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# The optimisation of microalgae growth in medium based on palm oil mill effluent (POME) from effluent of biogas plant of Felda Sg. Tengi palm oil mill

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**Abstract**

This paper reports on the effects of carbon dioxide ($CO_2$) concentration and agitation rate on microalgae growth in medium based on the effluent of the biogas plant of Felda Sg. Tengi Palm Oil Mill supplemented with palm oil mill effluent (POME) from their first facultative pond. Two variables which are $CO_2$ concentration ($X_1$) and agitation rate ($X_2$) are used to optimise the microalgae growth using a $2^2$ factorial design which was complemented to make a composite design. The linear regression model was used to analyse the results of the $2^2$ factorial experiment and determine whether it contains the maximum point. The quadratic regression model was used to analyse the results of the composite design experiments and optimise the microalgae growth based on maximum biomass concentration ($x_m$). The theoretical maximum point for $x_m$ from the quadratic regression model was predicted to be at 16.03% $CO_2$ concentration and 0.9 vvm agitation with the theoretical maximum $x_m$ predicted to be 42.6158 g/l.

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## 1.0 Introduction

The volume of $CO_2$ produced by the palm oil mills from its processes and from the utilisation of biogas to generate power can be reduced through sequestration by microalgae. These microalgae can be grown in palm oil mill effluent (POME) to biodegrade it.

Palm oil mill effluent (POME) in its original form, is a highly polluting waste water that contain thick brownish colloidal suspension and oil resulting from the palm oil mill processes. In 2004, the 381 palm oil mills in Malaysia produced 30 million tonnes of POME (Yacob et al., 2006). In the original design of the Felda Sg. Tengi palm oil mill, the POME in the microalgae pond feed was from the aeration pond of the POME treatment system. The biogas plant was a later addition, which was installed upstream of the first facultative pond, with the rest of the POME treatment system left as it was. In this biogas plant, the synthesis of biogas is from the digestion of organic substances and nutrients in POME by anaerobic bacteria.

The effluent of the biogas plant is then treated further in the old treatment system of Felda Sg. Tengi Palm Oil Mill which begins with the first facultative pond. At this point, some of the fresh POME from palm oil mill processing by-passes the biogas plant and flows directly into the first facultative pond. This may be due to the high generation rate of POME and limited capacity in the biogas plant. From the first facultative pond, the POME is then treated in the series of facultative ponds for several weeks. After that, the POME flows through the drain to the aeration pond. From the aeration pond the POME will be fed to the microalgae pond, and finally to the polishing pond before being discharged into open water bodies.

In the biogas plant, the strict anaerobic bacteria is an agent that synthesises biogas by utilising organic substances in a biodegradation process without the presence of oxygen. The economic feasibility of biogas synthesis led Felda Sg. Tengi Palm Oil Mill to install and run the biogas plant. The availability and abundance of biodegradable organic material in POME which is cheap, makes POME the selected raw material for use in biogas plant (Rahayu et al.,2015).

The maintenance cost for the biogas plant is also inexpensive. Methane makes up 55% of the biogas produced in closed anaerobic digesters with POME as feedstock (Sulaiman, 2007). The combustion of biogas with oxygen can be used to drive a gas turbine to generate electricity, which can be used in the palm oil mill. Alternatively, the biogas obtained from the

anaerobic digester of the biogas plant can be compressed in tanks and sold off, as in the case of Sg. Tengi Palm Oil Mill, to be used as a fuel for vehicles (Rahayu et al.,2015) .

The utilisation of methane through combustion will synthesise high amounts of $CO_2$. The emission of $CO_2$ will give a negative environmental impact called the greenhouse effect. To reduce the greenhouse effect to the environment, the $CO_2$ produced needs to be captured. The $CO_2$ capture and storage (CCS) is a procedure that trap and separate $CO_2$ from the industrial waste gas produced from the combustion of methane. The $CO_2$ is compressed and transported to a storage location for long-term isolation from the atmosphere. The ability of microalgae to capture $CO_2$ in photosynthesis under sunlight at 94% efficiency (Sayre, 2010), and the current use of microalgae by Felda Sg. Tengi Palm Oil Mill to bioremediate their POME after the aeration pond, makes it a choice to be studied for $CO_2$ capture by photosynthesis.

Microalgae have a photosynthetic productivity of 10–20% as compared to 1–2% of most terrestrial plants (Singh et al., 2005). It can be cultivated in wastewater and grows quite well as its required nutrients are abundantly available in wastewater. By performing photosynthesis, the microalgae can reproduce and grow in a short time and the duration for the experiment to grow microalgae in batch is taken as 7 days (Sheehan et al., 1998). In order to grow in photosynthetic mode, it also needs light and dissolved $CO_2$, which makes it as an aquatic carbon capture. $CO_2$ exists in lagoons, in non-gaseous form as bicarbonate and is utilised for algal growth (Sayre, 2010). $CO_2$ production in the palm oil mill varies with the variation in the combustion processes in the mill. This variation in $CO_2$ supply will give different $CO_2$ concentrations which in turn might have different effects on microalgae growth and $CO_2$ capture. Therefore, to ensure that microalgae can perform photosynthesis with the optimum $CO_2$ supply to the POME, the $CO_2$ concentration ($X_1$) in the supply to the POME medium need to be optimised by submitting it as a variable in a factorial design experiment. Also, in nature, microalgae grow in the upper layer of the pond, where it can receive sunlight to growth. The microalgae in the lower layer of the pond will not get much sunlight permeating through, which will cause failure in performing photosynthesis. Thus, the mixing in the pond or the culture system will ensure that all microalgae are receiving light. In addition, POME and microalgae need to be homogenised as the culture system contain nutrients for microalgae uptake, so the mixing system will help the microalgae to take-up nutrients by limiting the film boundary layer encompassing the cell. In this optimisation of microalgae growth, the mixing system is represented by the sparging rate of air-$CO_2$ mixture ($X_2$) in the cultivation flask. In this experiment all the microalgae in the cultivation flask receive equal amount of light.

## 2.0 Methodology

### *2.1 Materials*

*Microalgae species:* The microalgae *Chlorella sp*. is selected for $CO_2$ sequestration due to its high resistance to $CO_2$ which is up to 40%. For every 1 kg dry weight of microalgae produced, approximately 1.83 kg of $CO_2$ is required for the photosynthesis process (Brennan and Owende, 2010). An inoculum was grown at ambient temperature in Bold's Basal Medium (BBM) in a cultivation flask with 10, 000 lux of light supplied by fluorescent lamps and with constant sparging rate of air-$CO_2$ mixture (Andersen, 2005). The species was left to grow in the BBM for 4 days to ensure that the microalgae growth is at exponential phase.

*Bold's Basal Medium:* Bold's Basal Medium (BBM) was used as medium for microalgae inoculum growth. The BBM was prepared by mixing several nutrients, chemical solution and trace metals solution (Ahmad, 2015) .

*POME samples:* The POME samples were taken from the feed drain into the microalgae pond and from the facultative pond of the Felda Sg. Tengi Palm Oil Mill which are stored in a chiller at temperature of 4 °C.

### *2.2 Gas mixing system*

The $CO_2$ supplied were mixed with compressed air at pressure of 2 bar using pressure regulators. The sparging rates of the gas mixture to the medium in the flask were as in the factorial design, as representing agitation (Ahmad et al., 2015).

### 2.3 Lighting

The lighting chamber was supplied with 10,000 lux of lighting by fluorescent lamps. The light intensity was determined previously (Ahmad et al., 2015).

### 2.4 Centrifugation

Measured samples (~50 ml) obtained from the cultivation flasks were centrifuged twice at 10,000 rpm for 5 minutes each, with the supernatant separated each time and resuspending in distilled water. Then, the sediments were dried for 24 hours at 110 °C in an oven. The dried biomass was then weighed.

### 2.5 Microalgae cultivation

POME from the feed of the microalgae pond of Felda Sg. Tengi Palm Oil Mill, supplements consisting of POME from the first facultative pond of Felda Sg. Tengi Palm Oil Mill, and the prepared inoculum were mixed at volumes of 700 ml, 200 ml, and 100 ml respectively. The microalgae were cultivated in 1 L conical flasks in the lighting chamber with 10,000 lux lighting from fluorescent lamps. The flasks were fitted with silicone rubber stopper, equipped with inlet for sparging gas mixture reaching the bottom of the medium, and outlet for waste gases. The $CO_2$ and air were mixed in a gas mixing system and were connected to the sparging tube that sparged the gas mixture into the cultivation flask at a specific rate which can achieved by a control valve. The $CO_2$ concentrations and sparging rates used, were taken from the design of experiment based on factorial design. The microalgae were grown in the flask for one complete run in 7 days. Samples (~ca. 50 ml) were taken from the flask at 12 hours intervals. Each of the 50 ml sample was centrifuged twice at 10,000 rpm for 5 minutes. Then, it was dried in the oven at 110 °C for 24 hours before being weighed on weighing scale.

### 2.6 Mathematical method

*Factorial design:* This is the method used to design the experiments (Cochran and Cox, 1992). Two variables that were chosen namely, the $CO_2$ concentration in the gas mixture and the sparging rate. Each variable was assigned as variable $X_1$ and $X_2$, respectively and varies at two levels for use in the $2^2$ factorial experiments. For this design, there are 4 experiments that were run in duplicate for 7 days, as in Table 1.

*Logistic equation:* The Monod model is normally used to model biomass growth, but it considers only the effect of substrate limitations. For this experiment, the logistic equation was used to model the batch growth of microalgae biomass as it considers the effect of other limitations too (Maryam Ismail, 2010).

$$\frac{dx}{dt} = \mu_m x - \frac{\mu_m x^2}{x_m} \quad (1)$$

where $x$ is biomass concentration (g/l), $x_m$ is maximum biomass concentration (g/l), $\mu_m$ is the maximum specific growth rate. Integrating Eq. (1) and rearranging to obtain Eq. (2):

$$x = \frac{x_o x_m e^{\mu_m t}}{x_m - x_o + x_o e^{\mu_m t}} \quad (2)$$

Data from each experiment run were fitted with Eq. (2) to predict $x_m$ and $\mu_m$ using software Matlab R2013a.

*Linear regression:* The data of maximum biomass concentration and maximum growth rate were each then fitted with a linear regression as a function of the experimental variables.

$$y_n = a_o + a_i X_{in} + a_{i+1} X_{i+1} \quad (3)$$

where, $y_n$ is the maximum biomass yield or maximum growth rate of each experimental run, $X_{in}$ is the level of the $i^{th}$ experimental variable in the nth experimental run, $a_0$ is constant, $a_i$ is coefficient of the $i^{th}$ experimental variable.

*Composite design:* The factorial design was complemented with a centre point and the star points as in Table 2 to become a composite design which allows for curvature (Cochran and Cox, 1992).

*Quadratic response surface*: The composite design was then fitted with a quadratic equation (Eq. (4))to study the response surface and determine the theoretical maximum point.

$$y_n = b_o + \sum_{i=1}^{2} b_i X_{in} + \sum_{i=1}^{2} b_{ii} X_{in}^2 + \sum_{i=1}^{2} b_{ij} X_{in} X_{jn} \quad (4)$$

where $b_o$ is constant, $b_i$ is coefficient of the $i^{th}$ experimental variable, $b_{ij}$ is the coefficient of the square of th $i^{th}$ variable, and $b_{ij}$ is the coefficient of the product of the $i^{th}$ experimental variable and the $j^{th}$ experimental variable. The coefficients of the

quadratic equation are estimated using Design Expert 10.0.6 software.

**Table 1**: Factorial design experiment with its levels of variables.

| Run | $X_1$ | $X_2$ | $CO_2$ concentration (%) ($X_1$) | Sparging rate (v.v.m) ($X_2$) |
|---|---|---|---|---|
| 1 | -1 | -1 | 12 | 0.8 |
| 2 | +1 | -1 | 20 | 0.8 |
| 3 | -1 | +1 | 12 | 1.0 |
| 4 | +1 | +1 | 20 | 1.0 |

**Table 2**: Composite design with levels of variables in the experiment.

| Run | $X_1$ | $X_2$ | $CO_2$ (%) concentration ($x_1$) | Sparging rate (v.v.m) ($x_2$) |
|---|---|---|---|---|
| 5 | -1.414 | 0 | 10.34 | 0.9 |
| 6 | +1.414 | 0 | 21.65 | 0.9 |
| 7 | 0 | +1.414 | 16 | 0.76 |
| 8 | 0 | -1.414 | 16 | 1.04 |

## 3.0 Results & Discussions

### *3.1 Factorial design.*

From the factorial design, 4 experiments were run in duplicate. Data of each run of the $2^2$ factorial design were fitted with the logistic equation, giving a fit as exemplified by Fig. 1 for run 1, to obtain the values of $x_m$ and $\mu_m$ for every experiment as in Table 3.

The stationary phase must have been reached in each microalgae batch growth in order to obtain an accurate prediction of $x_m$. From the experimental results, it can be seen that some of the runs have not reached the stationary phase by 7 days.

The linear Eq. (3) was fitted to the data of maximum biomass yield $x_m$ in table 3 as a function of $X_1$ and $X_2$ giving values of coefficients of the linear equation as in Eq. (5).

$$x_m = -1.507 + 0.04244X_1 + 1.679X_2 \quad (5)$$

The magnitude of the coefficient of $X_1$($CO_2$ concentration) is very small compared to the intercept, indicating that the surface is a plateau with respect to $X_1$. Although the magnitude of the coefficient of $X_2$ (agitation) is big compared to the intercept, it was decided to complement the $2^2$ factorial experiment with the necessary experimental points to make it a composite design since variable $X_2$ (agitation) is related to mixing and the value of $X_2$ at which mixing begins to have adverse effect (by damaging the microalgae) might be very high.

*Composite design*: The values of $x_m$ and $\mu_m$ obtained by fitting the logistic model to the batch growth data of each of the 4 runs (in duplicate) of the additional points making the composite design are shown in Table 4.

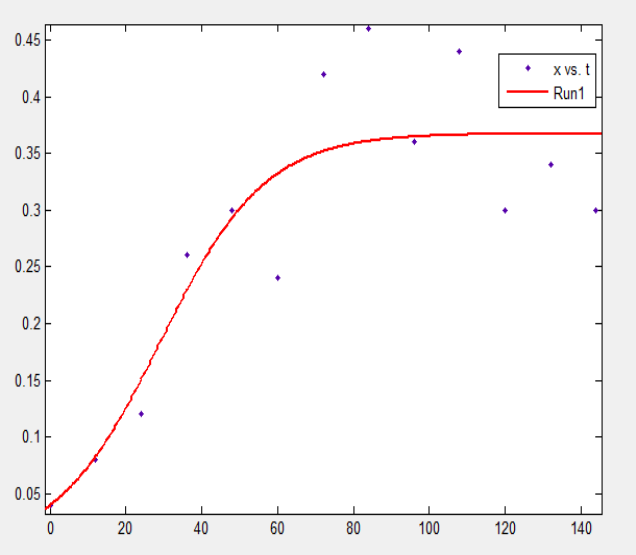


**Fig. 1**: Logistic model for data of run 1 with the variables set $X_1$= 12% $CO_2$ concentration and $X_2$= 0.8 vvm.

**Table 3**: Factorial design experiment result.

| Run | $X_1$ (%) | $X_2$ (vvm) | $x_m$ (g/l) | $\mu_m$ ($h^{-1}$) |
|---|---|---|---|---|
| 1 | 12 | 0.8 | 0.3676 | 0.07246 |
| 2 | 20 | 0.8 | 0.6637 | 0.05319 |
| 3 | 12 | 1.0 | 0.6601 | 0.03365 |
| 4 | 20 | 1.0 | 1.0430 | 0.03698 |

**Table 4**: Results of composite design.

| Run | $X_1$ (%) | $X_2$ (vvm) | $x_m$ (g/l) | $\mu_m$ ($h^{-1}$) |
|---|---|---|---|---|
| 5 | 10.34 | 0.9 | 0.7171 | 0.03445 |
| 6 | 21.65 | 0.9 | 1.846 | 0.02623 |
| 7 | 16 | 0.76 | 0.8217 | 0.03131 |
| 8 | 16 | 1.04 | 1.02 | 0.06657 |

*Quadratic response surface.* The combined results of the factorial design experiments and the additional experiments making the composite design were then fitted with a quadratic regression model to study the response surface and find the maximum point for $x_m$. The quadratic regression model was fitted to the data using design expert 10.0.6 software to give values of coefficients as in equation (6).

$$x_m = -2012.13199 + 41.36541\,X_1 + 3828.37046X_2 - 1.29217X_1^2 - 2126.68872\,X_2^2 + 0.054250\,X_1X_2 \quad (6)$$

The coefficients of quadratic regression equation (Eq. (6)) were used to find the coordinates of the point of the maximum yield using matrices (Himmelblau, 1970) as in Eq. (7).

$$X_{max} = -\left(\frac{1}{2}\right) B_{ii}^{-i} B_i^T \quad (7)$$

where $B_{ii} = \begin{bmatrix} b_{11} & \frac{b_{12}}{2} \\ \frac{b_{12}}{2} & b_{22} \end{bmatrix}$, $B_i = [b_1, b_2]$

$X_{max}$ is the matrix that gives the coordinates $X_1$ and $X_2$ of the maximum biomass concentration $x_m$.

From Eq. (7) the coordinates of the predicted maximum point are $X_1$=16.03% $CO_2$ concentration and $X_2$= 0.90 vvm agitation. The theoretical maximum biomass concentration, $x_m$ was found by putting the values of $X_1$ and $X_2$ at the maximum point into Eq. (7) to be at 42.6158 g/L. This is shown in Fig. 2 and 3. The regression performed by design expert software (Design Expert 10.6) converged but the optimised value of theoretical $x_m$ is too high, although the values of the coordinates for $X_1$ and $X_2$ are in the range of the experimental design. This may happen due to the experimental errors that have affected the results.

The prediction of linear regression model previously is accepted as the predicted maximum point of $x_m$ exists as shown in the quadratic response surface. From the result of optimization in Fig. 2 and Fig. 3, the area of response surface in red colour indicates that the maximum point is in the area of experiment from $2^2$ factorial design. The maximum point from $2^2$ factorial design is shown as in Fig. 2.

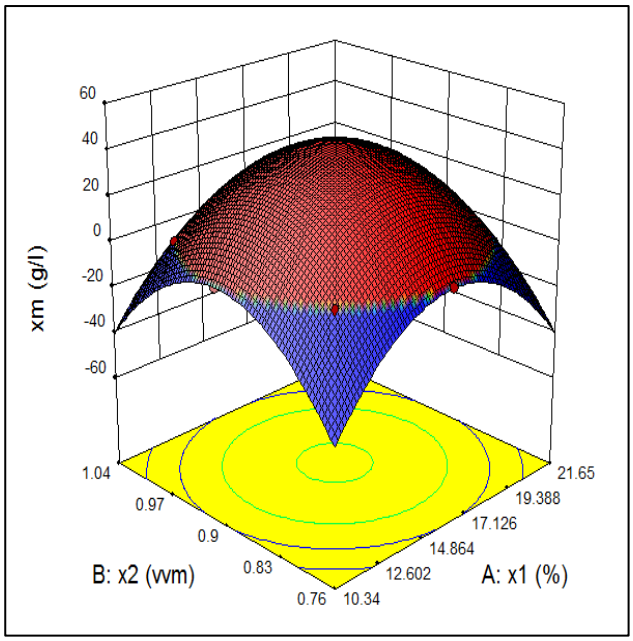


**Fig. 2:** Response surface of quadratic regression of maximum biomass concentrations.

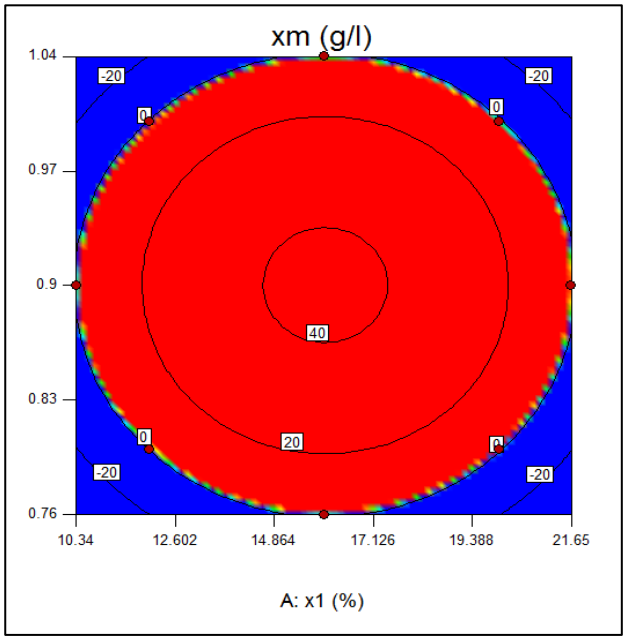


**Fig. 3**: The contour of the 3D graph of figure 2, showing the coordinates of $X_1$ $X_2$ at maximum biomass $x_m$.

The study of composite design that resulted from complementing the factorial design with the necessary points are shown in the area of response surface coated with the blue colour in Fig. 2 and 3.

From the estimation by composite design, the curvature shown indicates that there is no other maximum point existing outside the range of the levels $2^2$ factorial design. Therefore, the predicted maximum point of $x_m$ of 42.6158 g/Lat predicted coordinates of $X_1$= 16.03% $CO_2$ concentration and $X_2$= 0.90 vvm agitation is unique.

## 4.0 Conclusions

The objective of the experiment was to find the optimum level of dissolved $CO_2$ and the optimum level of agitation for maximum microalgae growth and $CO_2$ sequestration in POME from the effluent of Biogas Plant of Felda Sg. Tengi Palm Oil Mill. This objective has been achieved. The predicted maximum point of $x_m$ from composite design appears to be too high to be true, but it could not be experimentally tested due to time constrain. Nevertheless, it has been proven that microalgae sequester $CO_2$ in photosynthetic growth in POME from the effluent of Biogas Plant of Felda Sg. Tengi Palm Oil Mill to produce biomass. This finding has the potential of being useful in eliminating fully the $CO_2$ produced by Palm Oil Mills, besides remediating POME towards achieving zero BOD in the design of zero-waste Palm Oil Mills of the future and producing microalgae biomass as a side product.

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