

Growth Kinetics for Microalgae Grown in Palm Oil Mill Effluent (POME) medium at various CO₂ Levels

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Abstract. This paper sought to find the growth kinetic data of maximum specific growth rate ($\mu_{\max}$) and substrate saturation constant (K_s) for a microalgal reaction system over various dissolved CO₂ levels (0.04, 0.1, 0.3, 0.5, 0.8, 1.0, 5.0, 10.0% v/v) at a constant sparging rate of 1.2 vvm, by using logistic model and Monod kinetics. The reaction system consisted of microalgae growing in palm oil mill effluent (POME) medium in 1 L flask with constant light illumination and sparged with the specified CO₂ gas mixture. It is found from the experimental works that the values of $\mu_{\max}$ and K_s to be at 0.04958 h⁻¹ and 0.03523% (v/v) respectively. The results also showed that utilizing CO₂ levels (v/v) in the sparging gas mixture more than 1% (v/v) would not improve microalgae growth significantly as expressed in the values of specific growth rate μ . These data and information are critically important for bioreactor scaling up purposes, especially bioreactor system dedicated for microalgae products and CO₂ sequestration.

1. Introduction

The prospects of incorporating microalgal system into the palm oil industry for residual nutrient removal in palm oil mill effluent (POME) have been rigorously investigated, and the results are positively encouraging [1]. While the application of microalgae for bioremediation may be enticing, its potential for CO₂ sequestration is equally important to realize the zero-waste palm oil mill, where all greenhouse gases (GHG) produced are fully captured, and all the wastewater is fully recycled. POME can be used to generate renewable bioenergy such as biomethane and biohydrogen through anaerobic digestion [2]. May et al. [3] estimated that a palm oil mill with processing capacity of 60 tonnes/hr can generate a net profit of RM 3.8 mil per year from electricity generation using biogas produced from POME treatment. However, the final GHG discharged from the use of biomethane as fuel, carbon dioxide (CO₂), needs to be captured, along with all CO₂ produced elsewhere in the mill. Thus, it is envisioned that a microalgal system be incorporated into palm oil industry where bioremediation of residual nutrient in POME is coupled with sequestration of waste CO₂ gas [2,4].

Previous work by Adibah et al. [4] used factorial experimental designs to evaluate the microalgae biomass yield and the CO₂ recovery by microalgae grown in POME medium. They found that both the experimental variables of gas-mixture sparging rate (vvm) (representing mixing intensity) and the level of CO₂ (% v/v) in the CO₂-air gas mixture (approximating dissolved CO₂ – DCO₂) have significant main effects on biomass yield. Their conclusion adds to the evidence that aeration rate is proven to have influenced microalgal growth to a substantial extent [5,6] and the volume percentage of CO₂ in the sparging gas mixture can be increased to overcome the gas-liquid mass transfer limitation experienced in systems with inherently poor mass transfer capacity [6,7]. In this paper, we sought to find the overall maximum specific growth rate ($\mu_{\max}$) of the microalgae growing in POME medium over various levels of CO₂ at a constant sparging rate. The DCO₂ can be approximated from the respective levels of CO₂ content (% v/v) in the sparging gas mixture, because partial pressure of a gas



corresponds to its solubility in a liquid as predicted by Henry's law [8]. Determining $\mu_{\max}$ is of great importance in this case because it can be used for evaluating cell growth performance; which is especially useful in designing bioreactor systems. In addition, the kinetic data and information from this study might present valuable insights on the reaction system as well as imperative industrial implications.

2. Materials and methods

2.1. Microalgae culture

Chlorella sp. with identification number of UMACC324 was obtained from University of Malaya Algae Culture Collection (UMACC). The culture was maintained in 1 L flask in Bold's Basal Medium (BBM) [9] at ambient temperature with sterile air pump aeration.

2.2. Palm oil mill effluent (POME) sample

POME used in this experiment was obtained from the aeration pond of Sime Darby Bhd Mill (2°30'52.1"N101°53'34.3"E) in Sua Betong, Negri Sembilan, Malaysia. The POME was stored at 4°C until being used.

2.3. Experimental Set-up

The gas mixing system used in this study combined the air flow and gaseous CO₂ flow to make the gas mixture sparged into the culture. The pressure of each of the air flow and the CO₂ flow was regulated to be at 2 bars and the flow rate for each flow was controlled by a flow controller and measured by a flowmeter (model N082-03, Cole-Parmer, IL, USA) to give the required CO₂ content (% v/v) in the sparging gas mixture. The CO₂-air mixture entered the culture system from the bottom of the flask through a sparging tube with diameter of 1 mm. The stopper of the flask culture has one input tube for sparging and one output tube for venting. The concentration of DCO₂ in this study was approximated based on the CO₂ content (% v/v) of the sparging gas mixture, since it is recognized that the partial pressure of CO₂ gas will determine the solubility of the gas in the medium. In this regard, the composition of CO₂ in air was taken to be representative of the partial pressure of CO₂ as Dalton's law dictates that the percentage by volume of a gas in mixture corresponds with partial pressure it exerts [8]. The limitation of this approximation is in so far as that equilibrium is assumed to be achieved between DCO₂ and the CO₂ gas in the bubbles. The lighting chamber was an open-top box that was equipped with fluorescent bulbs on the inside walls that provided the light intensity of 10,000 lux. Figure 1 shows the experimental set-up for this study.

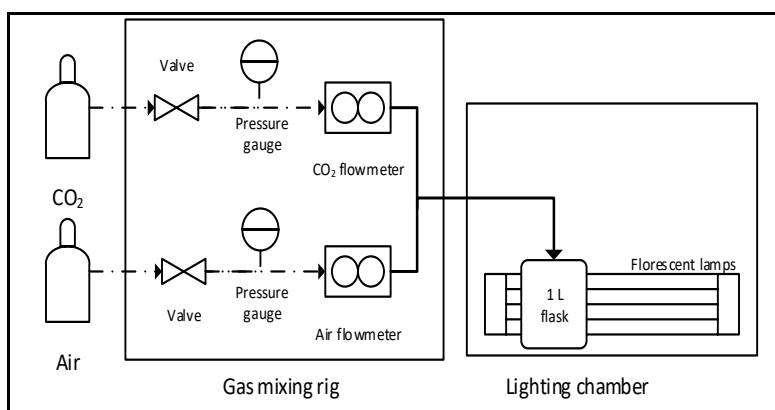


Figure 1. Experimental set-up for batch cultivation of microalgae in POME medium in 1 L flask with CO₂ supplementation. The set-up consists of a gas mixing rig which provides specified gas mixture of CO₂-air into a 1 L flask placed in a chamber with 10,000 lux illumination capacity.

2.4. Batch Cultivation of Microalgae in POME Medium in 1 L Flask

The medium was prepared by diluting 500 mL of unsterilized POME sample with 400 mL of distilled water [4]. A 10% (v/v) inoculum was then added, making the total volume of 1 L. The three-day old inoculum was prepared by the same technique as per microalgae maintenance procedure. Batch cultivation in 1 L flasks were carried out in duplicate. The flasks were placed in the lighting chamber and the cultivation proceeded up to seven (7) days. At interval of 12 hours, 30 mL sample was withdrawn, with a maximum of seven samples being drawn from a particular flask before the flask was discarded, and the sampling started on a new identical flask in the duplicate. The sample was centrifuged at 10,000 rpm for 15 minutes where the supernatant is discarded. The remaining pellet is then re-suspended in distilled water of the same volume and oven-dried in porcelain crucible at 100°C over 24 hours, and weighed to determine the cell dry weight (CDW) of the biomass.

2.5. Logistic Modeling

The logistic equation [10,11] was used to model the biomass growth profile of the microalgae in batch culture is shown in equation (1) and its integration gives equation (2); where X is actual biomass concentration (g/L), X_m is the maximum cell concentration (g/L), and μ is the specific growth rate (hr^{-1}) of the microalgae at the given level of CO_2 content. Denoting the actual microalgal biomass concentration at the beginning of the cultivation ($t=0$) as X_0 , the logistic equation (2) was fitted to the data of cell concentration X in time t from each batch cultivation experiment using curved fitting tool in MATLAB® R2013a (Mathworks, Natick, MA). Confidence bounds of 95% were taken into consideration to find the fit.

$$\frac{dX}{dt} = \mu X \left(1 - \frac{X}{X_m}\right) \quad (1)$$

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

2.6. Determination of Kinetic Parameters of Overall $\mu_{\max}$ and K_s

The μ (hr^{-1}) of the microalgae at a given level of CO_2 content increases with increasing level of CO_2 content, until the level of CO_2 content which gives the highest value of μ has been reached. Further increase in the level of CO_2 content beyond this point will not cause any increase in the value of μ . This highest value of μ is called the overall maximum specific growth rate (hr^{-1}) or $\mu_{\max}$. The saturation constant K_s is a constant in the Monod equation (3) [11] which has a value of half the value of the concentration of DCO_2 corresponding to the level of CO_2 content first giving $\mu_{\max}$; and S in the equation is the DCO_2 as represented by the level of CO_2 content (% v/v) in the sparging gas mixture.

$$\mu = \frac{\mu_{\max} S}{K_s + S} \quad (3)$$

In order to study the kinetics of the batch growth of the microalgae in POME medium, eight (8) batch culture experimental runs were conducted at eight (8) different levels of CO_2 in the sparging gas mixture namely 0.04, 0.1, 0.3, 0.5, 0.8, 1.0, 5.0, and 10.0% (v/v) at 1.2 vvm constant sparging rate. The Monod equation was used to model the growth kinetics and to find the value of overall $\mu_{\max}$ and the value of K_s . It was fitted to the values of μ determined from each batch cultivation experiment using the same MATLAB® curve fitting tool as per logistic equation previously. Minimum doubling time $t_{d(\min)}$ is then determined using equation (4) [11].

$$t_{d(\min)} = \frac{\ln 2}{\mu_{\max}} \quad (4)$$

3. Result and discussion

The growth profile of cultivation of *Chlorella* sp. in POME medium was determined at eight (8) different levels of CO_2 in the sparging gas mixture of 0.04, 0.1, 0.15, 0.2, 0.3, 0.5, 1.0, 5.0, and 10.0%

(v/v). Table 1 gives the biomass concentrations of the culture from samples taken at 12 hr intervals for seven days from the 1 L batch cultivation runs under 10,000 lux fluorescent light illumination, using constant sparging at 1.2 vvm with gas mixture different levels of CO₂ as above. The biomass growth data (table 1) was fitted into the logistic equation (2) to give the values of μ and X_m for each specific run characterized by the level of CO₂. Table 2 presents the values of μ and X_m for each batch of the cultivations. These data was then used to generate a plot of specific growth rate (μ) vs. the corresponding set point of CO₂ levels (S) by fitting it with the Monod equation (3) to give two important kinetic parameters namely maximum specific growth rate (μ_{max}) and substrate saturation constant (K_s). This was also done with aid of curve fitting tools in MATLAB® software. The plot can be seen in figure 2.

Table 1. Experimental data of growth in time (h) of *Chlorella* sp. growing in POME medium at various CO₂ levels (% v/v) in air.

CO ₂ %	Biomass Concentration (g/L)													
	0 h	12	24	36	48	60	72	84	96	108	120	132	144	156
0.04	0.20	0.30	0.22	0.30	0.36	0.44	0.52	0.56	0.52	0.54	0.58	0.56	0.58	0.64
0.10	0.30	0.35	0.37	0.43	0.47	0.48	0.47	0.53	-	0.50	0.50	0.48	0.50	0.53
0.30	0.20	0.43	0.45	0.47	0.57	0.70	0.65	0.62	0.69	0.80	0.77	0.77	0.73	0.77
0.50	0.20	0.30	0.38	0.36	0.38	0.42	0.45	0.48	0.48	-	0.48	0.54	0.46	0.48
0.80	0.20	0.33	0.37	0.40	0.40	0.43	0.50	0.67	0.50	0.53	0.50	0.43	0.50	0.48
1.00	0.30	0.38	0.44	0.46	0.50	0.53	0.56	0.60	0.60	0.56	0.53	0.53	0.50	0.52
5.00	0.20	0.23	0.30	0.33	0.36	0.37	0.37	0.40	0.40	0.43	0.40	0.39	0.40	-
10.0	0.10	0.30	0.37	0.43	0.48	0.50	0.57	0.67	0.73	0.81	0.82	0.82	0.83	0.80

Table 2. Specific growth rate μ and maximum biomass concentration X_m of *Chlorella* sp. growing in POME medium at different CO₂ level obtained from logistic modeling [equation (2)].

CO ₂ (% v/v)	μ (h ⁻¹)	X_m (g/L)	Goodness of Fit, R^2
0.04	0.02507	0.6461	0.9338
0.10	0.03764	0.5099	0.9303
0.30	0.04361	0.7533	0.9178
0.50	0.04429	0.4866	0.8991
0.80	0.05461	0.5066	0.7092
1.00	0.05051	0.5499	0.8171
5.00	0.04396	0.4065	0.9724
10.0	0.04785	0.8079	0.8925

From the result in table 2, it can be seen that the logistic equation found to fit the experimental data reasonably well based upon the value of R^2 . Most batches have near to unity R^2 values with only a few batches giving comparatively lower values. Similarly, the values of X_m also varies over different CO₂ levels with the highest is observed at 0.8079 g/L at 10% (v/v) CO₂ set point and the lowest is recorded at 0.4065 g/L at 5% (v/v) CO₂ set point. The X_m indicates the carrying capacity of the culture which can be defined as the maximum algal biomass concentration that can be supported by the culture at the particular experimental conditions [10]. Hence, the fluctuating pattern of X_m that can be observed from the lowest CO₂ set point to the highest is reasoned due to the variation in the availability of carbon source in the form of DCO₂ in these experiments. In addition, POME characteristics vary considerably with respect to variation in feedstock supply from plantation, rainfall pattern, and changing pattern of operation of the mill [12]. The aeration pond POME used in the experiment was taken from the same mill which is Sime Darby Berhad Sua Betong Mill, but obtained at different times during the course of the experiment. The resulting variation in POME characteristics actually can be visually observed while conducting the experiment as one batch is darker than the previous one and vice versa. This surely influenced the growth profile of the microalgae growing in POME, in addition to the influence of the variation in the availability of carbon sources in the form of DCO₂, which together translated in

the fluctuating pattern of X_m . The same reason also applies for the lack of fit in plot of μ vs. S (figure 2) that gives R^2 of 0.8321.

The K_S for this study is observed at 0.03523% (v/v CO_2 in gas mixture). This value provides a guideline at which the CO_2 concentration should be maintained in the feed as it characterizes the CO_2 concentration required to reach half of the $\mu_{\max}$. On the other hand, minimum doubling time $t_{d(\min)}$ for this study is 13.9804 h that is determined based on equation (4). These kinetic data of $t_{d(\min)}$ and $\mu_{\max}$ of microalgae growing in POME medium shows how fast is the microalgae growing in the system. The kinetic data gained from the system essentially gave a noticeably higher rate when compared to other *Chlorella* strains. Take for example, *Chlorella vulgaris* and *Chlorella kessleri* have $\mu_{\max}$ of 0.01220 h^{-1} and 0.01071 h^{-1} respectively [13]. However, there are also other studies that showed their value of $\mu_{\max}$ to be much lower. For instance, Kumar et al. [14] found $\mu_{\max}$ for *Chlorella sorokiniana* to be at 0.1 h^{-1} . These disparities are caused by different cultivation systems being used since growth rates are very test system specific. Thus, it is reasonable for each experimentations with dissimilar cultivation medium and bioreactor configuration to give different $\mu_{\max}$ values.

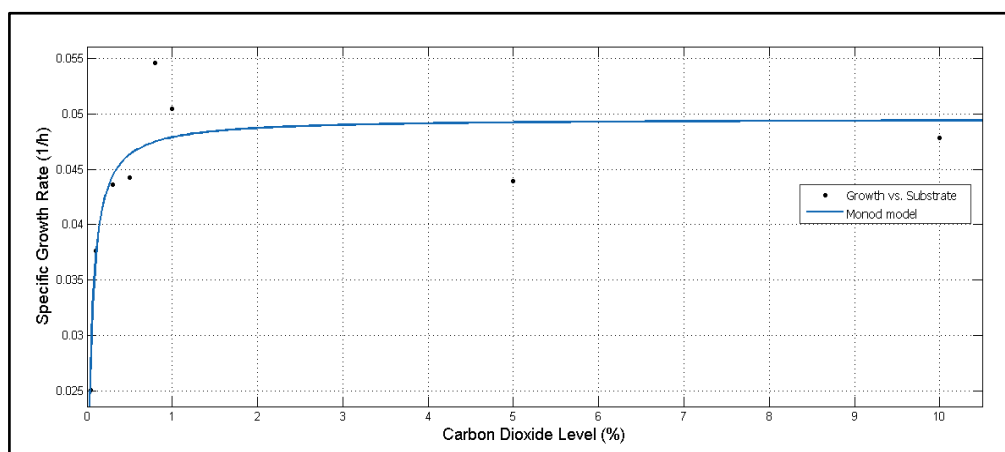


Figure 2. Plot of μ vs. CO_2 levels (S) obtained by fitting growth data against Monod equation (3). $\mu_{\max}$ and K_S found at 0.04958 h^{-1} and 0.03523% (v/v) respectively, with R^2 of 0.8321. The closed symbol ($\bullet$) denotes μ from logistic equation (2) (table 2) and solid blue line is the fitting curve.

Moreover, referring to plot of μ vs. S (figure 2), it is observed that the values of μ experienced a steep rise as the level of CO_2 increased from zero to 1% (v/v), followed by a very gentle slope as the level of CO_2 is increased further. So this implicates that, especially at industrial scale, for such a reaction system of microalgae growing in POME medium, it would not be economical to work at CO_2 levels higher than 1% (v/v), since it would not significantly improve the biomass performance as reflected in term of μ values.

4. Conclusion

The batch growth kinetics study conducted over various DCO_2 concentrations for a reaction system of microalgae growing in POME medium with 10,000 lux light illumination gave $\mu_{\max}$ and K_S at 0.04958 h^{-1} and 0.03523% (v/v) respectively. It is found that amid variations in POME characteristics due to varying environmental factors and processing conditions, the microalgae still able to grow very well, though it affects the μ values to some extent. It is also established that utilizing CO_2 levels higher than 1% (v/v) in the sparging gas mixture would not improve biomass performance as reflected in its μ values.

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References

- [1] Tao D G, Yaakob Z, Takriff M S, Salihon J and Abd Rahman M S 2016 Biomass production and nutrients removal by a newly-isolated microalgal strain *Chlamydomonas* sp in palm oil mill effluent (POME) *Int. J. Hydrogen Energy* **41** 4888–95
- [2] Lam M K and Lee K T 2011 Renewable and sustainable bioenergies production from palm oil mill effluent (POME): Win-win strategies toward better environmental protection *Biotechnol. Adv.* **29** 124–41
- [3] May C, Phaik P, Ti T, Eng C and Kit C 2013 Biogas from palm oil mill effluent (POME): Opportunities and challenges from Malaysia's perspective *Renew. Sustain. Energy Rev.* **26** 717–26
- [4] Ahmad A D, Salihon J and Tao D G 2015 Evaluation of CO₂ sequestration by microalgae culture in palm oil mill effluent (POME) medium *Adv. Mater. Res.* **1113** 311–6
- [5] Zhang K, Kurano N and Miyachi S 2002 Optimized aeration by carbon dioxide gas for microalgal production and mass transfer characterization in a vertical flat-plate photobioreactor *Bioprocess Biosyst. Eng.* **25** 97–101
- [6] Ryu H J, Oh K K and Kim Y S 2009 Optimization of the influential factors for the improvement of CO₂ utilization efficiency and CO₂ mass transfer rate *J. Ind. Eng. Chem.* **15** 471–5
- [7] Langley N M, Harrison S T L and van Hille R P 2012 A critical evaluation of CO₂ supplementation to algal systems by direct injection *Biochem. Eng. J.* **68** 70–5
- [8] Peavy H S, Dowe D R and Tchobanoglous G 1985 *Environmental Engineering* (Singapore: McGraw-Hill Inc.)
- [9] Andersen R A 2005 *Algal Culturing Techniques* (China: Elsevier Academic Press)
- [10] Kumar K and Das D 2012 Growth characteristics of *Chlorella sorokiniana* in airlift and bubble column photobioreactors *Bioresour. Technol.* **116** 307–13
- [11] Shuler M L and Kargi F 2002 *Bioprocess Engineering: Basic Concepts* (Pearson Education Inc.) p. 155
- [12] Cai T, Park S Y and Li Y 2013 Nutrient recovery from wastewater streams by microalgae: Status and prospects *Renew. Sustain. Energy Rev.* **19** 360–9
- [13] Zhao B and Su Y 2014 Process effect of microalgal-carbon dioxide fixation and biomass production: A review *Renew. Sustain. Energy Rev.* **31** 121–32
- [14] Kumar K, Dasgupta C N and Das D 2014 Cell growth kinetics of *Chlorella sorokiniana* and nutritional values of its biomass *Bioresour. Technol.* **167** 358–66