**OT.08**	**OT.12**	**OT.20**	**BD.21**
		4	**OT.04**	**OT.09**	**OT.13**	**OT.22**	**BD.22**
8	14.00-14.30	Awards + Closing					
9	14.30-end	Cultural Visit: Saung Angklung Udjo					

bio energy chemicals materials
BioEnChe 2013

October 10-11, Bandung, Indonesia

BIODIESEL
BD.19

International Seminar on Biorenewable Resources Utilization for Energy and Chemicals 2013
In conjunction with Chemical Engineering Seminar of Soehadi Reksowardojo 2013

Utilization of Palm Oil Mill Effluent for *Chlorella vulgaris* Cultivation Medium under Mixotrophic Condition as Feedstock of Biofuel

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Abstract. Indonesia is largest palm oil producer in the world. Increasing of palm oil influence palm oil mill effluent (POME) production. Several researcher reported that POME is a potential medium for microalgae to grow. Microalgae cultivated in mixotrophic condition has received attention as according to higher biomass and lipid productivity to provide biofuel feedstock. The aim of this research is to study growth of *Chlorella vulgaris* cultured in POME medium using different organic carbon source under mixotrophic condition. Carbon source (glucose, glycerol, and acetic acid) was added in 0-1.2gr/l concentration in 40% and 100% POME. Biomass was harvested using autoflocculation method, and dry biomass was extracted using ultrasound method to obtain lipid content. Our result revealed that *C. vulgaris* could grow on mixotrophic condition in POME medium and produce higher biomass and lipid content rather than autotrophic condition. Concentration of organic source also influenced in growth rate and biomass production. This provides a promising process to utilize POME and produce biomass as feedstock of biofuel from microalgae.

Keywords: *biofuel feedstock; Chlorella vulgaris; Mixotrophic cultivation; POME.*

1 Introduction

Indonesia is the largest producer of coconut palm in the world. In 2008, Indonesia produced 44% coconut palm of shared demand from around the world and from 2005 to 2008 the production rose up to 8.88% [1]. 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 0.66 ton as palm oil mill effluent [3]. Almost of POME in Indonesia is treated by using open anaerobic pond to reduce COD and BOD content.

One of potential source of biofuel is microalgae [4]. Several kind of microalgae can produce high biomass and accumulate high lipid in rapid time, and grow in non-arable land, even in wastewater.

Digested POME contains rich nutrient such as nitrogen and phosphorus that highly potential fertilizer for microalgae growth [5,6,7]. This medium could decrease fertilizer cost for microalgae cultivation, since cost of nitrogen and phosphorus fertilizer have almost doubled every year [8]. However, POME has high turbidity and dark color. This could be potential to inhibit light intensity and form mixotrophic condition.

Several microalgae can grow well in autotrophic condition, while the others grow in heterotrophic condition. Autotrophic condition is occured when microalgae utilize inorganic carbon source and light in photosynthetic reaction. In heterotrophic condition, microalgae utilize organic carbon source as energy source to form biomass, without light energy. However a mixing of these condition is called mixotrophic, when microalgae utilize organic and inorganic carbon source and light as energy to grow in a complex reaction [9,10]. Microalgae cultivated under mixotrophic condition utilize lower energy but accumulate higher biomass productivity [9,10,11].

Heredia-Arroyo et al. [12] cultivated *C. vulgaris* under mixotrophic condition using different glucose, glycerol, acetate, and mixture of those organic carbon, and revealed that lipid productivity was high by adding glucose and glycerol. But it still utilize synthetic fertilizer to grow. The purposes ot this research is to investigate growth, biomass and lipid accumulation of *Chlorella vulgaris* in Palm Oil Mill Effluent (POME) under mixotrophic condition.

2 Experimental Method

2.1 Cultivation Medium

Medium for cultivation is POME collected from PTPN VII 4th aerobic lagoon before released to environment. POME was filtered by using filter cloth to reduce total suspended solid. Medium was cooled for temperature 28^0C. POME in this research contained COD 1620 ppm and N total 284 ppm.

2.2 Culture *Chlorella vulgaris*

Chlorella vulgaris was purchased from BBPAP Jepara and cultivated in modified medium [13] consist of 40ppm urea, 20ppm TSP, 10ppm ZA, 1ppm $FeCl_3$ and 25 µg/l vitamin B12. Medium was used as control variable in autotrophic condition.

2.3 Cultivation Condition

Chlorella vulgaris was cultivated in different carbon source: D-glucose, crude glycerol, and $NaHCO_3$ with concentration (0,200,400,600,800,1000,1200 g/l). $NaHCO_3$ is used as sole inorganic carbon source to compare with other organic carbon source. In experiment, microalgae 10% v/v (0,7 OD_{680}) and 40%v/v POME wastewater was mixed and diluted using distilled water in 1L glass flask. Medium was conditioned in 3000 lux intensity, pH 6.8-7.2, 26-28^0C temperature, 2ppt salinity, and aerated using aquarium air pump to mix the medium.

2.4 Measurement

The concentration of biomass was measured using spectrophotometer Optima sp-300 at 680nm wavelength for 6 days. The optical density was plotted in dry biomass to make regression between optical density (y) and dry biomass (gr/l) (x).

Specific growth rate (μ) was calculated using equation from Putri et al. [6] in logaritmic growth phase (Eq 1).

$$\mu = \frac{\ln(OD_1) - \ln(OD_0)}{t - t_0} \quad (1)$$

OD_1 is optical density at last day of cultivation, OD_0 is optical density at first day of cultivation, t is end time of cultivation (day), and t_0 is begininng time of cultivation (day).

2.5 Harvestment

Medium was harvested using flocculation method. NaOH 0.5 M was used to increase the pH until reach 10.5, then the microalgal suspension was intensively mixed (1000 rpm) for 10 min followed by gentler mixing (250 rpm) for an additional 20 min. Subsequently, the suspension was settled for 30 min [14]. Biomass was dried at 55^0C tray dryer for 2 hours and the total biomass product was weighed as (X).

2.6 Lipid Extraction

Lipid extraction was applied using ultrasound extraction method [15]. Dry Biomass was extracted using n-hexane (1:10 gr/ml). Mix of dry biomass and n-hexane was placed in ultrasound assisted Branson 2510-DTH 40 kHz for 30 minutes and temperature 35^0C. N-hexane containing lipid was separated using whatman filter paper. Solvent was removed by distillation method until no more solvent collected. Dry residue was then extracted again for three replication. Total Lipid was weighed (W_2). Lipid content was calculated as equation 2.

$$L=\frac{W_2}{W_1}.100\% \quad (2)$$

L is lipid content (%), W_1 is total biomas product in dry basis (mg/l), W_2 is total lipid content (mg/l). Lipid productivity (Y,mg/l/day) was calculated using equation 3.

$$Y = \mu.L.W \quad (3)$$

Where Y is lipid productivity in mg/l/day, μ is specific growth rate of cultivation in d^{-1}, L is lipid content (%), and W is total dry biomass content (gr/l).

3 Result and Discussion

3.1 Biomass vs Optical Density

Relationship between DW (dry weight) and OD (optical density) in this research was described in Figure 1. The result was plotted in linear equation $y=1.481x + 0.0071$ ($R^2=0.9983$).

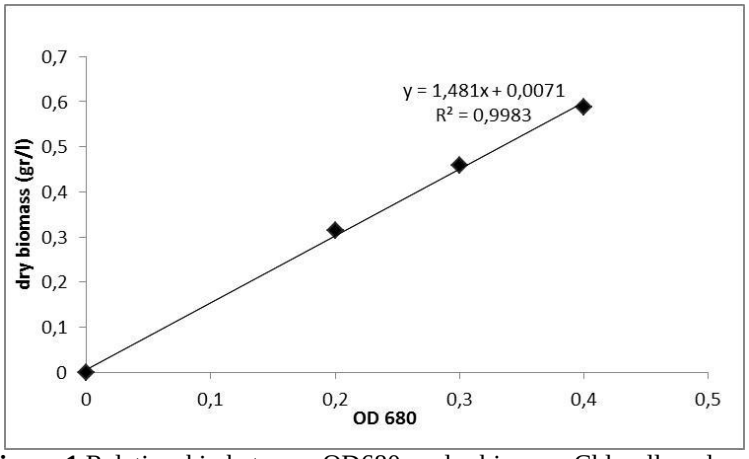


Figure 1 Relationship between OD680 vs dry biomass Chlorella vulgaris

This result was used to simplify measurement of dry biomass in cultivation.

3.2 Growth Rate and Biomass Production

3.2.1 Growth Rate

In D-glucose addition, highest growth rate was recorded from 1000 ppm. In glycerol addition, highest growth rate was recorded from 600 ppm. And by using $NaHCO_3$, highest growth rate was reached in 200 ppm addition. According to kind of carbon source, higher growth rate was obtained from glucose, followed by glycerol, and $NaHCO_3$ (Table 1).

Table 2 Growth rate (d-1) in 40% POME using different carbon source addition

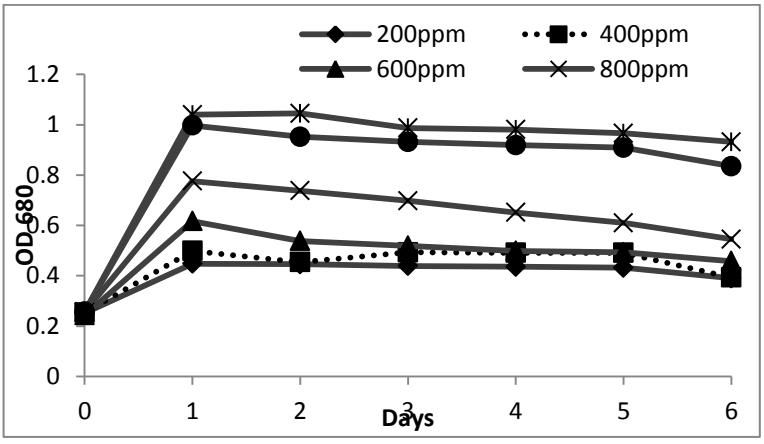
Source	Carbon concentration (ppm)					
	200	400	600	800	1000	1200
D-glucose	0.572	0.679	0.935	1.106	1.406	1.356
Glycerol	0.188	0.251	0.328	0.230	0.181	0.161
$NaHCO_3$	0.317	0.261	0.178	0.146	0.110	0.074

Due to different carbon and nitrogen ratio in medium, growth rate of microalgae was changed. Lack of carbon ratio seems to inhibit microalgae to grow. Moreover, 40% POME contains 648 ppm, it provides 243 ppm carbon (taken from 12/32 COD) and gave 2.15 C/N ratio. However optimum microalgae composition approximately 6.22 C/N ratio. It needs more carbon to grow. When more organic carbon source was added, (i.e. glucose 1000 ppm), it seems that medium meet the optimum required C/N ratio, and influenced growth rate.

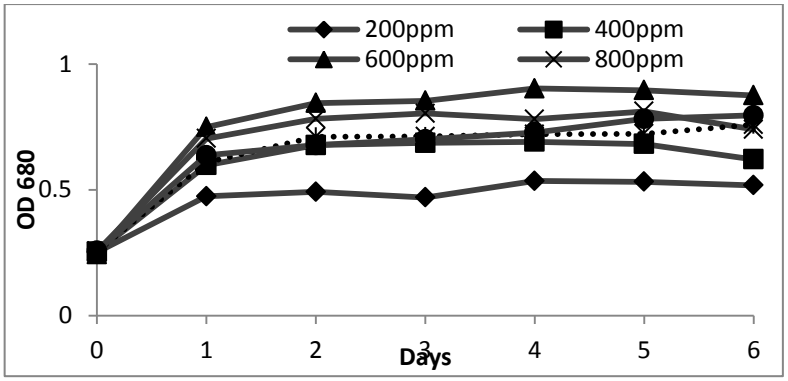
However in $NaHCO_3$ addition, growth rate was lower than other organic source. Microalgae seems not suitable to consume inorganic carbon source in mixotrophic condition. By adding 200 ppm and 400 ppm concentration, growth rate was slightly increased but drop when > 600 ppm was added. It seems that residual organic content in POME (i.e. acetic) was limiting growth of *C.vulgaris* rather than inorganic. When inorganic carbon was added, growth rate became lower due to inhibition factor. In addition, if the condition was changed to autotrophic, control medium, growth rate was recorded up to 0.354/ day, higher than in mixotrophic. This result revealed that inorganic condition is suitable for photosinthetic reaction.

When 1200 ppm of D-glucose was added, growth rate of *C. vulgaris* also decreased. Several researched informed that glucose could inhibit growth of microalgae in high concentration because excess carbon source could become toxic and C/N factor that far from microalgae demand 56/9 ratio [16].
However in glycerol addition, highest growth rate was recorded in 600 ppm. It seems that toxic matter in crude glycerol (i.e. residual methanol, FFA, etc) could inhibit growth of *C. vulgaris*.

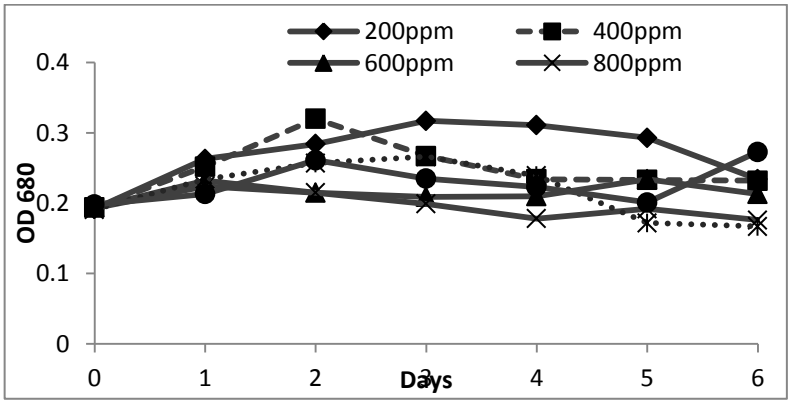
3.2.2 Biomass production



(a)



(b)



(c)

Figure 2 Growth phase of C. vulgaris in 40% POME (a) D-glucose

Microalgae cultivated in D-glucose addition gave high biomass product in day one, and decresase slowly (Figure 2). Highest biomass was produced from 1000 ppm D-glucose addition, in about 1.4 gr/ l dry weight, followed by 1200 ppm. While by using glycerol, highest biomass production was recorded from 600 ppm, in about 0,98 gr/l dry weight at 2nd day and drop in 3rd day. This phenomena revealed that D-glucose is easily to be utilized as organic carbon source rather than glycerol in mixotrophic condition [17].

When we use $NaHCO_3$ as source of carbon in mixotrophic condition, the biomass production is lower in average 0.39 gr/l due to limiation as mentioned in section 3.2.1. However when we use $NaHCO_3$ in autotrophic condition, biomass production reach 0.7 gr/l dry weight in 5th day due to photosynthetic reaction.

3.3 Lipid Content and Lipid Productivity

Highest growth rate from each organic carbon source was extracted. Higher lipid content obtained in D-glucose is higher than glycerol. Medium cointained 1000 ppm D-glucose gave 9,7% lipid content, while medium with 600 ppm glycerol gave 7,2% lipid content.

D-glucose 1000 ppm gave higher carbon content than 600 ppm glycerol. Higher C/N ratio in medium influence lipid content [18]. When nitrogen in medium tend to low, Microalgae tend to accumulate higher lipid due to stress environment. Nitrogen is needed to synthesize protein content in biomass. Lack of ratio of C/N will interupt metabolic reaction of cell, and tend to store energy, forming more lipid.

Table 3 Comparison of lipid productivity result

Medium	Growth rate (d^{-1})	Biomass content (g/L)	Lipid content (%)	Lipid productivity (mg/L/d)	Source
Digested Dairy Manure	0.474	1.7	14	11.3	[19]
MSG Wastewater	1.803	1.6	14	44	[20]
Artificial Wastewater	0.663	1.7	33	40	[21]
2^{nd} Municipal WW	0.458	0.67	31	22.9	[22]
40%POME + D-glu	1.406	1.43	9.7	195.02	This study
40%POME + Gly	0.328	0.98	7.3	23.46	This study

Lipid productivity was also calculated. By using 1000 ppm D-glucose and 600 ppm glycerol, lipid productivity reached 29.33 mg/l/day and 13.48 mg/l/day, respectively.

Several researchers also investigated growth of *Chlorella* under mixotrophic condition (Table 2). Highest lipid productivity was recorded from 40% POME + 1000 ppm D-glucose, followed by MSG wastewater.

Due to high price of D-glucose, it is not preferable to commercial production.
By cultivate microalgae in POME wastewater, nitrogen content also decreased due to biomass forming. According to Phang & Ong [23], a theoretical microalgae needs 9% nitrogen to form dry biomass. When POME medium contains 113,4 mg/l total nitrogen, 1,4 gr/ l *C. vulgaris* aproximately needs about 126 mg/l taken from medium.

4 Conclusion

Research was done by cultivating C.*vulgaris* in 40% POME and use different carbon source. Carbon concentration and kind of carbon source influenced in biomass, growth rate, and lipid accumulation. Addition of 1000 ppm D-glucose in medium gave 195.02 mg/l/day, while by using 600 ppm glycerol gave 23.46 mg/l/day lipid productivity. Mixotrophic condition of microalgae cultivation in POME medium could be alternative method to utilize POME and produce biofuel as renewable energy.

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