Enhancement of Biomass Production from Spirulina sp Cultivated in POME Medium

Hadiyanto, Muhamad Maulana Azimatun Nur, and Ganang Dwi Hartanto

Center of Biomass and Renewable Energy
Department of Chemical Engineering Diponegoro University,
Jl.Prof Soedarto Tembalang Semarang. Phone: (024) 7460058
***E-mail** : h.hadiyanto@undip.ac.id*

Abstract :

Indonesia is the largest producer coconut palm oil in the world. As increasing in production, the palm oil mill efluent are also produced in about 66% by FFB (Fresh Fruit Bunch). Palm oil mill effluent is usually processed by a traditional aerobic open lagoon or by anaerobic digestion. POMED (palm oil mill effluent digested) has a high nutrient content and it can be used as medium for microalgae cultivation. Spirulina sp is a kind of cyanobacteria contains high protein and a potential product for animal feed. This research purpose is to find optimum saving synthetic nutrient of Spirulina sp cultivated in POMED and to find optimum POMED concentration used for algae cultivation. Research was done in two steps. First step, Spirulina sp was cultivated in 20% POMED concentration with different saving nutrient (0%, 20%, 50%, 70%, and 90%). At second step, Spirulina sp was cultivated in different POMED concentration (10%, 20%, 30%, 40%, 50%, 60% v/v) for 16 days. At first 8 days, nutrient was added to medium. At second 8 days, the nutrient was not added to medium.. Optical density was monitored every day using spectrophotometer with wave length 680nm. At the end of cultivation, medium was filtered to obtain wet biomass (10% Total suspended solid). Spirulina sp can grow well in 20% POMED, save 50% from synthetic nutrient, and produced 5.93gr/l wet biomass for 9 days. Research was continued without adding nutrient and produced optimum biomass 9.8gr/l in 40% POMED for 13 days.

Keywords: optimum biomassa, POMED, saving synthetic nutrient, Spirulina sp

1. Introduction

Indonesia is the largest producer coconut palm in the world. In 2008, Indonesia produced 44% coconut palm shared demand from around the world **[1]**. At 2005 to 2008, the production obtained up to 8.88%. It is predicted that the production will grow in about 5.22% per annum (about 28.439 thousand tons) at 2010 to 2014. The fresh fruit bunch has a potention to be a palm oil mill effluent converted from FFB 66% along in process of palm oil.

Table 1. Comodities of Indonesia agriculture 2010-2014

Comodities	Year					Growth rate (%)/annum
	2010	2011	2012	2013	2014	
Coconut Palm	23.200	24.429	25.046	27.046	28.439	5.22
Rubber	2.681	2711	2741	2771	2801	1.10
Coconut	3.266	3290	3.317	3.348	3.380	0.86

Source: deptan, 2009 **[2]**

Almost of waste water coconut palm oil industries in Indonesia is processed in traditional open lagoon aerobic to decrease COD and BOD content. POME (Palm mill oil effluent) has organic matter, nitrogen, phosphorus, and minerals **[3, 4]**. This waste water can be used as source of microalgae medium after treated using bacteria activitiy in aerobic or anaerobic process **[5]**.

The research in POMED (Palm Mill Oil Effluent Digested) as medium of Chlorella vulgaris was done by Habib et al **[5]** for Zoo plankton Moina micrura at 10% concentration of POMED and the product contains high PUFA, EAA and essential mineral. Another research, Mayangsari **[6]**, was reported that S. platensis will need more time to obtain optimum growth if higher POME concentration is used as medium cultviation. Optimum growth S. platensis was obtained in 50% POME concentration and needs 134 days. The research was not purposed to obtain high biomass and save synthetic nutrient subtitued with POME.

Research of POMED as medium subtitution for synthetic nutrient is still low. Last research, Permatasari **[7]**, reported that S. platensis can grow in 90% POME and use 10% synthetic nutrient in photobioreactor and produces 0.267gr/l dry biomass in two weeks. The biomass is still low. In another related research, S. platensis was cultivated in soybean waste and obtain 0.9gr/l dry biomass in 5% concentration by modified CNP ratio of synthetic medium **[8]**. This research is potential if applied in POME, but there is problem in using low waste concentration. This research is purposed to find optimum saving synthetic nutrient as subtituent of POMED medium and to obtain optimum biomass using different POMED.

Table 2. Characteristic POME and POMED (*All in ppm except pH **[4,5]**

Parameter*	POME	POMED
pH	3.91-4.9	4-6
COD	83356	21227.5
TSS	49233.57	4798.5
Total N	1494.66	456
NH_3^-N	50.42	34.2
PO_4^-P	315.36	68.4
C:N:P Ratio	99.12: 4.74:1.0	116.37: 6.67:1.0

2. Material and Methods

2.1. POMED Waste

POMED waste was collected from PTPN 7 Lampung in 4th open pond lagoon. The waste then filtered to separate impurities.

2.2. Spirulina sp

Spirulina sp was collected from BPPT Jepara and cultivated in Bioprocess laboratory UNDIP. Algae was acclimated in 20% POME medium for 14 days. Spirulina sp is used as inocumum at 0.5 OD_{680}.

2.3. Growth Condition

Control synthetic nutrient was modified from Bangladesh synthetic nutrient No.3 **[9]** : 1gr/l NaHCO¬3, 50 ppm Urea, 10 ppm TSP and 50mcg/l B12 Vitamin. Spirulina sp was cultivated in 1L flask disk and agitated using aquarium water pump aeration. pH was adjusted in 9-10.5. Source of light was from flourescent lamp 4000-6000 lux intensity.

2.4. First Experiment

First experiment is purposed to cultivate Spirulina sp using 20% POMED at different saving synthetic nutrient (0%, 20%, 50%, 70%, 90%). The cultivation was done in 7 days.

2.5. Second Experiment

Second experiment is purposed to cultivate Spirulina sp in different POMED concentration (10%, 20%, 30%, 40%, 50%, 60%) and using optimum nutrient obtained from first experiment. The research was done in 16 days. At first 8 days, nutrient was added to medium. At second 8 days, the nutrient was not added to medium.

2.6. Measurement

Measurement was started from 0 day using spectrophotometer SP-300 wave length 680nm. Medium was measured in every day. Biomass was collected in second experiment, from first 8th days and 16th days by using filter cloth. Wet Biomass was recorded as 90% moisture content (10% Total Suspended Solid). Carbon, Nitrogen, and Phosphor was measured by Benfield and Randal method **[10]**

3. Result and Discussion.

3.1. Saving Syntheric Nutrient

At first experiment, Spirulina sp was cultivated in 20% POMED using different nutrient composition. Control medium is medium I, using fresh water and without reduce synthetic nutrient. The medium is measured by using optical density OD_{680} and obtained optimum growth rate from IV medium (50% reduction), followed by V medium (70% reduction) III (20% reduction), VI (90% reduction) and II (without reducing nutrien).

Habib et al. **[4]** explained that raw POME contains CNP ratio (weight) 99,12:4,7:1,0. Algae needs CNP ratio to grow 56:9:1 **[3]**. POMED used as 20% medium contains CNP ratio 19.2:1.68:0.2. To reach ideal CNP ratio, medium needs additional nutrient with CNP ratio 36.8:7.32:0.8. The ratio is taken from bicarbonate as source of carbon, urea as source of nitrogen, and triple super phosphate as source of phosporus.

Medium IV used 50% nutrient with 2 day addition as accumulated in one week i.e: 290ppm C, 38ppm N, and 5.2ppm P, respectively. This composition is already reach teoritical nutrition demand in medium. Assumed that air agitation from aquarium pump also contains C and N, so medium will complete to reach ideal CNP.

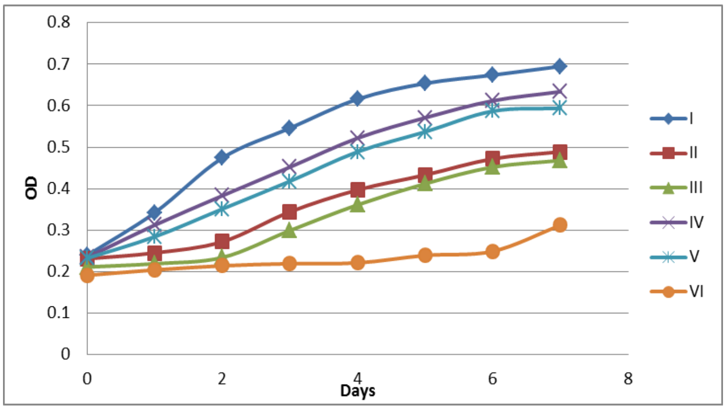


Figure 2. Growth phase Spirulina sp cultivated in 20% POMED under different saving synthetic medium

Next high growth rate is medium V with 70% reduction. This composition is little far from medium demand (36ppm C, 73ppm N, 7.8ppm P), but the growth rate is higher than medium III (30% reduction) with CNP contains in medium 400ppm C, 53ppm N, 72ppm P, and medium II (0% reduction). Along CNP in medium reach ideal condition, algal growth become stable. But exces nutrient in medium (i.e. medium III and II) and lack nutrient (medium VI) will influence growth condition.

Table 3. Result in different nutrition addition

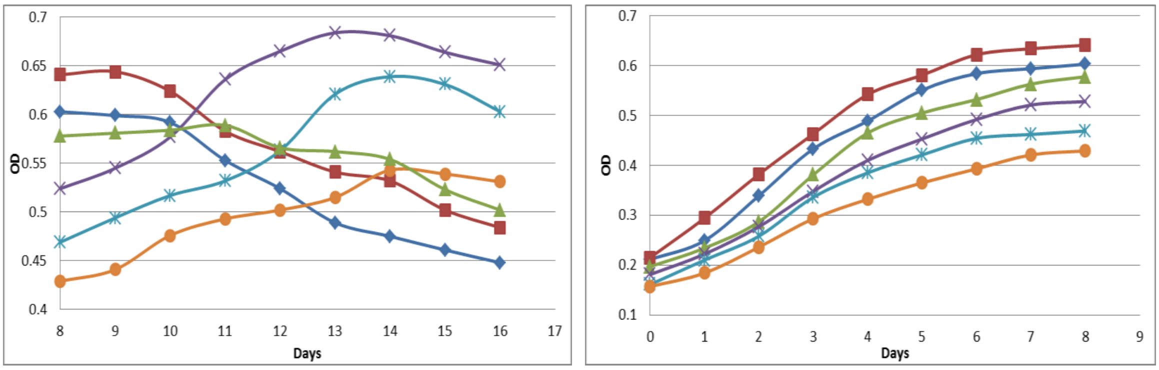
No	Media	Nutrien			Nutrien Reduction	Growth Rate /day
		NaHCO3	Urea	TSP		
I	Fresh water	1.2 gr/l	60ppm	20ppm	0%	0.152
II	POME 20%	1.2gr/l	60ppm	20ppm	0%	0.070
III	POME 20%	0.96 gr/l	40ppm	16ppm	20%	0.114
IV	POME 20%	0.6gr/l	25ppm	10ppm	50%	0.142
V	POME 20%	0.24gr/l	15ppm	6ppm	70%	0.134
VI	POME 20%	0.12gr/l	5ppm	2ppm	90%	0.107

Mun, et al. **[11]** explained that excess nutrient can lowering growth rate because not all nutrient can be absorbed to algae cell and nutrient could be converted to toxic mater. Chilmawati and Suminto **[12]** reported that medium contain excess nutrient or lack of nutrient can influence algal growth. Microalgae tend to need more time in adaptation phase, cells need enzym and substrate concentration to grow. Nutrient is diffused by algae because of different concentration in algae cell and medium. At medium IV, Spirulina sp has as higher concentration nutrient as algae concetration needed so the adaptation time become faster.

3.2. Optimum POMED Concetration

The research was splitted in two steps. Step one (8 days 1^{st}), Spirulina sp was cultivated in different POMED concentration but same synthetic nutrient 50% reduction. (500ppm $NaHCO_3$, 15ppm urea, 5ppm TSP) to obtain optimum biomass. Step two (8 days 2^{nd}), microalgae cultivation was continued without adding synthetic nutrient to determine optimum biomass produced from excess nutrient contained in medium.

At first 8 days measurement, optimum OD_{680} was recorded from medium II, followed by medium III and I. Optimum specific growth rate was recorded from medium II, I, and III. Different OD and specific growth rate occurred in medium I and III. Another medium remained specific growth rate decreased along OD value, respectively.



(a) (b)

Figure 3. Graphic of Spirulina sp growth phase in different POMED concentration. (a)growth phase at first step. (b)growth phase at second step. ◆ =10% POMED, ▰ =20% POMED, Δ=30% POMED, x=40% POMED, ж=50% POMED, ●=60% POMED.

At medium I, total CNP ratio reached 48.2:6.8:1. This ratio almost near CNP ideal algae 56:9:1 **[3]**. But the biomass and growth rate has little lower than medium II (CNP total ratio 31.9:4.6:1). Accordint to CNP total weight (in ppm), medium I has little source than medium II. Based from calculation, assumed that algae produced 7gr/l in 8 days, alge needs 3920 ppm C, 630 ppm N, and 70ppm P. Medium I only contained 823ppm C, 125,9ppm N and 17ppm P, nutrient supply is lower than medium II, although it has better CNP ratio.

Table 4. Result in Second Experiment

No.	Composition	1st step		2nd step		Optimum Growth Time (day)
		Total Biomas (g/l)	Growth rate (/day)	Total biomas (g/l)	growth rate (/day)	
I	10%	5.38	0.1313	4.43	-	8
II	20%	5.953	0.1365	5.28	-	9
III	30%	5.685	0.1305	5.55	-	11
IV	40%	4.697	0.1338	9.8	0.0314	13
V	50%	4.662	0.1336	8.7	0.0271	14
VI	60%	2.826	0.1257	6.83	0.0267	14

Comparing from medium III, medium I has lower biomass but has higher growth rate. It indicates that medium I has better adaptation time from the medium because of CNP ratio has better than medium III. But it has lower biomass because of nutrition supply is lower than medium III. In another point, medium III has more dark color than medium I, ligh intensity can not penetrate well in medium and interupt cultivation. Habib et al. **[4]** reported that POME above 20% interupt in Chlorella vulgaris growth to reach stationary phase.

Dark color in POME could also change medium become heterotrophic or mixotrophic condition. Meanwhile medium with lower dark color (i.e 10% or 20% POME concentration) tend to form mixotrophic condition. Spirulina platensis had been found to utilize organic carbon substrates for heterotrophic and mixotropic growth **[13]**. When microalgae grow in mixotrophic condition, light and organic carbon can be utilized as carbon source, but when microalgae grow in heterotrophic condition, organic carbon only the main of carbon source. Several researcher **[14,15]** also reported that microalgae has higher growth rate under mixotrophic condition (i.e Chlorella minutissima and Chlorella vulgaris) than heterophic and autotrophic condition. Another research, Anton et al. **[16]** also reported that algae can grow in optimum 14% POME, followed by 10%, 20% and 30%.

3.3. Spirulina sp Growth without Synthetic Nutrient

At second 8 days, cultivation continued without adding sythetic nutrition. Optimum biomass was collected from medium IV, with POMED 40% concentration and biomass 9.8gr/l, followed by medium V and VI. Another medium (I,II, and III) tend to reach death phase little faster than medium IV, V, and VI, respectively, based by specific growth rate. Along with higher excess nutrient containing in medium, algae still grow well and it can prolong from entering death phase.

Chilmawati and Suminto **[12]** reported that death phase occures when algae cell reach optimum production, culture can not maintain cell body because lack of nutrient in medium, and slowly lysis or dissapear into medium **[17]**. Biomass from 2nd step experiment has lower weight than biomass 1^{st} step. (see table 4).

Medium IV, V, and VI still have growing activities but slowly decrease from day 13, and 14. Medium IV has optimum biomass because there is balanced with nutrient supply and CNP ratio is better than medium V and VI. According to CNP excess, medium V and VI still much high nutrient in medium but biomass is low. There is could be dark color containing in medium prevent light to enter and lowering growth rate of Spirulina sp. Mayangsari **[6]** also reported that Spirulina platensis cultivated in higher POME concetration also needs more time to reach optimum production. Based by theotrical literature, Spirulina sp at medium V and VI could reach higher biomass and still needs more time to reach optimum production (more than 14th day), but excess of nutrient could be toxic in medium and prevent spirulina to grow.

4. Conclusion

Research was done by cultivating Spirulina sp under different saving synthetic nutrient and different POMED concentration. Spirulina sp can grow well in 20% POMED, save 50% from synthetic nutrient, and produced 5.93gr/l wet biomass for 9 days. Research was continued without adding nutrient and produced optimum biomass 9.8gr/l in 40% POMED for 13 days.

Acknowledgement

This work was supported by Maris Consortium and Bioprocess Laboratory Chemical Engineering Diponegoro Univeristy.

References

[1] Rupani PF, Singh RP, Ibrahim, MH, and Esa N.2010.Review of Current Palm Oil Mill Effluent (POME) Treatment Methods:Vermicomposting as a Sustainable Practice. World Applied Sciences Journal, 11: 70-81.

[2] Deptan. 2009. Rancangan Rencana Strategis Kementrian Pertanian : 2010-2014 www.deptan.go.id

[3] Phang SM, and Ong KC.1988. Algal biomass production in digested palm oil mill effluent. Biol. Wastes, 25: 177–191.

[4] Habib, MAB, Yusoff FM, Phang SM, Kamarudin MS, and Mohamed, S. 1998. Chemical characteristics and essential nutritients of agroindustrial effluents in Malaysia. Asian Fisheries Science, 11(3): 279-286.

[5] Habib MAB, Yusoff, FM, Phang, SM, Kamarudin MS, and Mohamed, S .2003.Growth and Nutritional Values of Moina micrura Fed on Chlorella vulgaris Grown in Digested Palm Oil Mill Effluent. Asian Fisheries Science 16 : 107-119.

[6] Mayangsari, Yunika. 2011. Produksi dan Karakterisasi Fikosianin dan Lipid Mengandung Asam Lemak Tidak Jenuh Majemuk dari Spirulina platensis yang Dibiakkan dalam Limbah Cair Pengolahan Kelapa Sawit. Master Thesis Jurusan Teknologi Pangan dan Hasil Pertanian. UGM Yogyakarta.

[7] Permatasari, Shinta. 2011. Production of Spirulina for Decreasing Pollution Level of Palm Oil Mill effluent in Contionus Photobioreactor. Department of Agricultural Technology. IPB.

[8] Jongkon P, Siripen T, and Richard DL. 2008. The optimum N: P ratio of kitchen wastewater and oil-extracted fermented soybean water for cultivation of Spirulina platensis: pigment content and biomass production. Int. J. Agric. Biol., 10: 437–441.

[9] Khatum R, Hossain MM, Begum SMS, Majid FZ.1994.Spirulina culture in Bangladesh V. Development of simple, inexpensive culture media suitable for rural or domestic level cultivation of Spirulina in Bangladesh. J. Sci. Ind. Res.,29: 163-166.

[10] Benefield, LD, and Randall, CW. 1980. Standard Methods for The Examination of Water and Wastewater. 18th Ed American Public Health Association, New York.

[11] Mun MD, Osborne LL, and Wiley MJ. 1989. Factors influencing periphyton growth in agricultural streams of central Illinois. Hydrobiologia 174:89-97.

[12] Chilmawati, Diana and Suminto. 2008. The Used of Different Culture Medium on the Growth of Chlorella sp. Jurnal Saintek Perikanan, 4(1): 42-49.

[13] Lodi, A., Binagi, L., De-Faveri, D., Carvalho, JCM., Converti, A., and Borghi, MD. Fed-batch mixotrophic cultivation of Arthospira platensis with carbon source pulse feeding. Annals of Microbiology 55(3), 181-185.

[14] Bhatnagar, A., Chinnasamy, S., Singh, M., Das, KC. Renewable biomass production by mixotrophic algae in the presence of various carbon source and wastewater. Applied Energy. Elsevier.

[15] Kong W, Song H, Cao Y, Yang H, Hua S, and Xia, C.2011.The characteristic of biomass production, lipid accumulation, and chlorophyll biosynthesis of Chlorella vulgaris under mixotrophic culvitation. African Journal of Biotechnology Vol.10(55), 11620-11630.

[16] Anton A, Kusnan M, and Hussin, ARM. 1994. Effects of palm oil mills effluent on algae. Proceedings of the Conference on Algal Biotechnology in the Asia-Pacific Region, Algal Biotechnology in the Asia-Pacific Region, Universiti Malaya, Kuala Lumpur, Malaysia:320-323.v Biological Wastes 25: 177-191.

[17] Fogg GE and Thake B. 1987. Algal cultures and phytoplankton ecology. University of Wisconsin Press.