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GROWTH KINETIC STUDY OF *CHLORELLA VULGARIS* USING LAB-SCALE AND PILOT-SCALE PHOTOBIOREACTOR: EFFECT OF CO_2 CONCENTRATION

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Abstract

In the present study, growth kinetic of *Chlorella vulgaris* was performed when the microalgae was cultivated with different concentrations of CO_2. The experiments were carried out using lab-scale and pilot-scale photobioreactors, and the growth results were analyzed using POLYMATH 6.0 with different growth kinetic models. The growth of the microalgae was found fitted well to the Richards growth model with attainable high R^2 values as demonstrated in all studied cases, in concert with low values of root mean squares deviation (RMSD) and variance. In addition, the output from the plots of experimental values versus predicted values and residual plots further confirmed the good fit of Richards model. The predicted specific growth rate from Richards model was similar to the experimental specific growth rate with deviation lesser than 5%. The attained results paved a preliminary prediction of microalgae growth characteristic when the cultivation is scaled-up to commercial scale.

Keywords: CO_2, Microalgae, Growth kinetic, Biofuels, Photobioreactor.

1. Introduction

For decades, the insidious negative impact of global warming due to increasing of CO_2 concentration in the atmosphere has continued to hamper the ecosystem of human and environment. This includes the occurrence of melting of arctic ice that reduces the natural habitat of polar bears, rising of sea level resulted to the

Nomenclatures

A	Asymptotic of ln X_t/X_o as t decreases indefinitely
B	Relative growth rate at time M (day^{-1})
C	Asymptotic of ln X_t/X_o as t increases indefinitely
k	Shape parameter
M	Time at which the maximum growth rate is reached (day)
n	Number of observation
R^2	Coefficient of determination
S^2	Variance
t	Residence time (day)
X_t	Biomass concentration at time t ($g\ L^{-1}$)
X_o	Initial biomass concentration ($g\ L^{-1}$)

Greek Symbols

λ	Lag phase (Gompertz model)
τ	Lag phase (Richard model)
μ	Specific growth rate (day^{-1})
μ_{max}	Maximum specific growth rate (day^{-1})
v	Shape parameter

Abbreviations

calc	Calculated data
obs	Observed data
RMSD	Root mean square deviation

inundation of low-lying islands, and frequent occurrence of droughts and desertification [1, 2]. The consequences of all these adverse phenomenon have intensified the development of carbon capture technologies to further reduce the emission of CO_2 to the atmosphere.

To date, the cultivation of microalgae for simultaneous CO_2 bio-fixation and biofuel production have gained increasing attention from researchers around the globe. Microalgae possess high photosynthetic rate which allows it to bio-fix CO_2 in a rate of 10-50 times higher than terrestrial plants [3, 4]. To bring the advantage further, the lipid and carbohydrate derived from microalgae biomass can be converted to biodiesel and bioethanol, respectively, which serve as renewable fuels that emits lesser CO_2 than fossil-fuel when combusted in heat engine [5]. Furthermore, it was reported that microalgae cells contain approximately 50% of carbon content, in which 1.8 kg of CO_2 are fixed by producing 1 kg of microalgae biomass [6]. Hence, this method is considered as a more feasible way to reduce the CO_2 concentration released to the atmosphere than other technologies, such as cryogenic fractionation and geological sequestration, which required high level of investment and technology.

In the present study, *Chlorella vulgaris* was cultivated using lab-scale 5 L and 100 L pilot-scale photobioreactor under different concentration of CO_2. The experimental results (biomass yield) were further analyzed using established growth kinetic models to further simulate the growth of the microalgae. It is hoped that the simulated result from the present study will help to understand

the growth behavior of the microalgae, especially when the cultivation is scaled-up to commercial scale.

2. Materials and Methods

2.1. Cultivation of *Chlorella vulgaris*

The growth conditions of *Chlorella vulgaris* in 5 L lab-scale and 100 L pilot-scale photobioreactor were reported in previous published works [1, 7, 8].

2.2. Kinetic growth model

Five non-linear mathematical models, namely Logistic, Gompertz, Modified Gompertz, Baranyi and Richards models were used to validate the experimental data of biomass production by *Chlorella vulgaris*. The experimental data were referring to the biomass yield of *Chlorella vulgaris* under the supplement of different concentration of CO_2 in lab scale 5 L photobioreactor [1] and pilot scale 100 L photobioreactor [7].

2.2.1. Logistic model

The logistic model describes the growth of microbial based on the initial population density, time, growth rate and final population density [9]. The original logistic function model was developed by Pearl and Reed (1977) [10]. The logistic model is expressed as follow [10]:

$$y = \frac{A + C}{1 + \exp^{-B(t-M)}} \tag{1}$$

where A is the asymptotic of ln X_t/X_o as t decreases indefinitely, C is the asymptotic of ln X_t/X_o as t increases indefinitely, B is the relative growth rate at time M (day^{-1}), t is the residence time (day), M is the time at which the maximum growth rate is reached (day), X_t is the biomass concentration at time t (g L^{-1}) and X_o is the initial biomass concentration (g L^{-1}).

2.2.2. Gompertz model

Gompertz model has been widely used in literature and many kinetic data was derived from this model. The model is represented as follow:

$$y = A + C\exp^{-\exp[-B(t-M)]} \tag{2}$$

where A is the asymptotic of ln X_t/X_o as t decreases indefinitely, C is the asymptotic of ln X_t/X_o as t increases indefinitely, B is the relative growth rate at time M (day^{-1}), t is the residence time (day), M is the time at which the maximum growth rate is reached (day), X_t is the biomass concentration at time t (g L^{-1}) and X_o is the initial biomass concentration (g L^{-1}). From the Gompertz model, two important parameters namely lag phase (λ) and specific growth rate (μ), can be derived as shown below:

$$\lambda = M - \frac{1}{B} \tag{3}$$

$$\mu = \frac{B \times C}{e}, e = 2.7182 \tag{4}$$

2.2.3. Modified Gompertz model

The Gompertz model was modified by Zwuetering et al. (1990) [11] which include three important biologically relevant parameters: lag phase, maximum specific growth rate and maximum biomass concentration [11].

$$y = C \exp^{-\exp[\frac{\mu_{max} \exp(1)}{C}(\lambda - t) + 1]} \tag{5}$$

where C is the asymptotic of ln X_t/X_o as t increases indefinitely, t is the residence time (day), μ_{max} is the maximum specific growth rate (day^{-1}), λ is the lag phase (day), X_t is the biomass concentration at time t ($g\ L^{-1}$) and X_o is the initial biomass concentration ($g\ L^{-1}$).

2.2.4. Baranyi model

In the early development of the Baranyi model, variation in cell population with time is described by a first order differential equation [12]. The model was further improved by introducing six biologically parameters by [13], but were later reduced to three parameters (lag phase, maximum specific growth rate and maximum cell population) by Baranyi (1997) [14] as shown below [13, 14]:

$$y = \mu_{max} A(t) - \ln\left[1 + \frac{\exp^{\mu_{max} A(t)} - 1}{\exp^{c}}\right] \tag{6}$$

where

$$A(t) = t + \frac{1}{\mu_{max}} \ln\left(\exp^{-\mu_{max} t} + \exp^{-\mu_{max} \lambda} - \exp^{-\mu_{max}(t+\lambda)}\right) \tag{7}$$

where C is the asymptotic of ln X_t/X_o as t increases indefinitely, t is the residence time (day), μ_{max} is the maximum specific growth rate (day^{-1}), λ is the lag phase (day), X_t is the biomass concentration at time t ($g\ L^{-1}$) and X_o is the initial biomass concentration ($g\ L^{-1}$).

2.2.5. Richards model

Richards model is a four-parameters model as shown below [15]:

$$y = A\left(1 + \upsilon \exp\left[k\left(\tau - t\right)\right]\right)^{(-1/\upsilon)} \tag{8}$$

where A is the asymptotic of ln X_t/X_o, t is the residence time (day), ν and k are shape parameter whereas τ is time at the inflexion point (lag phase). The specific growth rate, μ (day^{-1}), can be determined based on the following equation:

$$\mu = \frac{k}{2(\nu + 1)} \tag{9}$$

2.3. Analysis of microalgae growth kinetic using POLYMATH 6.0

A nonlinear regression technique was used to solve the growth models by using POLYMATH 6.0. The routine applied for each of the model consisted of the following steps: (1) the values for ln X_t/X_o was calculated based on the experimental data, (2) the data was loaded to the POLYMATH program, (3) the respective growth model was then inserted to the POLYMATH program under the 'Nonlinear' column, (4) all the dependent, independent and model variables are shown in the programme after inserting the respective model and checked carefully, (5) initial estimates of the parameters values were required and, (6) several statistic indicators were used to assess the quality of the estimated values and the regression models, such as coefficient of determination (R^2), variance (S^2), root mean square deviation (RMSD), graph (experiment versus predicted), residual plot and confidence intervals. The following guidelines were used in determining the goodness of fit of the developed kinetic model:

(a) R^2: The coefficient of determination is always used as an indicator to represent the preciseness of model in fitting the experimental data. A coefficient of determination close to one implies the correctness of the model. The coefficient of determination can be calculated based on the following equation:

$$R^2 = 1 - \frac{\sum_{i=1}^{n} \left(y_{i_{obs}} - y_{i_{calc}} \right)^2}{\sum_{i=1}^{n} \left(y_{i_{obs}} - \bar{y} \right)^2} \tag{10}$$

where,

$$\bar{y} = \frac{1}{n} \left(\sum_{i=1}^{n} y_{i_{obs}} \right) \tag{11}$$

'n' is referred as number of observation, 'obs' is referred as the observed data and 'calc' is referred as the calculated data.

(b) S^2 and RMSD: Similar to coefficient of determination, these two indicators are frequently used for comparing the accuracy of different models that represent the experimental data. A model with smaller variance and RMSD indicates the data are more accurate than a model with larger values of these indicators. The following equations describe the calculation of S^2 and RMSD:

$$S^2 = \frac{\sum_{i=1}^{n} \left(y_i - \bar{y} \right)^2}{n - 1} \tag{12}$$

$$RMSD = \frac{1}{n}\left(\sum_{i=1}^{n}\left(y_{i_{obs}} - y_{i_{calc}}\right)^2\right)^2 \quad (13)$$

where 'n' is referred as number of observation, 'obs' is referred as the observed data and 'calc' is referred as the calculated data.

(c) Graph: A plot of the calculated and experimental values of the dependent variables is created. If the plots show different trends, it usually implies an inappropriate model.

(d) Residual plot: Residual plot shows the difference between the calculated and experimental values of the dependent variables as function of experimental values. If the regression model represents the experimental values correctly, the residuals (errors) should be randomly distributed close to the line of x-axis.

3. Results and discussions

Mathematical modelling has been widely used to predict the trend of cell growth through estimation of maximum specific growth rate, lag phase and maximum cell concentration, which are essentially required in the study of microbial growth and for the use in industrial microbiology [9, 16]. Table 1 shows the R^2 values for various mathematical models tested to represent the kinetic growth of *Chlorella vulgaris* at different concentrations of CO_2. From this table, Richards model shows the highest R^2 values for all studied CO_2 concentration, in which the model was well accepted for all the case scenarios. In addition, the low RMSD and variance values shown by the Richards model in Tables 2 and 3, respectively, also indicated that the model fitted well to the experimental data. This is further confirmed by the plot of experimental value versus predicted value as shown in Figs. 1 to 5 (0.5% CO_2 supplement). From Fig. 5, the growth of *Chlorella vulgaris* predicted by the Richards model displayed a similar trend as the experimental values, which reflects that the model is accurately sufficient to describe the biomass productivity from the microalgae.

Although other growth models such as Gompertz, Logistic, modified Gompertz and Baranyi displayed high R^2 (R^2>0.95) and low RMSD, the growth trends predicted by these models did not fit well with the experimental values (Fig. 1 to Fig. 4) as compared to the values predicted by Richards model. This can be further observed in the residual plots as shown in Fig. 6 to Fig. 10. Residual plot indicates the difference between the predicted and experimental value and a good residual plot should has no random distribution pattern and the values are close to the X-axis. From Fig. 6 to Fig. 9, Gompertz, Logistic, modified Gompertz and Baranyi models showed a significant sigmoidal curves distribution along the X-axis, which indicates the inadequateness of the models in fitting the experimental values. Although the Richards model also showed a sigmoidal curve (Fig. 10), however the plot was more converged to the X-axis as compared with other models. This observation reflects the errors predicted by the Richard model were smaller than other growth models and acceptable to represent the growth of *Chlorella vulgaris* in the present study.

Table 1. R^2 values of different growth model for *Chlorella vulgaris* cultivation in lab-scale and pilot-scale.

CO_2 concentration	Gompertz	Logistic	Modified Gompertz	Baranyi	Richards
5 L (Indoor - 0.03% CO_2)	0.9866	0.9801	0.9866	0.9338	0.9967
5 L (Indoor - 0.5% CO_2)	0.9782	0.9630	0.9782	0.9847	0.9977
5 L (Indoor - 1% CO_2)	0.9936	0.9648	0.9794	0.9836	0.9967
5 L (Indoor - 2% CO_2)	0.9809	0.9427	0.9599	0.9667	0.9959
5 L (Indoor - 5% CO_2)	0.9885	0.9576	0.9724	0.9731	0.9929
100 L (Indoor -0.03% CO_2)[1]	0.9843	0.9814	0.9843	0.9816	0.9846
100 L (Indoor- 5% CO_2)[1]	0.9942	0.9889	0.9942	0.9924	0.9944
100 L (Indoor- 0.03% CO_2)[2]	0.9872	0.9885	0.9872	0.9837	0.9888
100 L (Outdoor-0.03% CO_2)[1]	0.9881	0.9930	0.9881	0.9871	0.9930

Note: [1]With sequential baffled device; [2]Without sequential baffled device

Table 2. RMSD values of different growth model for *Chlorella vulgaris* cultivation in lab-scale and pilot-scale.

CO_2 concentration	Gompertz	Logistic	Modified Gompertz	Baranyi	Richards
5 L (Indoor - 0.03% CO_2)	0.1156	0.1408	0.1156	0.2569	0.0109
5 L (Indoor - 0.5% CO_2)	0.0266	0.0347	0.0266	0.0223	0.0087
5 L (Indoor - 1% CO_2)	0.0152	0.0355	0.0272	0.0243	0.0109
5 L (Indoor - 2% CO_2)	0.0253	0.0437	0.0366	0.0333	0.0118
5 L (Indoor - 5% CO_2)	0.0208	0.0400	0.0323	0.0319	0.0164
100 L (Indoor -0.03% CO_2)[1]	0.0240	0.0261	0.0240	0.0260	0.0238
100 L (Indoor- 5% CO_2)[1]	0.0151	0.0209	0.0151	0.0173	0.0148
100 L (Indoor- 0.03% CO_2)[2]	0.0203	0.0193	0.0203	0.0229	0.0190
100 L (Outdoor-0.03% CO_2)[1]	0.0176	0.0136	0.0176	0.0184	0.0135

Note: [1]With sequential baffled device; [2]Without sequential baffled device

Table 3. Variance values of different growth model for *Chlorella vulgaris* cultivation in lab-scale and pilot-scale.

CO_2 concentration	Gompertz	Logistic	Modified Gompertz	Baranyi	Richards
5 L (Indoor - 0.03% CO_2)	0.2139	0.3571	0.2139	1.0561	0.0025
5 L (Indoor - 0.5% CO_2)	0.0140	0.0257	0.0140	0.0098	0.0016
5 L (Indoor - 1% CO_2)	0.0049	0.0270	0.0146	0.0116	0.0025
5 L (Indoor - 2% CO_2)	0.0136	0.0407	0.0263	0.0219	0.0029
5 L (Indoor - 5% CO_2)	0.0092	0.0342	0.0205	0.0200	0.0058
100 L (Indoor -0.03% CO_2)[1]	0.0141	0.0176	0.0141	0.0165	0.0147
100 L (Indoor- 5% CO_2)[1]	0.0056	0.0113	0.0056	0.0073	0.0057
100 L (Indoor- 0.03% CO_2)[2]	0.0101	0.0097	0.0101	0.0129	0.0094
100 L (Outdoor-0.03% CO_2)[1]	0.0092	0.0056	0.0092	0.0099	0.0056

Note: [1]With sequential baffled device; [2]Without sequential baffled device

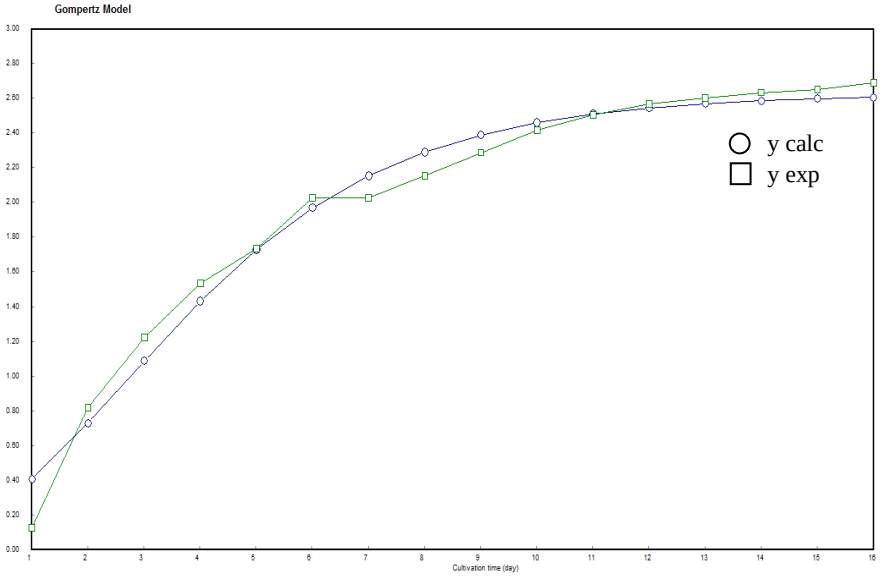

Fig. 1. Experiment versus predicted value (0.5% CO_2) for Gompertz model

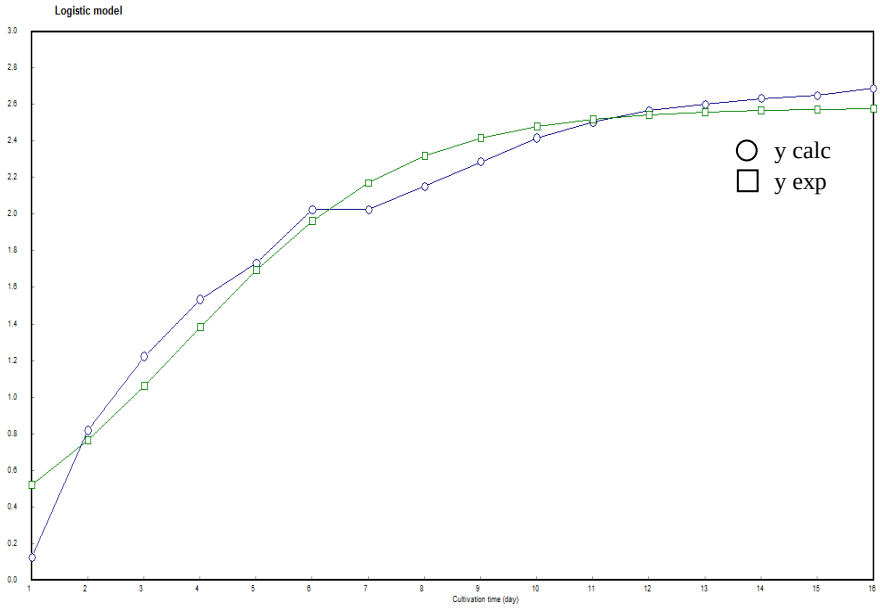

Fig. 2. Experiment versus predicted value (0.5% CO_2) for Logistic model

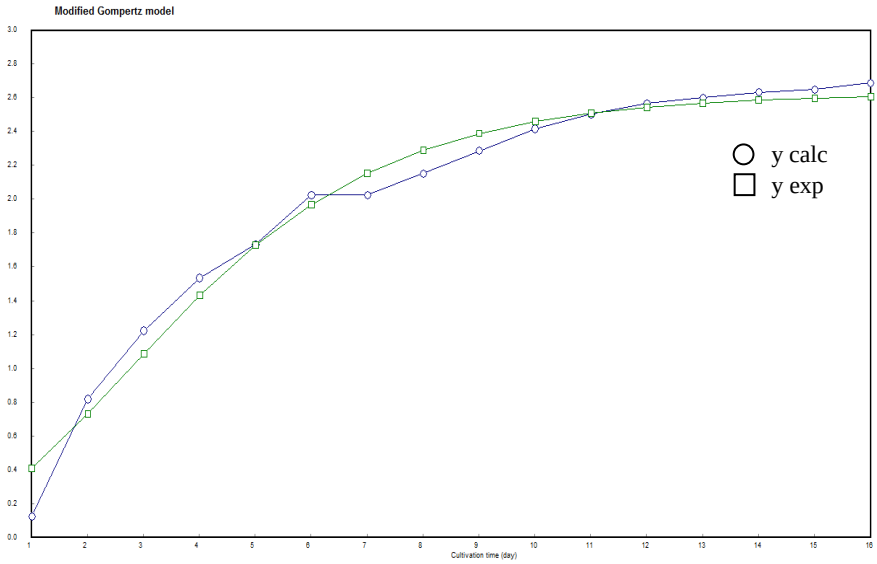

Fig. 3. Experiment versus predicted value (0.5% CO_2) for Modified Gompertz model

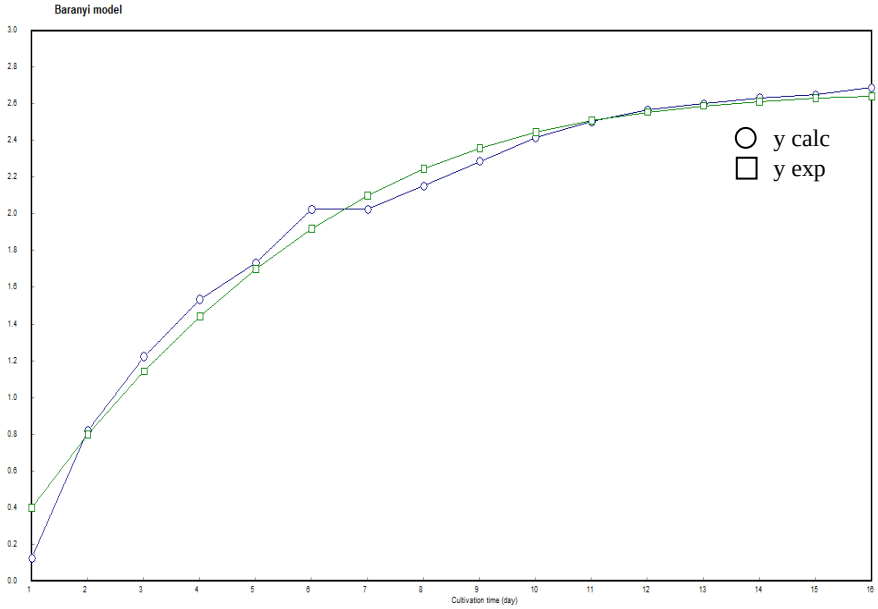

Fig. 4. Experiment versus predicted value (0.5% CO_2) for Baranyi model.

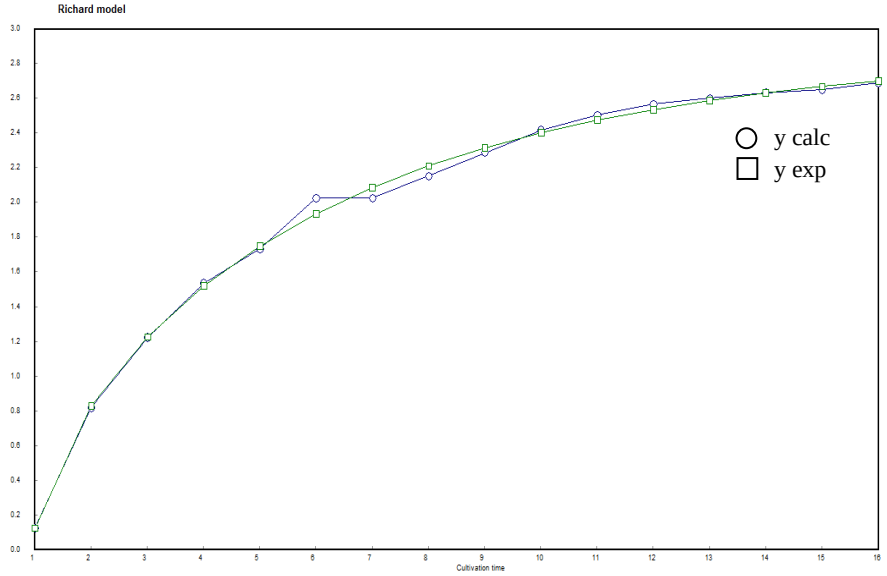

Fig. 5. Experiment versus predicted value (0.5% CO_2) for Richards model.

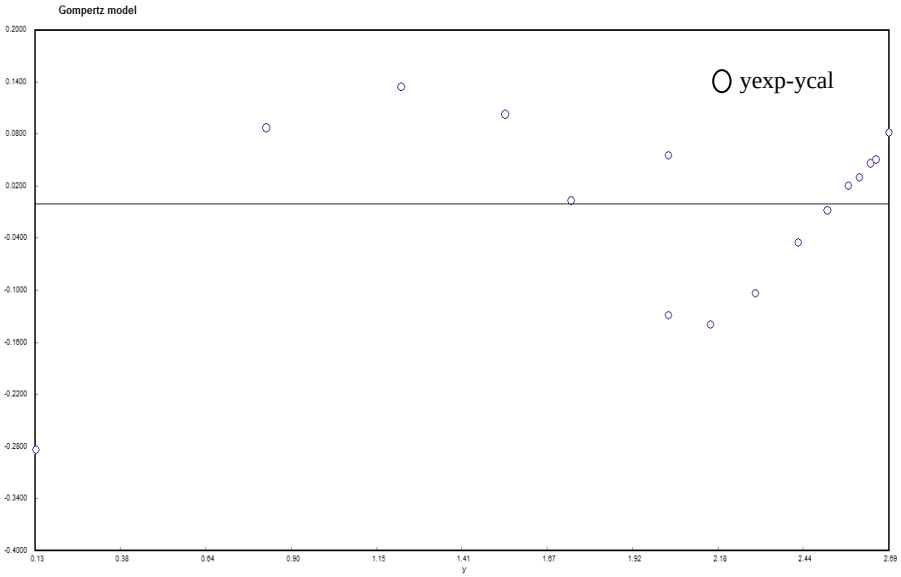

Fig. 6. Residual plots (0.5% CO_2) for Gompertz model.

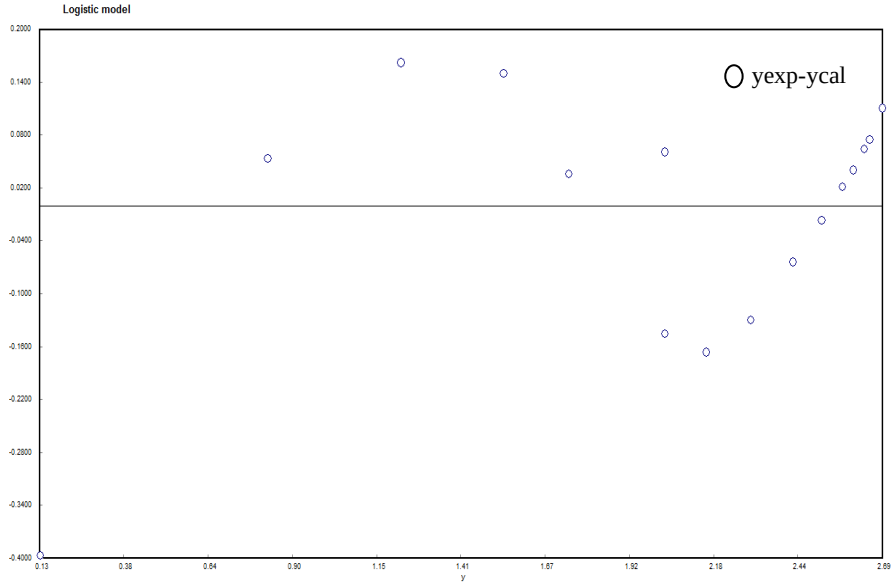

Fig. 7. Residual plots (0.5% CO_2) for Logistic model.

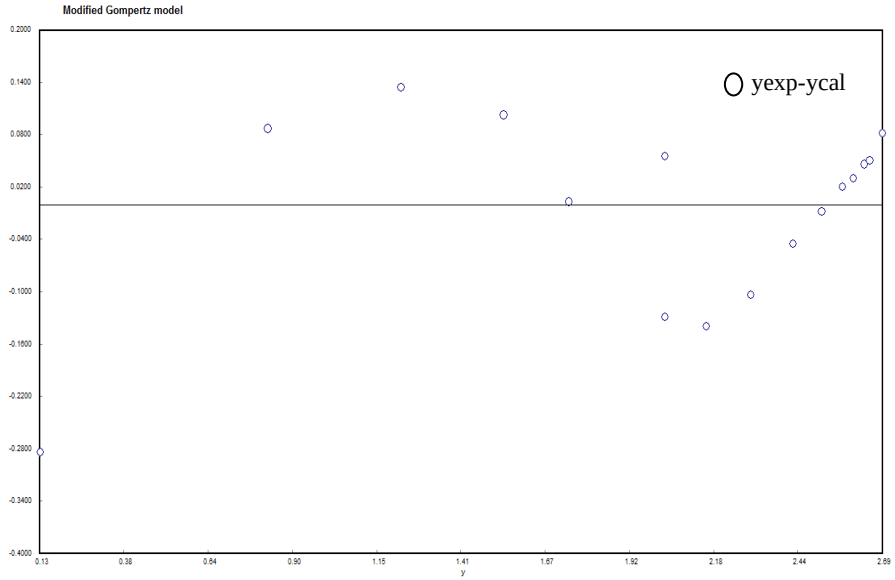

Fig. 8. Residual plots (0.5% CO_2) for Modified Gompertz model.

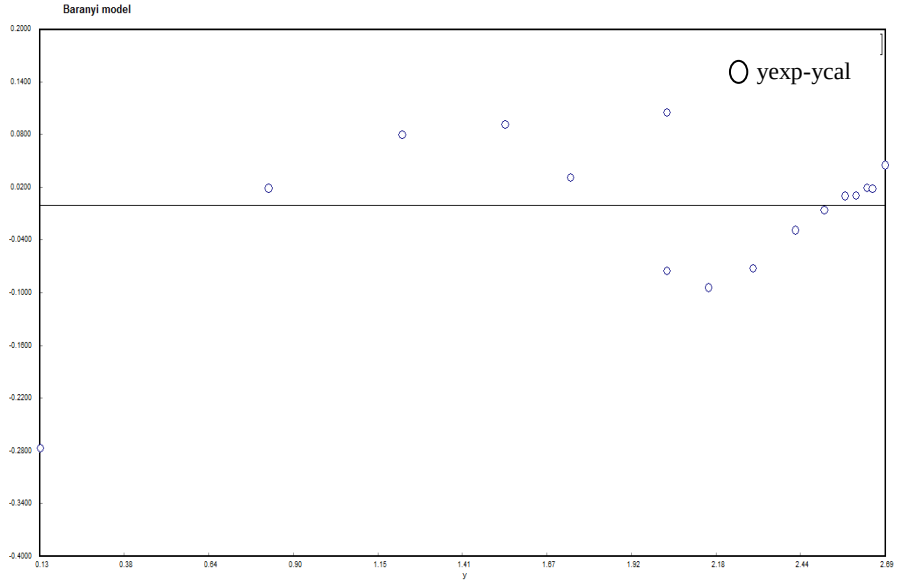

Fig. 9. Residual plots (0.5% CO_2) for Baranyi model.

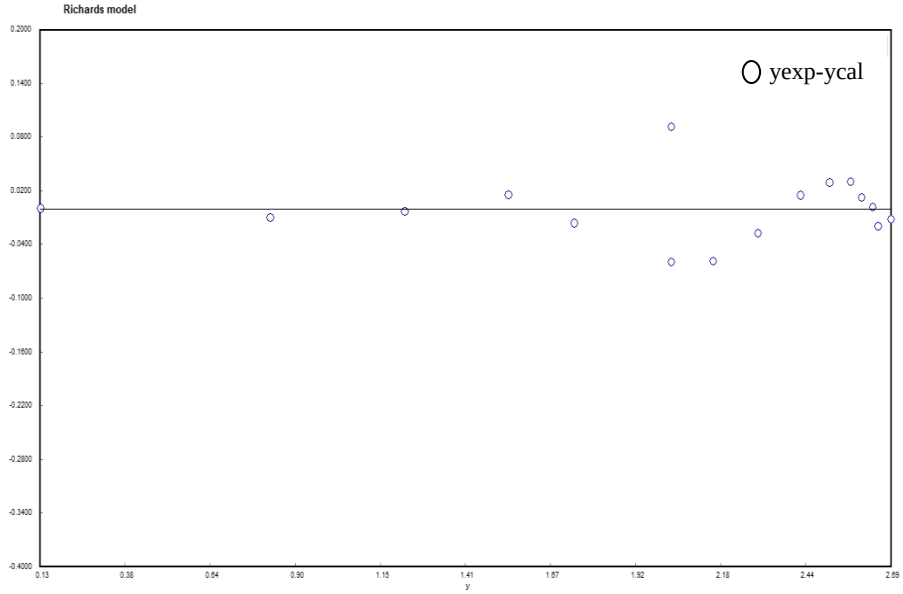

Fig. 10. Residual plots (0.5% CO_2) for Richard model.

The good fitting of *Chlorella vulgaris* growth data in Richards model under different case scenarios indicate that Richards model displayed a higher flexibility than Gompertz, Logistic, modified Gompertz and Baranyi model. In the case of Logistic model, in which the lag phase is not being considered, the model does not accurately to represent the microalgae growth in the present study. Although Gompertz and modified Gompertz defined the lag phase parameter, however the unsatisfactory fit of experimental data in the models could due to the

consideration of two fixed point of inflexion (less flexibility) of the models [17]. Apart from that, Baranyi model is a three-parameter model, which is lacking of one parameter as compared with Gompertz, Logistic, modified Gompertz and Richards model; has led to the inaccuracy to represent the experimental data. On the other hand, Richards model has a high flexibility due to the additional shape parameters that can make the Richards equation equivalent to the Logistic and Gompertz model. Varying the shape parameters allow the point of inflexion of the curve to be at any value between the minimum and upper asymptote [18].

Table 4 shows the parameters predicted from Richards' growth model based on several case scenarios. The predicted specific growth rate matched well with the experimental result, with less than 5% deviation except for the cultivation in 100 L photobioreactor under outdoor cultivation. From the lab-scale 5 L photobioreactor, by supplying 0.03% CO_2 (compressed air) resulted to the slowest growth rate of *Chlorella vulgaris* and longest lag-phase. The growth rate of *Chlorella vulgaris* was gradually increasing with CO_2 concentration, with the highest specific growth rate of 0.291 day^{-1} attained when supplying with 5% of CO_2. However, the lag-phase was not significantly reduced when increasing the CO_2 concentration. This might be due to a reasonable time length is required for *Chlorella vulgaris* to adapt to the new cultivation environment. This was clearly shown that with increasing of CO_2 concentration from 1% to 5%, the lag-phase of *Chlorella vulgaris* was still remained at 1.4-1.5 days.

On the other hand, the growth parameters depicted from Richards model demonstrated a clearer view on the scale-up cultivation of microalgae in 100 L photobioreactor. As stated in Table 4, the growth rates of *Chlorella vulgaris* cultivated in 100 L photobioreactor are significantly lower than the lab-scale 5 L cultivation. For example, the growth rate dropped 55.8% when supplied with 0.03% CO_2 concentration. In addition, the lag-phase was also prolonged to 5 days instead of 2 days. This observation was predominantly due to insufficient seed culture to initiate the fast growth of microalgae, but could be further improved through semi-batch cultivation as discussed in previous work [1]. In addition, the outdoor cultivation of *Chlorella vulgaris* exhibited the slowest growth rate among all the study cases, mainly due to temperature and weather fluctuation under outdoor condition [7].

Table 4. Growth parameters predicted from Richards model.

CO_2 concentration	τ (day)	Predicted specific growth rate (day^{-1})	Experiment specific growth rate (day^{-1})	% Different
5 L (Indoor - 0.03% CO_2)	2	0.154	0.156	1.28%
5 L (Indoor - 0.5% CO_2)	2	0.162	0.160	1.25%
5 L (Indoor - 1% CO_2)	1.5	0.193	0.190	1.58%
5 L (Indoor - 2% CO_2)	1.4	0.201	0.196	2.55%
5 L (Indoor - 5% CO_2)	1.4	0.291	0.283	2.83%
100 L (Indoor -0.03% CO_2)[1]	5	0.109	0.114	4.40%
100 L (Indoor -5% CO_2)[1]	4	0.133	0.135	1.48%
100 L (Indoor- 0.03% CO_2)[2]	5	0.109	0.106	2.83%
100 L (Outdoor -0.03% CO_2)[1]	6	0.089	0.083	7.22%

Note: [1]With sequential baffled device, [2]Without sequential baffled device

4. Conclusions

The growth of *Chlorella vulgaris* in the present study was found to fit well with Richards model across all the CO_2 concentration investigated. The Richards model displayed the highest R^2 value and lowest RMSD and variance values as compared with other growth kinetic models, such as Gompertz model, Logistic model, Modified Gompertz model and Baranyi model. In addition, the predicted specific growth rate by Richards model was close to the experimental specific growth rate with less than 5% deviation, except for microalgae cultivated in pilot-scale photobioreactor under outdoor environment. These findings are expected to contribute in the advancement of photobioreactor design and CO_2 mitigation by microalgae.

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