Optimisation of medium for microalgae growth based on facultative pond 6 POME of Sime Darby Sua Betong Palm Oil Mill

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Abstract— **This research study is about the optimisation of medium for microalgae growth based on facultative pond 6 POME of Sime Darby Sua Betong Palm Oil Mill. The idea is to treat Palm Oil Mill Effluent (POME) and sequester carbon dioxide (CO_2) that are released from the conversion of methane gas (CH_4) to CO_2. A strain of Chlorella sp. was used to treat POME and sequester CO_2 gas simultaneously. Six times diluted POME from facultative pond 6 was subjected to different CO_2 concentrations in the sparging gas mixture (x_1) and to different gas mixture sparging rates (x_2), to determine their optimum level of of dissolved CO_2 concentration, x1 and the level of sparging rate, x2 that gives a maximum biomass concentration and (X_m) and the maximum specific growth rate (μ_m) of microalgae in batch growth by using a Response Surface Method (RSM). The data obtained from the factorial experiments were fitted in logistic equation by using MATLAB R2014a software before performing Linear Regressions. Since the Linear Regression satisfies the requirement of containing the maximum in both cases, the 2^2 factorial experiments were complemented with the necessary points to make the Composite Design. The optimisation of microalgae growth was done using a Quadratic Equation to obtain the maximum point of the variables. From the result obtained, it was found that at sparging rate of 0.900 vvm and at CO_2 concentration of 16%, the maximum specific growth rate is predicted to be 4.17 h^{-1}.**

Keywords— CO_2 sequestration, microalgae, POME

I. INTRODUCTION

Nowadays, palm oil has become an important product in the world and Malaysia now has become second largerst producer of Crude Palm Oil (CPO) in the world which contributes 10.3% of oil and fat production. It produces also can produces more than 13 million tonnes per year of CPO and the oil palm plantation involves 11% of land area of Malaysia [1]. However, large production of palm oil produces CO_2 to the atmosphere and POME which caused a serious pollutants [2] towards the environments especially water bodies as it contains high amounts of Chemical Oxygen Demand (COD) and Biochemical Oxygen Demand (BOD) [3].

The process of extracting and producing CPO in palm oil generates two types of wastes which are solid waste and liquid waste, resulting from oil extraction, washing and cleaning process in the mills [4]. This waste is the POME. Apart from POME produced from palm oil productions, there are other waste generated that are considered as solid wastes which includes Empty Fruit Bunches (EFB), Oil Palm Trunks (OPT), Oil Palm Fronds (OPF) , Palm Pressed Fibres (PPF) and palm kernel shells [5].

POME produces CH_4 and CO_2 gases. The biogas can be combusted to drive gas turbines to generate electricity, giving more CO_2. Besides, CO_2 released are also released from the combustion of solid wastes in the palm oil mills. Thus, the final products discharged from the oil production process is CO_2. Therefore, a biotechnological techniques for CO_2 fixation need to be conducted in order to reduce CO_2 emission.

The high amount of CO_2 released need to be sequestered. Therefore, microalgae growth is one of the biological alternatives to sequester large amount of CO_2 released [5,6]. In order for the microalgae to grow, it needs a carbon source and an energy source which Nitrogen (N) and Phosphorus (P). However, carbon is the most important nutrient that must be supplied because it enhance algal growth and allow photosynthesis process to take places [8]. Therefore, the dissolved CO_2 act as carbon source and POME act as a medium for microalgae growth as it is rich of nitrogen (N) and phosphorus (P) as the nutrients [9].

The challenges faced in the CO_2 sequestration is the variation of composition of POME with the changes of rainfall pattern and operation patterns due to the variation of Fresh Fruit Bunches (FFB) supplies from the plantations [10]. This is because the variation will affect the performance of microalgae growth and the sequestration of CO_2 gas.

Long time ago, ponding systems are used to treat POME because of its easy operations [11]. Besides, this method also has been used in Malaysia since 1982 which is more than 85% of palm oil mills has been applied this method in order to treat POME [4] as it can handle the POME in large quantities [12]. Basically, it consists of series of ponds which are anaerobic, facultative (typically 6 ponds), aerobic and polishing pond [10]. In this work, facultative 6 pond will be used.

II. METHODOLOGY AND RESULTS

A. Microalgae species

Chlorella sp. (University of Malaya Algae Culture Collection) were grown at room temperature in 1L flask by using Bold's Basal Medium (BBM) [10] that act as the nutrient medium, sparged with air and illuminated with fluorescent bulbs (10,000 lux at the surface), and sub-cultured every seven days. Innocula were prepared by growing the microalgae in the same way and using it after three days of growth at 10% (v/v) [2].

B. The gas mixing and lighting chamber

Air were supplied by using compressor and filtered using air filtration. The pressure of CO_2 and compressed air were maintained at 2 bar by using pressure regulator. The flow rates were set according to factorial design. Both CO_2 and compressed air were mixed before it can be sparged into the cultivation flask.

C. Palm Oil Mill Effluent (POME)

POME samples were taken from facultative pond 6 of Sime Darby Bhd Sua Betong Mill and stored at 4 °C [14].

D. Microalgae cultivation in the flask

The medium was prepared in a 1L conical flask. POME from facultative pond 6 were diluted 6 times. A silicone rubber stopper was fitted to the flask and was placed inside the lighting chamber. The cultivation in the flask was let to react for 7 days of which for every run, a 50 mL sample was taken at every 12 hours of time interval.

One set of 2^2 factorial experiments were conducted on POME from facultative pond 6 which has been diluted with tap water involving only the variables % (v/v) CO_2 in sparging air- CO_2 mixture (x_1) and the gas mixture sparging rate (x_2), with level of Factorial Experiments [17] shown in Table 1.

Table 1: The level of variables in Factorial Experiments

Experimental Variables	Level of Experimental Variables			
	α= -1	α= 0	α= +1	Unit
CO_2 Concentration	12	16	20	%
Sparging Rate	0.8	0.9	1.0	vvm

Each of the batch growth experiment was run in duplicate. Starting with the first flask in the duplicate, on the first day of experiment until the seven day of experiment, a 50 ml of sample is taken at the interval of 12 hours. At the second day of the experiment, the duplicate sample was added to the original flask sample at 1L volume in order to maintain the volume of the sample until the last day that were not less than 300 mL.

For the sampling process, the sample was centrifuged at 10,000 rpm for 10 minutes where the supernatant was eliminated while the sediment was re-suspended to the same volume as a method of washing and centrifuged again. The washing step was repeated twice. The sediment was re-suspended to the same volume and transferred into a crucible and let dry at 105 °C for 24 hours. After 24 hours, crucible was weighed.

E. Mathematical Method

The Method of Factorial Experiments. The Method of Factorial Experiments was used to design the experiments [17]. For 2^2 factorial experiments, 4 experiments were run in duplicate over 7 days on combinations of levels of experimental variables as in Table 2.

The Logistic model. The equation to represent the model of microalgae growth at different conditions. It explains the characteristics sigmoidal curve of biomass in a batch culture and measure the change in biomass from the lag phase until it reached a stationary phase. The equation of logistic model is as followed:

$$\frac{dX}{dt} = \mu_m X - \frac{\mu_m X^2}{X_m} \quad (1)$$

where X is the biomass concentration (g/L) at time t, X_m is the theoretical maximum biomass concentration (g/L) and μ_m is the maximum specific growth rate (hr^{-1}).

By integrating equation (1), it will yields;

$$X = \frac{X_0 X_m e^{\mu_m t}}{X_m - X_0 + X_0 e^{\mu_m t}} \quad (2)$$

The results of all runs obtained from the experiment will be plotted to observe the growth curve of microalgae. Then, it will be fitted into the logistic equation (2) by using a MATLAB software to find the X_m and μ_m for each batch growth data. The value of each batch growth data in 2^2 factorial experiments is shown in table 2 and figure 1 shows a typical fit.

Table 2: Results of 2^2 Factorial Experiments

No	x_1	x_2	μ_m (hr^{-1})	X_m (g/L)
1	-	-	0.088	1.431
2	+	-	0.049	0.695
3	-	+	0.051	0.798
4	+	+	0.042	0.733

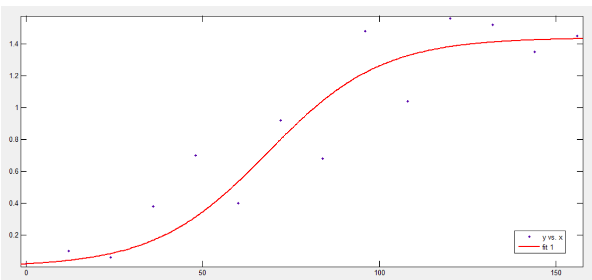

Figure 1: Graph plot of Run 1 of 2^2 factorial experiments [x_1 = 12% (v/v), x_2 = 0.8 vvm]

Linear Regression. The maximum specific growth rate results of the 2^2 Factorial Experiments in Table 3 were fitted with a linear regression to determine whether the area investigated contains the point of maximum specific growth rate (μ_m) giving equation (3):

$$\mu_m = 0.115305 - 0.0463x_1 - 0.00139x_2 \quad (3)$$

where μ_m is the value of μ_m of the n^{th} experiment, and x_i is the level of the i^{th} experimental variable at the n^{th} experiment.

In this experiment, since the coefficients of x_1 and x_2 of the maximum specific growth rate are small compared to the intercept. Thus, the area may contain the maximum point.

Composite Design. This 2^2 Factorial Experiment was then complemented with the additional experimental points needed as in Table 3 to make the Composite Design, giving results as in Table 4 [10].

Table 3: The level of variables in Composite Design

Experimental variable	Levels of experimental variables			
	α = -1.414	α = 0	α = +1.414	Unit
CO_2 Concentration (x_1)	10.34	16	21.65	%
Sparging rate (x_2)	0.76	0.9	1.04	vvm

Table 4: Results of Composite Design Experiments

No	CO_2 (x_1) [%(v/v)]	Sparging rate (x_2) [vvm]	μ_m (hr^{-1})
5	-1.414	0	0.057
6	+1.414	0	0.097
7	0	-1.414	0.094
8	0	+1.414	0.122

Quadratic Equation. The Quadratic Equation is the determination of levels of variables at maximum point. The maximum specific growth rate (μ_m) data of the Composite Design comprising of the experiments in Table 3 and the experiments in Table 4 were fitted with a quadratic response surface equation [4] as in equation (4):

$$\mu_m = b_0 + \sum_{i=1}^{2} b_i x_{in} + \sum_{i=1}^{2} b_{ii} x_{in}^2 + \sum_{i=1}^{2} b_{ij} x_{in} x_{jn} \quad (4)$$

where μ_m is the value μ_m in the nth experiment, b_0 is a constant, b_i is the coefficient of the i^{th} experimental variable, and b_{ij} is the coefficient of the product of the i^{th} experimental variable and j^{th} experimental variable.

By using a Design Expert-10 software, the values of the regression coefficients of the quadratic equation give a response surface as in Figure 2 which represent the maximum specific growth rate (μ_m). Then, these values were used in solving for the coordinates of the point of μ_m, using matrices [5], giving a predicted μ_m of 4.17 hr^{-1}.

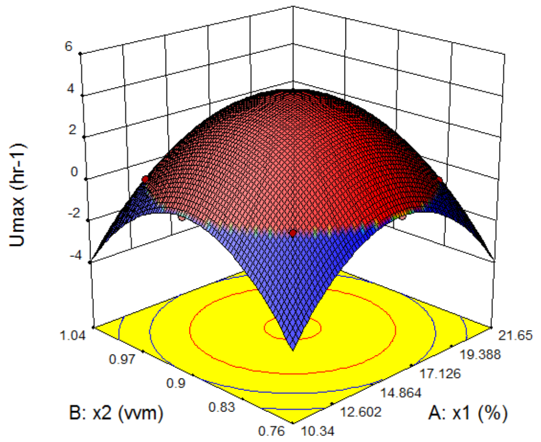

Figure 2: Quadratic response surface of the maximum growth rate (A is x_1, B is x_2, and μ_{max})

III. Discussion and Conclusion

From the result obtained, it was found that at sparging rate of 0.900 vvm and at CO_2 concentration of 16%, the maximum specific growth rate is predicted to be 4.17 h^{-1}. However the actual maximum specific growth rate, μ_m value was not obtained in experiments. Thus, the optimisation of maximum specific growth rate μ_m based on experimental design involving two variables which are CO_2 concentration (%) and sparging rate (vvm) complemented to make a Composite Design was successful.

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