Optimization of medium for microalgae growth based on aeration pond POME of Sime Darby Sua Betong Palm Oil Mill using four variables of CO_2 concentration, agitation rate, supplement concentration and dilution ratio

Nurul Afiqah Binti Mohd Ariffin, Prof Ir. Dr. Jailani Bin Salihon

Faculty of Chemical Engineering, Universiti Teknologi Mara (UiTM)

***Abstract— Chlorela sp.* is the type of microalgae chosen for this experiment. In this paper, effect concentration of carbon dioxide, agitation rate, supplement concentration and dilution ratio has been studied to optimize the growth of microalgae in a medium based on palm oil mill effluent (POME) from aeration pond. The experiment comprised of 16 runs. Each run required 7 days and sample were taken at 12 hours intervals. These expanded run represent the 8 extra point to complement the earlier 2^4 factorial experiment, to make the composite design. From the data obtained, optimization of microalgae was conducted to obtain the maximum growth rate (μ_{max}) and maximum biomass (X_{max}). The predicted maximum specific growth rate is 5.0029 h^{-1} with the optimum conditions 22% (v/v) for carbon dioxide concentration, 0.57vvm for agitation rate, 20.4% (v/v) and 22.06% (v/v) for supplement concentration and dilution rate respectively. The predicted maximum biomass is 283.7g/L with optimum condition for carbon dioxide concentration are 11.7% (v/v) for carbon dioxide concentration, 0.7vvm for agitation rate, 26.05% (v/v) and 56.79% (v/v) for supplement concentration and dilution ratio respectively.**

Keywords— Chlorella sp., microalgae, POME, optimization growth rate.

I. INTRODUCTION

Oil palm tree, the given the scientific name as *Elaeis guineensis* is one type of tropical palm plant which is originated from West Africa. The palm oil tree cultivation has changed from small scale of the crop production based in Africa and being commercialize around the world make it becoming the most profitable agricultural. This happens in duration less than 100 years. Based on history, British first introduced the oil palm trees as a decorative tree in early 1970's. However, the oil palm trees become one of the most important crops due to suitable climate in Malaysia. This also contribute in economic development [1].

Malaysia become one of the most palm oil producers in the world. About 4.5 million hectares of land in Malaysia is under palm cultivation. Malaysia Palm Oil Council (MPOC). Besides the contribution in economic growth, the rapid development of oil palm trees also contribute in environmental pollution due the waste product is produce in process of oil extraction. There are consist of several waste product on palm oil plant such as fibrous material such as empty fruit bunches (EFB), palm kernel shell and palm pressed fibres (PPF) and liquid discharge palm oil mill effluent (POME) [2].

One of the ponding treatment systems is by using aerobic system. Based on this systems, there are many advantages of this treatment of wastewater has been considered over biological treatments. Compare to the anaerobic treatment, aerobic treatment has a better process stability, a high effluent quality and required a smaller reactor sizes. With the presence of oxygen the aerobic biomass aerobic wastewater treatment systems will converts the organics in the wastewater into carbon dioxide (CO_2) and new biomass. Activated sludge and aerated stabilization basins (ASBs) are the most common types of aerated wastewater systems of the pulp and paper industry and also used in some municipalities [2].

Currently, one of the serious issue of environmental damage is CO_2 emission [3]. With this drastically increase result, the concern of serious uncontrolled CO_2 emissions to atmosphere grows, and currently there is a global push to limit the amount of CO_2 emission. One method that can be reduced the CO_2 emission is CO_2 sequestration. From an engineering point of view, CO2 show that it have high potential act as carbon source for microalgae growth as it is utilized during photosynthesis processes. The cultivation of microalgae needs sufficient CO2 for photosynthesis. Some research indicated that utilizing an atmosphere which contains sufficient of CO2 not only helps in growth of algae. It is also regulate the pH value and carbon balance [3].

Microalgae have received attention in research. It can grow in harsh conditions, either marine or wastewater [4]. POME always been observed as a highly polluting wastewater. In order to manage with the issues of palm oil effluent and CO2 emission, POME which is rich with nitrogen source (nutrient) can be used as medium for cultivation of microalgae [5]. However, for the microalgae growth needs an optimum condition for perfect growth. The problem statement for this research is how to bring the nutrient levels back to the levels which are optimum for microalgae growth. There are four main parameters that will be studied in order to reduce the problem for this research are CO2 concentration, agitation rate, supplement concentration and dilution ratio.

II. OBJECTIVE

The main aim of this study was to optimize the level of desired CO2 concentration in POME, the level of agitation rate, the level of supplement and the level of dilution, to maximum algae growth rate. Besides, to model the response based on the optimization experiment.

III. MATERIAL AND METHOD

A. Microalgae species

The type of microalgae used in this experiment is Chlorella sp. and it was grown at a room temperature in 1 L flask by using BBM as the nutrient medium. The microalgae were illuminated with fluorescent bulb at intensity of 10, 000 lux and it was sparged with air at different flowrates and different CO2 concentration [6]. Therefore, the medium is left for 3 to 4 days to ensure that the microalgae are already in exponential phase [7] before proceed cultivate for seven days in each run.

B. Palm Oil Mill Effluent (POME)

The sample of POME were collected from aeration pond from Sime Darby Sua Betong Palm Oil Mill. The supplement sample for the experiment was taken from facultative pond. Both of the samples stored at 4ºC until used.

C. Gas mixing system

Mixing is necessary to prevent the sedimentation of microalgae. In this experiment, the air was supplied by using compressor and the air was filtered by using air filtration to absorb moisture content in the air supply. By using the pressure regulators, carbon dioxide pressure and compressed air was maintained at 2 bar and the flow rates are being set referred to the factorial design. Both gases were mixed before it was sparged into the culture medium. A tube with a very small diameter (6mm) is used to ensure a smaller size of bubbles were produced to enhance mass flow rates and to improve the gas exchange between the culture medium and the air.

D. Lighting Chamber

Same with other plants, microalgae carried out photosynthesis process by breaking down inorganic carbon. Light is one of the source of energy, which initiate the reaction and in this concern, the light intensity need to be considered. The culture was provided with sufficient illumination by growing it inside a lighting chamber which is containing fluorescent bulbs. For this experiment, fluorescent lamp was used at with light intensity of 10,000 lux . The light intensity is maintained constant at a high value. This is because, the higher the values of light intensity, production of biomass is more when compared to a biomass production at low light intensity.

E. Centrifuge of algae

In order to separate the biomass from the culture medium, the samples were centrifuge twice at 10,000 rpm for 5 minutes. Then, the pallet was suspended in an equal volume of distilled water and dried in oven at 110ºC. The samples were dried for 24 hours in crucibles before it can be weighed.

F. Microalgae Cultivation in the Flask

The medium was prepared in a 1L conical flask. For every run, the sample was taken twice with 12 hour of time gap and each sample was taken at 50 mL. The sample was taken for seven days. In order to ensure the microalgae are having an equal mixing, the medium was prepared in duplicate.

Silicone rubber bung was fitted to the flask and the medium was placed inside the lighting chamber at 10, 000 1x. Tube with diameter of 6 mm was used to connect the medium to the gas mixer. The cultivation flask was sparged with a specified concentration of CO2 and specified sparging rate using gas mixing system. Then, the samples then were centrifuged twice at 10 000 rpm for 5 minutes.

A set of 2^4 factorial experiments were obtained from previous studies. This involving four variables which are CO_2 concentration (X1), agitation rate (X2), supplement concentration from facultative pond (X3) and dilution with distilled water (X4) with levels of experimental variable as shown in Table 1 [8]. The results of factorial experiment yield the predicted value of maximum specific growth rate (μ_{max}) and maximum biomass (X_{max}) as stated in Table 2 [8].

Table 1 : The levels of variables in Factorial experiments

Experimental variables	a = -1	a = 0	a = +1	Units
CO_2 (X1)	12	16	20	% (v/v)
Sparging rate (X2)	0.6	0.8	1	vvm
Supplement (X3)	10	20	30	% (v/v)
Dilution rate (X4)	10	20	30	% (v/v)

Table 2 : Results of 2^4 Factorial Experiments

Run	X1	X2	X3	X4	μ_{max} (h^{-1})	X_{max} (g/L)
1	-1	-1	-1	-1	0.059	1.059
2	1	-1	-1	-1	0.053	0.1595
3	-1	1	-1	-1	0.036	0.5575
4	1	1	-1	-1	0.032	0.3799
5	-1	-1	1	-1	0.031	0.9278
6	1	-1	1	-1	0.049	0.0315
7	-1	1	1	-1	0.032	0.5657
8	1	1	1	-1	0.030	0.5242

9	-1	-1	-1	1	0.030	0.4539
10	1	-1	-1	1	0.050	0.0575
11	-1	1	-1	1	0.016	0.5649
12	1	1	-1	1	0.481	0.3143
13	-1	-1	1	1	0.017	0.7083
14	1	-1	1	1	0.052	0.0336
15	-1	1	1	1	0.015	0.5383
16	1	1	1	1	0.440	0.4806

IV. RESULTS AND DISCUSSION

To obtain the result some mathematical analysis has been done in order to interpret the data collected throughout the experiment.

A. Linear regression.

Linear regression is the simplest and commonly used in analysis prediction. Regression estimates are used in data description and explanation of the relationship between one dependent variable and one or more independent variables. The result from Table 2 consisted of growth rate and biomass yields were fitted in Microsoft Excel 2013 to acquire linear regression equation. This method conducted to determine which data of growth rate and biomass have the criterion for the area containing the maximum yield (y_n, y_m), giving equation specific growth rate yield (1) and biomass yield (2).

$$y_m = 0.0349 + 0.0046x_1 - 0.0073\,x_2 - 0.0015\,x_3 - 0.005\,x_4 \qquad (1)$$

$$y_n = 0.439 - 0.179\,x_1 + 0.077\,x_2 + 0.057\,x_3 - 0.038\,x_4 \qquad (2)$$

Based on equation (1), since the coefficient of x_1 to x_5 are small compared to the constant and the error was less than 30% which accepted. Thus, the area investigated is a plateau which may contain the maximum. Besides, refer to the equation (2) the error was exceeding 30% then can be concluded it does not contain the maximum.

B. Logistic model.

Logistic Model is a growth rates mathematical explanation for a modest population in a limited space also with limited resources. Growth rate or logistic curve resulting in parabola form. Logistic model is similar with Monod model but it assume that growth limitations may be caused by other causes besides substrate limitations [9].

$$X = \frac{X_0 X_m e^{\mu m t}}{X_m - X_0 + X_0 e^{\mu m t}} \qquad (3)$$

Where X_0 is intial biomass concentration, X_m is maximum biomass concentration, μ_m is maximum growth rate and time taken, t.

C. Curve fitting.

MATLAB software version r2010a was used to fit the logistic model to the growth curve. The value of experimental yield and time was inserted in the command. In order to plot the graph, logistic equation was fit in the curve fitting tools (cftool). R-square value must be high in order to have better model fits of the data. R-square is a variance percent explained by the model. The fraction by which the variance of the error is less than the variance of dependent variable.

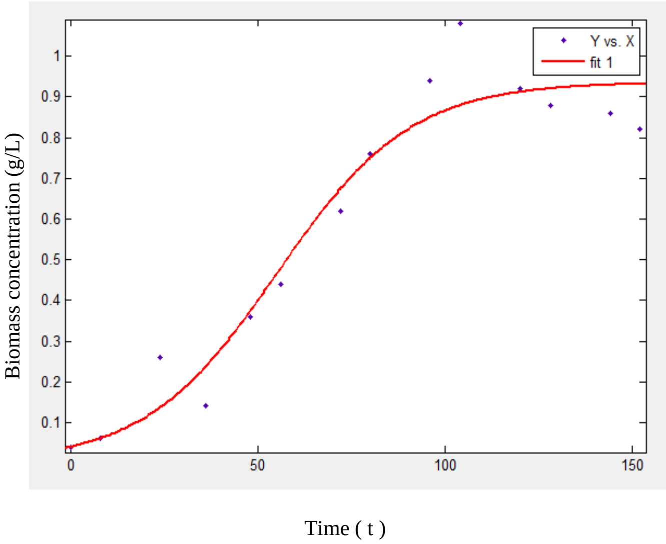

Fig. 1: Graph plot time (t) vs biomass concentration (g/L) of Run 17 of composite design of the experiment [x_1= 12%, x_2= 0.6vvm, x_3= 10% and x_4= 10%]

D. Composite design.

The continuation from 2^4 Factorial Experiment design was then complement with the additional experimental levels as shown in Table 3. The results of the composite design is recorded in Table 4.

Table 3 : The levels of variables in Composite design

Experimental variables	a = -2	a = 0	a = +2	Units
CO_2 (X1)	8	16	24	% (v/v)
Sparging rate (X2)	0.4	0.8	1.2	vvm
Supplement (X3)	0	20	40	% (v/v)
Dilution rate (X4)	0	20	40	% (v/v)

Table 4: Results of 2^4 Composite design experiment

Run	X1	X2	X3	X4	μ_{max} (h^{-1})	X_{max} (g/L)
17	-2	0	0	0	0.05636	0.936
18	+2	0	0	0	0.03214	0.346
19	0	-2	0	0	0.06287	0.7912
20	0	+2	0	0	0.04164	0.2131
21	0	0	-2	0	0.03417	0.6582
22	0	0	+2	0	0.05262	0.281
23	0	0	0	-2	0.02223	0.5034
24	0	0	0	+2	0.03731	0.5157

E. Maximum point determination.

Maximum yield point coordinate can be determined by partial derivative setting of the response function to zero. The maximum point can then be tested by using the second derivative. The first derivative for the simultaneous equations are solved based on the coordinates of maximum yield point. Equation (5) shows the form of the quadratic equation for 4-X variables.

$$y_N = b_0 + \sum_{i=1}^{4} i \; b_i x_{iN} + \sum_{i=1}^{4} i \; b_i x_{iN}^2 + \sum_{i=1}^{4} i \; b_i x_{iN} x_{jN} \quad (5)$$

Where y_n is the yield of the n^{th} experiment, the coefficient of the b_i is the i^{th} experimental variables and b_0 is a constant in the equation. Design Expert 10.0.6 was used to generate the coefficient from b_0 to b_{44}, using theory of least square method. Table 5 shows the coefficient of the quadratic regression equation for μ_{max} (h^{-1}) while table 6 shows for the X_{max} (g/L).

Table 5 : Coefficient quadratic regression equation for μ_{max} (h^{-1})

Coefficient	Value	Coefficient	Value
b0	0.034	b23	0.0081
b1	0.012	b24	0.009
b2	-0.016	b34	0.0099
b3	-0.004	b11	0.014
b4	0.00051	b22	0.022
b12	-0.043	b33	0.014
b13	-0.017	b44	0
b14	-0.0089		

Table 6 : Coefficient quadratic regression equation for X_{max} (g/L)

Coefficient	Value	Coefficient	Value
b0	0.46	b23	0.08
b1	-0.38	b24	0.2
b2	-0.055	b34	0.12
b3	-0.041	b11	0.13
b4	-0.086	b22	-0.0074
b12	0.58	b33	-0.04
b13	0.013	b44	0
b14	0.16		

Based on D.M. Himmelblau (1970) [10] in his book, the maximum yield can be solved by equation (6). However this equation solve using 4x4 inverse matrices method, equation (7) shows the form of the matrices. Rechecked the inverse matrix by following equation (8). The multiplication of matrix and inverse matrix must equal to identity. This applied to both of the specific growth rate and biomass yield.

$$X_{max} = -\left(\frac{1}{2}\right) B_{ii} B_i^{T} \quad (6)$$

$$A = \begin{bmatrix} b_{11} & \frac{b_{12}}{2} & \frac{b_{13}}{2} & \frac{b_{14}}{2} \\ \frac{b_{12}}{2} & b_{22} & \frac{b_{23}}{2} & \frac{b_{24}}{2} \\ \frac{b_{13}}{2} & \frac{b_{23}}{2} & b_{33} & \frac{b_{34}}{2} \\ \frac{b_{14}}{2} & \frac{b_{24}}{2} & \frac{b_{34}}{2} & b_{44} \end{bmatrix} \quad (7)$$

$$[A][A]^{-1} = I \quad (8)$$

The values of levels of the experimental variables at the maximum point has been calculated and summarized in Table 7 and Table 8.

Table 7 : Summarized result on maximum specific growth rate (μ_{max})

X	Experimental variables	Levels	Value of experimental variables	Units
X1	CO2	1.5	22	%(v/v)
X2	Agitation rate	-0.39	0.57	vvm
X3	Supplement	0.04	20.4	%(v/v)
X4	Dilution ratio	0.21	22.06	%(v/v)

Table 8 : Summarized result on maximum biomass (X_{max})

X	Experimental variables	Levels	Value of experimental variables	Units
X1	CO2	-1.086	11.56	%(v/v)
X2	Agitation rate	-0.076	0.78	vvm
X3	Supplement	1.069	26.05	%(v/v)
X4	Dilution ratio	3.119	56.79	%(v/v)

The values of X1 to X4 was substituted back into equation (5), follows each coefficient and resulting the maximum yield for both μ_{max} and X_{max}. The theoretical maximum specific growth rate yield is 5.009 h^{-1} and the maximum biomass yield is 283.69 g/L.

Based on the result, it shows that aerobic pond conditions were acceptable as the *Chlorella sp.* was well grown with the conditions. Besides, with the optimization of growth rate and biomass the aerobic pond can be controlled by supplemented the pond using medium from polishing pond. The value of maximum growth rate yield seems to be more acceptable compare to maximum biomass. This may cause from some experimental error during the experimental procedure.

V. CONCLUSION

In conclusion, this paper has achieve both the objective targeted in this study. The optimization of growth rate and biomass from Chlorella sp. was carried out using fermentation process with POME as a medium. This experiment varies the parameter in the fermentation process. The parameters are CO_2 concentration, agitation rate, supplement concentration and dilution ratio. The predicted maximum specific growth rate is 5.0029 h-1 with the optimum conditions 22% (v/v) for carbon dioxide concentration, 0.57vvm for agitation rate, 20.4% (v/v) and 22.06% (v/v) for supplement concentration and dilution rate respectively. The predicted maximum biomass is 283.7g/L with optimum condition for carbon dioxide concentration are 11.7% (v/v) for carbon dioxide concentration, 0.7vvm for agitation rate, 26.05% (v/v) and 56.79% (v/v) for supplement concentration and dilution ratio respectively. From the results, the growth rate seems to be more relevant compared to biomass. Therefore, it can be concluded the aerobic pond was acceptable as a medium and can control the supplement from polishing pond because the optimization has been achieve.

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References

[1] Awalludin, M. F., Sulaiman, O., Hashim, R., & Nadhari, W. N. A. W. (2015). An overview of the oil palm industry in Malaysia and its waste utilization through thermochemical conversion, specifically via liquefaction. Renewable and Sustainable Energy Reviews, 50, 1469-1484. doi: 10.1016/j.rser.2015.05.085

[2] Lim, S., & Teong, L. K. (2010). Recent trends, opportunities and challenges of biodiesel in Malaysia: An overview. Renewable and Sustainable Energy Reviews, 14(3), 938-954. doi: 10.1016/j.rser.2009.10.027

[3] Rahaman, M. S. A., Cheng, L.-H., Xu, X.-H., Zhang, L., & Chen, H.-L. (2011). A review of carbon dioxide capture and utilization by membrane integrated microalgal cultivation processes. Renewable and Sustainable Energy Reviews, 15(8), 4002-4012. doi: http://dx.doi.org/10.1016/j.rser.2011.07.031

[4] Maity, I.P.; Bundschuh, J.; Chen, C.Y.; Bhattacharaya, P. Microalgae for third generation biofuel production, mitigation of greenhouse gas emissions and wastewater treatment: Present and future perspectives—A mini review. Energy 2014, 78, 1–10.

[5] Resdi, R., Lim, J. S., Kamyab, H., Lee, C. T., Hashim, H., Mohamad, N., & Ho, W. S. (2016). Review of microalgae growth in palm oil mill effluent for lipid production. Clean Technologies and Environmental Policy, 18(8), 2347-2361. doi: 10.1007/s10098-016-1204-1

[6] Phukan M.M. *Microalgae Chlorella as a potential bio-energy feedstock.* 2011- Vol.88, 3307-3312.

[7] Matthew D. Rolfe, Christopher J. Rice, Sacha Lucchini, Carmen Pin, Arthur Thompson, Andrew D. S. Cameron, Mark Alston, Michael F. Stringer, Roy P. Betts, József Baranyi, Michael W. Peck, Jay C. D. Hinton J Bacteriol. 2012 Feb; 194(3): 686–701. doi: 10.1128/JB.06112-11

[8] Diyana N. (2016). *Effect of Supplement and Dilution on Microalgae Biomass Growth in Palm Oil Mill Effluent (POME) from Aeration Pond.* Unpublished.

[9] Maryam I. (2010). Modelling Of Batch Biopolymer Fermentation. Unpublished.

[10] D. M. Himmelblau(1970), Process analysis by statistical methods, John Wiley & Sons, Inc., New York.

[11] Ahmed, Y., Yaakob, Z., Akhtar, P., & Sopian, K. (2015). Production of biogas and performance evaluation of existing treatment processes in palm oil mill effluent (POME). Renewable and Sustainable Energy Reviews, 42, 1260-1278. doi: 10.1016/j.rser.2014.10.073

[12] Formighieri, C., Franck, F., & Bassi, R. (2012). Regulation of the pigment optical density of an algal cell: Filling the gap between photosynthetic productivity in the laboratory and in mass culture. Journal of Biotechnology, 162(1), 115-123. doi: http://dx.doi.org/10.1016/j.jbiotec.2012.02.021