Effect of supplement and dilution on microalgae biomass growth in POME from effluent of biogas plant of Felda Sg Tengi Palm Oil Mill

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***Abstract*— The purpose of this research is to investigate the effect of supplement and dilution of POME from biogas plant of Felda Sg Tengi Palm Oil Mill on the growth of microalgae species, *Chlorella sorokiniana*. The research is done by studying the main effect and interactive effect of four variables controlling the process on the maximum biomass concentration and maximum specific growth rate of microalgae in a batch fermentation. Linear regression was then used to determine the relationship between the variables and the objective function of maximum biomass concentration, and the objective function maximum specific growth rate. Four variables were introduced for 2^4 factorial experiment, namely carbon dioxide (CO2) concentration, gas mixture sparging rate, supplement concentration and dilution level. The data obtained from the experiment were fitted into logistic equation by using MATLAB R2013a software to get the value of both maximum specific growth rate and maximum biomass concentration. Then the main effect of each variable and the Interactive Effects between variables on the objective function maximum biomass concentration and maximum specific growth rate were evaluated by using Yate's Method. The results showed that the main effect of each variable is positive on both maximum biomass concentration and maximum specific growth rate. By increasing each of the variables from low level to high level will increase the biomass concentration. From regression evaluation, it showed that CO2 concentration has a very critical effect on biomass growth and on maximum specific growth rate, followed by gas mixture sparging rate, supplement concentration and dilution level. In conclusion, the study of CO2 sequestration by microalgae and the effect of supplement and dilution in POME on both maximum biomass concentration and maximum specific growth rate can be determined by this method.**

Keywords*— *POME, main effect, interactive effect, factorial, regression.

I. INTRODUCTION

The global and local demand had been increase significantly each year due to increment of researches, innovation and invention of oil-based product in many fields such as pharmaceutical industry, food technology and cosmetics. A studied had been conclude that the world demands of palm oil are mainly dominated by China, India and Middle East [1, 2]. Although high in demands can lead to stability and profitable economic, however, the environmental issue caused by huge amount of waste generated must be handle and control at all cost to ensure the health of environment and public are safe.

Palm oil industry is one of the leading agricultural commodities and the oldest industries exploited in Malaysia. The uniqueness of oil palm tree in the palm oil production is that the palm oil is not only obtained from fresh fruit bunch (FFB) but also located in its kernel. Due to this, there are many kind of fields with wide range of products involved in palm oil industry. Despite of different kind of oils produced in the industry, the number of palm oil production increase significantly due to high in demands each year, either the global demand or the local demand. Due to high amount of production produced, the amount of waste had generated uncontrollably.

POME is a wastewater generated by the palm oil mill that cannot been disposed into neither river nor lake due to high amount BOD and COD, thus, required a proper treatment according to the waste management and safety regulation as per declared by the authorities [3]. Ponding system, a low cost treatment system yet has a high degree of treatment, had introduced the used of biological treatment method [4]. Anaerobic digestion is the method used to decompose and treat the POME before discharged into the river as the safety limit is met [5].

Characterization of wastewater is a crucial part in wastewater treatment process so that the treated POME is able to meet the standard discharged limit set by the Department of Environment (DOE) authorities. One of the wastewater treatment process used is a ponding system. Ponding system, also known as oxidation pond has a general used and by mean can be either aeration, facultative or anaerobic. Although it is essentially employ biological treatment method, however, it is a viable low cost treatment system with reasonable degree of treatment and easily maintained as the technology required is relatively unsophisticated. Ponding system also able to be used as natural settling for sludge and suspended solid.

Methane, which a biogas produced by the reaction of anaerobic digestion with POME, is one of the greenhouse gases and can be used as a fuel source to the power plant [6]. However, the conversion of biogas methane as an energy source would produce carbon dioxide. Carbon dioxide is a greenhouse gas that required to be fixate to reduce the air pollution as well as global warming [7, 8]. One of the way to fixate the carbon dioxide is by biological sequestration. It is a method where carbon dioxide is capture by the plant and digested through photosynthesis, converting the carbon dioxide into oxygen.

Microalgae have been used as a culture sample due to its photosynthetic ability, however, required to be studied further to determine its suitable condition with the effect of different level of carbon dioxide, gas mixture sparging rate as well as the supplement concentration obtained from the POME and its water dilution.

II. METHODOLOGY

A. *Species of microalgae*

The type of microalgae species used in this experiment is Chlorella sp. It is inoculated in a 1L of flask by using Bold's Basal medium (BBM) as the culture media at room temperature. The microalgae are illuminated with fluorescent bulb at constant light intensity of 10000 lux. Four variables had been manipulated, mainly rate of sparging air, rate of carbon dioxide concentration, supplement concentration and dilution rate following both Yates's method and factorial design method. The inoculated microalgae were left for three to four days for propagating purpose before sub-cultured for seven days to ensure the microalgae were in exponential phase during experiments period.

B. *The BBM*

The BBM is a culture medium used to support the growth of the microalgae. It consists of highly enriched elements required by the bacteria to growth. The compositions of the medium were stated as the following table below [9]:

Component	Stock Solution	Quantity Used (mL)	Concentration (M)
Macronutrients			
1. $NaNO_3$	25.00	10	2.9×10^{-3}
2. KH_2PO_4	17.50	10	1.7×10^{-4}
3. $MgSO_4.7H_2O$	7.50	10	3.0×10^{-4}
4. K_2HPO_4	7.50	10	4.3×10^{-4}
5. $CaCl_2.2H_2O$	2.50	10	1.3×10^{-3}
6. NaCl	2.50	10	4.3×10^{-4}
Alkaline EDTA Solution			
1. EDTA	50.00	1	1.7×10^{-4}
2. KOH	31.00		5.5×10^{-4}
Acidified Iron Solution			
1. $FeSO_4.7H_2O$	4.98	1	1.8×10^{-5}
2. H_2SO_4			
Boron Solution			
1. H_3BO_3	11.42	1	1.8×10^{-4}
Trace Metals Solution			
1. $ZnSO_4.7H_2O$	8.82	1	3.1×10^{-5}
2. $CuSO_4.5H_2O$	1.57		7.3×10^{-6}
3. $MnCl_2.4H_2O$	1.44		4.9×10^{-6}
4. MoO_3	0.71		6.3×10^{-6}
5. $Co(NO_3)_2.6H_2O$	0.49		1.7×10^{-6}

Table 1: The composition of BBM medium

C. *The POME sample*

The POME sample is taken from the aeration and anaerobic pond of Felda Sg. Tengi Mill and being stored at 4°C inside the chiller. The sample from the anaerobic pond is used as the supplement and the amount of was varies according to factorial design.

D. *Microalgae cultivation in the flask*

The medium culture was prepare using dilution method by adding 1000 mL of POME from the aeration pond into 1000 mL of flask. The duration of each run is hold for seven days and the sampling method was as following chart.

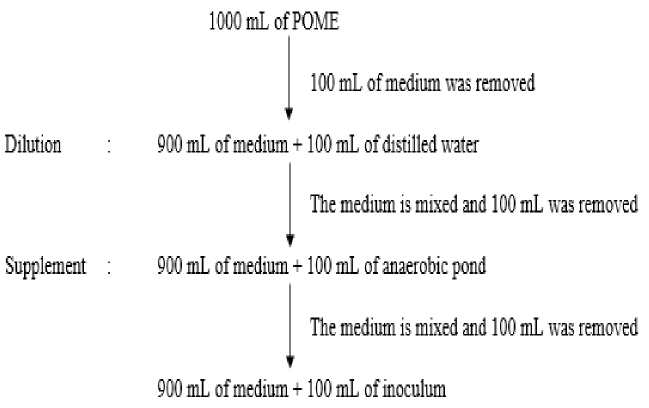

Fig. 1: The procedure for microalgae cultivation

E. *The Gas Mixing System*

Gas mixing is crucial to prevent the sedimentation of microalgae from occur. The air pumped by the compressor was filtered by using air filter before fed into the medium with the purpose to absorb the moisture content in the air supply. The pressure regulators were set at 2 bar to pump both carbon dioxide supply and compressed air supply, while the flowrates of both gases wat set according to the factorial experiment. The gases are mixed and sparged into the culture medium. Tube with small diameter in size were required to produce small sized-bubbles, enhancing the mass flowrates and improving the gas exchange between the culture medium and the air.

F. *Lighting*

Light is required by the microalgae to break down the inorganic compound through photosynthesis process. The intensity of light supplied to the microalgae is to be constant throughout the experiment and necessarily at high intensity to ensure the light able to penetrate through the cell culture, producing high amount of biomass. The fluorescent lamps with light intensity of 10 000 lux had been constantly used as the light source.

G. *Centrifugation of microalgae*

Biomass of microalgae from the culture medium is obtained by centrifuged 50 mL of the sample for 5 minutes at 10 000 rpm. The supernatant is removed while the sediment is suspended again with equal volume of distilled water as a washing method. The sample is centrifuged again and the washing method is repeated twice. The biomass is then weighed preliminarily and dried at 119°C for about 24 hours. The dried biomass is weighed again and the net weight is recorded.

H. *Mathematical method*

Factorial Design is mathematical method designed to determine the effects of number of experimental variables on the yield of the experiments to be investigated simultaneously. It enables the determination of 'main effects' and the 'interactive effects' of an experiment by changing the levels of the experimental variables from the lower level to the upper level [10]. The combination of these variables at different level would give the value of the biomass yield and the highest value would be taken as central point.

In this experiment, the initial central point, α_0 is taken from Diyana research paper [11]. The level of the factorial experiment is assigned to two levels, 2^n with the variables number of four. The levels are corresponded to both $\alpha = +1$ and $\alpha = -1$, where the α are referred to the multiplication factor for the level in experimental variables. The four variables involved are the rate of carbon dioxide, the rate of sparging air, the concentration of supplement and the dilution of water. Following the formula of factorial experiment (2^n), there are 16 runs and each runs will have 4 variables at different level alternately, depending on the order of Yates method. The factorial experiments make use of a mathematical method known as the Yates method to analyze the main effects and the interactive effects. The factorial experiment and Yate's order are both designed as the following Table 2 and Table 3.

Experimental variables	Level of experimental variables			
	$\alpha = -1$	$\alpha = 0$	$\alpha = +1$	Unit
Carbon dioxide (X_1)	12	16	20	%
Sparging air (X_2)	0.6	0.8	1.0	vvm
Supplement (X_3)	10	20	30	%
Water dilution (X_4)	10	20	30	%

Table 2: The factorial experiment design for the experiment

Run	Experimental variable			
	X_1	X_1	X_1	X_1
1	-	-	-	-
2	+	-	-	-
3	-	+	-	-
4	+	+	-	-
5	-	-	+	-
6	+	-	+	-
7	-	+	+	-
8	+	+	+	-
9	-	-	-	+
10	+	-	-	+
11	-	+	-	+
12	+	+	-	+
13	-	-	+	+
14	+	-	+	+
15	-	+	+	+
16	+	+	+	+

Table 3: The Yate's order of 4 variables

The Yates method is a mathematical method used to investigate and analyses the 'main effect' and the 'interactive effect' using the Yates algorithm. In order to use the Yates algorithm, the data set must be arranged in Yates order as demonstrated in Table 3.3. The yield response surface obtained from the Yates method is then will be used as optimization.

In order to describe the behavior of microalgae, a kinetic model has been used to determine the growth rate of biomass. The Logistic model has been used to explain the characteristics of biomass sigmoidal curve as well as to quantifies the change in biomass from the beginning of the exponential phase to the maximum cell growth. Similar to the Monod model, which is a model for a curves of non-linear microbial growth, however, the Logistic model assumes that there are other causes aside of substrate limitation involved in growth limitation. The Logistic model equation is stated in Equation 1.

$$x = \frac{X_0 X_m e^{\mu_m t}}{X_m - X_0 + X_0 e^{\mu_m t}} \quad \text{(eqn. 1)}$$

where; X_0 = Initial biomass concentration

μ_m = Maximum growth rate

X_m = Maximum biomass concentration

t = Time taken

Linear regression is one of the mathematical method used to fit the model to the data set. It is used to demonstrate the relationship between the concentration of biomass and the four variables, fitting the data into a linear function. The purpose of the method is to determine the optimum value of the variables involved for the maximum yield of microalgae as well as maximum growth rate of microalgae. The least squares regression is used to find the linear function by minimizing the sum of the squared deviations between the data and the model., creating the best-fitting line of the data. The base point location, which is maximum point can be estimated through the coefficient of the linear function. The coefficient with small values, which the gradient is closer to zero indicates the base point to be at maximum.

The linear regression for the biomass yield can be obtained using Data Analysis of Microsoft Excel software with the following Equation 2.

$$y_n = a_0 + a_i X_{in} + a_{ii} X_{iin} \quad \text{(eqn. 2)}$$

where; y_n = Yield of n^{th} experiment

X_n = Level of the i^{th} experimental variable of n^{th} experiment

a_0 = Constant number

a_i = Coefficient of the i^{th} experimental variable of n^{th} experiment

F-test analysis is the statistical test that has been used to test the overall significance for the regression model, which is both main effect and interactive effect significance against the experimental error as well as to compare the F-value obtained from the calculation with the F distribution table. If the F distribution table value is smaller than the F-value, the null hypothesis can be rejected.

III. RESULTS AND DISCUSSION

A. The main effect and interactive effect of maximum biomass concentration and maximum specific growth rate

Main effect

The main effect of CO2 concentration (X_1), gas mixture sparging rate (X_2), supplement concentration (X_3) and dilution level (X_4) for maximum biomass concentration are 0.9860, 0.1971, 0.2804 and 0.1288 respectively. The result shows that each of the variables are positive. Meanwhile, the main effect of X_1, X_2, X_3 and X_4 for maximum specific growth rate are 1.0076, 0.2479, 0.2181 and 0.0980 respectively. Likewise, the result shows that each of the variable are positive. Thus, by increasing each of the variables from low level to upper level will increase both the maximum biomass concentration and maximum specific growth rate.

Interactive effect: 2 variables

For biomass concentration, there are only one interactive effect of 2 variables that has negative value, which is X_2X_3 (sparging rate and supplement). While another five interactions of two variables, which are X_1X_2 (CO2 and sparging rate), X_1X_3 (CO2 and supplement), X_1X_4 (CO2 and dilution), X_2X_4 (sparging rate and dilution) and X_3X_4 (supplement and dilution) have positive values.

Different from maximum biomass concentration, maximum specific growth rate has two interactions of 2 variables that showed negative values, which are X_2X_3 interaction and X_3X_4 interaction. While the other interaction which are X_1X_2, X_1X_3, X_1X_4 and X_2X_4 have positive values. As the effect has positive value, both the maximum biomass concentration and maximum specific growth rate will increase when interaction variables increase from low level to upper level and vice versa.

Maximum biomass concentration (g/l)	Column				Effect	Identification
	1	2	3	4		
1.0333	1.8000	4.2467	7.9067	16.0234	2.0029	Grand Total
0.7667	2.4467	3.6600	8.1167	7.8900	0.9860	X_1
1.2667	1.6000	3.2500	-0.2267	1.5766	0.1971	X_2
1.1800	2.0600	4.8667	1.8033	2.9100	0.3638	X_1X_2
0.8667	1.4900	-0.3533	1.1067	2.2434	0.2804	X_3
0.7333	1.7600	0.1266	1.1367	1.7100	0.2138	X_1X_3
0.9000	2.0000	0.7900	0.5733	-0.6434	-0.0804	X_2X_3
1.1600	2.8667	1.0133	-1.2167	-1.8034	-0.2254	$X_1X_2X_3$
0.3300	-0.2666	0.6467	-0.5867	1.0300	0.1288	X_4
1.1600	-0.0867	0.4600	1.6167	2.0966	0.2621	X_1X_4
0.9000	-0.1334	0.2700	0.4799	0.7032	0.0879	X_2X_4
0.8600	0.2600	0.8667	0.2233	0.0366	0.0046	$X_1X_2X_4$
0.6600	0.8300	0.1799	-0.1867	0.4100	0.0513	X_3X_4
1.3400	-0.0400	0.3934	0.5967	0.8102	0.1013	$X_1X_3X_4$
1.2667	0.6800	-0.8700	0.2135	0.7368	0.0921	$X_2X_3X_4$
1.6000	0.3333	-0.3467	0.5233	0.5233	0.0654	$X_1X_2X_3X_4$

Table 4: Yate's method on maximum biomass concentration

Maximum specific growth rate (hr^{-1})	Column				Effect	Identification
	1	2	3	4		
0.9915	1.6850	3.9158	7.1839	15.3296	1.9162	Grand Total
0.6935	2.2308	3.2681	8.1457	8.0611	1.0076	X_1
1.0022	1.3961	3.3572	-0.0846	1.9834	0.2479	X_2
1.2286	1.8721	4.7885	2.0681	3.0899	0.3862	X_1X_2
0.7830	1.4849	-0.0716	1.0218	1.7444	0.2181	X_3
0.6130	1.8722	-0.0130	0.7226	1.5738	0.1967	X_1X_3
0.8576	2.2266	0.9769	0.8512	-0.4729	-0.0591	X_2X_3
1.0145	2.5619	1.0911	-1.3241	-1.9717	-0.2465	$X_1X_2X_3$
0.3534	-0.2980	0.5458	-0.6477	0.7837	0.0980	X_4
1.1315	0.2263	0.4760	1.4314	1.4900	0.1862	X_1X_4
0.8367	-0.1700	0.3873	0.0586	0.1728	0.0216	X_2X_4
1.0355	0.1570	0.3353	0.1142	0.0444	0.0055	$X_1X_2X_4$
0.6543	0.7781	0.5243	-0.0698	-0.1218	-0.0152	X_3X_4
1.5723	0.1989	0.3269	-0.0520	-0.2494	-0.0312	$X_1X_3X_4$
1.1944	0.9180	-0.5792	-0.1974	-0.3630	-0.0454	$X_2X_3X_4$
1.3675	0.1731	-0.7448	-0.1656	-0.1656	-0.0207	$X_1X_2X_3X_4$

Table 5: Yate's method on maximum specific growth rate

Interactive effect: 3 variables

For maximum biomass concentration, only one interactive effect of 3 variables that has negative value, which is $X_1X_2X_3$ (CO2, sparging rate and supplement) interaction while the rest of the other interaction which are $X_1X_3X_4$ (CO2, supplement, dilution), $X_1X_2X_4$ (CO2, sparging rate and dilution) and $X_2X_3X_4$ (sparging rate, supplement and dilution) have positive value. Different from maximum biomass concentration, the maximum specific growth rate has one interactions of 3 variables that showed positive values, which is $X_1X_2X_4$ while the rest of the other interaction which are $X_1X_2X_3$, $X_1X_3X_4$ and $X_2X_3X_4$ have positive value. From the result, it can be state that as the effect has positive value, both the maximum biomass concentration and maximum specific growth rate will increase when interaction variables increase from low level to upper level and vice versa.

Interactive effect: 4 variables

Last but not least, maximum biomass concentration has positive value of interactive effect of 4 variables, which means that by increasing each of the variables from low level to upper level will increase both the maximum biomass concentration and maximum specific growth rate. For maximum specific growth rate, the interactive effect of 4 variables is a negative value. As the effect has negative value, maximum specific growth rate will decrease when interaction variables increase from low level to upper level.

Data obtained from each run for both maximum biomass concentration and maximum specific growth rate have been analysed using linear regression method to demonstrate the relationship between the concentration of biomass and the four variables, fitting the data into a linear function. From the result, maximum biomass concentration has an equation of $y = -0.7414 + 4.9269X_1 + 0.7011X_2 + 0.6438X_3 + 0.1313X_4$. This is shown that CO2 concentration has a very critical effect on biomass growth and on maximum specific growth rate, followed by gas mixture sparging rate, supplement concentration and dilution level. Different from maximum biomass concentration, the maximum of specific growth rate has an equation of $y = -0.9201 + 6.1982X_1 + 0.5451X_2 + 0.4898X_3 + 0.6011X_4$. This is shown that CO2 concentration has a very critical effect on biomass growth and on maximum specific growth rate, followed by gas mixture sparging rate, dilution level and supplement concentration.

F-test analysis is done to test the significance of the main effect and interactive effect against experimental errors at chosen confidence level. F critical value has been chosen based on the degree of freedom of from both number of experimental runs and small significant value of the effect. The value with 99%, 97.5%, 95% and 90% significance level as listed in Table below.

$F_{15,5}$	99%, α = 0.01	97.5%, α = 0.025	95%, α = 0.05	90% , α = 0.1
Critical value of F	9.72	6.43	4.62	3.24

Table 6: F critical value for maximum biomass concentration

$F_{15,7}$	99%, α = 0.01	97.5%, α = 0.025	95%, α = 0.05	90% , α = 0.1
Critical value of F	6.31	4.57	3.51	2.63

Table 7: F critical value for maximum specific growth rate

From calculation, the F-ratio obtained for the maximum biomass concentration is 11.193 and like to occur by chance of a $p < 0.01$. For maximum specific growth rate, the F-ratio is statistically significant with value of 33.202 and likely to occur of a $p < 0.01$.

IV. CONCLUSION

In conclusion, the main and interactive effects can be analysed by using Yate's method for both maximum biomass concentration and maximum specific growth rate. Linear regression method can be used to demonstrate the relationship between both the concentration of biomass and the four variables as well as the maximum specific growth rate and the four variables, fitting the data into a linear function. From the equation, it can be concluded that that CO2 concentration has a very critical effect on biomass growth and on maximum specific growth rate. F-test analysis is used to show the significance of the main effect and interactive effect against experimental errors at chosen confidence level. Thus, it can be concluded that the study of CO2 sequestration by microalgae and the effect of supplement and dilution in POME on both maximum biomass concentration and maximum specific growth rate can be determined by this method. One recommendation is that the level of the variables need to undergo steepest ascent to obtained a better result.

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