



# Prospective peat swamp water as growth medium for microalgal cultivation and kinetic study

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**Abstract** Microalgae as source of renewable energy are very potential due to high biomass productivity and lipid content. The nutritious culture for microalgae cultivation, however, should be concerned to be affordable and feasible. Here, the utilization of peat swamp culture for microalgal cultivation was studied in comparison to the nutritious pure water. The effects of photoperiod and nitrogen sources on biomass were conducted as well. Compared to the commercial microalgae, the microalgae isolated from peat swamp showed excellent performance with the faster growth time of 10 days as well as higher biomass and its productivity of  $1.72 \text{ g L}^{-1}$  and  $0.16 \text{ g L}^{-1} \text{ d}^{-1}$ , respectively. Even for the commercial microalgae, the cultivation process using the peat swamp water led to increase in biomass by 17.2% and its productivity by 10% compared to that using the nutritious pure water. The proposed kinetic model with a modification to the modified Gompertz model showed an excellent prediction with the experimental data as  $R^2$  of 0.985 was obtained. The model could well envisage the initial biomass and lag phase compared to the original model. Hence, the model is deemed beneficial for the research development for implementation in high scale of microalgal cultivation.

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## 1. Introduction

In recent years, researches have focused on developing alternative sources of renewable energy for the limited sources of fossil fuels; nonetheless, fossil fuels also cause high  $\text{CO}_2$  emissions

and other environmental problems [1–3]. Bioethanol and biodiesel are some of prospective renewable energy sources to be implemented for their clean burning and low  $\text{CO}_2$  emissions [4–6]. Microalgae are the promising source of raw materials for renewable energy that can produce various types of biofuels, such as biodiesel and bioethanol [7–9]. Microalgae with the utilization of sunlight can convert  $\text{CO}_2$  into biomass in the form of lipids, carbohydrates, proteins and fatty acids [10,11]; whereas the typical biomass can be specifically converted into biodiesel or bioethanol [12,13]. Microalgae can be also used for

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various products, such as aquaculture feed, food supplements, cosmetics and pharmaceuticals [14,15].

The use of microalgae is also considered in terms of the high efficiency of photosynthetic conversion compared to other photosynthetic organisms [16]. Thus, it has the ability to thrive in diverse ecosystems and highly produce biomass [17–19]. The produced biomass may have a high lipid content to produce biodiesel or a high carbohydrate content to produce bioethanol depending the characteristic of microalgae [20–22]. The ability to produce high lipid content varies from species to species, especially green algae [10,23]. The substantial factors affecting the growth of microalgae are the medium composition (type of medium, nutrient or carbon and nitrogen sources), the light supply (photoperiod, light source and intensity) and the cultivation conditions (pH, oxygen removal, temperature, etc) [24–27].

To commercially produce biodiesel from microalgae, it is necessary to optimize the growth rate of microalgae in the cultivation process [25,28]. One of the problems faced in the development of microalgal cultivation is related to the appropriate growth media to increase the growth rate and also reduce the processing costs without additional nutrients and minerals [20,25,29,30]. Various mediums have been investigated among researchers to optimize the microalgal growth such as Walne medium, Bold Basal medium, BG11 medium, Allen medium, F-Si medium, SK medium [30–33]; such mediums are rich in nutrition or carbon and nitrogen source, but the cost is not cheaper. Peat swamp water has great potential as a medium for microalgal growth because the peat area extends in various parts of the world, such as in Central America, Africa and South East Asia [34,35]; with a total worldwide land area of more than 4 million km<sup>2</sup> [36]. Peat water has the high contents of organic substances and CO<sub>2</sub> gas. It contains sulfates (SO<sub>4</sub><sup>2-</sup>), chloride (Cl<sup>-</sup>), magnesium (Mg), aluminum (Al), iron (Fe), potassium (K), calcium (Ca), sodium (Na) and manganese (Mn) [37,38].

In microalgal cultivation, light intensity or photoperiod is one of parameters playing an important role in biomass production [26]. The effectiveness of light in the photosynthesis process needed by microalgae has a certain range or limit [39]. The photosynthetic process is more effective with a greater light intensity, but at very high light levels it can reduce the rate of the photosynthetic process [40]. The optimal conditions for light intensity vary dependent upon species, culture conditions, and culture density of algal species [18].

Another factor that also has a significant effect on the growth and biochemical composition of microalgae is the nutrition type [27,41] because of its role in growth and metabolism regulation [42]. Nitrogen is the main element in all structural and functional proteins such as peptides, energy transfer molecules, chlorophyll, enzymes and genetic components of microalgae [43]. Microalgae are capable of utilizing various forms of nitrogen, including the sources of ammonium, nitrite, nitrate and organic nitrogen such as urea [42,44]. Each nitrogen source is initially altered into the ammonium form and then assimilated to amino acids via various pathways [44]. Nitrogen becomes an important nutrient in comparison to other nutrients such as phosphorus in microalgae [45,46]; because it is an important constituent of the microalgal cell structure with the key components of protein, enzyme, amino acids, photosynthetic pigments and nucleic acids [47].

The cultivation process of microalgae to be implemented on a large scale is a challenge [48,49]. The fundamental basis in understanding and knowing the cultivation process requires a kinetic study [50]. Therefore, kinetic study is important in describing the productivity optimization of microalgae under various conditions [50,51]. Various kinetic models have been developed to study microalgae processes in their natural habitats; however, a growth kinetic model becomes an important point in an effort to scale-up the microalgal production [51,52].

This study aimed to explore the use of nutrient-rich peat water as a growth medium for microalgae *Chlorella* sp. The effects of the photoperiod and nitrogen source on the growth of these microalgae were also evaluated. To be applied on a large scale, the kinetics of the microalgal growth must be formulated to predict the production of microalgae. Through this study, hopefully the cultivation process in peat water rather than produces more microalgae, also consequences the accumulation of biomass and the formation of oxygen from the photosynthesis process, thus leading to a positive impact on the sustainability of the surrounding ecology.

## 2. Materials and methods

### 2.1. Microalgal culture and growth media

Microalgae *Chlorella* sp. was isolated from Rimbo Panjang Village, Kampar Regency, Riau, Indonesia and cultivated in the peat water culture obtained from the same place. Pre-culture microalgae *Chlorella* sp. was conducted using 95 ml of peat water and 5 ml of isolated microalgae *Chlorella* sp. placed in a 250 ml sample bottle equipped with special stirrer. Pre-culture was carried out for 7 days and stirred every 2 times a day. The type of the microalgae is hereinafter called as microalgae-peat swamp. The performance of the microalgae was also compared to the commercial one purchased in market, hereinafter called as microalgae-commercial.

### 2.2. Microalgal cultivation

For the first step of research, *Chlorella* sp. for type of microalgae-peat swamp and microalgae-commercial were cultivated to in the culture of peat swamp water (without nutrition) and pure water with the addition of nutrition to compare the performance of both microalgae types. There were four combinations of microalgae and culture. For the commercial microalgae, the pure water with additional nutrition and the peat swamp water without nutrition as type of culture were named as MCM-WN and MCM-PS, respectively. For the microalgae originating from peat swamp, the similar both types of culture were used namely MPS-WN and MPS-PS, respectively. The urea as nutrition (88 mg L<sup>-1</sup>) was used and diluted in pure water.

For the second evaluation, the microalgae-peat swamp were cultivated in three different photoperiods: 24:0, 16:8 and 12:12 h (light:dark) with a light intensity of 1500 lx to determine the effect of the photoperiod on biomass concentration and lipid content of microalgae. Light intensity was measured using a lux meter. Microalgal cultivation was carried out using a 250 ml sample bottle consisting of 90 ml of peat water and 10 ml of pre-cultured microalgae.

In the last stage, the effect of nitrogen source as additional nutrition in the culture media was studied. Here, three nitrogen sources of  $\text{NaNO}_3$ ,  $\text{NH}_4\text{Cl}$  and urea were used. The nutrient of  $\text{NaNO}_3$  (250 mg),  $\text{NH}_4\text{Cl}$  (157 mg) or urea (88 mg) was added to 1 L of peat water; as the nitrogen amount for each source equaled to 2.94 mM. Cultivation was carried out using a 250 ml sample bottle consisting of 90 ml of peat water and 10 ml of pre-cultured microalgae. Lighting with an intensity of 1500 lx was supplied.

### 2.3. Experimental analysis

Microalgal growth was regularly monitored at the 2-day intervals by measuring the optical density (OD) at 384 nm using a UV-VIS spectrophotometer (Easyspec, UV-Vis, Safas, Monaco, France). Some prepared solution containing certain amount of microalgae (in  $\text{g L}^{-1}$ ) was tested in UV-VIS to calibrate the optical density (OD) and biomass concentration. The linear correlation was evaluated by plotting OD versus biomass concentration; then the linear equation was generated. For this, the biomass concentration for the samples of cultivation was determined using the calibration curve and/or the linear equation. The biomass concentration (X) could be calculated by using Equation (1):

$$X(\text{g L}^{-1}) = (2.1086 \times \text{OD}_{384}) + 0.0058 (R^2 = 0.98) \quad (1)$$

The biomass productivity ( $\gamma$ ,  $\text{g L}^{-1} \text{d}^{-1}$ ) was examined by the following equation:

$$\gamma = \frac{\text{OD}_2 - \text{OD}_1}{t_2 - t_1} \quad (2)$$

and the specific growth rate ( $\mu$ ,  $\text{d}^{-1}$ ) in the exponential phase was calculated using Equation (3):

$$\mu = \frac{\ln(\text{OD}_2) - \ln(\text{OD}_1)}{t_2 - t_1} \quad (3)$$

where  $\text{OD}_1$  is optical density at the start point of the exponential phase,  $\text{OD}_2$  is optical density at the end point of the exponential phase,  $t_1$  is the start time and  $t_2$  is the end time of the exponential phase [53]. The gentle process of microalgae was used with filtration and drying method [21,54]. After filtration process using filter paper, the microalgae were dried in oven at 105 °C until the weight was constant.

Lipid extraction was carried out by the Bligh-Dyer method [55]. Nitrogen content was assessed by the cadmium reduction method [56]; while, the carbon one was evaluated by the Walkley-Black method [57]. Triplicate data were performed and the average data were presented in Figures and Tables. Statistical analysis was performed by using ANOVA at the 95% confidence level ( $p < 0.05$ ).

### 2.4. Kinetic study

The models to estimate the growth of microalgae used in this study were described as follows:

$$\gamma = \gamma_m (1 - \exp(-k.t)) \quad (4)$$

$$\gamma = \frac{\gamma_m^2 k.t}{1 + \gamma_m k.t} \quad (5)$$

$$\gamma = \gamma_m \exp \left[ -\exp \left[ \frac{r_m \cdot \exp(1)}{\gamma_m} (t_L - t) + 1 \right] \right] \quad (6)$$

$$\gamma = \frac{\gamma_m}{\left[ 1 + \exp \left[ \frac{4r_m \cdot (t_L - t)}{\gamma_m} + 2 \right] \right]} \quad (7)$$

Equation (4), (5), (6) and (7) are the kinetic models used to evaluate the data of growth, namely first exponential order (FO), second order (SO), modified Gompertz (MG) and modified logistic (ML), respectively. The FO model was based on the single order combined with exponential rate constant and while the SO model was based on the quadratic order combined with numerator of potential maximum parameter [58]. The MG and ML were modifications to the original Gompertz and Logistic models, respectively [59,60]. The symbol of  $\gamma$  is the concentration of microalgae ( $\text{g L}^{-1}$ ) produced after time  $t$  (d),  $\gamma_m$  is the potential maximum of microalgal production ( $\text{g L}^{-1}$ ),  $r_m$  is the maximum microalgal production rate ( $\text{g L}^{-1} \text{d}^{-1}$ ),  $k$  is the rate constant (units depending on equation),  $t$  is time (d) and  $t_L$  is time for lag phase (d).

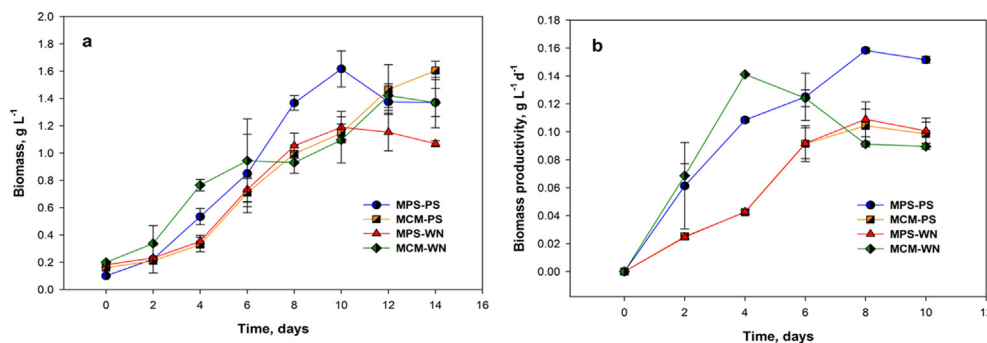
## 3. Results and discussion

### 3.1. Type of microalgae and culture media

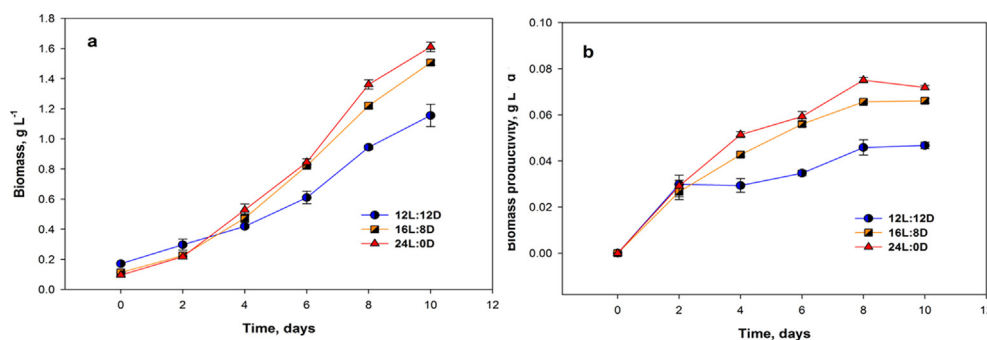
Fig. 1(a) and 1(b) present the kinetic profiles of biomass growth and biomass productivity for microalgae of peat swamp and the commercial one. Both types of microalgae were grown in both cultures' types of peat swamp and pure water with nutrient. In the beginning of process, the microalgae experienced the lag-phase up to 2 days to adapt in the culture. The lag-phase varied in the range of 1–8 days depending the type of microalgae and growth media used [61,62]. The biomass continued to have exponential growth up to 10–12 days and ran into stationary phase afterward. In Fig. 1(a), the microalgae of MPS-PS type showed the best performance indicated by highest biomass and fastest growth with a biomass value of  $1.72 \text{ g L}^{-1}$  for 10 days of growth. The results were also supported by the biomass productivity result with an optimum value of  $0.16 \text{ g L}^{-1} \text{d}^{-1}$  as shown in Fig. 1(b). The microalgae of MCM-PS type also showed good results with a maximum biomass growth of  $1.7 \text{ g L}^{-1}$  for 12 days and an optimum biomass productivity of  $0.14 \text{ g L}^{-1} \text{d}^{-1}$ . This revealed that non-nutritious peat media showed a potential culture. This was reasonable considering that the peat media has rich nutrient as reported [37]. The microalgae originating from peat swamp had a better growth adaptation in any cultures compared to the commercial one as higher biomass growth and productivity in both media of peat and pure water with nutrients were observed. It became possible because the microalgae are already accustomed to grow in wild environments; as reported, the isolated wild microalgae resulted in better growth [63,64]. In the next discussion, the usage of microalgae originating from peat and peat media was carried out on the effects of photoperiod and nutrient addition.

### 3.2. Effect of photoperiod and nutrient

Fig. 2(a) and 2(b) show the kinetic profiles of growth of microalgae and biomass productivity at various photoperiods. The longer light period led to better microalgal growth



**Fig. 1** Kinetic profiles of (a) biomass growth and (b) biomass productivity for (i) microalgae of peat swamp using peat swamp culture (MPS-PS); (ii) microalgae of commercial using culture of peat swamp (MCM-PS); (iii) microalgae of peat swamp using culture of pure water with nutrient (MPS-WN) and (iv) microalgae of commercial using culture of pure water with nutrient (MCM-WN). The cultivation was conducted at normal temperature and photoperiod of 24 h light and 0 h dark (24L:0D). The urea nutrient (88 mg L<sup>-1</sup>) was used for pure water media and without any nutrient addition for peat swamp media.



**Fig. 2** Kinetic profile of (a) biomass growth and (b) biomass productivity for photoperiod variations of (i) 12 h light and 12 h dark (12L:12D); (ii) 16 h light and 18 h dark (16L:8D) and (iii) 24 h light and 0 h dark (24L:0D) using microalgae of peat swamp and peat swamp culture (MPS-PS) without any nutrient addition.

performance. It was reported that the photoperiod length led to stimulate the precursors of carbohydrate synthesis [65]. The gap growth for the light period in the range of 12–16 h (0.35 g L<sup>-1</sup> increased threefold compared to the gap growth for it in the range of 16–24 h (0.1 g L<sup>-1</sup>). The insignificant effect of light after 16 h was possible due to the system of photosynthetic receptor as known as a photo-inhibition phenomenon [66]. Another reason might be related to the photo-oxidation occurred in the cell; as a consequence, the absorption of the excess light cannot be completely conducted into the photosynthetic apparatus [1]. On the other hand, more intensity of light caused the immediate interruption of the growth and even the death in very high intensity level [67].

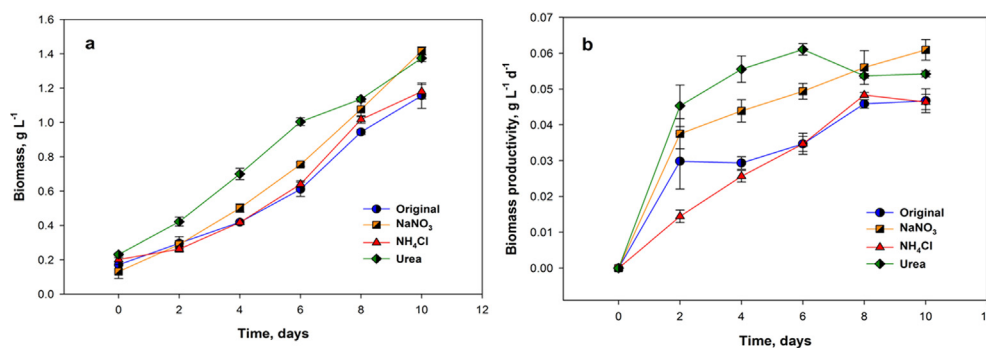
Fig. 3(a) and 3(b) show the kinetic profiles of growth of microalgae and biomass productivity at various nutrient additions. It was observed that the addition of nutrient shortened the lag phase and increased the growth of microalgae. However, the effect of NH<sub>4</sub>Cl on the growth was less apparent compared to the original media without nutrient. The growth of microalgae for both additional nutrients of NaNO<sub>3</sub> and urea were comparable regarding the value of biomass or maximum biomass productivity. However, as shown in Table 1, the maximum specific growth rate for NaNO<sub>3</sub> was little bit higher than the rate for urea. As reported [68], the highest microalgal growth was observed for the culture with yeast extract addition followed by peptone, urea and NaNO<sub>3</sub>. The yeast extract and

peptone are undesirable nutrients due to expensive nitrogen source; therefore, urea and NaNO<sub>3</sub> became potential nutrients to be used in large scale.

Table 1 shows the parameters of microalgae for various photoperiod and nutrient effects. The similar trends of biomass productivities were observed for either photoperiod or nutrient effects. However, the maximum specific growth trends for culture with NaNO<sub>3</sub> and urea were reversed with the maximum biomass productivity. For instance, at photoperiod of 24L:0D, the biomass productivity value for urea was 0.119 g L<sup>-1</sup> d<sup>-1</sup> compared to 0.086 g L<sup>-1</sup> d<sup>-1</sup> for NaNO<sub>3</sub>; on contrary, the higher value of specific growth rate for NaNO<sub>3</sub> (0.345 d<sup>-1</sup>) was obtained in comparison to its for urea (0.312 d<sup>-1</sup>).

Some results of lipid contents observed in this research are higher than the results reported with the range of 5–22% for *Chlorella* sp. type [69,70]. The trends for lipid content were also different with biomass productivity and specific growth rate. The lipid content for culture with NH<sub>4</sub>Cl was found highest compared to those with other nutrients, followed by original (without nutrient), NaNO<sub>3</sub> and urea. It was reasonable that the microalgae favorably assimilated nitrogen with a culture containing NH<sub>4</sub><sup>+</sup> in comparison to other sources such as nitrate or urea [71]. Xia et al. [72] reported that the cultivation with low pH could inhibit the biomass growth. Here, the cultivation with higher initial pH led to higher biomass growth as well as biomass productivity. On the other hand, the





**Fig. 3** Kinetic profile of (a) biomass growth and (b) biomass productivity using types of microalgae and culture of MPS-PS with/without nutrient of (i) original (without nutrient); (ii) NaNO<sub>3</sub>; (iii) NH<sub>4</sub>Cl and (iv) Urea. The cultivation was conducted at normal temperature and photoperiod of 12 light and 12 dark (12L:12D).

**Table 1** Parameters of growth of microalgae of peat swamp at various photoperiods and nutrients.

Parameters		Maximum biomass productivity, g L <sup>-1</sup> d <sup>-1</sup>	Specific growth rate, d <sup>-1</sup>	Initial pH	Final pH	Lipid content, %
Photoperiod	Nutrient					
12L:12D	Original	0.057	0.172	5.99	8.06	22.70
	NaNO <sub>3</sub>	0.061	0.210	7.14	8.69	21.21
	NH <sub>4</sub> Cl	0.043	0.144	4.34	5.69	25.85
	Urea	0.062	0.180	7.15	8.79	20.49
16L:8D	Original	0.064	0.209	5.85	8.35	20.90
	NaNO <sub>3</sub>	0.087	0.255	6.98	9.03	20.49
	NH <sub>4</sub> Cl	0.061	0.180	3.58	5.92	23.66
	Urea	0.092	0.236	7.39	9.08	19.44
24L:0D	Original	0.072	0.289	6.01	8.17	24.84
	NaNO <sub>3</sub>	0.086	0.345	7.08	8.32	24.01
	NH <sub>4</sub> Cl	0.062	0.269	4.35	5.51	28.78
	Urea	0.119	0.312	7.26	9.31	19.43

biomass concentration increased with the increasing pH; however, the lipid content was adjusted to 3 [71]. Similar findings were obtained here that higher lipid content was observed by using culture with lower initial pH. Furthermore, the culture with NH<sub>4</sub>Cl, besides having lowest initial pH, was also maintained in low pH during cultivation, thereby resulting in biomass containing highest lipid. On the other hand, culture with high pH led to change in the net charge on protein as well as partitioning behavior of protein, therefore causing an increase in protein solubility into aqueous phase [73].

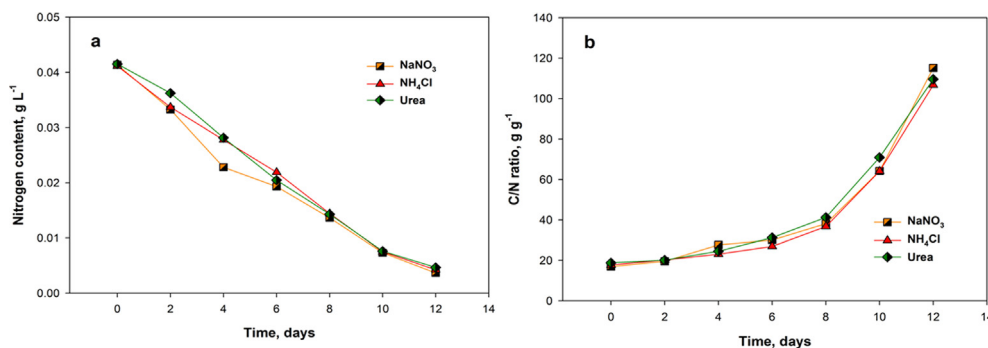
### 3.3. Effect of C/N ratio

Fig. 4(a) and 4(b) show the kinetic profiles of nitrogen content and C/N ratio at various nutrient additions. Similar trends were observed for cultivations in cultures with all three nutrients. The nitrogen was consumed by microalgae linearly up to 10 h and continued to drop slowly. As in the previous finding, the optimum biomass growth was obtained at the 12 h before stationary phase took place; it might be due to biomass inhibition occurred along with the small amount of nitrogen remained. As shown in Fig. 4(b), the exponential line was observed in the range 6–12 h. It indicated that the microalgae

were less likely to consume carbon than nitrogen, while nitrogen changes were relatively linear at the range time of cultivation. Whereas, the high carbon content promoted indistinctively the biomass growth though the cultivation with nitrogen resulted in a significant effect on the microalgal growth compared to the one with carbon such as glucose [68]. The higher final C/N ratio for culture with NaNO<sub>3</sub> followed by urea and NH<sub>4</sub>Cl confirmed the previous finding regarding higher biomass concentration and productivity for such sequent. Also, higher carbon consumed in the culture with NH<sub>4</sub>Cl led to higher lipid production; as reported, the high absorption of carbon to microalgae accumulated to high lipid production [74].

### 3.4. Comparison of biomass and lipid content to other studies

Table 2 shows the performance of *Chlorella* sp. isolated from various sources with different conditions of cultivation. *Chlorella* sp. Y8-1 with the additional nutrients increased the biomass and lipid content about two-folds [75]. The highest lipid content of 35.5% was achieved compared to others. It was plausible due to the presence of urea and sucrose as nutrients. It was reported that the sucrose led to increase in lipid;



**Fig. 4** Profiles of nitrogen content (a) and C/N ratio (b) during cultivation using microalgae type and culture of MPS-PS with nutrient addition of  $\text{NaNO}_3$ ,  $\text{NH}_4\text{Cl}$  and urea. The cultivation was conducted at normal temperature and photoperiod of 12 light and 12 dark (12L:12D).

**Table 2** Biomass and lipid content for *Chlorella* sp. isolated from various sources.

Microalgae types	Cultivation conditions	Biomass concentration ( $\text{g L}^{-1}$ )	Lipid content (%)	Reference
<i>Chlorella</i> sp. Y8-1 isolated from seawater around Taiwan	Walne medium 24L:0D Without nutrient	0.22	16.5	[75]
<i>Chlorella</i> sp. Y8-1 isolated from seawater around Taiwan	Walne medium 24L:0D 0.5 $\text{g L}^{-1}$ urea and 1 $\text{g L}^{-1}$ sucrose	0.45	35.5	[75]
<i>Chlorella</i> sp. isolated from University of Montreal biological station	Bold basal medium 14 $\text{W m}^{-2}$ fluorescent light intensity 2% $\text{CO}_2$	0.2	20.7	[32]
<i>Chlorella</i> sp. isolated from University of Montreal biological station	Bold basal medium 14 $\text{W m}^{-2}$ fluorescent light intensity 25 mM glycerol 2% $\text{CO}_2$	0.2	28.2	[32]
<i>Chlorella</i> sp. isolated from Ocean University of China	BG11 medium Light intensity of 30 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ $\text{K}_2\text{HPO}_4$ 32 $\mu\text{M}$ $\text{NaNO}_3$ 1.76 mM	1.9	23	[45]
<i>Chlorella</i> sp. isolated from collection of Taiwan Fisheries Research Institute	Aquaculture wastewater media $\text{NH}_4^+\text{-N}$ 5.6 $\text{mg L}^{-1}$ and $\text{NO}_3^-\text{-N}$ 12 $\text{mg L}^{-1}$ nutrient Boiler flue gas aeration 0.3 vvm	2.2	23.7	[78]
<i>Chlorella</i> sp. isolated from peat swamp	Peat swamp water media 24L:0D Without nutrient	1.61	24.8	This work
<i>Chlorella</i> sp. isolated from peat swamp	Peat swamp water media 24L:0D $\text{NH}_4\text{Cl}$ 0.16 $\text{mg L}^{-1}$ nutrient	1.41	28.8	This work

however, the nutrient is costly [76,77]. Other works of using *Chlorella* sp. led to the lipid content up to 28.2% [32]. The possible reason is that the use of glycerol and the presence of  $\text{CO}_2$  could increase the lipid content; furthermore, the bold basal medium that is not cheap is also rich in nitrogen content [76]. However, the biomass production for those researches was still below than 0.5  $\text{g L}^{-1}$ . The significant biomass concentration of up to about 2  $\text{g L}^{-1}$  was observed by other observa-

tions [45,78]. The increase in light intensity and the use of costly nutrient were conducted. Other work was also supplied by boiler flue gas that is rich in  $\text{CO}_2$  and nitrogen [78]. In this research, the microalgae isolated from peat swamp could result in increase in the biomass up to 1.5  $\text{g L}^{-1}$  without nutrient. The small additional  $\text{NH}_4\text{Cl}$  also increased the lipid content up to 28%. Thus, it shows the great performance of microalgae isolated from peat swamp.

### 3.5. Statistical analysis

Significant effect of microalgal types on the biomass productivity ( $p = 0.0213$ ) and specific growth ( $p = 0.021$ ) was observed. A marginally insignificant effect of microalgal types on the biomass concentration ( $p = 0.078$ ) was obtained; it was probably due to different biomass concentration at beginning. Similar observation was found in the effect of photoperiod with significant effect of biomass productivity ( $p = 0.0068$ ) and specific growth ( $p = 0.038$ ), and insignificant effect of biomass ( $p = 0.077$ ). For the effect of additional nutrient, all parameters of biomass ( $p = 0.00052$ ), biomass productivity ( $p = 0.0011$ ) and specific growth ( $p = 0.0031$ ) were significant. For the effect of photoperiod and nutrient on the lipid content, significant effects were obtained with the value of  $p = 0.038$  and  $p = 6 \times 10^{-5}$ , respectively.

### 3.6. Kinetic model of the microalgal growth

Kinetic model to predict the biomass growth and production is important to evaluate the lag phase, maximum specific growth and potential maximum biomass production; hence, the process can be implemented in industrial microbiology or large scale [79,80]. In the present study, the four models of first order (FO), second order (SO), modified Gompertz (MG) and modified Logistic (ML) were used to predict the microalgal growth. Fig. 5(a), 5(b), 5(c) and 5(d) present the kinetic profiles for biomass concentration using those models and Table 3 shows the predicted parameters for those models. The second order kinetic model showed a better result to fit the experimental data compared to the simple first order exponential model; however, the  $R^2$  values were still 0.917 and 0.924, respectively. As shown in Table 3, the values of potential maximum bio-

mass concentration ( $\gamma_m$ ) for both predictive models were distant from the actual data, i.e.  $1.47 \text{ g L}^{-1}$ . The stationary phase was also not recognized by the model. Both modified Gompertz and logistic models showed much better performance compared FO and SO models and accommodated the stationary stage. The values of potential maximum biomass ( $1.52 \text{ g L}^{-1}$  for MG and  $1.42 \text{ g L}^{-1}$  for ML) were very close to the actual data. Furthermore, the modified logistic model showed a better fit to experimental data than the modified Gompertz one with higher  $R^2$  (0.981 compared to 0.969). Lam et al. [81] modelled the biomass growth of *Chlorella vulgaris* using Richard, Gompertz and Logistic models. A very good fitting was obtained with the  $R^2$  values of about 0.98. However, the lag phase was ignored and the initial biomass was removed from the plot and their analysis. Similar finding was also obtained for the work [82] using logistic model, the lag phase was not accommodated in the prediction. Here, the models of MG and ML included both parameters. However, both models could not predict the initial biomass concentration. To accommodate this prediction, the extension to the models was then required.

To improve the previous model, the modification to the Gompertz equation was conducted. The initial biomass concentration was added into the equation, and the potential maximum of biomass concentration in the equation could be adjusted to the initial biomass value by subtraction. The modification of model is described as follows:

$$\gamma = \gamma_0 + (\gamma_m - \gamma_0) \exp \left[ -\exp \left[ \frac{r_m \cdot \exp(1)}{(\gamma_m - \gamma_0)} (t_L - t) + 1 \right] \right] \quad (8)$$

where  $\gamma_0$  is the initial biomass concentration ( $\text{g L}^{-1}$ ) and other symbols are the same as described in the previous equations.

Fig. 6 presents the kinetic profiles for biomass concentration using a modification model to the modified Gompertz

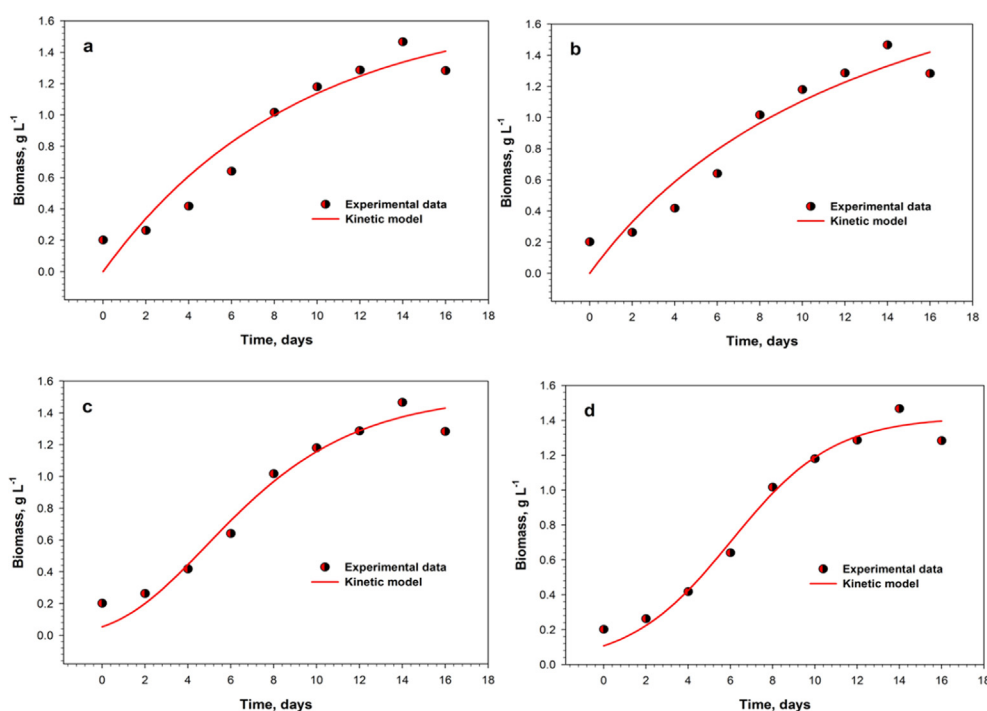
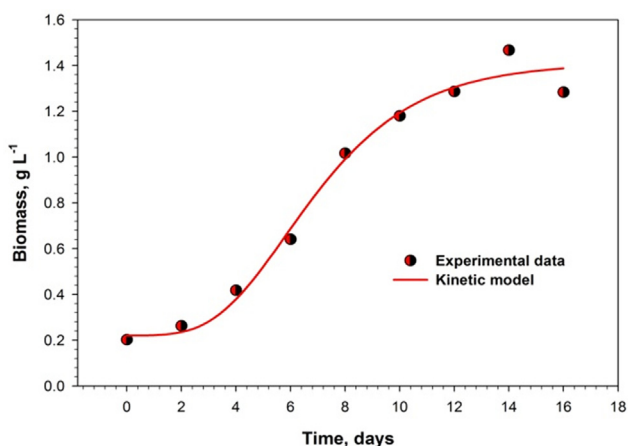


Fig. 5 Kinetic profile of biomass using (a) first order; (b) second order; (c) modified Gompertz; and (d) modified logistic.

**Table 3** Predicted parameters of various models for biomass concentration.

Parameter	Predicted value of various models			
	First order (FO)	Second order (SO)	Modified Gompertz (MG)	Modified Logistic (ML)
$\gamma_m$ (g L <sup>-1</sup> )	1.69	2.67	1.52	1.42
$r_m$ (g L <sup>-1</sup> d <sup>-1</sup> )	–	–	0.140	0.147
$t_L$ (d)	–	–	0.823	1.218
k	0.1119	0.0259	–	–
R <sup>2</sup>	0.917	0.924	0.969	0.981

**Fig. 6** Kinetic profile of biomass using a modification model to the modified Gompertz.**Table 4** Predicted parameters of proposed model for biomass concentration.

Parameter	Predicted value of proposed model
$\gamma_{m0}$ (g L <sup>-1</sup> )	0.22
$\gamma_m$ (g L <sup>-1</sup> )	1.412
$r_m$ (g L <sup>-1</sup> d <sup>-1</sup> )	0.168
$t_L$ (d)	3.215
R <sup>2</sup>	0.985

equation. Table 4 shows the predicted parameters of the proposed model for biomass concentration prediction. The kinetic model very well fitted the experimental data as shown in Fig. 6. High regression coefficient (R<sup>2</sup>) was obtained for this model with the value of 0.985. The model was also capable of envisaging the initial biomass concentration excellently. The stationary phase was also well modelled compared to the previous plotting using various models. This excellent prediction of experimental data is deemed very useful for the development of microalgal cultivation in higher scale or actual implementation.

#### 4. Conclusions

This study revealed that the microalgae originating from peat swamp showed great performance with highest biomass value of 1.72 g L<sup>-1</sup> and fastest growth time of 10 days. The commercial microalgae could also well stand for cultivation in peat

swamp (biomass of 1.6 g L<sup>-1</sup>) compared to that in the nutritious pure water (biomass of 1.36 g L<sup>-1</sup>). The microalgal biomass and its productivity increased significantly by 39.4% and 53%, respectively, while increasing light period from 12 h to 24 h. The presence of N source by using NaNO<sub>3</sub> in peat swamp culture resulted in highest biomass productivity of 0.086 g L<sup>-1</sup> d<sup>-1</sup> and specific growth of 0.345 d<sup>-1</sup> compared to its by using urea and NH<sub>4</sub>Cl. The highest lipid content of 28.78% was obtained in the cultivation using culture with NH<sub>4</sub>Cl. The modified Gompertz and logistic models showed the better prediction of biomass profiles compared to the first and second models. However, the extension model to the modified Gompertz equation showed an excellent prediction to the experimental data with R<sup>2</sup> of 0.985. Thus, this model is deemed useful for the implementation of the research in higher scale.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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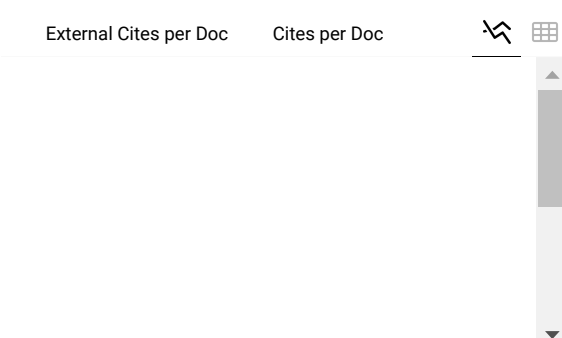
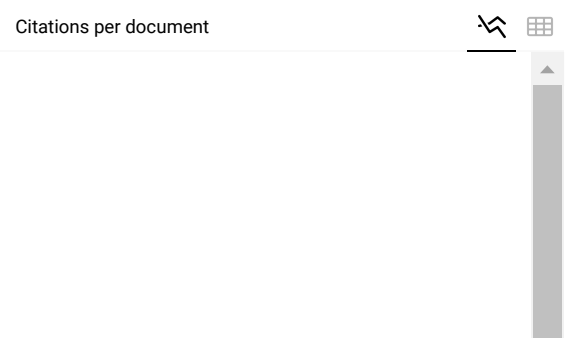
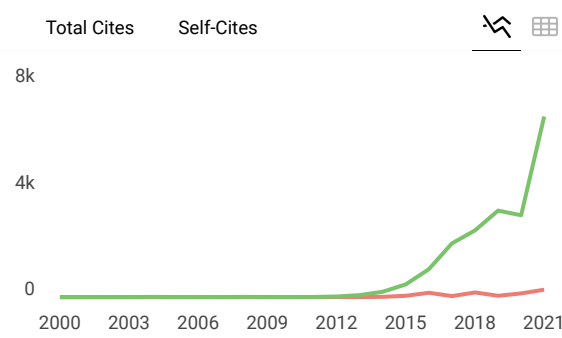
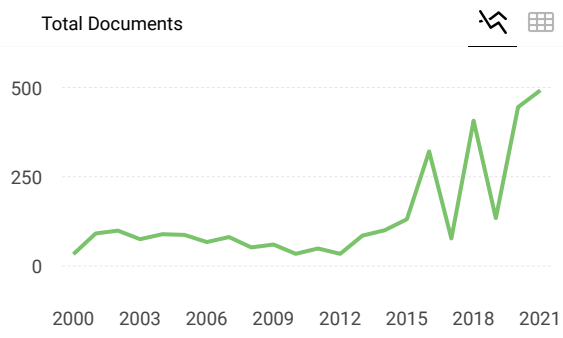
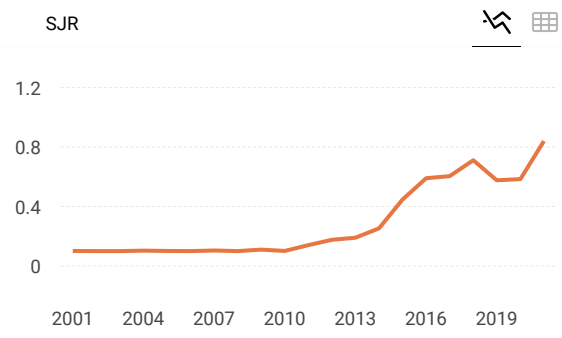


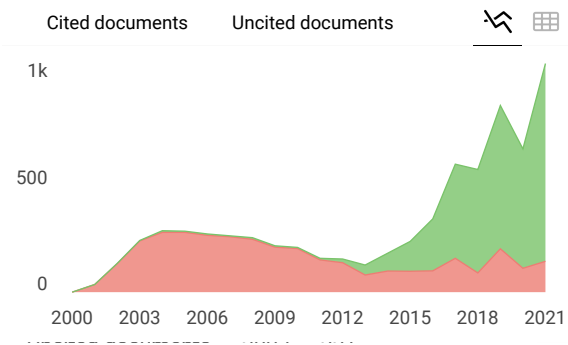
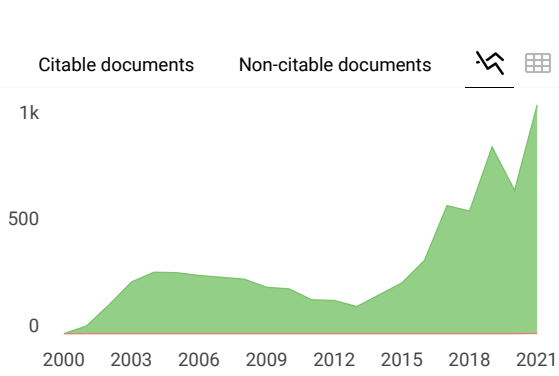
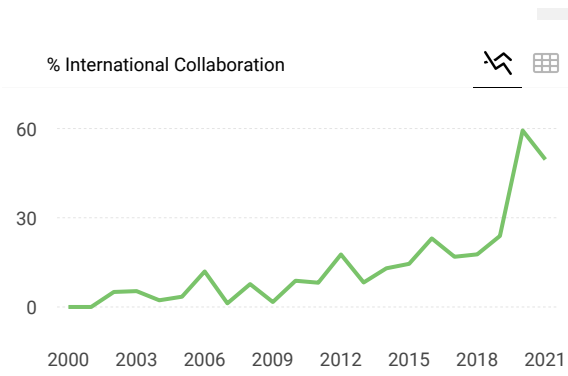
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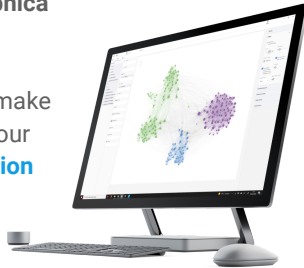
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