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Manipulation of Biochemical Compositions of *Chlorella* sp.

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Abstract. This work aimed to study the effect of several environmental parameters (light intensity, temperature, and aeration rate) on the accumulation of nutritional components and lutein production in a green microalgae *Chlorella* sp. It was proven in this work that the biochemical composition of *Chlorella* sp. could be manipulated through the control of environmental conditions during the cultivation. Six simple 2L bubble column photobioreactors installed in a well-controlled culture chamber was employed as a model system where temperature, light intensity, and aeration rate (u_{sg}) could be controlled in the range from 30-40ºC (± 0.5ºC), 10-30 kLux (± 0.1 kLux), and 0.5-1.5 cm/s (± 0.05 cm/s), respectively. Lipid and protein productivity were the most abundant at 35°C, 10 kLux and 1 cm/s, whereas carbohydrate productivity was found to be maximized at 30°C, 30 kLux and 0.5 cm/s. In addition, *Chlorella* sp. could also generate strong antioxidizing agents like lutein which was found to be mostly produced at 35°C, 10 kLux and 1 cm/s.

Keywords: *Chlorella* sp., biochemical compounds, lipid, protein, carbohydrate, lutein.

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1. Introduction

Chlorella has been widely known and cultured for a number of applications including food source for fish hatchery [1–3], animal food additives [4], human food supplementary [5–7], a biodiesel feedstock [8], and even a wastewater treatment agent. This microorganism can grow competently under non-strictly specified conditions. In other words, it can grow under temperature range of as wide as 4-35ºC, light intensity of 1,000-70,000 Lux, and aeration rate of 0-6 L/min [8–11]. This has made it one of the most versatile strains in algal culture industry. *Chlorella* can also be grown under different growth conditions to enable the accumulation of various specific components. For example, for a food source for fish hatchery or food additives, the alga is normally cultured under conditions urging the cell to accumulate high amount of protein. Seyfabadi et al. (2011)[12] revealed that at 25ºC with the light-dark cycle of 16:8, protein accumulation in *Chlorella vulgaris* was found to increase from 36±2.2 to 43±3 and 46±3.7 (%) with the increase of light intensity from 2,775 to 4,625 and 7,400 Lux, respectively. *Chlorella* UMACC 237 was reported to have the highest protein accumulation at 9ºC and light intensity of 3,108 Lux. [10]. As food, the cell must be cultured such that carbohydrate could be accumulated as much as practicable. On the other hand, if the product is biodiesel feedstock, the cell is stressed to produce the maximal amount of lipid. *Chlorella vulgaris* ESP-31 was reported to have the highest lipid accumulation at 25ºC and light intensity of 9 W/m^2 [13], while under the cultivation temperature and light intensity of 25ºC and 4,440 Lux, the cell could accumulate the highest amount of carbohydrate [14]. Other microalgae that have been studied for the accumulation of lipid, protein and carbohydrate regarding their cultivation conditions are such as *Nannochloropsis* sp. [15], *Isochrysis galbana* [15–16], *Choricystis minor* [17], *Spilurina platensis* [18], *Chaetoceros calcitrans f. pumilus* [19], *Amphora* sp. [20], *Dunaliella tertiolecta* [21], *Nannochloropsis oculata* CS-179 and *Isochrysis* sp. CS-177 [22].

In certain applications, high value products, e.g. antioxidants, might be needed and the alga must be grown in the right environment to be able to store such compounds [23]. Commercial lutein is produced from marigold but this suffers some disadvantages particularly long cultivation time and large area requirement. In this regard, microalgae can be an alternative source with comparable or even higher lutein productivity. For instance, lutein productivity from outdoors cultivation of *Muriellopsis* sp. was 180 $mg/m^2/d$ [24], approx. 11 times higher than that from marigold [25]. Several microalgae can effectively produce lutein such as *Scenedesmus almeriensis* [26–27], *Chlorella protothecoides* [28], *Chlorella zofingiensis*, *Chlorococcum citriforme* and *Neospongiococcus gelatinosum* [29].

Thailand also has its climate condition which is considered most suitable for the cultivation of several algal cultures, especially *Chlorella*. This work, hence, aimed to study the effect of several environmental parameters (light intensity, temperature, and aeration rate) on the accumulation of nutritional components and lutein production in a green microalgae *Chlorella* sp.

2. Materials and Methods

2.1. Operation of Bubble Column Photobioreactor

Each experiment was conducted with six sets of 2 L photobioreactor (bubble column) placed in a controlled chamber where temperature, light intensity and aeration were controlled. The control chamber included twelve compact fluorescence light bulbs (20 Watts) as a light source and a temperature control element. The temperature was controlled using an evaporative cooling and heater units. Light intensity and temperature were in the range from 10-30 klux (± 0.1 klux) and 30-40ºC (± 0.5ºC). A calibrated rotameter was used to control the volume of gas volumetric flow rate supplied to the system through a porous gas sparger at the base of the photobioreactors in the range from 0.5-1.5 cm/s (± 0.05 cm/s). Details of how the experiment was conducted (Design of Experiments, DOE) are provided in Fig. 1. The experiment was started by varying light intensity, and the best result was employed for the following experiments. The culture medium was the modified M4N [30–31]. Samples were collected daily in order to analyze the growth. After cell harvest (6 days), microalgal biomass was collected in order to analyze for the accumulation of nutritional components including total lipid, protein, carbohydrate, and lutein.

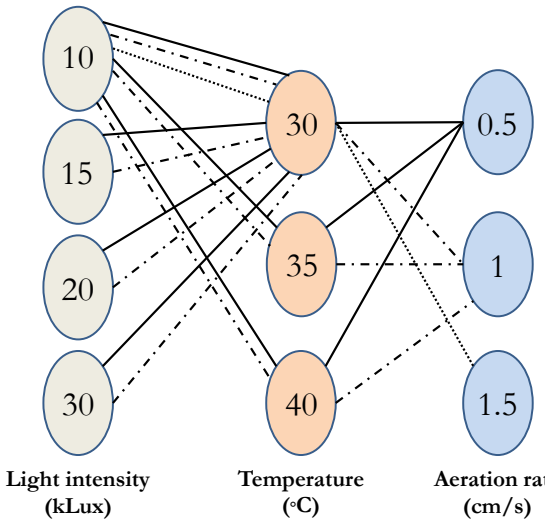

Fig. 1. Details of how this experiment was conducted: The different line type indicates how the matching between the various parameters was investigated.

2.2. Analyses

Total lipid: Biomass of microalgae in photobioreactor was harvested by centrifugation at 4,000 rpm, 10°C for 15 min (Kubota 7820). The cells were washed twice with deionized water. After drying the samples using freeze drier, the samples were pulverized in a mortar and extracted using a 2:1 (v/v) mixture of chloroform:methanol [32] by the typical soxhlet method.

Protein: Total nitrogen content of microalgal biomass was detected by an elemental analyzer (Perkin Elmer PE2400 Series II). The protein concentration of microalgae was estimated from the obtained nitrogen content according to the correlation reported in literature, i.e. protein concentration = nitrogen content x 4.44 [33].

Carbohydrate: Total carbohydrate concentration of microalgal biomass was considered simply as the remnant of the total biomass subtracted by the sum of lipid, protein, and ash.

Ash: Ash determination procedure according to Sluiter et al. (2005) [34] which is substantially similar to the ASTM Standard Method Number E1755-01 (Standard Method for the Determination of Ash in Biomass) was used to determine ash content in microalgae biomass.

Moisture: The moisture content of microalgal biomass was determined from the masses of microalgae before and after water evaporation. This method follows the procedure set out in the ASTM D 1762-84 (Reapproved, 2007) [35].

Lutein: Biomass was mixed with KOH and ethyl alcohol at the ratio of 1:0.6:10 (w/w/v). The mixture was shaken for 4 h after which ethyl alcohol (50 mL) was added. Lutein was extracted by solvent extraction with separatory funnel under dark place. Diethyl ether (80mL) and Na_2SO_4 (100 mL) were added. Low density lutein liquid was evaporated. Lutein was dissolved in ethyl alcohol where its quantity was analyzed by UV-VIS spectrophotometer (Agilant carry 60) at the wavelength of 478 nm.

3. Results and Discussion

3.1. How to Maximize Lipid Productivity

The cultivation of microalgae *Chlorella* sp. was conducted under several conditions. The alga seemed to grow best at the light intensity of approximately 10 kLux, temperature of 35°C and aeration at superficial gas velocity of 1 cm/s which yielded the highest lipid productivity of 37 mg/L/d. Moving away from this

optimal condition caused a decrease in lipid productivity, especially at high temperature, e.g. 38-40°C as shown in Fig. 2 where *Chlorella* grew considerably slowly. This corresponded well with the findings in literature which indicated that temperature higher than 37°C was not suitable for the growth of this algal species [36], and the suitable temperature range should be around 25-30°C [37–41]. Manipulating light intensity could facilitate the accumulation of lipid quite effectively. For instance, controlling the light intensity at 15 kLux could help enhance the lipid productivity to 36 mg/L/d when *Chlorella* sp. was cultivated at 30°C and aeration rate about 1 cm/s compared to 30 and 26 mg/L/d at light intensity of 20 and 10 kLux, respectively. In some cases, providing high light intensity might help the alga to better utilize light as a compensation for the poor circulation. This was observed when the aeration was poorly fixed at 0.5 cm/s (instead of 1 cm/s) and the lipid productivity could still be maintained at as high as 35 mg/L/d by increasing light intensity from 15 to 25 kLux (see Fig. 3).

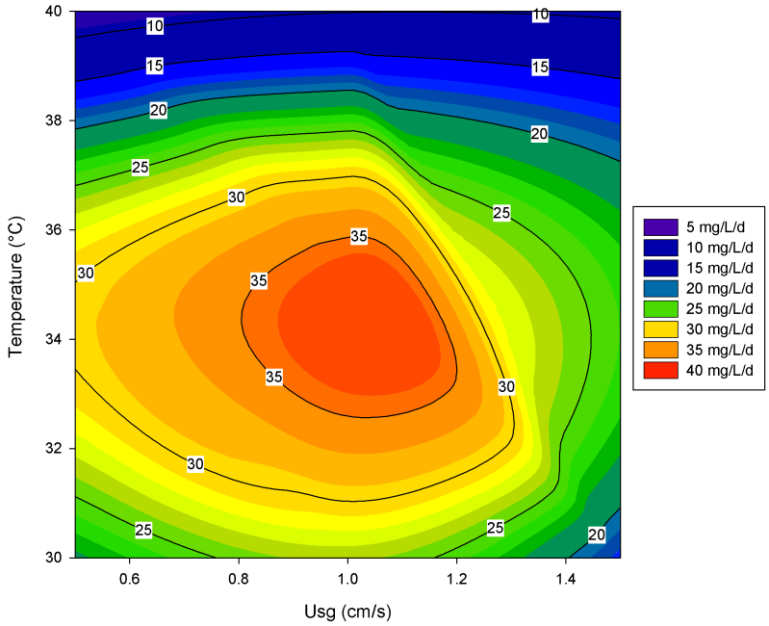

Fig. 2. Lipid productivity (mg/L/d) of *Chlorella* sp. (Light intensity=10 kLux).

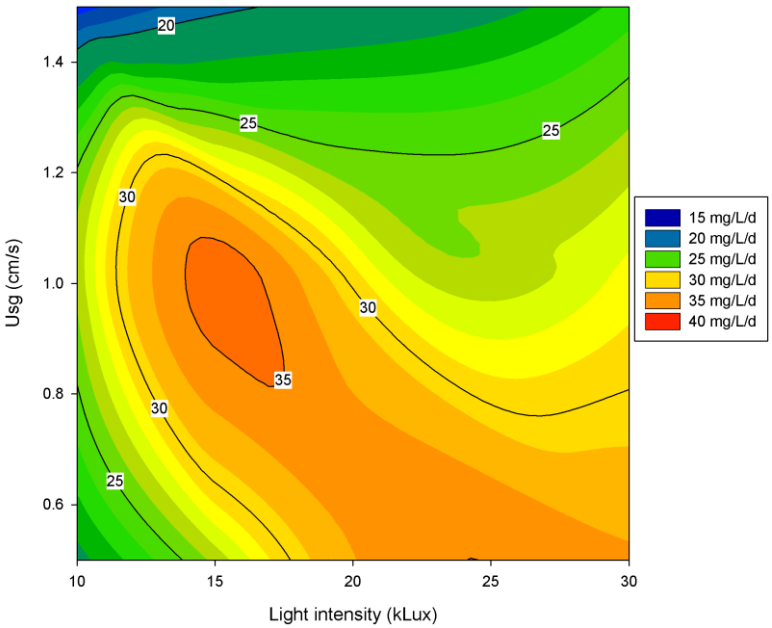

Fig. 3. Lipid productivity (mg/L/d) of *Chlorella* sp. (Temperature=30 °C).

3.2. How to Maximize Protein Productivity

Figure 4 illustrates that the highest protein productivity (89 mg/L/d) was achieved at 10 kLux, 35°C, and 1 cm/s (aeration). Protein productivity decreased significantly to 53 and 29 mg/L/d if temperature changed to 30 and 40°C, respectively. This was due to two major reasons. First, biomass productivity was the highest at 35°C at 195 mg/L/d. This dropped slightly to 133 mg/L/d at 30°C, but significantly dropped to 57 mg/L/d at 40°C. Secondly, the protein content at 35°C was relatively high at 45.6%wt when compared to 39.8% at 30°C. Although the maximum protein accumulation of 50.8% occurred at 40°C, the productivity was extremely low due to the poor growth rate. Figure 5 demonstrates further that inducing protein accumulation might be achieved by providing high light intensity at high circulation rate. For instance, increasing light intensity and aeration velocity from 15 to 30 kLux and 1 to 1.5 cm/s could notably enhance the protein productivity from 53 to 74 mg/L/d.

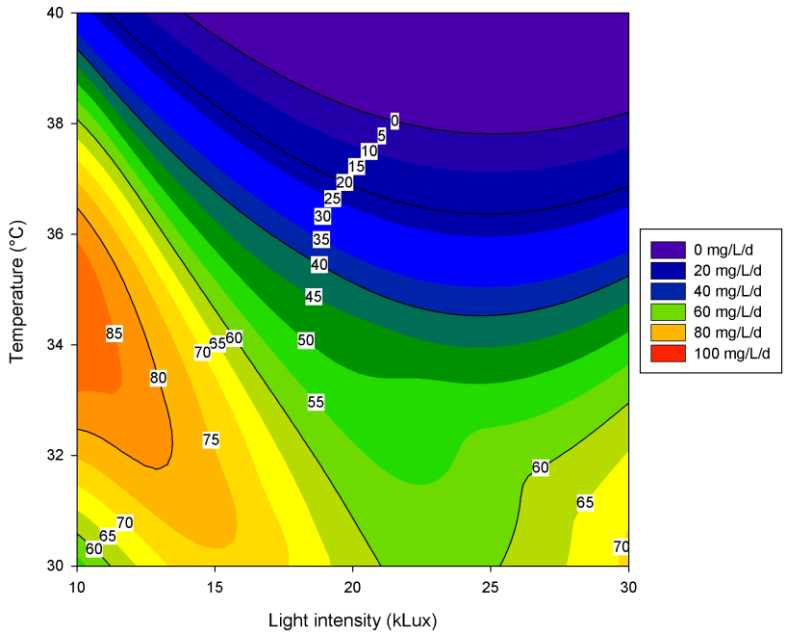

Fig. 4. Protein productivity (mg/L/d) of *Chlorella* sp. (Usg=1 cm/s).

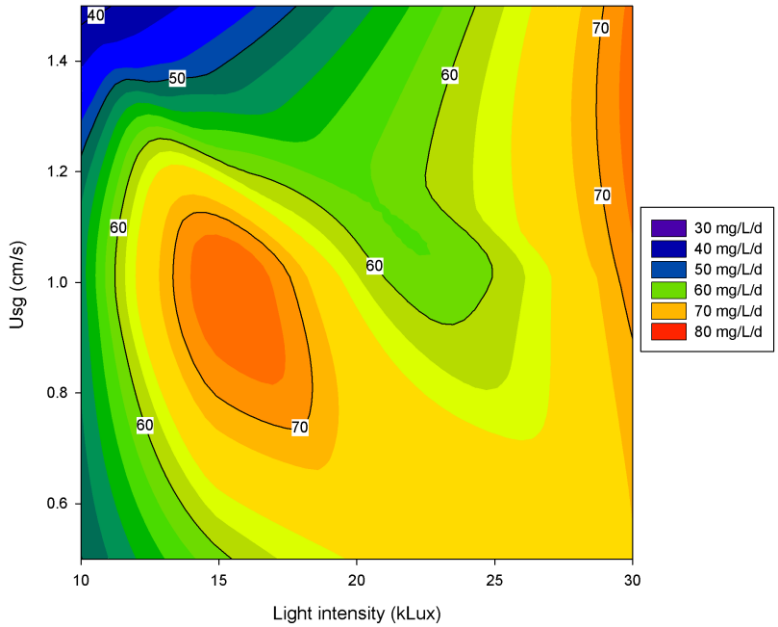

Fig. 5. Protein productivity (mg/L/d) of *Chlorella* sp. (Temperature=30 °C).

3.3. How to Maximize Carbohydrate Productivity

Figure 6 illustrates that the highest carbohydrate productivity of 60 mg/L/d could occur at two ranges of light intensity, i.e. at between 16 and 22 kLux, and at 30 kLux, both at 30°C and aeration velocity of 0.5 cm/s. When temperature and aeration were fixed at 30°C and 0.5 cm/s and the light intensity was reduced to 10 kLux, carbohydrate productivity decreased to 31 mg/L/d. At this low light intensity, there seemed to be an optimal range of temperature that could provide high carbohydrate content, i.e. between 33 to 36°C. Above and below this temperature range, carbohydrate productivity declined. At light intensity greater than 13 kLux, carbohydrate accumulated most at low temperature, and in this experiment, this was found at 30°C. Temperature greater than 33 or 34°C led to a decrease in carbohydrate productivity.

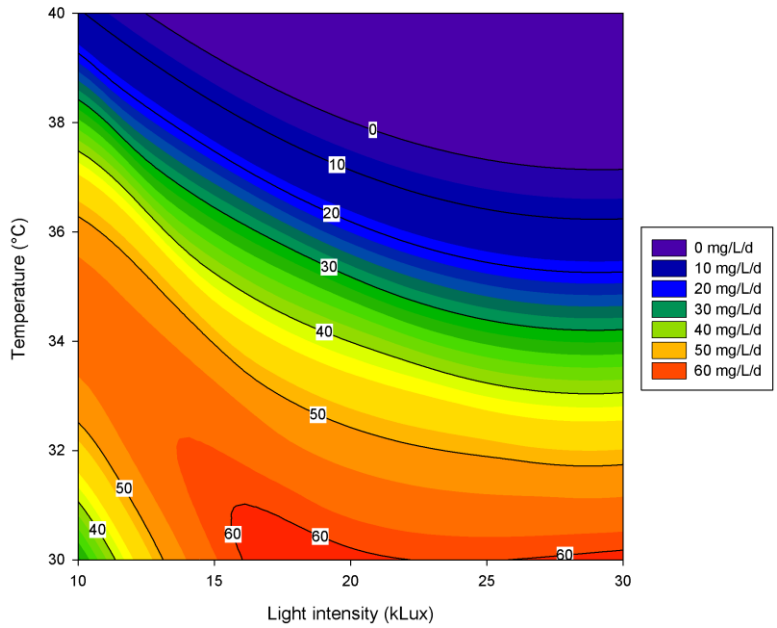

Fig. 6. Carbohydrate productivity (mg/L/d) of *Chlorella* sp. (Usg=0.5 cm/s)

3.4. How to Maximize Lutein Productivity

Lutein was among one of the most interesting biocomponents from *Chlorella* sp. considering its high price and the financial return rate. Lutein accumulation behavior was found to change with culture condition in a similar fashion to other nutritional compounds where the highest lutein productivity was 0.9 mg/L/d at 10 kLux, 35°C and aeration velocity of 1 cm/s. Lutein productivity seemed to decrease with increasing light intensity to greater than 10 kLux (Fig. 7). It was observed clearly that a decrease in temperature to 30°C lowered lutein productivity down to 0.3 mg/L/d. In fact, lutein was most accumulated when cells were exposed to a relatively high light intensity. Surprisingly, this same condition also resulted in the highest cell productivity indicating that cells were not in a stress condition. Sánchez et al. (2008b)[27] supported this finding and suggested that lutein was a primary metabolite of growth so the optimal condition for biomass productivity was the same with the optimal condition for lutein productivity.

Adjusting aeration could be a crucial factor for lutein accumulation (Fig. 8) whereas lutein decreased with aeration above or below 1 cm/s. To maintain high lutein productivity, temperature had to be controlled within the range of 33-37°C, aeration 0.8-1.2 cm/s, and with light intensity of 10 kLux. At these conditions, lutein productivity was in the range of 0.8-0.9 mg/L/d (270 mg/m²/d) which was about 16 times higher than that from marigold [42]. Table 1 illustrates the comparison of lutein yields from the various types of cultures which indicates that lutein from *Chlorella* was still not so high when compared with other algal species like *Chlorococcum citriforme*. However, *Chlorella* sp. is among the most common algal species in tropical area and the cultivation of such culture could be economically carried out which renders the production of lutein from such species more attractive.

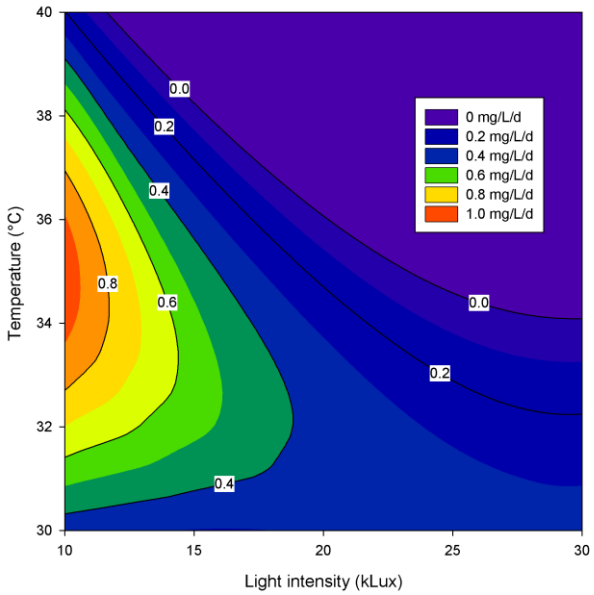

Fig. 7. Lutein productivity (mg/L/d) of *Chlorella* sp. (Usg = 1 cm/s).

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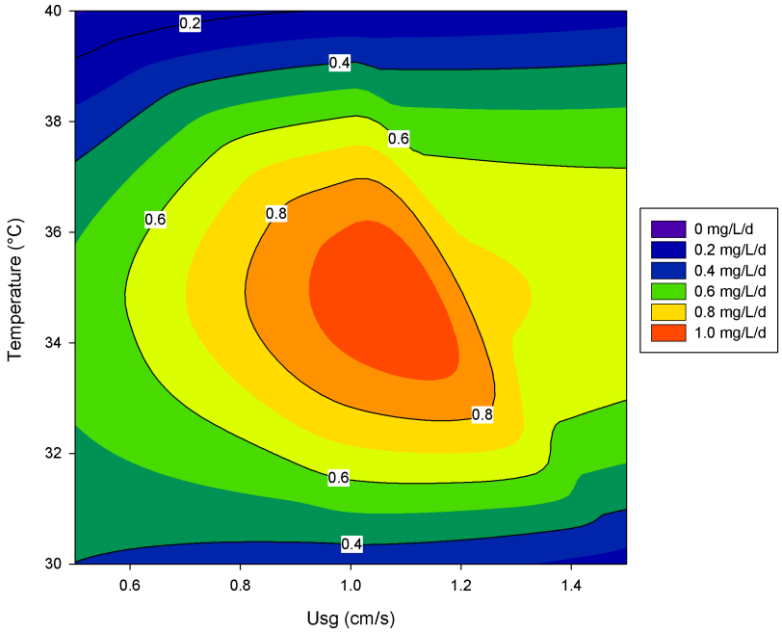

Fig. 8. Lutein productivity (mg/L/d) of *Chlorella* sp. (Light intensity = 10 kLux).

4. Conclusions

This work demonstrated that nutritional composition accumulated during the cultivation of *Chlorella* sp. could be adjusted, although slightly in some cases, by manipulating typical culture conditions at its most appropriate level, such as temperature, light intensity and aeration velocity. The selection of operating conditions therefore needs to be carefully considered to ensure that the final quality of the product could be achieved. For this work, the various cultivating conditions for the different purposes were examined and the summary of such conditions along with the comparison between the reported biochemical compositions from *Chlorella* spp. and those obtained from this work are provided in Tables 2 and 3, respectively.

Table 1. The comparison of lutein yields from the various types of cultures.

Biomass	Lutein content	Lutein productivity			References
	(mg/g dry biomass)	(g/rai/day)	(g/m^2/day)	mg/L/d	
Marigold	14.0	27	0.017	-	[42]
Muriellopsis sp.	4-6	160	0.10	-	[43]
	4.3	288	0.18	7.2	[24]
	5.5	336	0.21	0.8-1.4	[29]
Scenedesmus almeriensis	5.5	1,176	0.74	4.9	[26]
	4.5	464	0.29	-	[27]
Chlorella protothecoides	4.6	2,400	1.50	10.0	[28]
Chlorella zofingiensis	3.4	816	0.51	3.4	[29]
Chlorococcum citriforme	7.2	6,048	3.78	25.2	[29]
Neospongiococcus gelatinosum	7.6	4,032	2.52	16.8	[29]
Chlorella sp.	4.9	432	0.3	0.9	This work

Table 2. The summary of the conditions for each nutritional component of *Chlorella* sp.

Major Component	Maximal productivity (mg/L/d)	Optimal condition			Algal biomass productivity (mg/L/d)	Minor biocomponent productivity (mg/L/d)			
		Temperature (°C)	Light intensity (kLux)	Aeration velocity (cm/s)		Lipid	Protein	Carbohydrate	Lutein
Lipid	37	35	10	1	196	-	89	45	0.9
Protein	89	35	10	1	196	37	-	45	0.9
Carbohydrate	61	30	30	0.5	185	33	68	-	0.4
Lutein	0.9	35	10	1	196	37	89	45	-

Table 3. The comparison between the reported biochemical compositions from *Chlorella* spp. and those obtained from this work.

Strain	Operating parameter			Maximum biomass concentration (g/L)	Productivity (mg/L/d)			Reference
	Areation rate	Light intensity (Lux)	Temperature (°C)		Lipid	Protein	Carbohydrate	
Chlorella vulgaris ESP-31	300 rpm	3,057	25	0.17	23	17	6	[13]
Chlorella vulgaris	1200 (mL/min)	4,440	25	0.7	37	19	117	[14]
Chlorella vulgaris	6000 (mL/min)	2,220	22	0.86	13	-	-	[44]
Chlorella sp.	-	2,220	25±2	2	16	15	17	[45]
Chlorella vulgaris	2000 (mL/min)	18,500	25±1	1.48	40	26	26	[46]
Chlorella vulgaris	-	5,328	25±2	0.4	3	15	6	[47]
Chlorella sp.	1 (cm/s)	10,000	35±0.5	1.18	37	89	45	This work
Chlorella sp.	0.5 (cm/s)	30,000	30±0.5	1.11	33	68	61	This work

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