

## Preliminary study on the growth of *Tetraselmis suecica* in centred-light photobioreactor (CLPBR)

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**Abstract.** Over the past decade, there has been a sustained research on renewable fuel known as biofuel due to the reliance upon fossil fuel. Oil obtained from microalgae is a viable approach for biodiesel production to reduce the reliance on fossil fuel. In this study, the *Tetraselmis suecica* is cultivated in 5L centred-light Photobioreactor (CLPBR) to investigate the effect of pH and light intensity on cell concentration and lipid content of microalgae. Different pH (6.5, 8.5, 10.5) and light intensity (200 lux, 1000 lux, 2000 lux) were used to test their effect on algal growth and lipid production. The results showed that the highest cell concentration (4 mL/mL) and lipid content (18 %) was obtained at pH of 10.5. At 1000 lux light intensity, the highest cell concentration and lipid content was 0.898 mL/mL and 19% respectively. The two models that are tested to determine the growth kinetics are Logistic and Gompertz model. The  $R^2$  obtained from Logistic and Gompertz model was 0.8852 and 0.8736 respectively. The results demonstrate that both models were least accurate to predict the algal growth.

### 1. Introduction

The use of fossil fuel is unsustainable because of depleting resources, increasing greenhouse gas emission, increased population, and economy crisis. An alternative fuel which is renewable, low greenhouse gas emission, less toxic and low sulphur content preferred [1]. Biodiesel is one of the significant productions of biofuel which is a renewable fuel. The oil produced from microalgae has the ability to substitute the biodiesel. Microalgae are promising feedstock for sustainable production of biofuels and valuable chemicals [2]. The microalgae-based biofuels potentially employed in an economically effective and environmentally sustainable manner. Microalgae is a photosynthetic organism which synthesizes biochemical compounds such as proteins, lipids and carbohydrates [3]. Since they are highly resistant organisms, they can grow in water sources such as brackish, fresh and salty water [4]. The production of biofuel from microalgae was mainly considered due to its higher biomass and lipid productivity compared to other feedstocks [5].



In industrial application, the cultivation of algae needs to be in large scale for the high amount of biodiesel production. Thus, few methods were employed for the cultivation of algae such as open ponds, photobioreactor and hybrid system. Photobioreactor plays an important role in the production of environmentally friendly products from microalgae. Photobioreactor was highly recommended for the cultivation of microalgae because it has low risk contamination and the environmental conditions can be controlled [6]. The common types of photobioreactor used for cultivation are tubular, flat panel, airlift and bubble column reactors. Most commonly the lack of innate understanding on light distribution and cell growth in the reactor is one of the limitations in cultivation of microalgae. The most difficulties encountered are the light penetration to reach the cells and overgrowing of algae [7]. Thus, the best conditions for the algae growth and primary factors that leads to negative growth should be analysed carefully to cultivate microalgae.

Kinetic studies are done to predict the growth of algae in a medium. There are different phases in the growth of algae such as lag, exponential, stationary and death phase. The growth kinetic model is robust in describing the growth kinetics [8]. The kinetic growth of algae is usually studied by structured or non-structured models. The models such as Logistic and Gompertz used in this experiment is the example of non-structured models. These models explain as the biomass in the system increases, the specific growth coefficient linearly decreases [9]. The Logistic model is used more frequently than others to study the growth kinetics of algae. The S- shaped curve considered well fitted as the high correlation coefficients are obtained [10]. Gompertz model is also one of the three parameter model which is easier to use for predicting the growth of algae.

There are many researches that has been going on in cultivating microalgae in order to identify best conditions for microalgal growth and lipid production by controlling the light intensity, providing essential nutrients, carbon dioxide and temperature. In this study the growth kinetic of *Tetraselmis suecica* was determined using two kinetic models such as Logistic and Gompertz model. Different parameters such as pH, light intensity and carbon dioxide concentration was investigated to determine the algae growth and lipid production.

## 2. Materials and method

### 2.1. Cultivation medium preparation

Cultivation was carried out by using modified algae growth MLA medium that consist of following chemical composition (per litre of distilled water):  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  ( $49.9 \text{ g L}^{-1}$ ),  $\text{NaNO}_3$  ( $85.0 \text{ g L}^{-1}$ ),  $\text{K}_2\text{HPO}_4$  ( $6.96 \text{ g L}^{-1}$ ),  $\text{H}_3\text{BO}_3$  ( $2.47 \text{ g L}^{-1}$ ),  $\text{NaHCO}_3$  ( $16.9 \text{ g L}^{-1}$ ),  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  ( $29.4 \text{ g L}^{-1}$ ), 1 % (v/v) vitamins solution and 1 % (v/v) micronutrients solution. The vitamins solution was biotin ( $0.1 \text{ g L}^{-1}$ ), Vitamin B12 ( $0.1 \text{ g L}^{-1}$ ) and Thiamine HCl ( $0.1 \text{ g L}^{-1}$ ). The micronutrients solution was  $\text{Na}_2\text{EDTA}$  ( $4.36 \text{ g L}^{-1}$ ),  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  ( $1.58 \text{ g L}^{-1}$ ),  $\text{NaHCO}_3$  ( $0.60 \text{ g L}^{-1}$ ),  $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$  ( $0.36 \text{ g L}^{-1}$ ),  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  ( $1.0 \text{ g L}^{-1}$ ),  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  ( $2.2 \text{ g L}^{-1}$ ) and  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$  ( $1.0 \text{ g L}^{-1}$ ),  $\text{NaMoO}_4 \cdot 2\text{H}_2\text{O}$  ( $0.6 \text{ g L}^{-1}$ ) [11]. All chemicals used were analytical grade. Then, the reading of the dried biomass was recorded and cell concentration was calculated by weighing the falcon tubes after drying according to Eq. (1)

$$\text{Cell concentration (g/L)} = \frac{(W_1 - W_2)}{V} \quad (1)$$

Where  $W_1$  is weight of empty falcon tube,  $W_2$  is weight of falcon tube with dried biomass and  $V$  is volume of sample

A graph of optical density against cell concentration was plotted. The growth phase of microalgae was determined by recording the absorbance reading at 680nm every 24 hours.

## 2.2. Growth analysis

The standard curve is done to check the cell concentration by using the absorbance reading. *Tetraselmis suecica* is prepared in six different concentrations with total volume 50 mL in each 50 mL of falcon tube where the absorbance determined at the wavelength 680 nm by (HITACHI U1900) Japan spectrophotometer. The falcon tubes were dried in oven (60 °C) for 24 hours.

## 2.3. Cultivation of microalgae

The culture was cultivated in MLA medium with controlled environment temperature of  $25 \pm 3$  for 10 days. The cultivation is started by prepared the MLA medium with three different pH ( 6.5, 8.5 and 10.5) and three different light intensity (200 Lux, 1000 Lux and 2000 Lux) using LED light source. A volume of 1.8 mL of MLA medium and 200 mL of *Tetraselmis suecica* which was cultivated in 1L Erlenmeyer flask added in the CLPBR. Duplicate sample is taken at 24 hours interval for 10 days.

## 2.4. Biomass harvesting

After 10 days of cultivation, the *Tetraselmis suecica* samples were centrifuged at 5000 rpm for 5 minutes at 4°C. The pellets were rinsed twice with distilled water. The pellets were dried in oven (Binder BD 115) at 70 °C for 24 hours. After that, the dried biomass is crushed into the smaller size and the biomass content is calculated based on microalgae dry weight produced per litre (g/L).

## 2.5. Lipid extraction

The lipid was extracted from the microalgae biomass by using the Soxhlet extraction. The cell dried biomass was added into the cellulose thimble inside the Soxhlet extraction apparatus unit. A volume of 300 mL n-hexane are used to extract the lipid from the microalgae biomass. The lipid extraction was carried out for 5 hours till the solvent become colourless in the compartment that have cellulose thimble. After the lipid extraction the solvent was evaporated by using the rotary evaporator. The subsequent sample is dried in the oven at 70 °C for overnight. The lipid contents were weighed and determined gravimetrically using Eq. (2):

$$\text{Lipid content (\%)} = \frac{W_2 - W_1}{m} \times 100\% \quad (2)$$

Where,  $W_2$  is the weight of the empty beaker and lipid content (g),  $W_1$  is the weight of the empty beaker (g) and  $m$  is biomass of the microalgae used for the lipid determination (g).

## 2.6. Kinetic model

Gompertz model (Eq. 4) and Logistic model (Eq. 5) are used in this project to determine the kinetic behaviour of microalgae growth. Based on these two models, the  $R^2 > 0.95$  shows the best fitting to the experimental data for microalgae growth [12]. Specific growth rate,  $\mu$  was calculated using Eq. (3):

$$\text{Specific growth rate, } \mu = \frac{\ln(X_1/X_0)}{t_1 - t_0} \quad (3)$$

### Gompertz model:

$$C(t) = C_0 * e[-e(\mu * e^{1/C_0})] * (C_m - t) + 1 \quad (4)$$

### Logistic model:

$$C(t) = \frac{C_0 e^{\mu t}}{1 - \left(\frac{C_0}{C_m}\right)(1 - e^{\mu t})} \quad (5)$$

Where,  $C(t)$  is the growth kinetics,  $C_0$  is representing the initial of *Tetraselmi ssuecica* biomass ( $\text{mg L}^{-1}$ ),  $C_m$  is maximum biomass, ( $\text{mg L}^{-1}$ ),  $\mu$  is specific growth rate ( $\text{day}^{-1}$ ) and  $t$  is cultivation time (day)

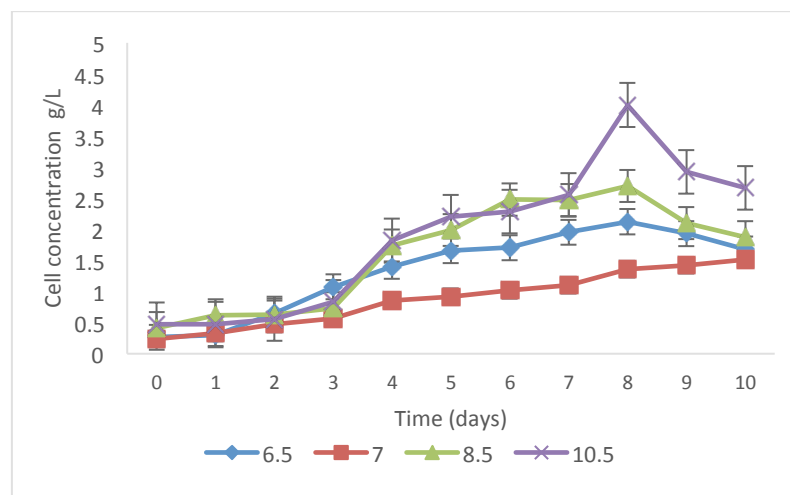
### 3. Results and discussion

#### 3.1. The growth analysis of microalgae

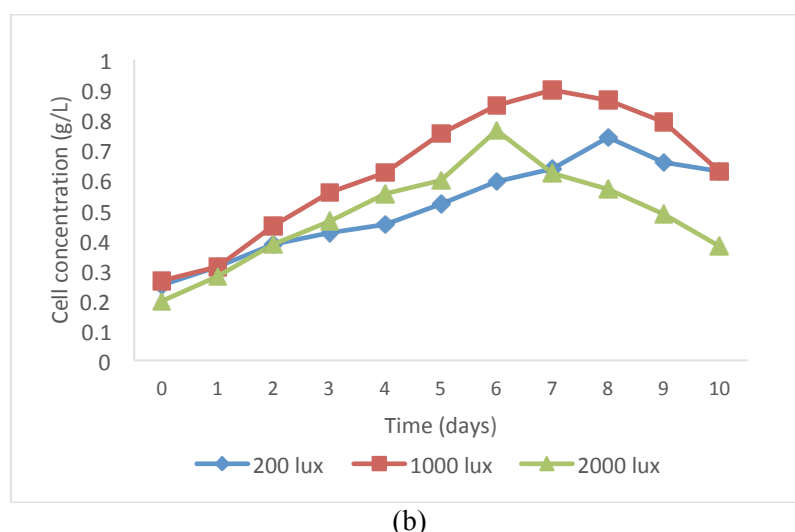
Figure 1 shows the growth curve of microalgae for 10 days at different pH and light intensity. The growth phase consists of lag, log, stationary and death phase. The lag phase can be called as induction phase where the newly introduced cells in a new culture starts to increase with short lag phase. There is no cell division, however the cells increase in size during this phase. The algal cells are metabolically active at this stage. The algal cells are starting to adapt in a new environment for growth. From the graph it can be observed that starting from day 0 to 2, the algal growth shows lag phase.

From day 3, the algal growth represents the log or exponential phase. At this stage, the cells start to divide, and the algal growth is rapid. The cell density increases as the cells started to adapt the new environment and consume the nutrients in the medium. The cells increase linearly with time as the cell divides at maximum rate [13]. This where the cells are metabolically active. But the duration of exponential phase for different conditions varied. However, usually the growth curve has a stationary phase where the growth curve becomes horizontal because the as the cells divide, the existing cells die at the same time. However, the graph does not project any stationary phase. After the exponential phase it enters the declining phase, where the cell division slows down as the nutrients, pH, carbon dioxide, light becomes limiting factor for growth of algae. This is where the competition for nutrients and other factors increases and the cell become less metabolically active.

In contrast, at pH 7 the cells were still at exponential phase at the end of day 10, which means the cells were still able to grow for longer period due to the nutritional availability in the growth medium. Investigation of *Chlorella* sp. for 12 days showed the growth curve till exponential phase by Wong *et al* [14]. Besides, growth of *Tetraselmis* sp. C7P4 at pH 7 was analysed where at the end of 15<sup>th</sup> day the microalgae was still at exponential phase [15]. However, another study showed the growth of *Tetraselmis* sp. C7P4 where at the end of 10<sup>th</sup> day the growth was till stationary phase [16]. The growth curve of light intensity also exhibited the normal growth curve at 200, 1000 and 2000 lux.



(a)



**Figure 1.** Cell concentration of *Tetraselmis suecica* at different conditions, a) pH and b) light intensity

### 3.2. Effect of different conditions on cell concentration and lipid production of microalgae

The variation in pH value in culture can affect cell metabolism and the growth of algae biomass. The pH 7 was used as a control for the cultivation of microalgae. From Figure 1(a), it can be observed that the cell concentration increases as the pH increases. On day 8, the lowest concentration was 1.356 mL/mL when the cultivation is done at pH of 7. At pH of 10.5, the highest cell concentration which is 4 mL/mL has been recorded. Mostly, the suitable pH for the growth of algae is neutral or alkaline condition. However, the pH required for the cultivation condition depends on the type of strain used. Table 1 shows the highest lipid content was 18% at pH of 10.5 and the lowest lipid content was 7.1% at pH of 8.5. It was reported that the alkaline condition can inhibit the microalgae growth and use the energy to form triacylglycerols (TAG) [14]. The cultivation of microalgae in alkaline medium increases the cell wall flexibility. High pH prevents the rupture of cell wall and autospores produced from cell is inhibited. Thus, this will increase the time for the algae to complete growth cell cycle [15].

The cultivation at different light intensity has been conducted in 5L CLPBR 12:12 hour (light : dark) cycle. Figure 1 (b) shows at light intensity of 1000 lux, the highest cell concentration which is 0.898 mL/mL was obtained on day 7. However, using 200 and 2000 lux of light intensity, the cell concentration was the same which is 0.621 mL/mL. Light is provided for the microalgae to activate photosynthesis and growth rate. Light distribution to the cells is divided into three distinct zones such as strong illumination zone, weak illumination zone and dark zone [16]. Strong illumination zone causes inhibitory effect for the cells. A study showed that in a flat panel reactor using  $1500 \mu\text{mol photon m}^{-2}\text{s}^{-1}$ , the biomass concentration was 1.8 g/L [17]. In a tubular reactor cultivating *Spirulina* sp. at 2000 lux gave 0.26g dry/L per day [18]. The growth of microalgae depends on the light intensity provided and the type of species used for cultivation. Huang et al., [19] reported that if the light intensity is very high, the growth of microalgae can be inhibited and cause photoinhibition as the light provided is converted into heat. If the light intensity is low, it can cause photolimitation which eventually kill the microalgae [20]. For lipid content, Table 1 shows the highest lipid content which is 19% was obtained at light intensity of 1000 lux. At 200 lux, the lowest lipid content of 5% is obtained. The difference in light intensity can alter the chemical composition of the microalgae. Low light intensity favours the formation of polar lipids while the high light intensity decreases the polar lipid and increase the neutral storage lipid most probably the triacylglycerol [21]. TAG production under high light conditions might serve as a protective mechanism for the cell [22].

**Table 1:** The lipid content of *Tetraselmis suecica* under different pH and light intensity

Parameters		Lipid content (%)
pH	6.5	16
	8.5	7.1
	10.5	18
Light intensity (lux)	200	5
	1000	19
	2000	7

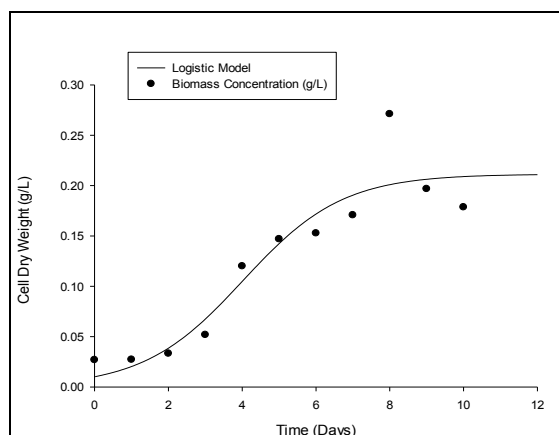
### 3.3. Kinetic model

Growth kinetics *Tetraselmis suecica* was determined by using Logistic and Gompertz models. Gompertz and logistic model was chosen because the growth fitted many organisms such as plant, bird, bacteria and so on. These models are frequently used sigmoid models to fit growth data since it is easy involving less correlated parameters. *Tetraselmis suecica* was grown in MLA medium with the basic environmental conditions such as pH 7, light intensity of 1000 lux, 5% carbon dioxide concentration and temperature of 30°C. These two models will describe the whole microalgae growth curve. Logistic model represents growth of microalgae based on time, growth rate, initial and final population density [10]. Table 2 represent the experimental growth kinetics where is the R and R<sup>2</sup> for predicted the cell growth kinetics. The R<sup>2</sup> is the coefficient of determination to indicate the model preciseness in fitting the experimental data. The value of R<sup>2</sup> which is close to 1 implies the accuracy of model. From these two models of the Logistic and Gompertz model, the R<sup>2</sup> that was obtained are 0.8852 and 0.8736 respectively. The high correlation coefficients represent the model fitted well to experimental data. However, the correlation coefficient obtained are not so close to 1 indicates that the model is not sufficient to describe the growth of *Tetraselmis suecica*. The low determination of R<sup>2</sup> indicated the Gompertz model is not sufficient to describe biomass production by *Tetraselmis suecica* in medium.

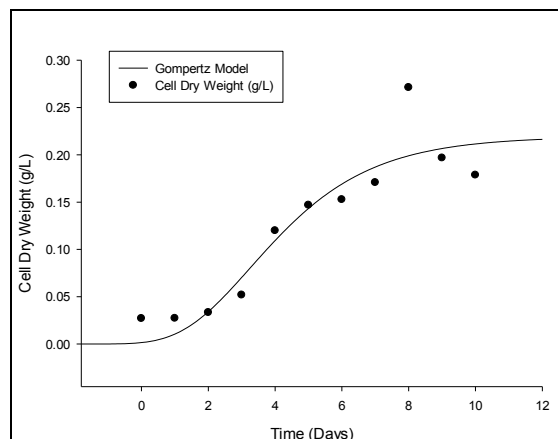
Figure 2 and 3 shows the Logistic and Gompertz model kinetic growth curve. The model might not sufficient enough to fit growth data because of its many re-parameterisations applied in different field of study for different types of growth [23]. These models do not suit to study growth data of *Tetraselmis suecica*. Similar result was found in the investigation of *Chlorella vulgaris* where the Gompertz and Logistic model does not describe the accuracy of data [24]. Lim et al., [24] studied the growth kinetics of *Chlorella* sp. using Richards, Gompertz and Logistic model. Richards model represents a good fit of data compared to Logistic and Gompertz model. However, a study conducted by Abinandon et al., described the growth kinetics efficiently using both Logistic and Gompertz model with *Chlorella* sp [25].

**Table 2:** The kinetic growth model of microalgae

Parameters	Models	
	Logistic	Gompertz
R	0.9409	0.9346
R <sup>2</sup>	0.8852	0.8736



**Figure 2:** Logistic model for *Tetraselmis suecica* kinetic growth.



**Figure 3:** Gompertz model for *Tetraselmis suecica* kinetic growth.

#### 4. Conclusion

In conclusion, this study showed that the growth of algae and lipid production is greatly influenced by the difference in pH and light intensity. The results revealed that the growth profiles are different for *Tetraselmis suecica* under these two conditions. From this study, the best pH for cultivation of *Tetraselmis suecica* in 5L CLPBR is 10.5 which gives the highest cell concentration and lipid content. Besides, the highest cell concentration and lipid content observed at 1000 lux of light intensity. It indicates that the light intensity of 1000 lux is more suitable for growth and lipid production of *Tetraselmis suecica*. The two growth kinetic models such as Logistic and Gompertz that have been tested gives  $R^2$  that is not so close to 1. It shows that both Logistic and Gompertz model is least to predict the growth kinetics of *Tetraselmis suecica* in 5 L CLPBR. For future recommendation to predict the growth of microalgae, the Logistic and Gompertz model that is modified can be used instead for better performance.

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