Observation of *Spirulina platensis* cultivation in a prototype household bubble column photobioreactor during 107 days

Shudi Zhang*, Fangfang Chen, Haiyue Pang, Yanfen Gao, Yonghuang Wen and Gueyhorng Wang

*Engineering Research Center of Natural Cosmeceuticals College of Fujian Province, Department of Public Health and Medical Technology, Xiamen Medical College, Xiamen, Fujian, PR China; †Shenzhen Space Food Analysis and Test Center Co. Ltd, Shenzhen, Guangdong, PR China; ‡Shenzhen Ludebao Health Food Co. Ltd, Shenzhen, Guangdong, PR China

**ABSTRACT**

Fresh *Spirulina* is more favorable than dried one, yet its ease of deterioration necessitates production of biomass that can be immediately consumed. We developed a prototype household photobioreactor and studied the semi-continuous cultivation and harvest conditions within 107 days. Three growth phases were observed featuring different growth rate and recovering time after harvest. Nutrients were consumed at rates of Fe > N > P > Mg > K. The pH mounted rapidly when below 9 and varied slowly between 9 and 10, whose variation positively correlated with that of biomass concentration. Bacterial load was at a constant level of about 2 × 10⁴ CFU, indicating good sanitary condition. Harvesting efficiency improved with greater biomass and filtration sack mesh number until reaching 3.5 g·L⁻¹ and 200, respectively. We hope this study can help the method development of fresh *Spirulina* cultivation in miniaturized systems.

**Introduction**

*Spirulina* has become one of the most well-known and useful cyanobacteria to mankind nowadays. Its versatility has been proven by its wide applicability in health food and supplements [1], animal feed [2], biofuel [3], cosmetics [4], wastewater treatment [5], pharmaceuticals [6], etc. Among all of its applications, food supplements may be the most prominent one. It contains high amounts of proteins, carbohydrates, vitamins, minerals, fatty acids, pigments and antioxidants and features important immune-modulating and bio-modulating effects [6], which makes it an ideal food for human beings.

Due to the perishable feature of fresh *Spirulina*, it is commonly dried after large-scale harvest and processed into the commercially available forms of powder, pellet, flake and capsule in order to prolong its shelf life from several hours to about one year. However, the product inevitably suffers from loss of main nutrients and bioactive compounds to a different extent (from 10% to 90%), including protein, sugar [7], phycobilin, lipid acid, β-carotene [8], total phenolic content, free radical scavenging activity [9–11] and so on. Moreover, dried *Spirulina* product can give out unpleasant flavor, which further hinders it from being popular among consumers [12]. Fresh *Spirulina*, on the other hand, is not faced with such problems, if it can be consumed immediately after being harvested. Photobioreactors (PBRs), in the forms of raceway pond, flat panel, bubble column, airlift, horizontal tube and stirred tank [13], makes production of fresh *Spirulina* possible. Although many studies have been carried out to improve the fresh *Spirulina* production in closed PBRs [14], yet quite few endeavors have been seen to develop PBRs that can run domestically. Considering the best way to consume *Spirulina* is dining immediately after harvest without any preservatives and other additives, the need of PBRs is prospective.

In order to maximize the *Spirulina* production by PBRs, the parameters that impact the *Spirulina* growth have been investigated by scientists, including different PBR forms [15], light source [16], culture media [17], carbon source [18], temperature [19] and so on. The physicochemical variables indicating the growth conditions have also been monitored, such as cell
mass/number concentration, cell morphology/size, pig-
mments, fatty acids, lipids, pH, \( pO_2 \), \( pCO_2 \) and so on
[20–22]. However, many of the current studies are
established on observations no longer than two
months, which may lack the information needed for
relatively long-term \textit{Spirulina} cultivation. Besides, stud-
ies monitoring the variation of inorganic salts during
\textit{Spirulina} cultivation are quite few [23], more related
efforts have been devoted to nutrient removal during
wastewater treatment [24–27], in which N and P nutri-
ents are the main factors to be monitored. Since the
inorganic salts are constantly consumed during culti-
vation, the knowledge of their concentration variations
must be acquired if a long-term \textit{Spirulina} cultivation
method is to be developed. Hence, we devised a pro-
totype household bubble column PBR in this study to
observe the relatively long-term (107 days) \textit{Spirulina}
cultivation conditions and found out the best way of
harvest.

\textbf{Materials and methods}

\textit{Layout of the \textit{prototype household bubble column PBR}}

The schematic diagram of the prototype household
bubble column PBR is shown in Figure 1. The whole
apparatus is enclosed in a 600 mm × 600 mm × 1370 mm cubic metal case with a movable front door
and an observatory window (label 1) mounted on it.
In the upmost part of the machine are the controlling
panel (label 2) and all the electronic devices connected
to its back side, including the power supply, light
source timer, air blower and its timer, water circulating
pump, water heating module, temperature sensing
module and ozone generator. Downwards is the upper
cover of the PBR column (label 3), with the water
pumping/discharging pipes, water heater, temperature
sensor and ozone outlet pipe mounted through it,
inserting into the PBR column (label 4) connected to
the cover. Note that there is a medium-sized hole on
the cover and correspondingly a lid covering it is
installed onto the end of the water discharging pipe
for the ease of \textit{Spirulina} harvesting, \( CO_2 \) aeration and
nutrients/water adding by simply taking off the lid.
The PBR column height is 800 mm and the diameter
is 300 mm, capable of loading 50 L culture medium in
it normally. Surrounding it are three LED light source
panels (38 mm × 52 mm) at the left, right and back
sides (label 5), the photon flux density of each of
which is about 420 \( \mu \text{mol·m}^{-2·s} \) when switched on.
On the bottom of the column fitted an aeration pipe
(label 6), which guides the air flow (7 \( \text{L·min}^{-1} \))
generated by the air blower lying behind the controlling
panel and blows bubbles into the culture medium. At
the bottommost is a water tap (label 7), which can
discard the waste water off when turned on. The PBR
column and the parts connecting to it are sterilized
using a disinfectant bought from a local market
before usage.

\textbf{Materials}

For \textit{Spirulina platensis} cultivation, \( \text{NaHCO}_3 \), \( \text{NaNO}_3 \), \( \text{NaCl} \),
\( \text{K}_2\text{HPO}_4 \), \( \text{K}_2\text{SO}_4 \), \( \text{MgSO}_4·7\text{H}_2\text{O} \), \( \text{EDTA} \) (ethylenedi-
aminetetraacetic acid), \( \text{CaCl}_2·2\text{H}_2\text{O} \), \( \text{FeSO}_4·2\text{H}_2\text{O} \), \( \text{H}_3\text{BO}_3 \),
\( \text{MnCl}_2·4\text{H}_2\text{O} \), \( \text{ZnSO}_4·7\text{H}_2\text{O} \), \( \text{CuSO}_4·5\text{H}_2\text{O} \), \( \text{Co(NO}_3)_2·6\text{H}_2\text{O} \),
(\( \text{NH}_4\))\text{MoO}_4·4\text{H}_2\text{O} \) were bought from Xilong Chemical
Co., Ltd (analytical grade, Guangdong, China), pure
water was acquired from Shenzhen Daneng yiliquan
Beverage Co., Ltd (Zhejiang, China), \( CO_2 \) supplement
cylinder was bought from Shenzhen Lunbo Technology
Co., Ltd (Guangdong, China), disinfectant fluid was
purchased from Jianzhisu Pharmaceutical Technology
Co., Ltd (Beijing, China); 100-mesh, 150-mesh, 200-mesh
and 300-mesh nylon filtration sacks of same volume
were acquired from Anhui Weilong Network Co., Ltd
(Anhui, China).

For the microbiological experiment, \textit{Plate count agar}
(PCA) was purchased from Beijing Aoboxing
Biotechnology Co., Ltd (Beijing China), KH$_2$PO$_4$, NaCl were bought from Guangdong Huankai Microbial Technology Co., Ltd (Guangdong, China), primary water was prepared by a water purifier (UPH-II-20T, Sichuan YOUPU Ultrapure Technology Co., Ltd, China).

**Strains and medium**

The strain *Spirulina platensis* 439 was obtained from Jiangxi Academy of Agricultural Sciences, China. The cultivation medium, which was a result of long-term amelioration of the Zarrouk's Medium [28] by the authors, was prepared (Table 1). The nutrient solutions were sterilized at 120 °C for 15 min before adding into the medium [15].

**Cultivation process**

The *Spirulina platensis* inoculum was carefully cultivated and kept in 2.5-L flasks until use. When inoculation to the PBR was to begin, the *Spirulina platensis* culture was filtered using a nylon filtration sack to about 50 g wet weight of *Spirulina platensis*, which was then transferred into the 50-L nutrient solution in the PBR column, resulting in a start-up wet weight biomass concentration ($C_{\text{biomass}}$) of about 1 g·L$^{-1}$.

During cultivation, the LED light source was operated at a daily 14 h:10 h light/dark cycle, and the air blower ran at a 20 min:10 min bubbling/resting cycle with an air flow rate of 7 L·min$^{-1}$. The cultivation temperature was monitored and kept within 30 ± 2 °C. 20 mL of culture liquid was sampled every weekday for further measurement, and harvesting was carried out intermittently when the UV-vis absorbance at 560 nm ($AB_{560\text{nm}}$) of the culture liquid was near 1000 (Table 2). Nylon filtration sack was utilized here to harvest *Spirulina platensis* by placing it between the water discharging lid and the PBR column and running the water circulating pump. According to the measurement result, the main nutrient salts (N, P, K, Mg, Fe) were replenished into the culture medium when signs of salt depletion or slow biomass growth were shown. Carbon source was supplied by external CO$_2$ aeration when pH was greater than 10, and the pH was mainly kept within a range of 9 to 10 (Table 3). The total volume of culture medium was kept constantly at 50 L.

During the cultivation period, an ozone sterilization experiment of the medium was carried out at day 77 with an ozone load of 1 g·h$^{-1}$. The total sterilization time was 1 h, and the medium was sampled every 15 min. After the sterilization, the microbiological condition of the medium was further monitored for 7 days to estimate the sterilization effect.

**Measurements**

Routine measurement of pH (PB-10, Satorius Scientific Instruments (Beijing) Co., Ltd, China) and $AB_{560\text{nm}}$ (UV-1102, Shanghai Tianmei Scientific Instrument Co., Ltd, China) of the sampled culture suspension were implemented for monitoring the carbon source and $C_{\text{biomass}}$. Afterwards, all the suspension was filtered and the resulted filtrate was subject to spectrophotometric analysis of N (Chinese National Standard GB 7480-1987), P (Chinese National Standard GB/T 5009.87-2016), and ICP-MS (inductively coupled plasma-mass spectrometry) analysis of Mg, K and Fe (ICAP Q, Thermo fishier Scientific, USA). All measurements were performed in triplicate.

Semi-continuous harvesting was carried out. When harvesting, the culture medium in the PBR column was pumped at a rate of 1.6 L·min$^{-1}$ and filtrated through a nylon filtration sack of certain mesh number, with the filtrate flowing back to the medium. After a measured period of time (so that the filtrated volume

| Ingredients        | Concentration (g·L$^{-1}$) |
|--------------------|-----------------------------|
| NaHCO$_3$          | 10.00                       |
| K$_2$HPO$_4$       | 0.50                        |
| NaNO$_3$           | 2.50                        |
| K$_2$SO$_4$        | 1.00                        |
| NaCl               | 0.50                        |
| MgSO$_4$·7H$_2$O   | 0.20                        |
| EDTA               | 0.84                        |
| CaCl$_2$·2H$_2$O   | 0.02                        |
| FeSO$_4$·2H$_2$O   | 0.02                        |
| H$_3$BO$_3$        | 2.80                        |
| MnCl$_2$·4H$_2$O   | 1.80                        |
| ZnSO$_4$·7H$_2$O   | 0.22                        |
| CuSO$_4$·5H$_2$O   | 0.05                        |
| Co(NO$_3$)$_2$·6H$_2$O | 4.4                    |
| (NH$_4$)$_6$Mo$_7$O$_2$·4H$_2$O | 0.01 |
can be calculated), the pump was stopped, and the retaining *Spirulina platensis* was collected for direct weighing (BSM2203, Shanghai Zhuojing Electronic Technology Co., Ltd, China) when no more liquid infiltrated through the sack.

The analysis of aerobic plate count of sampled culture liquid was carried out in accordance with Chinese National Standard GB 4789.2-2016 with all the agents and tools completely sterilized (MLS-3750, Sanyo electric Co., Ltd, Japan) before use.

### Data analysis

All the $C_{\text{biomass}}$ values were calculated via the software of Microsoft Excel after Eq. (1) had been fitted through the software of Origin 9 (OriginLab Co. Ltd, USA). All the variation trends of $C_{\text{biomass}}$, nutrient content, pH, aerobic plate count, harvest efficiency were pictured via the software of Origin 9 as well. The schematic diagram of the PBR was drawn via the software of Solidworks 2015 (Solidworks Co. Ltd, USA).

### Results and discussion

#### Relationship between biomass concentration and optical absorbance

In order to convert the absorbance data to the more practical form of $C_{\text{biomass}}$, we roughly estimate the relationship between them. On Day 41 we conducted 6 successive harvesting operations and measured the harvest amount of each operation and the medium absorbance after each harvest (Table 4). Note that the $C_{\text{biomass}}$ change was calculated via dividing the total harvest amount by the culture medium volume (50L). The concentration change versus AB 560nm was plotted in Figure 2(A). It shows good correlation between them ($R^2 = 0.9976$), which indicates the medium $C_{\text{biomass}}$ can be estimated by AB 560nm. We again measured the AB 560nm of the culture filtrate to be 0.010, which was supposed to contain no biomass, thus the equation can be rewritten to:

$$C_{\text{biomass}}(\text{gL}^{-1}) = 4.8872 \times \text{AB}_{560\text{nm}} - 0.0489 \quad (1)$$

Considering the moisture content of *Spirulina platensis* to be 85%–90% [29], the slope lies within the

---

### Table 2. Harvesting events throughout the 107 cultivation days.

| Day | $\text{AB}_{560\text{nm}}^1$ | $\text{AB}_{560\text{nm}}^2$ | Harvest (g) |
|-----|----------------------------|----------------------------|-------------|
| 15  | 0.580 ± 0.032              | 0.415 ± 0.029              | 46.213      |
| 27  | 1.747 ± 0.060              | 1.148 ± 0.039              | 155.334     |
| 28  | 1.221 ± 0.049              | 0.765 ± 0.030              | 132.196     |
| 31  | 1.38 ± 0.053               | 0.978 ± 0.035              | 123.652     |
| 34  | 1.092 ± 0.061              | 0.536 ± 0.024              | 157.525     |
| 41  | 1.261 ± 0.043              | 0.407 ± 0.028              | 209.245     |
| 45  | 0.91 ± 0.041               | 0.79 ± 0.022               | 36.458      |
| 48  | 1.3 ± 0.039                | 0.843 ± 0.032              | 149.134     |
| 49  | 0.853 ± 0.045              | 0.302 ± 0.020              | 146.283     |
| 56  | 0.853 ± 0.042              | 0.383 ± 0.026              | 140.139     |
| 61  | 1.38 ± 0.053               | 0.449 ± 0.027              | 303.497     |
| 64  | 0.982 ± 0.036              | 0.702 ± 0.038              | 80.843      |
| 69  | 1.240 ± 0.052              | 0.79 ± 0.028               | 117.755     |
| 77  | 1.112 ± 0.045              | 0.47 ± 0.021               | 211.235     |
| 92  | 0.879 ± 0.046              | 0.431 ± 0.021              | 106.736     |
| 107 | 1.306 ± 0.059              | 0.307 ± 0.026              | 290.874     |

1The subscripts 1 and 2 represent the $\text{AB}_{560\text{nm}}$ before and after harvest, respectively.

### Table 3. Nutrient replenishment and CO₂ aeration events throughout the 107 cultivation days.

| Day | NaCl | NaHCO₃ | NaNO₃ | K₂HPO₄ | K₂SO₄ | MgSO₄·7H₂O | FeSO₄·7H₂O | EDTA | pH¹ | pH₂² |
|-----|------|--------|-------|--------|-------|------------|------------|------|-----|------|
| 27  | \    | \      | \     | \      | \     | 5.19       | 0.78       | \    | 11.20±0.03| 9.47±0.02|
| 37  | \    | \      | 41.67 | \      | \     | \          | \          | \    | 10.14±0.02| 9.68±0.01|
| 45  | \    | \      | \     | \      | \     | \          | 0.78       | \    | 10.33±0.01| 9.27±0.02|
| 56  | \    | \      | \     | \      | \     | \          | \          | \    | 10.13±0.02| 8.00±0.01|
| 57  | 8.33 | \      | \     | \      | \     | \          | 2          | \    | \     | 9.67±0.03| 7.71±0.03|
| 61  | \    | \      | \     | \      | \     | \          | \          | \    | 10.06±0.02| 9.2±0.02 |
| 62  | \    | \      | 41.67 | 9.33   | 16.67 | 8          | \          | \    | \     | \    | \    |
| 77  | \    | \      | \     | \      | \     | \          | 2          | \    | \     | \    | \    |
| 82  | 8.33 | \      | 41.67 | 9.33   | 16.67 | 8          | 2          | 2    | 10.06±0.02| 9.2±0.02 |
| 92  | \    | 30     | \     | \      | \     | 3          | 0.5        | \    | \     | \    | \    |

¹The subscripts 1 and 2 represent the pH before and after CO₂ aeration, respectively.
range of 0.4887 to 0.7331 with respect to dry weight \( C_{\text{biomass}} \), which is close to the values in other studies where dry \( C_{\text{biomass}} \) was measured [30–32]. To test the applicability of Eq. (1), we applied it to the harvesting events listed in Table 2 to calculate the estimated harvests and compared them with real harvests, as shown in Figure 2(B). Again good linearity was acquired, so we utilized Eq. (1) to calculate medium biomass concentration in this study. Note that the equation was only a rough estimation since the \textit{Spirulina platensis} could not be completely removed from the filtration sack and no moisture content measurement was carried out, yet it was enough to track the growth condition of \textit{Spirulina platensis} in the study.

### Investigation of growth phases

We tracked the change of \( C_{\text{biomass}} \) and the concentration of the five nutrients N, P, Mg, K, Fe, with the results shown in Figure 3(A). Note that the nutrient concentration values have been normalized to the values of Day 1, and the harvesting and salts replenishing events have been marked out by yellow and purple bars, respectively. According to monitored growth data in Figure 3(A), 3 different characteristic growth phases were observed, namely latent phase (Day 3 to Day 22), exponential phase (Day 23 to Day 69), and decline phase (Day 70 to Day 107).

After inoculation, the \textit{Spirulina platensis} grew for two days and then fell into a relatively ‘dormant’ status, during which the \( C_{\text{biomass}} \) rarely changed. The first harvest was done in Day 15, after that \( C_{\text{biomass}} \) returned to original level and still remained relatively constant. The need of adaption time to the new cultivation environment was most likely the reason for this phenomenon, since the seed \textit{Spirulina platensis} was filtrated from a 2.5 L flask and moved to a 50 L PBR column during inoculation. It should be noted that the latent phase was quite long compared to other studies. For example, Ainas et al. also observed a latent growth period of about 10 days when they started \textit{Spirulina platensis} cultivation in a 0.2 L cylindrical PBR [33], which was shorter than the latent period found in this study. It may be due to the small PBR cultivation volume and the different experimental conditions applied therein; however, if the PBS is to be customized, the long latent phase is still a problem to be solved, which would be our next work to do.

From Day 23 to Day 69, because of its good adaptation to the new medium, the \textit{Spirulina} entered a rapid exponential growing period. According to the study done by Krishnamoorthy et al. [34], \textit{Spirulina} would reach a saturation concentration (wet basis) of about 8 g·L\(^{-1}\) in a customized 50 L rectangular PBR. In order to avoid the saturation effect during cultivation and maximize biomass production, we carried out semi-continuous harvest as soon as the \( C_{\text{biomass}} \) got around 6 g·L\(^{-1}\), as can be seen in Figure 3(A). Interestingly, by examining all the marked harvesting events on the \( C_{\text{biomass}} \) variation line, we can see that the \textit{Spirulina} growth would suspend for about 1 day rightly after harvesting. Moreira et al. [35] also

### Table 4. Harvesting experiment conducted on Day 41.

| Order | Harvest amount (g) | Cumulative amount (g) | Concentration change (g·L\(^{-1}\)) | \( A_{560nm} \) | Filtrated volume (L) |
|-------|--------------------|-----------------------|-------------------------------------|----------------|----------------------|
| 0     | 0.000              | 0.000                 | 0.000                               | 1.261 ± 0.049 | 0.000                |
| 1     | 39.038             | 39.038                | −0.781                              | 1.085 ± 0.041 | 7.800                |
| 2     | 35.918             | 74.956                | −1.499                              | 0.974 ± 0.059 | 8.500                |
| 3     | 39.968             | 114.924               | −2.298                              | 0.777 ± 0.051 | 10.920               |
| 4     | 32.058             | 146.982               | −2.940                              | 0.636 ± 0.034 | 10.920               |
| 5     | 33.728             | 180.710               | −3.614                              | 0.530 ± 0.047 | 15.600               |
| 6     | 28.538             | 209.248               | −4.185                              | 0.407 ± 0.037 | 18.720               |

Figure 2. Relationships between: (A) concentration change and \( A_{560nm} \); (B) real harvest and estimated harvest.
witnessed the same growth suspension phenomenon during semi-continuous cultivation of *Spirulina*. In their study, a 2-L vertical tubular PBR was utilized during the whole cultivation period of 55 days, and the biomass concentration would gain slowly or stay unchanged for a few days immediately after each harvest, which was not explained therein. After each harvest, the biomass concentration decreased, which would lead to the disruption of optimum *Spirulina* colony conditions; what is more, water replenishment was carried out during each harvest in order to keep the medium volume constant. As a consequence, the culture environment changed and it would take *Spirulina* some time to adapt to it.

Interestingly, *Spirulina* did not remain the same high growth rate throughout the exponential phase. Its growth slowed down from Day 41 to Day 56, indicated by more than two growth suspension days after harvest. To seek the potential reason, we checked the content of the five nutrients during this period and found that the content of Fe declined to lower than 0.1 mg·L⁻¹, which was considered to be responsible for the low growth recovery after harvest. Fe is an essential nutrient for algal growth, it is involved in chlorophyll and ferredoxin production, enzyme activities and so on [36], and can easily be the most limiting factor of growth. To remedy the situation, we implemented a salt replenishment on Day 57, then the *Spirulina* regained its fast-growing feature for the next 13 days (from Day 57 to Day 69), which verified the limiting effect of Fe depletion.

However, from Day 70 the growth again started to slow down, together with growth suspension time becoming longer and longer after each harvest until the end of the whole observation (Day 107). There was no shortage of nutrients during this period owing to regular nutrient replenishments (Table 3), so the *Spirulina* could be diagnosed to be on the way to the decline phase. With the ongoing cultivation process, the cumulatively supplied salts and excreted chemicals from *Spirulina* would gradually change the medium environment and eventually place the *Spirulina* in a stress state. According to a study done by Batac et al. [37], excessive Na and K ions could pose an inhibiting effect on the microalgae growth in closed cultivation systems [37]. On day 108, a large amount of foam floated on the surface of the culture medium, which had turned yellow and smelly, indicating the actual death phase in the growth curve and the end of measurement [38].
Study of nutrient consumption

Nutrients are crucial to the microalgae cultivation, since they are essential components of some bioactive molecules and participate in a large variety of biochemical processes [38]. To study the influence of nutrients on *Spirulina* growth, the variations of N, P, Mg, K, Fe content in the medium were measured (Figure 3A). All the salt contents remained unchanged during almost the whole latent phase, which was in accordance with the ‘dormant’ behavior of *Spirulina*. However, the nutrients started to be consumed after Day 17, which happened several days before the *Spirulina* began to grow rapidly. This may indicate that, when the *Spirulina* was adapting to the new medium, it would prepare itself for the fast growth by taking in the nutrients from the medium around it and waiting to be in the right condition, resulting in the time lag. Afterwards, the nutrient contents declined continuously at different speeds. To check the nutrient consumption rates more clearly and specifically, we selected the data segments in Figure 3(A) that showed a steady decreasing trend apart from the fluctuating ones (salt replenishment and so on), pieced them together and fitted them, leading to the result shown in Figure 3(B). According to the figure, the nutrient contents decreased quite linearly. The slopes of fitting were −0.0015, −0.0054, −0.0079, −0.0107 and −0.0429 for K, Mg, P, N and Fe, respectively, indicating the nutrient consumption rate ranking of Fe > N > P > Mg > K.

K almost remained unchanged after the fast consumption period. Krishnamoorthy et al. [34] cultivated *Spirulina* in a wastewater medium, which contained 9594 ppm of K, in a 50-L rectangular PBR, and found that 75% of K were absorbed by *Spirulina* eventually. They stated that K ion was mainly involved in osmotic regulation of microalgae, which would be greatly taken in *Spirulina* cell due to the osmotic pressure posed by the wastewater. It could also explain the low K absorption rate in this study, since the salinity of the medium is quite normal and *Spirulina* would face little osmotic pressure, and K would be needed only when new *Spirulina* cells were produced.

Mg is involved in the synthesis of chlorophyll, RNA and functional enzymes and influences bacterial permeability, which is a crucial element to microalgae growth [39]. In this study, Mg was consumed faster than K, but not too fast. Su et al. [39] investigated the influence of Mg concentration on the biomass productivity of another cyanobacterium of genus *Thermosynechococcus* in a 3.5-L flat plate PBR, and observed that the productivity improved only about 20% when the Mg content increased from 0.15 to 6.09 mmol·L⁻¹ [39]. It can be inferred that Mg is indeed an indispensible nutrient to microalgae, yet it is not quite a growth limiting factor, which supports the relatively low Mg consumption rate observed in this study.

P and N are also nutrients crucial to life since they are the major components of many bioactive molecules, such as proteins, DNA, etc. The concentration of these two nutrients exhibited similar variation trends (Figure 3A), and their relatively high decreasing rates were also close to each other (Figure 3B), with N slightly higher that P. According to the study by Krishnamoorthy et al., 84% of N and 90% of P were removed from the wastewater medium during *Spirulina* cultivation in a 50-L rectangular PBR, which is in accordance with our observation, though the P consumption was slightly higher than that of N therein [34].

Fe plays a very important role in photosynthesis reactions, DNA replication and repair, cell cycle progression, metabolic catalysis and lipid production; as a result, the demand for Fe in microalgae growth is great, making it a primary growth limiting factor [40]. In this study, Fe was consumed to a greater extent than the other nutrients, the depletion of which caused slower growth rate from Day 41 to Day 56 during the exponential phase, as discussed above. Kona et al. [40] also conducted an experiment to study the influence of iron concentration on microalgae growth in a 0.5-L PBR, and witnessed much greater biomass production when proper amount of Fe was added, compared with the control group (no Fe was added) where the biomass concentration barely increased. To sustain the cultivation in this study, nutrients were replenished intermittently. Interestingly, during Fe replenishment, the kinetics of Fe uptake from the medium showed a trend different from that of the other measured micronutrients. In the first replenishment (Day 37), 0.78 g FeSO₄·7H₂O was added into the 50L medium, but the Fe content did not increase at all. The reason may be the absence of a chelating agent in this salt addition, hence the added Fe²⁺ ions were precipitated in the high-pH liquid environment to the form of Fe(OH)₃, which features low solubility and bioavailability [36]. Consequently, we added FeSO₄·7H₂O together with EDTA in the following replenishment and an obvious increase in Fe content could be detected. However, immediately following it came the rapid decrease in Fe content, implying that the excessive Fe²⁺ ions could not survive long in liquid condition where pH rises constantly and not too much of Fe nutrient salt should be replenished at a time.
**Variation of pH during cultivation**

pH is also a parameter that should be taken seriously in *Spirulina* cultivation. It plays a significant role in protein functioning and pigment content, and an alkaline environment is preferred to maintain N and P nutrient content; on the other hand, lack of proper pH maintenance may result in system collapse and unexpected cell death [38]. pH also served as an indicator of carbon source conditions due to the interactions among CO$_2$, H$_2$CO$_3$, HCO$_3^-$ and CO$_3^{2-}$ in the medium [18,32], hence we also tracked the variation of pH during cultivation (Figure 4A). Generally, the pH value rose steadily between 9 and 10 at a relatively slow speed, indicating the continuous consumption of carbon source. Krishnamoorthy et al. [34] found that the medium pH varied between 9.1 and 10.4 in a 50-L rectangular PBR within 14 days, and Ainas et al. [33] also observed the same pH variation range in a 0.2-L cylindrical PBR within 70 days, all of which were consistent with our results. In order to keep the pH within the optimum range of 9–10 for *Spirulina* cultivation [32], we aerated the medium with CO$_2$ intermittently when the pH exceeded 10. By examining the whole pH variation trend, we can see that the value mounted quickly as soon as it fell below 9 after CO$_2$ aeration, resulting in the ‘sharp negative peaks’.

To further see the relationship between *Spirulina* growth and pH variation, we plotted the change rate of $C_{\text{biomass}}$ (g·L$^{-1}$·d$^{-1}$) and that of pH (d$^{-1}$) originating from Figure 4(A), with the result shown in Figure 4(B). As can be seen, the pH value was positively correlated with the biomass concentration of *Spirulina*, which can be explained by the following chemical equation:

\[
2H^+ + CO_3^{2-} \leftrightarrow H^+ + HCO_3^- \leftrightarrow H_2CO_3 (aq) \leftrightarrow CO_2 + H_2O \rightarrow C_4H_7O_4 + O_2 \uparrow
\]  

(2)

Microalgae consume CO$_2$ during the photosynthesis process, which makes the equilibrium of the above equation shift rightwards, resulting in the decrease in H$^+$ content and the increase in pH value. However, two different correlation trends were observed, with most of the values following Trend 1 and the data from the ‘sharp negative peaks’ following Trend 2 (Day 1, Day 60, Day 63 and Day 64). Since the most suitable pH range for *Spirulina* growth is within 9–10, the well adapted *Spirulina* might take in more carbon nutrient when the pH was below 9 after CO$_2$ aeration and adjust the medium to the best pH range, resulting in the phenomenon observed in this study.

**Bacterial load and ozone sterilization**

Typically, bacteria consisting of both harmful and beneficial species would grow along with microalgae, and most of them would exhibit symbiotic features and enormously help in the production of high-density biomass [41]. However, if *Spirulina* is to be harvested freshly and consumed immediately, the bacterial load should meet the criterion of no more than 1×10$^4$ CFU according to the Chinese food safety standard (DBS 44/006-2016). To check the medium microbiological condition and evaluate the necessity of sterilization, we measured the aerobic plate count of the medium under different ozone sterilization time (every 15 min in 1 h, ozone output 1 g·h$^{-1}$) on Day 77 (Figure 5A). The aerobic plate count, which was 1.855×10$^4$ CFU before sterilization, could not meet the above food safety standard, which necessitated the ozone sterilization conducted in this study. It declined gradually during the process, and 79.78% of the aerobic microorganism was eliminated after one hour of sterilization, which was below the demanded criterion. Chegukrishnamurthi et al. also implemented an ozonation experiment during *Chlorella vulgaris* cultivation in a 3.4 L airlift PBR, and a significant bacterial load reduction of 6.5 log was observed after 30 min of sterilization with an ozone load of 1 g·h$^{-1}$ [41]. The authors recorded higher sterilization efficiency, which may be due to the different microalgae cultivated and the much higher initial bacterial load (10$^{11}$ CFU) therein.

To further study how the condition would change after sterilization, we kept tracking the value for six more days after sterilization, which is shown in Figure 5(B). The microorganism continued to grow rapidly after sterilization and reached back to the original value within 4 days; however, its growth slowed down from then on and remained relatively constant below 2×10$^4$ CFU. It may be due to the high-pH environment condition of the medium, which can inhibit the growth of microorganism and make them stay at a low level.

According to the above results, the total plate count in the culture medium was constantly below 2×10$^4$ CFU, which, though slightly exceeded the food safety criterion, could still indicate its good sanitary condition. If immediate *Spirulina* consumption after harvesting is to be done, as the word ‘household’ means, ozone sterilization might be carried out in the medium, which would kill nearly 80% of the aerobic microorganism, and the good sanitation condition would remain within the next 4 days.
Factors influencing the harvesting efficiency

After the optimization of cultivation, the next harvesting conditions should also be investigated to provide guidance to customers. As illustrated above, we conducted a cumulative harvest experiment on Day 41 (Table 4, sack mesh number 200), which is a good chance to find the relationship between $C$ biomass and harvest efficiency. We measured the AB 560nm of the medium before and after each harvest, through which the average $C$ biomass,avg of the medium during each harvest can be calculated by Eq. (1). Then the harvest efficiency ($E_{\text{harvest}}$) can be calculated by:

$$E_{\text{harvest}}(\%) = \frac{C_{\text{biomass,avg}}}{m_{\text{filtration}}} \cdot \frac{V_{\text{filtration}}}{m_{\text{harvest}}}$$ (3)

where $V_{\text{filtration}}$ (L) and $m_{\text{harvest}}$ (g) represent the filtered medium volume and weight of collected *Spirulina* during each harvest, respectively. As can be seen in Figure 6(A), the filtration efficiency was relatively low when little biomass was present in the medium after cumulative harvest within one day (Day 41). As the biomass increased, $E_{\text{harvest}}$ rose accordingly and kept relatively constant when $C_{\text{biomass}}$ exceeded 3.5 g·L$^{-1}$. It may be explained that when there was more biomass in the medium, the chance of the mesh hole being blocked by *Spirulina* cells increased and improved the $E_{\text{harvest}}$; however, the shorter and smaller *Spirulina* cells were not affected and would penetrate the hole anyway, thus the $E_{\text{harvest}}$ did not increase with higher $C_{\text{biomass}}$. Moreover, we investigated the impact of sack mesh number on $E_{\text{harvest}}$ on Day 48 (Figure 6B). When a sack of small mesh number of 100 was utilized, $E_{\text{harvest}}$ could be as low as about 20%. It then rapidly increased with greater sack mesh number and stayed relatively constant between 80% and 90% when the mesh number exceeded 200. It may again be due to the thinner *Spirulina* cells which can penetrate the mesh holes regardless of the size. As a result, the optimum harvest condition in this PBR is using a sack of 200 mesh size when the $C_{\text{biomass}}$ reaches 3.5 g·L$^{-1}$.

Conclusions

It was observed that *Spirulina* growth featured 3 phases (latent-exponential-decline) according to the growth rate and the recovering time. Nutrients were consumed at different rates of Fe > N > P > Mg > K. pH normally varied within 9–10 and correlated positively to the variation trend of biomass concentration. Medium bacterial load declined by 79.78% after one
hour’s ozone sterilization, and grew back to the constant level of about $2 \times 10^4$ CFU. The best harvesting condition was using nylon sacks of 200 mesh number when the biomass concentration exceeded $3.5 \, \text{g} \cdot \text{L}^{-1}$.

**Data availability**

The data that support the findings of this study are available from the corresponding author, Shudi Zhang, upon reasonable request.

**Disclosure statement**

The authors declare no conflict of interests.

**Funding**

This study was funded by the Educational and Scientific Research Program for Young Scholar Sponsored by Educational Department of Fujian Province (JAT200733) and the Emerging and Future Industry Development Funds in Shenzhen (20170503163617880).

**References**

[1] Hosseini S, Shahbazizadeh S, Khosravi-Darani K, et al. Spirulina paltensis: food and function. CNF. 2013;9(3):189–193.

[2] Belay A, Kato T, Ota Y. Spirulina (arthrospira): potential application as an animal feed supplement. J Appl Phycol. 1996;8(4–5):303–311.

[3] Rempel A, de Souza Sossella F, Margarites AC, et al. Bioethanol from Spirulina platensis biomass and the use of residuals to produce biomethane: an energy efficient approach. Bioresour Technol. 2019;288:121588.

[4] Wang H-MD, Chen C-C, Huynh P, et al. Exploring the potential of using algae in cosmetics. Bioresour Technol. 2015;184:355–362.

[5] Zhou W, Li Y, Gao Y, et al. Nutrients removal and recovery from saline wastewater by Spirulina platensis. Bioresour Technol. 2017;245(Pt A):10–17.

[6] Khan Z, Bhadouria P, Bisen P. Nutritional and therapeutical potential of spirulina. Curr Pharm Biotechnol. 2005;6(5):373–379.

[7] Desmorieux H, Hernandez F. Biochemical and physical criteria of *spirulina* after different drying processes. In: Silva MA, Rocha SCS, editors. Proceedings of the 14th International Drying Symposium. São Paulo City, Brazil, 22–25 August 2004. UNICAMP, 2004. Vol. B: 900–907.

[8] Bennamoun L, Afzal MT, Léonard A. Drying of alga as a source of bioenergy feedstock and food supplement - a review. Renew Sust Energy Rev. 2015;50:1203–1212.

[9] Tello-Ireland C, Lemus-Mondaca R, Vega-Gálvez A, et al. Influence of hot-air temperature on drying kinetics, functional properties, colour, phycobiliproteins, antioxidant capacity, texture and agar yield of alga gracilaria chilensis. LWT - Food Sci Technol. 2011;44(10):2112–2118.

[10] Costa BR, Rocha SF, Rodrigues MCK, et al. Physicochemical characteristics of the spirulina sp. dried in heat pump and conventional tray dryers. Int J Food Sci Technol. 2015;50(12):2614–2620.

[11] Kuatrakul I, Kuarthongsri P, Yabuuchi C, et al. Sensory descriptive analysis and physicochemical properties of spirulina platensis from different drying processes: hot air drying and microwave vacuum drying. Curr Appl Sci Technol. 2017;17(2):191–199.

[12] Agustini T, Soetrisnanto D, Ma’ruf W. Study on chemical, physical, microbiological and sensory of yoghurt enriched by Spirulina platensis. Int Food Res J. 2017;24(1):367–371.

[13] Ting H, Halfeng L, Shanshan M, et al. Progress in microalgae cultivation photobioreactors and applications in wastewater treatment: a review. Int J Agri Bio Eng. 2017;10(1):1–29.

[14] Ma Z, Ahmed F, Yuan B, et al. Fresh living arthrospira as dietary supplements: current status and challenges. Trends Food Sci Technol. 2019;88:439–444.

[15] Oncel S, Sukan FV. Comparison of two different pneumatically mixed column photobioreactors for the cultivation of *Artrospira platensis (Spirulina platensis)*. Bioresour Technol. 2008;99(11):4755–4760.

[16] Xue S, Su Z, Cong W. Growth of Spirulina platensis enhanced under intermittent illumination. J Biotechnol. 2011;151(3):271–277.

[17] Soni RA, Sudhakar K, Rana RS. Comparative study on the growth performance of Spirulina platensis on modifying culture media. Energy Rep. 2019;5:327–336.

[18] Cao X, Xi Y, Liu J, et al. New insights into the CO$_2$-steady and pH-steady cultivations of two microalgae based on continuous online parameter monitoring. Algal Res. 2019;38:101370.

[19] Liu C, Zou D, Yang Y, et al. Temperature responses of pigment contents, chlorophyll fluorescence characteristics, and antioxidant defenses in *Gracilaria lemaneiformis* (Gracilariaceae, Rhodophyta) under different CO$_2$ levels. J Appl Phycol. 2017;29(2):983–991.

[20] Havlik I, Reardon KF, Únal M, et al. Monitoring of microalgae cultivation photobioreactors and applications of microalgal cultivations with on-line, flow-through microscopy. Algal Res. 2013;2(3):253–257.

[21] Havlik I, Lindner P, Scheper T, et al. On-line monitoring of large cultigens of microalgae and cyanobacteria. Trends Biotechnol. 2013;31(7):406–414.

[22] White S, Anandraj A, Bux F. PAM fluorometry as a tool to assess microalgal nutrient stress and monitor cellular neutral lipids. Bioresour Technol. 2011;102(2):1675–1682.

[23] Wu Y-H, Yu Y, Li X, et al. Biomass production of a *scenedesmus* sp. under phosphorous-starvation cultivation condition. Bioresour Technol. 2012;112:193–198.

[24] Praveen P, Loh K-C. Nutrient removal in an algal membrane photobioreactor: effects of wastewater composition and light/dark cycle. Appl Microbiol Biotechnol. 2019;103(8):3571–3580.

[25] Zhai J, Li X, Li W, et al. Optimization of biomass production and nutrients removal by Spirulina platensis from municipal wastewater. Ecol. Eng. 2017;108:83–92.

[26] Liu H, Chen H, Wang S, et al. Optimizing light distribution and controlling biomass concentration by continuously pre-harvesting *Spirulina platensis* for improving...
the microalgae production. Bioresour Technol. 2018;252:14–19.

[27] Yu H, Kim J, Lee C. Potential of mixed-culture microalgae enriched from aerobic and anaerobic sludges for nutrient removal and biomass production from anaerobic effluents. Bioresour Technol. 2019;280:325–336.

[28] Soni RA, Sudhakar K, Rana RS. Spirulina - from growth to nutritional product: a review. Trends Food Sci Technol. 2017;69:157–171.

[29] Patel P, Jethani H, Radha C, et al. Development of a carotenoid enriched probiotic yogurt from fresh biomass of spirulina and its characterization. J Food Sci Technol. 2019;56(8):3721–3731.

[30] Zeng X, Danquah MK, Zhang S, et al. Autotrophic cultivation of Spirulina platensis for CO₂ fixation and phycocyanin production. Chem Eng J. 2012;183:192–197.

[31] Leduy A, Therien N. An improved method for optical density measurement of the semimicroscopic blue green alga Spirulina maxima. Biotechnol Bioeng. 1977;19(8):1219–1224.

[32] Jung F, Jung CGH, Krüger-Genze A, et al. Factors influencing the growth of Spirulina platensis in closed photobioreactors under CO₂ - O₂ conversion. JCB. 2019;5(2):125–134.

[33] Ainas M, Hasnaoui S, Bouarab R, et al. Hydrogen production with the cyanobacterium Spirulina platensis. Int J Hydrog Energy. 2017;42(8):4902–4907.

[34] Krishnamoorthy S, Manickam P, Muthukaruppan V. Evaluation of distillery wastewater treatability in a customized photobioreactor using blue-green microalgae – laboratory and outdoor study. J Environ Manage. 2019;234:412–423.

[35] Moreira J, Terra A, Costa J, et al. Utilization of CO₂ in semi-continuous cultivation of Spirulina sp. and Chlorella fusca and evaluation of biomass composition. Braz J Chem Eng. 2016;33(3):691–698.

[36] Kean MA, Delgado EB, Mensink BP, et al. Iron chelating agents and their effects on the growth of Pseudokirchneriella subcapitata, Chlorella vulgaris, Phaeodactylum tricornutum and Spirulina platensis in comparison to Fe- EDTA. J. Algal Biomass Util. 2015;6(1):56–73.

[37] Batac CC, Gathercole NS, Maravilla AF, et al. Evaluation of different carbonate sources for bicarbonate-based integrated carbon capture and algae production system using Spirulina platensis. IOP Conf Ser Mater Sci Eng. 2020;778:012041.

[38] Hossain N, Mahlia TMI. Progress in physicochemical parameters of microalgae cultivation for biofuel production. Crit Rev Biotechnol. 2019;39(6):835–859.

[39] Su CM, Hsueh HT, Tseng CM, et al. Effects of nutrient availability on the biomass production and CO₂ fixation in a flat plate photobioreactor. Aerosol Air Qual Res. 2017;17(7):1887–1897.

[40] Kona R, Hemalatha M, Venu Srivastav K, et al. Regulatory effect of Fe-EDTA on mixotrophic cultivation of Chlorella sp. towards biomass growth and metabolite production. Bioresour Technol. 2017;244(Pt 2):1227–1234.

[41] Chegukrishnamurthi M, Shahabazuddin M, Sreevathsan S, et al. Ozonation as a non-thermal option for bacterial load reduction of chlorella biomass cultivated in airlift photobioreactor. J. Clean. Prod. 2020;276(123029):123029.